ISSN 0031 - 9465

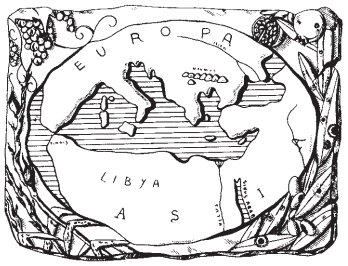
PHYTOPATHOLOGIA MEDITERRANEA

Plant health and food safety

Volume 64 • No. 2 • August 2025

The international journal of the
Mediterranean Phytopathological Union

FIRENZE UNIVERSITY PRESS



PHYTOPATHOLOGIA MEDITERRANEA

Plant health and food safety

The international journal edited by the Mediterranean Phytopathological Union
founded by A. Ciccarone and G. Goidànich

Phytopathologia Mediterranea is an international journal edited by the Mediterranean Phytopathological Union. The journal's mission is the promotion of plant health for Mediterranean climate and regions, safe food production, and the transfer of knowledge on diseases and their sustainable management.
The journal deals with all areas of plant pathology, including epidemiology, disease control, biochemical and physiological aspects, and utilization of molecular technologies. All types of plant pathogens are covered, including fungi, nematodes, protozoa, bacteria, phytoplasmas, viruses, and viroids. Papers on mycotoxins, biological and integrated management of plant diseases, and the use of natural substances in disease and weed control are also strongly encouraged. The journal focuses on phytopathology and closely related fields in the Mediterranean agro-ecological regions. The journal includes three issues each year, publishing Reviews, Original research papers, Short notes, New or unusual disease reports, News and opinion, Current topics, Commentaries, and Letters to the Editor.

PHYTOPATHOLOGIA MEDITERRANEA

The international journal of the Mediterranean Phytopathological Union

Volume 64, August, 2025

Firenze University Press

Phytopathologia Mediterranea. The international journal of the Mediterranean Phytopathological Union
https://www.fupress.com/pm
ISSN 0031-9465 (print) | ISSN 1593-2095 (online)
Published three times a year

Published by Firenze University Press

Firenze University Press
Università degli Studi di Firenze
via Cittadella, 7, 50144 Firenze, Italy
www.fupress.com

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Discovery of signature peptides through proteomic approach as potential biomarkers for root wilt infection in coconut trees

Perumalla Aman JAYAKER[1], Sundararajan VINO[1], Subramanian BABU[2,*]

[1] *School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore 632014, India*
[2] *VIT School of Agricultural Innovations and Advanced Learning (VAIAL), Vellore Institute of Technology, Vellore 632014, India*
*Corresponding author. E-mail: babu.s@vit.ac.in

Summary. Root wilt disease of coconut associated with phytoplasma presence is characterized by late symptoms in the field and hence disease detection has been challenging. Several attempts have been made in the past for detecting the infection, which included microscopic, histochemical, immuno assays and DNA based methods. However, the successful detection with precision and by a cost-effective simple assay is still not available. The current study used two-dimensional electrophoresis followed by mass spectrometric identification of the differentially or uniquely expressed proteins in the infected palms compared to healthy ones. Among the different proteins identified in the study, mannan endo-1,4-beta mannosidase and BTB/POZ domain and ankyrin repeat containing NPR2 proteins were selected. Bioinformatic analyses were carried out to characterize these proteins and the signature peptides with antigenic properties were determined. Biomarker protein structure prediction, homology modelling indicated the structure and function as well as uniqueness of these proteins. The sequences of these signature peptides are unique to these proteins and were found to be part of salicylic acid binding amino acid residues, thus involved in systemic acquired resistance against pathogens of plants. It is reported the procedure for obtaining signature peptides of potential biomarker proteins for detection of root wilt infection in coconut. The antibodies developed against these peptides would have more specificity for a precise detection of root wilt infection in coconut farms.

Keywords. Disease detection, phytoplasma, proteome biomarkers, root wilt, signature peptides.

Citation: Jayaker, P.A., Vino, S. & Babu, S. (2025). Discovery of signature peptides through proteomic approach as potential biomarkers for root wilt infection in coconut trees. *Phytopathologia Mediterranea* 64(2): 161-182. doi: 10.36253/phyto-15977

Accepted: May 1, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Assunta Bertaccini, Alma Mater Studiorum, University of Bologna, Italy.

ORCID:
PAJ: 0009-0000-5526-1708
SV: 0000-0002-0015-8460
SB: 0000-0003-2781-7136

INTRODUCTION

India accounts 32% of global coconut production. The Karnataka, Tamil Nadu, Kerala, and Andhra Pradesh are the major coconut growing states in India. Among them root wilt disease was reported in Tamil Nadu and Kerala. Similar diseases in coconut palms are also reported in other countries worldwide and described by various other names, including Bogia coconut

syndrome in Papua New Guniea (Miyazaki *et al*., 2018), Weligama coconut leaf wilt in Sri Lanka, Lethal bronzing in Texas, and lethal yellowing in Africa and Caribbean (Gurr *et al*., 2016).

Root wilt disease of coconut is associated with the presence of phytoplasmas and verified to be transmitted by *Haplaxius crudus* in the America continent while other planthoppers were only reported as infected with phytopalsmas (Dollet *et al*., 2020; Humphries *et al*., 2021; Bahder *et al*., 2023; Paredes Tomas *et al*., 2023; Fernandez-Barrera *et al*., 2024). Infected trees exhibit yellowing of leaves, flaccidity, necrosis of leaflets. Roots and inflorescence as well as leaf are rotting. The unopened pale-yellow spindle leaves are particularly susceptible to the disease presence (Ramjegathesh *et al*., 2019).

Bahder *et al.* (2020) characterized the lethal bronzing disease progression in date palms as early, moderate, and late-stage symptoms based on canopy discoloration at respective stages. However, irrespective of the disease stage, they observed the phytoplasma titer to be low in the leaves and high in the base of the trunk. Soto *et al.* (2020) recorded a higher phytoplasma titer leading to leaf collapse within four months.

Diagnosis of root wilt infection in coconut has been challenging for more than three decades. Microscopic observation of phloem tissues of infected plants (Solomon *et al*., 1983; Navratil *et al*., 2009) and histochemical analysis (Abdulsalam *et al*., 1993) were reported. ELISA based detection of phytoplasma proteins was developed later (Sasikala *et al*., 2001, 2005). Although universal primers for PCR amplification of phytoplasma genomic region was developed by Ceramic-Zagorac and Hiruki (1996), and a quantitative PCR system to amplify specific rRNA has been reported (Manimekalai *et al*., 2011).

However, the success rate in PCR based method is low due to the non-specific amplification in the complex metagenome of tender leaf tissue from phytoplasma infected coconut trees. Rather than targeting the organism for detection, proteomics offers an alternative strategy to identify differentially expressed proteins in the infected tissues which could serve as biomarkers of infection. The differentially expressed proteins may include i) pathogen derived proteins and or ii) host proteins in response to pathogen infection. In the current study, such biomarker proteins were specifically identified in the proteome of infected leaf samples. Signature peptides which are unique to the protein and do not show homology with any other peptides of other proteins were also analyzed and identified to improve the specificity of the detection technique.

MATERIALS AND METHODS

Sample collection

The tender leaf tissues from crown region of healthy and root wilt infected coconut trees were collected from two different coconut growing regions of Tamil Nadu, India namely Pollachi and Vellore, which are in the extreme west and north of the state with a distance of 267 miles. In each of these districts, root wilt infected and uninfected farms were chosen for sample collection. In Pollachi, healthy leaf tissues were from 'Tall × Dwarf' variety trees cultivated in Aliyar village and infected leaf tissues were from 'Chowghat orange dwarf' variety trees cultivated in Zamin Uthukuli village which are 15 miles away. In Vellore region, infected and healthy samples were collected from Pallikonda and Brahmapuram villages respectively, which are 17 miles away. Tender leaf tissues from the crown region of ten coconut trees from healthy and infected farms were collected. All the samples were carried to the laboratory in polyethylene bags in ice container. The samples were surface sterilized using 70% ethanol and stored at -80°C.

PCR confirmation of infected and healthy samples

DNA was extracted from tissue samples using the modified CTAB method as described by de Silva *et al.* (2023). Nested PCR was performed using two sets of primers namely, P1/P7 primers (Deng and Hiruki, 1991; Schneider *et al*., 1995) for the first PCR and R16F2n/R16R2 (Lee *et al*., 1995; Gundersen and Lee, 1996) for the second PCR. The 20 µL reaction mixture contained 2 µL of template DNA, 0.5 µM of each primer, 200 µM of dNTP and 10 µL of 1× master mix (Ampliqon, Denmark) and nuclease free water. The PCR conditions for P1/P7 primers are: initial denaturation step for 2 min at 94°C, followed by 40 cycles for 30 s at 94°C, annealing at 53°C for 30 s and extension at 72°C for 1.5 min, and final extension at 72°C for 10 min. The conditions for the second round of PCR with R16F2n/R16R2 primers are the same except 50°C for 30 s and 72°C for 1 min, for annealing and extension respectively. PCR reactions were carried out in a thermal cycler (BioRad, Singapore). The amplicons were analyzed in 1% agarose gel and stained with ethidium bromide.

Extraction of proteins from coconut leaf tissues

Phenol-SDS method: One gram of leaf tissue was homogenized using mortar and pestle in the presence

of liquid nitrogen followed by 5 mL of extraction buffer (0.1 M Tris HCl (pH 7.5), 30% (w/v) sucrose, 2% (w/v) SDS). The resulted slurry was transferred to sterile centrifuge tubes and subjected to vortexing with intermittent cooling on ice for 5 min. Equal volume of tris saturated phenol was added and contents were mixed by inverting the tubes up and down and allowed to stand on ice for 10 min and centrifuged at 12,000 rpm for 15 min. The organic phase obtained on top was collected in a separate tube to which 4 volumes of 0.1 M ammonium acetate in methanol was added and mixed by inverting the tubes followed by overnight incubation at -20°C. Samples were further centrifuged at 12,000 rpm for 15 min and the pellet was dissolved in 80% acetone containing 0.07% DTT vortexed and centrifuged at 12,000 rpm for 15 min. Acetone wash was repeated two more times. The pellet was air dried and dissolved in the rehydration buffer (8 M Urea, 2% CHAPS, 50 mM DTT, 0.2% ampholyte, bromophenol blue 20 µL). After sonication at 40% power rate, 5 sec burst with 10 sec time interval and incubated at 4°C overnight. Contents were centrifuged at 12,000 rpm for 15 min and the supernatant obtained was retained and stored at -80°C until further use.

2-D-Gel electrophoresis, Mass Spectrometry, and annotation

The quantified protein concentrations were normalized to 100 µg using rehydration buffer and subjected to IPG strip rehydration and first-dimension electrophoresis in a Protean IEF unit (Biorad, USA). Focusing of strips were programmed as follows: 50 µA limit per strip; 250 V for 15 min; 4000 V for 2 h; 4000 V for 5 h (20,000 Vh); 500 V hold step. The strips were equilibrated with buffers I with DTT and buffer II with iodoacetamide (Babu *et al*., 2005). The strips were placed on polyacrylamide separating gels with a high range protein molecular weight marker. SDS-PAGE was carried out in 12% and 15% separating gels at a constant voltage of 50 V. The electrophoresis continued until the dye front reached the bottom of gel. The gels were stained overnight with Coomassie brilliant blue R-250 followed by silver stain. Gel images were compared using PD Quest software and spot intensity was quantified using ImageJ software. Differentially expressed protein spots were selectively excised and subjected to MALDI TOF/TOF Mass spectrometry analysis at Sandor Lifesciences-Hyderabad, India. The spectrum search was performed using Mascot against Swissport 2019_09 -*Veridiplantae* database and protein hits were obtained at p value <0.05 confidence.

The functional annotation of the identified proteins was performed using QuickGo online server and MapMan "Bin" ontology in Mercator pipeline Version 3.0. The Conserved Domain Database, a component of NCBI's Entrez was used to annotate protein sequences and the conserved domains of the proteins were predicted. The data was used to draw a domain graph using TBtools for visualization.

Protein selection and in silico *characterization*

The differentially expressed proteins were analyzed using protein sequence motif search tool (https://www.genome.jp/tools/motif/MOTIF.html) of Prosite and Pfam databases. Based on the literature search on role of these proteins during plant-phytoplasma interactions, BTB/POZ domain and ankyrin repeat-containing protein NPR2 and Mannan endo-1, 4-beta-mannosidase were selected as biomarkers for phytoplasma disease in coconut.

The template for the identified query proteins was obtained by performing database search using NCBI-blastp tool (BLAST: Basic Local Alignment Search Tool (nih.gov)) against standard protein database, with Viridiplantae/Green plants (taxid:33090) as organism name. The templates were selected with sequence similarity above 45%. Further the protein structure was predicted *via* template-based homology modelling using Swiss modeller (https://swissmodel.expasy.org/). The alpha fold predicted structure obtained from uniport (UniProt)were considered for the biomarker protein having homology cover less than 40%.

Identification of signature peptides and their antigenicity

With a long-term objective developing antibodies for detection of root wilt infection in coconut trees, unique peptides in the biomarker with antigenicity were identified. The biomarker protein sequence was used to find potent antigens and subunit vaccines using VaXiJen v 2.0 server (VaxiJen (ddg-pharmfac.net)). Whereas the allergenicity was predicted using AllerHunter server (http://allerdictor.vbi.vt.edu/). Further the linear B-cell epitope prediction using the Immune Epitope Database (IEDB) (http://tools.immuneepitope.org/bcell/) module of Kolaskar and Tongaonkar (Kolaskar and Tongaonkar, 1990) was employed. With 75% accuracy, the results predicted the epitopes. Additionally, the IEDB was used to predict the characteristics of B-cell epitopes, including the predictions of Emini surface accessibility, Parker, Karplus and Schulz flexibility, and Bepipred linear epitope. The results also supported Chou and Fasman's prediction that the antigenic portions of the

proteins are restricted to beta turn regions. For a comprehensive compression, B-cell epitopes were further predicted based on artificial neural network http://crdd.osdd.net/raghava/abcpred/ABC_submission.html and BepiPred-2.0 (Jespersen *et al*., 2017). Further, the B-cell continuous epitopes were predicted using secondary structure of biomarker proteins in PDB format as input in ElliPro server (http://tools.iedb.org/ellipro/). Using a support vector machine tool (http://sysbio.unl.edu/SVMTriP/prediction.php) the predicted linear epitopes having similar amino acids with a length ranging from 10-15 residues were selected. The epitopes were manually searched against Uniport protein database to validate them as signature peptides specific to the biomarker protein. All the analysis was performed under unaltered default settings.

RESULTS

Collection of root wilt infected and healthy coconut leaf samples

The samples are collected from two different districts based on the field survey and observation of symptoms of the disease. The healthy and root wilt infected coconut trees and the wilting symptom on the infected leaves are shown in Figure 1. To confirm the infected and healthy situation of the trees from which samples were collected for proteome analysis, DNA was extracted from the tissues and PCR was performed using the above listed primers targeting the 16S rRNA region of phytoplasma genome, under nested PCR conditions. The expected amplicon of 1800 bp from PCR was used as template for the nested PCR with R16F2n/R16R2 which resulted in an amplicon size of 1250 bp (results not shown).

Differentially expressed proteins

Out of the two protein extraction methods followed, phenol-SDS method resulted in higher protein concentration in the samples indicating the efficiency of extraction and solubilization. Hence, for all the samples, proteins extracted by phenol-SDS method was used for two-dimensional gel electrophoresis. The two-dimensional electrophoresis gel images of proteins solubilized from healthy and infected leaf tissues are presented in Figure 2. SBA is the label given to all the differentially expressed protein spots from another replication (gel images not shown). Among all these samples, the number of differentially expressed proteins were 8 in leaf tissues from healthy leaves and 21 in infected leaves. MALDI-TOF/TOF MS analysis revealed the identity of the proteins and are listed in Table 1.

Compared to the infected leaf samples, the most abundant proteins in healthy leaf samples includes ubiquitin protein transferase, magnesium dependent protein

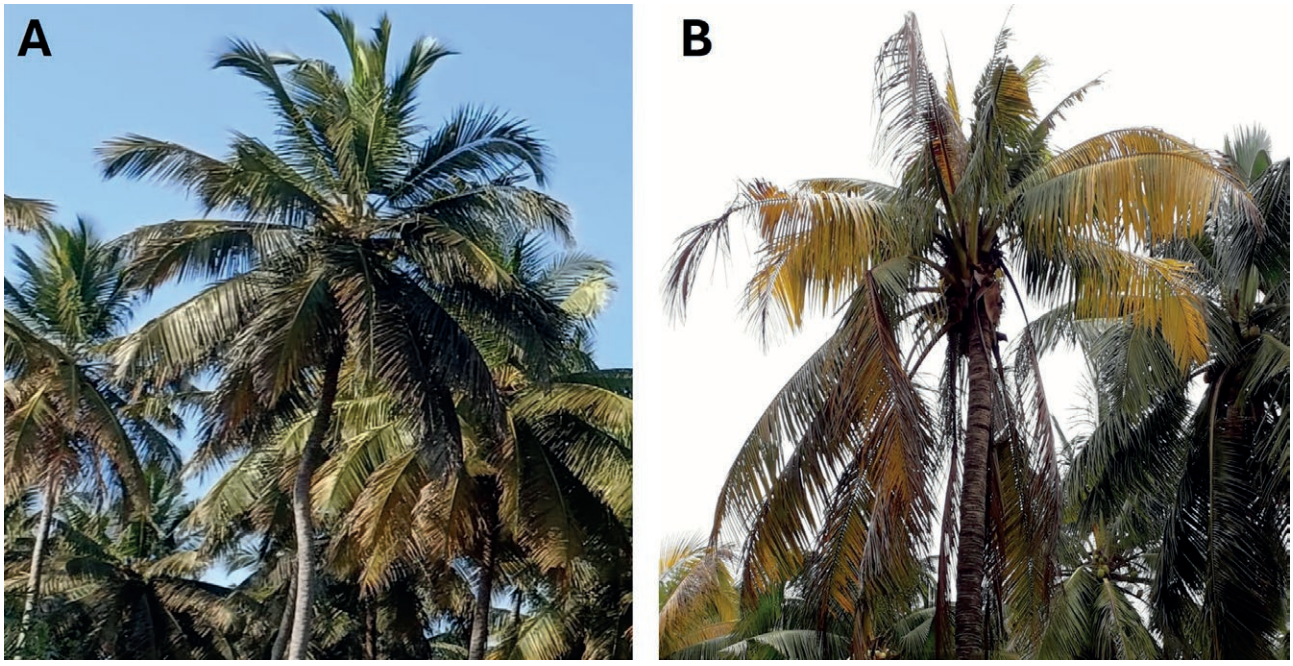


Figure 1. Field symptoms of root wilt infection in coconut. (A) Leaves of healthy coconut tree. (B) Leaves of root wilt infected coconut tree.

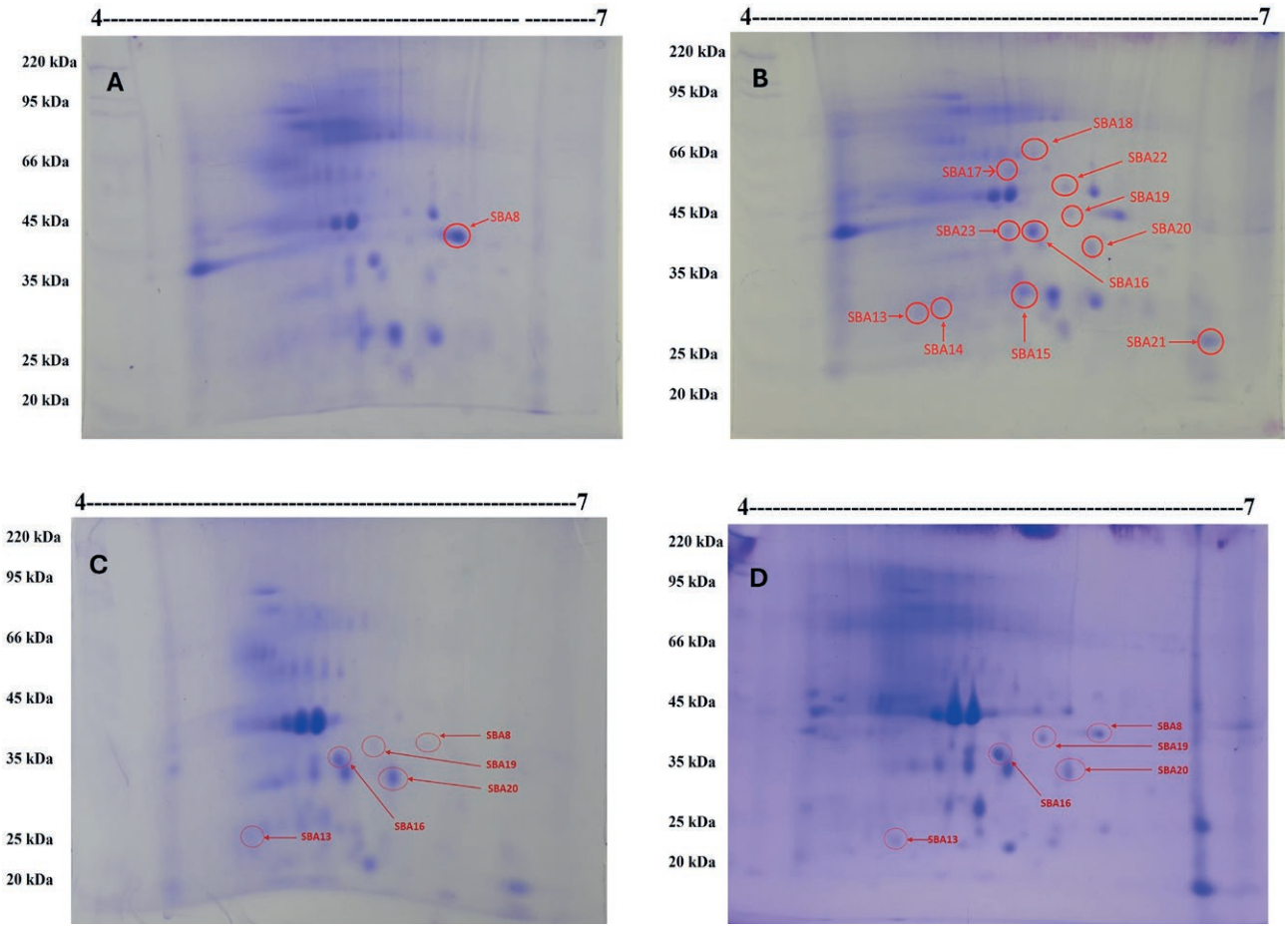


Figure 2. Two-dimensional electrophoretic analysis of healthy and infected coconut leaf proteome (Samples from Vellore and Pollachi, Tamil Nadu – Coommassie stained). (A) Leaf proteins from uninfected coconut trees. (B) Leaf proteins from root wilt infected coconut trees.

serine/threonine phosphatase, mannitol dehydrogenase, structural protein of cytoskeleton, co-localizing protein with bZIP18, in the nucleoplasm, GTPase activator and glycogen synthase, in addition to the house-keeping genes like DNA binding, RNA binding, photosynthesis, zinc ion binding. Table 1 reports the details of the mass spectrometry analysis.

The functional grouping of proteins (Figure 3) in the leaf tissues of healthy coconut trees indicated that 29% of proteins were found to be involved in protein modification, 20% in secondary metabolism, 10% in glycolysis, 10% in amino acid metabolism, 10% in cell wall organization and 26% in enzyme classification. In the leaf tissues of root wilt infected trees, 4% of proteins were found to be involved in processes like photosynthesis, glycolysis, nucleotide metabolism, DNA damage response, signaling, development, carbohydrate metabolism, stress response, C1-metabolism, and cell division. About 8% of proteins were related to hormone metabolism and RNA processing. About 12% were involved in protein modification and 20% in secondary metabolism. Among all identified proteins, homeobox-leucine zipper protein ROC4 was predicted as transcription factor responsible for drought resistance in rice. The majority of differentially expressed proteins were localised to cytoplasm followed by nucleus and chloroplast in both the root wilt infected and healthy coconut leaf samples (Figure 3).

Biomarker proteins

The conserved domains of all the identified proteins in both healthy and root wilt infected samples were predicted, which revealed the amino acid sequence associated with the predicted conserved domain cluster. The conserved domains of the differentially expressed proteins are presented in Figure 4. The amino acid residues not falling in any of the groups of conserved domain were used in identification of signature peptides. The two proteins *viz*., mannan endo-1, 4-beta-mannosidase and

Table 1. Differentially expressed proteins in healthy and root wilt infected coconut leaf tissues.

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA1	PUB73_ ORYSJ	U-box domain-containing protein 73 (*Oryza sativa Japonica* group)	K.AKADMSGLQR.S K.AKADMSGLQR.S R.MLDAGGMKMLR.G R.MLDAGGMKMLR.G R.QLSSDRLPMQK.M R.LTIILNLTMHR.Q K.NMEDFIERLR.Q R.LTIILNLTMHR.Q R.LPMQKMLQVER.I R.TLSDAFPAPTVRGR.C K.VASIVAVLSEFDMFR.K K.DTEVITEAATAILALYADGEGEQPAR.F	13	173	Healthy	Upregulating	Ubiquitin-protein transferase activity	36	64	4.80
SBA2	P2C44_ ORYSJ	Probable protein phosphatase 2C 44 *(Oryza sativa Japonica Group)*	K.DDISCIVIR.F K.ANLFCNILK.E K.DMWVANVGDSR.A+Oxidation (M) K.DDISCIVIRFR.C K.QLGPGGSTAVTAIVVDGK.D R.HVPINSSIEFVILASDGLWK.V R.AVVCERGAANQLTVDHEPHTTNER.Q	7	131	Healthy	Upregulating	Magnesium-dependent protein serine/threonine phosphatase activity	28	35	4.70
SBA3	MTDH_ MEDSA	Probable mannitol dehydrogenase (*Mucuna pruriens*)	K.YYGMTEPGK.H R.ENGDDDVSVK.I K.MHEINTAMER.L+2 Oxidation (M) K.HNITADIELIK.M -.MAKSPETELPLK.A K.ETQEMLDFCGK.H K.ETQEMLDFCGK.H+Oxidation (M) K.SPETELPLKAFGWAAR.D K.AFGWAARDTSGTLSPFHFSR.K K.LNGKLVTVGLPSKPLELSVFPLVAGR.K	10	142	Healthy	Upregulating	Mannitol dehydrogenase activity	32	39	5.00
SBA4	TBB2_ MAIZE	Tubulin beta-2 chain (*Zea mays*)	R.HGRYLTASAMFR.G+Oxidation (M) R.MMMTFSVFPSPK.V+2 Oxidation (M) R.LHFFMVGFAPLTSR.G+Oxidation (M) R.EILHIQGGQCGNQIGSK.F R.GLSMSSTFVGNSTSIQEMFR.R+2 Oxidation (M) R.EEYPDRMMMTFSVFPSPK.V+2 Oxidation (M) K.SSVCDIPPRGLSMSSTFVGNSTSIQEMFR.R+2 Oxidation (M)	7	61	Healthy	Upregulating	Structural constituent of cytoskeleton	34	50	5.10
SBA5	PP13_ TOBAC	Serine/threonine-protein phosphatase PP1 isoenzyme 3 (*Nicotiana sylvestris*)	IYGFYDECK IKYPENFFLLR QLCVASRDIFLK SSNPGKLVQLSESEIK QSLETICLLLAYKIK ILCMHGGLSPDLSSLDQIR NLPRPTAIPDTGLLCDLQRSDPGK	7	61	Healthy	Upregulating	Protein serine/_ threonine phosphatase activity	28	35	5.15

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA6	NEAP1_ ARATH	Nuclear envelope-associated protein (*Arabidopsis thaliana*)	R.MTQLGHQLDDLQR.G+Oxidation (M) .MSYSEKTTVDPLLR.D R.DMEIKEIRDLISEK.Q+Oxidation (M) K.LVSQEQSFLKETITR.K K.IVVSMSMLMLVVVSKR.-+2 Oxidation (M) K.IVVSMSMLMLVVVSKR.-+3 Oxidation (M) K.IQCSMLKQQLDDKTR.S K.IQCSMLKQQLDDKTR.S+Oxidation (M) K.LLEDVSPMKFERMNR.L+2 Oxidation (M) R.MTQLGHQLDDLQRGLSLR.E+ Oxidation (M) R.EQEDRMTQLGHQLDDLQR.G K.AGIGGMDSELQKLLEDVSPMK.F+ Oxidation (M) K.AGIGGMDSELQKLLEDVSPMK.F+2 Oxidation (M) K.FWDNSGFKIVVSMSMLMLVVVSK.R K.FWDNSGFKIVVSMSMLMLVVVSK.R+2 Oxidation (M) K.FWDNSGFKIVVSMSMLMLVVVSK.R+3 Oxidation (M)	16	114	Healthy	Upregulating	Colocalized with bZIP18 in the nucleoplasm	23	39	5.08
SBA7	RGAP4_ ARATH	Rho GTPase-activating protein 4 (*Arabidopsis thaliana*)	R.EEEEEEEERSEK.E R.INGENGQEEYIREELNK.G R.APSASATVFGVSTESMQLSYDTR.G K.EESADEEEEECAESVELVDIKK.S K.SSQSCHFPSPSSSSSTSCGGGNDGSNR.D	5	100	Healthy	Upregulating	GTPase activator activity	18	49	6.40
SBA8	SSG1_ SOLTU	Granule-bound starch synthase 1, chloroplasctc/ amyloplastic (*Solanum tuberosum*)	K.NCMSEELSWKEPAK.K K.GMNLIFVGTEVGPWSK.T R.GVDRVFVDHPMFLEK.V R.GVDRVFVDHPMFLEK.V + Oxidation (M) K.EFEQEIEQLEVLYPNK.A K.GVAKFNVPLAHMITAGADFMLVPSR.F + Oxidation (M) K.GSDILVAAIHKFIGLDVQIVVLGTGK.K K.FNVPLAHMITAGADFMLVPSRFEPCGLIQLHAMR.Y + 2 Oxidation (M)	8	69	Healthy	Upregulating	Glycogen (starch) synthase activity	39	67	6.30
SBA9	FEN1_ OSTLU	Flap endonuclease 1 (*Ostreococcus lucimarinus)*	K.EHGSIEKILEEIDTEK.Y R.LESFFGPPTIISSTIGKR.K R.VAIDASMHIYQFMMVVGR.Q + Oxidation (M) R.ELFKNPEVMDTTGIALSWK.A R.RVAIDASMHIYQFMMVVGR.Q K.AGLVWAVATEDMDTLTFAAPRLAR.N + Oxidation (M) R.VAIDASMHIYQFMMVVGRQGEQQLTNEAGEVTSHLQGMLNR.T	7	101	Infected	Upregulating	magnesium ion binding	28	43	4.30
SBA10	DHSO_ ARATH	Sorbitol dehydrogenase (*Arabidopsis thaliana*)	R.MKAVGICGSDVHYLK.T + Oxidation (M) K.VCLVGMGHGIMTVPLTPAAAR.E + 2 Oxidation (M) R.AEVGPETNVLVMGAGPIGLVTMLAAR.A R.CGGKVCLVGMGHGIMTVPLTPAAAR.E + 2 Oxidation (M) K.GGMSQGEGSKVEEENMAAWLVGINTLK.I + 2 Oxidation (M) R.AEVGPETNVLVMGAGPIGLVTMLAARAFSVPR.A	6	92	Infected	Upregulating	oxidative activity	27	40	4.50

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA12	TDC1_ ORYSJ	Tryotophan decarboxylase 1-like (*Oryza sativa Japonica* Group)	K.TGKAYVAHTVVGGR.F K.LWMVMRTYGVAK.L + Oxidation (M) R.SYLHKAVDFISDYYK.S R.FEVVVPRNFALVCFR.I R.ASPPTYSAPFDVTMKELR.S + Oxidation (M) K.NHASDSGEVTDLKDMQVGVGR.R K.WLMTCLDCTCLYVRDTHR.L R.LAGFDPANIRSIPTGAETDYGLDPAR.L	8	101	Infected	Upregulating	L-tryptophan decarboxylase activity	26	56	5.20
SBA13	Q0JJ01	BTB/POZ domain and ankyrin repeat-containing protein NPR2 *(Oryza sativa)*	K.EVDLNETPVTQNK.R R.RYFPNCSQVLDK.F R.STFFYNLFAARGR.G K.NSRGYTALHLAAMR.R K.SQPNEGDTVISDPVHEK.R K.SQPNEGDTVISDPVHEKR.V R.ADNSMFSILSSSSSSSPPPK.V R.ADNSMFSILSSSSSSSPPPK.V + Oxidation (M) K.VAMQIAQADTTPEFGIVPAASTSGK.L K.VAMQIAQADTTPEFGIVPAASTSGKLK.E R.EMIRKPMAVEDSVTSPLLADDLHMK.L	11	76.6	Infected	Upregulating	Salicylic acid signaling	39	69	5.9
SBA14	Q7Y0V9	Homeobox-leucine zipper protein ROC4 *(Oryza sativa)*	R.DQGITSASSTANMNCR.R R.DQGITSASSTANMNCR.R + Oxidation (M) K.LVGLTGNIGEDVHVMARK.S + Oxidation (M) K.QLADGVWAVVDVSADELMR.D + Oxidation (M) R.ESGIVIIDDGAALVETLMDER.R R.ESGIVIIDDGAALVETLMDERR.W	6	48.3	Infected	Upregulating	DNA-binding	25	87	6.5
SBA15	Q09FV4	ATP synthase subunit beta, chloroplastic (*Nandina domestica*)	R.TREGNDLYMEMK.E + Oxidation (M) R.INPTTSGPGVSALEEKNQGR.I K.GIYPAVDPLDSTSTMLQPR.I + Oxidation (M) R.GMEVIDTGAPLSVPVGGATLGR.I K.ELQDIIAILGLDELSEDDR.L R.MPSAVGYQPTLSTEMGSLQERITSTK.E + 2 Oxidation (M) K.MPNIYNALVVKGQDTVGQQINVTCEVQQLLGNNR.V	7	100	Infected	Upregulating	ATP binding	33	53	5.1
SBA16	Q9GI85	Maturase K *(Adesmia lanata)*	K.GLYRIK.Y R.MYQQNHLLLFANDSKK.N + Oxidation (M) R.QFISSLEDAETIKSFNNLR.S R.IKQLLSEHSFHFFWGGYISNVR.L	4	61	Infected	Upregulating	RNA-Binding	39	61	5.3
SBA17	D5JBX0	Germacrene A hydroxylase *(Helianthus annuus)*	K.LLTRPTSSKNR.L R.QAMNLAGYDVANKTK.L + Oxidation (M) R.ECRQAMNLAGYDVANK.T K.LDSLINNLVAEHTVSKSSK.V K.LPNGASHDQLDMTESFGATVQRK.T K.LPNGASHDQLDMTESFGATVQRK.T + Oxidation (M) R.LPIIGHMHHLIGTMPHRGVMDLAR.K + Oxidation (M) R.RMCPGSALGLANVQLPLANILYYFK.W + Oxidation (M)	8	80.5	Infected	Upregulating	heme binding	45	55	5.1

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA18	Q8GVE8	Phosphoenolpyruvate carboxylase 4 (*Arabidopsis thaliana*)	R.IVLGEVK.E R.RDEDNNK.L K.QLTSEISK.M K.LFEESQVGK.T K.VHNVTQLAR.S R.QKPTPVDEAR.A K.MPLEEALTLAR.T + Oxidation (M) R.AGLNIVEQSLWK.A R.MAGIEDTANLLEK.Q + Oxidation (M) R.EVGNPFMEKVER.I R.EVGNPFMEKVER.I + Oxidation (M R.AIPWVFAWTQTR.F R.AHLPACIDFGESRHTK.F R.NLMEEISGISCQHYR.S + Oxidation (M) K.EVSLLSRWMAIDLYIR.E K.ELMTTEKYVLVISGHEK.L R.SGLTSRGSFSSTSQLLLQR.K R.VVPLFETVNDLRAAGPSIR.K K.DARLALTSEHGKPCPGGTLR.V R.HTKFEIATTDYMPPNLQK.Q + Oxidation (M) K.QNEQDFSESDWEKIDNGSR.S R.MAGIEDTANLLEKQLTSEISK.M + Oxidation (M) K.SSSGIGHLRAIPWVFAWTQTR.F R.ILAQSALNLRMAGIEDTANLLEK.Q + Oxidation (M) R.EHIQKNHNGHQEVMVGYSDSGK.D + Oxidation (M) K.FTGKPLPLTCTPMKFGSWMGGDR.D R.GGSIGRGGGPTYLAIQSQPPGSVMGSLR.S + Oxidation (M) R.HSEALDAITTYLDMGTYSEWDEEKK.L	28	130	Infected	Upregulating	phosphoenolpyruvate carboxylase activity	50	62	5.40
SBA19	Q8W2X5	Flavanone 3-dioxygenase 2 *(Oryza sativa)*	K.YRSVWHR.A K.LYSDDPAKK.I R.AVVNSDRER.M K.AKLYSDDPAK.K R.EFFRLPAEEK.A K.EIIGTYCTEVR.E R.MSVASFLCPCNSVELGPAK.K + Oxidation (M) R.MSVASFLCPCNSVELGPAKK.L + Oxidation (M) R.ERMSVASFLCPCNSVELGPAK.K .MAAEAEQQHQLLSTAVHDTMPGK.Y + Oxidation (M) K.WIAVNPQPGALVINIGDQLQALSNGK.Y R.THGFFQVVNHGIDAALIASVMEVGREFFR.L + Oxidation (M)	13	102	Infected	Upregulating	Dioxygenase activity	38	39	5.60

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA20	F4JUY5	Gibberellic acid methyltransferase 1	R.YAVRAAADK.E K.GGVWIEGAEK.E K.DLVEFLKCR.K R.SLDEKVNGSSR.K K.YFAAGVPGSFYK.R K.RDGFNIPVYFR.T R.KYFAAGVPGSFYK.R K.SWNKGGVWIEGAEK.E R.LALSKPMLTTAINSIK.L + Oxidation (M) R.SLEHVLSMQGGEDDASYVK.N R.DGFNIPVYFRTTEEIAAAIDR.C R.ANYAQAGLKPIVQAYLGPDLTHK.L R.AAADKEILNNCFYHMIAVSAVR.V + Oxidation (M) K.NCYGPAARLALSKPMLTTAINSIK.L	7	55.5	Infected	Upregulating	gibberellin A9 carboxyl methyltransferase activity	36	18.7	5.70
SBA21	O65726	Squalene monooxygenase 1,2 *(Brassica napus)*	K.GLDEGSHIK.I R.NSIAPQVPLK.L R.QKASSLPNVR.L R.FFHNGRFVQR.L R.LLKPLGNLGDENK.V R.QGCFDYLSSGGFR.T K.ASSLPNVRLEEGTVR.S K.NLPSVSNGEMTSFVR.N + Oxidation (M) R.EPVRMMGEFMQPGGR.L + 2 Oxidation (M) K.AEGIGQMLSPTNAAAYR.K + Oxidation (M) R.MMGEFMQPGGRLMLSK.L K.EAMRQGCFDYLSSGGFR.T + Oxidation (M) R.MLLVFVLSWTIFHVNNRK.K + Oxidation (M) K.MLVPHLKAEGIGQMLSPTNAAAYR.K + Oxidation (M) K.NLPSVSNGEMTSFVRNSIAPQVPLK.L + Oxidation (M) R.KPMSATVNTLGNSFWQVLIASTDEAK.E + Oxidation (M) R.KPMSATVNTLGNSFWQVLIASTDEAKEAMR.Q + Oxidation (M)	17	100	Infected	Upregulating	Oxidoreductase	20	57	6.50
SBA22	P18026	Tubulin beta-2 chain *(Zea mays)*	R.MMMTFSVFPSPK.V + 2 Oxidation (M) R.LHFFMVGFAPLTSR.G + Oxidation (M) K.NSSYFVEWIPNNVK.S R.SLTVPELTQQMWDSK.N R.EILHIQGGQCGNQIGSK.F R.GLSMSSTFVGNSTSIQEMFR.R + 2 Oxidation (M) K.SSVCDIPPRGLSMSSTFVGNSTSIQEMFR.R + 2 Oxidation (M)	7	114	Infected	Upregulating	structural constituent of cytoskeleton	41	50	5.50

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBA23	A9RFQ5	Translation factor GUF1 homolog, chloroplastic *(Physcomitrella patens)*	K.FITENR.A R.GVVVYFR.V K.AANKGVDNGK.D K.IDLPGADPER.V K.VIASENIAAMRK.D R.NFSIIAHIDHGK.S K.AYSVGRALTQQLK.K R.SGSRYLHDLEAGR.S K.GYASMEYSVKGYR.E + Oxidation (M) R.EYGLDLITTAPSVVYR.V R.VDVPQDAFMAILRLEK.E + Oxidation (M) R.EIEEIIGLDCSEAILCSAK.E K.IPIQACIGSKVIASENIAAMR.K + Oxidation (M) R.REIEEIIGLDCSEAILCSAK.E	14	74.3	Infected	Upregulating	GTPase activity	30	82	5.10
SBA24	Q9FI48	Putative F-box protein (*Arabidopsis thaliana)*	K.ACLMSVNLHNHK.D + Oxidation (M) K.LMVWNPYLGQTR.W + Oxidation (M) K.IELNIVLWSKFLK.V K.KVAMVSELDIETCK.N + Oxidation (M) K.DNSKLMVWNPYLGQTR.W + Oxidation (M) -.MSTMSDLFPDLVEEILSR.V R.FYMGYDSNNNHKILWFSSMYR.E + 2 Oxidation (M) R.EEQLAVLFQKSDAYEMGIWITTK.I + Oxidation (M)	8	89.0	Infected	Upregulating	Unknown	39	44	5.90
SBSR10	Y1814_ ARATH	Uncharacterized Methyltransferase	K.NRGEK.K K.YAAQR.R R.QEIMR.Y R.VVFVSR.S K.EKLVLVR.A M.PMTVVSGR.F K.ACGLVNFTR.V K.NLRQEIMR.Y R.TPLVSFLYER.G	9	50	Healthy	Upregulating	methyltransferase activity	39	29	4.50
SBSR11	YCF4_ PELHO	Phytosystem I assembly protein	R.FLMK.D R.EIEQK.A R.RIFLR.F R.WGFPGR.N K.DIQSIR.I R.EGFYAR.R R.GQGAIPLTR.T K.EGIVCIFR.W R.IFLRFLMK.D	9	43	Infected	Upregulating	photosynthesis	21	66	5.00

(Continued)

Table 1. (Continued).

Protein ID	Accession No.	Protein	Peptides Matched	No. of peptides matched	Score	Sample type	On gel expression level	Molecular function	Experimental molecular mass (kDa)	Calculated molecular mass (kDa)	pI
SBSR12	MAN2_ ORYSJ	Mannan endo-1, 4-beta-mannosidase	K.VYLK.T R.LAAVR.Y R.QYVR.W K.FMTR.W K.SIDKK.H K.TLIMR.K R.DVLYR.A R.VSSMFR.T K.TLIMRK.N R.VSSMFRTAVSMGLTVCR.T	10	44	Infected	Upregulating	mannan endo-1,4-beta-mannosidase activity	50	44	5.30
SBSR13	CDA4_ ARATH	Probable inactive cytidine deaminase	M.TQQLK.F R.EEAASK.G K.FILTR.E R.ALTAANK.S K.SNAQYSK.C K.VSQEATAR.I R.APISGVQDAVLGLASSDR.I	7	51	Infected	Upregulating	zinc ion binding	27	33	5.70
SBSR14	PEN7_ ARATH	Putative pentacyclic triterpene synthase	R.SQYK.A K.KLIR.E R.HRTK.E K.QFPR.H K.EMLR.Y R.WPFK.K R.SMLIK.G R.GLVAAGK.T K.MDVER.L R.KEMLR.Y R.MQFLR.E K.WIIDR.G K.GYSFLR.K R.YIPIIK.N K.SACARAR.K R.GGATYTPLFGK.A K.TYQSYEPIR.R R.IMVDPDHDR.K	17	49	Infected	Upregulating	lanosterol synthase activity	89	27	5.90
SBSR15	YCF4_ CHLAT	Phytosystem I assembly protein	K.SDLIR.R K.AANLAR.F R.VIYLK.I K.EGINPR.R K.QDGIVR.I R.WGFPGK.N R.EIPLTR.I P.DPVLGSR.R K.SDLIRR.D K.EGINPRR.V R.VIYLKIK.G	11	62	Infected	Upregulating	photosynthesis	21	26	5.20

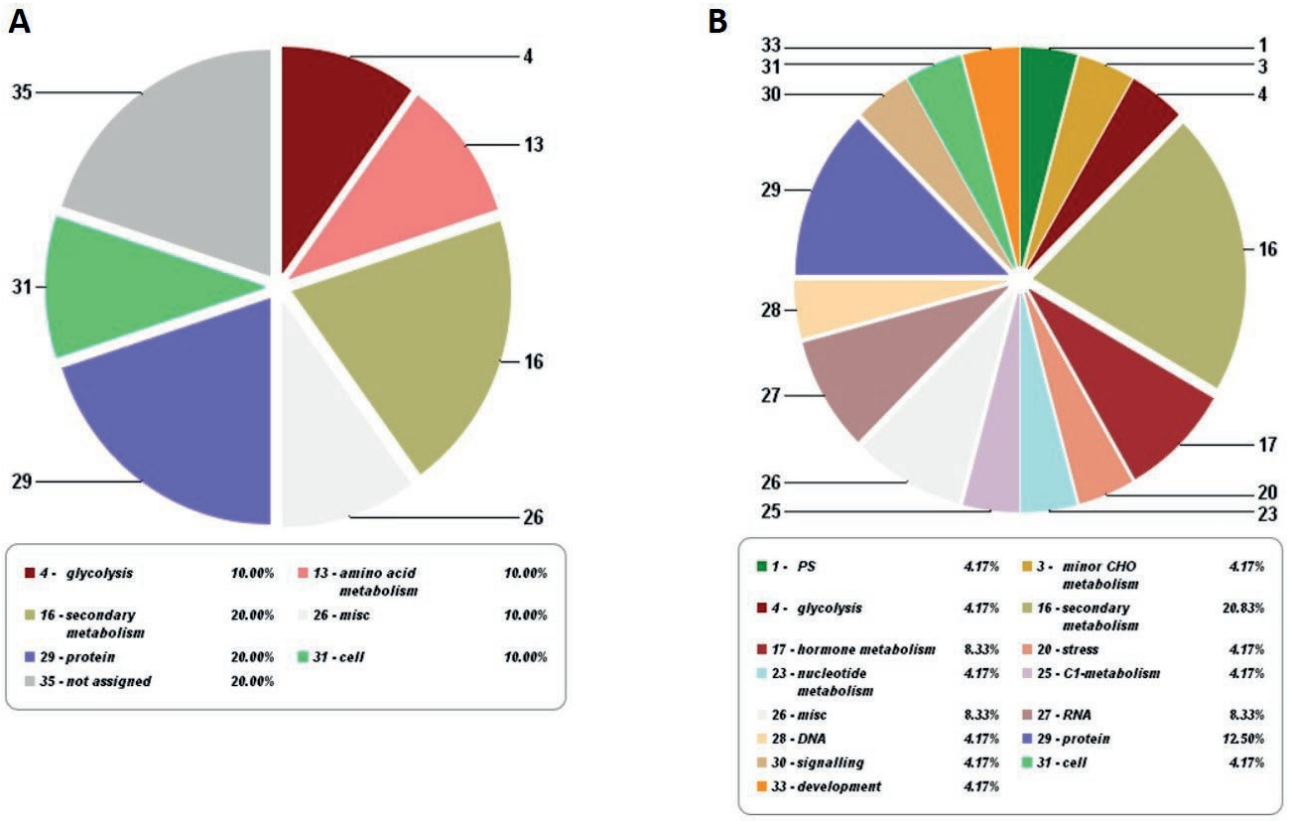


Figure 3. Functional classification of differentially expressed proteins in leaf tissues of coconut. (A) Leaf proteins from uninfected coconut trees. (B) Leaf proteins from root wilt infected coconut trees.

BTB/POZ domain and ankyrin repeat-containing protein NPR2 were selected as candidate biomarker proteins as they are functionally associated with phytoplasma infection in various plants. The conserved motifs in these potential biomarker proteins are presented in Figure 5.

The candidate protein sequence annotation, as determined by Prosite, revealed three motif profiles for the BTB/POZ ankyrin repeat protein: a circular ankyrin repeat region at amino acid positions 346-418, a BTB domain at positions 97-191, and an additional ankyrin repeat at positions 380-412. Pfam analysis disclosed six profiles for NPR1/NIM1-like defense protein C-terminal (422-626), domain of unknown function (276-323), ankyrin repeats (3 copies-323-407), multiple ankyrin repeats (350-392), ankyrin repeat (381-409), and BTB/POZ domain (97-194). The numbers indicate amino acid position in the protein sequence.

Gene ontology terms assigned by GO Central indicated the protein's involvement in defense responses to bacteria (GO:0042742), defense responses to fungi (GO:0050832), nuclear activity (GO:0005634), regulation of jasmonic acid-mediated signaling pathways (GO:2000022), and systemic acquired resistance mediated by salicylic acid signaling pathways (GO:0009862). Mercator annotation characterized its function as related to salicylic acid perception and signal transduction receptor protein (NPR3/4).

Furthermore, this study identified NPR-related peptides containing a conserved ethylene-responsive element-binding factor-associated amphipathic repression (EAR) motif sequence, notably VDLNE, which interacts with salicylic acid binding residues within NPR proteins such as VAMQIAQADTTPEFGIVPAASTSGK, TGKAYVAHTVVGGR, and RYFPNCSQVLDK. These residues have been reported to exhibit affinity towards salicylic acid. In our Mass spectrometry results (Table 1), the same peptides were observed in this BTB/POZ domain and ankyrin repeat-containing protein NPR2.

The biomarker protein mannan endo-1, 4-beta-mannosidase is functionally implicated in cell wall organization through modification and degradation facilitated by endo-beta-1, 4-mannanase activity on hemicellulose and heteromannan. Prosite profiling identified glycosyl hydrolases at amino acid positions 220-229, while Pfam

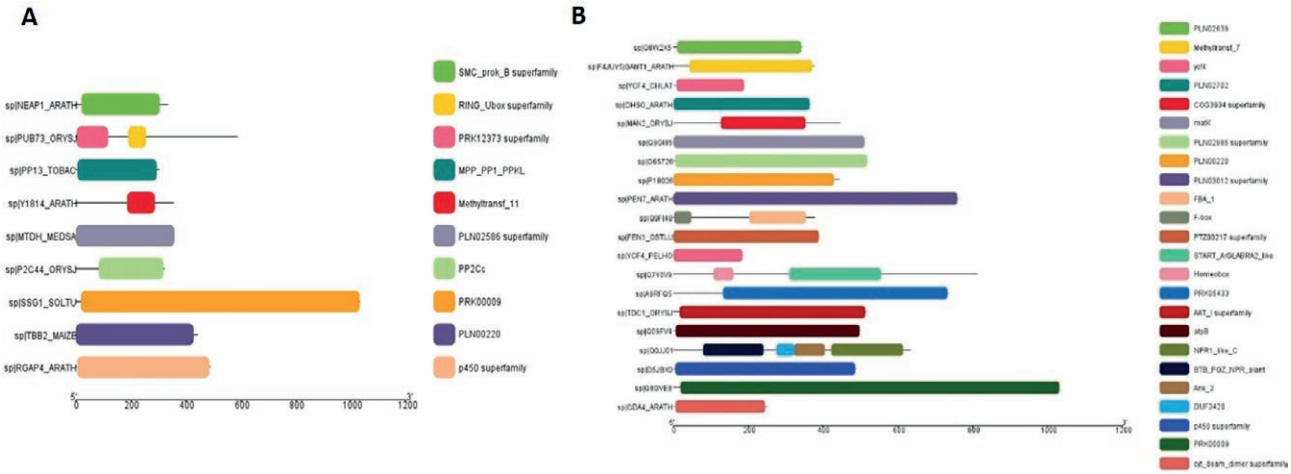


Figure 4. Conserved domains observed in the deferentially expressed coconut leaf proteins. (A) Leaf proteins from uninfected coconut trees. (B) Leaf proteins from root wilt infected coconut trees.

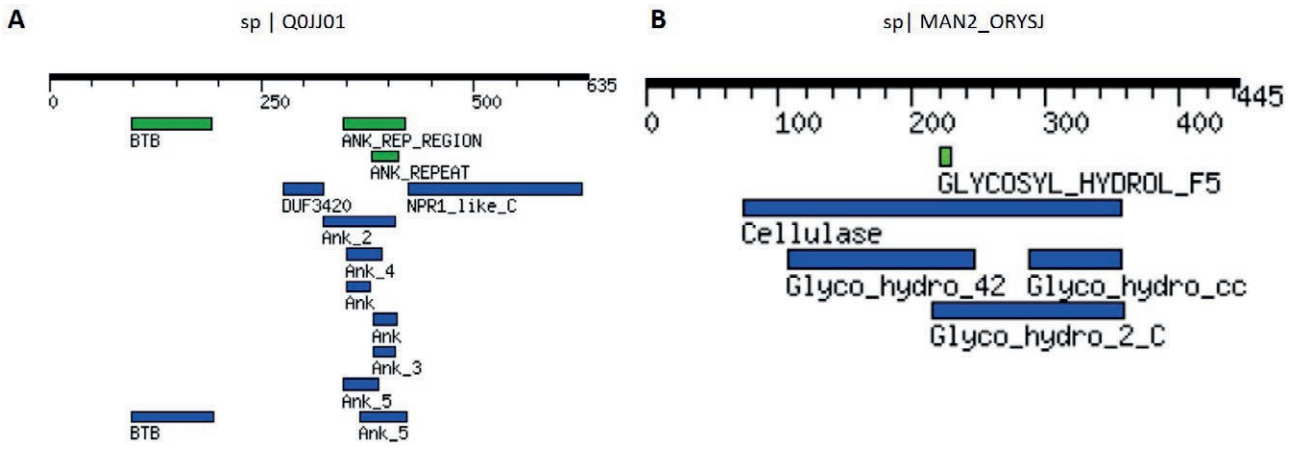


Figure 5. Conserved motif sites of the candidate biomarker proteins. (A) Leaf proteins from uninfected coconut trees. (B) Leaf proteins from root wilt infected coconut trees.

analysis detected cellulase (glycosyl hydrolase family 5 at positions 73-357, glycosyl hydrolase (288-357), beta-galactosidase (107-247), and glycosyl hydrolases family 2, TIM barrel domain family 5 signature (214-359).

Signature peptides and antigenicity

The biomarker protein sequences were subjected to BLAST searches against the NCBI-PDB database. The protein hit with the highest query coverage of 84% against endo-beta-mannanase (*Solanum lycopersicum*) (PDB ID: 1RH9_A) was selected and utilized as a template to build a model using SwissModel. However, for the biomarker protein containing the BTB/POZ domain and ankyrin repeats, the homology coverage was less than 40%. Therefore, the predicted structure from AlphaFold available in Uniprot was chosen instead.

The amino acid sequences of the selected biomarker proteins were submitted to the VaXiJen v 2.0 server, which aids in predicting potent antigens and subunit vaccines using default parameters, to determine antigenicity. Subsequently, the query sequences were screened against databases containing experimentally obtained antigenic residues (such as IEDB) along with various prediction modules (ABDpred, BepiPred-2.0, SVMTrip). Linear epitopes, as well as predictions for continuous and discontinuous epitopes were obtained from the analysis of the protein structure model using the Ellipro server. The predicted epitope sequences were

A

Bepipred Linear Epitope Prediction Average:0.491 Min:0.198 Max: 0.678		Kolaskar & Tongankar Antigenicity Average: 1.031 Min: 0.872 Min: 1.150		Parker Hydrophilicity Prediction Average: 1.752 Minimum: -2.386 Maximum: 5.907		Chou & Fasman Beta-Turn Prediction Average: 0.996 Min: 0.732 Max 1.541		Emini Surface Accessibility Prediction Average: 1.000 Min: 0.023 Max: 13.086		Karplus & Schulz Flexibility Prediction Average: 1.005 Min: 0.868 Max: 1.162		GRAVY score		Toxicity
Residue	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	
V	0.439	HSELTPVLEKCIRR	1.065	HSELTPVLEKCIRR	1.207	HSELTPVLEKCIRR	0.899	SELTPVLEKCIR	0.351	LTPVLEK	1.004	V L E K C I R R I A R S N L	-0.007	N o n - T o x i n
L	0.429	SELTPVLEKCIRRI	1.069	SELTPVLEKCIRRI	0.486	SELTPVLEKCIRRI	0.865	ELTPVLEKCIRR	0.513	TPVLEKC	1.0			
E	0.434	ELTPVLEKCIRRIA	1.072	ELTPVLEKCIRRIA	0.171	ELTPVLEKCIRRIA	0.81	LTPVLEKCIRRI	0.208	PVLEKCI	0.991			
K	0.427	LTPVLEKCIRRIAR	1.074	LTPVLEKCIRRIAR	-0.086	LTPVLEKCIRRIAR	0.825	TPVLEKCIRRIA	0.254	VLEKCIR	0.983			
C	0.426	TPVLEKCIRRIARS	1.057	TPVLEKCIRRIAR	1.036	TPVLEKCIRRIARS	0.885	PVLEKCIRRIAR	0.345	LEKCIRR	0.972			
I	0.429	PVLEKCIRRIARSN	1.047	PVLEKCIRRIARSN	1.164	PVLEKCIRRIARSN	0.928	VLEKCIRRIARSN	0.299	EKCIRRI	0.965			
R	0.467	VLEKCIRRIARSNL	1.061	VLEKCIRRIARSNL	0.357	VLEKCIRRIARSNL	0.861	LEKCIRRIARSN	0.648	KCIRRIA	0.965			
R	0.478	LEKCIRRIARSNLD	1.024	LEKCIRRIARSNLD	1.336	LEKCIRRIARSNLD	0.93	EKCIRRIARSNL	0.648	CIRRIAR	0.966			
I	0.498	EKCIRRIARSNLDN	0.99	EKCIRRIARSNLDN	2.493	EKCIRRIARSNLDN	0.999	KCIRRIARSNLD	0.625	IRRIARS	0.979			
A	0.532	KCIRRIARSNLDNV	1.028	KCIRRIARSNLDNV	1.671	KCIRRIARSNLDNV	0.982	CIRRIARSNLDN	0.503	RRIARSN	0.998			
R	0.560	CIRRIARSNLDNVSL	1.034	CIRRIARSNLDNVSL	1.729	CIRRIARSNLDNVS	0.012	IRRIARSNLDNV	0.696	RIARSNL	1.016			
S	0.564	IRRIARSNLDNVSL	1.022	IRRIARSNLDNVSL	0.971	IRRIARSNLDNVSL	0.969	RRIARSNLDNVS	1.331	IARSNLD	1.037			
N	0.574	RRIARSNLDNVSLD	1.002	RRIARSNLDNVSLD	2.257	RRIARSNLDNVSLD	1.04	RIARSNLDNVSL	0.56	ARSNLDN	1.039			
L	0.577	RIARSNLDNVSLDK	1.006	RIARSNLDNVSLDK	2.364	RIARSNLDNVSLDK	1.044	IARSNLDNVSLD	0.478	RSNLDNV	1.032			

B

Figure 6. Characterization of epitopes and immunogenic residues in protein BTB/POZ domain and ankyrin repeat-containing protein. (A) Characterization of epitopes. (B) Homologous immunogenic residues plotted from various epitope predictions.

manually searched against non-conserved regions of the query protein. Further, epitopes with high homology across predicted results, with lengths ranging from 12 to 14 amino acid residues were retained. The results of the predictions for BTB/POZ domain and ankyrin repeat-containing protein NPR2 are presented in Figure 6 and for mannan endo-1, 4-beta-mannosidase protein in Figure 7. Additionally, the Uniprot peptide search revealed epitopes showing specific hits against the query biomarker proteins.

The Protein Localization Prediction Server (LocTree-Protter) was used to characterize the amino acid sequence of mannan endo-1, 4-beta-mannosidase. The topology and annotations showed a predicted epitope on the extracellular matrix. Similarly, for the BTB/POZ domain and ankyrin repeat-containing NPR2 protein, the prediction indicated localization within the intracellular matrix. Both proteins exhibited the N-glyco motif (Figure 8).

The signature peptides identified in the study for use as biomarkers in the molecular detection of root wilt infection in coconut are VLEKCIRRIARSNL and HQVEGFEDAHRD respectively for the BTB/POZ domain and ankyrin repeat-containing protein, and for mannan endo-1, 4-beta-mannosidase.

DISCUSSION

Root wilt disease is estimated to cause yield loss of up to 968 million nuts in South India (Manimekalai *et al.*, 2010). The Central Plantation Crops Research Institute of the Indian Council for Agricultural Research has associated the coconut root wilt disease in Tamil Nadu and Kerala states of India with the presence of phytoplasmas belonging to the 16SrXI group and also developed PCR detection techniques. They have also suggested some insect vectors involved in transmission of the disease such as lace bug and plant hoppers. ELISA based detection assay was also developed (https://cpcri.icar.gov.in).

Diagnosis of root wilt infection in coconut has been challenging for more than three decades. Microscopic techniques like fluorescent microscopy (Hibben *et al.*, 1986), electron microscopy (Solomon *et al.*, 1999) were

A

Bepipred Linear Epitope Prediction Average:0.482 Min:0.198 Max: 0.685			Kolaskar & Tongankar Antigenicity Average: 1.025 Min: 0.876 Max: 1.216		Parker Hydrophilicity Prediction Average: 1.155 Min: -3.075 Max: 5.708		Chou & Fasman Beta-Turn Prediction Average: 0.977 Min: 0.736 Max: 1.315		Emini Surface Accessibility Prediction Average: 1.000 Min: 0.077 Max: 6.384		Karplus & Schulz Flexibility Prediction Average: 0.987 Min: 0.888 Max: 1.134		Gravy Score		Toxicity
Position	Residue	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	Peptide	Score	
353	H	0.522	GLSHQVE	1.07	EFGLSHQVEGFE	1.508	EFGLSHQVEGFE	0.916	LSHQVE	0.83	GLSHQVE	0.951	H		
354	Q	0.529	LSHQVEG	1.07	FGLSHQVEGFED	1.692	FGLSHQVEGFED	0.976	SHQVEG	0.996	LSHQVEG	0.962	Q		
355	V	0.558	SHQVEGF	1.047	GLSHQVEGFEDA	2.633	GLSHQVEGFEDA	0.981	HQVEGF	0.644	SHQVEGF	0.974	V		
356	E	0.578	HQVEGFE	1.024	LSHQVEGFEDAH	2.333	LSHQVEGFEDAH	0.93	QVEGFE	0.819	HQVEGFE	0.991	E		
357	G	0.595	QVEGFED	0.99	SHQVEGFEDAH	3.45	SHQVEGFEDAHR	0.96	VEGFED	0.79	QVEGFED	1.005	G		
358	F	0.593	VEGFEDA	0.997	HQVEGFEDAHRD	3.742	HQVEGFEDAHRD	0.962	EGFEDA	1.075	VEGFEDA	1.008	F	-1.6	Non-Toxin
359	E	0.578	EGFEDAH	0.957	QVEGFEDAHRDV	3.258	QVEGFEDAHRDV	0.925	GFEDAH	0.845	EGFEDAH	1.01	E		
360	D	0.579	GFEDAHR	0.961	VEGFEDAHRDVL	1.992	VEGFEDAHRDVL	0.893	FEDAHR	1.672	GFEDAHR	1.008	D		
361	A	0.561	FEDAHRD	0.959	EGFEDAHRDVLY	2.142	EGFEDAHRDVLY	0.946	EDAHRD	3.224	FEDAHRD	1.003	A		
362	H	0.529	EDAHRDV	1.001	GFEDAHRDVLYR	1.842	GFEDAHRDVLYR	0.963	DAHRDV	1.382	EDAHRDV	1.003	H		
363	R	0.517	DAHRDVL	1.058	FEDAHRDVLYRA	1.542	FEDAHRDVLYRA	0.888	AHRDVL	0.682	DAHRDVL	0.999	R		
364	D	0.509	AHRDVLY	1.1	EDAHRDVLYRAV	2.0	EDAHRDVLYRV	0.88	HRDVLY	1.058	AHRDVLY	0.99	D		

B

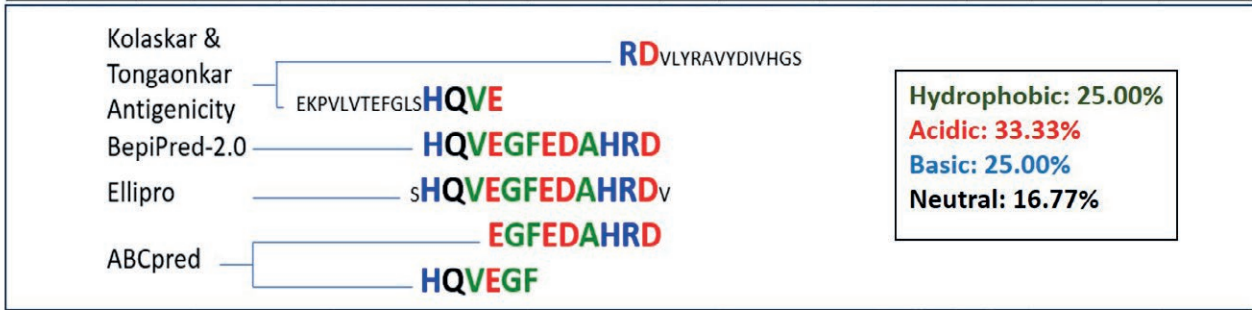


Figure 7. Characterization of epitopes and immunogenic residues in protein Mannan endo-1, 4-beta-mannosidase. (A) Characterization of epitopes. (B) Homologous immunogenic residues plotted from various epitope predictions.

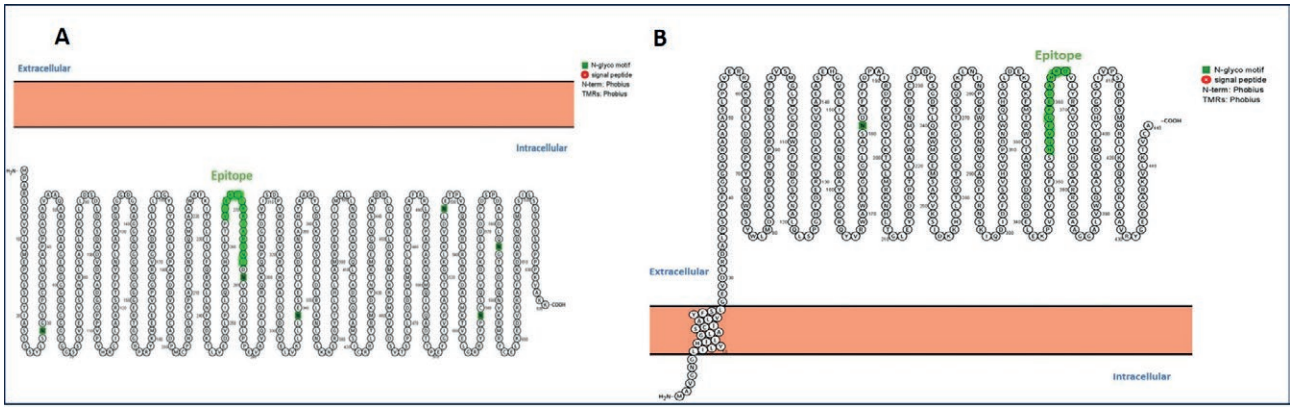


Figure 8. Graphical representation of biomarker proteins with predicted epitopes. A. Mannan endo-1, 4-beta-mannosidase protein sequence, topology and annotations depicts predicted epitope on intracellular matrix. B. BTB/POZ domain and ankyrin repeat-containing NPR2 protein sequence, topology and annotations depicts predicted epitope on intracellular matrix.

used in the past. Histochemical staining using 4,6, diamidino 2 phenylindole-2 hydroxychloride reaction indicated the presence of DNA in sieve tubes (Abdulsalam *et al.*, 1993). Thelly and Mohankumar (2001) observed lower H+ - ATPase in root wilt infected coconut leaves compared to healthy leaves and proposed H+ - ATPase as biochemical marker for early detection of the disease.

ELISA based detection of phytoplasmal proteins were developed later (Sasikala *et al.*, 2001). Due to the non-specificity that was observed in ELISA based detec-

tion, refinement of ELISA was done and used in the early detection of coconut wilt disease (Sasikala *et al*., 2005). Indirect ELISA using a polyclonal antibody was developed for detection of phytoplasma and it was reported to be capable of differentiating between phytoplasma-confirmed palms through PCR amplification of the phytoplasma-associated *secA* gene. (Kanatiwela-de Silva *et al*., 2019).

With the advent of PCR, universal primers for all phytoplasma were proposed (Ceramic-Zagorac and Hiruki, 1996) which was also used in detection of root wilt phytoplasma in coconut. The success was still 60% as the primers not always amplified the phytoplasma genomic region. The sample may consist of genomes of other microbes including viruses and bacteria in addition to the host genome. Homology of a few nucleotides in the 3' end of the primers in non-target DNA may mislead the output of the technique. Hence, nested-PCR was introduced (Lee *et al*., 1995) which made use of another set of genome specific primers and a second PCR reaction. This again consumed more time for detecting the presence of pathogen in the infected tissues but made the system more efficient.

In view of an improved detection strategy for phytoplasma associated coconut root wilt disease, Manimekalai *et al.* (2010) had developed a semi nested PCR primer pair observed no amplification in the healthy palm tissues which were asymptomatic. The same primer pair was used here to confirm the diseased and healthy coconut trees before proceeding to proteome analysis (Supplementary Figure 1). Similarly, this PCR analysis showed no amplification in asymptomatic tree samples except for two samples (Supplementary Figure 2). Nair *et al.* (2016) established a loop mediated isothermal amplification (LAMP) as a better method than conventional nested PCR. The infected samples resulted in ladder like bands, and the restriction digestion resulted in expected size fragments Ramjegathesh *et al.* (2019) developed qPCR assay combined with Taq Man probe. A 890 bp amplicon obtained from nested PCR was probed to specifically target a 69 bp region in 16S rRNA gene using Taq Man. Nested PCR followed by restriction analysis of 16S rRNA gene sequences was used to confirm Candidatus phytoplasma asteris group involved in the lethal wilt disease of coconut (Babu *et al*., 2021).

Instead of targeting a single gene (16S rRNA), targeting two genes including secA was used for detection of '*Candidatus* Phytoplasma palmicola' in Ecuatorial Guinea by Bertaccini *et al.* (2023) and '*Ca*. P. noviguineense' in Papua New Guinea by Dollet *et al.* (2022). De Silva *et al. (*2023) indicated in the nested PCR based detection that asymptomatic plants show no amplification. Hence, they optimized the primer pair combinations to result in 88 to 100% specificity in the detection of leaf wilt disease of coconut in Sri Lanka.

The two-dimensional electrophoresis is a high throughput analytical technique as it has potential in differentiating the proteome based on isoelectric point and molecular weight of individual proteins. The resulting differentially expressed proteins could be of pathogen origin in the infected tissues or host proteins produced in response to pathogen. Comparing the infected and uninfected coconut leaf tissues, it was possible to identify some differentially expressed proteins in replicated samples. Some of the previous studies on proteome analysis of plants infected with phytoplasma were successful in identifying the differentially expressed proteins indicating some of the proteins may be used as biomarkers for detection. Luge *et al.* (2014) identified defense related proteins and proteins involved in alpha-linoleic acid metabolism, that are upregulated in phytoplasma infected *Nicotiana occidentalis* plants. Cao *et al.* (2017) used transcriptome assisted proteomics in *Paulownia* seedlings infected with phytoplasma and identified many differentially expressed proteins with or without treatment using methyl methane sulfonate. By performing RT-qPCR, Margaria and Palmano (2011) correlated the protein expression level observed in proteome analysis of grapevine infected with phytoplasma, with the RNA concentration. Photosynthesis related proteins were found to be downregulated in plants infected with phytoplasma (Ji *et al*., 2009). However, in an iTRAQ quantitative proteomics study in *Ziziphus jujuba* – phytoplasma interaction, proteins involved in phenyl propanoid pathway and flavonoid biosynthesis were found to downregulated first and photosynthesis related proteins were downregulated later. This indicates that the phytoplasma downregulates the defence system of plants as the first thing during infection and later establishes to downregulate the photosynthesis. In another study, defense proteins were found to be induced in both susceptible and resistant varieties of Mexican lime infected with phytoplasma (Monavarfeshani *et al*., 2013). However, the time taken for the response in resistant plants was found to be less. Proteomics approach was used to identify candidate biomarkers for phytoplasma infection in sugarcane (Leetanasaksakul *et al*., 2022).

Previous reports on the role of some of the proteins observed in the present study are presented in Table 2. Tryptophan decarboxylase 1 – like protein was reported to play a role in disease resistance in rice plants against *Bipolaris oryzae*. Flavanone 3 – dioxygenase 2 is known for its role in plant interactions with phytoplasma and viruses. Putative penta cyclic triterpene synthase has been reported in Tanoak – Phytophthora interaction.

Table 2. Known roles of differentially expressed proteins in coconut trees under root wilt infection.

Protein ID	Accession No.	Protein Name	Superfamily	Expression	Plant-Pathogen	Reference
SBA12	tdc1_orysj	Tryptophan decarboxylase 1-like *Oryza sativa Japonica* Group	AAT_1 Aspartate aminotransferase	Upregulated upon infection showing resistance	*Oryza sativa -Bipolaris oryzae*	Ishihara *et al.*, 2011
				Over expressed TDC gene show resistance against whitefly	*Nicotiana – Bemisia tabaci*	Thomas *et al.*, 1995
SBA19	q8w2x5	Flavanone 3-dioxygenase 2 *Oryza sativa*	PLN02639 oxidoreductase	Flavonoid metabolism related protein upregulated upon infection	*Paulownia fortunei-'Candidatus* Phytoplasma asteris'	Wei *et al.*, 2017
					Vitis vinifera-"Flavescence dorée" phytoplasma	Margaria *et al.*, 2014
					Arabidopsis thaliana - Hyaloperonospora parasitica	Van Damme *et al.*, 2008
					Soybean mosaic virus resistance	Cheng *et al.*, 2010
SBSR14	pen7_arath	Putative pentacyclic triterpene synthase	PLN03012 Camelliol C synthase	Conserved amino acid residue aspartate "D" of DCTAE motif imply in β-amyrin synthesis	Oat	Salmon *et al.*, 2016
				Upregulated upon infection showing resistance	Tanoak - *Phytophthora ramorum*	Kasuga *et al.*, 2021
SBA20	F4JUY5	Gibberellic acid methyltransferase 1	SAM dependent carboxyl methyltransferase	Upregulated gene-Reduction in gibberellic acid levels	*Cocous nucifera*-Lethal yellowing phytoplasma	Nejat *et al.*, 2015
SBA13	q0jj01	BTB/POZ domain and ankyrin repeat-containing protein NPR2	NPR1_like_C/BTB_POZ_NPR_plant/ Ank_2/DUF3420	Upregulated NPR2 gene-Salicylic acid perception	*Arabidopsis thaliana- Pseudomonas syringae*	Dobón *et al.*, 2011
					Coconut-defense salicylic acid	Nic-Matos *et al.*, 2017
SBSR21	MAN2_ORYSJ	Mannan endo-1, 4-beta-mannosidase	COG3934 Glycoside hydrolase 5	Disease resistance	Wheat-*Fusarium graminearum/ Alternaria* sp.	Zhang *et al.*, 2020
					Transgenic tobacco-*Fusarium oxysporum*	Hoshikawa *et al.*, 2012
				Upregulated gene	Mexican lime trees- '*Candidatus* Phytoplasma aurantifolia=citri'	Mardi *et al.*, 2015
				Gene specifically expressed	Coconut-Lethal yellowing phytoplasma	Rajesh *et al.*, 2018
SBA14	q7y0v9	Homeobox-leucine zipper protein ROC4 *Oryza sativa*	Homeobox/ START_ArGLABRA2_like	Over expression	Rice-drought resistance/ Development of cuticular wax composition	Wang *et al.*, 2018

Three proteins such as gibberellic acid methyl transferase 1, BTB/POZ domain and ankyrin repeat containing protein NPR2 and mannan endo 1,4 mannosidase were previously reported in coconut – phytoplasma interaction and also in coconut salicylic acid defense pathway.

In the present study, mannan endo-1,4-beta mannosidase and BTB/POZ domain and ankyrin repeat containing NPR2 protein were identified as biomarker proteins for selection of signature peptides. Both were upregulated in the infected tissues. mannan endo-1,4-beta mannosidase is known to trigger defense against both fungal and bacterial pathogens in plants. Mannon oligosaccharides regulate stomata closure and cell death preventing the invasion of pathogens and also activate salicylic acid and jasmonic acid signaling pathways. BTB/POZ domain and ankyrin repeat containing NPR2 protein is a known activator of systemic acquired resistance (Boyle *et al*., 2009). Plant NPR1 and NPR2 are known to interact with promoter of pathogenesis related protein 1 (PR1) and NPR2 plays significant role in perception of salicylic acid (Canet *et al*., 2010; Backer *et al*., 2019). Both the proteins identified as potential biomarkers are well known in systemic acquired resistance in other plants.

Nevertheless, the protein biomarkers also have limitations due to their cross-reactivity of antibodies. Hence, after the identification of potential biomarker proteins through proteome analysis, bioinformatic analyses were carried out to identify the signature peptides. Signature peptides are unique tags of proteins and were initially developed for absolute quantification of a given protein in a mixture. Later, it was used for detection of a particular target protein (Geng *et al*., 2000). In both proteins under study, the signature peptides appeared to be part of salicylic acid binding residues, however the peptides are unique to each protein. The difference in peptide sequences also indicates that salicylic acid binding peptides are different between salicylic acid responsive proteins. The signature peptides to be conjugated with BSA or other carrier protein can be used for raising antibodies for further development of a simple lateral flow assay which will be useful for field level quick detection of coconut root wilt by immersing the strip in the sap obtained from the coconut crown leaves.

Since the proteins used to identify signature peptide biomarkers are upregulated proteins in infected symptomatic coconut trees, their upregulation in infected asymptomatic trees needs further evaluation. Hence the antibodies that would be developed using these signature peptides have to be tested in infected asymptomatic trees to develop them as biomarkers for early detection of the root wilt disease in coconut.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Vellore Institute of Technology, Vellore, India for the support in providing the lab and field facilities to carry out the research. The funding support from Science and Engineering Research Board (SERB) of Department of Science and Technology, Government of India (SERB Sanction order no.: No.EMR/2017/000829) is gratefully acknowledged.

AUTHOR CONTRIBUTIONS

P.A.J: Methodology, Investigation, Formal Analysis, Data Curation, Writing Original Draft. S.V: Conceptualization, Methodology, Writing – Review and Editing, Supervision, Funding Acquisition. S.B: Conceptualization, Supervision, Project Administration, Writing – Review and Editing, Funding Acquisition.

LITERATURE CITED

Abdulsalam K.S., Abael-Mageed M.I., Resk M.A., Nageeb M.A., 1993. The influence of oxytetracycline on Wijamed date palm trees. *Annals of Agricultural Sciences* 38: 301–309.

Babu M., Thangeswari S., Josephrajkumar A., Krishnakumar V., Karthikeyan A., Karun A., 2021. First report on the association of '*Candidatus* Phytoplasma asteris' with lethal wilt disease of coconut (*Cocos nucifera* L.) in India. *Journal of General Plant Pathology* 87: 16–23.

Babu S., Bansal V.K., Kav N.N.V., 2005. Proteome-level investigation of *Brassica carinata*-derived resistance to *Leptosphaeria maculans*. *Journal of Agricultural and Food Chemistry* 53: 313–324.

Backer R., Naidoo S., van den Berg N., 2019. The nonexpressor of pathogenesis-related genes 1 (NPR1) and related family: mechanistic insights in plant disease resistance. *Frontiers in Plant Science* https://doi.org/10.3389/fpls.2019.00102.

Bahder B.W., Soto N., Mou D.F., Humphries A.R., Helmick E.E., 2020. Quantification and distribution of the 16SrIV-D phytoplasma in the wild date palm, *Phoenix sylvestris*, at different stages of decline using quantitative PCR (qPCR) analysis. *Plant Disease* 104: 1328–1334.

Bahder B.W., Zumbado Echavarria M.A., Barrantes Barrantes E.A., Helmick E.E., Bartlett C.R., 2023. A new species of planthopper in the genus *Shellenius*

(Hemiptera: Fulgoroidea: Derbidae) from palms in Costa Rica. *Zootaxa* 5306: 571–585.

Bertaccini A., Contaldo N., Feduzi G., Andeme A.M.E., Yankey E.N., Rovesti L., 2023. Molecular identification of '*Candidatus* phytoplasma palmicola' associated with coconut lethal yellowing in Equatorial Guinea. *Annals of Applied Biology* https://doi.org/10.1111/aab.12854.

Boyle P., Su E.L, Rochon A., Shearer H.L., Murmu J., Despres C., 2009. The BTB/POZ domain of the *Arabidopsis* disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function. *Plant Cell* 21: 3700-3713.

Canet J.V., Dobon A., Roig A., Tornero P., 2010. Structure-function analysis of npr1 alleles in *Arabidopsis* reveals a role for its paralogs in the perception of salicylic acid. *Plant Cell and Environment* https://doi.org/10.1111/j.1365-3040.2010.02194.x.

Cao X., Fan G., Dong Y., Zhao Z., Deng M., Liu W., 2017. Proteome profiling of *Paulownia* seedlings infected with phytoplasma. *Frontiers in Plant Science* https://doi.org/10.3389/fpls.2017.00342.

Ceramic-Zagorac P., Hiruki C., 1996. Comparative molecular studies on aster yellows phytoplasmas. *Acta Horticulturae* 377: 266–276.

Cheng H., Yang H., Zhang D., Gai J., Yu D., 2010. Polymorphisms of soybean isoflavone synthase and flavanone 3-hydroxylase genes are associated with soybean mosaic virus resistance. *Molecular Breeding* 25: 13–24.

De Silva P.R., Perera C.N., Bahder B.W., Attanayake R.N., 2023. Nested PCR-based rapid detection of phytoplasma leaf wilt disease of coconut in Sri Lanka and systemic movement of the pathogen. *Pathogens* 12: 294.

Deng S.J., Hiruki C., 1991. Ampllification of 16s rRNA genes from culturable and nonculturable mollicutes. *Journal of Microbiological Methods* 14: 53–61.

Dobón A., Canet J.V., Perales L., Tornero P., 2011. Quantitative genetic analysis of salicylic acid perception in *Arabidopsis*. *Planta* 234: 671–684.

Dollet M., Fabre S., Beaumont M., Barnabe C., Namaliu Y., Bourdeix R., 2022. The phytoplasma associated with Bogia coconut syndrome in Papua New Guinea is a new phytoplasma in the group of the lethal yellowing syndromes (LYTS) of coconut and other palms. *Tropical Plant Pathology* 47: 530–552.

Dollet M., Fidelis E.G., Dos Passos E., Da Silva F., Aberlenc H.P, Bartlett C.R., 2020. Derbid planthoppers (Hemiptera: Fulgoroidea: Derbidae) associated with coconut and oil palm in Brazil. *Neotropical Entomology* 49: 722–738.

Fernández-Barrera M., Córdova-Lara I., Chan-Rodríguez J.L., Castillo-Vera A., Blanco-Rodríguez E., Saenz-Carbonell L., 2024. Detection of 16SrIV-A phytoplasma DNA in *Colpoptera* sp. (Hemiptera: Nogodinidae) insects in Yucatan Peninsula, Mexico. *Brazilian Journal of Biology* 84: 1–8.

Geng M., Ji J., Regnier F.E. 2000. Signature-peptide approach to detecting proteins in complex mixtures. *Journal of Chromatography A* 870: 295–313.

Gundersen D.E., Lee I-M., 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathologia Mediterrania* 35: 114–151.

Gurr G.M., Johnson A.C., Ash G.J, Wilson B.A.L., Ero M.M., You M.S., 2016. Coconut lethal yellowing diseases: A phytoplasma threat to palms of global economic and social significance. *Frontiers in Plant Science* 7: 1–21.

Hibben C.R., Lewis C.A., Castello J.D., 1986. Mycoplasma like organisms, cause lilac witches' broom. *Plant Disease* 70: 342–345.

Hoshikawa K., Endo S., Mizuniwa S., Makabe S., Takahashi H., Nakamura I., 2012. Transgenic tobacco plants expressing endo-β-mannanase gene from deep-sea *Bacillus* sp. JAMB-602 strain confer enhanced resistance against fungal pathogen (*Fusarium oxysporum*). *Plant Biotechnology Reports* 6: 243–250. https://doi.org/10.1007/s11816-012-0219-2.

Humphries A.R., Ascunce M.S., Goss E.M., Helmick E.E., Bartlett C.R., Bahder B.W., 2021. Genetic variability of *Haplaxius crudus*, based on the 5′ region of the cytochrome C oxidase subunit I gene, sheds light on epidemiology of palm lethal decline phytoplasmas. *PhytoFrontiers* 1: 127–134.

Ishihara A., Nakao T., Mashimo Y., Murai M., Ichimaru N., Miyagawa H., 2011. Probing the role of tryptophan-derived secondary metabolism in defense responses against *Bipolaris oryzae* infection in rice leaves by a suicide substrate of tryptophan decarboxylase. *Phytochemistry* 72: 7–13.

Jespersen M.C., Peters B., Nielsen M., Marcatili P., 2017. BepiPred-2.0: improving sequence based B-cell epitope prediction using conformational epitopes. *Nucleic Acid Research* 45: W24–W29. https://doi.org/10.1093/nar/gkx346.

Ji X., Gai Y., Zheng C., Mu Z., 2009. Comparative proteomic analysis provides new insights into mulberry dwarf responses in mulberry (*Morus alba* L.). *Proteomics* 9: 5328–5339.

Kanatiwela-de Silva C., Damayanthi M., de Silva N., Wijesekera R., Dickinson M., Udagama P., 2019. Immunological detection of the Weligama coconut

leaf wilt disease associated phytoplasma: Development and validation of a polyclonal antibody based indirect ELISA. *PLoS One* 14: 1–15.

Kasuga T., Hayden K.J., Eyre C.A., Croucher P.J.P., Schechter S., Garbelotto M., 2021. Innate resistance and phosphite treatment affect both the pathogen's and host's transcriptomes in the Tanoak – *Phytophora ramorum* pathosystem. *Journal of Fungi* 7: 198. https://doi.org/10.3390/jof7030198.

Kolaskar A.S., Tongaonkar P.C., 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Letters* 276: 172–174.

Lee I–M., Bertaccini A., Vibio M., Gundersen D.E., 1995. Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. *Phytopathology* 85: 728–735.

Leetanasaksakul K., Roytrakul S., Phaonakrop N., Kittisenachai S., Thaisakun S., Soulard L., 2022. Discovery of potential protein biomarkers associated with sugarcane white leaf disease susceptibility using a comparative proteomic approach. *Peer J* 10: e12740.

Luge T., Kube M., Freiwald A., Meierhofer D., Seeműller E., Sauer S., 2014. Transcriptomics assisted proteomic analysis of *Nicotiana occidentalis* infected by '*Candidatus* Phytoplasma mali' strain AT. *Proteomics* 14: 1882–1889.

Manimekalai R., Nair S., Soumya V.P., Roshna O.M., Thomas G.V., 2011. Real-time PCR technique-based detection of coconut root (wilt) phytoplasma. *Current Science* 101: 1209–1212.

Manimekalai R., Soumya V.P., Sathish Kumar R., Selvarajan R., Reddy K., Baranwal V.K., 2010. Molecular detection of 16SrXI group phytoplasma associated with root (wilt) disease of coconut (*Cocos nucifera*) in India. *Plant Disease* 94: 636–636.

Mardi M., Karimi Farsad L., Gharechahi J., Salekdeh G.H., 2015. In-Depth Transcriptome sequencing of Mexican lime trees infected with *Candidatus* phytoplasma aurantifolia. *PLoS One* 10: e0130425. https://doi.org/10.1371/journal.pone.0130425.

Margaria P., Ferrandino A., Caciagli P., Kedrina O., Schubert A., Palmano S., 2014. Metabolic and transcript analysis of the flavonoid pathway in diseased and recovered N ebbiolo and B arbera grapevines (*Vitis vinifera* L.) following infection by F lavescence dorée phytoplasma. *Plant Cell and Environment* 37: 2183–2200. https://doi.org/10.1111/pce.12332.

Margaria P., Palmano S., 2011. Response of the *Vitis vinifera* L. cv. 'Nebbiolo' proteome to "flavescence dorée" phytoplasma infection. *Proteomics* 11: 212–224.

Miyazaki A., Shigaki T., Koinuma H., Iwabuchi N., Rauka G.B., Namba S., 2018. '*Candidatus* phytoplasma noviguineense', a novel taxon associated with bogia coconut syndrome and banana wilt disease on the island of New Guinea. *International Journal of Systematic and Evolutionary Microbiology* 68: 170–175.

Monavarfeshani A., Mirzaei M., Sarhadi E., Amirkhani A., Nekouei M.K., Salekdeh G.H., 2013. Shotgun proteomic analysis of the Mexican Lime tree infected with '*Candidatus* Phytoplasma aurantifolia'. *Journal of Proteome Research* 12: 785–795.

Nair S., Manimekalai R., Raj P.G., Hedge V., 2016. Loop mediated isothermal amplification (LAMP) assay for detection of coconut root wilt disease and arecanut yellow leaf disease phytoplasma. *World Journal of Microbiology and Biotechnology* 32: 108.

Navratil M., Safarova D., Valova P., Franova J., Simkova M., 2009. Phytoplasma associated with witches'-broom disease of *Ulmus minor* MILL. in the Czech Republic: Electron microscopy and molecular characterization. *Folia Microbiologia* 54: 37–42.

Nejat N., Cahill D.M., Vadamalai G., Ziemann M., Rookes J., Naderali N., 2015. Transcriptomics-based analysis using RNA-Seq of the coconut (*Cocos nucifera*) leaf in response to yellow decline phytoplasma infection. *Molecular Genetics and Genomics* 290: 1899–1910. https://doi.org/10.1007/s00438-015-1046-2.

Nic-Matos G., Narváez M., Peraza-Echeverría S., Saenz L., Oropeza C., 2017. Molecular cloning of two novel *NPR1* homologue genes in coconut palm and analysis of their expression in response to the plant defense hormone salicylic acid. *Genes and Genomics* 39: 1007–1019.https://doi.org/10.1007/s13258-017-0566-z.

Paredes-Tomás C., Luis-Pantoja M., Rodríguez-Tapia J.L., Bertaccini A., 2023. *Haplaxius crudus* transmission of '*Candidatus* Phytoplasma palmae' to coconut seedlings in Cuba. *Phytopathogenic Mollicutes* 13: 151–156.

Rajesh M.K., Rachana K.E., Kulkarni K., Sahu B.B., Thomas R.J., Karun A., 2018. Comparative transcriptome profiling of healthy and diseased Chowghat Green dwarf coconut palms from root (wilt) disease hot spots. *European Journal of Plant Pathology* 151: 173–193.

Ramjegathesh R., Karthikeyan G., Balachandar D., Ramaraju K., Rajendran L., Samiyappan R., 2019. Nested and TaqMan ® probe based quantitative PCR for the diagnosis of '*Ca*. Phytoplasma' in coconut palms. *Molecular Biology Reports* 46: 479–488.

Salmon M., Thimmappa R.B., Minto R.E., Melton R.E., Hughes R.K., Osbourn A., 2016. A conserved amino acid residue critical for product and substrate speci-

ficity in plant triterpene synthases. *Proceedings of the National Academy of Sciences USA* 113: E4407–4414. https://doi.org/10.1073/pnas.1605509113.

Sasikala M., Chithra K.R., Solomon J.J., Rajeev G., 2001. Development of DAC Indirect ELISA for the rapid detection of coconut root (wilt) disease. *Coconut Research and Development Journal* 17: 34.

Sasikala M., Prakash V.R., Sapna V.P., Mayilvaganan M., Nair L.S., 2005. Refinement of ELISA and its use in early detection of coconut root (wilt) disease. *Coconut Research and Development Journal* 21: 37–44.

Schneider B., Seemüller E., Smart C.D., Kirkpatrick B.C., 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or Phytoplasmas. In: Razin S., Tully J.G., Eds., Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I. Academic Press, San Diego, 369–380. http://dx.doi.org/10.1016/B978-012583805-4/50040-6

Solomon J.J., Govindankutty M.P., Nienhaus F., 1983. Association of mycoplasma-like organisms with the coconut root (wilt) disease in India. *Journal of Plant Diseases and Protection* 90: 295–297.

Solomon J.J., Nair C.P.R., Srinivasan N., Gunasekaran M., Sasikala M., 1999. Coconut root (wilt) – The malady and remedy. *Journal of Plantation Crops* 27: 71–92.

Soto N., Humphries A.R., Mou D.F., Helmick E.E., Glover J.P., Bahder B.W., 2020. Effect of oxytetracycline-hydrochloride on phytoplasma titer and symptom progression of the 16SrIV-D phytoplasma in cabbage palms from Florida. *Plant Disease* 104: 2330–2337.

Thelly T.M., Mohankumar C., 2001. H+-ATPase as a biochemical marker for early detection of root (wilt) disease in coconut palms (*Cocos nucifera* L.). *Indian Journal of Biochemistry and Biophysics* 38: 199–202.

Thomas J.C., Adams D.G., Nessler C.L., Brown J.K. and Bohnert H.J., 1995. Tryptophan decarboxylase, tryptamine and reproduction of the whitefly (*Bemisia tabaci*). *Plant Physiology* 109: 717–720.

Van Damme M., Huibers R. P., Elberse J., Van den Ackerveken G., 2008. Arabidopsis *DMR6* encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. *The Plant Journal*. https://doi.org/10.1111/j.1365-313X.2008.03427.x.

Wang Z., Tian X., Zhao Q., Liu Z., Li X., Bu Q., 2018. The E3 ligase DROUGHT HYPERSENSITIVE negatively regulates cuticular wax biosynthesis by promoting the degradation of transcription factor ROC4 in rice. *Plant Cell* 30: 228–244. https://doi.org/10.1105/tpc.17.00823.

Wei Z., Wang Z., Li X., Zhao Z., Deng M., Dong Y., Fan G., 2017. Comparative proteomic analysis of *Paulownia fortunei* response to phytoplasma infection with dimethyl sulfate treatment. *International Journal of Genomics* 1–11. 10. https://doi.org/1155/2017/6542075.

Zhang S.B., Zhang W.J., Li N., Zhai H-C., Lv Y-Y., Cai J-P., 2020. Functional expression and characterization of an endo-1,4-β-mannosidase from *Triticum aestivum* in *Pichia pastoris*. *Biologia* 75: 2073–2081. https://doi.org/10.2478/s11756-020-00525-8.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

Gnomoniopsis paraclavulata, a previously unrecorded causal agent of oak decline in Italy

Simone MAVICA, Giuseppa Rosaria LEONARDI*, Giancarlo POLIZZI, Dalia AIELLO

Department of Agriculture, Food and Environment, University of Catania, 95123, Via S. Sofia 100, Catania, Italy
*Corresponding author. Email: giuseppa.leonardi@phd.unict.it

Summary. Oak trees (*Quercus pubescens*) showing symptoms of twig and branch dieback, internal wood necroses, and decline, were surveyed in a public park located in Catania province (eastern Sicily, Italy). *Gnomoniopsis*-like fungi were consistently isolated from symptomatic wood tissues. Based on morphology and phylogenetic analyses of ITS, *tef1* and *tub2* loci, the fungi were identified as *Gnomoniopsis paraclavulata*. A pathogenicity test was conducted by inoculating stems of 1-year-old oak seedlings with mycelium plugs of a representative *G. paraclavulata* isolate. Three months after inoculation, internal necrosis around inoculation points and twig dieback were observed. Colonies of *G. paraclavulata* were reisolated from necrotic tissues of inoculated plants, fulfilling Koch's postulates. This is the first report of *G. paraclavulata* causing dieback and decline on *Q. pubescens* trees.

Keywords. Fungal disease, *Quercus pubescens*, dieback, wood necrosis.

Citation: Mavica, S., Leonardi, G.R., Polizzi, G. & Aiello, D. (2025). *Gnomoniopsis paraclavulata*, a previously unrecorded causal agent of oak decline in Italy. *Phytopathologia Mediterranea* 64(2): 183-190. doi: 10.36253/phyto-16184

Accepted: June 16, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Luisa Ghelardini, University of Florence, Italy.

ORCID:
SM: 0009-0008-0073-2478
GRL: 0000-0002-4676-5100
GP: 0000-0001-8630-2760
DA: 0000-0002-6018-6850

INTRODUCTION

Quercus L., (*Fagaceae*), is an important plant genus that is widespread in forest ecosystems (Tantray *et al*., 2017). Oak forests are abundant in the southern regions of Italy, particularly around Mount Etna in Sicily, making these forests significant areas for biodiversity (La Mantia *et al*., 2003; Gianguzzi *et al.,* 2015). In 2022 and 2023, symptoms of defoliation and dieback of twigs and branches were observed on *Q. pubescens* trees in a public park in Catania (37°32'22.5"N 15°01'46.6"E). These oak trees have considerable social and ecological importance, as they commemorate an historical lava flow from Mount Etna in 1669 that caused significant damage to the area (Bottari *et al*., 2022). In literature oak decline is reported as a serious ecologically important disease that is spreading in many Italian regions, being a disease syndrome that is affected by climate change, drought, wildfire, and forest mismanagement (Linaldeddu *et al*., 2014, 2017; Scortichini, 2025). Since the incidence of the declined oaks was very high in our surveys, the aim of this study was to characterize the causal agent of *Q. pubescens* decline in Catania province, providing morphological and molecular characterizations of the pathogen.

MATERIALS AND METHODS

Diseased samples were collected from 20 plants and transferred to the laboratory of the Department of Agriculture, Food and Environment, University of Catania. Wood fragments between symptomatic and healthy tissues were used for isolations onto potato dextrose agar (PDA) amended with streptomycin sulfate (100 mg L^{-1}). After incubation at 25 ± 1 °C in the dark for 7 d, *Gnomoniopsis*-like colonies were consistently isolated, with an isolation frequency of 70%. A total of eight colonies were selected to obtain single spore isolates, and these were stored in the fungal collection of the Plant Pathology Section, Department of Agriculture, Food and Environment, University of Catania.

A representative isolate (Q9) was used to study culture characteristics morphology (colony and conidium morphology). Conidial masses were placed on microscope slides with drops of 100% lactic acid, and covered with a coverslip, to measure conidium dimensions at 100× magnification, with a Zeiss Axiolab 5 microscope and Zeiss Axiocam 208 colour camera, using the software Zen Core (v.35.96.03000). Five of the collected isolates (Q3, Q4, Q7, Q9 and Q17) were grown on PDA for 14 d, and genomic DNA was extracted from each isolate after scraping the mycelium with a sterile scalpel, and using the Wizard Genomic DNA Purification Kit (Promega Corporation). The extracted DNA was stored at 4°C for further analyses. The polymerase chain reaction (PCR) was carried out in a total volume of 25 µL, using One Taq® 2× Master Mix with Standard Buffer (BioLabs), according to the manufacturer's instructions. The following loci were amplified and sequenced: the complete internally transcribed spacer region (ITS1-5.8S-ITS2) rDNA gene with the primers ITS5 and ITS4 (White *et al*., 1990), an approx. 0.7 kb fragment of the translation elongation factor 1 alpha (*tef1*) with primers EF1-688F (Alves *et al*., 2008) and EF-2 (O'Donnell *et al*., 1998), and an approx.0.5 kb fragment of the partial beta tubulin gene (*tub2*) with the primer Bt2a and Bt2b (Glass and Donaldson, 1995). PCR conditions were set as follows: 30 s at 94°C; 35 cycles each of 30 s at 94°C; 1 min at 52°C (ITS) or 54°C (*tef1* and *tub2*); 1 min at 68°C; and a final cycle for 5 min at 68°C. PCR products were visualized on 1% agarose gels (90 V for 40 min), that were stained with GelRed® Nucleic Acid GelStain (Biotium) to confirm the presence and size of PCR products. Amplicons were purified and sequenced in both direction by Macrogen Inc., Seoul, South Korea. The DNA sequences generated were assembled with Lasergene SeqMan Pro (DNASTAR), and were deposited in GenBank (https://www.ncbi.nlm.nih.gov/) (Table 1).

The phylogenetic analyses were performed using Maximum Likelihood (ML) and Maximum Parsimony (MP) methods. Thirty-seven reference sequences were retrieved according to the studies of the *Gnomoniopsis* by Jiang *et al*. (2021), Bashiri and Abdollahzadeh (2024), and Li *et al*. (2025), including *Apiognomonia errabunda* AR 2813 used as outgroup, and the five representative isolates from the present study were included. Sequence alignments for phylogenetic analyses were produced with the server version of MAFFT (https://mafft.cbrc.jp/alignment/server/), and were checked and refined using BioEdit Sequence alignment Editor 7.7.1.0 (Hall, 1999). Three loci (ITS, *tef1*, *tub2*) were concatenated to a combined matrix using Phyutility v. 2.2 (Smith and Dunn, 2008). Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis, 2006), as implemented in raxmlGUI 2.0 (Silvestro and Michalak, 2012), using the ML + rapid bootstrap setting and the GTRGAMMA+I substitution model. Bootstrap analyses were carried out with 1,000 bootstrap replicates. Maximum parsimony (MP) bootstrap analyses were performed with Phylogenetic Analyses Using Parsimony (PAUP) v. 4.0a169 (Swofford, 2002). A total of 1,000 bootstrap replicates were implemented using five rounds of heuristic search with random sequence addition, followed by tree-bisection-reconnection (TBR) branch swapping. The MULTREES option was enabled, the steepest descent option was disabled, the COLLAPSE command was set to MINBRLEN, and each replicate was limited to 1 million rearrangements. All molecular characters were treated as unordered and assigned equal weight, with gaps considered as missing data. For evaluation and interpretation of bootstrap support, values between 70% and 90% were considered moderate, above 90% as high, and 100% as the maximum.

Pathogenicity tests were conducted on 12 one-year-old potted healthy *Q. pubescens* plants. Mycelial plugs (5 mm diam.), from colonies of isolate Q9 grown for 7 d at 25 ± 1 °C, were inoculated onto the plant stems that had been previously surfaced sterilized with 70% ethanol solution and wounded with a sterile cork borer (6 mm diam.). Inoculation controls were similarly treated with sterile PDA. After inoculation, the plant wounds were sealed with Parafilm®, and the plants were then maintained at 25 ± 1°C in growth chamber. Symptoms were evaluated after three months, and re-isolation from symptomatic lesions was performed. Thus, single colonies were transferred onto MEA plates, and conidia were observed under light microscope after 25 d.

Table 1. Information on fungal isolates deposited in GenBank and used in the phylogenetic analyses in the present study.

Fungal species	Isolate ID[a]	Host	Location	GenBank accession numbers[b]		
				ITS	*tef1*	*tub2*
Apiognomonia errabunda	AR 2813	*Fagus sylvatica*	Switzerland	DQ313525	-	EU219134
Gnomoniopsis agrimoniae	MFLUCC 14–0844 T	*Agrimonia eupatoria*	Italy	-	MF377585	-
Gnomoniopsis alderdunensis	CBS 125680 T	*Rubus parviflorus*	USA	GU320825	GU320801	GU320787
Gnomoniopsis angolensis	CBS 145057 T	unknown	Angola	MK047428	-	-
Gnomoniopsis chamaemori	CBS 804.79	*Rubus chamaemorus*	Finland	GU320817	GU320809	GU320777
Gnomoniopsis chinensis	CFCC 52286 T	*Castanea mollissima*	China	MG866032	MH545370	MH545366
Gnomoniopsis clavulata	CBS 121255	*Quercus falcata*	USA	EU254818	GU320807	EU219211
Gnomoniopsis castanopsidis	CFCC 54437 T	*Castanopsis hystrix*	China	MZ902909	MZ936385	-
Gnomoniopsis comari	CBS 806.79	*Comarum palustre*	Finland	EU254821	GU320810	EU219156
Gnomoniopsis daii	CFCC 54043 T	*Castanea mollissima*	China	MN598671	MN605519	MN605517
Gnomoniopsis diaoluoshanensis	SAUCC DL0963 T	*Castanopsis chinensis*	China	ON753744	ON759769	ON759777
Gnomoniopsis fagacearum	CFCC 54316 T	*Lithocarpus glaber*	China	MZ902916	MZ936392	MZ936408
Gnomoniopsis flava	CFCC 71563 T	*Castanopsis carlesii*	China	PV257808	PV268106	PV339811
Gnomoniopsis fragariae	CBS 121226	*Fragaria vesca*	USA	EU254824	GU320792	EU219144
Gnomoniopsis guangdongensis	CFCC 54443 T	*Castanopsis fargesii*	China	MZ902918	MZ936394	MZ936410
Gnomoniopsis guttulata	MS 0312	*Agrimonia eupatoria*	Bulgaria	EU254812	-	-
Gnomoniopsis hainanensis	CFCC 54376 T	*Castanopsis hainanensis*	China	MZ902921	MZ936397	MZ936413
Gnomoniopsis idaeicola	CBS 125672	*Rubus* sp.	USA	GU320823	GU320797	GU320781
Gnomoniopsis lithocarpi	SAUCC YN0743 T	*Lithocarpus fohaiensis*	China	ON753749	ON759765	ON759783
Gnomoniopsis macounii	CBS 121468	*Spiraea* sp.	USA	EU254762	GU320804	EU219126
Gnomoniopsis mengyinensis	SAUCC MY0293 T	*Castanea mollissima*	China	ON753741	ON759766	ON759774
Gnomoniopsis occulta	CBS 125677	*Potentilla* sp.	USA	GU320828	GU320812	GU320785
Gnomoniopsis paraclavulata	CBS 123202	*Quercus alba*	USA	GU320830	GU320815	GU320775
Gnomoniopsis paraclavulata	CBS:121912 T	*Quercus alba*	USA	MH863162	-	-
Gnomoniopsis paraclavulata	66G	*Quercus robur*	Poland	MZ078654	MZ078875	MZ078820
Gnomoniopsis paraclavulata	CBS 115312	*Quercus* sp.	Netherlands	EU254840	-	EU219236
Gnomoniopsis paraclavulata	**Q3**	*Quercus pubescens*	Italy	**PV628520**	**PV646569**	**PV646574**
Gnomoniopsis paraclavulata	**Q4**	*Quercus pubescens*	Italy	**PV628521**	**PV646570**	**PV646575**
Gnomoniopsis paraclavulata	**Q7**	*Quercus pubescens*	Italy	**PV628522**	**PV646571**	**PV646576**
Gnomoniopsis paraclavulata	**Q9**	*Quercus pubescens*	Italy	**PV628523**	**PV646572**	**PV646577**
Gnomoniopsis paraclavulata	**Q17**	*Quercus pubescens*	Italy	**PV628524**	**PV646573**	**PV646578**
Gnomoniopsis quercicola	IRAN 4313C T	*Quercus brantii*	Iran	OR540614	OR561996	OR561907
Gnomoniopsis racemula	CBS 121469 T	*Chamerion angustifolium*	USA	EU254841	GU320803	EU219125
Gnomoniopsis rosae	CBS 145 085 T	*Rosa* sp.	New Zealand	MK047451	-	-
Gnomoniopsis rosae	TMR4	unknown	unknown	OR095582	-	OR094914
Gnomoniopsis rossmaniae	CFCC 54307 T	*Castanopsis hainanensis*	China	MZ902923	MZ936399	MZ936415
Gnomoniopsis sanguisorbae	CBS 858.79	*Sanguisorba minor*	Switzerland	GU320818	GU320805	GU320790
Gnomoniopsis silvicola	CFCC 54418 T	*Quercus serrata*	China	MZ902926	MZ936402	MZ936418
Gnomoniopsis smithogilvyi	CBS 130190 T	*Castanea* sp.	Australia	JQ910642	KR072534	JQ910639
Gnomoniopsis tormentillae	CBS 904.79	*Potentilla* sp.	Switzerland	EU254856	GU320795	EU219165
Gnomoniopsis xunwuensis	CFCC 53115 T	*Castanopsis fissa*	China	MK432667	MK578141	MK578067
Gnomoniopsis yunnanensis	SAUCC YN1659 T	*Castanea mollissima*	China	ON753746	ON759771	ON759779

[a] Isolates and sequences generated in the present study are shown in bold font; T = Isolates linked to type specimens.
[b] ITS, internal transcribed spacer; *tef1*, translation elongation factor 1-α; *tub2*, beta-tubulin.

RESULTS AND DISCUSSION

Incidence of the disease was estimated at approx. 20% on about 1,000 plants, with a mortality rate of 5%. Cross sections of stems and branches of affected trees revealed internal necroses, and in the severe cases irregular necroses extending to the external wood (Figure 1). Isolated colonies on PDA were 70 mm diam. after 15 d at 25°C, with sparse aerial mycelium and irregular margins forming a concentric ring, and developing a lobed rosette-like appearance. Colonies on malt extract agar (MEA) were >90 mm diam. after 23 d at 25°C, and were brownish, forming solitary, erumpent, pulvinate, conidiomata exuding pale creamy conidial masses (Figure 2, a and b). Conidia were oval to oblong, straight or slightly curved, aseptate, and hyaline, with dimensions of (min, average – SD, average + SD max; length/width ratio) (4.5–) 6.6–8.3 (–9.4) × (2.3–)3.1–4.3(–5.2) µm, l/w = (1.3–)1.7–2.4(–3) (n = 100) (Figure 2c).

BLASTn searches of ITS, *tef1* and *tub2* sequences showed 100% identity with those of *Gnomoniopsis paraclavulata* Sogonov (*Gnomoniaceae, Diaporthales*) isolate 477E (GenBank accession No. MZ078819.1). According to *Index Fungorum* (https://www.indexfungorum.org/Names/Names.asp; Accessed May 4, 2025), a total of 52 *Gnomoniopsis* species have been previously described, with sequence data available only for 32 of these taxa. Of the 1735 characters (550 from ITS, 700 from *tef1*, and 485 from *tub2*) of the combined matrix used for phylogenetic analyses, 633 were parsimony informative (105 from ITS, 332 from *tef1*, and 196 from *tub2*), 223 were parsimony-uninformative and 879 were constant. The ML tree (-lnL = 15318.023949) obtained by RAxML is shown in Figure 3. ML analysis resulted in a tree topology similar to that revealed by MP analysis. The present study isolates were placed within the clade of *G. paraclavulata*, with maximum and medium support (100% ML, 83% MP).

Gnomoniopsis paraclavulata is phylogenetically close to the recently described *G. quercicola*, for which sequence data are available only for one isolate (IRAN 4313C). In accordance with Bashiri and Abdollahzadeh (2024), some nucleotide differences were observed between the present study *G. paraclavulata* isolates and that of *G. quercicola* in all three loci ITS (four substitutions, one deletions/insertions), *tef1* (38 substitutions, four deletions/insertions), and *tub2* (36 substitutions). However, the present study isolates differed from those of *G. paraclavulata* included in the analyses for nucleotide substitutions and insertion/deletions within the *tef1* introns (four substitutions and three deletions/insertions with *G. paraclavulata* 66G, and 18 substitutions and three deletions/insertions with *G. paraclavulata* CBS 123202).

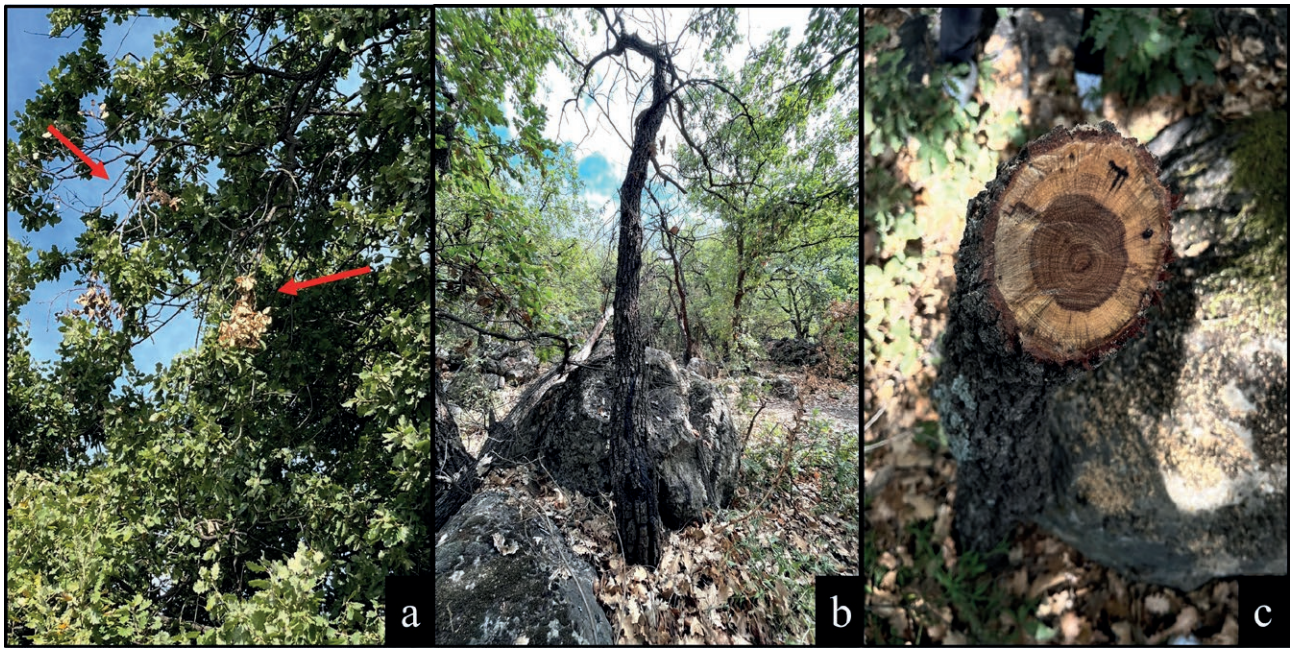


Figure 1. Decline of pubescent oak trees caused by *Gnomoniopsis paraclavulata* in a public park located in Catania, Italy. (a**)** Dieback of twigs showing defoliation. (b) Severe dieback of oak tree. (c) Cross-section of the trunk of a declining tree, with regular brown necrosis and irregular black streaking in the inner wood tissues.

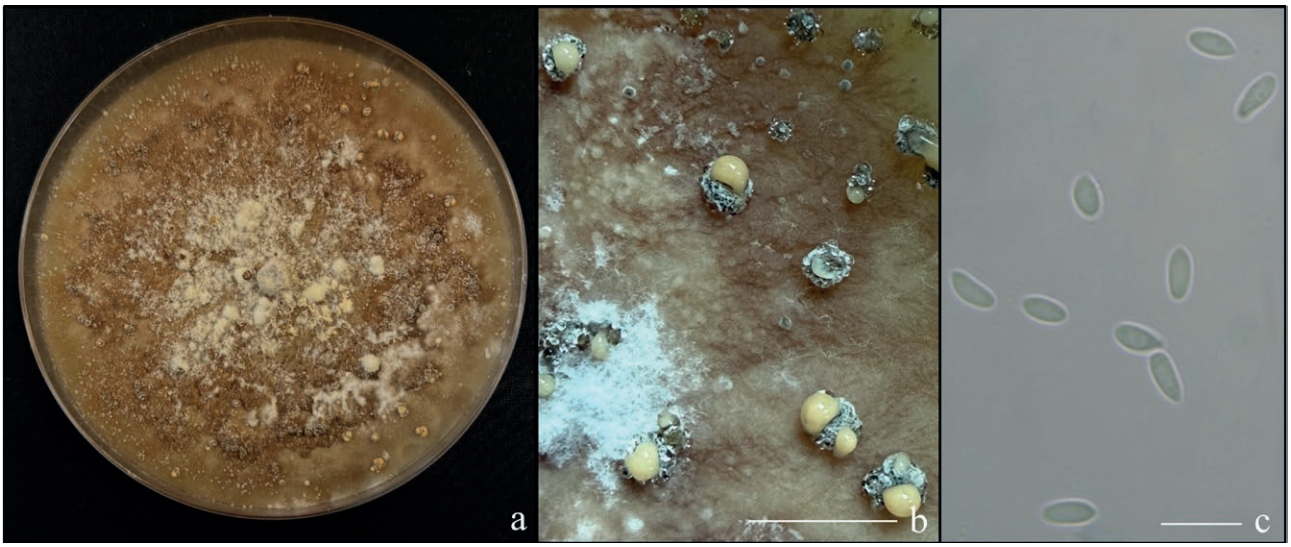


Figure 2. Morphology of *Gnomoniopsis paraclavulata* in culture. (a) Colony of isolate Q9 on MEA (after 23 d at 25 °C). (b) Conidiomata formed on MEA (after 23 d at 25°C). (c) Conidia, that are oval to oblong, straight or slightly curved, aseptate, from culture (MEA, 30 d). Scale bars: (b) = 1 mm, and (c) = 10 μm (c).

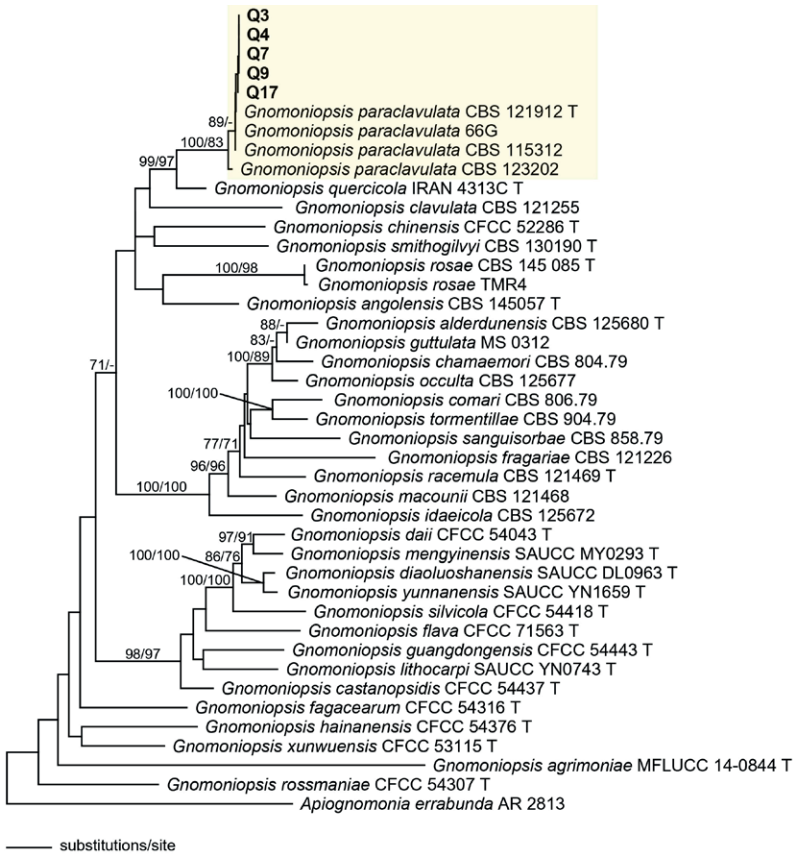


Figure 3. Phylogram of the best ML tree (-lnL = 15318.023949) revealed by RAxML from an analysis of the combined ITS-*tef1*-*tub2* matrix of *Gnomoniopsis*, showing (in bold font) the phylogenetic position of the present study isolates from diseased *Quercus pubescens*, *Apiognomonia errabunda* AR 2813 is the outgroup to root the tree. Maximum Likelihood (ML) and Maximum Parsimony (MP) bootstrap support >70% are given at first and second positions, respectively, above the tree branches. T = ex-type isolates.

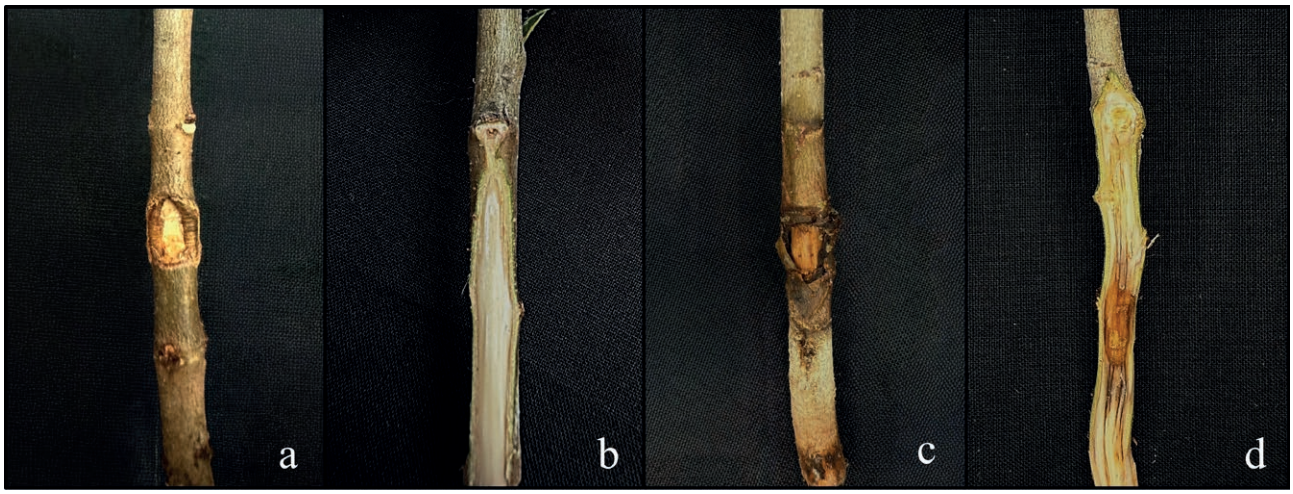


Figure 4. Pathogenicity test on pubescent oak plants. External (a) and internal (b) tissues of control stems, with no symptoms around the agar inoculation points. Stem inoculated with *Gnomoniopsis paraclavulata* isolate Q9, showing an externally brown to black lesion (c), and an internal necrotic lesion (d) with streaking extending upward and downward from the inoculation point, at 3 months after inoculation.

After three months, bark was removed from the stems of inoculated plants, and symptoms of wood discolouration and internal necroses extending upward and downward from each inoculation site were observed (Figure 4), as well as dieback of the basal twigs. Re-isolations were conducted, and these resulted (after 7 d) in colonies resembling *Gnomoniopsis paraclavulata*. Morphology and conidia of colonies isolated from the inoculated plants matched the originally inoculated isolate of *G. paraclavulata*. These results fulfil Koch's postulates for the inoculated fungus.

Gnomoniaceae (e.g., *Gnomoniopsis* spp.) have been reported among the most common pathogenic fungal genera associated with oak tree decline, causing symptoms of canker, gummosis, dieback, wilting, wood discolouration, and necroses (Moricca and Ragazzi, 2008; Sogonov *et al*., 2008; Walker *et al*., 2010; Jiang *et al*., 2021). *Gnomoniopsis paraclavulata* was first discovered on overwintered leaves of *Q. alba* in the United States of America (Sogonov *et al*., 2008), whereas in Italy, this fungus was occasionally isolated from branches with dieback symptoms from oak forests where *Q. pubescens* was also present, but without assessing its pathogenicity (Pinna *et al*. 2019). *Gnomoniopsis paraclavulata* was also isolated from the bodies of the black-banded oak borer (*Coraebus florentinus* Herbst) (Pinna *et al*., 2019), which infests oak species (Sallé *et al*., 2014; Gallardo *et al*., 2018; Cárdenas and Gallardo, 2018). Other studies have reported *G. paraclavulata* causing decline on *Q. robur* in Poland (Jankowiak *et al*., 2022), and *G. quercicola*, phylogenetically close to *G. paraclavulata*, as the most common fungus associated with decline of oak trees (*Q. brantii*, *Q. infectoria*, and *Q. libani*), along with other fungi including *Alloeutypa*, *Botryosphaeria*, *Cytospora*, *Didymella*, *Kalmusia*, and *Neoscytalidium* in Iran (Bashiri and Abdollahzadeh, 2024). The present study is the first to report *G. paraclavulata* causing dieback and internal necrosis on *Q. pubescens*.

FUNDING

This study was funded by Piano di incentivi per la ricerca di Ateneo DIME-SIECO 2024-2026 University of Catania (Italy).

DATA AVAILABILITY

Nucleotide sequences of this study are deposited in NCBI GenBank and the accession number are reported in the text.

LITERATURE CITED

Alves A., Crous P.W., Correia A.C.R.B., Phillips A.J.L., 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* 28(1): 1–13. http://www.fungaldiversity.org/fdp/sfdp/28-1.pdf

Bashiri S., Abdollahzadeh J., 2024. Taxonomy and pathogenicity of fungi associated with oak decline in northern and central Zagros forests of Iran with

emphasis on coelomycetous species. *Frontiers in Plant Science* 15: 1377441. https://doi.org/10.3389/fpls.2024.1377441

Bottari C., Capizzi P., Martorana R., Azzaro R., Branca S., ... Pecora E., 2022. Diagnostic multidisciplinary investigations for cultural heritage at etna volcano: A case study from the 1669 eruption in the mother church at the old settlement of Misterbianco. *Remote Sensing* 14(10), 2388. https://doi.org/10.3390/rs14102388

Cárdenas A.M., Gallardo P., 2018. Assessment and monitoring damage by *Coraebus florentinus* (Coleoptera: Buprestidae) in Mediterranean oak forests. *Open Journal of Ecology* 8(5): 324. https://doi.org/10.4236/oje.2018.85020

Gallardo P., Cárdenas A.M., Soriano J.M., 2018. Long-term assessment of selective pruning of *Quercus* species for controlling populations of *Coraebus florentinus* (Coleoptera: Buprestidae) in Mediterranean forests. *Forests* 9: 1–14. https://doi.org/10.3390/f9020049

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied Environmental Microbiology* 61: 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Gianguzzi L., Papini F., Cusimano D., 2015. Phytosociological survey vegetation map of Sicily (Mediterranean region). *Journal of Maps 12*(5), 845–851. https://doi.org/10.1080/17445647.2015.1094969

Hall T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Jankowiak R., Stępniewska H., Bilański P., Taerum S.J., 2022. Fungi as potential factors limiting natural regeneration of pedunculate oak (*Quercus robur)* in mixed-species forest stands in Poland. *Plant Pathology* 71(4), 805-817. https://doi.org/10.1111/ppa.13529

Jiang N., Voglmayr H., Bian D.R., Piao C.G., Wang S.K., Li Y., 2021. Morphology and phylogeny of *Gnomoniopsis* (Gnomoniaceae, Diaporthales) from Fagaceae leaves in China. *Journal of Fungi* 7(10): 792. https://doi.org/10.3390/jof7100792

La Mantia T., Cullotta S., Garfi G., 2003. Phenology and growth of *Quercus ilex* L. in different environmental conditions in Sicily (Italy). *Ecologia Mediterranea* 29(1): 15–25.

Li Y., Li A., Jiang N., 2025. Identification of the nut rot pathogen affecting *Castanopsis carlesii* based on morphological and phylogenetic analyses. *Forests* 16(4): 627. https://doi.org/10.3390/f16040627

Linaldeddu B.T., Maddau L., Franceschini A., 2017. First report of *Diplodia corticola* causing canker and dieback of *Quercus ilex*, *Q. petraea* and *Q. suber* in Corsica (France). *Plant Disease* 101: 256. https://doi.org/10.1094/PDIS-07-16-1076-PDN

Linaldeddu B.T., Scanu B., Maddau L., Franceschini A., 2014. *Diplodia corticola* and *Phytophthora cinnamomi*: the main pathogens involved in holm oak decline on Caprera Island (Italy). *Forest Pathology* 44: 191–200. https://doi.org/10.1111/efp.12081

Moricca S., Ragazzi A., 2008. Fungal endophytes in Mediterranean oak forests: a lesson from *Discula quercina*. *Phytopathology* 98: 380–386. https://doi.org/10.1094/PHYTO-98-4-0380

O'Donnell K., Kistler H.C., Cigelnik E., Ploetz R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences* 95(5): 2044–2049.

Pinna C., Linaldeddu B.T., Deiana V., Maddau L., Montecchio L., Lentini A., 2019. Plant pathogenic fungi associated with *Coraebus florentinus* (Coleoptera: Buprestidae) attacks in declining oak forests. *Forests* 10(6), 488. https://doi.org/10.3390/f10060488

Sallé A., Nageleisen L.M., Lieutier F., 2014. Bark and wood boring insects involved in oak declines in Europe: current knowledge and future prospects in a context of climate change. *Forest Ecology and Management* 328: 79–93. https://doi.org/10.1016/j.foreco.2014.05.027

Scortichini M., 2025. Similarities and differences among factors affecting complex declines of *Quercus* spp., *Olea europaea*, and *Actinidia chinensis*. *Horticulturae* 11: 325. https://doi.org/10.3390/horticulturae11030325

Silvestro D., Michalak I., 2012. RaxmlGUI: A graphical front-end for RAxML. *Organisms, Diversity & Evolution* 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0

Smith S.A., Dunn C.W., 2008. Phyutility: A phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* 24(5): 715–716. https://doi.org/10.1093/bioinformatics/btm619

Sogonov M.V., Castlebury L.A., Rossman A.Y., Mejía L.C., White J.F., 2008. Leaf-inhabiting genera of the Gnomoniaceae, Diaporthales. *Studies in Mycology* 62: 179. https://doi.org/10.3114/sim.2008.62.01

Stamatakis E., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22(21): 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swofford D.L., 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0a165. Sinauer Associates, Sunderland, Massachusetts, USA.

Tantray Y.R., Wani M.S., Hussain A., 2017. Genus *Quercus*: an overview. *International Journal of Advance Research in Science and Engineering* 6(8): 1880–1886.

Walker D.M., Castlebury L.A., Rossman A.Y., Sogonov M.V., White J.F., 2010. Systematics of genus *Gnomoniopsis* (Gnomoniaceae, Diaporthales) based on a three-gene phylogeny, host associations and morphology. *Mycologia* 102: 1479–1496. https://doi.org/10.3852/10-002

White T.J., Bruns T., Lee S.J.W.T., Taylor J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (Eds.), *PCR Protocols: a guide to methods and applications*. Academic Press, New York: 315–321. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

First report of *Biscogniauxia mediterranea* causing cankers on almond trees (*Prunus dulcis*)

Ana FAUSTINO[1,2,3]*, Cláudia MARINHO[1,2], M. Margarida OLIVEIRA[4], Maria do ROSÁRIO FÉLIX[5], Liliana MARUM[1,2]

[1] *Alentejo Biotechnology Center for Agriculture and Agro-Food (CEBAL)/ Polytechnic University of Beja (IPBeja), 7801-908 Beja, Portugal*
[2] *MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, CEBAL, 7801-908, Beja, Portugal*
[3] *MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal*
[4] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade NOVA de Lisboa, Plant Functional Genomics Laboratory, 2780-157 Oeiras, Portugal*
[5] *MED - Mediterranean Institute for Agriculture, Environment and Development & CHANGE - Global Change and Sustainability Institute, Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal*
*Corresponding author. E-mail: ana.faustino@cebal.pt

Summary. *Biscogniauxia mediterranea* is the causal agent of charcoal disease in *Quercus suber*, the main species of the dynamic ecosystem, known as "Montado", in the Alentejo region, Portugal. In the last years, almond orchards have been introduced in this region due to water availability through the Alqueva dam and the possibility of mechanical harvest. The high-density planting associated with mechanized harvesting and irrigation systems observed in these new orchards can potentiate the appearance of new diseases. In a survey conducted in March 2022, symptomatic diseased trees from Soleta and Vairo cultivars were detected in Beja, Portugal. From this material, we have isolated numerous cultures and could identify *B. mediterranea* from all individuals analyzed by molecular and morphological techniques. Pathogenicity tests were performed in almond plant material and successfully reisolated from lesions, confirming Koch's postulates. Phylogenetics analyses demonstrated the similarity between our sequences and sequences from *Quercus suber* worldwide. To our knowledge, this is the first report of *B. mediterranea* causing diseases on almond trees (*Prunus dulcis*) in Portugal and worldwide.

Keywords. *Biscogniauxia mediterranea*, *Prunus dulcis*, charcoal disease, pathogenicity, Koch's postulates.

OPEN ACCESS

Citation: Faustino, A., Marinho, C., Oliveira, M. M., Félix, M. do R. & Marum, L. (2025). First report of *Biscogniauxia mediterranea* causing cankers on almond trees (*Prunus dulcis*). *Phytopathologia Mediterranea* 64(2): 191-197. doi: 10.36253/phyto-16049

Accepted: May 1, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

ORCID:
AF: 0009-0004-6912-3962
CM: 0000-0001-9035-8070
MMO: 0000-0002-0095-1952
MRF: 0000-0003-3870-5033
LM: 0000-0002-6938-479X

INTRODUCTION

Almond (*Prunus dulcis* Mill. D. A. Webb) is an important nut crop originating from southwest Asia and grown in other regions including the Mediterranean basin (Tomishina *et al*., 2022). In Portugal, almond trees have been

traditionally cultivated in Douro/Trás-os-Montes and Algarve regions (respectively, north and south Portugal). Almond production in Portugal has recently increased, with introduction of commercial cultivars adapted for an irrigated system in new regions, such as Alentejo and Beira Interior (Faustino *et al*., 2022). The Alentejo region is known for its agro-forestry-pastoral "Montado" ecosystem, characterized by presence of *Quercus* species such as *Q. suber* and *Q. ilex*. One of the most frequent diseases affecting *Quercus* trees in this region is charcoal canker, caused by the fungus *Biscogniauxia mediterranea*.

This disease is characterized by necrotic cankers on host stems and branches (Henriques *et al*., 2016). When plants are under stress, *B. mediterranea* can rapidly colonize xylem and bark tissues, resulting in the production of large cankers. These accelerate decline and eventually cause tree death. Ascospores (the main *B. mediterranea* inoculum) are widely disseminated via airborne dispersal and insect transmission (Henriques *et al*., 2014).

Genetic diversity within *B. mediterranea* populations from single stromata, and from different hosts and geographic locations, has been related to high rates of sexual reproduction and the heterothallic mating system of *B. mediterranea* (Vannini *et al*., 1999; Henriques *et al*., 2014). This pathogen has been reported in other woody hosts, including *Erica multiflora* (Yangui *et al*., 2019), *Olea europaea* (Gharbi *et al*., 2020) in Tunisia, and *Amygdalus scoparia* in Iran (Rostamian *et al*., 2016). However, *B. mediterranea* has not been previously recorded in *Prunus dulcis*.

The genetic heterogeneity and biology of *B. mediterranea,* and current climatic change scenarios, are factors that could favour pathogen occurrence and spread to new hosts. The main goal of the present research was to confirm almond (*Prunus dulcis*) as a new host of *B. mediterranea*, and to morphologically and molecularly characterize this pathogen.

MATERIALS AND METHODS

During a survey conducted in March 2022, several symptomatic almond trees with severe orange/black exudates on trunks were observed in an almond orchard in Beja, Portugal (Figure 1, A and B). Samples were collected from branches and trunks of three symptomatic trees each of Vairo and Soleta cultivars. These samples were fractionated into small fragments, and were then

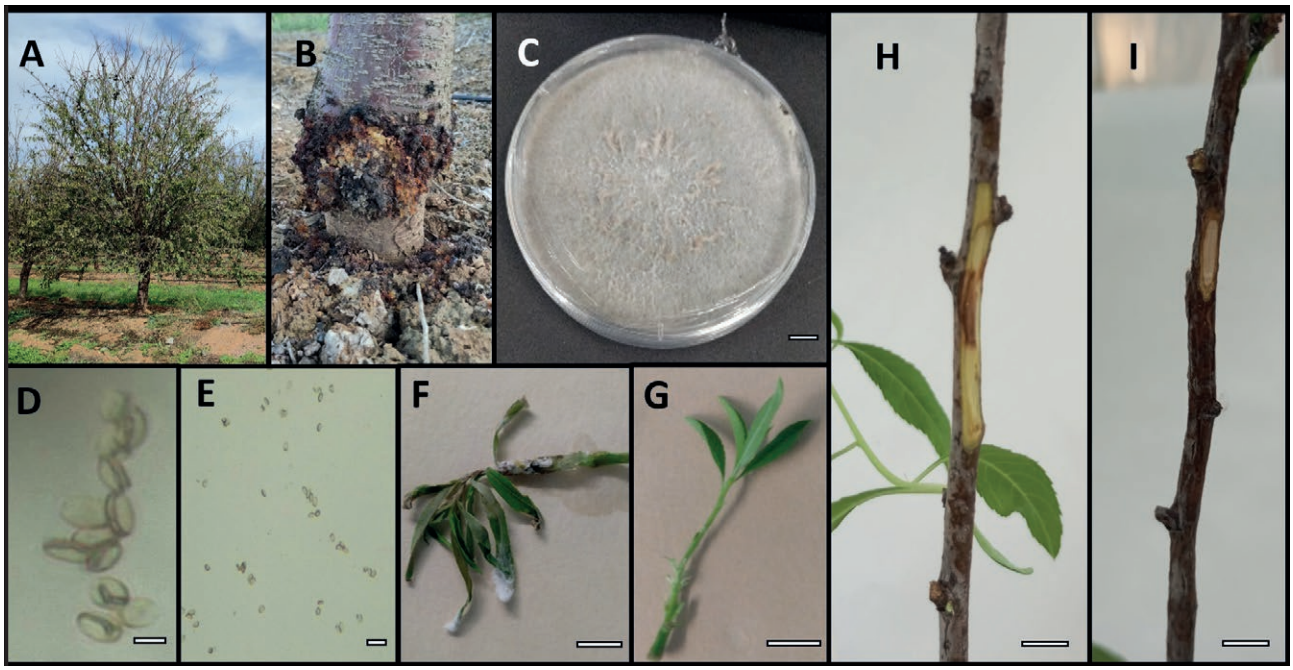


Figure 1. Aspects of a diseased almond tree, *Biscogniauxia mediterranea* morphology, and pathogenicity testing. (**A**) Symptomatic almond tree (*Prunus dulcis*). (**B**) Detail of an almond tree with severe orange and black exudate on the trunk. (**C**) Colony of *B. mediterranea* isolated from the almond tree, and growing in Petri dish containing PDA. (**D** and **E**) Microscope images of conidia. (**F**) Image of an *in vitro*-grown almond shoot, 1 week after inoculation with a *B. mediterranea* isolate. (**G**) Control *in vitro*-grown almond shoot 1 week after wounding and inoculation with a sterile PDA plug. (**H**) Almond tree stem with a necrotic lesion, 1 month after inoculation with a *B. mediterranea* isolate. (I) Control almond tree stem 1 month after wounding and inoculation with a sterile PDA plug. (Scale bars: 1 cm in C, F, G, H, and I; 40 μm in E; 100 μm in D).

surface disinfected to suppress epiphytic microorganisms (Varanda *et al*., 2016). Surface disinfection included a series of 3 min immersions in 96% ethanol, 3% sodium hypochlorite solution, 70% ethanol, and then ultra-pure water, under a sterile laminar airflow environment. The samples were then incubated on Potato Dextrose Agar (PDA; VWR Portugal), in Petri dishes, at 25ºC for 5 d. Resulting colonies were sub-cultured to fresh PDA, and were then incubated (as above) for 10 d. Microscope examinations were carried out using a Leica DM750 light microscope, and relevant images were captured using a Leica ICC50W digital camera and Leica Application Suite LAZ EZ v. 3.4.0 software.

To obtain genomic DNA, fungal mycelium was suspended in 1× TE (100× Stock solution) (Sigma), then frozen at -20ºC for 3 min., thawed at 75ºC, and the vortexed for 2 min. These steps were repeated three times. The final thawing was extended to 15 min, followed by a 5 min centrifuge spin at 15700 g (Paiva, 2011).

For PCR amplification, the internal transcribed spacer (ITS) region was targeted with primer pair ITS-1/ITS-4 (White *et al*., 1990), and the amplified fragments were sequenced by Sanger (STABVIDA, Portugal). The sequences obtained were analyzed using the BioEdit Sequence Alignment Editor v.7.2.3 (Hall, 1999), and were compared with homologous sequences deposited in the National Center for Biotechnology Information (NCBI) platform.

Biscogniauxia mediterranea isolates P.V.Fs.R3.3 (GenBank ID OR908437), P.S.Hs.R1.1 (GenBank ID PQ728784), P.V.Hs.R2.4 (GenBank ID PQ728785), P.V.Hs.R3.1 (GenBank ID PQ728786), and P.S.Hs.R2.4 (GenBank ID PQ728787) from the present study were selected for molecular characterization using a second primer pair targeting the translation elongation factor, EF1-728F/EF1-986R, as described by Carbone and Kohn (1999). Phylogenetic analyses were carried out using sequences of *Biscogniauxia* spp. deposited in GenBank (NCBI). The DNA sequences were aligned using the BioEdit software, and the alignments were concatenated in NEXUS format with partitions for each gene using the SequenceMatrix program v. 1.7.8 (Vaidya *et al*., 2011). The phylogenetic tree was constructed using the maximum likelihood method in IQ-TREE (Nguyen *et al*., 2015), with automatic selection of evolutionary models and support calculated by 1000 bootstrap replications. The tree was visualized and annotated in the iTOL v7 (Letunic and Bork, 2024).

Isolate P.V.Fs.R3.3 of *B. mediterranea* was used for pathogenicity tests. A preliminary assay was carried out by inoculating ten shoots of an *in vitro*-growing bitter almond cultivar each with a mycelium plug harvested from actively-growing colonies on PDA. The inoculum was applied to stems that were previously wounded with scalpel-made cuts. Assessments were made 1 week after *in vitro* inoculations. A second experiment was carried out by inoculating ten *ex-vitro* almond trees (commercially purchased 1-year-old potted plants) with isolate P.V.Fs.R3.3. All inoculated material was collected from potted plants after 1 month, and the lengths of wood discolouration from the inoculation points were measured. In these experiments, the inoculation controls were ten shoots of *in vitro*-grown plants, or ten potted plants that were similarly wounded, but were inoculated with sterile PDA plugs. To determine fulfillment of Koch's postulates, fungal re-isolations were made from the edges of necrotic lesions (as described above), and fungal identities were confirmed using colony morphology and ITS region sequencing. Statistical significance of variations in mean lesion lengths from the second pathogenicity assay was assessed using Statistica 7's one-way analysis of variance (ANOVA) (StatSoft, Inc., 2004).

RESULTS AND DISCUSSION

Analyses of results obtained for both cultivars (three trees of each cultivar) demonstrated that *B. mediterranea* was the main identified fungus (32%) amongst 87 isolates obtained, followed by *Alternaria* spp. (21%), *Fusarium* spp. (18%), and *Trichoderma* spp. (13%).

The BLASTn analysis of the sequence obtained from isolate P.V.Fs.R3.3 showed 99.82% nucleotide for ITS region similarity with the *B. mediterranea* reference strain GenBank ID JQ781799 from Portugal, and 99.68% TEF-1 similarity with GenBank ID MZ221965. Morphology of colonies on PDA and conidia of the isolates identified as *B. mediterranea* was assessed (Figure 1, C, D, and E). As described by Henriques *et al.* (2014), the colour of the aerial mycelium of colonies on PDA varied from white to grayish, to smoke grey. Some isolates had brownish colouration, eventually producing dark exudates. Isolate P.V.Fs.R3.3 (GenBank ID OR908437) produced white colonies on PDA (Figure 1 C).

The phylogenetic analyses demonstrated the similarity of the sequences with other reported sequences of *B. mediterranea* (Figure 2). All the isolates used in this study are in the same cluster as the other *B. mediterranea* isolates and are separated from the other *Biscogniauxia* spp., except for *B. rosacearum*, which is in the same cluster as *B. mediterranea*. This can be explained by the proximity of *B. rosaceaum* to *B. mediterranea* already described in previous studies considering morphological, cultural, and molecular data (Raimondo

Table 1. Isolates (species and codes), hosts, origins, and GenBank accession numbers of the *Biscogniauxia* species and strains used in phylogenetic analyses.

Species	Isolate ID	Host	Origin	GenBank accession number ITS	GenBank accession number TEF-1
Biscogniauxia mediterranea	Bm04.001	*Quercus suber*	Portugal	KM216752	KM216788
	Bm06.003	*Quercus suber*	Morocco	KM216753	KM216789
	Bm07.003	*Quercus suber*	Portugal	KM216754	KM216790
	Bm09.001	*Quercus suber*	Tunisia	KM216755	KM216791
	Bm10.006	*Quercus suber*	Portugal	KM216757	KM216793
	Bm10.012	*Quercus rotundifolia*	Portugal	KM216758	KM216794
	Bm10.016	*Quercus suber*	Italy	KM216759	KM216795
	Bm12.005	*Quercus suber*	Portugal	KM216766	KM216802
	Bm12.015	*Quercus suber*	Tunisia	KM216769	KM216805
	Bm12.022	*Quercus suber*	Portugal	KM216771	KM216807
	Bm12.023	*Eucalyptus globulus*	Portugal	KM216772	KM216808
	Bm12.024	*Quercus suber*	Portugal	KM216774	KM216810
	Bm12.039	*Quercus suber*	France	KM216780	KM216816
	Bm13.004	*Quercus suber*	France	KM216781	KM216817
	Bm13.007	*Quercus suber*	Algeria	KM216782	KM216818
	P.S.Hs.R1.1	*Prunus dulcis*	Portugal	PQ728784	PV963833
	P.V.Hs.R2.4	*Prunus dulcis*	Portugal	PQ728785	PV963834
	P.V.Hs.R3.1	*Prunus dulcis*	Portugal	PQ728786	PV963835
	P.S.Hs.R2.4	*Prunus dulcis*	Portugal	PQ728787	PV963836
	P.V.Fs.**R3.3**	***Prunus dulcis***	**Portugal**	**OR908437**	**PV963837**
Biscogniauxia formosana	*B. formosana*	Bark of *Quercus* sp.	Taiwan	JX507802	-
Biscogniauxia atropunctata	*B. atropunctata*	Wood	USA	JX507799	-
Biscogniauxia maritima	*B. maritima*	Plant leaf	South Korea	MT269517	-
Biscogniauxia arima	*B. arima* EF026150	Wood	Mexico	EF026150	.
Biscogniauxia rosacearum	*B. rosacearum*	*Prunus dulcis*	Iran	MZ190891	-
Biscogniauxia mummularia	*B. mummularia*	*Fagus sylvaticus*	Italy	AJ246230	-
Biscogniauxia anceps	*B. anceps*	Wood	Spain	OQ990096	-
Outgroups					
Xylaria hypoxylon	*Xylaria hypoxylon*	*Fagus sylvatica*	Germany	AM993138	-

et al., 2016; Sohrabi *et al*., 2022). A similar result was observed in our phylogenetic analysis, where *B. rosacearum* clustered together with all *B. mediterranea* isolates and with high genetic similarity to our isolate P.S.Hs. R1.1. Between our isolates obtained from almond trees, slight differences in genetic sequences were observed, although the isolates P.V.Hs.R2.4, P.V.Fs.R3.3, and P.S.Hs.R2.4 were genetically identical.

In the preliminary assay, the ten *in vitro* plants inoculated with *B. mediterranea* developed superficial and internal brown-black discolourations (Figure 1 F), with white mycelium growing from the points of inoculation to the stems and leaves 1 week after inoculations. *Biscogniauxia mediterranea* was then re-isolated from the lesions from all the inoculated plants, and grew on PDA with a 100% re-isolation rate, confirming Koch's postulates and demonstrating pathogenicity of *B. mediterranea* to almond trees (*Prunus dulcis*). The *in vitro* control plants presented healed wounds without any disease symptoms (Figure 1 G). In the second pathogenicity assay, using potted almond trees inoculated with *B. mediterranea*, internal wood discolourations were observed in nine of the ten inoculated almond trees at 1 month after inoculation. These lesions varied in length from 0.3 cm to 8.8 cm (mean = 2.18 cm.), upward and downward from the points of inoculation (Figure 1 H). The control almond trees presented healed wounds without any wood discolouration (Figure 1 I). Statistically significant differences ($P \leq 0.013$) were detected between plants inoculated with *B. mediterranea* and the non-inoculated controls, where no pathogen re-isolations were detected. For re-isolation from material inoculated with *B. mediterranea*,

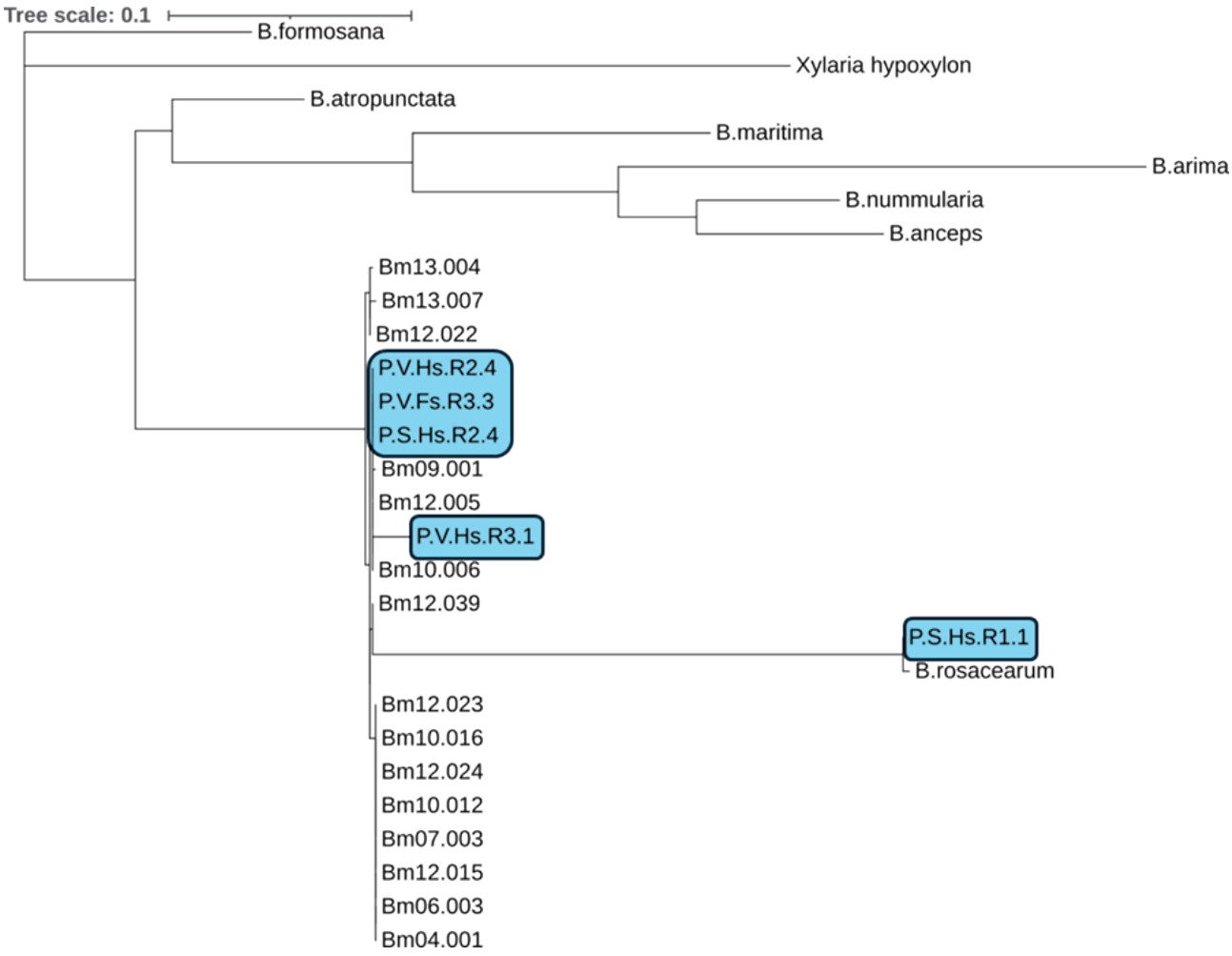


Figure 2. Phylogenetic tree based on Bayesian Inference analyses of combined ITS and TEF-1 sequence data. Almond tree isolates obtained in the present study are shown in blue boxes. The scale bar shows expected changes per site.

the fungi *Boeremia exigua* (5%), *Diaporthe* sp. (5%), *Didymella pomorum* (20%), *Epicoccum nigrum* (5%), *Wilsonomyces carpophilus* (5%), *Alternaria alternata* (10%), *Neofusicoccum parvum* (5%), and *Botryosphaeria dothidea* (5%) were isolated. Inoculation controls yielded similar re-isolation proportions of these fungi, including *Boeremia exigua* (8%), *Diaporthe* sp. (4%), *Didymella pomorum* (20%), *Alternaria alternata* (14%), *Neofusicoccum parvum* (8%), *Botryosphaeria dothidea* (8%), and other species. Despite the necrotic lesions observed on woody stems, we speculate that the phytosanitary status of the almond trees may have influenced the rates of re-isolation of *B. mediterranea* in the second pathogenicity assay.

In conclusion, identification of isolate P.V.Fs.R3.3, based on morphological and molecular data (obtained by sequencing the ITS and TEF-1 genes), has demonstrated that almond trees are a new host for *B. mediterranea* in Portugal, and probably elsewhere. Furthermore, the pathogenicity tests showed that *B. mediterranea* can cause interior wood discolouration lesions in almond plants. The high genetic variability in this fungus is likely to allow it to adapt to new hosts and environmental conditions, demonstrating the importance of edaphoclimatic conditions to the phytosanitary status of potential host plants. Further research is required to determine the impacts of *B. mediterranea* on the health, productivity, and longevity of almond orchards.

FUNDING

This publication was funded by Portuguese National Funds through FCT under the project UIDB/05183 and a PhD study grant of A. Faustino UI/BD/153511/2022.

ACKNOWLEDGMENTS

This study was carried out in the scope of Inov-Amendo-AL: Microenxertia in vitro de amendoeiras selecionadas para a promoção do amendoal no Alentejo (ALT20-03- 0246-FEDER-000068), supported by Program Alentejo 2020 through the European Fund for Regional Development (ERDF), within the scope of the Collective Action Support System – Transfer of scientific and technological knowledge – Domain of Competitiveness and Internationalization. The authors also acknowledge FCT for funding the R & D Units UIDB/05183/2020 to the Mediterranean Institute for Agriculture, Environment and Development (MED), and UIDB/04551/2020 (DOI 10.54499/UIDB/04551/2020) to GREEN-IT Bioresources for Sustainability, as well as LA/P/0121/2020 to Global Change and Sustainability Institute (CHANGE), the Contrato – Programa of L. Marum (CEECINST/00131/2018), and a PhD study grant of A. Faustino (UI/BD/153511/2022, https://doi.org/10.54499/UI/BD/153511/2022). The authors also acknowledge Paula Nozes (Instituto Politécnico de Beja) for technical support and availability of microscopic equipment.

LITERATURE CITED

Carbone I., Kohn L. M., 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051.

Faustino A., Pires R.C., Caeiro S., Rosa A., Marreiros A., … Marum L., 2022. Micrografting in almond (*Prunus dulcis*) Portuguese varieties for production of disease-free plants in International Symposium on *In Vitro* Technology and Micropropagated Plants. *Acta Horticulturae* 1359: 165–172. https://doi.org/10.17660/ActaHortic.2023.1359.20.

Gharbi R., Ennouri K., Bouazizi E., Cheffi M., Triki M.A., 2020. First report of charcoal disease caused by *Biscogniauxia mediterranea* on *Olea europaea* in Tunisia. *Journal of Plant Pathology* 102: 961. https://doi.org/10.1007/s42161-020-00531-4.

Hall T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Ser.* 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008.

Henriques J., Nóbrega F., Sousa E., Lima A., 2014. Diversity of *Biscogniauxia mediterranea* within Single Stromata on Cork Oak. *Journal of Mycology* 324349. https://doi.org/10.1155/2014/324349.

Henriques J., Nóbrega F., Sousa E., Lima A., 2016. Analysis of the genetic diversity and phylogenetic relationships of *Biscogniauxia mediterranea* isolates associated with cork oak. *Phytoparasitica* 44: 19–34. https://doi.org/10.1007/s12600-015-0503-0.

Letunic I., Bork P., 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research* 52(W1): W78–W82. http://doi.org/10.1093/nar/gkae268.

Nguyen L. T., Schmidt H. A., von Haeseler A., Minh B. Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. https://doi.org/10.1093/molbev/msu300.

Paiva D., 2011. Detecção de *Fusarium circinatum* Nirenberg & O'Donnell em coníferas da região central de Portugal. Master Thesis, University of Coimbra, Coimbra, Portugal, 64 pp.

Raimondo M. L., Lops F., Carlucci A., 2016. Charcoal Canker of Pear, Plum, and Quince Trees Caused by *Biscogniauxia rosacearum* sp. nov. in Southern Italy. *Plant Disease* 100(9): 1813–1822. https://doi.org/10.1094/PDIS-09-15-1037-RE.

Rostamian M., Kavosi M.R., Bazgir E., Babanezhad M., 2016. First report of *Biscogniauxia mediterranea* causing canker on wild almond (*Amygdalus scoparia*). *Australasian Plant Disease Notes* 11: 30. https://doi.org/10.1007/s13314-016-0219-0.

Sohrabi M., Mohammadi H., Armengol J., León M., 2022. New report of *Biscogniauxia rosacearum* as a pathogen on almond trees in Iran. *Journal of Plant Diseases and Protection* 129: 411–417. https://doi.org/10.1007/s41348-022-00582-y.

Tomishima H., Luo K., Mitchell A. E., 2022. The almond (*Prunus dulcis*): Chemical properties, utilization, and valorization of coproducts. *Annual Review of Food Science and Technology* 13(1): 145–166. https://doi.org/10.1146/annurev-food-052720-111942.

Vaidya G., Lohman D.J., Meier R., 2011. SequenceMatrix: concatenation software for the last assembly of multigene datasets with character set and codon information. *Cladistics* 27: 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x.

Vannini A., Mazzaglia A., Anselmi N., 1999. Use of random amplified polymorphic DNA (RAPD) for detection of genetic variation and proof of the heterothallic mating system in *Hypoxylon mediterraneum*. *European Journal of Plant Pathology* 29(3): 209–218. https://doi.org/10.1046/j.1439-0329.1999.00145.x.

Varanda C.M.R., Oliveira M., Materatski P., Landum M., Clara M.I.E., Félix M.R., 2016. Fungal endophytic communities associated to the phyllosphere of grave-

pine cultivars under different types of management. *Fungal Biology* 120(12): 1525–1536. https://doi.org/10.1016/j.funbio.2016.08.002.

White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols, a Guide to Methods and Applications (MA Innis, DH Gelfand, JJ Sninsky, J White, ed.), Academic Press, San Diego, CA, USA, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1.

Yangui I., Boutiti Z. M., Messaoud C., Ben Jamaâ M. L., Vannini A., Vettraino A. M., 2019. First report of *Biscogniauxia mediterranea* causing canker on *Erica multiflora* L. in Tunisia. *Journal of Plant Pathology* 101: 1273. https://doi.org/10.1007/s42161-019-00339-x.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Short Notes

First report of cereal yellow dwarf virus (CYDV-RPS) on maize in Bosnia and Herzegovina

Amani BEN SLIMEN[1], Armin VUKOJEVIC[2], Mirsad MUJKOVIC[2], Emina KRAJINA-IBRULJ[2], Toufic ELBEAINO[1,3,*]

[1] *Department of Integrated Pest Management, Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy*
[2] *Department of quality control of seeds, pest diagnosis and the presence of GMOs of Federal Institute of Agriculture, Sarajevo, Butmirska cesta 18, Ilidza 71210, Bosnia & Herzegovina*
[3] *National Research Council of Italy (CNR), Institute for Sustainable Plant Protection (IPSP), Naples, Italy*
*Corresponding author. E-mail: elbeaino@iamb.it

Summary. A survey was conducted in Bosnia and Herzegovina in 2023, to investigate the presence of several important viruses affecting cereals, particularly those associated to cereal yellow dwarf viruses (CYDVs) and barley yellow dwarf viruses (BYDVs). Sixty leaf samples were collected, including 47 from maize plants (*Zea mays* L.) and 13 from barley plants (*Hordeum vulgare* L.), from across four grain-producing regions (Odzak, Sarajevo, Gornji Vakuf and Ilidza). Assessments for both groups of viruses, using ELISA and RT-PCR assays, detected CYDV in one maize sample (hybrid BC 418B) out of the 60 samples assessed. Nucleotide sequence analysis of the RT-PCR amplicon (2476 bp) of Bosnian isolate from maize hybrid BC418B (GenBank no. PV476203) showed that the isolate had 99.1% similarity with the CYDV RPS Mexican isolate (RPV-Mex-1; GenBank no. NC002198). This is the first report of the presence of CYDV-RPS in Bosnia and Herzegovina.

Keywords. Maize, barley, yellow dwarf viruses, RT-PCR.

OPEN ACCESS

Citation: Ben Slimen, A., Vukojevic, A., Mujkovic, M., Krajina-Ibrulj, E. & Elbeaino, T. (2025). First report of cereal yellow dwarf virus (CYDV-RPS) on maize in Bosnia and Herzegovina. *Phytopathologia Mediterranea* 64(2): 199-203. doi: 10.36253/phyto-16200

Accepted: July 6, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Arnaud G Blouin, Institut des sciences en production végétale IPV, DEFR, Agroscope, Nyon, Switzerland.

ORCID:
ABS: 0000-0001-8070-5120
AV: 0009-0008-2710-0892
MM: 0009-0009-2435-769X
EK-I: 0009-0008-9415-2115
TE: 0000-0003-2211-7907

INTRODUCTION

Yellow dwarf viruses (YDVs) are among the most economically damaging and widespread viruses affecting cereal crops, often leading to significant yield losses (Rybicki, 2015). Some viruses containing "yellow dwarf" in their names do not infect cereals, and conversely, some cereal-infecting viruses lack "yellow dwarf" in their names (e.g., barley virus G). The present study specifically addressed viruses that infect cereals and cause yellow dwarf diseases, particularly viruses within two YDV groups: barley yellow dwarf viruses (BYDV, *Tombusviridae*: *Luteovirus*) and cereal yellow dwarf viruses (CYDV, *Solemoviridae*: *Polerovirus*).

As studies of these two groups have evolved, their classification underwent numerous revisions (Rochow, 1969; Rochow and Muller, 1971; Jin *et*

al., 2004; Zhang *et al*., 2009; Jarosova *et al*., 2013). Taxonomy was initially based on specific virus vectors, clustering all in the *Luteoviridae* which is no longer recognized. BYDV and CYDV complexes included diverse species, *i.e*. the four species that were originally discovered as BYDVs, BYDV-Pav, BYDV-MAV, and BYDV-RMV (now reclassified as maize yellow dwarf virus-RMV; MYDV-RMV), and BYDV-RPV reclassified into CYDV-RPV (Rochow, 1969; Rasochova and Miller, 1997). Further viruses were later identified, including BYDV-GAV, BYDV-SGV, BYDV-KerII and BYDV-KerIII in the United States of America, and BYDV-PAS as a new variant now considered as a diverse species deriving from BYDV-PAV (Wang *et al*., 2001; Zhang *et al*., 2009; Jarosova *et al*., 2013). Similarly, another CYDV virus called CYDV-RPS was separated from the initially found CYDV-RPV (Jarosova *et al*., 2013).

Names for some of these viruses have changed. Formerly known BYDV-GPV is now identified as a wheat yellow dwarf virus under the designation *Polerovirus* WYDVGPV (Cheng *et al*., 1996; Wang *et al*., 1998; Zhang *et al*., 2009). Following sequencing of their genomes, these two groups (CYDVs and BYDVs) were shown to be distantly related. Specifically, CYDV-RPV is more closely related to Potato leafroll virus (PLRV) and Beet western yellows virus (BWYV) than to BYDV-PAV. This led to reclassification of CYDV-RPV and CYDV-RPS into *Polerovirus* (Krueger *et al*., 2013; Delfosse *et al*., 2021), while the BYDVs were assigned to *Luteovirus* (D'Arcy *et al.,* 2000; Ali *et al.,* 2014; Scheets *et al*., 2020).

From 2023, the International Committee on Taxonomy of Viruses (ICTV) implemented a new nomenclature system that reorganized the BYDV-MAV and BYDV-PAV viruses as, respectively, *Luteovirus mavhordei* and *Luteovirus pavhordei*. BYDV-SGV, BYDV-PAS, BYDV-kerII, and BYDV-kerIII have been given the species names of, respectively, *Luteovirus sgvhordei*, *Luteovirus pashordei*, *Luteovirus kerbihordei*, and *Luteovirus kertrihordei*. Similarly, the species name of CYDV-RPS is *Polerovirus CYDV-RPS,* and the species name for CYDV-RPV is *Polerovirus CYDV-RPV* (ICTV, 2024).

BYDVs and CYDVs have +ssRNA genomes, ranging in size from 5.5 to 6 kb, each with five to eight ORFs depending on the genus and isolate (Domier and D'Arcy, 2008). Transmitted by aphids (Lister and Ranieri, 1995), YDVs cause epiphytotic outbreaks in nearly all small grain cereal-producing regions, leading to host symptoms including yellowing, stunting, and\or reddening of leaves, depending on the host (Oswald and Houston, 1953; Zitter, 2001; Ali *et al*., 2018; Trzmiel, 2020). Many of these viruses have been reported in various Eastern European countries, but none have been reported in Bosnia and Herzegovina (BiH) (Jarosova *et al*., 2013; Kakareka *et al*., 2020; Trzmiel and Hasiow-Jaroszewska, 2023).

Because of the economic significance of cereal production in BiH (BHAS, 2014) and potential presence of these viruses in maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.), the present study was conducted to determine virus occurrence in cereal-producing regions of this country.

MATERIALS AND METHODS

Origins of plant material

In the 2023 growing season, a total of 60 leaf samples were collected from four cereal-producing regions of BiH. The sampling locations were in Odzak (29 samples), Butmir (four samples), Otes (11 samples), Bojnik (11 samples) and Gornji Vakuf (five samples). Among the samples, 47 were from maize of five distinct varieties. These were two hybrid varieties for which the specific variety names were unidentified, but the plants were within the maturity classes FAO 400 (11 samples) and FAO 500 (15 samples). Additionally, the sample set comprised hybrid BC678 (five samples), hybrid BC418B (eight samples), and Pajdas (eight samples). Furthermore, 13 barley plants of variety Tuna were also included in the collection. The collected leaves had symptoms indicating virus infections, including yellowing and stunting. After collection, the samples were stored at -80°C for further analyses.

DAS-ELISA, RT-PCR and RT-qPCR assays

All samples were first screened for the presence of BYDVs and CYDVs in a Double-Antibody-Sandwich Assay (DAS-ELISA) (Clark and Adams, 1977), and using polyclonal antibodies to detect the serologically known BYDV-B subgroup (BYDV-PAV) (IgG: Art. No. 140115), the BYDV-F subgroup (BYDV-MAV) (IgG: Art. No. 140215), BYDV-RPV (now CYDV-RPV) (IgG: Art. No. 140615), using the commercial ELISA of BIOREBA AG, Reinach, Switzerland (Derron *et al*., 1986; Ayala *et al*., 2001). The samples were assessed alongside an internal positive control of infected material (BYDV-PAV, Art. No. 140153; BYDV-MAV, Art. No. 140253; CYDV-RPV, Art. No. 140653) and were analyzed using a Multiread 400 Microplate Reader (Biochrom) at 405 nm. Two molecular assays (RT-PCR and RT-qPCR) were subsequently carried out on reverse-transcribed total nucleic acids extracted from leaves, as described by Foissac *et al.* (2001). PCR was carried out using specific prim-

Table 1. List of the specific primer sequences designed and used in RT-PCR for detecting CYDV-RPV and CYDV-RPS in this study.

Virus	Primers Sequence (5′ to 3′)	Amplicon size
CYDV-RPS	RPS-F1: CTCTTGTGACGAGTGAGCACAA RPS-R1: GTCAATCCGAAAGTCATCCCA	1395 bp
	RPS-F2: TGGGATGACTTTCGGATTGAC RPS-R2: GCTCAGTTATCTTTTGTGGTTATGCC	1117 bp
CYDV-RPV	RPV-F1: AAGACATCGAAGACGAGTCGGGAA RPV-R1: ACGTTTCCCAACTTAACTCACCT	794 bp
	RPV-F2: AGGTGAGTTAAGTTGGGAAACGT RPV-R2: ACGCCRGGTACTCGTTGAGCTAA	719 bp

ers for BYDV isolates (BYDV-MAV, BYDV-PAV, MYDV-RMV, BYDV-SGV) and CYDV-RPV (Deb and Anderson, 2008; Balaji *et al.,* 2003). The PCR products were electrophoresed on a 1.2% TAE agarose gel. Amplicons of positive samples were ligated into a pGEM-T Easy vector (Promega) and were transformed into *Escherichia coli* DH5α-competent cells, following the manufacturer's instructions. Three clones containing the expected size of the DNA inserts were sent for sequencing (Eurofins Genomics).

Further RT-PCR assays were carried out using four sets of specific primers targeting two overlapping parts in the RNA-dependent RNA polymerase P1-P2 fusion protein, where CYDV-RPV and CYDV-RPS show most differences. Two primer pairs were designed specifically for CYDV-RPS, based on the alignment of the available sequence isolates retrieved from GenBank, while the other two primer pairs targeted CYDV-RPV using the same approach (Table 1). Following the same procedure as for the previous RT-PCR assays, three clones from each amplification were sent for sequencing (Eurofins Genomics).

RESULTS AND DISCUSSION

DAS-ELISA conducted on barley and maize samples yielded one positive reaction, suggesting the presence of CYDV-RPV in a maize sample (of maize hybrid BC 418B). The RT-PCR and RT-qPCR assays generated positive reactions to CYDVs using the universal primers (RPV-CP-F/RPV-CP-R) (Balaji *et al.,* 2003) from this sample from maize hybrid BC 418B. The nucleotide sequence analysis of the three PCR DNA clones (332 bp) obtained from the infected maize sample showed one sequence type, which in BLASTN analysis had 98.8% similarity with *Polerovirus* isolate CYDV-RPS Mex-1 (AF235168) and 92.8% similarity with *Polerovirus* isolate CYDV-RPV TR-2 (KR005847). Given the slight differences in similarities, this sequence alone was not sufficient to confirm whether the infection was due to CYDV-RPS or CYDV-RPV. The subsequent RT-PCRs were conducted on the same infected maize sample using specific pairs of primers for each of CYDV-RPV and CYDV-RPS. Only RPS1 and RPS2 primers amplified distinct amplicons, in contrast to the specific RPV primers where no amplifications were observed. The three clones obtained for each of the two CYDV-RPS amplicons showed identical sequences and complete alignment; upon merging, they generated a consensus sequence of 2,477 nucleotides in length. BLASTN analysis of the consensus sequence showed that it shared 99.1% nucleotide similarity with CYDV-RPS Mex-1 isolate (AF235168), and 99% similarity at amino acid level to RNA-dependent RNA polymerase P1-P2 fusion protein (AAF62532) of the same CYDV RPS isolate.

When compared to available European CYDV-RPS isolate sequences, the sequence from the single maize sample showed 96.5% similarity to the Estonian isolate Olustvere1-O (MK012664), and 96.2% similarity to the Irish La3a isolate (OQ686645). The newly identified sequence from the present study, named CYDV-RPS BiH isolate, has been deposited in GenBank under accession number PV476203.

In the ELISA test, the CYDV-RPV antibodies used were unable to distinguish between the CYDV-RPV and CYDV-RPS species. A similar limitation was also reported by Miller *et al.* (2002). Consequently, it was necessary to use additional diagnostic assays and sequencing analyses to accurately determine the viral species responsible for the YD infection.

Earlier CYDV-RPS detections, such as those from Mexico (Miller *et al*., 2002) and Iran (Rastgou *et al*., 2005), relied on RT-PCR with CYDV-RPV primers. The recent CYDV-RPS discoveries have mostly been attributed to High-Throughput Sequencing (HTS) techniques, with reports from the United Kingdom (Pallett *et al*., 2010), the United States of America (Malmstrom *et al*.,

2017), the Czech Republic (Singh *et al*., 2020), Estonia (Somera *et al*., 2021), and Ireland (Byrne *et al*., 2024).

This is the first report of a CYDV-RPS in BiH. Further investigations are required to assess the virus's prevalence in this country, as well as its potential correlation to the symptoms observed in the maize hybrid BC 418B.

ACKNOWLEDGEMENT

This study was supported by the project "PHYTO BiH" (AID 011681), funded by the General Directorate for Development Cooperation of the Italian Ministry of Foreign Affairs and International Cooperation.

AUTHOR CONTRIBUTIONS

A.B.S.: writing original manuscript, visualization, validation, methodology, formal analysis, review and editing. A.V.: writing original manuscript, visualization, methodology, review. M.M.: writing, review and editing, visualization, administration. E.K.I.: investigation, visualization, methodology. T.E.: review and editing, visualization, supervision, resources and funding acquisition. All authors read, revised, and approved the final manuscript of this paper.

LITERATURE CITED

Ali M., Hameed S., Tahir M., 2014. Luteovirus: insights into pathogenicity. *Archives of Virology* 159: 2853–2860.

Ali M., Anwar S., Shuja M.N., Tripathi R.K., Singh J., 2018. The genus *Luteovirus* from infection to disease. *European Journal of Plant Pathology* 151: 841–860.

Ayala L., Henry M., González de León D., van Ginkel M., Mujeeb-Kazi A., … Khairallah M., 2001. A diagnostic molecular marker allowing the study of *Thinopyrum intermedium* derived resistance to BYDV in bread wheat segregating populations. *Theoretical and Applied Genetics* 102: 942–949.

Balaji B., Bucholtz D.B., Anderson J.M., 2003. *Barley yellow dwarf virus* and *Cereal yellow dwarf virus* quantification by real-time polymerase chain reaction in resistant and susceptible plants. *Phytopathology* 93: 1386–1392.

Byrne S., Schughart M., Ballandras V., Carolan J.C., Sheppard L., McNamara L., 2024. The first survey using high-throughput sequencing of cereal and barley yellow dwarf viruses in Irish spring and winter barley crops. *Irish Journal of Agricultural and Food Research* 63(1): 1–16

Cheng Z., XiaoYuan H., CaiCeng, C., GuangMin X., MaoSeng W.M., Jie Z., … GuangHe Z., 1996. Creating new transgenic wheat germplasm resistant to BYDV by applying gene engineering technology. *Plant Protection* 22(3): 18–20

Clark M.F., Adams A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475–483.

D'Arcy C.J., Domier L.L., 2000. Family Luteoviridae. In: *Virus Taxonomy: Seventh Report of the International Committee on the Taxonomy of Viruses* (MHV van Regenmortel, CM Fauquest, DHL Bishop, EB Carstens, MK Estes, SM Lemon, J. Maniloff, MA Mayo, DJ McGeoch, CR Pringle, and RB Wickner, ed.). Academic Press, San Diego, 1162 pp.

Deb M., Anderson J.M., 2008. Development of a multiplexed PCR detection method for *Barley* and *Cereal yellow dwarf viruses*, *Wheat spindle streak virus*, *Wheat streak mosaic virus* and *Soil-borne wheat mosaic virus*. *Journal of Virological Methods* 148: 17–24.

Delfosse V.C., Barrios Barón M.P., Distéfano A.J., 2021. What we know about poleroviruses: advances in understanding the functions of polerovirus proteins. *Plant Pathology* 70: 1047–1061.

Derron J.O., Gugerli P., Hani A., Widmer H., 1986. Caractérisation du virus de la jaunisse nanisante de l'orge (BYDV) en Suisse. *Revue Suisse d'Agriculture* 18(4) : 233–237.

Domier L.L., D'Arcy C.J., 2008. Luteoviruses. In: *Encyclopedia of Virology*. (Mahy B.W.J., van Regenmortel M.H.V. ed.). Elsevier Ltd, 231–238.

Foissac X., Svanella-Dumas L., Gentit P., Dulucq M.J., Candresse T., 2001. Polyvalent detection of fruit tree *Tricho*, *Capillo* and *Foveavirus* by nested RT-PCR using degenerated and inosine containing primers (DOP RT-PCR). *Acta Horticulturae* 550: 37–43.

International Committee on Taxonomy of Viruses, 2024. *Virus Taxonomy: 2023 release*. https://ictv.global/taxonomy.

Jarosova J., Chrpova J., Sip V., Kundu J.K., 2013. A comparative study of the *Barley yellow dwarf virus* species PAV and PAS: distribution, accumulation and host resistance. *Plant Pathology* 62: 436–443.

Jin Z., Wang X., Chang S., Zhou G., 2004. The complete nucleotide sequence and its organization of the genome of Barley yellow dwarf virus-GAV. *Science in China Series C: Life Sciences* 47: 175–182.

Kakareka N.N., Volkov Y.G., Sapotskyi M.V., Tolkach V.F.,

Shchelkanov M.Y., 2020. Viruses of cereal crops and their vectors in the south of the Russian Far East. *Sel'skokhozyaistvennaya Biologiya* (*Agricultural Biology*) 55(3): 439–450.

Krueger E., Beckett R., Gray S., Miller W. A., 2013. The complete nucleotide sequence of the genome of Barley yellow dwarf virus-RMV reveals it to be a new Polerovirus distantly related to other yellow dwarf viruses. *Frontiers in Microbiology* 4:205

Lister R.M., Ranieri R., 1995. Distribution and economic importance of *Barley yellow dwarf virus*. In: *Barley Yellow Dwarf: 40 Years of Progress*. (D'Arcy C.J., Burnett P.A., ed.). American Phytopathological Society Press, St. Paul, Minnesota, 29–53.

Malmstrom C.M., Bigelow P., Trebicki P., Busch A.K., Friel C., … Alexander H.M., 2017. Crop-associated virus reduces the rooting depth of non-crop perennial native grass more than non-crop-associated virus with known viral suppressor of RNA silencing (VSR). *Virus Research*, 241: 172–184.

Miller W.A., Liu S., Beckett R., 2002. Barley yellow dwarf virus: Luteoviridae or Tombusviridae? *Molecular Plant Pathology* 3: 177–183.

Oswald J.W., Houston B.R., 1953. Host range and epiphytology of the cereal yellow-dwarf disease. *Phytopathology* 43: 309–313.

Pallett D.W., Ho T., Cooper I., Wang H., 2010. Detection of *Cereal yellow dwarf virus* using small interfering RNAs and enhanced rate with *Cocksfoot streak virus* in wild cocksfoot grass (*Dactylis glomerata*). *Journal of Virological Methods* 168(2): 223–227.

Rasochova L., Miller W. A., 1997. Barley Yellow Dwarf Viruses. *Annual Review of Phytopathology* 35: 167–190.

Rastgou M., Khatabi B., Kvarnheden A., Izadpanah K., 2005. Relationships of *Barley yellow dwarf virus*-PAV and *Cereal yellow dwarf virus*-RPV from Iran with viruses of the family *Luteoviridae*. *European Journal of Plant Pathology* 113(3): 321–326.

Rochow W. F., 1969. Biological properties of four isolates of barley yellow dwarf virus. *Phytopathology* 59(11): 1580–1589.

Rochow W. F., Muller I., 1971. A fifth variant of barley yellow dwarf virus in New York. *Plant Disease Reporter* 55(10): 874–877.

Rybicki E.P., 2015. A top ten list for economically important plant viruses. *Archives of Virology* 160: 17–20.

Scheets K., Miller W.A., Somera M., 2020. Abolish the family *Luteoviridae* (*Tolivirales*) and move its genera to the familes *Tombusviridae* (*Tolivirales*) and *Solemoviridae* (*Sobelivirales*). *International Committee Taxonomy Viruses* 2020: 1–10.

Singh K., Jarosova J., Fousek J., Chen H., Kundu J.K., 2020. Virome identification in wheat in the Czech Republic using small RNA deep sequencing. *Journal of Integrative Agriculture* 19(9): 1825–1833.

Somera M., Massart S., Tamisier L., Soovali P., Sathees K., Kvarnheden A., 2021. Corrigendum: A survey using high-throughput sequencing suggests that the diversity of cereal and barley yellow dwarf viruses is underestimated. *Frontiers in Microbiology* 12: 772637.

Trzmiel K., 2020. Occurrence of Wheat dwarf virus and Barley yellow dwarf virus species in Poland in the spring of 2019. *Journal of Plant Protection Research* 60(4): 345–350.

Trzmiel K., Hasiow-Jaroszewska B., 2023. Molecular characteristics of *Barley yellow dwarf virus*-PAS, the main causal agent of barley yellow dwarf disease in Poland. *Plants* 12(19): 3488.

Wang M.B., Cheng Z., Keese P., Graham M.W., Larkin P.J., Waterhouse P. M., 1998. Comparison of the coat protein, movement protein and RNA polymerase gene sequences of Australian, Chinese, and American isolates of barley yellow dwarf virus transmitted by *Rhopalosiphum padi*. *Archives of Virology*, 143: 1005–1013.

Wang X., Chang S., Jin Z., Li L., Zhou G., 2001. Nucleotide sequences of the coat protein and readthrough protein genes of the Chinese GAV isolate of Barley yellow dwarf virus. *Acta Virologica* 45: 249–252

Zhang W., Cheng Z., Xu L., Wu M., Waterhouse P., Zhou G., 2009. The complete nucleotide sequence of the barley yellow dwarf GPV isolate from China shows that it is a new member of the genus Polerovirus. *Archive Virology* 154(7):1125–1128.

Zitter T., 2001. *Virus Problems of Sweet Corn*. Department of Plant Pathology Cornell University Ithaca, New York 14853, https://www.academia.edu/78639988/Virus_Problems_of_Sweet_Corn.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Botryosphaeriaceae fungi associated with apricot dieback and gummosis in Türkiye

Mukaddes KAYIM[1,*], Marcelo I. BUSTAMANTE[2], Ali ENDES[3], Akif ESKALEN[2]

[1] *Department of Plant Protection, Faculty of Agriculture, University of Çukurova, Balcalı 01330, Adana, Türkiye*
[2] *Department of Plant Pathology, University of California, Davis CA 95616, California, USA*
[3] *Department of Plant Protection, Faculty of Agriculture, University of Yozgat Bozok, Yozgat, Türkiye*
*Corresponding author: mkayim@cu.edu.tr

Summary. Apricot (*Prunus armeniaca* L.) is an important and widely grown fruit crop in Türkiye. In the last 15 years, symptoms of branch dieback and gummosis have been observed in commercial apricot orchards. A survey conducted in 2015 across 44 apricot orchards in the Adana and Mersin provinces revealed consistent infections caused by *Botryosphaeriaceae* fungi. From symptomatic branch samples (n = 232), a total of 128 fungal isolates with botryosphaeriaceous morphology were recovered, representing an incidence of 55.2%. Preliminary morphological identifications suggested the presence of three species. Representative isolates from each morphological group were identified as *Diplodia seriata* (n = 10), *Neofusicoccum parvum* (n = 7), and *Lasiodiplodia mediterranea* (n = 1), based on phylogenetic analyses of nucleotide sequences from the ribosomal DNA internal transcribed spacer, beta-tubulin and translation elongation factor 1-alpha genes. Mycelium growth rates were different among the three species, and the optimal growth temperatures were estimated as 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea*. Pathogenicity of the identified fungi was confirmed on 2-year-old 'Tyrinthe' apricot seedlings, with *L. mediterranea* being the most virulent, followed by *D. seriata*, and *N. parvum*. This is the first report of *D. seriata*, *N. parvum*, and *L. mediterranea* causing branch dieback and gummosis of apricot in Türkiye.

Keywords. *Diplodia*, etiology, *Lasiodiplodia*, *Neofusicoccum*, *Prunus armeniaca*

OPEN ACCESS

Citation: Kayim, M., Bustamante, M. I., Endes, A. & Eskalen, A. (2025). *Botryosphaeriaceae* fungi associated with apricot dieback and gummosis in Türkiye. *Phytopathologia Mediterranea* 64(2): 205-218. doi: 10.36253/phyto-15991

Accepted: July 20, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Lizel Mostert, Faculty of AgriSciences, Stellenbosch, South Africa.

ORCID:
MK: 0000-0003-0309-0390
MIB: 0000-0001-7887-7230
AE: 0000-0003-4815-5864
AE: 0000-0002-8829-7413

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is an economically important and widely grown fruit tree in Türkiye. Although commercial production of apricot is concentrated in the provinces of Malatya, Mersin, Elazığ, Iğdır, and Kahramanmaraş (Hasdemir, 2022), cultivation in the eastern Mediterranean region of Türkiye has expanded over the past 20 years to meet the demands for early-season fresh fruit consumption. Apricots are main-

ly consumed fresh and dried, as well as in processed forms including as jam, juice, and baby food. Türkiye accounts for 25.2% of the global apricot production area, covering 141,851 ha, and contributes 21.7% of total global yield, amounting to 803,000 metric tons annually (FAOSTAT, 2024). More than half of Türkiye's apricot production originates from the Eastern Anatolia and Mediterranean regions. In 2022, total world apricot production area was 562,000 ha, with total fruit yield of 3.7 million metric tons.

Several biotic factors may limit apricot production. Among these, diseases such as dieback and gummoses on trunks and scaffolds of trees have been identified as potential threats to apricot orchards in the East Mediterranean Region of Türkiye. The causal agents of dieback in stone fruit crops (*Prunus* spp.) have been partially attributed to be fungi within the *Botryosphaeriaceae,* which are distributed in many countries, and affect numerous tree species which potentially serve as inoculum sources for apricot orchards (Zhang *et al*., 2021). For example, *Diplodia seriata* has been reported affecting apricot in Wisconsin, United States of America (Smith and Stanosz, 2006), South Africa (Damm *et al*., 2007) and Canada (Ellouze and Ilyukhin, 2024). *Botryosphaeria dothidea* has been detected affecting Japanese apricots in Taiwan (Ko *et al*., 2010). In the Czech Republic, species of *Diplodia* and *Dothiorella* were recorded associated with apricot decline (Spetik *et al*., 2024). In Iran, apricot dieback and gummosis were determined to be caused by *B. dothidea*, *D. seriata, Lasiodiplodia theobromae*, *Neofusicoccum mangiferae*, and *N. parvum*, among other pathogens (Soltaninejad *et al*., 2017). In Türkiye, *Neoscytalidium dimidiatum* has been reported as the causative agent of shoot blight, dieback, and canker on apricot trees in Central Anatolia (Oksal *et al*., 2020).

Apricot orchards were established in southern Türkiye between 2005 and 2014, using European varieties for early table fruit production. Within a few years after planting, several young trees showed sudden dieback and branch canker, while trees more than 10 years old were also affected. Since apricot orchards were planted near other stone fruit orchards, including plums and nectarines which were known to be infected by *D. seriata*, *L. theobromae*, *L. pseudotheobromae*, and *N. parvum* (Endes *et al*., 2016; Endes and Kayim, 2022), it can be hypothesized that apricot trees were also infected by *Botryosphaeriaceae* fungi. Consequently, the objectives of the present study were: (i) to identify the fungi associated with dieback and gummosis of apricot trees in Türkiye; and (ii) to determine their pathogenicity and virulence on apricot.

MATERIALS AND METHODS

Field sampling and isolation of fungi

Field sampling was conducted in 44 apricot orchards with histories of branch dieback and gummoses. The orchards were located in the provinces of Adana (n = 25) and Mersin (n = 19). In each orchard, trees were inspected across diagonal transects, following the method described by Lazarov and Grigorov (1961). Common symptoms, including dieback, gummoses, and wood cankers were recorded. Symptomatic branches (lengths 20 to 70 cm) and sections of main trunks (lengths 5 to 20 cm) were collected and transported to the laboratory. Fungal isolations were carried out from symptomatic plant samples (n = 232), after surface disinfection using 1% sodium hypochlorite for 5 min followed by four rinses in sterile distilled water. The samples were then dried on clean filter papers, and four to five pieces (1 to 10 mm^2) were excised and placed onto Petri dishes containing potato dextrose agar amended with 0.01% tetracycline (PDA-tet) (Sigma-Aldrich). Plates were then incubated in the dark at 25±1°C for 4 to 7 d. Subcultures of isolates from fungi growing from tissue pieces were made by transferring hyphal tips onto fresh PDA–tet plates. Single conidium cultures from representative isolates of each pathogen species were obtained according to Choi *et al.* (1999), for molecular identification and morphological characterizations. Fungal colonies were sub-cultured on fresh PDA, and then stored in 30% glycerol in a −80°C deep-freeze for long term storage as fungal plugs.

Morphological characterization of isolated fungi

Fungal isolates identified to species level were further studied for their cultural and conidial characteristics as previously described (Damm *et al*., 2007, Phillips *et al*., 2013). All selected isolates (n = 16) were first grown on PDA or 3% oat meal agar (OMA) and incubated at 25°C under a 12 h daily photoperiod for 30 days, to induce conidium production (Amponsah *et al*., 2008; Wang *et al*., 2011). During the incubation period, colony morphology was observed for cultures on PDA. Conidium size (length and width) of each isolate was determined for 50 conidia, using a compound microscope camera (Olympus Bx51).

Molecular identification of isolated fungi

Total genomic DNA from 18 fungal isolates was extracted using a commercial kit following the manu-

facturer's protocol (Qiagen GmbH). Polymerase chain reactions (PCR) were carried out using the primer pairs ITS4/ITS5 (White *et al*., 1990), Bt2a/Bt2b (Glass and Donaldson, 1995), and 688F/1251R (Alves *et al*., 2008) to amplify, respectively, the ribosomal DNA internal transcribed spacer (ITS), the beta-tubulin (*tub2*), and translation elongation factor 1-alpha (*tef1*) gene regions. PCRs were run in a T100 thermocycler (Bio-Rad). Each PCR reaction had a total volume of 50 µL, that consisted of 25 µL of DNA polymerase master mix (GoTaq® Green MasterMix 2X, Promega), 18.6 µL of nuclease-free water, 1.2 µL of each primer (10 µM), and 4 µL of DNA template (approx. 100 ng). The PCR parameters included a 3 min preheating at 95°C, followed by 35 cycles each of 30 s at 95°C for denaturation, 45 s at 49°C for ITS and *tef1* annealing or 58°C for *tub2*, and 60 s at 72°C, with a final extension for 7 min at 72°C. The PCR products were visualized by electrophoresis in 1% agarose gels, and were purified for sequencing with the QIAquick PCR Purification Kit (Qiagen). Sanger sequencing was carried out by Genoks (Ankara, Türkiye). Obtained sequences were assembled using BioEdit software (version 7.2.5). Consensus sequences were compared with those deposited in the NCBI GenBank database using BLAST (version 2.0) searches. All isolates (n = 18) were first sequenced for ITS. From groups sharing identical ITS sequences, representative isolates were further sequenced for *tub2* (n = 8), and only one was sequenced for *tef1* (isolate MEKA194). Two sequence databases were created, one consisting of ITS and *tub2* sequences and the second consisting of ITS, *tub2* and *tef1* sequences, incorporating reference strains of species that showed similarity percentages greater than 97% in the BLAST search results (Tables 1 and 2). Alignments were performed by locus in MAFFT 7 (Katoh *et al*., 2019), and were manually trimmed on BioEdit 7 (Hall, 1999). Alignments were combined into one, and phylogenetic analyses were performed using maximum parsimony (MP) in MEGA 11 (Tamura *et al*., 2021) and maximum likelihood (ML) in IQ-TREE 2 (Minh *et al*., 2020), each with 1,000 bootstrap replicates. The MP phylogenies were generated using the tree-bisection-regrafting algorithm at search level 1, with initial trees obtained through the random addition of sequences across ten replicates. Tree length, consistency index (CI), retention index (RI), and composite index (CI) values were recorded for each analysis. For ML analyses, the dataset was partitioned by locus to identify the best-fit models, based on the PartitionFinder algorithm (Lanfear *et al*., 2012), resulting in two and three partitioned subsets. Settings for ML analysis were as described elsewhere (Bourret *et al*., 2018). The resulting trees were then compared, and support values were combined for nodes sharing identical topology using Inkscape 0.92 (http://inkscape.org).

Effects of temperature on isolate mycelium growth

Representative isolates of *Diplodia seriata* (AYKA244, MEYKA116, and MEYKA124), *Neofusicoccum parvum* (MEKA205) and *Lasiodiplodia mediterranea* (MEKA194) were cultured on PDA for 48 h at 25°C in continuous darkness to obtain actively growing mycelium. Mycelium plugs (each 4 mm diam.) were made around colony margins using a sterile cork borer, and were transferred to new Petri dishes each containing 20 mL of PDA. A plug was placed at the centre of each plate with the mycelium facing the medium surface. Plates were then incubated in darkness at different temperatures (5, 10, 15, 20, 25, 30, or 35°C) for 48 h. Resulting colony diameters were then measured using a caliper, with two (horizontal and vertical) measurements that were averaged for each colony. The experiment was repeated twice, with five replicate plates for each isolate. The optimal growth temperature (T_{opt}) and maximum colony growth (Y_{max}) were estimated for each replicate using the Analytis beta model, following Moral *et al*. (2012). Data obtained were subjected to analysis of variance (ANOVA) using generalized linear models in InfoStat (version 2008). Normality and homoscedasticity of data were checked and corrected when necessary, and means were compared using Fisher's LSD test (α = 5%).

Table 1. Locations, frequencies of orchards, plant samples, and *Botryosphaeriaceae* isolates recovered from apricot trees in Türkiye.

Provinces	Surveyed orchards (n)	Collected samples (n)	Frequency of isolates recovered (n)			
			D. seriata	*N. parvum*	*L. mediterranea*	Total *Botryosphaeriaceae*
Adana	25	135	41	35	0	76
Mersin	19	97	35	16	1	52
Total	44	232	76	51	1	128

Table 2. Conidium dimensions of isolates of *Botryosphaeriaceae* species obtained from samples from apricot trees affected by dieback and gummosis in Türkiye.

Isolate[a]	Conidium length (L) × width (W) (µm) [b]	Mean ± SD (µm)[c]	L/W ratio ± SD[d]
D. seriata			
AYKA374.3	(22.5–)24.6-27.5 × (11.5–)12.5-15.0	24.4 ± 0.2 × 12.6 ± 0.1	1.9 ± 0.02
AYKA374.1	(20.0–)24.1-27.0 × (10.0–)12.5-14.5	24.2 ± 0.2 × 12.4 ± 0.2	2.0 ± 0.03
AYKA374.2	(22.5–)24.5-27.0 × (10.5–)12.3-13.3	24.3 ± 0.2 × 12.2 ± 0.1	2.0 ± 0.02
MEYKA124	(17.3–)23.0-29.5 × (8.0–)10.0-12.5	23.4 ± 0.4 × 9.9 ± 0.1	2.4 ± 0.04
AYKA244	(19.0–)25.3-29.5 × (8.0–)10.8-13.3	25.3 ± 0.3 × 10.9 ± 0.2	2.3 ± 0.02
MEYKA116	(23.3–)25.4-29.8 × (10.0–)11.3-13.3	25.9 ± 0.2 × 11.4 ± 0.1	2.3 ± 0.02
MEYKA39	(18.5–)23.8-30.0 × (7.5–)10.0-13.0	23.7 ± 0.4 × 10.0 ± 0.2	2.4 ± 0.04
MEYKA117	(22.8–)25.4-29.3 × (8.8–)11.3-13.3	25.7 ± 0.2 × 11.3 ± 0.1	2.3 ± 0.02
CBS 112555[T]	(21.5–)22.0-28.0) × (11.0–)11.5-15.5	24.9 ± 1.9 × 12.9 ± 1.1	1.9
N. parvum			
MEKA205	(13.8–)18.8-23.8 × (3.8–)6.3-8.8	19.2 ± 0.4 × 6.0 ± 0.2	3.2 ± 0.05
AKKA308.2	(12.5–)18.9-29.0 × (3.8–)6.3-13.5	19.1 ± 0.6 × 6.9 ± 0.4	2.9 ± 0.07
AKKA308.6	(11.3–)18.3-23.8 × (3.8–)5.8-8.5	18.3 ± 0.5 × 5.8 ± 0.2	3.2 ± 0.05
AKKA308.4	(12.5–)20.1-23.8 × (3.8–)6.0-8.0	19.4 ± 0.4 × 5.9 ± 0.2	3.3 ± 0.05
AKKA308.3	(11.3–)18.1-24.0 × (3.8–)5.3-8.0	17.7 ± 0.5 × 5.5 ± 0.2	3.3 ± 0.06
AKKA308.5	(12.3–)18.1-24.0 × (4.0–)6.3-8.0	18.2 ± 0.5 × 6.0 ± 0.1	3.1 ± 0.08
AKKA308.1	(12.3–)19.4-24.0 × (3.8–)6.1-8.0	18.7 ± 0.4 × 5.9 ± 0.1	3.2 ± 0.07
CMW 9081[T]	(12.0–)15.0-24.0 × 4.0-6.0	16.9 × 5.4	3.1
L. mediterranea			
MEKA194	(18.5–)26.9-34.0 × (10.8–)15.0-20.5	26.4 ± 0.6 × 14.9 ± 0.3	1.8 ± 0.02
CBS 137784[T]	(26.3–)30.6(–37) × (13.5–)16.1(–18)	30.6 ± 2.8 × 16.1 ± 0.9	1.9 ± 0.20

[a] Type-material strains are noted with a superscript T. Data were obtained from Linaldeddu *et al.* (2015), Phillips *et al.* (2013), and Slippers *et al.* (2004).
[b] Minimum values are shown in parentheses followed by median and maximum values in length and width of 50 conidia from each isolate.
[c] SD = Standard deviation.
[d] Average length/width ratio.

Pathogenicity tests

To assess fulfillment of Koch's postulates, 2-year-old apricot seedlings ('Tyrinthe') were grown in a greenhouse at the Faculty of Agriculture of Cukurova University, and were inoculated with isolates of *D. seriata* (isolate MEYKA117), *N. parvum* (isolate MEKA205), or *L. mediterranea* (isolate MEKA194). Mycelium plugs were obtained with a sterile cork borer from the margin of a colony of each isolate after incubation for 5 d on PDA. The surfaces of the inoculation points on the stems of the seedlings, 10cm above the graft unions, were disinfected with 70% alcohol. The inoculations were each performed by making a 4 mm diam. wound on the disinfected stem with a cork borer, then placing the mycelium plug into the wound, and then wrapping with Parafilm to prevent secondary fungal contamination. Each isolate was inoculated into five apricot seedling replicates. Inoculation controls consisted of PDA plugs without mycelium. Inoculated and control seedlings were then incubated in a greenhouse for 3 months. Disease progression on the seedlings was observed, and lengths of streaking lesions were measured after the 3 month period. The experiment was conducted twice. Re-isolations from the margins of developed lesions were carried out onto PDA-tet. Analyses of variance (ANOVA) of the lesion length data were carried out in SPSS (IBM SPSS statistical software, v20.0), and means were compared using Fisher's LSD test at 5% of significance (Gomez and Gomez, 1984).

RESULTS

Field sampling, sample collections, and isolation of fungi

The field survey revealed branch dieback of young trees with lack of leaves, canker, and gummoses of

trunks (Figure 1 A, B and C). Sparse branching was observed in all trees exhibiting dieback. The most prevalent symptoms were wedge-shaped dark brown discolourations towards the xylem of cankered mature branches, with necroses (Figure 1 B) and cracking of the bark, often associated with gumming (Figure 1 C). Disease incidence varied from 1% to 50% among the affected orchards.

Sampled orchards (n = 44) yielded a total of 232 symptomatic branch samples, that were further inspected and analyzed in the laboratory. Isolations from margins of internal necrotic lesions of symptomatic samples gave

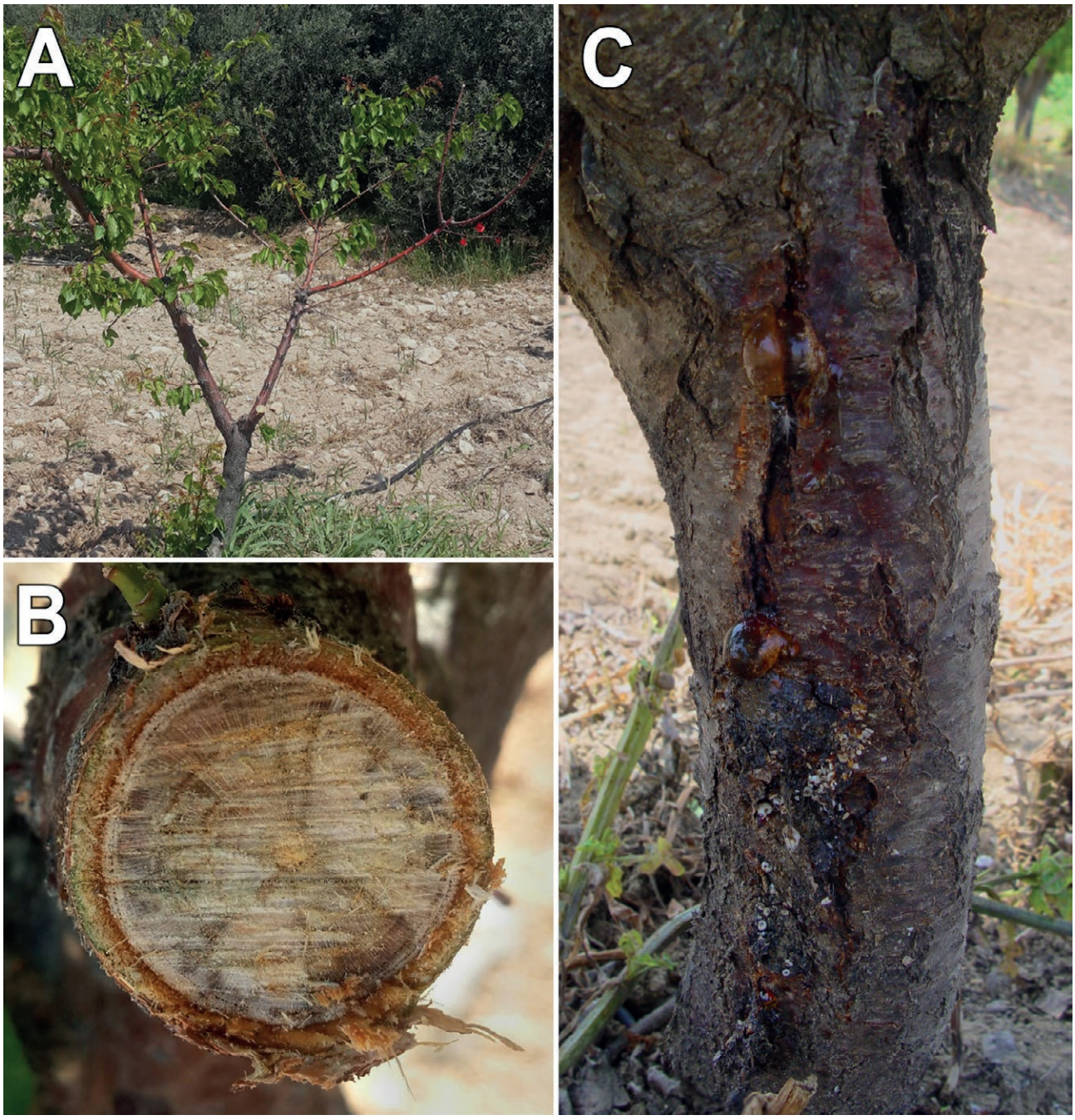


Figure 1. Symptoms of apricot dieback and gummosis in Türkiye. A, branch dieback on a juvenile tree showing lack of green leaves. B, cross section of a cankered mature branch showing wood discolouration and necroses. C, trunk gummosis on a mature tree.

consistent recovery (n = 128) of fungi exhibiting *Botryosphaeriaceae* morphology, representing 56.3% of samples from Adana and 53.6% from Mersin (Table 1). Based on colony morphology, fungal isolates were preliminarily identified up to genus level, as *Diplodia* (n = 76), *Neofusicoccum* (n = 51), or *Lasiodiplodia* (n = 1). While isolates with *Botryosphaeriaceae* morphology were consistently recovered from 55.2% of the apricot symptomatic samples, other recovered isolates morphologically identified (but not further analyzed) were *Alternaria* (n = 18), *Aspergillus* (n = 14), *Aureobasidium* (n = 8), Basidiomycetes (n = 8), *Colletotrichum* (n = 6), *Fusarium* (n = 12), *Nigrospora* (n = 2), *Penicillium* (n = 8), and *Trichoderma* (n = 8).

Morphological characterization of isolates

Isolates identified as *Diplodia* sp. (n = 10) developed white to gray colonies that became dark olivaceous gray to black with age.

One group of *Diplodia* isolates had fluffy aerial mycelium that started white gray and turned olivaceous gray to black with age, whereas the second group had flat olivaceous gray mycelium that became gray with time. All isolates produced large amounts of pycnidia on PDA and OMA at 25°C under a 12 h daily photoperiod within 25 d. Conidia were initially hyaline, aseptate, ellipsoid or cylindrical, each with a round apex and truncate base, turning brown once they matured and rarely having a single septum in some isolates. Conidium dimensions ranged from 17.3 to 30.0 μm in length (means 23.4 to 25.7μm) and from 7.5 to 15.0 μm in width (means 9.9 to 12.6 μm), with minor differences between isolates (Table 2).

The isolates preliminarily identified as *Neofusicoccum* sp. initially grew as white mycelium on PDA, turning to pale olivaceous gray and later black with age. A few isolates produced pycnidia after 30 d, and these structures were infrequent on PDA and OMA. Immature conidia were hyaline, aseptate, and fusiform with round apices, turning light brown when mature, and with one to two septa. Conidium dimensions ranged from 11.3 to 29 μm in length (means 17.7 to 19.2 μm) and from 3.8 to 13.5 μm in width (means 5.8 to 6.9 μm) (Table 2).

The isolate of *Lasiodiplodia* sp. developed abundant dense aerial mycelium on PDA that turned from white and gray to dark olivaceous gray after 15 d. Abundant pycnidia were observed after 25 d. Immature conidia were hyaline, aseptate, thick-walled, ellipsoid or cylindrical with round apices, and turned dark brown when mature, each with one septum and longitudinal striations. Conidium sizes ranged from 26.3 to 37 μm in length (mean 26.4 μm) and from 10.8 to 20.5 μm in width (mean 14.9 μm) (Table 2).

Molecular identification of fungi

Resulting sequences ranged from 505 to 582 bp for ITS, from 433 to 436 bp for *tub2*, and 377bp for *tef1*. Sequences were deposited in GenBank (Tables 3 and 4). The alignment of the first dataset (ITS-*tub2*) consisted of 37 taxa and 996 characters (including gaps). The MP analysis yielded one most parsimonious tree (tree length = 321, CI = 0.74, RI = 0.95, RC = 0.70), with an identical topology to that of the ML analysis. The MP bootstrap values were therefore combined into the ML phylogram (Figure 2). Selected isolates obtained from symptomatic apricot trees formed highly supported clades with reference strains of *Diplodia seriata* (84%/97% bootstrap values for ML/MP analyses, respectively), and *Neofusicoccum parvum* (78%/84%). The alignment of the second dataset (ITS-*tub2*-*tef1*) consisted of 22 taxa and 1,227 characters (including gaps). The maximum parsimony analysis yielded one most parsimonious tree (tree length = 420, CI = 0.75, RI = 0.77, RC = 0.58), with a comparable topology to that of the ML analysis. The bootstrap values were therefore combined into the ML phylogram (Figure 3). The *Lasiodiplodia* sp. isolate obtained from a symptomatic apricot tree formed a well-supported cluster with reference strains of *Lasiodiplodia mediterranea* (97%/95.9%).

Effects of temperature on mycelium growth

All isolates of *D. seriata* and *N. parvum* were able to grow at temperatures between 5°C and 35°C. The isolate of *L. mediterranea* grew only between 10°C and 35°C. The growth of the three tested isolates of *D. seriata* was not significantly different (P = 0.9649) at any of the temperatures; therefore, these data were averaged. The optimal growth temperatures (T_{opt}) and maximum colony growth values (Y_{max}) calculated by the model were significantly different ($P < 0.0001$) among species. The mean estimated T_{opt} were 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea* (Figure 4). Likewise, the calculated Y_{max} values were 40.5 mm for *D. seriata*, 56.2 mm for *N. parvum*, and 75.5 mm for *L. mediterranea*.

Pathogenicity tests

The three assessed isolates (*D. seriata*, *N. parvum*, *L. mediterranea*) were pathogenic on the main stems of 2-year-old 'Tyrinthe' apricot seedlings. This was evident by internal vascular lesions after peeling off the external layers of the inoculated stems and the presence of

gumming around the inoculated areas after a period of 3 months of incubation in the greenhouse. Mock inoculated plants remained symptomless. The fungal isolates used in the trial were successfully re-isolated from the margins of the lesions on the respectively inoculated seedlings, at rates that ranged from 66.7% to 100%, whereas no fungal colonies were recovered from mock inoculated plants. The mean lesion lengths caused by the fungi and the controls were significantly different ($P < 0.05$) (Figure 5). The isolate of *L. mediterranea* was the most virulent (mean lesion length = 193.4 mm), with the most extended lesions developing with gummoses around the inoculation points, followed by *D. seriata* (mean lesion length = 94.2 mm), and *N. parvum* (mean

Table 3. GenBank accession numbers of *Diplodia* and *Neofusicoccum* strains and isolates used in phylogenetic analyses.

Species[a]	Strain/isolate[b]	Host/substrate	Location	GenBank accession number	
				ITS	*tub2*
Botryosphaeria dothidea†	CBS 115476^T	*Prunus* sp.	Switzerland	AY236949	AY236927
Diplodia africana	CBS 120835^T	*Prunus persica*	South Africa	EF445343	KF766129
D. citricarpa	CBS 124715^T	*Citrus* sp.	Iran	KF890207	KX464784
D. corticola	CBS 112549^T	*Quercus suber*	Portugal	AY259100	DQ458853
D. malorum	CBS 124130^T	*Malus sylvestris*	Portugal	GQ923865	MT592507
D. mutila	CBS 136014^T	*Populus alba*	Portugal	KJ361837	MG015815
D. rosulata	CBS 116470^T	*Prunus africana*	Ethiopia	EU430265	EU673132
D. sapinea	CBS 121105	*Prunus persica*	South Africa	EF445339	KX464806
D. sapinea	CBS 393.84^T	*Pinus nigra*	Netherlands	DQ458895	DQ458863
D. scrobiculata	CBS 118110^T	*Pinus banksiana*	USA	AY253292	AY624258
D. scrobiculata	CBS 119939	*Pinus radiata*	Italy	MT587353	MT592500
D. seriata	**AYKA244**	***Prunus armeniaca***	**Adana, Türkiye**	**KX244800**	**KX259183**
D. seriata	CBS 112555^T	*Vitis vinifera*	Portugal	AY259094	DQ458856
D. seriata	CBS 121110	*Prunus armeniaca*	South Africa	EF445308	MT592554
D. seriata	CBS 124137	*Prunus domestica*	Bulgaria	MT587384	MT592559
D. seriata	**MEYKA116**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244797**	**KX259180**
D. seriata	**MEYKA117**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244798**	**KX259181**
D. seriata	**MEYKA124**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244799**	**KX259182**
Neofusicoccum australe	CMW 6837^T	*Acacia* sp.	Australia	AY339262	AY339254
N. dianense	CGMC C3.20082^T	*Eucalyptus urophylla* × *E. grandis*	China	MT028605	MT028937
N. illicii	CGMCC 3.18310^T	*Illicium verum*	China	KY350149	KY350155
N. kwambonambiense	CBS 123639^T	*Syzygium cordatum*	South Africa	EU821900	EU821840
N. luteum	CBS 562.92^T	*Actinidia deliciosa*	New Zealand	KX464170	KX464968
N. mediterraneum	CBS 121718^T	*Eucalyptus* sp.	Greece	GU251176	GU251836
N. nonquaesitum	CBS 126655^T	*Umbellularia californica*	USA	GU251163	GU251823
N. parvum	**AKKA308.1**	***Prunus armeniaca***	**Adana, Türkiye**	**MH221120**	**MH221122**
N. parvum	**AKKA308.2**	***Prunus armeniaca***	**Adana, Türkiye**	**MH221121**	**MH221123**
N. parvum	CBS 117923	*Guava* sp.	Venezuela	MT587517	MT592733
N. parvum	CMW 9071	*Ribes* sp.	Australia	EU339552	AY236909
N. parvum	CMW 9081^T	*Populus nigra*	New Zealand	AY236943	AY236917
N. parvum	**MEKA205**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244807**	**MG970283**
N. ribis	CBS 115475^T	*Ribes* sp.	USA	AY236935	AY236906
N. ribis	CBS 121.26	*Ribes rubrum*	USA	AF241177	AY236908
N. stellenboschianum	CBS 110864^T	*Vitis vinifera*	South Africa	AY343407	KX465047
N. vitifusiforme	CBS 110887^T	*Vitis vinifera*	South Africa	AY343383	KX465061
N. yunnanense	CGMC C3.20080	*Eucalyptus urophylla* × *E. grandis*	China	MT028672	MT029004
N. yunnanense	CGMC C3.20083^T	*Eucalyptus globulus*	China	MT028667	MT028999

[a] Outgroup is marked with a dagger symbol (†).
[b] Isolates obtained in this study are highlighted in bold font. Type-material strains are accompanied by a superscript T.

Table 4. GenBank accession numbers of *Lasiodiplodia* strains and isolates used in phylogenetic analyses.

Species[a]	Strain/isolate[b]	Host/substrate	Location	GenBank accession number		
				ITS	*tef1*	*tub2*
Diplodia mutila†	CBS 136014^T	*Populus alba*	Portugal	KJ361837	KJ361829	MG015815
Lasiodiplodia brasiliensis	CMM 4015^T	*Mangifera indica*	Brazil	JX464063	JX464049	n/a
L. cinnamomi	CFCC 51997^T	*Cinnamomum camphora*	China	MG866028	MH236799	MH236797
L. crassispora	CBS 118741^T	*Santalum* sp.	Australia	DQ103550	DQ103557	KU887506
L. gilanensis	CBS 124704^T	*Citrus* sp.	Iran	GU945351	GU945342	KU887511
L. gonubiensis	CBS 115812^T	*Syzygium cordatum*	South Africa	AY639595	DQ103566	DQ458860
L. hormozganensis	CBS 124709^T	*Olea* sp.	Iran	GU945355	GU945343	KU887515
L. laeliocattleyae	CBS 130992^T	*Mangifera indica*	Egypt	JN814397	JN814424	KU887508
L. lignicola	CBS 134112^T	Dead wood	Thailand	JX646797	KU887003	JX646845
L. lignicola	CGMCC 3.18061	Woody branch	China	KX499889	KX499927	KX500002
L. macrospora	CMM 3833^T	*Jatropha curcas*	Brazil	KF234557	KF226718	KF254941
L. mahajangana	CBS 124925^T	*Terminalia catappa*	Madagascar	FJ900595	FJ900641	FJ900630
L. mediterranea	CBS 137783^T	*Quercus ilex*	Italy	KJ638312	KJ638331	KU887521
L. mediterranea	CBS 137784	*Vitis vinifera*	Italy	KJ638311	KJ638330	KU887522
L. mediterranea	**MEKA194**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244816**	**PV239496**	**PV239497**
L. parva	CBS 456.78^T	Cassava-field soil	Colombia	EF622083	EF622063	KU887523
L. pseudotheobromae	CBS 116459^T	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111
L. pseudotheobromae	CBS 130991	*Mangifera indica*	Egypt	MT587433	MT592145	MT592629
L. subglobosa	CMM 3872^T	*Jatropha curcas*	Brazil	KF234558	KF226721	KF254942
L. theobromae	CBS 164.96^T	Fruit on coral reef coast	New Guinea	AY640255	AY640258	EU673110
L. viticola	CBS 128313^T	*Vitis vinifera*	USA	HQ288227	HQ288269	HQ288306
L. vitis	CBS 124060^T	*Vitis vinifera*	Italy	KX464148	MN938928	KX464917

[a] Outgroup is marked with a dagger symbol (†).
[b] Isolates obtained in this study are highlighted in bold font. Type-material strains are accompanied by a superscript T.

lesion length = 85.0 mm). Of the three species, *D. seriata* caused the least amount of gummosis, but caused significantly longer lesions than *N. parvum*.

DISCUSSION

In this study, three different species of *Botryosphaeriaceae*, including *Diplodia seriata*, *Lasiodiplodia mediterranea*, and *Neofusicoccum parvum*, were confirmed as causal agents of branch dieback and gummosis in commercial apricot orchards in the Adana and Mersin provinces of Türkiye. Among these pathogens, *D. seriata* and *N. parvum* have cosmopolitan distribution and broad host ranges, including apricot (Gure *et al*., 2005; Damm *et al*., 2007; Slippers *et al*., 2007; Abdollahzadeh *et al*., 2010; Linaldeddu *et al*., 2015; Soltaninejad *et al*., 2017). *Lasiodiplodia mediterranea* is known to have narrower host range and more limited geographical distribution (Linaldeddu *et al*., 2015; Wiseman *et al*., 2017).

The present study results indicated that *D. seriata* is the most abundant species affecting apricot trees in both Adana and Mersin in Türkiye. This is consistent with previous studies that reported predominance of *D. seriata* from symptomatic *Prunus* hosts in South Africa and Canada (Damm *et al*., 2007; Slippers *et al*., 2007 Ellouze and Ilyukhin, 2024). In Türkiye, *D. seriata* has previously been reported affecting commercial nectarine, plum (Endes *et al*., 2016; Endes and Kayim, 2022), and citrus orchards (Kurt *et al*., 2025) located in Adana and Mersin.

The second most frequently isolated species was *N. parvum*, one of the most common *Botryosphaeriaceae* affecting woody hosts (Sakalidis *et al*., 2013). In Türkiye, *N. parvum* has also been reported on almond and plum (Kayim *et al*. 2015; Endes and Kayim, 2022), as well as on grapevine (Akgül *et al*., 2014), pear (Kurbetli *et al*., 2020), walnut (Kara *et al*., 2021), and citrus (Kurt *et al*., 2025).

Lasiodiplodia mediterranea was the least frequently isolated species, represented by a single isolate recovered from the Mersin province. To date, *L. mediterranea* has been reported in Italy, Algeria and the Pacific Northwest of the United States of America, with a narrow host range including *Citrus sinensis*, *Quercus ilex*, *Vaccinium corymbosum*, and *Vitis vinifera* (Linaldeddu *et al*., 2015;

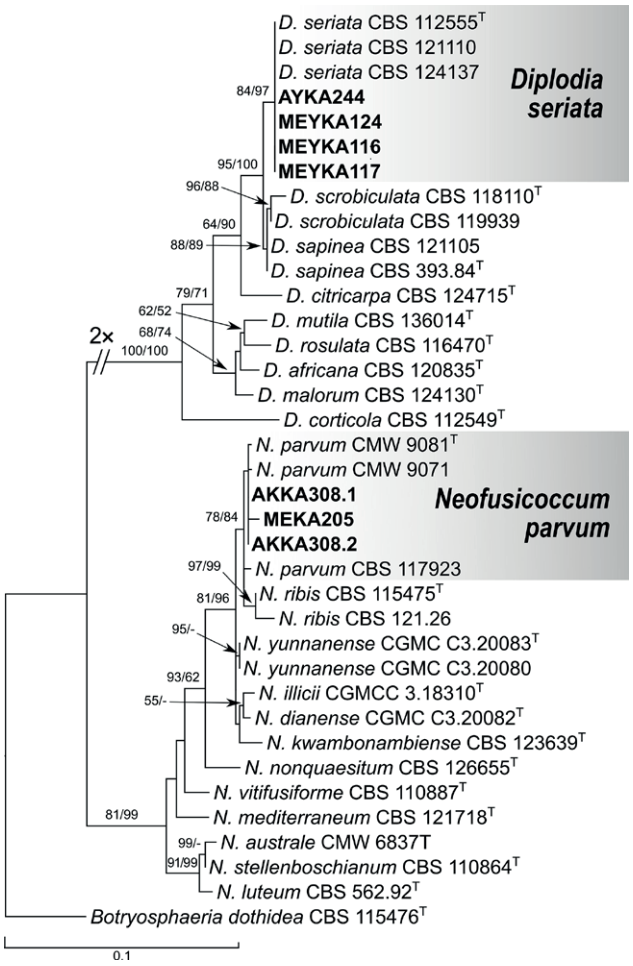


Figure 2. Maximum likelihood phylogenetic inference for isolates of *Diplodia seriata* and *Neofusicoccum parvum* obtained from apricot trees with dieback and gummosis symptoms in Türkiye, compared to reference strains of closely related species. The phylogenetic tree was inferred from a combined dataset of ITS and *tub2* sequences and rooted with *Botryosphaeria dothidea* (CBS 115476). Numbers above branches represent maximum likelihood and maximum parsimony bootstrap values from 1,000 replicates. Type-material strains are accompanied by a superscript T. Scale bar represents nucleotide substitutions per site.

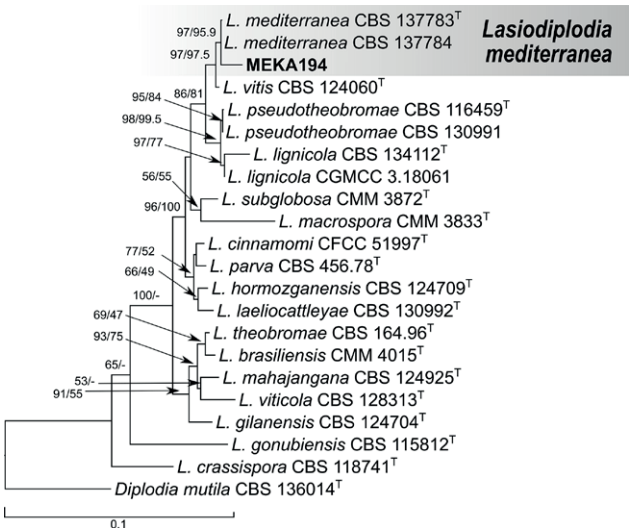


Figure 3. Maximum likelihood phylogenetic inference of a single isolate of *Lasiodiplodia mediterranea* (MEKA194) obtained from a symptomatic sample of apricot dieback in Türkiye, compared to reference strains of closely related species. The tree was inferred from a combined dataset of ITS, *tub2*, and *tef1* sequences and rooted with *Diplodia mutila* (CBS 136014). Numbers above branches represent maximum likelihood and maximum parsimony bootstrap values from 1,000 replicates. Type-material strains are accompanied by a superscript T. Scale bar represents nucleotide substitutions per site.

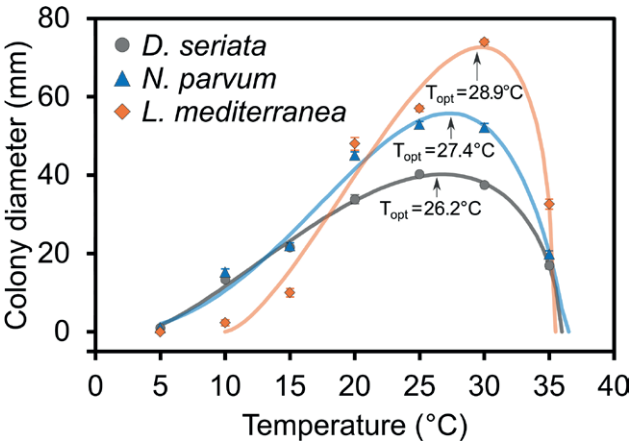


Figure 4. Mean colony diameters for representative isolates of *Diplodia seriata*, *Neofusicoccum parvum*, and *Lasiodiplodia mediterranea* causing apricot dieback and gummosis in Türkiye. Data points represent mean values of ten replicates measured after 48 h on PDA, fitted with nonlinear regression curves using the Analytis beta model. Vertical bars = standard errors. Topt = optimal growth temperature.

Wiseman *et al*., 2017). The present study provides a new geographical record and a new host record for *L. mediterranea*, in Türkiye and affecting apricot. Occurrence of this pathogen is likely due to the introduction of infected plant material.

Identification of *Botryosphaeriaceae* species currently relies on morphological observations coupled with DNA sequence analyses (Slippers *et al*., 2005; Phillips *et al*., 2013). In the present study, phylogenetic analyses using ITS, *tub2* and *tef1* sequences revealed well-supported clusters between the Turkish isolates and reference strains of *D. seriata*, *N. parvum* and *L. mediterranea* (Figures 2 and 3). This aligns with previous studies that utilized the same DNA barcodes to accurately

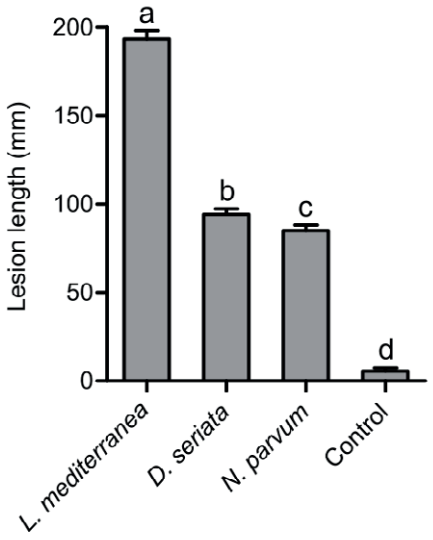


Figure 5. Mean lesion lengths (mm) caused by four *Botryosphaeriaceae* species inoculated into the main stems of 2-year-old 'Tyrinthe' apricot seedlings. Means accompanied by different letters are different according to Fisher's LSD test ($P < 0.05$).

identify *Botryosphaeriaceae* species (López-Moral *et al*., 2019; Gusella *et al*., 2022). Morphological characters were also important to confirm the species identification. The *D. seriata* isolates showed two distinct morphologies based on mycelium texture and colour when cultured at 25°C on PDA, which is consistent with previous studies (Moral *et al*., 2010). Conidium features of colour, shape and dimensions matched with the type strain of *D. seriata* (Table 2; Phillips *et al.,* 2013). The isolates identified as *N. parvum* and *L. mediterranea* had colonies that were consistent with descriptions of those species (Phillips *et al*., 2013; Linaldeddu *et al*., 2015). Conidium characteristics (size, shape, and colour) matched respective descriptions of *N. parvum* and *L. mediterranea* (Table 2; Burgess *et al*., 2006; Alves *et al*., 2008; Abdollahzadeh *et al*., 2010; Wang *et al*., 2011; Chen *et al*., 2014; Linaldeddu *et al*., 2015).

Studying effects of temperature on mycelium growth of plant pathogens assists understanding their distributions and abundance in geographical areas (Úrbez-Torres *et al*., 2006). Results of the present study showed that the three pathogens had different growth patterns across the assessed temperatures (Figure 4). At low temperatures (5, 10, and 15°C), the *D. seriata* and *N. parvum* isolates had similar growth rates, which were greater than for *L. mediterranea*. In contrast, at higher temperatures (25, 30, and 35°C), *L. mediterranea* grew more rapidly than *N. parvum,* while *D. seriata* grew more slowly than *L. mediterranea* or *N. parvum*. On the other hand, estimated optimum temperatures for mycelium growth were 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea*. This suggests that the *D. seriata* and *N. parvum* isolates were better adapted to cool environments than *L. mediterranea*, which may explain the higher frequency of *D. seriata* and *N. parvum* in the surveyed apricot orchards. Similar results have been reported elsewhere, with similar growth ranges for *D. seriata* (Jacobs and Rehner 1998; Copes and Hendrix 2004) and optimum growth at 26.8°C (Úrbez-Torres *et al*., 2006). For *N. parvum*, optimum temperatures have previously been estimated to be 25°C (Espinoza *et al*., 2009; Thomidis *et al*., 2011; Ismail *et al*., 2013; Chen *et al*. 2014), 28.2°C (Úrbez-Torres *et al*., 2006), and 30°C (Puig *et al*., 2021; Martino *et al*. 2024). For *L. mediterranea*, the optimum temperatures have been determined to be between 25°C and 30°C (Linaldeddu *et al*., 2015). The sampled apricot orchards are located in regions with temperate climate, characterized by warm to hot, dry summers, occasional droughts, and mild, wet winters. These conditions resemble those of other climatic zones where *Botryosphaeriaceae* are adapted, posing significant challenges for fruit crop growers in different countries (Phillips *et al*., 2013). Therefore, the occurrence of *D. seriata*, *N. parvum* and *L. mediterranea* affecting apricot trees in Türkiye is not surprising. In addition, due to ongoing climate change, temperatures are expected to increase during the coming decades, accompanied by decreased precipitation, conditions that could create increasingly stressful environments for agriculture, and likely leading to more intense expression of diseases (Altın and Barak, 2017; Zittis *et al*., 2022). These temperature changes could alter the distribution of the *Botryosphaeriaceae* pathogens in Türkiye, with *Lasiodiplodia* spp. potentially increasing in abundance relative to *Diplodia* and *Neofusicoccum* spp. (Batista *et al*., 2021).

Results from pathogenicity tests showed that the *D. seriata*, *N. parvum* and *L. mediterranea* isolates were pathogenic to 2-year-old apricot seedlings, with *L. mediterranea* being the most virulent species, followed by *D. seriata* as intermediate, and *N. parvum* as the least aggressive species (Figure 5). These results align with previous studies that have described *Lasiodiplodia* species causing more severe symptoms than *Diplodia* and *Neofusicoccum* species (Úrbez-Torres and Gubler 2009). Conversely, other studies described *N. parvum* as a more aggressive pathogen than *D. seriata* (Úrbez-Torres and Gubler 2009; Reis *et al*., 2020). Differential levels of aggressiveness among isolates of *D. seriata* and *N. parvum* have been documented previously (Qiu *et al*., 2016; Trotel-Aziz *et al*., 2022; Fernandez *et al*., 2023), a phenomenon highly influenced by environmental factors. Therefore, it is likely that environmental conditions in southern Türkiye exacerbate

symptoms caused by *D. seriata* rather than those caused by *N. parvum* in apricot trees.

In conclusion, *D. seriata*, *N. parvum*, and *L. mediterranea* were identified as causal agents of branch dieback and gummosis of apricot in Türkiye. Detection of these pathogens highlights the need for effective management strategies to reduce their impacts on apricot production. Further research should focus on preventative management strategies for these pathogens, which could include assessments of pruning wound protection using synthetic and biological products.

ACKNOWLEDGEMENTS

This research was supported by Grant 114O048 from The Scientific and Technological Research Council of Türkiye. Part of this work was also conducted as a Ph.D. thesis, supported by TÜBİTAK.

LITERATURE CITED

Abdollahzadeh J., Javadi, A., Mohammadi Goltapeh E., Zare R., Phillips A.J.L., 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. *Persoonia* 25: 1–10. https://doi.org/10.3767/003158510X524150

Akgül D.S., Savaş N.G., Eskalen A., 2014. First report of wood canker caused by *Botryosphaeria dothidea*, *Diplodia seriata*, *Neofusicoccum parvum*, and *Lasiodiplodia theobromae* on grapevine in Turkey. *Plant Disease* 98: 568. https://doi.org/10.1094/PDIS-07-13-0726-PDN

Altın T.B., Barak B., 2017. Trends and changes in tropical and summer days at the Adana Sub-Region of the Mediterranean Region, Southern Turkey. *Atmospheric Research* 196: 182–199. https://doi.org/10.1016/j.atmosres.2017.06.017

Alves A., Crous P.W., Correia A., Phillips A.J.L., 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* 28: 1–13.

Amponsah N.T., Jones E.E., Ridgway H.J., Jaspers, M.V., 2008. Production of *Botryosphaeria* species conidia causing grapevine green shoots. *New Zealand Plant Protection*-Se. 61: 301–305. https://doi.org/10.30843/nzpp.2008.61.6840

Batista E., Lopes A., Alves A., 2021. What do we know about Botryosphaeriaceae? An overview of a worldwide cured dataset. *Forests* 12: 313. https://doi.org/10.3390/f12030313

Bourret T.B., Choudhury R.A., Mehl H.K., Blomquist C.L., McRoberts N., Rizzo D.M., 2018. Multiple origins of downy mildews and mito-nuclear discordance within the paraphyletic genus *Phytophthora*. *PLOS ONE* 13(3):e0192502. https://doi.org/10.1371/journal.pone.0192502

Burgess T.I., Barber P.A., Mohali S., Pegg G., de Beer W., Wingfield M.J. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* 98: 423–435. https://doi.org/10.1080/15572536.2006.11832677

Chen S.F., Morgan D.P., Hasey J.K., Anderson K., Michailides T.J., 2014. Phylogeny, morphology, distribution, and pathogenicity of *Botryosphaeriaceae* and *Diaporthaceae* from English walnut in California. *Plant Disease* 98: 636–652. https://doi.org/10.1094/PDIS-07-13-0706-RE

Choi Y.W., Hyde K.D., Ho W.H., 1999. Single spore isolation of fungi. *Fungal Diversity* 3: 29–38.

Copes W.E., Hendrix F.F., 2004. Effect of temperature on sporulation of *Botryosphaeria dothidea*, *B. obtusa*, and *B. rhodina*. *Plant Disease* 88: 292–296. https://doi.org/10.1094/PDIS.2004.88.3.292

Damm U., Crous P.W., Fourie P.H., 2007. Botryosphaeriaceae as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov.. *Mycologia* 99: 664–680. https://doi.org/10.1080/15572536.2007.11832531

Ellouze W., Ilyukhin E. 2024. First report of *Diplodia seriata* associated with canker and dieback diseases of apricot and nectarine trees in Ontario, Canada. *J. of Plant Pathology* 106: 759–760. https://doi.org/10.1007/s42161-023-01579-8

Endes A., Kayim M., Eskalen A., 2016. First report of *Lasiodiplodia theobromae*, *L. pseudotheobromae*, and *Diplodia seriata* causing bot canker and gummosis of nectarines in Turkey. *Plant Disease* 100: 2321. https://doi.org/10.1094/PDIS-01-16-0036-PDN

Endes A., Kayim M., 2022. Morphological and Molecular Characterization of Botryosphaeriaceae Species Associated with Dieback and Gummosis on Plum Trees in Turkey. *Comptes Rendus de l'Acad émie Bulgare des Sciences* 75 (2): 295–302. https://doi.org/10.7546/CRABS.2022.02.16

Espinoza J.G., Briceño E.X., Chávez E.R., Úrbez-Torres J.R., Latorre B.A., 2009. *Neofusicoccum* spp. associated with stem canker and dieback of blueberry in Chile. *Plant Disease* 93: 1187–1194. https://doi.org/10.1094/PDIS-93-11-1187

FAOSTAT. 2024. https://www.fao.org/faostat/en/7-14-2024 data/QCL.

Fernandez O., Lemaître-Guillier C., Songy A., Robert-Siegwald G., Lebrun M.-H., ... Fontaine F., 2023. The combination of both heat and water stresses may worsen Botryosphaeria dieback symptoms in grapevine. *Plants* 12: 753. https://doi.org/10.3390/plants12040753

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied Environmental Microbiology* 61: 1323–1329. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Gomez K.A., Gomez A.A., 1984. Statistical Procedures for Agricultural Research. 2nd ed. Wiley, New York, USA.

Gure A., Slippers B., Stenlid J., 2005., Seed-borne *Botryosphaeria* spp. from native *Prunus* and *Podocarpus* trees in Ethiopia, with a description of the anamorph *Diplodia rosulata* sp. nov. *Mycological Research* 109: 1005–1014. https://doi.org/10.1017/S0953756205003266

Gusella G., Lawrence D.P., Aiello D., Luo Y., Polizzi G., Michailides T. J., 2022. Etiology of Botryosphaeria panicle and shoot blight of pistachio (*Pistacia vera*) caused by Botryosphaeriaceae in Italy. *Plant Disease* 106: 1192–1202. https://doi.org/10.1094/PDIS-08-21-1672-RE

Hall T.A., 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Hasdemir M., 2022. Ürün raporu kayısı 2022. *Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü*. 36 pp, https://arastirma.tarimorman.gov.tr/tepge/Menu/37/Urun-Raporlari

Inderbitzin P., Bostock R.M., Trouillas F.P., Michailides T.J., 2010. A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. *Mycologia* 102: 1350–1368. https://doi.org/10.3852/10-006

Ismail A.M., Cirvilleri G., Lombard L., Crous P.W., Groenewald J.Z., Polizzi G., 2013. Characterization of *Neofusicoccum* species causing mango dieback in Italy. *Journal of Plant Pathology* 95: 549–557.

Jacobs K.A., Rehner S.A., 1998. Comparison of cultural and morphological characters and ITS sequences in anamorphs of *Botryosphaeria* and related taxa. *Mycologia* 90:601–610. https://doi.org/10.1080/00275514.1998.12026949

Kara M., Soylu E.M., Soylu S., Uysal A., Kurt Ş., 2021. First report of *Neofusicoccum parvum* causing branch dieback on *Juglans regia* in Turkey. *Journal of Plant Pathology* 103: 335. https://doi.org/10.1007/s42161-020-00662-8

Katoh K., Rozewicki J., Yamada K.D., 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108

Kayim M., Endes A., Eskalen A., 2015. First report of *Neofusicoccum parvum* and *Diplodia* sp. associated with wood canker and dieback on almond in Turkey. *XVIII. International Plant Protection Congress*. 24–27 August, 2015 Berlin, Germany. https://ec.europa.eu/eip/agriculture/en/event/international-plant-protection-congress-ippc-2015.html

Ko Y., Liu, C.W., Chen S.S., Chiu K.Y., Sun Y.W., Maruthasalam, S. 2010. First report of gummosis diseases of Japanese apricot caused by *Botryosphaeria dothidea* in Taiwan. *Plant Disease* 95: 77. https://doi.org/10.1094/PDIS-05-10-0384

Kurbetli I., Aydoğdu M., Sülü G., Woodward S., Demirci F., 2020. A potential role for *Botryosphaeria parva* (anamorph *Neofusicoccum parvum*) in plane tree (*Platanus orientalis*) decline in Istanbul, Turkey. *Forest Pathology* 50: e12653. https://doi.org/10.1111/efp.12653

Kurt Ş., Uysal A., Guarnaccia V., Martino I, Soylu E. M., Oğuz M., 2025. Molecular identification and pathogenicity of Botryosphaeriaceae species associated with citrus wood diseases in the eastern Mediterranean region of Türkiye. *Journal of Plant Pathology* 107: 1077–1089. https://doi.org/10.1007/s42161-025-01877-3

Lanfear R., Calcott B., Ho S.Y. W., Guindon S., 2012. Partition Finder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses, *Molecular Biology and Evolution* 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Lazarov A., Grigorov P., 1961. Karantina na Rastenijata. Sofia, Bulgaria: Zemizdat.

Linaldeddu B.T., Deidda A., Scanu B., Franceschini A., Serra S., ...Phillips A.J.L., 2015. Diversity of Botryosphaeriaceae species associated with grapevine and other woody hosts in Italy, Algeria and Tunisia, with descriptions of *Lasiodiplodia exigua* and *Lasiodiplodia mediterranea* sp. Nov. *Fungal Diversity* 71: 201–214. https://doi.org/10.1007/s13225-014-0301-x

López-Moral A., Lovera M., Raya M. del C., Cortés-Cosano N., Arquero O., ...Agustí-Brisach C., 2019. Etiology of branch dieback and shoot blight of English walnut caused by Botryosphaeriaceae and *Diaporthe* species in southern Spain. *Plant Disease* 104: 533–550. https://doi.org/10.1094/PDIS-03-19-0545-RE

Martino I., Lione G., Garbelotto M., Gonthier P., Guarnaccia, V., 2024. Modeling the effect of temperature

on the severity of blueberry stem blight and dieback with a focus on Neofusicoccum parvum and cultivar susceptibility. *Horticulturae* 10: 363. https://doi.org/10.3390/horticulturae10040363

Minh B.Q., Schmidt H.A., Chernomor O., Schrempf D., Woodhams M.D., ...Lanfear R., 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution* 37(5): 1530–1534. https://doi.org/10.1093/molbev/msaa015

Moral J., Muñoz-Díez, C., González N., Trapero A., Michailides T.J., 2010. Characterization and pathogenicity of Botryosphaeriaceae species collected from olive and other hosts in Spain and California. *Phytopathology* 100: 1340–1351. https://doi.org/10.1094/PHYTO-12-09-0343

Moral J., Jurado-Bello J., Sánchez M.I., de Oliveira R., Trapero A., 2012. Effect of temperature, wetness duration, and planting density on olive anthracnose caused by *Colletotrichum* spp. *Phytopathology* 102: 974–981. https://doi.org/10.1094/PHYTO-12-11-0343

Oksal E., YiğitT., Özer G., 2020. First report of *Neoscytalidium dimidiatum* causing shoot blight, dieback and canker of apricot in Turkey. *Journal of Plant Pathology* 102: 579–580. https://doi.org/10.1007/s42161-019-00467-4

Phillips A.J.L., Alves A., Abdollahzadeh J., Slippers B., Wingfield M.J., ... Crous P.W., 2013. The Botryosphaeriaceae: genera and species known from culture. *Studies in Mycology* 76: 51–167. https://doi.org/10.3114/sim0021

Puig A. S., Keith, L. M., Matsumoto, T. K., Gutierrez, O. A., Marelli, J. P. 2021. Virulence tests of *Neofusicoccum parvum*, *Lasiodiplodia theobromae*, and *Phytophthora palmivora* on *Theobroma cacao*. *European Journal of Plant Pathology* 159: 851–862. https://doi.org/10.1007/s10658-021-02210-1

Qiu M., Steel C., Ash G., Savocchia S., 2016. Effects of temperature and water stress on the virulence of *Botryosphaeriaceae* spp. causing dieback of grapevines and their predicted distribution using CLIMEX in Australia. *Acta Horticulturae* 1115: 171–182. https://doi.org/10.17660/ActaHortic.2016.1115.26

Reis P., Gaspar A., Alves A., Fontaine F., Lourenço I., ... Rego C., 2020. Early season symptoms on stem, inflorescences and flowers of grapevine associated with *Botryosphaeriaceae* species. *Plants* 9: 1427. https://doi.org/10.3390/plants9111427

Sakalidis M.L., Slippers B., Wingfield B.D., Hardy G.E.S.J. Burgess T.I., 2013. The challenge of understanding the origin, pathways and extent of fungal invasions: global populations of the *Neofusicoccum parvum–N. ribis* species complex. *Diversity and Distributions* 19: 873–883. https://doi.org/10.1111/ddi.12030

Slippers B., Johnson G.I., Crous P.W., Coutinho T.A., Wingfield B.D., Wingfield M.J., 2005. Phylogenetic and morphological re-evaluation of the *Botryosphaeria* species causing diseases of *Mangifera indica*. *Mycologia* 97: 99–110. https://doi.org/10.1080/15572536.2006.11832843

Slippers B., Smit W.A., Crous P.W., Coutinho T.A., Wingfield B.D., Wingfield M.J., 2007. Taxonomy, phylogeny and identification of Botryosphaeriaceae associated with pome and stone fruit trees in South Africa and other regions of the world. *Plant Pathology* 56: 128–139. https://doi.org/10.1111/j.1365-3059.2006.01486.x

Smith D.R., Stanosz G.R., 2006. A species-spesific PCR assay for detection of *Diplodia pinea* and *D. scrobiculata* in dead red and jack pines with collar rot symptoms. *Plant Disease* 90: 307–313. https://doi.org/10.1094/PD-90-0307

Soltaninejad N., Mohammadi H., Massumi H., 2017. Isolation, identification and pathogenicity of Botryosphaeriaceae and *Phaeoacremonium* species associated with decline of *Prunus* species in Iran. *Journal of Plant Pathology* 99: 571–581. https://doi.org/10.4454/JPP.V9913.3937

Tamura K., Stecher G., Kumar S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version11. *Molecular Biology and Evolution* 38(7): 3022–3027. https://doi.org/10.1093/molbev/msab120

Thomidis T., Michailides T.J., Exadaktylou E., 2011. *Neofusicoccum parvum* associated with fruit rot and shoot blight of peaches in Greece. *European Journal of Plant Pathology* 131: 661–668. https://doi.org/10.1007/s10658-011-9840-0

Trotel-Aziz P., Robert-Siegwald G., Fernandez O., Leal C., Villaume S., ... Fontaine F., 2022. Diversity of *Neofusicoccum parvum* for the production of the phytotoxic metabolites (-)-terremutin and (*R*)-mellein. *Journal of Fungi* 8: 319. https://doi.org/10.3390/jof8030319

Úrbez-Torres J.R., Leavitt G.M., Voegel T.M., Gubler W.D., 2006. Identification and distribution of Botryosphaeria species associated with grapevines cankers in California. *Plant Disease* 90: 1490–1503. https://doi.org/10.1094/PD-90-1490

Úrbez-Torres J.R., Gubler W.D., 2009. Pathogenicity of Botryosphaeriaceae Species Isolated from Grapevine Cankers in California. *Plant Disease* 93: 584–592. https://doi.org/10.1094/PDIS-93-6-0584

Wang F., Zhao L., Li G., Huang J., Hsiang, T., 2011. Identification and characterization of *Botryosphaeria* spp.

causing gummosis of peach trees in Hubei province, central China. *Plant Disease* 95: 1378–1384. https://doi.org/10.1094/PDIS-12-10-0893

White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*, (Innis M.A , Gelfand D.H., Snisky J.J., White T.J. ed.). Academic Press, San Diego, CA, USA, 315–322.

Wiseman M.S., Serdani M., Putnam M.L., 2017. A New Cane Dieback Disease of Northern Highbush Blueberry in the United States Caused by *Lasiodiplodia mediterranea*. *Plant Disease* 101(7): 1317–1317. https://doi.org/10.1094/PDIS-01-17-0028-PDN

Zhang W., Groenewald J.Z., Lombard L., Schumacher R.K., Phillips A.J.L., Crous P.W., 2021. Evaluating species in Botryosphaeriales. *Persoonia* 46: 63–115. https://doi.org/10.3767/persoonia.2021.46.03

Zittis G., Almazroui M., Alpert, P., Ciais P., Cramer W., ... Howari F., 2022. Climate change and weather extremes in the Eastern Mediterranean and Middle East. *Reviews of Geophysics* 60: e2021RG000762. https://doi.org/10.1029/2021RG000762

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Occurrence of *Grapevine fanleaf virus* in Algerian vineyards, and complete genome sequencing

Nour Elhouda LAIDOUDI[1,*], Osamah ALISAWI[2], Bilal YAHIAOUI[3], Anfel DJENAOUI[4], Imane MAHDID[4], Adel BACHIR[4], Francesca DE LUCA[5], Elena FANELLI[5], Angelantonio MINAFRA[5], Naima MAHFOUDHI[6], Arezki LEHAD[4]

[1] *Laboratory of applied Microbiology, Department of Agronomy, Faculty of Natural and Life Sciences, Ferhat Abbas University, Setif, Algeria*
[2] *Department of Plant Protection, Faculty of Agriculture, University of Kufa, Najaf, Iraq*
[3] *Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas University, Setif, Algeria*
[4] *Phytopathology and molecular biology laboratory, National high school of agronomy, Belfort, Elharrach, Algiers, Algeria*
[5] *Istituto per la Protezione delle Piante, UOS-Bari, Consiglio Nazionale delle Richerche (C.N.R.), Via Amendola 122/D, 70126 Bari, Italy*
[6] *Laboratoire de Protection des Végétaux, Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray, 2049 Ariana, Tunisia*
*Corresponding author: nouelhouda.laidoudi@univ-setif.dz

Summary. Prevalence and genetic diversity of *Grapevine fanleaf virus* (*Nepovirus foliumflabelli,* GFLV) were determined in vineyards and grape varieties in Algeria. Samples (414) from different cultivars and viticulture areas were screened using DAS-ELISA and partially confirmed by RT-PCR, revealing 21% infection incidence. In Ahmer Bou Amer the greatest incidence of infection was recorded (61%). Some vines, confirmed to be GFLV-infected, had characteristic symptoms of leaf yellowing, chloroses, and mosaic patterns, reducing vine vigour and fruit quality. High throughput sequencing and bioinformatics analyses of a single GFLV-infected accession obtained a nearly complete grapevine fanleaf virus RNA1 consensus sequence of 5,979 nt, and an RNA2 with complete consensus sequence of 3,711 nt. *Grapevine yellow speckle viroid*, *Hop stunt viroid* and other viruses were also identified in the 'background' virome. Phylogenetic analyses of an amplified fragment of the GFLV coat protein gene from some of the accessions indicated close genetic relationships between Algerian and Russian/United States of America GFLV isolates, suggesting potential shared origins or transmission pathways. These results emphasize the need for implementing strict phytosanitary measures (e.g. use of virus-free planting material) to mitigate GFLV spread and its detrimental effects on grapevine production in Algeria.

Keywords. Grapevine, GFLV, DAS-ELISA, High Throughput Sequencing, RT-PCR.

OPEN ACCESS

Citation: Laidoudi, N. E., Alisawi, O., Yahiaoui, B., Djenaoui, A., Mahdid, I., Bachir, A., De Luca, F., Fanelli, E., Minafra, A., Mahfoudhi, N. & Lehad, A. (2025). Occurrence of *Grapevine fanleaf virus* in Algerian vineyards, and complete genome sequencing. *Phytopathologia Mediterranea* 64(2): 219-228. doi: 10.36253/phyto-15993

Accepted: July 23, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Nihal Buzkan, Kahramanmaraş Sütçü Imam University, Türkiye.

ORCID:
NEL: 0009-0006-6594-9411
OA: 0000-0002-8344-1113
BY: 0000-0003-0823-9860
AD: 0009-0004-1790-1320
IM: 0009-0006-9417-757X
BA: 0009-0005-6483-2222
FDL: 0000-0002-6646-8066
EF: 0000-0002-3132-3908
AM: 0000-0002-0547-7129
NM: 0000-0001-5157-5099
AL: 0000-0001-6653-4425

INTRODUCTION

Grapevine is the most important fruit crop in the world, with cultivation area of 6.7 million hectares producing 74 million tons of grapes annually. In Algeria, viticulture is carried out on 64,720 ha yielding 627,325 tons each year (FAOSTAT, 2022). Grapevines are susceptible to a several pests and pathogens, among which infectious intracellular agents (viruses, viroids, phloem and xylem limited prokaryotes) cause significant crop losses. Among diseases caused by viruses, the most important are grapevine degeneration and decline caused by nepoviruses, leafroll disease, the rugose wood complex and fleck disease (Fuchs, 2024).

Grapevine fanleaf virus (*Nepovirus foliumflabelli,* GFLV) is one of the 15 viruses implicated in fanleaf degeneration, a severe and widespread grapevine disease. (Schmitt-Keichinger *et al*., 2017). This virus can reduce vineyard yields, fruit quality, and decrease vine lifespans (Krebelj *et al*., 2015). GFLV is a member of the *Nepovirus* (*Secoviridae*), and is transmitted by the nematode *Xiphinema index* (M'rabet Samaali *et al*., 2024), in a nonpersistent, noncirculative manner (Demangeat, 2007; Fuchs *et al*., 2017).

Symptoms of GFLV infections appear in early spring and are in two main syndromes; leaf discolouration (mosaic patterns, vein discolouration, chloroses) and leaf malformation (deformation, close veins, serrated edges, enlarged petiolar sinuses) (Cigsar *et al*., 2003; Schmitt-Keichinger *et al*., 2017). Affected shoots exhibit fasciations, double nodes, and short internodes, with zigzag growth patterns. The fruit bunches on infected vines have clusters with small, unevenly ripened berries, which are few and irregular (Digiaro *et al*., 2017).

GFLV has a genome consisting of two single-stranded positive-sense RNAs (Vigne *et al*., 2008; Sanfaçon et *al*., 2009). Each RNA contains a single open reading frame (ORF) translated as a single polyprotein matured into functional products. The RNA 1 polyprotein (P1) with 7342 nt encodes the five proteins, protease cofactor (1A), helicase (1BHe), genome-linked protein (1CVPg), protease (1DPro), and RNA-dependent RNA polymerase (1EPol) (Vigne *et al*., 2004b). The RNA 2 polyprotein (P2), consisting of length 3774 nt, encodes protein $2A^{HP}$, designated as a putative homing protein necessary for the replication of RNA2, the movement protein (2BMP), and the coat protein (2Ccp) (Vigne *et al*., 2008; Mekuria *et al*., 2009). Both RNAs are polyadenylated at their 3' ends, and have a small covalently attached VPg protein (Mekuria *et al*., 2009; Elbeaino *et al*., 2014).

The GFLV genome has been characterized. High-throughput sequencing (HTS) of GFLV isolates from infected vines in the Champagne region of France showed that most grapevines were infected with multiple genetically diverse variants (Kubina *et al*., 2022). Additionally, *de novo* assembly of three GFLV genomes, along with those of other viruses and viroids, was achieved from four RNAseq datasets using HTS in Colmar, France (Hily *et al*., 2018). The complete RNA1 and RNA2 sequences of a new GFLV isolate (GFLV-SDHN) from northeastern China were determined, revealing unique genetic features, including a distinct insertion and significant sequence divergence compared to other isolates, along with evidence of a recombination event in the RNA2 2AHP region (Zhou *et al*., 2017).

In addition to virus pathogens, viroids are also important infectious agents affecting grapevine (Di Serio *et al*., 2017). Recently, six grapevine infecting viroid were known in the Mediterranean basin (Kaponi *et al*., 2024)

Based on coat protein (CP) genes, genetic diversity of GFLV has been was determined in South Africa (Liebenberg *et al*., 2009), Russia (Porotikova *et al*., 2021), China (Zhou *et al*., 2015), Tunisia (Fattouch *et al*., 2005) and France (Vigne *et al*., 2004a), but genetic diversity of GFLV in Algerian isolates is unknown, although some studies have hinted at presence of the virus in the center and west of Algeria (Tahirine *et al*., 2020).

HTS is an technology for analyzing genomic data (Mokili *et al*., 2012; Massart *et al*., 2014; Brister *et al*., 2015), and identifying virus pathogens *de novo* (Adams *et al*., 2009; Kreuze *et al*., 2009; Barba *et al*., 2014; Al Rwahnih *et al*., 2015). A study on Iranian grapevine cultivars by using HTS identified thirteen viruses and viroids, with grapevine red blotch, satellite, grapevine leafroll associated virus 1, and *Grapevine fanleaf virus* dominating (Gholampour *et al*., 2024).

The main objectives of the present study were to determine the presence and distribution of GFLV among different grapevine cultivars from multiple commercial vineyards, including autochthonous and Saharan grapevine accessions in Algeria, and to compare the phylogenetic relationships within the Algerian isolates with those from other countries.

MATERIALS AND METHODS

Virus sources

The field study and sample collections were carried out during autumn 2021 and winter 2022, in the major grapevine growing areas in western (Aïn Temouchent, Mascara, Sidi Belabes and Mostaganem) and central (Algiers, Tizi-Ouzou, Medea, Blida and Boumerdes) regions of Algeria (Figure 1). A total of 414 samples were

collected from individual vines of 24 different grapevine varieties, including commercial, autochthonous, and Saharan varieties. Mature canes were randomly collected. Each sample, consisting of four dormant cuttings, was split into two subsamples for serological and molecular analyses, and were stored at 4°C. During sampling, some randomly collected grapevine plants exhibited characteristic symptoms of GFLV infection. Approx. 20% of the selected samples showed visible symptoms, and these symptomatic vines were photographed for further documentation.

DAS-ELISA tests

To assess prevalence of GFLV viruses in Algeria, all the samples were tested by double sandwich ELISA (DAS-ELISA) (Adams and Clark, 1977) using the GFLV Commercial kit (Bioreba AG), following the manufacturer's protocol. Extracts were obtained by decorticating phloem tissue macerated in PBS buffer using 2 g per 10 mL. Absorbance was recorded at 405 nm using an automatic microplate reader.

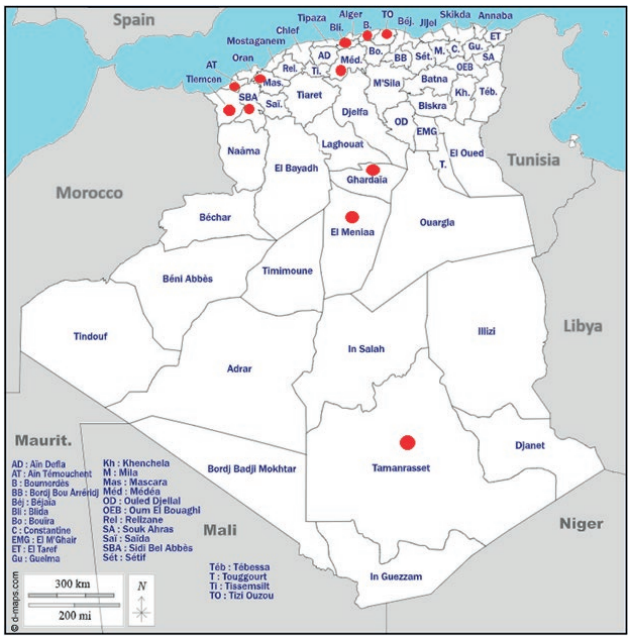


Figure 1. Map of Algeria showing the primary grapevine sampling regions for GFLV assessments. The red circles represent the locations where the samples were collected.

Molecular detection of viruses

Total nucleic acid extraction. Positive samples shown by DAS-ELISA were used for the subsequent molecular analyses, and total nucleic acids were extracted using the protocol of Gambino *et al.* (2008), with modifications. Phloem tissue (0.2 g) was ground in liquid nitrogen, then homogenized with 1 mL of grinding buffer (2% CTAB, 2,5 PVP 40, 2M NaCl, 100mM Tris-HCl pH 8, 25mM EDTA pH 8) prewarmed to 65°CThe solution was then centrifuged, and the supernatant was recovered. An equal volume of a mixture chloroform/isoamyl alcohol (24:1) was added to each tube and shaken for 45 min at low speed. After centrifugation for 15 min at 13,000 rpm, the supernatant from each tube was collected in a new tube. The precipitations were carried out in 2/3 volume of cold isopropanol (-20°C), with incubation for 20 min at -20°C followed by 10 min centrifugation at 13,000 rpm. The washing step was then carried out using ethanol 70%. The resulting pellet was re-suspended in 100 µL of sterile distilled water, and then stored at (-20°C).

Reverse transcription and amplification. Denaturation of total RNA was carried out 70°C by mixing 10 µL of each sample with 1 µL of random primer and 5.5 µL of sterile water. Reverse transcription was carried out using MMLV reverse transcriptase (200 U μL^{-1}). The cDNA was amplified using the Taq polymerase (HOT FIREPol DNA polymerase) and the primer pairs C3310 5′-GATGGTAACGCTCCCGCTGCTCTT-3′ and H2999 5′-TCGGGTGAGACTGCGCAACTTCCTA-3′ designed by MacKenzie *et al.* (1997) were used to amplify a fragment of 312 bp of the coat protein (CP) gene region. PCR cycling conditions used for amplification were: an initial denaturation at 90°C for 12 min, followed by 35 cycles each of denaturation at 90°C for 30s, 52°C annealing for 45 s and 72°C for 1 min, and final elongation at 72°C for 7 min. The PCR was hot-started at 92°C for 10 min, and completed by a 10 -min elongation step at 72°C. Amplified products were electrophoresed on 1.5% agarose gels in 1× tris-acetic-EDTA pH 8.0, and were visualized with gel red under UV light.

Sequence analyses. The amplified true size products were gel-purified using the NucleoSpin Gel & PCR Clean-up Mini kit (MACHEREY-NAGEL), according to the manufacturer's protocol. The purified DNA fragments were then cloned into the pGEM-T Easy Vector System II (Promega) using T4 DNA ligase. The ligation products were then transformed into Escherichia coli JM109 competent cells. Plasmid DNA from positive clones were isolated using the QIAprep Spin Miniprep Kit (Qiagen), following the manufacturer's instructions. The positive clones were sent for sequencing to Eurofins Genomics (Germany), and T7 and SP6 primers were used to obtain the entire sequence for each fragment.

High throughput sequencing

Total RNA extraction and Illumina-HTS. A single grapevine cane sample from the autochthonous cultivar 'Cherchalli' (ALG101) was selected for high-throughput sequencing to obtain the complete virome of the virus in the sample, which was known to be infected by GFLV. The sample was placed in Eppendorf tubes and immersed in RNALater solution, and then sent to the South Korean company JS-link, for further HTS production. QIAGEN's (Hilden, Germany) RNeasy® Plant Mini Kit was used to extract RNA from the plant sample, As part of the Illumina HTS library preparation, total RNA sequencing was carried out using the TruSeq Total RNA Library Prep Kit. After RNA quality was checked with an Agilent 2100 Expert Bioanalyzer (Agilent), the sequences from the RNA library were generated using NovaSeq 6000, 2× 100PE (Illumina, Inc.). Raw reads were trimmed (Kearse *et al*., 2012b) using Trimmomatic-0.39 and BBduk v 37.22 in Geneious Prime® 2024.0.5 (Kearse *et al,*. 2012a)

Map to reference. Mapper Geneious RNA was used in map to reference runs to map RNASeq data to reference sequences of principal grapevine affecting viruses (Sensitivity: Medium-Low). A consensus sequence was extracted from the RNA clean reads after mapping against suspected virus genomes using Geneious Prime® 2024.0.5 (Kearse *et al*., 2012a). The complete RNA reads were also mapped against a concatenated sequence (76,145,671 nt long), representative of all the 5040 plant virus sequences in GenBank. Results are displayed in a report that includes assembled reads, total used reads, coverage, and pairwise identity (Khaffajah *et al*., 2022).

Phylogenetic analysis. Phylogenetic analysis of the CP gene of the retrieved GFLV genome was carried out with sequences from different origins downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The alignment was carried out using MEGA11, and the phylogenetic relationships were constructed by the neighbor-joining method, with 1,000 bootstrap replications (Tamura *et al*., 2021), with *Grapevine fleck virus* (GFKV) isolate MT48 (NC_003347.1) as the out-group (Figure 3).

RESULTS

Symptoms caused by GFLV

Most of the surveyed vineyards, monitored across various regions in central Algeria during the spring of 2022 exhibited a range of disease symptoms, including leaf deformation, vein banding, mottling, proliferation, and yellow mosaic. The most common symptoms observed were leaf yellowing, vein banding, and deformation (Figure 2). The grapevine cultivars sampled in this study and the sampling locations are listed in Table 1.

In 'Seibel', the leaves each had fanleaf deformation, which is a symptom specific for GFLV. This symptom, along with chloroses, can reduce photosynthetic efficiency of grapevines, reducing overall vigour and productivity. 'Ahmer Bou Amer' exhibited yellow chloroses and mosaic patterns on the leaves, with irregularly distributed discolouration across the leaf surfaces.

GFLV incidence in Algerian grapevines

Results showed that GFLV was present in 87 (21%) of the samples tested by DAS-ELISA assay. The greatest incidence (61%) was observed in the variety 'Ahmer Bou Amer', followed by 'Carignon' (45%), 'Alicante' (40%), 'Muscat Italy' (28%), 'Cardinal' (27%), 'Cinsault' (26%), 'Seibel' (21%), 'Michel Palieri' and 'Ladhari' (20%), 'Gros Noir' (10%), 'Dattier' (6%), and 'Muscat' (5%) (Table 1). In contrast, the varieties 'Mersguerra', 'Valensi', 'Metllili', 'Sebseb', and 'Issers' were free of GFLV (Table 1). In

Figure 2. Grapevine leaf symptoms observed in Centre Algeria. (A) fanleaf with yellow mosaic, (B) chlorosis, and (C) vein discolouration with leaf malformation and yellowing.

Algeria, GFLV incidences were 59% in the central region and 41% in the western region. However, samples from the southern region (Sahara region Tamanrassetm, El Meniaa and Ghardaia) were negative for GFLV (Table 1).

Sequences analysis of the CP gene

Based on geographic origin and grapevine cultivar, 12 GFLV ELISA-positive samples were selected for further RT-PCR analyses. These samples included three from cultivars 'Muscat', 'Ahmer Bou', 'Amer' or 'Seibel' from the central region, and 'Dattier' from the western region. Among these, five cultivars were selected for variability analyses of a 312 bp fragment of the cloned CP gene sequences, to determine genetic diversity across different regions and grapevine varieties. The obtained sequences were submitted to GenBank, and accession numbers was assigned as: 'Ahmer Bou Amer', PP983215; 'Muscat Italy', PP983212; and 'Seibel', PP983213; from central Algeria: 'Dattier', PP983214) from the western region: and 'Cherchalli', PP976050 from central Algeria. These five sequences were selected for NGS analyses.

Table 1. Grapevine virus infection rates of GFLV detected in Algeria using double antibody sandwich (DAS-ELISA).

Grapevine cultivar	Number of samples collected from main viticulture regions in Algeria			Number of infected samples	Infection rate (%)
	Center	Western	South		
Ahmer Bou Amer	34	-	-	21	61.8
Dattier	15	-	-	2	6.7
	-	30	-	1	
Michel Palliri	10	-	-	2	20.0
Muscat Italie	28	-	-	8	28.6
Gros Noir	19	-	-	2	10.3
	-	10	-	1	
Seibel	28	-	-	6	21.4
Cardinal	27	-	-	9	27.0
	-	10	-	1	
Red Globe	20	-	-	0	0.0
Issers	4	-	-	0	0.0
Ladhari	-	20	-	4	20.0
Carignon	-	20	-	9	45.0
Cinsault	-	60	-	16	26.7
Alicante	-	10	-	4	40.0
Mersguerra	10	-	-	0	0.0
Muscat	-	20	-	1	5.0
Valensi	-	20	-	0	0.0
Sebseb	-	-	2	0	0.0
Metlili	-	-	4	0	0.0
Tazrouk Elkahla	-	-	1	0	0.0
Tazrouk Elbeidha	-	-	1	0	0.0
Tazrouk Sans Pepin	-	-	1	0	0.0
Dattier De Beirouth	-	-	1	0	0.0
Vigne Commun	-	-	1	0	0.0
Unknown	-	-	8	0	0.0
Total		414		87	21.0

To infer the phylogenetic relationship of the Algerian isolates, five Algerian coat protein (CP) gene sequences were aligned with homologous sequences from other countries, including 13 from France, two from Russia, eight from the United States of America, one from Iran, and one from Türkiye, all of which were retrieved from the GenBank database. The resulting phylogenetic tree showed that most Algerian isolates, particularly 'Seibel'" and 'Ahmer Bou Amer', exhibited close genetic relationship with European isolates, particularly those from Russia. The 'Muscat' and 'Dattier' isolates clustered with United States of America isolates, while the isolate GFLV ALG 101 clustered with the France isolate (Figure 3).

HTS analysis

The Illumina platform generated 47,034,642 short reads of a total of 101 bases. In Geneious software, all RNA reads were paired and mapped against suspected virus and viroid genomes. Against *Grapevine fanleaf virus* RNA1, 228,166 reads (0.48%) were assembled into a nearly complete consensus sequence contig of 5,979 bp. Two parts were missing, which were not covered by the reads. The first part was 15 bp between 5,980 and 5,994 bp, and the second was 73 bp between 6,801 and 6,873 bp. The coverage was 98.8%, and the GC content was 46.8%. The sequence covered 5,721 bp of 6,855 bp of the whole polyprotein region. This sequence was deposited in GenBank under accession number PP976049, and named ALG100. The partial genome portion reconstructed by reads assembly had one open reading frame coding for RNA helicase and RNA dependent RNA polymerase with length 5,721 nt, which encodes 1,907 amino acids residues. The assembled reads were 301,093 against the sequence of *Grapevine fanleaf virus* RNA2 (0.64%), and produced a complete consensus sequence of 3,711 bp. The coverage was 97.5% and the GC content was 49.9%. The sequence was 3,333 bp long, that encodes 1,111 residues representing the polyprotein P2 region, and was deposited in GenBank under accession number PP976050, and named ALG101. The P2 sequence codes for a typical Nepovirus subgroup A polyprotein and coat protein. In addition to the complete GFLV genome, two other viroids were identified as *Grapevine yellow*

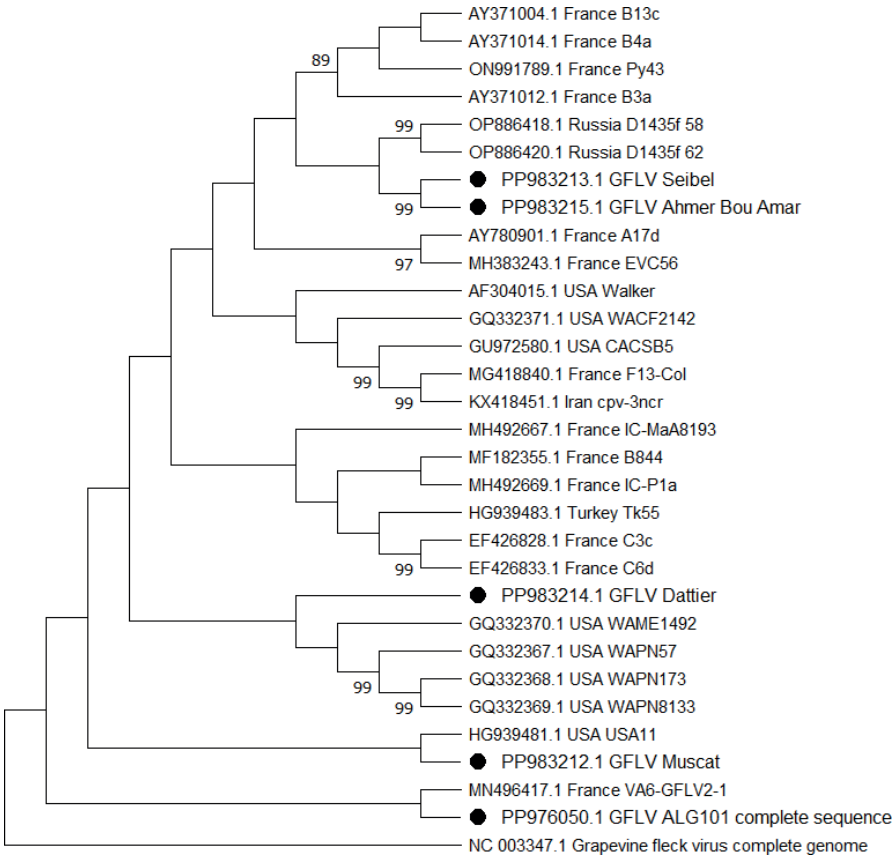


Figure 3. Phylogenetic tree of *Grapevine fanleaf virus*, constructed with sequences of a 312nt fragment of the viral capsid protein (CP), constructed using the neighbor-joining method with 1,000 bootstrap replicates. ● indicates Algerian isolates.

speckle viroid (PP977156) and *Hop stunt viroid* isolate (PP977157). Furthermore, *Grapevine Rupestris stem pitting-associated virus (GRSPaV)* and *Grapevine Pinot gris virus (GPGV)* were also detected. These viruses will be further characterized in a separate study.

DISCUSSION

The survey of vineyards across regions in Algeria revealed prevalence of symptoms suggestive of viral infections in grapevines. The observed symptoms, including leaf deformation, vein banding, mottling and yellow mosaic, were characteristic of virus etiology, aligning with reports from other viticulture regions.

These symptoms are often associated with the *Grapevine fanleaf virus* (GFLV), which have been widely documented as threats to grapevine health and productivity (Andret-Link *et al.,* 2004; Martelli, 2014). The widespread occurrence of these symptoms in Algeria may be influenced by environmental factors and agricultural practices that favour virus persistence and transmission. North Algeria has a moderate climatic conditions and intensive viticulture, which together can create conducive environments for GFLV spread through vectors such as the nematode *Xiphinema index* (Martelli, 2014; El Sayed *et al*., 2023). Additionally, the lack of rigorous virus-free certification programmes for planting material could contribute to dissemination of infected propagation stocks (Panno *et al*., 2021). The observed symptoms in the grapevine samples examined in the present study indicated infections caused only by GFLV. The key symptoms identified included chlorosis or yellowing along the leaf veins and edges, with some cases exhibiting distinct mosaic patterns (Martin *et al*., 2021; Kubina *et al*., 2022). These symptoms were particularly pronounced in 'Seibel' and 'Ahmer Bou Amer' grapevine cultivars.

DAS ELISA test results revealed an infection rate of 21% out of 414 samples, and demonstrated significant presence of GFLV within the surveyed regions. Previously, Tahirine *et al.* (2020) reported a GFLV infection rate of 47% in central and western Algeria. The observed decrease in infection incidence in the present study could

be due to the different vineyards sampled or the limited distribution of the virus in propagation materials.

This study has shown significant regional variations in GFLV incidence across central, western, and southern Algeria. The central region had the greatest infection rate at 59% (out of 159 samples). This could be due to environmental factors or agricultural practices in this region that are conducive to GFLV transmission. In contrast, the western region had a lower GFLV infection rate of 41% (out of 210 samples). While still considerable, this reduced incidence may indicate differences in viticultural practices, or lower presence of GFLV transmission vectors (*Xiphinema index*) (Everaert *et al.,* 2024). In the southern region, no GFLV infections were detected, suggesting that high temperatures may play a significant role in suppressing or eliminating the virus. This observation aligns with the principle underlying thermotherapy, a widely used method for the elimination of grapevine viruses through exposure to elevated temperatures (Panattoni and Triolo, 2010; Miljanić *et al*., 2022; El Sayed *et al*., 2023).

It is important to note that ELISA- and PCR-based methods are complementary in grapevine virus diagnostics. ELISA is widely used as a cost-effective and high-throughput tool for large-scale screening, while PCR offers increased sensitivity and can confirm infections, particularly in asymptomatic plants or when virus titers are low (Erilmez and Kaya, 2016; Vigne *et al*., 2018). In the present study, ELISA was employed to estimate GFLV prevalence across the sampled regions, and then RT-PCR was applied to a subset of samples for molecular confirmation and subsequent sequencing of viruses. Five GFLV isolates were chosen to represent diverse grapevine cultivars and geographic regions, and the phylogenetic analyses showed an absence of correlation between clustering and geographic origins of the samples. The Algerian isolates clustered in different clades, revealing sequence variability among each other and indicating potential differences at origins and spread of a panmictic population. The 'Seibel' and 'Ahmer Bou Amer' isolates grouped with Russian isolates; while 'Dattier', 'Musca't and GFLV ALG 101 isolates grouped with several isolates from the United States of America. These phylogenetic relationships demonstrated that the Algerian GFLV isolates were characterized by high diversity in this country.

A primary objective of the present study was to generate the first complete genome sequences of GFLV from Algeria, using Next-Generation Sequencing (NGS). This technique is important for detecting grapevine viruses due to its high sensitivity and throughput, as well as its capacity to concurrently identify known and novel viruses (Al Rwahnih *et al*., 2015). The resulting genomic data provide a valuable reference for future molecular and epidemiological studies.

Algerian GFLV isolates exhibited considerable genetic similarities to isolates from Russia, the United States of America, and France. These genetic relationships may be related to the past introductions of GFLV-infected propagation material from different viticultural regions, allowing dissemination of the virus across geographically distant host populations. Therefore, it is important to ensure that grafting materials, including scions and rootstocks, which are key sources of GFLV dissemination, are free of viruses. Rigorous procedures must be emphasized throughout production of virus-free plant material to prevent virus transmission. Understanding the genetic diversity within viral populations also facilitates creation of effective diagnostic tools that are essential for pathogen-free plant programmes. Furthermore, analysis of nematode distribution, specifically *Xiphinema index*, is essential for controlling GFLV dissemination in viticulture systems. The present preliminary investigation will continue, aiming to provide insights into the genetic population structure of GFLV in Algeria, and to support development of effective virus containment and management strategies.

FUNDING

This research was funded by the Algerian Ministry of Higher Education and Scientific Research (MESRS) Directorate-General of Scientific Research and Technological Development (DGRSDT), and the Algero-Tunisian project "INNOVITIS".

LITERATURE CITED

Adams AN., Clark MF., 1977. Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses. *Journal of General Virology* 34: 475–483. https://doi.org/10.1099/0022-1317-34-3-475

Adams I.P., Glover R.H., Monger W.A., Mumford R., Jackeviciene E., … Boonham N., 2009. Next-generation sequencing and metagenomic analysis: a universal diagnostic tool in plant virology. *Molecular Plant Pathology* 10: 537–545. https://doi.org/10.1111/j.1364-3703.2009.00545.x

Al Rwahnih M., Daubert S., Golino D., Islas C., Rowhani A., 2015. Comparison of Next-Generation Sequencing Versus Biological Indexing for the Optimal Detection of Viral Pathogens in Grapevine. *Phytopa-*

thology 105: 758–763. https://doi.org/10.1094/PHYTO-06-14-0165-R

Andret-Link P., Laporte C., Valat L, Ritzenthaler C., Demangeat G., Vigne E., ... Fuchs M., 2004. Grapevine fanleaf virus: still a major threat to the grapevine industry. *Journal of Plant Pathology* 86: 183–195.

Barba M., Czosnek H., Hadidi A., 2014. Historical Perspective, Development and Applications of Next-Generation Sequencing in Plant Virology. *Viruses* 6: 106–136. https://doi.org/10.3390/v6010106

Brister J.R., Ako-adjei D., Bao Y., Blinkova O., 2015. NCBI Viral Genomes Resource. *Nucleic Acids Research* 43: D571–D577. https://doi.org/10.1093/nar/gku1207

Cigsar I., Digiaro M., Gokalp K., Ghanem-Sabanadzovic NA., De Stradis A., ...Martelli G.P., 2003. Grapevine deformation virus, a novel nepovirus from Turkey. *Journal of Plant Pathology* 85: 183–191.

Demangeat G., 2007. Transmission des Nepovirus par les nématodes Longidoridae. *Virologie* 11: 14. https://doi.org/10.1684/vir.2007.0102

Di Serio F., Izadpanah K., Hajizadeh M., Navarro B., 2017. Viroids Infecting the Grapevine. In: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. (Meng B., Martelli G.P., Golino D.A., Fuchs M. ed.). Cham, Switzerland, Springer International Publishing AG, pp 373–392.

Digiaro M., Elbeaino T., Martelli G.P., 2017. Grapevine fanleaf virus and Other Old World Nepoviruses. In: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. (Meng B., Martelli G.P., Golino D.A., Fuchs M. ed.). Cham, Switzerland, Springer International Publishing AG, pp 47–82.

El Sayed T., Saker K., El Shorbagy A., Mandour A., Alajouz M., Soliman A., 2023. Characterization and Elimination of Grapevine fanleaf virus Using Thermotherapy in Combination with Meristem Tip Culture. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology* 15: 101–117. https://doi.org/10.21608/eajbsc.2023.312619

Elbeaino T., Kiyi H., Boutarfa R., Minafra A., Martelli G.P., Digiaro M., 2014. Phylogenetic and recombination analysis of the homing protein domain of grapevine fanleaf virus (GFLV) isolates associated with 'yellow mosaic' and 'infectious malformation' syndromes in grapevine. *Archives of Virology* 159: 2757–2764. https://doi.org/10.1007/s00705-014-2138-8

Erilmez S., Kaya A., 2016. Comparison of DAS-ELISA and RT-PCR methods for the diagnosis of grapevine viruses. *Bitki Koruma Bülteni* 56: 297–302.

Everaert E.A., Viaene N., Quataert P., Haegeman A., De Jonghe K., 2024. Towards Improved Nepovirus Detection and Identification in *Xiphinema* Nematodes. *PhytoFrontiers™ PHYTOFR*-03-24-0018-R. https://doi.org/10.1094/PHYTOFR-03-24-0018-R

Fattouch S., Acheche H., M'hirsi S., Mellouli L., Bejar S., Marrakchi M., Marzouki N., 2005 RT-PCR–RFLP for genetic diversity analysis of Tunisian Grapevine fanleaf virus isolates in their natural host plants. *Journal of Virological Methods* 127: 126–132. https://doi.org/10.1016/j.jviromet.2005.03.008

FAOSTAT, 2022. FAOSTAT: Crops and livestock products – Grapevines. Food and Agriculture Organization of the United Nations. Available at https://www.fao.org/faostat/en/#data/QCL/visualize. Accessed 12 Aug 2024

Fuchs M., 2024. Grapevine viruses: Did you say more than a hundred? *Journal of Plant Pathology* 107: 217–227. https://doi.org/10.1007/s42161-024-01819-5

Fuchs M., Schmitt-Keichinger C., Sanfaçon H., 2017. A Renaissance in Nepovirus Research Provides New Insights Into Their Molecular Interface With Hosts and Vectors. *Advances in Virus Research* 67: 61–105.

Gambino G., Perrone I., Gribaudo I., 2008. A Rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis* 19: 520–525. https://doi.org/10.1002/pca.1078

Gholampour Z., Zakiaghl M., Asquini E., Moser M., Gualandri V., Mehrvar M., Si-Ammour A., 2024. Application of High-Throughput Sequencing for Comprehensive Virome Profiling in Grapevines Shows Yellows in Iran. *Viruses* 16: 204. https://doi.org/10.3390/v16020204

Hily J-M., Candresse T., Garcia S., Vigne E., Tannière M., ...Lemaire O., 2018. High-Throughput Sequencing and the Viromic Study of Grapevine Leaves: From the Detection of Grapevine-Infecting Viruses to the Description of a New Environmental Tymovirales Member. *Frontiers in Microbiology* 9: 1782. https://doi.org/10.3389/fmicb.2018.01782

Kaponi M., Kyriakopoulou P.E., Hadidi A., 2024. Viroids of the Mediterranean Basin. *Viruses* 16:612. https://doi.org/10.3390/v16040612

Kearse M., Sturrock S., Meintjes P., 2012a The Geneious 6.0.3 Read Mapper. *Auckland, New Zealand: Biomatters, Ltd.*

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Drummond A., 2012b. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Khaffajah B., Alisawi O., Al Fadhl F., 2022. Genome sequencing of eggplant reveals *Eggplant mild leaf*

mottle virus existence with associated two endogenous viruses in diseased eggplant in Iraq. *Archives of Phytopathology and Plant Protection* 55: 1930–1943. https://doi.org/10.1080/03235408.2022.2123601

Krebelj AJ., Čepin U., Ravnikar M., Novak M.P., 2015. Spatio-temporal distribution of Grapevine fanleaf virus (GFLV) in grapevine. *European Journal of Plant Pathology* 142: 159–171. https://doi.org/10.1007/s10658-015-0600-4

Kreuze J.F., Perez A., Untiveros M., Quispe D., Fuentes S., Barker I., Simon R., 2009. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: A generic method for diagnosis, discovery and sequencing of viruses. *Virology* 388: 1–7. https://doi.org/10.1016/j.virol.2009.03.024

Kubina J., Hily J.M., Mustin P., Komar V., Garcia S., … Vigne E., 2022. Characterization of Grapevine Fanleaf Virus Isolates in 'Chardonnay' Vines Exhibiting Severe and Mild Symptoms in Two Vineyards. *Viruses* 14: 2303. https://doi.org/10.3390/v14102303

Liebenberg A., Freeborough M.J., Visser C.J., Bellstedt D.U., Burger J.T., 2009. Genetic variability within the coat protein gene of Grapevine fanleaf virus isolates from South Africa and the evaluation of RT-PCR, DAS-ELISA and ImmunoStrips as virus diagnostic assays. *Virus Research* 142: 28–35. https://doi.org/10.1016/j.virusres.2009.01.016

MacKenzie D.J., McLean M.A., Mukerji S., Green M., 1997. Improved RNA Extraction from Woody Plants for the Detection of Viral Pathogens by Reverse Transcription-Polymerase Chain Reaction. *Plant Disease* 81: 222–226. https://doi.org/10.1094/PDIS.1997.81.2.222

Martelli G.P., 2014. Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology* 96(1) Suppl., 136 pp.

Martin I.R., Vigne E., Velt A., Hily J-M., Garcia S., … Schmitt-Keichinger C., 2021. Severe Stunting Symptoms upon Nepovirus Infection Are Reminiscent of a Chronic Hypersensitive-like Response in a Perennial Woody Fruit Crop. *Viruses* 13: 2138. https://doi.org/10.3390/v13112138

Massart S., Olmos A., Jijakli H., Candresse T., 2014. Current impact and future directions of high throughput sequencing in plant virus diagnostics. *Virus Research* 188: 90–96. https://doi.org/10.1016/j.virusres.2014.03.029

Mekuria T.A., Gutha L.R., Martin R.R., Naidu R.A., 2009. Genome Diversity and Intra- and Interspecies Recombination Events in *Grapevine fanleaf virus*. *Phytopathology*® 99: 1394–1402. https://doi.org/10.1094/PHYTO-99-12-1394

Miljanić V., Rusjan D., Škvarč A., Chatelet P., Štajner N., 2022. Elimination of eight viruses and two viroids from preclonal candidates of six grapevine varieties (*Vitis vinifer*a L.) through in vivo thermotherapy and in vitro meristem tip micrografting. *Plants* 11: 1064. https://doi.org/10.3390/plants11081064

Mokili J.L., Rohwer F., Dutilh B.E., 2012. Metagenomics and future perspectives in virus discovery. *Current Opinion in Virology* 2: 63–77. https://doi.org/10.1016/j.coviro.2011.12.004

M'rabet Samaali B., Loulou A., MougouHamdane A., Kallel S., 2024. Acquisition and transmission of Grapevine fanleaf virus (GFLV) by *Xiphinema index* and *Xiphinema italiae* (*Longidoridae*). *Journal of Helminthology* 98: e26. https://doi.org/10.1017/S0022149X24000154

Panattoni A., Triolo E., 2010. Susceptibility of grapevine viruses to thermotherapy on in vitro collection of Kober 5BB. *Scientia Horticulturae* 125: 63–67. https://doi.org/10.1016/j.scienta.2010.03.001

Panno S., Caruso A.G., Bertacca S., Pisciotta A., Di Lorenzo R., … Davino S., 2021. Genetic structure and molecular variability of grapevine fanleaf virus in sicily. *Agriculture* 11(6): 496. https://doi.org/10.3390/agriculture11060496

Porotikova E., Terehova U., Volodin V., Yurchenko E., Vinogradova S., 2021. Distribution and genetic diversity of grapevine viruses in Russia. *Plants* 10: 1080. https://doi.org/10.3390/plants10061080

Sanfaçon H., Wellink J., Le Gall O., Karasev A., van der Vlugt R., Wetzel T., 2009. Secoviridae: a proposed family of plant viruses within the order Picornavirales that combines the families Sequiviridae and Comoviridae, the unassigned genera Cheravirus and Sadwavirus, and the proposed genus Torradovirus. *Archives of Virology* 154: 899–907. https://doi.org/10.1007/s00705-009-0367-z

Schmitt-Keichinger C., Hemmer C., Berthold F., Ritzenthaler C., 2017. Molecular, Cellular, and Structural Biology of Grapevine fanleaf virus. In: *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. (Meng B., Martelli G.P., Golino D.A., Fuchs M. ed.). Cham, Switzerland, Springer International Publishing AG, pp. 83–107

Tahirine M., Louanchi M., Aitouada M., 2020. Actualisation à la révélation de deux virus responsables à la maladie du court noué de la vigne du genre Vitis dans la région Centre et Ouest de l'Algérie par voie sérologique et biochimique. *Journal Algérien des Régions Arides (JARA)* 14: 150–158

Tamura K., Stecher G., Kumar S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version

11. In: *Molecular Biology and Evolution* (F.U. Battistuzzi, ed.) 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Vigne E., Bergdoll M., Guyader S., Fuchs M., 2004a. Population structure and genetic variability within isolates of Grapevine fanleaf virus from a naturally infected vineyard in France: evidence for mixed infection and recombination. *Journal of General Virology* 85: 2435–2445. https://doi.org/10.1099/vir.0.79904-0

Vigne E., Komar V., Fuchs M., 2004b. Field Safety Assessment of Recombination in Transgenic Grapevines Expressing the Coat Protein Gene of Grapevine fanleaf virus. *Transgenic Research* 13: 165–179. https://doi.org/10.1023/B:TRAG.0000026075.79097.c9

Vigne E., Marmonier A., Fuchs M., 2008. Multiple interspecies recombination events within RNA2 of Grapevine fanleaf virus and Arabis mosaic virus. *Archives of Virology* 153: 1771–1776. https://doi.org/10.1007/s00705-008-0182-y

Vigne E., Garcia S., Komar V., Lemaire O., Hily J.M., 2018. Comparison of Serological and Molecular Methods With High-Throughput Sequencing for the Detection and Quantification of Grapevine Fanleaf Virus in Vineyard Samples. *Frontiers in Microbiology* 9: 2726. https://doi.org/10.3389/fmicb.2018.02726

Zhou J., Fan X., Dong Y., Zhang Z ping., Ren F., Hu G., 2015. Detection and genetic variation analysis of grapevine fanleaf virus (GFLV) isolates in China. *Archives of Virology* 160: 2661–2667. https://doi.org/10.1007/s00705-015-2566-0

Zhou J., Fan X., Dong Y., Zhang Z., Ren F., Hu G., Li Z., 2017. Complete nucleotide sequence of a new variant of grapevine fanleaf virus from northeastern China. *Archives of Virology* 162: 577–579. https://doi.org/10.1007/s00705-016-3101-7

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Cellulose synthase gene expression profile and physiological responses of tomato cultivars exposed to virus and salt stresses

Samra MİRZAYEVA[1], Irada HUSEYNOVA[1], İbrahim Özer ELİBÜYÜK[2], Canan YÜKSEL ÖZMEN[3], Ali ERGÜL[3,*]

[1] *Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of Azerbaijan Republic, Baku, Azerbaijan*
[2] *Faculty of Agriculture, Department of Plant protection, Ankara University, Ankara, Türkiye*
[3] *Biotechnology Institute, Ankara University, Ankara, Türkiye*
*Corresponding author. E-mail: ergul@ankara.edu.tr

Summary. Plants are exposed to adverse growth conditions, and have developed mechanisms to adapt and survive under abiotic and biotic stresses. The plant's response to the combined effects of biotic and abiotic stress represents a highly complex phenomenon, involving intricate interactions between the host plant and associated pathogens, further modulated by the intensity, duration, and type of environmental stressors. Tomato production can be severely affected by tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV), and salt stress inhibits tomato crop productivity, although molecular regulation controlling tomato resistance to salt stress remains unclear. The cellulose synthase (*Ces*) and cellulose synthase-like (*Csl*) gene families control biosynthesis of cellulose and hemicellulose in plant cell walls, and *Ces/Csl* genes are also involved in resistance against abiotic and biotic stresses, including those from viruses and salt. To gain understanding of the molecular basis of combined viruses (TYLCV/ToCV) and salt stresses on the tomato cultivars Money Maker and Yegana, comparative analyses of four cellulose synthase genes (*CesA/Csl*) were carried out using Quantitative Reverse Transcription Polymerase Chain Reaction (*RT -qPCR*). Tomato physiological parameters, including relative water content, specific leaf weight, leaf area, and dry biomass, were also assessed. *CesA/Csl* genes (*Ces-A2, Csl-D3,2, Csl-D3,1, Csl-H1*) were up-regulated in virus-infected plants. These genes, associated with the biosynthesis of *CesA/Csl* genes are probably pivotal in defense mechanisms against TYLCV/ToCV. Relative water content in plants subjected to combined ToCV and salt stresses were similar to those observed in non-inoculated controls. Congruence between the outcomes of these analyses and physiological studies indicates that the Yegana tomato cultivar may be as sensitive to these stresses as the Money Maker cultivar. This research emphasizes the importance of up-regulating specific genes, namely *Csl-D3,1*, *Csl-D3,2*, and *Ces-A2*, to confer host resistance to the complex effects of salt and virus stresses. This study will facilitate development of stress-resistant tomato plants, and contribute to elucidating the molecular mechanisms of *CesA/Csl* genes in abiotic and biotic stress situations.

Keywords. *Solanum lycopersicum* L., tomato yellow leaf curl virus, tomato chlorosis virus, cellulose synthase genes, RT-qPCR.

OPEN ACCESS

Citation: Mirzayeva, S., Huseynova, I., Elibüyük, İ. Ö., Yüksel Özmen, C. & Ergül, A. (2025). Cellulose synthase gene expression profile and physiological responses of tomato cultivars exposed to virus and salt stresses. *Phytopathologia Mediterranea* 64(2): 229-244. doi: 10.36253/phyto-15444

Accepted: July 14, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Assunta Bertaccini, Alma Mater Studiorum, University of Bologna, Italy.

ORCID:
SM: 0000-0002-1693-4487
IH: 0000-0003-3336-2203
İÖE: 0000-0002-2603-5813
CYÖ: 0000-0002-4421-1358
AE: 0000-0002-1205-268X

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown food crops, and is consumed as fresh or processed food products (Li *et al*., 2021; Roșca *et al*., 2023). World tomato production in 2021 was 189.1 million metric tons (FAOSTAT, 2022), and diseases caused by viruses cause reductions in crop yields and impair fruit quality, causing important economic losses (Jones and Naidu, 2019).

Tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) are pathogens that can cause serious losses in tomato production (Chinnaraja *et al*., 2016; Jin *et al*., 2020) which continue to spread throughout many countries (Abd El Rahman *et al*., 2024).

TYLCV (*Begomovirus*, *Geminiviridae*) affects more than 20 tomato cultivars, leading to severe productivity reducing host symptoms including yellowing, curling size reduction of leaves, stunted plant growth and early flower shedding shedding (Moriones and Navas-Castillo, 2000; Huang *et al*., 2016; Desbiez *et al*., 2018; Verdin *et al*., 2018). TYLCV ranks third among viruses reducing tomato production (Ong *et al*., 2020), and most tomato cultivars are very susceptible to this virus (Mugiira *et al*., 2011). ToCV (*Crinivirus*, *Closteroviridae*) has also emerged as an important pathogen, now recorded in 35 countries. Besides tomatoes, ToCV can infect other economically important vegetable crop plantss and many wild hosts (Fiallo-Olivé and Jesús Navas-Castillo, 2019; Louro *et al*., 2000; Elsharkawy *et al*., 2022). The first symptoms of ToCV infections are formation of interveinal chloroses on lower leaves, which then progress to upper leaves of infected plants (Kwon *et al*., 2024). Despite substantial impacts of ToCV on crop yields, resistant or tolerant tomato germplasms have not been well-documented, and the genetic basis of resistance to ToCV remains poorly understood (Gao *et al*., 2024).

The early detection of TYLCV and ToCV is critical for their effective management. Several approaches have been developed to control these two pathogens, including physical barriers and the applications of insecticide chemicals. Genetic engineering strategies have also been investigated (Tabein, 2021), with the most effective strategy for managing TYLCV and ToCV involving transfer of virus resistance genes from wild *Solanum* species into susceptible tomato cultivars.

Among abiotic stressors, high soil salinity is an increasing concern, with more than a third of irrigated areas already affected, and estimates suggesting that by 2050, more than half of the world's cropland will be affected by high salinity (FAO, 2011; Zelm *et al*., 2020; Zhao *et al*., 2021). Besides affecting morphological and physiological status of crop plants, many studies have shown that high salt concentrations cause biochemical and molecular imbalances, resulting in low plant productivity (Kusvuran *et al*., 2016). In general, salinity stress determines changes in gene expression in tomato plants, but information on these effects is still limited, and most investigations have been on changes in genes associated with transcription factors (Devkar *et al*., 2020), and studies on effects of salinity on tomato gene expression have been conducted on particular cultivars (Roșca *et al*., 2023). These results suggested changes in the expressions of genes involved in transport activity, cell wall construction, secondary metabolites, and protein synthesis. Most tomato cultivars are known to have the genetic potential to tolerate mild to moderate salt stress (Ibrahim, 2018; Alam *et al*., 2021; Guo *et al*., 2022), and knowledge of salinity effects on tomato plants is an asset in selection of appropriate crop practices to fulfill demands of tomato markets (Roșca *et al*., 2023).

The cellulose synthase (*Ces*) and cellulose synthase-like (*Csl*) gene families within the *Ces* gene superfamily are central to the biosynthesis of cellulose and hemicellulose in plant cell walls (Cao *et al*., 2019). *CesA* and *Csl* genes are key regulators in the synthesis of plant cell wall polysaccharides, which are essential for plant adaptation to abiotic stresses (Wang *et al*., 2022). Recent research has indicated that salt stress negatively affects cell wall synthesis, including *Ces* complexes (*CSCs*), *CesA*, and *Csl* genes (Maksup *et al*., 2020), leading to alterations in the expression patterns of related genes (Shafi *et al.,* 2019). In TYLCV-infected tomato plants, however, the availability of *Csl* genes to strengthen host immune systems and maintain crop productivity has been reported (Huang *et al*., 2022). Nevertheless, the *CesA/Csl* family genes have not been fully characterized in *Solanaceae* species, particularly tomatoes. Song *et al*. (2019) identified a total of 38 *CesA/Csl* protein-encoding genes in tomatoes, and characterized these based on phylogenetic, gene structure, chromosome distribution, and localization, and then deduced protein sequences.

Given the growing concern about global climate variability, there is urgent need to expand knowledge of the interactions of combined biotic and abiotic stresses in plants. Plants are exposed to many biotic and abiotic stressors throughout their life cycles, and these factors activate physiological and molecular defense mechanisms that provide viability withstand these stressors (Zhang and Sonnewald, 2017). The primary objective of the present study was to analyze expression profiles of four *cellulose synthase* (*CesA*) and *cellulose synthase-like* (*Csl*) genes in the virus sensitive tomato cultivar Money Maker (MK, UK) and virus-susceptible/unknown culti-

var Yegana (YG, AZ), which were exposed to combined salt and TYLCV and ToCV stresses, using Quantitative Reverse Transcription Polymerase Chain Reaction (*RT-qPCR*) assessments. To increase understanding of the defense responses of tomato plants to salinity stress during TYLCV and ToCV infections, effects on four key physiological parameters (relative water content, specific leaf weight, leaf area, dry biomass) were also assessed.

MATERIALS AND METHODS

Plant material and single-leaflet grafting of TYLCV and ToCV

Seeds of the virus-susceptible cultivar Money Maker (MK, UK, Milc *et al.,* 2019) were sourced from the Institute of Biotechnology at Ankara University, Türkiye, while seeds of the virus/salt tolerant or sensitive cultivar Yegana (YG, Azerbaijan) were obtained from the seed bank of the Research Institute of Crop Husbandry, Ministry of Agriculture of the Azerbaijan Republic. Tomato seeds were germinated in plastic vials containing a mixture of peat (90%), perlite (10%), and vermiculite (70%). Germination took place in an insect-free growth chamber maintained at a 26°C 16 h light and 20°C 8 h dark cycle, and relative humidity ranging between 60–70% (Çevik *et al*., 2019). The plants were watered daily at the same time each day according to the moisture condition of the plant gowth medium.

Each experiment was conducted with three technical and biological replicates. Tomato leaf samples which were infected with isolates of TYLCV (GenBank accession number MK238543) or ToCV (MK248741) (Fidan and Sarıkaya, 2020), and were showing characteristic symptoms, were collected from greenhouses in the Kumluca region of Antalya (Türkiye) in June 2022. Presence of these viruses in the tomato leaf samples was confirmed using the polymerase chain reaction (PCR) method with specific primers (BC-36 and BC-37/ BC-40 and BC-41 for nested PCR; AV632, AC950, and AC1048 for duplex PCR) (Martinéz-Culebras *et al*., 2001; Dovas *et al*., 2007), and were subsequently utilized as inoculation material for single-leaflet grafting (ToCV by nested PCR and TYLCV by duplex PCR). Single-leaflet grafting involved small incisions on the stem of each recipient seedling by removing the first leaf from the node. Leaflets obtained from diseased plant samples containing TYLCV and ToCV were used as inocula for grafting, and also as positive inoculation controls for further grafting confirmation reactions, as described by Lee *et al.* (2017). The grafted leaflets were misted with steril distiled water multiple times each day to prevent wilting. After 21 d the success grafting transmission rates were 97.6% for TYLCV and 89.3% for ToCV (Çevik *et al*., 2019). Grafting leaflets collected from virus-free tomato plants were included as experimental controls. Four weeks after inoculation (4 w.p.i.), grafting transmission was confirmed through molecular detection of TYLCV using a duplex PCR assay (Martinéz-Culebras *et al*., 2001), and of ToCV using nested PCR reactions (Dovas *et al*., 2007).

Table 1. Tomato cultivars and treatment abbreviations.

No.	Cultivar	Treatment	Abbreviation
1	Money Maker (MK)	TYLCV, Salt	MK-TYLCV-S
2	Money Maker (MK)	ToCV, Salt	MK-ToCV-S
3	Money Maker (MK)	Control	MK-C
4	Yegana (YG)	TYLCV, Salt	YG-TYLCV-S
5	Yegana (YG)	ToCV, Salt	YG- ToCV-S
6	Yegana (YG)	Control	YG-C

The plants were subsequently, divided into three groups: one group remained as the experimental controls (non-inoculated, healthy); the second group consisted only of virus-infected plants (MK-ToCV-1st d or MK-TYLCV-1st d, YG-ToCV-1st d or YG-TYLCV-1st d; d = day); the third group was infected with the viruses and was also subjected to salt stress (MK-ToCV-S-21std or MK-TYLCV-S-21std, YG-ToCV-S-21std or YG-TYLCV-S-21std) (Table 1). Each biological replicate consisted of a group of 15 tomato seedlings per treatment. For molecular analyses (including RT-qPCR), leaf tissue samples were collected from these groups of plants at 4 w.p.i. Five to six severely infected leaves were harvested from multiple individual seedlings within each 15-plant group, and were pooled to constitute a single biological replicate. After virus inoculations, at 4 w.p.i., the tomato plants were exposed to salt stress.

TYLCV and ToCV grafting confirmation with duplex and nested PCR

Detection of ToCV. For confirmation of ToCV, two -step PCR assays were carried out, including the reverse transcription polymerase chain reaction (RT-PCR) and nested PCR. The first step of the RT-PCR was conducted using BC-36 and BC-37 primer pairs. Additionally, a primer designed for *heat shock protein 70 (HSP70*), the highly conserved gene region, was used for PCR amplification. For nested PCR, specific primer pairs designed by Dovas *et al*. (2007) (BC-40 and BC-41) were utilized (Table 2).

Total RNA was extracted from 30 to 50 mg of fresh leaf tissue using the Tri-Reagent solution (Biorad). RNA

Table 2. BC-36, BC-37/BC-40 and BC-41 primers used in nested PCRs, and AV632, AC950, and AC1048 primers used in duplex PCR reactions (Dovas *et al*., 2007; Aboul-Maaty and Oraby, 2019)

ToCV				
Type of PCR	Primer	Sequence (5'.....3')	Product size (bp)	Reference
First Step Nested PCR	BC 36-F	5'GG(G/T)TT(A/G)GA(G/T)TT(C/T)GGTACTAC-3'	587	Dovas *et al*., 2007
	BC 37-R	5'-CC(G/T) CCACCAAA(A/G)TCGTA-3'		
Second Step Nested PCR	BC 40-F	5'-GG TTTGGATTTTGGTACTACTAGT-3'	463	Dovas *et al*., 2007
	BC41-R	5'- AAACTGCCTGCATAAAGTCT C- 3'		
TYLCV				
Type of PCR	Primer	Sequence (5'.....3')	Product size (bp)	Reference
Duplex PCR	AV632-F	5′-CCG GTG TTG TGC GTT GTG TTA G-3′	462	Aboul-Maaty and Oraby, 2019
	AC950-F	5′-TGA AGG AGC AGT GTY TGY TG-3′		
	AC1048-R	5′- GGA TTA GAG GCA TGC GTA CAT-3′	135	

quality and amounts were measured using 1% agarose gel electrophoresis and a spectrophotometer (ND-1000, NanoDrop Technologies). Isolated RNA samples were stored at −80°C until the nested PCR step. In nested PCR reactions, ToCV-infected (GenBank accession number: MK248741) (Fidan and Sarıkaya, 2020) samples from the Kumluca region of Antalya (Türkiye) were used as positive controls. The PCR reactions were each conducted with three technical replicates, and sterile distilled water was used as the negative control. PCR products were separated on a 1% agarose gel containing ethidium bromide (0.5 μg mL^{-1}) alongside a 100-bp (basepair) DNA ladder (Invitrogen), and were visualized under an ultraviolet light using a gel documentation system (Uvitek).

Detection of TYLCV. Typical symptoms of TYLCV infections in the plants appeared 10 to 14 d post-infection (Çevik *et al*., 2019), and grafting transmission was confirmed 4 w.p.i. using specific primers in a duplex PCR. The duplex PCR was carried out in accordance to the method described by Martinéz-Culebras *et al.* (2001).

Primers AV632, AC950, and AC1048 were used for detection of TYLCV (Table 1) (Brown *et al*., 1996; Martinéz-Culebras *et al*., 2001). Total DNA was extracted from 50 mg of fresh leaf tissue using CTAB solution (Aboul-Maaty and Oraby, 2019). DNA quality and concentration were assessed utilizing 1% agarose gel electrophoresis and a spectrophotometer (NanoDrop ND-1000). Isolated DNA samples were stored at −80°C until the duplex PCR step.

In duplex PCR reactions, TYLCV-infected samples (GenBank accession number MK238543) (Fidan and Sarıkaya, 2020) from the Kumluca region of Antalya (Türkiye) were used instead of DNA as a positive control, while sterile distiled water was used as a reaction negative control. The PCR reactions were each carried out with three technical replicates.

PCR products were controlled within 1% agarose gel electrophoresis with a 100 bp DNA ladder (Invitrogen), and were visualized under an ultraviolet light using a gel documentation imaging device (Uvitek).

Salt stress treatments

Salt effects on tomato cultivars were examined using the method developed by Gharsallah *et al*., (2016). Electrical conductivity (EC) of NaCl solutions was measured with a conductivity meter (Thermo Corporation). Salt stress was applied for 21 d, with the salt treatment initiated on day one with 50 mM of NaCl solution (6 dS m^{-1}), then later increased to 100 mM (12 dS m^{-1}) on day 2, and then to 150 mM (15 dS m^{-1}) on day three. Three biological replicates were used for each of the two tomato cultivars, and each replicate comprised 15 plants. Control plants of each cultivar were grown under identical conditions (non-inoculated, healthy), and were irrigated with Hoagland 's nutrient solution (Hoagland and Arnon 1950) at the same time each day, as for the salt-stressed tomato cultivars.

Primer design and RT-qPCR analyses

The National Center for Biotechnology Information (NCBL) Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast) was used to design the *CesA/Csl* primers and designed gene-specific primers that were used to produce a PCR product of approx. 200–300 bp in tomato cultivars. The designed primer information

(gene name, gene ID, NCBI reference sequence and primer sequence) is outlined in Table 3.

Three biological replications were used for RNA extraction. Initially, five to six severely infected leaves from salt-stressed tomato cultivars were collected for each biological replication and were frozen in liquid nitrogen. The sample tissues were then homogenized in sterilized porcelain mortars and a Tri-Reagent solution (Biorad) protocol was used. Concentrations of RNA were measured using a NanoDrop spectrophotometer and 1% agarose gel electrophoresis. The RT-qPCR reactions were carried out in a Light Cycler 480 (Roche), for the genes *Ces-A2, Csl-D3,2, Csl-D3,1,* and *Csl-H1,* with three biological and three technical replicates.

The RT-qPCR method was carried out in two steps. In the first, cDNAs were synthesized using the first-strand cDNA synthesis kit (Roche, Cat no: 04897030001), following the manufacturer's protocol. Standard curves (with respective efficiency and slope values close to 2.2 and –3.2) for each primer were generated using six serial dilutions (ranging from 1/10 to 1/100,000) of a control (non-inoculated, healthy) cDNA. cDNA samples were then used as templates to quantify target gene expression levels. Non-inoculated, healthy samples were used as a control group in the reactions.

In the second step, the RT-qPCR reactions were each carried out in a 12 µL mixture containing 1.8 to 2.0 µL of forward and reverse primer (10 pmol), 3 µL cDNA (500 ng μL^{-1}), 5 µL LightCycler® 480 SYBR Green I Master (Roche) and ddH_2O. The amplification reaction commenced with an initial denaturation at 95°C for 10 min. This was followed by denaturation (10 s at 95°C), annealing (1 min at 52-58°C, according to the optimized annealing temperature (Tm) of the primer), and elongation (1 min at 72°C) steps conducted through 45 cycles. The specificity of amplification was examined through a melting curve analysis after the last cycle. For each gene, cycle threshold (Ct) values were obtained for infected-salt stressed and control samples.

Gene expression values were normalized to the expression of *Solanum lycopersicum* housekeeping gene actin-7-like (Gene ID: LOC101262163; Klay *et al*., 2014) in all samples. Relative expression levels were calculated using the REST 2009 software program, according to the delta delta-Ct ($2^{-\Delta\Delta CT}$) algorithm as described by Livak and Schmittgen (2001).

Table 3. Primer information for the RT-qPCR.

No	Gene ID	Gene name/gene name abbreviation in this study	NCBI Reference Sequence	Forward Primer Sequence (5'...3')	Reverse Primer Sequence (5'...3')
1	Solyc07g051820	XM_004243439.4, **Csl-H1**	*Solanum lycopersicum cellulose synthase-like* protein H1(LOC101259456), mRNA	ACCACCGTATACCGACTCCA	TCGATGCACCGTCGTCTGAG
2	Solyc06g097050	XM_00423523.4, **Csl-D3,1**	*Solanum lycopersicum cellulose synthase-like* protein D3 (LOC101247596), mRNA	TGCGACGAGGTGATTCAGAC	GAGGCCGTCCATTCTTCACA
3	Solyc08g076320	XM_004245868.4, **Csl-D3,2**	*Solanum lycopersicum cellulose synthase-like* protein D3 (LOC101249747), transcript variant X1, mRNA	ACAACTCCGAGGCAATCAAG	CGGAAGAGACAACCAGTCCC
4	Solyc12g056580	XM_004252522.4, **Ces-A2**	*Solanum lycopersicum cellulose synthase* A catalytic subunit 2 [UDP-forming] (LOC101260024), mRNA	ATGGATCCTGCTGCCCTTGG	TGGGGCGAGGAGGAAAAGAC
5	*Solyc11g005330*	Q96483m **Actin**	*Solanum lycopersicum actin-7-like* gene (ID: LOC101262163	TGTCCCTATTTACGAGGGTTATGC	CAGTTAAATCACGACCAGCAAGAT

Measurements of plant physiological parameters

Physiological measurements were made for three groups of plants: virus (TYLCV and ToCV) infected plants, salt stressed and virus (TYLCV-S and ToCV-S)

infected plants, and healthy plants (controls). All physiological measurements were carried out on three replicates, including both biological and technical replicates. Relative water contents (RWC) of virus-infected and healthy tomato samples were determined using the method outlined by Tambussi *et al.* (2005).

To measure dry biomass (DB) of tomato leaves, samples of uniform size were collected from infected and healthy (non-inoculated) leaf samples. The samples were then weighed on an electronic balance, using the method described by Grünzweig *et al.* (1999).

Leaf area (LA) measurements were carried out for diseased and non-inoculated samples. These were determined from each leaf length and diameter (widest part), as described by Grünzweig *et al.* (1999).

Leaf water deficits were assessed through a series of steps. Initially, leaf primary weights were recorded. The leaves were then soaked in water for 1 h, and leaf wet weights were measured. The change in weight before and after soaking provided measures of water deficit in the leaves, indicating each plant's ability to retain water.

For all physiological measurements, statistical analyses were carried out to assess the differences between the experimental controls and treatment groups, using a two-way analyses of variance (ANOVA), with mean separation at $P \leq 0.005$.

RESULTS

ToCV and TYLCV detection in virus inoculated plants

ToCV detection. From the first step of nested PCR, a 587 bp band was amplified in all samples, and a 463 bp band of the *HSP70* gene was also amplified as a result of nested PCR in all samples and the positive controls (Figure 1, a and b).

TYLCV detection. In agarose gel electrophoresis, 462 bp bands were observed in all tomato samples. However, no band was observed in the healthy (non-inoculated) samples and negative controls. Tomato samples from Kumluca (Türkiye) used as positive controls also exhibited bands of 462 bp (Figure 1, c).

RT-qPCR analyses

These results indicated different patterns of increases or decreases in gene expression under salt stress conditions. The expression profiles of the four assessed *CesA/Csl* genes were all low. Greatest statistically significant ($P \leq 0.05$) up-regulation (64 fold change) was

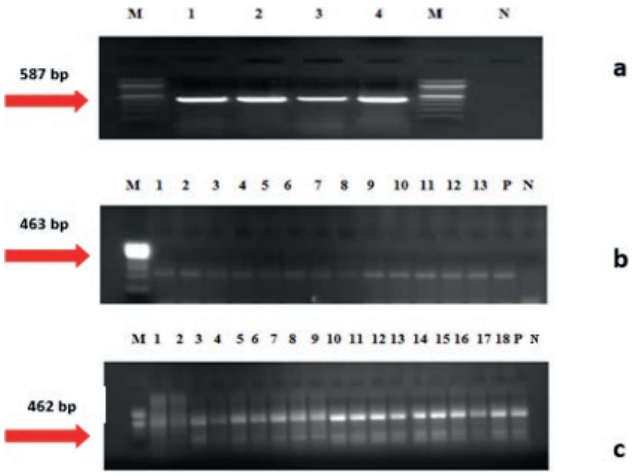


Figure 1. Molecular detection of tomato chlorosis virus and tomato yellow leaf curl virus. 1 to 4, ToCV infected MK tomato cultivar (first step of nested PCR). b, 1 to 8, ToCV infected YG cultivar, 9 to 13, ToCV infected MK cultivar (second step of nested PCR). c, 1 to 9 TYLCV, infected YG cultivars, 10 to 18, TYLCV infected MK cultivar (duplex PCR). In each gel, M is the 100 bp DNA ladder (Biolabs), N is the negative control of the PCR reaction, and P is the positive control.

detected for the MK-ToCV-S-21st d cultivar in the *Csl-D3,1* gene, and the greatest down-regulation (-23 fold change) was detected for the MK-ToCV-1st d cultivar in the *Ces-A2* gene.

For the *Csl-H1* gene, a comparative analysis between the 1st and 21st days showed no statistically significant changes in gene expression among the tomato cultivars. However, the YG-ToCV-21st d cultivar exhibited down-regulation at all time points, except for the 1st day of gene expression, where a 2-fold change was observed (Figure 2).

For the *Ces-A2* gene, the YG-TYLCV-1st d cultivar had down-regulation (-3.67 fold change) on the 1st day, and up-regulation (3-fold change) on the 21st day. Aside from this result, there were no statistically significant gene expression differences between the different time points for the *Ces-A2* gene. Similar to the *Csl-H1* gene, the greatest down-regulation (-23 fold change) in the *Ces-A2* gene occurred at the 1st day, specifically in the MK-ToCV-1st d cultivar (Figure 2).

The *Csl-D3,1* gene exhibited noteworthy expression variations between the 1st and 21st days in both tomato cultivars. This gene was down-regulated in both cultivars on the 1st day, but was up-regulated on the 21st day. The MK-ToCV-1st d cultivar was down-regulated (-2 fold change) on the 1st day, and was strongly up-regulated (64 fold change on the 21st day. This could have been linked to heightened sensitivity of the MK tomato cul-

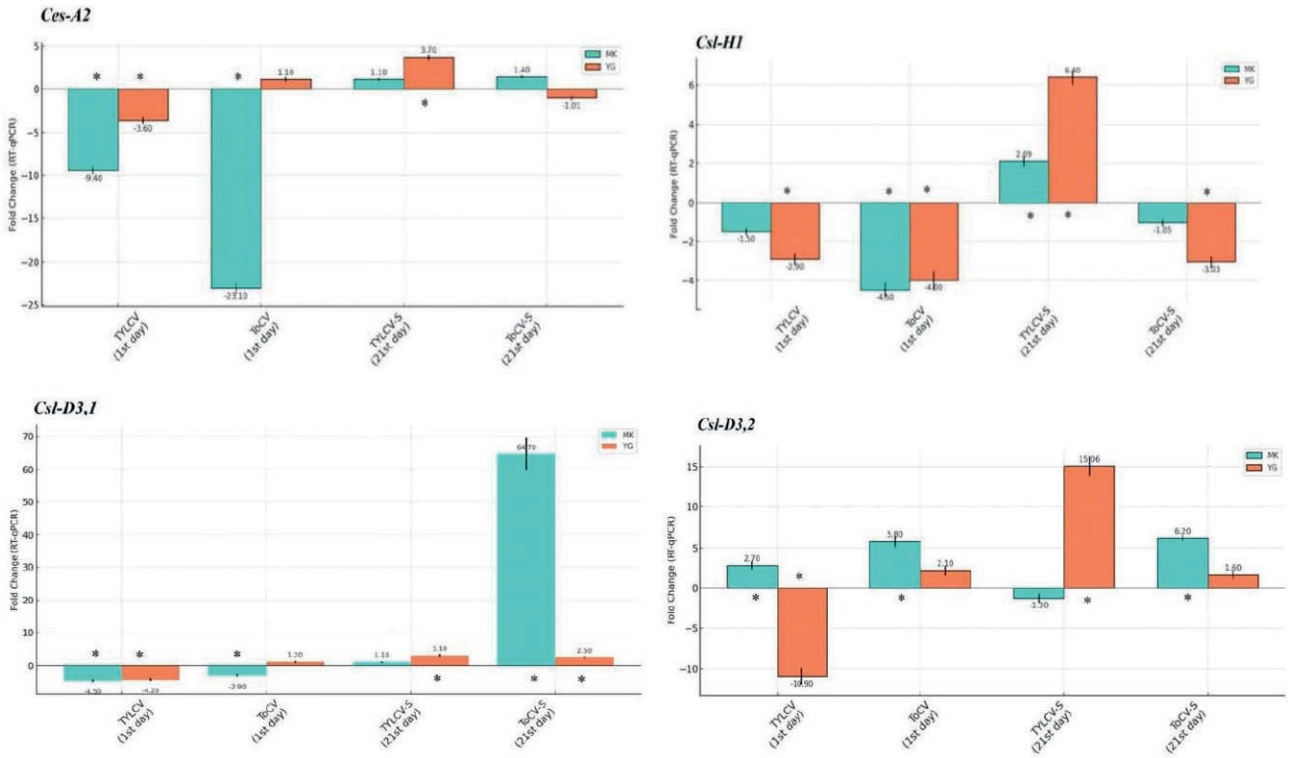


Figure 2. Mean relative gene expression (fold changes) of *CesA/Csl* genes (t-test; $P \leq 0.05$). Bars indicate standard errors of means. Asterisks indicate significant differences (t-tests; $P \leq 0.05$) in gene expressions.

tivar on the 21st day compared to the 1st day, implying that the *Csl-D3,1* gene was an indicator for sensitivity in this cultivar.

For the *Csl-D3,2* gene, the YG-TYLCV-1st d cultivar was down-regulated (-10 fold change) on the 1st day, and up-regulated (15 fold) on the 21st day (YG-TYLCV-S-21st d). This increased gene expression was greatest in the YG cultivar exhibiting on the 21st day in comparison to the 1st day. In contrast, observed other gene expression changes (*Csl-H1* and *Ces-A2*) did not show clear correlations with the sensitive/tolerant differentiation that was observed in both the MK-ToCV-S-21st d or MK-TYLCV-S-21st d, and the YG-ToCV-S-21st d or YG-TYLCV-S-21st d cultivars (Figure 2).

Physiological measurements

In the MK and YG cultivars, reductions in RWC were observed in leaves infected with TYLCV and ToCV compared to non-inoculated (healthy) samples. Although there were slight increases in the salt/virus samples compared to non-inoculated ones, these were slight. Specifically, the mean RWC was 74% in the non-inoculated group (MK-C), 70% in MK -TYLCV-S-21st d plants, and 65% in the MK -TYLCV-S-1st d plants. In the MK cultivar infected with ToCV, RWC were 79% in MK-C plants, 72% in MK -ToCV-S-21st d group plants, and 61% in MK -ToCV-S-1st d group plants. Similar results were also recorded in the YG cultivar. In that case, RWC was 59% in the YG-C variant, 53% in YG -TYLCV-S-21st d group plants, and 44% in YG-TYLCV-S-1st d. Compared to YG-C (63%), YG -ToCV-S-21st d exhibited 59%, and YG -ToCV-1st d plants showed 47% RWC (Figure 3).

When the DB percentages were evaluated, they increased by approx. 1.3-fold (20%) in the MK -TYLCV-S-1st d cultivar compared to the MK-C plants, and by approx. 1.6-fold (25%) in MK -TYLCV-S-21st d. In MK -ToCV-S-1st d, this indicator exhibited an approx. 1.2-fold increase (25%), and in the MK-ToCV-S-21st d group an approx. 1.5-fold increase (31%). DB percentage was also assessed in the Yegana cultivar, where YG-TYLCV-S-21st d samples showed an approx. 1.1-fold increase (19%) compared to YG-C samples, while an approx. 1.4-fold increase (26%) was observed only in YG-TYLCV-1st d plants (Figure 4).

In the water deficit analyses, an increase was observed both in the virus-infected and dual stressed plants, compared to non-inoculated plants. The water

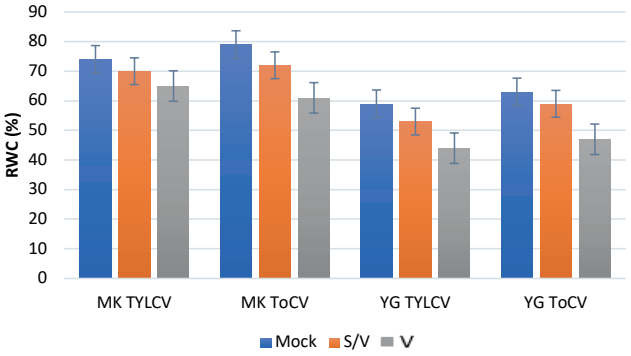


Figure 3. Mean relative water content (%) in MK and YG tomato cultivars exposed to combined virus (TYLCV and ToCV) and salt stress: Non-inoculated, ("Mock") controls, S/V = salt plus virus treatment, V = virus only (TYLCV or ToCV) treatment. Bars indicate standard errors of means.

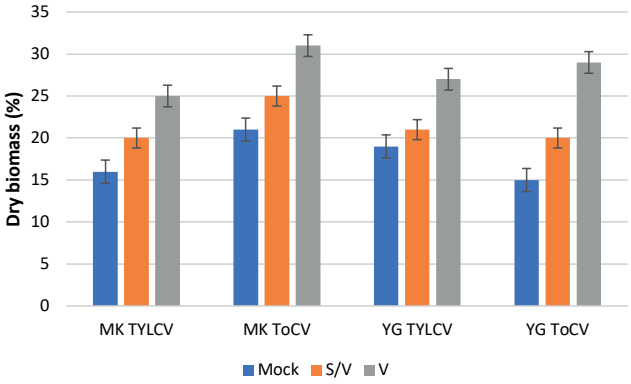


Figure 4. Mean dry biomass (%) of MK and YG tomato cultivars exposed to combined virus (TYLCV and ToCV) and salt stress. Non-inoculated ("Mock") healthy controls, S/V = salt plus virus treatment, V = virus only (TYLCV or ToCV) treatment. Bars indicate standard errors of means.

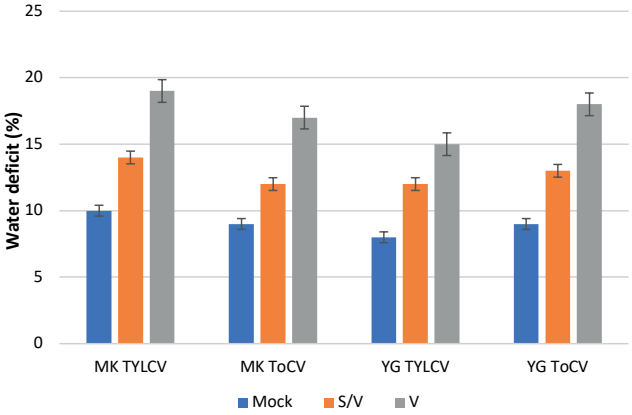


Figure 5. Mean water deficit (%) in MK and YG tomato cultivars exposed to combined virus (TYLCV or ToCV) and salt stress. Non-inoculated ("Mock") healthy controls, S/V = salt plus virus treatment, V = virus only (TYLCV or ToCV) treatment. Bars indicate standard errors of means.

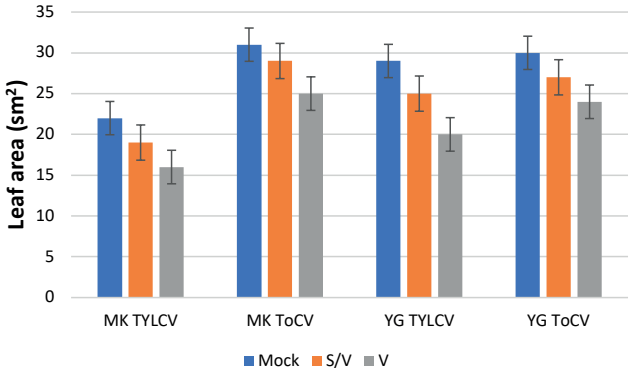


Figure 6. Mean leaf area (sm2) of MK and YG tomato cultivars exposed to virus (TYLCV or ToCV) and salt stress. Non-inoculated "Mock" healthy controls, S/V =salt plus virus treatment, V = virus only (TYLCV or ToCV) treatment. Bars indicate standard errors of means.

deficit percentage was 10% in the MK-C cultivar, 14% in MK-TYLCV-S-21st d plants, and 19% in MK-ToCV-S-1st d samples. Water deficit was 15% in MK -ToCV-S-1st d samples, and 12% in MK-ToCV-S-21st plants. The water deficit in YG-TYLCV-1st d plants was 12% and, and 15% in YG-TYLCV-S-21st d samples. As well, in YG -ToCV-1st d and YG-ToCV-S-21st d samples, these parameters were, respectively, 13% and 18%. Accordingly, the equivalent control plants gave, respectively, 8% and 9% water deficits (Figure 5).

For both cultivars, LA decreases were observed in samples in comparison to the non-inoculated control. These reductions were particularly noticeable in the combined stress group of plants compared to the healthy plants. Specifically, in the MK cultivar, LA was 22 cm^2 and 31 cm^2 in non-inoculated plant leaves. This parameter decreased to 19 cm^2 for MK-TYLCV-S-21st d, and 29 cm^2 for the MK-ToCV-S-21st d plants, and was 16 cm^2 in MK-TYLCV-S-1st d plants and 25 cm^2 in the MK-ToCV-S-1st d plants. During the experiments, the LAs of the YG cultivar in different groups of plants changed as follows: 29 cm^2 and 30 cm^2 in control plant leaves, 20 cm^2 YG-TYLCV-1st d plants and 24 cm^2 in the YG-ToCV-1st d plants, 25 cm^2 in YG-TYLCV-S-21st d plants, and 27 cm^2 YG-ToCV-S-21st d plants (Figure 6).

DISCUSSION

Abiotic and biotic stresses have been shown to stimulate the expression of *CesA/Csl* genes in plants (Sha-

rif *et al*., 2021). However, there has been an absence of research on *CesA/Csl* genes in response to combined salt and virus stresses in the *Solanaceae* plants, and particularly in tomatoes.

It is well-established that salt (abiotic) stress can alter plant cell wall polysaccharide synthesis (Peng *et al*., 2019). Many reports have shown that cell walls have prominent roles in sensing salt stress (Zheng *et al*. 2019). Additionally, disruptions to cell wall polysaccharide synthesis enzymes have been linked to reduced salt tolerance (Zhang *et al*., 2016; Kesten *et al*., 2019). For example, mutations in the *CesA6* gene in *Arabidopsis* disrupt cellulose synthesis and lead to reduced salt tolerance (Endler *et al*., 2014, 2015; Kesten *et al*., 2019). Similarly, *CesA* genes have been shown to be differentially expressed under salt stress in barley, highlighting the important role of cellulose in maintaining cell wall integrity against salt permeability (Ueda *et al*., 2007).

In previous studies of TYLCV-infected tomato plants, *Csl* and *CesA* genes were found to be up-regulated (Chen *et al*., 2013; Choe *et al*., 2021), while *CesA* genes were highly down-regulated in response to TYLCV infection (Seo *et al*., 2018). In the present study, TYLCV-infected cultivars subjected to salt stress for 21 days exhibited a down-regulation of the *Csl-H1* gene when compared with the 1st day, which was consistent with the results of Seo *et al*. (2018). It has been suggested that the down-regulation of these genes may play trigger reduced growth and leaf curling in TYLCV-infected plants (Li *et al*., 2019). However, the down-regulation fold changes were similar and of low magnitude between the MK and YG tomato cultivars experiencing salt stress, and infected by TYLCV and ToCV, for the *Csl-H1* gene. Also, various transcription factors and some hormones have been shown to affect cellulose biosynthesis in salt stressed plants (Dabravolski and Isayenkov, 2023). *Csl* genes related to cellulose biosynthesis were also down-regulated, suggesting that these genes can be negative modulators of salt tolerance (Zhang *et al*., 2019). From this perspective, the present study results related to this gene are also consistent with previous studies. However, the other *Csl* gene *Csl-D3,1* exhibited up-regulation in both TYLCV- and ToCV-infected tomato cultivars (except for YG-ToCV, 1st day) as duration of salt stress increased (21st day), compared with day 1.

The present study has demonstrated significant up-regulation in the other three *CesA/Csl* genes (with the exception of the *Csl-H1*) after 21 days of salt stress. This up-regulation was particularly prominent in the MK ToCV-infected tomato cultivar. In the YG cultivar, the *Csl-D3,1* gene was 2-fold increased on the 1st day, and was 64-fold up-regulated on the 21st day. Similarly, to these results, the *Csl-D3,1* and *Csl-D3,2* genes were up-regulated in TYLCV-infected tomato cultivars (Choe *et al*., 2021). In the MK TYLCV-infected cultivar, no significant up-regulation was observed between the 1st and 21st days of salt stress. Nonetheless, considering the up-regulated profiles of the four genes which assessed, it can be inferred that these genes contribute to tomato response to TYLCV, irrespective of time (1st day or 21st day). Particularly noteworthy was the extreme down-regulation of the *Csl protein G2* gene (*CslG2; Solyc07g043390*) observed in TYLCV-infected plants. Chantreau *et al*. (2015) have suggested that the constitutive overexpression of the *Csl* gene can mitigate the severity of TYLCV symptoms, enhance disease tolerance, and increase productivity in TYLCV-infected tomato plants.

RNA-Seq analyses of uninfected and TYLCV-infected tomato cultivars were conducted by Seo *et al*. (2018) to investigate 38 *CesA/Csl* genes. The *Ces* family gene (*Csl-H1g043390.2.1*), a homolog of the *AtCESA8* gene, was found to be highly down-regulated among the genes implicated in TYLCV infection outcomes. This result underscores the critical role of cellulose in provoking stunted growth and leaf curls in response to TYLCV infections. Symptoms of TYLCV infections may involve substantial down-regulation of the *Ces* family gene to reduce cellulose levels. In the present study experiments, and in alignment with previous results, both tomato cultivars exhibited down-regulation of the *Ces-A2* and *Csl-D3,2* genes at the 1st day compared to the 21st day of salt stress. On the 21st day, an up-regulation profile was observed only for the *Csl-D3,2* gene in both tomato cultivars. It can therefore be postulated that the *Csl-D3,2* gene is linked to salt stress, directly or indirectly. Up-regulation was especially observed for the *Csl-D3,2* gene in the MK ToCV-infected (21st day) plants, with a 64-fold change. In the MK cultivar, no significant up-regulation differences were detected between days 1 and 21 of salt stress. Up-regulation of *CesA* (*Solyc03g005450.2.1*) in response to salt stress in tomato plants has also been previously documented (Renau-Morata *et al*., 2017).

Tomato plants infected with TYLCV and ToCV have been subjected to drought stress for 25 days, resulting in increased expression of *Ces-A2, Csl-D3,2* and *Csl-H1* genes, especially in the MK cultivar (Mirzayeva *et al*., 2023). Similarly, in the present study, both TYLCV- and ToCV-infected MK plants had increaseed *Csl-D3,2* and *Ces-A2* gene expressions (21st day of salt stress). However, unlike in drought stress (Mirzayeva *et al*., 2023), the *Csl-H1* gene was down-regulated in all the virus infected MK cultivar. This suggests that this gene may

be expressed differently under drought and salt stress.

Plant cell walls predominantly consist of polyphenolic compounds, including lignin and polysaccharides such as cellulose, pectin, and hemicellulose, which form primary plant biomass (Hu *et al*., 2018). Cell walls also have key roles in plant growth and maturation, as they determine cell shape and size, providing essential structural support. Additionally, cell walls act as a defense mechanism against environmental stressors (Malinovsky *et al*., 2014; Le Gall *et al*., 2015).

For the MK and YG tomato cultivars infected with TYLCV/ToCV without salt stress, high coefficients were detected in these plants during physiological measurements. Statistically significant reductions in mean dry weights were observed in both experimental groups (virus-grafted and virus plus salt-treated plants) of the MK and YG cultivars compared to non-inoculated controls. Leaf tissues had reduced biomass in response to TYLCV and ToCV inoculation, indicating that virus infections enhanced plant endurance under salt stress conditions, with leaflet biomass allocation in TYLCV- and ToCV-grafted plants allowing host adaptation to saline environments. Assessments of physiological water balance parameters in healthy and virus TYLCV- and ToCV-grafted tomato plants previously revealed substantial differences in plant their responses to salt stress (Gharsallah *et al*., 2020).

When TYLCV- and ToCV- infected samples with salt stress applied were examined as another experimental group, significant reductions were detected in leaf area in the MK and YG cultivars for virus-treated plants. Similarly, LA decreased by approx. 6 cm^2 in these plants compared to experimental controls. The experiments demonstrated LA increases in salt-treated plants in both cultivars, with the plants responding positively to salinity. However, the LA was reduced in salt-treated plants in both MK and YG cultivars. LA measurements were taken for both control plants and those exposed to salt stress, showing this parameter gradually increased. However, the LA of control plants was greater under favorable conditions than in salt-stressed plants (Bacha *et al*., 2017).

Sandy *et al*. (2014) measured 20 to 25% reductions plant dry mass in drought-treated tomato plants. Transgenic overexpressing genes *SlSOS2* and *LeNHX2* exhibited increased growth and biomass production when cultivated in an NaCl-rich condition compared to their wild-type counterparts (Maach *et al*., 2021; Roșca *et al*., 2023). Although tomato is categorized as moderately salt-tolerant, salt accumulation in the soil can reduce plant production. This was evident for plants grown in compost conditions and those treated with mineral fertilizers, which had increased nutrient content, indicating adaptation to salt stress (Savy *et al*., 2022). These results indicate that tomato defence systems adapt to unfavourable growth conditions by activation from viruses and from high soil salt contents.

Wang *et al*. (2021) demonstrated that moderate salt stress inhibited tomato leaf growth, degree of inhibition increasing with time. Also, tomato plants subjected to salinity stress had reduced height and decreased leaf area. *Taraxacum officinale* and *Ambrosia artemisiifolia* had weak correlations with other parameters. Conversely, LA in *Tilia tomentosa* and *Aesculus hippocastanum* were positively correlated with specific leaf weights (SLW) but negatively correlated with specific leaf area (SLA). Furthermore, fractal dimensions (FD) in *T. tomentosa* and *A.s hippocastanum* were negatively correlated with SLA values, while FDs in *T. officinale* and *A. artemisiifolia* were negatively correlated with SLWs (Terada *et al*., 2021).

Relative water content (RWC) is an indicator of cellular water status, and is associated with abiotic stress tolerance. Reductions in RWC were observed in virus-inoculated tomato plants, and RWC was reduced in plants exposed to salt stress compared to the controls. Patane *et al*. (2022) showed that RWC values exceeding 75% were normal even under drought stress conditions. Hosseini *et al*. (2018) found that experimental plants exhibited reduced water content when exposed to a combination of drought stress and cucumber mosaic virus (CMV) compared to non-inoculated cultivars. Silencing of *SlCBL3-1* also reduced shoot and root growth, as well as RWC (Hosseini *et al*., 2018). In the present study, reductions in RWC were observed in TYLCV/ToCV-infected tomato cultivars MK and YG. The combined stresses reduced shoot fresh and dry weights, leaf area, and RWC in all cultivars assessed by Zhou *et al*. (2017). Increased RWC contributes to reductions in osmotic stress during the recovery periods (Tiwari *et al*., 2016), with leaf RWC being greatest in control plants and least in salt-exposed plants (Tanveer *et al*., 2019). Salt stress also severely inhibits seedling growth and biomass accumulation (Parvin *et al*., 2019, 2020). Restrictions in plant growth caused by salt have been attributed to salt-mediated reductions in cell growth. In addition to ion toxicity, salinity induces osmotic stress by altering water potential in growth media and within plants (Raziq *et al*., 2022).

Osmotic stress in tomato plants is evident through reduced leaf RWC, as high NaCl concentrations can damage root systems, leading to reduced water absorption (Zeng *et al*., 2011). Nahar *et al*. (2016) reported that salinity enhances proline levels, potentially increasing stress resistance by maintaining osmotic potential, promoting leaf expansion, enhanc-

ing stomatal conductance, and facilitating photosynthesis. Zhao *et al*. (2021) showed that root water and osmotic potentials improved in tomato plants exposed to salinity stress following biochar applications, and leaf RWC increased after biochar application under salinity stress. Water deficit is detrimental to plant growth, primarily due to reductions in RWC and water potential (Diouf *et al*., 2018). In the present study increased water deficits were detected in SV and V tomato plants. TYLCV- and ToCV-infected plants of the MK and YG cultivars had increased RWC in response to salt stress, although these approached levels similar to those of non-inoculated samples. Previously research revealed that tomato seeds exhibited maximum RWC when exposed to *Trichoderma viride* in a non-saline MS medium, but displayed minimum RWC when subjected to 100 mM NaCl (Metwally and Shereen S., 2023). TYLCV infection resulted in a 58% reduction in plant height when the plants were adequately watered, although infection did not affect numbers of leaves (Botto *et al*., 2023). Decreased shoot fresh and dry weights, leaf area, and RWC were also recorded in several cultivars under combined stresses (Zhou *et al*., 2017). These results show that plants resist salt stress do so by producing particular proteins (Maach *et al*., 2020).

CONCLUSIONS

While previous studies have explored the physiological and biochemical processes of tomato plants under various biotic and abiotic stress factors, there has been absence of research reports on the intricate interplay between biotic stress, such as that caused by virus infections, and abiotic stress, particularly salinity.

The present study has addressed this knowledge gap by utilizing virus susceptible tomato cultivars which were subjected to TYLCV and ToCV inoculations through single-leaflet grafting. The experimental design involved exposure to virus infections and salt stress to investigate the potential role of *CesA/Csl* genes biosynthesis in modulating host plant defense mechanisms. The study also aimed to assess the physiological effects of combined biotic and abiotic stress in tomato leaf tissues. The results highlight key physiological responses and gene expression patterns under the combined stress conditions. Alignment between gene expression data and physiological measurements indicates that the YG tomato cultivar was as sensitive to salt and virus stresses as the MK cultivar. The results also emphasize the importance of up-regulating specific genes, including *Csl-D3,1*, *Csl-D3,2*, and *Ces-A2*, which contribute to resistance against the combined effects of salt and virus stresses during dual stress exposure. Almost similar results were obtained during physiological measurements in MK and YG cultivars. As a result of TYLCV/ToCV infections and the combined effects of virus and salt stress, key physiological parameters, including relative water content, dry biomass, water deficit (WD), and leaf area, exhibited high variation coefficients in experimental plants. An exception was observed in relative water content measurements, where plants subjected to combined ToCV and salt stress exhibited values close to those of non-inoculated controls. This stability indicates a distinct physiological adaptation in response to the combined ToCV and salt stress conditions. However, when the broader physiological and gene expression data indicate that the YG tomato cultivar was similarly sensitive to stress conditions as the MK cultivar, suggesting that both tomato cultivars are similarly affected by virus and salt stresses.

This research provides insights into the molecular and physiological responses of tomato plants to the concurrent challenges of virus infection and salt stress. The observed regulation of specific *CesA/Csl* genes, along with distinct physiological alterations, highlight the complex interplay between biotic and abiotic stress signalling pathways. This study advances the understanding of stress adaptation mechanisms, and has potential to inform development of resilient tomato cultivars through targeted breeding or biotechnological approaches aimed at enhancing tolerance to multiple stress factors.

FUNDING

This research was financially supported by a grant from the Islamic Development Bank (ISDB) "Postdoc" Scholarship programme, Scholarship No. 600047690 (2022).

AUTHORS CONTRIBUTIONS

S.M., conceptualization, investigation and visualization, data analyses, drafting of original draft manuscript; İ.H., author of the idea project topic; İ.Ö.E., provided infected plant collections; C.Y.Ö., methodology, statistical analyses, revised original manuscript; A.E., methodology, conducted experiments, provided laboratory and the necessary chemical reagents. All authors read and approved the final version of the manuscript.

LITERATURE CITED

Abd El Rahman H., Nasr-Eldin M. A., Abo-Elmaaty S. A., Abdelwahed M. A., ElHefnawi M., El Fiky A. M., Soliman; E. R., 2024. Partial Genome Detection, Characterization of TYLCV (MZ546492) Infecting tomato plants and sirna sequences detection for alternative control strategy. *Egyptian Journal of Botany* 64(1): 211–223. https://doi.org/10.21608/ejbo.2023.208980.2321

Aboul-Maaty N., Oraby H., 2019. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre* 43(1): 25–35. https://doi.org/10.1186/s42269-019-0066-1

Alam M.S., Tester M., Fiene G., Mousa M.A., 2021. Early growth stage characterization and the biochemical responses for salinity stress in tomato. *Plants* 10(4): 712. https://doi.org/10.3390/plants10040712

Bacha H., Tekaya M., Drine S., Guasmi F., Touil L., Enneb H., Triki T., Cheour F., ... Ferchichi A., 2017. Impact of salt stress on morpho-physiological and biochemical parameters of *Solanum lycopersicum* cv. Microtom leaves. *South African Journal of Botany* 108(1): 364–369. https://doi.org/10.1016/j.sajb.2016.08.018

Botto C.S., Mati S., Moine A., Chitarra W., Nerva L., D'Errico C., Pagliarani C., ... Noris Emanuela., 2023. Tomato yellow leaf curl sardinia virus ıncreases drought tolerance of tomato. *International Journal Molecular Sciences* 24(3): 2893–2909. https://doi.org/10.3390/ijms24032893.

Brown R., Wyatt H., Price J., Kelly F., 1996. Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress. *European Respiratory Journal* 9(5): 334–339. https://10.1183/09031936.96.09020334

Cao S., Cheng H., Zhang J., Aslam M., Yan M., ... Qin Y., 2019. Genome-wide ıdentification, expression pattern analysis and evolution of the Ces/Csl gene superfamily in pineapple (*Ananas comosus*). *Plants* 8(11): 275. https://doi.org/10.3390/plants8080275

Chantreau M., Chabbert B., Billiard S., Hawkins S., Neutelings G., 2015. Functional analyses of cellulose synthase genes in flax (*Linum usitatissimum*) by virus-induced gene silencing. *Plant Biotechnology Journal* 13(9): 1312–1324. https://doi.org/10.1111/pbi.12350.

Chen T., Lv Y., Zhao T., Li N., Yang Y., ... Zhang B., 2013. Comparative transcriptome profiling of a resistant vs. susceptible tomato (*Solanum lycopersicum*) cultivar in response to infection by tomato yellow leaf curl virus. *PloS One* 8(11): e80816. https:// doi.org/10.1371/journal.pone.0080816

Chinnaraja C., Ramkissoon A., Ramsubhag A., Jayaraj J., 2016. First report of Tomato yellow leaf curl virus infecting tomatoes in Trinidad. *Plant Diseases* 100(4): 1958. https://doi.org/10.1094/PDIS-04-16-0446-PDN

Choe S., Choi B., Kang J.H., Seo J.K., 2021. Tolerance to tomato yellow leaf curl virus in transgenic tomato overexpressing a cellulose synthase-like gene. *Plant Biotechnology Journal* 19(4): 657–659. https://doi.org/10.1111/pbi.13539

Çevik B., Kıvrak H., Şahin-Çevik M., 2019. Development of a graft inoculation method and a real-time RT-PCR assay for monitoring Tomato chlorosis virus infection in tomato. *Journal of Virological Methods* 265(5): 1–8. https://doi.org/10.1016/j.jviromet.2018.12.004

Dabravolski S.A., and Isayenkov S.V., 2023. The regulation of plant cell wall organisation under salt stress. *Frontiers in Plant Science* 14(1): 1118313. https://doi.org/10.3389/fpls.2023.1118313

Desbiez C., Verdin E., Moury B., Lecoq H., Millot P., ... Huseynova I., 2018. Prevalence and molecular diversity of the main viruses infecting *Cucurbit* and *Solanaceous* crops in Azerbaijan. *European Journal of Plant Pathology* 153(2): 1–11. https://link.springer.com/article/10.1007%2Fs10658-018-1562-0

Devkar V., Thirumalaikumar V. P., Xue G.-P., Vallarino J. G., Turečková V., ... Balazadeh S.,2020. Multifaceted regulatory function of tomato SlTAF1 in the response to salinity stress. *New Phytology* 225(4): 1681–1698. https://doi.org/10.1111/nph.16247

Diouf I.A., Derivot L., Bitton F., Pascual L., Causse M., 2018. Water deficit and salinity stress reveal many specific qtl for plant growth and fruit quality traits in tomato. *Environmental and Experimental Botany* 9(6): 279. https://doi.org/10.3389/fpls.2018.00279

Dovas C., Katis N., Avgelis A., 2002 Multiplex detection of criniviruses associated with epidemics of a yellowing disease of tomato in Greece. *Plant Disease* 86(12): 1345–1349. https://doi.org/10.1094/PDIS.2002.86.12.1345

Elsharkawy M.M., El-Okkiah S., Elsadany A.Y., Bedier M.Y., Omara R.I., ...Abdelkhalek A., 2022. Systemic resistance induction of tomato plants against tomato mosaic virus by microalgae. *Egyptian Journal of Biological Pest Control* 32(1): 1–7. https://doi.org/10.1186/s41938-022-00538-2

Endler A., Kesten C., Schneider R., Zhang Y., Ivakov A., ... Persson S., 2014. *Alfalfa cellulose synthase* gene expression under abiotic stress: a hitchhiker's guide to RT-qPCR Normalization. *PLoS One* 162(6): 1353–1364. https://doi.org/10.1371/journal.pone.0103808

Endler A., Kesten C., Schneider R., Zhang Y., Ivakov A., … Persson S., 2015. A mechanism for sustained cellulose synthesis during salt stress. *Cell* 162(6): 1353–1364. https://doi.org/10.1371/journal.pone.0103808

FAO 2011. *The state of the world's land and water resources for Food and Agriculture (SOLAW)—managing systems at risk*. Abingdon: Food and Agriculture Organization of the United Nations and Earthscan.

FAOSTAT 2022. Crops and livestock products. Available at: https://www.fao.org/faostat/en/#data/QCL.

Fiallo-Olivé E., Navas-Castillo J., 2019. Tomato chlorosis virus, an emergent plant virus still expanding its geographical and host ranges. *Molecular Plant Pathology* 20(9): 1307–1320. https://doi.org/10.1111/mpp.12847

Fidan H., Sarıkaya P., 2020. Virus diseases in eggplant (*Solanum melongena*) cultivation in Antalya province. *Mediterranean Agricultural Sciences* 33(1): 27–35. https://doi.org/10.29136/mediterranean.642226 (in Turkish)

Gao W., Wang Z., Dong C., Wei K., Chen Y., Qiu Z., Wang., X., 2024. Screening resistant germplasms and quantitative trait locus mapping of resistance to Tomato chlorosis virus. *International Journal of Molecular Sciences* 26(5): 2060. https://doi.org/10.21203/rs.3.rs-4235788/v1

Gharsallah C., Fakhfakh H., Grubb D., Gorsane. F., 2016. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants* 8(1): plw055. https://doi.org/10.1093/aobpla/plw055

Gharsallah C., Gharsallah S., Werghi S., Mehrez M., Fakhfakh H., Gorsane F., 2020. Tomato contrasting cultivars responses under combined salinity and viral stresses. *Physiology and Molecular Biology of Plants* 26(7): 1411–1424. https://doi.org/10.1007/s12298-020-00835-w.

Grünzweig J., Katan J., Ben-Tal Y., Rabinowitch H., 1999. The role of mineral nutrients in the increased growth response of tomato plants in solarized soil. *Plant and Soil* 206(1): 21–27. https://doi.org/10.1023/A:1004321118896

Guo M., Wang X.-S., Guo H.-D., Bai S.-Y., Khan A., … Li J.-S., 2022. Tomato salt tolerance mechanisms and their potential applications for fighting salinity: *A review. Frontiers in Plant Science* 13(1): 949541. https://doi.org/10.3389/fpls.2022.949541

Hoagland D.R., Arnon D.I., 1950. The water culture method for growing plants without soil. California Agricultural Experimental Station Circular, University of California, Berkeley 347(2): 1–32. https://doi.org/10.1371/journal.pone.0103808

Hosseini S., Reza Z., Mostafa Y., Khayyat M., 2018. Effects of Cucumber Mosaic Virus infection and drought tolerance of tomato plants under greenhouse conditions: Preliminary results. *Journal of Berry Research* 8(3): 1–8. https://doi.org/10.3233/JBR-170285

Hu H., Zhang R., Feng S., Wang Y., Wang Y., … Peng L., 2018. Three AtCesA6-like members enhance biomass production by distinctively promoting cell growth in *Arabidopsis*. *Plant Biotechnology Journal* 16(5): 976–988. https://doi.org/10.1111/pbi.12842

Huang H., Zhao S., Chen J., Li T., Guo G., Xu M., Liao X. 2022. Genome-wide identification and functional analysis of *Cellulose synthase* gene superfamily in *Fragaria vesca*. *Frontiers in Plant Science* 13(1): 1044029.

Huang Y., Ma H.-Y., Huang W., Wang F., Xu Z-S., Xiong A.-S., 2016. Comparative proteomic analysis provides novel insight into the interaction between resistant vs susceptible tomato cultivars and TYLCV infection. *BMC Plant Biology* 16(1): 162. https://doi.org/10.1186/S12870-016-0819-Z

Ibrahim A.M., 2018. Salt tolerance of some tomato (*Solanum lycoversicum* L.) cultivars for salinity under controlled conditions. *American Journal of Plant Physiology* 13(2): 58–64. https://doi.org/10.3923/ajpp.2018.58.64

Jin F.M., Song J., Xue J., Sun H.B., Zhang Y., Wang S., Wang Y.H., 2020. Successful generation of anti-ToCV and TYLCV transgenic tomato plants by RNAi. *Biology of Plant* 64(1): 490–496. https://doi.org/10.32615/bp.2020.069

Jones R. A., Naidu R. A., 2019. Global dimensions of plant virus diseases: current status and future perspectives. *Annual Review of Virology* 6(1): 387–409. https://doi.org/10.1146/annurev-virology-092818-015606

Kesten C., Menna A., Sanchez-Rodrıguez C., 2017. Regulation of cellulose synthesis in response to stress. *Current Opinion in Plant Biology* 40(3): 106–113. https:// doi.org/10.1016/j.pbi.2017.08.010

Klay I., Pirrello J., Riahi L., Bernadac A., Cherif A., Bouzayen M., Bouzid S., 2014. Ethylene response factor *Sl-ERF.B.3* ıs responsive to abiotic stresses and mediates salt and cold stress response regulation in tomato. *Scientific World Journal* 1(1): 12. https://doi.org/10.1155/2014/167681

Kusvuran S., Kiran S., Ellialtioglu S. S., 2016. Antioxidant enzyme activities and abiotic stress tolerance relationship in vege crops. In: *Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives*. (A. K. Shanker, C. Shanker, ed.). InTech 8(12): 481–506. https://doi.org/10.5772/62235

Kwon S. J., Lee Y. J., Cho Y. E., Byun H. S., Seo J. K., 2024. Engineering of stable infectious cDNA constructs of a fluorescently tagged tomato chlorosis virus. *Virology* 16(1): 125. https://doi.org/10.1038/ijir.2012.45

Le Gall., Philippe F., Domon J., Gillet F., Pelloux J., Rayon C., 2015. Cell wall metabolism in response to abiotic stress. *Plants* 4(1): 112–166. https://doi.org/10.3390/plants4010112

Lee H., Kim M., Choi H., Kang J., Ju H., Seo J., 2017. Efficient transmission and propagation of tomato chlorosis virus by simple single-leaflet grafting. *Plant Pathology Journal* 33(3): 345–349. https://doi.org/10.5423/PPJ.NT.02.2017.0039

Li N., Wu X., Zhuang W., Xia L., Chen Y., Wu C., et al., 2021. Tomato and lycopene and multiple health outcomes: Umbrella review. *Food Chemistry* 343(3): 128396. https://doi.org/10.1016/j.foodchem.2020.128396

Li T., Huang Y., Xu Z.S., Wang F., Xiong A.S., 2019. Salicylic acid-induced differential resistance to the tomato yellow leaf curl virus among resistant and susceptible tomato cultivars. *BMC Plant Biology* 19(1): 173. https://doi.org/10.1186/s12870-019-1784-0

Livak K., Schmittgen T., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4): 402–408. https://doi.org/10.1006/meth.2001.1262

Louro D., Accotto G., Vaira A., 2000. Occurrence and diagnosis of tomato chlorosis virus in portugal. *European Journal of Plant Pathology* 106(6): 589–592. https://doi.org/10.1023/A:1008738130592

Maach M., Baghour M., Akodad M., Gálvez F.J., Sánchez M.E., … Rodríguez-Rosales M.P., 2020. Overexpression of *LeNHX4* improved yield, fruit quality and salt tolerance in tomato plants (*Solanum lycopersicum* L.). *Molecular Biology Reports* 47(4): 4145–4153. https://doi.org/10.1007/s11033-020-05499-z

Maach M., Rodríguez-Rosales M., Venema K., Akodad M., Moumen A., Skalli A., Baghour M., 2021. Improved yield, fruit quality, and salt resistance in tomato co-overexpressing *LeNHX2* and SlSOS2 genes. *Physiology and Molecular Biology of Plants* 27(4): 703–712. https://doi.org/10.1007/s12298-021-00974-8

Maksup S., Sengsai S., Laosuntisuk K., Asayot J., Pongprayoon W., 2020. Physiological responses and the expression of cellulose and lignin associated genes in Napier grass hybrids exposed to salt stress. *Acta Physiologiae Plantarum* 42(7): 1–12. https://doi.org/10.1007/s11738-020-03092-2

Malinovsky F., Fangel J., Willats W., 2014. The role of the cell wall in plant immunity. *Frontiers in Plant Science* 5(6): 178–190. https://doi.org/10.3389/fpls.2014.00178

Martínez-Culebras P., Font I., Jordá C., 2001. A rapid PCR method to discriminate between tomato yellow leaf curl virus isolates. *Annals of Applied Biology* 139(2): 251–257. https://doi.org/10.1111/j.1744-7348.2001.tb00401.x

Metwally R., Shereen S., 2023. Alleviation of the adverse effects of NaCl stress on tomato seedlings (*Solanum lycopersicum* L.) by *Trichoderma viride* through the antioxidative defense system. *Botanical Studies* 64(4): 1–18. https://doi.org/10.1186/s40529-023-00368-x

Milc J., Bagnaresi P., Aragona M., Valente M.T., Biselli C., … Pecchioni N., 2019. Comparative transcriptome profiling of the response to *Pyrenochaeta lycopersici* in resistant tomato cultivar Mogeor and its background cultivar-susceptible Moneymaker. *Functional & Integrative Genomics* 19(5): 811–826. https://doi.org/10.1007/s10142-019-00685-0

Mirzayeva S., Huseynova I., Özmen C.Y., Ergül A., 2023. Physiology and gene expression analysis of tomato (*Solanum lycopersicum* L.) exposed to combined-virus and drought stresses. *Plant Pathology Journal* 39(5): 466–485. https://doi.org/10.5423/PPJ.OA.07.2023.0103

Moriones E., Navas-Castillo J., 2000. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Research* 71(2): 2123–2134. https://doi.org/10.1016/S0168-1702(00)00193-3

Mugiira R.B., Arama P.F., Macharia J.M., Gichimu B.M., 2011. Antibacterial activity of foliar fertilizer formulations and their effect on ice nucleation activity of *Pseudomonas syringae* pv garcae Van Hall; the causal agent of Bacterial Blight of Coffee. *International Journal of Agricultural Research* 6(7): 550–561. https://doi.org/10.3923/ijar.2011.550.561

Nahar K., Hasanuzzaman M., Alam M., Rahman A., Suzuki T., Fujita M., 2016. Polyamine and nitric oxide crosstalk: Antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems. *Ecotoxicology and Environmental Safety* 126(1): 245–255. https://doi.org/10.1016/j.ecoenv.2015.12.026

Ong S.N., Taheri S., Othman R.Y., Teo C.H., 2020. Viral disease of tomato crops (*Solanum lycopesicum L.*): an overview. *Journal of Plant Diseases and Protection* 127(10): 725–739. https://doi.org/10.1007/s41348-020-00330-0

Parvin K., Hasanuzzaman M., Bhuyan M., Nahar K., Mohsin S.M., Fujita M., 2019. Comparative physiological and biochemical changes in tomato (*Solanum*

lycopersicum L.) under salt stress and recovery: Role of antioxidant defense and glyoxalase systems. *Antioxidants* 8(9): 350. https://doi.org/10.3390/antiox8090350

Parvin K., Nahar K., Hasanuzzaman M., Bhuyan M.B., Mohsin S.M., Fujita M., 2020. Exogenous vanillic acid enhances salt tolerance of tomato: Insight into plant antioxidant defense and glyoxalase systems. *Plant Physiology and Biochemistry* 150(1): 109–120. https://doi.org/10.1016/j.plaphy.2020.02.030

Patanè C., Cosentino S., Romano D., Toscano S., 2022. Relative water content, proline, and antioxidant enzymes in leaves of long shelf-life tomatoes under drought stress and rewatering. *Plants* 11(22): 3045–3046. https://doi.org/10.3390/plants11223045

Peng X., Pang H., Abbas M., Yan X., Dai X., Li Y., Li Q., 2019. Characterization of *Cellulose synthase-like D (CSLD*) family revealed the involvement of *PtrCslD5* in root hair formation in *Populus trichocarpa*. *Scientific Reports* 9(1): 1452. https://doi.org/10.1038/s41598-018-36529-3

Raziq A., Wang Y., Mohi Ud Din A., Sun J., Shu S., Guo S., 2022. A comprehensive evaluation of salt tolerance in tomato (*Var. Ailsa Craig*): responses of physiological and transcriptional changes in RBOH's and ABA biosynthesis and signalling genes. *International Journal of Molecular Science* 23(3): 1603. https://doi.org/10.3390/ijms23031603.

Renau-Morata B., Molina R.V., Carrillo L.J., Cebolla-Cornejo J., Sánchez-Perales M.,... Nebauer S.G., 2017. Ectopic expression of *CDF3* genes in tomato enhances biomass production and yield under salinity stress conditions. *Frontiers in Plant Science* 3(13): 660–678. https://doi.org/10.3389/fpls.2017.00660

Roșca M., Mihalache G., Stoleru V., 2023. Tomato responses to salinity stress: From morphological traits to genetic changes. *Frontiers in Plant Science* 14(1): 1118383. https://doi.org/10.3389/fpls.2023.1118383

Sandy C.J., Gosling S.D., Koelkebeck T., 2014. Psychometric com-parison of automated versus rational methods of scale abbreviation: Anillustration using a brief measure of values. *Journal of Individual Differences* 35(4): 221–235. https://doi.org/10.1027/1614-0001/a000144

Savy D., Cozzolino V., Vinci G., Verrillo M., Aliberti A., ... Piccolo A., 2022. Fertilisation with compost mitigates salt stress in tomato by affecting plant metabolomics and nutritional profiles. *Chemical and Biological Technologies in Agriculture* 9(1): 12–30. https://doi.org/10.1186/s40538-022-00373-5

Seo J.K., Kim M.K., Kwak H.R., Choi H.S., Nam M., ... C. Jung, 2018. Molecular dissection of distinct symptoms induced by tomato chlorosis virus and tomato yellow leaf curl virus based on comparative transcriptome analysis. *Virology* 516(1): 1–20. https://doi.org/10.1186/s40538-022-00373-5

Shafi A., Gill T., Zahoor I., Ahuja P.S., Sreenivasulu Y., Kumar S., Singh A.K., 2019. Ectopic expression of SOD and APX genes in *Arabidopsis* alters metabolic pools and genes related to secondary cell wall cellulose biosynthesis and improve salt tolerance. *Molecular Biology Reports* 46(2): 1985–2002. https://doi.org/10.1007/s11033-019-04648-3

Sharif R., Liu P., Wang D., Jin Z., Uzair U., Yadav V., Li Y., 2021. Genome-wide characterisation and expression analysis of *cellulose synthase* genes superfamily under various environmental stresses in *Cucumis sativus* L. *New Zealand Journal of Crop and Horticultural Science* 49(3): 127–150. https://doi.org/10.1080/01140671.2021.1926291

Song X., Xu L., Yu J., Tian P., Hu X., Wang Q., Pan Y., 2019. Genome-wide characterization of the cellulose synthase gene superfamily in *Solanum lycopersicum*. *Gene* 688(10): 71–83. https://doi.org/10.1016/j.gene.2018.11.039

Tabein S., 2021. Evaluation of two nontransformative approaches in triggering RNAi against tomato yellow leaf curl virus. *Plant Protection (Scientific Journal of Agriculture)* 44(4): 77–88. https://doi.org/10.22055/ppr.2021.17177

Tambussi E., Nogués S., Araus J., 2005. Ear of durum wheat under water stress: water relations and photosynthetic metabolisms. *Planta* 221(3): 446–458. https://doi.org/10.1007/s00425-004-1455-7

Tanveer M., Shahzad B., Sharma A., Khan E.A., 2019. 24-Epibrassinolide application in plants: An implication for improving drought stress tolerance in plants. *Plant Physiology and Biochemistry* 135(2): 295–303. https://doi.org/10.1016/j.plaphy.2018.12.013.

Terada N., Sanada A., Koshio K., Habibi N., Sediquim N., 2021. Effects of salinity on growth, physiological and biochemical responses of tomato. *Journal of the International Society for Southeast Asian Agricultural Sciences* 27(2): 14–28. https://doi.org/10.1007/s10811-018-1619-7

Tiwari S., Lata C., Chauhan P., Nautiyal C., 2016. *Pseudomonas putida attunes* morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiology and Biochemistry* 99(1): 108–117. https://doi.org/10.1016/j.plaphy.2015.11.001

Ueda A., Yamamoto-Yamane Y., Takabe T., 2007. Salt stress enhances proline utilization in the apical region of barley roots. *Biochemical and Biophysical*

Research Communications 355(1): 61–66. https://doi.org/10.1016/j.bbrc.2007.01.098

Verdin E., Desbiez C., Wipf-Scheibel C., Gognalons P., Kheyr-Pour A... Huseynova I., 2018. First report of tomato yellow leaf curl virus infecting tomato in Azerbaijan. *Journal of Plant Pathology* 100(2): 335. https://doi.org/10.1007/s42161-018-0050-x

Wang W., Cai L., Long Z., Zhang X., Zhao F., 2021. Effects of non-uniform salt stress on growth, yield, and quality of tomato. *Journal of the International Society for Southeast Asian Agricultural Sciences* 27(5): 545–556. https://doi.org/10.1080/00380768.2021.1966834

Wang J., Li J., Lin W., Deng B., Lin L., ... Ma X., 2022. Genome-wide identification and adaptive evolution of *CesA/Csl* superfamily among species with different life forms in *Orchidaceae*. *Frontiers in Plant Science* 13(1): 994679. https://doi.org/10.3389/fpls.2022.994679

Zelm E., Zhang Y, Testerink C., 2020. Salt Tolerance Mechanisms of Plants. *Annual Review of Plant Biology* 71(1): 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005.

Zeng C., Liu L., Wang B., Wu X., Zhou Y., 2011. Physiological effects of exogenous nitric oxide on *Brassica juncea* plants under NaCl stress. *Biology of Plants* 55(2): 345–348. https://doi.org/10.1007/s10535-011-0051-5

Zhang H., Sonnewald U., 2017. Differences and commonalities of plant responses to single and combined stresses. *The Plant Journal* 90(5): 839–855. https://doi.org/10.1111/tpj.13557

Zhang S.S., Sun L., Dong X., Lu S.J., Tian W., Liu J.X., 2016. Cellulose synthesis genes *CESA6* and *CSI1* are important for salt stress in *Arabidopsis*. *Journal of Integrative Plant Biology* 58(7): 623–626. https://doi.org/10.1111/jipb.12442

Zhao S., Zhang Q., Liu M., Zhou H., Ma C., Wang P., 2021. Regulation of plant responses to salt stress. *International Journal of Molecular Science* 22(9): 4609. https://doi.org/10.3390/ijms22094609

Zhou R., Yu X., Ottosen C.-O., Rosenqvist E., Zhao L., ... Wu Z., 2017. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biology* 17(1): 24. https://doi.org/10.1186/s12870-017-0974-x

Zheng M., Liu X., Lin J., Liu X., Wang Z., ... Ni Z., 2019. Histone acetyltransferase *GCN 5* contributes to cell wall integrity and salt stress tolerance by altering the expression of cellulose synthesis genes. *The Plant Journal* 97(3): 587–602. https://doi.org/10.1111/tpj.14144

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Survey of banana bunchy top virus in southern Mozambique

Sandra Carvalho I. Mussa BARROS[1], Antonia DOS REIS FIGUEIRA[2,*], Antonia Thalyta Lopes SILVEIRA[2], Edson A. POZZA[2]

[1] *Research Department in Agricultural Sciences, Pedagogical University of Maputo (UP-Maputo), Maputo, Mozambique*
[2] *Department of Plant Pathology, Federal University of Lavras, Minas Gerais State, Lavras, 37203-202, Brazil*
*Corresponding author. E-mail: antonia@ufla.br

Summary. Banana bunchy top virus (BBTV) has been recently recorded in Mozambique and threatens economic viability of banana production in this country. Research on BBTV in the region is still initial, and collective effort is required to support efficient control measures. Surveys were made in four administrative areas of the Chókwè district, Gaza Province, to determine the incidence and distribution of the virus. Samples were collected from two banana cultivars in 23 production fields where the plants had characteristic virus symptoms. DNA was extracted from these samples and subjected to BBTV detection using polymerase chain reaction technology. BBTV was detected in 19 of the 23 sampled fields, with overall mean incidence of 54.3%, and minimum incidence of 20%. No infected plants were found in the fields of one farm. These results highlight the urgency to initiate strategies for control of BBTV in Mozambique.

Keywords. BBTV incidence, BBTV distribution, viral.

OPEN ACCESS

Citation: Barros, S. C. I. M., Dos Reis Figueira, A., Silveira, A. T. L. & Pozza, E. A. (2025). Survey of banana bunchy top virus in southern Mozambique. *Phytopathologia Mediterranea* 64(2): 245-254. doi: 10.36253/phyto-16676

Accepted: August 10, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Assunta Bertaccini, Alma Mater Studiorum, University of Bologna, Italy.

ORCID:
SCIMB: 0000-0002-1398-7188
ADRF: 0000-0002-2589-1394
ATLS: 0000-0001-9044-7284
EAP: 0000-0003-2813-584X

INTRODUCTION

Bananas are of great socioeconomic importance to Mozambique, as this fruit generate income and jobs, and are an important food source. According to the Ministry of Agriculture and Rural Development (MADER), Mozambique produces approx. 500,000 tons of bananas per year, with production from two sectors: family production (74.5% of total production) and commercial production (25.5%) (Diário Econômico, 2021).

Among factors restricting banana production, banana bunch top disease (BBTD), caused by *Babuvirus musae* (syn. banana bunch top virus, BBTV), is one of the most economically important diseases. BBTV is one of the 100 most important invasive plant pathogens and is subject to strict quarantine measures within the International Plant Protection Convention (Kumar *et al.*, 2011; Global Invasive Species Database, 2025). BBTD was first reported in Fiji in 1879, in Egypt in 1900, and then in Sri Lanka and Australia in 1913

(Magee, 1927), and is currently present in Southeast Asia, the South Pacific, India and Africa (Bouwmeester *et al*., 2023; Ocimati *et al*., 2024).

From 1913 to 1920, banana plantations in Australia were destroyed by BBTD (Magee, 1927; Hooks *et al*., 2009). In the 1990s, the first serious BBTD epidemic in Africa caused drastic reductions in banana production in the Nkhatabay and Nkhotakota districts of Malawi, where planted areas reduced from 3,500 ha to approx. 800 ha (Soko *et al*., 2009; Kumar *et al*., 2011). In 2016, the disease was identified in Mozambique, in the Chókwè district, in the Primeira Zona area of an irrigated region. There are no precise estimates of yield losses due to BBTD in Mozambique, but reductions 90% have been reported in susceptible cultivars (e.g. 'AAA-Cavendish'), due to severe BBTD (IPPC, 2016).

BBTV belongs to *Nanoviridae* family, *Babuvirus* genus and has a circular and multipartite ssDNA genome in six components, each separately encapsidated within an isometric virion of diameter 17-19 nm. These components are: DNA-R (Master Rep); DNA-S (coat protein); DNA-C (cell cycle binding protein); DNA-M (movement protein); DNA-N (nuclear transport protein) and DNA-U3 which encodes a protein of unknown function (Thomas *et al*., 2021).

Symptoms caused by BBTV on banana plants include discontinuous stripes and dark green spots on leaf blades, central veins and petioles, chlorosis and drying of leaf margins. Other characteristic symptoms: plant stunting, due to short and narrow leaves at the top of plants ("rosette"), lack of fruit production and fruit bunches, which are not suitable for consumption (Kumar *et al*., 2015; Food and Agricultural Organization, 2018).

Spread of BBTV over long distances, within and among countries and continents, occurs through infected planting material (suckers or tissue cultured plants). Dissemination of the virus over short distances can occur through infected planting material and the banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae). This aphid transmits BBTV in a circulative, non-propagative manner, while there is no evidence for mechanical virus transmission (Bressan and Watanabe, 2011; Watanabe and Bressan, 2013; Qazi, 2016; Thomas *et al*., 2021). Although *P. nigronervosa* was initially reported in associations with hosts belonging to *Zingiberales* and *Arecales*, morphological and morphometric studies have confirmed that the aphids found on these plants are of different species (Bhadra and Agarwala, 2010; Thomas *et al*., 2021). *P. nigronervosa* has high specificity for *Musa* spp. hosts and has been found in almost all banana-producing countries (Kumar *et al*., 2011). Transmission of BBTV by aphids to other hosts, such as *Heliconia* sp., *Alpinia galanga* and *Curcuma longa*, has been recently reported (Ngatat *et al*., 2022; Mendoza *et al*., 2024).

Information on BBTD in Mozambique has not been previously reported, so a survey was carried out to identify and determine the epidemiology of this virus in the region, aiming to acquire information for development of effective management strategies, particularly for the small-scale agriculture system in Mozambique. The study used polymerase chain reaction (PCR) tests for detection of BBTV, and to determine incidence and distribution of BBTD in Chókwè district of Gaza Province.

MATERIALS AND METHODS

Sample collection

Sample collections were carried out from 11 farms the Chókwè district, Gaza province, Mozambique (Figure 1), in January 2020. The Chókwè district is in the south of the Gaza province, close to the middle course of the Limpopo River (24°05' to 24°48' south latitude, 32°33' to 33°35' east longitude). Gaza province is bordered to the north by the Limpopo River, which separates it from the districts of Massingir, Mabalane and Guijá; to the south by the Bilene district and the Mazimuchope River; to the east by the Chibuto district; and to the west by the districts of Magude and Massingir.

The present study was carried out on farms that produced bananas, regardless of size (including large and small producers). For each collection site, termed a field, the geographic coordinates, average temperature, altitude, crop size, banana cultivar and presence of the *P. nigronervosa* vector were recorded. Banana leaves were collected at each site (sample size calculated as described below). Leaf samples were placed in zip lock plastic bags containing silica gel, and were transported to the laboratory of the Biotechnology Center of the Eduardo Mondlane University of Mozambique (UEM) for DNA extraction.

Sampling

To create the representative samples, BBTV foci were chosen in the four administrative areas (Macarretane, Chókwé-Sede, Lionde and Xilembene) of the Chókwè district, Gaza province, according to data from the MADER of Mozambique.

The estimated variable for disease incidence of Cooke (1998) was used to calculate the minimum num-

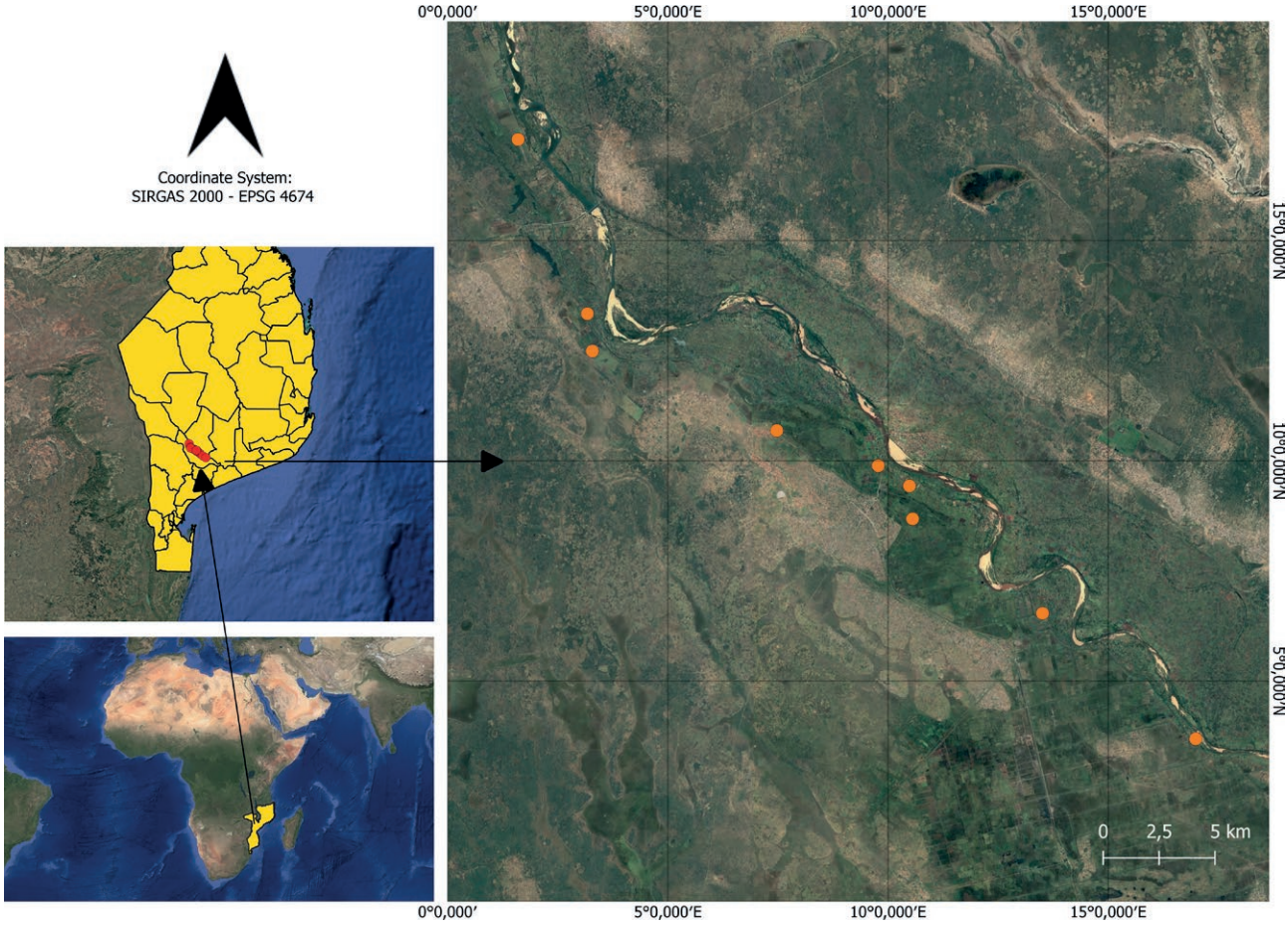


Figure 1. Maps of Africa and Mozambique, and a satellite image indicating sample collection sites for the 11 farms in the Chókwè district, Gaza province, Mozambique analyzed in this study. The orange dots and latitude and longitude co-ordinates for the satellite image show the geographic where the samples were collected.

ber of samples per location in each administrative area. This equation is:

$$n = \frac{Z^2.p.q.N}{d^2.(N-1)} + Z^2.p\,q$$

where:
n = the number of samples to be collected
Z = the tabulated value, referring to the abscissa of the standard normal curve, with a 90% confidence level (in this case, 1.65)
p = 0.6, an estimate of the probability of finding BBTV in the sample, as determined by the virology laboratory at the Federal University of Lavras
q = equal to 1-p
N = the population size or total number of plants of each banana producer's field, in the administrative area of the Chóckwè district, according to MADER data; and
d = the sampling error (the error attributed to the probability of finding or not finding a BBTV in the collected sample).

BBTV detection

DNA extractions

To extract DNA in the UEM laboratory, the protocol described by Lodhi *et al.* (1994) was used, with minor modifications. Fresh plant tissue (0.35 g) was macerated in liquid nitrogen, and then 1.5 mL of cetyl trimethylammonium bromide (CTAB) buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl; 80 mM Na_2SO_3; 2% polivinilpirrolidone and 2% CTAB) containing 0.2% β mercaptoethanol was added to macerated plant tissue mixture. This was then transferred to a 1.5 mL capacity sterilized microcentrifuge tube, and 750 µL of extraction buffer was added. The tubes were then incubated

at 60°C for 30 min., and 750 µL of chloroform: isoamyl alcohol solution (24:1) was added to each tube, and the tubes were centrifuged at 12,000 rpm for 10 min. at 4°C. The aqueous phase (supernatant) in each tube was transferred to new microcentrifuge tube, and the DNA was precipitated by adding 0.6 volumes of isopropanol and incubating at -20°C for 1 h. After centrifugation at 12,000 rpm for 10 min, the supernatant was discarded, and DNA was washed with 500 µL of 70% ethanol and the resuspended in 100 µL of 1× Tris-EDTA buffer. The tubes containing the extracted DNA were placed in dry ice and transported to the Laboratory of Molecular Virology, Department of Phytopathology, Federal University of Lavras (UFLA), Brazil for further analyses.

Polymerase chain reaction (PCR)

PCR was used to confirm the identity of the BBTV with primers flanking the region of the S gene encoding the capsid protein: BBTV 67SF and BBTV 784SR (Barros *et al*., 2024). Each DNA amplification reaction was carried out with 2.5 µL of 10× PCR buffer, containing 15 mM $MgCl_2$, 2.0 µL of 10 mM dNTPs, 1.0 µL of the forward primer and 1.0 µL of the reverse primer at a concentration of 10 µM, 0.25 µL of Taq DNA polymerase (Cellco, BRA), 1.0 µL of DNA (30 ng μL^{-1}), plus water for a total reaction volume of 25 µL. The amplification was carried out with the following program: 94°C for 3 min, followed by 35 cycles each of 94°C for 30 sec, 49°C for 30 sec, and 72°C for 60 sec. with a final extension of 72°C for 5 min. The amplified products were analyzed by electrophoresis in a 0.7% agarose gel and contrasted with Gel Red (Biotium®). The gel bands were visualized and documented using MiniBis Pro photodocumenter (DNR Bio-Imaging Systems®).

Data analysis

Microsoft Excel® software was used to organize the data, create tables, and calculate the mean incidence of BBTD with the following equation:

$$ID = \frac{UI}{UO}.100\%$$

where:
ID = incidence of disease (%)
IU = number of units affected by BBTD
UO = total number of plants assessed.

The assumptions of the analysis of variance were evaluated from the residuals of the three assays carried out. The residuals did not present normal distributions according to the Shapiro–Wilk test ($P < 0.05$), and the homogeneity of variances was violated. Nonhomogeneous residuals ($P < 0.05$) were identified by the ncvTest function and the Durbin-Watson independence test, where the residuals were correlated and there was no independence ($P < 0.05$). The analysis was carried out using the dwtest function of the lmtest package for the R statistical program.

The nonparametric Kruskal–Wallis test was used with the Kruskal test function in the statistical program R (R Core Team, 2021). This test allows comparison of three or more groups of independent samples and is used in cases where ANOVA requirements are not met (Kruskal and Wallis, 1952). The Dunn test was then applied with *P* value adjustment by Bonferroni (Dunn, 1961) with the rstatix package, through the Dunn test function in the R statistical program.

Histograms and maps of the 11 farms in the Chókwè district were created with the ggplot2 package for the R statistical program. For multiple comparisons (compare_means) between farms, the ggpubr package for R was used.

RESULTS

Detection of BBTV in collected samples

Presence of BBTV was confirmed in the Chókwè district of southern Mozambique. This region has farmers who practice subsistence agriculture, harvesting bananas for their personal needs, and commercial producers. A total of 175 plant samples were collected from 11 farms. Regardless of the presence of disease symptoms, all the collected samples were analyzed by PCR. Infected (positive) samples produced amplification bands each of 718 bp, as expected for the BBTV.

Incidence of BBTV in the collected samples ranged from zero (*i.e*., no infection) to 100%. The obtained PCR bands are illustrated in Figure 2, and incidence of BBTV are outlined in Table 1.

Incidence and distribution of BBTV

The greatest incidence of positive BBTV samples was recorded from Farm 2 (100%), followed by Farm 1 (84%), Farm 11 (83%), Farm 5 (80%) and Farm 3 (78%). The other farms had BBTV incidence levels lower than 70%, with the lowest incidence recorded on Farm 9 (20%) (Figure 3). BBTV was detected in most of the farms sampled, with the small family farms hav-

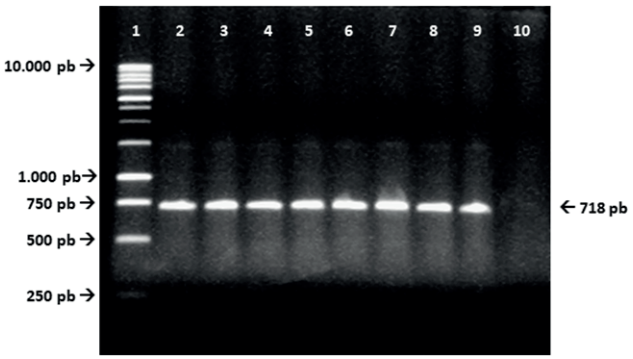


Figure 2. Electrophoretic analysis of PCR products obtained from BBTV diagnostic tests on DNA samples extracted from banana trees suspected to be infected by the virus. Lane 1:1 kb marker; lanes 2 to 9: BBTV positive samples; lane 10: negative control.

ing greatest proportions of BBTV positive samples (102 infected out of 116 samples). However, BBTV was not detected in Farm 6 and in two fields (Field 2 of Farm 4; Field 3 of Farm 10) (Table 1). The sampled farms had average temperature of 24.2°C, average monthly precipitation of 833.5 mm, and altitudes from 20 to 37 m a.s.l. Banana production in the region is predominantly of cv. 'Dwarf Cavendish' and 'Williams' (AAA genome). *Pentalonia nigronervosa* (the aphid vector of BBTV) was found only at one farm (Farm 5).

Based on the results of Kruskal-Wallis test, incidence of the BBTV was significantly different between most of the surveyed farms ($P < 0.05$). Dunn tests indicated significant differences for 28 combinations, while 23 were not significant ($P > 0.05$), with similar incidence levels (Table 2) The greatest differences in incidence ($P < 0.0001$) were observed between farms: 1 and 6, 2 and 4, 2 and 6, 2 and 8, 2 and 9, 2 and 10, and 6 and 11. The Dunn tests also showed that when Farm 6 was compared to Farms 4, 8, 9 10, there were no statistically significant differences ($P > 0.05$).

It is newsworthy to point out that Farm 6, where BBTV was not detected, was a commercial area with high productivity, in which intense control methods were used, (including roguing and establishing planted areas with suckers indexed as virus-free). On the other hand, the sampled Farms that showed highest incidences

Table 1. BBTV detection results from banana samples collected in the Chókwè district, Gaza province, Mozambique.

Farms	Field	N° of collected samples	BBTV infected samples		Altitude (m a.s.l.)	Area of banana crop (ha)	Banana cultivar
			N°	%			
Farm 1	1	10	7	70	37	<1	'Dwarf Cavendish'
	2	10	10	100	37	<1	Dwarf Cavendish
	3	6	5	83	37	<1	Dwarf Cavendish
Farm 2	1	10	10	100	37	<1	Dwarf Cavendish
	2	10	10	100	29	<1	Dwarf Cavendish
	3	6	6	100	29	<1	Dwarf Cavendish
	4	10	10	100	29	<1	Dwarf Cavendish
Farm 3	1	9	5	56	31	<1	Dwarf Cavendish
	2	10	10	100	31	<1	Dwarf Cavendish
Farm 4	1	8	4	50	37	55	Dwarf Cavendish
	2	4	0	0	37	35	Williams
Farm 5	1	10	8	80	33	2	Dwarf Cavendish
Farm 6	1	7	0	0	29	30	Williams
	2	3	0	0	29	20	Williams
Farm 7	1	3	2	67	35	2	Dwarf Cavendish
Farm 8	1	10	3	30	33	<1	Dwarf Cavendish
Farm 9	1	10	2	20	26	<1	Williams
Farm 10	1	10	4	40	26	<1	Dwarf Cavendish
	2	2	1	50	34	<1	Williams
	3	3	0	0	34	<1	Williams
Farm 11	1	10	8	80	34	<1	Dwarf Cavendish
	2	10	7	70	31	<1	Dwarf Cavendish
	3	4	4	100	20	<1	Dwarf Cavendish
Total	23	175	116	54.3			

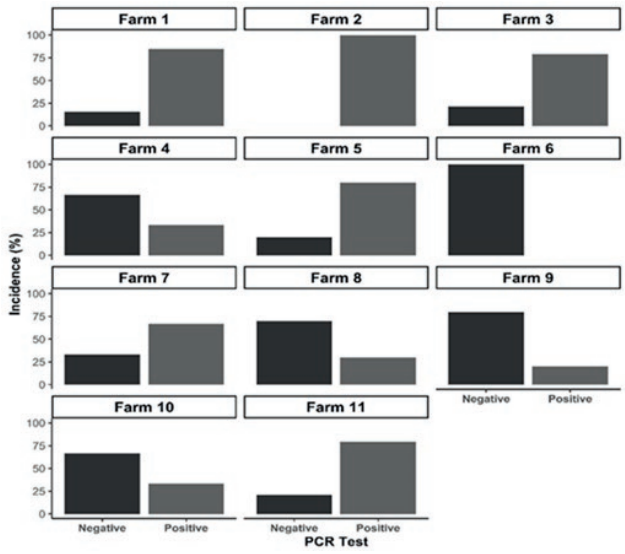


Figure 3. Average percentage of BBTV infected (positive) and healthy (negative) plants from banana trees as indicated by PCR assays for the virus in 11 farms (fields) in the Chóckwè district.

of BBTV belonged to farmers with small areas of bananas, who do not use the technologies indicated for the management of viral diseases.

DISCUSSION

Presence of BBTV in Mozambique, as shown in this study, represents a significant threat to banana cultivation in Mozambique, especially in the currently affected south region. BBTV presence was detected by PCR, using primers designed to flank the S gene region, in every sampled area, except for one commercial farm (Farm 6). On this farm, practices have been adopted to control BBTD, including appropriate roguing of affected plants and use of suckers with certified health.

Although no statistically significant differences (Dunn tests) were found when the results from Farm 6 were compared to those for Farms 4, 8, 9, and 10, BBTV was not present at Farm 6, but was detected on 4 other farms, at incidences from 20% to 30%. Therefore, the epidemiological significance of these incidences cannot be considered as equal. At Farm 6, no BBTV spread would be likely as long as the virus was not introduced. As aphids transmit and transport BBTV, incidences equal to or greater than 20% are likely to act as sources of inoculum for dissemination through whole farms in short periods. These sources would provide imminent risks for banana producers (Almeida *et al*., 2009; Dato *et al*., 2021; Qazi, 2016).

Soil and climate conditions in the Chókwè district promote favorable environments for the propagation of the vector *P. nigronervosa* and BBTV. Other studies have analyzed the climatic conditions that favor the dissemination of BBTV in banana plantations in different regions of the African continent (Ocimati *et al*., 2024; Bouwmeester *et al*., 2023), and the present study data corroborate the reported virus incidences. Greater numbers of fields infested by the aphid vector were expected, but the data were collected during January, which is the rainy season in the region. Presence of the aphid vector in only one farm probably demonstrates the negative correlation between vector and precipitation, as observed by Niyongere *et al.* (2013).

High BBTV incidence at Farms 1, 2, 3, 5, 7 and 11 could have been because these farms are in areas where

Table 2. Probabilities(indicated by Dunn tests) for positive incidence of BBTV in banana plant samples from 11 farms.

Farm	1	2	3	4	5	6	7	8	9	10	11
1	-	**0.0165***	0.6395^{ns}	**0.0019****	0.7636 ^{ns}	**<0.0001****	0.4771 ^{ns}	**0.0018****	**0.0003****	**0.0011****	0.6302 ^{ns}
2		-	**0.0049****	**<0.0001****	**0.0075****	**<0.0001****	**0.0008****	**<0.0001****	**<0.0001****	**<0.0001****	**0.0048****
3			-	**0.0134***	0.9739 ^{ns}	**<0.0001****	0.6928 ^{ns}	**0.0121***	**0.0029****	**0.0087****	1,0000 ^{ns}
4				-	**0.0357***	0.0545 ^{ns}	0.3506 ^{ns}	0.9025 ^{ns}	0.5219 ^{ns}	1.0000 ^{ns}	**0.0083****
5					-	**0.0004****	0.7290 ^{ns}	**0.0318***	**0.0101***	**0.0271***	0.9785^{ns}
6						-	**0.0104***	0.0767^{ns}	0.1675 ^{ns}	**0.0500***	**<0.0001****
7							-	0.3173 ^{ns}	0.1706 ^{ns}	0.3268 ^{ns}	0.6685 ^{ns}
8								-	0.6506 ^{ns}	0.8909 ^{ns}	**0.0076****
9									-	0.4984 ^{ns}	**0.0016****
10										-	**0.0050****
11											-

This table outlines *P* values from two-by-two comparisons of PCR test for presences of BBTV on 11 farms in Chókwè district, Gaza province, Mozambique. Statistically significant probabilities are indicated (at, respectively, $P < 0.05$ (*) or $P > 0.01$ (**).

semi-commercial fields predominate, and where only tall banana varieties are produced rather than shorter varieties (e.g. 'William'). Robson *et al.* (2006) believe that *P. nigronervosa* prefers small varieties, where it is mainly found. The rise of monoculture in these places could also favor the insect. Regardless of plant size, all banana varieties are susceptible to BBTV, although the banana genotypes with genome A are more susceptible than genotypes with genome B (Niyongere *et al*., 2011).

Low incidences of BBTV in other farms (4, 8, 9, and 10) could be because these fields contained at least two banana varieties ('William' and 'Cavendish'), and were also surrounded by other crops, especially leafy vegetables. In addition to the use of tall banana trees which are unfavorable to the vector, the aphids are important pests of vegetables grown in that region. According to Zawadneak *et al.* (2015), aphids are major pests of vegetables. However, when vegetable producers control these insects using chemical pesticides, it is likely that some sprays will drift into banana plantations in neighboring areas.

Different scenarios can be proposed for the introduction of BBTV into Mozambique. The virus may have been disseminated by aphids from countries neighboring Mozambique (Kenyon *et al*., 1997; Jooste *et al*., 2016; Shimwela *et al*., 2022). A second scenario is that vegetative banana propagation enhances virus dissemination via infected suckers. Therefore, virus incidence in the Chókwè district could be associated with some local growers acquiring virus-infected banana suckers from neighboring countries that have previously reported BBTV occurrence (EPPO, 2021).

Exchange of banana propagation material, generally common among local producers, could have caused the high BBTV incidence levels observed in Chókwè, and increased by *P. nigronervosa* transmitting the virus. This movement of infected material from one area to another may have contributed to the rise in the BBTD incidence in many places where BBTV was detected (Kumar *et al*., 2015; Djailo *et al*., 2016; Kolombia *et al*., 2021; Magee, 1927). The aphid vector is the primary source of inoculum for the dissemination over long distances, favoring spread of BBTD (Wall, 2016).

The major socio-economic factors that contribute to the dissemination of BBTD include: lack of necessary finance to buy chemical products for the control of *P. nigronervosa*; resistance from local family producers to removal of infected plant material; high workforce costs and lack of resources; time required to eradicate large numbers of plants; and the scarcity of workers to answer the seasonal plant culture requirements. These factors do not generally affect large quantity of commercial vegetable producers, as they are likely to adopt effective management strategies involving chemical control of vectors and roguing of virus-infected plants. For low-scale producers to recover productive banana yields, competent authorities are required for educating growers of the efficacy of consistent roguing and virus disease management (Omondi *et al*., 2020).

Niyongere *et al.* (2013) investigated the dissemination of BBTV from the banana crops of small farmers in Burundi, that were established using sucker from mother plants and seedlings produced in tissue cultures laboratories from different cultivars, and from areas localized at different distances from inoculum sources. They observed that at 9 months after planting, in the crops established inside areas affected with BBTD, incidence of the virus varied between 22% and 56% and was most prominent with diminishing inoculum distance. These authors observed that the BBTD incidence increased during 2007 to 2009, having varied in intensity according to banana cultivar.

Safari Murhububa *et al.* (2021), working in the Democratic Republic of the Congo, showed that banana plants infected with BBTV, independent of genotype, were attractive to wingless and winged *P. nigronervosa* that could carry a significant virus loads. Plantains, which are a triploid (AAB) *Musa paradisiaca* hybrids derived from mixing *M. acuminada* × *M. balbisiana*, have also been more attractive to aphids than dessert bananas which are strict *Musa acuminata* triploids (AAA), independent from host infection stage. Increased attraction of aphids by different varieties of bananas could be due to increased quantities of volatile organic compounds expelled by some genotypes, independent from plant growth stage. In the same study, *P. nigronervosa* preferred to settle in infected banana trees (apparently, less susceptible to BBTV) than on 'Cavendish' dessert banana (Su *et al*., 1993; Hooks *et al*., 2009). Dessert bananas are susceptible to many diseases due to their narrow genetic bases (Safari Murhububa *et al*., 2021).

Once present in banana crops, dissemination of BBTV is difficult to prevent, even with intense roguing practices, and once established, BBTD was never been eradicated from any country (Jones, 2009). However, if appropriate measures are not taken, banana cultivation is impracticable when this disease becomes established in a particular region. For improved control of BBTD, roguing must be accompanied by other practices, including monitoring of plantings and use of planting material that is free from viruses.

The present study has shown that there was wide distribution of BBTV across farms in Chókwè, highlighting the potential for BBTD to spread across all banana-producing regions in Mozambique. BBTD is circum-

scribed to the provinces of Maputo, Gaza, and Zambezia. Therefore, in addition to eliminating infected plants from the Chókwè region, development and enforcement of educational programs by extension practitioners, as well as local farmer use of indexed virus-free plants of acceptable banana varieties, are all essential for avoiding the rapid dissemination of BBTV. Data provided by this study give information on occurrence and distribution of BBTV in banana crops in Mozambique, and is background knowledge for developing BBTD management strategies actions that must be taken implemented through extension practitioners, technicians, and production activities of local banana farmers.

CONCLUSIONS

BBTV was found in 116 samples from ten farms in the Chókwè district, confirming presence this virus in more than 82% of the sampled fields. The average proportion of infection was over 54% (ranging from 20% to 100%). BBTV was absent only in the banana plantations of one farm, indicating that when BBTD control measures are carried out rigorously the disease can be controlled among other infected areas. This knowledge highlights the importance of banana plantation management for effective control of BBTV.

ACKNOWLEDGMENTS

The authors thank the Coimbra group of Brazilian universities, the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for their support in this project, as well as the Pedagogical University of Mozambique and the Ministry of Agriculture and Rural Development of Mozambique (Department of Plant Health). Assistance from the Biotechnology Laboratory of the Eduardo Mondlane University of Mozambique was provided for nucleic acid extractions from banana plant tissues.

LITERATURE CITED

Almeida R.P., Bennett G.M., Anhalt M.D., Tsai C.W., O'Grady P., 2009. Spread of an introduced vector-borne banana virus in Hawaii. *Molecular Ecology* 18(1): 136–146. https://doi.org/10.1111/j.1365-294X.2008.04009.x

Barros S.C.I.M., Figueira A.D.R., Silveira A.T.L., 2024. Molecular Characterization of Isolates of the Banana Bunchy Top Virus (BBTV) from the District of Chókwè, Mozambique. *Applied Sciences* 14(10): 4291. https://doi.org/10.3390/app14104291

Bhadra P., Agarwala B.K., 2010. A comparison of fitness characters of two host plant-based congeneric species of the banana aphid, *Pentalonia nigronervosa* and *P. caladii*. *Journal of Insect Science* 10(1): 140. https://doi.org/10.1673/031.010.14001

Bressan A., Watanabe S., 2011. Immunofluorescence localisation of *Banana bunchy top virus* (family Nanoviridae) within the aphid vector, *Pentalonia nigronervosa*, suggests a virus tropism distinct from aphid-transmitted luteoviruses. *Virus Research* 155(2): 520–525. https://doi.org/10.1016/j.virusres.2010.12.005

Bouwmeester H., Blomme G., Omondi A.B., Ocimati W., 2023. Banana bunchy top disease in Africa—predicting continent-wide disease risks by combining survey data and expert knowledge. *Plant Pathology* 72(8): 1476–1490. https://doi.org/10.1111/ppa.13764

Cooke B.M., 1998. Disease assessment and yield loss. In: *The Epidemiology of Plant Diseases* (D.G. Jones, ed.). Springer, Dordrecht, 1th ed., pp. 42–72. https://doi.org/10.1007/978-94-017-3302-1_3

Dato K.M.G., Dégbègni M.R., Atchadé M.N., Zandjanakou Tachin M., Hounkonnou M.N., Aman Omondi B., 2021. Spatial parameters associated with the risk of *banana bunchy top disease* in smallholder systems. *PLoS One* 16(12): e0260976. https://doi.org/10.1371/journal.pone.0260976

Diário Econômico, 2021. Moçambique Reforça Produção da Banana. Available at: https://www.diarioeconomico.co.mz/2021/04/05/negocios/agronegocios/mocambique-reforca-producao-da-banana/. Accessed October 5, 2021.

Djailo B.D., Lokana J., Ngama F., Nkosi B.I., Blomme G., 2016. Systemicity of *banana bunchy top viral* infection in the Kisangani region of the Democratic Republic of Congo. *African Journal of Agricultural Research* 11(7): 527–532. https://doi.org/10.5897/AJAR2015.10641

Dunn O.J., 1961. Multiple comparisons among means. *Journal of The American Statistical Association* 56(293): 52–64.

EPPO, 2016. Global Database. Banana bunchy top virus (BBTV00). Available at: https://gd.eppo.int/taxon/BBTV00/distribution. Accessed July 15, 2021.

Food and Agricultural Organization, 2018. Fighting the threats of banana bunchy top disease in Africa. Rome, Italy.

Global Invasive Species Database, 2025. 100 of the World's Worst Invasive Alien Species. Available at: http://www.iucngisd.org/gisd/100_worst.php. Accessed August 9, 2025.

Hooks C.R.R., Manandhar R., Perez E.P., Wang K.H., Almeida R.P.P., 2009. Comparative susceptibility of two banana cultivars to *Banana bunchy top virus* under laboratory and field environments. *Journal of Economic Entomology* 102(3): 897–904. https://doi.org/10.1603/029.102.0306

IPPC, 2016. Confirmation of occurrence of Banana Bunch Top Disease (BBTV) in Mozambique. Available at: https://www.ippc.int/es/countries/mozambique/pestreports/2016/12/confirmation-of-occurrence-of-banana-bunch-top-disease-bbtv-in-mozambique-1/. Accessed June 20, 2021.

Jones D.R., 2009. Disease and pest constraints to banana production. *Acta Horticulturae* 828: 21–36. https://doi.org/10.17660/ActaHortic.2009.828.1

Jooste A.E.C., Wessels N., Van der Merwe M., 2016. First Report of *Banana bunchy top virus* in Banana (*Musa* spp.) from South Africa. *Plant Disease* 100(6): 1251. https://doi.org/10.1094/PDIS-12-15-1422-PDN

Kenyon L., Brown M., Khonje P., 1997. First report of *banana bunchy top virus* in Malawi. *Plant Disease* 81(9): 1096–1096. https://doi.org/10.1094/PDIS.1997.81.9.1096A

Kolombia Y.A., Oviasuyi T., Dzola A.K., Ale Gonh-Goh A., Atsu T., Oresanya A., … Kumar P.L., 2021. First report of *banana bunchy top virus* in banana (Musa spp.) and its eradication in Togo. *Plant Disease* 105(10): 3312. https://doi.org/10.1094/PDIS-03-21-0473-PDN

Kruskal W.H., Wallis W.A., 1952. Use of ranks in one-criterion variance analysis. *Journal of The American Statistical Association* 47(260): 583–621. https://doi.org/10.1080/01621459.1952.10483441

Kumar P.L., Selvarajan R., Iskra-Caruana M.L., Chabannes M., Hanna R., 2015. Biology, etiology, and control of virus diseases of banana and plantain. *Advances in Virus Research* 91: 229–269. https://doi.org/10.1016/bs.aivir.2014.10.006

Kumar P.L., Hanna R., Alabi O.J., Soko M.M., Oben T.T., Vangu G.H.P., Naidu R.A., 2011. *Banana bunchy top virus* in sub-Saharan Africa: investigations on virus distribution and diversity. *Virus Research* 159(2): 171–182. https://doi.org/10.1016/j.virusres.2011.04.021

Lodhi M.A., Ye G.N., Weeden N.F., Reisch B.I., 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter* 12: 6–13. https://doi.org/10.1007/BF02668658

Magee C.J.P., 1927. Investigation on the bunchy top disease of bananas. *Bulletin of The Council for Scientific and Industrial Research* 30: 1-88. Retrieved June 20, 2021, from https://www.musalit.org/seeMore.php?id=12192

Mendoza N.A.P., Mendoza J.V.S., Thomas J.E., Dela Cueva F.M., 2024. Alternative hosts of banana bunchy top virus in the Philippines and the first evidence of seed transmission of BBTV. *Frontiers in Plant Science* 15: 1467331. https://doi.org/10.3389/fpls.2024.1467331

Safari Murhububa I., Tougeron K., Bragard C., Fauconnier M.L., Bisimwa Basengere E., Walangululu Masamba J., Hance T., 2021. Banana tree infected with *banana bunchy top virus* attracts *Pentalonia nigronervosa* aphids through increased volatile organic compounds emission. *Journal of Chemical Ecology* 47(8): 755–767. https://doi.org/10.1007/s10886-021-01298-3

Ngatat S., Hanna R., Lienou J., Ghogomu R.T., Nguidang S.P.K., Enoh A.C., … Kumar P. L., 2022. Musa germplasm A and B genomic composition differentially affects their susceptibility to banana bunchy top virus and its aphid vector, *Pentalonia nigronervosa*. *Plants* 11(9): 1206. https://doi.org/10.3390/plants11091206

Niyongere C., Losenge T., Ateka E.M., Ntukamazina N., Ndayiragije P., Simbare A., … Blomme G., 2013. Understanding banana bunchy top disease epidemiology in Burundi for an enhanced and integrated management approach. *Plant Pathology* 62(3): 562–570. https://doi.org/10.1111/j.1365-3059.2012.02676.x

Niyongere C., Ateka E., Losenge T., Blomme G., Lepoint P., 2011. Screening Musa genotypes for banana bunchy top disease resistance in Burundi. *Acta Horticulturae* 897: 439–447. https://doi.org/10.17660/ActaHortic.2011.897.60

Ocimati W., Ogwal G., Tazuba A.F., Kubiriba J., Tugume J., Erima R., … Blomme G., 2024. Mapping the vulnerability of banana production landscapes in Uganda to banana bunchy top disease. *Frontiers in Agronomy* 6: 1401478. https://doi.org/10.3389/fagro.2024.1401478

Omondi B.A., Soko M.M., Nduwimana I., Delano R.T., Niyongere C., Simbare A., … Staver C., 2020. The effectiveness of consistent roguing in managing banana bunchy top disease in smallholder production in Africa. *Plant Pathology* 69(9): 1754–1766. https://doi.org/10.1111/ppa.13253

Qazi J., 2016. *Banana bunchy top virus* and the bunchy top disease. *Journal of General Plant Pathology* 82: 2–11. https://doi.org/10.1007/s10327-015-0642-7

R Core Team, 2021. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical*

Computing. Available at: https://www.scirp.org/reference/referencespapers.aspx?referenceid=2631126. Accessed June 20, 2021.

Robson J.D., Wright M.G., Almeida R.P., 2006. Within-plant distribution and binomial sampling of *Pentalonia nigronervosa* (Hemiptera: Aphididae) on banana. *Journal of Economic Entomology* 99(6): 2185–2190. https://doi.org/10.1093/jee/99.6.2185

Shimwela M.M., Mahuku G., Mbanzibwa D.R., Mkamilo G., Mark D., Mosha H.I., ... Kumar P.L., 2022. First report of *banana bunchy top virus* in banana and plantain (*Musa* spp.) in Tanzania. *Plant Disease* 106(4): 1312. https://doi.org/10.1094/PDIS-07-21-1387-PDN

Soko M.M., Dale J., Kumar P.L., James A., Izquierdo L., Fiaboa K., ... Mugin J., 2009. Banana bunchy top disease survey reports, previous control efforts and the way forward. *Bvumbwe Agricultural Research Station PO Box* 5748: 171–182.

Su H.J., Wu R.Y., Tsao L.Y., 1993. Ecology of banana bunchy top virus desease. In: *Proceedings of The International Symptosium on Recent Development in Banana Cultivation Technology*. INIBAPASPNET, Los Bonos, Philippines, p. 308–312.

Thomas J.E., Gronenborn B., Harding R.M., Mandal B., Grigoras I., Randles J.W., ... ICTV Report Consortium., 2021. ICTV virus taxonomy profile: *Nanoviridae*. *Journal of General Virology* 102(3): 001544. https://doi.org/10.1099/jgv.0.001544

Wall G., 2016. *Banana bunchy top virus* prevention and control. *Western Pacific Tropical Research Center*. Available at: https://cnas-re.uog.edu/wp-content/uploads/2016/06/Banana-Bunchy-Top-Virus-Prevention-and-Control-1.pdf

Watanabe S., Greenwell A.M., Bressan A., 2013. Localization, concentration, and transmission efficiency of *Banana bunchy top virus* in four asexual lineages of *Pentalonia* aphids. *Viruses* 5(2): 758–776. https://doi.org/10.3390/v5020758

Zawadneak M.A.C., Schuber J.M., Medeiros C.S.R.A., 2015. Olericultura: pragas e inimigos naturais. *Senar* Curitiba, Paraná. Available at: https://seer.ufal.br/index.php/era/article/view/9581. Accessed June 12, 2021.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Survey for *Pyrenophora teres* and *Ramularia collo-cygni* in barley grain from Italy

Emilio BALDUCCI[1], Francesco TINI[1], Laura ROEHRIG[2], Giovanni BECCARI[1,*], Andrea ONOFRI[1], Massimo MONTANARI[3], Ilaria ALBERTI[4], Antonio PRODI[5], Neil D. HAVIS[2], Paolo BENINCASA[1], Lorenzo COVARELLI[1]

[1] *Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121, Perugia, Italy*
[2] *Scotland's Rural College (SRUC), King's Buildings Campus, West Mains Road, Edinburgh, EH9 3JG, Scotland, UK*
[3] *Council for Agricultural Research and Economics (CREA) Research Centre for Cereal and Industrial Crops, Via di Corticella 133, 40128, Bologna, Italy*
[4] *Council for Agricultural Research and Economics (CREA) Research Centre for Cereal and Industrial Crops, Via G. Amendola 82, 45100, Rovigo, Italy*
[5] *Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Viale G. Fanin 44, 40127, Bologna, Italy*
*Corresponding author. Email: giovanni.beccari@unipg.it

Summary. A 2 year study (2019/2020 and 2020/2021 growing seasons) investigated the occurrence and distribution of the barley pathogens *Pyrenophora teres* and *Ramularia collo-cygni* (*Rcc*) in grain in Italy. *Pyrenophora teres* occurs as two forms causing different host symptoms, *P. teres* f. *teres* (*Ptt*), causing net form net blotch and *P. teres* f. *maculata* (*Ptm*) causing spot form net blotch. *Ramularia collo-cygni* causes Ramularia leaf spot. *Pyrenophora teres* and *R. collo-cygni* cause significant economic damage to barley crops, and their seed-borne stages make their control challenging. Distribution of these two pathogens across different geographic regions was examined in 99 barley grain samples collected from northern, central or southern Italy, characterized by different climatic conditions. Fungal isolates from barley grains onto potato dextrose agar were identified using morphology and polymerase chain reaction (PCR) assays with species-specific primers, and amounts of fungal DNA in grain were quantified using quantitative real-time PCR (qPCR). Traditional isolation methods showed presence of *Ptt*, but did not provide information to assess *Rcc* distribution and infection levels, as no *Rcc* isolates were observed. The qPCR assays also revealed presence of *Rcc* over the two growing seasons, and amounts of *Ptt* DNA were greater than for *Rcc*, particularly in malting barley varieties in2019/2020. Presence of *Ptm* DNA within grain was detected in only two of the 99 grain samples.

Keywords. Cereals, net blotch, Ramularia leaf spot, qPCR.

OPEN ACCESS

Citation: Balducci, E., Tini, F., Roehrig, L., Beccari, G., Onofri, A., Montanari, M., Alberti, I., Prodi, A., Havis, N. D., Benincasa, P., & Covarelli, L. (2025). Survey for *Pyrenophora teres* and *Ramularia collo-cygni* in barley grain from Italy. *Phytopathologia Mediterranea* 64(2): 255-269. doi: 10.36253/phyto-15998

Accepted: August 16, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Andy Tekauz, Cereal Research Centre, Winnipeg, MB, Canada.

ORCID:
EB: 0000-0002-8656-7870
FT: 0000-0002-6427-8234
LR: 0000-0001-7743-9300
GB: 0000-0002-9227-9023
AO: 0000-0002-6603-329X
MM: 0000-0002-9891-6425
IA: 0000-0001-8803-718X
AP: 0000-0002-7221-7271
NH: 0000-0002-2824-8679
PB: 0000-0001-8502-8026
LC: 0000-0002-4568-6160

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a well-adapted cereal crop across many food grain production areas, and barley grain is extensively used for human food, animal feed and malt production (Sharma and Gujral, 2010) and has promise for use in plant-based material engineering (Puglia *et al*., 2020). In 2022, barley was one of the most widely cultivated cereals in Italy with approx. 1.2 million tons produced from 270,000 ha (FAOSTAT 2022). Italy ranks tenth among European countries for barley production (EUROSTAT 2022).

Fungal pathogens can adversely affect barley production (Retman *et al*., 2022). Breakdown of host resistance, development of pathogen insensitivity to fungicides, and increasing of pathogens previously considered of minor importance, all contribute to pathogen threats to the quantity and quality of world barley production (Laitila *et al*., 2007; Walters *et al*., 2012; Singh *et al*., 2023). Among fungal diseases, net blotch (NB), caused by *Pyrenophora teres* Drechs. (*Pt*) [Anamorph *Drechslera teres* (Sacc.) Shoemaker], is one of the most common and widespread in the world (Weibull *et al*., 2003; Liu *et al*., 2011). Similarly, Ramularia leaf spot (RLS), caused by *Ramularia collo-cygni* (Sutton et Waller) (*Rcc*), has been reported in most temperate regions, and has become an important disease of barley in Europe and South America (Walters *et al*., 2008; Havis *et al*., 2015; Matzen *et al*., 2024). *Pyrenophora teres,* previously known as *Helminthosporium teres* (Sacc.), is a pathogen that can occur in two different forms: *P. teres* f. *teres* (*Ptt*) and *P. teres* f. *maculata* (*Ptm*) (Smedegård-Petersen, 1971). The two forms are morphologically similar, and are differentiated by the symptoms they induce on host leaves. In detail, *Ptt* causes the net form of net blotch (NFNB), characterized by elongated and dark-brown lesions. Necrosis develops along leaf veins, occasionally forming transverse necrotic lesions that create net-like patterns (Liu *et al*., 2011). On the other hand, *Ptm* causes the spot form of net blotch (SFNB), as circular leaf lesions surrounded by chlorotic areas (Liu *et al*., 2011). The two forms have genetic and pathogenic differences (Campbell *et al*., 2002; Rau *et al*., 2003; Wu *et al*., 2003; Ellwood *et al*., 2019; Oğuz *et al*., 2019).

Ramularia collo-cygni, the causal agent of RLS, was first reported in Italy by Cavara (1893), but significance of RLS has only been recognized in recent decades (Sachs, 2006). This delayed recognition can be attributed to the conflation of RLS with physiological leaf spots, with other diseases, and with the rapid senescence of crops (Havis *et al*., 2015). However, typical RLS symptoms are necrotic reddish-brown square spots on leaves, visible on both sides of leaf blades and restricted by veins, and generally surrounded by chlorotic areas (Sutton and Waller, 1988; Havis *et al*., 2015). The fungus is hemibiotrophic, eliciting visible symptoms on the leaves after an asymptomatic endophytic phase (Newton *et al*., 2010), with symptoms generally appearing during final stages of crop development.

Both *Pt* and *Rcc* cause important annual yield losses in barley, and are primarily managed through chemical control (Matzen *et al*., 2024). Chemical control in integrated management is crucial, particularly in seasons when conditions favor the pathogens. Widespread resistance to major fungicide classes (QoIs, DMIs, and SDHIs) used in cereal cultivation across Europe (Sierotzki *et al*., 2007; Matusinsky *et al.,* 2010; Mair *et al.,* 2016; Rehfus *et al*., 2016; Rehfus *et al*., 2019), highlights the urgent need for preventive and integrated disease management strategies.

Both pathogens have multiple inoculum survival strategies, including overwintering on crop residues and on several grass species. These pathogens also rely on multiple mechanisms for inoculum dispersal. They spread through airborne spores, and can be seed-borne. In addition, their presence in grain can be related to the development of severe disease in seedlings which progresses to whole plants if climatic conditions are favourable (Havis *et al*., 2006; Carmona *et al*., 2008; Tini *et al.,* 2022). For this reason, a primary disease management option for farmers is use of cereal seed that is free from fungal infections (Walters *et al*., 2012).

Molecular techniques that identify and quantify pathogen DNA have increased the surveillance options for *Pt* and *Rcc*, providing tests for monitoring plant material (Bates *et al*., 2001; Matusinsky *et al*., 2011). Since cereal seed is exchanged on a global basis, and seed-borne inoculum is generally considered the primary factor for distribution of pathogens (Dussart *et al*., 2020; Backes *et al.,* 2021; Khaledi *et al*., 2024). Seed treatments with fungicides are generally considered effective for prevention of disease caused by *Pt* (McLean and Hollaway, 2019). However, their efficacy depends on several factors, including pathogen sensitivity to the fungicide, the intrinsic activity of the chemical compounds, and the uniformity of seed coverage (Reis *et al*., 2012). Seed treatments are also important for reducing presence of *Rcc*, but active ingredients employed in foliar applications have limited efficacy because this fungus is deep-seated withing barley seeds (Erreguerena *et al*., 2025).

Presence of *Pt* and *Rcc* has been reported in almost all temperate barley production areas (Walters *et al*., 2008; Backes *et al*., 2021). For this reason, consistent phytosanitory monitoring of grain is important. Recent

investigations of barley grain health in Italy have focused on presence/absence of mycotoxin-producing *Fusarium* (Morcia *et al*., 2016; Beccari *et al*., 2016; 2017; 2018). Despite the first report of *Rcc* in Italy by Cavara (1893), and records of presence of *Pt* (Rau *et al*., 2003; Tini *et al*., 2022) in grain harvested in some areas, no research has been conducted on national occurrence of these pathogens in this country.

The present study aimed to conduct a national survey of barley grain collected in the main Italian production areas during the growing seasons of 2019/2020 and 2020/2021, focusing for the first time on occurrence and distribution of *Pt* and *Rcc*. Samples were collected from the three barley production macro-areas of northern central and southern Italy. Fungal isolations from grain were carried out, and the fungal isolates exhibiting *Pt* or *Rcc* colony morphologies were confirmed through PCR assays using species-specific primers. DNA extracted from barley grain samples was then analyzed using quantitative PCR (qPCR) to detect infected grain samples and to quantify the amount of fungal DNA in each.

MATERIALS AND METHODS

Barley grain sampling

A total of 99 barley grain samples, of which 43 were collected in 2019/2020 and 56 in the 2020/2021 (hereafter designated respectively as GS1 and GS2), were assessed in the present survey. Chemically untreated grain samples (1 kg) were collected at harvest from several Italian barley production areas to cover as many of these areas as possible, also based on the availability of samples that were from individual cultivated fields. All sampled cultivars were from fields where, as usual in Italy, the crops were sown in late autumn, although some of the cultivars are classified as spring types. Among the samples, 47 were from the northern macro-area, 37 from the central area, and 15 were from the southern area (only sampled in GS2).

Southern Italy is an area mostly dedicated to durum wheat; consequently, the barley cultivation has reduced. No samples were collected from four southern regions (Molise, Campania, Basilicata and Calabria). This division into three macro sampling areas covered the varying climatic conditions across Italy, ranging from the cool wet climates of North Italy to the warmer and drier climates of South Italy (Fratianni and Acquaotta, 2017).

Tables S1 and S2 provide comprehensive details of all the analyzed samples, including places of origin, cultivars, and intended uses. Each barley grain sample was divided into two equal sub-samples of 500 g each. One sub-sample was used for fungal isolations onto potato dextrose agar (PDA), while the other sub-sample was finely ground with a laboratory blender (Retsch GM200) for qPCR analysis. Both sub-samples were stored at 4°C until the analyses were carried out.

Pathogen isolations from barley grain and molecular identification

For all samples, evaluations of the isolation incidence of *Pt* and *Rcc* were made using the methods described by Beccari *et al*. (2018). Barley grain (30 g) from each sub-sample for fungal isolation was surface sterilized using a solution containing water, ethanol (95%, Sigma Aldrich), and sodium hypochlorite (7%, Carlo Erba Reagents) (82:10:8, v/v/v) for 2 min, and then rinsed in sterile deionized water for 1 min. From each sub-sample, 100 grains were randomly selected and equally divided into ten Petri dishes (90 mm diam.) containing PDA (Bio life Italiana) supplemented with streptomycin sulphate (0.16 g L^{-1} Sigma Aldrich). These Petri dishes were then incubated at 22°C in the dark for 5 d, and each grain was then visually assessed. Prevalence of the two pathogens was assessed for each Petri dish, as the number of grains yielding particular fungi divided by the total number of grains per dish. After the morphological screening, all fungal colonies resembling *Pt* or *Rcc* were transferred onto PDA and placed at 22°C in the dark. The morphotypes for *Pt* and *Rcc* were selected based, respectively, on the descriptions of Champion (1997) and McGrann *et al*. (2017). After 14 d incubation, the colonies for each grain sample were assigned to the *Pt* or *Rcc* specific morphotypes based on their colour and shape, as shown in Figure S1. A purified mono-hyphal fungal culture for each isolate was obtained using a stereomicroscope (SZX, Olympus). This selection resulted in two representative pure cultures for each sample.

To confirm the morphological screening observations, a representative colony of the morphotype in each sample was subjected to DNA extraction (Beccari *et al*., 2017), and identified by PCR. For *Pt* resembling colonies, species-specific primers (Table 1) were used to identify the isolates and discriminate between *Ptt* and *Ptm*. As described in Poudel *et al*. (2017), the PCR protocol consisted of an initial denaturation step at 95°C for 7 min, followed by 35 cycles each of denaturation at 95°C for 30 sec, annealing at either 60°C or 62°C (primer temperatures listed in Table 1) for 30 sec, and extension at 72°C for 20 sec. The final extension step was at 72°C for 7 min. The amplified DNA fragments were visualized in a 2% agarose gel containing 500 μL L^{-1} of FireRed

(Applied Biological Materials) in TAE 1× buffer. Electrophoresis was carried out for 40 min at 100 V using an electrophoresis apparatus. A HyperLadder 100–1000 bp (Bioline Meridian Bioscience) was used to determine the sizes of the amplified fragments. DNA fragments were observed using an ultraviolet transilluminator (Uvitec Ltd) after the electrophoretic runs.

As no fungal isolates morphologically related to *Rcc* were visually identified and obtained directly from the grain, no PCR assays were carried out to identify or confirm this fungus.

Quantification of Pt *and* Rcc *in barley grain using qPCR*

Total DNA from 4 g of finely ground barley grain was extracted (Parry and Nicholson, 1996, with the modifications of Beccari *et al*., 2019). Total DNA concentrations were determined with a NanoDrop™ One© Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific) and brought to 25 ng μL^{-1} for further analysis. Subsequently, the amounts of *Ptt, Ptm* and *Rcc* DNA were determined.

Although only *Ptt* was isolated and molecularly identified among the *Pt* isolates, all samples were assessed for both forms of the pathogen using qPCR, following the protocol of Tini *et al.* (2022). To create standard curves for the qPCR assay, DNA was extracted from the *Ptt* strain PTT-S (Tini *et al*., 2022) and the *Ptm* strain PSG521 (isolated from barley leaves and molecularly identified as previously described), as well as from healthy barley grain (cv. Atomo). The qPCR reactions for standard curves were performed using the species-specific primers PttQ4-F and PttQ4-R for *Ptt*, PtmQ8-F and PtmQ8-R for *Ptm*, and Hor-1F and Hor-2R for barley (sequences shown in Table 1). DNA from fungal strains was diluted over a range from 30 ng to 0.3 pg, while DNA from barley was diluted from 100 ng to 0.1 pg (Beccari *et al*., 2019). The qPCR mix was composed of 2.5 µL of total DNA, 6 µL of SYBR® Select Master Mix CFX (Thermo Fisher Scientific), 1.5 µL of 2 µM of each primer, and 0.5 µL of sterile water (5prime) in a total reaction volume of 12.5 µL. The analyses were carried out using a CFX96 real-time PCR detection system (Bio-Rad) following the following programme: 50°C for 2 min, 95°C for 10 min, 45 cycles each of 95°C for 15 s, 61°C for 1 min, heating at 95°C for 10 s, cooling at 65°C for 5 s, and finally an increase to 95°C of 0.5°C every 5 s, with measurement of fluorescence. The standard curves were plotted on an Excel worksheet using logarithmic values of known DNA quantities and corresponding cycle threshold (Ct) values. The linear equation ($y = -mx + q$), R^2 value, and reaction efficiency ($10^{(-1/m)}$) were calculated for each curve. The sample analyses followed the same protocol described (above) for standard curve realization. Three different reactions per sample were separately carried out: one for *Ptt*, one for *Ptm* and one for barley. *Pt* biomass was expressed as the ratio of pg of fungal DNA to ng of barley DNA.

For *Rcc*, DNA extracted from grain sub-samples (Parry and Nicholson, 1996; Covarelli *et al*., 2015; Beccari *et al*., 2019) was subjected to a qPCR assay following the protocol of Taylor *et al.* (2010). Pure DNA of the *Rcc* strain SC19 (Roehrig and Havis, personal communication, 2022) was diluted to concentrations from 2 ng to 0.002 pg (10-fold dilution), and standard curves were created using the method of Beccari *et al.* (2019). The qPCR reactions were carried out using RamF6 and RamR6 species-specific primers and the molecular bea-

Table 1. Primers used in this study for PCR and qPCR assays.

Target	Primer	Sequence (5' - 3')	Annealing T(°C)	Use	Reference
Pyrenophora teres f. *teres*	PttQ4-F	CGTCCCGCCGAAATTTTGTA	60	qPCR	Poudel *et al*., 2017
	PttQ4-R	CAAGGACTTACGCGCTCAAA			
P. teres f. *teres*	PttQ6-F	TCAGAATACTCCGCGGACTC	60	PCR	
	PttQ6-R	GTCCGCCATTGTCTAGCACTC			
P. teres f. *maculata*	PttQ8-F	ACGCTAAGACCACCTCGTTT	60	qPCR	
	PttQ8-R	TCGACCAGAGAGAGCACAAA			
P. teres f. *maculata*	PttQ9-F	AATGCTCAATTCTGGTGGCG	62	PCR	
	PttQ9-R	TGTTCGAGTGCAAACTTGGG			
Ramularia collo-cygni	RamF6	CGTCATTTCACCACTCAAG	55	qPCR	Taylor *et al*., 2010
	RamR6	CCTCTGCGAATAGTTGCC			
	Ram6 (probe)	GCGATTCCGGCTGAGCGGTTCGTCATCGCG			
Barley (host)	Hor1-F	TCTCTGGGTTTGAGGGTGAC	61	qPCR	Nicolaisen *et al*., 2009
	Hor2-R	GGCCCTTGTACCAGTCAAGGT			

con probe Ram6 (Table 1). The qPCR assays were carried out in an AriaMx Real-Time PCR System (Agilent) according to the following cycle: an initial hot start of 10 min at 95°C, followed by 50 cycles each at 95°C for 20 s, 55°C for 20 s and 72°C for 20 s, and a final extension step at 95 °C for one min. As described (above) for *Pt*, the fungal biomass was expressed as the ratio of pg of fungal DNA to ng of barley DNA. Both assays were carried out in two technical replicates for each sample.

A qualitative approach was also applied to the results of these qPCR assays to establish the presence or absence of pathogen DNA in the analysed samples. This approach enabled quantification of the incidence of infected samples for each pathogen, using the following formula:

qPCR incidence value (%) = (number of pathogen positive samples / total samples analysed) × 100.

Classifying the samples based on the end-use of different barley cultivars (malt or feed), data from qPCR assays were examined to detect potential differences among the samples in quantities of fungal DNA and incidence of the two pathogens.

Statistical analyses

Incidence data for each pathogen (*Pt* isolated on PDA) were analyzed using a Generalized Linear Model (GLM) with a binomial error and a logit link. Scale parameters were added to account for over/under-dispersion (quasi-binomial model); for seasons (GS1 and GS2), and macro-areas (northern, central entre or and southern Italy), were introduced as the explanatory factors. Quantitative data (qPCR) were analyzed with a heteroscedastic linear model with GS and macro-areas as the explanatory factors and allowing for different variance per macro-area and GS (generalized least square, GLS, fitting; Pinheiro and Bates, 2000).

The qPCR incidence value data (qualitative approach) were also analyzed with a GLM with binomial error and logit link; GS, macro-areas and use were introduced as the explanatory factors. For GLM fits, back-transformed means with 'delta' standard errors were derived. Both for GLM and GLS fits, back-transformed means (for GLM fits) and means (for GLS fits) were submitted to pairwise comparisons (i.e. between different GS, macro-areas, malt or feed use) were analyzed using a generalized linear contrast testing procedure, with single-step multiplicity adjustment (Bretz *et al*., 2011). All analyses were carried out with the R statistical environment (vers. 4.2.3; R Core Team, 2023,) together with the packages 'emmeans' (Lenth, 2022).

Correlations between numbers of *Ptt* colonies isolated and amounts (pg) of *Ptt* DNA from the grain samples were assessed using the Pearson correlation coefficient (r).

RESULTS

Fungal isolations from barley grain

Fungal isolations, conducted by the direct plating of barley grains onto PDA, are summarized in Figure 1, expressed as incidence (%), considering all of Italy (TOTAL) and the three macro-areas (NORTH, CENTRE and SOUTH). These results showed presences only of *Pt,* whereas *Rcc* was not isolated with this method (Figure S1). Presence of other fungal species, such as rapidly growing *Alternaria* and *Fusarium* spp., was also observed.

All fungal colonies phenotypically identified as *Pt* were subjected to molecular identification using PCR, demonstrating detection only of *Ptt* and absence of *Ptm*.

Analysis of the season averages showed that overall incidence of samples positive for *Ptt* were not different ($P > 0.05$) between the two GS, and were less than 2%. In GS1, greatest *Ptt* incidence was detected in central Italy (greatest incidence = 17%, average = 2.1%; $P = 0.0054$), whereas in the GS2, the north region had greater presence of the pathogen (greatest incidence = 16%, average = 3.1%; $P < 0.0001$), compared to the central and southern regions where no differences were detected.

Detection of Ptt *and* Rcc *in barley grain using qPCR*

To overcome challenges associated with the direct isolation of fungi from grain, qPCR assays were used to assess occurrence of *Ptt, Ptm* and/or *Rcc* on barley grain coming from the different Italian regions. Quantification of fungal DNA in the grain is a reliable estimate of pathogen amounts within barley samples.

The coefficients of determination (R^2) for the four standard curves were 0.99 for *P. teres* f. *teres* and *R. collo-cygni*, and 0.98 for *P. teres* f. *maculata* and barley. Reaction efficiencies, calculated from the linear equations of the standard curves, were 100% for barley, 101% for *P. teres* f. *teres*, 98.2% for *P. teres* f. *maculata*, and 97.4% for *R. collo-cygni*. Dissociation curve analysis confirmed the presence of specific amplification products in pure fungal DNA (standard curves) and in DNA extracted from infected samples. No target amplifications were detected in negative controls.

Considering the total samples in the GS1, the results showed a significantly more *Ptt* DNA in grain compared

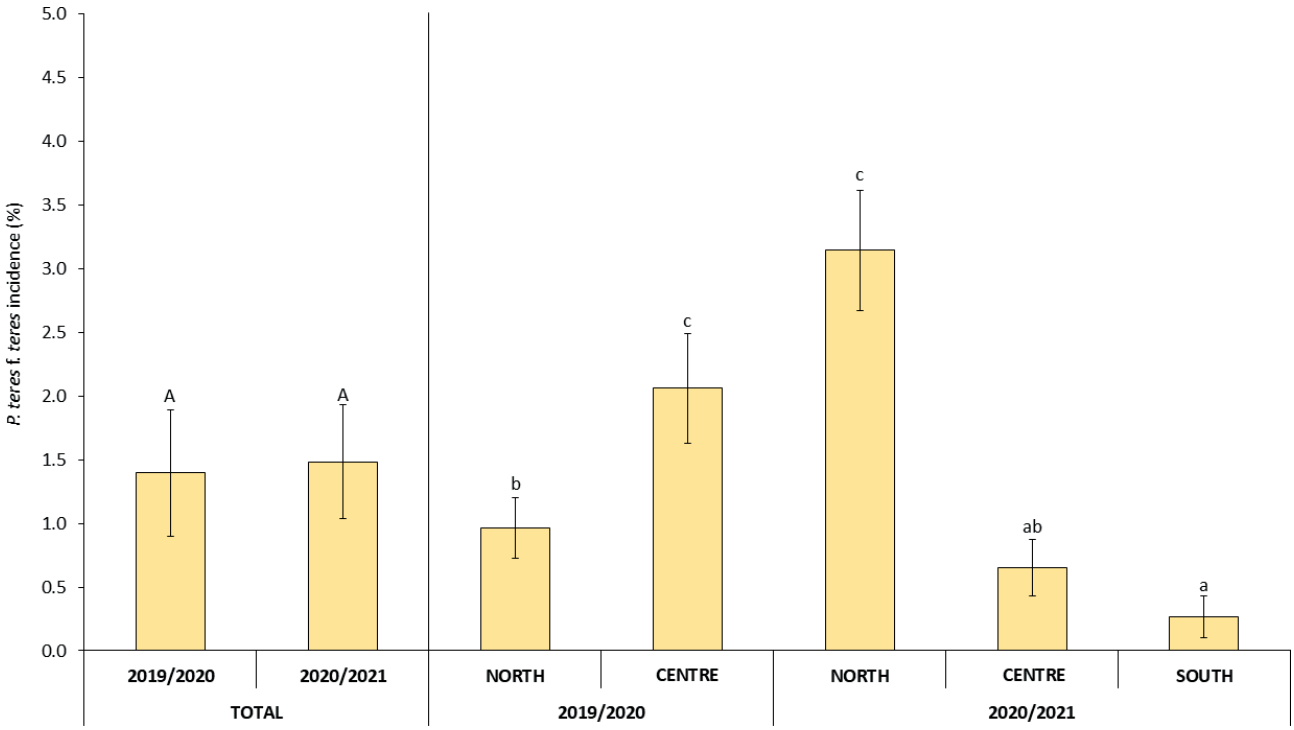


Figure 1. Mean incidences of *Pyrenophora teres* f. *teres* colonies isolated from barley grain collected from different Italian macro-areas in two growing seasons. Columns represent average incidence of *P. teres* f. *teres* (% = number of isolated fungal colonies/100 grains; ± standard error), including the total average for all samples (TOTAL) and samples from each Italian macro-area (NORTH, CENTRE or SOUTH) in the two growing seasons (the South macro-area was analysed only during the second season). Different letters indicate differences ($P \leq 0.05$) pathogen incidence between the two growing seasons (uppercase) and macro-areas (lowercase) (Tukey HSD tests).

to *Rcc* ($P = 0.0048$). For regions, samples from northern and central Italy had greater amounts of *Ptt* DNA than that of *Rcc* (Figure 2). In GS2, no statistically significant differences in DNA accumulation were detected between the two pathogens, and no significant macro-area effects were detected. However, an exception was found for samples from the North and the Centre of Italy, where greater amounts of *Rcc* DNA were detected than for southern Italy (Figure 2).

Only two samples (12.2 and 13.2; Table S2), showed presence of *Ptm* DNA, indicating presence of this pathogen in the grain. However, due to its sporadic occurrence, this form of the pathogen was excluded from further analyses and statistical comparisons.

Comparing the two growing seasons, *Ptt* DNA was generally found in greater amounts in GS1 than in GS2. In contrast, DNA amounts for *Rcc* were similar across the two GS, except for the differences observed in the central samples with greater amounts in GS2. In addition, in GS2, the general trend was similar to that of GS1. In detail, notable distinctions in samples from the North area were observed, where *Ptt* had greater (though not statistically so) accumulation than *Rcc*. Conversely, in central Italy, no differences between the two pathogens were detected in the two seasons.

For *Ptt*, it was possible to study correlations between numbers of colonies isolated on PDA and the amounts of pathogen DNA (pg) quantified in each sample, where a weak correlation ($r = 0.32$; Figure S2) was detected. Considering the difficulty of direct fungal isolations from grain, this study used qPCR assays to determine presence or absence of the pathogens in each barley grain sample. This allowed calculation of qPCR incidence, expressed as proportions (%) of positive samples for each macro-area GS1 and GS2 (Table 2). A national map of Italy was generated (Figure 3) to illustrate the distributions of each pathogen.

Incidence analysis showed no statistically significant differences in the samples from GS1. However, in GS2, *Rcc* was the most frequently detected pathogen in the analysed samples, especially in samples from the Centre of Italy (70%; Table 2). Additionally, 50% of all samples in the two GS showed no presence of *Ptt* or *Rcc* DNA, while 13% of the samples were found to be co-infected by these two pathogens.

The data were also analysed to investigate the occurrence of fungal infections in samples coming from malt

Table 2. Mean incidence (%) of *Pyrenophora teres* f. *teres* and *Ramularia collo-cygni* in different Italian macro-areas during two growing seasons [2019/2020 (GS1) and 2020/2021 (GS2)], as detected by quantitative PCR assays. In GS1, samples from the South macro-area were not collected. Different letters accompanying means indicate differences ($P \leq 0.05$) between the two growing seasons (uppercase) and the three acro-areas (lowercase) (Tukey HSD tests). SE = standard error; MCT= multiple comparison test.

Growing season	Areas	Species	Mean incidence (%)	SE	MCT
2019/2020	Total	*P. teres* f. *teres*	30.2	± 7.09	AB
		R. collo-cygni	32.6	± 7.23	AB
2020/2021	Total	*P. teres* f. teres	21.4	± 6.11	A
		R. collo-cygni	42.9	± 6.25	B
2019/2020	North	*P. teres* f. teres	30.8	± 8.79	ab
		R. collo-cygni	38.5	± 8.79	b
	Centre	*P. teres* f. teres	29.4	± 10.86	ab
		R. collo-cygni	23.5	± 10.86	ab
2020/2021	North	*P. teres* f. teres	19.1	± 9.78	ab
		R. collo-cygni	42.9	± 9.78	bc
	Centre	*P. teres* f. teres	30.0	± 10.02	ab
		R. collo-cygni	70.0	± 10.02	c
	South	*P. teres* f. teres	13.3	± 11.57	ab
		R. collo-cygni	6.7	± 11.57	a

or feed barley cultivars (Figure 4). For *Rcc*, no statistically significant differences were detected between the two GS, either for DNA amounts or incidence of infected samples.

For *Ptt*, significant differences were found in GS2. Considering all the samples, the malt barley cultivars had greater accumulation of *Ptt* DNA than the feed cultivars, although no differences were detected for incidences. Specifically, in GS2, malt samples from the northern Italy had 100% *Ptt* incidence rate (Figure 4B), and greater DNA amounts (Figure 4A) than for samples from the other two regions.

DISCUSSION

The present study aimed to investigate the presence and the distribution of *Rcc* and *Pt* in Italy by analyzing barley grain samples collected in two growing seasons from different production areas across the north, centre and south of the country characterized by distinct climatic conditions. Two diagnostic methods were used [isolations on PDA and DNA analyses (qPCR) from barley grains], which confirmed presence of *Ptt* (PDA + qPCR) and *Rcc* (qPCR only) in the different sampling Italian areas, and also showed presence of *Ptm*, although in a limited number of samples.

Previous studies have been carried out to understand the biology and epidemiology of RLS and NB, highlighting the possible ways these diseases are transmitted and the importance of the seed-borne pathogen transmission (Deadman and Cooke, 1987; Havis *et al*., 2014). For both diseases, host seeds have been shown as primary sources of inoculum, and are a key intervention point for preventing disease in crops. Havis *et al.* (2006) reported that the seed-borne nature of *Rcc* represented a major threat to barley production, as this fungus could be present in the crops when each growing season starts and before symptoms develop.

Fungal isolations directly from barley grain onto PDA showed that this method did not provide information on the distribution and infection levels of *Rcc*, as no *Rcc* isolates were obtained. Isolating *Rcc* on artificial media is challenging and labour-intensive, especially when dealing with seeds. The fungus produces small conidiospores and has slow growth on artificial media, which often leads to it being overgrown by other common saprophytes (Frei and Gindrat, 2000; Frei *et al*., 2007). The method outlined by Makepeace *et al.* (2008) remains the predominant approach for successful isolation of *Rcc* from barley leaves, but this method cannot be easily applied for isolations from seeds.

Isolation onto PDA was effective for *Ptt*. The obtained isolates were subsequently subjected to molecular analyses to confirm their identities, and determine which of the two forms of the *P. teres* was present in Italian barley seeds. These results showed that all the isolates in GS1 and GS2 were *Ptt*. Previous studies on barley leaves and grain, conducted in Italy, Finland and Algeria, have also detected high incidence of this pathogen form (Rau *et al*., 2003; Lammari *et al*., 2020; Tini *et al*., 2022). These results align with the hypothesis of one form prevailing over the other in specific geographic areas, likely due to the strong relationship between pathogen form, climatic conditions, and the barley cultivars. (Arabi *et al*., 1992; Dokhanchi *et al*., 2021; Ahmed Lhadj *et al*., 2022).

In the present study, *Ptt* was obtained from seed from all sampling areas, with no statistically significant differences between the two sampling seasons. Close analysis showed that greatest incidence was in the samples from central Italy in GS1 and northern Italy in GS2. Samples from southern Italy had the least *Ptt* infection levels. This is a preliminary indication, because grain from the southern region were only included in GS2. Consistent but low incidence of *Ptt* (< 3%) occurred in all three regions. Champion (1997) concluded that pathogens were at consistently low incidence levels in cereal seed.

The present study also evaluated fungal DNA accumulation for pathogens directly in barley grain. Pres-

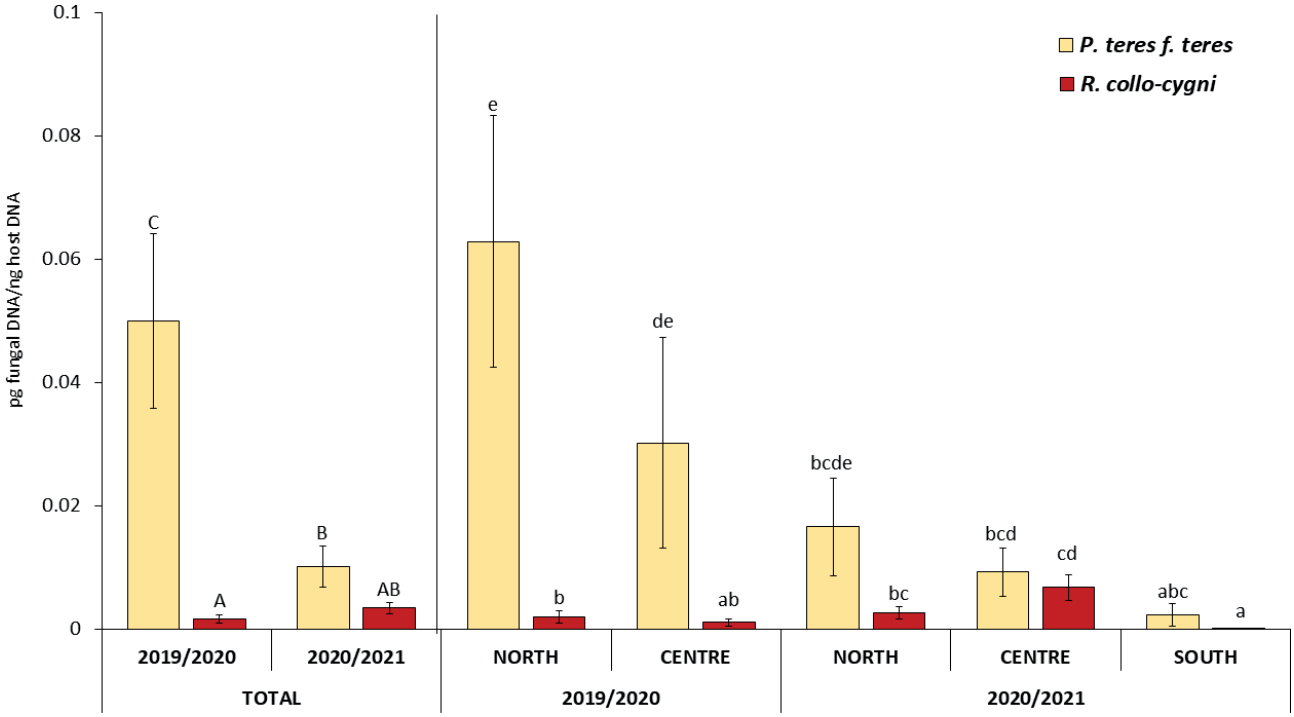


Figure 2. Mean amounts (± standard errors) of DNA of *Pyrenophora teres* f. *teres* and *Ramularia collo-cygni* in barley grain samples during two growing seasons (GS), from overall Italian samples (TOTAL) and from different Italian macro-areas (NORTH, CENTRE and SOUTH). In GS1, samples from the South macro-area were not collected. Different letters indicate differences ($P \leq 0.05$) between the TOTAL (uppercase) and different macro-areas (lowercase) sampled in the two growing seasons (Tukey HSD tests).

ence of *Rcc* in the grain samples was demonstrated using qPCR. Generally, fungal DNA was recorded at levels below 0.006 pg of fungal DNA per ng of barley DNA, with no statistically significant differences observed between the surveyed years and geographical regions. The exception was in GS2, where less *Rcc* DNA was recorded in samples from the south regions, confirming the value of molecular diagnostic tools for detecting fungal infections in grain. The results also emphasize the importance of the seed-borne stage of *Rcc,* as a major inoculum source for pathogen dissemination (Havis *et al*., 2006; Nyman *et al*., 2009; Clemente *et al*., 2014; Havis *et al*., 2015).

Without producing symptoms, *Rcc* can move from one seed generation to the next, and colonize emerging leaves of host plants (Nyman *et al*., 2009; Matusinsky *et al*., 2011). Despite the lack of significant differences in DNA levels observed in the present study, and conflicting results in the literature regarding correlation between amounts of fungal DNA in grain and severity of resulting infections, quantifying the presence of pathogen DNA in grain remains a key factor for determining pathogen occurrence distribution across specific areas (Havis *et al*., 2006; Taylor *et al*., 2010; Matusinsky *et al*., 2011). Oxley and Havis (2010) detected variations in *Rcc* contamination in grain collected during the 2006/2007 growing season in England and Scotland, and categorized contamination, as high (> 5 pg fungal DNA) or low (< 5 pg fungal DNA). This, and reports from other barley-growing regions (Matusinsky *et al*., 2011; Pereyra *et al*., 2017; Kildea *et al*., 2024) suggest that in regions where the *Rcc* is not endemic, contamination levels in grain should be kept under control. The present study has shown that in Italy *Rcc* is common in barley grain, but is present at low DNA levels.

For *Ptt*, in both seasons assessed, greater DNA amounts were recorded in samples from northern and central Italy. In the one season of sampling conducted in the southern region, low amounts of DNA were detected. *Ptt* has been identified as a pathogen significantly affecting barley crops across Europe, with a well-established correlation with the climatic conditions in northern regions (Arabi *et al*., 1992; Serenius *et al*., 2007; McLean *et al*., 2009; Jalli, 2011). The generally moist climate in North and Central Italy probably promotes *Ptt* infection and disease development. This observation aligns with other cereal grain surveys in Italy focusing on *Fusarium* and *Alternaria*, where high infection rates have been associated with moist climatic conditions (Beccari *et al*., 2020; Senatore *et al*., 2023). Differences in regional cli-

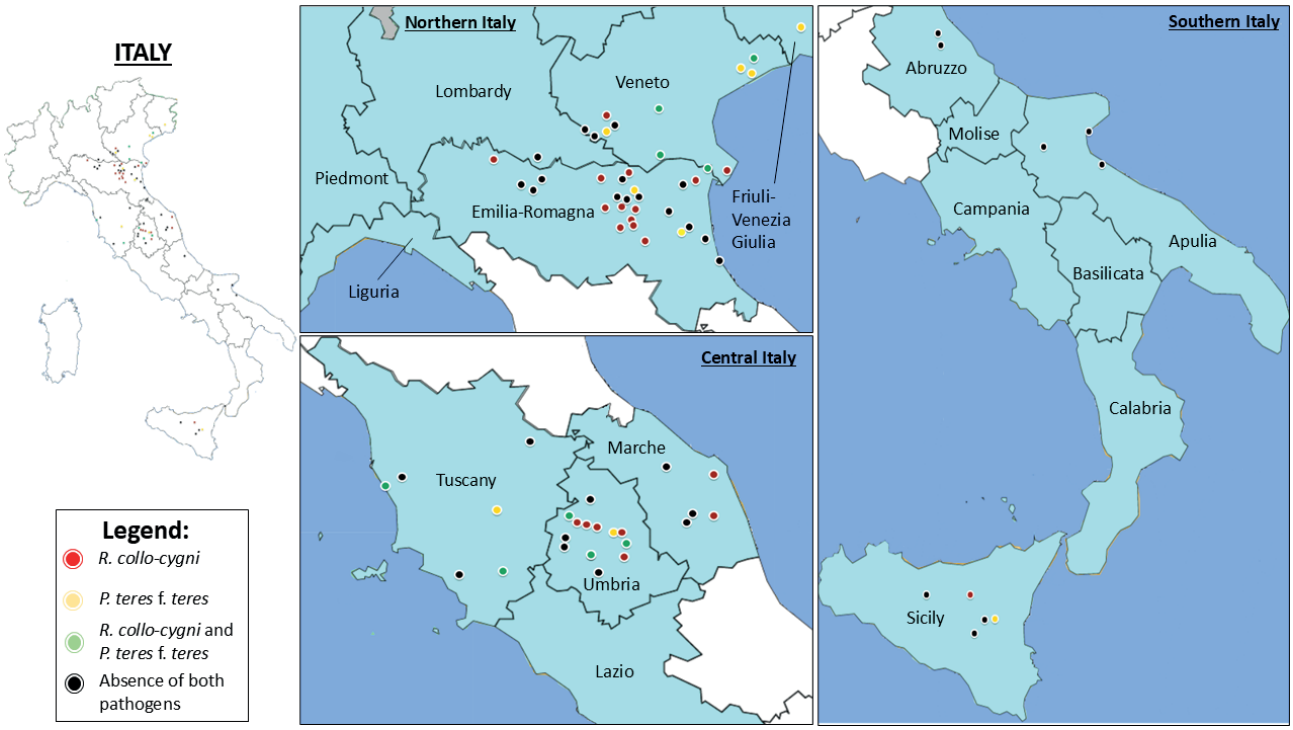


Figure 3. Maps of Italy showing distribution of *Pyrenophora teres* f. *teres* and *Ramularia collo-cygni* (and absence of both pathogens) in the 2019/2020 and 2020/2021 growing seasons (GS) as detected by quantitative PCR assays carried out on barley grain. The red dots show barley grain samples positive for *R. collo-cygni*; the light-yellow dots show samples positive for *P. teres* f. *teres*; the green dots samples positive for both fungi; the black dots show samples where the two pathogens were not detected.

mate in Italy probably account for variability in the presence of *Ptt* from year to year, but this may also be affected by use of susceptible cultivars (Bates *et al.*, 2001). Traditionally, the presence of *Ptt* is assessed on leaves. However, the present study took a novel approach, by focusing on detection of this seed-borne pathogen in grain. This shift in perspective has highlighted a critical and often overlooked stage in *Ptt* epidemiology. The present study results have shown that presence of *Ptt* in grain followed a geographical gradient from the north to south Italy, likely influenced by climatic conditions.

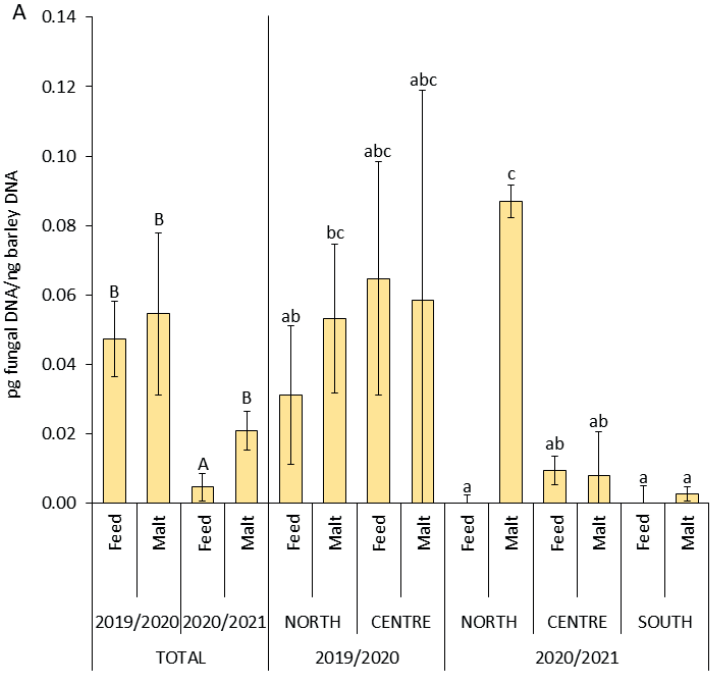
Also, for *Ptt*, fungal DNA quantification in the barley grain allowed comparison with detection on PDA. The results showed a weak correlation of r = 0.32, probably because of some samples exhibiting high variability between the two methods. Bates *et al.* (2001) also measured differences in individual samples when comparing these two detection methods.

Comparison between *Ptt* and *Rcc* DNA levels was, nevertheless, conducted, generally showing greater amounts of *Ptt* than *Rcc* across the different regions. Statistically significant differences were observed in the first year, during which the two macro-areas had greater DNA levels for *Ptt* than for *Rcc*.

Examination of DNA extracted from grain also showed presence of *Ptm* DNA in two samples from northern Italy in GS2. These results align with previous observations of coexistence of both forms in some production environments, and indicate the seed-borne nature of this form of *Pt* (McLean *et al.*, 2009; Liu *et al.*, 2011). Because of this low and sporadic detected presence, data on *Ptm* were not included in any comparisons or analyses. However, *Ptm* presence should not be disregarded, and should be monitored routinely.

Given the nearly constant DNA presence and the general difficulties encountered in direct seed isolations, the results of analyses conducted by qPCR assays were used to quantify infection incidence in the different seed samples. This allowed development of a standardized method to assess numbers of infected samples for both pathogens in all of the seed sample, providing preliminary information on occurrence and distribution of the two pathogens (qPCR incidence value). Significant differences were detected from GS2, when *Rcc* was the most widespread pathogen in the samples despite the low amounts of fungal DNA detected. Samples from central Italy had the greatest incidence of *Rcc* DNA presence (70%). In GS2, when samples from southern

A



B

Growing season	Areas	Use (n° samples)	*Ptt* incidence (%)	SE	MCT
TOTAL	2019/2020	Feed (27)	29.63	±8.955	A
		Malt (16)	18.52	±11.968	A
	2020/2021	Feed (37)	18.52	±5.698	A
		Malt (19)	25.93	±11.370	A
2019/2020	NORTH	Feed (14)	28.57	±12.073	b
		Malt (12)	33.33	±13.608	b
	CENTRE	Feed (13)	30.77	±12.801	b
		Malt (4)	25.00	±21.651	ab
2020/2021	NORTH	Feed (17)	0.00	±0.001	a
		Malt (4)	100.00	±0.003	c
	CENTRE	Feed (18)	27.78	±10.557	b
		Malt (2)	50.00	±35.355	abc
	SOUTH	Feed (2)	0.00	±0.004	a
		Malt (13)	15.39	±10.007	ab

Figure 4. Mean amounts of *Pyrenophora teres* f. *teres* DNA accumulation (A), and mean incidence of infected barley grain samples (B) across different feed and malt cultivars sampled in two growing seasons (GS1 = 2019/2020 and GS2 = 2020/2021). Columns in A represent mean DNA amounts (± standard errors) in barley samples with different end uses (feed or malt), in general (TOTAL) and within Italian macro-areas (NORTH, CENTRE or SOUTH) in both seasons. In GS1, samples from the South macro-area were not collected. The table (B) shows mean data of incidence (%) of infected samples in the two barley types, in general (TOTAL) and in the different macro-areas during the two growing seasons. Different letters indicate differences ($P \leq 0.05$) between the two surveyed seasons (uppercase) and in the individual macro-areas (lowercase) (Tukey HSD tests). SE= standard error; MCT = multiple comparison test.

Italy were included in the analysis, *Rcc* was of low incidence (6%). This may be due to the generally dry climatic conditions that are typical of southern Italy, which may have limited development of fungal infections and DNA during crop cycles. Other projects and studies (AHDB, 2018; Hoheneder *et al.,* 2021) have suggested a link between rainfall, temperature, and RLS, highlighting how prolonged drought periods create unfavourable conditions for disease, and indicate that extent and duration of leaf wetness are key factors for both *Pt* and *Rcc* outbreaks. McGrann *et al.* (2015) also showed that increased drought tolerance increased plant resistance to *Rcc*. For *Ptt*, incidence of positive samples (qPCR), at low but constant levels (< 30%) was recorded in the different macro-areas in both assessed growing seasons. In contrast to DNA quantification, the climatic conditions of the production areas did not seem to influence infection incidence. These results agree with those of Ronen *et al.* (2019), who reported no connection between pathogen infection and eco-geographical conditions in a study conducted in a Mediterranean basin area. In general, 50% of the analyzed samples were infected by one of the two pathogens (*Ptt* or *Rcc*), while 13% were infected by both fungi. These results also show that seed and grain exchanged among Italian barley growing areas is not likely to be free from infections by *Pt* and *Rcc*.

An additional analysis categorizing the samples based on their end use was carried out, to understand differences between barley cultivars. The results showed significant differences both for amounts of fungal DNA and the proportion of infected samples. For *Ptt*, generally high DNA presence and incidence were observed in malting cultivars in the second year. This trend was also slightly evident in the first season, in samples from North Italy, where malting barley cultivars had greater incidence and amounts of *Ptt* DNA than feed barley samples. Interaction between pathogens and different barley genotypes is well known (Liu *et al.*, 2011), and the present study results indicate greater susceptibility of malting barley, which usually composed of two-row cultivars. Previous studies have also observed increased susceptibility in field experiments, both for symptoms and accumulation of *Ptt* DNA in grain (Burlakoti *et al.*, 2017; Tini *et al.*, 2022). In the present study, *Rcc* appeared to be equally widespread in all samples, with no noticeable effects based on the end-use or barley type.

The present study has highlighted presence of the pathogens *Ptt* and *Rcc* in a majority of barley samples collected across Italy. The study also demonstrated the value of qPCR carried out directly on grain for this type of investigation. Despite positive interactions with seasonal and macro-area sampling factors, both pathogens were detected across the different barley growing macro-areas. *Rcc* more consistently present than *Ptt,* while *Ptt* generally had greater DNA amounts than *Rcc* especially in GS1. This indicates possible association of the pathogen with climatic conditions. Considering the increasingly restrictive EU directives regarding crop protection using pesticides, the increasing threats of fungicide resistance, and the overcoming of host varietal resistances, robust integrated disease management is essential in barley production. Routine monitoring of seed health and quality, and knowledge of interactions between pathogens, host and pedoclimatic conditions, are crucial for development of successful integrated disease management in barley crops.

ACKNOWLEDGEMENTS

The authors thank Dr Edoardo Ceccomori, Ms Maria Vittoria Consalvi, and Mr Luca Ceccarelli for their excellent technical assistance in this study.

LITERATURE CITED

AHDB, 2018. Development of UK wide risk forecast scheme for Ramularia leaf spot in barley. UK's Agriculture and Horticulture Development Board (AHDB). Available at: https://ahdb.org.uk/ramularia-leaf-spot-in-barley. Accessed October 21, 2024.

Ahmed Lhadj W., Boungab K., Righi Assia F., Çelik Oğuz A., Karakaya A., Ölmez F., 2022. Genetic diversity of *Pyrenophora teres* in Algeria. *Journal of Plant Pathology* 104(1): 305–315. https://doi.org/10.1007/s42161-021-01010-0.

Arabi M.I., Barrault G., Sarrafi A., Albertini L., 1992. Variation in the resistance of barley cultivars and in the pathogenicity of *Drechslera teres* f. sp. *maculata* and *D. teres* f. sp. *teres* isolates from France. *Plant Pathology* 41(2): 180–186. https://doi.org/10.1111/j.1365-3059.1992.tb02336.x.

Backes A., Guerriero G., Ait Barka E., Jacquard C., 2021. *Pyrenophora teres*: taxonomy, morphology, interaction with barley, and mode of control. *Frontiers in Plant Science* 12: 614951. https://doi.org/10.3389/fpls.2021.614951.

Bates J.A., Taylor E.J.A., Kenyon D.M., Thomas J.E., 2001. The application of real-time PCR to the identification, detection and quantification of *Pyrenophora* species in barley seed. *Molecular Plant Pathology* 2(1): 49–57. https://doi.org/10.1046/j.1364-3703.2001.00049.x.

Beccari G., Arellano C., Covarelli L., Tini F., Sulyok M., Cowger C., 2019. Effect of wheat infection timing on Fusarium head blight causal agents and secondary metabolites in grain. *International Journal of Food Microbiology* 290: 214–225. https://doi.org/10.1016/j.ijfoodmicro.2018.10.014.

Beccari G., Caproni L., Tini F., Uhlig S., Covarelli L., 2016. Presence of *Fusarium* species and other toxigenic fungi in malting barley and multi-mycotoxin analysis by liquid chromatography–high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry* 64(21): 4390–4399. https://doi.org/10.1021/acs.jafc.6b00702.

Beccari G., Prodi A., Senatore M.T., Balmas V., Tini F., … Covarelli L., 2020. Cultivation area affects the presence of fungal communities and secondary metabolites in Italian durum wheat grains. *Toxins* 12(2): 97. https://doi.org/10.3390/toxins12020097.

Beccari G., Prodi A., Tini F., Bonciarelli U., Onofri A., … Covarelli L., 2017. Changes in the Fusarium head blight complex of malting barley in a three-year field experiment in Italy. *Toxins* 9(4): 120. https://doi.org/10.3390/toxins9040120.

Beccari G., Senatore M.T., Tini F., Sulyok M., Covarelli L., 2018. Fungal community, Fusarium head blight complex and secondary metabolites associated with malting barley grains harvested in Umbria, central Italy. *International Journal of Food Microbiology* 273: 33–42. https://doi.org/10.1016/j.ijfoodmicro.2018.03.005.

Bretz F., Hothorn T., Westfall P., 2011. *Multiple Comparisons Using R*. CRC Press Boca Raton, FL. https://doi.org/10.1201/9781420010909.

Burlakoti R.R., Gyawali S., Chao S., Smith K.P., Horsley R.D., … Neate S.M., 2017. Genome-wide association study of spot form of net blotch resistance in the Upper Midwest barley breeding programs. *Phytopathology* 107(1): 100–108. https://doi.org/10.1094/PHYTO-03-16-0136-R.

Campbell G.F., Lucas J.A., Crous P.W., 2002. Evidence of recombination between net- and spot-type populations of *Pyrenophora teres* as determined by RAPD analysis. *Mycological Research* 106(5): 602–608. https://doi.org/10.1017/S0953756202005853.

Carmona M., Barreto D., Moschini R., Reis E.M., 2008. Epidemiology and control of seed-borne *Drechslera*

teres on barley. *Cereal Research Communications* 36: 637–645. https://doi.org/10.1556/CRC.36.2008.4.13.

Cavara F., 1893. Ueber einige parasitische pilze auf dem cetreide. *Zeitschrift für Pflanzenkrankheiten* 3(1): 16–26.

Champion R., 1997. *Identifier les Champignons Transmis par les Semences*. INRA, Paris, France, 398 pp.

Clemente G., Quintana S., Aguirre N., Rosso A., Cordi N., Havis N.D., 2014. State of art of *Ramularia collo-cygni* (leaf spot of barley) in Argentina and detection and quantification of *R. collo-cygni* by real-time PCR in barley plantlets and seeds treated with fungicide. In: *Proceedings Conference of the European Foundation for Plant Pathology* 8–13 September, 2014, Kraków, Poland, 116-117 (abstract).

Covarelli L., Beccari G., Prodi A., Generotti S., Etruschi F., ... Mañes J., 2015. *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture* 95(3): 540–551. https://doi.org/10.1002/jsfa.6772.

Deadman M. L., Cooke B.M., 1987. Effects of net blotch on growth and yield of spring barley. *Annals of Applied Biology* 110(1): 33–42. https://doi.org/10.1111/j.1744-7348.1987.tb03230.x.

Dokhanchi H., Arzanlou M., Abed-Ashtiani F., 2021. Sexual recombination and genetic diversity in Iranian populations of *Pyrenophora teres*. *Journal of Phytopathology* 169(7-8): 447–460. https://doi.org/10.1111/jph.13001.

Dussart F., Creissen H.E., Havis N.D., 2020. *Ramularia collo-cygni* - An enemy in waiting. In: *eLS*, John Wiley and Sons, Ltd (Ed.). https://doi.org/10.1002/9780470015902.a0028896.

Ellwood S.R., Piscetek V., Mair W.J., Lawrence J.A., Lopez-Ruiz F.J., Rawlinson C., 2019. Genetic variation of *Pyrenophora teres* f. *teres* isolates in Western Australia and emergence of a *Cyp51A* fungicide resistance mutation. *Plant Pathology* 68(1): 135–142. https://doi.org/10.1111/ppa.12924.

Erreguerena I.A., Quiroz F.J., Cambareri M., Pereyra S., Havis N.D., Carmona M.A., 2025. Assessing the impact of Ramularia leaf spot on barley: prospects for fungicide protection strategies and weather-based prediction models in Argentina. *Plant Pathology* 74(3): 858–872.

EUROSTAT, 2022. Statistical office of the European Communities (EUROSTAT). Available at: https://ec.europa.eu/eurostat/databrowser/view/tag00051/default/table?lang=en&category=t_agr.t_apro.t_apro_cp. Accessed January 4, 2024.

FAOSTAT, 2022. Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). Available at: https://www.fao.org/faostat/en/#data/QCL. Accessed January 4, 2024.

Fratianni S., Acquaotta F., 2017. The climate of Italy. In: *Landscapes and Landforms of Italy* (M. Soldati, M. Marchetti, ed.). *World Geomorphological Landscapes*. Springer Cham. https://doi.org/10.1007/978-3-319-26194-2_4.

Frei P., Gindrat D., 2000. Le champignon *Ramularia collo-cygni* provoque une forme de grillures sur les feuilles d'orge d'automne et de graminées adventices. *Revue Suisse d'Agriculture* 32: 119–125.

Frei P., Gindro K., Richter H., Schürch S., 2007. Direct-PCR detection and epidemiology of *Ramularia collo-cygni* associated with barley necrotic leaf spots. *Journal of Phytopathology* 155(5): 281–288. https://doi.org/10.1111/j.1439-0434.2007.01228.x.

Havis N.D., Brown J.K.M., Clemente G., Frei P., Jedryczka M., ... Hess M., 2015. *Ramularia collo-cygni*-An emerging pathogen of barley crops. *Phytopathology* 105(7): 895–904. https://doi.org/10.1094/PHYTO-11-14-0337-FI.

Havis N.D., Nyman M., Oxley S.J.P., 2014. Evidence for seed transmission and symptomless growth of *Ramularia collo-cygni* in barley (*Hordeum vulgare*). *Plant Pathology* 63(4): 929–936. https://doi.org/10.1111/ppa.12162.

Havis N.D., Oxley S.J.P., Piper S.R., Langrell S.R.H., 2006. Rapid nested PCR-based detection of *Ramularia collo-cygni* direct from barley: rapid nested PCR-based detection direct from barley. *FEMS Microbiology Letters* 256(2): 217–223. https://doi.org/10.1111/j.1574-6968.2006.00121.x.

Hoheneder F., Hofer K., Groth J., Herz M., Heß M., Hückelhoven R., 2021. *Ramularia* leaf spot disease of barley is highly host genotype-dependent and suppressed by continuous drought stress in the field. *Journal of Plant Diseases and Protection* 128(3): 749–767. https://doi.org/10.1007/s41348-020-00420-z.

Jalli M., 2011. Sexual reproduction and soil tillage effects on virulence of *Pyrenophora teres* in Finland. *Annals of Applied Biology* 158(1): 95–105. https://doi.org/10.1111/j.1744-7348.2010.00445.x.

Khaledi N., Zare L., Hassani F., Osroosh S., 2024. Comparison of diagnostic methods, virulence and aggressiveness analysis of *Pyrenophora* spp. in pre-basic seeds in the barley fields. *Tropical Plant Pathology* 49: 304–316. https://doi.org/10.1007/s40858-023-00631-3

Kildea S., Mulhare J., Zia R., Hutton F., Creissen H., 2024. Presence and prevalence of *Ramularia collo-cygni* SDHI resistance in Irish barley seed. *Journal of Plant Diseases and Protection* 131: 1233–1238.

Laitila A., Kotaviita E., Peltola P., Home S., Wilhelmson A., 2007. Indigenous microbial community of barley greatly influences grain germination and malt quality. *Journal of the Institute of Brewing* 113(1): 9–20. https://doi.org/10.1002/j.2050-0416.2007.tb00250.x.

Lammari H.I., Rehfus A., Stammler G., Fellahi Z.E.A., Benbelkacem A., Benslimane H., 2020. Occurrence and frequency of spot form and net form of net blotch disease of barley in Algeria. *Journal of Plant Diseases and Protection* 127(1): 35–42. https://doi.org/10.1007/s41348-019-00278-w.

Lenth R., 2022. *Emmeans: Estimated Marginal Means, aka Least-Squares Means.* R package version 1.7.4-9990003. Available at: https://github.com/rvlenth/emmeans. Accessed December 28, 2024.

Liu Z., Ellwood S.R., Oliver R.P., Friesen T.L., 2011. *Pyrenophora teres*: Profile of an increasingly damaging barley pathogen. *Molecular Plant Pathology* 12(1): 1–19. https://doi.org/10.1111/j.1364-3703.2010.00649.x

Makepeace J.C., Havis N.D., Burke J.I., Oxley S.J.P., Brown J.K.M., 2008. A method of inoculating barley seedlings with *Ramularia collo-cygni*. *Plant Pathology* 57: 991–999. https://doi.org/10.1111/j.1365-3059.2008.01892.x.

Mair W.J., Deng W., Mullins J.G., West S., Wang P., ... Lopez-Ruiz F.J., 2016. Demethylase inhibitor fungicide resistance in *Pyrenophora teres* f. sp. *teres* associated with target site modification and inducible overexpression of Cyp51. *Frontiers in Microbiology* 7, 1279. https://doi.org/10.3389/fmicb.2016.01279.

Matusinsky P., Svobodova-Leisova L., Gubis J., Hudcovicova M., Klcova L., ... Minarikova V., 2011. Impact of the seed-borne stage of *Ramularia collo-cygni* in barley seed. *Journal of Plant Pathology* 93(3): 679–689.

Matusinsky P., Svobodova-Leisova L., Mariks P., Tvaruzek L., Stemberkova L., ... Spitzer T., 2010. Frequency of a mutant allele of cytochrome b conferring resistance to Qol fungicides in the Czech population of *Ramularia collo-cygni*. *Journal of Plant Diseases and Protection* 117, 248–252. https://doi.org/10.1007/BF03356369.

Matzen N., Weigand S., Bataille C., Kildea S., Havis N., ... Jørgensen L.N., 2024. EuroBarley: control of leaf diseases in barley across Europe. *Journal of Plant Diseases and Protection* 131: 1239–1244. https://doi.org/10.1007/s41348-023-00852-3.

McGrann G.R.D., Havis N.D., 2017. Ramularia leaf spot: a newly important threat to barley production. *Outlooks on Pest Management* 28: 65–69. https://doi.org/10.1564/v28_apr_05.

McGrann G.R.D., Steed A., Burt C., Goddard R., Lachaux C., ... Brown J.K.M., 2015. Contribution of the drought tolerance-related Stress-responsive NAC1 transcription factor to resistance of barley to Ramularia leaf spot. *Molecular Plant Pathology* 16(2): 201–209. https://doi.org/10.1111/mpp.12173.

McLean M.S., Howlett B.J., Hollaway G.J., 2009. Epidemiology and control of spot form of net blotch (*Pyrenophora teres* f. *maculata*) of barley: A review. *Crop and Pasture Science* 60(4): 303. https://doi.org/10.1071/CP08173.

McLean M.S., Hollaway G.J., 2019. Control of net form of net blotch in barley from seed-and foliar-applied fungicides. *Crop and Pasture Science* 70: 55–60. https://doi.org/10.1071/CP18142.

Morcia C., Tumino G., Ghizzoni R., Badeck F.W., Lattanzio V.M.T., ... Terzi V., 2016. Occurrence of *Fusarium langsethiae* and T-2 and HT-2 Toxins in Italian Malting Barley. *Toxins* 8(8): 247. https://doi.org/10.3390/toxins8080247.

Newton A.C., Fitt B.D.L., Atkins S.D., Walters D.R., Daniell T.J., 2010. Pathogenesis, parasitism and mutualism in the trophic space of microbe–plant interactions. *Trends in Microbiology* 18(8): 365–373. https://doi.org/10.1016/j.tim.2010.06.002.

Nicolaisen M., Suproniene S., Nielsen L.K., Lazzaro I., Spliid N.H., Justesen A.F., 2009. Real-time PCR for quantification of eleven individual *Fusarium* species in cereals. *Journal of Microbiological Methods* 76(3): 234–240. https://doi.org/10.1016/j.mimet.2008.10.016.

Nyman M., Havis N.D., Oxley S.J.P., 2009. Importance of seed-borne infection of *Ramularia collo-cygni*. *Aspects of Applied Biology* 92: 91–96.

Oğuz A.Ç., Ölmez F., Karakaya A., 2019. Genetic diversity of Net Blotch Pathogens of barley in Turkey. *International Journal of Agriculture and Biology* 21: 1089–1096.

Oxley S.J.P., Havis N.D., 2010. Managing *Ramularia collo-cygni* through varietal resistance, seed health and forecasting. HGCA Project report (463).

Parry D.W., Nicholson P., 1996. Development of a PCR assay to detect *Fusarium poae* in wheat. *Plant Pathology* 45(2): 383–391. https://doi.org/10.1046/j.1365-3059.1996.d01-133.x.

Pereyra S., Erreguerena I., Couretot L., Pérez C., Palladino C., Havis N. D., 2017. Upsurge of Ramularia leaf spot in South America. In: *International Workshop on Barley Leaf Diseases*, 2o. Rabat, Morocco: The International Center for Agricultural Research in the Dry Areas (ICARDA), April 5–7, 2017.

Pinheiro J., Bates D., 2000. Mixed-Effect Models in S and S-plus. In: *Journal of The American Statistical Asso-*

ciation Vol. 96. https://doi.org/10.1007/978-1-4419-0318-1.

Poudel B., Ellwood S.R., Testa A.C., McLean M., Sutherland M.W., Martin A., 2017. Rare *Pyrenophora teres* Hybridization Events Revealed by Development of Sequence-Specific PCR Markers. *Phytopathology* 107(7): 878–884. https://doi.org/10.1094/PHYTO-11-16-0396-R.

Puglia D., Luzi F., Lilli M., Sbardella F., Pauselli M., ... Benincasa P., 2020. Straw fibres from barley hybrid lines and their reinforcement effect in polypropylene-based composites. *Industrial Crops & Products* 154: 112736. https://doi.org/10.1016/j.indcrop.2020.112736.

R Core Team, 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/. Accessed December 28, 2023.

Rau D., Brown A.H.D., Brubaker C.L., Attene G., Balmas V., ... Papa R., 2003. Population genetic structure of *Pyrenophora teres* Drechs. The causal agent of net blotch in Sardinian landraces of barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 106(5): 947–959. https://doi.org/10.1007/s00122-002-1173-0.

Rehfus A., Matusinsky P., Strobel D., Bryson R., Stammler G., 2019. Mutations in target genes of succinate dehydrogenase inhibitors and demethylation inhibitors in *Ramularia collo-cygni*. *Journal of Plant Diseases and Protection* 126: 447–459. https://doi.org/10.1007/s41348-019-00246-4.

Rehfus A., Miessner S., Achenbach J., Strobel D., Bryson R., Stammler G., 2016. Emergence of succinate dehydrogenase inhibitor resistance of *Pyrenophora teres* in Europe. *Pest Management Science* 72: 1977–1988. https://doi.org/10.1002/ps.4244.

Reis E.M., Danelli A., Casa R.T., 2012. Fungicides, seed dresser adjuvants and storage time in the control of *Drechslera teres* in barley seeds. *Summa Phytopathologica* 38: 187–191. https://doi.org/10.1590/S0100-54052012000300001.

Retman S., Melnichuk F., Kyslykh T., Shevchuk O., 2022. Complex of barley leaf spots in Ukraine. *Chemistry Proceedings* 10(1). https://doi.org/10.3390/IOCAG2022-12290.

Ronen M., Sela H., Fridman E., Perl-Treves R., Kopahnke D., ... Harel A., 2019. Characterization of the Barley Net Blotch Pathosystem at the Center of Origin of Host and Pathogen. *Pathogens* 8(4): 275. https://doi.org/10.3390/pathogens8040275.

Sachs E, 2006. The history of research into Ramularia leaf spot on barley. *Proceedings of the 1st European Ramularia Workshop,* pp 9-15, Gottingen, Germany.

Senatore M.T., Prodi A., Tini F., Balmas V., Infantino A., ... Beccari G., 2023. Different diagnostic approaches for the characterization of the fungal community and *Fusarium* species complex composition of Italian durum wheat grain and correlation with secondary metabolite accumulation. *Journal of the Science of Food and Agriculture* 103(9): 4503–4521. https://doi.org/10.1002/jsfa.12526.

Serenius M., Manninen O., Wallwork H., Williams K., 2007. Genetic differentiation in *Pyrenophora teres* populations measured with AFLP markers. *Mycological Research* 111: 213–223. https://doi.org/10.1016/j.mycres.2006.11.009.

Sharma P., Gujral H.S., 2010. Milling behavior of hulled barley and its thermal and pasting properties. *Journal of Food Engineering* 97(3): 329–334. https://doi.org/10.1016/j.jfoodeng.2009.10.025 .

Sierotzki H., Frey R., Wullschleger J., Palermo S., Karlin S., ... Gisi U., 2007. Cytochrome b gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. *Pest Management. Science* 63: 225–233. doi: 10.1002/ps.1330

Singh B.K., Delgado-Baquerizo M., Egidi E., Guirado E., Leach J. E., ... Trivedi P., 2023. Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology* 21: 640–656. https://doi.org/10.1038/s41579-023-00900-7.

Smedegård-Petersen V., 1971. *Pyrenophora teres* f. *maculata* f. nov. and *Pyrenophora teres* f. *teres* on barley in Denmark. In: *Årsskrift, Kongelige Veterinær- og Landbohøjskole* 124, 144.

Sutton B.C., Waller J.M., 1988. Taxonomy of *Ophiocladium hordei*, causing leaf lesions on Triticale and other *Gramineae*. *Transactions of the British Mycological Society* 90(1): 55–61. https://doi.org/10.1016/S0007-1536(88)80180-3.

Taylor J.M.G., Paterson L.J., Havis N.D., 2010. A quantitative real-time PCR assay for the detection of *Ramularia collo-cygni* from barley (*Hordeum vulgare*). *Letters in Applied Microbiology* 50(5): 493–499. https://doi.org/10.1111/j.1472-765X.2010.02826.x.

Tini F., Covarelli L., Ricci G., Balducci E., Orfei M., Beccari G., 2022. Management of *Pyrenophora teres* f. *teres*, the causal agent of net form net blotch of barley, in a two-year field experiment in central Italy. *Pathogens* 11(3): 291. https://doi.org/10.3390/pathogens11030291.

Walters D.R., Avrova A., Bingham I.J., Burnett F.J., Fountaine J., Havis N.D., ... Newton A.C., 2012. Control of foliar diseases in barley: towards an integrated approach. *European Journal of Plant Pathology* 133(1): 33–73. https://doi.org/10.1007/s10658-012-9948-x.

Walters D.R., Havis N.D., Oxley S.J.P., 2008. *Ramularia collo-cygni*: The biology of an emerging pathogen of barley. *FEMS Microbiology Letters* 279(1): 1–7. https://doi.org/10.1111/j.1574-6968.2007.00986.x.

Weibull J., Walther U., Sato K., Habekuß A., Kopahnke D., Proeseler G., 2003. Chapter 8-Diversity in resistance to biotic stresses. In: *Developments in Plant Genetics and Breeding* (R. von Bothmer, T. van Hintum, H. Knüpffer, K. Sato, ed.), Elsevier, Denmark, Europe, 7, 143–178. https://doi.org/10.1016/S0168-7972(03)80010-5.

Wu H.L., Steffenson B.J., Zhong S., Li Y., Oleson A.E., 2003. Genetic variation for virulence and RFLP markers in *Pyrenophora teres*. *Canadian Journal of Plant Pathology* 25(1): 82–90. https://doi.org/10.1080/07060660309507052.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Pseudomonas putida has potential for biological control of bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*

Gizem ERYİGİT[1], Hatice OZAKTAN[1]*, Utku SANVER[2]

[1] *Ege University Faculty of Agriculture Department of Plant Protection, İzmir, Türkiye*
[2] *Siirt University Faculty of Agriculture Department of Plant Protection, Siirt, Türkiye*
*Corresponding author. Email: hatice.ozaktan@ege.edu.tr

Summary. Bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*, is a serious disease that causes yield and quality losses. There has been increased focus on biological control agents as alternatives to chemical pesticides in plant disease management. In this study, 313 endophyte and epiphyte bacterial isolates, from tomato plants sampled from different locations in Turkey, were assessed for their potential for plant growth promotion and biocontrol efficacy against *X. euvesicatoria*. Results obtained from *in vitro* assays were evaluated using the weighted ranking method, and 15 isolates were selected for *in planta* biocontrol evaluation against *X. euvesicatoria*. In efficacy tests, bacteria were introduced into tomato plants by biopriming of seeds or by spraying whole plants. The two most effective isolates reduced bacterial spot by 40–45% after seed biopriming, and 30–41% from shoot application, compared to the non-treated experimental controls. Sequence analysis using 16S rRNA primers identified one representative isolate (coded KD 91/1) as *Pseudomonas putida*. Tomato plants bioprimed with KD 91/1 through seed treatment had greatest biomass compared to that for the other tested bacteria. The population of *P. putida* KD 91/1 in tomato tissues after pathogen inoculation was approx. 7.2×10^4 cfu g^{-1} in shoots and 1×10^5 cfu g^{-1} in roots. This study indicates that antagonistic *P. putida* isolates are promising candidates for biological control of *X. euvesicatoria*.

Keywords. Plant growth promotion, antagonistic bacteria, endophytes, biocontrol agents.

Citation: Eryiğit, G., Ozaktan, H., & Sanver, U. (2025). *Pseudomonas putida* has potential for biological control of bacterial spot of tomato, caused by *Xanthomonas euvesicatoria*. *Phytopathologia Mediterranea* 64(2): 271-284. doi: 10.36253/phyto-15347

Accepted: August 18, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Ali Siah, UMR 1158 BioEcoAgro, Yncréa HdF, Lille, France.

ORCID:
GE: 0000-0001-6161-5285
HO: 0000-0001-9971-6508
US: 0000-0001-5373-2924

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important edible vegetable, with a 42.3% share of vegetable market and production of 13.2 million tons in Turkey, (FAO, 2022). Tomato production is affected by biotic factors that cause yield and quality losses, and bacterial diseases are important contributors to economic losses. Tomato bacterial spot, caused by *Xanthomonas* spp. (*X. perforans*, *X. gardneri, X. euvesicatoria* and *X. vesicatoria*), is a serious disease that impacts tomato production in many countries (Sharma and Bhattarai, 2019). This disease can cause up to 50% crop losses in

tomatoes, and prevents marketability of fresh tomatoes (Balogh *et al*., 2003).

Symptoms of bacterial spot can be observed on tomato plant leaves, stems, and fruits. The lesions are initially green then turn brown, and are irregular necrotic spots often surrounded by large chlorotic areas. Combined large lesions appear as leaf blight, and later, defoliation may occur. Necrotic lesions and cracks can occur along stems. Fruit lesions are first small with scalded appearance in water, then enlarge and become brown and scab-like rough spots. Fruit lesions can facilitate secondary fruit infections caused by entry of fungi and bacteria (Ritchie, 2000).

Conventional methods for controlling bacterial spot of tomato include application of copper-based pesticides and use of resistant varieties (Sahin and Miller, 1998). However, tomato breeding efforts involving wild lines and varieties resistant to bacterial spot have largely been unsuccessful, and there are no available tomato varieties with resistance to this disease (Sharma and Bhattarai, 2019). Efficacy of copper preparations used against the disease is rapidly decreasing due to pathogen evolution (Martin *et al*., 2004; Abbasi *et al*., 2015), and use of chemical pesticides for disease control has posed environmental hazards and caused accumulation of potentially toxic substances in human food chains.

Therefore, use of chemical pesticides for crop disease management should be minimized, and to achieve this, plant protection alternatives, such as biocontrol, must be identified (Pertot *et al*., 2016). Beneficial bacteria can directly enhance of plant growth, mitigate biotic and abiotic stresses (Khan *et al*., 2016; Tiwari *et al*., 2016), provide nutrients to agricultural crops, and stimulate plant growth by producing phytohormones, improving soil structure, bioaccumulation of inorganic compounds, and bioremediation (Mahmood *et al*., 2016). Beneficial bacteria are used in agricultural crops for biocontrol of plant pathogens through various mechanisms. Microbial biological control agents interact with plants through direct mechanisms, such as competing with targeted pathogens for ecological niches or substrates, or by producing antimicrobial metabolites that inhibit pathogen development. These agents may also exert indirect effects by inducing resistance in host plants against a broad spectrum of pathogens (Compant *et al*., 2005; Köhl *et al*., 2019).

El-Hendawy *et al*. (2005) demonstrated that tomato leaf, root, soil, and seed treatments with *Rahnella aquatilis* reduced severity of bacterial spot caused by *X. campestris* pv. *vesicatoria*. (Mirik *et al*., 2008) also showed that three *Bacillus* sp. solates (designated M1-3, M3-1 and H8-8) from rhizospheres of greenhouse and field-grown pepper plants, reduced disease caused by *X. axanopodis* pv. *vesicatoria* by 11 to 62% in greenhouse-grown pepper plants, and by 38 to 67% in field-grown plants. In addition (Shrestha *et al*., 2014) reported that lactic acid bacteria reduced bacterial spot of pepper by 57-73% under greenhouse conditions and 70–94% in the field. (Pajčin *et al*., 2020) showed that *Bacillus velezensis* IP22, isolated from fresh cheese and grown in a laboratory-scale bioreactor, suppressed pepper plant symptoms caused by *X. euvesicatoria*.

The present study aimed to assess endophyte and epiphyte bacteria from healthy tomato plants for biocontrol potential against *X. euvesicatoria* spot of tomato. bacteria associated with healthy tomato plants were isolated and characterized, and isolates were assessed for disease reduction and plant growth promotion potential. Selected isolates that showed were then assessed for tomato growth enhancement and biocontrol potential *in planta*. Identification of one of these isolates was determined using gene barcoding analysis.

MATERIALS AND METHODS

Plant material, and isolations of bacteria

Epiphytic and endophytic bacteria were isolated from root, stem, and leaf samples from 127 healty tomato plants collected from 40 different locations in the Izmir, Manisa, and Aydın provinces of Turkey.

Endophytic bacteria were isolated from internal tissues of roots, leaves, and stems of the healthy plants, following tissue surface sterilization with sequential immersion in 70% ethanol for 5 min, then 5% sodium hypochlorite solution for 10 min, followed by three rinses with sterile distilled water. Bacterial isolates were obtained using two techniques: either triturating leaves or imprinting stem and root tissues onto tryptic soy agar (TSA). A tissue surface sterility test was carried out on each sample to verify the elimination of surface microorganisms. In cases where no bacterial growth occurred in the sterility test, the isolated bacteria were classified as endophytes (Nejad and Johnson, 2000).

Epiphytic bacteria were isolated from the surfaces of roots, leaves, and stems of healthy tomato plants, and were then each suspended in 100 mL of phosphate buffer. Following extraction on a rotary shaker for 30 min at 120 rpm, ten-fold serial dilutions (10^{-1} to 10^{-3}) were prepared, and 0.1 mL of each dilution was spread onto triplicate TSA plates. The plates were then incubated at 24°C for 48 h (Akköprü and Ozaktan, 2018). The isolates obtained were then preserved in nutrient broth plus 20% glycerol at at -86°C.

A highly aggressive strain of *Xanthomonas euvesicatoria* strain183 (*X. euvesicatoria*) was obtained from the Bacteriology Laboratory of the Department of Plant Protection at Ege University, İzmir, Türkiye. This strain is known to cause bacterial leaf spot on tomatoes.

Characterization of bacterial isolates

Hydrogen cyanide (HCN) production from glycine was assessed by culturing bacteria on 10% tryptic soy agar (TSA) supplemented with glycine (4.4 g L^{-1}). Cyanogenesis was detected using 0.5% picric acid and 2% sodium carbonate (Na_2CO_3), (Bakker and Schippers, 1987). Filter paper impregnated with the reagents was affixed to the underside of Petri dish lids, and results were evaluated after 5 d incubation at 24±2°C. Colour change in the filter paper from yellow to orange-brown indicated the production of HCN. Indole acetic acid (IAA) production was quantified as described by Bric *et al.* (1991). Individual isolates were cultivated in their respective media supplemented with 100 µg mL^{-1} of L-tryptophan at 30°C for 48 h. Following cultivation, the cultures were centrifuged at 8000 rpm for 10 min. Supernatant (2 ml) from each culture was combined with 4 mL of Salkowski reagent (composed of 150 mL of concentrated H_2SO_4, 7.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$, and 250 mL of distilled water). The development of a pink colour indicated IAA production. Siderophore production was assessed using Chrome Azurol S (CAS) blue supplemented agar in Petri plates, as described by Klement *et al.* (1990). Isolates were cultivated on TSA medium for 24 h at 24±2 °C, then suspended in sterile water to achieve an optical density (OD_{600}) of 0.1. A 2 µL aliquot of each culture was inoculated onto CAS-blue agar plates. The plates were incubated for 48 h at 24±2 °C and observed daily for formation of yellow-orange haloes around colonies, with presence of a yellow-orange halo indicating siderophore production, and halo diameter was measured. Activities of 1-aminocyclopropane-1-carboxylate (ACC) deaminase for bacterial isolates were screened on sterile minimal DF salt media, utilizing ACC as the sole nitrogen source. DF salt media were prepared as described by Saravanakumar and Samiyappan (2007). A 2 µL aliquot of each culture was inoculated into the DF media, the plates were incubated for 72 h at 24±2 °C, and growth of bacterial colonies was observed. Growth on DF medium was interpreted as indicative of ACC deaminase positivity. Isolates were also screened on NBRIP agar plates for capacity to solubilize inorganic phosphate (Nautiyal, 1999). Bacterial cultures were inoculated at the centres of agar plates, which were then incubated for 3 d at 30°C. Presence of clear zones (halo zones) surrounding colonies was recorded, indicating phosphate solubilization ability. Antibiosis activity against *X. euvesicatoria* was assessed as outlined by Jetiyanon and Kloepper (2002).

In vitro *activity of isolates against* Xanthomonas euvesicatoria

In vitro activity was assessed on King's B medium (KBM). A suspension of *X. euvesicatoria* (10^8 cfu mL^{-1}; OD_{600} = 0.1) was prepared and was streak inoculated with a sterile swab into Petri dishes containing KBM. After a 30 min incubation, the plates were dot inoculated with candidate bacteria, and then incubated at 24°C for 48 h. Diameters of *X. euvesicatoria* inhibition zones formed around dot inoculation points were then measured. A modified weighted ranking method was used for assessments of bacterial isolate inhibition of *X. euvesicatoria*. This facilitated numerical classification of bacterial strains by weighting each of several traits based on relative importance, and integrating these weightings with other weighted characteristics. Data obtained were subjected to variance analysis, which enabled quantitative comparisons among all items within the test (Michelson *et al.*, 1958; Akbaba and Ozaktan, 2018). The modified "weighted-rankit" method was applied to 313 bacterial isolates obtained from tomato plant tissues. This approach was adapted to evaluate the diverse bacteria for; ability to produce siderophores, exhibit ACC deaminase activity, produce indole 3-acetic acid (IAA) and hydrogen cyanide (HCN), solubilize phosphates, and inhibit *X. euvesicatoria* growth, with different levels of significance for each factor. Fifteen isolates that were characterized through *in vitro* tests (above) were selected for testing against *X. euvesicatoria*, based on their weighted-average rankings (Table 1). These isolates were also preserved at -80°C in liquid nutrient broth plus 20% glycerol, for long-term storage.

In planta *assessments of antagonistic bacteria against* Xanthomonas euvesicatoria

An *in planta* experiment with 15 selected bacterial isolates was carried out by applying seed coating and plant leaf sprays, applied to tomato variety SC2121. This variety is well-regarded due to its early maturation, good adaption for field cultivation, appropriateness for direct human consumption, and its round, red, and thin skinned fruit (Turfan and Düzel, 2023).

The experiment was conducted in a growth chamber, set to a daily regime of 16 h light at 25°C and 8 h dark at 22°C, and lasted for 45 d.

Tomato seeds were sterilized in a solution of 1% sodium hypochlorite for 1 min, and then rinsed three times with sterile distilled water. Antagonistic bacteria and *X. euvesicatoria* were incubated in KBM medium at 24°C for 24 to 48 h (Schaad *et al*., 2001). For seed bacterization treatments, bacterial colonies were suspended in carboxymethyl cellulose (CMC) at 1% v/v, and inoculum was adjusted to OD_{600} = 0.1 using a spectrophotometer (PG Instruments T60 UV/VIS). The seeds were then soaked for 30 min in inoculum solutions, each containing an antagonistic bacterium suspension amended with carboxymethyl cellulose. The experimental control treatment applied 1% CMC to seeds. The seeds were then allowed to dry on sterile blotting paper for 24 h in a microbial-free cabinet before planting. After completing bacterization, the seeds inoculated with the candidate bacteria were individually planted at approx. 1 cm depth into 10 cm^2/500 cm^3 plastic pots containing sterile peat (TS1 Klasmann-Deilman GmbH, Germany). This substrate had electrical conductivity of 35 mS m^{-1}, pH of 6.5, nutrient composition of 14:10:18 (N:P:K), and density of 1.0 kg m^3 (Bolat *et al*., 2022).

The pots were then placed in the growth chamber. When resulting plants reached true leaf stage second applications of respective antagonistic bacteria were applied by spraying onto the plant surfaces. Isolates of antagonistic bacteria were incubated in KBM at 24°C for 24 to 48 h. Bacterial suspensions were then prepared in sterile distilled water and adjusted to OD_{600} = 0.1 (1×10^8 cfu mL^{-1}) using the UV-visible spectrophotometer (above). The suspensions were then sprayed onto tomato plants (at 3rd true leaf stage) using a hand nozzle sprayer. One to two days later the plants were inoculated with *X. euvesicatoria* (Lwin and Ranamukhaarachchi, 2006), and inoculation contol plants were treated with distilled water.

For *X. euvesicatoria* inoculum, colonies of the pathogen were grown on KB medium at 25°C for 48 h. Bacterial suspension was prepared as suspension in sterile distilled water, and with concentration adjusted to approx. 10^8 cfu mL^{-1} (OD_{600} = 0.35) using the UV-visible spectrophotometer (above) (Klement *et al*., 1990). The 3rd-leaf tomato seedlings were sprayed with *X. euvesicatoria* suspension applied to the undersides of their leaves, because *X. euvesicatoria* infiltrates through leaf stomata which are at high densities on the underside leaf surfaces. The seedlings were then placed in a controlled environment cabinet at 24°C and of 95-100% relative humidity for 2 to 3 d. They were then transferred to a transparent cabinet to preserve high relative humidity (95–100%). After inoculation, the plants were placed in a growth chamber for 2 weeks to observe any disease symptoms that may develop. Tomato plants that were not treated with bacteria and were inoculated with the pathogen were used as experimental controls. Tomato plants that were not treated with antagonistic bacteria or *X. euvesicatoria* were used as experimental controls. The experiment was structured in a completely randomized design with ten replicates, with one plant of each treatment assigned to each replicate. Two weeks after *X. euvesicatoria* inoculation, disease severity was evaluated using a 0 to 4 scale, where 0 = no symptoms, 1 = 1 to 5 lesions on leaves, 2 = multiple lesions and merged lesions on leaves, 3 = merged lesions and necrotic leaves, and 4 = dead leaves, as a modification of the scale described by Al-Dahmani *et al*. (2003). Disease indices (DI) were determined using a formula based on that of Townsend and Heuberger (1943), as DI = [Σ (number of plants in the rating × rating number)/(total number of plants × highest rating)] × 100. This experiment was conducted twice.

Colonization of tomato by antagonistic bacteria and Xanthomonas euvesicatoria

Based on the *in vitro* and *in planta* experiments, and on availability of plant resources and growth chamber capacity, two bacterial strains were chosen that gave significant antagonism against *X. euvesicatoria*. The strain population densities in roots and shoots of tomato plants growing under conditions described above and for 30 d after planting. The two selected bacterial isolates were made resistant to rifampicin (200 ppm), labelled, and tomato seeds were treated with the respective modified strains (Kloepper *et al*., 1980). Colonies were selected that had resistance to rifampicin and typical bacterial colony size and appearance to bacteria. Rifampicin resistance was checked by subject the modified isolates to 10, 50, 100 or 200 ppm concentrations of rifampicin. Isolate purification was achieved using KBM supplemented with 200 ppm of rifampicin.

Time-dependent colonization of tomato plants with beneficial bacteria in the presence and absence of *X. euvesicatoria* was carried out at sampling intervals, including seedling cotyledon stage, first true leaf stage, second true leaf stage, as well as at 24 h, 48 h , 7 d, and 14 d post- *X. euvesicatoria* inoculation. Commencing at the seed bacterization stage, 1 g of plant material (root or shoots) was taken from each assessed plant at each sampling time, and this was rinsed in 100 mL of sterile water for 15 min. A dilution series was prepared from the washing water for each seedling, and was subsamples were inoculated onto KBM supplemented with 200 ppm of rifampicin), Resulting colonies were counted, and the bacterial populations in 1 g of plant tissue (cfu g^{-1} of plant tissue) were determined. The experiment was of randomized plot design

with three replicates, and with one tomato seedling in each replicate for each sampling period.

Identification of two antagonistic bacteria through sequencing of the bacterial 16S rRNA gene

To extract DNA, antagonistic bacterial isolates KD15/1 and KD91/1 were grown on KBM for 24 to 48 h at 24°C. DNA was obtained from resulting bacterial suspensions using boiling lysis. Sterile distilled water (OD_{600} = 0.1) was used to prepare the bacterial suspensions, and the suspensions were centrifuged at 15,000 g for 10 min. The resulting pellets were each suspended in 40 µL of ultrapure water and heated at 100°C for 10 min. The suspensions were then cooled on ice and centrifuged at 15,000 g for 10 s. These resulting the pellets were stored at -20°C. The extracted DNA was used as templates for PCR amplification (Omar *et al*., 2014) . To amplify approx. 1460 base pairs of 16S rDNA, the universal primers 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) were used in the PCR (Hodkinson and Lutzoni, 2009). Each final PCR mixture included 100 ng of DNA extract, 10× Taq KCl reaction buffer, 1 mM of each primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 1 unit of Taq DNA polymerase (recombinant, 5 U μL^{-1}). The following steps were used in a thermocycler for amplification: 35 cycles, each with an initial denaturation at 95°C for 3 min, followed by denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min, followed by a final extension at 72°C for 5 min using a thermal cycler. The PCR products were then analysed by electrophoresis on a 1.5% agarose gel in 0.5× TAE buffer containing 50× Tris-acetate-EDTA, and were stained with nucleic acid staining solution at 20,000× concentration. The 80 V setting was applied to the gel for 90 min, and the resulting DNA bands plus a 1 kb DNA ladder were visualized under UV light. The amplified products were then purified using a QIAquick Gel Extraction Kit (QIAGEN). Sequencing was performed by MedSanTek Company (Turkey). Sequence editing was carried out using MEGA v10. DNA sequences were analysed using BLASTn software (http://blast.ncbi.nlm.nih.gov/) and were compared with GenBank sequences. The 16S rRNA sequences of the antagonistic bacteria used in this study were submitted to the GenBank database with relevant assigned accession numbers.

Statistical analyses

The antagonistic bacterial population data were first transformed using log root and shoot CFU g^{-1} values, before averaging. All data were analysed using the "agricolae" package in the R statistical programming language. The data obtained were subjected to analysis of variance (ANOVA), with the population data transformed to log (CFU g^{-1}). Means were compared using Duncan's multiple range test at $P < 0.05$, and standard deviations of means were calculated.

RESULTS

In vitro *PGPR and biocontrol assessments of antagonistic bacterial isolates against* Xanthomonas euvesicatoria

A total of 313 endophyte or epiphyte bacterial isolates were obtained from tomato leaf or stem tissues, and were assessed *in vitro* for their antagonistic activity against *X. euvesicatoria*, and for aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, siderophore, indole 3-acetic acid (IAA) and hydrogen cyanide (HCN) production, and to solubilize phosphates (Supplementary Table 1). From these assessments, the 15 isolates were selected for further bioassays (Table 1).

In planta *biocontrol efficacy of bacterial isolates against* Xanthomonas euvesicatoria

Of the 15 potential antagonistic bacterial isolates assessed against *X. euvesicatoria*, isolates isolates KD 4/5 and KD 15/2 were the most disease suppressive producing overall mean disease severities of, respectively, 33 and 34% (Table 2).

In the seed treatment assays, isolate KD 15/2 was the most disease suppressive (Figures 2 and 3). From seed bacterization and spray applications, isolates KD 15/2, KD 84/3, and KD 91/1 gave the greatest pathogen disease suppression (Table 3)

Colonization of tomato plants by antagonistic bacteria and Xanthomonas euvesicatoria

Isolates KD 15/2 and KD 91/1, labelled with rifampicin resistance (200 ppm) and affected systemic transport and colonization at different stages of tomato plant depending on time. At 24 h after applications of the labelled isolates KD 15/2 and KD 91/1 to tomato seeds, the beneficial bacteria were detected at populations of 7×10^9 cfu g^{-1} for KD 15/2 and 8.6×10^8 cfu g^{-1} for KD 91/1. Time-dependent changes in the population densities of the beneficial bacteria in roots and shoots of tomato plants treated with KD 15/2, KD 91/1, KD 15/2 + *X. euvesicatoria*, or KD 91/1 + *X. euvesicatoria* are

Table 1. Characteristics (including mean parameters) of 15 bacterial isolates selected for further *in planta* evaluation in this study.

Isolate code	Location obtained	Growth habit	HCN [a]	IAA [b]	SID [c]	ACC [d]	PS [e]	XE-In [f]
KD4/5	Eğlenhoca/Karaburun/İZMİR	Endophyte	-	125	4.25	+	1.00	10.00
KD7/1	Eğlenhoca/Karaburun/İZMİR	Epiphyte	-	137	3.25	+	10.25	7.25
KD7/2	Mordoğan/Karaburun/İZMİR	Epiphyte	-	114	3.25	+	4.75	10.00
KD10/3	Mordoğan/Karaburun/İZMİR	Endophyte	-	132	5.00	+	3.50	8.25
KD15/1	Ildır/Çeşme/İZMİR	Epiphyte	-	353	2.75	+	4.00	8.25
KD15/2	Ildır/Çeşme/İZMİR	Epiphyte	-	275	2.50	+	3.50	8.25
KD17/2	Ildır/Çeşme/İZMİR	Epiphyte	+	75	12.25	+	1.50	9.00
KD25/2	Nohutalan/Urla/İZMİR	Epiphyte	+	217	4.50	+	3.50	6.00
KD63/2	Dalama/Efeler/AYDIN	Epiphyte	-	256	8.50	+	0.00	6.00
KD80/2	Kınık Ovası/Kınık/İZMİR	Endophyte	-	484	15.00	+	7.75	2.75
KD84/3	Bölcek/Bergama/İZMİR	Endophyte	-	343	10.50	+	5.50	4.00
KD88/1	Yanıkköy/Menemen/İZMİR	Epiphyte	-	167	6.25	+	2.00	8.50
KD88/2	Yanıkköy/Menemen/İZMİR	Endophyte	-	311	4.00	+	1.00	3.00
KD91/1	UTEAM/Menemen/İZMİR	Endophyte	-	191	8.75	+	4.00	8.25
KD97/1	Musahoca/Kırkağaç/MANİSA	Endophyte	-	278	4.00	+	5.00	8.25

[a] Hydrogen cyanide production. [b] Indole acetic acid production (ppm)., [c] Siderophore production (mm), [d] ACC (1-Aminocyclopropane-1-carboxylic acid) deamilase activity, [e] Phosphate solubilization (mm), [f] *Xanthomonas euvesicatoria* inhibition zone (mm). All tests were set up in three replicates and repeated twice.

Table 2. Mean disease severities (DS) caused by *Xanthomonas euvesicatoria* on tomato plants inoculated with different bacterial isolates. Means accompanied by the same letters are not significantly different ($P< 0.05$).

Isolate Code	1. Trial DS (%)	2. Trial DS (%)	Mean DS (%)
Untreated Control	61.66 ± 12.07 a	56.45 ± 7.82 a	59.06 ± 10.12 a
KD 4/5	35.62 ± 5.50 cd	30.62 ± 6.80 de	33.12 ± 6.54 de
KD 7/1	56.95 ± 10.82 a	34.16 ± 3.82 bcde	45.56 ± 14.11 b
KD7/2	35.12 ± 7.64 cd	30.83 ± 6.03 cde	32.97 ± 7.05 de
KD10/3	43.25 ± 9.33 bc	35.83 ± 4.89 bcde	39.54 ± 8.19 bcd
KD15/1	34.87 ± 9.21 cd	34.87 ± 10.34 bcde	34.87 ± 9.53 cde
KD15/2	31.62 ± 12.22 d	37.12 ± 5.56 bcd	34.37 ± 9.66 cde
KD17/2	51.00 ± 19.59 ab	39.78 ± 4.65 b	45.39 ± 15.01 b
KD25/2	36.12 ± 10.23 cd	38.75 ± 4.41 b	37.43 ± 7.78 cde
KD63/2	58.12 ± 10.77 a	53.33 ± 8.95 a	55.72 ± 9.95 a
KD80/2	53.12 ± 10.31 ab	36.45 ± 5.57 bcde	44.79 ± 11.75 b
KD84/3	30.50 ± 7.95 d	30.00 ± 11.33 e	30.25 ± 9.53 e
KD88/1	44.79 ± 9.27 bc	36.25 ± 3.95 bcde	40.52 ± 8.20 bc
KD88/2	53.75 ± 9.86 ab	37.87 ± 4.50 bc	45.81 ±11.04 b
KD91/1	51.25 ± 10.85 ab	31.25 ± 6.58 cde	41.25 ± 13.47 bc
KD97/1	37.50 ± 8.83 cd	39.79 ± 7.76 b	38.64 ± 8.18 bcd

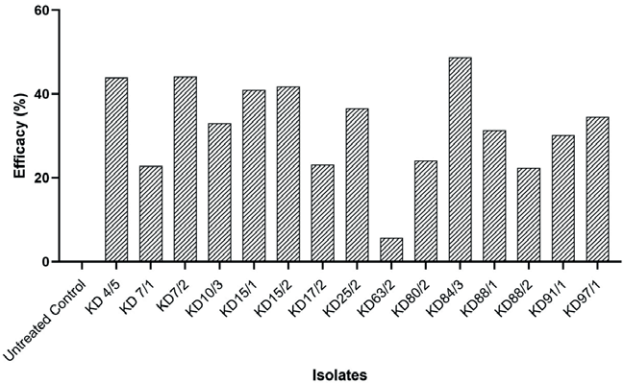


Figure 1. Efficacy percentages for 15 bacterial isolates against *Xanthomonas euvesicatoria*, after applications to surfaces of tomato plants.

shown in Figure 4. At the seedling cotyledon stage, bacterial populations of $6.2 \times 10^{4\text{-}5}$ cfu g^{-1} of internal shoot tissue were detected, and $3.2 \times 10^{5\text{-}6}$ cfu g^{-1} roots. This indicates that isolates KD 15/2 and KD 91/1 colonized plant tissues from seeds to the developing plants. At the second true leaf stage, the bacterial isolates maintained populations of 2.9×10^4 to 2.5×10^6 cfu g^{-1} in shoots and root tissues. For the shoot inoculations, at 24 h after *X. euvesicatoria* inoculation, isolate KD 15/2 was detected at 4×10^3 cfu g^{-1} in plant shoots and 1.1×10^4 cfu g^{-1} in roots. At 72 h after *X. euvesicatoria* inoculation, isolate KD 91/1 maintained a population of 7.2×10^4 cfu g^{-1} plant in the plant shoots and 1×10^5 cfu g in roots. In general, the bacterial isolates successfully colonized tomato roots and shoots in the presence and absence of *X. euvesicatoria*.

Table 3. Mean disease severities (DS) caused by *Xanthomonas euvesicatoria* on tomato plants after seed bacterization with different bacterial isolates. Means accompanied by the same letters are not significantly different ($P < 0.05$).

Isolate Code	Trial 1 DS (%)	Trial 2 DS (%)	Mean DS (%)
Untreated Control	61.67 ± 12.07 a	56.45 ± 7.82 ab	59.06 ± 10.12 a
KD 4/5	46.37 ± 17.54 cde	50.00 ± 4.16 bcde	48.18 ± 12.55 cd
KD 7/1	53.12 ± 15.09 abc	58.12 ± 4.85 a	55.62 ± 11.21 ab
KD7/2	52.29 ± 14.94 abc	51.70 ± 9.90 abcd	52.00 ± 12.34 bc
KD10/3	52.41 ± 5.48 abc	53.12 ± 7.93 abc	52.77 ± 6.64 abc
KD15/1	49.83 ± 17.41 bcd	44.37 ± 7.73 e	47.10 ± 13.41 cd
KD15/2	30.62 ± 9.72 f	34.16 ± 4.19 f	32.39 ± 7.51 e
KD17/2	46.04 ± 8.12 cde	49.58 ± 6.93 bcde	47.81 ± 7.57 cd
KD25/2	50.20 ± 9.28 abcd	55.50 ± 4.60 ab	52.85 ± 7.63 abc
KD63/2	60.62 ± 6.62 ab	56.12 ± 5.11 ab	58.37 ± 6.20 ab
KD80/2	51.25 ± 12.07 abcd	44.75 ± 7.26 de	48.00 ± 10.25 cd
KD84/3	40.20 ± 8.21 def	46.25 ± 11.85 cde	43.22 ± 10.40 d
KD88/1	54.29 ± 9.42 abc	37.50 ± 5.10 f	45.89 ± 11.34 cd
KD88/2	59.04 ± 11.87 ab	53.62 ± 10.67 ab	56.33 ± 11.33 ab
KD91/1	36.50 ± 6.36 ef	34.25 ± 4.04 f	35.37 ± 5.32 e
KD97/1	44.37 ± 3.54 cde	52.08 ± 7.41 abc	48.22 ± 6.90 cd

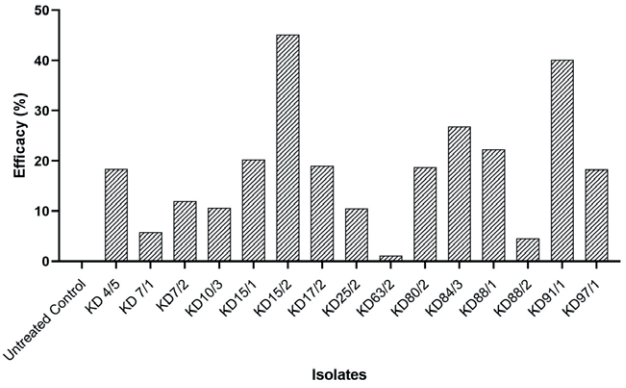


Figure 2. Efficacy percentages from 15 different bacterial isolates applied as seed bacterization treatments against *Xanthomonas euvesicatoria.*

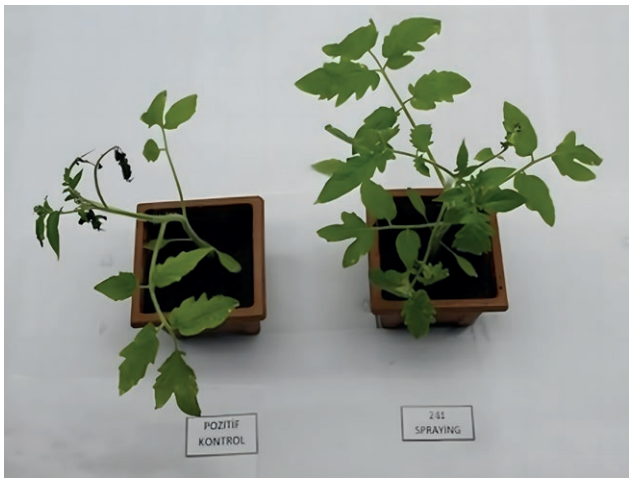


Figure 3. Tomato plants inoculated with *Xanthomonas euvesicatoria* which were either untreated (experimental control, A) or treated with bacterial isolate KD 91/1 (B).

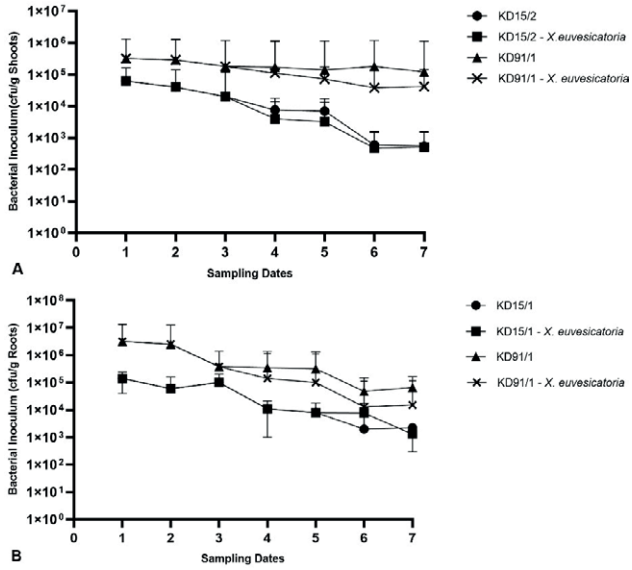


Figure 4. Time-dependent variations of mean populations of different bacteria applied to tomato plants as shoot inoculum (A) or root inoculum (B). Error bars indicate standard errors. Different letters denote significant differences at $P < 0.05$.

Molecular identification of bacteria that reduced Xanthomonas euvesicatoria *infections*

Molecular identifications of six bacteria that were most inhibitory to *X. euvesicatoria* in *in planta* tests are shown in Figure 5, the BLAST analyses of the sequence results in the NCBI database are outlined in Table 4. According to the sequence results, three isolates were identified as *Pseudomonas putida* (KD 4/5, KD 7/2, KD 91/1), one isolate as *Enterobacter aerogenes* (KD 15/1), one isolate as *Enterobacter cloacae* (KD 15/2), and one isolate as *Pantoea* spp. (KD 84/3).

DISCUSSION

Xanthomonas euvesicatoria causes significant yield losses in most tomato-growing regions (Potnis *et al.*, 2015). Important bacterial plant pathogens have devel-

Figure 5. Gel image at 1460 bp after amplification with primer pairs 27F/1492R from six isolates of bacteria that inhibited *Xanthomonas euvesicatoria* infections of tomato plants.

Table 4. Diagnosis and NCBI reference numbers for six bacterial isolates that reduced *Xanthomonas euvesicatoria* infections of tomato plants.

Isolate Code	Bacteria species	Reference Similarity	Reference Access No.	NCBI Access No.
KD 4/5	*Pseudomonas putida*	99.1	MH488985.1	OM218731
KD 7/2	*Pseudomonas putida*	93.0	KF478210.1	OM219206
KD 15/1	*Enterobacter aerogenes*	96.7	LN623608.1	OM219003
KD 15/2	*Enterobacter cloacae*	95.4	MG274270.1	OM219024
KD 84/3	*Pantoea ananatis*	93.0	MN641907.1	PP824970
KD 91/1	*Pseudomonas putida*	94.0	MF683391.1	OM219060

oped resistance to long-used chemical controls (Lucas, 2011), so management of economically important diseases has become increasingly challenging, particularly because of scarcity of effective compounds (Bailey, 2010). Therefore, biological control using microbial antagonists as alternatives to chemicals has been proposed as a positive alternative, to reduces risks chemical contamination in ecosystems and human food chains. Biological control is less affected by pest resistance development than conventional chemicals, and has advantages of minimal or zero residual toxicity and environmental pollution (Gardener and Fravel, 2002; Lazarovits *et al*., 2014; Stenberg *et al*., 2021). In recent years, research on identification and characterization of potential biocontrol agents, such as PGPRs and microbial endophyte antagonists against plant pathogens, has increased considerably.

Approximately 2–5% of rhizosphere bacteria promote plant growth (Glick, 1995). Most plant growth promoting rhizobacteria (PGPRs) belong to *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonas*, and *Bacillus* (Goswami *et al*., 2016). The most common bacterial endophytes belong to *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea*, and *Microbacterium* (Santoyo *et al*., 2016).

The present study assessed isolates of endophytic and epiphytic bacteria obtained from 127 different tomato plant samples from 40 different locations. Among the 313 bacterial isolates obtained, three of the six bacterial isolates shown to be most effective against *X. euvesicatoria in planta* belonged to *Pseudomonas*, two to *Enterobacter*, and one to *Pantoea*, and these results reflect previous reports. The 15 most inhibitory endophyte and epiphyte bacterial isolates were evaluated for effects on disease incidence by evaluating the *in vitro* biocontrol and plant growth-promoting parameters using weighted grading. The chosen isolates were introduced into the pathosystem by host plant seed biopriming or shoot spraying. Among selected isolates, isolates KD 91/1 and KD 15/2 reduced the severity of bacterial spot caused by *X. euvesicatoria* on tomato plants after both treatment types. *In vitro*, isolates KD 91/1 and KD 15/2 gave greatest inhibition of *X. euvesicatoria*. Inhibition zones result from production of volatile antimicrobial compounds (Raza *et al*., 2016) and is a primary antagonistic mechanism (Talibi *et al*., 2014). The 15 selected potential biocontrol agents exhibited biocontrol activity and plant growth-promoting properties, including production of IAA, siderophores, and phosphate solubility. IAA promotes plant growth, whereas siderophore and phosphate solubility help nutrient accumulation in plants, creating nutrient scarcity in soil for pathogens (Wahab *et al*., 2024). These properties of bacterial endophytes indirectly inhibit pathogen growth and promote host plant growth (Kashyap *et al.,* 2019). Of the two isolates, KD 91/1 was the most active *in vitro*, especially for siderophore production, by forming the largest yellow-orange inhibition zones in CAS blue agar.

Bacteria can enhance plant growth and stimulate production of plant growth-promoting substances, including cytokinins and indole acetic acid (Arkhipova *et al*., 2005; Sandhya *et al*., 2017). Bacterial endophytes may produce ACC deaminase, which produces ammonia and α-ketobutyrate from the ethylene precursor ACC, which can promote plant growth under nitrogen-limiting conditions (Afzal *et al*., 2019). The ability of isolate KD 91/1 to produce ACC deaminase and IAA at a high

rate (191 ppm) was probably for greater host plant biomass compared to the other 14 bacterial isolates and the untreated negative controls.

When the 15 potential biocontrol bacteria selected were evaluated for *in planta* biocontrol and plant growth promotion, the greatest effects were detected from isolate KD 91/1. Tomato seed and plant foliar applications gave, respectively, 30 and 40% increases, respectively. Isolate KD 91/1 was identified as a *Pseudomonas* sp. by molecular diagnosis. Plant phyllospheres are predominantly inhabited by *Pseudomonas* spp. (Delmotte *et al*., 2009; Maignien *et al*., 2014) because of their abilities to use effectors to leak water from cells to apoplasts (Xin *et al*., 2016), and to synthesize biosurfactants to increase water availability on leaf surfaces (Hernandez and Lindow, 2019).

Pseudomonas putida is widely recognized as non-hazardous to human and environmental health. Strain KT2440 of *P. putida* is classified by the FDA as HV1 certified, signifying its safety for use inP1 or ML1 environments (Kampers *et al*., 2019), which indicates low-risk for human exposure. The environmental compatibility and efficacy of *P. putida* in bioremediation have been demonstrated in previous studies (Xue *et al*., 2022; Tasleem *et al*., 2023). However, close examination reveals contradictions and important facts. Although *P. putida* is generally considered safe, clinical isolates of this species have been reported, albeit infrequently. These clinical strains possess genes associated with survival under oxidative stress, resistance to biocides, and toxin/antitoxin systems, potentially enhancing capacity to colonize and persist within human tissues (Molina Delgado *et al*., 2016). Furthermore, while *P. putida* shares 85% of its coding regions with the opportunistic pathogen *P. aeruginosa*, it lacks virulence factors such as exotoxins and type III secretion systems (Udaondo *et al*., 2016).

Pseudomonas isolates have been observed to directly inhibit growth of various pathogens such as *X. campestris* pv. *vesicatoria* and *P. syringae* pv. *glycinea* in laboratory and field experiments (Völksch and May, 2001; Abo-Elyousr and El-Hendawy, 2008). In the present study, no significant reduction in disease severity caused by *X. euvesicatoria* was found when bacterial isolates KD 4/5 and KD 7/2 were applied to tomato seeds by biopriming, whereas they reduced the disease severity by an average of 43% when applied by spraying on plant leaves. Molecular identifications of isolates KD 4/5 and KD 7/2 showed them to be *Pseudomonas*. Isolate KD 91/1 (*P. putida*) was an endophyte isolated from tomato roots. Compared to non-endophytic bacteria, endopytes enter and colonize their original host more readily than non-endophytes, as they are adapted to hosts without stimulating defense mechanisms. Endophytes also compete with pathogens that infect plants and survive in plant tissues, endophyte colonization prevents pathogenic organisms from entering plant tissues (Martinez-Klimova *et al*., 2017). For example, *P. putida* WCS358 effectively mitigated Fusarium wilt in radish by competing for iron through siderophore production, whereas *P. putida* RE8 induced systemic resistance against the disease (de Boer *et al*., 2003). Additionally, *P. putida* strains have demonstrated capacity to employ contact-dependent mechanisms, including the type IVB secretion system, to eliminate competing bacterial species and safeguard plants from pathogens, such as *Ralstonia solanacearum* (Purtschert-Montenegro *et al*., 2022).

Pantoea ananatis, while predominantly identified as a plant pathogen, also has PGPR characteristics. This bacterium has diverse ecological functions, including plant growth enhancement, and has potential as a biological control agent. Some strains of this bacterium have been shown facilitate plant growth in *Solanum tuberosum* (potato) and *Capsicum annuum* (pepper) (Coutinho and Venter, 2009). Additionally, *Pa. ananatis* can infect monocotyledonous and dicotyledonous plants, causing leaf blotches and die-back. The bacterium is responsible for diseasesmany economically important crop plants including *Zea mays* (maize), *Oryza sativa* (rice), *Allium cepa* (onion), and *Citrullus lanatus* (melon). symptoms of these diseases vary depending on host plant, leading to diverse agricultural challenges. For example, *Pa. ananatis* causes center rot in onions, which can result in substantial yield reductions and postharvest losses (De Maayer *et al*., 2010). This pathogen also has toxicity towards specific human cell lines, including glioblastoma cells, underscoring its relevance (Polidore *et al*., 2021).

Enterobacter cloacae, a known PGPR, exerts several effects that can be beneficial or detrimental. As a PGPR, *E. cloacae* facilitates plant growth by enhancing nutrient uptake, improving stress resistance, and promoting overall growth, thereby contributing to sustainable agriculture. However, similar to many non-native rhizobacteria, when this bacterium is introduced into ecosystems unintended consequences can result, including alteration of local microbiomes and disruption of ecosystem functions (dos Santos *et al*., 2020; Moore *et al*., 2022). Conversely, *E. cloacae* is acknowledged as a significant opportunistic pathogen in humans. It can have multidrug resistance, frequently attributed to the production of chromosomally-encoded AmpC β-lactamase, which complicates therapeutic interventions (Davin-Regli and Pagès, 2015). This organism has been associated with hospital-acquired infections, including those of urinary tracts and respira-

tory systems, and can be disseminated throughout hospital settings, often leading to considerable healthcare challenges including outbreaks in intensive care units (John *et al*., 1982; Moradigaravand *et al*., 2016).

Enterobacter aerogenes can be PGPR and an opportunistic pathogen with considerable implications. This bacterium functions as a PGPR by producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces plant ethylene levels, thereby enhancing plant growth under stress conditions such as in saline alkali environments. The bacterium can also synthesize indole-3-acetic acid (IAA), which facilitates root elongation and branching, which are important for plant survival under drought stress (Jochum *et al*., 2019). Furthermore, *E. aerogenes* has ability to solubilize phosphate and produce siderophores, which contribute to improved plant nutrient availability and uptake, ultimately leading to enhanced plant productivity and yields (Liu *et al*., 2019). Conversely, *E. aerogenes* is recognized as an opportunistic pathogen associated with hospital-acquired infections, particularly in immunocompromised patients. It has been implicated in various clinical conditions, including pneumonia, bacteremia, and urinary tract infections. The bacterium can have multidrug resistance, which complicates clinical treatment options (Davin-Regli and Pagès, 2015).

For these reasons, *P. anantis*, *E. cloacae* and *E. aerogenes* are not recommended as biological control agents, despite their efficacy in controlling *X. euvesicatoria* and their beneficial impacts on plant growth.

In general, bacterial populations in plant rhizoplanes are in the range of 10^5 to 10^7 cfu g^{-1} root fresh weight (Goel *et al*., 2017). Populations of endophytes can vary depending on bacterial species, plant genotypes, plant tissue, and environmental conditions (Rosenblueth and Martínez-Romero, 2006). In the present study, bacterial population of 8.6×10^8 cfu g^{-1} seed was determined after coating *P. putida* KD 91/1 bacterial isolates onto tomato seeds. Average bacterial population of $3 \times 10^{4\text{-}5}$ to $2.5 \times 10^{4\text{-}6}$ cfu g^{-1} plant tissue was determined within tissues of tomato shoots and roots during the cotyledon and second true leaf stages. At 72 h after pathogen inoculation, the isolate KD 91/1 maintained a population of 7.2×10^4 cfu g^{-1} in shoots and 1×10^5 cfu g^{-1} in roots. Overall, *P. putida* KD 91/1 successfully colonized tomato roots and shoots in the presence and absence of *X. euvesicatoria*. Root colonization ability is a prerequisite and determinant for biocontrol agent activity and efficacy (Cavaglieri *et al*., 2005). The enhanced colonization ability of *P. putida* KD 91/1 may have contributed to biocontrol efficacy. *Pseudomonas rhodesiae* and *Pantoea ananatis*, selected for their consistent colonization of pepper stems, have been shown to mitigate disease severity caused by *Xanthomonas axonopodis* pv. *vesicatoria* and to induce systemic host resistance (Kang et al., 2007).

CONCLUSIONS

Previous research has suggested that particular antagonistic isolates may be suitable candidates for biological control of *X. euvesicatoria*. However, further research is required to gain understanding of the interactions between this pathogen, host plants, and antagonistic bacteria. Effectiveness of biocontrol against *X. euvesicatoria* should also be confirmed through molecular gene expression experiments and detection of genes involved in plant resistance induction.

ACKNOWLEDGEMENTS

The authors thank Professor Ayse GUL for providing plant chamber conditions.

FUNDING

This study was supported by national grants from the Ege University Office of Scientific Research Projects (BAP) (FDK-2019-20622).

AUTHOR CONTRIBUTIONS

GE carried out the growth chamber and colonization studies. HO participated in the study design and performed the statistical analysis. HO conceived the study and participated in its design and coordination; US performed the statistical analysis, sequence alignment, and drafted the manuscript. All authors have read and approved the final manuscript.

LITERATURE CITED

Abbasi P.A., Khabbaz S.E., Weselowski B., Zhang L., 2015. Occurrence of copper-resistant strains and a shift in *Xanthomonas* spp. causing tomato bacterial spot in Ontario. *Canadian Journal of Microbiology* 61: 753–761.

Abo-Elyousr K.A.M., El-Hendawy H.H., 2008. Integration of *Pseudomonas fluorescens* and acibenzolar-S-methyl to control bacterial spot disease of tomato. *Crop Protection* 27: 1118–1124.

Afzal I., Shinwari Z.K., Sikandar S., Shahzad S., 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. *Microbiological Research* 221: 36–49.

Akbaba M., Ozaktan H., 2018. Biocontrol of angular leaf spot disease and colonization of cucumber (*Cucumis sativus* L.) by endophytic bacteria. *Egyptian Journal of Biological Pest Control* 28: 1–10.

Akköprü A., Ozaktan H., 2018. Identification of rhizobacteria that increase yield and plant tolerance to angular leaf spot disease in cucumber . *Plant Protection Science* 54(2): 67–73.

Al-Dahmani J.H., Abbasi P.A., Miller S.A., Hoitink H.A.J., 2003. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. *Plant Disease* 87: 913–919.

Arkhipova T.N., Veselov S.U., Melentiev A.I., Martynenko E. V, Kudoyarova G.R., 2005. Ability of bacterium *Bacillus subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. *Plant and Soil* 272: 201–209.

Bailey K.L., 2010. Canadian innovations in microbial biopesticides. *Canadian Journal of Plant Pathology* 32: 113–121.

Bakker A.W., Schippers B.O.B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. *Soil Biology and Biochemistry* 19: 451–457.

Balogh B., Jones J.B., Momol M.T., Olson S.M., Obradovic A., … Jackson L.E., 2003. Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. *Plant Disease* 87: 949–954.

Bolat I., Bakır A.G., Korkmaz K., Gutiérrez-Gamboa G., Kaya O., 2022. Silicon and nitric oxide applications allow mitigation of water stress in Myrobalan 29C rootstocks (*Prunus cerasifera* Ehrh.). *Agriculture* 12: 1273–1283.

Bric J.M., Bostock R.M., Silverstone S.E., 1991. Rapid *in situ* assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology* 57: 535–538.

Cavaglieri L., Orlando J., Rodriguez M.I., Chulze S., Etcheverry M., 2005. Biocontrol of *Bacillus subtilis* against *Fusarium verticillioides in vitro* and at the maize root level. *Research in Microbiology* 156: 748–754.

Compant S., Duffy B., Nowak J., Clément C., Barka E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* 71: 4951–4959.

Coutinho T.A., Venter S.N., 2009. *Pantoea ananatis*: An unconventional plant pathogen. *Molecular Plant Pathology* 10: 325–335. https://doi.org/10.1111/j.1364-3703.2009.00542.x.

Davin-Regli A., Pagès J.M., 2015. *Enterobacter aerogenes* and *Enterobacter cloacae*; Versatile bacterial pathogens confronting antibiotic treatment. *Frontiers in Microbiology* 6: 392–402.

De Boer M., Bom P., Kindt F., Keurentjes J.J.B., van der Sluis I., … Bakker P.A.H.M., 2003. Control of Fusarium wilt of radish by combining *Pseudomonas putida* strains that have different disease-suppressive mechanisms. *Phytopathology* 93: 626–632.

De Maayer P., Chan W.Y., Venter S.N., Toth I.K., Birch P.R.J., … Coutinho T.A., 2010. Genome sequence of *Pantoea ananatis* LMG20103, the causative agent of Eucalyptus blight and dieback. *Journal of Bacteriology* 192: 2936–2937. https://doi.org/10.1128/JB.00060-10.

Delmotte N., Knief C., Chaffron S., Innerebner G., Roschitzki B., … Vorholt J.A., 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences* 106: 16428–16433.

Dos Santos R.M., Diaz P.A.E., Lobo L.L.B., Rigobelo E.C., 2020. Use of plant growth-promoting rhizobacteria in maize and sugarcane: characteristics and applications. *Frontiers in Sustainable Food Systems* 4: 136–151.

El-Hendawy H.H., Osman M.E., Sorour N.M., 2005. Biological control of bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* by *Rahnella aquatilis*. *Microbiological Research* 160: 343–352.

FAO, 2022. *Statistical Yearbook*. Available at: https://www.fao.org/faostat/en/#home.

Gardener B.B.M., Fravel D.R., 2002. Biological control of plant pathogens: research, commercialization, and application in the USA. *Plant Health Progress* 3: 17–32.

Glick B.R., 1995. The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41: 109–117.

Goel R., Kumar V., Suyal D.K., Dash B., Kumar P., Soni R., 2017. Root-associated bacteria: rhizoplane and endosphere. In: *Plant-Microbe Interactions in Agro-Ecological Perspectives: Volume 1: Fundamental Mechanisms, Methods and Functions*, Springer, 161–176 pp.

Goswami D., Thakker J.N., Dhandhukia P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food & Agriculture* 2: 1127500–1127514.

Hernandez M.N., Lindow S.E., 2019. *Pseudomonas syringae* increases water availability in leaf microenvironments via production of hygroscopic syringafactin. *Applied and Environmental Microbiology* 85: 14-19.

Hodkinson B.P., Lutzoni F., 2009. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163–180.

Jetiyanon K., Kloepper J.W., 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biological Control* 24: 285–291.

Jochum M.D., McWilliams K.L., Borrego E.J., Kolomiets M. V., Niu G., … Jo Y.K., 2019. Bioprospecting plant growth-promoting rhizobacteria that mitigate drought stress in grasses. *Frontiers in Microbiology* 10.

John J.F., Sharbaugh R.J., Bannister E.R., 1982. *Enterobacter cloacae*: bacteremia, epidemiology, and antibiotic resistance. *Reviews of Infectious Diseases* 4: 13–28. https://doi.org/10.1093/clinids/4.1.13.

Kampers L.F.C., Volkers R.J.M., Martins dos Santos V.A.P., 2019. *Pseudomonas putida* KT 2440 is HV 1 certified, not GRAS. *Microbial Biotechnology* 12: 845–848.

Kang S., Cho H.S., Cheong H., Ryu C.M., Kim J.F., Park S.H., 2007. Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). *Journal of Microbiology and Biotechnology* 17(1): 96–103.

Kashyap B.K., Solanki M.K., Pandey A.K., Prabha S., Kumar P., Kumari B., 2019. *Bacillus* as plant growth promoting rhizobacteria (PGPR): a promising green agriculture technology. In: *Plant Health Under Biotic Stress*: Volume 2: Microbial Interactions, Springer, 219–236 pp.

Khan A., Zhao X.Q., Javed M.T., Khan K.S., Bano A., … Masood S., 2016. *Bacillus pumilus* enhances tolerance in rice (*Oryza sativa* L.) to combined stresses of NaCl and high boron due to limited uptake of Na+. *Environmental and Experimental Botany* 124: 120–129.

Klement Z., Mavridis A., Rudolph K., Vidaver A., Perombelon M.C.M., … Rudolph K., 1990. Inoculation of plant tissues. In: *Methods in Phytobacteriology*, Akadémiai Kiadó, Budapest, 94–124 pp.

Kloepper J.W., Schroth M.N., Miller T.D., 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology* 70: 1078–1082.

Köhl J., Kolnaar R., Ravensberg W.J., 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. *Frontiers in Plant Science* 10: 845–864.

Lazarovits G., Turnbull A., Johnston-Monje D., 2014. Plant Health Management: Biological Control of Plant Pathogens. In: *Encyclopedia of Agriculture and Food Systems*; Elsevier Ltd.: London, UK, 2014; pp. 388–399.

Liu J., Tang L., Gao H., Zhang M., Guo C., 2019. Enhancement of alfalfa yield and quality by plant growth-promoting rhizobacteria under saline-alkali conditions. *Journal of the Science of Food and Agriculture* 99: 281–289. https://doi.org/10.1002/jsfa.9185.

Lucas J.A., 2011. Advances in plant disease and pest management. *The Journal of Agricultural Science* 149: 91–114.

Lwin M., Ranamukhaarachchi S.L., 2006. Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. *International Journal of Agriculture and Biology* 8: 657–660.

Mahmood A., Turgay O.C., Farooq M., Hayat R., 2016. Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology* 92(8): 112–126.

Maignien L., DeForce E.A., Chafee M.E., Eren A.M., Simmons S.L., 2014. Ecological succession and stochastic variation in the assembly of *Arabidopsis thaliana* phyllosphere communities. *mBio* 5: 10–1128.

Martin H.L., Hamilton V.A., Kopittke R.A., 2004. Copper tolerance in Australian populations of *Xanthomonas campestris* pv. *vesicatoria* contributes to poor field control of bacterial spot of pepper. *Plant Disease* 88: 921–924.

Martinez-Klimova E., Rodríguez-Peña K., Sánchez S., 2017. Endophytes as sources of antibiotics. *Biochemical Pharmacology* 134: 1–17.

Michelson I.F., Lachman W.H., Allen D.D., 1958. The use of the "Weighted-Rankit" method in variety trials. *ASHS Proceedings* 71: 334–338.

Mirik M., Aysan Y., Cinar O., 2008. Biological control of bacterial spot disease of pepper with *Bacillus* strains. *Turkish Journal of Agriculture and Forestry* 32: 381–390.

Molina Delgado L., Udaondo Z., Duque E., Fernández M., Bernal Guzmán P., … Ramos J.L., 2016. Specific Gene Loci of Clinical *Pseudomonas putida* Isolates. *Plos ONE* 11(1): 147–171.

Moore J.A.M., Abraham P.E., Michener J.K., Muchero W., Cregger M.A., 2022. Ecosystem consequences of introducing plant growth promoting rhizobacteria to managed systems and potential legacy effects. *New Phytologist* 234(6): 1914–1918.

Moradigaravand D., Reuter S., Martin V., Peacock S.J., Parkhill J., 2016. The dissemination of multidrug-

resistant *Enterobacter cloacae* throughout the UK and Ireland. *Nature Microbiology* https://doi.org/10.1038/nmicrobiol.2016.173.

Nautiyal C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters* 170: 265–270.

Nejad P., Johnson P.A., 2000. Endophytic bacteria induce growth promotion and wilt disease suppression in oilseed rape and tomato. *Biological Control* 18: 208–215.

Omar B.A., Atif H.A., Mogahid M.E., 2014. Comparison of three DNA extraction methods for polymerase chain reaction (PCR) analysis of bacterial genomic DNA. *African Journal of Microbiology Research* 8: 598–602.

Pajčin I., Vlajkov V., Frohme M., Grebinyk S., Grahovac M., … Grahovac J., 2020. Pepper bacterial spot control by *Bacillus velezensis*: bioprocess solution. *Microorganisms* 8: 1463.

Pertot I., Puopolo G., Giovannini O., Angeli D., Sicher C., Perazzolli M., 2016. Advantages and limitations involved in the use of microbial biofungicides for the control of root and foliar phytopathogens of fruit crops. *Italus Hortus* 23: 3–12.

Polidore A.L.A., Furiassi L., Hergenrother P.J., Metcalf W.W., 2021. A phosphonate natural product made by *Pantoea ananatis* is necessary and sufficient for the hallmark lesions of onion center rot. *mBio* 12: 1–18. DOI: 10.1128/mBio.03402-20.

Potnis N., Timilsina S., Strayer A., Shantharaj D., Barak J.D., … Jones J.B., 2015. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology* 16: 907–920.

Purtschert-Montenegro G., Cárcamo-Oyarce G., Pinto-Carbó M., Agnoli K., Bailly A., Eberl L., 2022. *Pseudomonas putida* mediates bacterial killing, biofilm invasion and biocontrol with a type IVB secretion system. *Nature Microbiology* 7: 1547–1557.

Raza W., Ling N., Yang L., Huang Q., Shen Q., 2016. Response of tomato wilt pathogen *Ralstonia solanacearum* to the volatile organic compounds produced by a biocontrol strain *Bacillus amyloliquefaciens* SQR-9. *Scientific Reports* 6: 24856.

Ritchie, 2000. Bacterial spot of pepper and tomato. *The Plant Health Instructor*. https://doi.org/10.1094/PHI-I-2000-1027-01.

Rosenblueth M., Martínez-Romero E., 2006. Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions* 19: 827–837.

Sahin F., Miller S.A., 1998. Resistance in *Capsicum pubescens* to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. *Plant Disease* 82: 794–799.

Sandhya V., Shrivastava M., Ali S.Z., Sai Shiva Krishna Prasad V., 2017. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russian Agricultural Sciences* 43: 22–34.

Santoyo G., Moreno-Hagelsieb G., del Carmen Orozco-Mosqueda M., Glick B.R., 2016. Plant growth-promoting bacterial endophytes. *Microbiological Research* 183: 92–99.

Saravanakumar D., Samiyappan R., 2007. ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. *Journal of Applied Microbiology* 102: 1283–1292.

Schaad N.W., Jones J.B., Chun W., 2001. *Laboratory Guide for the Identification of Plant Pathogenic Bacteria*. American Phytopathological Society (APS Press). USA, 373pp.

Sharma S., Bhattarai K., 2019. Progress in developing bacterial spot resistance in tomato. *Agronomy* 9: 26.

Shrestha A., Kim B.S., Park D.H., 2014. Biological control of bacterial spot disease and plant growth-promoting effects of lactic acid bacteria on pepper. *Biocontrol Science and Technology* 24: 763–779.

Stenberg J.A., Sundh I., Becher P.G., Björkman C., Dubey M., … Jonsson M., 2021. When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science* 94: 665–676.

Talibi I., Boubaker H., Boudyach E.H., Ait Ben Aoumar A., 2014. Alternative methods for the control of postharvest citrus diseases. *Journal of Applied Microbiology* 117: 1–17.

Tasleem M., El-Sayed A.-A.A.A., Hussein W.M., Alrehaily A., 2023. *Pseudomonas putida* metallothionein: structural analysis and implications of sustainable heavy metal detoxification in Madinah. *Toxics* 11: 864.

Tiwari S., Lata C., Chauhan P.S., Nautiyal C.S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiology and Biochemistry* 99: 108–117.

Townsend G., Heuberger J., 1943. Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter* 27: 340–343.

Turfan N., Düzel Ö., 2023. Investigation of Foliar L-Glutamic Application on the Resistance to the Capacity of the SC2121 Tomato Variety (*Solanum lycopersicum*) L.) to Long-Term Salinity Stress. *Yuzuncu Yıl University Journal of Agricultural Sciences* 33: 327–337.

Udaondo Z., Molina L., Segura A., Duque E., Ramos J.L., 2016. Analysis of the core genome and pangenome of *Pseudomonas putida*. *Environmental Microbiology* 18: 3268–3283.

Völksch B., May R., 2001. Biological control of *Pseudomonas syringae* pv. *glycinea* by epiphytic bacteria under field conditions. *Microbial Ecology* 41: 132–139.

Wahab A., Bibi H., Batool F., Muhammad M., Ullah S., ... Abdi G., 2024. Plant growth-promoting rhizobacteria biochemical pathways and their environmental impact: a review of sustainable farming practices. *Plant Growth Regulation* 104: 637–662.

Xin X.-F., Nomura K., Aung K., Velásquez A.C., Yao J., ... He S.Y., 2016. Bacteria establish an aqueous living space in plants crucial for virulence. *Nature* 539: 524–529.

Xue Y., Qiu T., Sun Z., Liu F., Yu B., 2022. Mercury bioremediation by engineered *Pseudomonas putida* KT2440 with adaptationally optimized biosecurity circuit. *Environmental Microbiology* 24: 3022–3036.

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

New opportunity for early on-site detection of *Plasmopara viticola* by qPCR assay

Roberta CORONELLI, Giuseppe INCAMPO, Davide CORNACCHIA, Flora SPATARO, Francesco FARETRA, Stefania POLLASTRO*, Donato GERIN

Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari 70126, Italy
*Corresponding author. Email: stefania.pollastro@uniba.it

Summary. *Plasmopara viticola,* the causal agent of grapevine downy mildew, is a widespread and significant plant pathogen. A quantitative PCR (qPCR) assay using a portable thermocycler was developed to enable rapid and early on-site detection of *P. viticola*. The internal transcribed spacer 1 (*ITS1*) region was selected as the target, and the specific primer pairs PLAV19 was designed. The assay was optimized using traditional thermocyclers, testing three different primer concentrations (100, 200, and 300 nM), and two annealing temperatures (58°C and 60°C). Optimal conditions were 200 nM primer concentration and an annealing temperature of 60°C. Under these parameters, the assay yielded a limit of detection (LoD) of 1.5 fg/µL and a limit of quantification (LoQ) of 15 fg/µL for *P. viticola* DNA (strain PLVDisspa1), showing consistent performance across both thermocyclers. Specificity tests confirmed no cross-reactivity with DNA from common grapevine-associated microorganisms, biocontrol agents, other Oomycetes and several grapevine cultivars. The PLAV19 primer set was further validated on DNA extracted from healthy, artificially inoculated, and naturally infected grapevine tissues, including samples exhibiting nonspecific leaf symptoms and latent bunch infections. Three DNA extraction protocols were evaluated to validate the extraction method, and one of these was shown to be suitable for on-field applications. The developed assay was a reliable diagnostic tool for the early detection and monitoring of *P. viticola* under field conditions, with potential applications in disease forecasting and sustainable management of grapevine downy mildew.

Keywords. Downy mildew, *Vitis vinifera*, SYBR Green, grapevine, *ITS1*, portable lab station.

OPEN ACCESS

Citation: Coronelli, R., Incampo, G., Cornacchia, D., Spataro, F., Faretra, F., Pollastro, S., & Gerin, D. (2025). New opportunity for early on-site detection of *Plasmopara viticola* by qPCR assay. *Phytopathologia Mediterranea* 64(2): 285-299. doi: 10.36253/phyto-16604

Accepted: August 21, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

ORCID:
RC: 0009-0003-6187-6735
GI: 0009-0008-6424-0667
DC: 0009-0004-4605-7640
FF: 0000-0003-1063-0607
SP: 0000-0001-8806-1905
DG: 0000-0002-6520-2311

INTRODUCTION

Grapevine (*Vitis vinifera L.*), a Eurasian species, is among the most extensively cultivated plants worldwide due to its high economic significance (McGovern *et al.,* 2017). Grapevine is highly susceptible to a broad spectrum of phytopathogens, including fungi, bacteria, viruses, viroids, and phytoplasmas. One of the major threats is the Oomycete *Plasmopara viticola* Berl. & de Toni, the causal agent of downy mildew. It can affect all the green parts of vines,

causing severe yield losses and a reduction in berries quality (Toffolatti *et al*., 2018). Additionally, berries affected less early have purplish to blackish, depressed ("push"), non-fruiting spots. In this case, the presence of " brown rot" on berries can be observed (Koledenkova *et al.,* 2022).

Several studies revealed that different varieties of grapevine show different levels of resistance and susceptibility to downy mildew (Heyman *et al*., 2021; Toffolatti *et al*., 2012). Generally, the American grapevine species, *Vitis riparia*, *V. rupestris*, *V. rotundifolia* and *V. amurensis* appear to be more resistant to the disease than *V. vinifera,* perhaps due to their longer coevolution with the pathogen (Boso *et al*., 2014). However, variation has been recorded also among different varieties of *V. vinifera*, and even clones of the same variety (Yu *et al*., 2012). Importantly, on resistant varieties nonspecific small necrotic areas can be observed as symptoms of *P. viticola* infections (Sotolář *et al*., 2007). Nevertheless, the pathogen can evolve more aggressive strains, potentially leading to the manifestation of non-specific symptoms in resistant plants, and progressively diminishing their resistance (Delmas *et al.,* 2016, Gouveia *et al*., 2024).

Studies conducted through the analysis of internal transcribed spacer 1 (*ITS*), β-tubulin (*TUB*), actin (*ACT*), and cytochrome b (*cytb*) on genetic characterization of *P. viticola* isolates collected from Canada and the United States led to the identification of four distinct intraspecific clades: 1) *riparia*, found on *V. riparia* and 'Chancellor', and interspecific hybrids; 2) *aestivalis*, found on *V. aestivalis, V. labrusca, V. vinifera* and interspecific hybrids; 3) *vinifera*, found on *V. aestivalis, V. cinerea, V. vulpine* and interspecific hybrids; 4) *quinquefolia*, found on *Parthenocissus quinquefolia* (Rouxel *et al*., 2013, 2014; Fontaine *et al*., 2021). These studies laid the foundation for identifying the clades of *P. viticola*, establishing it as a species complex. In addition to its genetic complexity, the epidemiology of *P. viticola* plays a critical role in the development and spread of the disease. Indeed, *P. viticola* can undergo multiple infection cycles within a single growing season, particularly under favourable environmental conditions such as high humidity, frequent rainfall, and moderate temperatures (Khatal *et al.,* 2023). Primary infections by *P. viticola* oospores result in the formation of sporangia containing zoospores, which are dispersed by rain splash onto the leaf surface, where they germinate developing hyphae colonizing the host tissue (Burruano *et al.,* 2000; Rossi *et al.,* 2008, 2013). Sporangia formed on infected tissues give rise to secondary infections. Consequently, the early and specific detection of *P. viticola*-complex is crucial for timely deployment of effective disease management strategies to avoid yield loss.

Several methods are available for detecting *P. viticola*. Traditional techniques, such as microscopic examination of infected leaves (Toffolatti *et al*., 2007), are limited by their inability to detect and quantify low concentrations of the pathogen. In contrast, molecular methods offer higher specificity and sensitivity, allowing even quantitative detection of the pathogen. Real-time PCR and digital droplet PCR (ddPCR) were developed for the detection of several plant pathogens, such as *Apiospora marii*, *Aspergillus carbonarius*, *A. niger*, *Erwinia amylovora*, *Monilinia* spp., *P. viticola* (syn. *Diaporthe ampelina*), *Verticillium dahliae* and *V. longisporum* (Valsesia *et al.,* 2005; Palumbo *et al*., 2016; Santander *et al*., 2019; Si Ammour *et al.,* 2020; Raguseo *et al*., 2021; Wang *et al*., 2022; Yang *et al.,* 2023; Agnusdei *et al*., 2024; Sánchez-Zelaia *et al*., 2024; Fedele *et al*., 2025; Incampo *et al*., 2025; Heger *et al*., 2025; Muthukumar *et al.,* 2025). In the case of *P. viticola*, they are mainly proposed for oospores quantification in diseased, senescent grapevine leaves (Si Ammour *et al*., 2020); for oospores and sporangia detection in soil, leaf litter, and asymptomatic leaf samples (Yang *et al*., 2023); for quantify the fungicide resistance (Huang *et al.,* 2023); for investigation the relationship between oospore density in vineyard leaf litter and primary infection incidence (Fedele *et al*., 2025), and for early detection and quantification of airborne inoculum of the pathogen in spore trap (Muthukumar *et al.,* 2025).

Despite the proven reliability of molecular techniques in providing both quantitative and qualitative information, their application typically requires laboratory equipment, trained personnel, and extended processing times. In recent years, various rapid pathogen detection methods have been developed, including lateral flow devices (Immuno Strip, Pocket Diagnostic), loop-mediated isothermal amplification (LAMP) (DeShields *et al*., 2018), and chlorophyll fluorescence (Chl-F) imaging (Rodríguez-Moreno *et al*., 2008). Specifically, *P. viticola* has been successfully detected using both LAMP (Marimuthu *et al*., 2020; Kong *et al*., 2016; Douillet *et al*., 2022) and Chl-F imaging (Cséfalvay *et al*., 2009).

While these methods offer advantages in terms of speed and user-friendliness, they are limited to providing high-quality results. Consequently, there is growing interest in the development of portable quantitative PCR (qPCR) systems that enable on-field diagnostics without compromising analytical accuracy and reliability. Several portable PCR protocols have already been validated for the detection of various plant pathogens (Koo *et al*., 2013; DeShields *et al*., 2018; Nguyen *et al*., 2018). The primary objective of this study was to design and validate a rapid, sensitive, and field-deployable real-time

PCR assay, employing SYBR Green fluorescence chemistry, for the on-field early detection of *P. viticola* in samples displaying typical and atypical symptoms.

MATERIALS AND METHODS

Pathogen isolate and growing conditions

The reference isolate of *P. viticola,* PLVDisspa1 originally isolated from grapevine leaves of 'Primitivo' was used to design the primer. Other fungal (*Alternaria alternata, Aspergillus carbonarius, Aspergillus niger, Botrytis cinerea, Cladosporium fulvum, Cylindrocarpon destructans* (syn. *Ilyonectria destructans*)*, Cytospora vitis, Diplodia seriata, Erysiphe necator*, *Eutypa lata, Neofusicoccum parvum, Penicillium expansum, Phaeomoniella chlamydospora, Phaeoacremonium aleophilum* (syn. *Phaeoacremonium minimum*)*, Phomopsis viticola* (syn. *Diaporthe ampelina*)*, Rosellinia necatrix* (syn. *Dematophora necatrix*), *Trichoderma asperellum*, *T. atroviride* and *T. gamsii*) and bacterial isolates (*Bacillus amyloliquefaciens*, *B. subtilis*, *Pseudomonas syringae* pv. *syringae* and *Xylella fastidiosa* subsp. *fastidiosa ST1*) commonly associated with grapevines as well as the Oomycetes *Phytophthora infestans* and *Pythium oligandrum* were used for specificity assay. All isolates were stored in 15% of glycerol at -80°C in the culture collection of our department except for *P. viticola* and *E. necator* that were inoculated on 'Baresana' fresh leaves placed on water agar medium (0.8% agar Oxoid No. 3 L^{-1}) incubated in a growth chamber at 25°C under a 16/8 h light/dark photoperiod and routinely refreshed. Other fungal and bacterial isolates were routinely grown at 25°C under darkness, respectively on potato dextrose agar (PDA: infusion from 200 g peeled and sliced potatoes kept at 60°C for 1 h, 20 g dextrose, adjusted at pH 6.5, and 20 g agar Oxoid No. 3 per liter) and nutrient agar (NA; 8 g nutrient broth and 20 g agar Oxoid No. 3 per liter) and PD3 (Pierce disease 3, Davis, 1980).

Designs for primer sets

To identify the most suitable region for primer design, the Internal Transcribed Spacer 1 (*ITS1*) region was the target selected. The *ITS1* sequence of the isolate PLVDisspa1 was aligned with 158 *ITS1* sequences from various *P. viticola* isolates available in GenBank (https://www.ncbi.nlm.nih.gov). Multiple sequence alignment was performed using Clustal Omega (version 1.2.2; https://www.ebi.ac.uk) to identify conserved regions shared across all isolates. These conserved regions were subsequently used to design a primer set using Primer3Plus version 3.3.0 (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi), following the guidelines outlined in the Droplet Digital PCR Application Guide (Bio-Rad, www.bio-rad.com, last accessed April 29th, 2025). The resulting primer set, designated PLAV19, was evaluated for in silico specificity using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast; Ye *et al.,* 2012) against the non-redundant nucleotide database. The selected primers were custom synthesized by Macrogen Inc. (Seoul, South Korea).

DNA extraction

Genomic DNA (gDNA) from PLVDisspa1 and *E. necator* was obtained by collecting mycelium directly from infected leaves, followed by extraction using the CTAB protocol. gDNA of the other fungal isolates was extracted from 3-day-old cultures grown on PDA overlapped by sterile cellophane disks (De Miccolis Angelini *et al.,* 2010). The DNA of *Bacillus* spp. and *P. syringae* pv. *syringae* was extracted from a cell pellet of 2 mL of nutrient broth (NA without agar) culture, obtained after 16 h of incubation at 25°C under shaking (200 rpm) (Nigro *et al.,* 2005). The DNA of different grapevine varieties was extracted from 500 mg of leaves after their homogenization in extraction bags (Bioreba AG, Reinach, Switzerland) with the Homex apparatus (Bioreba AG), using a CTAB based protocol (Doyle and Doyle, 1987). The DNA of *Xylella fastidiosa* subsp. *fastidiosa* ST1 was kindly supplied by the researchers of the DiSSPA SELGE Official Laboratory for quarantine plant pathogens. The DNA of *P. viticola* isolates from Lombardia was kindly supplied by S.L. Toffolatti (Department of Agricultural and Environmental Sciences, University of Milan, Italy). The quality and quantity of all DNA extracts was assessed using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA), while the concentration of double-stranded DNA (dsDNA) was specifically determined using a Qubit 2.0 fluorometer (Life Technologies Ltd., Paisley, UK).

qPCR assay optimization

For the optimization of the qPCR assay, the DNA extracted from the PLAVDisspa1 strain was used. Three distinct PLAV19 concentrations (100, 200, and 300 nM) and two annealing temperatures (58°C and 60°C) were evaluated. This optimization process was performed on the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA).

Each 20 µL reaction mixture contained 2× SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Labo-

ratories), one of three primer concentrations (100, 200, or 300 nM for each primer), 2 µL of DNA template, and ultrapure nuclease-free water to the final volume. The qPCR cycling conditions included an initial denaturation at 95°C for 3 min., followed by 35 cycles of denaturation at 95°C for 10 sec., annealing at 58°C or 60°C and extension at 72°C both for 30 seconds. A melting curve analysis was subsequently performed from 65°C to 95°C, with 0.5°C increments every 5 seconds, to assess the specificity of the PCR products. Amplification data were analysed using CFX™ Manager Software (version 3.1, Bio-Rad Laboratories). Key performance metrics, including the quantification cycle (Cq), amplification curve slope, reaction efficiency (E), relative fluorescence units (RFU), and coefficient of determination (R^2), were determined to evaluate the assay's consistency and reliability under the tested conditions. The optimization reactions were conducted in biological triplicates to ensure reproducibility, and a no-template control (NTC), consisting of ultrapure sterile water, was included in each run to monitor for potential contamination and nonspecific amplification. Cq values were determined using the baseline settings automatically assigned by the instrument. The baseline was defined over the initial cycles where no significant increase in fluorescence signal was observed, thereby excluding background noise. Resulting Cq values from technical triplicates were averaged to represent each biological replicate, and these averages were used for all subsequent statistical analyses. The resulting Cq values were exported to Excel (version 2021, Microsoft Corp., Redmond, WA, USA) for construction of the calibration curve.

Sensitivity and specificity of detections using a conventional and a portable thermocycler

The sensitivity of the qPCR assays was assessed using PLVDisspa1 DNA at an initial concentration of 1.5 ng/µL, as measured by Qubit 2.0 fluorometer (Life Technologies Ltd., Paisley, UK) with the dsDNA BR Assay kit (Thermo Fisher Scientific Inc.), followed by seven10-fold serial dilutions down to 150 ag/µL. A linear regression analysis was performed by plotting the Log_{10} of DNA concentration against both the corresponding Cq values obtained from qPCR performed using CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and Hyris bCUBE, a compact and portable real-time PCR (Generon S.p.a., Modena, Italy), in order to evaluate assay performance under both laboratory and field-compatible conditions. From this analysis, the coefficient of determination (R^2) and the slope were calculated. To assess the analytical sensitivity, the limit of detection (LoD) and limit of quantification (LoQ) were determined based on ten replicate measurements. Precision was further evaluated by performing the assay on the same samples across independent runs on different days, allowing assessment of both intra- and inter-assay variability.

Specificity of the PLAV19 primer was evaluated using DNA from a broad range of grapevine-associated pathogens, biocontrol agents other *P. viticola* isolates collected from different locations across the Apulia, Calabria and Lombardia regions, and the Oomycetes *P. infestans* and *P. oligandrum*. DNA from the *V. vinifera* cultivars 'Gaglioppo', 'Greco Bianco', 'Negroamaro', 'Nero d'Avola', 'Primitivo', 'Red Globe', 'Sugar Crisp', 'Sugar One', and 'Timco' was also used. In all amplifications, the reference strain PLVDisspa1 was used as positive control (PC) and ultrapure water as NTC.

Methods for validation of in-field DNA extractions

Three DNA extraction methods were compared. The CTAB based method (1) (Doyle and Doyle (1987), was used as laboratory control, while the other two methods (2 and 3) herein set up were carried out using the portable laboratory for qPCR (Generon S.p.a.), including specific equipment for extraction (centrifuge, dry bath, vortex) and the portable real-time Hyris bCUBE.

The protocol 2 was a chloroform-based method, in which a total of 500 mg of grapevine tissue was homogenized in extraction bags using a manual homogenizer (Bioreba AG) and 5 mL of CTAB extraction buffer. The samples were incubated for 15 min at 65°C in a heat block. Subsequently, 1 mL of chloroform was added, followed by centrifugation at 7,200 g for 5 min. The supernatant was carefully collected, and 0.6 volumes of cold isopropanol were added to precipitate nucleic acids. The mixture was incubated on ice for 5 min and then centrifuged for 10 min at 7,200 g. The resulting pellet was resuspended in 20 µL of nuclease-free water (Qiagen, Venlo, The Netherlands). The protocol 3 was performed following the same initial steps as previously described, homogenizing 500 mg of plant tissue in Bioreba extraction bags (Bioreba AG) using CTAB buffer and incubating the samples for 15 min at 65°C in a heat block. Subsequently, 1 mL of isopropanol was added to the lysate, followed by centrifugation at 7,200 g for 10 min. The supernatant was discarded, and the pellet was resuspended at 100 µL in nuclease-free water.

Validation of the molecular diagnostic method

The validation of the molecular assay was performed using DNA extracted from: i) leaf samples from artificial

inoculation; ii) leaf and bunch samples natural infected; iii) a homemade siliconized sampling tape for trap spore artificially contaminated with PLVDisspa1 sporangia; iv) leaves with atypical symptoms and berries with "brown rot" symptoms from the field; v) leaves with necrotic spots bordered of a purple line never associated to downy mildew. DNA was extracted by using all three protocols.

For the artificial contamination, symptomatic leaves were used to collect the sporangia, which were counted and diluted up to 10^6 sporangia mL^{-1}. Subsequently, asymptomatic leaves were collected, using a sterile blade, from vines cuttings maintained in greenhouses of our department and 50 mg aliquots were transferred into 2 mL Eppendorf tubes. Ten-fold dilutions up to 10^0 sporangia mL^{-1} were prepared, and 1 mL of each concentration was added to 50 mg of health leaf tissue. The homemade siliconized sampling tape (1x2 cm) for trap spore was also artificially contaminated with 80 µL of each sporangial suspension of 10-fold serial dilution (10^6 to 10^0) and allowed to dry before DNA extraction. To assess the potential interference of the sample matrix to which sporangia were added, control reactions were performed using sporangia pure extracts. While to evaluate the impact of inhibitory substances on the qPCR reaction, the final three dilutions (10^2, 10^1, and 10^0) were spiked into unripe grape samples, known to be rich in polyphenolic compounds, and subsequently subjected to extraction using the three methods. The coefficient of variation (CV) of the reactions was calculated. Leaves of cultivar Primitivo (Bari, Apulia, Italy), showing unspecific necrotic spots, and bunches of cultivar Italia (Canosa di Puglia, Apulia, Italy), showing brown rot symptoms, were collected in June 2025. Finally, the gDNA was extracted and amplified with PLAV19 using CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and Hyris bCUBE qPCR thermocycler (Generon S.p.a.).

To ensure the reliability of the protocol, both repeatability and reproducibility were evaluated. Repeatability was assessed by performing three measurements on the same sample by a single operator using the same instrument. Reproducibility was evaluated by performing the same measurements with two different operators using two equivalent instruments. For both assessments, the CV was calculated.

Statistical analysis

The Cq values obtained from the qPCR optimization tests were analysed using a two-way ANOVA (Costat software, Cohort, Monterey, CA, USA) to evaluate the effects of primer concentration (100, 200, and 300 nM) and annealing temperature (58°C and 60°C). Additionally, a separate two-way ANOVA was conducted to determine the influence of these two factors on relative fluorescence units (RFUs). To evaluate the agreement between the Cq values obtained from the CFX96™ Real-Time PCR Thermal Cycler and the Hyris bCUBE, a Pearson correlation analysis was performed using the same software. Finally, a one-way ANOVA was carried out to evaluate statistical differences in Cq values among the three DNA extraction methods.

RESULTS

Generation of primer sets

Assessing the inter-isolate variability among all *ITS1* sequences of *P. viticola* available in GenBank (accessed on April 28th, 2025), a region was identified allowing the design of the primer set (Table 1). In detail, considering the partial *ITS1* sequence of *P. viticola* as reference (NCBI Accession No.: JF897782.1), the PLAV19 set includes the region from base 126 to base 261 (Table 1).

The results of the *in-silico* specificity analysis of the primers showed 100% identity with *P. viticola* sequences, including those from other countries such as Australia (MG552098.1), Brazil (MH310113.1), China (KM279691.1), India (ON183962.1), and the United States (MK345987.1).

The *in silico* specificity was proved against the Non-Redundant Nucleotide sequence database and in deep detail against the main grape-associated microorganisms, such as *A. alternata*, *Aspergillus* spp., *Bacillus* spp., *B. cinerea*, *C. fulvum*, *C. destructans*, *C. vitis*, *D. seriata*, *E. lata*, *N. parvum*, *P. expansum*, *P. chlamydospora, P. minimum, P. viticola, P. syringae, R. necatrix, Trichoderma* spp., *E. necator,* and *X. fastidiosa* subsp. *fastidiosa*, the other Oomycetes *P. infestans* and *P. oligandrum*, and grapevine.

Optimization of qPCR assays

The results of the qPCR optimization using the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) are presented in Figure 1.

Table 1. Primer set generated with this study.

Set	Primer		Amplicon size (bp)
	Name	Sequence (5'-3')	
PLAV19	Forward	GTAGCTTACCCTGCACCAC	136
	Reverse	TCTTCATCGATGCCAGAACC	

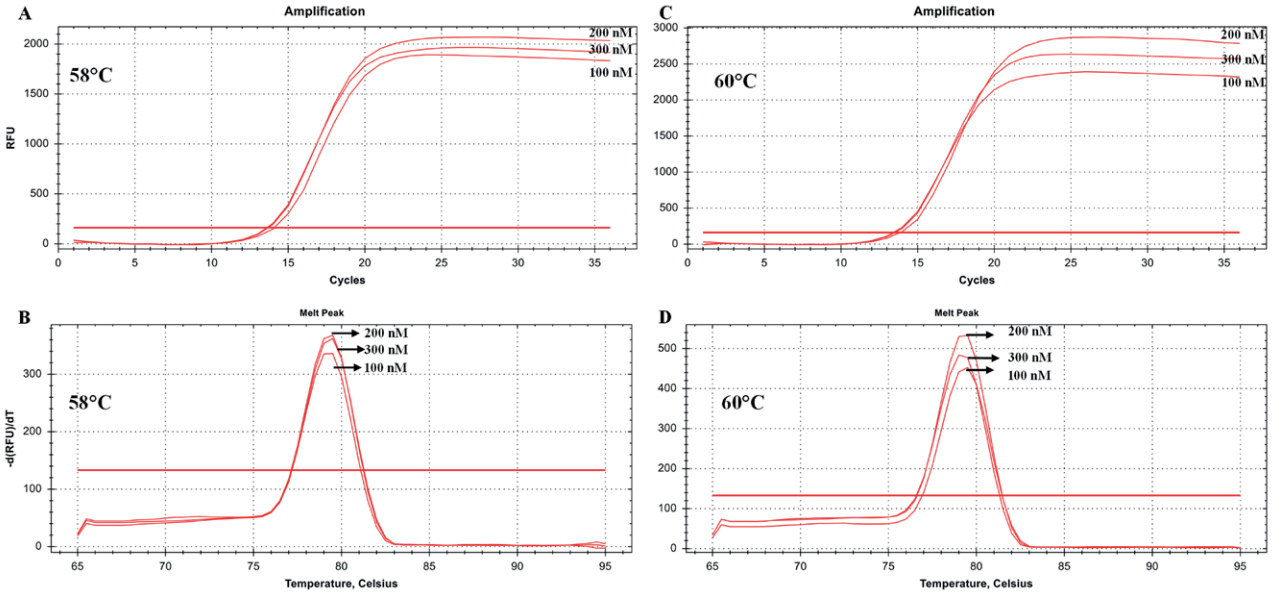


Figure 1. Optimization of qPCR conditions; in A 58°C of annealing temperature and three concentrations (100, 200, and 300 nM, in C the same concentrations at annealing temperature of 60°C. Panels B and D show the respective melt curves corresponding to the different temperatures and concentrations tested. For each tested conditions, was reported the mean of three biological replicate.

The Cq (quantification cycle) values remained consistent across all tested primer concentrations (F = 5.24x10^{-29}, p = 1.000) and annealing temperatures (ANOVA, F = 0.102 p = 0.904), with no statistically significant differences.

In contrast, analysis of RFUs revealed notable differences. At an annealing temperature of 58°C, the highest fluorescence was observed with a primer concentration of 200 nM, yielding an average RFU of approximately 2,000 (Figure 1A). At 60°C, RFU values increased further, up to 2,900 (Figure 1C). A two-way ANOVA confirmed statistically significant effects of both temperature (F = 555.17, p < 0.0001) and primer concentration (F = 129.96, p < 0.0001) on RFU values. Moreover, a significant interaction between temperature and concentration was observed (F = 9.70, p = 0.0031), indicating that the impact of primer concentration on RFU was temperature dependent. Based on these findings, the optimal qPCR conditions were determined to be a primer concentration of 200 nM and an annealing temperature of 60°C. Across all experimental conditions, the melting peak was consistently observed at 79.5°C, confirming the high specificity of the amplification reaction (Figure 1 B and D).

Sensitivity and specificity assays

A 10-fold serial dilution of PLVDisspa1 DNA, ranging from 1.5 ng μL^{-1} to 150 ag μL^{-1}, was tested using the PLAV19 primer set in qPCR assays (Figure 2).

On the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories), PLAV19 successfully detected DNA concentrations as low as 1.5 fg μL^{-1}, corresponding to a Cq value of 33.5. Across the dynamic range of 1.5 ng μL^{-1} to 1.5 fg μL^{-1}, the assay exhibited excellent linearity (R^2 = 0.998), with a slope of -3.375, corresponding to a mean PCR efficiency of 97.9%. Similarly, the Hyris bCUBE (Generon S.p.a.) demonstrated the capability to detect 1.5 fg μL^{-1} with a Cq value of 32.0. Within the same dynamic range, this platform also displayed strong linearity (R^2 = 0.992) and a slope of -3.478, equivalent to a PCR efficiency of 93.6%. Notably, the Hyris bCUBE (Generon S.p.a.) yielded slightly earlier Cq values at higher DNA concentrations. For instance, a concentration of 1.5 ng μL^{-1} was detected at a Cq of 11, compared to 13.5 on the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories). Despite this difference in amplification kinetics, the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) exhibited a light higher linearity.

The strong positive correlation observed between Cq values from the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and the Hyris bCUBE (Generon S.p.a.) (Pearson's r = 0.99, p = <0.001) highlights the comparable performance and high reproducibility of the two qPCR thermocyclers.

The analytical sensitivity of the assays was determined by calculating the LoD and LoQ. The LoD was

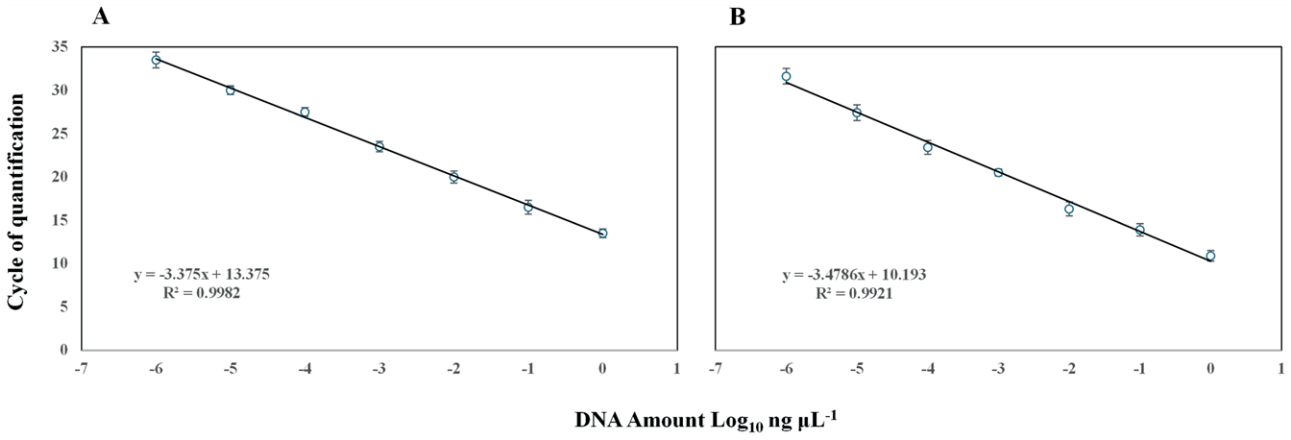


Figure 2. Linear regression analysis obtained from qPCR assays performed with different concentrations of PLVDisspa1 DNA, amplified using the PLAV19 primer. Panel A shows results from the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories), while Panel B shows results from the Hyris bCUBE system (Generon S.p.a.).

determined empirically as the lowest concentration at which the target was reliably distinguishable from the background signal and was found to be 1.5×10^{-6} (1.5 fg) for both the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and the Hyris bCUBE (Generon S.p.a.) platforms. The LoQ was calculated from ten replicate measurements at low concentrations, with all replicates exhibiting a CV below 20%, resulting in a LoQ of 1.5×10^{-5} (15 fg). This criterion ensures that concentrations at or above the LoQ can be quantified with acceptable precision and accuracy. Precision was further evaluated through intra- and inter-assay analyses. Intra-assay precision was assessed by measuring replicates of the same sample within a single run, yielding CV of 1%. Inter-assay precision was determined by comparing measurements of the same sample across independent runs performed on different days, yielding a CV of 3%. These results confirm that the assay is reproducible and robust, providing reliable quantitative measurements across the tested concentration range.

Specificity testing for both qPCR assays was performed using DNA at a concentration of 20 ng μL^{-1} from a range of fungal and bacterial species, as well as various grapevine cultivars. The results of the specificity assays were conducted on both the thermocyclers confirmed that no reaction occurred using the DNA from the panel of microorganisms different from *P. viticola* and grape genotypes (Table 2).

Reference DNA PLVDisspa1 yielded Cq values of 13.5 and 11.0 for CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and Hyris bCUBE (Generon S.p.a.), respectively. Similarly, the other 9 *P. viticola* isolates yielded Cq in the range 13.1-13.8 for CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and in the range 10.7-11.3 by using Hyris bCUBE thermocycler (Generon S.p.a.) Table 2.

Extraction protocol

Three protocols were compared. Four criteria were considered: solvents required, the total nucleic acid yield, the double-stranded DNA (dsDNA) concentration, and overall processing time. The classical CTAB method (1) Doyle and Doyle (1987) was used as reference. For protocol 2, the extraction involved several solvents, including CTAB, chloroform, and isopropanol. The total time required to extract DNA from a single sample was approximately 37 minutes, broken down as follows: 2 minutes for sample disruption using the Bioreba homogenizer (Bioreba AG), 15 minutes of incubation, 5 minutes of chloroform treatment, 5 minutes at –20°C with isopropanol, and 10 minutes of centrifugation. The total nucleic acid yield was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc.), while the concentration of double-stranded DNA (dsDNA) was specifically determined using a Qubit 2.0 fluorometer (Life Technologies Ltd.). The results, summarized in Table 3, showed a total nucleic acid concentration of 350 ng μL^{-1} and a dsDNA concentration of 22 ng μL^{-1}. In the protocol 3, the use of solvents was lower, as it involved only CTAB and isopropanol. The DNA extraction process was faster, requiring a total of 27 minutes, distributed as follows: 2 minutes for sample disruption using the Bioreba homogenizer (Bioreba AG), 15 minutes of incubation, and 10 minutes of centrifugation in isopropanol. The total nucleic acid concentration was 200 ng μL^{-1}, while the double-stranded DNA (dsDNA)

Table 2. Panel of microorganism used for specificity assay.

Species	Isolate/ variety	Cq	
		1	2
Plasmopara viticola	PLVDisspa1	13.5 ± 0.5	11.1 ± 0.3
	PLV_puglia1	13.2 ± 0.4	10.7 ± 0.3
	PLV_puglia2	13.5 ± 0.5	11.1 ± 0.2
	PLV_puglia3	13.8 ± 0.6	11.3 ± 0.4
	PLV_calabria1	13.6 ± 0.3	11.1 ± 0.3
	PLV_calabria2	13.2 ± 0.6	10.8 ± 0.3
	PLV_calabria2	13.1 ± 0.6	10.8 ± 0.3
	CAS_A_6(PN)	13.4 ± 0.6	10.9 ± 0.3
	GH_NO	13.5 ± 0.5	11.1 ± 0.3
	LONG_A2(VI)	13.2 ± 0.4	10.7 ± 0.3
Bacterial isolates			
Bacillus amyloliquefanciens	D747_Disspa	-	-
Bacillus subtilis	QST713	-	-
Pseudomonas syringae pv. *syringae*	Pssy_1	-	-
Xylella fastidiosa subsp. *fastidiosa*	ST1	-	-
Fungal isolates			
Alternaria alternata	Alt_Disspa		-
Aspergillus carbonarius	Ac9_Disspa	-	-
Aspergillus niger	An2_Disspa	-	-
Botrytis cinerea	SAS 56_Disspa	-	-
Cladosporium fulvum	Cladsp2	-	-
Cylindrocarpon destructans	Cd_2_Disspa	-	-
Cytospora vitis	Cv1_Disspa	-	-
Diplodia seriata	Ds_Disspa	-	-
Erysiphe necator	EN_1_Disspa	-	-
Eutypa lata	EL_1_Disspa	-	-
Neofusicoccum parvum	DiSSPA_NP1	-	-
Penicillium expansum	Pspp1	-	-
Phaeomoniella chlamydospora	Pch1_Disspa	-	-
Phaeoacremonium aleophilum	PcAl_1_Disspa	-	-
Phytophthora infestans	Phy_1	-	-
Pythium oligandrum	M1	-	-
Phomopsis viticola	PhomV_1_Disspa	-	-
Rosellinia necatrix	Rn_1_Disspa	-	-
Trichoderma asperellum	ICC012	-	-
Trichoderma atroviridae	SC1	-	-
Trichoderma gamsii	ICC080	-	-
***Vitis* varieties**			
Vitis vinifera	Gaglioppo	-	-
	Greco Bianco	-	-
	Negramaro	-	-
	Nero d'Avola	-	-
	Primitivo	-	-
	Red Globe	-	-
	Sugar Crisp	-	-
	Sugar One	-	-
	Timco	-	-
Vitis rupestris × *Vitis berlandieri*	140 RU	-	-
Vitis berlandieri × *Vitis rupestris*	1103	-	-

1, CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories); 2, Hyris bCUBE (Generon S.p.a.).

Table 3. Comparison of solvents, time and amount of DNA extracted by three methods.

Extraction protocols	Solvents	Time (minutes)	Total nucleic acid (ng μL^{-1})	dsDNA (ng μL^{-1})
1	CTAB, Chloroform, Isopropanol	110	750 ± 10	65 ± 6
2	CTAB, Chloroform, Isopropanol	37	350 ± 8	22 ± 5
3	CTAB, Isopropanol	27	200 ± 5	16 ± 3

*Values are presented as mean ± standard error (SE) of three independent biological replicates, each measured in triplicate. SE reflects variability only among biological replicates and does not include technical variation.

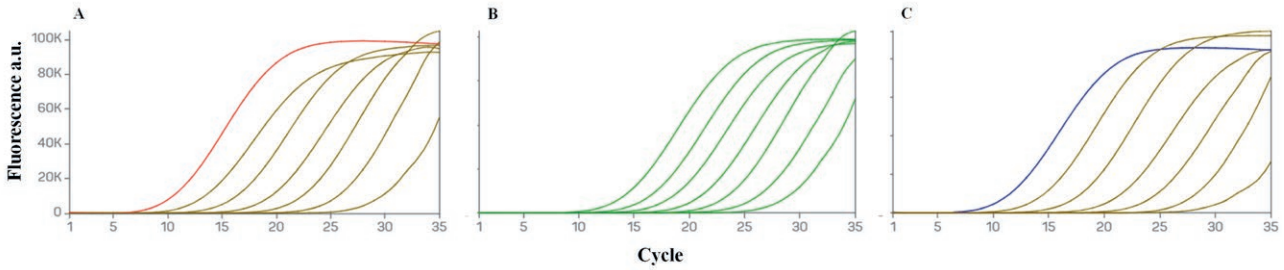


Figure 3. Validation of PLAV19 results of different sporangia suspension 10^6 to 10^0 added to leaves, extracted with protocol 1 (A), protocol 2 (B) and protocol 3 (C).

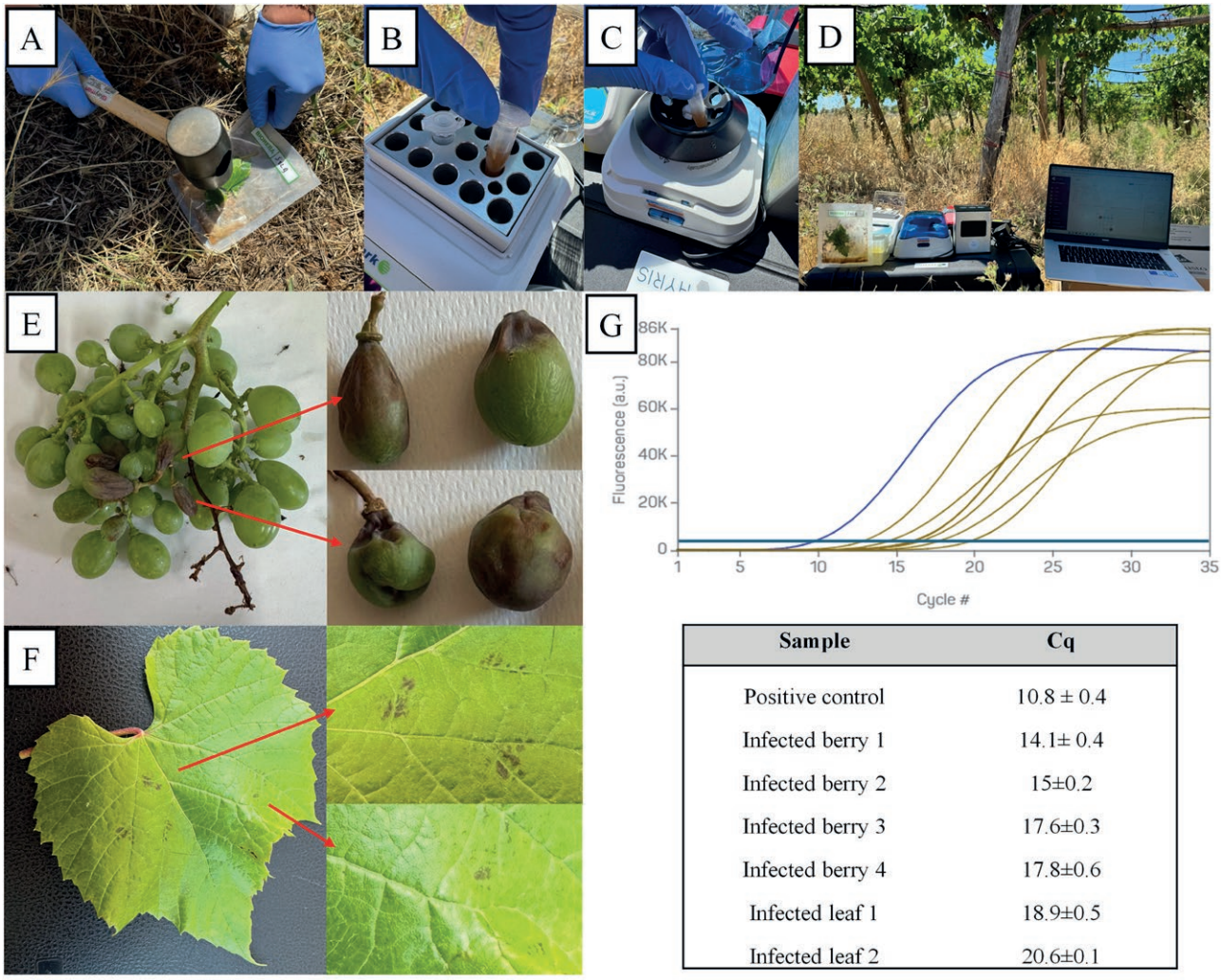
concentration was 16 ng μL^{-1}. For comparison, the control protocol (CTAB) yielded a total nucleic acid concentration of 750 ng μL^{-1} and a dsDNA concentration of 65 ng μL^{-1}.

Validation of the molecular diagnostic

To validate the diagnostic method, an initial evaluation was carried out by amplifying DNA extracted from grapevine leaves artificially inoculated with known quantities of *P. viticola* sporangia. The assay was performed using the portable thermocycler Hyris bCUBE (Generon S.p.a.) to determine the limit of detection (LoD) for sporangia, comparing the performance of the three DNA extraction protocols.

The results showed that the quantification cycle (Cq) values were consistent across all extraction methods (Figure 3). Notably, each protocol achieved a detection limit of 10^0 sporangia, indicating equivalent sensitivity regardless of the extraction protocol employed. Statistical analysis confirmed the absence of significant differences among the protocols ($F = 0.020$, $P = 0.980$), supporting the reliability and comparability of the three extraction protocols for the sensitive detection of *P. viticola* sporangia. Furthermore, analysis of CV was performed to assess the potential interference of sample matrices. Compared to the controls, all three extraction methods showed a CV of approximately 2%, confirming the absence of significant interference from components external to the DNA of *P. viticola* sporangia. Similarly, testing the potential impact of polyphenolic compounds from unripe grapes yielded comparable CV values (~2%) across all three methods.

These results enabled the selection of the most rapid DNA extraction method (3) suitable for early in-field detection of *P. viticola*, optimizing both efficiency and practicality for field applications. Similar results were noted for DNA extracted from strips added with different concentrations of sporangia. PLAV19 was capable of detecting up to 10^0 sporangia on the strip. Moreover, during the validation phase using samples collected from various vineyards across Apulia, Calabria and Lombardia regions, the assay demonstrated robust performance. Notably, it successfully detected *P. viticola* in leaf samples exhibiting unspecific symptoms of downy mildew, as well as in berries with brown rot (Figure 4 E and F). Figure 4 G illustrates the corresponding Cq values obtained from field-collected samples, further confirming the assay's sensitivity and reliability under real-world conditions. Leaves with necrotic spots bordered by purple line, a smptoms never associated to downy mildew, confirmed to be always negative to the test. Furthermore, method repeatability and reproducibility were assessed by calculating the CV%. The CV for repeatability was around 1%, while for reproducibility it was approximately 3%, indicating very low variability and confirming the robustness of the method.



Sample	Cq
Positive control	10.8 ± 0.4
Infected berry 1	14.1± 0.4
Infected berry 2	15±0.2
Infected berry 3	17.6±0.3
Infected berry 4	17.8±0.6
Infected leaf 1	18.9±0.5
Infected leaf 2	20.6±0.1

Figure 4. Validation assay of PLAV19 with naturally infected samples. From A to D steps are carried out in the field. In E symptoms on berries (brown rot); in F unspecific symptoms on leaves. In G the corresponding Cq values detected in qPCR assay with Hyris bCUBE (Generon S.p.a.) thermocycler on berries and leaves.

DISCUSSION

This study focused on the development of a portable SYBR Green-based qPCR molecular diagnostic assay for the on-site detection of *P. viticola* and to our knowledge it is the first for downy mildew detection and quantification in grapevines.

The PLAV19 primer set, targeting specifically the *ITS1* region of *P. viticola* within the base 126 to 261, was designed and although statistical analysis revealed no significant differences in quantification cycle (Cq) values across the tested conditions, significant differences in RFUs were observed in relation to both primer concentrations and temperatures resulting 200 nM primer concentration and 60°C the annealing temperature the best condition to be used.

Both *in-silico* analysis and *in vivo* specificity assay confirmed the reliability and usability of the primer set PLAV19, enlarging so the ones available for *P. viticola* detection and quantification. Their specificity was proved also for additional grapevine cultivars ('Gaglioppo', 'Greco Bianco', 'Negroamaro', 'Nero d'Avola', 'Primitivo', 'Red Globe', 'Sugar Crisp', 'Sugar One', and 'Timco') as well as for other important pathogens of grapes such as *A. carbonarius* responsible for Ochratoxin A contamination of wine (Pollastro *et al.*, 2005) and fungi reported as Grapevine Trunk diseases causal agents (Gramaje *et al.*, 2018). The PLAV19 specificity was proved for the first time also against important bacterial pathogens i.e. *X. fastidiosa* subsp. *fastidiosa* (Cornara *et al.*, 2025) and *P. syringae* pv. *syringae* (Gerin *et al.*, 2019), as well as several biocontrol agents currently

applied against Grapevine Trunk Disease (*Trichoderma* spp.), bunch roots (*Bacillus* spp.), and downy mildew (*P. oligandrum*). All reactions were terminated at 35 Cq to achieve an optimal balance between sensitivity, specificity, and rapid processing time suitable for field monitoring applications.

DNA extraction methods recover a crucial role into a qPCR assay. Indeed, if the quality and quantity of DNA extraction was low, the qPCR can yield false negative results, since the quantity of possible inhibitors can alter sensibility in the qPCR assays (Nourrisson *et al.,* 2020). In this study, three DNA CTAB-based extraction methods were evaluated. Two simplified CTAB-based methods herein proposed for the first time were compared to the reference protocol detailed by Doyle and Doyle (1987) with the aim of identify the most suitable in the balancing the following four criteria: solvents required, total nucleic acid yield, double-stranded DNA (dsDNA) concentration, and overall processing time, respect to the performance characteristics as detailed in EPPO PM7/98 and PM7/7 (2021; 2024). As expected, the standard CTAB method yielded the highest concentrations of total nucleic acids and of dsDNA but required multiple handling steps, laboratory-specific materials, and the use of solvents, such as chloroform particularly and isopropanol. Unsing method 2 in which the modification affects the processing time (37 minutes) a halved amount of total nucleic acid and a third amount of dsDNA was obtained, but non-beneficial in respect to the solvents used. In protocol 3, the main modification consists in removal the chloroform step by adding isopropanol directly to the lysate and removing it by a 10 min-centrifugation. It proved to be the most suitable for field applications (less than 30 minutes) and the most ecofriendly avoiding the use of the potential carcinogenic solvent chloroform. Additionally, it is chipper comparing the commonly used total DNA extraction procedures CTAB- or Kit- based and proposed in qPCR for *P. viticola* sporangia, oospores and mycelium detection in different matrices as leaves, litter, air and spore-trap (Valsesia *et al*., 2005; Piccolo *et al*., 2012; Si Ammour *et al*., 2020; Huang *et al*., 2023; Yang *et al*., 2023; Fedele *et al*., 2025; Muthukumar *et al*., 2025). The total amount of DNA and dSDNA resulted in about a quarter of the reference protocol, resulting in quite aligned with the concentration commonly used in qPCR for *P. viticola* and so not requiring the additional step of DNA dilution usually made in lab (i.e. Fedele *et al*., 2025). Two different extraction methods from grape leaves (Xin *et al*., 2003) and from air samples collected by the spore trap (Rogers *et al*., 2009) were proposed by Kong *et al*. (2016) in *P. viticola* LAMP based detection, with the first one certainly easy to be applied on-site and the second one longer and requiring lab-equipment. Also, Marimuthu *et al*. (2020) extract DNA according to the long-used CTAB protocol proposed by McDermott *et al*. (1994) confirming several limitations to the on-field application. Differently, a simple rapid DNA extraction procedure based on cell lysis in potassium hydroxide (KOH), was compared with the CTAB protocol described in Douillet *et al*. (2022) in both LAMP and ddPCR. In our work, two different Thermal cyclers the CFX96™ Real-Time PCR Thermal Cycler (Bio-Rad Laboratories) and the portable Hyris bCUBE (Generon S.p.a.) never used in the qPCR reported for *P. viticola* detection and quantification were compared. Both systems demonstrated high linearity, with R^2 values of 0.998 and 0.992, respectively, across a dynamic range from 1.5 ng μL^{-1} to 1.5 fg μL^{-1}, resulting better performing to the LAMP and ddPCR (Douillet *et al*., 2022). The analytical sensitivity expressed as limit of detection (LoD) for both thermocyclers was established at 1.5 fg μL^{-1}, corresponding to mean Cq values of 33.5 for the CFX96™ and 32.0 for the Hyris bCUBE. No potential unspecific target until the 35 Cq were also observed. The Cq values obtained using the CFX96™ and the Hyris bCUBE showed a Pearson's r = 0.99 (p <0.001) indicating an extremely strong and statistically significant positive correlation between the results obtained with the two thermal-cyclers. The LoD value is on average 10 times lower than that reported for LAMP based portables (Marimuthu *et al*., 2020; Kong *et al.,* 2016; Douillet *et al*., 2022) being so the technique more sensible for pathogen detection on-field. The LoQ was calculated at 15 fg, these findings are consistent with the sensitivity levels reported for other qPCR assays developed for the detection of fungal pathogens such as *Monilia* spp. and *Verticillium* spp. (Raguseo *et al*., 2021; Wang *et al*., 2022), and also for the oomycete *P. viticola* (Valsesia *et al.,* 2005; Si Ammour *et al*., 2020; Yang *et al*., 2023; Huang *et al*., 2023; Fedele *et al*., 2025; Muthukumar *et al*., 2025) but to our knowledge it is the first time for a portable qPCR for this pathogen. Moreover, the CV were examined to assess whether the presence of sample matrices could interfere with the qPCR assays. Across all three extraction methods, the CV remained around 2% relative to the controls, indicating that components external to the *P. viticola* sporangia DNA did not significantly affect the reaction. Similarly, the inclusion of polyphenolic-rich unripe grape tissue did not alter the CV, which remained consistently low (~2%), further supporting the robustness of the extraction methods against potential matrix-related inhibition. Overall, precision evaluated through both intra- and inter-assay analyses yielded CVs below 20%, confirming that the proposed

assay is reproducible and robust, providing reliable quantitative measurements across the tested concentration range. The demonstrated high specificity, combined with a detection sensitivity of 1.5 fg (Cq 32), ensures excellent assay performance. Stopping amplification at Cq 35 led to complete the reaction in 75 min improving the on-field portability of the assay. Considering the performance herein obtained using the DNA extracts with the three methods in terms of analytical sensitivity, analytical specificity and risks of false-positive and false-negative no statistical differences in the qPCR results were observed. Consequently, the protocol identified as protocol 3 is proposed for on-site applications.

The availability of portable diagnostic methods represents a valuable opportunity to accelerate pathogen monitoring and support more effective and sustainable plant disease management strategies.

For *P. viticola*, the use of molecular tools enables the early detection of airborne inoculum such as sporangia, oospores and mycelium and is also proposed for monitoring population variability in terms of fungicide resistance (Massi *et al*., 2021). This early warning capability is crucial for implementing targeted proactive and reactive protective measures, thereby minimizing the impact of the disease on vineyards, the reliance on chemical treatments and consequently reducing the risk of acquire resistance to fungicides. The molecular method herein developed can be integrated with existing forecasting models to enhance the accuracy of *P. viticola* prevention strategies (Puelles *et al*., 2024). Our results lay the foundation for the implementation of reliable and portable diagnostic tools, offering an effective solution for real-time *P. viticola* monitoring also integrating spore trap air sampling and so providing real evidence of pathogen presence essential for verifying model accuracy, calibration, and updating. Additionally, the portable qPCR herein presented proved to be applied on necrotic non sporulated spots frequently detected on some resistant grapevine genotypes in Apulian and Calabrian vineyards, confirming the usefulness of this device in detection and quantification of this important Oomycete also in unusual conditions, and resulting useful at evaluating the risk of infection and of overcoming genetic resistance in the new grapevine genotypes. These findings corroborate the requiring of knowledge in these new scenarios opening new epidemiological questions.

FUNDING

This work was partially carried out in the framework of the projects Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

ACKNOWLEDGMENTS

Roberta Coronelli is a PhD candidate at the University of Bari granted by UE under the Program “DM 352/2022 innovative doctoral programs with industrial connotation”, Project Code PID2021-126005OBI00 (MCIN/AEI/10.13039/501100011033/FEDER, UE). CUP H91I22000070007. The grant was co-funded by Sysman Progetti & Servizi Srl.

LITERATURE CITED

Agnusdei A., De Miccolis Angelini R. M., Faretra F., Pollastro S., … Gerin D., 2024. *AcOTApks* Gene-Based Molecular Tools to Improve Quantitative Detection of the Mycotoxigenic Fungus *Aspergillus carbonarius*. *Foods* 14 (1): 65. https://doi.org/10.3390/foods14010065

Boso S., Alonso-Villaverde V., Gago, P., Santiago J. L., … Martínez M. C., 2014. Susceptibility to downy mildew (*Plasmopara viticola*) of different Vitis varieties. *Crop Protection* 63: 26–35. https://doi.org/10.1016/j.cropro.2014.04.018

Burruano S., 2000. The life cycle of *Plasmopara viticola*, cause of downy mildew of vine. *Mycologist* 14(4): 179–182. https://doi.org/10.1016/S0269-915X(00)80040-3

Cornara D., Boscia D., D’Attoma G., Digiaro M., Ligorio A. M., … Saponari M., 2025. An integrated strategy for pathogen surveillance unveiled *Xylella fastidiosa ST1* outbreak in hidden agricultural compartments in the Apulia region (Southern Italy). *European Journal of Plant Pathology* 171(2): 277–285. https://doi.org/10.1007/s10658-024-02945-7

Cséfalvay L., Di Gaspero G., Matouš K., Bellin D., Ruperti B., … Olejníčková J., 2009. Pre-symptomatic detection of *Plasmopara viticola* infection in grapevine leaves using chlorophyll fluorescence imaging. *European Journal of Plant Pathology* 125: 291–302. https://doi.org/10.1007/s10658-009-9482-7

Davis M. J., 1980. Isolation Media for the Pierce’s Disease Bacterium. *Phytopathology* 70: 425. https://doi.org/10.1094/Phyto-70-425

De Miccolis Angelini R. M., Habib W., Rotolo C., Pollastro S., … Faretra F., 2010. Selection, characterization

and genetic analysis of laboratory mutants of *Botryotinia fuckeliana* (*Botrytis cinerea*) resistant to the fungicide boscalid. *European journal of plant pathology* 128: 185–199. https://doi.org/10.1007/s10658-010-9643-8

Delmas C. E., Fabre F., Jolivet J., Mazet I. D., Richart Cervera S., Deliere L., ... Delmotte F., 2016. Adaptation of a plant pathogen to partial host resistance: selection for greater aggressiveness in grapevine downy mildew. *Evolutionary Applications* 9(5): 709–725. https://doi.org/10.1111/eva.12368

DeShields J. B., Bomberger R. A., Woodhall J. W., Wheeler D. L., Moroz N., ... Tanaka, K., 2018. On-site molecular detection of soil-borne phytopathogens using a portable real-time PCR system. *Journal of visualized experiments* (132): 56891. https://doi.org/10.3791/56891

Douillet A., Laurent B., Beslay, J., Massot M., Raynal M., ... Delmotte F., 2022. LAMP for in-field quantitative assessments of airborne grapevine downy mildew inoculum. *Journal of Applied Microbiology* 133(6): 3404–3412. https://doi.org/10.1111/jam.15762

Doyle J. J., Doyle J. L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin 19*(1): 11–15.

EPPO 2021. Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity (PM 7/98 (5)). *EPPO Bulletin* 51: 468–498. https://doi.org/10.1111/epp.12780S.

EPPO. 2024. Use of EPPO Diagnostic Standards (PM 7/76 (6)). *EPPO Bulletin* 54: 312–316. https://doi.org/10.1111/epp.13046

Fedele G., Maddalena G., Furiosi M., Rossi V., Toffolatti S. L., Caffi T., 2025. Relationship between the oospore dose in the leaf litter above the vineyard ground and primary infections by *Plasmopara viticola*. *Frontiers in Plant Science* 16: 1524959. https://doi.org/10.3389/fpls.2025.1524959

Fontaine M. C., Labbé F., Dussert Y., Delière L., Richart-Cervera S., ... Delmotte F., 2021. Europe as a bridgehead in the worldwide invasion history of grapevine downy mildew, *Plasmopara viticola*. *Current Biology* 31(10): 2155–2166. https://doi.org/10.1016/j.cub.2021.03.009

Gerin D., Cariddi C., de Miccolis Angelini R. M., Rotolo C., Dongiovanni C., Pollastro S., 2019. First report of Pseudomonas Grapevine Bunch Rot caused by *Pseudomonas syringae pv. syringae*. *Plant Disease* 103(8): 1954–1960. https://doi.org/10.1094/PDIS-11-18-1992-RE

Gouveia C., Santos R. B., Paiva-Silva C., Buchholz G., Malhó R., Figueiredo A. 2024. The pathogenicity of *Plasmopara viticola*: a review of evolutionary dynamics, infection strategies and effector molecules. BMC *Plant Biology* 24(1): 327. https://doi.org/10.1186/s12870-024-05037-0

Gramaje D., Urbez-Torres J. R., Sosnowski M. R., 2018. Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. *Plant disease* 102(1): 12–39. https://doi.org/10.1094/PDIS-04-17-0512-FE

Heger L., Sharma N., McCoy A. G., Martin F. N., Miles L. A., Chilvers M. I., Miles T. D., 2025. Multiplexed real-time and digital PCR tools to differentiate clades of *Plasmopara viticola* causing downy mildew in grapes. *Plant Disease Ja*. https://doi.org/10.1094/PDIS-01-25-0173-SR

Heyman L., Höfle R., Kicherer A., Trapp O., Ait Barka E., Töpfer R., ... Höfte M., 2021. The durability of quantitative host resistance and variability in pathogen virulence in the interaction between European Grapevine cultivars and *Plasmopara viticola*. *Frontiers in Agronomy* 3: 684023. https://doi.org/10.3389/fagro.2021.684023

Huang X., Wang X., Zhou L., Kong F., Liu Y., Wang Z., Zhang H., 2023. TaqMan-MGB PCR Method for Rapid Detection of QoI Fungicide Resistance in Chinese Populations of *Plasmopara viticola*. *Plant Disease* 107(10): 3007–3013. https://doi.org/10.1094/PDIS-12-22-2954-RE

Incampo G., Chiaromonte E., Cornacchia D., De Miccolis A., Pollastro S., Gerin D., 2025. Development of qPCR and ddPCR-based diagnostic tools for detection and quantification of *Apiospora marii*. *Plant Disease* (ja). https://doi.org/10.1094/PDIS-03-25-0635-SR

Khatal M. P., Narute T. K., Sonawane R. B., ... Bhalerao V. K., 2023. Effect of weather parameters on the growth and development of downy mildew of grape caused by *Plasmopara viticola*. *Journal of Agrometeorology* 25(4): 610–612. https://doi.org/10.54386/jam.v25i4.2063

Koledenkova K., Esmaeel Q., Jacquard C., Nowak J., Clément C., ... Ait Barka E., 2022. *Plasmopara viticola* the causal agent of downy mildew of grapevine: from its taxonomy to disease management. *Frontiers in Microbiology* 13: 889472. https://doi.org/10.3389/fmicb.2022.889472

Kong X., Qin W., Huang X., Kong F., Schoen C. D., ... Zhang H., 2016. Development and application of loop-mediated isothermal amplification (LAMP) for detection of *Plasmopara viticola*. *Scientific reports* 6(1): 28935. https://doi.org/10.1038/srep28935

Koo C., Malapi-Wight M., Kim H. S., Cifci O. S.,Vaughn-Diaz V. L., Ma B., ... Han A., 2013. Development of

a real-time microchip PCR system for portable plant disease diagnosis. *PloS one 8*(12): e82704. https://doi.org/10.1371/journal.pone.0082704

Marimuthu K., Ayyanar K., Varagur Ganesan M., Vaikuntavasan P., Uthandi S., ... Nagaraj G., 2020. Loop-mediated isothermal amplification assay for the detection of *Plasmopara viticola* infecting grapes. *Journal of Phytopathology* 168(3): 144–155. https://doi.org/10.1111/jph.12866

Massi F., Torriani S. F., Borghi L., Toffolatti S. L., 2021. Fungicide resistance evolution and detection in plant pathogens: *Plasmopara viticola* as a case study. *Microorganisms* 9(1): 119. https://doi.org/10.3390/microorganisms9010119

McDermott J. M., Brandle U., Dutly F., Haemmerli U. A., Keller S., Muller K. E., Wolfe M. S., 1994. Genetic variation in powdery mildew of barley: Development of RAPD, SCAR, and VNTR Markers. *Phytopathology* 84(11): 1316–1321. https://doi.org/10.1094/Phyto-84-1316

McGovern P. E., Jalabadze M., Batiuk S., Callahan M. P., Smith K. E., ... Lordkipanidze D., 2017. Early Neolithic wine of Georgia in the South Caucasus. *Proceedings of the National Academy of Sciences of the United States of America* 114: E10309–E10318. https://doi.org/10.1073/pnas.171472811

Muthukumar G., Kamalakannan A., Johnson I., Kamaraj P., Muthuvel I., ... Angayarkanni T., 2025. Early detection and quantification of airborne inocula of *Plasmopara viticola* causing grapevine downy mildew using impaction spore trap. *Physiological and Molecular Plant Pathology* 102842. https://doi.org/10.1016/j.pmpp.2025.102842

Nguyen P. L., Sudheesh P. S., Thomas A. C., Sinnesael M., Haman K., ... Cain K. D., 2018. Rapid detection and monitoring of *Flavobacterium psychrophilum* in water by using a handheld, field-portable quantitative PCR system. *Journal of Aquatic Animal Health* 30(4): 302–311. https://doi.org/10.1002/aah.10046

Nigro F., Gallone P., Romanazzi G., Schena L., Ippolito A., ... Salerno M. G., 2005. Incidence of *Verticillium* wilt on olive in Apulia and genetic diversity of *Verticillium dahliae* isolates from infected trees. *Journal of Plant Pathology* 87: 13–23. https://www.jstor.org/stable/41998203

Nourrisson C., Brunet J., Flori P., Moniot M., Bonnin V., Delbac F., ... Poirier P., 2020. Comparison of DNA extraction methods and real-time PCR assays for the detection of *Blastocystis* sp. in stool specimens. *Microorganisms 8*(11): 1768. https://doi.org/10.3390/microorganisms8111768

Palumbo J. D., O'Keeffe T. L., Fidelibus M. W., 2016. Characterization of *Aspergillus* section *Nigri* species populations in vineyard soil using droplet digital PCR. *Letters in applied microbiology* 63 (6) :458–465. https://doi.org/10.1111/lam.12667

Piccolo S. L., Alfonzo A., Conigliaro G., Moschetti G., Burruano S., ... Barone A., 2012. A simple and rapid DNA extraction method from leaves of grapevine suitable for polymerase chain reaction analysis. *African Journal of Biotechnology* 11(45): 10305. https://doi.org/10.5897/AJB11.3023

Pollastro S., Dongiovanni C., De Miccolis Angelini R. M., Abbatecola A., de Guido M. A., Lepore A., ... Faretra F., 2005. Occurrence and distribution of ochratoxin-producing fungi in vineyards in South of Italy. In Atti *International Workshop: Ochratoxin A in grapes and wine: prevention and control* (pp. 61–61). https://doi.org/10.3390/toxins2040840

Puelles M., Arbizu-Milagro J., Castillo-Ruiz F. J., ... Peña J. M., 2024. Predictive models for grape downy mildew (*Plasmopara viticola*) as a decision support system in Mediterranean conditions. *Crop Protection* 175: 106450. https://doi.org/10.1016/j.cropro.2023.106450

Raguseo C., Gerin, D., PollastroS., Rotolo,C., Rotondo,P. R., ... De Miccolis Angelini R.M., 2021. A Duplex-Droplet Digital PCR Assay for Simultaneous Quantitative Detection of *Monilinia fructicola* and *Monilinia laxa* on Stone Fruits. *Frontiers in Microbiology* 12: 747560. https://doi.org/10.3389/fmicb.2021.747560

Rodríguez-Moreno, L., Pineda, M., Soukupová, J., Macho, A. P., Beuzón, C. R., Barón, M., ... Ramos, C. 2008. Early detection of bean infection by *Pseudomonas syringae* in asymptomatic leaf areas using chlorophyll fluorescence imaging. *Photosynthesis research* 96: 27–35. https://doi.org/10.1007/s11120-007-9278-6

Rogers S. L., Atkins S. D., West J. S., 2009. Detection and quantification of airborne inoculum of *Sclerotinia sclerotiorum* using quantitative PCR. *Plant Pathology* 58: 324–331. https://doi.org/10.1111/j.1365-3059.2008.01945.x

Rossi V, Caffi T, Bugiani R, Dellavalle D. 2008. Estimating the germination dynamics of *Plasmopara viticola* oospores using the hydrothermal time. *Plant Pathology* 57: 216–226. https://doi.org/10.1111/j.1365-3059.2007.01738.x.

Rossi V, Caffi T, Gobbin D. 2013. Contribution of molecular studies to botanical epidemiology and disease modelling: grapevine downy mildew as a case-study. *European Journal of Plant Pathology* 135: 641–54. https://doi.org/10.1007/s10658-012-0114-2.

Rouxel M., Mestre P., Baudoin A., Carisse O., Delière L., Ellis M. A., Gadoury D., Lu J., ... Delmotte F., 2014.

Geographic distribution of cryptic species of *Plasmopara viticola* causing downy mildew on wild and cultivated grape in eastern North America. *Phytopathology* 104(7): 692–701. https://doi.org/10.1094/PHYTO-08-13-0225-R

Rouxel M., Mestre P., Comont G., Lehman B. L., Schilder A., ... Delmotte F., 2013. Phylogenetic and experimental evidence for host-specialized cryptic species in a biotrophic oomycete. *New Phytologist* 197(1): 251–263. https://doi.org/10.1111/nph.12016

Sánchez-Zelaia H., Nanni I., Oggiano I., Hernández M., Díez-Navajas A., ... Collina M., 2024. Droplet Digital PCR: A New Molecular Method to Detect G1105S/V Mutations in *Plasmopara viticola* CesA3 Gene. *Biology* 13: 919. https://doi.org/10.3390/biology13110919

Santander R. D., Meredith C. L., Aćimović S. G., 2019. Development of a viability digital PCR protocol for the selective detection and quantification of live *Erwinia amylovora* cells in cankers. *Scientific Reports* 9(1): 11530. https://doi.org/10.1038/s41598-019-47976-x

Si Ammour M., Bove F., Toffolatti S. L., ... Rossi V., 2020. A real-time PCR assay for the quantification of *Plasmopara viticola* oospores in grapevine leaves. *Frontiers in Plant Science* 11: 1202. https://doi.org/10.3389/fpls.2020.01202

Sotolář R., 2007. Comparison of grape seedlings population against downy mildew by using different provocation methods. *Notulae Botanicae Horti Agrobotanici Cluj-Napoc* 35: 61–68. https://doi.org/10.15835/nbha352223

Toffolatti S. L., Russo G., Campia P., Bianco P. A., Borsa P, ... Sierotzki H., 2018. A time-course investigation of resistance to the carboxylic acid amide mandipropamid in field populations of *Plasmopara viticola* treated with anti-resistance strategies. *Pest management science* 74(12): 2822–2834. https://doi.org/10.1002/ps.5072

Toffolatti S. L., Serrati L., Sierotzki H., Gisi U., Vercesi A., 2007. Assessment of QoI resistance in *Plasmopara viticola* oospores. *Pest Management Science: Formerly Pesticide Science* 63(2): 194–201. https://doi.org/10.1002/ps.1327

Toffolatti S. L., Venturini G., Maffi D., Vercesi A., 2012. Phenotypic and histochemical traits of the interaction between Plasmopara viticola and resistant or susceptible grapevine varieties. BMC Plant Biology 12: 1–16. https://doi.org/10.1186/1471-2229-12-124

Valsesia G., Gobbin D., Patocchi,A., Vecchione A., Pertot I., Gessler C., 2005. Development of a high-throughput method for quantification of *Plasmopara viticola* DNA in grapevine leaves by means of quantitative real-time polymerase chain reaction. *Phytopathology* 95(6): 672–678. https://doi.org/10.1094/PHYTO-95-0672

Wang D., Jiao X., Jia H., Cheng S., Jin X., ... Su X., 2022. Detection and quantification of *Verticillium dahliae* and *V. longisporum* by droplet digital PCR versus quantitative real-time PCR. *Frontiers in Cellular and Infection Microbiology* 12: 995705. https://doi.org/10.3389/fcimb.2022.995705

Xin Z., Velten J. P., Oliver M. J., Burke J. J., 2003 High-throughput DNA extraction method suitable for PCR. *BioTechniques* 34: 820–824, 826. https://doi.org/10.2144/03344rr04

Yang L., Chu B., Deng J., Yuan K., Sun Q., Ma Z., 2023. Use of a real-time PCR method to quantify the primary infection of *Plasmopara viticola* in commercial vineyards. *Phytopathology Research* 5(1): 19. https://doi.org/10.1186/s42483-023-00178-w

Ye J., Coulouris G., Zaretskaya I., Cutcutache I., Rozen S., Madden T. L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC bioinformatics* 13: 1–11. https://doi.org/10.1186/1471-2105-13-134

Yu Y., Zhang Y., Yin L., Lu J., 2012. The mode of host resistance to *Plasmopara viticola* infection of grapevines. *Phytopathology* 102(11): 1094–1101. https://doi.org/10.1094/PHYTO-02-12-0028-R

Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Abstracts

Abstracts of invited Keynote lectures, Keynote and Oral presentations and Poster papers presented at the 17th Congress of the Mediterranean Phytopathological Union, July 6–10, 2025, in Bari, Italy

This Issue of *Phytopathologia Mediterranea* firstly outlines a summary of the Congress, and then publishes abstracts of all the invited Keynote papers, and offered Oral and Poster papers, that were presented at the Congress.

Preface

The 17th International Congress of the Mediterranean Phytopathological Union (MPU) was headlined as addressing "New Phytopathology Frontiers of Research and Education for Plant Health and Food Safety". The Congress was organized by the Mediterranean Phytopathological Union (MPU), and the Mediterranean intergovernmental organization CIHEAM Bari, International Centre for Advanced Mediterranean Agronomic Studies.

The Congress Plenary Session was opened with welcoming remarks by Dr Biagio Di Terlizzi, Director of CIHEAM Bari; Prof. Dimitri Tsitsigiannis, President of MPU; and Dr Ugo Della Marta, Director General of the Directorate for Food Hygiene, Safety and Nutrition (DGISAN) of the Italian Ministry of Health.

The Congress was attended by more than 350 participants from 38 countries: 19 countries in the Mediterranean region (Albania, Algeria, Croatia, Egypt, France, Greece, Israel, Italy, Jordan, Lebanon, Montenegro, Morocco, Palestine, Portugal, Slovenia, Spain, Syria, Tunisia, Turkey), eight elsewhere in Europe (Belgium, Germany, Hungary, Latvia, Lettonia, North Macedonia, Serbia, The Netherlands), and 11 other countries (Australia, Chile, Congo, Georgia, New Zealand, Pakistan, Reunion Island, South Africa, United Arab Emirates, United Kingdom, United States of America).

The congress was aimed to promote knowledge exchange and dialogue between science, institutions, industry and civil society, reinforcing a vibrant, connected, and forward-looking scientific community. Among these, beside researchers of the 15 National Plant Pathology or Plant Protection societies that are MPU members, people also attending included representatives from: IPPC-FAO, FAO-RNE, EUPHRESCO, Ministry of Agriculture, Food Sovereignty and Forests (MASAF), Directorate General for Food Hygiene, Food Safety and Nutrition (DGISAN), EPPO, EFSA, the National Research Council (CNR), the Apulia Region, the European Commission, and numerous universities and research centers.

Papers presented at the Congress included: 14 Keynote lectures, 36 Session Keynote papers, and research papers as 88 oral and 180 poster presentations, in a total of in 18 Congress sessions. Beyond its scientific scope, the Congress was a platform to strengthen existing partnerships and forge new collaborations across borders and research disciplines. This included links with the International Society for Plant Pathology, the International Society for Mycotoxicology, EUPHRESCO, and CIHEAM Bari, supporting advancements in plant health sciences as a pillar of the One Health approach recognizing the links between human, animal, plant, and environmental health.

CIHEAM Bari took active steps to make the Congress accessible to young researchers, especially from non-European regions. This included reduced registration and accommodation fees for students (including PhD candidates) and early career scientists.

An all-day field trip on 10 July included observations of ancient olive trees infected by *Xylella fastidiosa* and the characteristic sites of Alberobello, providing a unique opportunity to connect field and territory observations with the latest research and disease mitigation strategies.

ISSN 0031-9465 (print) | ISSN 1593-2095 (online) | DOI: 10.36253/phyto-16678

To recognize scientific excellence of the early career researchers, MPU presented awards for the oral presentations by Marah Abukhmaish (Palestine), Serafina Amoia (Italy) and Marco Crudele (Italy), and the poster papers of Martini Florian (Belgium), Luiza Sánchez-Pereira (Spain), and Aleksandra Susnjar (Republic of Serbia). The Congress also hosted the award ceremony for the 4th edition of the video competition *Plant Health TV: Promoting the Importance of Plant Health Research*. Organized by EUPHRESCO III, CIHEAM Bari, the Plant Biosecurity Research Initiative (PBRI), IPPC, and CABI, this competition highlights global efforts in plant health research. The award recipient was Spyridoula Dimitropoulou, for the video "Saving Greece's plane trees: digital technologies for early detection of *Ceratocystis platani*". The award included a 2-week internship in Australia, sponsored by the PBRI.

In its concluding session, the congress identified key frontiers and pressing challenges in plant pathology that must be addressed, including:

- The integration of Plant Health within the One Health framework.
- The role of climate change in driving the emergence and spread of new pests and diseases.
- The urgent need for predictive tools and early warning systems.
- The integration of digital technologies, genomics, and field ecology into plant health research and practice.
- The necessity of addressing biosecurity vulnerabilities and regulatory gaps.
- The imperative of safeguarding global food security and biosecurity in increasingly complex and interconnected supply chains.

To respond effectively to these developments and challenges, interdisciplinary and collaborative educational models across all professional levels are essential. Such models are crucial for training a new generation of plant pathologists equipped with the necessary skills, technological expertise, and cross-sectoral understanding.

Furthermore, strong support for early career researchers is vital to ensure continuity, innovation, and the advancement of knowledge in the field.

In this context, the Mediterranean Phytopathological Union (MPU), in collaboration with national, regional, and international networks, aims to significantly strengthen interdisciplinary and cross-border cooperation in the field of plant health, while fostering meaningful dialogue among the scientific community, regulatory bodies, and society at large.

Photographs from the Congress are available at: https://ciheambaricongressmpu2025.org/photo-gallery/

Editorial disclaimer

The Co-Editors-in-Chief of Phytopathologia Mediterranea present these Congress Abstracts with the proviso that all abstracts are published as submitted by the respective paper authors. The abstracts have not been refereed using the normal standard peer review processes applied for research papers in this journal.

Opening Plenary Session

KEYNOTE LECTURES

The past, present, and future for the Mediterranean Phytopathological Union and Phytopathologia Mediterranea. L. MUGNAI[1], A. M. D'ONGHIA[2], R. E. FALLOON[3], G. SURICO[1]. [1]*Department of Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, University of Florence,* P.le *delle Cascine 18, Florence, Italy.* [2]*CIHEAM-Bari, Via Ceglie 9, Valenzano, Italy.* [3]*154 Hackthone Road, Christchurch 8022, New Zealand.* E-MAIL: laura.mugnai@unifi.it

This Congress marks the 65th anniversary of foundation of *Phytopathologia Mediterranea,* by the eminent Italian plant pathologists Antonio Ciccarone and Gabriele Goidanich, from Italy. With colleagues from Israel, France and Portugal, these pioneers aimed to create a medium for publishing high quality research papers, enhancing cooperation in meeting the increasing plant health challenges in the ecophysiological areas surrounding the Mediterranean Sea. Two years later, these people also decided to enrich the project by providing opportunity for researchers to meet and collaborate, by organizing congresses, workshops, and joint research planning. These objectives were achieved by establishing the Mediterranean Phytopathological Union (MPU), which became the owner and editor of *Phytopathologia Mediterranea*. Since then, 16 Congresses have been successfully held, and the 17th being held in July 2025 with the theme "New Phytopathology Frontiers of Research and Education for Plant Health and Food Safety." This Congress aims to highlight education and research as essential tools to address emerging challenges in phytopathology and food safety, while raising public awareness and contributing to the achievement of the Sustainable Development Goals (SDGs). In 2024, the MPU General Assembly restructured the Union by extending membership to researchers who are members of any national society of phytopathology or related fields within the Mediterranean region, provided that these societies have formal agreements with MPU. Now the Union includes 15 National societies from Croatia, Egypt, Greece, Israel, Italy, Palestine, Bosnia and Herzegovina, Serbia, Slovenia, Portugal, Spain, and Türkiye. This reform broadened MPU's vision to include individual researchers from non- Mediterranean countries, as well as international societies and organizations involved in plant health across Mediterranean ecological zones. The revised Statute establishes an Advisory Committee to represent this expanded research community and actively promotes the global dissemination and coordination of plant health research. It fosters collaboration with other networks, such as International Society of Plant Pathology, Arab Society of Plant Protection, International Society of Mycotoxicology, EUPHRESCO, while encouraging active involvement in European initiatives like EUPHRESCO III. *Phytopathologia Mediterranea* remains a key part of this initiative, with all volumes now available as open access and online, and with an Impact Factor in the first quartiles of relevant subject areas. The Journal is overseen by two Editors-in-Chief, from Italy and New Zealand, and by researchers from 14 different countries. The journal gets value from having a non-profit online editor and publisher, Firenze University Press. Looking ahead, *Phytopathologia Mediterranea* is strengthening its role by expanding international visibility, increasing reviewer engagement, and encouraging submissions on emerging plant diseases and food security, while maintaining non-profit open access status. The journal maintains a rigorous and transparent editorial processes, with artisanal care in manuscript selection and quality control. The Mediterranean Phytopathological Union and *Phytopathologia Mediterranea* have enabled two generations of plant pathologists to communicate research outcomes. The future of the MPU and its journal will be supported by a continuing commitment to knowledge sharing and research collaboration, and to maintaining the objectives and foresight of the people who established the Union and journal.

Plant Pathology is important in today's world. G. SURICO. *Department of Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, University of Florence,* P.le *delle Cascine 18, Florence, Italy.* E-MAIL*:* giuseppe.surico@unifi.it

In December 2021, the American Phytopathological Society (APS) conducted a survey to identify and rank key discoveries in plant pathology that have had broad impacts on science and practical disease management during the past half century. Based on the responses received, the working group has chosen about fifteen of the most important discoveries of all: they ranged from the discovery of the Ti plasmid up to the Type III secretion system; from the deployment and management of host resistance genes up to the utilization of biological controls and suppressive soils. However, despite the acquisition of this and much other knowledge, agricultural production losses due to diseases remain, today, very high. Indeed, losses have increased compared to 50 years ago. Despite this scenario, world public opinion is

pushing for a substantial reduction in the use of pesticides in agriculture. Will we be able to defend plants from diseases with means other than those predominantly used today? Will we be able to practice our profession as Frederick L. Wellman defined it more than 50 years ago?: "*All the academic duties, reading, teaching and research, in a plant pathologist's life have one central purpose, and that is to control plant diseases*". Recently a group of our colleagues commissioned by the APS identified 17 major themes among the most important ones to investigate. One of them was the following*: "Early detection, identification, diagnosis, surveillance, and forecasting of emerging, and reemerging plant diseases and economically important plant diseases, including development toolkits for rapid, sensitive, specific, dependable, portable, economic, and accurate diagnostics, development and application of new technologies, and systems for global surveillance of plant diseases*". Twelve years have passed since the outbreak of the *Xylella* epidemic on olive trees in Italy. The disease probably appeared in Southern Italy in 2008 and only 5 years later the bacterium (*Xylella fastidiosa* subsp. *pauca*) was isolated from infected olive tissues. By that year the disease had already spread over an area of about 8,000 hectares. The event had a huge impact on the population. Considering the particular importance of the affected plant and of the drastic measures taken to try and block the disease, everyone felt obliged to have their say and the scientific element has become almost a secondary object in a scenario that immediately appeared very complex. To date, despite the containment measures adopted, the disease has killed hundreds of thousands of plants, has spread for tens of kilometers beyond the original infection focus, and the presence of two other subspecies, *fastidiosa* and *multiplex*, has been recently diagnosed. Two other topics that need to be remembered are artificial intelligence and combining the human, animal and plant medicine into one. Regarding the first point, it is superfluous to note that artificial intelligence is also coming to agriculture, the slowest sector in welcoming the innovations of the intellect, and it is doing so in an increasingly pervasive way so much so that everything or almost everything could change in a few years for example in the fields of diagnostics, prediction systems, and disease management strategies. The MPU could help to answer some or all of the questions raised earlier, during this Congress or even later, by setting up special study commissions. We just have to want it.

Plant Health research coordination: an international endeavor. B. GIOVANI[1], L. MUGNAI[2], J. ZORRILLA-FONTANESI[1], A.M. D'ONGHIA[3]. [1]*Euphresco, 21 Bd Richard Lenoir, 75011 Paris, France.* [2]*University of Florence,* P.le *delle Cascine 28, 50144 Florence, Italy.* [3]*CIHEAM-Bari, via Ceglie 9, 70010 Valenzano, Bari, Italy.* E-MAIL: bgiovani@euphresco.net

Plant health is a key factor in any strategy to achieve food security, protect the environment and biodiversity and facilitate safe trade and is an essential pillar of the bioeconomy. It is not possible to avoid all the challenges to plant health posed by global trade, increasing travel activities and climate change. However, it is possible to optimise strategies to address these challenges with effective coordination and cooperation. Research plays a key role in underpinning phytosanitary activities, ranging from pest risk analysis, regulation, surveillance, taxonomy, diagnostics and mitigation measures. Research also helps to maintain and develop scientific expertise and infrastructure that support plant health. The need to revive the scientific basis of the phytosanitary field has been a priority since the EPPO Madeira Declaration. Upon a request of the EC Council Working Party of the Chief Officers of Plant Health Services (COPHS), the Euphresco network for phytosanitary research coordination and funding started in 2006 to support phytosanitary research programme owners and programme managers to develop and take advantage of synergies amongst national research programmes and activities. The success of Euphresco as a primarily European network for phytosanitary research coordination has set the ground for discussions on the development of initiative(s) to address the needs of other regions of the world and global phytosanitary research coordination. In 2019, during the Meeting of the Agricultural Chief Scientists of the G20, the importance 'to strengthen research collaboration to develop effective measures against transboundary pests and to participate in joint funding networks such as Euphresco' was noted. In 2020, the article 'Science diplomacy for plant health', co-authored by experts from the IPPC, several Regional Plant Protection Organizations (RPPOs), NPPOs, research coordination networks and research organizations, highlighted the need for '*a global network for phytosanitary research coordination that can shape research agendas across countries and accelerate the development of science to support regulatory phytosanitary decision makers*'. In 2021, the IPPC Strategic Framework 2020-2030 was adopted at the 15th session of the Commission on Phytosanitary Measures (CPM-15). Global phytosanitary research coordination is one of the development programmes identified, and it is expected that by 2030 '*possibilities for establishing an international research col-*

laborative structure have been explored and, if appropriate, the structure has been established'. The presentation will showcase the role of Euphresco as a platform for phytosanitary research coordination and collaboration and how the network has evolved since 2006. Activities dedicated to the Mediterranean region will be presented and explained.

Plant Health for all: strengthening global phytosanitary capacity through the IPPC Plant Health campus. S. BRUNEL. *IPPC Officer in Charge for day-to-day matters, Implementation and Facilitation Unit Lead.* E-MAIL: sarah.brunel@fao.org

Plant health is the first line of defense against threats to food security, biodiversity, and safe trade. Yet many countries face critical capacity gaps in their ability to manage pests, prevent incursions, and meet international standards. The IPPC Plant Health Campus was created to change that. The IPPC Plant Health Campus is a transformative global learning platform developed by the International Plant Protection Convention (IPPC) in collaboration with the FAO eLearning Academy and funded by the European Union. It is the first initiative of its kind to offer free, structured, and standardized training in phytosanitary practices, accessible both online and offline to users around the world—including those in remote or under-resourced areas. The platform offers multilingual certified e-learning courses, interactive tools, downloadable IPPC guides, and practical training materials designed to strengthen technical and operational capacities across the global plant health community. As public good, the Campus is democratizing access to critical knowledge, reducing disparities, and fostering equitable development. A distinctive feature of the Campus is its tailored learning pathways, built around real-world roles within National Plant Protection Organizations (NPPOs) and other regulatory or technical agencies. These pathways guide learners through structured modules on priority topics including NPPO management, pest risk analysis, market access, import and export procedures, inspection and surveillance, stakeholder engagement, emergency response, wood packaging materials, and resource mobilization. To date, IPPC Campus courses have been completed over 7,000 times by users in more than 100 countries, demonstrating strong global uptake. Learners receive digital badges or certificates upon completion, validating their new competencies and supporting their professional development. These credentials contribute to building a more skilled, recognized, and mobile plant health workforce. Courses are currently offered in English and French, with selected modules available in Spanish. The IPPC welcomes in-kind and financial contributions to support translation into additional UN languages, expanding accessibility for a truly global audience. Beyond the public sector, the Campus is increasingly seen as a tool for the next generation of plant health professionals. Several universities are exploring the integration of IPPC-certified courses into their agricultural and phytosanitary curricula, embedding international standards and best practices into formal education systems. To amplify its reach, the IPPC has mobilized regional champions who actively promote the Campus in national and international fora, including conferences and media interviews. Their efforts are part of a growing global campaign to raise awareness and drive engagement with the platform. By making expert-developed, standardized training universally available, the IPPC Plant Health Campus strengthens the implementation of International Standards for Phytosanitary Measures (ISPMs), promotes safe international trade, and builds more resilient, knowledge-driven phytosanitary systems. Ultimately, the Campus plays a vital role in advancing the Sustainable Development Goals, particularly in areas such as food security, environmental protection, and inclusive economic development. The IPPC Plant Health Campus is not just a training tool—it is a global investment in a safer, healthier, and more sustainable future.

CIHEAM Bari Educational Programmes to strengthen plant health research in the Mediterranean Region. A.M. D'ONGHIA. *International Centre for Advanced Mediterranean Agronomic Studies, Via Ceglie, 9-70010 Valenzano, Italy.* E-MAIL: donghia@iamb.it

Plant pests pose a major threat to food security and rural livelihoods across the Mediterranean, where agriculture remains a cornerstone of socioeconomic stability. The United Nations designated 2020 as the International Year of Plant Health and 12 May as the International Day of Plant Health, underscoring the vital role of plant protection in global sustainability efforts. CIHEAM Bari has been at the forefront of plant health research and capacity building for over 35 years, fostering scientific innovation, training, and international cooperation across the Mediterranean and neighbouring regions. Its research activities mainly focus on advanced diagnostic methodologies and epidemiological studies of high-impact pests. The institute has also pioneered the integration of smart technologies into pest risk analysis, early warning systems, and predictive modelling—sup-

porting the implementation of precision surveillance and targeted intervention strategies. CIHEAM Bari has made substantial investments in higher education and research, significantly contributing to the development of a regional scientific community in plant health. Since 1985 about 4000 former students were trained in Plant Health through Master, Master of Science, Advanced Specialized Courses & PhD programs—mainly supported by scholarships from the Italian Cooperation—with the aim to prepare young generation towards professional, research and academic careers in plant health. MSc and PhD students conduct original research on regionally relevant phytosanitary challenges, gaining strong technical expertise as well as critical thinking and problem-solving skills. Since 2000, around 60 of 400 MSc graduates have completed PhDs in collaboration with Italian and international universities: 42% from Maghreb, 39% from the Near and Middle East, 7% from the Balkans, and 12% from EU. This sustained academic effort has led to about 300 scientific publications in peer-reviewed journals and conference proceedings. Research thematic areas were mainly in plant pathology, followed by entomology and post-harvest, while research topics were focusing on characterization, diagnosis, surveillance and control. CIHEAM Bari also plays a strategic role in aligning phytosanitary regulations with EU standards, contributing to food safety, facilitating international trade, and promoting agricultural sustainability. With support from international cooperation, modern research facilities and equipment have been provided to enable young scientists to carry out studies in their home countries, helping to limit the so-called "brain drain". A notable example is the PHYTO BiH project, which supports Bosnia and Herzegovina in aligning its regulatory framework with EU legislation. Similar initiatives in Near Eastern and African countries aim to enhance national systems for managing phytosanitary risks and emergency responses. To advance plant health research in the Mediterranean and beyond, strengthening both national capacities and regional collaboration, is essential. In 2021, CIHEAM Bari and Euphresco launched the first coordinated initiative to establish a shared research agenda: *The Plant Health Research Priorities for the Mediterranean Region*. This laid the foundation for the ongoing EUPHRESCO III project, fostering long-term partnerships and aligning priorities on a global scale. Through its integrated approach—combining research, education, policy support, and international collaboration—CIHEAM Bari continues to serve as a regional leader in building a resilient, cooperative, and forward-looking phytosanitary system for the Mediterranean and beyond.

Transboundary plant pathogens threatening crops and natural landscapes in the Mediterranean region.
T. YASEEN, Y. AHMED. *FAO regional office for the Near East and North Africa, United Nations, El Islah El Zeraey St., Ad Doqi, Al Giza, Giza Governorate 3751311, Egypt.* E-MAIL: thaer.yaseen@fao.org

The Mediterranean region, recognized for its rich agricultural heritage and ecological diversity, is becoming increasingly vulnerable to the emergence and spread of transboundary plant pathogens. These pathogens, including fungi, bacteria, and viruses, can cross national borders and rapidly establish in new environments. They pose serious threats to crop productivity, food security, rural livelihoods, ecosystem integrity, and the economic stability of the region. The accelerated pace of globalization, combined with climate change and the intensification of agricultural trade, has significantly increased the transboundary movement and establishment of plant pathogens, thereby overwhelming conventional national phytosanitary systems. The international exchange of planting materials remains a key pathway for the unintentional introduction and spread of invasive and emerging plant diseases. In the Mediterranean context, climate variability, particularly rising temperatures and altered precipitation patterns, is expanding the geographical distribution, seasonality, and persistence of numerous pathogens. This trend is heightening phytosanitary risks across borders. Despite notable initiatives and efforts undertaken by individual countries, responses to transboundary plant pathogens remain constrained by limited technical capacities, fragmented surveillance systems, inadequate quarantine infrastructure, and insufficient risk perception. Many countries encounter difficulties in timely detection, accurate diagnosis, and effective containment of outbreaks. The lack of harmonized phytosanitary protocols and regional data-sharing mechanisms further impedes coordinated responses. Notably, the complexity and cross-border nature of many plant diseases make it difficult for any single country to manage them effectively in isolation. Recent incursions of high-impact pathogens such as *Xylella fastidiosa*, *Fusarium oxysporum* f. sp. *cubense*, and *Candidatus* Liberibacter asiaticus (citrus greening) illustrate the urgent need for collective action, as these diseases threaten critical crops like olives, bananas, and citrus. Addressing the transboundary nature of these threats demands a science-driven, collaborative approach that extends beyond national boundaries. Priority actions include: (i) strengthening early warning and diagnostic systems through advanced molecular tools and remote sensing technologies; (ii) promoting integrated disease manage-

ment practices tailored for Mediterranean agro-ecosystems; (iii) enhancing regional collaboration through joint surveillance platforms and coordinated emergency response frameworks; and (iv) investing in interdisciplinary education, capacity building and training to build resilient and responsive plant health systems. Integrated pest and disease management (IPM), plant breeding for resistance, and nature-based solutions are key to reducing pathogen spread and impact while ensuring environmental sustainability. Regional mechanisms must also support applied research, comprehensive risk assessment, and real-time data exchange to expedite decision-making. This presentation will provide an overview of the current status and emerging trends in transboundary plant pathogens affecting the Mediterranean, drawing on recent case studies and field-based experiences. It will emphasize the importance of regional cooperation, sustainable management practices, and science-based plant health practices. In summary, transboundary plant pathogens represent a complex and evolving threat to agricultural productivity, biodiversity, and natural landscapes across the Mediterranean region. Addressing this challenge aligns with FAO's mandate to promote sustainable plant health systems and requires the integration of cutting-edge research, evidence- based policy, and sustained regional cooperation. Building a resilient Mediterranean plant health system calls for a shared vision, strong political will, sustained investment in innovation, and collaboration.

General overview of the EU policies for Plant health with a focus on *Xylella fastidosa*. M.B. MARQUEZ GARCIA. *European Commission – DG SANTE, Unit G1 – Plant Health.* E-MAIL: maria.marquez-garcia1@ec.europa.eu

In the EU, Regulation (EU) 2016/2031, known as the Plant Health Regulation, establishes the framework for protecting plant health in the European Union, aiming to prevent the introduction and spread of plant pests and diseases that can have significant impact on agriculture, natural ecosystems and biodiversity. This Regulation defines the different categories of regulated plant pests in the EU, enhances prevention an early detection of Union quarantine pests by establishing the obligation for Member States to create multiannual survey programmes spanning 5-10 years, while requiring annual surveys for those pests whose potential economic, environmental or social impact is the most severe, the priority pests. Moreover, it requires Member States to implement measures to eradicate Union quarantine pests, including the creation of demarcated areas, composed by infected zone and buffer zone, to prevent the spread of the pest to the rest of the Union territory. *Xylella fastidiosa* is listed as Union quarantine pest in Part B of Annex II of Commission Implementing Regulation (EU) 2019/2072, as a pest known to be present in the EU territory. Due to its economic, environmental and social impact, this pest is also listed as a priority pest in Commission Delegated Regulation (EU) 2019/1702. Since the first finding of *Xylella fastidiosa* in the EU in 2013, the Commission has put in place specific measures to control this pest and has updated them following the scientific developments. Prevention and early detection are key pillars in the fight against this pest. Today, the measures to prevent the introduction into and the spread within the EU are detailed in Commission Implementing Regulation (EU) 2020/1201. The regulation, amended in September 2024, includes the obligation for all Member States to carry out annual surveys and have prepared contingency plans and for those Member States where the pest is present, the regulation details the eradication, and when eradication is no longer possible, the containment measures that have to be taken to prevent the further spread of the pest. Moreover, it also details the rules for the movement of plants known to be host of *Xylella fastidiosa* within the Union and the requirements for the introduction of these plants from third countries. Based on the annual survey campaigns in all Member States, *Xylella fastidiosa* is known as absent in 23 Member States and present in 4 Member States where the affected areas have been demarcated for the control of the pest and they are currently under eradication or containment approach.

Prevention, preparedness and response to plant disease outbreaks in the Mediterranean Basin. A. VICENT. *Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Spain.* E-MAIL: vicent_antciv@gva.es

Plant health should be a priority in the Mediterranean Basin, particularly for the citrus sector, which now faces major threats from pests and diseases. The EU has implemented a risk-based regulatory framework through Regulation (EU) 2016/2031 and Implementing Regulation (EU) 2019/2072. These regulations establish specific requirements for the import, movement of plants, and surveillance of the territory, aiming to prevent the introduction and spread of harmful organisms. Risk analysis plays a central role in this preventive strategy, allowing authorities to evaluate the likelihood and impact of pest and disease incursions and to define appropriate

phytosanitary measures. This framework is supported by risk-based surveillance, epidemiological modeling, contingency planning and social engagement, forming a comprehensive strategy for preparedness and response. Two major threats to citrus crops—*Phyllosticta citricarpa*, the fungus causing citrus black spot (CBS), and '*Candidatus* Liberibacter spp.', associated with huanglongbing (HLB)—are used here as case studies to illustrate this approach. These pathogens are classified as priority 'pests' under Implementing Regulation (EU) 2019/1702 due to their potential socioeconomic and environmental impacts, which mandates, reinforced surveillance and contingency plans, among other measures. Risk assessment studies on *P. citricarpa*, have underpinned EU regulations, despite opposition from other studies claiming that Mediterranean climates are unsuitable for CBS development. However, subsequent research and the recent outbreak in Tunisia, have confirmed that CBS epidemics can occur under Mediterranean conditions. Surveillance is another key pillar of the strategy. EU Member States are required to conduct surveys aligned with the phytosanitary status of each region. Tools like the Pest Survey Toolkit developed by EFSA provide methodological support for risk-based and statistically sound survey planning. Epidemiological models enhance outbreak preparedness by simulating disease spread and evaluating control strategies. For HLB, models incorporating spatial variability, vector dynamics, climate conditions, and management actions have shown that, without intervention, the disease could spread rapidly, becoming unmanageable. However, early detection and aggressive vector control could slow its progression, though such strategies may rely on insecticides not currently authorized in the EU. Contingency plans are critical for effective responses to pest and disease introductions. These plans outline procedures, responsibilities, and resources needed to contain or eradicate pests and diseases. For CBS and HLB, the Spanish Ministry of Agriculture has developed dedicated contingency plans. Simulation exercises, such as one held in Valencia in 2021 under the Pre-HLB project, tested these plans with positive feedback from stakeholders and provided opportunities for improvement. Finally, social acceptance plays a decisive role in the success of outbreak management plans. Resistance from producers due to lack of information can undermine control efforts. A survey among citrus sector actors in Spain revealed good awareness of HLB but limited knowledge of the contingency plan. The study highlighted the need to involve stakeholders early in the planning process to ensure better compliance and implementation. In summary, the EU's integrated, risk- based approach—combining legal regulations, early detection, epidemiological modeling, contingency planning and stakeholder engagement—is essential for protecting citrus crops from phytosanitary threats. Achieving this goal requires strong coordination among Mediterranean countries.

Plant quarantine challenges under climate change anxiety. S.M. ABDEL-MOMEN. *Former Minister of Agriculture and Land Reclamation of Egypt; MPU Delegate of the Egyptian, Phytopathological Society; Agricultural Research Center, Giza, Egypt.* E-MAIL: salah1993@yahoo.com

Several studies indicated the adverse effects of the climatic changes on most, if not all, elements of the agricultural production. Arable lands, water resources and microorganisms are also subjected to be adversely affected due to their exposure to the changeable weather factors. Plant quarantine as the safe guard of the plant wealth and as the regulator of the exportation and importation of the agricultural commodities is expected to be highly affected and challenged by the climatic Changes. The expected increase in the bio-diversity of the harmful and useful microorganisms will be a challenge facing the regulations of plant quarantine and consequently the trade and movement of commodities among countries. Some secondary pests could be altered to be major ones and new races of a pest could emerge causing new problems that need to be managed in certain methods and new regulations. From a first-hand experience and as being an editor of a scientific journal, several first reports and observations of pests on new hosts. This could be due to the change in virulence of the pathogen or the host due to the exposure to the stress of the climatic change. Also, the quantity and quality of several crops were affected in some seasons and this was with a correlation to the climatic changes. Also, geographic regions were effective in such new observations confirming the role of the climatic changes. The aforementioned challenges and observations are a burden on scientists working in the agricultural field. To face this burden a multidisciplinary team work should cooperate together through integrated and comprehensive work plan. The plan should include elements that mitigate the effects of the climatic changes on all the inputs and outputs of the sustainable agriculture process. Modern approaches for pest detection and identification should be applied. Also, pest dispersal monitoring regional network are needed to put the strategy of facing the current and expected challenges. Moreover, information exchange systems including developing

stress-tolerant crop varieties, reporting new diseases, pest races and bio-types in addition to climate changes information among the Mediterranean countries need to be established. Based on the obtained data, a resilient and accurate strategy will be developed to face the threat of the climatic changes in each country due to its local conditions and in cooperation with whatever progress occurring in neighboring Mediterranean countries. Also, the obtained data will be considered to make the suitable regulations of plant quarantine according to conditions of each country aiming at enhancing exportations and importations of the agricultural commodities among countries. In parallel with, frequent scurvies and monitoring are needed to revise and regulate the plant quarantine policies and regulations.

Fungi and mycotoxins crucial role in food and feed safety. P. BATTILANI. *Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29122 Piacenza, Italy.* E-MAIL: paola.battilani@unicatt.it

Mycotoxin contamination is considered the main chronic health risk coming from food and a quite similar impact can be described for feed. Even if three main fungal genera are reported as including mycotoxin producing species, *Fusarium, Aspergillus* and *Penicillium*, at least two more should be added because their metabolites are listed among mycotoxins and regulated or candidates for regulation, *Claviceps* and *Alternaria*. This results in a wide range of ecological and host crops requirements, with room for their action worldwide, in many different crops. Most mycotoxin- producing fungi are weak parasites and their main impact on agricultural products is not due, or only partially due, to yield reduction, but rather to cryptic health risks for consumer, due to symptomless contamination, so that grain and fruit are apparently healthy, but containing toxic metabolites. Maize is one of the best crops to consider when discussing mycotoxin/when it comes to mycotoxins. It has a wide use for animal feeding, but also has several food or food industry destinations. It is a perfect host for several fungal species, belonging to at least 3 of the main fungal genera including mycotoxin producers. Therefore, depending on the growing area, at least one fungus can grow and cause mycotoxin contamination. *Aspergillus flavus* merits to be mentioned, being the main producer of aflatoxin B1, the most toxic naturally occurring compound, carcinogenic, teratogenic and immunosuppressor, just to mention some known effects. Also nuts and cotton are among the host crops. Recently, aflatoxin contamination was also reported in fresh fruits, also used as ingredients for baby food, rising further concerns and challenges for the crop chain management. Dairy animals fed with mycotoxin contaminated products, like maize or derived products, excrete in milk a modified form of the toxin, aflatoxin M1, almost equally toxic, contributing to human exposure through food. Tropical and subtropical areas climate optimise *A. flavus* activity, and stress conditions, like heat and water stress, are conducive for aflatoxin production, suggesting climate change as favourable for the increase of contamination, both in terms of the amount quantified in each product and the widening of suitable geographic areas and crops for *A. flavus* occurrence. Maize is not only consumed as grain and related processed product, but also as silage and similar fermented outcomes, giving room to other less studied fungi and metabolites, sometimes with fatal effect on animals. Therefore, it can be surely affirmed that mycotoxin producing fungi play a crucial role in food and feed safety, emphasised by climate change. All these different aspects make it clear that mycotoxin producing fungi challenge food safety and food security with different relevance depending on the geographic area. Consequently, it is more and more urgent to increase the attention towards this hidden challenge to increase resilience and adaptation and optimising mitigation of climate change effects.

Low-risk active substances and plant protection products: a possible opportunity. P. CAVALLARO. *Ministero della Salute, Rome, Italy.* E-MAIL*:* p.cavallaro@sanita.it

One of the main ways of protecting plants and plant products against harmful organisms (insects, mites, fungi – including those producing mycotoxins toxic to humans - weeds) as well as improving agricultural production, is the use of plant protection products, which are one of the two main categories of "pesticides" (the other being biocides). These products can be based on chemical active substances or microorganisms (bacteria and viruses) that perform the aforementioned protective/healing action against the harmful organisms. Since, in addition to the benefits for agricultural production, they can cause potential damage to human health and the environment, these products are subject to a strict authorization procedure that requires a careful assessment of the risks for humans, animals and the environment. This authorization procedure involves first, at EU level, the active substances that are subjected to a rigorous approval procedure coordinated by EFSA, which leads to their inclusion in a positive list and

afterwards, at National level, the plant protection products that contain such substances. Only plant protection products containing approved substances may be authorised by Member States. The EU evaluation process of active substances, which began in 1993 with Directive 91/414/EEC, has led to the decommissioning of a large part of the active substances previously approved in Member States, many of which have profiles of high acute and/or chronic toxicity for humans and the environment. Regulation (EC) 1107/2009, in order to allow the availability of products useful for supporting agricultural production, introduced the concepts of "low-risk" active substances and biocontrol agents (or biopesticides), and introduced measures to facilitate the approval of said active substances, often based on microorganisms or substances of natural origin, and the authorization of the related plant protection products by the Member States. Given that biopesticides are not currently a defined group at the level of substances and products in EU legislation, these are often so-called "low risk", although there is no automatic rule of attribution and they must always meet the approval criteria. To be considered low-risk, substances must always meet the criteria defined in Regulation (EU) 1432/2017, which amends Annex II, point 5 of Regulation (EC) 1107/2009. Biopesticides are generally intrinsically less harmful/toxic than usual substances and cause less environmental load or pollution, their control nature is preventive, they work through different modes of action compared to chemical products and can be used up to harvest since they usually do not have residue problems. However, adequate incentives must be developed so that farmers gain confidence and familiarity with the use of these substances. Of the 1,470 substances present in the EU database, only 80 are low-risk substances, most of which are microorganisms while almost 1,000 are substances that have been withdrawn from the market.

Smart digital and artificial intelligence technologies for plant disease detection, territory surveillance and control measures. D. TSITSIGIANNIS. *Department of Crop Science, Iera Odos 75, Agricultural University of Athens, Athens, 11855, Greece.* E-MAIL: dimtsi@aua.gr

The escalating global demand for safe and sustainable food has necessitated the development of smart, digital, and integrated plant protection strategies. These strategies aim to enhance the resilience of agricultural systems through early detection, precise intervention, and data-driven decision-making. Our research focuses on advancing integrated pest management (IPM) by leveraging smart digital technologies, Internet of Things (IoT) sensors, artificial intelligence (AI), and innovative plant protection products to address plant disease management in key crops. To accelerate the prognosis of pest outbreaks, early warning systems are being developed utilizing IoT sensors and predictive models. These systems monitor environmental parameters like humidity and temperature as well as plant disease epidemiological parameters, providing real-time data that, when integrated with forecasting models, can predict disease outbreaks. Such predictive capabilities enable farmers to implement preventive measures proactively, reducing the reliance on reactive treatments. Modern diagnostic tools employ AI, particularly convolutional neural networks (CNNs), to analyze images of plant tissues captured via smartphones, drones, or proximal sensing cameras. These tools can facilitate early identification of plant diseases, enabling timely interventions. Additionally, spectral and hyperspectral imaging technologies, deployed via unmanned aerial vehicles (UAVs)/drones or satellites like European Space Agency's (ESA) Sentinel- 2, detect disease symptoms not visible to the human eye, enhancing surveillance capabilities over large agricultural areas. Geographic Information Systems (GIS) further support spatial analysis, mapping disease outbreaks and identifying high-risk zones for targeted management. Our research also explores the discovery and evaluation of novel plant protection products (PPPs) such as biocontrol agents, including beneficial fungal strains, bacteria, and yeasts, to control plant pests at both pre- and post-harvest stages. Innovative prototype sprayers equipped with variable rate control systems have been developed to optimize PPP application. These sprayers adjust the application rate based on real-time data, ensuring that pesticides are applied only where needed, thus minimizing chemical use and environmental impact. Coupled with Decision Support Systems (DSS), which integrate weather, soil, and crop data, these technologies provide farmers with actionable recommendations on crop management, irrigation, pesticide use, and harvest timing, promoting sustainable agricultural practices. Recognizing the importance of knowledge dissemination, our research includes capacity-building activities aimed at training farmers, agronomists, and other stakeholders in the adoption of smart digital and AI agricultural technologies. Furthermore, policy recommendations derived from data collected through early warning, detection, and response systems support European Union goals of reducing pesticide use and managing plant disease outbreaks. The integration of AI, IoT, and biocontrol agents into plant protection strategies offers a transformative approach to agriculture. By enabling early and accurate

disease detection, efficient use of agrochemicals, and informed decision-making, these technologies contribute to reducing crop loss, improving yield, and promoting sustainable agricultural practices. Our research underscores the potential of smart digital technologies in fostering regenerative, resilient, and sustainable agriculture.

*The presented research has received funding from the European Union's Horizon Europe research and innovation programmes under grant agreement No 101134750 (*https://stella-pss.eu/*).*

Integrating Plant Health into One Health through One Biosecurity. P.E. HULME. *Centre for One Biosecurity Research, Analysis & Synthesis, Lincoln University, New Zealand*. E-MAIL: philip.hulme@lincoln.ac.nz

Invasive alien species are arguably one of the most complex and undervalued emerging biological threat to global health. It is increasingly recognised that human activities have spread pathogens, parasites and their vectors across the world fuelling multiple epidemics and pandemics. However, the considerable impact invasive alien pests, pathogens and weeds have on ecosystem services supporting food and water security and good quality of life remain poorly quantified. As a result, biosecurity interventions essential to prevent, contain or eradicate these biological threats remain chronically under resourced and poorly implemented in most parts of the world. The transboundary nature of biological invasions is facilitated by the international movement of people and goods. Transport networks enable invasive species to rapidly progress from a local problem to a global crisis. Thus, failure to manage a biological threat in one nation can lead to a cascade of economic and environmental consequences across the globe. The current limited regional and global governance of biological invasions undermines any approach to deliver One Health. Embedding biosecurity more strongly within the One Health framework will be the catalyst for a more holistic approach to the management of biological invasions that ensures a common suite of tools, regulatory approaches and policy frameworks are integrated across the human, animal, plant and ecosystem health sectors. Although One Health and biosecurity both aim to protect the health of people, animals, and ecosystems from biological hazards, the two fields remain heavily siloed across distinct policy and research domains. One Health has yet to fully integrate environmental perspectives especially biological invasions, into its workplan while biosecurity lacks an effective inclusion of social and health sciences, further hindering collaboration. This holistic approach— recently termed "One Biosecurity"—requires a step change in how biological invasions are managed. This includes overcoming and crossing disciplinary barriers to strengthen surveillance systems, extend risk assessments to encompass the breadth of potential impacts, ensure open data sharing, and coordinate effective public engagement. One Biosecurity offers a vital interdisciplinary framework that bridges human, animal, plant, and ecosystem health sectors, fostering a stronger connection between biosecurity and One Health. This comprehensive approach spans the entire biosecurity continuum, from pre-border intelligence scans to border inspections and post-border incursion management, enabling more effective responses to the threats posed by biological invasions. By uniting these efforts, One Biosecurity could engage a broader group of multilateral organizations, bring together diverse stakeholders, and implement balanced strategies that better safeguard human health, agriculture, production systems, and the natural environment. It is in the interest of each individual government to support the biosecurity of its neighbouring countries, of its regional partners, and, indeed, globally to effectively address the threat of invasive alien species. Integrated governance is critical to enable the international community to act collectively to prevent local biological threats from escalating into global emergencies. Such coordination will be critical to underpinning a One Health framework which ultimately can prevent and mitigate health threats that emerge at the interface between humans, animals, plants, and the environment.

Plenary session - Xylella fastidiosa in the Euro-Mediterranean countries: Disclosing new genetic diversity and innovations in diagnostics and disease management

SESSION KEYNOTES

Insect vectors of *Xylella fastidiosa* in the EU: an overview. C. LAGO. *IFISC-(CSIC-UIB), Campus Universitat de les Illes Balears Carretera de Valldemossa, km 7,5 Edificio Científico- Técnico, 07122 Palma de Mallorca, Islas Baleares, Balearic Islands, Spain*. E-MAIL: claralago@ifisc.uib-csic.es

Xylella fastidiosa (*Xf*) is a xylem-limited, plant-pathogenic bacterium responsible for severe diseases in a wide range of economically important crops such as olives, grapevines, almonds, and citrus. This bacterium relies on insects as vectors to spread through ecosystems, mak-

ing them a critical component in *Xf* epidemiology and management. Putative vectors of *Xf* feed exclusively on xylem sap, a characteristic shared by insects belonging to the order Hemiptera, suborder Cicadomorpha, including the superfamilies Cercopoidea (spittlebugs), and the family Cicadellidae, subfamily Cicadellinae (sharpshooters) and to less extent Cicadoidea (cicadas). In the Americas, sharpshooters are the primary vectors of *Xf*, while in Europe, the main vectors are spittlebugs (Hemiptera: Aphrophoridae). The meadow spittlebug, *Philaenus spumarius*, has been identified as the most relevant vector of *Xf* in Europe. Additionally, the recent detection of *Draeculacephala robinsoni* (Hemiptera: Cicadellidae: Cicadellinae), a sharpshooter native to the Americas, raises new concerns and underscores the need for continuous monitoring of potential vector introductions. This presentation provides an overview of insect vectors of *Xf* in the EU. Key aspects of vector biology, ecology, and behaviour will be addressed, including *Xf* transmission, life cycle, host plant preferences, dispersal behaviour, and vector control strategies aimed at preventing disease spread. We will specifically highlight studies carried out by our research group over recent years, in combination with the available literature on European vectors. Our studies on the population dynamics and distribution of the main vectors of *X. fastidiosa* in olive groves in the Iberian Peninsula will be summarized. Furthermore, our studies show that the dispersal abilities of spittlebugs have revealed that adult spittlebugs are capable of travelling long distances—far greater than previously assumed. This, combined with the fact that they remain infectious for life, suggests that reducing nymphal populations is essential to manage *Xf* vectors and avoid disease spread. In addition, our research on spittlebug development and its correlation with the temperature enabled us to develop a Growing Degree Day (GDD) model to predict egg hatching and vector phenology under different climatic scenarios. This model has been used to develop a decision-support tool for optimizing the timing and effectiveness of vector control measures across various agroecosystems. Furthermore, experimental insights such as Electrical Penetration Graph (EPG) studies analysing feeding behaviour of *P. spumarius* and its relationship to pathogen transmission will be briefly presented. Finally, our studies on ground cover crops have led to the identification of repellent, trap, and attractive plants. Based on our findings we propose a combined "push-pull" strategy to manage *P. spumarius* populations: repellent plants used to deter vectors from the main crop (push), while trap crops attract vectors to specific areas (pull), facilitating their elimination. We also explored the use of entomopathogenic fungi (EF), analysing both lethal and sublethal effects against *P. spumarius*, and discuss the potential of EF as a sustainable alternative for *Xf* vector control.

Current status of *Xylella* in the Mediterranean with a focus on the evolution in Apulia, Italy. D. BOSCIA. *CNR – Institute for Sustainable Plant Protection, Via Amendola 122/D, 70126 Bari, Italy.* E-MAIL: donato.boscia@cnr.it

The discovery of an outbreak of *Xylella fastidiosa* in Puglia in 2013 represented the first case of established bacterial infections in EU. The pathogen is regulated as quarantine organism in EU and in the EPPO region (listed in the EPPO A2 list). Its finding in association with the olive quick decline syndrome, the devastating epidemic disease affecting this major Mediterranean crop, prompted the European Union to issue a strict regulation that, among the prevention measures, obliged all member states to carry out annual monitoring campaigns. Unexpectedly in the framework of this surveillance program several bacterial outbreaks were discovered mainly in southern Countries, affecting different crop species and caused by genetically different strains. Investigations showed they were mostly related to inadvertent introductions from north America, occurred recently or in the past decades. Differently from the situation determined by the spread of isolates harboring the *pauca* ST53 genotype in Puglia, in these outbreaks strains belonging to other subspecies with much limited impact have been characterized (i.e. infections were overlooked until sampling and laboratory tests led to its detection). These include outbreaks reported in Italy, in France, Portugal and Spain, caused by strains belonging to the subspecies *multiplex* and, to a lesser extent, also to the subspecies *fastidiosa*. Therefore, the first detection in Italy in 2013 resulted in a much more complex scenario than expected. Due to the emergence on olives, the Apulian authorities implemented a particularly intense monitoring program with over 1.2 million plants sampled and analyzed in the past 11 years. A program that probably represents an *unicum*, which is not sustainable in the long term, but it is certainly a useful case study to clarify the true status of the presence of *X. fastidiosa* in Europe. From the initial outbreak in Gallipoli (southwest of the Salento peninsula), the spread of the *pauca* isolates occurred at high rate until 2018, then decreased significantly, even so, currently, the demarcated infected area corresponds to the 40% of the region (about 8,000 km^2). Moreover, in 2024, while monitoring the Xf-free area, in the central part of the region outbreaks associ-

ated to two other subspecies were discovered: *multiplex*, with isolates harboring the genotype ST26, and *fastidiosa*, with isolates harboring the genotype ST1. In the first case, infections affect almost exclusively almond trees, apparently not showing any specific or manifest Xylella-symptomatology. The occurrence of ST26-infected trees occurs as spotted infected trees in three areas distant some km each other (up to 50km), currently over 35,000 samples analyzed approx. 600 plants tested positive. The demarcation is not yet definitive as the delimiting survey is still ongoing and, in some area, it overlaps the demarcated areas established for the concomitant occurrence of isolates of the other subspecies. All this, combined with the absence of specific symptoms, leads to the hypothesis that it is due to an old introduction that, having gone unnoticed, has been able to establish itself and spread undisturbed for years. The situation is different for the outbreak of the *fastidiosa*, discovered following a program of monitoring of insect vectors. In this case, the analysis of about 50,000 samples (mainly grapes and almond) has allowed to delimit the outbreak in a relatively small area, about 8 km^2, surrounded by a 2.5 km wide belt without the presence of the bacterium. In this case, inspections revealed the occurrence of severely affected grapes, suggesting that the management of this outbreak should be carefully designed. Overall, the scenario emerged in Apulia confirms how complex and heterogeneous the occurrence of the bacterium is, suggesting to adopt targeted management measures adapted case by case rather than a unique approach.

BIOVEXO: Can we find solutions to protect plants against *Xylella*? S. COMPANT, THE BIOVEXO CONSORTIUM. *Bioresources Unit, AIT Austrian Institute of Technology, Konrad Lorenz Strasse 24, 3430 Tulln, Austria.* E-MAIL: stephane.compant@ait.ac.at

Xylella fastidiosa is increasingly causing diseases in olive trees and various crops in the Mediterranean region. It has wiped out a number of olive and almond groves in Italy and Spain in only a few years. Unfortunately, the climate of the southern European Union is ideal for *Xylella*. Due to its rapid transmission across cultivation areas, *Xylella fastidiosa* is projected to cause yield losses of 35-70% in olive and 13% in almond harvests. Currently, there are no products available on the market that have been proven to be effective against *Xylella fastidiosa*, which is spread by xylem- feeding insects–notably the spittlebug *Philaenus spumarius*. Several chemical insecticides are authorized to control xylem-feeding insects. For products allowed in organic farming, only temporary authorizations, for limited periods, have been obtained. In BIOVEXO, 11 partners from 5 different countries aim to develop environmentally sustainable and economically viable plant protection solutions to control *Xylella* and its insect vector. After 4 years of the BIOVEXO project, several biopesticides have been upscaled and formulated. They are still currently being assessed and applied via foliar spray or drip irrigation on largescale field trials (olive and almond trees) as preventive and curative approaches. They are also applied in an integrated pest management program in Italy (Apulia). Results obtained so far show that some pesticides reduce the insect vector populations, while some others reduce *Xylella* symptoms on plants such as olive trees, not almonds, and only under certain conditions as assessed after 3.5 years of application. In the case of biopesticides reducing *Xylella* symptoms, only symptoms have been recorded as reduced in curative approaches, while the pathogen population seems not to be affected. A further assessment in the last year of the BIOVEXO project should be carried out to confirm these results obtained so far. However, data from field applications also show biostimulant effects against abiotic stress and an increase in plant vigor, particularly on olive trees treated with some BIOVEXO products. Results from toxicity and genotoxicity tests as well as from life cycle assessment and the shelf life of products showed further that biopesticides can be relevant for the market. Putative mechanisms of action of biopesticides targeting *Xylella* or the insect vector involve direct biocontrol properties, biostimulant effects on olive trees, and induced systemic plant resistance against *Xylella*. Insect and plant microbiomes following biopesticide applications are also currently analyzed. All the results suggest multiple potential future applications of products, i.e., as biostimulants to improve plant growth, protect plants against abiotic stress, or as biopesticides to reduce *Xylella* symptoms and vector populations.

This research has received funding from European Union's Horizon 2020 research and innovation program under grant agreement no. 887281.

ORAL PRESENTATIONS

Grapevine recovery from Pierce's disease under Mediterranean conditions. O. BAHAR[1], M. VANUNU[1,2], O. DROR[1], E. AMZALLAG[1,2], Y. OMER[1], T. ZAHAVI[3], N. MAOZ[4]. [1]*Department of Plant Pathology and Weed Research, Agricultural Research Organization – Volcani Institute, Rishon LeZion, 7505101, Israel.* [2]*The Robert H.*

Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel. [3]*Extension Service, Ministry of Agriculture, Kiryat Shemona, Israel.* [4]*Israel Wine Grapes Board, Israel.* E-MAIL: ofirb@agri.gov.il

Pierce's disease (PD) of grapevine, caused by *Xylella fastidiosa* (Xf), results in systemic colonization of the xylem and often leads to chronic infections. Although recovery from PD has been documented in California vineyards, where some infected vines were shown to clear the infection, the underlying mechanisms remain poorly understood. Notably, despite the growing number of PD reports from other regions over the past decade—including Europe and the Middle East—recovery has neither been systematically reported nor studied outside California. To investigate whether recovery from PD can occur under Mediterranean climate we inoculated Cabernet sauvignon and Chardonay grapevines at different time points from May to September and examined infection and disease symptoms for two consecutive years. During the dormancy period, grapevines were placed in two locations representing different growing climates in Israel. Infection and symptoms incidence were over 80% when inoculation was done in May or July, while only ~60% infection (with no symptoms) was seen in September inoculations. Over 90% of the plants inoculated in September recovered from the infection, in contrast to ~20% of the plants inoculated in May. Cultivar or dormancy location did not significantly affected recovery. These results demonstrate that PD recovery occurs under Mediterranean climate and confirm previous findings that inoculation timing is a critical determinant of disease outcome. Together with studies evaluating recovery in vineyards, these results will help define critical inoculation windows associated with chronic infection, which will be instrumental in designing effective PD management strategies tailored to Mediterranean climates.

This research was financially supported by the Chief Scientist of the Ministry of Agriculture of Israel, the Israel wine grape board and the BeXyl project (Beyond Xylella*, Integrated Management Strategies for Mitigating* Xylella fastidiosa *Impact in Europe; grant 101060593, from European Union's Horizon Europe "Food, Bioeconomy Natural Resources, Agriculture and Environment" Programme.*

Impact of suboptimal and extreme temperatures on *Xylella fastidiosa* survival in the Mediterranean area. M. ROMÁN-ÉCIJA, C. OLIVARES-GARCÍA, B.B. LANDA, J.A. NAVAS-CÓRTES. *Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), 14004 Córdoba, Spain.* E-MAIL: mromanecija@ias.csic.es

Temperature plays a key role in the biology and ecology of *Xylella fastidiosa* (*Xf*). Previous studies have shown that *Xf* displays a broad optimal temperature range which, combined to its adaptability to diverse environments, increases the risk of establishment and spread in Mediterranean climate- type regions. However, the effects of extreme temperatures on *Xf* survival remain poorly understood. This study evaluated whether different thermal treatments (TT) (from -20 to 4°C and from 40 to 50°C) at varying exposure times have a lethal or bacteriostatic effect on several *Xf* strains of different subspecies. To assess cell survival bacterial growth and biofilm formation were monitored, and live and dead cells were quantified using viability-qPCR after TT. Our results showed that *Xf* was able to survive TT below 4°C, including freezing temperatures, maintaining among 21 and 45% of viable cells, enabling the recovery of growth and biofilm formation once incubated under favourable conditions of 28°C. In contrast, TT at 40°C had a bactericidal effect after 24 to 72 hours of exposure. Treatments above 42°C with shorter exposure times had variable results. Even though cell viability was significantly reduced (survival rate from 0.99 to 7.38% after incubation exposure of 60 minutes), a substantial number of cells remained viable, although with growth reductions of 87– 96% compared to untreated controls. These findings provide valuable information for the development of more accurate risk maps and to support the development and implementation of TT in nurseries as a phytosanitary measure.

This research was financially supported by BeXyl (Grant ID 101060593, EU-Horizon Europe), PIE202240E067 and KODA Projects, and the PTI-SolXyl of CSIC.

Preliminary transcriptomic analyses reveal *in vitro* and *in planta* overexpression of various bacteriocins in *Xylella fastidiosa*. S.S. AMOIA, M. SAPONARI, P. SALDARELLI, A.M. LIGORIO, C. DEL GROSSO, G. LOCONSOLE, G. D'ATTOMA, D. BOSCIA, A. GIAMPETRUZZI. *National Research Council (CNR), Institute for Sustainable Plant Protection, Via Amendola 165/A Bari, Italy.* E- MAIL: serafinaserena.amoia@cnr.it

Xylella fastidiosa (*Xf*) is a xylem-limited phytobacterium of worldwide importance, associated with severe plant diseases, including Olive Quick Decline Syndrome

(OQDS). Despite extensive research on plant responses to *Xf* infection, bacterial gene expression dynamics within the host remain still poorly explored. A pilot application of dual RNA sequencing (dRNA-seq) was conducted to profile bacterial transcripts in xylem-enriched tissues of a naturally infected olive tree. A gene encoding a colicin V-like bacteriocin (*cvaC-1*), a pore-forming toxin, emerged as the most highly regulated, surpassing even housekeeping genes. The overexpression of a Blp family class II bacteriocin was also observed in RNA-seq libraries obtained from *in vitro*-grown *Xf* strains. This result was validated in a time-course experiment by testing total RNA extracts of artificially inoculated olive, citrus, and periwinkle plants using the newly developed probe-based RT-qPCR assay targeting *cvaC-1*. Notably, the test showed to be more sensitive and accurate than the conventional qPCR, widely employed for bacterial DNA detection, also being able to distinguish between growing and non-growing bacterial cells in tissues, particularly for those plant matrices from which it is laborious to retrieve *Xf* colonies on artificial media. These data support the use of *cvaC-1* as a novel biomarker to be applied in the research context for an earlier *Xf* detection, to track the bacterial progression within plants, thus predicting the pathogen-host compatibility, and to assess the impact of antimicrobial compounds. This work contributes to a better understanding of *Xf* interaction mechanisms with host plants and endophytic microbial communities.

This research was partially supported by the Regione Puglia (DD no. 495 del 14/10/2015 and no. 279 del 9/8/2016, Progetto di ricerca Linea B-STIPXYT and by Ministero dell'agricoltura, della sovranità alimentare e delle foreste in the framework of the Research Project 1LIVEXYLELLA - ID01- Trasmissione D.M. n. 664519 del 28/12/2022 (Tecnologie portatili e protocolli innovativi per la diagnosi ultrasensibile di Xylella fastidiosa *direttamente in piante e vettori).*

Sustainable management of *Xylella fastidiosa* through innovative delivery of plant extracts. D. SCHIAVI[1], M.A. MUAWIYA[1], L. FELICI[1], C. MICCOLI[1], E. GHERBALI[1], D. RONGAI[2], M.G. DI SERIO[2], R. RONCHETTI[3], P. WOJTYLO[3], S. GIOVAGNOLI[3], E. CAMAIONI[3], V. TAGLIAVENTO[4], G.M. BALESTRA[1-4]. [1]*Dipartimento di Scienze Agrarie e Forestali, Università degli Studi della Tuscia, Viterbo, Italy.* [2]*CREA-IT PE Centro di ricerca Ingegneria e Trasformazioni agroalimentari, Pescara, Italy.* [3]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Perugia, Italy.* [4]*Phy.Dia. srl, Viterbo, Italy.* E-MAIL: balestra@unitus.it

Bioactive compounds from agricultural waste could represent a valuable tool in the management of plant health issues if paired with targeted delivery systems which enhance antimicrobial properties, plant absorption, and chemical stability. This research has been evaluating a plant protection strategy against *Xylella fastidiosa* subsp. *pauca* (*Xfp*) that combines natural molecules with nanoscale delivery technologies. In particular, pomegranate peel extract (PGPE) demonstrated the capability to reduce the growth of *Xfp in vitro* starting from 0.5% w/v. PGPE was evaluated through endotherapy and root application, exploring its biostimulant and eliciting activity on olive plants. Experiments were conducted both on 2-year-old olive plants and on cuttings of 'Leccino' and 'Ogliarola'. Parameters evaluated at different time points included Nitrogen Balance Index (NBI), root-to-total plant weight ratio, and soil pH, while the expression of genes related to photosynthetic, plant defence and polyphenol pathways were analyzed in leaves. Sap from the cuttings was also extracted, and the main metabolites were identified through UHPLC/HRMS. Furthermore, the expression of genes involved in oxidative stress response and hormone biosynthesis was evaluated in root tissues. Results revealed both biostimulant and resistance activation in plants treated with our formulations, especially at 30 days post-treatment. Interestingly, significant differences were observed among the two tested cvs, suggesting specific responses to the treatments. Preliminary experiments on artificial *Xfp*-pathosystems, using tobacco plants, are under evaluation to determine the control efficacy of the proposed approaches on the disease progression as well as open field trials in *Xfp* naturally infected areas.

This research was financially supported by MASAF, project "Approcci Nanotecnologici per un Controllo Sostenibile e Innovativo di Xylella *(ANCoSIX)" CIG B1C5F6D998 CUP J83C22001990005.*

Integration of membrane-based DNA extraction with LAMP for rapid detection of *Xylella fastidiosa* in olive trees. W. MELLIKECHE[1], R. CARACCIOLO[2], M. COPPOLA[2], G. ARCOLEO[2], M. GALLO[1], A.M. D'ONGHIA[1], F. VALENTINI[1]. [1]*International Centre for Advanced Mediterranean Agronomic Studies, Via Ceglie, 9-70010, Valenzano (BA), Italy.* [2]*Enbiotech SRL, Via Masuccio Salernitano, 28-84012, Angri (SA), Italy.* E-MAIL: mellikeche@iamb.it

Early and accurate detection of plant pathogens is critical for managing outbreaks of destructive diseases such as Olive Quick Decline Syndrome (OQDS) in olive trees,

caused by *Xylella fastidiosa*. This study evaluates a novel diagnostic protocol that integrates a membrane-based DNA extraction method with real-time loop-mediated isothermal amplification (LAMP) to enhance sample handling and enable rapid pathogen detection. Naturally infected olive stem imprints on nitrocellulose were stored at room temperature and tested after their extraction by real-time LAMP over five months. These samples were compared with extracts obtained from conventional plant material stored at +4 °C and –20 °C. Validation of the LAMP assay combined with the membrane-based extraction method was performed in parallel with a reference qPCR method. Results showed that the membrane-based extraction method maintained high detection accuracy, while enhancing biosafety, facilitating sample handling, and reducing processing time. Furthermore, the real-time LAMP method achieved 100% diagnostic specificity and high sensitivity with a detection limit of 10^2 cfu/mL. This low-resource, storage-friendly protocol enhances LAMP's potential for routine detection of *X. fastidiosa* in field surveys, nurseries and control checkpoints, particularly within surveillance.

POSTER PRESENTATIONS

Early detection of *Xylella fastidiosa*-induced symptoms through hyperspectral imaging using a phenotyping platform. V. EGEA-COBRERO, J.A. JIMÉNEZ-BERNI, R. CALDERÓN, P.J. ZARCO-TEJADA, M. ROMÁN-ÉCIJA, G. LEÓN-ROPERO, A. HORNERO, J.A. NAVAS-CORTÉS, B.B. LANDA. *IAS-CSIC, Institute for Sustainable Agriculture - Spanish National Research Council, Av. Menendez Pidal s/n, 14004 Córdoba, Spain.* E-MAIL: vegea@ias.csic.es

Xylella fastidiosa (*Xf*) detection is hindered by asymptomatic infections, long incubation periods and non-specific symptoms, allowing infected plants to spread the pathogen before visible symptoms appear. A phenotyping platform that integrates a motorized vertical sliding mechanism, an illumination device, and a high-resolution hyperspectral imaging camera was designed to monitor and quantify physiological and biochemical responses in plants after *Xf*- infection under controlled conditions. *Xf* subsp. *fastidiosa* (IVIA5770) was inoculated in two commercial grapevine cultivars and incubated under full and deficit irrigation and three temperature-CO_2 combinations simulating current and future climate change scenarios. Hyperspectral images spanning 272 bands in the VNIR range (400 to 1000 nm) with 6 nm spectral and 3.5 mm spatial resolutions were acquired at 2- and 4- months post-inoculation. Additionally, *Xf*-infection was monitored via qPCR analysis of leaf petioles. An image processing pipeline was developed, including smoothing and segmentation techniques to extract pure vegetation pixels using thresholding within regions of interest in supervised classification algorithms. Furthermore, 70 vegetation spectral indices (VIs) were calculated and integrated into machine learning classification models to distinguish between *Xf*- inoculated and non-inoculated plants. Preliminary results show that VIs captured treatment- related differences, with tree-based and gaussian-based models achieving over 70% validation accuracy in detecting early *Xf*-infections. This phenotyping platform holds great potential for early detection of *Xf* at asymptomatic stages and can facilitate the selection of *Xf*-resistant genotypes in breeding programs, providing at the same time valuable information on grapevine physiological responses under various environmental stress conditions including future climate change scenarios.

Research funded by BeXyl (Grant ID 101060593, EU-Horizon Europe), PIE202240E067-CSIC, and QUAL21_023 IAS Projects and the PTI-SolXyl-CSIC.

Integrating commercial fertilizers into IPDM strategies mitigates Olive Quick Decline Syndrome in Southern Italy. C. DEL GROSSO[1,2], M. SAPONARI[2], P. SALDARELLI[2], D. PALMIERI[1], G. ALTAMURA[3], R. ABOU KUBAA[2], A. PASTORINI[1], F. DE CURTIS[1], G. LIMA[1]. [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100, Campobasso, Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council (CNR), 70126 Bari, Italy.* [3]*CRSFA- Centro Ricerca, Sperimentazione e Formazione in Agricoltura Basile Caramia, 70010 Locorotondo, Italy.* E-MAIL: lima@unimol.it, carmine.delgrosso@cnr.it

The management *of Xylella fastidiosa* subsp. *pauca* (*Xfp*) remains a major challenge due to the limited availability of effective antimicrobial, few chemical control options, and increasing restrictions on the use of traditional bactericides, particularly high dose of copper-based compounds, in the European Union. Therefore, alternative approaches, such as the evaluation of certain systemic fertilizers/biostimulants improving plant disease resistance and/or displaying bactericidal side effects, are increasingly being explored. This study investigates the antibacterial side effects of certain commercial fertilizers containing low concentration of copper and/or zinc complexed with phosphites and/or bioavailable silicon. These compounds were initially selected through in vitro assays for

their activity against *Xfp*. Greenhouse trials confirmed the effectiveness of these products in reducing disease severity and pathogen populations. In open field trials, when included into an Integrated Pest and Disease Management (IPDM) strategy, including agronomic practices and insect vector control measures, these treatments effectively reduced symptoms and improved fruit yield. Beyond their antimicrobial properties, they may also enhance plant immunity, as similar compounds are known to trigger defense mechanisms in other pathosystems. The results suggest that selected systemic fertilizers with antimicrobial properties offer a promising and sustainable approach to *Xfp* control, reducing the reliance on traditional bactericides, and improving crop resilience. This strategy is particularly important to safeguarding olive production in *Xfp*- affected areas such as Apulia, where centuries-old olive groves represent not only an agricultural resource but also an invaluable cultural heritage. Further research is needed to optimize and confirm their long-term effectiveness under broader field conditions.

This research was financially supported by INTEGROLIV project "Integrated Eco-friendly Approach for the Containment of Xylella fastidiosa *and for the Regeneration of Olive Growing and the Environment (Project Code H33C22000860001) - Minister of Agriculture, Food Sovereignty and Forests Italy (MASAF), D.M. n. 664829 del 29/12/2022 – Research Topic 2: Investigations and tests to identify methods of control of* Xylella fastidiosa. *The findings presented are based on a study accepted for publication in Plant Disease DOI:* https://doi.org/10.1094/PDIS-08-24-1770-RE.

Management of *Xylella fastidiosa* disease in almond plants using a peptide that interferes with twitching motility. L. MOLL[1], M. PLANAS[2], L. FELIU[2], L. DE LA FUENTE[3], B.B. LANDA[1], E. MONTESINOS[4], E. BADOSA[4], A. BONATERRA[4]. [1]*Institute for Sustainable Agriculture (IAS) of the Spanish National Research Council (CSIC), Spain.* [2]*LIPPSO, Department of Chemistry, Campus Montilivi, University of Girona, 17003 Girona, Spain.* [3]*Department of Entomology and Plant Pathology, Auburn University, AL 36849 Auburn, Alabama, United States of America.* [4]*Laboratory of Plant Pathology, Institute of Food and Agricultural Technology-CIDSAV, Campus Montilivi, University of Girona, 17003 Girona, Spain.* E-MAIL*:* ljmoll@ias.csic.es

Xylella fastidiosa is a xylem-limited bacterial pathogen that poses a major threat to global agriculture, severely affecting economically important crops such as almond. Within the plant xylem, the bacterium displays a dual lifestyle, alternating between sessile microbial aggregates and planktonic cells that move by twitching motility. Movement is mediated by type IV pili which is crucial for systemic colonization of the host. Although previous studies have demonstrated that peptides can modulate key biological processes in *X. fastidiosa*, their impact on motility had not yet been investigated. In this work, we evaluated the effect of both previously identified peptides and newly designed analogs on *X. fastidiosa* motility *in vitro*, and explored their protective potential against almond leaf scorch disease. By measuring the twitching fringe width and through microfluidic chamber observations, we demonstrated that BP100 significantly inhibits twitching motility. Interestingly, transmission electron microscopy observations showed that BP100-treated cells exhibited type IV pili that were similar in frequency, length, and morphology to those of non-treated controls, suggesting that the inhibition was not due to structural defects. Furthermore, endotherapy application of BP100 to almond plants inoculated with *X. fastidiosa* under greenhouse conditions resulted in a significant reduction of bacterial populations and xylem vessels were less severely affected, which correlated with a decrease in disease symptoms. Overall, our findings reveal that BP100 not only disrupts the motility of *X. fastidiosa,* but also contributes to disease mitigation *in planta*, highlighting its potential as a promising tool for the management of almond leaf scorch.

This work was supported by grants from the European Commission BeXyL (grant 101060593) and from the Spain Ministerio de Ciencia e Innovación (MCIU)/AEI and EU FEDER (TED2021-130110B-C43, PID2022- 140040OB-C21 and C22). L. Moll was a recipient of a research grant from Spain MCIU (Ref. FPU19/01434 and EST22/00007).

Exploring xylem-Inhabiting bacteria in synthetic communities for the control of vascular diseases in olive trees. L. ALONSO-VILLAR[1], N.J. FLORES-DUARTE[2], M. ROMÁN-ÉCIJA[1], M.P. VELASCO-AMO[1], C. OLIVARES-GARCÍA[1], J.A. NAVAS-CORTÉS[1], C. HARO[1], L. MOLL[1], B.B. LANDA[1]. [1]*Department of Crop Protection, Institute for Sustainable Agriculture, Spanish National Research Council, Córdoba, Spain.* [2]*Department of Microbiology and Parasitology, University of Seville, Seville, Spain.* E-MAIL*:* lalonso@ias.csic.es

Infections caused by the fungus *Verticillium dahliae* (*Vd*) and the bacterium *Xylella fastidiosa* (*Xf*) pose major threats to economically important crops such as olive (*Olea europaea* L.). In the context of the European Green Deal and its emphasis on sustainable agriculture, endophytic microorganisms are emerging as a promis-

ing strategy for the biological control of plant pathogens. In particular, synthetic microbial communities (SynComs) composed of xylem-inhabiting bacteria from olive trees can offer a novel approach for the control of these vascular plant pathogens. In this work, over 100 bacterial strains isolated from olive xylem were characterized for phenotypic and genetic traits to assess their potential as biocontrol agents and/or plant growth promoters. The analyzed traits included: (i) carbon source assimilation and tolerance to abiotic stresses (antibiotics, low pH, salinity); (ii) plant growth- promoting features (siderophore production, indole-3-acetic acid synthesis, nitrogen fixation); (iii) antimicrobial enzyme production (amylase, chitinase, protease, etc.); and (iv) in vitro inhibition of *Vd* and *Xf*. Compatibility assays among some of the most promising strains enabled the design of two SynComs, each consisting of three compatible strains. SynCom biocontrol activity is currently being tested against *Vd* and *Xf* in vitro using dual culture plates and microfluidic chambers simulating xylem vessels. Their *in planta* efficacy is being assessed in various pathosystems: *Xf* in *Nicotiana benthamiana* (model plant), *Xf* in olive, and *Vd* in olive. Additionally, the capacity of the SynComs bacterial members as well as both pathogens to systemically colonize xylem vessels after endotherapy application will be monitored via species-specific qPCR.

This research was financially supported by projects MCIN/AEI/10.13039/501100011033 (PID2020- 114917RB-100), MICIU/AEI/10.13039/501100011033 "European Union Next Generation EU/PRTR" (TED2021-130110BC41), MCIN/AEI/10.13039/501100011033 Grant IJC2019-040423-I from the Spanish Ministry of Science and Innovation (AEI); project BeXyl (Grant ID 101060593, EU-Horizon Europe) and Qualifica Project QUAL21_023 IAS.

Genomic characterization and phylogenetic analysis of *Xylella fastidiosa* subsp. *fastidiosa* strains from Cova da Beira region, Portugal. E. GARCIA[1,2], A.K. KAHN[3], C. RODRIGUES[2], A. CAMELO[1,4], C. ESPÍRITO SANTO[1,4], H.D. COLETTA- FILHO[5], R.P.P. ALMEIDA[3], J. COSTA[1,2]. [1]*University of Coimbra, Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, Coimbra, Portugal.* [2]*Instituto Pedro Nunes, Laboratory for Phytopathology, Coimbra, Portugal.* [3]*Department of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, CA 94720, USA.* [4]*CATAA - Centro de Apoio Tecnológico Agro-Alimentar, Castelo Branco, Portugal.* [5]*Centro APTA Citros Sylvio Moreira, Instituto Agronômico de Campinas, Cordeirópolis, SP, Brazil.* E-MAIL: jcosta@uc.pt

The emergence of *Xylella fastidiosa*, a xylem-limited bacterial pathogen with a broad host range, represents a significant threat to plant health and agricultural sustainability in Mediterranean ecosystems. In this study, six *X. fastidiosa* subsp. *fastidiosa* strains isolated from the Beira Interior region of Portugal, an important area for fruit production, were analysed. Whole-genome sequencing and phylogenetic analyses were performed to genetically characterize these strains and determine their origin. All strains belong to sequence type ST1, known to infect economically important crops such as cherry, plum, almond, and grapevine. The isolates were obtained from both cultivated (*V. vinifera*) and native (*Cytisus* sp.) plant species, indicating the potential role of wild vegetation as a reservoir for the pathogen. Phylogenetic reconstruction suggests a single introduction event linked to California, USA, with molecular clock analysis estimating the introduction between 2002 and 2016, with the strains forming a clade that diverged circa 2020. The increasing number of demarcated areas where *X. fastidiosa* has been detected in Portugal, along with the co- occurrence of subsp. *fastidiosa* and *multiplex* highlight the need for sustained surveillance efforts in both agricultural and unmanaged ecosystems. Moreover, the extent of *X. fastidiosa*'s establishment and its long-term ecological and economic impact on Portuguese landscapes remain largely unknown, emphasizing the necessity of intensified research efforts. The findings of this study provide insights into the pathogen's introduction in Portugal and reinforce the importance of proactive measures to protect Mediterranean agroecosystems.

This work was financed by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project XylOut (PTDC/ASP-PLA/3145/2021), by the BeXyl Project (HORIZON, id: 101060593), and supported by the R&D Unit Centre for Functional Ecology—Science for People and the Planet (CFE) UIDB/04004/2020 and Associated Laboratory TERRA LA/P/0092/2020 financed by FCT/MCTES through national funds (PIDDAC).

Screening the response of Greek olive cultivars to *Xylella fastidiosa* infections. M.M. MATHIOUDAKIS[1], N. KAVROULAKIS[1], G. KOUBOURIS[1], A.M. LIGORIO[2], V. CAVALIERI[2], R. SPANÒ[2], P. SALDARELLI[2], M. SAPONARI[2]. [1]*Institute of Olive tree, Subtropical Crops and Viticulture, Plant Pathology Laboratory, Karamanlis Ave. 167, GR-73134, Chania, Crete, Greece.* [2]*National Research Council -Institute for Sustainable Plant Protection, Via Amendola, 122/D Bari, 70126, BA, Italy.* E-MAIL: maria.saponari@cnr.it

In this work we evaluated the response of the major Greek olive cultivars to the infections caused by *Xylella fastidiosa* subsp. *pauca (Xfp)*. Isolates of this subspecies have been found to severely impact olives, with severe desiccations on the canopies leading to tree decline in highly susceptible cultivars. Huge research efforts are devoted to screen the response to the infections of a large number of cultivars representing the vast Mediterranean olive germplasm. In the framework of the projects CERTIFIED NURSERIES[a] and XYLEVA[b] a collaboration was established between the Greek and Italian research centers to assess the vulnerability of Greek cultivars to *Xfp*. Briefly, plants of the cultivars Adramitini, Kolovi, Throubolia N. Aegean, Amfissis, Chalkidikis, Gaidourelia, Throuba Kritis, Throuba Thassou and Tsounati, are currently under testing. For the first three cultivars, phenotypic assessment and diagnostic tests have been completed at 2 years post-inoculation, while they are still ongoing for the remaining cultivars. For these three cultivars systemic infections were detected in 80- 100% of the replicates, similar to that of the susceptible control, the cultivar Ogliarola salentina, which also showed defoliation, shoot dieback and complete desiccation of the plants. Although, none of the infected plants of the three N. Aegean cultivars showed severe symptoms as those reported in the control, defoliation and shoot dieback were recorded, with slightly higher impact on Throubolia N. Aegean. It should be remarked that in the cv Adramitini a slower rate of plant colonization and symptoms progression were recorded. Assessments are ongoing for the remaining cultivars.

This research has been (a) co-financed by the European Agricultural Fund for Rural Development of the European Union and Greek national funds through the Action 2 "Rural Development Program for Greece 2014-2020" (project code and acronym: M16ΣΥΝ2-00156, CERTIFIED NURSERIES), and (b) co-funded by the North Aegean Region under the programming contract "Research and information actions to enhance the preparedness of the North Aegean Region regarding the immediate eradication of the pathogen Xylella fastidiosa *in case of detection (XYLEVA).*

Evaluation of molecular tests for the determination of the *Xylella fastidiosa* subspecies: results from a European test performance study and a proficiency test. T.M. RAAYMAKERS, R.A.M. VREEBURG, A.A.L.A.M. VAN DUIJNHOVEN, M.A.W. VOGELAAR, M. BERGSMA-VLAMI. *Netherlands Institute for Vectors, Invasive plants and Plant Health (NIVIP), National Plant Protection Organization (NPPO), Netherlands Food and Consumer Product Safety Authority (NVWA), Geertjesweg 15, 6706 EA Wageningen, The Netherlands.* E-MAIL: r.a.m.vreeburg@nvwa.nl

Xylella fastidiosa (*Xf*) is an European Union (EU) priority pest with a broad host range. Plants can be infected with Xf for prolonged periods before symptoms develop. Symptomless plants are a source for introduction and spread of the bacteria. The detection of *Xf* has been harmonized among the EU national reference laboratories (NRLs). Until recently, *Xf* subspecies determination required a laborious multilocus sequence typing (MLST) test. Over the past few years, two real-time PCR tests have been published that focus on the *Xf* subspecies designation. These real-time PCRs offer several advantages when compared with the MLST test: firstly, they should increase the sensitivity of detection, secondly, they decrease the analysis time and costs for subspecies designation. Furthermore, subspecies determination is more easily achieved in plant extracts. Available validation data was limited, an international test performance study was therefore organized by the Netherlands Institute for Vectors, Invasive plants and Plant health (NIVIP) in its role as EU Reference Laboratory for pests of plants on bacteria (EURL) to evaluate the novel real-time PCRs. The results showed that these tests are fit for purpose and addition of both real-time PCR tests to EU legislation (Annex IV B of Commission Implementing Regulation (EU) 2020/1201) was recommended and implemented. Finally, a proficiency test on the determination of *Xf* subspecies was organized by NIVIP to assess the diagnostic competence of EU NRLs. The results revealed a high level of accuracy among participating laboratories.

This research was financially supported by EU, grant number 101143941.

***In vitro* screening of antimicrobial potential of *Myrtus communis* extract against *Xylella fastidiosa* subsp. *pauca, multiplex* and *fastidiosa*.** G. INCAMPO*, M. MOUROU*, D. CORNACCHIA, V. MONTILON, D. GERIN, F. FARETRA, F. NIGRO, S. POLLASTRO. *Department of Soil, Plant and Food Sciences-University of Bari Aldo Moro, via Amendola 165/A 70126 Bari, Italy* E-MAIL: francesco.faretra@uniba.it. *These two authors contributed equally to this work

Xylella fastidiosa is a Gram-negative bacterium responsible for severe damage to several economically important crops worldwide. The absence of effective and sustainable management strategies highlights the urgent need for innovative antibacterial solutions. Screening

plant-derived extracts for antimicrobial activity may offer promising alternatives for the control of xylem-colonizing pathogens, including *X. fastidiosa*. In this study, *in vitro* well diffusion and broth dilution assays were performed to evaluate the antimicrobial activity of *Myrtus communis* extract against *X. fastidiosa* subspecies *pauca*, *multiplex*, and *fastidiosa*, all reported in Apulia. Clear inhibition zones (ranging from 4 to 20 mm) were observed for all tested subspecies compared to the control. Moreover, *Myrtus* extract significantly reduced the growth of *X. fastidiosa* subsp. *pauca* by 45% after 336 hours in liquid cultures. Similar inhibitory effects were recorded for the other subspecies: a remarkable 75% growth reduction for subsp. *multiplex* and a 40% reduction for subsp. *fastidiosa*, relative to their respective controls. These findings suggest that *Myrtus communis* extract exhibits notable antimicrobial potential against different *X. fastidiosa* subspecies. However, further investigations under in vivo conditions are necessary to validate its efficacy and assess the feasibility of incorporating such natural products into integrated management strategies for *Xylella*-associated diseases.

This research was financially supported by INTEGROLIV project "Integrated Eco-friendly Approach for the Containment of Xylella fastidiosa and for the Regeneration of Olive Growing and the Environment (Project Code H33C22000860001) - Ministry of Agriculture, Food Sovereignty and Forests Italy (MASAF), D.M. n. 664829 del 29/12/2022 – Research Topic 2: Investigations and tests to identify methods of control of Xylella fastidiosa. The Authors thank Prof.ssa G. De Fonzo and Prof. F. Caponio for supplying Myrtus communis extracts.

***In vitro* high-throughput screening of the antimicrobial activity of different compounds against *Xylella fastidiosa* subsp. *pauca*.** C. DEL GROSSO[1], L. GRANDI[2], T. LOMBARDI[3], G. D'ATTOMA[1], N. SCHMITT[2], V. R. DE MICHELE[2], M. SAPONARI[1]. [1]*Institute for Sustainable Plant Protection, National Research Council (CNR), Bari, Italy.* [2]*Invaio Sciences International GmbH, Basel, Switzerland.* [3]*Invaio Sciences Italy SRL, Milan, Italy.* E-MAIL: carmine.delgrosso@cnr.it

In recent years, the distribution and host range of *Xylella fastidiosa* (*Xf*) have expanded significantly, with severe outbreaks reported in Europe. The subspecies *pauca* (*Xfp)* is considered the most dangerous, as it causes olive quick decline syndrome (OQDS) devastating olive trees in southern Italy. Despite the severe impact of this pathogen, there are no active substances officially approved to directly cure *Xf* infections in plants. Management strategies are limited to preventive actions, vector control, and eradication measures, underlining the urgent need for new therapeutic options. In this context, our research aimed to identify and evaluate new compounds with antimicrobial and antibiofilm activities against *Xfp*. To address the challenges posed by the slow growth of *Xfp*, we optimized high-throughput screening protocols to efficiently assess bactericidal and biofilm-inhibiting effects of a broad panel of products, including metal ions, micronutrients, antibiotics, and phenolic compounds. Most products showed a dose–response effect. Among micronutrients and phenolic compounds, $CuSO_4{\cdot}5H_2O$, Dentamet®, pyrocatechol, and 4-methylcatechol displayed the highest bactericidal and antibiofilm activity. Antibiotics demonstrated strong bacteriostatic effects and effectively inhibited biofilm formation. Some metal ions, such as $CoCl_2$, $K_2B_4O_7{\cdot}4H_2O$, and $MnSO_4{\cdot}H_2O$, significantly impacted bacterial cell viability but did not completely kill *Xfp*. Regarding biofilm formation, some ions inhibited biofilm, while others promoted it, such as $Ca(NO_3)_2{\cdot}4H_2O$ and Na_2MoO_4, which enhanced bacterial growth. This study provides valuable insights into the potential use of these compounds for managing *Xf* infections. The methodologies employed offer a reliable platform for profiling antimicrobial activity and identifying effective compounds for future therapeutic strategies.

Preliminary screening of new olive genotypes for resistance to *Xylella fastidiosa*. A. CARLUCCI[1], M.L. RAIMONDO[1], G. RICCIARDI[1], M.G. MOREA, F. INGROSSO[2], G. VERGARI[2], F. PENNETTA[2], F. LOPS[1]. [1]*Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Italy.* [2]*Centro Studi Olea, Lecce, Italy.* E-MAIL: antonia.carlucci@unifg.it

In the last decade, olive groves in Apulia have faced a significant threat from the quarantine bacterium *Xylella fastidiosa* subsp. *pauca* (XF). It is feared that the spread of this bacterium will expand from Salento to northern Apulia, mainly through insect vectors. Despite intense control efforts by the SSF of the Apulia Region, the XF

infection has also spread to the province of Bari. Among various control approaches being tested, genetic resistance shows promise for significant results. The present work aims to evaluate 608 new olive genotypes collected over 30 years through the crossing of numerous olive varieties. In October 2016, these genotypes were transplanted to a private farm in the Monteroni (LE), a major area infected by XF. No chemical control and fertilization treatments were carried out except to light pruning when it has been necessary. After almost eight years, 522 plants survived, from which samples were collected and analysed for the presence of the bacterium using the EPPO protocol. From the initial pool of 163 genotypes (31.23%) found not to be infected by XF at the first sampling, 45 (8.62%) were identified as XF-resistant. The non-infected olive trees were carefully observed for symptoms of other common olive diseases such as peacock spot, Cercospora leaf spot, and knot disease. Preliminary findings suggest that genetic resistance could effectively limit the spread of XF in Apulian olive groves. This study aims to offer new potentially resistant olive genotypes to olive growers of Mediterranean basin, raising their hopes for the future.

This research was financially supported by MASAF D.D. 646715/16.12.2022 – Research project "Fenotipizzazione di genotipi di olivo resistenti a Xylella Fastidiosa e messa a punto di un modello di gestione agronomica ad elevata sostenibilità – GENFORAGRIS".

Spectral signatures to reveal *Xylella fastidiosa* infection in the resistant olive cultivar 'Leccino'. E. CHIAROMONTE[1], M. MAGARELLI[1], V. MONTILON[1], A. AGNUSDEI[1], P. LUCCHESE[1], S. TANGARO[1,2], S. POLLASTRO[1], F. NIGRO[1]. [1]*Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, via Amendola 165/A, 70126- Bri, Italy.* [2]*National Institute for Nuclear Physics (INFN), Bari Section, Via A. Orabona 4, Bari, 70125, Italy.* E-MAIL: franco.nigro@uniba.it

The containment of *Xylella fastidiosa*, the causal agent of Olive Quick Decline Syndrome (OQDS), depends largely on the early detection of infected plants, particularly asymptomatic ones, including recently infected individuals or resistant cultivars such as *Olea europaea* cv. Leccino. Given the xylem-limited lifestyle of the pathogen and its ability to impair water flow through biofilm formation, this study applied hyperspectral imaging to identify infection- specific spectral signatures. Spectral data were acquired using an ASD FieldSpec® 4 spectroradiometer. To standardize the acquisition procedure, initial comparisons were made between spectra collected in the field and under controlled laboratory conditions. A preliminary analysis of 1350 spectra from 150 leaves revealed no significant statistical differences between environments, supporting the use of laboratory settings for calibration. Subsequently, spectral profiles were obtained from two sets of 70 leaves each-originating from infected and healthy branches of olive trees growing in the same field within an infected area. Infection status was confirmed via qPCR using the Harper protocol, with DNA extracted from individual leaves. For each leaf, three spectra were recorded from the basal, central, and apical regions to determine the most responsive area to infection-induced spectral variation. Data were analyzed using a Random Forest classifier to identify key wavelengths associated with infection. The portability and resolution of the spectroradiometer, combined with the results obtained under controlled conditions, suggest that this non-destructive approach can be adapted for rapid field screening of trees in quarantine zones.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Field-deployable molecular diagnostic platform for *Xylella fastidiosa* detection. A. PUCA, A.L. PEPORI, F. PECORI, S. TORRE, F. SEBASTIANI, A. SANTINI, N. LUCHI. *National Research Council, Institute for Sustainable Plant Protection (CNR-IPSP), Via Madonna del Piano 10, 50127 Sesto Fiorentino, FI, Italy.* E-MAIL: alessandropuca@cnr.it

Xylella fastidiosa (*Xf*) is a quarantine pathogen threatening several plants of economic and social importance in Europe. Accurate surveillance of *Xf* is crucial for planning phytosanitary measures to eradicate or contain outbreaks. Given the numerous potential hosts and the possible lack of visible symptoms, effective surveillance requires Point-of-Care (POC) evaluations using fast, sensitive, and specific tests. To this aim, we tested a new pipeline using the portable bCUBE device (Hyris, London, UK) for real-time PCR (qPCR) and isothermal amplification (LAMP) to detect Xf in naturally infected plants. We validated the qPCR and LAMP bCUBE systems using *in vitro* assays with DNA extracted from pure cultures, and compared them to standard labora-

tory instruments. No statistical differences were found in qPCR Ct values. For LAMP assays, the bCUBE was slightly faster and more effective in detecting low DNA concentrations of the target *Xf*. Our workflow could enhance POC monitoring, leading to better and faster data collection and aiding in rapid response for *Xf* containment.

This research was financially supported by the Ministero dell'agricoltura, della sovranità alimentare e delle foreste through the Research Project 1LIVEXYLELLA - ID01 - Trasmissione D.M. n. 664519 del 28/12/2022 (Tecnologie portatili e protocolli innovativi per la diagnosi ultrasensibile di Xylella fastidiosa direttamente in piante e vettori).

Evaluation of olive genotypes response to quick decline syndrome caused by X*ylella fastidiosa* subsp. *pauca* in the Apulian outbreak zone. A.M. SAVOIA[1], M. CARLUCCI[1], P.G. LUCCHESE[1], V. FANELLI[1], I. MASCIO[1], F.L. AURELIO[1], M.M. MIAZZI[1], A. PACIFICO[1], C. MONTEMURRO[1,2], F. NIGRO[1]. [1]*Department of Soil, Plant and Food Sciences (DiSSPA), Via Giovanni Amendola 165/A, University of Bari Aldo Moro, Bari, 70126, Italy.* [2]*Spin Off Sinagri s.r.l., Via Giovanni Amendola 165/A, University of Bari Aldo Moro, Bari, 70126, Italy.* E-MAIL: michele.savoia@uniba.it

Xylella fastidiosa subsp. *pauca* ST53 (*Xfp*) is a Gram-negative bacterium responsible for olive quick decline syndrome (OQDS). *Xfp* is a highly destructive pathogen transmitted by xylem- sap feeding insects. Currently, no effective curative treatments are available. One of the most significant advances in combating OQDS has been the identification of olive cultivars exhibiting resistance to *Xfp* and the subsequent replanting with resistant genotypes. In this context, the aim of this study was to identify new resistant/tolerant genotypes (RTG) to *Xfp* and to investigate their genetic relationships with a broad panel of 244 cultivars from the Mediterranean region. Sixteen RTG olive genotypes were monitored over six years to assess symptom severity and bacterial load. A set of nine SSR markers was used to analyze genetic relationships between the RTG genotypes and widely cultivated Mediterranean varieties, enabling synonym identification (via LRM analysis) and phylogenetic clustering (using Unweighted Neighbor-Joining analysis). Additionally, paternity tests were performed to determine the parentage of unknown genotypes. Data on bacterial population dynamics and symptom expression revealed considerable variability among genotypes, indicating different levels of susceptibility and potential resilience. Phylogenetic analysis grouped most RTG genotypes near the resistant cultivars 'Leccino' and 'FS17', suggesting significant genetic affinity. Some RTG genotypes also showed close genetic relationships with foreign cultivars such as the Albanian 'Kalinjot' and the Greek 'Leucocarpa', as well as with local Apulian cultivars. These findings underscore the potential of both local and broader Mediterranean olive germplasm as valuable sources of resistance to *Xfp*.

This research was financially supported by RIGENERA (Approcci IntegRati per il mIgliorамento GENEtico, la selezione e l'ottenimento di materiali vegetali Resistenti a Xylella fastidiosA*) (CUP: H93C22000750001); NOVIXGEN (Nuove prospettive di sviluppo per l'Olivicoltura italiana attraverso la valorizzazione della biodiversità e la selezione di materiale GENetico d'olivo tollerante/resistente a XYlella fastidiosa e azioni mirate a prevenire il possibile impatto sulla Viticoltura (CUP: C83C22001280006).*

INTEGROLIV: an integrated and eco-friendly approach for containing *Xylella fastidiosa* and regenerating the olive growing systems. G. LIMA[1], R. CASTORIA[1], F. DE CURTIS[1], D. PALMIERI[1], G. IANIRI[1], S. MARINO[1], A. PASTORINI[1], C. ALTOMARE[2], P. SALDARELLI[3], C. DEL GROSSO[3], M. MORELLI[3], F. CARAGLIA[3], A.M. MINCUZZI[3], P. DE BELLIS[2], G. BLEVE[2], A. GALLO[2], M. CIUFFO[3], F. LIMONGELLI[2], A.M. TARANTINI[2], A.M. SALZANO[4], C. D'AMBROSIO[4], G. RENZONE[4], F. NIGRO[5], F. FARETRA[5], A. IPPOLITO[5], S. POLLASTRO[5], R.M. DE MICCOLIS[5], O. POTERE[5], M. MOUROU[5], R. MARRA[6], S.L. WOO[7], V. LANZOTTI[6], N. LOMBARDI, M. SINNO[7], C. DONGIOVANNI[8], M. DI CAROLO[8], M. TAGLIENTE[8], M. CARRIERI[8], D. PERRELLI[8], M. CASETTA[8], R. ARGENTIERI[8]. [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy,* [2]*Institute of Food Production Sciences, National Research Council (CNR), 70126 Bari, Italy,* [3]*Institute for Sustainable Plant Protection, National Research Council (CNR), 70126 Bari, Italy,* [4]*Institute for Animal Production System in Mediterranean Environment, National Research Council (CNR), 80055 Portici (NA), Italy,* [5]*Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy,* [6]*Department of Agricultural Sciences, University of Naples "Federico II", 80055 Portici (NA), Italy,* [7]*Department of Pharmacy, University of Naples "Federico II", 80131 Napoli, Italy,* [8]*Research, Experimentation and Training Center in Agriculture "Basile Caramia", 70010 Locorotondo (BA), Italy*, E-MAIL: lima@unimol.it

The spread of *Xylella fastidiosa* subsp. *pauca* ST53, responsible for Olive Quick Decline Syndrome (OQDS),

has severely damaged olive cultivation in Salento (Southern Italy), causing dramatic economic and environmental losses. Although the implementation of containment measures reduced the rate of spread of the pathogen, the request of a control tools for infected plants is still urgent, particularly to preserve centuries-old native olive trees, which shape the Apulian landscape. To date, replanting with resistant cultivars remains the most effective strategy to promote the recovery in affected areas. The INTEGROLIV project (D.M. MASAF No. 664829 of 29/12/2022) addresses this crucial challenge by promoting a multidisciplinary and eco-friendly strategy. The project aims to identify, evaluate, and transfer innovative solutions to mitigate the impact of *Xylella fastidiosa* in infected areas. Selected antibacterial products are being tested *in vitro*, in greenhouse, and in the field to be successively included into new sustainable management protocols, targeting both the pathogen and its main vector, *Philaenus spumarius*. Advanced biochemical and molecular- genetic techniques are also employed to investigate the direct and indirect modes of action of the most promising products, with the goal of optimizing their effectiveness and ensuring environmental and economic sustainability. By selecting and validating novel strategies, INTEGROLIV aims to support olive grove regeneration, improve the resilience of traditional cultivars, ensure a sustainable future for olive growing in areas affected by *Xylella fastidiosa* and contribute to the long-term conservation of the Mediterranean olive agroecosystem.

This research was financially supported by INTEGROLIV project "Integrated Eco-friendly Approach for the Containment of Xylella fastidiosa *and for the Regeneration of Olive Growing and the Environment (Project Code H33C22000860001) - Minister of Agriculture, Food Sovereignty and Forests Italy (MASAF), D.M. n. 664829 del 29/12/2022 – Research Topic 2: Investigations and tests to identify methods of control of* Xylella fastidiosa.

From Risk to Response: The BeXyl Project's Integrated Approach to *Xylella fastidiosa* in Europe. B.B. LANDA, THE BEXYL CONSORTIUM. *Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), 14004 Córdoba, Spain*. E-MAIL: blanca.landa@csic.es

The BeXyl project is a Horizon Europe-funded multiactor initiative aimed at strengthening research and innovation around the priority quarantine pathogen *Xylella fastidiosa* (*Xf*). By offering interdisciplinary, stakeholder-driven solutions, BeXyl addresses three main objectives: (i) enhancing capacities for prevention, monitoring, and response to *Xf*; (ii) improving control measures, especially through biological approaches; and (iii) providing scientific support for EU and associated countries' plant health policies. Aligned within the EU Plant Health Law, BeXyl focuses on reinforcing the two fundamental pillars of plant protection: prevention and control. The project assesses the risk of future *Xf* outbreaks under current and climate change scenarios (WP1), improves early detection through enhanced surveillance and border inspection (WP2), and ensures phytosanitary standards in plant trade via thermal treatments (WP3). Restoration of affected areas is supported by the development of resistant host plant varieties (WP4). In parallel, the project advances innovative biological control strategies targeting insect vectors (WP5) and *X. fastidiosa* itself (WP6). These efforts are integrated into decision-support tools and protocols tailored for both conventional and organic production systems (WP7). Furthermore, BeXyl integrates ecological and socioeconomic factors (WP8) to support the effective adoption of management strategies. Through dedicated efforts in research, innovation, and knowledge transfer (WP9), the project contributes to the EU Green Deal by supporting the transition toward sustainable, resilient agriculture and forestry. BeXyl ultimately aims to improve crisis preparedness, help prevent new *Xf* outbreaks, and mitigate the spread and impact of current infestations across Europe.

BeXyl Project is financed by HORIZON-CL6-2021-FARM-2FORK-01 (Grant 101060593).

Bacterial Communities of *Philaenus tesselatus*. S. BOUHACHEM[1,2], C. NACCACH[3], R. ABOU KUBAA[4], M. MEZGHANI[3]. [1]*Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray, University of Carthage, Tunis, Tunisia*. [2]*GVRF Laboratoy INRGREF,* [3]*Faculté des Sciences de Tunis, Université El Manar, Tunisia,* [4]*Department of Plant Pathology, University of California, Davis, CA, USA*. E-MAIL: bouhachem.sonia@inrat.ucar.tn/bouhachems@gmail.com

Philaenus tesselatus is a potential vector of *Xylella fastidiosa*, a highly dangerous bacterium that poses a serious threat to olive trees and other fruit crops in the Mediterranean region. Given the growing interest in microbiota-mediated vector control strategies, microorganisms associated with insects are being explored as alternative biocontrol agents due to their ability to influence host-parasite interactions. To investigate the composition and structure of the symbiotic microbiota in *P. tesselatus*, we collected ten adult specimens from each of five

host plants—*Rumex crispus, Cirsium arvense, Glebionis segetum, Smyrnium olusatrum,* and *Daucus carota*—in the Cap Bon region of Tunisia. The microbial community was then analyzed using next-generation sequencing (NGS) of 16S rRNA gene amplicons. Amplicon Sequence Variants (ASVs) were classified into a total of 17 phyla, 34 classes, 82 orders, 114 families, 195 genera, and 74 species. Among these, 44 ASVs were consistently detected across all samples and considered as core microbiota, representing a substantial fraction of the total microbial community. The bacteriome of *P. tesselatus* comprised 20 distinct bacterial phyla, with *Proteobacteria*, *Cyanobacteria*, *Bacteroidota*, *Actinobacteriota*, *Bdellovibrionota*, and *Firmicutes* being the most abundant. The most well-represented bacterial orders include *Pseudomonadales*, Chloroplast (derived from symbiotic plant-associated bacteria), *Rhizobiales*, *Burkholderiales*, *Rickettsiales*, *Sphingomonadales*, *Sphingobacteriales*, Flavobacteriales, and Enterobacteriales. Notably, key symbionts were found were primarily found within the orders *Rickettsiales* (*Wolbachia*, *Rickettsia*, *Candidatus* Megaira), *Sphingobacteriales*, *Enterobacteriales* (*Rosenbergiella*), *Flavobacteriales*, and *Burkholderiales* (*Duganella*), with corresponding genera/species identified. Significant variation in endosymbiotic bacterial communities was observed across specimens collected from different host plant, highlighting the crucial role of host plant species and environmental conditions on microbial community composition. These findings provide valuable insights into the ecological of *P. tesselatus*, suggesting that host-associated microbial diversity may play a key role in the insect's biology and potential as a disease vector.

CONCURRENT SESSION B1— Plant viruses and phytoplasma diseases in a changing climate

SESSION KEYNOTES

Success and challenges: the new diagnostic era using High Throughput Sequencing. M. AL RWAHNIH. *Department of Plant Pathology, Foundation Plant Services, University of California, Davis, USA.* E-MAIL*:* malrwahnih@ucdavis.edu

Foundation Plant Services (FPS), located in Davis, California, USA, is a source of elite propagation materials of grape, Prunus, pistachio, olive, strawberry, rose and sweetpotato cultivars as a member of the US National Clean Plant Network (NCPN). NCPN clean plant centers such as FPS are responsible for conducting diagnostic testing, therapeutics, and propagation, and distribution of certain vegetatively propagated speciality crops. FPS also facilitates the introduction, quarantine, testing and release of material under a Controlled Import Permit from the United States Department of Agriculture, Animal Plant Health Inspection Service, Plant Protection and Quarantine (USDA- APHIS-PPQ). When FPS was established in 1958, conventional techniques for detection of plant viruses relied on biological indexing using indicator plants. Diagnostic methods advanced to immunodetection (like ELISA) and then polymerase-chain reaction (PCR) testing, though indexing was still considered the "gold standard". FPS has pioneered the use of high throughput sequencing (HTS) as a diagnostic method, which is now approved by USDA-APHIS-PPQ for release of plant material from quarantine and by California Department of Food and Agriculture for use in grape and fruit tree certification programs. HTS for virus detection and characterization in plants was first used at FPS in 2007 and compared to biological indexing and other conventional methods beginning in 2012. After demonstrating that HTS was more effective at detecting virus infection than biological indexing, FPS validated its protocols and took part in an interlaboratory validation study to demonstrate reproducibility across labs. After this rigorous validation of HTS protocols, FPS received approval to utilize this technology on regulated grape and fruit tree plant materials in 2021. To date, the USA is one of only a few countries who have adopted HTS in quarantine and certification programs, although other countries have been evaluating the technology and the academic reference literature continues to grow. HTS adoption has been slow, primarily due to challenges in standardizing preparation and evaluation methods. Human error caused by fatigue due to repetitive processes can be alleviated by using automated sample processing equipment, which allows for high sample throughput and consistent results. A laboratory utilizing HTS must have a skilled bioinformatician to align and analyze the complex sequence data that is generated. A trained virologist must review the data provided by the bioinformatician and consider whether viruses detected are evidence of real infections, or if they are artifacts of cross-contamination or non-host contribution to the sample. Using HTS, new viruses are being discovered at an unprecedented rate. It is important that when a new virus is detected when testing plant material using HTS, detection is confirmed with a second testing method such as PCR, graft transmissibility is demonstrated, and scientists remain in regular and clear communication with regulatory agencies to assess the threat to plant health.

ORAL PRESENTATIONS

Comparative analysis of Stolbur Phytoplasma genomes highlights key genomic features of the novel 16SrX-II-P Stolbur pathogen. R. TOTH[1], B. HUETTEL[2], O. EINI[3], M. VARRELMANN[3], M. KUBE[1]. [1]*Integrative Infection Biology Crops-Livestock, University of Hohenheim, 70599 Stuttgart, Germany.* [2]*Max-Planck Genome Centre Cologne, Max-Planck-Institute for Plant Breeding Research, 50829 Cologne, Germany.* [3]*Department of Phytopathology, Institute of Sugar Beet Research, 37079 Göttingen, Germany.* E-MAIL: rafael.toth@uni-hohenheim.de

Stolbur phytoplasmas (Mollicutes) are a heterogeneous group of insect-transmitted phytopathogenic bacteria that cause several diseases in crops including grapevine, potato, tomato, corn, and sugar beet. Since the 1990s, it has been known that stolbur phytoplasmas are also present in sugar beets diseased by syndrome "basses richesses", caused by the '*Candidatus* Arsenophonus phytopathogen' (γ-Proteobacteria). Both pathogens are transmitted to sugar beet and other crops by the cixiid *Pentastiridius leporinus* L. The ongoing epidemic in Germany is driven by a novel, poorly characterised phytoplasma of the 16SrXII-P subgroup. We determined and analysed the complete genome of the 16SrXII-P phytoplasma strain GOE from a metagenomic high-molecular-weight DNA template obtained from infected *P. leporinus* individuals using single-molecule real-time sequencing (PacBio). The metagenomic dataset was filtered for phytoplasma reads via taxonomic binning. *De novo* assembly was performed on the selected reads. The circular GOE chromosome was compared with other stolbur genomes regarding phylogeny, genome organization, encoded metabolism and virulence factors. The results suggest that 16SrXII-P phytoplasmas represent a new phytoplasma species. GOE is characterised by the smallest size and lowest G+C content compared to other complete stolbur genomes. While stolbur phytoplasmas share conserved membrane proteins and minimal metabolism of phytoplasmas, they differ in encoding a riboflavin kinase, suggesting a lost pathway outside the 16SrXII and 16SrXIII group. In addition, virulence factor analysis showed that GOE encodes a complete *tra5*-transposon forming a phytoplasma-pathogenicity island since it harbours important effector proteins. These findings emphasise the diversity within the 16SrXII group and improve the understanding of phytoplasma evolution.

This research is part of the project "Differentiation of pathogens and course of infection in SBR-associated bacterioses in sugar beet to derive resistance testing methods to ensure yield stability" which is coordinated by the "Gemeinschaft zur Förderung von Pflanzeninnovation e. V." (GFPi) and founded by the Federal Ministry of Economic Affairs and Climate Protection (BMWK) (funding no. 22943 N).

'*Candidatus* Phytoplasma phoenicium', a causal agent of stone fruits diseases and a target for surveillance. F. QUAGLINO[1], Y. ABOU JAWDAH[2], A. ALMA[3], P.A. BIANCO[1], P. CASATI[1], E. CHOUEIRI[4], M. KUBE[5], M. MOLINO LOVA[6], R. TEDESCHI[3]. [1]*Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Università degli Studi di Milano, 20133 Milan, Italy.* [2]*Department of Agricultural Sciences, Faculty of Agricultural and Food Sciences, American University of Beirut, 11-0236 Riad El-Solh 1107-2020, Beirut, Lebanon.* [3]*Department of Agricultural, Forest and Food Sciences, Università degli Studi di Torino, 10095 Grugliasco (TO), Italy.* [4]*Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287, Zahlé, Lebanon.* [5]*Department of Integrative Infection Biology Crops-Livestock, University of Hohenheim, 70599 Stuttgart, Germany.* [6]*AVSI Foundation, Jounieh, Ghadir, Lebanon.* E-MAIL: piero.bianco@unimi.it

A disease affecting almond trees, characterized by symptoms such as small yellowish leaves, shoot proliferation, and dieback, was first observed in Lebanon during the 1990s. In 2001 it was reported as being associated with phytoplasmas belonging to the pigeon pea witches'-broom (PPWB) group (16SrIX). In 2002 other authors carried out a survey in Lebanon showing the association of Almond Witches'-broom disease (AlmWB) with a phytoplasma from a newly subgroup 16SrIX-B, later classified as '*Candidatus* Phytoplasma phoenicium' and detected in both Lebanon and Iran.From 2010 to 2015, a scientific cooperation project between Lebanese and Italian institutions was funded with the aim to investigate the host range of the pathogen, the geographic distribution of the disease, and the identification of potential insect vectors. These studies revealed that the epidemiological cycle of AlmWB involves *Asymmetrasca decedens* (Hemiptera: Cicadellidae), possibly responsible for almond-to-almond transmission of '*Ca*. P. phoenicium'. In addition, two species of *Tachycixius*, *T. viperinus* and *T.* cf. *cypricus* (Hemiptera, Cixiidae), were identified as potential vectors responsible for transmitting the phytoplasma from weed hosts to almond trees. Further studies, including draft genome sequencing, demonstrated that '*Ca*. P. phoenicium' (i) infects almond, peach, nectarine, and apricot in both Lebanon and Iran; (ii) the genome encodes membrane proteins and effector-like

proteins that may play a role in host- pathogen interactions and pathogenicity. In 2017, the European and Mediterranean Plant Protection Organization (EPPO) published a pest risk analysis of '*Ca.* P. phoenicium', and the EUPHRESCO network supported the project "DIPCAPP" (Development of reliable protocols for the detection and identification of '*Ca.* P. phoenicium).

Insights into Wisteria vein mosaic virus: an evolutionary framework for understanding the emergence of new viral threats. M. MORELLI, G. D'ATTOMA, P. SALDARELLI, A. MINAFRA. *CNR, Institute for Sustainable Plant Protection (IPSP), Via Amendola 122/D, 70126 Bari, Italy.* E-MAIL: massimiliano.morelli@cnr.it

Wisteria vein mosaic virus (WVMV, *Potyvirus wisteriae*), a member of the *Potyvirus* genus, is the causative agent of Wisteria vein mosaic disease (WMD), a severe infection threatening wisteria plants, ornamentals highly valued worldwide. Likely originating in East Asia, WVMV was first officially documented in the United States, and subsequently reported in multiple countries. In Italy, the virus was first identified as the WVMV Bari isolate, and its complete genome was sequenced using NGS barcoding technology. Phylogenetic analysis via PhyML, corroborated by clustering algorithms, revealed a distinct division into two phylogenetic groups. The dominant clade included isolates from *Wisteria* spp., while the minor clade comprised three genetically divergent strains, with sequence identity close to the species boundary, infecting herbaceous non-wisteria hosts. Molecular clock estimates, based on the Relative Time Dated Tips (RTDT) approach, placed the divergence of these lineages around the 17th century. Network inference further validated the genetic separation between the two host-related groups and indicated the potential existence of intermediate variants occurring in the host jump transition. Population genetic analyses demonstrated a pronounced genetic divergence, reinforced by significant permutation tests. The fixation index (*F*ST) and migrant number (*N*m) indicators pointed to limited gene flow and well- structured populations. Negative neutrality tests and a low d*N*/d*S* ratio suggested that purifying selection is actively removing deleterious mutations. This study proposes an evolution-based framework approach, using the intriguing evolutionary dynamics of WVMV as a case study to explore how other overlooked potyviruses or newly emerging viruses could spread and infect economically important crops.

RNA-Seq transcriptomic analysis reveals genetic mechanisms enhancing virus tolerance in grafted *Cucumis melo* onto *C. melo* cv. *Barattiere*. M. CRUDELE, M. MARASHI, R.M. DE MICCOLIS ANGELINI, M. MASTROCHIRICO, T. MASCIA. *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.* E-MAIL: marco.crudele@uniba.it

Tomato leaf curl New Delhi virus is an emerging begomovirus listed in the EPPO Alert-list 2, first reported in 2015 in Southern Italy on Sicilian courgette, and particularly harmful to cucurbit crops, often leading to complete production losses. RNA-Seq analyses of *C. melo* cv. Retato Standard F1 (RSF1) (susceptible), not grafted and grafted onto *C. melo* cv. Barattiere (B) (tolerant), was performed to study mechanisms conferring tolerance in grafted plants. A total of 189,419,643 Illumina short reads (2 x 150 bp) were generated and mapped to the *C. melo* reference genome. By comparing grafted *vs* ungrafted and inoculated *vs* mock-inoculated plants, a total of 7,962 differentially expressed genes (DEGs) (FC $\geq |2|$, FDR ≤ 0.05) were identified. ToLCNDV infection in grafted plants strongly induced the expression of genes involved in defense mechanisms (e.g., pathogen-induced protein CuPi1 and acidic endochitinase precursor) and the activation of 798 genes involved in signal reception and transduction, response to pathogen (e.g., Major Latex Protein 28 and 149-like isoform X1) and stress factors (e.g., protein RADIALIS-like 1 and 4). DEGs activated by grafting included genes exclusively or more strongly induced after virus infection. Among them, several genes were involved in stress response and oxidative stress (e.g., protein RADIALIS-like 1 and glutaredoxin C11 and C13) and in the biosynthesis of volatile terpenoids and aromatic compounds (e.g., nerolidol synthase 1- like and benzyl alcohol O-benzoyltransferase), respectively. This study provides insights genetic mechanisms underlying virus tolerance in RSF1 plants grafted onto B plants and into molecular interactions between ToLCNDV and *C. melo*.

This research was financially supported by National Recovery and Resilience Plan, Mission 4, Component 2: "From Research to Business" – Investment 3.3. This study was carried out within the Regione Puglia Administration under Rural Development Program 2014–2020, Project 'Biodiversity of Apulian fruit vegetables (BiodiverSO Karpos)', Measure 10, Sub measure 10.2, Operation 1 "Program for the conservation and the valorization of the genetic resources in agriculture" (DDS n. 04250178565) and Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032

17/06/2022, CN00000022).

Biological characterization of beet western yellows virus-pepper isolate. N. BUZKAN[1], B.B. ARPACI[2], C. VILLEROY[3], V. ZIEGLER-GRAFF[4], V. BRAULT[3]. [1]*Plant Protection Department, Agriculture Faculty, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, Türkiye.* [2]*Horticulture Department, Agriculture Faculty, Kilis Yedi Aralik University, Kilis, Türkiye.* [3]*INRAE, Colmar, France.* [4]*CNRS-IBMP, Strasbourg, France.* E-MAIL: nbuzkan@ksu.edu.tr; nbuzkan@gmail.com

Beet western yellows virus (BWYV) infecting over 150 plant species from 23 dicotyledonous families is the most well-known member of the genus *Polerovirus* in the family *Solemoviridae*. An isolate of this virus was first described in Turkey, which represents the first polerovirus reported to infect cultivated pepper naturally and to cause a severe disease. This polerovirus displays sequence similarities with BWYV and is distantly related to the Chinese and American isolates from sugar beet and lettuce. This study investigated the host range of BWYV- pepper and -sugar beet isolates. Whole peppers collected from symptomatic plants were used as virus source in aphid (*Myzus persicae* Sulzer) transmission assays onto healthy peppers. Virus purification was performed from peppers to use as inoculum source in aphid transmission tests while the purified BWYV-sugar beet was already available in stock. Non viruliferous aphids (*ca*. 200 individuals) were fed on an artificial medium containing the purified virus preparations through stretched parafilm for 24 hours. Then, groups of 10 aphids were transferred onto healthy sugar beet (*Beta vulgaris*), pepper (*Capsicum annuum*), *Claytonia* spp., spinach (*Spinacia oleraceae*) and pea (*Pisum sativum*) plants at 2- to 4-true leaf stage for 6 days. Virus presence was checked by ELISA using a turnip yellows virus (polerovirus) antiserum combined with molecular detection. Results showed that BWYV-pepper and -sugar beet isolates could not infect sugar beet and pepper respectively, although both isolates were successfully transmitted onto other hosts in the experiments.

This research was granted by TUBITAK- FRANCE PHC BOSPHORUS (221 N 050).

Biological characterization of tomato fruit blotch virus (genus *Blunervirus*, family *Kitaviridae*) a "HTS-borne" plant virus. A. TIBERINI[1], A. SYBILSKA[2], M. LUIGI[1], F. TARCHI[3], A. TAGLIENTI[1], D. LUISON[1], F. FAGGIOLI[1], S. SIMONI[3], M. LEWANDOWSKI[2], S. BERTIN[1]. [1]*Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Rome, Italy.* [2]*Department of Plant Protection, Warsaw University of Life Sciences, Nowoursynowska St. 159, 02-776 Warsaw, Poland.* [3]*Council for Agricultural Research and Economics, Research Centre for Plant Protection and Certification, Via Lanciola 12/a, 50125 Florence, Italy.* E-MAIL: antonio.tiberini@crea.gov.it

High-throughput sequencing (HTS) is a powerful tool for detecting and identifying viruses in plant hosts, regardless of their genome nature or structure. HTS also provides insights into virus population structure, ecology, and evolution. Despite the exponential growth of HTS data, many newly identified viruses remain biologically uncharacterized, and their epidemiological significance is unassessed. The Euphresco project "Valorisation of HTS output data in view of a timely risk assessment of regulated or emerging plant viruses (VALORHIGHTS)", recently started, aims to provide biological information to support the evaluation of phytosanitary risks posed by novel or poorly characterized viruses. Tomato fruit blotch virus (ToFBV - *Blunervirus solani*; genus *Blunervirus*, family *Kitaviridae*) is a recently identified virus causing uneven ripening and dark spots on tomato fruits. First identified by HTS in 2018 in Italy, ToFBV shows a recurrent emergence trend and is included in the EPPO Alert list. Recent experiments demonstrated the virus's replication and translocation within the host plant and identified potential weed hosts. Additionally, HTS data mining of existing RNAseq datasets from ToFBV-infected tomato samples suggested a mite as a potential vector. The sequences retrieved were taxonomically assigned to the tomato russet mite (TRM) *Aculops lycopersici* (Tyron). The presence of this mite was confirmed at virus outbreak sites. Two independent transmission trials unequivocally identified the mite as the vector of ToFBV. These findings provide new insights into the potential spread of ToFBV and inform the development of effective control and surveillance strategies.

Witches' broom disease of lime in the Arabian Peninsula: a multiscale study of pathogen dynamics, climate influence, and trade-driven spread. A.M. AL-SADI[1,2], A.M. AL-SUBHI[2], A.G. AL-GHAITHI[2], R.A. AL-YAHYAI[2]. [1]*College of Agriculture, University of Al Dhaid, Sharjah, P.O. Box 27272, United Arab Emirates.* [2]*Department of Plant Sciences, Sultan Qaboos University, P.O. Box 34, Al Khod 123, Sultanate of Oman.* E-MAIL: alsadi@uodh.ac.ae

Witches' Broom Disease of Lime (WBDL), caused by *'Candidatus* Phytoplasma aurantifolia', has led to severe losses in lime production across the Middle East. In our study, we investigated the molecular, ecological, and environmental factors influencing the spread and severity of WBDL, with a particular focus on the Arabian Peninsula. Through field surveys conducted across 18 districts in Oman and the UAE, we documented widespread phytoplasma infection, with symptom severity varying by climate. Witches' broom symptoms were most pronounced in subtropical areas, while trees in arid and semi-tropical zones showed milder symptoms despite harboring the pathogen. Molecular analysis revealed significantly higher phytoplasma titers in symptomatic leaves compared to asymptomatic ones. We detected elevated expression of the phytoplasma effector gene *SAP11WBDL*, which correlates with the downregulation of plant defense-related pathways, particularly those involving jasmonate and salicylic acid signaling. Our findings confirm that *SAP11* interferes with TCP transcription factors, promoting excessive shoot proliferation, which in turn increases phytoplasma accumulation and enhances transmission by the leafhopper vector *Hishimonus phycitis*. In parallel, we examined historical trade patterns and phytosanitary practices in the region. The results indicate that the introduction and spread of WBDL were facilitated by increased international trade and the movement of infected plant material and insect vectors. Our study highlights the urgent need for enhanced biosecurity measures, including stricter quarantine protocols and coordinated regional surveillance, to mitigate the further spread of WBDL and protect citrus production systems in the Middle East.

Development of *turn-back* loop-mediated isothermal amplification assay for the detection of *Tomato fruit blotch virus*. S. Y. ATEŞ[1], B. ÖZGÖREN[2]. [1]*Ministry of Agriculture and Forestry, Izmir Agricultural Quarantine Directorate 35230 Izmir, Türkiye.* [2]*DOSA Bilgi ve Bilim Teknolojileri Ltd.Şti., Ege Üniversitesi Teknopark 35100 Izmir, Türkiye.* E-MAIL: songulyalcinates@outlook.com

Tomato fruit blotch virus (ToFBV-*Blunervirus solani*) is a new emerging virus in tomato and was added to the EPPO's alert list as of 2024-01. As it is a new pathogen, its epidemiology and rate of spread are still unknown. Currently, ToFBV is only detected by RT-PCR which is time consuming. In this study, a novel turn-back loop primer-accelerated LAMP (TLAMP), for reliable and sensitive detection of the virus directly from field samples at quarantine laboratories, was developed. Basic Local Alignment Search Tool (BLAST), Clustal Omega and PrimerExplorer v5 were used to design primers targeting conserved regions of RNA3 and RNA4, enabling the detection of a wide range of ToFBV isolates. They were successfully used for colorimetric TLAMP and fluorometric TLAMP methods in several laboratory assays. Additionally, BLAST and laboratory studies showed that there was no cross-reaction with other viruses such as tomato brown rugose fruit virus and tomato spotted wilt virus, commonly infecting tomato plants.

POSTER PRESENTATIONS

Detection of grapevine virus A in grapevine in Tekirdağ province of Türkiye. S.D. NOGAYLAR, H. İLBAĞI. *Department of Plant Protection, Faculty of Agriculture, Tekirdağ Namık Kemal University, 59030, Tekirdağ, Türkiye*. E-MAIL: hilbagi@nku.edu.tr

Grapevine virus A (GVA) is a type of member of the genus *Vitivirus*, family *Betaflexiviridae*. It is associated with the Kober stem grooving disease of the Rugose wood complex (RW) and is transmitted by grafting, mealybugs, and scale insects. The virus is among the most frequently identified viruses in vineyards worldwide; however, no comprehensive study has been associated with GVA in Tekirdağ province to determine its prevalence. In this study, extensive surveys were conducted to investigate the presence and prevalence of GVA at the end of summer 2024 in the vineyards of Tekirdağ province, Trakya, which has the largest germplasm and collection vineyard in Turkey. The surveys involved the collection of a total of 139 symptomatic and asymptomatic leaf samples from autochthonous and foreign grapevine cultivars. Leaf tissues from all samples were screened using DAS-ELISA, and then subjected to amplify a 429 bp using a specific primer of GVA-coat protein (CP) gene by RT-PCR assay. None of the autochthonous and foreign grapevine cultivars, such as 'Semillion', 'Merlot', 'Gamay', 'Hamburg Misketi', 'Alphonso Lavalle', 'Cinsaut', 'Adakarası', 'İlkeren', 'Cabernet Sauvignon' were found to be infected with GVA. However, both DAS-ELISA and RT-PCR assays revealed that only 'Yapıncak' autochthonous cultivar showed a positive reaction in 9 out of 139 leaf samples. As a result of this study, the infection rate of GVA was found to be 6.47% in the commercial vineyards of Tekirdağ province of Trakya, Turkey.

This research was financially supported by Tekirdağ Namık Kemal University, The Scientific Research Projects Coordination Unit (NKU-BAP, Project No: NKUBAP.03.YL.24.554).

Investigation of maize dwarf mosaic virus in maize and common reed in Türkiye. H. İLBAĞI, N. ÇETİN. *Department of Plant Protection, Faculty of Agriculture, Tekirdağ Namık Kemal University, 59030, Tekirdağ, Türkiye.* E-MAIL: hilbagi@nku.edu.tr

Maize (Zea mays L.) is one of the most important cereals for human and animal consumption and is grown for grain, forage, and silage in the Trakya region of Türkiye. Viral diseases negatively affect maize production yearly by causing significant yield loss in maize fields worldwide. Among maize-infecting viruses, maize dwarf mosaic virus (MDMV) is one of the economically important viruses that cause yield loss in maize. Our study aimed to determine the presence of maize dwarf mosaic virus in both maize and common reed. By this aim, 34 maize and 19 reed samples were collected from Tekirdağ province of Trakya in 2023. Leaf tissues from symptomatic and asymptomatic plants were first screened for the presence of maize dwarf mosaic virus (MDMV) by the DAS-ELISA test. Subsequently, all leaf samples were tested to amplify a 720 bp using the primer pair designated from the NIb gene region of the MDMV genome. The ELISA and RT-PCR results revealed that 7 out of 34 maize and 10 out of 19 reeds were found to be infected with MDMV. PCR amplicons were subsequently sequenced and subjected to a BLASTn search to identify MDMV sequences. Multiple nucleotide sequence alignments of Turkish maize and reed MDMV isolates revealed that the highest level of identity was 85.37–76.77% and 86.36–76.77% with Golestan and SP isolates from Iran and Spain, respectively. Amino acid multiple sequence alignments of Turkish maize and reed isolates with other published MDMV isolates revealed that the highest level of identity was 95.93–66.66% with TR34, TekirdagE41, TekirdagK43, and TekirdagT42 isolates from Türkiye. The nucleotide-based phylogenetic tree clustered with MDMV isolates from Türkiye. The amino acid-based phylogenetic tree clustered with MDMV isolates from Türkiye, Germany, Hungary, Bulgaria, and Iran.

This research was financially supported by Tekirdağ Namık Kemal University, The Scientific Research Projects Coordination Unit (NKU-BAP, Project No: NKUBAP.03.GA.21.289).

Heat shock proteins 70 in viruses: diversity and evolution. A. MAACHI. *Regional Center of Agricultural Research of Agadir, National Institute of Agricultural Research (INRA), Inezgane, 86350, Agadir, Morocco.*

The heat shock proteins 70 (Hsp70) are a family of conserved ubiquitously proteins, reported from all living organisms. They play a key role in performing chaperoning functions, helping to protect cells from adverse effects of physiological stress. Hsp70 consists of two high conserved domains, a nucleotide binding domain at the N-terminal, and a substrate binding domain at the C-terminal. In viruses, hsp70 were previously reported from closteroviruses, however, little is known on their abundance, diversity, and evolution. In this work, we searched for hsp70s from virus protein database from the National Center for Biotechnology Information. Hsp70s retrieved belonged mainly to viruses from two different classes: Alsurivicetes (viruses reported to infect plants) and Megaviricetes (Giant viruses reported to infect protists). In the megavirids, multiple copies of hsp70 were recovered from theHir genomes suggesting either gene duplication or multiple acquisition events. A correspondence analysis based on the similarity matrix among these proteins showed two different clusters, one grouped hsp70s from giant viruses, and the other, grouping the hsp70 from plant viruses, suggesting different evolutionary origins of these proteins. The relationship among virus hsp70s were uncovered based on the phylogenetic trees, showing that the hsp70 from plant viruses exhibited more diversification, suggesting higher evolutionary rates, and higher genetic diversity in comparison to the ones from megaviricids. Our results show that the hsp70 presence is restricted in viruses, suggesting the occurrence of few gene transfer events of these proteins between viruses and their hosts.

Sanitary status of onion (*Allium cepa*) in Tunisia. S. NAHDI[1,2], W. ABIDI[1], B. M'RABET SAMAALI[3], M. BRAHAM[3], S. MATIC[4,5]. [1]*Ecole Supérieure d'Agriculture du Kef, ESAK, rte de Dahmani Boulifa 7100 Kef - KEF, Le Kef, Université de Jendouba, Jendouba, Tunisia.* [2]*Laboratoire de Protection des Végétaux (LPV), INRAT, Université de Carthage, Rue Hédi Karray, 1004 El Menzah, Ariana, Tunisia.* [3]Laboratoire des Analyses Virologiques, *Direction Générale de la Santé Végétale et du Contrôle des lntrants Agricoles,* 30 Rue Alain Savary, Le belvédère, Tunis 1002, Tunisia. [4]*Institute for Sustainable Plant Protection, National Research Council of Italy (IPSP-CNR), Strada delle Cacce 73, 10135 Turin, Italy.* [5]*Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Ed. 5, 90128 Palermo, Italy.* E-MAIL: nehdimah@yahoo.fr

Similar to other vegetatively propagated crops, onion

(*Allium cepa* L.) is highly vulnerable to viral infections that can accumulate over successive cycles, leading to yield and quality losses. In Tunisia, despite the crop's economic importance, little information is available about the diversity of viruses affecting onion. Until recently, only onion yellow dwarf virus (OYDV) had been officially reported, although field observations often show virus-like symptoms. To investigate this gap, a survey was conducted in spring 2023 to assess the presence of other viruses in symptomatic onion plants. A total of 111 leaf samples were collected from five major onion-producing regions (Sousse, Mahdia, Gabes, Kebili, and Cap Bon). Samples were tested using DAS-ELISA for Leek yellow stripe virus (LYSV), garlic common latent virus (GarCLV), and three allexiviruses (garlic Virus A (GarV-A), garlic Virus B (GarV-B), garlic virus C (GarV-C)). Results showed that 75 out of 111 samples (67.6%) were infected with at least one virus. GarCLV was mostly prevalent (72.05%), followed by GarV-C (27%), GarV-A (21.61%), LYSV (4.5%), and GarV-B (9%). Mixed infections were more common than single infections. These findings reveal a broader viral diversity in Tunisian onions than previously reported. Further investigations involving high-throughput sequencing, vector identification, and epidemiological studies are necessary to enhance our understanding of the onion virome and to support the development of integrated and sustainable disease management strategies.

Response of Local Tomato Ecotypes from the Campania Region (Italy) to Infections by Potato virus Y (C-to strain) and Potato Spindle Tuber Viroid. L. VACCARO[1], R. MARZIALE[2], R. SPANÒ[2], D. ALIOTO[3], B. NAVARRO[2], F. DI SERIO[2], T. MASCIA[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", 70126 Bari, Italy.* [2]*Institute for the Sustainable Protection of Plants, National Research Council (CNR) Bari, Italy.* [3]*Department of Agriculture, University of Naples Federico II, 80055 Portici, Naples, Italy.* E-MAIL: lorenza.vaccaro@uniba.it

Tomato (*Solanum lycopersicum*) is one of the most important solanaceous crops due to its economic and nutritional relevance. However, it is susceptible to a wide range of plant pathogens, including a recombinant strain of potato virus Y (PVY^{C-}to) and potato spindle tuber viroid (PSTVd), both of which are considered major threats to tomato cultivation. PVY^{C}-to, a single-stranded RNA virus (family *Potyviridae)*, induces symptoms such mosaic, chlorosis, and vein necrosis in horticultural crops, leading to significant production losses. PSTVd, a nuclear- replicating viroid (family *Pospiviroidae),* elicits stunting and leaf curling in tomato plants. This study aimed to assess the susceptibility of five local tomato ecotypes from the Campania region (Corbarino, Vesuviano, Giallo determinato da Serbo, Pizzutello, and San Marzano) to PVY^{C}-to and PSTVd infections. Visual symptom assessment at 14 and 28 days post-inoculation (dpi) and quantification of virus and viroid accumulation using qDot-Blot or RT-qPCR showed that all ecotypes were susceptible to PVY^{C}-to and PSTVd infections, although the severity of symptoms and the accumulation level of the virus/viroid in the infected tissues was differed depending on the ecotype. Notably, Corbarino and San Marzano showed mild symptoms and reduced pathogen accumulation upon PVY^{C}-to and PSTVd infection, respectively, revealing a certain level of tolerance. In contrast, the ecotypes Giallo determinato da serbo and San Marzano were particularly susceptible to PSTVd and PVY^{C-}to, respectively. The local tomato ecotypes identified in this study may serve as valuable resources to further explore the molecular pathways underlying tomato defence responses against PVY^{C}-to and PSTVd infections.

This research was financially supported by European Union - Next Generation EU, Missione 4 Componente 2, investimento 1.1 - 'progetti di ricerca di rilevante interesse nazionale (PRIN), project PRIN-2022 "Dissection of molecular mechanisms underlying tolerance to virus and viroid infection in grafted tomato plants – DiVInGrafT (ID 2022BZW9PF; CUP H53D23005160006 and CUP B53D23017480006).

First Report of Citrus Leaf Blotch Virus on Nagami Kumquat in Turkey. B. FIDANCI-AVCI[1], N. ÖNELGE[1], A. ESKALEN[2]. [1]*Department of Plant Protection, Cukurova University, Adana, Turkey.* [2]*Department of Plant Pathology, University of California, Davis, CA, USA.* E-MAIL: aeskalen@ucdavis.edu

Nagami kumquat (*Fortunella margarita*) production has expanded in Turkey due to its agronomic potential and growing market demand. In May 2022, during a survey in commercial orchards in Mersin Province, symptoms consistent with bud union disorder were observed in Nagami kumquat trees. Molecular testing of symptomatic and asymptomatic samples excluded common citrus pathogens, including Citrus psorosis virus, Citrus exocortis viroid, and Hop stunt viroid. RT-PCR assays using specific primers revealed the presence of Citrus leaf blotch virus (CLBV), with expected amplicons obtained for both coat protein (CP) and RNA-dependent RNA polymerase (RdRp) genes only in symptomatic trees.

Sequencing confirmed CLBV identity, showing 96–97% nucleotide identity with GenBank sequences. Graft-inoculation onto Etrog citron and Dweet tangor confirmed pathogenicity, producing chlorotic blotching and stem pitting symptoms. This is the first confirmed report of CLBV in citrus in Turkey. Its likely introduction through infected grafting material highlights the importance of virus-tested propagation programs. Considering climate change and increasing movement of plant material, further surveys are needed to assess CLBV's distribution and potential impact on citrus production in the region.

Dynamics of the Flavescence dorée spread in South Tyrol (northern Italy). S. OETTL[1], Y. REYES-DOMINGUEZ[1], F. PERNTER[2], A. SIMONCELLI[2], A. GALLMETZER[1]. [1]*Laimburg Research Centre, Laimburg 6 – Pfatten/Vadena, 39040 Auer/Ora, BZ, Italy.* [2]*Office for Fruit and Viticulture and Phytosanitary Service, Brennerstraße 6, 39100 Bozen/Bolzano, BZ, Italy.* E-MAIL: sabine.oettl@laimburg.it

Flavescence dorée, a serious and rapidly spreading threat to European viticulture, is caused by a phytoplasma associated with the 16SrV group (FDp). Although the disease has been known in Europe and neighbouring wine regions for decades and has been monitored in South Tyrol (northern Italy) since the early 2000s, the first outbreak in the province did not occur until 2016. Since then, its prevalence has increased significantly. As part of a province- wide monitoring program led by the phytosanitary service, over 5,500 leaf samples from symptomatic grapevines have been tested for FDp. Over the past nine years, the number of FDp-positive samples has risen steadily, with nearly 30% of symptomatic grapevines testing positive in 2024. The continued spread of the disease into new areas further exacerbates the situation. Due to the high phytosanitary risk, several municipalities have been designated as infestation zones, with legally mandated control measures implemented against *Scaphoideus titanus*, the insect vector responsible for transmission. A subset of 327 FDp-positive samples collected between 2016 and 2024 was further characterized using the method of Rossi et al. (2019). All grapevine-derived samples from South Tyrol were assigned to the *dnaK1* genotype, consistent with the FD-D strain found in other Italian wine-growing regions. However, seven samples from natural FDp reservoir plants, *Clematis vitalba* and *Alnus* spp., were classified either as *dnaK2* or *dnaK3* genotypes. These findings underscore the need for continued surveillance and the implementation of effective management strategies to mitigate the spread of this destructive disease.

Carbon dot-mediated dsRNA delivery enhances RNAi efficacy against cucumber green mottle mosaic virus in susceptible and resistant cucumber. L. VELASCO, E. MARTÍNEZ, D. JANSSEN. *Instituto Andaluz de Investigación y Formación Agraria (IFAPA), 29140 Churriana, Málaga, Spain. IFAPA, 04745 La Mojonera, Almería, Spain.* E-MAIL: leonardo.velasco@juntadeandalucia.es

In this study, a dsRNA-based system for controlling cucumber green mottle mosaic virus (CGMMV) in cucurbits using carbon dots (CDs) or chitosan nanoparticles (ChNPs) as delivery nanoparticles were developed. In growth room trials, two cucumber varieties we evaluated: (i) CGMMV-susceptible and (ii) partially resistant. Three formulations were tested: (1) naked dsRNAs targeting CGMMV movement (MP) and coat (CP) viral transcripts, (2) dsRNA-CDs (1:0.5 w/w), and (3) dsRNA-chitosan nanoparticles (ChNPs) (1:0.5 ratio). Water-treated (mock) and uninoculated groups served as positive and negative controls, respectively. In susceptible plants, untreated inoculated controls exhibited symptoms, being severe in 11/12 individuals. Naked dsRNA reduced symptom severity (5/12 mild, 7/12 asymptomatic) and viral load by 25%, while dsRNA-CDs achieved 9/12 asymptomatic plants with >50% viral load reduction. DsRNA-ChNPs showed limited efficacy (3/12 severe symptoms) and no significant reduction in viral titers. Resistant plants remained asymptomatic across treatments but displayed distinct viral load patterns: dsRNA-CDs and dsRNA-ChNPs reduced titers by 68% and 81%, respectively, compared to untreated resistant controls. These results demonstrate that CD-complexation enhances dsRNA stability and uptake, providing effective protection against CGMMV in susceptible cultivars. The unexpected efficacy of ChNPs in resistant plants needs further investigation in nanoparticle-plant interactions. This nanotechnology-driven approach offers a promising strategy for integrating RNAi with host resistance in cucurbit disease management.

This research was financially supported by grant PID2021-125787OR-C32 financed by MICIU/AEI/10.13039/501100011033 and FEDER, EU.

Breaking innate resistance: transgenic RNAi strategies against tomato brown rugose fruit virus unintentionally promote viral systemic movement in tobacco hosts. L. VELASCO, E. MARTÍNEZ, C. RANGEL, D. JANSSEN. *Instituto Andaluz de Investigación*

y Formación Agraria (IFAPA), 29140 Churriana, Málaga, Spain. IFAPA, 04745 La Mojonera, Almería, Spain. E-MAIL: leonardo.velasco@juntadeandalucia.es

In our investigation of RNA interference-based resistance strategies against tomato brown rugose fruit virus (ToBRFV), we engineered transgenic *Nicotiana tabacum* lines expressing hairpin-derived dsRNAs targeting the coat protein (CP) and movement protein (MP) genes. These plants are intended as potential sources of dsRNAs, siRNAs and miRNAs for topical applications. Through primer walking, we identified the insertion loci, ensuring that transgene integration didn't disrupt endogenous genes - a critical consideration given tobacco's natural resistance against this pathogen. ToBRFV-mechanical inoculation in wild-type phenotype induces necrotic local lesions and limited systemic spread, with RT-qPCR revealing low viral titers in distal tissues. Our transgenic lines, however, subverted this pattern. Contrary to anticipated enhanced resistance, CP/MP-expressing plants displayed absence of local necrosis post-inoculation, accompanied by dramatic systemic spread - distal tissue viral loads averaged 570-fold compared to non-transgenic controls. This pattern suggests our engineered constructs might interfere with tobacco's native defense signaling pathways rather than directly inhibiting viral replication. Deep sequencing of small RNAs (18-24 nt) from the transgenic plants revealed construct-specific activity patterns. In CP-targeting lines TOCP2 and TOCP4, we detected modest siRNA production (139 and 169 CP-derived siRNAs per million total sRNAs, respectively). MP-targeting lines TOMP6 and TOMP14 exhibited markedly higher siRNA generation (1,245 and 2,676 MP-derived siRNAs per million), potentially reflecting differences in hairpin structure stability or transcript processing efficiency. Remarkably, the MP lines' heightened siRNA production didn't correlate with improved viral containment, suggesting ToBRFV might employ RNAi-independent movement mechanisms in transgenic plants, or that viral suppressors neutralize RNAi effects.

This research was financially supported by grant PID2021-125787OR-C32 financed by MICIU/AEI/10.13039/501100011033 and FEDER, EU.

Experimental evolution of sweet potato leaf curl deltasatellite 1 in *Ipomoea setosa* and Nicotiana benthamiana. J.N. JAÉN-SANJUR, J. NAVAS-CASTILLO, E. FIALLO-OLIVÉ. *Instituto de Hortofruticultura Subtropical y Mediterránea 'La Mayora' (IHSM-UMA-CSIC), Consejo Superior de Investigaciones Científicas, Algarrobo-Costa, Málaga, Spain.* E-MAIL: efiallo@eelm.csic.es

Deltasatellites are circular single-stranded DNA molecules found in association with several begomoviruses, relying on their helper viruses for replication, movement, encapsidation, and transmission by *Bemisia tabaci*. Deltasatellites are approximately one-fourth the size of a typical begomovirus genome component. The genomes of deltasatellites do not encode any proteins, contain an adenine-rich region, a conserved satellite region, and two stem-loop structures. In nature, deltasatellites have been identified in association with both monopartite and bipartite begomoviruses in the Old and New Worlds, infecting both cultivated and wild plant species. Experimentally, some deltasatellites have been shown to be trans-replicated by their naturally associated helper virus and also by various begomoviruses and even curtoviruses. Typically, deltasatellites do not alter the symptomatology induced by their helper viruses, and their impact on viral DNA accumulation varies depending on the specific virus–host plant combination. To assess the adaptation of sweet potato leaf curl deltasatellite 1 (SPLCD1) to different plant hosts, serial passage evolution experiments were conducted in *Ipomoea setosa* and *Nicotiana benthamiana*. Six plants of each species were agroinoculated with SPLCD1 infectious clones, followed by five successive passages using veneer grafting every four weeks. At each passage, young leaves were collected for DNA extraction, rolling circle amplification, and cloning. Sequencing and bioinformatic analyses of six clones per plant and passage were performed to investigate genetic changes associated with host adaptation. The results obtained from this study will be discussed, providing insights into the evolutionary dynamics of deltasatellites in different plant hosts.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101000570 (VIRTIGATION), the Ministerio de Ciencia e Innovación from Spain/AEI/ERDF (PID2021-128445OA-I00/AEI/10.13039/501100011033), as well as from the Ifarhu- Senacyt Scholarship Program from the Government of Panama.

Identification of whitefly-associated begomovirus 7 in *Achyranthes sicula* and its pathogenicity in tomato. E. DE LA LASTRA, I. TRUJILLO-ARTERO, E. FIALLO-OLIVÉ, J. NAVAS-CASTILLO. *Instituto de Hortofruticultura Subtropical y Mediterránea 'La Mayora' (IHSM-UMA-CSIC), Consejo Superior de Investigaciones Científicas, Algarrobo-Costa, Málaga, Spain.* E-MAIL: jnavas@eelm.csic.es

During systematic surveys to detect begomoviruses (genus *Begomovirus*, family *Geminiviridae*) in crops and wild plants in the province of Málaga (southern Spain), *Achyranthes sicula* plants were found to be infected with a begomovirus identified as whitefly-associated begomovirus 7 (WfaBV7). This virus had previously only been isolated from adults of the whitefly *Bemisia tabaci* MED, and its plant host was unknown. Phylogenetically, WfaBV7 is related to monopartite begomoviruses that infect tomato in several islands of southeastern Africa. An infectious clone of a WfaBV7 isolate from *A. sicula* was obtained and successfully agroinoculated into *Nicotiana benthamiana*, *A. sicula*, and *tomato plants*. In tomato, WfaBV7 caused mild leaf curling and a significant reduction in fruit yield. In *N. benthamiana*, WfaBV7 was able to act as a helper virus for sweet potato leaf curl deltasatellite 1. Interestingly, all attempts to transmit WfaBV7 between tomato plants using *B. tabaci* MED, MEAM1, and SSA2 as vectors were unsuccessful. Comparative analysis of the nucleotide sequences encoding the capsid protein (CP) of all available WfaBV7 isolates revealed the presence of a unique triplet of nucleotides not found in CP genes of phylogenetically related begomoviruses. Since the CP is known to be involved in vector transmission of begomoviruses, we investigated whether the additional amino acid encoded by this nucleotide triplet could be related to the lack of transmissibility by whiteflies. To this end, an infectious clone of a mutant WfaBV7 lacking this triplet was generated. The results of transmission assays performed with the mutant virus will be discussed.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101000570 (VIRTIGATION).

Viroid and Virus Infections in Tunisian Pistachio Orchards. M. ELAIR[1], C.E. KOURAICHI[2], M. DIGIARO[3], N. MAHFOUDHI[1]. [1]*Laboratoire de Protection Des Végétaux, Université de Carthage, Institut National de La Recherche Agronomique de Tunisie, Rue Hedi Karray, 1004 ElMenzah, LR16INRAT04, Tunis, Tunisia.* [2]*Institut Supérieur des Sciences Agronomiques de Chott Mariem, Université de Sousse, B.P 47, 4042 Chott Mériem.* [3]*Istituto Agronomico Mediterraneo di Bari, Via Ceglie 9, 70100, Valenzano, Bari, Italy.* E-MAIL: manel_elair@hotmail.com

Pistachio (*Pistacia vera* L.) is an economically important crop in Tunisia, but its production is increasingly threatened by viral and viroid infections. In spring 2022, field surveys were conducted in the main pistachio-growing areas of Morneg, Sidi Bouzid, and Gafsa to investigate the presence of pistachio ampelovirus A (PAVA), hop stunt viroid (HSVd), and citrus bark cracking viroid-pistachio (CBCVd-pis). While chlorosis and leaf deformation were common symptoms, stunting and bushy growth were observed less frequently. A total of 144 leaf samples, collected from various cultivars and pollinator trees, were analyzed using RT- PCR. Of the analyzed samples, 116 (80.5%) tested positive for at least one of the targeted pathogens. Mixed infections of HSVd and CBCVd-pis were detected in 41% of samples, while PAVA was present in 15.9%. The highest infection rate was recorded in Sidi Bouzid (84.7%), followed by Morneg (84.2%) and Gafsa (70.7%). CBCVd-pis was the most prevalent pathogen, with incidence rates of 73.1% in Sidi Bouzid, 60.9% in Gafsa, and 57.8% in Morneg. In Morneg, female cultivars exhibited a higher infection rate (88%) compared to pollinator trees (77%), with frequent co-infections by both viroids. Among pollinator plants, 50% were infected with HSVd and 63.6% with CBCVd-pis. The higher incidence of HSVd and CBCVd-pis compared to PAVA may reflect efficient transmission through pruning tools and potential pollen-mediated dissemination. Further research is needed to understand their epidemiology, incidence, and symptom severity. Tunisia is currently implementing a certification program to produce virus- and viroid-free pistachio propagation material.

Preliminary identification of a novel Maculavirus infecting peach in California using High- Throughput Sequencing. R.A. KUBAA[1], A. OURO-DJOBO[2], K.A. STEVENS[1], O.J. ALABI[2], M. AL RWAHNIH[1,3]. [1]*Department of Plant Pathology, University of California, Davis, CA 95616, U.S.A.* [2]*Department of Plant Pathology & Microbiology, Texas A&M AgriLife Research and Extension Center, Weslaco, TX 78596, U.S.A.* [3]*Department of Plant Protection, School of Agriculture, The University of Jordan, Amman, Jordan.* E-MAIL: raboukubaa@ucdavis.edu

The application of High-Throughput Sequencing (HTS) in routine virus screening has significantly improved the detection of known and novel viruses in vegetatively propagated crops. In 2020, Foundation Plant Services (FPS) at the University of California, Davis, received a peach selection from a public breeding program for evaluation and testing. Leaf tissue from grafted plants was analyzed via HTS as part of the plant health diagnostic pipeline. Bioinformatics analysis revealed the presence of a previously uncharacterized RNA virus showing

sequence similarity to members of the family *Tymoviridae*. Further analysis by HTS, followed by RT-PCR and rapid amplification of cDNA ends (RACE), enabled the complete sequencing of the viral genome which is 6,664 nucleotides long (excluding the poly(A) tail), and confirmed its affiliation with the genus *Maculavirus*, which includes monopartite, positive-sense single-stranded RNA viruses. The infected plant exhibited no visible symptoms at the time of sampling, suggesting a latent or asymptomatic infection. Preliminary genomic analysis indicated that the virus has a two-ORF genome organization and contains conserved domains such as methyltransferase, helicase, and RNA-dependent RNA polymerase. Phylogenetic trees based on both ORFs placed the virus within the *Maculavirus* clade. Subsequent RT-PCR testing confirmed the virus in both the scion and rootstock of grafted plants, while original rootstocks tested negative, indicating transmission occurred via bud grafting. This study highlights the importance of integrating HTS into certification programs for early detection of latent viral infections that may otherwise go unnoticed by conventional methods.

Emergence of Paprika mild mottle virus infecting pepper (*Capsicum annum* L.) crops in Türkiye. N. BUZKAN[1], B.B. ARPACI[2]. [1]*Plant Protection Department, Agriculture Faculty, Kahramanmaraş Sütçü Imam University, Kahramanmaraş, TÜRKİYE.* [2]*Horticulture Department, Agriculture Faculty, Kilis Yedi Aralik University, Kilis, TÜRKİYE.* E-MAIL: nbuzkan@ksu.edu.tr; nbuzkan@gmail.com

During August 2013 onwards, foliar (unfamiliar curling, distortion, yellowing) and fruit (reduced size, discoloration, mottle, pitting) symptoms were identified in pepper crops (*Capsicum annuum* L.) in Aegean region in Turkey. Fruit and leaf samples were tested in DAS-ELISA for the most frequent viruses such as potato virus Y (PVY), tobacco etch virus (TEV), cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV) and cucurbit aphid-borne yellows virus (CABYV) in Turkey. The samples resulting positive for CABYV were subjected to RT-PCR with the general polerovirus primer pair. Direct sequencing of PCR amplicons and BLASTn search proved the presence of paprika mild mottle tobamovirus (PaMMV) in a mixed infection nature with a polerovirus detected. Extensive survey was later carried out in protected and open-field grown pepper plants out in 11 provinces throughout Thrace, eastern Mediterranean, Southeast Anatolia, Aegean, eastern Black Sea regions from june to august in 2013 – 2022. A total of 478 samples were tested with RT-PCR with primer pairs amplifying the corresponding region to the coat protein (CP) gene of PaMMoV. Only samples from northern Aegean region resulted positive for PaMMV. The nucleotide sequences corresponding to 451 nt-long the CP gene of the Turkish PaMMV isolates showed 97.7% sequence similarity with PaMMV pepper strain P11 from Spain. PaMMV as an emergent virus is firstly detected in pepper crops in Turkey and the results support its widespread in the region due to the infected seeds sold by local seed companies.

This research was partially granted by KSU BAP 2021/2-28D.

Image-based high-throughput phenotyping of grafted tomato plants upon virus or viroid infection. R. MARZIALE[1], L. VACCARO[2], R. SPANO[1], G. BUBICI[1], M. CHIUMENTI[1], A. PETROZZA[3], F. CELLINI[3], T. MASCIA[2], B. NAVARRO[1], F. DI SERIO[2]. [1]*Institute for Sustainable Plant Protection, National Research Council of Italy CNR, Via Amendola 165/A, 70126, Bari, Italy.* [2]*Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, 70126, Bari, Italy.* [3]*Research Centre of the Lucana Agency for Development and Innovation in Agriculture (ALSIA) Metapontum Agrobios, Metaponto, Italy.* E-MAIL: robertamarziale@cnr.it

Grafting is an agronomic technique used as a sustainable integrated strategy for the management of biotic and abiotic stresses. Besides improving the performance of several horticultural crops, grafting can enhance the tolerance to viral infections in tomato. Whether grafting may interfere also with viroid infections in this host plant is still unknown. Here, we investigated by high-throughput phenotyping (Scananalyzer 3D system) the responses of grafted and non-grafted tomato plants to infections with potato spindle tuber viroid (PSTVd) or a recombinant strain of potato virus Y (PVY^{c}-to), which elicit stunting and leaf curling or severe leaf distortion and mosaic in this host, respectively. Two tomato varieties, Manduria (Ma) and UC82 (UC), tolerant and highly susceptible to PVY^{c}-to infection, respectively, were tested. Non-grafted, self-grafted UC (UC/UC) and UC grafted onto Ma (UC/Ma) plants were mechanically inoculated with PVY^{c}-to, PSTVd or mock-inoculated. Quantitative data of morphological parameters were acquired at 9 time points up to 36 dpi. PCA analysis showed that grafting has a global effect on PSTVd and PVY^{C}-to infection, with a slightly positive effect of UC/Ma with respect to self-grafted tomato plants. Moreover, a delay in viroid-induced stunting and a reduced accumulation of the viroid were

observed in the grafted plants. A slight increase in plant area, silhouette and solidity values were measured in grafted-PVY^{c}-to plants compared to the non-grafted counterparts starting at 15 dpi, associated with the lower viral load. These preliminary results show that high-throughput phenotyping is a promising tool to estimate tomato response across PVY^{C}-to and PSTVd infections.

This research was financially supported by European Union-NextGeneration EU, Missione 4 'istruzione e ricerca', Componente 2, investimento 1.1 'progetti di ricerca di rilevante interesse nazionale (PRIN), project PRIN-2022 "DiVInGraft: dissection of molecular mechanisms underlying tolerance to virus and viroid infection in grafted tomato plants", ID: 2022BZW9PF, CUP B53D23017480006 and CUP H53D23005160006.

Characterization of two isolates of Bougainvillea chlorotic vein banding virus in Italian Bougainvillea plants. A. BEN SLIMEN, R. YAZBECK, T. ELBEAINO, M. DIGIARO. *International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM IAMB), Bari, Italy.* E-MAIL: b.slimen@iamb.it

Despite their global distribution, ornamental Bougainvillea plants have received limited attention in virological studies. In this study, fifty-eight Bougainvillea samples, both symptomatic and asymptomatic, were collected from various nurseries across Apulia, Southern Italy. To investigate the presence of viruses, total RNA was extracted from pooled samples and subjected to high-throughput sequencing (HTS) using the Illumina platform. Analysis of the assembled contigs from the HTS data led to the identification of two distinct isolates of *Bougainvillea chlorotic vein banding virus* (BCVBV), genus *Badnavirus*, known to infect bougainvillea. Complete viral genomes were reconstructed by assembling contigs and bridging sequence gaps through PCR using newly designed primers. The first isolate, designated BCVBV Apulia 1 (PQ682518), comprised 8,694 nucleotides and showed highest sequence identity with the Brazilian isolate BCVBV-UNB-01 (MK473389), sharing 81.6% nucleotide and 84.0% amino acid identity. The second isolate, BCVBV Mini-Thai (PQ682517), was 8,822 nucleotides long and displayed high sequence identity to the Malaysian isolate BCVBV-UKM (MK816926), with 98.5% nucleotide and 98.7% amino acid identity. Genome structure analysis confirmed the typical BCVBV organization, featuring four open reading frames. Phylogenetic analysis placed the two Italian isolates in separate clades. Subsequent screening of individual samples revealed the presence of BCVBV in 12 out of 58 samples (20.7%), with the Apulia 1 isolate in 7 samples (12.1%) and BCVBV Mini-Thai in 5 samples (8.6%). This study represents the first report of BCVBV in Italy and Europe, identifying two genetically distinct strains and underscoring the need for further investigation into the epidemiology and impact of this virus.

This research was financially supported by the Regional Project "Pro.Di.Qua.Vi." *(Trasferimento di Protocolli per organismi da quarantena e nocivi e per la selezione di materiali sanitariamente migliorati per il vivaismo pugliese). Misura 16 Cooperazione – Sottomisura 16.2).*

CONCURRENT SESSION B2 – Emerging and re-emerging wood and vascular diseases of grapevine

SESSION KEYNOTES

Wood and vascular diseases of grapevine: emerging and re-emerging problems. L. MARTÍN. *Centro de Investigaciones Científicas y Tecnológicas de Extremadura (CICYTEX) Instituto de Investigaciones Agrarias Finca La Orden - Valdesequera, Área de Protección Vegetal, Autovía A-5 Km 372, 06187 Guadajira, Badajoz, España.* E-MAIL: laura.martin@juntaex.es

Vitis vinifera is one of the oldest and widely cultivated fruit crops globally, with over 7 million hectares under cultivation. In Europe, wine production constitutes a major socio-economic sector. Grapevines host a complex microbiota, including more than 900 fungal species reported to interact within the *Vitis* environment. Among these fungal community there are several well-known phytopathogenic fungi that cause diseases affecting leaves (e.g., downy and powdery mildew), fruit (e.g., grey mold and bunch rot), and roots (e.g., white rot and wilt). However, increasing attention is now being directed toward root and vascular diseases caused by soilborne fungi, particularly those affecting young vines. Decline syndromes in young grapevines have raised special attention among plant pathologists. These include root rot caused by *Fusarium* spp., black-foot disease caused by *Cylindrocarpon*-like species, and Petri disease. Both black-foot and Petri disease are part of the Grapevine Trunk Diseases (GTDs) complex. Since the early 2000s, the restriction of certain chemical treatments in Europe due to environmental and health concerns has coincided with the resurgence of GTDs, now considered as one of the major threats to the sustainability of viticulture. GTDs comprise a diverse group of diseases affecting both mature vineyards (e.g., Esca complex, Eutypa dieback, Botryosphaeria dieback, Diaporthe dieback) and

young plantations (e.g., Petri disease, black-foot disease). These diseases are chronic and often asymptomatic for years post-infection. However, they ultimately reduce vineyard longevity, yield, and grape quality, leading to plant death and decreased economic profitability of wine industry. The etiology of GTDs is complex, involving a wide range of taxonomically unrelated fungal pathogens—up to 30 genera and more than 100 species—isolated from the perennial wood of grapevines. While *Vitis vinifera* cultivars exhibit variable levels of tolerance and activate specific defense responses to vascular pathogens, complete resistance to GTDs has not been described. Recent advances in molecular technologies, particularly next-generation sequencing (NGS), have significantly enhanced our understanding of the grapevine mycobiome. Fungal community composition varies across the grapevine's vegetative cycle, between cultivars and plant tissues, and as a function of plant age. Additionally, microbial diversity is shaped by biogeographical factors and vineyard management practices. These insights highlight the complexity of fungal communities and emphasize the importance of understanding their ecological roles—including trophic modes and guild affiliations—as pathogens, endophytes, saprobes, or potential biocontrol agents. This growing knowledge provides a foundation for the development of innovative and sustainable disease management strategies. By integrating beneficial microorganisms, bioactive compounds, and improved agronomic practices, we can more effectively address wood and vascular diseases in vineyards and support growers in reducing losses caused by fungal pathogens.

This research was financially supported by MIP3 project (El manejo integrado de enfermedades y malas hierbas en los sistemas agroforestales de Extremadura para un desarrollo sostenible: prevención, supervisión y control). FEDER Junta de Extremadura 2024-2026.

Grapevine Flavescence dorée: an update. E. ANGELINI. *CREA Centro di Ricerca per la Viticoltura e l'Enologia, Via XXVIII Aprile 26, 31015 Conegliano, TV, Italy.* E-MAIL: elisa.angelini@crea.gov.it

Flavescence dorée (FD) is a quarantine disease of grapevine associated to the presence of FD phytoplasmas (FDp). It is efficiently transmitted from vine to vine by *Scaphoideus titanus* Ball., a leafhopper of American origin, imported to Europe in the last century. FD is strongly epidemic in Central-Southern Europe, occurring from Portugal to Romania. Damages are very serious, spanning from lower production to the death of the plants. Not all the varieties show the same susceptibility, with most of the rootstocks and a very few *Vitis vinifera* cultivars being less susceptible. Until few years ago the epidemiologic cycle FDp - *S. titanus* - grapevine has been thought to be the only one, while currently there are many scientific reports that demonstrated the presence of several secondary host plants and other secondary vectors, although with lower epidemiologic impact. The genetic variability of the pathogen plays an utmost role in FD epidemiology, with only a few isolates being epidemic, because transmitted by *S. titanus*. The main strategies to control the disease and the vector are mandatory and rely on insecticide treatments against the vector, uprooting of the infected plants, and planting of healthy material. However, several studies are ongoing to find out most sustainable and durable control strategies. Vibrational mating disruption of vector is one of these possibilities, since males and females communicate and mate via vibrations. It was demonstrated to be effective in laboratory conditions, and field trials are ongoing. Stimulation of plant natural defences is also a very interesting issue, however so far successful trials are missing. Gene silencing, based on RNA interference, can be used for controlling vectors: experiments with FD vectors (*S. titanus* and *Euscelidius variegatus*) were carried out in laboratory conditions and showed decreased fitness and lower egg development. Natural antagonists to the vector are not present in Europe. Some trials with the natural endosymbiont *Asaia* sp., already present in European *titanus* populations, gave good results in the model FD vector *E. variegatus*, leading to significantly reduced phytoplasma acquisition. Also a few studies on the resistance and susceptibility of grapevine varieties, against the vector and against the pathogen, are in progress, but they proceed slowly due to the complexity of the pathogen, which is not cultivable *in vitro* and transmissible only *in vivo* by means of the vector. Quantitative Trait Loci identification is indeed ongoing by classical approaches, such as breeding and phenotyping, and by modern tools, such as transcriptomic and Next Generation Sequencing. The genetic traits responsible for susceptibility and resistance to FD are thus not yet known, however their discovery could help a lot in producing grapevine plants resistant to the disease, by means of conventional breeding, genetic editing, and cisgenesis techniques.

ORAL PRESENTATIONS

Long-term establishment and active colonization of *Trichoderma atroviride* SC1 and *Pseudomonas chlo-*

***roraphis* M71 in the trunk of Esca-affected grapevine after their injection.** G. BRUSSI[1], G. PUOPOLO[1,2], S. DI MARCO[3], L. MUGNAI[4], I. PERTOT[1,2]. [1]*Center Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Italy;* [2]*Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige, Italy;* [3]*Institute of BioEconomy, National Research Council, Bologna, Italy;* [4]*Plant Pathology and Entomology Section, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), University of Florence, Florence, Italy.* E-MAIL: greta.brussi@unitn.it

The increasing difficulties in protecting plant health pave the way for integrating endotherapy as a sustainable alternative, especially in contexts where conventional treatments are complex and/or ineffective, such as the Esca Complex, one of the primary wood diseases affecting grapevines. Despite numerous benefits, endotherapy is rarely used due to high application costs, the limited availability of effective formulations, and unclear outcomes. In this scenario, the use of plant-beneficial microorganisms for endotherapy could be a promising method for controlling plant diseases since this approach benefits from the plant's active and persistent colonisation of these microorganisms. Additionally, it enables them to reach areas that would otherwise be inaccessible to the passive movement of injected compounds, eliminating the need for multiple applications throughout the year. This work aims to verify if the plant-beneficial microorganisms *Pseudomonas chlororaphis* M71 and *Trichoderma atroviride* SC1 can systemically establish themselves once injected, both in rooted cuttings in the greenhouse and grapevine plants in the vineyard. Experiments were conducted to evaluate the colonisation efficiency, translocation within the grapevine plant, long-term persistence, and protection against the main Esca pathogens. The results confirmed the successful establishment of both microorganisms, with extensive translocation throughout the grapevine tissues, providing reassurance about the method's effectiveness. Long- term colonisation results showed that these plant-beneficial microorganisms could persist in the trunk for at least 60 days. Further investigation is needed to optimise injection protocols and assess the practical applications of this method under field conditions.

From *in vitro* screening to vineyard evaluation of endophytic biocontrol bacteria targeting *Fomitiporia mediterranea*, a key pathogen of esca, a grapevine trunk disease. O. MESGUIDA[1,2], R. TRAVADON[1], A. YACOUB[1], M. MESLIER[1], S. COMPANT[3], S. GODIN[1], M. LE BECHEC[1], JEAN-YVES BERTHON[2], E. ATTARD[1], P. REY[1]. [1]*E2S UPPA, CNRS, IPREM, Université de Pau et des Pays de l'Adour, 64000 Pau, France.* [2]*GreenCell: Biopôle Clermont-Limagne, 63360 Saint Beauzire, France.* [3]*Center for Health & Bioresources, Bioresources Unit, AIT Austrian Institute of Technology, Konrad Lorenz Str. 24, Tulln 3430, Austria.* E-MAIL: ouiza.mesguida@univ-pau.fr

Esca is a complex grapevine trunk disease that represents a major challenge to viticulture worldwide. Since the banning of sodium arsenite in European countries, the last approved pesticide, no curative treatment is available for its control. In this context, our study aimed to (i) select bacterial biological control agents (BCAs) effective against *Fomitiporia mediterranea* (Fmed), a major pathogen associated with Esca, (ii) investigate their modes of action, and (iii) evaluate their efficacy under field conditions. A stepwise *in vitro* screening of increasing complexity was performed to evaluate the inhibitory potential of 58 grapevine endophytic bacteria against Fmed. Three strains *Pseudomonas lactis* SV9, *Pseudomonas paracarnis* S45, and *Paenibacillus polymyxa* SV13 which exhibited a strong antifungal activity against Fmed were selected. Whole-genome and metabolome analyses of the three strains revealed that their biocontrol activity operates through both direct and indirect mechanisms. The efficacy of *P. paracarnis* S45 and *P. polymyxa* SV13 was evaluated in a vineyard trial by injecting them into the trunks of Sauvignon Blanc grapevines exhibiting Esca symptoms. Bacterial injections reduced the severity of Esca without affecting grape quality. The abundance of *Bacillus* increased in grapevines injected with *P. polymyxa* SV13, which correlated negatively with the abundance of *Phaeomoniella* spp. and *Diplodia* spp.; a negative correlation between Pseudomonas spp. and *Fomitiporia* spp. was observed in grapevines injected with *P. paracarnis* S45. Furthermore, *P. paracarnis* S45 improved the defense mechanisms of the grapevine. These results highlight the potential of both strains as effective biological control agents for the management of Esca.

This work was supported by the Excellence Initiative of Université de Pau et des Pays de l'Adour–I-Site E2S UPPA [Project Biovine, seed funding], a French "Investissements d'Avenir" program and by the Industrial Chair "WinEsca" funded by the ANR (French National Research Agency, grant number ANR-22CHIN-0002-01), and the JAs Hennessy & Co and GreenCell companies.

One formulation for the control of downy mildew and grapevine trunk diseases in vineyard: The "no-copper" challenge of the Natural Agro project. V. MONDELLO, M. BOUVY, L. PARENT, J.F. GUISE, P. TROTEL-AZIZ, F. FONTAINE. *University of Reims Champagne-Ardenne, Research Unit Induced Resistance and Plant Protection – RIBP UCS INRAE 1488, Building 18, 51100 Reims, France.* E-MAIL: florence.fontaine@univ-reims.fr

The concerns about the impact of plant protection products (PPPs) on environment and human health lead the industry in finding new more sustainable ways to control diseases. This is particularly urgent in viticulture where the massive use of cupric-based PPPs has led to high level of copper contamination in soils and water. For these reasons, copper is now considered as "candidate for substitution" (art. 24 EC Regulation n. 1107/2009) by the European Community (EC) and its use is now limited at max. 28 kg·ha^{-1} in 7 years (1981/2018 EC regulation). In this framework, the European LIFE project namely NaturalAgro (2023-2028), involving France, Italy and Portugal, aims to homologate one formulation for the control of two major grapevine diseases: the downy mildew and GTDs. The formulation is based on the carrier molecule hydroxyapatite (Microsap®), plant extracts and will contain no copper. The potential PPP will be tested, with different strategies according to the targeted disease, in several vineyards located in the three NaturalAgro project countries. Here we present some preliminary results obtained during our in vitro and in planta tests especially against some Esca-, Eutypa- and Botryosphaeria-dieback associated pathogens. These results were useful to identify the best formulation among those tested, differing in both components and relative ratios. The chosen potential PPP seems to be promising in the protection of pruning wounds from GTD-pathogens infections. Experimental plots in French vineyard have been initiated in 2024 and for 4 years.

"Natural Agro" is financed by the CINEA LIFE 2022 Horizon 2020 Framework under the specific programme LIFE-2022-SAP-ENV-ENVIRONEMENT (GA No 101113781).

Exploring soil properties influence on Esca Disease dynamics: insights from a Bolgheri vineyard. F. BIGAZZI[1], G. CARELLA[1], S. DEL DUCA[2], S. MOCALI[2], S. PRIORI[3], F. VITALI[2], L. MUGNAI[1]. [1]*Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section, P. le delle Cascine 28, 50142 Florence, Italy.* [2]*Council for Agricultural Research and Economics - Research Centre for Agriculture and Environment (CREA-AA), Via di Lanciola 12/A, 50125 Florence, Italy;* [3] *University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), via San Camillo de Lellis, 01100 Viterbo, Italy.* E-MAIL: francesco.bigazzi1@unifi.it

Esca complex of diseases remains one of the most damaging grapevine disorders, yet its causal factors are not fully understood. While numerous fungi are involved, and heavy rainfall in May and June is known to exacerbate foliar symptoms, the role of soil physicochemical properties in disease expression remains poorly investigated. This study aims to explore the influence of soil properties on leaf symptoms expression by comparing vineyard areas with high and low disease incidence over a five-year period. The research was conducted in a 1.5-hectare 'Cabernet Sauvignon' vineyard in Tuscany (Italy). Electromagnetic induction (EMI) sensor was employed to map soil spatial variability through soil electrical conductivity (ECa). Four sampling points were selected for profile excavation based on the combination of disease incidence and ECa. Soil features revealed significant differences between high- and low-incidence areas. Low-incidence soils showed clay-loam texture, high lime content, alkaline pH (8.3–8.5), and low assimilable manganese (6.8–12.3 mg kg^{-1}). In contrast, high-incidence soils showed deep soils with sandy-clay-loam texture, low lime content (< 3%), sub-alkaline pH (7.8–8.1), greater fertility (higher nitrogen) and good amount of organic matter. Assimilable manganese was slightly higher in high-incidence soils (13–18 mg kg^{-1}). These findings suggest that soil structure, pH, and organic matter buffering capacity may significantly influence micronutrient availability, particularly manganese, potentially enhancing the degradative activity of *Fomitiporia mediterranea*, a key Esca pathogen. This could result in increased production of byproducts and toxins, contributing to foliar symptoms. This study highlights the critical role of soil properties in Esca disease dynamics.

Grapevine trunk diseases in Greece: disease incidence and fungi involved in discrete geographical zones and varieties. S.I. TESTEMPASIS[1], E.A. MARKAKIS[2,3], G.I. TAVLAKI[3], S. SOULTATOS[2,3], C. TSOUKAS[4], A. SAMARAS[1], D. GKIZI[4], A. TZIMA[4], E. PAPLOMATAS[4], G.S. KARAOGLANIDIS[1]. [1]*Aristotle University of Thessaloniki, School of Agriculture, Faculty of Agriculture, Forestry and Natural Environment, 54124 Thessaloniki, Greece.* [2]*School of Agricultural Sciences, Hellenic Mediterranean University, Stavromenos 71004, Heraklion, Crete,*

Greece. [3]*Institute of Olive Tree, Subtropical Crops and Viticulture, Hellenic Agricultural Organization "DIMITRA", Kastorias 32A, 71307, Heraklion, Crete, Greece.* [4]*Agricultural University of Athens, School of Plant Sciences, Department of Crop Science, Iera Odos 75, Votanikos, 11855 Athens, Greece.* E-MAIL: markmanos@hmu.gr

The main objective of the present study was to estimate disease parameters and isolate fungi associated with grapevine trunk diseases (GTDs) in Greece. In total, 310 vineyards in different geographical regions in northern, central, and southern Greece were surveyed, and 533 fungal strains were isolated from diseased vines. Morphological, physiological and molecular analyses revealed that isolates belonged to 35 distinct fungal genera. The GTDs-inducing population structure differed significantly among the discrete geographical zones. *Phaeomoniella chlamydospora* (26.62%, *n*=70), *Diaporthe* spp. (18.25%, *n*=48) and *Fomitiporia mediterranea* (10.27%, *n*=27) were the most prevalent in Heraklion, whereas *Diplodia seriata*, *Alternaria* spp., *P. chlamydospora* and *Fusarium* spp. were predominant in Nemea. In Amyntaio and Kavala, *D. seriata* was the most frequently isolated species (>50% frequency). Multi-gene (*rDNA-ITS*, *LSU*, *tef1-α*, *tub2* και *act*) sequencing of selected isolates, followed by pathogenicity tests, revealed that *Neosetophoma italica*, *Seimatosporium vitis*, *Didymosphaeria variabile* and *Kalmusia variispora* caused wood infection, with the former being the most virulent. To the best of our knowledge, this is the first report of *N. italica* associated with GTDs worldwide. This is also the first record of *K. variispora*, *S. vitis* and *D. variabile* associated with wood infection of grapevine in Greece. The potential associations of disease indices with vine age, cultivar, GTD-associated population structure and the prevailing meteorological conditions in different viticultural zones in Greece are presented and discussed.

This research was financially supported by Greek national funds through the Public Investments Program (PIP) of the General Secretariat for Research & Technology (GSRT), under the Emblematic Action "Routes of Vineyards", Grant No. 6070.03.

POSTER PRESENTATIONS

Tolerance of cultivars and hybrid grapes against trunk diseases. A. CSÓTÓ[1], N. LAURINYECZ[1], A. NAGY[1], N. RAKONCZÁS[2], K.E. NÉMETH[3], C. NÉMETH[4], Z. A. NAGY[4], A. CSIKÁSZ-KRIZSICS[5], E. SÁNDOR[6]. [1]*Institute of Plant Protection, Faculty of Agricultural and Food Science and Environmental Manage-ment, University of Debrecen, H-4032, Debrecen, Böszörményi út 138, Hungary.* [2]*Institute of Horticultural Science, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, H-4032, Debrecen, Böszörményi út 138, Hungary.* [3]*Research Institute for Viticulture and Oenology Kecskemét, Hungarian University of Agriculture and Life Sciences, H-6000 Kecskemét, Hungary.* [4]*Research Institute for Viticulture and Oenology Badacsony, Hungarian University of Agriculture and Life Sciences, H-8263 Badacsonytomaj, Hungary.* [5]*Research Institute for Viticulture and Oenology, University of Pécs, H-7634 Pécs, Hungary.* [6]*Institute of Food Science, Faculty of Agricultural and Food Science and Environmental Manage-ment, University of Debrecen, H-4032, Debrecen, Böszörményi út 138, Hungary.* E-MAIL: csoto.andras@agr.unideb.hu

Grapevine trunk diseases are among the most significant problems of grape plant protection worldwide, and there is no adequate curative procedure for its treatment. In Hungary, little is known about the resistance of the varieties in the environmental conditions of the region, neither that of domestic cultivars nor that of breeding lines. In our studies, we assessed the annual frequency of symptoms in 2022, and the degree of plant loss before that, for 305 varieties of four Hungarian grape variety collections. We compared the order of susceptibility of dominant domestic and internationally significant varieties. We formed groups of the varieties based on their descent as follows i) monophyletic varieties of *Vitis vinifera* origin and interspecific hybrids, and ii) monophyletic varieties of *V. vinifera* origin, Asian (*V. amurensis*) and American (*V. labrusca*, *V. riparia*, *V. rupestris*) origin hybrids. The tolerance of the resulting groups was compared based on their mortality. Our susceptibility results for the international varieties are supported by the previous literature data. Compared to the symptoms of the international varieties, the main Hungarian variety 'Juhfark' is the most resistant, while 'Furmint' can be classified as the most sensitive. The mortality of interspecific hybrids is significantly lower than that of monophyletic varieties, and the resistance of species hybrids derived from *V. amurensis* proves to be outstanding even within this group.

Bacteria and fungi colonizing grapevine wood issues: Taxa diversity and interactions in the context of grapevine trunk diseases. R. HAIDAR, A. YACOUB, O. MESGUIDA, L. LEYMARIE, E. ATTARD, R. GUYONEAUD, P. REY. *Université de Pau et des Pays de l'Adour / E2S-UPPA/ CNRS, Institut des Sciences Analytiques et de Physicochimie pour l'Environnement et les Matériaux - UMR 5254, Pau, F-64013 France.* E-MAIL: rana.haidar@univ-pau.fr

Pathogenic fungi are considered as the major cause of Grapevine Trunk Diseases (GTDs) which threat wine and table grape industries worldwide. While fungal diversity in different cultivars is well studied, less information are available about bacterial diversity in grapevine wood. The function of these bacteria and their interactions with wood-inhabiting fungi is relatively less studied. Using metabarcoding, we identified the bacterial and fungal taxa composition of microbiota colonizing different wood tissues of five grapevine varieties (Ugni Blanc, Tannat, Baroque, Gros menseng and Arriloba) from the Nouvelle-Aquitaine region in France. The results of next-generation sequencing are under analysed. These data will provide a better understanding of bacterial and fungal diversity, and their distribution in the various tissues of grapevine cultivars. To the best of our knowledge, this is the first report on the diversity of cultivable endophytic bacterial in the wood of these grapevine varieties. By using cultural dependent methods, 135 bacteria from the different grapevine varieties were isolated and assigned to bacterial species based on the 16S rRNA genes. 39 bacterial strains issues from different tissues of different cultivars and showed different ability to degrade cellulose, were selected to study their interaction, *in vitro*, with four of major pathogenic fungi of GTDs (*Fomitiporia mediterranea*, *Neofusicoccum parvum*, *Diplodia seriata* and *Eutypa lata)*. Diverse types of bacterial/fungal interactions were observed which depended on bacterial and/or fungal species. However, three bacterial strains (*Bacillus amyoliquefaciens*, *B. subtilis* and *Priestia megaterium*) have showed a great potential as biocontrol agents against the studied fungi of GTDs.

This research was financially by the Industrial Chair "WinEsca" funded by ANR (French National Research Agency), the JAs Hennessy & Co and the GreenCell companies and the fnancial support of the E2S-UPPA Project.

New potential BCA from table grape microbial community for Grapevine Trunk Diseases (GTDs) management. D. CORNACCHIA[1], F. DALENA[1], D. SALAMONE[1], A. AGNUSDEI[1], P. TANCREDI[1], R.M. DE MICCOLIS ANGELINI[1], G.L. BRUNO[1], F. MANNERUCCI[1], D. GERIN[1], F. FARETRA[1], S. MAVICA[2], D. AIELLO[2], S. POLLASTRO[1]. [1]*Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, Italy.* [2]*Department of Agriculture, Food and Environment, University of Catania, via S. Sofia, 100, Catania, Italy.* E-MAIL: francesco.faretra@uniba.it; dalia.aiello@unict.it

In this work, the antagonistic activity of members of table-grape microbial community, isolated from Apulia and Sicily, and the registered *Bacillus amyloliquefaciens* (D747), *Bacillus subtilis* (QST713), *T. atroviridae SC1, T. asperellum* ICC012 and *T. gamsii* ICC080 was assessed against Grapevine trunk diseases (GTDs) associated pathogens *Neofusicoccum parvum*, *Eutypa lata*, *Diplodia seriata*, *Cylindrocarpon destructans*, *Phaeomoniella chlamydospora, Phaeoacremonium minimum*, *Diaporthe neoviticola.* In dual culture assay, almost all *Trichoderma* strains differently affected the growth of tested pathogens, showing greater effectiveness than *T. gamsii* ICC080 and *T. asperellum* ICC012, and similar activity to *T. atroviridae SC1*. Previously, *Aphanocladium album MX95* showed a deadlock at mycelial contact with *F. mediterranea*. *Clonostachys rosea* GP80 inhibited the growth of *P. minimum*. *B. amyloliquefaciens* (D747) and *B. subtilis* (QST713) showed a limited effectiveness against *N. parvum* and *E. lata*. Furthermore, the ability of the BCA to produce volatile organic compounds (VOCs) was evaluated. All the *Trichoderma* tested were effective against *P. chlamydospora*, *C. destructans* and *P. minimum*. The *Trichoderma* strains FV145 and FV185 were the best performant, showing similar activity to *T. atroviridae SC1*. The growth of *C. destructans* and *P. chlamydospora* was affected by *A. album* and *C. rosea*. *B. amyloliquefaciens* (D747) showed a moderate effectiveness against *P. chlamydospora*, *C. destructans* and *P. minimum,* while *B. subtilis* (QST713) light reduced the growth of *P. chlamydospora* and *C. destructans*. Moreover, *Trichoderma* TCH4 altered the colour of *N. parvum* and *D. seriata* mycelia. These results highlight the potential of native microorganisms as effective biocontrol agents against GTDs.

This research was financially supported by the Project "New Therapeutic Approaches to Reinforce the natural Grapevine micrbiomE against Grapevine Trunk Diseases (TARGET_GTDs)", P2022ENPCL, PNRR Missione 4 "Istruzione e Ricerca" - Componente C2 Investimento 1.1, "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)".

Studying the evolution of Flavescence dorée symptomatology for the assessment of an early detection strategy. R. PINI, C. AGLIETTI, G. CARELLA, F. BIGAZZI, A. ROSSI, L. MUGNAI. *Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section, University of Florence,* P.le *delle Cascine 28, 50144 Florence, Italy.* E-MAIL: chiara.aglietti@unifi.it

Flavescence dorée (FD) is an invasive grapevine disease reported in many European countries. Despite the quarantine measures mandatory for its control, concerning FD epidemic outbreaks continue to be reported. Investigating the FD symptoms development in 'Sangiovese', the most typical variety of the Chianti area in Tuscany, is useful to reduce delays between symptoms manifestation and management actions. In this work the evolution of FD was analyzed in three Sangiovese vineyards, where the development of symptoms was followed for 1 year, registering the presence and classifying each kind of symptom observed on leaves, shoots and trunks. Every 15 days, samples of each part of the analyzed plants were collected from each vineyard and tested for the presence of FD with a species-specific LAMP assay. The presence of vector insects, *Scaphoideus titanus* and *Dictyophara europaea*, was monitored to confirm flight periods and to correlate insect presence with disease incidence and severity. The higher percentage of positives to FD was found from leaf samples collected from shoots with zigzagging tips and curled leaves, while the lowest was observed in samples taken from shoots and leaves with a marbled lamina. The higher percentage of inoculum of FD was registered from mid-June to late July, while most insects were found between late July and end of August. Early symptom monitoring showed to be of great help, along with confirming sample positivity through molecular testing by the first ten days of July, to enable the early removal of infected plants before the arrival of adult *S. titanus*.

This research was partly financially supported by Enbiotech s.r.l.

Presence of GTD pathogens in young vineyards in Montenegro. B. KANDIĆ[1], V. MONDELLO[2], J. LATINOVIĆ[1], F. FONTAINE[2], N. LATINOVIĆ[1]. [1]*University of Montenegro, Biotechnical Faculty, Mihaila Lalića 15, 81 000 Podgorica, Montenegro.* [2]*University of Reims Champagne-Ardenne, INRAE, RIBP USC 1488, 51100 Reims, France.* E-MAIL: kandic.b@ucg.ac.me

Grapevine trunk diseases (GTDs) are caused by several fungi belonging to different genera and not phylogenetically related. They are widespread in all grapevine cultivation countries, causing significant economic losses. Due to the scarce knowledge about the presence of GTDs in Montenegro, a survey for the presence of these diseases in vineyard was carried out from 2021 to 2024. A total of 72,500 vines in 15 different vineyards were inspected. During the survey, samples of asymptomatic vines were uprooted and analyzed to verify the presence of any internal GTD-like symptoms. A total of 10 samples from 13 different varieties were collected and processed in the laboratory of the Biotechnical Faculty in Podgorica. Generally, the survey for the GTD presence in the 15 Montenegrin vineyards showed the absence of foliar symptoms which resembles GTDs. However, both cross and longitudinal sections of the sampled vines highlighted the presence of black spots and small brown necrosis, especially in the wood of the upper third of the vine while the middle part and the grafting point did not show any symptoms of internal necrosis. The morphological and molecular identification of isolates obtained from asymptomatic grapevine plants showed the presence of GTD- associated pathogens *Phaeomoniella chlamydospora*, *Diaporthe ampelina* and pathogens from the family *Botryosphaeriaceae*. The acquired results indicate the presence of latent infections by GTD pathogens in young grapevine plantings in Montenegro.

This research was financially supported by the Project "Biofungicides application in agriculture and urban areas (BIOAPP)", funded by Ministry of Education, Science and Innovation of Montenegro and abstract was created as a result of the ERASMUS + KA 171 project implemented between the University of Montenegro and the University of Reims Champagne-Ardenne.

***In vitro* insights into manganese-driven wood degradation by *Fomitiporia mediterranea*: are micronutrients abiotic triggers for foliar symptoms in Esca complex?** F. BIGAZZI, G. CARELLA, L. MUGNAI. *Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section,* P.le *delle Cascine 28, 50142 Florence, Italy.* E-MAIL: francesco.bigazzi1@unifi.it

Fomitiporia mediterranea (Fmed) is a basidiomycete fungus having a relevant role in grapevine wood tissue degradation associated with Esca complex of diseases and of its typical foliar symptoms. While abiotic factors such as soil drought suppress symptom expression, fertilization with specific microelements appears to exacerbate it, suggesting that soil chemistry plays an understudied role in disease dynamics. This study focuses on manganese (Mn), hypothesizing that its bioavailability in soils—through accumulation in vine tissues— could stimulate Fmed wood-degrading capacity by enhancing Mn-dependent enzymatic activity. We therefore evaluated whether Mn supplementation influences Fmed growth, wood decay, and secretome composition. Fmed was cultured on water agar with graded Mn concentrations. Mycelial growth rates and biomass production were

monitored on vine sawdust, while wood degradation was assessed via mass loss in pre-weighed wood chips. Results revealed statistically significant differences in fungal growth, biomass, and wood degradation between Mn-supplemented and control substrates. Moderate Mn enrichment accelerated mycelial growth, increased biomass production, and intensified wood mass loss compared to controls. However, elevated Mn concentrations suppressed fungal development, indicating a toxicity threshold. Visual analyses showed distinct color changes in the fungal colony under Mn-enriched conditions, suggesting altered metabolite profiles. These findings suggest that Mn availability modulates Fmed degradative activity, with optimal levels promoting enzymatic processes critical to wood decay. These results highlight the need to investigate micronutrient bioavailability as a potential abiotic driver of Esca disease progression, with Mn emerging as a key factor influencing pathogenesis through modulation of fungal enzymatic activity and wood degradation processes.

Phomopsis cane and leaf spot of grapevine is associated with Diaporthe dieback: first report in Italy. G. CARELLA[1], F. BIGAZZI[1], G. DARDANI[2], V. GUARNACCIA[2,3], L. MUGNAI[1]. [1]*Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant pathology and Entomology section,* P.le *delle Cascine 28, 50142 Florence, Italy.* [2]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy;* [3]*Interdepartmental Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* E-MAIL: giuseppe.carella@unifi.it

Phomopsis cane and leaf spot (PCLS), a disease caused by *Diaporthe* spp., is often considered secondary in viticulture. Recent studies classify *Diaporthe* species among Grapevine Trunk Diseases agents, causing Diaporthe dieback. This pathogen disrupts vascular tissues, obstructing sap flow and inducing spur decline. A three-year monitoring study (2022–2024) was conducted in a 20-year-old Cabernet Sauvignon vineyard located in San Casciano (Florence, Italy) to assess cortical necrosis incidence and severity. A representative number of plants were selected, and unproductive spurs—characterized by extensive tissue necrosis and PCLS symptoms—were sampled to assess the association between spur mortality and the presence of *Diaporthe* spp. in the spurs wood. Fungal isolates obtained from the selected spurs were characterized morphologically and through a multi-locus phylogenetic analyses based on different genomic regions: internal transcribed spacer (ITS), *translation elongation factor-1α* (*EF1-α*), *beta-tubulin* (*TUB2*) and *calmodulin* (*CAL*) genes were used. Results indicate that in 2022 spur dieback reached 69% of the vines spurs, with an incidence of PCLS of approximately 96%, while in 2023 spur dieback was 56% and PCLS 77%, and in 2024 the values were 41% and 31%. Across all three years, the ratio of spur mortality related to PCLS incidence remained consistently around 71%, underscoring the pivotal role of *Diaporthe ampelina* inoculum in the manifestation of Diaporthe dieback in vineyards, even if also Botryosphaeriaceae species were isolated. This study demonstrates the significant contribution of *D. ampelina* inoculum in PCLS in spur dieback, emphasizing the need for targeted management strategies to mitigate inoculum-driven disease spread.

Recent advances on emerging vascular pathogens of grapevines in Eastern and Southern Mediterranean countries. E. CHOUEIRI[1], F. JREIJIRI[1], T. ELBEAINO[2], R. ABOU KUBAA[3], P. SALAR[4], X. FOISSAC[4]. [1]*Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287, Zahlé, Lebanon.* [2]*CIHEAM Bari, Istituto Agronomico Mediterraneo di Bari, Italy.* [3]*Department of Plant Pathology, University of California, Davis, USA.* [4]*University of Bordeaux, INRAE, Fruit Biology and Pathology, Villenave d'Ornon, France.* E-MAIL: echoueiri@lari.gov.lb

Eastern and southern Mediterranean countries are illustrious for the cultivation of grapevine, since the old antiquity. Emerging vascular pathogens of grapevines, including viral and phytoplasma diseases, significantly reduced grape yield and quality, in addition to shortening the productive life of grapevine canes, which prompted to conduct field surveys in grapevine growing areas over the past few decades in Lebanon, Syria, Palestine, Jordan, Tunisia, Morocco, Algeria, and Egypt. Bright yellow leaves, yellow mosaic, veinal mottling, and the malformed shoots, were the most common symptoms caused by GFLV in the vineyards. ToRSV and ArMV were rarely reported producing symptoms that resemble those described for fanleaf. Leaf reddening/yellowing with leafroll symptoms was consistently observed in different areas, mainly affected by GLRaV-1, GLRaV-2 and GLRaV-3, whereas other relevant viruses such as GLRaV-4 strain 6, GLRaV-5 and GLRaV-7 were less frequent. Rugose wood, corky bark and GVA were the most observed among the infrequent cases. In addition to the presence of GVB and GRSPaV, few cases of GPGV,

GVL and GVD were also reported. Later, typical grapevine yellows (GY) symptoms were observed in Lebanon, Jordan and Syria caused by '*Candidatus* Phytoplasma solani' associated with grapevine bois noir, '*Candidatus* Phytoplasma omanense', '*Ca.* P. aurantifolia', '*Ca.* P. asteris' and clover proliferation group (16SrVI). Finally, the heavy widespread of mealybug infestations and the outbreak of GY epidemic could be a real risk in the Arab vineyard agroecosystems. The use of certified seedlings and monitoring strategies are essential to prevent spread of epidemic viruses and phytoplasmas.

Genome and genetic diversity of *Lasiodiplodia* species associated with Mango dieback and stem-end rot diseases in Côte d'Ivoire. Y.S. YEO[1,2,3], A. DEREEPER[3], D. KONE[1,2], D. FERNANDEZ[3]. [1]*UPR of Plant Physiology and Pathology, University Félix HOUPHOUËT-BOIGNY, UFR Biosciences, 22 BP 582 Abidjan 22, Côte d'Ivoire.* [2]*Wascal/African Center of Excellence in Climate Change, Biodiversity and Sustainable Agriculture, University Félix HOUPHOUËT-BOIGNY, 01 BP V 34 Abidjan 01, Côte d'Ivoire.* [3]*UMR PHIM (Plant Health Institute), Univ. Montpellier, IRD, CIRAD, INRAE, Institute Agro, Montpellier, France.* E-MAIL: diana.fernandez@ird.fr

Mango (*Mangifera indica*) is a highly value fruit in the global export market. However, its production faces significant biotic and abiotic challenges, notably Mango Dieback (MD) and Stem-End Rot (SER), which affect orchard yield and postharvest quality. To investigate the prevalence and fungal diversity associated with these diseases, 92 mango orchards were randomly surveyed across four agro-ecological zones (AEZ) in Côte d'Ivoire during 2020 and 2021. Symptomatic mango organs were sampled, leading to the identification of 17 fungal genera based on morphological traits. Among these, *Lasiodiplodia* (*Botryosphaeriaceae* family) was the most prevalent, with 49% isolation frequency in MD and 74% in SER cases. Multilocus sequence analysis targeting the rDNA internal transcribed spacer (ITS) and the translation elongation factor 1-alpha (*tef1-α*) gene revealed six *Lasiodiplodia* species: *L. pseudotheobromae*, *L. euphorbicola*, *L. brasiliensis*, *L. caatinguensis*, *L. theobromae*, and *L. citricola* associated to MD and SER. Genotyping-by-Sequencing (GBS) combined with Principal Coordinate Analysis (PCoA) and sparse Non-Negative Matrix Factorization (sNMF) identified 4 distinct genetic groups among 150 isolates of the AEZ *Lasiodiplodia* populations. Species assignment by GBS did not fully match ITS and *tef1-α* phylogenetic assignment. Genome sequencing of representative *Lasiodiplodia* spp. isolates using Illumina HiSeq and subsequent annotation through multiple public databases allow comparative analysis, revealing both shared and species-specific gene clusters. These findings will enhance our understanding of disease dynamics in Côte d'Ivoire and will provide insight into the molecular determinants of *Lasiodiplodia* virulence, paving the way for developing effective disease management strategies.

This research was financially supported by the Regional Scholarship and Innovation Fund (Rsif) programme of the Partnership for Skills in Applied Sciences, Engineering and Technology (PASET).

Trunk disease and dieback pathogens in Australian almond orchards. B.J. OSWALD[1,2], E.S. SCOTT[3], L. CARVALHAIS[2], M.R. SOSNOWSKI[1,3], T.J. WIECHEL[4], S. KREIDL[4], L. TESORIERO[5], O.A. AKINSANMI[2]. [1]*South Australian Research & Development Institute, GPO Box 397, SA 5001.* [2]*The University of Queensland, Centre for Horticultural Science, QAAFI, Brisbane, Australia.* [3]*School of Agriculture, Food and Wine, The University of Adelaide, SA 5005.* [4]*Agriculture Victoria, AgriBio Centre, Bundoora, Victoria.* [5]*NSW Department of Primary Industries, Ourimbah, Australia.* E-MAIL: brittany.oswald@sa.gov.au

Several fungal species cause trunk diseases in almond trees. Common symptoms include necrosis of the wood, discolouration of vascular tissues, gumming, and dieback of limbs from the point of infection. Infection may kill primary branches and entire trees if left untreated. Samples collected from symptomatic trees across Australia from 2015 to 2025 yielded numerous fungal species. Fungi were identified using ITS, TEF1α, β tubulin and GAPDH gene regions as required. Botryosphaeriaceae species were the most prevalent trunk disease pathogens, with *Diplodia seriata* being the most common. Less commonly isolated species included *Botryosphaeria dothidea*, *Lasiodiplodia* spp., *Dothiorella* spp., and *Neofusicoccum* spp. Other genera isolated included *Colletotrichum, Collophora, Cytospora*, as well as species of families *Diaporthaceae* and *Diatrypaceae*. The distribution of these trunk disease pathogens differed among the growing regions. Detached branch assays and in planta experiments have shown that 18 Botryosphaeriaceae and 7 Diaporthaceae isolates are pathogenic to cvs Price and Nonpareil. Experiments towards developing a better understanding of how extrinsic factors influence disease severity are ongoing. Water-deficit stress applied to young trees in controlled conditions had no significant effect on the susceptibility of cv. Nonpareil to *D. seria-*

ta. The experiment is being repeated. Disease severity in detached branches inoculated with *D. seriata, Neofusicoccum australe* and *C. acutatum* varied with incubation temperature. Of the six almond cultivars used in the experiment, none was consistently less susceptible to any of the three pathogens. Results from this research are helping to improve our understanding of fungal trunk disease pathogens and the factors that influence disease severity.

Evaluation of selected fungal biological control agents for the protection of grapevine pruning wounds against *Diplodia seriata*. A. FLOUDAS[1], S. TESTEMPASIS[1,2], A. FLARI[1], E. DIMOU[1], A. ELEFTHERIADOU[1], G.S. KARAOGLANIDIS[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Plant Pathology Laboratory, Thessaloniki, Greece.* [2]*Department of Agriculture, School of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece.* E-MAIL: gkarao@agro.auth.gr

Grapevine Trunk Diseases (GTDs) are among the most destructive grapevine diseases, causing significant economic losses due to vineyard decline and replanting costs. The lack of effective chemical measures highlights the urgent need for environmentally and consumer-friendly alternatives. Biological Control Agents (BCAs) offer a promising, sustainable approach, targeting multiple GTD-associated fungi. This field study was conducted in the viticultural region of Nemea to evaluate the efficacy of four BCAs - *Trichoderma atroviride*, *T. citrinoviride*, *T. ghanense*, *Talaromyces pinophilus*—and a mixture (*T. ghanense* + *T. pinophilus*) in controlling infections by *Diplodia seriata*, a key GTD pathogen in Greece. Two Greek grapevine cultivars with contrasting susceptibility - Roditis (susceptible) and Limnio (tolerant) were used. BCAs were applied as conidial suspensions on fresh pruning wounds, followed 24 hours later by artificial inoculation with *D. seriata*. Wounds were covered for two days to maintain moisture and promote infection. After six months, samples were collected and analyzed using a culture-based method. Pathogen reisolation was conducted on PDA medium, and all obtained fungal isolates were identified molecularly. The results revealed that *T. pinophilus* and its mixture with *T. ghanense* provided strong protection in the susceptible cultivar Roditis, substantially reducing pathogen recovery. Conversely, *T. atroviridae* was more effective in the tolerant cultivar Limnio, indicating a potential cultivar- specific interaction. All BCAs demonstrated the ability to colonize and persist on pruning wounds. These findings reinforce the potential of BCAs as effective, sustainable tools for GTD management and support their integration into future vineyard protection strategies.

This research was funded by the European project SHIELD4GRAPE (HORIZON-CL6-2023-BIODIV-01, NUMBER 101135088).

CONCURRENT SESSION C1 – Phytophthora–prominent, emerging threats to crops and forest ecosystems

SESSION KEYNOTES

***Phytophthora* in different ecosystems of the Mediterranean Basin: agriculture, horticulture, forestry, natural environments, and emerging *Phytophthora* threats from other regions.** A. PEREZ-SIERRA[1], T. JUNG[2], M. HORTA JUNG[2], J. BAKONY[3], D. COOKE[4], T. DOĞMUŞ-LEHTIJÄRVI[5], R. LAHLALI[6], I. MILENKOVIC[7], C. MORALES-RODRIGUEZ[8], S. MORICCA[9], J. OLIVA[10], B. SCANU[11], A. SOLLA[12], A. VANNINI[8], S. WOODWARD[5], S.O. CACCIOLA[13]. [1]*Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Bitecnología, Moncada, Valencia, Spain.* [2]*Department of Forest Protection and Wildlife Management, Phytophthora Research Centre, Mendel University in Brno, 613 00 Brno, Czech Republic.* [3]*HUN-REN Centre for Agricultural Research, Plant Protection Institute, Fehérvári str. 132-144, 1116 Budapest, Hungary.* [4]*The James Hutton Institute, Plant Pathology, Invergowrie, Invergowrie, Dundee, United Kingdom of Great Britain and Northern Ireland, DD2 5DA.* [5]*Isparta University of Applied Sciences, Yenisarbademli Vocational School, 32950, Isparta, Türkiye.* [6]*Phytopathology Unit, Department of Plant Protection and Environment, Ecole Nationale d'Agriculture de Meknès, Km 10, Rte Haj Kaddour, BP S/40, Meknes 50001, Morocco.* [7]*Faculty of Forestry, University of Belgrade, 11030 Belgrade, Serbia.* [8]*Department for Innovation in Biological, Agro-food, and Forestry Systems, Tuscia University, Viterbo, Italy.* [9]*Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Plant Pathology and Entomology Section, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy.* [10]*Department of Agricultural and Forest Sciences and Engineering, University of Lleida, Lleida, Spain.* [11]*Department of Agricultural Sciences, University of Sassari, Viale Italia 39A, 07100 Sassari, Italy.* [12]*Faculty of Forestry, Universidad de Extremadura, Avenida Virgen del Puerto 2, 10600 Plasencia, Spain.* [13]*Department of Agri-*

culture, Food and Environment (Di3A), University of Catania, Via Santa Sofia, 100, 95123 Catania, Italy. E-MAIL: perez_anasie@gva.es

The oomycete *Phytophthora* is one of the most important genera in plant pathology, with 260 species currently known, 60 descriptions in progress and an estimated number of 400 to be discovered worldwide. The social and economic impacts of *Phytophthora* in agriculture, horticulture, forestry and its wider environment are significant. It can cause important losses in crops and plant production, alters the structure of forests and has a negative impact on the landscape and biodiversity of natural ecosystems. The Mediterranean Basin is particularly vulnerable with many important crops, endemic plants and biodiversity that are at risk to diseases caused by *Phytophthora*. Several studies have demonstrated that these pathogens move accidentally mainly with infected plant material and infested soil through worldwide trade, and they are introduced into new plantations or restoration projects with devastating consequences. Moreover, *Phytophthora* can be spread by natural water courses and irrigation water. Other drivers are the introduction into environments with highly susceptible hosts, intensive cultivation, stressed hosts, climate change or the combination of all of them. Usually, *Phytophthora* is studied in one type of system, either agriculture, horticulture or forestry, however, these systems are interconnected and the spread between systems threatens crop health, plant production and biodiversity simultaneously. In many cases, the devastating effects are caused by exotic *Phytophthora* species, which are introduced into new areas where they have not coevolved with the hosts, becoming invasive even if in their natural habitats are unnoticeable. Understanding the interactions between *Phytophthora* species, their hosts, and the environment is crucial to mitigating their impact. Data is needed to start understanding *Phytophthora* interactions across landscapes and, in recent years, major surveys have been carried out around the world to find the origin of some of the most damaging *Phytophthora* species. These studies indicate most *Phytophthora* species are not native to Europe. Moreover, they have resulted in the description of a high number of species that are present in different geographical areas, which could thrive and be a possible threat to plants in the Mediterranean Basin. Evidence suggests there are still many unknown species whose potential impact cannot be predictable, and biosecurity measures and practices are essential to minimize the risk of introduction and spread of new or already introduced species. There are important biosecurity challenges with asymptomatic dispersal of the pathogen and the broad host ranges. Therefore, future research should focus on improving surveillance, early detection, advanced diagnostics, data sharing, crop breeding for resistance, environmentally friendly control measures and good practices to safeguard global plant health and biodiversity, with particular attention to vulnerable regions, such as the Mediterranean Basin.

Phytophthora in public gardens- risks and their mitigation (Phyto-gard EUPHRESCO project).

F. DRIZOU[1,2], D. FREDERICKSON MATIKA[3], S. GREEN[3], F. LA SPADA[4], S.O. CACCIOLA[4] , G.J. BILODEAU[5] , M. GARBELOTTO[6], E. MARKELLOU[7], A. VICHOU[7], M. TRIVIZA[7] ,J. ZAJC ZUNIC[8], E. KOVACEC[8], M. HRABETOVA[9], S. PECKA[9], R.CAMPBELL[10]. [1]*Institute of Mediterranean Forest Ecosystems, Athens, 115 28, Greece;*[2] *Royal Horticultural Society, Department of Plant health, RHS Garden Wisley, Woking, Surrey, GU 23 6QB, UK;* [3]*Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK;* [4]*Department of Agriculture, Food and Environment (Di3A), University of Catania, Via S. Sofia 100, 95123 Catania, IT;*[5]*Canadian Food Inspection Agency, Ottawa, Canada, K2H 9P9, CA;* [6]*Department of ESPM, University of California, Berkeley, CA 94720;* [7] *Benaki Phytopathological Institute, Scient. Directorate of Phytopathology, Kifisia, Athens 145 61, Greece;* [8]*Agricultural Institute of Slovenia, Plant Protection Department, Ljubljana, SI-1000, Slovenia;* [9]*Landscape Research Institute, Pruhonice 252 43, Czech Republic;* [10]*SASA, Roddinglaw Road, Edinburgh, Scotland.* E-MAIL*:* fdrizou@elgo.gr

Public gardens are characterised by a wide range of plant species, including rare plant specimens and collections. They have a high volume of incoming, including imported, plant stock and increasing numbers of international visitors annually. All these factors increase the risk of introduction and transmission of potentially pathogenic *Phytophthora* species. The Euphresco 'Phytogard' project, involves ten international partners, aiming to establish the main *Phytophthora* spp. found in selected public gardens and risk factors associated with their distribution within the garden, including association with incoming plants. All partners in the project collect and analyse samples with the same set of shared protocols, previously developed within ID-Phyt. Soil, water and plant samples from two gardens and each of their nurseries, were collected by each collaborating partner over two years. Sample analysis consisted of DNA extraction from the samples and nested PCR to verify the presence (or absence) of *Phytophthora* DNA. All *Phytophthora*-

positive samples were processed using an established metabarcoding approach involving Illumina sequencing. Our results indicate the presence of *Phytophthora* spp. in both garden and nursery in more than half of the samples. We detected differences in the total number of Oomycetes found in each country as well as in the predominant *Phytophthora* spp., probably related to both climatic conditions and host species present. The abundance of Oomycetes and *Phytophthora* spp. was related to both plant-host genera and the type of sample. The present project provided new records of *Phytophthora* species in both plant and soil samples. Overall, this work showed that gardens are hot spots of *Phytophthora* diseases. Disease mitigation could be improved by both stakeholder and public awareness. Improvement of best practice throughout the process of plant procurement, planting and garden management, is a key factor to prevent *Phytophthora* diseases.

Early detection of *Phytophthora* in EU and third country nurseries and traded plants (ID-PHYT). S. GREEN[1], D. FREDERICKSON MATIKA[1], D.E.L. COOKE[2], P.J.A. COCK[2], L. BARWELL[3], B. PURSE[3], S.J. FRANKEL[4], J. DEL CASTILLO MÚNERA[5], S. ROONEY-LATHAM[6], C. BLOMQUIST[6], L. CULLEN[7], M. DESTEFANIS[7], A. BRECHON[7], R. O'HANLON[7], B. O'LOINSIGH[7], D. KIZIS[8], E. MARKELLOU[8], K.E. VICHOU[8], A. SANTINI[9], D. MIGLIORINI[9], A. PEPORI[9], E. HILAIRE[10], J. AGUAYO[10]. [1]*Forest Research, Northern Research Station, Roslin, Midlothian, UK.* [2]*The James Hutton Institute, Invergowrie, Dundee, UK.* [3]*The Centre for Ecology & Hydrology, Wallingford, Oxfordshire, UK.* [4]*USDA Forest Service, Pacific Southwest Research Station, Albany, CA, USA.* [5]*University of California, Davis, CA, USA.* [6]*California Department of Food & Agriculture, Sacramento, CA, USA.* [7]*Department of Agriculture, Food and the Marine (DAFM), Backweston, IE.* [8]*Benaki Phytopathological Institute (BPI), Athens, GR.* [9]*Institute for Sustainable Plant Protection – National Research Council (CNR), Sesto fiorentino, IT.* [10]*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Malzéville, FR.* E-MAIL: sarah.green@forestresearch.gov.uk

Invasive *Phytophthora* pathogens are causing significant economic damage to agricultural, horticultural and forestry crops worldwide, as well as ecological damage to native plant species in wider environments. Traded plants are a well-documented pathway for *Phytophthora* pathogens, facilitating their spread both nationally and internationally. The goal of the ID-PHYT project was to develop a co-ordinated strategy for the early detection of *Phytophthora* pathogens in plant nurseries and traded plants for planting across EU and third countries to inform best practice. Protocols for nursery sampling and detection of *Phytophthora* using an eDNA metabarcoding method plus a traditional baiting method were successfully shared and validated across project teams in six partner countries (FR, GB, GR, IE, IT, USA). The final nursery sampling dataset consisted of 1011 samples with replicates collected from thirteen plant nurseries across six countries. This included 647 root samples and 364 water samples with 627 samples analysed by baiting and 384 samples analysed by metabarcoding. Sample metadata and a set of key nursery management data were also collected for downstream analyses conducted using hierarchical Bayesian mixed models. A high diversity of *Phytophthora* (65 known *Phytophthora* species including quarantine-regulated species and some first country records) was detected across the 13 sampled nurseries. *Phytophthora* was found in the irrigation water at several of the nurseries highlighting water management as a key priority area for improvement. High risk hosts with consistent Phytophthora associations included *Fagus, Ligustrum, Thuja, Lavandula, Quercus* and *Choisya* spp. Other nursery risk factors which increased the likelihood of *Phytophthora*-positive samples included reliance on greater than 50% imported plant stock and growing a high diversity of plant genera. Analyses were also able to identify *Phytophthora* species' sensitivities to substrate (water versus root), nursery latitude and detection method (baiting versus metabarcoding) which assists understanding of their lifestyle and habit and sensitivity to detection method, facilitating further prediction of risk. One outcome of the project was the co-design of a concise, best-practice guidance document based on scientific evidence translated from English into partner country languages and disseminated through each country's trade association channels. The best practice guidance highlights key plant biosecurity considerations for growers and focuses on the need to understand high risk hosts and pathways, improved water management and plant growing conditions, awareness of symptoms and the importance of having staff trained in plant health knowledge.

ORAL PRESENTATIONS

***Phytophthora palmivora*: A Serious Challenge for South African Papaya Growers.** F. JAMI[1], W.J. BOTHA[1], M. SCHOEMAN[2], M. DANEEL[2]. [1]*Agricultural Research Council, Plant Health and Protection,*

Roodeplaat, Gauteng. [2]*Agricultural Research Council, Tropical and Subtropical Crops, Mbombela, Mpumalanga.* E-MAIL: JamiF@arc.agric.za

Phytophthora palmivora is an Oomycete pathogen widespread in tropical and subtropical regions, causing severe diseases such as fruit rot, bud rot, blight, cankers, and root rot. In South Africa, papaya is grown by both smallholder and commercial farmers, particularly in the Limpopo and Mpumalanga provinces. In 2019, widespread symptoms of crown and root rot were observed in South African papaya orchards, leading to significant tree collapse and loss of fruit production. This study aimed to identify and characterize *Phytophthora* species infecting papaya in South Africa, as well as to investigate the distribution and aggressiveness of identified species. Samples were collected from six commercial orchards, and diseased stems and soil samples were analysed, resulting in isolation of only one *Phytophthora* species. Molecular identification using rDNA-ITS sequencing confirmed the species identification as *P. palmivora*, and pathogenicity tests revealed the pathogen's aggressiveness on papaya fruits. This is the first report of *P. palmivora* in South Africa, where it is a regulated pathogen. Given its destructive potential, *P. palmivora* poses a serious threat to the papaya industry and other crops such as macadamia, citrus, and avocado. Understanding the distribution of *P. palmivora* and its potential introduction routes is critical for implementing effective management strategies to protect South African agriculture from further damage.

Emerging *Phytophthora* species in natural and agricultural ecosystems: a case study of wild and cultivated olives in Sardinia, Italy. A. DEIDDA, A. BRANDANO, C. MORITTU, G.G.A. SATTA, B. SCANU. *Department of Agricultural Sciences, Viale Italia 39/a, University of Sassari, 07100 Sassari, Italy.* E-MAIL: adeidda2@uniss.it

The genus *Phytophthora* includes numerous species widely recognised as destructive pathogens in agricultural and natural ecosystems worldwide. Several species have recently emerged as major threats to agricultural tree crops, particularly in areas with Mediterranean and semi-arid climates. Among these, cultivated olive has increasingly suffered from wilting and mortality in several olive- growing countries. Since 2022, severe decline and mortality of wild olive trees, traditionally used as rootstocks for cultivated olive, have been observed in Sardinia (Italy). Affected trees exhibited leaf chlorosis, wilting, shoot blight, defoliation and root necroses. Multiple *Phytophthora* taxa were isolated from rhizosphere soil and their pathogenicity on wild olive was confirmed. Similarly, since 2010, *Phytophthora*-induced decline has been reported in cultivated olive in the region, particularly in newly established commercial plantations. In addition to crown symptoms resembling those on wild olive, trees exhibited root and collar rot, with necrotic lesions often girdling the stem. Based on morphological and molecular analyses, isolates from infected tissues were identified as *P. heterospora*, a sister species of *P. palmivora*. More recently the number of *Phytophthora* species detected from young olive groves increased, including *P. citrophthora*, *P. inundata, P. nicotianae* and *P. niederhauserii*. Notably, *P. niederhauserii* and *P. citrophthora* were also detected in potted olive saplings coming from nurseries and ready for outplanting. These findings provide novel insights into the diversity and impact of *Phytophthora* species on wild and cultivated olives, highlighting the role of intensified cultivation practices and uncontrolled plant movement driving the spread of these pathogens.

This research was financially supported by the project entitled 'Studio e sperimentazione di misure di contrasto per arginare la diffusione di specie di Phytophthora *negli olivastri del centro Sardegna – DGR N. 32/19 DEL 25/10/2022' funded by the Sardinian Regional Government.*

Influence of environmental and biological factors on *Phytophthora acerina* pathogenicity in almonds. B.J. OSWALD[1,2], E.S. SCOTT[3], L. CARVALHAIS[2], M.R. SOSNOWSKI[1,3], O.A. AKINSANMI[2]. [1]*South Australian Research & Development Institute, GPO Box 397, SA 5001.* [2]*The University of Queensland, Centre for Horticultural Science, QAAFI, Brisbane, Australia.* [3]*School of Agriculture, Food and Wine, The University of Adelaide, SA 5005.* E-MAIL: brittany.oswald@sa.gov.au

Phytophthora root rot, crown rot and trunk cankers in almond trees are caused by several *Phytophthora* spp., which are widespread in Australian orchards. Symptoms include limb dieback, chlorosis, gummosis, woody tissue discolouration and necrosis, root necrosis, tree decline and death, ultimately resulting in significant loss. *Phytophthora acerina* was the most commonly identified species, most frequently isolated from the trunks of mature trees. Other isolated species included *P. cactorum, P. hedraiandra, P. multivora* and *P. niederhauserii*. Current research aims to understand how biotic and abiotic factors influence disease severity. Experiments assessed the susceptibility of commonly planted rootstocks and almond cultivars to *P. acerina*, and the

influence of water-deficit, temperature, pruning, and carbohydrate and lignin content on disease severity. These investigations revealed that although there was some variation, all almond cultivars tested were highly susceptible to infection. Lesions caused by *P. acerina* were shorter in trees subjected to deficit irrigation and longest at 30°C, compared to well-watered trees and lower temperatures, respectively. Further experiments showed that grafted nursery material subjected to pruning was more susceptible to colonisation by *P. acerina* than non-pruned trees. Experiments investigating rootstock susceptibility are underway, and compositional analysis is ongoing to determine how carbohydrates and lignin influence colonisation in almond wood, and results will be presented. An improved understanding of the effect of these factors on the severity of *Phytophthora* diseases in almonds will aid in developing effective management strategies to enhance orchard longevity and profitability.

Detection of *Phytophthora* and other oomycete pathogens in UK peat-free growing media and implications for plant health. D. FREDERICKSON MATIKA[1], S. GREEN[1], D. CRISP[1], K. SCHIFFER-FORSYTH[2], M. ELLIOT[3], P.J.A. COCK[4]. [1]*Forest Research, Northern Research Station, Roslin, Midlothian, EH25 9SY, UK.;* [2]*INRAE Grand Est-Nancy, 54280 Champenoux, France;* [3]*Royal Botanic Gardens Edinburgh, 20a Inverleith Row, Edinburgh, EH3 5LR;* [4]*Strathclyde Institute of Pharmacy & Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, G4 0RE, UK.* E-MAIL: debbie.frederickson@forestresearch.gov.uk

Peat-based growing media products are being phased out in the UK. Alternative constituents may be at elevated risk of harbouring oomycete plant pathogens. Thirty-six peat-free growing media samples were tested for oomycete pathogens by baiting and DNA metabarcoding. As evidence of live pathogens present, one *Phytophthora* species, *P. bilorbang,* was detected by baiting of wood chips and bark, and eight species of *Pythium, Phytopythium* and *Elongisporangium,* including four species not previously reported in the UK, were also baited. DNA of eleven *Phytophthora* species were detected by Illumina metabarcoding of the ITS1 across a range of samples including *P. citrophthora*. *P. cactorum* and *P. idaei* in compost mixes and *P. citrophthora* and *P. cinnamomi* in bark. Coir samples were found to contain DNA of *P. crassamura/ megasperma* andalso *Peronosclerospora,* a genus of tropical downy mildew pathogens. Of particular concern, DNA of six *Phytophthora* pathogens, including *P. bishii,* a pathogen of strawberry not previously reported in the UK, were detected in imported, recycled coir previously used for strawberry production. We discuss the implications of the species detected for plant health and the need for further evidence- gathering to strengthen biosecurity.

This research was financially supported by FPPH DEFRA, UK & Scotland's PHC/ Scottish Government.

Silent Invaders: unmasking *Phytophthora* diversity in Mediterranean nurseries and gardens. A. VICHOU[1], M. TRIVIZA[1], E. FOTOPOULOU[1], T. MARGARITOPOULOU[1], D. FREDERICKSON MATIKA[2], F. DRIZOU[3], B. GUILLAUME[4], I. FIRTH[4], E. PAPLOMATAS[5], E. MARKELLOU[1]. [1]*Laboratory of Mycology, Benaki Phytopathological Institute, St. Delta 8, Kifissia 145 61, Greece;* [2]*Forest Research, Northern Research Station, Bush Estate, Roslin, Midlothian, Scotland EH25 9SY;* [3]*Laboratory of Plant Pathology – Protection of Forest Ecosystem, Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 11528 Athens, Greece;* [4]*Ottawa Plant Laboratory, Canadian Food Inspection Agency, 1400 Merivale Rd, Ottawa, ON K1A 0Y9, Canada;* [5]*Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos Str., 118 55, Athens, Greece.* E-MAIL: k.vichou@bpi.gr

Oomycete diseases, especially those caused by *Phytophthora* species, present a serious and growing threat to nurseries and gardens worldwide, often spreading silently through asymptomatic plants. With nearly 200 recognized species and an exceptionally wide host range, *Phytophthora* is behind some of the most destructive plant diseases globally. From 2021 to 2023, an extensive survey across Greece aimed to map the incidence and diversity of *Phytophthora* species in both symptomatic and asymptomatic plants. A total of 2,607 samples—including rhizosphere soil and irrigation water from ponds and canals—were collected from 34 plant genera, sourced from nurseries in Greece, France, and Italy, as well as public and private Greek gardens. Baiting assays produced 763 oomycete isolates, from which 129 were selected according to specific criteria for molecular identification. Targeting three genetic loci, 99 strains were confirmed as *Phytophthora* spp., representing 11 species and one interspecific hybrid. An additional 27 isolates were classified as *Phytopythium* spp., and three as *Pythium* spp. *Phytophthora nicotianae* emerged as the most prevalent species (35.36%), isolated from the rhizosphere of 20 host plants. Other species, such as *P. virginiana*, *P. parsiana*, *P. lacustris*, and the hybrid *P. lacustris* × *riparia*, were found exclusively in irrigation water. ITS motif

analysis revealed that even single nucleotide polymorphisms can effectively distinguish among *Phytophthora* species. Furthermore, under the Euphresco project "Phyto-gard" and national research initiatives, high-throughput sequencing (NGS) targeting the ITS region was performed on 163 samples, greatly enhancing detection sensitivity and offering a broader, more comprehensive view of oomycete diversity.

Survival of oomycetes and fungi during composing of horticultural waste: implications for compost biosafety. L. GUIDONI, A. VANNINI, C. MORALES-RODRIGUEZ. *Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, 01100 Viterbo, Italy.* E-MAIL: vannini@unitus.it

European countries are reducing peat use in horticulture due to its environmental impact. Research has demonstrated that compost can effectively replace peat without compromising the growth and development of potted horticultural plants. However, alternatives must meet strict quality standards, particularly regarding the risk of contamination by plant pathogens. Reliable diagnostic methods are essential to assess the survival of pathogens during composting. This study evaluated the survival of *Phytophthora cinnamomi*, a model plant pathogen, in woody chips from artificially inoculated *Castanea sativa* saplings used as bulking agents in composting. Detection methods included pure culture isolation, quantitative PCR (qPCR), and High Throughput Sequencing (HTS). At the start of composting, the pathogen was easily detected using baiting and molecular barcoding. By the end of the maturation phase, *P. cinnamomi* was no longer detectable by any method, indicating that effective composting can eradicate the pathogen. HTS also identified DNA from plant pathogenic fungal genera naturally present in green waste and bulking materials throughout the composting process. These findings support the development of diagnostic pipelines for compost biosafety certification. Overall, the study demonstrates that high-quality, pathogen-free compost can be produced from recycled horticultural waste without the need for complex or expensive composting infrastructure.

Unveiling intraspecific variability and population structure of the oak-pathogen *Phytophthora quercina* using genotyping-by-sequencing. A. BRANDANO[1], M. MULLETT[1,2], T. JUNG[2], M. HORTA-JUNG[2], A. DEIDDA[1], B. SCANU[1]. [1]*Department of Agricultural Sciences, University of Sassari, Viale Italia 39A, 07100 Sassari, Italy.* [2]*Department of Forest Protection and Wildlife Management, Phytophthora Research Centre, Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic.* E-MAIL: abrandano@uniss.it

Phytophthora quercina is an important root pathogen of oak trees. Its geographic distribution seems to be limited to Europe, although there have been reports in Asia Minor, North Africa and America. In this study a total of 75 isolates of *P. quercina* were selected to investigate its population structure and the outcrossing level through genotyping-by-sequencing (GBS) analysis. The STRUCTURE analysis suggested that eight clusters best fit the dataset, whereas the Bayesian Information Criterion from K-means clustering in Discriminant Analysis of Principal Components (DAPC) supported five groups. Among these, some clusters were consistent in sharing the same host (e.g. *Quercus suber*), while others had the same geographic origin (e.g. Portugal) but different hosts. Some clonal lineages were consistent sharing both the same host and geographic origin (e.g. *Q. suber* – Sardinia, Italy; *Q. suber* – Spain; or *Quercus robur* – Central Europe). Network analysis and the index of association show that this homothallic, selectively inbreeding *Phytophthora* species has undergone a significant amount of outcrossing, which may has generated distinct subgroups of admixed individuals. The estimation of the degree of linkage disequilibrium revealed that the results for these populations were significantly lower than those for a clonal. This study provides new insights into the phylogeography and evolutionary history of *P. quercina*, which seem to be mainly driven by the diversification of host oak species.

Big problems, big solutions: holistic approach and Integrated Pest Management for *Phytophthora cinnamomi* in the FAGESOS Project. C. MORALES-RODRIGUEZ, D. LIBERATI, R. CACCIA, A. VANNINI. *Department for Innovation in Biological, agro-food and forest systems (DIBAF) Tuscia University. Via San Camillo de Lellis snc, VT, Italy.* E-MAIL: cmorales@unitus.it

Phytophthora cinnamomi is a highly invasive soil-borne oomycete responsible for root rot in a wide range of woody species. In Mediterranean ecosystems, it poses a serious threat to biodiversity and forest stability, particularly affecting *Quercus ilex*, *Q. suber*, and *Castanea sativa*. The pathogen is facilitated by rising temperatures and changing precipitation patterns, which expand its geographic range and intensify disease outbreaks. In

September 2022, the European 101074466—LIFE21-CCA- IT-LIFE FAGESOS project was launched. The project has among its objectives the development of IPM protocols for the mitigation of damage caused by *P. cinnamomi* in *Quercus ilex*, *Q. suber*, and *C. sativa* as well as its large-scale application. By the end of the project, we expect to treat up to 1,070 ha (7 demonstrative sites) and to protect up to 18,119 ha of vulnerable areas in total. The project adopts a holistic and ecosystem-based approach, combining chemical, biological, and cultural practices over two consecutive years of treatment. In 2024, the first treatment cycle was applied across seven demonstration sites in different Mediterranean forest contexts. The protocol focuses on reducing pathogen inoculum in the soil, enhancing populations of beneficial microorganisms, and stimulating the plant's natural defense mechanisms. The contribution will present preliminary results of this initial phase. The LIFE FAGESOS project offers a model for integrated forest health management, promoting resilience against emerging diseases in the context of climate change.

This research was financially supported by the LIFE FAGESOS, co-funded by the European LIFE Programme, under grant agreement 101074466—LIFE21-CCA-IT-LIFE FAGESOS.

POSTER PRESENTATIONS

Disentangling the influence of Mediterranean *Quercus* species on *Phytophthora cinnamomi* infectivity. R. LÓPEZ, M.T. HIDALGO, M. GARCÍA, M. S. SERRANO. *Department of Agronomy, Campus de Rabanales Edif. C-4, University of Córdoba, Córdoba, 14071, Spain.* E-MAIL: a12semom@uco.es

Mediterranean oak forests exhibit high *Quercus* species diversity with varying response against the pathogen *Phytophthora cinnamomi (Pc)*. Recent studies using excised twigs inoculation have shown that *Q. pyrenaica* (*Qp*) was as sensitive to *Pc* as *Q. ilex (Qi)*, whereas *Q. coccifera (Qc)* and *Q. faginea (Qf)* appeared more tolerant to infection. However, little is known about how these species could influence on *Pc* infective capacity. To address this, the ability of *Qc, Qf*, and *Qp* root exudates to stimulate zoospore release of *Pc* was assessed through *in vitro* experiments, using *Qi* as a reference. Aerial mycelium of *Pc* growin in Pea- Broth was transferred to glass beakers containing sterilized saline solution to stimulate sporangial production. Seedling rootlets from each species were immersed into the liquid and incubated in the dark at 23°C for 48 hours. Three beakers with one plant each were prepared per species along with three more beakers without plants as controls. Zoospore production was quantified in a Neubauer chamber. The full experiment was repeated twice. Results showed that *Qi, Qc* and *Qp* induced significantly higher zoospores production than the controls whereas *Qf* did not. Additionally, although all species showed a similar effect to *Qi*, the infectivity of *Pc* was significantly less stimulated by *Qf* compared to *Qp*. These findings suggest that the high ability to induce *Pc* infective zoospores exhibited by sensitive *Quercus* species and even by the tolerant *Qc*, puts the survival of Mediterranean oak forests at greater risk.

Characterization of *Phytophthora* and *Pythium* species causing root rot in olive trees in Morocco. I. LEGRIFI[1,2], A. LAZRAQ[2], S. AMIRI[1], A. TAHIRI[1], R. LAHLALI[1]. [1]*Phytopathology Unit, Department of Plant Protection, Ecole Nationale d'Agriculture de Meknès, Km 10, Rte Haj Kaddour, BP S/40, Meknes 50001, Morocco.* [2]*Laboratory of Functional Ecology and Environmental Engineering, Sidi Mohamed Ben Abdellah University, Route d'Imouzzer, P.O. Box 2202, Fez 30000, Morocco.* E-MAIL: rlahlali@enameknes.ac.ma

The olive tree (*Olea europaea*) is a vital fruit crop in Morocco; however, a widespread decline due to root rot has been observed in several regions. This study aimed to identify and characterize the oomycete species associated with olive root rot. During the 2021 and 2022 growing seasons, symptomatic root tissues and soil samples were collected, leading to the identification of 10 oomycete species through morphological characterization and sequencing of the internal transcribed spacer (ITS) region of rDNA. Seven species belonged to *Phytophthora* (*P. palmivora, P. plurivora, P. acerina, P. oleae, P. cactorum, P. gonapodyides,* and *P. megasperma*), while three species were from *Pythium sensu lato* (*P. schmitthenneri, P. aphanidermatum,* and *P. irregulare*). A pathogenicity assay using soil inoculation on one-year- old olive seedlings (cv. Picholine Marocaine) revealed that all species were pathogenic, causing root rot, vascular discoloration, and wilting, with successful re-isolation of the pathogens from necrotic roots, fulfilling Koch's postulates. These findings highlight the complex etiology of olive root rot, as multiple species induce similar symptoms, and represent the first detailed report of *Phytophthora* and *Pythium s.l.* species associated with this disease in Morocco.

Gene expression dynamics in olive drupes (*Olea europaea* L.) under *Phytophthora oleae* infection: RNA-Seq

insights for sustainable management. F. LA SPADA[1], S. CONTI TAGUALI[1], M. RIOLO[1], R. PARLASCINO[1], C. BUA[1], M.C. TAMBE'[1], A. PANE[1], G. DIONISIO[2], S.O. CACCIOLA[2]. [1]*Department of Agriculture, Food and Environment Di3A, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy.* [2]*Department of Agroecology, Research Center Flakkebjerg, Aarhus University, Slagelse, 4200, Denmark.* E-MAIL: olga.cacciola@unict.it

Rot of drupes caused by *Phytophthora oleae* is an emerging disease of olive (*Olea europaea* L.) in humid environments. This study assessed the effectiveness of two biocontrol means, a culture filtrate of *Trichoderma atroviride* and a biocontrol agent (BCA), a cell suspension of the yeast *Candida oleophila*, in controlling this disease. To better understand the mechanism of action of these two biocontrol means, the transcriptional reprogramming of the host as a consequence of *P. oleae* infection and treatments with either *T. atroviride*-culture filtrate or *C. oleophila* cells was investigated. Both treatments significantly reduced disease severity compared to the untreated control, with a reduction in the relative AUDPC of 52 and 56 percent, respectively. Transcriptomic analyses of olive drupes revealed noticeable changes of gene expression following pathogen infection and biocontrol applications. Infection with *P. oleae* alone modulated the expression of more than 2,200 genes, many of which were linked to defense regulation mechanisms, oxidative stress response, and hormonal signalling pathways. Treatment of non-infected drupes with either biocontrol means modulated genes involved in immune priming, stress responses, and cell wall remodelling. When applied to *P. oleae* infected drupes, both treatments influenced key defense related pathways, including jasmonic acid signaling and reactive oxygen species regulation, suggesting enhanced or redirected defense mechanisms. These findings highlight the bi-directional mode of action of both *T. atroviride* culture filtrate and *C. oleophila* as biocontrol means of rot of olive drupes, i.e. suppressing the disease development and modulating the expression of host genes promoting disease resistance.

This research was inspired by the projects "Nuove soluzioni tecnologiche per la filiera degli agrumi – NewCitrusTech" (cod. 1.3.1, Misura 19 PSR 2014/2022 – CLLD GAL Eloro) and "Azioni Innovative per la Produttività del Distretto dell'Ortofrutta di Qualità – INNOVAPROD" (cod. 1.3.2, Misura 19 PSR 2014/2022 CLLD).

Biotic and abiotic factors involved in kiwifruit decline syndrome (KVDS) in Spain. A. PÉREZ-SIERRA, C. SALAS SIERRA, M.A. CAÑAVERAS GÓMEZ, J.L. MIRA, M.R. MARTÍNEZ CUENCA, A. PRIMO CAPELLA, A. VICENT. *Instituto Valenciano de Investigaciones Agrarias, Carretera cv-315 km 10,7, 46113 Moncada, Valencia, Spain.* E-MAIL: perez_anasie@gva.es

Since 2020 decline symptoms have been observed in kiwifruit plantations in Valencia (Spain). Kiwifruit plants exhibited progressive dieback and died during summer. Initial investigations showed that a root rot was always associated with the decline. The symptoms were consistent with Kiwifruit Vine Decline Syndrome (KVDS), a disease of unknown aetiology affecting kiwifruit production in Italy since 2012. The aim of this study was to investigate the cause of the decline. A survey was conducted in 2024 in seven plantations were decline symptoms were detected. Soil and roots samples were collected. Isolations were performed on agar media and using baiting techniques and. In total, nine genera of fungi were recovered from the roots and four genera of oomycetes were recovered from roots and soil. The results were on agreement with the results obtained in Italy suggesting that the decline observed in Valencia could be the KVDS previously observed in Italy. As oomycetes have been shown to play an important role on the decline in Italy, an initial pathogenicity test was performed with four of the oomycetes detected: *Phytophthora nicotianae* and three *Phytopythium* species: *Ph. heliciodes, Ph. litorale* and *Ph. vexans*. One year old plants of two different rootstocks (*A. deliciosa* and *A. macrosperma*) were used. The results obtained indicated that this is a complex disease where abiotic factors could also be involved, and it is critical to identify the cause of this decline to develop appropriate control measures and to prevent the further spreading of the KVDS to new areas.

This research was financially supported by Proyecto MORIAkiwi (PID2023-147748OR-I00) from Ministerio de Ciencia e Innovación de España y la Agencia Estatal de Investigación.

***Phytophthora hedraiandra* on the move: detection and characterization on imported *Hydrangea macrophylla* in Apulia, Southern Italy.** M. CARLUCCI[1], C. CARBOTTI[1], P.G. LUCCHESE[1], S. CONVERTINI[2,3], A. PACIFICO, F. NIGRO[1]. [1]*Department of Soil, Plant and Food Sciences (DiSSPA), Via Giovanni Amendola 165/A, University of Bari - Aldo Moro, Bari, 70126, Italy.* [2]*Reagri srl, via Chiatona 62, 74016 Massafra (TA).* [3]*Agrin scarl, via xxv Aprile 13, 36055 Nove (VI).* E-MAIL: franco.nigro@uniba.it

The oomycete genus *Phytophthora* comprises highly destructive primary plant pathogens with significant economic impact. Moreover, *Phytophthora* species are responsible for extensive losses in ornamental plant production worldwide. Global trade facilitates the spread of these pathogens and the emergence of new hybrids suggests that novel *Phytophthora* species are evolving, likely in nursery environments where related but geographically isolated species come into contact. In spring 2024, several cases of decline and mortality of *Hydrangea macrophylla* were observed in nurseries in Apulia (southeastern Italy) on plants imported from Germany. Symptoms included collar and root rot, gradual wilting, leaf chlorosis, and stem necrosis. Isolations performed on potato dextrose agar (PDA) yielded colonies morphologically referable to *Phytophthora* spp., characterized by slow-growing, rosaceous to petaloid colony patterns on PDA, and the production of globose to broadly ovoid, caducous, papillate sporangia with short pedicels, typical of *P. hedraiandra*. Multilocus phylogenetic analysis of two isolates, based on ITS, TEF1α, TUB2, and COX1 sequences, confirmed their identity as *Phytophthora hedraiandra*. Pathogenicity tests conducted by artificial inoculation of three-year-old *H. macrophylla* plants fulfilled Koch's postulates. The introduction of this pathogen through imported plants highlights the urgent need for the production of certified plant material, enhanced surveillance at points of entry, and improved local propagation practices to reduce the risk of introducing new invasive pathogens.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Essential oils against *Phytophthora infestans:* A natural alternative to prevent potato from late blight disease. F. MARTINI[1,2,3], J. MUCHEMBLED[2], C. BURGEON[1,4], E. GONTIER[3], M.H. JIJAKLI[4], M-L. FAUCONNIER[1]. [1]*Laboratory of Chemistry of Natural Molecules, UMRt BioEcoAgro INRAE-1158, Gembloux Agro-Bio Tech, Liege University, Passage des Déportés 2, 5030 Gembloux, Belgium.* [2]*Plant Pathology and Biocontrol Team, UMRt BioEcoAgro INRAE-1158, ISA JUNIA, 2 rue Norbert Ségard, 59000 Lille, France.* [3]*Laboratory of Plant Biology and Innovation, BIOPI-UPJV, UMRt BioEcoAgro INRAE-1158, UFR Sciences of University of Picardie Jules Verne, 22 rue Saint Leu, 80000 Amiens, France.* [4]*Laboratory of Integrated and Urban Plant Pathology, UMRt BioEcoAgro INRAE-1158, Gembloux Agro-Bio Tech, Liege University, Passage des Déportés 2, 5030, Gembloux, Belgium.* E-MAIL: florian.martini@uliege.be

The intensive use of synthetic and mineral (notably copper sulfate) pesticides depletes resources, pollutes water and threatens human health and ecosystems. As such, new and more sustainable alternatives for crop protection are urgently needed. This study explores the use of essential oils (EOs) – plant secondary metabolites known for their diverse biological activities – as natural alternatives for controlling potato late blight, a disease caused by the oomycete *Phytophthora infestans*. For this purpose, several EOs were chemically analysed, emulsified in water with Tween 20 and tested *in vitro* against three *P. infestans* genotypes (EU-13-A2, EU-36-A2, EU-37-A2). Oils rich in phenylpropanoids such as oregano, clove or cinnamon completely inhibited both mycelium growth and spore germination from 1 $\mu L\ mL^{-1}$, more efficiently than terpene-rich oils (bergamot, rosemary). *Ex vivo* assays on potato leaves confirmed the increasing susceptibility of three different potato varieties (Bintje, Fontane, Carolus) known to exhibit respectively no, low and high resistance against the disease. This highlights how varietal selection plays another key role in disease management. Phytotoxicity assessments showed no visible lesions or significant reduction in chlorophyll fluorescence caused by any EO under 2.5 $\mu L\ mL^{-1}$, although chlorosis was observed at higher concentrations. *In planta* experiments are currently underway to evaluate the protective properties of essential oils together with how they could induce plant systemic resistance. Together, these findings, together with deep comprehension of cellular sites and modes of action, will reveal promising EO for the development of effective and non-toxic biofungicides, paving the way for future applications in sustainable managment of late blight.

CONCURRENT SESSION C2 – Major diseases that affect economic crops in the Arab and Mediterranean countries

SESSION KEYNOTES

Prevalence of emerging vascular diseases of stone fruit trees in Arab countries and progress in control strategies. E. CHOUEIRI. *Department of Plant Protection, Lebanese Agricultural Research Institute, Tal Amara, P.O. Box 287, Zahlé, Lebanon.* E-MAIL: echoueiri@lari.gov.lb

Stone fruit trees are a major industry in the Arab region characterized by climatic conditions favorable for fruit species providing economic, social and environmental services. Emerging vascular diseases had great influence on the quality and yield of these crops. Over 10 viruses have been reported in the Arab countries to affect stone fruits with a wide range of symptoms that consisted of mosaic, line patterns, mottling, yellow spots, distortion of the leaves, leaf shot-hole, fruit abnormalities, etc. The more common viruses and viroids detected in most Arab countries to infect stone fruits were PNRSV, PDV, ApMV, ACLSV, PPV, PLMVd and HSVd, whereas other viruses such as ApLV, APLPV, PBNSPaV were less widespread. Phytoplasma-like symptoms such as leaf yellowing/reddening and rolling, and witches'-broom were observed, where '*Candidatus* Phytoplasma solani' infected plum, cherry and peach and '*Ca.* P. omanense' on plum in Jordan. In peach and plum orchards, the disease incidence ranged from 25% to 55% and from 15% to 55%, respectively, with a disease incidence of around 60% in sweet cherry. In addition, disease symptoms resembled those caused by phytoplasmas confirmed the presence of aster yellows phytoplasma (16SrI) affecting peach trees. In Egypt, European stone fruit yellows (ESFY) producing leaf curling, yellowing and fruit malformation caused serious economical losses on apricots and peaches. In Lebanon, almond witches' broom associated with '*Ca.* P. Phoenicium' destroyed more than 100,000 of almond and peach trees. Proliferation of slender shoots at several points on the main trunk of affected trees, or from the roots appeared on almond trees. Witches' broom and proliferation, perpendicular development of many auxiliary buds on the branches, leaf size reduction and yellowing (pale green), phyllody, stunted growth with affected fruit quality and yield reduction reaching 70%-100% and general decline of affected trees were also encountered. Intensified symptoms appeared after excessive pruning of infected trees. Infected almond trees generally die 3 to 4 years after the appearance of the first symptoms. Recently, *Xylella fastidiosa* has been reported on almonds with symptoms such as leaf scorch in Lebanon. However, monitoring efforts for *X. fastidiosa* in Palestine, Jordan and Morocco on stone fruit trees have been conducted, providing confirmation that these important horticultural crops are still free of *X. fastidiosa*. Control strategies have been initiated through several practices such as prohibiting imports of plant material from high risk countries, production of pathogen-free propagation materials, avoiding grafting scions from infected trees and strengthening human capacity in pathogens diagnosis. Large-scale surveys are of utmost importance to detect newly established outbreaks, particularly for diseases that can be transmitted by insect vectors. Unfortunately, in most Arab countries, information on natural vectors of emerging vector-borne diseases is still limited or incomplete. Intensive training of technical staff and surveys to investigate the identity and distribution of the insect vectors and their natural hosts all over these countries is essential. Field surveys and continuous monitoring to assess the sanitary status of stone fruit crops and implement appropriate measures to limit the spread of these emerging vascular diseases is badly needed.

Management of major viral and fungal diseases affecting temperate food legume crops in the Arab and Mediterranean countries. S. G. KUMARI[1], S.-A. KEMAL[2]. [1]*International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol Station, Beqa'a Valley, Zahle, Lebanon.* [2]*ICARDA, Rabat, Morocco.* E-MAIL: s.kumari@cgiar.org

The production, productivity and quality of temperate food legumes (faba bean, Kabuli chickpea, lentil, and field pea) are affected by several viral and fungal diseases in the Arab and Mediterranean countries. Surveys conducted over the past three decades have shown that the most important diseases affecting food legumes are wilt/root rot disease complex caused by *Fusarium* spp., *Rhizoctonia* and *Pythium* spp., and foliar diseases. In a few countries, parasitic nematodes (*Heterodera* and *Pratylenchus* spp.), and Ascochyta blights (*Didymella* spp.) are the major diseases of chickpea, faba bean and lentil, whereas faba bean suffers from chocolate spot (*Botrytis fabae*) and rust (*Uromyces viciae-fabae*). Viruses are emerging production constraints of food legumes where Faba bean necrotic yellows virus (FBNYV), Chickpea chlorotic stunt virus (CpCSV), Beet western yellows virus (BWYV), Bean yellow mosaic virus (BYMV), and Pea seed-borne mosaic virus (PSbMV) are the major ones. These viruses are affecting food legumes individually or as mixed infections. The importance of food legume viruses is mainly associated with increased in aphid activities due to changes in climate and cropping system in the regions. A positive linear correlation has been observed between virus prevalence and aphids that can transmit these viruses. The main aphid species in legume fields were *Acyrthosiphon pisum* (Harris), *Aphis craccivora* Koch. and *Aphis fabae* Scopoli. In addition, several wild species (annual or perennial) were found infected with these viruses and may play an important role in the spread of these viruses. In recent

years, virus epidemics have been reported in some Arab countries (such as Egypt, Tunisia, Syria, and Jordan), sometimes causing considerable yield reduction. Significant progress has been made in managing fungal diseases by integrating two or more management options, such as development resistant varieties, production of healthy seeds, adjusting planting dates, seed treatments, selective use of fungicide sprays, and cultural practices that reduce the impact on food legume productivity and quality. Over the past two decades, faba bean genotypes resistant to FBNYV and BLRV, chickpea genotypes resistant to CpCSV and BWYV, and lentil genotypes resistant to FBNYV, BLRV and PSbMV have been successful identified. In addition, a relatively quick and simple plastic house technique was developed to identify resistant genotypes on the basis of relative virus movement and multiplication using Tissue blot immunoassay (TBIA). However, management options remain limited for emerging viruses, the emergence of virulent pathogens mainly for Ascochyta blight on chickpea, and complex soil-borne diseases caused by several parasitic pathogens and nematodes. Further efforts are still needed to develop varieties with multiple disease resistance, integrate new management options supported by digital innovations, establishing regional networks for food legume disease research for development, capacity development, and utilize modern tools, such as diagnostic tools, AI-based early warning and detection systems. Furthermore, more farmer-led participatory research for development is needed to effectively address the key plant health challenges arising from climate and farming systems changes facing food legume production systems in the Arab and Mediterranean countries which is leading to cereal monocropping.

ORAL PRESENTATIONS

Peacock's eye disease of olive, do we finally understand how to manage it effectively? D. EZRA[1], D. YGZAO[1,2], Y. GILAT[1,2], D. SHTIENBERG[1]. [1]*Department of Plant Pathology and Weed Research, Institute of Plant Protection, Agricultural Research Organization – Volcani Institute, Rishon LeZion,7528809 Israel.* [2]*Robert H. Smith Faculty of Agriculture, Food and Environment, the Hebrew University of Jerusalem, Rehovot, Israel.* E-MAIL: dezra@volcani.agri.gov.il

Peacock's eye disease, also known as olive leaf spot, is caused by the fungus *Venturia oleaginea* and affects susceptible olive varieties globally. The disease symptoms appear as dark green to black spots on leaves, leading to defoliation, twig desiccation, and significantly reduced tree productivity in subsequent seasons. Traditionally, chemical fungicides, mainly copper-based compounds, are applied to manage this disease. However, in recent years, olive growers in Israel have experienced severe disease outbreaks despite frequent fungicide applications (five to eight times per season). Our research aimed to develop a decision support system (DSS) to implement effective control measures against peacock's eye disease in Israel. We investigated the pathogen's biology and epidemiology, focusing on environmental conditions conducive to disease development. Our findings and the result of chemical control experiments we conducted over the past five years, led to the development of a DSS we named "Tavazit". This system facilitates precise and limited fungicide applications based on specific temperature and rainfall events, optimizing disease management strategies. Proper treatment for disease control based on the "Tavazit" DSS, over subsequent years, increased both the quantity of fruit yield and the quality of oil obtained from them, compared to untreated control trees. Implementing such DSS approaches aligns with integrated pest management principles, promoting sustainable agriculture by reducing unnecessary chemical usage and mitigating environmental impact.

This research was partially financially supported by the Israeli Council of Fruit Trees and by the Chief Scientist Ministry of Agriculture and Food Security.

Deciphering the European vine code: phenotypic and genetic insights into resistance to mildew. G. MADDALENA[1], V. RICCIARDI[2], O. FAILLA[1], G. DE LORENZIS[1], S.L. TOFFOLATTI[1]. [1]*Department of DiSAA, Via G. Celoria 2, Università degli Studi di Milano, Milano, MI 20133, Italy.* [2]*IBBR, National Research Council CNR, Via Ugo La Malfa, 153, 90146 Palermo, PA, Italy.* E-MAIL: giuliana.maddalena@unimi.it

The sustainable management of grapevine diseases, such as downy mildew and powdery mildew, remains a major challenge for viticulture. Breeding varieties with genetic resistance provides an effective strategy for reducing chemical inputs. Although resistance loci to downy mildew (*Rpv*) and powdery mildew (*Ren/Run*) have primarily been introgressed from wild North American and Asian *Vitis* species, their use in breeding programs often results in the introduction of undesirable traits that compromise wine quality. Despite genetic erosion caused by human selection, exploring resistance within the Eurasian grapevine (*Vitis vinifera*) offers a promising alter-

native for breeding programs. Recent analysis has shown the potential of *V. vinifera* accessions from the Caucasus, which harbor a rich biodiversity, as source of disease resistance without compromising oenological quality. In this study, 88 *V. vinifera* accessions (wild and cultivated) from the Caucasus and neighboring regions were phenotypically evaluated for resistance to downy and powdery mildews over three consecutive years. Phenotypic screening allowed the identification of seven downy mildew-resistant and 31 powdery mildew-resistant accessions. A Genome-Wide Association Study (GWAS) performed on selected individuals revealed four novel resistance loci: *Rpv36* and *Rpv37* (downy mildew) and *Ren14* and *Ren15* (powdery mildew), located in genomic regions enriched with biotic stress response genes. These findings identify key targets for breeding disease-resistant grapevines within the *V. vinifera* species. Advanced tools like CRISPR and RNA interference could further enhance these loci potential, reducing chemical use and supporting sustainable viticulture. Ultimately, this approach promises lower production costs and improved environmental sustainability in the wine industry.

This research was financially supported by the European Union within the project titled 'FREECLIMB – Fruit Crops Adaptation To Climate Change In The Mediterranean Basin' under the Program Partnership For Research And Innovation In The Mediterranean Area (PRIMA), and the project titled 'INNOVINE - Combining innovation in vineyard management and genetic diversity for a sustainable European viticulture' (Grant agreement ID: 311775) under the Specific Programme 'Cooperation': Food, Agriculture and Biotechnology."

Characterization of Tunisian *Phyllosticta citricarpa* population and assessment of the effect of three *Trichoderma* species against the Citrus Black Spot. S. MANNAI[1], C. JEANDEL[2], J. AGUAYO[2], R. IOOS[2], N. BOUGHALLEB-M'HAMDI[1] [1]*InstitutSupérieurAgronomique de Chott Mariem, Laboratory of Plant Pathology, LR21AGR05 University of Sousse, 4042 Sousse, Tunisia.* [2]*ANSES Plant Health Laboratory, mycology unit, USC INRAE 1480, Domaine de Pixérécourt, 54220 Malzéville, France*. E-MAIL: n.boughalleb2017@gmail.com

Citrus black spot (CBS) caused by *Phyllosticta citricarpa* was first reported in Tunisia in 2019, making the first occurrence of this disease under a Mediterranean climate. *P. citricarpa* is a heterothallic fungus capable of sexual reproduction only when two mating types are present. Tunisian population revealed that strains belong to a unique mating type (MAT1.1.1). The aim of future investigations was to find some integrated tools for the management of this fungus. In this sense, three *Trichoderma* species were evaluated against *P. citricarpa* development. The *in vitro* results obtained exhibited the effectiveness of these antagonists in reducing the mycelial growth of *P. citricarpa,* with varying levels of inhibition. *Trichoderma* species showed the highest mycelial growth inhibition with 54.33%, noted for *P. citricarpa* isolate CBS1 treated with *T. atroviride* (A3); 51.76 and 53.51% for *P. citricarpa* isolate CBS4 in presence of *T. atroviride* and *T. asperellum,* respectively. The *in vivo* assay on *Citrus limon* fruits confirmed the reduction in lesions development caused by the four *P. citricarpa* isolates. In fact, the reduction ranged from 28.28 to72.83% for *P. citricarpa* CBS3 isolate treated with *T. kunmingense* and *T. atroviride,* respectively. Based on the current results, the *Trichoderma* species used reduced *P. citricarpa* mycelial growth *in vitro* and the lesions appearance due to *P. citricarpa* on *Citrus lemon* variety Eureka. Efficacy varies depending on the type of antagonist species and pathogen isolates. Therefore, these compounds have shown promising potential and could be used for CBS control.

This research was financially supported by was supported by grant of the European Food Safety Authority (EFSA) "GP/EFSA/ALPHA/2019/04 Reduce risk assessment uncertainty: suitability of Mediterranean citrus production areas for Phyllosticta citricarpa" *and Ministry of Agriculture, Water Resources and Fisheries-Tunisia.*

Identification of chickpea genotypes with multiple resistance to fusarium wilt and Ascochyta blight. F.Z. IBN EL MOKHTAR[1,2], I. MAAFA[3], M. MITACHE[2,4], F. BELBOU[1,5], H. HOUMAIRI[2], O. IDRISSI[4], S. KRIMI BENCHEQROUN[1]. [1]*Plant Pathology Laboratory, Regional Center of Agricultural Research of Settat, National Institute of Agricultural Research (INRA), Morocco.* [2]*Agri-Food and Health Laboratory, Hassan First University, Faculty of Science and Technology of Settat, Morocco.* [3]*International Center for Agricultural Research in the Dry Areas (ICARDA), Agdal, Rabat 10080, Morocco.* [4]*Laboratory of Food Legumes Breeding, Regional Center of Agronomic Research of Settat, National Institute of Agronomic Research, Avenue Ennasr, BP 415 Rabat Principale, 10090, Morocco.*[5]*Biology department, Cadi Ayyad University, Faculty of Science Semlalia, Marrakech. Morocco.* E-MAIL: sanae.krimibencheqroun@inra.ma

Chickpea (*Cicer arietinum* L.) is an important legume crop valued for its high protein content and soil-enriching nitrogen fixation. However, its production is threatened by biotic stresses, particularly Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *ciceris*

and Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. These diseases, especially under favorable environmental conditions, cause substantial yield losses and lack effective treatments, making genetic resistance the most viable solution. A total of 216 chickpea genotypes, including landraces and breeding lines, were evaluated for their resistance to FW and AB. FW screening was conducted in a sick plot field trial and a controlled growth chamber, using a completely randomized design with two replications. AB screening was conducted in a field trial (augmented RCBD) and a greenhouse trial (completely randomized design with two replications). Significant genotype, environment, and genotype-by- environment interaction effects ($P < 0.001$) were observed for both diseases. Using the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) with a 30% selection intensity, 30 genotypes were identified as highly resistant to FOC, and 40 genotypes as moderately resistant to AB. Resistance levels varied between environments, highlighting environmental influence on disease expression. Notably, six genotypes exhibited resistance to both FOC and AB. The identified genotypes offer promising material for breeding programs aimed at developing dual-resistant chickpea cultivars. These efforts can enhance disease resilience and promote sustainable chickpea production.

POSTER PRESENTATIONS

Wheat Fusarium crown rot occurrence and disease strategy management in Algeria. H. BOUREGHDA, K. HACHEFA, K. DJEMAOUI. *Ecole Nationale Supérieure Agronomique (ENSA), Département de Botanique, Laboratoire de Phytopathologie et Biologie Moléculaire, Hassan Badi street, El Harrach, Algiers, Algeria*. E-MAIL: hou.boureghda@gmail.com

Crown rot (CR), caused mainly by *Fusarium* spp., is a globally widespread disease of wheat. CR may affect yield quantity and quality due to the accumulation of mycotoxin in wheat grains. Surveys conducted in Algeria have revealed the prevalence of CR in all cereal-growing regions in the north of the country, with *F. culmorum* being the dominant associated species. Within the framework of integrated disease management, the behavior of 17 durum and bread wheat varieties grown in Algeria were evaluated over two growing seasons (2022/23 and 2023/24) at the seedling (greenhouse) and maturity (field conditions) stages. The results showed that the bread wheat varieties were more resistant than durum wheat at both stages, with varying disease indices. At the seedling stage, disease indices ranged from 1.05 to 2.80. The bread wheat variety 'Rmada' was found to be the most resistant, while the durum wheat varieties 'Boussalem' and 'Manssourah' were considered the most susceptible. At maturity stage, disease indices ranged from 0.71 to 1.74, with Rmada recording the lowest index. In contrast, durum wheat varieties 'GTA' and 'Semito' were found to be the most susceptible. A strong positive correlation ($r=0.71$, $P \leq 0.05$) was observed between the behavior of the varieties at both stages over the two years. In conclusion, the variety 'Rmada', thanks to its behavior at both growth stages, represents a promising source for CR control and should be recommended in wheat breeding programs.

Diversity of fungal pathogens linked to dieback and canker diseases in Moroccan fruit trees. K. GOURA[1,2], N. EL ALAMI[2], A. TAHIRI[1], S. AMIRI[1], R. LAHLALI[1]. [1]*Phytopathology Unit, Department of Plant Protection, Ecole Nationale d'Agriculture de Meknès, Km 10, Rte Haj Kaddour, BP S/40, Meknes 50001, Morocco.* [2]*Moulai Ismail University, Faculty of Sciences, Meknes 50000, Morocco.* E-MAIL: rlahlali@enameknes.ac.ma*;* atahiri@enameknes.ac.ma

Fruit tree trunk diseases, caused by a wide range of fungal pathogens, affect the vascular tissues and bark of fruit trees, posing a major challenge to orchard management globally. This study aimed to investigate the diversity and distribution of trunk diseases affecting various fruit tree crops in the main production regions of Morocco. Symptomatic trunk samples were collected from infected trees, and the causal agents were identified through morphological and molecular phylogenetic analysis, targeting the internal transcribed spacer (ITS) region and the β-tubulin (β-tub) gene. The findings revealed a diverse range of fungal species associated with trunk diseases, including genera such as *Neoscytalidium*, *Lasiodiplodia*, *Botryosphaeria*, *Diplodia*, *Neofusicoccum*, *Diaporthe*, *Cytospora*, *Curvularia*, *Fusarium*, *Sordaria*, and *Biscogniauxia*. Some pathogens were found to be prevalent across different tree species and regions, indicating their widespread impact on orchard health. Pathogenicity tests confirmed that all isolates could induce characteristic lesions on inoculated shoots, with *Lasiodiplodia theobromae*, *Neoscytalidium hyalinum*, and *Diplodia insularis* causing the largest lesions. This study provides the first comprehensive report on species associated with apple, peach, cherry, plum, almond, and persimmon trees in Morocco, offering new species records and critical insights for developing effective control

strategies.

Olive diseases worldwide. V. SERGEEVA. *Western Sydney University Locked Bag 1797, Penrith NSW 2751, Australia.* E-MAIL: sergeeva@tpg.com.au

Plant diseases are of global concern and exact a heavy toll on food crop production. Olives are now one of the most extensively cultivated fruit crops in the world. Olive groves can be found in 60 countries around the world. However, growers face challenges due to many different pathogens that infect olive trees, reducing yields and increasing production costs. Harmful agents of the olive tree include fungi, bacteria, phytoplasm, viruses and virus-like agents attacking all parts of the plant. Different types of damage can be expressed qualitatively by describing the symptoms, or quantitatively through assessment of crown condition. The number of these occurrences has dramatically increased in recent years, causing serious damage to olive production. More than one factor can affect the health of a tree at any one time and differentiation can be made between the primary disease, which first and foremost affects the health of the tree, and the secondary disease, which has a less important influence and usually affects trees already weakened by an existing factor. An especially important concept is that olive-tree care attempts to manage plant health rather than just control disease problems. Due to the growth of the olive industry, it has become even more important to address the issues of product quality and the adoption of integrated pest and disease management. A proper understanding of olive variety, grove design, climate, soils and grove management are vitally important to the nurturing of trees, which affects health, quality and yield. A Guide to Olive Pests and Diseases published in 2024.

Sources of inoculum of *Alternaria* species associated with apple leaf and fruit spot disease. Z. EL KHATTABI, C. MORAGREGA, I. LLORENTE. *Plant Pathology, Institute of Food and Agricultural Technology, Universitat de Girona, C/ Maria Aurèlia Capmany, 61, 17003 Girona. Spain.* E-MAIL: zohra.elkhattabi@udg.edu

In recent years, a new apple disease caused by *Alternaria* has been reported in Europe, causing significant production losses. In Catalonia, this emerging disease was first detected in 2006 in commercial apple orchards in the province of Girona. Since then, it has progressively spread, leading to production losses ranging from 10% to 40%. The disease is usually associated with the *Alternaria alternata* and *Alternaria arborescens* species complex. To develop an effective disease management program, it is essential to assess the inoculum potential. This requires identifying the specific sources of inoculum production within the apple orchards. This study aims to identify the sources of inoculum and model its production in apple orchards in Girona, with the objective of contributing to the development of effective management tools. The main sources of pathogen inoculum in apple orchards were identified through periodic sampling of leaf litter, canopy leaves, shoots, buds, and flowers. Additionally, the pathogenicity of the isolates obtained from these samples was assessed. The results indicated that *Alternaria* inoculum was produced in all examined organs; however, it was most abundant in leaf litter and other dead organic material on the soil.

This research was financially supported by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" under Grant PID2021-126505OB-I00 and by Agaur, Generalitat de Catalunya (Grant 2022 FISDU 00051).

RNAi-mediated silencing of *AS1* and *B3* genes to reduce grapevine susceptibility to *Plasmopara viticola*. L. BOLOGNA, G. MADDALENA, E. SERGI, G. DE LORENZIS, S.L. TOFFOLATTI. *Department of DiSAA, Via G. Celoria 2, Università degli Studi di Milano, MI 20133, Italy.* E-MAIL: lucia.bologna@unimi.it

Downy mildew, caused by *Plasmopara viticola,* is one of the most widespread and economically impactful grapevine diseases in Europe and is primarily managed through fungicide applications. The increasing awareness of the negative impact of fungicides on human health and the environment is driving efforts to find more sustainable disease management strategies. RNA interference (RNAi) is emerging as a promising transgene-free biotechnological tool for pest management. In this study, RNAi was applied to protect grapevine from downy mildew by silencing two specific genes (*AS1* and *B3*), both implicated in nitrogen metabolism, which are associated with grapevine susceptibility to *P. viticola*. In the experimental setup, two susceptible cultivars, Pinot noir and Merlot, were sprayed with dsRNA specifically designed to silence the expression of the two genes. Plants treated with dsRNA targeting a gene not involved in nitrogen metabolism and with sterile water were used as controls. Leaves were sampled at 1, 3, 5, 7, and 14 days after treatment; three disks were obtained from each leaf and inoculated with a *P. viticola* sporangia suspension at a fixed concentration. A visual assessment of the inoculated disks was performed to evaluate disease severity by calculating the infection index per-

centage. Preliminary data indicate that the dsRNA-*VvB3* treatment significantly reduced the infection index by day five post-treatment compared to both dsRNA-*VviAS1* and control groups, although overall infection levels were modest, potentially due to non-ideal plant vigor. Future research will consider the use of in vitro-grown plants under controlled environmental conditions to further validate these promising findings.

This research was financially supported by Fondazione Cariplo.

Impact of cropping practices on dry root rot and soil microbial diversity in durum wheat in semi-arid regions of Morocco. H. EL WAZZIKI[1], B. EL YOUSFI[1], A. EL FADI[1,2]. [1]*Laboratory of Cereal Pathology, Regional Center of Agricultural Research of Settat, National Institute of Agricultural Research (INRA), Avenue Ennasr, BP 415 Rabat Principal, Rabat 10090, Morocco.* [2]*Natural Resources Engineering and Environmental Impacts Team, Multidisciplinary Research and Innovation Laboratory, Polydisciplinary Faculty of Khouribga, Sultan Moulay Slimane University of Beni Mellal, B.P.145, Khouribga 25000, Morocco.* E-MAIL: hanane.elwazziki@inra.ma; hananelwazziki@gmail.com

Dry root rot is one of the most important wheat diseases worldwide, including Morocco. This study aimed to evaluate the health status of five no-till soils compared to a conventionally tilled soil in terms of dry root rot severity, microbial biodiversity, and the potential inoculum level of *Fusarium culmorum*. To assess disease severity, a root rot-susceptible variety 'Ourgh' was grown under greenhouse conditions in the six natural soils collected from three regions (Abda, Chaouia, and Ourdigha), each with different previous crops (lentils, fallow, wheat, barley, and forage). Disease severity was assessed at flowering on roots and stem internodes, and microbial biodiversity was estimated using microbial counting techniques. These assessments were also performed after artificial inoculation with *F. culmorum*. Root rot symptoms, confirmed by isolation of pathogen from infected roots and stems, were observed in all six soils at varying levels. Three pathogenic genera were identified: *Fusarium* spp., *Alternaria* spp., and *Bipolaris* (*B. sorokiniana*), with *Fusarium* spp. being present in all soils. Untilled soil showed higher microbial diversity than the conventionally tilled soil, which was dominated by bacteria, except in soil from Ourdigha with barley as the previous crop, where fungal and bacterial levels were similar. Inoculation increased microbial diversity and disease severity, and reduced plant height without significantly affecting dry biomass. Except for this latter soil, microbial profiles shifted post-inoculation, with soils becoming either bacterial- dominant or balanced. Soil type, texture, and crop history (except barley) had no significant impact on microbial biodiversity. These findings highlight potential natural resistance mechanisms against *F. culmorum* for future biological control strategies.

This research was financially supported by the National Institute of Agricultural Research (INRA) of Morocco through the Aridoculture Centre (Settat) to conduct this study.

Assessing the Impact of *Fusarium culmorum* on agronomic performance and DON contamination in stems and grains of nine durum wheat varieties in Tunisia. S. GUERMECH[1,2,3], M. MASIELLO[4], S. SOMMA[4], S.M. SANZANI[3], A. IPPOLITO[3], A. MORETTI[4], S. GARGOURI[2]. [1]*Faculty of Science of Tunis, University of Tunis El Manar, Tunis, Tunisia.* [2]*Plant Protection Laboratory, National Institute of Agricultural Research of Tunisia, Carthage University, Menzah, Tunisia.* [3]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.* [4]*Institute of Sciences of Food Production, National Research Council of Italy (CNR- ISPA), Bari, Italy.* E-MAIL: guermechsalma@gmail.com

Fusarium culmorum, the main causal agent of Fusarium Crown Rot (FCR) in Tunisia, represents a serious phytopathological threat under favorable environmental conditions. Its ability to produce the harmful deoxynivalenol (DON) mycotoxin, which can accumulate in plant tissues and grains, raises major concerns for human and animal health. Despite efforts over recent decades to breed resistant wheat varieties, many modern durum wheat varieties remain susceptible to FCR. This study aimed to evaluate the susceptibility of durum wheat varieties, widely grown in Tunisia to FCR. During two growing seasons (2020–2022), a field trial was conducted in Tunisia using artificial inoculation with *F. culmorum* at sowing. Agronomic traits such as tiller and plant density, kernels per spike, and grain yield were evaluated. FCR symptoms were present in all tested varieties, with significant differences in disease incidence, severity, and yield loss. The varieties Carioca, Maali, and Karim showed the highest incidence and severity, while Dhahbi, though susceptible, experienced the most severe yield loss (up to 42% in 2021). Strong correlations were observed between the incidence and severity of FCR and DON concentration in stems, and between the frequency of *F. culmorum* and DON concentration in grains. In addition, both latter parameters and FCR incidence were

correlated with yield loss. On the other hand, a weak correlation was observed between DON concentrations in stems and grains, indicating limited translocation of the toxin. These findings highlight the impact of *F. culmorum* on wheat health, yield, and mycotoxin contamination, emphasizing the importance of resistant selection and integrated disease management.

Study of the behavior of different potato varieties in response to infection with major viruses in most regions of Algeria. L. ALLALA-MESSAOUDI[1], I. MOURAKEB[2], M. BENDANBRI[2], M.S. SHAHID[3]. [1]*Ecole Nationale Supérieure Agronomique; department of botanique, Hassen Badi, 16200 El Harrach Alger.* [2]*Département de semences et plants, laboratoire de technologie de production de plants. Institut Technique des Cultures Maraichères et industrielles, BP50 Route de Moretti. Staoueli, Alger.* [3]*Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University. Al-Khoud PC 123, Oman.* E-MAIL: linda.allala@edu.ensa.dz

In Algeria, viruses are among the most important biotic factors that significantly influence the growth and productivity of potato crops, both in terms of quality and quantity. This study is part of an investigation into the behavior of potato cultivars in response to viral infections, conducted under controlled conditions and in open fields. The objectives are twofold: first, to update data on the prevalence of the major potato-associated viruses (PVY, PVX, PVA, PVS, and PLRV); and second, to provide insights into the degree of resistance or susceptibility expressed by three registered potato cultivars (Kennebec, Désirée, and Red Pontiac) in the region. The results showed that most of the studied viruses were present, with the highest infection rates for PVX and PVY viruses in open fields, where infection rates reached 95% and 82%, respectively. However, under controlled conditions using an insect-proof film, the infection rates of these two viruses were significantly reduced, with PVX at 37% and PVY at 49%. Furthermore, the findings indicate that Kennebec is more resistant to PVY, despite a relatively high infection rate of 68%, while Red Pontiac proved to be the most susceptible of the other three varieties tested.

Pathogenic, morphologic, and genetic studies of *Colletotrichum* spp. associated with anthracnose of citrus in Algeria. S. ALI-AROUS[1], M. MEZIANE[1], K. DJELOUAH[2], S.M. SANZANI[3]. [1]*Laboratory of production and protection of crops of Chlef region, Faculty of Nature and Life Sciences, University of Hassiba Ben Bouali, Chlef, Algeria.* [2]*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Mediterranean Agronomic Institute of Bari, Valenzano, Bari, Italy.* [3]*Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, Bari, Italy.* E-MAIL: s.aliarous@univ-chlef.dz

The economic importance of citrus fungal diseases is constantly increasing, and they are caused by several species, some of which may be linked to the widespread citrus decline observed in Algerian citrus areas. Citrus anthracnose is associated with mild to severe symptoms on leaves, fruits, and twigs, likely due to host susceptibility, climatic conditions, and possibly affected species. For many years, this species was known as *Colletotrichum gloeosporioides*. The latter was genetically characterized by using the universal ITS regions as a marker, and it was the only species reported to be associated with citrus anthracnose in Algeria. By isolating 58 fungal colonies from infected leaves, fruits, and twigs collected from citrus orchards, four different isolates of Colletotrichum (CHDZ-1, CHDZ-2, CHDZ-3 and CHDZ- 4) were distinguished from different aboveground parts of infected citrus fruits of different species and cultivars grown in the Chlef Valley. Based on Koch's postulate, all of them were involved in the development of the disease. Multi-locus sequencing analyses of the internal transcribed spacer ITS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) revealed that species belonging to the *C. gloeosporioides* species complex were associated with the disease. Further molecular sequencing of other genomic regions is recommended for the precise identification of these *Colletotrichum* isolates. During this research, we observed severe anthracnose infection in the most visited citrus region, causing visible damage to all aerial parts of trees. Special attention must be paid to this emerging disease to prevent its fatal establishment.

The foliar black spot caused by *Neodidymelliopsis ranunculi* (*Ascomycota*: *Didymellaceae*) a new fungal disease in the Algerian citrus orchards. S. ALI-AROUS[1], K. DJELOUAH[2], A. HOUARI[1], O.J. ALABI[3], M. SÉTAMOU[4], S.M. SANZANI[5]. [1]*Laboratory of Production and Protection of Crops of Chlef region, University of Hassiba Ben Bouali, Chlef, Algeria.* [2]*CIHEAM Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy.* [3]*Department of Plant Pathology & Microbiology, Texas A&M AgriLife Research & Extension Center, Weslaco, TX 78596, USA.* [4]*Texas A&M University-Kingsville Citrus Center,*

Weslaco, Texas 78599, USA. [5]*Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi di Bari Aldo Moro, Via Amendola 165/A, Bari, Italy.* E-MAIL: s.aliarous@univ-chlef.dz

During routine surveys in the summer of 2017, severe leaf defoliation was observed in four citrus orchards, two sweet orange ('Thompson Navel' and 'Washington Navel') and two mandarin ('Nova' and 'Oro Grande'), located in the Chlef valley in the northwestern Algeria, one of the most important citrus region of the country. Visual inspection of defoliated green leaves and leaves on tree canopy revealed unusual symptoms consisting of greasy black protuberant spots on the abaxial side and yellowing on the adaxial surface. A quick survey was conducted to determine the disease incidence. The percentage of trees infected with the disease reached 50% and 35% in sweet orange and mandarin orchards, respectively. The incidence of the diseased leaves ranged from 30% to 60% for sweet orange, while 50–70% of leaves showed disease symptoms in mandarin orchards. Morphological identification such as size, shape, status of otiole(s) and aggregation of pycnidia then a multilocus sequencing approach was used for identity confirmation based on the 5.8S region of rDNA with flanking internal transcribed spacers (ITS), a portion of the 28S large subunit of rDNA (LSU), part of RNA polymerase second largest subunit (RPB2) and of β-tubulin gene (TUB2). The outcome of the identification process has associated this disease to the fungus *Neodidymelliopsis ranunculi*. Koch's postulates also confirmed *N. ranunculi* to be the causal agent. This is the first report of black spot symptoms caused by *N. ranunculi* on citrus in Algeria.

Recent findings of new citrus tristeza virus strains in the Chlef valley, Algeria. M. MEZIANE, S.A. AROUS, N.A. BENTURQUIA. *Research Laboratory of Crop Production and Protection, Hassiba Benbouali University of Chlef. Algeria.* E-MAIL: m.meziane@univ-chlef.dz

Citrus production plays a vital economic role in the Chlef Valley. However, this important crop faces ongoing challenges from diseases that threaten yield, fruit quality, and the long-term sustainability of orchards. For decades, several citrus orchards have shown decline and severe symptoms associated with tristeza disease, caused by the new, virulent exotic VT strain first reported in 2019, affecting both old and young orchards. To update the current distribution and strain occurrence of this disease, an additional survey was conducted in the valley in 2021. Samples collected from various citrus areas were tested using DTBIA/DAS-ELISA. The survey revealed an increased infection rate of 4.24%, up from 3.21% in 2019. Molecular genotype characterization using multiple molecular markers and CP gene sequencing confirmed the presence of the preexisting T30 and VT genotypes. In addition, two new genotypes were detected in the S1 and T3 strains. This study confirmed the widespread dissemination of the virulent VT genotype and the emergence of new alien strains. The other CTV isolates were found to be Mediterranean, with a nucleotide identity of 98.6% to 99.1% compared to the worldwide T30 mild CTV isolates recorded in the Mitidja region. These findings raise serious concerns: the discovery of new strains alongside the continued spread of the severe VT genotype, combined with the presence of efficient aphid vectors, poses a significant threat to the citrus industry in Algeria. This situation requires proactive measures by the National Phytosanitary Services, including extending surveillance to other citrus production regions.

First report of *Septoria pistaciarum* on pistachios in Basilicata, Italy. M. GALLO[1], W. MELLIKECHE[1], R. DHAHRI[1], V.M. RICCIUTI[2], A.M. D'ONGHIA[1], F. VALENTINI[1]. [1]*Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari (CIHEAM Bari), Via Ceglie 9, 70010 Valenzano, Bari, Italy.* [2]*Azienda agricola - Pistacchio di Stigliano,* C.da *Sauro – Capalbi, 75018 Stigliano, Matera, Italy.* E-MAIL: gallo@iamb.it

In May–June 2024, leaf spots were observed on 'Bianca' pistachio trees from a Stigliano (MT) farm, Basilicata, Italy. Leaves showing distinct red-brown spots with black margins were brought to the laboratory for isolation. Small portions (0.2–0.3 cm^2) of symptomatic leaves were surface-sterilised, air-dried in a laminar flow hood, and placed on antibiotic-amended Potato Dextrose Agar (PDA), then incubated at 25°C for 7–10 days. Slow-growing, *Septoria*- like colonies were transferred to PDA plates, and pure cultures were obtained from single hyphae. The isolate produced sulcate and immersed grey/black colonies. A yellow mucilaginous matrix exuding from black conidiomata, often covered by white mycelium, was observed. Conidia were hyaline, curved to falcate, with 1–5 septa, measuring (21.8–) 45.2 ± 8.5 (–64.4) × (1.7–) 2.9 ± 0.5 (–4.2) µm. A comparison with data from the literature confirmed the identification of *Septoria pistaciarum* Caracc. Traditional identification was further confirmed by sequencing the ITS region of rDNA. A BLAST search using the ITS sequence of the *S. pistaciarum* isolate revealed an exact match

with several reference sequences in the database, mainly from pistachio hosts. Pathogenicity tests were conducted on two-year-old pistachio plants, and the fungus was successfully re-isolated from leaf lesions, fulfilling Koch's postulates. While *S. pistaciarum* had previously been isolated in Sicily, this is the first confirmed occurrence on the continental mainland of Italy.

Preventing food-borne threats at the source: A one health approach to crop and animal Biosecurity. F. ALI[1], M. SADIQ[2], I. AHMAD[2]. [1]*Pet Street Veterinary Clinic Abu Dhabi UAE.* [2]*University of Agriculture Peshawar Pakistan.* E-MAIL: drfarzand_2011@yahoo.com

Ensuring food safety begins long before animal production starts in the field, with healthy plants. As a veterinarian, the link between plant health and animal health is clear: contaminated crops can lead to unsafe feed, compromised animal health, and ultimately impact human food safety. Recent advances in phytopathology offer new tools to detect and control plant diseases that threaten the safety and quality of crops used in animal feed and the broader food chain. By integrating veterinary and plant sciences, we can develop more effective strategies to prevent foodborne illnesses, reduce toxin exposure (such as mycotoxins), and enhance biosecurity. Strengthening education and collaboration across disciplines is key to training professionals who can address these challenges holistically. This approach supports a safer, more sustainable food system, one where plant, animal, and human health are protected together.

Incidence and severity of early blight affecting *Solanaceae* family crops. R. DUMBADZE, G. MEPARISHVILI, L. GORGILADZE, N. JABNIDZE, L. KOIAVA, M. MURADASHVILI. *Department of Plant Diseases Monitoring, Diagnostics and Molecular Biology, Institute of Phytopathology and Biodiversity, Batumi Shota Rustaveli State University, Kobuleti 6200, Georgia.* E-MAIL: rusudandumbadze6@gmail.com

In the conditions of today's globalization, there is a great danger that the spread of some highly harmful microorganisms will take a very wide scale and adapt to different ecological conditions in many regions. Some microorganisms were not previously considered to be among the causes of diseases threatening agricultural crops, but today they are already considered important phytopathogens that significantly reduce the quantity and quality of crops. Among them, *Alternaria* fungi are noteworthy, which cause especially great damage to the crops of the family of *Solanaceae*. They cause spots of Potatoes, Tomatoes, Eggplants, Peppers, Tobacco - leaves, stems, tubers and fruits. In 2024, the monitoring of the crops of the family of *Solanaceae* was carried out in the municipalities of the Adjara region, to detect *Alternaria* spp. Private homesteads and farms were visited during the vegetation period of the plant through a scientific expedition. About 186 disease samples were collected and analysed. The Incidence and Severity of *Alternaria* spp. was uneven on Solanaceae crops, the Incidence on potatoes was 35%, and the severity was 25%, on tomatoes 30% and 20%, the disease was spread with the same frequency on eggplant and pepper, and it was 20% and 20%, It should be noted that the mentioned disease was not observed at all on tobacco. The identification of *Alternaria* spp. isolates was performed exclusively based on morphological criteria, with the most significant characteristics being the morphology of the conidia and the formation.

This research was financially supported by Targeted the scientific research projects of BSU, Grant number - № 06-01/25

CONCURRENT SESSION C3 – Huanglongbing of citrus and bacterial plant diseases

SESSION KEYNOTES

Sustainable control of bacterial diseases: Current advances and safety concerns. E. STEFANI. *Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, Pad. Besta, 42122 Reggio Emilia, Italy.* E-MAIL: emilio.stefani@unimore.it

Bacterial diseases represent a challenge in the current scenario of climate change, innovative agricultural systems, increased consciousness of sustainable production, need to minimise chemical inputs into the environment. Basically, the management of bacterial diseases rely on a plethora of copper compounds, a few antibiotics (where allowed), and, in case of insect transmission, use of insecticides. Emerging technologies, such as CRISPR and TALEN gene editing or the use of RNAi vectors, offer the potential to develop disease resistant plants or to modify the plant response to a pathogen attack; however, both technologies raise ethical concerns for their possible off-target effects, potential unintended consequences and unforeseen and unpredictable impacts on human health and the environment. The use

of copper-based pesticides has been thoroughly reduced, and the European Commission adopted the regulation (EU) 2018/1981 to restrict copper use in agricultural systems (i.e. only uses resulting in a total application of maximum 28 kg of copper per hectare over a period of 7 years are authorised), and copper compounds are candidates for substitution. Then, the biocontrol of bacterial diseases appears the most prospective approach to ensure crop productivity, remuneration to farmers, environmental safety and consumers' satisfaction. Many beneficial microbes are described in the scientific literature as possible solutions for farmers: basically, these are bacteria and fungi; additionally, bacteriophages are described as prospective biocontrol agents that can be used to control phytopathogenic bacteria. The biosafety of plant pathogen biocontrol agents is a crucial consideration for those researchers and companies intending to develop commercial biopesticides as these agents, while generally considered safe, once introduced into the environment to control diseases may pose potential risks, such as unintended effects on non-target organisms, host-switching, and the potential for the biocontrol agent to become a new pathogen. Therefore, no authorisation will occur in the EU if the putative microbial biocontrol agent is suspected to be pathogenic to humans or terrestrial vertebrates, if it has effects on other non-target organisms and if it has toxicological effects on humans, animal health and the environment. Currently no guidance is available on the risk assessment for microbial consortia specifically developed for plant protection purpose: indeed, despite microbiome manipulation in agricultural systems may provide a system approach to increase sustainability and safety, studies on microbial dynamics in the topsoil are scarce. Bacteriophages as anti-bacterial agents are generally considered non-toxic, safe for users and target-specific, leading to them being possibly used as biopesticides in the coming future: a document issued by OECD is currently available to provide guidance for a regulatory framework. Finally, nanoparticles are being explored as antimicrobial agents, nanocarriers and microbe-mediated particles, despite there are insufficient reliable ecotoxicological data for risk assessment purposes and to establish safety doses. To conclude, microbial antagonists, bacteriophages, nanoparticles and RNAi vectors as putative and prospective plant protection agents are under evaluation for their biosafety: indeed, their foreseen increasing use requires additional studies to confirm safety for consumers and lack of possible adverse effects to non-target organisms and the environment, prior to their registration as plant protection products.

ORAL PRESENTATIONS

Exploring the potential of antimicrobial peptides, bacteriophages, and bacteriocins in managing bacterial diseases, with a focus on *Xylella fastidiosa*. M. SABRI[1], O. CARA[1,2], F. VALENTINI[1], A. DE STRADIS[3], C.D. CALVANO[4], A. M. BIANCO[4], K. EL HANDI[1], T. ELBEAINO[1,5]. [1]*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM BARI), Bari, Italy.* [2]*University of Bari, Department of Soil, Plant and Food Science, Bari, Italy.* [3]*Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Bari, Italy.* [4]*University of Bari, Department of Chemistry, Bari, Italy.* [5]*Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Portici, Italy.* E-MAIL: elbeaino@iamb.it

The increasing threat of *Xylella fastidiosa* (*Xf*), alongside other significant bacterial plant pathogens affecting Mediterranean agriculture, such as *Erwinia amylovora* (*Ea*), *Agrobacterium tumefaciens* (*At*), and *Xanthomonas campestris* pv. *campestris* (*Xcc*), which cause leaf scorch, fire blight, and black rot diseases, respectively, highlights the need for sustainable, effective, and eco-friendly control strategies. Our research investigates the use of antimicrobial peptides (AMPs), bacteriophages (phages), and lactic acid bacteria-derived bacteriocins as advanced nanobiocontrol approaches to combat these pathogens. At the AMPs level, potent antimicrobial agents against *Xf* subspecies *pauca (Xfp)*, including Nisin A from *Lactococcus lactis*, and Ascaphin-8, DASamP2 from frog skin, and MK-45, MR-53, MW-56, MG-58 from *Leuconostoc mesenteroides* strain MS4 have shown strong bactericidal activity against *Xfp* with different lethal concentrations. Notably, Nisin A rapidly disrupted bacterial membranes, effectively controlling infections in *Nicotiana benthamiana* with systemic translocation lasting up to nine days. Similarly, the four *Leuconostoc mesenteroides* MS4-derived bacteriocins were efficient at 0.2–0.4 mg/mL to combat *Xfp* infections *in planta*, proving to be cost-effective, eco-friendly biocontrol agents. Furthermore, a newly identified lytic bacteriophage (MATE 2) exhibited broad-spectrum activity against *Xfp* and *Xcc*, a major cruciferous crop pathogen. When combined with *L. lactis* and Nisin A, MATE 2 showed a synergistic effect, reducing *Xcc* infection symptoms in broccoli by 71%, outperforming individual treatments (64% and 38%). Additionally, a novel bacteriophage targeting *Ea* effectively reduced fire blight disease in pear plants, highlighting phage-based strategies' broader poten-

tial. These outcomes demonstrate that nano-biocontrol agents; bacteriocins, bacteriophages, and AMPs, can serve as sustainable, eco-friendly alternatives to chemical pesticides, offering a multifaceted approach to managing *Xfp* and other bacterial plant diseases while promoting agricultural sustainability.

This research was financially supported by the Italian Ministry of Agriculture, Food Sovereignty and Forestry (MASAF), in the frame of project "Approcci Nanotecnologici per un Controllo Sostenibile e Innovativo di Xylella fastidiosa *"ANCOSIX", CUP n. J83C22001990005.*

***Ralstonia pseudosolanacearum*, an emerging threat in Europe.** R.A.M. VREEBURG, M.J.C. PEL, T.M. RAAYMAKERS, M. BERGSMA-VLAMI. *Netherlands Institute for Vectors, Invasive plants and Plant Health (NIVIP), National Plant Protection Organization (NPPO), Netherlands Food and Consumer Product Safety Authority (NVWA), Geertjesweg 15, 6706 EA Wageningen, The Netherlands.* E-MAIL: r.a.m.vreeburg@nvwa.nl

Until recently, *Ralstonia pseudosolanacearum* was absent from outdoor agricultural systems and natural aquatic environments in Europe and was only reported in restricted greenhouse cultivation systems, such as in the greenhouse cultivation of roses in the Netherlands and some other European countries. Since a few years now, *R. pseudosolanacearum* has been frequently found in Europe: in imported plant material (*e.g.* ginger and curcuma), plants cultivated outdoors or under semi- protected conditions (*e.g.* tunnels) - often after importation of infected plant material, and in waterways. Given the broad range of host plants that can be infected by *R. pseudosolanacearum* major awareness is currently needed in Europe. Potato is a major arable crop in Europe and a known host of *R. solanacearum*, we therefore studied the virulence of three *R. pseudosolanacearum* strains in potato. When potato plants are inoculated with *R. pseudosolanacearum*, they develop symptoms at high temperatures (28°C). At lower temperatures (20°C) however, less or no symptoms develop while the bacteria could be found back in the progeny tubers, increasing the risk for spread of the bacteria. Genomic analysis of recent European isolates shows that there is a high genetic diversity among the *R. pseudosolanacearum* strains that have been found in Europe. This makes it difficult to predict how well the virulence results obtained with the three tested *R. pseudosolanacearum* strains can be generalized to all *R. pseudosolanacearum* strains.

Population Genetic signatures revealed contrasted outbreak histories of bacterial diseases: the case of Bacterial wilt, Huanglongbing and Citrus Canker. G. CELLIER. *Anses-Plant Health Laboratory, Saint Pierre F97410 – Reunion Island.* E-MAIL: gilles.cellier@anses.fr

As long-distance traveling of human beings and movement of goods drastically increase over time in relation to globalization of trade and exchanges, and so is the spread of bacterial pathogens and associated infectious diseases across the globe. Key for improved disease control lies into acquiring a thorough knowledge on factors shaping pathogen populations at fine scales and how they interact with their environment. In order to show much more clearly how infectious agents are spreading and evolving other than genetic drift alone, phylogenetic and epidemiological techniques are often use. Bacterial lineage-centered molecular genotyping techniques, such as multilocus variable number of tandem repeats analysis (MLVA). They are of interest especially when they provide high throughput, a sound phylogenetic signal, and a resolution fitting the spatiotemporal scale investigated. In the case of complex plant pathogens, such as the *Ralstonia solanacearum* species complex (RSSC), the '*Candidatus* liberibacter spp.' species complex associated with the huanglongbing disease, and *Xanthomomonas citri* pv. *citri*, several studies achieved molecular characterization of outbreak strains during the last decade, and brought light on its epidemiology. Nevertheless, selecting proper genetic marker and analytical algorithm is vital to apply molecular genetics in a given biological population. We will present examples of epidemiological results produced within the framework of these three pathosystems, which revealed the need for a proper sampling and methodological approach, along with the adoption of complementary multiscale analyses from gene to genome in order to apprehend the evolution complexity as a whole.

Citrus huanglongbing in California: current status and progress. D. GUTIERREZ[1], L. KUMAGAI[1], K. OKASAKI[1], D. MORGAN[1], G. VIDALAKIS[2]. [1]*California Department of Food and Agriculture, Sacramento, CA 95814, United States of America.* [2]*Department of Microbiology & Plant Pathology, Citrus Clonal Protection Program, University of California, Riverside, CA 92521, United States of America.* E-MAIL: vidalg@ucr.edu

The spread of *Diaphorina citri*—the Asian citrus psyllid (ACP) and vector of Huanglongbing (HLB)— has posed a persistent threat to California's citrus industry. First identified in San Diego County in 2008, ACP

has since established itself throughout all major citrus-producing areas. The first detection of HLB occurred in 2012 in Los Angeles County. By 2024, state agencies tested more than 650,000 citrus trees and 330,000 psyllid samples, confirming and removing over 9,800 HLB-positive trees from urban landscapes across six southern California counties. In response, quarantine zones now span over 6,215 square kilometres, limiting the movement of nursery stock and commercial fruit to mitigate further spread. The California Department of Food and Agriculture (CDFA), in collaboration with the USDA and the Citrus Pest and Disease Prevention Committee, manages a $47 million program encompassing detection, regulatory, and biological control strategies. Targeted surveys, incorporating travel and human activity data, inform inspection priorities. Since 2011, more than 31 million *Tamarixia radiata* (*Eulophidae*) parasitoids have been released to suppress ACP populations, particularly around new HLB finds and high-risk corridors. Regulatory zones were restructured in 2017 to restrict the movement of citrus from southern to central California, while nursery stock production is confined to protective structures with mandatory testing. Ongoing efforts include public outreach campaigns, homeowner engagement, and multi-agency collaborations to eliminate unmanaged citrus. Future program goals include scaling HLB diagnostic capacity through private and industry-led laboratories, while state and federal funding continues to drive research in detection, epidemiology, and sustainable management of the HLB-ACP complex.

Whole Genome Sequencing and Fourier transform Infra-Red typing bring new insights to Portuguese *Erwinia amylovora* population diversity. D. McGUIRE[1,2], C. FERREIRA[1], L. DUARTE[1], A. MADURO[1], C. ALMEIDA[3], H. OLIVEIRA[4], L. CRUZ[1,2]. [1]*National Institute for Agrarian and Veterinarian Research, Oeiras, Portugal.* [2]*BioSystems & Integrative Sciences Institute (BioISI), University of Lisbon, Lisbon, Portugal.* [3]*INIAV - National Institute for Agrarian and Veterinarian Research, Vairão, Portugal;* [4]*CEB - Centre of Biological Engineering, University of Minho, Braga, Portugal.* E-MAIL: leonor.cruz@iniav.pt

Following the first outbreaks of fire blight affecting Portuguese pear orchards in 2010, intensive surveys and control of nursery materials resulted in the collection of a large set of strains of *Erwinia amylovora*. Isolates obtained from distinct apple, pear and quince varieties, from different production areas were identified following EPPO standard PM7/20(3). Characterization of *E. amylovora* pathogenicity and virulence features, assessed through biological tests by inoculation of apical pear twigs and immature fruits, displayed distinct virulence levels, even within the same CRISPR group. A set of 44 selected isolates was sequenced by Illumina Whole Genome Sequencing (WGS) and exhibited the expected conservative patterns with high levels of similarity for the whole set (>99.87%). In an attempt to understand these differences in virulence patterns, the bacterial collection was further characterized by Fourier transform infrared spectroscopy (FTIR). FTIR showed reproducibility and congruence of cluster composition for the biomolecules within the wavelength range of 800-1300 cm^{-1}, including lipids, proteins and polysaccharides. As expected, *E. tasmaniensis* and *E. pyrifoliae* grouped as outliers after clustering, by Euclidean UPGMA. Interestingly, the Portuguese strains of *E. amylovora* revealed specific features distinct from the reference strain CFBP 1430. Integrative analysis of WGS data and FTIR high-resolution fingerprinting profiles unveiled the diversity associated with the expression of disease severity, often hidden by the homogeneity of WGS profiles. These diagnostic and epidemiological features derived from differential expression of specific biomolecules, may contribute to better understanding new outbreaks and alternative therapies.

*This research was financially supported by national funds through PRR – Recovery and Resilience Plan, under the project PRR-C05-i03-I-000179-LA2.3- BioFago, by UIDB/04046/2020 and UIDP/04046/2020 Centre grants from FCT, Portugal (to BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal. DOI 10.54499/UIDB/04046/2020 (*https://doi.org/10.54499/UIDB/04046/2020*). Support was also provided by INIAV as part of the Euphresco Project Consortium 2023-A-454 -Whole genome sequencing in identification of plant pathogenic bacteria (PPBSeq).*

***Xanthomonas euroxanthea* expands its host range: first occurrence in sunflower crops in Bulgaria.** E. GARCIA[1,2], F. SOARES[1,2], C. RODRIGUES[2], J. TROVÃO[1,2], J.F. POTHIER[3], A. CAMELO[1,4], C. ESPÍRITO SANTO[1,4], D. DRAGNEV[5], E. PETROVA[5], J. COSTA[1,2], S. BOBEV[5]. [1]*University of Coimbra, Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, Coimbra, Portugal.* [2]*Instituto Pedro Nunes, Laboratory for Phytopathology, Coimbra, Portugal.* [3]*Environmental Genomics and Systems Biology Research Group, Institute of Natural Resource Sciences, Zurich University of Applied Sciences (ZHAW), Wädenswil, Switzerland.* [4]*CATAA - Centro de Apoio Tecnológico Agro-*

Alimentar, Castelo Branco, Portugal. [5]*Agricultural University - Department of Phytopathology, Plovdiv, Bulgaria.* E-MAIL: jcosta@uc.pt

Sunflower (*Helianthus annuus*) is a major oilseed crop globally, with Bulgaria being the leading producer within the European Union. During a 2021 field survey in Kavarna (Dobrich district, Bulgaria), sunflower plants exhibiting necrosis and longitudinal cracking of the petiole were observed. Microscopic examination suggested a bacterial infection, prompting further etiological investigation. Strains belonging to the genus *Xanthomonas* were isolated, and phylogenetic and phylogenomic analyses revealed their clustering with *Xanthomonas euroxanthea* CPBF 424^T, a pathogenic strain identified in walnut buds in Portugal, responsible for walnut bacterial blight. The sunflower isolate exhibited five of eight *X. euroxanthea*-specific markers (XEA4-XEA8), a pattern also observed in strains from *Solanum lycopersicum*, *Phaseolus vulgaris*, and rainwater sources, suggesting host adaptation and a potential emerging lineage. This is the first report of *X. euroxanthea* affecting sunflower. Given sunflower's economic importance and widespread cultivation in Mediterranean countries, this study highlights the need for monitoring the pathogen's distribution and potential impact on sunflower production. As many sunflower crops are also used in farming systems for crop rotation systems, alternating with rice, beans, or maize, early detection and management strategies are crucial to mitigate risks associated with its spread to other plant hosts.

The initial steps (field sampling and pathogen isolation) were funded by the Agricultural University (Plovdiv, Bulgaria). The rest of the activities were funded by the project CULTIVAR (CENTRO-01-0145-FEDER-000020), co- financed by the Regional Operational Programme Centro 2020, Portugal 2020, and the European Union, and by national funds through FCT - Fundação para a Ciência e a Technology, I.P., under the project DOI: 10.54499/PTDC/ASP-PLA/3145/2021.

POSTER PRESENTATIONS

Survey of bacteria affecting stone fruits and almond in Montenegro. T. POPOVIĆ[1], J. ADAMOVIĆ[2], A. PROKIĆ[2], N. ZLATKOVIĆ[3], A. OBRADOVIĆ[2]. [1]*Administration of Food Safety, Veterinary and Phytosanitary Affairs, Serdara Jola Piletića 26, Podgorica, Montenegro.* [2]*Faculty of Agriculture, University of Belgrade, Nemanjin 6, Belgrade, Serbia.* [3]*Institute for Plant Protection and Environment, Teodora Drajzera 9, Belgrade, Serbia.* E-MAIL: tamara.popovic@ubh.gov.me

The production of stone fruits and almonds in Montenegro is often compromised by various pathogenic bacteria. The infection and disease spread are facilitated by favourable environmental conditions as well as extensive cultivation practices and inadequate control measures. Symptomatic samples of stone fruit and almond plant material were collected from different locations across Montenegro during the 2017–2020 survey. Not all of them resulted in bacterial isolation indicating that some symptoms could not be a reliable diagnostic criterion. From apricot, peach and sweet cherry samples 48 strains were isolated, and based on their morphological, pathogenic, biochemical, and molecular characteristics identified as *Xanthomonas arboricola* pv. *pruni* (Xap). Additionally, 29 strains, identified as *Pseudomonas syringae* pv. *syringae* (Pss), were isolated from apricot, peach, nectarine, sweet cherry, Japanese plum, and almond, suggesting a wider spread of this pathovar across the country. No mixed infections were registered. Phenotypic and genetic variations within the Pss population could be a consequence of multiple strain origin, and adaptation to different hosts and climatic conditions. In contrast, the genetic homogeneity observed in the Xap population suggests a spread of the local population without new introductions. To prevent spread of Xap to other areas and susceptible hosts, strict phytosanitary measures are implemented. However, Pss as a wide host range pathogen should be controlled by cultural practices. All tested strains showed *in vitro* resistance to copper sulphate and sensitivity to streptomycin sulphate, indicating that integrated management strategies have to be implemented to protect orchard productivity and stone fruit yield.

This research was supported by Administration of Food Safety, Veterinary and Phytosanitary Affairs, Montenegro, partly funded by Ministry of Science and Technological Development, Montenegro; and the Ministry of Education, Science and Technological Development, Republic of Serbia and University of Belgrade, Faculty of Agriculture (Contract No. 451-03-137/2025-03/200116).

First report of *Pectobacterium carotovorum* causing potato soft rot in Georgia. M. MURADASHVILI[1], C. LOMBAERS[2], M. KRIJGER[2], P. VAN DER ZOUWEN[2], G. MEPARISHVILI[1], Z. SIKHARULIDZE[1], R. DUMBADZE[1], J.M. VAN DER WOLF[2]. [1]*Batumi Shota Rustaveli State University, Institute of Phytopathology and Biodiversity, Kobuleti, 6200, Georgia.* [2]*Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands.* E-MAIL: makamuradashvili25@yahoo.com

Today, bacterial diseases still remain a significant limiting factor for potato harvest in Georgia. During the 2022 year has been revealed that potato soft rot diseases caused by the *Pectobacterium carotovorum* in the various municipalities of Georgia: Marneuli, Kobuleti Tsalka, and Akhaltsikhe. Under the research were observed many cases of rotted potato tubers with cream and dark black colored tissue, as in the field, also storage. Since 2020, potato soft rot and blackleg diseases, caused by *Dickeya solani* were particularly prevalent in the regions of Khulo, Akhaltsikhe, Akhalkalaki, and Kobuleti (Muradashvili *et al*., 2023). 19 isolates from collected 28 samples were identified as a gram-negative, facultative anaerobes. They have produced cavities on the crystal violet pectate media (Perombelon & Van Der Wolf, 2002) were negative for oxidase, urease, and indole production, did not produce acid from dulcitol or sorbitol, could not utilize malonate and citrate. The isolates were catalase positive, and were able to grow at 37°C. Pathogenicity tests were conducted by the fulfill Koch's postulates. Observed potato soft rot similar tissue maceration on the inoculated potato slices and whole potato tubers and causal pathogen were reisolated. 11 isolates from them were selected for genetic study. DNA was extracted from test bacterial isolates and analyzed using gapA PCR with the primer pair gapA-7-F/gapA-938-R (Cigna *et al*., 2017). The amplicon size was 932 bp. GapA gene sequence alignment fragments showed a uniform group of isolates 100% identical to gapA sequences of *Pectobacterium carotovorum* strains MBr2, isolated from *Brassica rapa* subsp. *chinensis* in Taiwan (OR651974), and strain ZB18121, isolated from China (OP793220). The gapA sequences of the strains from Georgia are represented by accession numbers: PP060483; PP060484; PP060485; PP060486; PP060487; PP060488; PP060489; PP060490; PP060491; PP060492; PP060493. This is the first report of *Pectobacterrium carotovorum* causing potato soft rot in Georgia.

This work was provided by Shota Rustaveli National Science Foundation (SRNSF) of Georgia [grant № FR- 21-1778].

First report of *Pseudomonas avellanae* causing hazelnut decline in Serbia. T. POPOVIĆ MILOVANOVIĆ[1], S. LORETI[2], P. MILOVANOVIĆ[3], A. JELUŠIĆ[4], R. ILIČIĆ[5], M. SCORTICHINI[6]. [1]*Institute for Plant Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia.* [2]*CREA - Research Centre for Plant Protection and Certification, Via Carlo Giuseppe Bertero 22, 00156 Rome, Italy.* [3]*Agrounik doo, Krnješevačka BB, 22310 Šimanovci, Serbia.* [4]*University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11030 Belgrade, Serbia.* [5]*University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia.* [6]*CREA - Research Centre for Olive, Fruit and Citrus Crops, Via di Fioranello, 52, I-00134 Rome, Italy.* E-MAIL: tanjaizbis@gmail.com

During July 2024, a decline was observed on hazelnut (*Corylus avellana* L.) cultivar Tonda di Giffoni, 5-years old. Developed symptoms coming out when trees started to yield in form of rapid wilting of leaves on branches. Diseased stems exposed a brown discoloration of vascular tissue. Isolations from wood tissue resulted in the forming of large, convex, levan-type, mucoid, cream-whitish *Pseudomonas*-like bacterial colonies on Nutrient agar supplemented with 5% sucrose. Results of LOPAT tests (Levan positive, Oxidase negative, Potato soft rot negative, Arginine dehydrolase negative, Tobacco hypersensitivity positive) ranged isolates into *Pseudomonas syringae* group Ia. Pathogenicity test was performed on shoots of potted 1-year-old hazelnut (Tonda di Giffoni) plants inoculated by bacterial suspension through leaf scars in the early autumn. First necrosis at the points of inoculation was observed after ten days, and continued to spread longitudinally through the stem, reaching a length of 15–20 cm after two months. Koch's postulates were fulfilled by reisolation of the same pathogen from the inoculated stems. Genetic identification was performed by sequencing of *gyrB* (DNA gyrase subunit B) gene. Nucleotide BLAST analysis of the partial *gyrB* gene sequence of the two Serbian isolates showed 100% identity with *Pseudomonas avellanae* strains from the NCBI database. Phylogenetic analysis indicated genetic homogeneity among the tested and five reference *P. avellanae* strains (BPIC631, CFBP4960, CIP 105176T, NCPPB 3491, and NCPPB 4222) and confirmed identification by placing them within the same tree cluster. This finding indicates on possibility of wider spread of this bacterium in the territory of EU.

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia [contract numbers 451-03-136/2025-03/200010, 451-03-136/2025-03/200053].

CONCURRENT SESSION D1 – Nematodes of crops and forestry

SESSION KEYNOTES

Microbiome-assisted agriculture for the management of nematode pests in vegetable crops. S. MOLINARI.

Institute for Sustainable Plant Protection (IPSP), National Research Council of Italy (CNR), Via G. Amendola 122/D, 70126 Bari, Italy. E-MAIL: sergio.molinari@cnr.it

Plant Microbiome is the collective communities of plant-associated microorganisms, also referred as plant second genome. Such microorganisms colonize the rhizosphere that is the zone of soil influenced by plant roots. Plant microbiome in rhizosphere can improve root architecture, enhance nutrient uptake and tolerance to environmental stresses, and stimulate plant immune system. Benefits from enrichment of soil by rhizosphere microorganisms in agricultural practices have brought to the development of a more sustainable Microbiome-Assisted Agriculture (MAA) to establish low input and low impact crop systems. Massive use of chemical fertilizers and pesticides has caused farmers to face severe problems of soil degradation associated with decline in quality and yield in crop production. Soil is a non-renewable resource on human time scales with high vulnerability to degradation processes such as erosion, depletion of the soil organic carbon (SOC) pool and loss in biodiversity, in soil fertility caused by nutrient scarcity and uptake difficulty, acidification and salinization. Natural undamaged soils are rich in beneficial rhizosphere microbes, such as arbuscular mycorrhizal fungi (AMF), opportunistic fungi, plant growth promoting rhizobacteria (PGPR), that are specifically selected by plants to have benefits in terms of growth and tolerance to abiotic and biotic stresses. One of the main tools for regeneration of degraded soils is the addition of suitable microbiome-generating formulations in the attempt to establish beneficial relationships in the rhizosphere between microorganisms and roots for having higher yield and product quality. Different commercial microbiome-generating formulations or low scale green waste composts have been used in our laboratory to test their activity as biofertilizers and biocontrol agents (BCAs) against attacks of soil-borne parasites, such as plant parasitic nematodes, to vegetable plants. Pilot programs involve vegetable crop plants grown in pots or small plastic containers located in glasshouses under controlled environmental conditions, and single pre- treatments before artificial nematode inoculation. Commercial formulations are differently effective as BCAs, whilst a specific low scale food waste compost has been found to be both a good fertilizer and bio-stimulant against root-knot nematodes (RKNs). Best formulations can reduce infection factors to the 40-50% of controls. Paramount to have an effective increase of pest resistance under our experimental conditions is the amounts of added formulations with respect to plant species, age and size. Inhibitory compounds are used to select specific microorganisms and study their specific role. Controls of nematode infection involves mycorrhization of roots with the activation of plant immune system, monitored through detection of defense gene expressions and enzyme activities. Target genes are those encoding for pathogenesis-related proteins (PR-proteins) and enzymes regulating the Reactive Oxygen Species (ROS) production and degradation. Primed plants react to pest attack by high and prompt defenses, such as Hypersensitive Reaction characterized by cell death. Microbiome-generating formulations and composts are currently being studied as agro-products that can associate bio-fertilizing and bio-stimulating effects. Moreover, composted materials have their own microbial and fungal community and may enhance soil microbial life of plants to which they are added for benefits in terms of improvement in bulk density, water content, and nutrient availability.

This research was financially supported in part by the Agritech National Research Center and received funding from the European Union Next-Generation EU (Piano Nazionale Di Ripresa E Resilienza (Pnrr)—Missione 4 Componente 2, Investimento 1.4—D.D. 1032 17/06/2022, CN00000022), Spoke8.

The pinewood nematode - a nightmare for Europe. I. ABRANTES, L. FONSECA. *University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Associate Laboratory for Sustainable Land Use and Ecosystem Services-TERRA, Department of Life Sciences, Calçada Martim de Freitas, P-3000 456 Coimbra, Portugal.* E-MAIL: isabel.abrantes@uc.pt

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of the pine wilt disease (PWD), a quarantine organism by the European and Mediterranean Plant Protection Organisation (EPPO), is recognised worldwide as a major forest pest, endangering European pine forests and the forestry industry. The PWD results from a complex interaction among three organisms: the PWN, its insect vector (*Monochamus* spp.), and a conifer tree. The nematode is the common element in this interaction, while insect vectors and host trees vary from one location to another. This nematode is originated from North America and in the early 20th century has spread first to Japan and later to China, Korea, and Taiwan. In Europe, it was first identified in mainland Portugal, in 1999, and later in Spain and Madeira Island. More recently, it was reported in the Republic of Armenia (Asia). The nematode is transmitted to new hosts by insects, mainly from the genus *Monochamus* (Coleoptera: Cerambycidae), being *M. gal-*

loprovincialis the most widespread species in Europe. The primary susceptible hosts of PWN are coniferous species within the genus *Pinus*, which is widely distributed across the Northern Hemisphere including European species such as *P. mugo* (dwarf mountain pine), *P. nigra* (black pine), *P. pinaster* (maritime pine) and *P. sylvestris* (Scots pine). Nevertheless, the list of susceptible plants also includes coniferous species from the genera *Abies*, *Chamaecyparis*, *Cedrus*, *Larix*, *Picea*, and *Pseudotsuga*. After being transmitted to the host tree by the insect vector, the PWN reproduces and migrates through the vascular system, causing the tree's death. Symptoms in infected plants include reduced resin exudation, yellowing and wilting of needles, and partial or total drying of the crown. The spread of the PWN into non- native areas occurs predominantly due to human activity. Uncontrolled dispersion of this nematode in Europe could lead to severe economic consequences for the forestry industry. Furthermore, the PWN spread could be exacerbated by climate change, which has altered environmental conditions in ways that facilitate the establishment and expansion of both the nematode and its vector. Warmer temperatures, increased droughts, and milder winters create favourable conditions for the nematode reproduction and increase tree susceptibility. Severe symptoms of PWD are typically observed in regions where average summer temperatures exceed 20°C. As climate change continues to progress, the expansion of PWN infected areas across Europe is expected. Urgent adaptive management strategies (mechanical, cultural, biotechnical, chemical, biological, or genetic) are needed. The outbreaks in Europe, along with their negative economic, ecological, and social impacts, serve as a warning to guard against the introduction of invasive alien species into new ecosystems.

This research was financially supported by FCT - Fundação para a Ciência e Tecnologia*, I.P., in the framework of the Project UIDB/04004/2025 - Centre for Functional Ecology - Science for the People & the Planet and* Instituto do Ambiente, Tecnologia e Vida.

ORAL PRESENTATIONS

Status of plant-parasitic nematodes associated with Citrus in Morocco: spotlight on *Tylenchulus semipenetrans* and a call for Integrated Management. B. ZOUBI[1,2,3], A. QADDOURY[2], A. IRAQI HOUSSEINI[1], K. KHFIF[1], F. MOKRINI[3]. [1]*Laboratory of Biotechnology, Environment, Agri-Food, and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohammed Ben Abdellah University, Fez 30050, Morocco.* [2]*Center of Agrobiotechnology and Bioengineering, Research Unit Labeled CNRST, Cadi Ayyad University, Marrakech 40000, Morocco.* [3]*Biotechnology Unit, National Institute for Agricultural Research, INRA-Rabat, Rabat 10080, Morocco.* E-MAIL: fmokrini.inra@gmail.com

Plant-parasitic nematodes (PPNs) represent a major threat to citrus production worldwide being particularly problematic in Moroccan orchards. Among them, *Tylenchulus semipenetrans*, the causal agent of citrus slow decline, is considered the most destructive species due to its widespread distribution and significant impact on tree vigor, fruit yield, and quality. As a semi-endoparasitic nematode, it colonizes citrus roots, leading to gradual weakening of the tree and long-term yield reduction. To address the lack of recent data on the distribution and diversity of citrus-associated nematodes in Morocco, extensive surveys were conducted between 2018 and 2021 across major citrus-growing regions including Gharb, Moulouya, Souss-Massa, Tadla, Loukkos, Haouz, Marrakech-Safi, Béni Mellal-Khénifra, and Berkane. Soil and root samples collected during the 2018- 2020 campaigns were analyzed for nematode identification and soil physicochemical characterization. A total of 11 genera and 10 species of PPNs were identified. *T. semipenetrans* showed the highest prevalence and reached infestation rates of up to 100% in regions such as Gharb, Loukkos, Moulouya, Souss-Massa, and Tadla. The survey carried out in 2021 reaffirmed these findings, highlighting *T. semipenetrans* as the dominant species in Souss-Massa (88%), Gharb (67%), Berkane (63%), Marrakech-Safi (62%), and Béni Mellal-Khénifra (57%). The distribution of PPNs was significantly correlated with soil physicochemical properties such as soil texture, pH levels, and mineral content. The findings highlight the urgent need for region-specific integrated nematode management programs, incorporating monitoring, sustainable biocontrol approaches, and agronomic practices tailored to local soil and climate conditions.

Evaluation of *in vitro* nematocidal effects on *Meloidogyne incognita* of laboratory extracted and commercial essential oils of *Artemisia annua*. E. AJOBIEWE[1,2], E. FANELLI[2], M.P. ARGENTIERI[3], F. DE LUCA[2]. [1]*Department for Humanistic, Scientific and Social Innovation, University of Basilicata, Via Lanera 20, 75100 Matera, Italy.* [2]*IPSP, National Research Council CNR, Via Amendola 122/D, 70126 Bari, Italy.* [3]*Department of Pharmacy-Drug Sciences, University of Bari Aldo Moro, Bari 70125, Italy.* E-MAIL: francesca.deluca@cnr.it

Root-knot nematodes, *Meloidogyne* spp., are considered the most harmful nematodes because of their worldwide occurrence and heavy yield losses caused to a large range of crops. In the last two decades, many experiments have been conducted using essential oils (EOs) against different genera of plant parasitic nematodes. Sweet wormwood, *Artemisia annua* shows great potential of application to produce new nematocidal products due to the presence of a high number of bioactive phytochemicals. The aim of this work is to evaluate the effects against *M. incognita* of laboratory extracted and commercial EOs of *A. annua* collected from different geographical locations. One EO extracted from dried leaves of *A. annua* from South Italy and one commercial EO purchased from India were tested on juveniles (J2s) of *M. incognita*. J2s were exposed for 24 and 48 h to 1000, 2000 and 4000 µg mL^{-1} concentrations of both EOs in an orbital incubator at 70 rpm, at 25 °C. J2 mortality caused by the lab-extracted EO was about 18% at 4000 µg mL^{-1} concentration after 24 h, and 44% after 48 h. J2 mortality caused by the commercial EO was 38% at both 1000 and 2000 µg mL^{1} after 24 h, and 48% at 2000 µg mL^{-1} after 48 h. Our findings show that the activity profiles of *Artemisia* EOs could be associated with distinct phytochemical compositions, different geographical origin and season of collection. The chemical profiles of the EOs and the molecular mechanisms they trigger in J2s are being studied.

This work was supported by funding provided by the National Research Centre for Agricultural Technologies (Agritech), within the National Recovery and Resilience Plan (PNRR) - Mission 4 Comp. 2, Investment 1.4 financed by the European Union – NextGenerationEU (D.D. 1032 of 17/06/2022, CN00000022).

Investigating biotic and abiotic factors causing a hitherto unknown disorder of olive trees in Southern Italy. C. DONGIOVANNI[1], M. CASETTA[1], L. MANCO[1], M. TAGLIENTE[1], M. CARRIERI[1], G. PERRONE[1], D. PERRELLI[1], M. DI CAROLO[1], R. ARGENTIERI[1], F. DE LUCA[2], E. FANELLI[2], A. TROCCOLI[2], M. CONTE[1], D. BOSCIA[2], M. SAPONARI[2]. [1]*Research, Experimentation and Training Center in Agriculture "Basile Caramia", 70010 Locorotondo (BA), Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council (CNR), 70126 Bari, Italy.* E-MAIL: enzadongiovanni@crsfa.it

Olive trees showing extensive leaf yellowing, reddening of the foliage followed by defoliations have been observed at the end of the autumn or in the winter in several new olive plantations in the Southern part of Apulia on FS17 and Leccino. Only these 2 cultivars resistant to *Xylella fastidiosa* are authorized for planting to restore the devastation caused by the *Xf* epidemic until 2024. Visual inspections and sampling were conducted for 2 consecutive years in 20 three-four years old groves that showed this unusual discoloration of the canopy affecting a limited number of trees or very large areas of the groves. Two or three symptomatic plants per orchard were completely uprooted and mycological analysis were carried out on roots and crown; phytoparasitic nematodes were searched on roots and in the soil. Indeed, samples included symptomatic and asymptomatic leaves and soil for chemical analysis. *Meloidogyne javanica* (root-knot nematodes, RKNs) was the most frequently species of plant parasitic nematodes detected on roots. *Cylindrocarpon* spp. *Fusarium* spp., *Macrophomina phaseolina*, *Rhizoctonia solani* were the mainly soil fungi pathogens detected in highly RKN-infested plants. On the other hand, chemical analyses revealed high sodium content (3062 to 9267 mg kg^{-1}) and potassium deficiency (0.16 to 0.45%) in symptomatic leaves, regardless of the content of these elements in the soil, most likely, as a consequence of the impairment of the root systems caused by the nematode and fungal occurrence. Overall, the symptoms on canopies were more severe than those in soil, with high values of total (351-372 g kg^{-1}) and active (53-135 g kg^{-1}) calcium carbonate.

This research was financially supported by Reach-Xy project "Research actions for reducing the impact on agricultural and natural ecosystems of the harmful plant pathogen Xylella fastidiosa" *funded by Legge di Bilancio del 30 Dicembre 2021, N. 234.*

***In vivo* biocontrol efficacy of *Streptomyces violascens* AS2 strain against *Meloidogyne* spp. in tomato.** L. AZLAY[1,2], E.H. MAYAD[2], M. BARAKATE[1]. [1]*Laboratory of Water Sciences, Microbial Biotechnologies and Natural Resource Sustainability; Microbial Biotechnology, Agro-Sciences and Environment & CNRST Labeled Research Unit No. 4, Faculty of Sciences Semlalia, Cadi Ayyad University, 40000 Marrakesh, Morocco.* [2]*Laboratory of Biotechnologies and Valorization of Natural Resources, Department of Biology, Faculty of Sciences Agadir, Ibn Zohr University, Agadir, Morocco.* E-MAIL: l.azlay.ced@uca.ac.ma

Conventional agriculture relies on chemical inputs, disrupting soil health and biodiversity. Climate change has exacerbated ecological collapse. Biocontrol offers an eco-friendly approach by harnessing natural antagonistic mechanisms, notably through filamentous actino-bacteria that produce bioactive compounds. Their application to plant-parasitic nematodes, a major agricultural

threat, demonstrates a significant potential of population suppression. The adoption of actinobacteria-based biocontrol supports sustainable practices, ensuring long-term food security without compromising ecosystem integrity. In the current study, 14 extracts of Actinobacteria were screened *in vitro* for their nematocidal activity against *Meloidogyne* spp. Afterwards, suitable aqueous and ethyl acetate extracts of *Streptomyces violascens* were selected for *in planta* assays to: (i) assess the *in vivo* bionematocidal activity against root-knot nematodes (RKNs), (*Meloidogyne* spp.); (ii) evaluate the plant growth-promoting effect on tomato (*Solanum lycopersicum*); at a gradient of concentration ranging from 400 to 1000 µg ml^{-1} . After 3 months of treatment, the *in vivo* trials showed that *S. violascens* aqueous and ethyl acetate extracts significantly reduced the disease index at 1000 µg mL^{-1} , by reducing gall formation by about 84% and 69 %, respectively, along with about 65% and 40% decrease in egg numbers, and a substantial decrease of 78% and 54% in second-stage juveniles numbers in the root systems.. *S. violascens* treatment also resulted in a significant enhancement of plant-growth parameters and photosynthetic pigments, indicating a positive impact on overall plant health and vigor. The present findings suggest that the tested actinobacterial extracts could serve as a sustainable resource for managing RKNs, offering a viable alternative to conventional chemical nematicides.

This work was financially supported by the CNRST Labeled Research Unit N°4 grant and the Moroccan (CNRST)- Italian (CNRi) Scientific and Technical Cooperation Program.

New solution to control nematodes affecting key vegetables: Reklemel™ (fluazaindolizine). A. FENIO[1], T. THODEN[2], S. YANNIS[3], S. ELLIS[4], M. BRADASCIO[5], M. ALIQUO[6]. [1]*IFS Field Scientist, Corteva Agriscience Italia srl, via dei Comizi Agrari 10, Cremona, Italy.* [2]*Global Biology Program Leader, Corteva Agriscience Germany GMbH.* [3]*Zonal Biology Leader, Corteva Agriscience Hellas S.A.* [4]*Global Regulatory Ecotoxicologist, Corteva Agriscience Limited UK.* [5]*Country Regulatory Lead Italy, Corteva Agriscience Italia srl.* [6]*Country Marketing Manager, Corteva Agriscience Italia srl.* E-MAIL: antonino.fenio@corteva.com

Phytopathogenic nematodes cause significant damage to many agricultural crops, especially horticultural ones. The most widespread species in Italy are those belonging to the root-knot genus *Meloidogyne* and the cyst-forming *Globodera* and *Heterodera*, to which some invasive alien species have recently been added. The damage caused to crops and the impact on farmers income is increasing. The intensification of nematode activity can be traced back to multiple factors such as the lack of availability of effective means of defense, limitations in their use, the emergence of resistance and more recently the effects of climate change. To address these problems farmers have resorted to soil fumigation creating the conditions for the impoverishment of biodiversity and consequent impact on the health of the soil itself. Reklemel is a new sulfonamide nematicide developed by Corteva Agriscience and applied at lower rates than many traditional nematocides. It provides a novel mode of action which specifically targets plant-parasitic nematodes which is vital importance to extending the usefulness of nematode resistance management programs. Nematodes affected by Reklemel show a significant decrease in their activity and ability to infect plant roots which has a notable benefit on plant health and crop yield. Within our talk, we will present also recent data on the compatibility of Reklemel, with various beneficial organism including free-living nematodes, nematode & disease suppressive soil fungi as well as beneficial soil bacteria.

This research was financially supported by Corteva Agrisciences

POSTER PRESENTATIONS

Molecular nematode community analysis reveals novel and known foes in Portuguese vineyards. P. FRAGA[1], J. WILTEN[2], L. CONCEIÇÃO[1], M.J. CUNHA[3], A. BERTRAN[2]. [1]*University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Department of Life Sciences, P-3000 456 Coimbra, Portugal.* [2]*HLB B.V. R&D Molecular Diagnostics Department, Wageningen, The Netherlands.* [3]*Polytechnic University of Coimbra, Research Centre for Natural Resources Environment and Society (CERNAS), High School of Agriculture, Bencanta, P-3045-601 Coimbra, Portugal.* E-MAIL: luci@zoo.uc.pt

Concerning vineyards in Portugal, the knowledge of plant parasitic nematodes (PPN) affecting this important crop is scarce. To survey PPN and free-living nematodes associated to this crop, 13 root-adjacent soil samples from vineyards in Portugal were collected and their nematode communities extracted. In collaboration with University of Coimbra, HLB B.V. received nematode communities and performed DNA isolation, full-length 18S rDNA SSU amplification, Nanopore sequencing, nematode SSU database searches and data analysis for these 13 samples. Primer sequences were trimmed

from the demultiplexed data using Porechop and used as input for the Decona pipeline. Further, reads were filtered, clustered (min. 20 reads per cluster) and polished cluster consensus sequences generated. The BLAST analysis of the reads against a nematode SSU database comprising more than 5000 sequences retrieved 151 amplified sequence variants (ASVs) matching 125 distinct taxa. The average number of mapped reads per sample with a minimum identity percentage of 97% was 67,240 (avg. 42 ASVs per sample) and the avg. read quality (mean Q) was 17.85. Further, beta diversity and Shannon's biodiversity indices were calculated and nematode community similarity across samples was assessed based on PCoA, NMDS and hierarchical clustering methods. *Helicotylenchus* spp. and *Tylenchulus semipenetrans* were found in 10 and 11 of the 13 samples, respectively. *Xiphinema pachtaicum*, *Ditylenchus destructor* and *Meloidogyne hapla* were also found. Based on the NGS data, molecular detection tests are being developed for each of the 5 species of the genus *Helicotylenchus* present in the samples.

This work was supported by FCT - Fundação para a Ciência e Tecnologia*, I.P., in the framework of the Project UIDB/04004/2025 - Centre for Functional Ecology - Science for the People & the Planet and Instituto do Ambiente, Tecnologia e Vida.*

Resistance of *Solanum linnaeanum* to *Meloidogyne* spp. B. SIOPA, L. CONCEIÇÃO. *University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Department of Life Sciences, P-3000 456 Coimbra, Portugal.* E-MAIL: luci@zoo.uc.pt

Root-knot nematodes (RKNs), *Meloidogyne* spp., are important crop pests that cause severe losses in crop production worldwide, reducing both productivity and crop quality. Some species are considered quarantine organisms by the European and Mediterranean Plant Protection Organization. The development of nonchemical and sustainable management strategies to reduce nematode damage is crucial. *Solanum linnaeanum* was already considered resistant to *M. chitwoodi*, with an average of 519 small galls/plant and with 45% adult nematodes, all males, inside the roots. The resistance of this *Solanaceae* species was assessed against the most common RKN species, *M. hapla*, *M. incognita* and *M. javanica*. Five replicates of *S. linnaeanum* and 5 replicates of *S. lycopersicum* cv. Coração de Boi (control) were inoculated with a suspension of eggs and juveniles of each RKN species. Two separated bioassays were performed for each RKN species. Gall index, the Bridge and Page rating chart and reproduction factor are being estimated and will be presented. The use of *S. linnaeanum* as a new source of resistance is a good alternative for the management of RKNs in search of nonchemical and sustainable strategies to protect crops.

This work was supported by FCT - Fundação para a Ciência e Tecnologia*, I.P., in the framework of the Project UIDB/04004/2025 - Centre for Functional Ecology - Science for the People & the Planet and Instituto do Ambiente, Tecnologia e Vida.*

PINEMARK – Pine resistance/susceptibility proteomic biomarkers to *Bursaphelenchus xylophilus* and *B. mucronatus* infection under climate change scenarios. H. SILVA[1], J.M.S. CARDOSO[1], B. MANADAS[2], L. FONSECA[1,3]. [1]*Centre for Functional Ecology - Science for People and the Planet, TERRA Associate Laboratory, Faculty of Science and Technology, University of Coimbra, Portugal.* [2]*CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.* [3]*FITOLAB - Laboratory for Phytopathology, Instituto Pedro Nunes (IPN), Coimbra, Portugal.* E-MAIL: hugoffs.95@gmail.com

The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease, is a quarantine organism in Europe. Its closely related species, *Bursaphelenchus mucronatus*, which shares similar ecological and morphological features with PWN, is considered to have low pathogenicity but is also capable of killing trees under specific environmental conditions. Climate changes have raised concerns about how these nematode species would interact with stressed pine hosts. Understanding these interactions is crucial for developing successful management strategies. This study aims to assess the effects of drought and high temperatures on the proteomic profiles of pine species with differing susceptibilities to *B. xylophilus* infection and to identify susceptibility and resistance biomarkers. These objectives have been approached through comparative proteomics of PWN and *B. mucronatus*-infected and non-infected *Pinus pinaster* (highly susceptible) and *P. pinea* (low susceptible) under different temperatures (25–30°C) and varying water availability conditions (well-watered/water-stressed). The identification of differentially expressed proteins and molecular pathways associated with pines response to infection will reveal resistance and susceptibility biomarkers, providing new insights into host-nematode interactions. Identified host resistance and susceptibility biomarkers will be useful for selecting more resistant trees to nematode infection under climate change

scenarios and improving the management strategies for this important forest disease.

This research was financially supported by FEDER-Portugal 2020, COMPETE 2020 and by FCT projects, PineWALL, PTDC/ASP-SIL/3142/2020; UIDB/04004/2025 (DOI: 10.54499/UIDB/04004/2025); UIDP/04004/2025 (DOI: 10.54499/UIDP/04004/2025); TERRA - LA/P/0092/20205 and Instituto do Ambiente, Tecnologia e Vida. Hugo Silva (Grant - 2023.03527.BD) is funded by FCT, European Social Fund (ESF), under the Programa Demografia, Qualificações e Inclusão (PDQI) – Portugal2030.

Effect of temperature and water availability on *Bursaphelenchus xylophilus* and *B. mucronatus* pathogenicity. H. SILVA[1], J.M.S. CARDOSO[1], B. MANADAS[2], L. FONSECA[1,3]. [1]*Centre for Functional Ecology - Science for People and the Planet, TERRA Associate Laboratory, Faculty of Science and Technology, University of Coimbra, Portugal.* [2]*CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Portugal.* [3]*FITOLAB - Laboratory for Phytopathology, Instituto Pedro Nunes (IPN), Coimbra, Portugal.* E-MAIL: hugoffs.95@gmail.com

Pine wilt disease, caused by pinewood nematode (*Bursaphelenchus xylophilus*), poses a significant threat to forests, particularly under climate change scenarios. This study evaluated the pathogenicity of *B. xylophilus* and its closely related species, *B. mucronatus* that is a species with similar morphological and ecological traits although considered as a weaker pathogen. Impact of these nematodes was assessed in *Pinus pinaster* and *P. pinea* under different temperature and water availability conditions. Two- to three-year-old seedlings of both pine species were inoculated with 3,000 nematodes (*B. xylophilus* or *B. mucronatus*) and exposed to 2 temperature conditions (25°C and 30°C) and 2 water availability regimes (water- stressed and well-watered). Disease symptoms were monitored for 40 days, and soil water content was recorded. At the end of monitoring, nematodes were extracted from aerial parts and roots, and quantified per gram of dry wood. Results showed that *P. pinea* exhibited no symptoms, and few nematodes of both species were recovered. However, *P. pinaster* was highly susceptible to *B. xylophilus*, showing symptoms few days after inoculation with high number of nematodes recovered. In *B. mucronatus*-inoculated *P. pinaster*, heavy symptoms appeared at 30°C under water-stressed conditions, with high nematode populations recovered. This study highlights the significance of temperature and water availability in determining the pathogenicity of *B. xylophilus* and *B. mucronatus*. Additionally, it reveals that *B. mucronatus*, despite being considered a relatively weak pathogen, may exhibit high pathogenicity to *P. pinaster* under certain conditions, suggesting that this nematode species could emerge as a significant threat to pine forests in a context of climate change.

This research was financially supported by FEDER-Portugal 2020, COMPETE 2020 and by FCT projects, PineWALL, PTDC/ASP-SIL/3142/2020; UIDB/04004/2025 (DOI: 10.54499/UIDB/04004/2025); UIDP/04004/2025 (DOI: 10.54499/UIDP/04004/2025); TERRA - LA/P/0092/20205 and Instituto do Ambiente, Tecnologia e Vida. Hugo Silva (Grant - 2023.03527.BD) is funded by FCT, European Social Fund (ESF), under the Programa Demografia, Qualificações e Inclusão (PDQI) – Portugal2030.

Diversity and role of plant-parasitic and free-living nematode communities in Italian vineyards. A. VOVLAS[1], A. TROCCOLI[1], E. FANELLI[1], V. PAPESCHI[2], F. DE LUCA[1]. [1]*IPSP, National Research Council CNR, Via Amendola 122/D, 70126 Bari, Italy.* [2]*Servizio Tecnico di Condifesa Lombardia Nord Est, Via Malta 12, Brescia, Italy.* E-MAIL: alessiovovlas@cnr.it

Nematodes represent a diverse and widespread group of invertebrates, largely studied for their economic and ecological relevance. Their identification is important to arrange the proper management strategies that should be designed to maintain soil health and fertility, as well. A wide survey was conducted in 2024 in North Italy to study plant parasitic (PPNs) and free-living nematodes (FLNs) associated with *Vitis vinifera* vineyards. This study aimed to determine the prevalence and distribution of PPNs and FLNs in vineyards. Moreover, explanatory variables were used, to describe the influence of soil, climate and agricultural management factors in structuring the variation of nematode community composition. Fifty- three sampling sites from ten different vineyards were surveyed, and nematode species and genera were identified using an integrative approach. A total of approximately 3000 soil nematode individuals were found belonging to 16 different genera. The diversity of nematode communities associated with soil samples was determined using several ecological indices such as Dominance, Shannon-Wiener Index, Eveness, Margaleff, Equitability, Enrichment Index (EI), and Maturity Index (MI). The Nematode Indicator Joint Analysis (NINJA) tool, structural nematode dynamics, Correspondence and Food web analyses were also applied. The results obtained provide novel insights into the biodiversity and dynamics of terrestrial nematodes associated with Italian vineyards. The contribution of

such biodiversity as an effective soil health bioindicator will be presented.

This research was partially funded by NEMAGEST project funded by Lombardy Region (Bando 2018 per Progetti di ricerca in campo agricolo e forestale – d.d.s. n. 4403 del 28/03/2018).

Morphological and molecular characterization of reniform nematodes in the olive orchard of central Italy.
A. VOVLAS[1], E. FANELLI[1], A. TROCCOLI[1], E. AJOBIEWE[1,2], F. DE LUCA[1]. [1]*IPSP, National Research Council CNR, Via Amendola 122/D, 70126 Bari, Italy.* [2]*Department of European and Mediterranean Cultures, University of Basilicata, Via Lanera, 10, 75100 Matera, Italy.* E-MAIL: alessiovovlas@cnr.it

Reniform nematodes belonging to the genus *Rotylenchulus* are semi-endoparasites that affect the roots of various herbaceous and woody plants. The genus *Rotylenchulus* Linford & Oliveira, 1940 comprises 10 valid species, showing high intraspecific variability of some diagnostic features, making identification, based only on morphology, a difficult task. Nematode surveys of plant-parasitic nematodes in Italy, undertaken during the last few years on several crops, including sugarcane, sorghum, maize, grapevine, olive, and wild olive have revealed feeder-root infections and heavy soil infestations of *Rotylenchulus* spp. The main objective of this study was to investigate the biodiversity of reniform nematodes in olive orchards of central Italy, conducted with a traditional management strategy. Reniform nematodes recovered from two olive groves were characterized using a polyphasic approach, combining morphological and molecular characterization. Morphological and morphometric analyses identified at species level reniform nematodes in both sampling sites as *Rotylenchulus macrodoratus*. Molecular analyses, based on D2-D3 segments of 28S rRNA gene and mitochondrial COI confirmed the high intraspecific and interspecific variability within Italian *Rotylenchulus* populations. Blast search based on the D2-D3 sequences revealed that one population showed a 97-98% similarity with the corresponding Italian populations of *R. macrodoratus* from GenBank, while the second population showed 95% similarity suggesting the occurrence of a species complex for *R. macrodoratus* or a high intraspecific variability. Further molecular analyses are ongoing to correctly identify these nematodes that are frequently ignored or misdiagnosed as pests, and this may potentially pose a threat to food security.

This research was financially supported by European Union's Horizon Europe research and innovation programme "Soil O-Live", under grant agreement No. 101091255 (Soil Deal for Europe – HORIZON-MISS- 2021-SOIL-02-03) [AV] and partially by Agritech National Research Center funding from the EU Next- Generation EU (Piano Nazionale di Ripresa e Resilienza (PNRR) – Missione 4 Comp. 2, Invest 1.4 – D.D. 1032 17/06/2022, CN00000022) [EA].

Nematocidal activity of the alpine medicinal plant *Peucedanum ostruthium* against the root-knot nematode *Meloidogyne incognita*.
P. VERONICO[1], D. LODDO[2], M.P. ARGENTIERI[3], T. D'ADDABBO[1]. [1]*Institute for Sustainable Plant Protection, National Council of Research, via Amendola 122/d, 70126 Bari, Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council, Viale dell' Università 16, 35020 Legnaro, Italy.* [3]*Department of Pharmacy—Drug Sciences, University of Bari, via Orabona 4, 70125 Bari, Italy.* E-MAIL: pasqua.veronico@cnr.it

Restrictions on the use of chemical pesticides have raised growing interest in the study of plant-derived nematocidal compounds. The potential nematocidal properties of *Peucedanum ostruthium* (*Apiaceae*) were tested by 2% ethanol plant extracts *in vitro* and in pot experiments. Infective juveniles (J2s) were exposed for 4, 8, 24, 48 and 96 h to 62.5, 125, 250, 500, 1000 and 2000 µg mL^{-1} concentrations of both root and leaf extracts, including distilled water and a chemical nematicide as controls. Nematode mortality reached more than 98% after 24-hour treatment with 500, 1000 and 2000 µg mL^{-1} solutions of root extract and 94% after 48-hour treatment with 1000 and 2000 µg mL^{-1} solutions of leaf extract. To assess the ability to infect, juveniles were exposed for 24 h to the LD50 of both extracts, then rinsed and inoculated in tomato plants kept in growth chamber. Thirty days after inoculation, nematode infection was significantly reduced in roots inoculated with root extract treated J2s. The suppressiveness of *P. ostruthium* extracts was also investigated by irrigating tomato plants in 50 mL pots with 5 mL of 4000 µg mL^{-1} solutions at inoculation and 2 weeks later. At the end of nematode life cycle, infestation on tomato roots was reduced and, particularly, a significant reduction of the eggs/egg mass numbers was observed. Results from this study show a high nematocidal potential of *P. ostruthium* root extract, which can be eexploited forthe formulation of new effective nematicides.

This study was funded by PNRR Agritech, task 3.2.5 'Nature-based solutions for natural resources and environment protection'.

Toxicity of Asteraceae plants from the Anatolian region to the Root-Knot Nematode *Meloidogyne incognita*. G. AYDINLI[1], T. D'ADDABBO[2], M. KOÇ[3], M.P. ARGENTIERI[4], S. MENNAN[5], P. VERONICO[2]. [1]*Ondokuz Mayıs University, Bafra Vocational High School, Samsun, Türkiye.* [2]*Institute for Sustainable Plant Protection, National Council of Research, Bari, Italy.* [3]*Ankara Yıldırım Beyazıt University, Public Health Institute, Department of Traditional, Complementary and Integrative Medicine, Ankara, Türkiye.* [4]*Department of Pharmacy—Drug Sciences, University of Bari, Bari, Italy.* [5]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Plant Protection, Samsun, Türkiye.* E-MAIL: pasqua.veronico@cnr.it

Asteraceae plants from the endemic flora of the Anatolian region are a reservoir of still unexplored sources of bioactive compounds that may be used for the control of root-knot nematodes (*Meloidogyne* spp.). The aim of this study was to assess the toxicity to the infective juveniles (J2s) of the root-knot nematode *M. incognita* of 2 extracts from Asteraceae spp.: one from *Helichrysum noeanum* and one from *Tanacetum nitens*. The nematode J2s were exposed for 24 and 48 h to 62.5, 125, 250, 500, 1000 and 2000 μg mL^{-1} concentrations of each plant extract, including distilled water and a 2% ethanol solution as controls. J2 motility and mortality were checked microscopically at the end of each exposure time. About 90% of the *M. incognita* J2s were immobilized by a 24-hour exposure to the 3 and 2 highest concentrations of *T. nitens* and *H. noeanum* extracts, respectively. At the same concentrations, all the J2s treated with H. *noeanum* extract recovered their mobility after a 24-hour permanence in water, while 13–26% mortality rates occurred for the J2s exposed to T. *nitens* extract. Nematode mortality reached 93 and 55% after 48-hour treatment with 1000 μg mL^{-1} solution of *T. nitens* and *H. noeanum*, respectively. Both extracts did not show any toxicity to J2s when used at concentrations ≤ 250 μg mL^{-1}. The results indicate a higher suitability of *T. nitens* plant extracts to a potential formulation of nematocidal products. This study was carried out within the frame of a bilateral project CNR-TUBITAK "The nematocidal potential of endemic species of Asteraceae in Turkiye"

CONCURRENT SESSION D2 – The role of pathogens in forest tree mortality in times of global change

SESSION KEYNOTES

Climate acts as an environmental filter to forest pathogens. M. CABALLOL[1,2,3], M.A. REDONDO[4], N. CATALÁN[5], T. CORCOBADO[6,7], T. JUNG[6], B. MARÇAIS[8], I. MILENKOVIĆ[6,9], M. NEMESIO-GORRIZ[10], J. STENLID[4], J. OLIVA[1,2]. [1]*Department of Agricultural and Forest Sciences and Engineering, University of Lleida, 25198, Lleida, Spain.* [2]*Joint Research Unit CTFC – AGROTECNIO-CERCA, 25198, Lleida, Spain.* [3]*Current address: Forest Science and Technology Centre of Catalonia (CTFC), 25280, Solsona, Spain.* [4]*Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 750 07, Uppsala, Sweden.* [5]*Institute of Environmental Assessment and Water Research, IDAEA-CSIC, 08034, Barcelona, Spain.* [6]*Mendel University in Brno, Faculty of Forestry and Wood Technology, Department of Forest Protection and Wildlife Management, Phytophthora Research Centre, 613 00, Brno, Czech Republic.* [7]*Austrian Research Centre for Forests (BFW), Seckendorff-Gudent-Weg 8, 1131, Vienna, Austria.* [8]*Université de Lorraine - Inrae, UMR Interactions Arbres/Microorganismes, 54000, Nancy, France.* [9]*University of Belgrade-Faculty of Forestry, 11030, Belgrade, Serbia.* [10]*Forestry Development Department, Teagasc, D15DY05, Dublin, Ireland.* E-MAIL: maria.caballol@ctfc.cat

Global change and forest pathogens pose a combined and often synergistic threat to natural ecosystems worldwide. The number of reports of forest diseases and tree mortality has increased worldwide over the past decades. Global change has contributed to this increase by introducing forest pathogens to new areas and favouring their range expansion due to changes in the climate. Understanding how the environment influences the distribution of forest pathogens is paramount given the upraising effects of climate change. Many studies have predicted an expansion of the distribution of forest pathogens with increasing temperatures. However, predictions based on single-species models considering pathogens as independent and static entities may not fully represent the complexity of their interactions in nature. The functional trait-based approach has emerged as a powerful tool for explaining community patterns and could be used to predict how pathogen communities may respond to environmental changes based on their functional traits. While this approach has been applied to plant-associated microbes such as bacteria and mycor-

rhizal and endophytic fungi, the role of climate on plant pathogens has been rarely explored from a community ecology perspective. The genus *Phytophthora* comprises some of the most damaging plant pathogens of agricultural, forestry and natural ecosystems. The ecologically diverse array of species included in this genus provides a good opportunity to study how the environment influences the composition of communities. We explored the role of climate in the assembly of *Phytophthora* species at >250 river sites across two gradients, a latitudinal gradient spanning from Mediterranean to Arctic conditions, and an altitudinal gradient including the Spanish Pyrenees. *Phytophthora* communities were obtained by sequencing the full internal transcribed spacer (ITS) region of river filtrates. We tested whether the biogeography and seasonal variation of *Phytophthora* was linked to climatic variables and whether other environmental factors such as water chemistry and vegetation diversity played a role. In this talk, we present how climate acts as an environmental filter for the assembly of *Phytophthora* communities. We show how different mechanisms occur in southern and northern latitudes, and how functional traits related to morphological and physiological characteristics of *Phytophthora* species explain community assembly processes. At northern latitudes, the cold climate restricts species diversity and acts as a filter for *Phytophthora* distribution, selecting species with a low minimum temperature limit for growth. By contrast, at southern latitudes, a dry climate poses a strong environmental filter for *Phytophthora* communities which results in the dominance of drought-tolerant species with thick oospore walls and a high optimum temperature for growth, and a high maximum temperature limit for growth. The ability to survive under cold conditions seems to be key for colonising northern latitudes while the ability to cope with dry conditions seems crucial for colonising southern latitudes. These findings indicate that considering functional traits can improve the predictability of how pathogens will respond to future climate change scenarios.

Forest pathogens in the era of global change: disrupting the holobiont and reshaping ecosystems. C. MORALES-RODRIGUEZ. *Department for Innovation in Biological, agro-food and forest systems (DIBAF) University of Tuscia. Via San Camillo de Lellis snc, VT, Italy.* E-MAIL: cmorales@unitus.it

Forest ecosystems are under mounting pressure from global change, which acts through a convergence of drivers such as climate shifts, biological invasions, increased human mobility, and landscape fragmentation. These factors do not merely add new stressors; they profoundly reshape the structure and function of ecological networks, including those that govern the relationships between forest trees and their associated microorganisms. The increasing frequency and severity of tree mortality events linked to pathogens underscore the need for a deeper and more integrated understanding of forest health. In this context, the holobiont framework, provides an innovative perspective for interpreting the outcomes of host-microbe-pathogen interactions in dynamically changing environments. Global change can disrupt these interactions, tipping the balance between symbiosis, commensalism, and pathogenicity. Climate-induced shifts in temperature regimes are driving the expansion of geographic ranges and phenological shifts in pathogens. Simultaneously, biological invasions introduce novel species that can destabilize established microbial communities, with cascading effects on ecosystem resilience. Understanding how these microbial networks respond to global change is essential for predicting emerging disease outbreaks and implementing effective forest management strategies. This contribution emphasizes the need to transcend classical paradigms, advocating for a more systems-level approach that integrates microbial ecology, climate science, invasion biology, and adaptive forest management. Such approaches are increasingly facilitated by advances in high-throughput sequencing, metagenomics, and modelling tools that can unravel the complexity of host–microbiota–pathogen systems. As a representative example, we explore the chestnut forest system of central Italy, which has experienced the compounding effects of global change drivers over recent decades. In this system, the introduction of the gall wasp *Dryocosmus kuriphilus* has indirectly influenced the fungal communities already present, such as *Gnomoniopsis castanea*, concurrently altering the native microbial communities associated with chestnut tissues. In addition to the effect of the introduction of a new species, we will see the influence of global warming on the distribution of soilborne pathogens such as Phytophthora, which cause chestnut ink blight. These changes have led to unexpected disease patterns and declines in tree vitality, illustrating how biotic and abiotic stressors can synergistically destabilize forest holobionts. In conclusion, forest pathogens should be studied within the broader context of microbial and environmental interactions that define the holobiont. Addressing forest health in the Anthropocene requires not only pathogen surveillance but also a comprehensive understanding of the host-associated microbiota and its role in ecosystem resilience. By embracing this integrated framework, researchers and

forest managers can better anticipate future disease scenarios and design more effective mitigation strategies.

The rise of emerging pathogens in a changing world: the case of *Cryptostroma corticale*. R. SCHLOESSER[1,2], S. BIEN[2], J. BUSSKAMP[2], G.J. LANGER[2], E.J. LANGER[3], L. GHELARDINI[4,5], A.L. PEPORI[1], A. SANTINI[1]. [1]*IPSP, National Research Council CNR, Via Madonna del Piano 10, 50127 Sesto Fiorentino, FI, Italy.* [2]*NW-FVA, Northwest German Forest Research Institute, Grätzelstraße 2, 37079 Göttingen, Germany.* [3]*University of Kassel, Department of Ecology, Heinrich-Plett-Straße 40, D-34132 Kassel, Germany.* [4]*University of Florence, Department of Agriculture, Food, Environment and Forestry (DAGRI), Florence, Italy.* [5]*National Biodiversity Future Center (NBFC), Palermo, Italy.* E-MAIL: rebekkaschloesser@cnr.it

Climate change is progressively stressing and debilitating trees, encouraging the emergence of pathogens previously considered secondary as, for example, *Diplodia sapinea, Biscogniauxia nummularia, B. mediterranea* and *Cryptostroma corticale*. *C. corticale*, the causal agent of the Sooty Bark Disease (SBD), has been present in Europe at least since the middle of the 20th century. The pathogen had been reported from several central European countries in the 1950s and 1960s, causing mostly minor and localised damage, however, started causing large scale damages after the extreme drought years 2018-2020. According to literature, the pathogen emerges in cyclic patters following years with above-average summer temperatures. SBD mainly causes damage on *Acer pseudoplatanus* but can also infect other native *Acer* species as well as *Aesculus hippocastanum*. As temperatures are expected to rise further, especially in the Mediterranean area, further outbreaks of secondary pathogens can be envisaged in the future. Since the start of the latest large-scale outbreak of SBD in Europe, interest in SBD has increased, and several papers regarding dispersion and potential management have been published. The disease has also come to the attention of the general public, as the spores produced by *C. corticale* can cause an allergic reaction in humans referred to as 'maple bark strippers' disease. As breakouts of SBD have been reported from many different regions, it was of interest to investigate whether *C. corticale* might have an endophytic life stage and thus be dispersed much farther than observed by symptomatic trees.

This research was financially supported by the Hessian Ministry of the Environment, Climate Protection, Agriculture, and Consumer Protection and located at the Northwest German Forest Research Institute (NW-FVA), and by the Phytosanitary service of the Regione Toscana, Italy.

ORAL PRESENTATIONS

Integrative analysis of bacterial pathogens causing acute oak decline on Portuguese fagaceae forests. L. CRUZ[1,2], L. DUARTE[1], D. McGUIRE[1,2], A. MADURO[1], E. SOUSA[1,3], P. NAVES[1,3]. [1]*National Institute for Agrarian and Veterinarian Research, Oeiras, Portugal.* [2]*BioSystems & Integrative Sciences Institute (BioISI), University of Lisbon, Lisbon, Portugal.* [3]*GREEN–IT – Bioresources for Sustainability.* E-MAIL: leonor.cruz@iniav.pt

Since the first report of acute oak decline agent *Brenneria goodwinii* affecting *Quercus suber* in Portugal (2018), an increasing number of similar findings on other Fagaceae species, namely *Quercus ilex* and *Quercus pyrenaica*, have been observed in distinct geographic areas of the country. Over 100 samples collected from trunks, twigs, acorns and insects from the Buprestidae, Dermestidae and Cerambycidae (Coleoptera) families were laboratory tested using isolation and biomolecular tools to identify the putative agents involved in this decline syndrome. Additionally, cross pathogenicity tests were made for selected strains under confinement conditions on healthy plantlets of *Q. suber*, Q. *ilex* and Q. *pyrenaica* using reference strains of *B. goodwinii* and *Lonsdalea* spp., as controls. Whole Genome Sequencing (WGS) was further implemented on a set of strains selected based on the host species, location and pathogen. Relative frequency of positive isolation and PCR tests showed differences between host species. *Q. ilex* displayed the lowest infection level (5.71%), *Q. suber* 14.29% and *Q. pyrenaica* showed the highest disease rate (17.24%). These tests highlighted the presence of distinct bacterial pathogens affecting stands and isolated trees, while cross pathogenicity tests disclosed specific host-pathogen interaction patterns. Additionally, WGS confirmed the presence, among other bacteria, of pathogenic strains of *Lonsdalea iberica*, *B. goodwinii* and *Rahnella victoriana,* affecting these hosts. This study brings to light relevant epidemiological aspects of acute oak decline, contributing to clarify the presence, diversity and risk of dispersion of these pathogens in south-western Europe Mediterranean forests, where distinct Fagaceae species coexist.

This research was financially supported by the project MoniTREEng - POCI-07-62G4-FEDER-181575 (funding program "Portugal 2020" REACT-EU), and the Portuguese FCT

*- Fundação para a Ciência e a Tecnologia, I.P., through the R&D Unit "GREEN-IT - Bioresources for Sustainability" (UIDB/04551/2020 and UIDP/04551/2020). Work supported by UIDB/04046/2020 and UIDP/04046/2020 Centre grants from FCT, Portugal (to BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal. DOI 10.54499/UIDB/04046/2020 (*https://doi.org/10.54499/UIDB/04046/2020*). Support was also provided by INIAV as part of the Euphresco Project Consortium 2023-A-454 -Whole genome sequencing in identification of plant pathogenic bacteria (PPBSeq).*

Phylogenetic structure, host abundance, and microclimate predict establishment of Fusarium dieback - invasive shothole borers in California. S. LYNCH[1], M. BUSTAMANTE[1], K. ELFAR[1], C. CARACHURE[1], A. ADASKAVEG[1], J. KABASHIMA[2], C. SHOGREN[2], A. ESKALEN[1]. [1]*University of California, Davis, United States of America;* [2]*University of California, Agricultural and Natural Resources, University of California, United States of America.* E-MAIL: sclynch@ucdavis.edu

Pine ghost canker is a recently described disease affecting multiple pine species in urban forests of Southern California. Symptoms include wedged cankers with irregular margins and cryptic discoloration on cross-sections of branches, which can lead to severe dieback and potentially tree death. In this study, we identified and characterized five *Neofusicoccum* species (*N. luteum*, *N. mediterraneum, N. parvum, N. stellenboschianum*, and *N. vitifusiforme*) as the primary etiological agents of pine ghost canker. These pathogens were consistently isolated from multiple symptomatic pine samples (n = 41) and identified by morphology and phylogenetic analyses using four DNA barcodes (rDNA ITS, tef1, tub2, and rpb2). Pathogenicity was confirmed on healthy branches of 15- year-old Monterey pines, where the five *Neofusicoccum* species, caused vascular lesions that were not significantly different in length. Secondary fungi (*Diaporthe, Diplodia, Neopestalotiopsis*, and *Pestalotiopsis* spp.) were also recovered from symptomatic tissues but did not cause vascular lesions in pathogenicity tests. The optimal temperature for mycelial growth of *N. luteum* and *N. parvum* was 30°C, whereas for *N. mediterraneum, N. stellenboschianum* and *N. vitifusiforme*, it was 25°C. All five species were able to resume growth at room temperature (20°C) after showing no growth during a 7-day exposure to 5°C and 40°C. This study constitutes the first report of *N. luteum, N. stellenboschianum*, and *N. vitifusiforme* causing pine ghost canker in California. Environmental factors such as warmer temperatures, irrigation, and pest infestations are discussed as drivers of disease expression in pine trees. Management practices are also proposed.

First detection of acute oak decline associated bacteria in holm oak of Salento: environmental drivers and disease implications. A. BENE[1], G. CARLUCCIO[1], M. VERGINE[1], E. SABELLA[1], A.G. DELLE DONNE[2], L. DE BELLIS[1], A. LUVISI[1]. [1]*Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100 Lecce, Italy.* [2]*Department of Agriculture, Rural Development and Environment, Sec. Osservatorio Fitosanitario,70125 Bari, Italy.* E-MAIL: alessandro.bene@unisalento.it

Acute Oak Decline is a complex disease that affects oak trees, including Holm oak (*Quercus ilex* L.), representing a new challenge for the protection of European and Mediterranean forests. Pathogens related to AOD in Holm oak were analysed in the Salento peninsula, southern Italy, in two different contexts: urban and rural environment. The results showed a difference in symptomatology where urban trees showed crown desiccation and insect attacks, such as *Kermes* spp. and *Nidularia* spp., while rural trees showed characteristic AOD symptoms, including vertical bark cracks with dark exudates, larval galleries, and insect exit holes associated with Buprestidae. In addition, the presence of three key disease bacteria *Brenneria goodwinii*, *Gibbsiella quercinecans*, and *Rahnella victoriana* was confirmed, but only in samples from rural trees. Pathogenicity tests confirmed *B. goodwinii* and *G. quercinecans* as the causative agents of AOD in Holm oak, having fulfilled all of Koch's postulates. In addition, phylogenetic tests showed a high genetic similarity between these strains and those widespread in Europe and Iran, suggesting the possibility of wide-spread dissemination via vector insects or plant material. Thus, while bacteria play a fundamental role, the multifactorial nature of AOD imposes the need to investigate all other possible abiotic, such as drought and poor soil conditions, and biotic actors as well. Future investigations should focus on monitoring the spread of AOD, determining its impact on Mediterranean forests.

Hidden phytosanitary threat within internationally exchanged tree seed. I. FRANIĆ[1], M. CLEARY[2], R. ESCHEN[3], A. PEREZ-SIERRA[4], S. PROSPERO[1]. [1]*Swiss Federal Research Institute WSL, Birmensdorf, Switzerland.* [2]*Swedish University of Agricultural Sciences, Alnarp, Sweden.* [3]*CABI, Delémont, Switzerland.* [4]*Instituto Valenciano*

de Investigaciones Agrarias, Valencia, Spain. E-MAIL: iva.franic@wsl.ch

Although several major tree disease outbreaks in forests and plantations have been caused by pathogens that are also known as seed-borne, tree seeds are generally considered a low-risk pathway for introducing plant-pathogenic fungi, and their movement remains largely unregulated. However, our recent analysis of the international tree seed trade revealed that approximately 2.5 million kilograms of tree seeds are exchanged between countries each year. Due to climate change- driven seed shortages, we anticipate that the volume of international seed trade will increase in the future. This is particularly concerning because our recent molecular studies, which examined multiple tree species originating from several continents, demonstrated that tree seeds harbour diverse fungal communities, including known pathogens. Furthermore, these seed-associated fungal communities were primarily structured by the host species rather than environmental factors, indicating strong host specificity, likely due to confirmed frequent vertical transmission. These findings suggest a significant risk of pathogen introduction through seed trade, with a higher likelihood of establishment in new areas if suitable host species are present. Nonetheless, further research is needed to understand the impact of seed-borne fungi in new environments and to develop effective risk management strategies.

POSTER PRESENTATIONS

Emerging fungal pathogens associated with holm oak decline in Southern Italy. C. DEL GROSSO[1,2], D. PALMIERI[1], L. MARCHESE[1], L. MELISSANO[3], G. LIMA[1]. [1]*Department of Agricultural, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council (CNR), 70126 Bari, Italy.* [3]*Department of Agriculture, Environment and Rural Development, Sustainable Management and Protection of Natural and Forest Resources, Apulia Region, 70100 Bari, Italy.* E-MAIL: lima@unimol.it, carmine.delgrosso@cnr.it

Holm oak (*Quercus ilex* L.) forests, iconic elements of the Mediterranean landscape, are increasingly threatened by complex decline phenomena. Since 2016, extensive dieback symptoms, such as cankers, branch necrosis, and crown thinning, have been observed in holm oak stands across the Salento Peninsula (Southern Italy), raising concerns about forest resilience under climate change. This study aimed to identify fungal isolates associated with holm oak decline and assess their pathogenic potential. Several fungal species of the Botryosphaeriaceae family, as in particular *Diplodia corticola* (*Dc*), *Diplodia quercivora* (*Dq*), and *Neofusicoccum vitifusiforme* (*Nv*), were identified. Pathogenicity tests on artificially stressed and inoculated holm oak seedlings revealed that *Dc* and *Dq* induced severe subcortical and leaf margin necrosis, while *Nv* induced similar but milder symptoms, suggesting its behaviour as a weak pathogen. These findings confirm the important role of *Botryosphaeriaceae* in holm oak decline but report for the first time *Dq* and *Nv* as *Q. ilex* pathogens in Italy. These pathogens may act within a broader decline syndrome in which abiotic stress factors, particularly alternating periods of droughts, heavy rainfall and heat waves, severely compromise tree vitality, favouring opportunistic infections. Furthermore, recent reports identifying bacterial agents associated with Acute Oak Decline (AOD) in the same geographical area, highlight the need for integrated and multidisciplinary approaches to monitor and manage this complex forestry issue and to develop the most appropriate strategies to counteract holm oak decline in Mediterranean forests.

The findings presented are based on a study published in Journal of Fungi, DOI: https://doi.org/10.3390/jof10010035.

Plasticity responses of *Hymenoschyphus fraxineus* populations to thermal stress in the Italian peninsula. L. STAZIONE[1*], C. AGLIETTI[1*], A.L. PEPORI[2], F. PECORI[2], G. GRAHAM[1], L. GHELARDINI[1], A. SANTINI[2]. [1]*Department of Agricultural, Food, Environmental and Forest Sciences and Technologies (DAGRI), University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy.* [2]*National Research Council (CNR), Institute for Sustainable Plant Protection, Via Madonna del Piano, 10, 50019 Sesto Fiorentino, Italy.* E-MAIL: leoneldaniel.stazione@unifi.it. **Equal contribution*

Global change is a complex trend affecting the spread and establishment of non-native species. Among these, the invasive fungal pathogen *Hymenoscyphus fraxineus* was reported as a challenge threatening natural ash (*Fraxinus* sp.) populations of the whole Europe. In Italy, *H. fraxineus* was recently reported on *Fraxinus excelsior* at the southward of the country, being able of colonizing mediterranean habitats previously considered far from the growth optimum of the species. There is a gap of knowledge about *H. fraxineus* adaptability and distribution in Italy, also due to the highly fragmented geo-

graphic presence of the host, especially in the south of the country. The aim of this work is to characterize populational adaptive traits of *H. fraxineus* along the Italian peninsula. Samples were collected from symptomatic *F. excelsior* along the distribution of ash populations and fungal strains were identified by sequencing of nuclear and mitochondrial loci. The phenotypic responses were studied to assess optimal range and thermal stress tolerance by testing the mycelial growth of each strain against heat and cold thermal treatments, as well as their virulence by inoculation into the host tissues. Preliminary results clarify the possibility of further spread of the pathogen in the Mediterranean area, contributing to the understanding of the distribution and potential adaptive responses of invasive fungal pathogens in the context of fluctuating temperatures. Results will be useful to build an effective prevention and forest management strategy, helping the conservation of ash biodiversity richness at the southern edge of the species' natural distribution.

This research was financially supported by European Union - Next- GenerationEU - National Recovery and Resilience Plan (NRRP) – MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, CALL PRIN 2022 PNRR D.D. 1409 14-09-2022 – SAVEASH CUP N.B53D23032110001.

Preliminary findings of ophiostomatoid fungi from bark beetle galleries of the Bakhmaro Resort Forest, Georgia. T. ABRAMISHVILI[1], D. GAGANIDZE[1], M. GIORBELIDZE[1], A. DADEGASVILI[1], N. KHARABADZE[2], M. BURJANADZE[2], J. FOIT[3], K. TOMANKOVA[4]. [1]*Department of Plant Pest Diagnostic, State Laboratory of Agriculture, Tbilisi, Georgia.* [2]*Vasil Gulisashvili Institute of Forestry, Agricultural University of Georgia, Tbilisi, Georgia.* [3]*Department of Forest protection and management of environmental, Mendel University, Brno, Czech Republic.* [4]*Central Institute for Supervising and Testing in Agriculture, Olomouc, Czech Republic.* E-MAIL: tea.abramishvili@sla.gov.ge

Bark beetles are dangerous pests of forest crops and vectors of pathogens causing phytopathogenic fungal diseases. They introduce the fungal spores with their body into the passages made in the plant, where they cover the vascular fiber cones and hinder the physiological processes. Finally, the plant withers. The aim of the study is to detect and identify bark beetles as vectors of phytopathogenic fungi and the fungi spread by them in the coniferous forests of the resort zone Bakhmaro of Guria region of Western Georgia. The bark beetles and fungi were identified by morphological and molecular methods (sequencing the ITS 1 and ITS2 2 regions of the ribosomal RNA operon 5.8S gene). Seven bark beetle species were identified: *Ips typographus*, *Cryphalus* sp., *Cryphalus asperatus, Phytoptorus polygraphus*, *Phytokteines spinidens, Pityophtorous* sp., *Cryphalus piceae*. From these species and their galleries, 114 fungal isolates were isolated: *Leptographium* sp. Was 55.2%, *Ophiostoma piceae* and *Ophiostoma ainoae* were each 3.5%. *Graphilbum* sp. was 15%. *Trichoderma* sp. 18,4% and *Lecanicillium* sp. 4,3%. This research first recorded two species of *Ophiostoma—O. piceae* and *O. ainoae*, as well as representatives of *Leptographium* and *Graphilbum* genera on spruce, in galleries of bark beetles. These findings expand the known diversity of ophiostomatoid fungi in the Caucasus region and underscore the need for continued monitoring of vector-pathogen relationships in Georgian coniferous forests, especially under climate-induced stress conditions.

Emerging association between *Neofusicoccum parvum* and the invasive *Ailanthus altissima*: a first report from Italy. I. GIUBILEI, M.I. DRAIS, D. COS, A. MAZZAGLIA. *Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy.* E-MAIL: irene.giubilei@unitus.it

Ailanthus altissima (Tree of Heaven) is among the most aggressive invasive plant species worldwide, capable of outcompeting native flora and altering ecosystem dynamics. In 2024, a widespread dieback phenomenon affecting *A. altissima* was observed in Rome province, Italy. Symptomatic trees exhibited canopy decline, wood discoloration, and necrotic lesions. Fungal isolates obtained from infected tissues were identified morphologically and molecularly as *Neofusicoccum parvum*, an aggressive pathogen within the Botryosphaeriaceae family. The identity of the fungus was confirmed through multilocus sequencing (ITS, tef1-α, tub2, and LSU), and its pathogenicity was validated via inoculation trials, which reproduced disease symptoms and fulfilled Koch's postulates. This represents the first report of *N. parvum* infecting *A. altissima* in Italy. The occurrence of this pathogen on an invasive host species raises intriguing questions about potential biocontrol implications. As *N. parvum* is known for its virulence on a broad range of woody plants, its natural emergence on *A. altissima* may offer novel avenues for controlling this difficult-to-eradicate species. These findings provide new insights into the pathogen's ecology and underscore the importance of monitoring emerging host-pathogen interactions, particularly in

the context of invasive species management and changing environmental conditions.

This work was conducted within the framework of the Ministry for University and Research (MUR) initiative "Department of Excellence" (Law 232/2016) DAFNE Project 2023-27 "Digital, Intelligent, Green and Sustainable" (acronym: D.I.Ver.So*).*

Impact of climate change on olive dieback diseases in Tunisia and beneficial effect of *Bacillus velezensis* OEE1 in biocontrol of fungal pathogens. M.A. TRIKI, M. CHEFFI, Y. GHARBI, K. ENNOURI, S. KRID, M. ENNOURI. *Laboratory of Genetic Resources of Olive tree: Characterization, Valorization and Phytosanitary Protection, Olive Tree Institute, University of Sfax, Road of the airport km 1.5, P.O. Box 1087, Sfax, 3000, Tunisia.* E-MAIL: mohamedalitriki2@gmail.com

Olive tree (*Olea europaea* L.) is of great economic importance in Tunisia. Field surveys showed increased incidence of severe dieback and mortality of olive trees increased in many olive- growing area. These regions might be particularly affected by climate change, which could have extensive impact on the olive agrosystem including the development of diseases. Olive dieback is characterized by rapid browning of leaves, dieback of shoots, twigs and branches followed by death of the entire tree. Laboratory diagnosis and pathogenicity tests, revealed the occurence of pathogens in branches and occasional attacks by soil-borne fungi. *Neofusicoccum australe*, *Phoma fungicola*, *Nigrospora* sp., *Biscogniauxia mediterranea* and *Lasiodiplodia theobromae* were detected in branches. These species are reported for the first time to occur in olive trees in Tunisia. To limit the use of agrochemicals against these pathogens, different biocontrol strategies have been developed using endophytic bacteria. *Bacillus velezensis* OEE1 represented an efficient and effective alternative in the control of these pathogens as it was able to reduce mycelial growth up to 60% either by diffusible or volatile compounds. The mechanisms used by this strain include competition for space and nutrients (siderophore), production of cell wall degrading enzymes (chitinase and glucanase) and several antimicrobial compounds (fungicin, utirin, surfactin, bacillomycin, macrolactin).
B. velezensis OEE1 can also activate the plant's defensive responses. Besides biocontrol ability, *B. velezensis* OEE1 is an effective plant growth promoting bacteria due to atmospheric nitrogen fixation, phosphate solubilisation and phytohormone production.

Holm Oak (*Quercus ilex*) decline in Apulia, Southern Italy, a phytopathological overview. P.G. LUCCHESE, E. CHIAROMONTE, D. SALAMONE, E. TARASCO, S. POLLASTRO, F. FARETRA, F. NIGRO. *Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, Italy.* E-MAIL: stefania.pollastro@uniba.it

Holm oak (*Quercus ilex*) is a keystone species in Mediterranean agroecosystems, valued for its ecological, ornamental, and landscape functions. Over the last decade, widespread and progressive decline has been observed in Apulia (southern Italy). This study aims to investigate the underlying causes of this phenomenon. Systematic surveys and sample collections were carried out across different Apulian locations. Fungal isolates were identified by morphological and molecular techniques; bacterial communities were characterized, and insect presence was monitored in urban settings. Multiple xylem-inhabiting fungi were identified, notably *Diplodia corticola*, a known canker pathogen of *Quercus* spp. Bacterial strains of the genera *Brenneria* and *Gibbsiella* were also isolated from symptomatic tissues. Concurrently, infestations by phytophagous insects such as *Kermes vermilio* and *Nidularia pulvinata* were frequently observed. These findings, along with meteorological data under analysis, suggest a potential link between drought stress and disease severity. Preliminary evidence indicates a complex etiology, with holm oak decline likely resulting from the interplay of biotic (fungi and insects) and abiotic (climatic) stressors. The study highlights the importance of integrated monitoring strategies to address forest tree decline in the context of global change.

Funding: This research was supported by Agritech National Research Center and funded by the European Union – NextGenerationEU (PNRR – Mission 4, Component 2, Investment 1.4, D.D. 1032 17/06/2022, CN00000022).

Genetic characterization and evolution dynamics of *Ophiostoma novo-ulmi* on wych elm along the Italian peninsula. A.L. PEPORI, H. BERTO, S. TORRE, S. VILLA, C. DE QUATTRO, F. SEBASTIANI, F. PECORI, A. SANTINI. *National Research Council, Institute for Sustainable Plant Protection, Via Madonna del Piano 10, 50127 Sesto Fiorentino, FI, Italy.* E-MAIL: alessia.pepori@cnr.it

Dutch elm disease (DED), caused by the fungus *Ophiostoma ulmi* and later *Ophiostoma novo- ulmi*, is one of the most aggressive plant diseases that has been devas-

tating elm populations throughout Europe for almost a century. Two subspecies of *O. novo-ulmi* are present in Europe: subsp. *novo-ulmi* (formerly Euro-Asiatic race) and subsp. *americana* (formerly North American race). Since the 1980s, hybrids between these subspecies have also been found. *Ulmus glabra* Hudson (wych elm) is present along the Italian peninsula in small, scattered populations, separated by hundreds of kilometres. The survival of these populations is at risk due to the spread of DED and for the changing climatic conditions. Wych elm is a species with average DED resistance, but which suffers considerable damage under stress conditions. A sampling of symptomatic plants was carried out throughout the Italian peninsula to perform the genetic characterisation of the *O. novo-ulmi* populations and to monitor the relative abundance of the two subspecies in selected populations of *U. glabra*. We also compared two sets of samples available at the IPSP-CNR from the pre-epidemic phase (2001) and the epidemic phase (2013). Sixty-three isolates of *O. novo-ulmi* obtained from different elm populations were selected and genetically characterised. The results of this study are providing a clear and detailed picture of how these two subspecies and their hybrids evolved in this area. Information regarding the evolution of *O. novo-ulmi* populations, the dynamics of the spread over time and space were analysed and suggestions for future scenarios were developed.

This research was financially supported by European Union - Next- GenerationEU - National Recovery and Resilience Plan (NRRP) – MISSION 4 COMPONENT 2, INVESTIMENT N. 1.1, CALL PRIN D.D. MUR n. 104 del 2/2/2022 - Project "MONTANA: Ulmus glabra protection in Italian peninsula", 2022SFNMYC.

Etiology of Pine Ghost Canker in Southern California Urban Forests. L. SHANNON[1], B. MARCELO[1], E. KARINA[1], C. CARLOS[1], A. ADAM[1], K. JOHN[2], S. CHRISTOPHER[2], E. AKIF[1]. [1]*University of California, Davis, United States of America;* [2]*University of California, Agricultural and Natural Resources, University of California, United States of America.* E-MAIL: sclynch@ucdavis.edu

Pine ghost canker is a recently described disease affecting multiple pine species in urban forests of Southern California. Symptoms include wedged cankers with irregular margins and cryptic discoloration on cross-sections of branches, which can lead to severe dieback and potentially tree death. In this study, we identified and characterized five *Neofusicoccum* species (*N. luteum*, *N. mediterraneum, N. parvum, N. stellenboschianum*, and *N. vitifusiforme*) as the primary etiological agents of pine ghost canker. These pathogens were consistently isolated from multiple symptomatic pine samples (n = 41) and identified by morphology and phylogenetic analyses using four DNA barcodes (rDNA ITS, tef1, tub2, and rpb2). Pathogenicity was confirmed on healthy branches of 15-year-old Monterey pines, where the five *Neofusicoccum* species, caused vascular lesions that were not significantly different in length. Secondary fungi (*Diaporthe, Diplodia, Neopestalotiopsis*, and *Pestalotiopsis* spp.) were also recovered from symptomatic tissues but did not cause vascular lesions in pathogenicity tests. The optimal temperature for mycelial growth of *N. luteum* and *N. parvum* was 30°C, whereas for *N. mediterraneum, N. stellenboschianum* and *N. vitifusiforme*, it was 25°C. All five species were able to resume growth at room temperature (20°C) after showing no growth during a 7-day exposure to 5°C and 40°C. This study constitutes the first report of *N. luteum, N. stellenboschianum*, and *N. vitifusiforme* causing pine ghost canker in California. Environmental factors such as warmer temperatures, irrigation, and pest infestations are discussed as drivers of disease expression in pine trees. Management practices are also proposed.

CONCURRENT SESSION E1 – Recent and future developments in plant pathogen diagnostics

SESSION KEYNOTES

Using High Throughput Sequencing to resolve cases of mistaken identity. A.R. FOWKES[1,2], N. BOONHAM[1,2], A. FOX[1]. [1]*Fera Science Ltd, York Biotech Campus, York, United Kingdom, YO41 1LZ.* [2]*Newcastle university, Newcastle upon Tyne, Tyne and Wear, United Kingdom, NE1 7TU.* E-MAIL: aimee.fowkes@fera.co.uk

Traditionally, virus discovery, has been driven by viruses causing charismatic, conspicuous symptoms on hosts. Viruses were characterised by host range, serology, physical characteristics etc. Partial sequence data was only generated from the advent of PCR based methodologies. Since 2009, use of high throughput sequencing (HTS) has enabled detection and molecular characterisation of novel viruses based on sequence alone. Disconnect between pre- and post-sequencing eras has led to accidental 'rediscovery' of viruses, which has the potential to lead to unnecessary regulation or incorrect conclusions on host ranges and distribution. Three case studies will be presented where HTS has been used to resolve mistaken identity. (1) Resolving the "rediscovery" of

plantain virus X is an ideal example, where sequencing of original isolates of the virus allowed for taxonomic clarification, information on host range, geographic distribution and removal of pending regulation. However, this represents an ideal scenario where the virus was preserved in an accessible collection, after forty years the virus was detected in plants at the same location and the willingness of laboratories to share their data and collaborate to form a comprehensive study on this virus. (2) In Ullucus, Andean potato latent virus and potato leafroll virus have previously been reported, based on serological testing. Contemporary serology testing also suggested the presence of these regulated viruses. Yet, testing by HTS identified novel viruses closely related to these viruses, suggesting a cross reaction with the antisera. In contrast to plantain virus X, lack of original material has so far prevented further work to obtain sequence from the original isolates and do comparison of sequence. (3) In UK pea crops, HTS informed surveillance demonstrated presence, distribution and impact of turnip yellows virus in peas, a virus not previously associated with this host in the UK. As HTS is increasingly used for virus detection and discovery, but the biological relevance and context is lacking behind due it's costly and time-consuming nature, what tools can we use to determine which viruses are of most concern and therefore where we should allocate resources. Increased use of HTS in virome studies will result in new virus findings, but these give little context required for risk assessment. As we continue to use HTS, how can we gather more contextual information to better inform identified viruses?

The evolution of diagnostics for graft-transmissible diseases of citrus from a citrus germplasm quarantine program perspective. G. VIDALAKIS. *Department of Microbiology & Plant Pathology, Citrus Clonal Protection Program, University of California, Riverside, CA 92521, United States of America.* E-MAIL: vidalg@ucr.edu

Over the past century, the diagnostics for graft-transmissible diseases of citrus have evolved from visual symptom identification to advanced molecular and in silico tools. This transformation is embodied in the evolution of the Citrus Clonal Protection Program (CCPP) at the University of California, Riverside, which has served as a global model for germplasm quarantine, diagnostics, and the distribution of pathogen-tested citrus propagative materials. Early diagnostic efforts, dating back to the 1930s, relied on visual inspection of field symptoms such as psorosis bark scaling. The development of biological indexing in the 1950s marked a major advancement, enabling earlier symptom expression on a range of indicator plants under controlled environmental conditions. However, bioindexing posed inherent challenges, including the need for extensive greenhouse space, highly trained personnel, and high variability in results across different programs and geographic regions. With the introduction of ELISA and sPAGE in the 1970s and PCR-based methods in the 1990s, diagnostics became more specific and scalable. Today, high-throughput sequencing and bioinformatics platforms such as EDNA and MiFi have introduced a paradigm shift in pathogen detection, enabling rapid, sensitive, and comprehensive screening of citrus germplasm. Despite these technological advances, the integration of classical and novel diagnostic tools remains essential to confirm ambiguous results and ensure regulatory compliance. At the CCPP, the diagnostic strategy goes far beyond selecting the "best" test. It implements a multi-layered system that combines bioindexing with molecular tools and continuous innovation, including instruments engineering, qPCR assays updates and validation, synthetic internal standards, bulk sampling, LAMP field diagnostics, controlled environment agriculture, indicator optimization, and light spectrum manipulation to enhance symptom expression. An example of this evolution is the development of a high thruput samples processing system and two universal SYBR® Green RT-qPCR assays for eight citrus viroids, which replaced biological indexing as the official California Department of Food and Agriculture test under the state's mandatory Citrus Nursery Stock Pest Cleanliness Program. More recently, EDNA-MiFi probes were developed for 20 graft-transmissible citrus pathogens. These assays enable simultaneous screening of multiple pathogens without the need for individual tests, reducing costs and increasing efficiency. They were validated according to the MIQE guidelines to ensure reproducibility, sensitivity, and specificity. Assay optimization included melting temperature analysis, degenerate primer design, and compatibility with phenol-chloroform, spin column, and magnetic bead RNA extraction—methods tailored to high-throughput environments. These advancements have supported tens of thousands of viroid tests, achieved a 0% viroid infection rate in commercial nurseries by 2023, led to the discovery of citrus viroid VII, and increased the CCPP's quarantine testing and germplasm introduction capacity by 300%. Lab operations emphasize meticulous sampling, preparation, and workflow design to prevent cross-contamination. These include one-directional sample movement, clean/dirty zones, equipment swab-testing and decontamination, and use of internal synthetic stand-

ards. Sample pooling and targeted tissue selection ensure reliable results, especially for asymptomatic or low-titer infections. This presentation offers a retrospective of citrus diagnostics through the lens of the CCPP's evolution, highlighting how research, standardized protocols, infrastructure, and multidisciplinary collaboration ensure the sustainability of citrus quarantine diagnostics.

Rapid detection of mycotoxigenic *Aspergillus* spp. associated with pistachios using Loop mediated isothermal amplifications (LAMP). W. MELLIKECHE[1], A. RICELLI[2], M. ABUKHMAISH[1], R. CARACCIOLO[3], M. GALLO[1], C. CASINI[3], G. COLELLI[4], F. VALENTINI[1], A.M. D`ONGHIA[1]. [1]*International Center for Advanced Mediterranean Agronomic Studies, Bari, Italy.* [2]*National Research Council- Institute of Molecular Biology and Pathology (CNR-IBPM), Rome, Italy.* [3]*Enbiotech SRL, Palermo, Italy.* [4]*Department of Agriculture, Food, Natural resources & Engineering, University of Foggia, Foggia, Italy.* E-MAIL*:* mellikeche@iamb.it

Aspergillus species create major postharvest problems due to the food losses caused by their mere presence and the hazardous mycotoxins they produce. The major mycotoxins associated with these species are aflatoxin B1 (AFB1), produced mainly by *A. flavus* and *A. parasiticus*, and ochratoxin A (OTA), produced mainly by *A. carbonarius*. In this study, we developed three rapid detection assays for the aforementioned species based on Loop-mediated isothermal amplification (LAMP). Three assays were developed targeting genes from mycotoxin production clusters, two of them are species-specific targeting genes pks and aflT for *A. carbonarius* and *A. flavus*, respectively. The third assay is generic to detect aflatoxigenic Aspergilli associated with pistachios including *A. flavus, A. parasitius* and *A. nomius*. Result visualization was done in real-time via detection of fluorescent signals. The method developed showed high sensitivity and specificity with detection limits of 0.3 pg/reaction of DNA for the *A. carbonarius* assay and 0.03 pg/reaction for the other two assays. The assays were further implemented on inoculated nuts, including pistachios and almonds, after a one- step crude DNA extraction. These tests revealed a detection level of 0.5 spore/g that shows the effectiveness of LAMP as a rapid method for detecting potentially toxigenic *Aspergillus* spp. directly from food. The validation of the assays included tests on a larger scale that further confirmed their sensitivity and specificity, as well as enabled the production of ready-to-use LAMP kits. These kits are easy to use and aim to simplify the screening of food samples in order to monitor the presence of specific *Aspergillus* contaminations.

Application of isothermal amplification and high throughput sequencing methods for enhanced virus detection in yam. G. SILVA[1], R. FESTUS[1,2], R. PREMPEH[3], M.D. QUAIN[3], S.E. SEAL[1]. [1]*Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK;* [2]*current address: Department of Agricultural Science and Plant Protection, Mississippi State University, Mississippi, MS 39762, USA;* [3]*Council for Scientific and Industrial Research-Crops Research Institute, Fumesua, P.O. BOX 3785, Kumasi, Ghana.* E-MAIL: g.silva@gre.ac.uk

Yam (*Dioscorea* spp.) productivity is severely compromised by viruses and the lack and high cost of clean planting material. Propagation through 'seed' yam tubers encourages the recycling of infected planting materials, increasing virus incidence and yield losses, a common issue for vegetatively propagated crops. In Ghana, yam farmers often use low-quality, virus-infected planting material from their own or neighbouring farms. The only effective control method is to use virus-free seed yam. The Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) developed an aeroponics and hydroponics system to enhance seed yam production. This system has a high multiplication rate, generating thousands of plantlets from a single plant. However, virus titres in the plantlets are reduced but not eliminated, complicating a reliable virus detection and making it difficult to ensure that planting material is virus-free. This could lead to false virus-negative certification of planting materials, spreading infected materials and threatening crop production and food security. We have developed and evaluated isothermal amplification- and high throughput sequencing (HTS)-based diagnostic tests for virus detection in various crops. The yam tests have been transferred to CSIR-CRI, improving their capacity in diagnostics and seed certification and contributing towards the production and sustainable supply of high-quality seed yams. We will discuss the potential wider application of these tests and the factors influencing their future deployment in support of seed systems of root and tuber crops.

Early diagnostics of *Erysiphe corylacearum* causing emerging powdery mildew on hazelnut. S. MATIĆ[1,2], C. D'ERRICO[2], M. BARONE[1], S. DAVINO[1], E. NORIS[2], M. MOIZIO[3], G. MASOERO[4]. [1]*Department of Agricul-*

tural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, edificio 5, 90128 Palermo, Italy. [2]*Institute for Sustainable Plant Protection, National Research Council, Strada delle Cacce 73, 10135 Turin, Italy.* [3]*SAGEA Centro di Saggio s.r.l., 12050 Castagnito d'Alba, CN, Italy.* [4]*Accademia di Agricoltura di Torino, Via Andrea Doria 10, 10123 Turin, Italy.* E-MAIL: slavica.matic@unipa.it

Erysiphe corylacearum has recently emerged as the causal agent of a new and dangerous powdery mildew affecting hazelnut in northern Italy and other European regions. After shifting to hazelnut as a new host in Asia, the fungus rapidly spread to several European countries within a few years. Compared to the common hazelnut powdery mildew caused by *Phyllactinia guttata*, this pathogen poses a greater threat due to its substantial impact on yield and hazelnut quality. The disease's emergence in Italy revealed distinct *E. corylacearum* subgroupings, population expansion, and high genetic similarity with recently identified isolates from other regions. Effective containment requires early and reliable detection, ideally before symptom onset. In this study, we evaluated innovative, field-deployable diagnostic methods, including isothermal amplification, NIRS / Raman spectroscopic analyses, and foliar pH measurement. These techniques, integrated with portable devices capable of cloud data storage and mobile phone-based remote monitoring, enabled rapid, non-destructive detection of the disease within 30 minutes directly in the field. Both isothermal amplification and spectroscopy-based approaches showed high accuracy in distinguishing between asymptomatic and symptomatic plants before visible symptoms appeared. Our findings suggest that these methods, through measurements of amplification curves or spectral patterns, offer a promising basis for non-destructive diagnosis of emerging hazelnut powdery mildew and potentially other plant diseases. The portability and ease of use of these systems allow their application directly by farmers, without the need for laboratory expertise, making them highly useful in supporting timely disease control.

This research was financially supported by the Cassa di Risparmio di Cuneo (CRC) Foundation (LODICAM project).

Unmasking the hidden microbiome of *Fagus sylvatica*: Combining PNA clamps and targeted Primers to explore seasonal variation in endophytic diversity. I. GIUBILEI[1], S. TURCO[1], L. MAHAWAR[2], B.R. ALBRECTSEN[2], A. MAZZAGLIA[1]. [1]*Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy.* [2]*Department of Plant Physiology, Umeå Plant Science Centre (UPSC), Umeå University, Umeå, Sweden.* E-MAIL: irene.giubilei@unitus.it

Climate change is reshaping forest ecosystems, threatening the health and stability of key species like *Fagus sylvatica*. Understanding the dynamics of plant-microbiome interactions is essential, as these microbes influence plant resilience and stress response. This study evaluated the efficiency of Peptide Nucleic Acid (PNA) clamps and selective primers in enhancing bacterial and fungal metagenomic profiling, and explored seasonal dynamics of the endophytic community in *F. sylvatica*. Amplicon sequencing was performed using Illumina technology, targeting the bacterial 16S rRNA gene (V3-V4 region) and the fungal internal transcribed spacer regions (ITS1 and ITS2). The application of PNA clamps significantly improved bacterial detection by reducing host DNA interference and increasing microbial read diversity. Among fungal targets, the ITS1 region amplified with the ITS1F/ITS2 primer set proved most efficient, offering higher taxonomic resolution and greater community richness than other approaches. Seasonal shifts were observed in both bacterial and fungal communities, with notable differences in composition, diversity, and relative abundance between early and late season samples. Certain bacterial (e.g., *Pseudomonas*, *Sphingomonas*) and fungal taxa (e.g., *Stemphylium*, *Epicoccum*) showed clear seasonal enrichment patterns. Interestingly, the increasing abundance and complexity of fungal communities later in the season may contribute to the improved performance of PNA clamps, suggesting the microbial load could influence host DNA suppression efficiency. These findings confirm the effectiveness of PNA clamps and selective primers in refining endophytic profiling and reveal seasonal shifts in the *F. sylvatica* microbiome. These insights deepen our understanding of plant-microbe interactions and support strategies for forest resilience under changing environmental conditions.

This research was funded by AGRITECH PNRR SPOKE 7.

Detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds: Challenges encountered and methodology approaches to address them. M.C. HOLEVA, P.E. GLYNOS, C.I. REPPA, I. DERVISI, C.D. KARAFLA. *Benaki Phytopathological Institute, Scientific Directorate of Phytopathology, Laboratory of Bacteriology, 8 Stefanou Delta str., GR 14561, Kifissia, Greece.* E-MAIL: m.holeva@bpi.gr

Seed-transmitted bacterial pathogens are of major concern in plant health, as they cause crop diseases with high economic impact, especially when they belong to regulated pathogens and phytosanitary measures are applied. Contaminated or infected seeds can be a major pathway for bacterial pathogens to enter a new area and spread in long distances. Due to the possible variability of treatment efficacy of the commercially produced seed against bacterial pathogens, it is crucial to employ sensitive, specific and robust diagnostic methodologies to detect and exclude contaminated/infected seed lots. Currently, the official seed testing methods are based on standard (validated) protocols relying primarily on the isolation of bacterial pathogens from seeds on semi-selective media. However, this approach can be hampered by factors such as: the outgrowth of the pathogen by other microbes that are present in the tested seeds, the possible viable but nonculturable cell (VBNC) status of the pathogen, the low pathogen population in the seeds and the interference of seed coating in the isolation procedure. In the present study, results of laboratory efforts to address such diagnostic difficulties and assess the presence of live pathogens in seed samples employing a grow-out assay and a PCR-based approach will be presented, focusing on the bean pathogen *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. The bean crop is of high economic importance for several Regional Units in Greece in which beans constitute a protected designation of origin (PDO) product. Exclusion of contaminated/infected bean seeds is essential to preserve local varieties and achieve increased productivity of enhanced quality.

Monitoring *Erwinia amylovora* spread in apple orchards: development of a detection protocol from corbicular pollen. B. VICELLI[1,2], V. GUALANDRI[1,2], T. FATTORE[1], C. CAINELLI[1], G. PUOPOLO[2,3]. [1]*Fondazione Edmund Mach, Centro di Trasferimento Tecnologico, Via E. Mach 1, 38098 San Michele all'Adige, Italy.* [2]*Centro Agricoltura Alimenti Ambiente (C3A), Università degli Studi di Trento, Via E. Mach 1, 38098, San Michele all'Adige, Italy.* [3]*Fondazione Edmund Mach, Centro di Ricerca e Innovazione, Via E. Mach 1, 38098 San Michele all'Adige, Italy.* E-MAIL: bianca.vicelli@fmach.it

Fire blight, a destructive disease caused by *Erwinia amylovora* (Ea), significantly threatens global apple and pear production. Since honeybees play a key role in Ea transmission within orchards, a qPCR-based protocol has been developed for the early detection of Ea from corbicular pollen. Uninfected pollen was inoculated with a cell suspension of strain Ea21 (rifampicin-resistant) at different concentrations (from 1×10^8 to 1×10^0 CFU mL^{-1}). Uninoculated pollen was used as an untreated control. One gram of spiked samples was serially diluted and plated on Nutrient Agar, which was amended with rifampicin for the CFU counting. Concurrently, 30 g of spiked samples were homogenised in 120 mL of 0.85% (w/v) NaCl and 0.001% (v/v) TWEEN®80 and incubated on ice under orbital shaking (140 rpm). After 1 h, samples were centrifuged to remove impurities. The supernatants were immediately collected and centrifuged. The resulting pellets were suspended in Tris-HCl (pH 8) buffer and exposed to thermal shock. Total DNA was extracted with DNeasy® mericon® Food kitand amplified in qPCR according to EPPO standard PM 7/20. Following validation, the robustness of this protocol was meticulously tested on corbicular pollen collected in two Trentino valleys. In 2022 and 2023, over 200 corbicular pollen samples from Valsugana and Val di Non were analysed in real-time, ensuring the protocol's reliability and accuracy. The protocol developed in this study has the potential to serve as a crucial predictive tool in the future. It could provide an early warning system for potential Ea infection in apple orchards, offering hope for more effective disease management strategies.

POSTER PRESENTATIONS

Chlorophyll spectroscopy and fluorescence for the racial characterization of yellow rust in wheat. M.T. GARCIA-LOPEZ[1], M.F. RUZ-RUIZ[1], J.R. PORRAS-PEREZ[2], J.C. SILLERO-SANCHEZ[2], J.A. JIMENEZ-BERNI[1]. [1]*AgroPhenoLab, Institute for Sustainable Agriculture-Spanish National Research Council (IAS-CSIC), Cordoba, Spain.* [2]*Breeding and Biotechnology Area, Andalusian Institute of Agricultural Research and Training (IFAPA). Alameda del Obispo Center, Cordoba, Spain.* E-MAIL: tgarcialopez@ias.csic.es

Yellow rust (Yr), a fungal disease caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), affects both durum and bread wheat. Recently, new Asian *Pst* races have caused significant losses in European crops, even in previously resistant varieties. This polycyclic disease progresses rapidly when conditions are favourable, as occurred in spring 2024. Our study characterized different Yr races in 52 near-isogenic lines (NILs) of the susceptible 'Avocet' cultivar, each containing a single Yr resistance gene. We also evaluated bread wheat varieties (*Triticum aestivum* L.) with known resistance genes. Plants were visually assessed four times during spring using the Cobb

severity scale. Simultaneously, we measured flag leaf photosynthetic activity (4 repetitions/NIL) with a PhotosynQ fluorometer and micro-plot spectral reflectance (3 repetitions/NIL) using an SVC HR-1024i spectroradiometer. We observed significant differences in disease severity among NILs: some showed susceptibility, others displayed hypersensitive responses with cellular necrosis, and some remained resistant. Several genotypes with medium severity allowed us to correlate symptom evolution with spectral and photosynthetic parameter changes, demonstrating a direct relationship with visual observations. Developing phenomic tools for early detection of new races of this virulent pathogen is crucial for implementing fast screening, providing objective assessments applicable to large-scale studies and resistance gene identification research in cereals. Moreover, these methodologies could also be applicable in the context of precision agriculture where early detection and disease mapping can enable mitigation measures.

This research was financially supported by research projects PID2020-118650RR-C32 and PID2020- 118650RR-C33 from MICIU/AEI/10.13039/501100011033. M. Teresa García López's contract is part of grant JDC2022-048362-I, from MICIU/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR.

Development of an isothermal recombinase polymerase amplification assay for the field deployable detection of Begomoviruses. B. DAVENPORT[1], M. AMATO[2], N. WHITE[1], A. HARNESS[1], H. FALZON[1], K. RAU[1]. [1]*Research and Development, Agdia, Inc. Elkhart, IN 46514, United States of America.* [2]*Managing Director, Agdia-EMEA, Soisy-sur-Seine 91450, France.* E-MAIL: marcos.amato@agdia-emea.com

The Begomovirus genus is the largest of all plant viruses comprising over 450 recognized species infecting several economically important fiber, root, and vegetable dicotyledonous crops. Begomoviruses are persistently transmitted by whiteflies, distributed worldwide with higher prevalence in tropical and subtropical regions, and consist of single stranded circular DNA genomes with both monopartite and bipartite organization. High rates of genomic recombination and population introduction through global trade can lead to issues with established diagnostic methods missing new isolates, variants, or even species. To circumvent both novel speciation detection and rigorous lab-based testing, a novel recombinase polymerase amplification (RPA) assay was developed and validated for the detection of Begomoviruses. The developed RPA reaction utilizes a crude plant extract, runs at a single operating temperature for 20 minutes, and generates real-time amplification data. The assay has successfully identified 56 isolates from 28 diverse species and is predicted to be able to detect over 350 species based on insilico analysis. The RPA was screened against 42 potential host species to assess performance. The validated test provides a novel tool in screening suspect plants for several Begomoviruses in a single field deployable reaction.

Development and validation of a field-based colorimetric lamp assay for the detection of *Clavibacter michiganensis* in tomato plants. G. MERMIGKA[1,2], M. MEGARITI[3], M.G. PAGOULATOU[1], E. GIZELI[3,4], D.E. GOUMAS[1,2]. [1]*Hellenic Mediterranean University (HMU), School of Agricultural Sciences, Department of Agriculture, Laboratory of Biotechnological Applications and Phytopathology, GR71004, Heraklion, Greece.* [2]*Hellenic Mediterranean University (HMU), University Research Centre, Institute of Agri-Food and Life Sciences, GR71410, Heraklion, Greece.* [3]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece.* [4]*Department of Biology, University of Crete, Heraklion, Greece.* E-MAIL: dgoumas@hmu.gr

Point-of-care (POC) diagnostics are revolutionizing the detection of plant pathogens by enabling rapid, on-site identification without the need for specialized laboratories. One of the tools used for this purpose is Loop-mediated isothermal amplification (LAMP). LAMP is a powerful molecular technique increasingly used in pathogen control for its rapid, sensitive, and specific detection of plant pathogens. Unlike traditional PCR, LAMP operates at a constant temperature, eliminating the need for expensive thermocycling equipment, which makes it ideal for field-based applications. In this study, we have developed and evaluated a portable colorimetric LAMP assay for the detection of *Clavibacter michiganensis* (Cm) in tomato plants. Initially, we designed a set of primers for micA gene that shows high specificity for this subspecies and tested them against genomic DNA (gDNA) isolated from Cm and *C. sepedonicus*. Next, we evaluated a) the detection limit of Cm in plant extracts spiked with bacteria, b) the specificity in lysates spiked with Cm and other bacteria frequently occurring in tomato plants under field conditions, c) the effect of plant lysates from different tomato varieties in Cm detection and d) the detection of Cm in artificially infected tomato plants. The minimum concentration that we could consistently detect Cm was 10^5 CFU while we were able to detect Cm

as soon as four days post infection in the stems of the infected plants. Altogether, from our findings we can conclude that the developed method for Cm detection can be used as a reliable tool for the early detection of the pathogen.

The study was part of the project "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP"- TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative Agriculture and Food Industry.

Advanced diagnostic strategies for the detection and identification of seed-borne viruses at ICARDA to enhance seed health security. S.G. KUMARI, A. MOUKAHEL. *Seed Health/Virology Laboratory, International Center for Agricultural Research in the Dry Areas (ICARDA), Terbol Station, Beqa'a Valley, Zahle, Lebanon.* E-MAIL: s.kumari@cgiar.org

ICARDA's Seed Health Laboratory (ICARDA-SHL) is responsible for the monitoring, clearance, and documentation of the safe germplasm movement at the Center. The laboratory processes over 50,000 samples annually for seed-borne pathogens/pests, for the purpose of short- and long-term conservation and global distribution. This rigorous screening process is essential for preventing the spread of seed-borne pests across borders, especially viruses that can be transmitted further by insect vectors upon introduction. This has made the management of seed-borne viruses more challenging, as infected seeds can introduce the virus into new areas and lead to infections in subsequent growing seasons. Early detection and accurate diagnosis of viral diseases are critical for implementing effective control measures and strategies. In this context, ICARDA-SHL is actively engaged in the development, validation and dissemination of advanced diagnostic tools and protocols for germplasm health testing, facilitating technology transfer to NPPOs. Accordingly, ICARDA-SHL has developed and applied various molecular tests for the detection and identification of seed-borne legume viruses, including IC-RT-PCR, uniplex RT-PCR and multiplex RT-PCR in a single direct test. These advanced diagnostic techniques allow rapid analysis of large numbers of samples at a relatively low cost, high throughput, short execution times and enhanced accuracy, thus ensuring the availability of certified seeds, which are key strategies to mitigate the spread of seed-borne legume viruses that cannot be controlled by conventional plant treatments. In this presentation, validated molecular tools for the detection and identification of selected legume viruses (e.g. AMV, CMV, BYMV, PSbMV) applied by ICARDA-SHL will be described.

Molecular diagnostics for greenhouse populations of *Alternaria* spp. (*Ascomycota*, *Pleosporaceae*) on tomatoes. L. DUČKENA[1,2], N. BESSADAT[2,3], N. BATAILLÉ-SIMONEAU[2], F. BASTIDE[2], B. HAMON[2], M. KOPPEL[4], K. LOIT[4], N. RASIUKEVICIUTE[5], G. BIMSTEINE[1], P. SIMONEAU[2]. [1]*Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies, Liela Street 2, LV- 3001, Jelgava, Latvia*. [2]*University of Angers, Institut Agro, INRAe, UMR 1345 IRHS, SFR 4207 QUASAV, Beaucouzé Cedex, 49070, France*. [3]*Laboratory of Applied Microbiology, University of Oran1 Ahmed Ben Bella, BP 1524, El M'Naouer, 31000, Oran, Algeria*. [4]*Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr.R. Kreutzwaldi 1, 51006, Tartu, Estonia*. [5]*Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kauno Street 30, LT-54333, Babtai, Lithuania*. E-MAIL: Lilija.Duckena@lbtu.lv

Diseases caused by fungal pathogens, including *Alternaria* spp., are one of the major limiting factors in the production of tomato (*Solanum lycopersicum* L.) due to devastating yield losses. Fungi from the genus *Alternaria* are characterized by overlapping of the morphological characters among several species; therefore, DNA-sequence based identification is required for accurate species identification that is critical for the development of disease management strategies. In our study, a total of 896 plant samples with typical disease symptoms caused by *Alternaria* spp. were collected from greenhouses in Latvia, Lithuania, and Estonia between 2022 and 2024. Recovered *Alternaria* spp. strains were further identified at the species level using a multigene phylogenetic framework. Initial identification of fungal strains based on the internal transcribed spacer regions 1 and 2 and intervening 5.8S nrDNA (ITS) confirmed the occurrence of four sections from the genus *Alternaria*, namely *Alternaria*, *Porri*, *Infectoriae,* and *Ulocladioides*. Small-spored *Alternaria* strains from the section *Alternaria* were further analysed based on RNA polymerase II second largest subunit (*rpb2*), putative F-box-domain- containing protein (*ASA-10),* and putative histone-like transcription factor (*ASA-19*), and *Alternaria arborescens* and *A. alternata* species complexes along with *A. postmessia* were identified on tomato. Further analysis of large-spored *Alternaria* spp. (section *Porri*) based on a multi-locus phylogeny of ITS, *rpb2,* glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), translation elongation factor 1-α

(*tef1*), and Alternaria major allergen gene (*alt a 1*) confirmed the occurrence of five *Alternaria* species on tomato in the Baltic region, including novel pathogens that represent two previously undescribed taxa of the genus *Alternaria*.

This research was financially supported by the ERAF project No. 1.1.1.8/1/24/I/002 "LBTU Doctoral Support and Development Initiative".

A multi-method approach for detection of *Tomato brown rugose fruit virus*. E. DEL ÁNGEL DE LA CRUZ ARÉVALO[1], J. GARITA-CAMBRONERO[2], P. OLIVARES PACHECO[3], A. DE FRANCESCO[3], A.P. FERNÁNDEZ-GETINO GARCÍA[1]. [1]*Dirección Técnica de Evaluación de Variedades y Laboratorios, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain.* [2]*Instituto Tecnológico Agrario de Castilla y León (ITACyL), Subdirección de Investigación y Tecnología Área de Innovación y Optimización de Procesos Laboratorio de Biología Molecular y Secuenciación, Valladolid, Spain.*[3]*Asociación Nacional de Obtentores Vegetales (ANOVE), I+D en Protección Vegetal, Madrid, Spain.* E-MAIL: agustina.defrancesco@anove.es

Tomato brown rugose fruit virus (ToBRFV) is an emerging plant virus posing a significant threat to tomato and pepper production worldwide. To enhance early and accurate detection, we developed and evaluated an optimized diagnostic workflow combining multiple methods: LAMP, RT-qPCR, and bioassay, tailored for use across different stages of diagnosis, from greenhouse pre-screening to precise molecular confirmation and direct detection in host plants. The work was carried out with ToBRFV naturally infected seeds. The sensitivity of the bioassay was compared with RT-qPCR, contributing to the bioassay method validation. A LAMP protocol, adapted from a published seed detection method, showed improved performance under our lab conditions starting from seed extracts instead of isolated RNA. A standard curve generated from serial dilutions of crude seed extracts and purified RNA showed consistent RNA detection up to RT-qPCR Ct values of ~26, while LAMP detected infections up to the 10^{-5} dilution of crude extracts (Ct ≈ 30.85), surpassing purified RNA in sensitivity. The LAMP method will be further assessed for its viability in greenhouse pre- screening in terms of speed, cost, and diagnostic accuracy. Comparing sensitivities, both bioassay and LAMP exhibited comparable limits of detection, detecting up to one infected seed in a 400 seed lot. The RT-qPCR is the most sensitive method, detecting one ToBRFV- infected seed in a total of 1000 seed lot. These findings underscored the potential of bioassays in discerning virus infectivity, although further validation and standardization are necessary. A final diagnostic series will be proposed for efficient ToBRFV detection.

This research was financially supported by Project CON23-316. Convenio entre la Agencia Estatal Consejo Superior de Investigaciones Científicas, M.P., a través del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, y la Asociación Nacional de Obtentores Vegetales, para la realización de investigaciones en materia de sanidad de semillas. BOE-A-2024-4494.

Development of a portable real-time PCR and digital droplet PCR assay for *Cercospora beticola* detection on beet. M. CRUDELE, C. LAGUARDIA, P.R. ROTONDO, S. LAERA, T. MASCIA, R.M. DE MICCOLIS ANGELINI, F. FARETRA. *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.* E-MAIL: ritamilvia.demiccolisangelini@uniba.it

Cercospora beticola, the causal agent of Cercospora leaf spot, is one of the most destructive pathogens affecting leaf beet (*Beta vulgaris*). The pathogen spreads rapidly under warm and humid conditions and frequently coexists in mixed infections with *Phoma betae*. Disease management is challenging, particularly due to the emergence and spread of fungicide resistance in the pathogen populations. Rapid and accurate pathogen detections is essential for the development of effective and sustainable strategies of disease control. This study aimed at developing new sensitive and specific TaqMan probe-based assays for rapid detection and quantification of *C. beticola* using portable real-time qPCR systems for in-field diagnostic tests, and digital droplet PCR (ddPCR) for lab tests. The specificity of both qPCR and ddPCR was confirmed against a panel of fungal and bacterial species commonly associated with beet crops. Sensitivity was calculated as the mean of three independent experiments. The limit of detection for the ddPCR assay was 10 pg of DNA, while qPCR was 100-fold more sensitive, quantifying up to 0.1 pg of fungal DNA. To enable in-field detection, different protocols for rapid DNA extraction from fresh leaves, crop residues, and seeds using commercial kits (Biomeme Sample Homogenization and M1 Sample Prep Cartridge kit for DNA; Sigma-Aldrich REDExtract-N-Amp Plant PCR Kit; and BN QuickPick Plant DNA) were tested and compared in qPCR assays. The developed methods for early detection and quanti-

fication of *C. beticola* provide valuable tools for enhancing epidemiological studies that can contribute to design appropriate strategies for the disease management.

This research was financially supported by National Recovery and Resilience Plan, Mission 4, Component 2: "From Research to Business" – Investment 3.3. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Investigating microbiome shifts in tomato plants under pathogen pressure. E. FRANCOMANO[1], M.M. ACI[1], M. CASUSCELLI[1], N. MOHAMED[1], S. MOSCA[1], L. SCHENA[1], A. MALACRINÒ[1,2]. [1]*Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, 89124 Reggio Calabria, Italy.* [2]*Department of Biological Sciences, Clemson University, Clemson, SC, USA.* E-MAIL: edda.francomano@unirc.it

The plant microbiome plays a critical role in maintaining plant health and productivity, influencing plant tolerance to both biotic and abiotic stressors. Previous research has shown that the plant microbiome composition often changes when plants are exposed to stressors. However, it is still unclear whether this response is stressor-specific and can be used to predict the occurrence of a specific stressor. Here, we set up a microcosm system using tomato as model plant to test the response of the leaf bacterial microbiome to the presence of different belowground pathogens (*Pseudomonas syringae* pv. *tomato*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Alternaria alternata*). Through amplicon sequencing, machine learning, and null-model analysis, we found that different belowground pathogens can indeed leave a signature on the leaf bacterial microbiota, either on its composition or on the ecological relationships within the microbiome. This combined approach is helping us to better understand plant-microbiome interactions under stress, to develop new tools to reliably predict the occurrence of biotic stressors in the field, and to deploy the next generation of microbiome-based plant health management strategies.

This research was financially supported by the Next Generation EU - Italian NRRP, Mission 4, Component 2, Investment 1.5, call for the creation and strengthening of 'Innovation Ecosystems', building 'Territorial R&D Leaders' (Directorial Decree n. 2021/3277) - project Tech4You - Technologies for climate change adaptation and quality of life improvement, n. ECS0000009. AM was supported by the Italian Ministry of University and Research (MUR) through the PRIN 2022 PNRR program (project P2022KY74N, financed by the European Union - NextGenerationEU). This work reflects only the authors' views and opinions, neither the Ministry for University and Research nor the European Commission can be considered responsible for them.

Early and sensitive detection of C*eratocystis ficicola* in *Ficus carica*: a new Taqman real-time PCR approach. C. CARBOTTI[1], M. CARLUCCI[1], R. SPANÒ[2], P.G. LUCCHESE[1], A. PACIFICO[1], F. NIGRO[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy.* [2]*National Research Council, Institute for Sustainable Plant Protection, Via Amendola, 122/D 70126 Bari, Italy.* E-MAIL: franco.nigro@uniba.it

Ceratocystis ficicola is a soil-borne pathogen that infects the roots, the trunk and main stem of susceptible hosts, causing vascular wilt and tree death. It was first identified as the causal agent of Ceratocystis canker in fig (*Ficus carica*) in Japan and subsequently reported in Greece and Italy. Diagnostic procedures for *C. ficicola* are not yet fully standardized. In this study, specific primers and a TaqMan real-time PCR assay were designed and implemented to develop a rapid, sensitive molecular diagnostic tool for *C. ficicola* detection. Prior to assay validation, the identity of the fungal isolates was confirmed by evaluating key morphological traits, including the size and shape of ascomata and conidia, and by amplifying the internal transcribed spacer (ITS) region using primers ITS5 and ITS4. Assay validation was conducted on a panel of fungal isolates collected from healthy and diseased *F. carica* plants. Additional tests were performed on symptomatic, asymptomatic, and artificially inoculated plant and soil samples, demonstrating the assay's ability to reliably detect the pathogen. The assay exhibited high sensitivity, detecting as little as 0.2 pg μL^{-1} of *Ceratocystis* DNA both in water and in plant DNA extracts. These findings indicate that the molecular method developed, utilizing specific primers and a TaqMan probe, offers a reliable, sensitive, and time-efficient tool for *C. ficicola* detection. Implementation of this assay will facilitate improved monitoring of the pathogen's distribution, particularly in the Apulian region, and support timely management strategies to mitigate its spread.

The DIFENDO project: Advanced diagnostics for plant health in agriculture. A. TAGLIENTI, S. BERTIN, F. COSTANTINI, N. PUCCI, P.F. ROVERSI. *National*

Reference Institute for Plant Protection (CREA Research Centre for Plant Protection and Certification), Via C.G. Bertero 22, 00156 Rome. E-MAIL: anna.taglienti@crea.gov.it

The control of plant pests is becoming increasingly challenging due to rising threats posed by food demand and security, global trade, and climate change. Key factors for such control include (i) accurate and rapid detection tests, which are crucial for the appropriate application of phytosanitary measures, and (ii) implementation of natural compounds as eco-friendly biopesticide alternatives to agrochemicals, which are currently subjected to limitations in the European Union (EU Sustainable Use of Pesticides Directive 2009/128/EC). The "DIFENDO project – *Advanced diagnostics for plant health in agriculture*", coordinated by National Reference Institute for Plant Protection (CREA-DC) and financed by the Italian Ministry of Agriculture (MASAF) aims at achieving advancements in both fields. Tests will be developed for the application of innovative tools to be used on-site (i.e., fields, entry points), based on advanced biosensors and molecular diagnostics. The proposed technologies include Lab-on- Chip systems for pest detection via real-time amplification, nanopore-based high-throughput sequencing, e-nose, and microfluidic based sensing devices. All these tools will be connected to a platform for data transmission and storage, allowing for a rapid and comprehensive setting up of both preventive measures and outbreak response. The project also aims at defining testing protocols and platforms for the evaluation of natural compounds for pest control within the framework of Integrated Pest Management. The effectiveness of natural compounds (i.e., plant biomass extracts, essential oils, hydrosols) will be tested through the quantification of the target pest in the experimental plants, using advanced diagnostic systems such as digital PCR and microfluidic networks.

Phylogenetic relationships of *Alternaria* species infecting apple trees in northeastern Spain. M.VILA DE VARGAS, M. PLA, A. NADAL, I. LLORENTE, C. MORAGREGA. *Institute of Agri-food Technology (INTEA), Carrer Maria Aurèlia Capmany, 61. University of Girona, 17003 Girona, Spain.* E-MAIL: marc.viladevargas@udg.edu

In recent years, an emerging apple tree disease characterized by lesions on leaves and fruits has been reported. This disease is of increasing importance in both Australia and various regions of Europe (Italy, France, and Spain). The disease symptoms were first detected in apple orchards in the Girona region (Catalonia, northeastern Spain) in 2013. Within two years, the disease had spread to up to 20% of 'Golden' and 'Gala' apple orchards, and since then, its impact on production losses has persisted. This disease has been attributed to a species complex within *Alternaria* section *Alternaria*, though the specific species involved in Catalonia outbreak remain unknown. Due to its recent emergence and the limited information available regarding its etiology and epidemiology, there are currently few detection and management methods, posing a potential threat to the major apple-producing regions of Europe. To develop a qPCR-based tool for detecting pathogenic *Alternaria* species affecting apple trees, an analysis of the species diversity associated with the disease in Catalonia was conducted. This analysis was based on four newly developed molecular markers that allow for the distinction of phylogenetic lineages within *Alternaria* sect. *Alternaria*. Sixty *Alternaria* isolates from the INTEA-UdG collection, obtained from different symptomatic apple cultivars from various orchards in Girona, were selected for analysis. Isolates included a range of morphotypes and varying levels of pathogenicity/virulence. This study evaluated the suitability of the molecular markers for identifying the *Alternaria* species present in apple orchards and will provide a basis for the development of pathogen detection techniques.

This research was financially supported by PID2021-126505OB-I00, MCIN/AEI/10.13039/501100011033 and by FEDER A way to make Europe; and by the predoctoral grant PRE2022-104041, financially supported MICIU/AEI/10.13039/501100011033 and by FSE+.

Study of some Calabrian olive cultivars for the selection of genotypes resistant to *Verticillium dahliae*. A. MUTO, V. VIZZARRI, M. HUSSAIN, A. IULIANO, A. DE MARCO, I. DE ROSE, F. POLIZZO, E. PERRI, E. SANTILLI. *CREA-Research Centre for Olive, Fruit and Citrus Crops, Via Settimio Severo, 83 87036 Rende (CS), Italy.* E-MAIL: elena.santilli@crea.gov.it

Verticillium dahliae, is one of the most devastating soilborne fungi of the olive tree (*Olea europaea* L.) worldwide. The olive sector plays a crucial role in Calabria's agribusiness; in fact, it is Italy's second-largest olive-growing region, just behind Puglia. However, no information regarding resistant or sensitive Calabrian cultivars affected by *V. dahliae* is available. Yet, our understanding of the defence mechanisms that operate at the root level to explain tolerance to this disease is incom-

plete. This project seeks to explore the molecular networks that contribute to the root defence mechanisms in Calabrian olive cultivars, both tolerant and susceptible, when faced with *Verticillium*. For this study, six Calabrian olive cultivars (two years old), along with non-Calabrian cultivars serving as controls, were selected for inoculation with a strain of *V. dahliae* classified as defoliating (D) at specific time intervals of 0, 2, 7, and 15 days under controlled experimental conditions (three for each time point and conditions). The disease progression of inoculated and control (non-inoculated) plants from each cultivar was first evaluated by scoring the severity and incidence of disease. Next, we quantified *V. dahliae* DNA using real-time PCR to correlate the phenological data obtained. Finally, the expression over time of nine defence-related genes and ten genes associated with the phenylpropanoid pathway was also examined under the conditions reported above. The results obtained showed links between tolerance's level to this pathogen and specific root defence mechanisms at the phenological and genetic levels.

This research was financially supported by the Ministry of University and Research TECH4YOU innovation ecosystem project DM 1049 of the 23/06/2022 and by Project INDIOL PSR CALABRIA 2014/2020 MIS. 16 INTERVENTION 16.2 decree Prot. no. 286253 of 20/06/2022

Loop-mediated isothermal amplification for seed and surface detection of ToBRFV. R. CARACCIOLO[1], E. TROIANO[1,2,3], S. LO CASTRO[1], G. ARCOLEO[1], G. PARRELLA[2]. [1]*Enbiotech srl, Via Masuccio Salernitano 28, 84012 Angri, SA, Italy.* [2]*Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR),* P.le *Enrico Fermi 1, 80055 Portici, NA, Italy.* [3]*University of Naples "Federico II", Department of Biology, Via Cinthia 26 - 80126 Naples, Italy.* E-MAIL: r.caracciolo@enbiotech.eu

Loop-mediated isothermal amplification (LAMP) is a molecular diagnostic method that enables rapid and specific detection of plant pathogens. Tomato brown rugose fruit virus (ToBRFV) is a virus that significantly impacts tomato and pepper production worldwide. The focus of this work was to evaluate the ToBRFV detection by Reverse Transcription-LAMP (RT- LAMP) in tomato and pepper seeds, and on environmental surfaces such as benches, plexiglass, glass, cardboard, and metal. Seeds were artificially contaminated at 1% and 0.1% of infection levels using a homogenate obtained from ToBRFV naturally infected leaves, previously checked for virus infection. Different surfaces were directly contaminated using homogenates obtained from 1 % of infected seeds. RNA extraction was performed following a rapid protocol based on seeds grindings and surface swab smearing, followed by RT-LAMP analysis conducted on ICGENE system. An RT-qPCR validate protocol was used to compare the efficiency and sensitivity of the proposed RT-LAMP method, using in parallel the total RNA extracted and purified from the same samples. ToBRFV was detected on all contaminated seeds and surfaces with both molecular methods, showing the same efficiency and sensitivity for both methods compared. The results of this study demonstrate the efficiency and sensitivity of LAMP technology in detecting ToBRFV in different matrices, offering a practical and rapid solution for tomato and pepper seed health screening and environmental monitoring. The user-friendly format of the diagnostic tool facilitates rapid on-site testing, thereby enabling timely interventions that minimise the risk of spreading ToBRFV in vulnerable agricultural systems.

Development of Real-time LAMP assay for the rapid detection of *Colletotrichum acutatum* in olive. F.Z. SENNOUN[1], W. MELLIKECHE[1], R. CARACCIOLO[2], A.M. D'ONGHIA[1], F. VALENTINI[1], M. GALLO[1]. [1]*International Centre for Advanced Mediterranean Agronomic Studies, Via Ceglie, 9-70010, Valenzano (BA), Italy.* [2]*Enbiotech SRL, via Del Bersagliere, 45-90143, via Masuccio Salernitano, 28-84012, Angri (SA), Italy.* E-MAIL: sennounfatmazohra@gmail.com

Olive anthracnose (OA) caused by *Colletotrichum* spp., is a significant disease that affects olive cultivation worldwide, resulting in considerable yield losses and a deterioration in olive oil organoleptic qualities. Effective management of OA requires an integrated approach with a strong focus on the accurate and precise detection of the causal agent, using specific and sensitive diagnostic tools. Among these, loop-mediated isothermal amplification (LAMP) has emerged as one of the simplest, fastest, and most accurate methods. In this study, a characterization of *Colletotrichum* spp., isolated from samples collected in Maruggio area in southern Apulia (Italy), was conducted. Six isolates were obtained from symptomatic olive drupes and identified morphologically and molecularly as *Colletotrichum acutatum sensu stricto* (s.s.). A LAMP assay was subsequently developed for the direct detection of this causal agent. The primers were designed to target the conserved β-tubulin 2 (TUB2) gene, and result visualization was carried out in real time through the detection of fluorescent signals. The assay exhibited high specificity and sensitivity with

no cross-reaction observed and a detection limit of 1 ng DNA/mL. To further validate the assay for direct pathogen detection, it was applied to inoculated olive drupes following a one-step crude DNA extraction protocol. This method successfully detected *C. acutatum* s.s. at concentrations as low as 5 spores per gram, confirming LAMP as an effective, rapid, and sensitive tool for direct pathogen detection, even in complex matrices like olive.

Beyond *Colletotrichum* identification: species diversity, host association patterns and geographical distribution. I. MARTINO[1], C. GIORDANO[1], P. TALHINHAS[2], A.P. RAMOS[2], T. NASCIMENTO[2], A. PRODI[3], R. BARONCELLI[3,4], D.C. SPADARO[1,5], V. GUARNACCIA[1,5]. [1]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* [2]*LEAF-Linking Landscape, Environment, Agriculture and Food Res. Centre and Terra Associate Lab., Instituto Superior de Agronomia, Universidade de Lisboa, Lisbon, Portugal.* [3]*Department of Agricultural and Food Sciences (DISTAL), University of Bologna, Viale Fanin 42, 40127 Bologna, Italy.* [4]*Center for Studies on Bioinspired Agro-Environmental Technology, Università of Naples Federico II, 80055, Portici, Italy.* [5]*Interdepartmental Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* E-MAIL: ilaria.martino@unito.it

Colletotrichum spp. include important plant pathogens affecting several plant hosts worldwide. Recent re-evaluations in taxonomy, and possible incorrect genomic sequences available in public databases, have led to uncertainties in *Colletotrichum* species identification, raising concerns about the reliability of both past and new records. The CLARITY project, funded by EFSA, aims to resolve these ambiguities by improving species identification, clarifying their real geographical distribution and host associations, and contributing to effective management strategies. To achieve this, *Colletotrichum* isolates from European and global culture collections are being reassessed using molecular and morphological approaches to validate taxonomic identities. Additionally, extensive field surveys across the Mediterranean basin are investigating *Colletotrichum* diversity in various hosts, including tropical and subtropical fruit crops, apples, strawberries, ornamentals and native plants. In parallel, a large-scale analysis of genetic metadata is underway, integrating type specimens, past records and newly obtained isolates. Nearly 80 *Colletotrichum* isolates have been retrieved from European culture collections and over 600 were collected from surveys conducted in Greece, Italy, Portugal and Spain. Robust phylogenetic analyses based on multi-locus sequence typing (MLST) are ongoing to provide accurate species identification, with preliminary data showing a predominance of *C. acutatum* (18.5%), *C. boninense* (26.1%) and *C. gloeosporioides* (51.3%) species complexes. Whole genome sequencing (WGS) will be applied to selected isolates for improved taxonomic resolution and functional genomic insights. The combined results will enhance our knowledge on taxonomy, evolution, geographical distribution and host association patterns of *Colletotrichum* spp., contributing to the development of a comprehensive database for accurate species identification.

This research was financially supported by CLARITY, a project granted from the European Food Safety Authority (EFSA): "GP/EFSA/PLANTS/2023/06 - Improving the knowledge on the European distribution of plant pathogenic species of the genus Colletotrichum, recently subject to taxonomical changes".

CONCURRENT SESSION E2 – Integrated pest management: from plant resistance to chemical control

SESSION KEYNOTES

A key to sustainable management of the grapevine downy mildew pathogen. F. DELMOTTE. *INRAE, Bordeaux Sciences Agro, SAVE, ISVV, F-33140, Villenave d'Ornon, France.* E-MAIL: francois.delmotte@inrae.fr

Europe is the historical cradle of viticulture, but cultivated grapevine has been increasingly threatened by pathogens of American origin. The invasive oomycete *Plasmopara viticola* causes downy mildew, one of the most devastating grapevine diseases worldwide. Current strategies for controlling *P. viticola* primarily relies on fungicide applications. Genetic analyses revealed that all invasive *P. viticola* populations stem from a single North American lineage associated with V. aestivalis. After its introduction into Europe, this lineage spread globally through secondary introductions, underscoring the importance of stricter regulation of international plant material movement to prevent further introductions of downy mildew. As an alternative to fungicide-based control, we are exploring two strategies: deploying disease-resistant grapevine varieties and disrupting the pathogen's sexual reproduction cycle. Our efforts have focused on understanding the dynamics of primary inoculum resulting from sexual reproduction, which play a critical role in initiating disease outbreaks. For the first time in

oomycetes, the locus that determines mating types was described. We then applied ddPCR to describe oospore distribution in vineyard soils. We found higher accumulation of oospores below vines than between rows, consistent with the distribution of grapevine leaf litter which accumulates at the base of the vines in autumn. Finally, we found that removing grapevine leaves containing the pathogen's oospores can significantly delay disease onset in the following season. Taken together, these results confirm that management strategies targeting the disruption of the pathogen's sexual cycle could significantly enhance the control of downy mildew epidemics. The adoption of disease-resistant varieties (DRV) represents another promising innovation to significantly reduce fungicide use. However, while DRV are planted, *P. viticola* populations are demonstrating rapid adaptability, successfully overcoming the grapevine resistances. Promoting sustainable resistance management therefore requires a deep understanding of the genetic interactions between the plant and the pathogen. We explore pathogen adaptation and provided the first description of candidate effectors of *P. viticola* involved in the interaction with 3 resistance factors of grapevine. We discovered that strains overcoming Rpv3 and Rpv12 shared deletions that removed several effector genes. In contrast, the breakdown of Rpv10 appears to be driven by a dominant suppressor locus, likely introduced through a secondary introduction event of the pathogen into Europe. These findings illustrate the evolutionary diversity of virulence strategies used by *P. viticola*, from gene deletions to admixture-driven adaptation. The discovery of these avriulence loci has opened the door to the design of new molecular tools to monitor the evolution of grapevine downy mildew populations adapting to partial resistance of grapevine.

Diversity-Driven strategies to enhance wheat resistance to major fungal diseases in Tunisia. S. BEN M'BAREK[1,2,3], M. LARIBI[2,4], W. ABDEDAYEM[2,5], H. KOUKI[2], S. ARFAOUI[6], J. NASRI[1], S. HAMZA[5], P. KARISTO[7], A. MIKABERIDZE[8], M. PATPOUR[9], M.S. HOVMØLLER[9], M. RAHMATOV[10], S.E. STRELKOV[4], C.P. SANSALONI[11], S. DREISIGACKER[11], P.K. SINGH[11], C. SAINT PIERRE[11], K. AMMAR[11], A. YAHYAOUI[12]. [1]*Regional Field Crops Research Center of Beja, Beja, Tunisia.* [2]*IRESA-Wheat Septoria Precision Phenotyping Platform, Tunis, Tunisia.* [3]*Laboratory of 'Appui à la Durabilité des Systèmes de Production Agricole Dans la Région du Nord-Ouest', Higher School of Agriculture of Kef (ESAK).* [4]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.* [5]*Department of agronomy and plant biotechnology, National Agronomic Institute of Tunisia, Tunis, Tunisia.* [6]*National Institute of Field Crops, Tunisia.* [7]*Plant Health, Natural Resources Institute Finland (Luke), Jokioinen, Finland.* [8]*School of Agriculture, Policy and Development, University of Reading, Reading, UK.* [9]*Department of Agroecology, Aarhus University, Slagelse, Denmark.* [10]*Department of Plant Breeding, Swedish University of Agricultural Sciences, P.O Box 190, SE-23422 Lomma, Sweden.* [11]*International Maize and Wheat Improvement Center (CIMMYT) km, 45 Carretera México-Veracruz El Batan, Texcoco CP56237, Mexico.* [12]*Borlaug Training Foundation, Colorado State University, Fort Collins, CO, United States.* E-MAIL: *sarrah.bm2712@gmailcom*

Tunisia, a major durum wheat producer in the Mediterranean, faces recurring threats from foliar fungal diseases such as *Zymoseptoria tritici* (causal agent of Septoria tritici blotch, STB), *Pyrenophora tritici-repentis* (tan spot), and the re-emergent *Puccinia graminis* f. sp. *tritici* (wheat stem rust), although sporadic, is a major concern. In the context of limited resistant cultivars and high reliance on fungicides, a shift toward integrated pest management (IPM) is essential. We present a multi- layered, resistance-driven strategy combining high-throughput field phenotyping, seedling screening under controlled conditions, genotyping, genome-wide association studies (GWAS), and ecological field practices to identify and deploy durable resistance. Since 2015, a Septoria precision phenotyping platform—established by CIMMYT in collaboration with Tunisian research institutions—has screened up to 20,000 wheat accessions from CIMMYT annually and collaborators at two disease hotspot locations under natural and artificial inoculations. This platform accelerates the identification of resistant germplasm to feed breeding pipelines. In parallel, a USDA Mediterranean collection was phenotyped for STB and tan spot, genotyped with the 90K SNP array, and analyzed via GWAS, identifying marker-trait associations (MTAs) and highlighting eight Tunisian landraces as promising donors for tan spot resistance. For stem rust, race phenotyping revealed the dominance of the Sicily race in Tunisia, and seedling screens against Ug99 and related races showed resistance in only 9.4% of Mediterranean accessions, indicating the need for more widespread resistance introgression. To explore sustainable disease management strategies and reduce fungicide inputs, field experiments mixing the susceptible durum wheat variety Karim with 25% of resistant cultivars (Salim or Monastir) significantly reduced STB severity and enhanced both grain yield and thousand-kernel weight, underscoring the potential of varietal mixtures

as an effective approach to disease control. This multi-layered strategy provides a robust IPM framework that reduces dependence on chemical control, harnesses valuable genetic resources, and supports the development of resilient wheat cultivars adapted to Tunisia's evolving disease landscape. During my talk, I will present these key findings and their implications for breeding and disease management.

ORAL PRESENTATIONS

Resistance to fungicides in apple powdery mildew and eco-friendly management alternatives. L. GUR[1,2], A. MESHOOLAM[1,3], E. KURZBAUM[1,3], Y. COHEN[4], Y. KINRIECH[1], Y. YANOVSKY[1], S. OVADIA[5], M. REUVENI[1]. [1]*Shamir Research Institute, University of Haifa, Katzrin 1290000, Israel.* [2]*Institute of Evolution (IOE), University of Haifa, Haifa 3103301, Israel.* [3]*Tel-Hai College, Kiryat Shmona 1220800, Israel.* [4]*Bar-Ilan University, Ramat Gan 5290002, Israel.* [5]*S.H.F, Agricultural consulting and development services, Karmei Yosef 9979700.* E-MAIL: liorgur@gri.org.il

Apple powdery mildew, caused by *Podosphaera leucotricha*, is a major disease of apple worldwide. It infects leaves, flowers, and fruits, leading to significant yield losses. Disease management relies primarily on fungicides such as demethylation inhibitors (DMIs) and respiration inhibitors (SDHIs, QoIs), which increase resistance risks. Although reduced fungicide efficacy has been documented, no genetic mutations for resistance have been detected in *P. leucotricha*. Israel's climate, with rainy springs and dry summers, favors disease development and necessitates frequent fungicide applications. In this study, we assessed *P. leucotricha* resistance to fungicides in field trials, laboratory assays, and genetic analyses. In orchard trials conducted in the Mediterranean climate of northern Israel, penconazole (DMI) and kresoxim-methyl (QoI) performed poorly (20–30% efficacy) compared to trifloxystrobin (QoI). In leaf disc bioassays, EC90 values were 30 and 75 ppm ai for penconazole and kresoxim-methyl, respectively, compared to 2.1 ppm for trifloxystrobin. No resistance-related mutations were detected, suggesting alternative resistance mechanisms. Preventive foliar sprays in the greenhouse and orchard with macro- and micronutrients reduced disease incidence by 94–97% and severity by 77–83%. Hot air (180°C) applied with the aid of a tractor-towed heat blower reduced disease incidence and severity by 50–70%. Combining reduced fungicide applications with heat treatment resulted in 60–90% and 85-90% reduction in disease incidence and severity, respectively, suggesting similar or better efficacy compared to conventional treatments. This study provides the first characterization of *P. leucotricha* resistance in Israel and highlights eco-friendly strategies for reducing fungicide reliance.

This research was supported by the Ministry of Agriculture and Food Security, and the Plants Production & Marketing Board, Israel.

Integrated control of *Coniella granati*: fungicide efficacy and the role of cultivar resistance. P. MARINO, A. CARLUCCI, M.L. RAIMONDO, F. LOPS. *Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), Via Napoli 25, 71121, Foggia, FG, Italy.* E-MAIL: francesco.lops@unifg.it

Pomegranate (*Punica granatum* L.) is gaining increasing importance in Italy, particularly in southern regions such as Apulia, Basilicata, and Sicily, due to favorable climatic conditions. This study aimed to evaluate the effectiveness of different chemical strategies against *Coniella granati*, the causal agent of pomegranate stem and crown canker in the Mediterranean area. The experimental work was divided into two phases, with an initial in vitro screening followed by field trials. The in vitro tests assessed the antifungal activity of 13 commercial formulations applied at three different concentrations. Only a few active ingredients showed effective inhibition of pathogen growth, including Tebuconazole and Dodine. These fungicides were subsequently selected for field applications, with symptom assessments carried out at regular intervals on three commonly cultivated varieties—Jolly Red, Ako, and Wonderful—at two commercial orchards in Apulia. Tebuconazole proved to be the most effective treatment in reducing disease severity, as indicated by the lowest AUDPC values, whereas Dodine showed less consistent results. Additionally, controlled inoculations revealed significant differences in varietal susceptibility: Wonderful exhibited the lowest level of infection, while Jolly Red appeared to be the most sensitive. This study highlights the value of an integrated management approach, combining the targeted selection of the most effective chemical active ingredients with varietal resistance screening as key tools for the sustainable control of *C. granati*, in alignment with the principles of the European Green Deal.

Integrated management of grapevine downy mildew: from disease forecasting to optimized fungicide resist-

ance management. B. LECCHI[1], G. MADDALENA[1], P. BORSA[2], M. COATTI[2], L. BORGHI[3], S.L. TOFFOLATTI[1]. [1]*Università degli Studi di Milano, Dipartimento di Scienze Agrarie eentali, DiSAA, Via Celoria 2, 20133, Milan, Italy.* [2]*Syngenta Italia, Viale Fulvio Testi 280/6, 20126 Milan, Italy.* [3]*Syngenta Crop Protection AG, Schaffhauserstrasse 101, 4332 Stein, Switzerland.* E-MAIL: beatrice.lecchi@unimi.it

Grapevine downy mildew poses one of the main challenges in modern viticulture, emphasizing the need for sustainable and integrated management strategies. While precision agriculture offers valuable tools for optimizing fungicide use and improving disease control efficacy, it often overlooks the critical importance of fungicide selection based on resistance monitoring. This study integrated three key components of disease management: (i) validation of the EPIcure forecasting model to optimize treatment timing; (ii) assessment of anti-resistance strategies within two different fungicide application programs, aimed at ensuring effective crop protection and limiting the spread of resistant strains; and (iii) development of monitoring methods to quantify resistant individuals using cell sorting techniques. Field trials were conducted during the 2023 and 2024 seasons in two commercial vineyards located in Northern Italy (Veneto and Friuli-Venezia Giulia). Untreated control plots and plots treated according to anti-resistance strategies were established. Throughout the seasons, disease severity was assessed, and leaf samples were collected for fungicide resistance monitoring through bioassays and molecular analyses. Results demonstrated the forecasting model's reliability in identifying periods of high infection risk and confirmed the effectiveness of anti-resistance strategies, even in the presence of strains exhibiting multiple resistance to different classes of single-site fungicides. Moreover, cell sorting showed promising potential for resistance monitoring, although further methodological optimization is required to enhance its efficiency.

This research is carried out within the framework of an industrial PhD program co-funded by the Ministry of University and Research and by Syngenta Italia.

Beyond the berries: unlocking the biopesticide potential of strawberry tree leaves. F.J. CEBALLOS-BURGOS[1,2], J. MARTINS[1,3], J. CANHOTO[1,2], C. MALEITA[1,2]. [1]*University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.* [2]*University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Rua Sílvio Lima, Pólo II –Pinhal de Marrocos, 3030-790 Coimbra, Portugal. 3University of Coimbra, Molecular Physical-Chemistry R&D Unit, Department of Chemistry.* E-MAIL: jhoannacevallosb@gmail.com

Alongside fruit production, plants synthesize a diverse array of chemical compounds enabling them to adapt to and resist environmental threats. Domestication and selection for consumer-favoured traits (e.g., fruit size, flavour, yield) have often inadvertently compromised the natural defences of cultivated plants against pests, requiring active intervention by farmers. Wild or less domesticated plants, with their retained resistance mechanisms, offer a potential solution. The strawberry tree (*Arbutus unedo*), resilient to challenging environments like marginal lands and drought, is valued for its nutraceutical fruits and its production of phenolic compounds, including arbutin and hydroquinone. While its pharmacological benefits for human health and its antimicrobial properties are known, its efficacy against agricultural biotic stressors has not been extensively explored. Therefore, this research investigated the *in vitro* efficacy of *A. unedo* leaf extracts and/or arbutin/hydroquinone against the root-knot nematode *Meloidogyne incognita* (among the global plant parasitic nematodes with the greatest economic impact), the oomycete pathogen *Phytophthora cinnamomi* (causal agent of crown and root wilting in over 5000 plant species), and the fungal pathogens *Zymoseptoria tritici* (causing septoria tritici blotch in wheat) and *Venturia inaequalis* (causal agent of apple scab), some of the most serious agricultural threats. *M. incognita* mortality and hatching inhibition assays were performed, and growth reduction was measured in solid medium for the fungal and oomycete pathogens. The findings reveal that aqueous and methanolic leaf extracts and arbutin and/or hydroquinone significantly induce nematode mortality and inhibit hatching, as well as inhibit the growth of the tested fungal and oomycete pathogens.

This research was financially supported by FEDER funds through the Portugal 2020 (PT 2020), COMPETE 2020 and by the Portuguese Foundation for Science and Technology (FCT), under contracts UIDB/00102/2020, UIDP/00102/2020 (CERES), and UIDB/04004/2025, UIDP/04004/2025 (CFE).

The prolonged effect of biochar application during the nursery stage on plant diseases. O. FRENKEL[1], T. SAMUCHA[1], G. MORDUKHOVICH[1], A.K. JAISWAL[1],

E.R. GRABER[2], Y. ELAD[1]. [1]*Department of Plant Pathology and Weed Research, 68 Hamacabim st. Rishon Lezion, Israel.* [2]*Department of Soil Chemistry, Plant Nutrition and Microbiology, 68 Hamacabim st. Rishon Lezion, Israel.* E-MAIL: omerf@volcani.agri.gov.il

Biochar is widely recognized as a soil amendment with potential to suppress plant pathogens. However, its effectiveness in disease suppression is highly dependent on factors such as feedstock type and, particularly, application rate-beneficial effects that are often observed within a narrow dosage range. Given the difficulty of removing biochar once applied to soil, its targeted use in nursery systems and soilless media presents a more controlled and sustainable approach to managing plant diseases. This study aimed to evaluate the duration of induced systemic resistance triggered by nursery-stage biochar application and its potential to suppress both foliar fungal pathogens and soilborne pathogens. Eucalyptus-derived biochar at a 3% concentration was incorporated into the root plugs of commercial tomato trays, which were then transplanted into biochar-free greenhouse soil. The induced resistance effect persisted for over 60 days post-transplanting, resulting in more than a 50% reduction in *Botrytis cinerea* damage of leaves. Comparable suppression was observed in cucumber leaves infected with *Podosphaera xanthii*, the causal agent of powdery mildew, in a field trial. In contrast, suppression of the soilborne pathogen *Pythium aphanidermatum* was effective only after a pre-activation period of over 14 days, during which biochar interacted with the growth substrate and reshaped the microbial community within it. These findings highlight the strategic potential of biochar use in nursery production systems to extend disease resistance beyond early growth stages, offering a precise and sustainable tool for integrated plant disease management.

Development of novel ddPCR assays for detection and quantification of SDHI resistance in *Botrytis cinerea*. G. SOFIANOS[1], K. MAVRIDIS[2], N. KRASAGAKIS[3], E. MARKAKIS[3], J. VONTAS[2], G. KARAOGLANIDIS[1]. [1]*Laboratory of Plant Pathology, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece;* [2]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, GR-700 13 Heraklion, Crete, Greece,* [3]*Department of Agriculture, School of Agricultural Sciences, Hellenic Mediterranean University, Stavromenos,71004 Heraklion, Greece.* E-MAIL: gkarao@agro.auth.gr

Botrytis cinerea is a high-risk plant pathogen for resistance development and, indeed, fungal populations resistant to fungicides have often been observed in the fields, throughout the world. Since resistance development reduces the control efficacy of botryticides, it is vital that quick and efficient resistance monitoring programs be developed and implemented in the control strategies. Unfortunately, typical approaches of fungicide resistance detection take time and often lack the ability of sensitive and accurate quantification. Digital droplet PCR (ddPCR) could constitute the ideal diagnostic tool, since it can be used both to identify and quantify alterations in genomic sequences, even in large pooled samples, while it may provide high specificity and sensitivity. In this study, we tested the applicability of ddPCR in analyzing the most common, and functionally validated, SDHIs resistance mutations in B. cinerea, namely H272R, H272Y N230I, P225F and I274V. Method validation was performed using control samples possessing the mutations at known concentrations, and DNA extracted from pooled conidia samples with various mutations' ratio, mimicking environmental sample collections. Consequently, a new ddPCR detection tool was established that could effectively detect, even at very low frequencies (reaching a detection limit of 0.5%), widespread SDHI-resistance conferring mutations in *B. cinerea* environmental samples.

This research was financially supported by "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP - TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative "Agriculture and Food Industry".

POSTER PRESENTATIONS

From field to gene: insights into zoxamide resistance management in *Plasmopara viticola*. M. PERACCHI, B. LECCHI, G. PIGNI, G. MADDALENA, S.L. TOFFOLATTI. *Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali – DISAA, Via Celoria 2, 20133 Milan, Italy.* E-MAIL: mattia.peracchi@unimi.it

Fungicides are essential tools in the management of grapevine downy mildew, a disease caused by the oomycete *Plasmopara viticola*, a biotrophic and polycyclic pathogen with a high risk of developing resistance. Zoxamide, a fungicide with a low to moderate risk of selecting resistant strains, acts by inhibiting tubulin polymerization, with resistant strains characterized by a missense mutation at amino acid position 239 of the β-tubulin

gene. This six-year study (2017–2022) aimed to investigate the emergence and distribution of zoxamide-resistant *P. viticola* strains under different field conditions and fungicide use histories. Sensitivity was assessed through biological assays on the oospores and complemented by molecular screening for resistance-associated mutations. A total of 126 samples were collected from 57 vineyards, mostly located in North-Eastern Italy. The majority (90%) showed full sensitivity to zoxamide (EC_{50} < 0.2 mg L^{-1}; EC_{95} and MIC < 10 mg L^{-1}). Thirteen samples exhibited reduced sensitivity (MIC >100 mg L^{-1}), but only two showed a consistent presence (24–33%) of resistant oospores at 100 mg L^{-1}. Resistance was mainly observed in vineyards where zoxamide had been applied four or more times per season. Multiple polymorphisms at codon 239 of the β- tubulin gene were identified through sequencing, including the known C239S and C239G mutations and a potentially novel C239T variant. These findings highlight the role of selection pressure in shaping resistance in *P. viticola* populations and emphasize the need to limit the number of zoxamide applications per season. The observed genetic variability also calls for further research into the fitness and epidemiology of resistant strains to inform sustainable resistance management strategies.

This research is carried out within the framework of an industrial PhD programme confounded by the ministry of university and research and by Gowan company.

Elucidating the key determinants affecting Italian *Pyricularia oryzae* genetic diversity. S. BOSCO[1], F. SILLO[2], P. RUFFA[1], D. TENNI[3], P. ABBRUSCATO[4], D. TORELLO MARINONI[1], M. ROMANI[3], M. MEZZALAMA[1,5], V. GUARNACCIA[1,5], D. SPADARO[1,5]. [1]*Dept. Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Paolo Braccini, 2, 10095, Grugliasco, TO, Italy.* [2]*Institute for Sustainable Plant Protection, National Research Council (CNR-IPSP), Strada delle Cacce, 73, 10135, Turin, TO, Italy.* [3]*Ente Nazionale Risi, Centro Ricerche Sul Riso, Strada per Ceretto, 4, 27030 Castello d'Agogna, PV, Italy.* [4]*Nuova Genetica Italiana, Piazza XX Settembre, 2, 22079, Villa Guardia, CO, Italy.* [5]*Interdepartmental Centre for Innovation in the Agro-environmental Sector (AGROINNOVA), Largo Paolo Braccini, 2, 10095, Grugliasco, TO, Italy.* E-MAIL: davide.spadaro@unito.it

Rice blast, caused by *Pyricularia oryzae* Cavara, is the most damaging disease in Italy. The use of resistant cultivars represents a sustainable method to minimize yield loss, however high genomic plasticity of the pathogen promotes rapid adaptation to host resistance. Understanding *P. oryzae* population structure is fundamental to assess its genetic variability and to devise durable management practices. While the first population study in Italy dates to 2005, updated knowledge of local *P. oryzae* diversity is lacking. In this study, we genotyped the population structure of 242 isolates collected from rice panicles in five Italian regions (Piedmont, Lombardy, Veneto, Emilia-Romagna, and Sardinia) in 2011–2012 and 2020–2022 using SSR markers. The potential occurrence of sexual reproduction was determined by linkage disequilibrium and mating type assessment, displaying the exclusive presence of the MAT1-1 idiomorph, thus confirming the clonal structure of the Italian *P. oryzae* population. Cluster analysis of 200 distinct multilocus genotypes (MLGs) via DAPC, UPGMA, and STRUCTURE allowed to identify five genetic clusters. Molecular variance and genetic divergence analyses revealed that geographic origin and sampling year significantly affected population structure. Correlation analysis in isolates from Piedmont highlighted combined effects of climatic factors and geographic origin on local allelic diversity. Virulence testing of cluster-representative strains suggested the disappearance of a less virulent cluster over time. These findings provide novel insights into the temporal and spatial dynamics of the Italian *P. oryzae* population in Italy.

This research was financially supported by the "REACT-EU – PhD programmes on innovation and green topics" funded by NOP Research and Innovation 2014-2020. Part of this work was also granted by the European Commission – NextGenerationEU, Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy", code n. IR0000005.

The potassium phosphite-based fungicide CANON® controls basil downy mildew under greenhouse conditions in Israel. N. NITZAN, H. GOREN. *The Valley of Springs Research & Extension Center, Israel, M. P. Beit She'an.* E-MAIL: nitzan_nadav@yahoo.com

Sweet basil downy mildew (BDM), caused by *Peronospora belbahrii*, is the most destructive disease of commercially produced sweet basil in Israel, and unless properly managed, may lead to 100% yield reduction. Four experiments were conducted over three growing seasons (fall 2021, fall 2022 and spring 2023) under commercial-like conditions to determine the efficacy of the phosphonic acid-based fungicide Canon® following a conservative, seven-day protective spraying protocol.

While Canon did not eradicate the pathogen, treated plants had 41 to 99.9% less ($P < 0.05$) disease incidence and / or severity than the untreated control plants. The disease scores on plants treated with Canon were similar ($P > 0.05$) to an alternation of Revus® / Cabrio® in fall 2021; less ($P < 0.05$) than Revus in fall 2022 and higher ($P < 0.05$) than an alternation of Acrobat® / Cabrio at the end of the spring 2023 experiment. The inhibitory effect of Canon, its short pre-harvest application interval and low cost compared to other fungicides make it an excellent addition and a potential replacement for controlling BDM under greenhouse conditions that should be further explored.

GrapeBreed4IPM: Developing sustainable solutions for viticulture through multi-actor innovation targeting breeding for integrated pest management. S.E. LEGLER[1], V. MANSTRETTA[1], K. AVIA[2], M. DRESSLER[3], L. HAUSMANN[4], G. DI GASPERO[5], B. LAURENT[6], M. DE LA FUENTE[7]. [1]*Horta srl, Piacenza, IT 29122, Italy.* [2]*INRAE, Université de Strasbourg, UMR SVQV, 68000 Colmar, France.* [3]*University of Ludwigshafen, Winecampus, D-67059 Ludwigshafen, Germany.* [4]*Julius Kuhn Institute (JKI), Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany.* [5]*Istituto di Genomica Applicata, 33100, Udine, Italy.* [6]*IFV, UMT SEVEN, Blanquefort, France.* [7]*Spanish Wine Technology Platform – CEIGRAM - Universidad Politécnica de Madrid, Madrid, Spain.* E-MAIL: s.legler@horta-srl.com

Viticulture is an economically and culturally important in the EU, highly dependent on the use of Plant Protection Products, in order to protect plants from diseases. The sector faces significant environmental pressures, aiming for a transformation towards sustainability and ecosystem preservation in line with the goals set by the European Union. Advances in grapevine breeding gave origin to Disease Resistant Varieties (DRV), also known as PIWI, which open the possibility to drastically reduce the use of fungicides in vine management. However, the reduced need for fungicides cannot be easily implemented and requires holistic pest control strategies and varieties adapted to future climate conditions are also needed. To address these issues, the GrapeBreed4IPM project focus on developing grapevine varieties with enhanced resistance to fungal diseases, adapted to local environmental and pedoclimatic conditions, aiming to provide tools to reduce the reliance on the application of fungicides. Methods like for example genomic selection are used to improve and accelerate the breeding process. In addition, the project is tailoring existing digital tools and developing guidelines and best practices for Integrated Pest Management (IPM) specifically for the management of DRV, thus equipping farmers and advisors with the tools needed to optimize pest control while minimizing chemical usage. Within the project, Horta customized its Decision Support System for sustainable management of grapevine specifically for PIWI varieties by calibrating the plant growth model and the downy and powdery mildew models and validating them in a three-year vineyard trial in the Colli Piacentini viticultural area.

This research was financially supported by the European Union as part of the Project GrapeBreed4IPM, grant agreement 101132223.

Foundation Plant Services: member of US national clean plant network. M. AL RWAHNIH. *Department of Plant Pathology, Foundation Plant Services, University of California, Davis, USA.* E-MAIL: malrwahnih@ucdavis.edu

Located in Davis, California, USA, Foundation Plant Services is one of thirty-three US clean plant centers that make up the US National Clean Plant Network (NCPN). The NCPN can be used as a model of successful implementation of a coordinated clean plant strategy that supports producers and improves food security. The seven specialty crops within NCPN are berries, citrus, fruit trees, grapes, hops, roses, and sweet potatoes. These crops are clonally propagated, which assures identical genetic horticultural characteristics in propagated progeny, but can easily spread pathogens including viruses, viroids, phytoplasma, and bacteria if the mother plant is infected. These pathogens can have detrimental effects on performance and yield of infected plants, and the only cure is complete removal of the plant. It is important to limit pathogen spread and therefore reduce economic impact due to crop loss by screening for pathogens in plant stocks before using them for propagation. NCPN centers provide pathogen diagnostic and elimination services and maintain and distribute virus-tested plant material. In total, NCPN centers distribute on average over 37 million propagative units each year, allowing producers around the US to plant fields, orchards, and vineyards with clean material. Estimates of economic impact for grape and fruit tree centers supplying virus-tested plant material show a return on public investment dollars ($) ranging from 10:1 to 150:1.

Evaluation of *Pisum sativum* resistance to *Didymella pinodes* after gamma irradiation in ex- planta bioassays. E. SARRI[1], A. KATSILEROS[1], S. MIGARDOU[2], P. VILIOTIS[1], I. SIDIROPOULOS[1], D. SIFNAIOS[1], P. DIAMANTIS[1], N. SKLAVOUNOS[2], P. BEBELI[1], D. KIZIS[2], E. TANI[1]. [1]*Laboratory of Plant Breeding and Biometry, Department of Crop Science, Agricultural University of Athens, Athens, Greece.* [2]*Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute (BPI), Athens, Greece.* E-MAIL: d.kizis@bpi.gr, etani@aua.gr

Ascochyta blight caused by one or more pathogenic fungi including *Didymella pinodes*, *Ascochyta pisi*, and *Phoma pinodella*, is a devastating field pea disease, characterized by leaf, stem, and pod lesions. Plant breeding focuses on developing new crop varieties that fulfill nutritional requirements for humans and livestock while adapting to environmental challenges. A key tool in modern genetic improvement is random mutagenesis via gamma- irradiation, a non-GMO method that generates genetic variability and accelerates the identification of genotypes with desirable traits. This study examined the resistance of the M2 generation of forage pea variety 'Dodoni' (*Pisum sativum* L. var. *arvense* 'Dodoni') to *Didymella pinodes* CBS 251.47 strain. The M2 plants originated from M0 seeds treated with 100 Gy gamma radiation and grown in the greenhouse during 2023-2024. Resistance to the fungus was evaluated through a detached-leaf bioassay using mycelium agar slabs for artificial infection, in Petri dishes, under controlled conditions. Leaf photos were captured on the 3rd and 5th days post-infection. Image analysis was conducted to assess the extent of leaf lesions and calculate the Diseased Area and Disease Severity Index. After extensive screening in both greenhouse-grown (16 families x 24 plants) and field conditions (100 families x 24 plants), three M2 families showed statistically significant differences in resistance compared to non- irradiated controls. Additionally, within these families, plants exhibited statistically significant differences in yield, with increased pod number and higher fresh and dry weight. Further evaluation of selected genotypes will enable the development of varieties towards enhanced productivity and pathogen resistance.

Different susceptibility of local Apulian and Calabrian grapevines to downy mildew and characterization of *Plasmopara viticola* populations. R. CORONELLI[1], D. GERIN[1], F. SPATARO[1], S. TOFFOLATTI[2], M. PERACCHI[2], B. LECCHI[2], R.M. DE MICCOLIS ANGELINI[1], F. FARETRA[1], S. POLLASTRO[1]. [1]*Department of Plant Soil and Food Sciences, University of Bari Aldo Moro Via G. Amendola, 165A/ 70126 Bari, Italy.* [2]*Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi di Milano, Via Celoria 2 20133 Milan, Italy.* E-MAIL: donato.gerin@uniba.it

Downy mildew, a severe grapevine disease worldwide, is caused by different cryptic species of *Plasmopara viticola*. Several approaches are under study to reduce the impact of chemicals in the disease management, including the selection of resistant genotypes for grapevine breeding. In this study, the susceptibility of six different local wine grape cultivars was assessed on leaves and bunches, in 2023, a year particularly favourable to the downy mildew epidemy. Based on McKinney's index (MKI: 37.3 to 52.6%), Marchione, Moscato reale, Maresco and Moscato di Terracina were more susceptible than Moscato di Trani, Trebbiano and Verdeca, classified as resistant ones (MKI: never exceeding 16.6%). Additionally, the genetic variability of *P. viticola* populations infecting grapevine in Apulia, Calabria and Lombardia was evaluated. Sporangia were collected from oil-spots in 26 vineyards (15 Apulia, 11 Calabria), during 2023 and 2024 years. The CAPS analysis was carried on restriction enzyme *AseI* profiles of the amplified DNA using the ITS1-O/ITS2, according to Rouxell *et al.* (2013). A total of 130 isolates and 20 DNA samples from oil spots sampled in Lombardia were analysed. All belong to the clade *aestivalis*, as also confirmed by BLASTn analysis of the ITS partial sequence of few representative isolate of Apulia and Calabria regions. These preliminary results suggest that the resistant behaviour of analysed grapevine genotypes is limited to *P. viticola* clade *aestivalis* and highlight the opportunity to preserve the local biodiversity for possible introduction of new *P. viticola* clades, currently absent.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. The Authors thanks Dr. P. Venerito and P.A. L. Catucci of the Centro di Ricerca, Sperimentazione e Formazione in Agricoltura "Basile Caramia", Locorotondo (BA) responsible of the ex-situ repository of grapevine biodiversity.

Evaluation of an organic biostimulant on ZYMV infection and behaviour of its aphid vector in *Cucurbita pepo* L. C.L. CORRADO[1,3], T. TUNGADI[2], L. DONATI[1], T. BRUCE[2], A. TAGLIENTI[1], S. BERTIN[1]. [1]*Council for Agricultural Research and Economics–Research Centre for Plant Protection and Certification, 00156 Rome, Italy.* [2]*School of Life Sciences, Keele University, Keele, ST5 5BG, United Kingdom.* [3]*Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro, 5, 00185 Rome, Italy.* E-MAIL: carlalibia.corrado@crea.gov.it

Biostimulants are substances that enhance plant growth and nutrient uptake by improving plant or rhizosphere characteristics (EU Regulation 1009/2019). This study assessed the effect of a commercial biostimulant containing alfalfa, brown algae, and molasses extracts on the interaction among zucchini (*Cucurbita pepo*), zucchini yellow mosaic virus (ZYMV), and one of its aphid vectors *Myzus persicae*. Biostimulant-treated plants showed enhanced vegetative growth (e.g., leaf number, biomass) in healthy conditions and improved reproductive traits (e.g., flowers, fruits) in infected conditions. Repeated biostimulant applications reduced ZYMV titre over time and slowed symptoms progression. The pathogenesis-related gene 1 (PR1), associated with systemic acquired resistance, and the peroxidase gene (POD), involved in the oxidative stress pathway, was upregulated in biostimulant-treated ZYMV-infected plants, suggesting a role of biostimulants in priming the plant defence mechanisms. Aphid choice tests using detached leaves showed fewer *M. persicae* settling on treated plants, regardless of infection status. When caged on potted plants, aphids exhibited lower survival and fecundity on treated plants. Volatile organic compounds (VOCs) were collected from the plants, and GC-MS analysis revealed differences in volatiles blend composition between treated and untreated plants. The same volatile samples were used in olfactometer assays, whose results indicated that aphids preferred untreated plants, suggesting a possible repellent effect due to biostimulant-induced VOC production. Overall, the obtained results suggest that biostimulant application may reduce ZYMV impact by enhancing plant defence and limiting vector performance, offering a promising tool for IPM strategies for cucurbit crops.

This research was financially supported by National Operational Programme (PON) Research and Innovation 2014/2020—Action IV.5—PhD projects on green issues, "Education and research for recovery— REACT-EU".

Ecological approaches to grapevine protection: preliminary considerations on the potential of stilbenes. A. ŠUŠNJAR, S. LAZIĆ, J. EĆIMOVIĆ, S. VUKOVIĆ, A. ŽUNIĆ, D. ŠUNJKA. *Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia.* E-MAIL: dragana.sunjka@polj.edu.rs

The grapevine has developed various adaptative mechanisms in response to environmental fluctuations and growth-threatening factors, with phytopathogenic infections being among the most significant. Their occurrence and severity are primarily influenced by climatic conditions and the plant's natural resistance. In this context, secondary metabolism serves as a key mechanism to mitigate or delay damage. Secondary metabolites synthesized in response to phytopathogenic infections are classified as phytoalexins. During interactions with plant pathogens, phytoalexins exhibit inhibitory effects, and their synthesis is triggered by the plant-microorganism interaction. This suggests that their presence is directly linked to resistance. Among these compounds, resveratrol, a stilbenoid organic compound, plays a crucial role in the grapevine's immune system. Its presence has been detected in most grape varieties at varying concentrations, with each cultivar differing in its ability to synthesize and accumulate this compound. The initial resveratrol concentrations are typically low, but synthesis increases significantly upon pathogen infection. Whether the infection is halted at an early stage or will continue to spread depends on subsequent weather conditions, physical barriers such as the berry skin thickness, leaf surface, etc., along with the synthesized amount of resveratrol. This study investigates the potential fungitoxic effect of resveratrol on *Botrytis cinerea* spores in laboratory conditions. The aim is to determine whether and to what extent the resveratrol application affects spore viability and to what extent the plant's natural defense mechanism can be efficient.

This research was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grants No. 451-03-65/2024-03/ 200117 and No. 451-03-66/2024-03/ 200117) and joint research project Serbia-Slovenia grant No. 337-00-110/2023-05/8.

Food safety assessment of strobilurin and carboxamide fungicides in some fruits and vegetables. S. LAZIĆ, S. VUKOVIĆ, A. ŠUŠNJAR, J. EĆIMOVIĆ, A. ŽUNIĆ, D. ŠUNJKA. *Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia.* E-MAIL: dragana.sunjka@polj.edu.rs

The cultivation of fruits and vegetables requires intensive protection against harmful organisms. Crops, such as strawberries, raspberries, lettuce and spinach are particularly vulnerable to diseases, primarily caused by various fungal infections. Due to persistent presence of pathogenic fungi throughout the growing season, frequent applications of plant protection products are often necessary. At the same time, food safety has become an increasingly critical global concern, with pesticide residues posing a major potential risk to human health. This risk is further exacerbated by the short growing cycles of these crops and the timing of pesticide applications, which often coincide with the ripening stage. Consequently, assessing the potential health risks of pesticide residues is essential, particularly for fresh produce consumed directly. In this study, we evaluated the health risks associated with residues of pesticides belonging to the chemical groups of strobilurins and carboxamide in strawberries, raspberries, lettuce and spinach. Field experiments were conducted following EPPO standards, with a plant protection product (PPP) containing boscalid and pyraclostrobin applied at recommended rates. Sampling was performed according to pre-harvest intervals. Fungicide residues were analyzed usinga validated QuEChERS-based method followed with HPLC. The Hazard Quotient (HQ) was calculated using the Estimated Daily Intake (EDI) and the relevant Acceptable Daily Intake (ADI) value. In order to evaluate risk assessment, as the worst-case scenario, the maximum residue levels of pesticides obtained one hour after the application, were used. The results suggest that the consumption of these fruits and vegetables does not pose a significant health risk to humans.

This research was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grants No. 451-03-65/2024-03/ 200117 and No. 451-03-66/2024-03/ 200117) and joint research project Serbia-Slovakia grant No. 337-00-3/2024-05/06.

Differential molecular responses of pear and apple cultivars in response to *Erwinia amylovora* causal agent of fire blight disease. Y. GHARBI, E. BOUAZIZI, M.A. TRIKI. *Laboratory of Olive Genetic Resources: Characterization, Valorization and Phytosanitary Protection, Institute of the Olive Tree, University of Sfax, B.P 1087, Sfax, Tunisia.* E-MAIL: yaakoub.gharbi@yahoo.com

A collection of pear and apple cultivars adapted to Tunisia's growing conditions were evaluated for resistance to fire blight disease (FBD) caused by *E. amylovora*. In this context, the molecular response of local and foreign pears and apple cultivars to FBD was investigated using an artificial infection model and monitoring of defense-related genes using Q-RT-PCR. Based on the evaluation of 8 cultivars, 6 were classified as susceptible (Meski, Anna, Williams, Bouguedma, Alexandrine, Meski Ahrech), and 2 as resistant (Lurka and Ambri) to FBD. The expression levels of genes coding for chalcone synthase (CHS), phenylalanine ammonia-lyase (PAL), and coronatine-insensitive protein (COI) were higher in the resistant cultivars, indicating that early induction of the phenylpropanoid pathway plays a crucial role in resistance against *E. amylovora* and FBD symptom mitigation. The resistant cultivar exhibited early upregulation of catalase (CAT) alongside enhanced superoxide dismutase (SOD) activity, triggering a hypersensitive response (HR) at infection sites that effectively limited pathogen spread. In addition, simultaneous early up-regulation of chitinase and β-1,3-glucanase genes correlated with reduced host susceptibility and FBD symptom severity in the resistant cultivars Lurka and Ambri. These findings suggest that the resistant cultivars Lurka and Ambri represent valuable genetic material for replanting programs or potential use as rootstocks for susceptible varieties. Future studies should evaluate grafted pear and apple hybrids of these resistant cultivars to assess the heritability of this resistance trait.

Improvement of olive anthracnose management in prefecture of Chania, Crete. A. PAPAGEORGIOU[1], A. KARAGIANNI[2], C. STEPPAS[3], M.M. MATHIOUDAKIS[2], D.I. TSITSIGIANNIS[1], A.L. LAGOPODI[3]. [1]*Department of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, Agricultural University of Athens, Athens, 11855, Greece.* [2]*Institute of Olive Tree, Subtropical Crops & Viticulture (ELGO-DIMITRA), Plant Pathology Laboratory, Karamanlis Ave. 167, Chania, Crete, 73134, Greece.* [3]*Aristotle University of Thessaloniki, School of Agriculture, Plant Pathology Laboratory, P.O. Box 269, Thessaloniki, 54124, Greece.* E-MAIL: lagopodi@agro.auth.gr

In previous years, temperatures rise combined with high relative humidity, led to serious outbreaks of the olive anthracnose disease in Greece. In the frame of the GleoliveTreat project, an integrated olive anthracnose management strategy was implemented in Chania prefecture of Crete. It included an integrated management model adapted to the local conditions indicated by meteorological data, the pathogen's population profile in the area of action, and specific phenological olive stages in pilot groves (conventional, organic). Sprays were applied from

the beginning of blooming and during autumn, using selected pesticides that were evaluated in previous studies. The weather conditions during 2023–2024 did not favor the appearance of olive anthracnose, but in combination with entomological infestations, the development of rots from other fungi was not successfully prevented with most predominant species in genera of *Fusarium* and *Alternaria*. The causative species of anthracnose, endemic to the region is *Colletotrichum acutatum*. However, this pathogen participated in the complex of olive fruit rots by only 3% of the total isolates tested. The sprays did not significantly reduce the rates of rotting, which is possibly due to the use of formulations that targeted *Colletotrichum* species, while other pathogens prevailed during the application period. The implementation of an integrated pest management system is difficult to be applied due to the natural topography of the area making it imperative to investigate additional means of treatment. Precision agriculture approaches seem to be the most appropriate tools to manage anthracnose and other olive fruit rots in mountainous areas' olive groves.

This research has been co-financed by the European Agricultural Fund for Rural Development of the European Union and Greek national funds through the Action "Rural Development Program for Greece 2014-2020", under the call (project code: M16ΣΥΝ2-00188).

UCB-1 Pistachio: a resilient choice for orchard rootstock or landscape planting. M. AL RWAHNIH, R. ABOU KUBAA. *Department of Plant Pathology, Foundation Plant Services, University of California, Davis, USA.* E-MAIL: malrwahnih@ucdavis.edu

UCB-1 pistachio rootstock is a hybrid from the controlled cross of the *Pistacia atlantica* by *Pistacia integerrima*, selected at University of California, Berkeley, USA. Seeds have been produced in California since the 1960s, with current UC production at Foundation Plant Services in Davis, California. The UCB-1 hybrid is used as a rootstock for *Pistacia vera* for commercial nut production. Individually, *P. atlantica* and *P. integerrima* were investigated as pistachio rootstocks and compared to the UCB-1 seedlings. Each of these *Pistacia* species exhibit useful traits for pistachio production: *P. integerrima* is tolerant of Verticillium, and is the more vigorous of the two, while *P. atlantica* is more tolerant of cold and salinity. The UCB- 1 hybrid exhibits a combination of these beneficial traits, having tolerance to Verticillium, salinity, cold, and drought. The highest yields of 'Kerman' *P. vera* scion were from trees on UCB-1 rootstock. For decades, UCB-1 has been the most used rootstock in California pistachio orchards because of these attributes. Its resilience factors have also led to the use of ungrafted UCB-1 as an ornamental tree in the US, where it is planted in yards and along roadsides in arid environments. When allowed to grow as a landscape tree instead of grafting UCB-1 is deciduous, with a beautiful red hue to new growth in the spring, and often dazzling red, orange, and yellow colours in the fall.

Application of phage display for the selection of antibodies for the identification of defence- related proteins in plants. L. ZAGORCHEV, I. ALBANOVA, D. TEOFANOVA. *Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov blvd., 1164, Sofia, Bulgaria.* E-MAIL: lzagorchev@biofac.uni-sofia.bg

Proteins play a pivotal role in plant disease resistance, serving key functions in pathogen recognition, intracellular signalling, and defense activation. Resistant genotypes typically exhibit distinct protein expression profiles compared to susceptible varieties, particularly during or following pathogen challenge. These differential expression patterns enable resistant plants to more effectively detect, respond to, and combat pathogenic threats. Therefore, the identification and characterization of specific resistance-associated proteins is crucial in understanding the molecular basis of disease resistance and screening diverse genotypes for resistance. Antibodies represent a powerful tool for studying protein expression and localization. While phage display technology offers a valuable alternative to traditional immunization methods, it still requires highly purified antigen preparations. In the present study, a novel approach was developed for rapidly selecting antibodies against putative disease-related proteins using phage display libraries. Our strategy employed alternating rounds of positive and negative selection to select antibodies targeting antigens present in the treated samples (e.g., infected plants), while excluding those present in the control. To validate this methodology, we utilized a *Solanum lycopersicum* cultivar, resistant to *Cuscuta campestris*, a holoparasitic plant with significant economic impact. Following four selection rounds, we successfully selected a polyclonal phage-conjugated antibody suspension, specific to a protein antigen, exclusively present only in infected plants.

This research was financially supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No BG-RRP-2.004-0008.

Sustainable pest and weed management strategies for enhancing olive production and quality. ABDEL KADER EL HAJJ. *Lebaa station, Lebanese Agricultural Research Institute.* E-MAIL: ak.hajj@lari.gov.lb

The olive oil industry has a long-standing history and remains a crucial agricultural sector, particularly in Mediterranean regions. However, olive production faces significant challenges due to pest infestations and weed competition, which can negatively impact yield and quality. This review highlights challenges from pests and weeds affecting yield and quality and explores sustainable pest and weed management strategies aimed at enhancing olive production while maintaining environmental and economic sustainability. Integrated pest and weed management approaches and smart farming technologies are discussed. Emphasis on minimizing chemical herbicides and pesticides reliance through the use biological, cultural, the sterile insect techniques, and biopesticides and inert materials. Future research should optimize strategies for diverse olive-growing regions. By integrating modern agricultural techniques with traditional practices, olive growers can achieve higher productivity while ensuring sustainable land use.

Screening of Tunisian cucurbit accessions for resistance to virus and fungal diseases. M. ELBEKKAY, H. HAMZA, C. DESBIEZ, N. DJEBALI. *Institut of Arid Region, Medenine, Tunisia.* E-MAIL: mokhtra123ira@gmail.com

The evaluation of resistance of local melon, watermelon and squash cultivars (cv.) to seven viruses and two fungal pathogens *Fusarium oxysporum* f. sp. *melonis* and *Podosphaera xanthii* was made. The results showed that 26 out of 44 cultivars evaluated, exhibited resistance for at least for one of the studied pathogens. The local cucurbit cultivars were generally resistant to MNSV and ToLCNDV in comparison to commercial cultivars. Specifically, melon cultivars M5 and M18, and squash cv. C46, C60 and C65 were resistant to WMV. Watermelon cultivars P15 and P24 along with squash cultivar C60 exhibited resistance to CMV. Partial resistance to MWMV was observed in melon cultivars M35 and M129, watermelon cultivars P35 and P55, and squash cultivar C65. Only C65 squash cv. was resistant to PRSV. For fungal pathogens, the local melon cultivars M6, M26, M58, M120, M131 were resistant to race 1 of FOM, and all studied cultivars were susceptible to race 2 of this fungus. Two local melon cultivars M120 and M122 were resistant to *P. xanthii* races 1, 2 and 3. The wild desert bitter watermelon was resistant to WMV, MNSV, CMV, MWMV and ToLCNDV, but not to PRSV. This study demonstrated that the Tunisian cucurbit genetic resources present valuable material for improving resistance to viral and fungal diseases in these crops.

CONCURRENT SESSION F1 – Biocontrol of plant pathogens: recent advances and future challenges

SESSION KEYNOTES

Developing microbial biocontrol agents: a long road full of question and exclamation marks. G. PUOPOLO[1,2]. [1]*Centre Agriculture Food Environment (C3A), University of Trento, San Michele all'Adige, Italy;* [2]*Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy.* E-MAIL: gerardo.puopolo@unitn.it

Our journey as researchers, from the isolation of microbial biocontrol agents (mBCAs) to their final registration and application in agriculture, is a rollercoaster of uncertainty and excitement. The first significant challenge we face is selecting the target plant pathogen and the crops it infects. This decision is crucial, as it determines whether the chosen plant pathogen aligns with the interests of companies. Once this hurdle is crossed, we encounter the question of where to search for new mBCAs. Typically, we isolate potential mBCAs based on the environmental niche and epidemiological parameters of the target plant pathogen. However, evaluating their efficacy in plant protection can yield surprises, and none of the isolated microorganisms could be effective in controlling the target plant pathogens. Recently, coupling classical isolation procedures with metagenomic analyses is a practical approach for focusing attention on specific microbial taxa associated with controlling plant pathogens. Screening for effective mBCAs may also lead to disappointment; indeed, even if they prove to be effective, some microorganisms must be discarded due to concerns primarily related to their impact on human health, their ability to grow cost- effectively, and their resistance to environmental stressors. Thus, incorporating these aspects into the design of the screening strategies could reduce the number of microorganisms that need to be evaluated for efficacy in controlling the target plant pathogens. Once we identify the right mBCA, we enter the phase we enjoy most: characterising its mode of action. We extensively use *in vitro* methodologies to assess the mBCA's capacity to produce antibiotics, lytic enzymes, phytohormones, plant resistance elicitors and

siderophores that can be involved in the direct and/or indirect control of plant pathogens. Recently, particular attention has been given to the capacity of mBCAs to release Volatile Organic Compounds (VOCs) that can have a toxic effect on plant pathogens. Moreover, microbial VOCs can also prevent plant pathogen infections by triggering resistance mechanisms in plants. Nowadays, we combine *in vitro* data with findings generated through omics technologies. Metabolomics and transcriptomics can provide a clearer understanding of how our selected mBCA can effectively control plant pathogens. These omics technologies could provide indications of genes and secondary metabolites, specifically expressed and/or produced during the microbe- microbe and plant-microbe interactions. Subsequently, we take our chosen mBCAs into the field, designing and formulating them appropriately. To do this, we will continue characterising our mBCAs by assessing their ability to resist desiccation, wash-off, and UV light. Based on this, we will select the most effective additives to protect our mBCAs against these factors. Finally, we could dedicate the last part of our efforts to providing all the data related to the ecotoxicity of the selected mBCAs. This phase is crucial as it ensures that our mBCAs are not only effective in controlling plant pathogens but also safe for the environment. All this information will be helpful for their registration and introduction of our mBCAs into the global market.

This research was financially supported by Autonomous Province of Trento in scope of L.P. No. 6/1999 with determination. No. 275 - 29/05/2024. (Ref.: project TERPENE ALPHA)

Biological induced resistance in plants against pathogens: recent advances and future trends. A. SIAH. *JUNIA-BioEcoAgro, Lille, France*. E-MAIL: ali.siah@junia.com

In the current context promoting agroecology and sustainable agriculture, looking for eco-friendly control strategies of plant diseases is strongly encouraged. Plant resistance inducers from biological origin are considered as promising biocontrol tools that can fit with these challenges. These compounds, also referred to as bio-elicitors, are agents that confer improved protection to pathogen or pest attacks by inducing host defense mechanisms. They are effective against a wide range of plant pathogens, including viruses, bacteria, fungi, oomycetes, and nematodes. The mode of action of these compounds relies on the activation of the plant immune system rather than direct antimicrobial activity against phytopathogens. Plant resistance inducers of natural origin include living microorganisms, plant extracts, microbial cell-wall extracts, microbial metabolites, minerals, and ions. Plant immunity mechanisms involve at the biochemical level the activation of several defense responses, including reactive oxygen species metabolism, pathogenesis-related protein synthesis, as well as phenylpropanoid and octadecanoid pathways. However, plant defense pathways triggered and the level of observed protection can vary depending on the considered compound, the targeted pathosystem, and the environmental conditions. Plant immunity can occur at two distinct scales, either locally (local acquired resistance, LAR) or systemic (systemic acquired resistance, SAR). LAR is expressed by cells, tissues, or even organs that have been treated by an elicitor, whereas SAR occurs in parts of the plants spatially distant from the treated tissues or organs. LAR is often a prerequisite to the expression of SAR, and SAR has been characterized as broader and more long-lasting than LAR. Other characteristic features of SAR are that it depends on the perception of salicylic acid (SA) and results on the expression of PR-protein encoding genes. Beside SAR, the systemic expression of induced resistance can also consist of induced systemic resistance (ISR), triggered at the root level by certain beneficial microorganisms occurring in the rhizosphere (such as plant growth-promoting bacteria, PGPR; plant growth-promoting fungi, PGPF; and arbuscular filamentous fungi, AMF). Although ISR also leads to a broad and long-lasting induced resistance, it relies on jasmonic acid (JA) perception and generally does not result in PR-protein biosynthesis. The compounds inducing the plant immune defenses may be distinguished into two categories, including elicitors, that directly activate host defense responses after their application, and priming agents, that require additional signals, such as pathogen recognition, to trigger full defense responses. Primed plants may develop a stronger and/or faster response pattern than so- called naïve (unprimed) plants. They can also detect the pathogen invasion at a lower threshold and hence react in a more sensitized way. Finally, primed plants can exhibit other response networks, involving specific defense pathways. An overview of the recent advances on plant resistance inducers as well as future challenges to promote their development and wide use in disease management programs will be discussed.

ORAL PRESENTATIONS

Root colonization by the oomycete biocontrol agent *Pythium oligandrum* in various French and world

vineyards and protection of grapevines exposed to biotic and abiotic stresses. L. CHABOUSSIE[1,2], A. YACOUB[1], J-F. GUISE[2], E. ATTARD[1], P. TROTEL-AZIZ[2], F. FONTAINE[2], P. REY[1]. [1]*Université de Pau et des Pays de l'Adour, CNRS, IPREM UMR 5254, 64000 Pau, France.* [2]*Université de Reims Champagne-Ardenne, INRAE, RIBP USC 1488, 51100 Reims, France.* E-MAIL: lisa.chaboussie@univ-pau.fr

Grapevine trunk diseases (GTDs) are complex and pose a significant threat to viticulture worldwide. This study investigates the potential of the oomycete *Pythium oligandrum*as a biocontrol agent to protect grapevines. Colonisation of grapevine roots by *P. oligandrum* was assessed in 11 cultivars from diverse wine-growing regions across France, Austria, Hungary, Iran, Israel, Italy, South Africa and Spain. Results showed that, with the exception of South Africa, most grapevine roots were colonised by *Pythium* spp. with echinulated oospores. ITS-region sequencing identified 90% of these strains as *P. oligandrum*. Amplification of the elicitin-like genes encoding the proteins, oligandrin and the cell wall protein (POD1), which are essential for the induction of systemic plant resistance, showed that the majority (80%) of these strains possessed both genes. Associated rhizospheric microbial communities (i.e. bacteria, fungi and oomycetes) have also been studied. In parallel, one Po strain was applied to grapevine cuttings later infected by *Neofusiccocum parvum*, a GTD- pathogen, with or without additional abiotic stresses (e.g. water deficit and heat shock). Grapevines colonised with *P. oligandrum* showed a 41% reduction in internal necrosis caused by *N. parvum* compared to control plants. However, under abiotic stresses, the protection provided by the biocontrol agent was no greater than under normal conditions. This research highlights the potential of *P. oligandrum* as an effective biocontrol agent and provides insights into its mechanism of action and its role in improving grapevine resistance to fungal diseases and abiotic stresses.

Environmental isolates of *Pseudomonas* spp. inhibit *Armillaria mellea* and promote microbiome- induced growth in olive plants. M.M. ACI[1], G.E. AGOSTEO[1], G. PELLE[1], V. CIANCI[1], M.G. LIDESTRI NICOSIA[1], A. MALACRINO[1,2], L. SCHENA[1]. [1]*Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, 89124 Reggio Calabria, Italy.* [2]*Department of Biological Sciences, Clemson University, Clemson, SC, USA.* E-MAIL: miyassa.aci@unirc.it

Modern agriculture faces significant challenges in managing soil-borne pathogens such as *Armillaria mellea*, a destructive pathogenic fungus that causes root rot in several plant species. Harnessing the soil microbiomes offers a promising alternative to overcome the limitations of chemical control methods. In this study, four *Pseudomonas* isolates, selected from a wider panel of 155 strains isolated from soil, demonstrated a strong *in vitro* efficacy against *A. mellea*; three isolates inhibited fungal growth, and one prevented rhizomorph formation. Whole genome sequencing revealed gene clusters related to the biosynthesis of antifungal compounds and siderophores, implicating these as likely mechanisms for pathogen suppression and plant growth promotion. Microcosms experiments and amplicon metagenomic analysis revealed that the presence of the biocontrol agents increased plant biomass, while exerting only limited effects on the bacterial communities associated with olive plant roots and rhizosphere. Through structural equation modelling, we found that biocontrol agents influence plant biomass both directly and indirectly through the modulation of root and rhizosphere microbiota. These bacterial isolates offer an alternative approach to manage soilborne pathogens like *A. mellea*, promoting plant health and growth with minimal impact on the ecosystem.

This research was financially supported by i) PSR Calabria 2014/2020 Misura 16.2. Prevenzione e contrasto alla diffusione del marciume radicale fibroso da Armillaria in olivicoltura (ARMISTOP) and ii) Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Biocontrol potential of *Bacillus halotolerans* against *Fusarium oxysporum* f. sp. *radicis- cucumerinum* under normal and saline soil conditions. F. SEVDALI[1], A. MONKA[1], M. MARINAKOU[1], E. POULAKI[1], P.C. TSALGATIDOU[2], E. PAPLOMATAS[1], A. VENIERAKI[1]. [1]*Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.* [2]*Department of Agriculture, University of Peloponnese, 24100 Kalamata, Greece.* E-MAIL: venieraki@aua.gr

Root and stem rot caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) is a serious threat to

cucumber cultivation worldwide, often leading to substantial yield losses. With the limited efficacy of chemical control methods, biological control strategies offer promising alternatives. This study evaluated the biocontrol potential of two endophytic *Bacillus halotolerans* strains (Cal.l.30 and Cal.f.4) against FORC under greenhouse conditions. Cucumber plants were treated with foliar applications of Cal.l.30, Cal.f.4, and their combination, with repeated treatments every 10 days over a 30-day period. Plants treated with the bacterial strains exhibited a delayed onset of disease symptoms and achieved approximately 67% protection compared to untreated controls. Additionally, the experiments were repeated under increasing soil salinity levels up to 200 mM NaCl. Under salinity stress, treatments with Cal.f.4 and the Cal.l.30 + Cal.f.4 mixture conferred enhanced protection against FORC, even under combined abiotic (salinity) and biotic (pathogen) stress conditions. The bacterial strain Cal.f.4 demonstrated a protective effect on FORC-infected plants grown under saline conditions, reducing overall disease and abiotic stress symptomatology by approximately 20%, and enhancing the production of healthy fruits by 30% compared to the negative control. Molecular analyses indicated the upregulation of defense-related gene expression in treated plants. These findings highlight the potential of *B. halotolerans* strains not only as effective biocontrol agents against FORC but also as enhancers of plant resilience under salinity stress. The results support their integration into sustainable disease management programs, especially in agricultural systems increasingly affected by soil salinization.

This research was financially supported by the project "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP - TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative "Agriculture and Food Industry".

Biological control of *Podosphaera xanthii* in cucumber by *Bacillus halotolerans* Cal.l.30: activation of plant defense pathways and role of secondary metabolites. T. MARGARITOPOULOU[1], S. NASTOS[1], K. KOTSARIDIS[2], M. MARATOS[1], P.C. TSALGATIDOU[3], A. VENIERAKI[4], E. MARKELLOU[1]. [1]*Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 14561 Athens, Greece.* [2,1]*Laboratory of Virology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 14561 Athens, Greece.* [3]*Department of Agriculture, University of Peloponnese, 24100 Kalamata, Greece.* [4]*Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece.* E-MAIL: th.margaritopoulou@bpi.gr

Powdery mildew (PM), caused by *Podosphaera xanthii*, is a major constraint in cucumber (*Cucumis sativus* L.) cultivation worldwide. The extensive use of fungicides underscores the need for sustainable biological control strategies. This study evaluated three endophytic *Bacillus* strains — Cal.r.19 (*B. subtilis*), Cal.r.29 (*B. velezensis*), and Cal.l.30 (*B. halotolerans*) — isolated from *Calendula officinalis*, for their efficacy against PM. Greenhouse assays showed that Cal.l.30 consistently achieved a reduction of over 90% in disease severity without phytotoxic effects. In *in vivo* assays performed on cucumber cotyledons, the infection process and morphological development of *P. xanthii* were microscopically monitored in the presence of the biological control agents. Observations focused on pathogen structures such as spore germination, mycelial growth, and conidiophore formation, allowing for the assessment of the inhibitory effects exerted by the beneficial *Bacillus* strains at the early stages of infection. Additionally, gene expression analyses revealed that Cal.l.30-treated plants exhibited significantly higher expression of defense-related genes (PR-1, PR-4, WRKY, MAPK), indicating the activation of induced systemic resistance (ISR). Moreover, Cal.l.30 produces secondary metabolites with fungicidal activity. Protein–ligand docking studies suggest that bacillaene analogues interact with fungal ribosomal subunits, leading to the disruption of protein expression. This interference affects the functional activity of the *P. xanthii* effector protein RPS27A, thereby inhibiting fungal pathogenicity. These findings highlight a dual- action mechanism involving both direct antagonism and host defense priming. *Bacillus halotolerans* Cal.l.30 emerges as a strong candidate for the biocontrol of obligate pathogens such as *P. xanthii,* supporting the integration of endophytic beneficial bacteria into cucurbit disease management programs.

This research was financially supported by the project "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP - TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative "Agriculture and Food Industry".

Harnessing nave rhizosphere soil microbial communities to support traditional almond cultivation in Morocco. Z. BOUABIDI[1], A. MOSCA[2], D. NICOTRA[2], G. DIMARIA[2], F. MODICA[2], M.E. MASSIMINO[2], L.E.

VISÔTTO[3], M. DOUMA[1,4], N. MANAUT[4], V. CATARA[2]. [1]*Natural Resources Engineering and Environmental Impacts Team, Multidisciplinary Research and Innovation Laboratory, Polydisciplinary Faculty of Khouribga, Sultan Moulay Slimane University, BP 145, 25000 Khouribga, Morocco.* [2]*Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy.* [3]*Laboratory of Biochemistry and Molecular Genetics, Federal University of Viçosa, campus Rio Paranaíba. Road MG 230 Km 7, LAE Building, Room 121, Zip code 38810-000, Rio Paranaíba – MG, Brazil.* [4]*Laboratory of Water Sciences, Microbial Biotechnologies, and Natural Resources Sustainability, Faculty of Science Semlalia, Cadi Ayyad University, BP 2390, Marrakech 40000, Morocco.* E-MAIL: zinebbouabidi3@gmail.com

Almond (*Prunus dulcis* Mill.) cultivation in Morocco, particularly of traditional varieties such as Beldi, plays a vital role in supporting rural livelihoods and preserving agrobiodiversity. In many regions of the country, almonds are still propagated by seed, a practice that not only sustains genetic diversity but also reflects deep-rooted cultural traditions. However, climate variability, soil degradation, and plant diseaseS threaten the resilience and productivity of these systems. Native Mediterranean plants, known for their adaptability, may harbor beneficial microbiota with the potential to support almond growth and health. To investigate this potential, rhizospheric soils from five plant species were sampled, including almond trees and four wild Mediterranean species commonly associated with almond cultivation areas in Morocco. Total genomic DNA was extracted and subjected to amplicon-based metagenomic sequencing, targeting bacterial 16S rRNA and fungal ITS regions. Significant differences in bacterial richness and fungal diversity were detected using the Observed and Shannon α-diversity metrics, respectively. Similarly, β-diversity analysis revealed a clear differentiation of samples based on the plant species from which the soils were collected. A collection of cultivable microorganisms was established from all soils using multiple culture media. Bacterial isolates were screened for functional traits related to plant growth promotion, including salinity tolerance, phosphate solubilization, and siderophore production. Both bacterial and fungal isolates were assessed for antagonistic activity against phytopathogens. The results are expected to provide insights into plant-specific microbiomes in Moroccan soils and facilitate the selection of new microbial inoculants to enhance seed germination, as well as seedling growth and health.

This research was financially supported by: The Italian Ministry of Foreign Affairs and International Cooperation (MAECI) grant for the academic year 2024-25.

Isolation and characterization of Psa-bacteriophages from infected kiwifruit orchards in Portugal: potential for biocontrol of *Pseudomonas syringae* pv. *actinidia*. E. GIMRANOV[1,2,3], J. AZEREDO[2], C. SANTOS[1], L. MOURA[3]. [1]*Biology Department, Faculty of Science, University of Porto (FCUP), 4169-007 Porto, Portugal.* [2]*Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal.* [3]*CISAS—Centre for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, 4900-347 Viana do Castelo, Portugal.* E-MAIL: up201804355@edu.fc.up.pt

Bacterial canker is a severe disease of *Actinidia* species. This disease is caused by *Pseudomonas syringae* pv. *actinidiae* (Psa), which is an extremely virulent pathogen compromising kiwi production worldwide. Efficient treatments are unavailable, and the recurrent use of copper-based compounds led to Psa-resistant populations. The bacteriophages represent a safer and sustainable solution for Psa control. The aim of this study is to isolate and characterize a population of Psa-bacteriophages from infected kiwifruit orchards in North and Central Portugal and evaluate their potential as biocontrol agents against Psa. To achieve this goal, bacteriophages were isolated from different samples. Bacteriophages lytic activity was characterized against representative Psa-lineages through efficiency of plating and inhibition curves. The most promising-phages were selected for transmission-electron-microscopy and genome sequencing. Finally, *in vitro* assay to assess the impact of bacteriophages on Psa concentration in surface of kiwifruit leaves, as well as stability under different pH levels (5–8), temperatures (5–35°C), and UVA radiation exposure (180 min). In total, 41 bacteriophages were isolated, among which two were lytic and other lysogenic. Three types of tail morphologies were observed (*Podovirus*, *Myovirus* and *Siphovirus*). Only Brt_Psa3 demonstrated strong activity and specificity (91 % of the bacterial collection). Genomic analysis supports its safe application as it does not possess any bacterial virulence genes. *In vitro* experiments demonstrated significant reduction of Psa levels on kiwifruit surfaces and tolerance to different environmental conditions, highlighting the bacteriophage's therapeutic potential. Future work should focus on optimizing bacteriophage formulations and conducting extensive field trials.

This research was financially supported by predoctoral grant the Fundação para a Ciência e Tecnologia (FCT) with the reference 2021.07616.BD.

From Lab to plant phenotyping platform: a stepwise pipeline for the selection of high-performing antagonistic bacteria against tomato soil-borne pathogens.
C. VINCENZO[1,2], P. TRIPODI[1], N. LOMBARDI[2], C. PANE[1]. [1]*Council for Agricultural Research and Economics (CREA), Research Centre for Vegetables and Ornamental Crops, Pontecagnano Faiano, Italy;* [2]*Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy.* E-MAIL: cono.vincenzo@crea.gov.it*;* cono.vincenzo@unina.it

Biological control is a valuable tool for the sustainable management of soil-borne diseases in tomato. Digital technologies can play a key role in accelerating the screening of new, high- performing microbial biocontrol agents. In this study, a collection of eleven endophytic bacterial strains, isolated from tomato and compatible with *Trichoderma* spp. (with a potential for future consortium formulation), were evaluated against the tomato fungal pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Sclerotium rolfsii*. The assessment included both *in vitro* and *in vivo* assays, and biochemical characterization. The final stage of the evaluation was conducted using the PlantEye 500 multispectral dual scanner embedded within a plant phenotyping platform to acquire the plant's phenomic response to the microbial treatments. The experimental design included potted tomato plants 'Crovarese' (n=234) distributed among 11 microbial treatments along with non- treated and healthy controls, each with six replicates under three different conditions: infection with *F. oxysporum* f. sp. *lycopersici*, infection with *S. rolfsii*, and no infection. Multivariate analysis of 20 digitally computed traits identified *Peribacillus* spp. C5NA and *Neobacillus* spp. TR12 as the most effective against *F. oxysporum* f. sp. *lycopersici,* while *Peribacillus* spp. TR2 and C6 showed significant efficacy against *S. rolfsii*. Additionally, *Microbacterium* sp. TR9 was found to enhance digital biomass, plant height and NDVI, and to increase biofertilizing properties (ammonia production, nitrogen fixation, phosphate solubilization) and hormone-like activity (indoleacetic acid production), highlighting its potential as a promising biostimulant. Overall, the use of phenomics supported the selection of specialized and effective microbial strains for biocontrol applications.

This work was supported by the Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Evaluation of potential biological control agents against *Colletotrichum godetiae* and *C. nymphaeae,* the main causal agents of olive anthracnose in southern Spain.
L. SÁNCHEZ-PEREIRA[1], B.I ANTÓN-DOMÍNGUEZ[1], A. TRAPERO[1], C. AGUSTÍ-BRISACH[1]. [1]*Departamento de Agronomía (Unidad de Excelencia 'María de Maeztu' 2020-2024), ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, España.* E-MAIL*:* z22sapel@uco.es

The use of beneficial microorganisms as biological control agents (BCAs) has been proposed as an alternative to control olive anthracnose, caused by *Colletotrichum* species. The antagonistic effects of 21 BCAs against *C. godetiae* (Col-511) and *C. nymphaeae* (Col-151) were evaluated. Copper sulfate (2000 mg/L) and Serenade ASO® (*Bacillus subtilis*, 8 mL L-1) were included for comparison. Their inhibitory effect on mycelial growth and conidial production of both pathogens were assessed using dual culture assays *in vitro*. Bioassays on detached fruits and potted plants of cv. Arbequina were conducted to determine their effects on disease progression, severity, and incidence. Six BCAs suppressed Col-511 growth between 31 and 43%, while 12 inhibited Col-151 growth between 32 and 56%. Sixteen BCAs reduced Col-511 conidial production by >50%, but only five achieved >80% inhibition for Col-151. Copper sulfate and Serenade ASO® were the most effective *in vitro*. Five BCAs reduced disease progression by >30% in detached olives inoculated with Col-511; however, reductions did not exceed 23% in fruits inoculated with Col-151 compared to the untreated control. The three most effective BCAs (*Bacillus* sp. PV-700, *Bacillus* sp. PV-1394 and *Myrothecium inundatum* PV-329) from the detached fruits assay were further evaluated in potted plants. *In planta*, they reduced disease incidence between 32.9-57.8% for both *Colletotrichum* species compared to the untreated control, whereas copper sulfate reduced disease incidence by 60% and 50% for Col-511 and Col-151, respectively. These results will allow the selection of potential BCAs for further investigation of their mechanisms of action against the disease.

This research has been funded by the Spanish Ministry of Science and Innovation and State Research Agency (project PID2021-123645OA-I00 'BIOLIVE'), co-financed by the European Union ERDF Funds. L. Sánchez-Pereira is the holder of a grant for 'Formación de Personal Investigator' (FPI; contract no.

PRE2022-101542). B.I. Antón- Domínguez was the holder of a FPI grant (contract no. PRE2020-096038) during all the experimental period. We gratefully acknowledge financial support from the State Research Agency through the Severo Ochoa and María de Maeztu Program for Centers and Units of Excellence in R&D (Ref. CEX2019-000968-M). The authors thank F. Luque, M.C. Saigner, A. Caneo and F. González for their technical assistance in the laboratory.

POSTER PRESENTATIONS

Integrating biostimulation and plant pathogen control: endophytic *Trichoderma* strains boost yield and disease resistance in field crops. A. CSÓTÓ[1], P. RICZU[2], A. ZABIÁK[3], L. KARAFFA[4], E. SÁNDOR[3]. [1]*University of Debrecen, Faculty of Agricultural and Food Science and Environmental Management, Institute of Plant Protection, H-4032 Debrecen, Böszörményi út 138., Hungary.* [2]*KITE Zrt., Innovation Directorate, H- 4181 Nádudvar, Bem J. u. 1., Hungary.* [3]*University of Debrecen, Faculty of Agricultural and Food Science and Environmental Management, Institute of Food Science, H-4032 Debrecen, Böszörményi út 138., Hungary.* [4]*University of Debrecen, Faculty of Science and Technology, Department of Biochemical Engineering, H-4032, Debrecen, Egyetem tér 1, Hungary.* E-MAIL: karaffa@agr.unideb.hu

The biostimulant potential of endophytic *Trichoderma* fungi in field conditions presents a viable option for sustainable agriculture. This research examined the impact of foliar spraying with a formulated consortium of *Trichoderma afroharzianum* TR04 and *T. simmonsii* TR05 on maize and sunflower fields in Hungary. The treatment significantly enhanced plant photosynthetic activity, evidenced by an increase in the number of viable leaves by up to 6.7% along with a rise in the SPAD index, indicating chlorophyll content, by up to 19.1% relative to the control. Under extreme drought conditions, maize yield doubled, increasing from 0.587 t/ha to 1.62 t/ha ($P < 0.001$). Additionally, consistent increases in harvested seed moisture content and sunflower plant height were observed. *In vitro* studies revealed that these *Trichoderma* strains demonstrated effective mycoparasitism against *Fusarium* and *Aspergillus* species, suggesting a significant potential to reduce mycotoxin contamination in field crops. Field applications significantly reduced the incidence of *Fusarium* head blight in wheat. This study supports the use of foliar applications of well-selected endophytic *Trichoderma* biostimulants to enhance crop yield, quality, and abiotic stress tolerance in both monocot maize and dicot sunflower crops, offering a sustainable and effective strategy for modern agriculture.

Biocontrol of tomato diseases by alfalfa extracts. A. TAGLIENTI[1], A. TAVA[2], S. BERTIN[1], E. BIAZZI[2], C. VINCENZO[3,4], C.L. CORRADO[1], S. SIMONI[1], C. PANE[4]. [1]*CREA Research Centre for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Rome, and Via Lanciola 12A, 50125 Florence, Italy.* [2]*CREA Research Centre for Animal Production and Aquaculture, Viale Piacenza 29, 26900, Lodi (LO), Italy.* [3]*Department of Agricultural Sciences, University of Naples Federico II, Piazza Carlo di Borbone 1, 80055 Portici (NA), Italy.* [4]*CREA Research Centre for Vegetal and Ornamental Crops, Via Cavalleggeri 51, 84098 Pontecagnano Faiano (SA), Italy.* E-MAIL: anna.taglienti@crea.gov.it

The control of plant pathogens by compounds extracted from plants is increasingly popular as a sustainable alternative to agrochemicals, which are currently subjected to limitations in the European Union; in fact, regulatory frameworks aim at reducing the risks and impacts of synthetic pesticides in terms of toxicity, environmental pollution, and the development of resistance. In this context, plant extracts are gaining interest because of their effects on plant fitness and tolerance to biotic stressors. Flavonoids and saponins extracted from alfalfa, and prosapogenins obtained by alkaline hydrolysis of saponins, were the subjects of this study. The flavonoid extract was mainly composed of apigenin and chrysoeriol glycosides, while saponins contained bidesmosides of medicagenic and zanhic acids, and prosapogenins were made up only of the corresponding monodesmosidic compounds. An evaluation of their biopesticidal potential towards two fungi and a virus affecting tomato, as well as their possible undesired effects, was performed. Prosapogenins inhibited both the mycelial growth and conidia germination of *Alternaria alternata* and *Botrytis cinerea,* and showed lesion- containment efficacy against alternariosis and grey mould. The flavonoid mixture decreased the tomato spotted wilt orthotospovirus titer by 10^{-3}-fold one-month post-inoculation, with a concurrent regression of symptoms. The assessment of non-target effects towards two beneficial mites, *Phytoseiulus persimilis* and *Amblyseius swirskii*, indicated that the treatments were not toxic, while some impact on fertility was observed especially with flavonoid extract on *A. swirskii*. The biopesticide activity against fungi and viruses in tomato, and their safe toxicity profile, support the application of these extracts in Integrated Pest Management programs.

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2,

INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Assessment of a *Streptomyces* strain and its metabolites against fungal pathogens on wheat and tomato. T. CONTE[1], M.G. MOREA[1], G. RICCIARDI[1], J. G. ZORRILLA[2], R. M. VARELA[2], F. A. MACIAS[2], M. MASI[3], A. CIMMINO[3], A. CARLUCCI[1]. [1]*Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia*, *Foggia*, *FG 701122, Italy.* [2]*Grupo de Alelopatıa (GAC), Department of Organic Chemistry, University of Cadiz, Puerto Real, 11510, Spain.* [3]*Department of Chemical Sciences, University of Napoli Federico II, University Complex of Monte Sant'Angelo, Naples, NA 80126, Italy.* E-MAIL: thomas.conte@unifg.it

One of the most difficult challenges for modern agriculture is providing sustainable production capable of meeting the demands of a growing global population, which is expected to reach 9.8 billion people by 2050. In the past, chemical management helped achieve high productivity levels, but nowadays the environmental, economic and ethical issues associated with this approach underscore the need to find sustainable alternatives for plant protection against fungal diseases. In particular, natural products derived from microorganisms are potential tools against plant pathogens. Among these microorganisms, the genus *Streptomyces* is known for its capability to produce many largely unexplored bioactive compounds with potential antifungal activity. Through research activities carried out under both *in vitro* and *in vivo* conditions, four *Streptomyces* strains were screened for their potential use as Biocontrol Agents (BCAs). Among them, the strain *Strep_22* significantly reduced the growth of ten fungal pathogens (*Athelia rolfsii*, *Fusarium graminearum*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Verticillium dahliae*). Moreover, the culture filtrate of the same *Streptomyces* strain was able to protect the roots of wheat and tomato and, at the same time, to promote plant growth. As a subsequent step, the biological activities of metabolites isolated from this same strain following chromatographic methods have also been explored.

Use of agricultural waste to control *Phytophthora cinnamomi* root disease on *Quercus ilex*. R. LÓPEZ[1], M.T. HIDALGO[1], M. GARCÍA[1], R. RODRÍGUEZ-ARCOS[2], A. JIMÉNEZ-ARAUJO[2], M.S. SERRANO[1]. [1]*Department of Agronomy, Campus de Rabanales Edif. C-4, University of Córdoba, Córdoba, 14071, Spain.* [2]*Department of Food Phytochemistry, Campus de la Universidad Pablo de Olavide, Edif. 46, Instituto de la Grasa-CSIC, Sevilla, 41013, Spain.* E-MAIL: a12semom@uco.es

The circular economy could turn agricultural waste into innovative biocontrol tools. We studied the effect of two organic extracts enriched in bioactive compounds obtained from by- products of asparagus (spears) and date (seeds), as well as the residues of both species, on *Phytophthora cinnamomi* (*Pc*) root disease development *in planta*. Extracts were chosen by their known effectiveness on inhibition of *Pc* sporangial production. Residues previously shredded and extracts diluted in water were added separately at 0.05% (w/v) to a substrate (sand-peat 1:1 vol) infested with 250 chlamydospores of *Pc* per gram of dry soil and 10 pots per treatment were planted with 1-year-old *Quercus ilex* seedlings. Pots with infested and uninfested substrate were also prepared as controls. All pots were placed in a shade house and subjected to waterlogging 2 days per week. Fourteen weeks later, seedlings growing in infested and untreated substrate showed high levels of root symptoms, whereas plants growing in substrate treated with both extracts and residues showed a significant reduction of symptom severity, being the organic extract of date seeds the most effective treatment. It is highlighted that there are not significant differences between extract and residues for each crop although they were tested at the same dose regardless of their phytochemical concentration. Results suggest that soil application of asparagus spears and date seeds extract, and especially residues, should be considered for the reduction of the incidence of *Pc* root disease affecting *Quercus*.

Research funded by Emergia Program (Call 2021).

Potential antagonism and plant growth promoting capacities of two endophytic actinobacteria strains isolated from Moroccan aromatic and medicinal plants. EZ. OUBASSOU[1], L. AZLAY[1], Y. OUAHMANE[1], M. BAZ[1], A. JAMJARI[1], A. BERR[3], M. BARAKATE[1,2]. [1]*Laboratory of Water Sciences, Microbial Biotechnologies and Natural Resource Sustainability; Microbial Biotechnology, AgroSciences and Environment & CNRST Labeled Research Unit N° 4, Faculty of Sciences Semlalia, Cadi Ayyad University, 40000 Marrakesh, Morocco.* [2]*Biodiversity and Plant Sciences Program, AgroBioScience Department, College of Agriculture and Environmental Sciences, Mohammed VI Polytechnic University (UM6P), 43150 Benguerir, Morocco.* [3]*Institut de Biologie Moléculaire des*

Plantes (IBMP), CNRS, Université de Strasbourg, 12, rue du Général Zimmer, 67084 Strasbourg, France. E-MAIL: mbarakate@uca.ac.ma

Since the discovery of actinomycin, *Actinobacteria* have provided many important compounds of high commercial value and continue to be routinely screened for new bioactive substances that are investigated for their agricultural applications. However, the intensive application of pesticides for crop protection can affect the environment and the health of humans and animals. Thus, the present work aims to assess the effectiveness of endophytic actinobacteria isolated from some interesting medicinal plants as biocontrol agents against *Pectobacterium* spp. and *Streptomyces scabies* which are the agents of soft rot and common scab, respectively and constitute a severe problem for potato crops. From a total of forty-four endophytic actinobacteria isolated from Aromatic and Medicinal plants, which were screened for their ability to inhibit *in vitro* different strains of *Pectobacterium* sp. and *S. scabies,* as well as for their PGPR traits, two *Streptomyces* isolates (A28 and A36) were selected for their *in vitro* antimicrobial activity against both pathogens and their ability to produce hydroxamate- type siderophores. *In vivo*, they significantly reduced ($P < 0.05$) the severity of soft rot on potato slices of cv. Desiree caused by *P. carotovorum* and *P. atrosepticum*. Furthermore, culture filtrate application of *Streptomyces* sp. A28 on potato tubers reduced the severity and incidence of the disease, as a preventive measure under storage conditions. This prevention was accompanied by the induction of defense enzymes related to oxidative metabolism and the phenylpropanoid pathway. Our data suggests that *Streptomyces* sp. A28 could be a promising natural biocontrol agent against both *Pectobacterium* spp. and *S. scabies*, and act as a biostimulant, to partially substitute the use of synthetic chemicals.

This research was financially supported by the CNRST Labeled Research Unit N°4 grant, and the Partnership Hubert Curien (PHC) Maghreb (PHC 23MAG09).

Biocontrol potential of bacterial strains against root and stem rot of cucumber. S.K. SOULTATOS[1,2], N.E. KRASAGAKIS[1,2], M. PAPADAKI[2], V. MICHALOPOULOU[3,4], P.F. SARRIS[3,4,5], E.A. MARKAKIS[1,2]. [1]*Institute of Olive Tree, Subtropical Crops and Viticulture, Hellenic Agricultural Organization "DIMITRA", Kastorias 32A, 71307, Heraklion, Crete, Greece.* [2]*School of Agricultural Sciences, Hellenic Mediterranean University, Stavromenos 71004, Heraklion, Crete, Greece.* [3]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology – Hellas, Heraklion, Crete, Greece.*[4]*Department of Biology, University of Crete, Heraklion, Crete, Greece.* [5]*Biosciences, University of Exeter, Exeter, United Kingdom.* E-MAIL: markmanos@hmu.gr

Root and stem rot of cucumber (*Cucumis sativus* L.) caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*F.o.r.c.*) is one of the most severe diseases of cucumber, with no effective chemical control available. In this study, we evaluated the suppressive effect of 31 bacterial strains isolated from the rhizosphere of halophytes in Crete (Greece) against root and stem rot of cucumber. Preliminary evaluation of bacterial strains *in-vitro* revealed that 17 strains inhibited fungal growth significantly (≥20%). These most effective bacterial strains were further evaluated thoroughly in dual-culture (confrontation tests) and dual-plate (volatile tests) assays *in vitro*. Fungal parameters such as growth rate, hyphal width and sporulation of the pathogen were assessed. Confrontation tests revealed that 13 bacterial strains inhibited fungal growth rate and 6 decreased hyphal width significantly, whereas none of the tested strains inhibited sporulation of the pathogen. Volatile tests revealed that 5 strains were capable of inhibiting fungal growth, 8 strains decreased hyphal width and 5 strains decreased *F.o.r.c.* conidial production significantly. Based on *in vitro* assays, the 10 most effective bacterial strains were selected and evaluated furtherly for their efficiency to protect cucumber against root and stem rot, by conducting *in planta* bioassays. The results indicated that one bacterial strain (code SRL871) reduced all disease parameters significantly compared to the positive control plants. Decreased, symptom severity was associated with lower plant colonization levels by the fungus and increased plant fresh weight.

The present study was conducted within the framework of the project entitled: «Innovations in Plant Protection for sustainable and environmentally friendly pest control» (Acronym InnoPP, Code Act TAA: TAEDR-0535675) «Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan»

Potential of indigenous biological agents for the control of grapevine trunk diseases in montenegrin viticulture. N. LATINOVIĆ[1], J. LATINOVIĆ[1], B. KANDIĆ[1], A. JELUŠIĆ[2], T. POPOVIĆ MILOVANOVIĆ[3]. [1]*University of Montenegro, Biotechnical Faculty, Bulevar Mihaila Lalića 1, 81000 Podgorica, Montenegro.* [2]*Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1, 11030 Belgrade, Serbia.* [3]*Institute for Plant*

Protection and Environment, Teodora Drajzera 9, 11040 Belgrade, Serbia. E-MAIL: tanjaizbis@gmail.com

Grapevine trunk diseases **(**GTDs) are a group of diseases caused by a large number of fungal phytopathogens that infect and colonize the woody tissue of grapevine, causing chronic or apoplectic decline of plants, and as such posing a serious threat to viticulture. Among the most virulent pathogens are *Neofusicoccum parvum*, the causal agent of Botryosphaeria dieback, and *Fomitiporia mediterranea* considered as the main causal agent of grapevine rot within the Esca disease complex. This study aimed to isolate and select potent indigenous bacteria collected from Montenegrin vineyards to serve in the control of GTDs. Isolations were performed from soil samples taken in a zone of grapevine root (Montenegro) on NA after soil was dissolved in water and heating at 80°C for 20 min. A total of 262 isolates were tested for antagonistic activity in a direct *in vitro* test on PDA. The appearance of a clear zone was checked after fungi development. Results showed that 32 Montenegrin isolates inhibited the growth of *N. parvum* by 34.0–65.0%, and *F. mediterranea* by 34.8–71.0%. Neighbour- joining phylogenetic analysis based on the 16S rRNA (primers P0/P6) sequences differentiated those isolates into six clusters but lacked the resolution to differentiate between several species. Six groups were as follows: I (21 isolates: *Bacillus amyloliquefaciens/B. velezensis/B. siamensis/B. subtilis*), II (2: *B. subtilis/B. velezensis/B. tequilensis*), III (5: *B. halotolerans/B. mojavensis/B. subtilis/B. velezensis/B. tequilensis/B. spizizenii*), IV (1: *B. licheniformis/B. paralicheniformis*), V (2: *B. cereus/B. thuringiensis/B. albus/B. anthracis*), VI (1: *Paenibacillus peoriae/P. polymyxa*). Sequencing of housekeeping genes would provide more accurate identification.

This work was supported by the Ministry of Education, Science and Innovation of Montenegro [Project "Biofungicides application in agriculture and urban areas" - BIOAPP] and Ministry of Science, Technological Development and Innovation of the Republic of Serbia [contract numbers 451-03-136/2025-03/200010, 451-03-136/2025-03/200053].

Effectiveness of Tunisian Bacteria B8, B9, and B15 in controlling *Diplodia seriata in vitro* and *in planta* in grapevine. H. TRABELSI[1,2], L. KALAI GRAMI[2], Y. ZAOUALI[1], C. MESSOUAD[1], F. FONTAINE[3], A. BEN GHNAYA-CHAKROUN[1]. [1]*University of Carthage, INSAT, Laboratory of nanobiotechnologies LR17ES22, Department of Biology, North Urbain Center BP 676, Tunis 1080, Tunisia.* [2]*Laboratory of Biotechnology Applied to Agriculture, National Institute of Agricultural Research of Tunisia, INRAT, 2049 Hedi Karray Street, Tunis, Tunisia.* [3]*University of Reims Champagne-Ardenne, Induced Resistance and Bioprotection of Plants Research Unit (RIBP) – USC INRAE 1488, BP1039, Reims Cedex 2, France*. E-MAIL: hiba.trabelsi@insat.u-carthage.tn

Grapevine trunk diseases (GTDs), such as Esca and Botryosphaeria dieback, pose a major threat to viticulture worldwide. In Tunisia, little is known about these diseases, but their incidence is increasing, and no effective treatment currently exists. *Diplodia seriata* is a fungal pathogen and one of the etiological agents of Botryosphaeria dieback in grapevines. This study evaluates the effectiveness of three bacteria (B8, B9, B15), isolated from Tunisian grapevines, in controlling *Diplodia seriata* strain *Ds type18*. The bacteria were tested for their ability to colonize grapevine vitroplants, inhibit fungal growth *in vitro*, and reduce disease severity *in planta* (detached canes assay). The results show that B8, B9, and B15: *i)* successfully colonized grapevine vitroplants from roots to leaves; *ii)* Inhibited *Ds type18 in vitro* (64.69 %, 51.25 %, and 56.3 % inhibition, respectively); *iii)* significantly reduced disease severity *in planta* (54 %, 47 %, and 44 % protection, respectively). These findings highlight the potential of these bacteria as promising biocontrol agents for *Diplodia seriata*. Future studies should focus on field applications, bioformulation and ecological assessments.

Development of sustainable activity of some plant extracts against the fire blight. S. LAALA[1], D. BOUAICHA[1,3], S. CHAMAT[2], H. DJABALI[1], A. KERROUM[1], F. VALENTINI[3]. [1]*Laboratory of Phytopathology and Molecular Biology, Botany Department, Higher National Agronomic School (ENSA), EL-Harrach, Algiers, Algeria.* [2]*Laboratory of chemistry, Research Center Scientific and Technical in Analyzes Physico-chemical (CRAPC), Tipaza, Algeria.* [3]*CIHEAM Bari, Centre International de Hautes Etudes Agronomiques Méditerranéennes, via Ceglie 9, 70010 Valenzano (BA), Italy*. E-MAIL: samia.laala@edu.ensa.dz

Fire blight is a serious disease in many countries, and even a small outbreak could have a significant economic impact on a newly affected country, due to potential restrictions on international plant trade. The development of sustainable, effective, and eco-friendly compounds to contain and counteract pathogen development is a key goal of phytopathological research, which focuses on identifying natural molecules or compounds effective against phytopathogenic bacteria. There are several

research studies in literature on extracts and/or compounds of plant origin that are effective in counteracting pathogens and pests that have subsequently been used in the open field in integrated pest management programs. The present study investigates the potential efficacy of four plant extracts (pomegranate, onion, eucalyptus, and garlic) for the control of *Erwinia amylovora*. The selected plants were processed using the maceration technique to obtain aqueous and organic extracts. These extracts were evaluated for their antibacterial activity *in vitro* against *E. amylovora* through disc diffusion, well diffusion, and agar paper tests. Additionally, *vivo* assays were conducted on immature pear fruits and detached pear leaves. From the results obtained, it was found that pomegranate ethanolic extract showed increased and remarkable antimicrobial activity against the pathogen during the biological test (disc diffusion test, well diffusion test and agar paper test) and bioassay (immature pear fruit and detached pear leaves) on reference strains. Pomegranate extract contains a high concentration of bioactive compounds.

Comparative evaluation of biological control agents against *Colletotrichum* species causing olive anthracnose in Italy and Spain. L. SÁNCHEZ-PEREIRA[1], M. RIOLO[2], C. AGUSTÍ-BRISACH[1], S.O CACCIOLA[2]. [1]*Department of Agronomy, ETSIAM, University of Córdoba, Campus de Rabanales, C4, 14071 Córdoba, Spain.* [2]*Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy.* E-MAIL: z22sapel@uco.es

Olive (*Olea europaeae*) anthracnose, caused by *Colletotrichum* species, is a major disease of this crop worldwide, particularly across the Mediterranean Basin. This study evaluated six potential biological control agents (BCAs; *Aureobasidium pullulans* PV-1033; *Bacillus amyloliquefaciens* PV-700; *Epicoccum nigrum* RB4C; *Phoma* sp. PV-375; *Trichoderma asperellum* IMI393899; *T. atroviride* TRICH-S) against representative isolates of the main *Colletotrichum* species causing the disease in Italy and Spain: *Colletotrichum acutatum* C9D2C and UWS149, *C. godetiae* OLP12, OLP16, Col-508, Col-511 and *C. nymphaeae* Col-151 and Col-466. Copper sulfate (2 g L^{-1}) and *Bacillus subtilis* (Serenade ASO®, 8 mL L^{-1}) were used as reference treatments. *In vitro* assays assessed the BCAs' inhibitory effects on mycelial growth and their production of volatile organic compounds (VOCs). All BCAs significantly inhibited mycelial growth in dual cultures and produced VOCs that suppressed the growth of all *Colletotrichum* species. TRICH-S and IMI393899 showed the strongest inhibition in both tests. The pathogenicity of all the *Colletotrichum* isolates was previously assessed by inoculating detached 'Arbequina' fruits. All isolates developed symptoms, with disease incidence ranging from 41.67% to 98.33%. The most virulent *Colletotrichum* isolates (C9D2C, OLP12, Col-511, and Col-151) were used to test the effect of the BCAs on disease development in detached fruits. To this end, BCA suspensions were applied by spraying 4 days and 24 hours prior to pathogen inoculation. IMI393899 was the most effective against C9D2C and OLP12; PV-700 against Col-511; and RB4C and PV-700 against Col-151. These findings identify promising BCAs for further *in planta* evaluation and investigation of their mechanisms of action.

This research has been funded by the Spanish Ministry of Science and Innovation and State Research Agency (project PID2021-123645OA-I00 'BIOLIVE'), co-financed by the European Union ERDF Funds. L. Sánchez- Pereira is beneficiary of a grant for 'Formación de Personal Investigator' (FPI; contract no. PRE2022- 101542). We gratefully acknowledge financial support from the State Research Agency through the Severo Ochoa and María de Maeztu Program for Centres and Units of Excellence in R&D (Ref. CEX2019-000968- M). This study was also inspired by the projects "Nuove soluzioni tecnologiche per la filiera degli agrumi – NewCitrusTech" (cod. 1.3.1, Misura 19 PSR 2014/2022 – CLLD GAL Eloro) and "Azioni Innovative per la Produttività del Distretto dell'Ortofrutta di Qualità – INNOVAPROD" (cod. 1.3.2, Misura 19 PSR 2014/2022 CLLD). The authors thank F. Luque, M.C. Saigner, F. González, Cristian Bua and Sebastiano Conti Taguali for their technical assistance in the laboratory.

Multifunctional bio-based strategies for sustainable control of postharvest fungal pathogens in citrus: yeasts, fermentates and edible coatings. M. RIOLO[1], F. LA SPADA[1], S. CONTI TAGUALI[1], R. PARLASCINO, C. BUA, M.C. TAMBE', A. PANE[1], G.MECA[2], S.O.C CACCIOLA[1]. [1]*Department of Agriculture, Food and Environment, University of Catania, 95123 Catania, Italy;* [2]*Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicente Andrés Estellés s/n, 46100-Burjassot, Spain.* E-MAIL: mario.riolo@unict.it

Postharvest rots significantly impact the shelf life and marketability of citrus fruit in the Mediterranean region. In this study, different methods based on biocontrol agents (BCAs) and bio-formulations to control key postharvest pathogens were evaluated. The yeast *Candida oleophila* strain O was tested against *Penicillium digitatum*, the causal agent of green mold in citrus, using *in*

vivo assays simulating all stages of the postharvest supply chain. To optimize the treatment, the yeast applied to various citrus varieties before, during, and after pathogen inoculation. *Candida oleophila* showed an efficacy comparable to imazalil, the most popular fungicide used for post-harvest treatments of citrus fruit. Transcriptomic analyses of fruits treated with the yeast revealed activation of defense genes, including PAL, β-1,3-glucanases, and peroxidase, indicating multiple mechanisms of action, competition with or antagonism against the pathogen and induction of plant resistance. Cell-free supernatants (CFSs) from fermentates of *Lactiplantibacillus plantarum* and the cyanobacterium *Arthrospira platensis* inhibited toxigenic fungi, such as *Fusarium graminearum* and *Penicillium expansum,* both *in vitro* and *in vivo* on various agricultural products including lemons. The antifungal properties of CFSs were mainly due to acidic metabolites, such as lactic acid, benzoic acid, and DL-3-phenyllactic acid. Finally, chitosan-based, biodegradable-edible fruit coatings activated by nesting the chitosan film with antifungal agents derived from citrus fruit peel were developed. Bioactive coatings effectively prevented Penicillium rots and improved UV shielding. BCAs, bioactive fermentates and biodegradable-edible coatings can be exploited as part of integrated and sustainable management strategies of post-harvest decay of citrus fruits.

This research was inspired by the projects "Nuove soluzioni tecnologiche per la filiera degli agrumi – NewCitrusTech" (cod. 1.3.1, Misura 19 PSR 2014/2022 – CLLD GAL Eloro) and "Azioni Innovative per la Produttività del Distretto dell'Ortofrutta di Qualità – INNOVAPROD" (cod. 1.3.2, Misura 19 PSR 2014/2022 CLLD).

Exploring the potential of mycoviruses as new biological control agents (BCAs) against the grey mould fungus *Botrytis cinerea*. S. LAERA, M. CRUDELE, C. ROTOLO, M. MARASHI, L. VACCARO, P.R. ROTONDO, D. DI COSMO, T. MASCIA, F. FARETRA, R.M. DE MICCOLIS ANGELINI. *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.* E-MAIL: ritamilvia.demiccolisangelini@uniba.it

Botrytis cinerea is the fungus that causes grey mould, a major disease affecting a wide range of host plants, both in the field and postharvest, leading to significant economic losses. Several mycoviruses are known to infect fungal plant pathogens, offering a potential new approach for biocontrol. In this study, we investigated the phylogenetic relationships of mycoviruses previously identified through a metagenomic analysis in *B. cinerea*, the presence of viruses putatively associated to host hypovirulence in individual isolates of the fungus, and their possible influence on the host phenotype. The analysis was performed on 11 (+) ssRNA viruses and revealed a great diversity and the phylogenetic relationships of the identified mycoviruses with known viruses within the *Hypoviridae*, *Fusariviridae*, and *Mitoviridae* families. The presence of eight viruses was checked by RT-PCR in 28 *B. cinerea* isolates, revealing single or multiple viral infections. Among the tested viruses, Botrytis cinerea mymonavirus 1 (BcMyV1) was selected for further analysis. Virus-free isogenic fungal lines were generated by antibiotic treatments combined with hyphal tipping. *In vitro* assays in virus-infected and virus-free isogenic lines revealed that BcMyV1 did not affect colony growth and spore germination. On artificially inoculated grape berries, a slightly reduced virulence of the virus-infected isolate compared to the virus-free isogenic lines was observed but this difference was not statistically significant. The results obtained in this study contribute to the understanding of mycovirus diversity in *B. cinerea* and highlight the potential use of mycoviruses as new candidate biological control agents against grey mould.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

A new approach for the obtention of *Ascophyllum nodosum* stimulants for plant defense and improved seedling development. G. OJEDA[1,2], J. ARAUJO[2], J. COTAS[3], L. PEREIRA[3], F.J. CEBALLOS-BURGOS[4,5], C. MALEITA[4,5], K. BAHCEVANDZIEV[6], A. SOBRAL[2]. [1]*Escuela de Ciencias Agrícolas, Pecuarias y del Medio Ambiente ECAPMA, Universidad Nacional Abierta y a Distancia UNAD, Cl. 14 Sur # 14-23, Bogotá, DC, Colombia.* [2]*Coimbra Chemistry Centre, Department of Chemistry, University of Coimbra, Coimbra, Portugal.* [3]*CFE—Centre for Functional Ecology: Science for People & Planet, Marine Resources, Conservation and Technology—Marine Algae Lab, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal.* [4]*University of Coimbra, Centre for Functional Ecology - Science for People & the Planet (CFE), Department of Life Sciences, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal.* [5]*University of Coimbra, Chemical Engineering and Renewable Resources for Sustainability (CERES), Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal.* [6]*Research Centre for Natural Resources, Environment and Society (CERNAS), Coimbra Agriculture School, Polytech-*

nic of Coimbra, Bencanta, Coimbra, 3045-601, Portugal. E-MAIL: gerardo.ojeda@qui.uc.pt

Due to its bioactive compounds, extracts from the brown algae *Ascophyllum nodosum* (ASn) can be exploited as plant nutritional supplements, acting as biostimulants for plant defence induction. Even though abundant literature reports the effects of *A. nodosum*-based products on plant growth and protection, studies involving simple, low-temperature extraction methods are scarce. We compared two extraction methods to obtain a plant biostimulant to promote plant growth and to induce resistance against the root-knot nematode *Meloidogyne incognita*, an important pest for agriculture worldwide. The protocol we followed was: (a) Biomass: 10 g of *A. nodosum* biomass dried at 60°C. (b) Extraction method: (1) Soxhlet (water, 100°C) – AsnH2500, (2) drip column (water, 20°C) – AsnG2500. (c) Experimental design: 3 treatments (control, AsnH2500, AsnG2500). (d) Seed growth: *Brassica rapa* cv. cymosa (25/dish, 10 days at 21.6°C). (e) *M. incognita* mobility: 50 nematodes*4 replicates incubated at 24°C for 72 h. (f) Chemical characterization: F-TIR and GC/MS analysis. As a final result we could see that the ASnH2500 (high temperature) and ASnG2500 (low temperature) extracts increased the roots, hypocotyl, and total dry weight of *B. rapa* compared to the control treatment. In addition, several compounds responsible for promoting growth, modulating defense, and stress responses were detected. Therefore, it is possible to obtain multipurpose biofertilizers useful for improving plant growth and inducing plant resistance against pests and environmental stress at low temperatures. Although the nematostatic effect of extracts on *M. incognita* was not significant, it opens a way to obtain new valuable extracts under different temperatures.

This research was financially supported by Universidad Nacional Abierta y a Distancia – UNAD (Colombia), University of Coimbra and Coimbra Agriculture School - Polytechnic of Coimbra (Portugal). At UC, this research was financially supported by FEDER funds through the Portugal 2020 (PT 2020), COMPETE 2020 and by the Portuguese Foundation for Science and Technology (FCT), under contracts UIDB/00102/2020, UIDP/00102/2020 (CERES), and UIDB/04004/2025, UIDP/04004/2025 (CFE).

Evaluation of biostimulants and beneficial bacteria for controlling Pistachio decline. W. ROUISSI[1], I. OUERGHI[1,2], I. HEMISSI[1], A. CHELLI CHAABOUNI[1]. [1]*National Institute of Agronomic Research of Tunisia, University of Carthage, Tunis, Tunisia.* [2]*Faculty of science, Manar University, Tunis, Tunisia.* E-MAIL: wafa.r24@hotmail.com

In recent years, the experimental pistachio orchards at INRAT's Agricultural experimentation Unit in Mornag have faced phytosanitary problems, leading to tree decline and reduced productivity. Isolation, Microscopic and molecular identification showed that damages are mostly due to *Alternaria alternata* and *Neofusicoccum mediterraneum.* To address these challenges sustainably, biostimulants were tested as a potential solution to improve tree resilience and enhance yield and quality. *In vivo* trial was conducted on a 33-year-old orchard on Pistachio trees cv. Mateur grafted onto *Pistacia vera* and *Pistacia atlantica* rootstocks, to evaluate the combined effect of two biostimulant products, PLANTA and EPSOMIT, under three treatments: rainfed control (T0), irrigated (T1), and PLANTA-EPSOMIT combined treatment (T2) with regular phytosanitary monitoring. A pre-treatment with Methyl- thiophanate was applied on all the orchard. Observations conducted after treatments during filed inspections showed no new cases of plant dieback and a significant reduction in necrotic leaves previously caused by Alternaria. Absence of infected fruits on bunches of trees treated with Planta-Epsomit comparing to the control T0 was also recorded. Additionally, PLANTA and EPSOMIT effectiveness against *Neofusicoccum mediterraneum was assessed in vitro*, comparing to plant growth-promoting bacteria (B1, B2, and B3). The results revealed that both PLANTA and B3 exhibited the highest antifungal activity, by reducing significantly the fungal mycelium growth by 65% and 50% respectively. This highlights B3 as a promising biological control agent, suggesting that beneficial bacteria and biostimulants, could play a key role in mitigating pistachio decline while reducing reliance on chemical treatments.

This research was financially supported by INRAT and AGRIUP – Distribution society.

Kinetics of lipopeptide production from the supernatant of *Bacillus methyltrophicus* strain TEB1 and dose-response effect of these lipopeptides on the fungus *Pleunodomus tracheiphilus*. L. KALAI-GRAMI[1,2], I. BEN SLIMENE[2], O. NAILI[1], F. LIMAM[2], M. MNARI HATTAB[1], M. RABEH HAJLAOUI[1]. [1]*Laboratoire de Biotechnologie Appliquée à l'Agriculture, INRA, Hedi Karray, Tunis, Tunisia.* [2]*Laboratoire des Substances Bioactives, CBBC, Hammam-Lif, Tunisia.* E-MAIL: Kalai_leila@yahoo.fr

Citrus mal secco is a highly destructive vascular disease caused by the fungus *Pleunodomus tracheiphilus* which has a relevant economic impact on the lemon (Citrus lemon) industry in the mediterranean region. A lipopeptide-producing endophytic strain *Bacillus methyltrophicus* TEB1, obtained from citrus leaves, exhibited a good *in vitro* activity against *P. tracheiphilus* in dual cultures as well as with the well diffusion method. In this study, we compared the Pt antifungal activity of the supernatant of TEB1 spore harvested on Difco sporulation media, with that of vegetative cell harvested on LB medium. We also investigated the optimal time course of lipopeptide production from vegetative cell supernatant as well as the minimum inhibitory concentration (MIC) of these lipopeptides against the fungus *P. tracheiphilus* and their dose-response effect on the germination and morphology of conidia. Results showed that TEB1 vegetative cells exhibited higher inhibition than spores. Lipopeptide production from vegetative cell supernatant started at the early growth phase and reached a plateau after 36 h of culture reducing the mycelium growth by 80%. The lipopeptide extract harvested at the stationary phase efficiently inhibited *P. tracheiphilus* mycelial growth and the MIC values displaying 50 and 90% inhibition of conidial germination were approximately 47.5 and 100 mg mL^{-1}, respectively. Furthermore, morphological analysis showed that an increase in the concentration of the lipopeptide extract till 3 mg mL^{-1} induced 10% swelling and 3% crumbling of fungal conidia while an increase to 5 mg mL^{-1} resulted in 40% swelling and 20% crumbling.

Identification and characterization of autochthonous microbial communities' inhabiting Apulian vineyards soil for natural improving grapevine health. A. AGNUSDEI, E. CHIAROMONTE, M. MOSCHETTA, D. SALAMONE, V. MONTILON, S. POLLASTRO, D. GERIN, F. FARETRA. *Department of Soil, Plant and Food Sciences, University of Bari, Via G. Amendola, 165A/ 70126 Bari, Italy.* E-MAIL: donato.gerin@uniba.it

Microorganisms can benefit plant health by enhancing plant nutrient-use efficiency and protecting plants against biotic and abiotic stresses. This study aimed to isolate and characterize as plant growth promotion and biocontrol potential autochthonous microbial communities from soil samples of two vineyards located in Rutigliano (BA; vineyard 1) and Ginosa marina (TA; vineyard 2). A total of No. 16 bacterial isolates, selected as the most abundant morphologies (8 in both vineyards representing 5.3×10^5 CFU g^{-1} of dry soil in vineyard 1 and 4.8×10^5 CFU g^{-1} in vineyard 2) on nutrient agar (NA) and starch casein agar (SCA) were identified by morphotypes and 16SrDNA region sequencing, and characterized for indole 3-acetyc acid (IAA) production, phosphate solubilisation, copper sensitivity and salt tolerance. *Pseudomonas* sp., *Raoultella* sp. and *Enterobacter* produced the higher amount of IAA (1.5, 0.9 and 2.2 mg L^{-1}), while *Advenella* sp., *Raoultella* sp. and *Enterobacter* were more able to solubilize phosphate (solubilization index≥3). Finally, almost all isolates were inhibited by CuSO4•5H2O at the concentrations over 300 μg L^{-1} and by >2% NaCl concentration, while two *Pseudomonas* sp. isolates, *Raoultella* sp., *Pseudarthrobacter* sp. and *Cupriavidus* sp. were able to grow also in presence of 4% of NaCl. Their ability to inhibit *Dematophora necatrix* was also assessed in dual culture assay. Additionally, a beneficial like *Pseudomonas* sp. reduced the colony growth of the fungus for ~ 40% by both direct- and VOCs mediated-antibiosis. This work proposed a high-throughput approach to screening soil microorganisms with beneficial potential.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Effect of LED UV-C rays on ToBRFV infected tomato seeds. L. DONATI[1], S. BOLLANTI[2], D. MURRA[2], P. DI LAZZARO[2], L. FRY[3], A. MANGLLI[1], D. LUISON[1], A. TIBERINI[1]. [1]*CREA Research Centre for Plant Protection and Certification, Via C.G. Bertero 22, 00156 Rome, Italy;* [2]*ENEA, Nuclear Department, Fusion and Technology for Nuclear Safety and Security, Frascati Research Center, via E. Fermi 45, 00044 Frascati, Italy.* E-MAIL: livia.donati@crea.gov.it

Tomato brown rugose fruit virus-ToBRFV (*tobamovirus fructirugosum*) (genus *tobamovirus*, family: Virgaviridae) is an important crop pathogen for tomato industries, with several outbreaks worldwide. ToBRFV is mechanically transmitted, also through contaminated seeds, promoting further dissemination and spread to new growing areas. ToBRFV is located only on the seed coat and infect emerging seedlings. Several papers describe physical methods such as UV radiation to stimulate the hormetic effect in plants, but little is known regarding their use in the treatment of seeds and plant material to inac-

tivate any pathogens present on the surface. In tomato, it has already been demonstrated that hormetic UV-C treatments on seeds can control diseases caused by different fungi, but no positive results have been obtained against viral pathogens. In this work, the effects of UV-C radiation at doses 12 kJ/m^2 emitted by light-emitting diode (LED) arrays on ToBRFV-contaminated seeds were evaluated, to assess both the virucide and inactivation effect. After the irradiations, the viral titre was assessed by TaqMan qPCR assay and the virus infectivity by bioassay on both tobacco indicator plants through mechanical inoculation. Biostimulation parameters (% germination, root and hypocotyl length, DW, FW and free water of seedlings) were evaluated during germination. The hormetic effect was evaluated using the MPM-100 which measures the quantities of chlorophylls, flavonols and anthocyanins in irradiated plants compared to control plants. Although UV-C treatment had no effect in reducing the viral titre of seeds, ToBRFV inactivation was observed for the highest dose (12kJ m^2) preventing their transmission to the indicator plants.

Selection of *Bacillus* spp. for the control of *Sclerotinia sclerotiorum* and growth promotion in common bean. A.C.M. SANTOS[1], E.A. LOPES[1], W.V. CUNHA[2], Z. BOUABIDI[3], L.E. VISÔTTO[1]. [1]*Federal University of Viçosa - Campus Rio Paranaíba. Road MG 230 Km 7, Zip code 38810-000, Rio Paranaíba – MG, Brazil.* [2]*University of Patos de Minas, Via Maj. Gote, 808 - Caiçaras, Zip code 38700-207, Patos de Minas - MG, Brazil.* [3]*Natural Resources Engineering and Environmental Impacts Team, Multidisciplinary Research and Innovation Laboratory, Polydisciplinary Faculty of Khouribga (FPK), Sultan Moulay Slimane University, BP 145, 25000 Khouribga, Morocco.* E-MAIL: zinebbouabidi3@gmail.com

Some bacteria suppress the development of phytopathogenic fungi and can contribute to integrated pest management. The aim of this study was to select isolates of *Bacillus* spp. with antagonistic potential for the control of *Sclerotinia sclerotiorum*. The suppression of mycelial growth and inhibition of the germination of sclerotia of *S. sclerotiorum,* by diffusible and volatile substances produced by 12 isolates of *Bacillus* spp., were evaluated. Among the isolates, GB01, GB05, GB13, GB14 and GB16 produced diffusible substances capable of inhibiting mycelial growth by 39.0; 23.7; 19.6; 26.5 and 15.3%, respectively. Isolates GB10, GB01, GB14 and GB16 inhibited by 47.1; 60.0; 69.0 and 100% the germination of sclerotia at an incubation temperature of 20°C and 47.1; 60.0; 69.0 and 100.0% at 28°C, respectively. Volatile organic compounds produced by bacterial isolates did not reduce the development of *S. sclerotiorum* and the germination of sclerotia. The isolates GB01, GB14 and GB16, selected in the in vitro tests, were evaluated for their ability to promote the growth of beans and reduce the incidence of white mold. The percentage of diseased plants varied between 0% (isolated GB14) and 12.5% (negative control). No bacterial isolate promoted bean growth. The isolates GB01, GB14 and GB16 from *Bacillus* spp. have potential for use in the biocontrol of *S. sclerotiorum*, however, it is necessary to establish standards that suit the particularities regarding the adaptation of these microorganisms to the environment.

Science-based innovative solutions transfer for chestnut blight biocontrol by hypovirulence in Portugal. E. GOUVEIA[1,2], J. ARAÚJO[2], V. COELHO[2]. [1]*CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300- 253 Bragança, Portugal.* [2]*Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300- 253 Bragança, Portugal.* E-MAIL: egouveia@ipb.pt

Chestnut blight (CB) is associated with *Cryphonectria parasitica* (Murill) Barr. is introduced and established in Europe since 1938. In Portugal was introduced only in 1989, one of the last European countries where the disease was established as a rapid and devastating epidemic. Twelve years were enough to the disease be present in all chestnut areas of Portugal that cover more than 50 thousand hectares. Eradication, sanitary, physical and cultural measures didn't stop the spread and didn't control the disease. The imminent catastrophic situation of the chestnut ecosystem mobilizes regional entities and farmers for more efficient control means be applied. Biological control of CB by hypovirulence is associated with RNA virus (Cryphonectria Hypovirus 1 - CHV1) that infect *C. parasitica* which reduces sporulation and virulence. This method is considered very efficient and recommended by EFSA to be applied when the natural hypovirulence is rare or not present. In this work we present the long, hard and persistent work, of which we are proud and pleased for having made possible the treatment of CB based on the principles of biological control by hypovirulence. After being officially authorized in 2015, and still in practice, more than 150 thousand chestnuts trees are treated in more than 7 thousand plots' and have participated in this program more than 3 thousand chestnut producers. In addition to healing cankers and tree recovery and the sustainability of the method a second level of outcomes was the successful transfer technology and large application in the field by

the farmers, the end users who can put hypovirulence in practice.

This research was financially supported by Instituto Politécnico de Bragança, Individual farmers and Municipalities of chestnut areas in Portugal.

Evaluation of *Dunaliella salina* extracts as potential antifungal and antioxidant agents *in vitro*. S. AOUZAL[1,2], FZ. IBN MOKHTAR[1,3], A. KHTIRA[1,4], D. PLISCHKE[2], S. KRIMI BENCHEQROUN[1]. [1]*Plant Protection Laboratory, Regional Center of Agricultural Research of Settat, National Institute of Agricultural Research, Avenue Ennasr, BP 415 Rabat Principal, 10090 Rabat, Morocco.* [2]*Viride Maroc SARL, Av. El Aargoub 01-7724, 73000 Dakhla, Morocco.* [3]*Laboratory of Agri-Food and Health, Faculty of Sciences and Techniques, Hassan First University, B.P. 539, 26000 Settat, Morocco.* [4]*Laboratory of Functional Ecology and Environmental Engineering. Faculty of Sciences and Technology, Sidi Mohamed Ben Abdallah University. Fes. Morocco.* E-MAIL: sanae.krimibencheqroun@inra.ma

Dunaliella salina is a microalga known for its wide range of bioactive compounds. In this study, the antioxidant and antifungal properties of various *D. salina* extracts were evaluated *in vitro*. Antifungal activity was assessed against *Fusarium oxysporum* (FOC) and *Ascochyta rabiei* (AB) using the broth microdilution technique to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC). Antioxidant activity was measured through DPPH, ABTS, and total phenolic content (TPC) assays. Statistical analysis revealed significant differences among the extracts in both antifungal and antioxidant activities. Among the three tested extracts, extract 1 was the only one exhibiting fungicidal effect against FOC with a MIC of 1.25 mg mL^{-1} and an MFC of 5 mg mL^{-1}. Extracts 2 and 3 demonstrated fungistatic activity against both pathogens. In antioxidant assays, extract 1 also demonstrated the highest activity across DPPH, ABTS, and TPC tests with the values of 37.77%, 0.42 mg TE mg^{-1}, and 35.37 µg GAE mg^{-1}, respectively. These findings highlight the promising antifungal and antioxidant potential of *D. salina* extracts, suggesting their possible use in agricultural and pharmaceutical applications. Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for these activities.

This research was financially supported by: Viride Maroc company.

***In planta* evaluation of bacterial isolates as biological agents against *Clavibacter michiganensis* and assessment of their plant growth promotion properties.** G. MERMIGKA[1,2], M. PANIS[1], M.G. PAGOULATOU[1], V. MICHALOPOULOU[3], P. SARRIS[3,4], D.E. GOUMAS[1,2]. [1]*Hellenic Mediterranean University (HMU), School of Agricultural Sciences, Department of Agriculture, Laboratory of Biotechnological Applications and Phytopathology, GR71004, Heraklion, Greece,* [2]*Hellenic Mediterranean University (HMU), University Research Centre, Institute of Agri-Food and Life Sciences, GR71410, Heraklion, Greece,* [3]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece,* [4]*Department of Biology, University of Crete, Heraklion, Greece.* E-MAIL: dgoumas@hmu.gr

The use of beneficial microbes has emerged as a promising, sustainable alternative to traditional chemical inputs, harnessing the power of naturally occurring microorganisms to enhance crop growth and resilience. These microbes work symbiotically with plants to improve nutrient uptake but also to bolster plant defenses by suppressing soil-borne pathogens and pests, thus decreasing reliance on chemical pesticides. In our laboratory, we have isolated various bacteria belonging to the *Bacillus* spp. and evaluated their ability to inhibit *in vitro* the growth of the phytopathogen *Clavibacter michiganensis* (Cm). Among these bacteria, eight showed a more profound effect against Cm. The aim of this study was to evaluate these bacteria on Cm-infected plants. For this purpose, tomato seedling plants were root-drenched with bacterial suspensions of each one of the eight bacterial species. Five days later, the plants were inoculated with Cm, placed in a growth chamber, and the symptoms were monitored for a period of 30 days. In parallel with the above experiments, healthy tomato plants were root-drenched with each of the bacterial suspensions and used to evaluate the plant growth promoting features of these treatments. From the latter experiments, we found that the bacterial strains used had no effect on the phenotypical characteristics of the plants. Concerning the effect of the bacteria on the Cm infection, we found that one strain had a statistically significant delay in the onset of the infection symptoms. This strain will be used in future experiments under greenhouse conditions to further evaluate its effect against Cm.

The study was part of the project "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP"- TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative Agriculture and Food Industry.

Biocontrol over chemicals: native microorganisms combat soilborne pathogens in arid agriculture. A.M. AL-SADI[1,2], B.A. HALO[2], R.A. AL-YAHYAI[2], R. VELAZHAHAN[2]. [1]*College of Agriculture, University of Al Dhaid, Sharjah, P.O. Box 27272, United Arab Emirates.* [2]*Department of Plant Sciences, Sultan Qaboos University, P.O. Box 34, Al Khod 123, Sultanate of Oman.* E-MAIL: alsadi@uodh.ac.ae

Soilborne pathogens, particularly *Pythium aphanidermatum*, cause severe damping-off in cucumber and tomato crops and are a major constraint to vegetable production in the GCC countries. This study evaluated the biocontrol potential of indigenous endophytic and rhizospheric microorganisms isolated from local environments, including desert plants, and vegetable rhizospheres. Several fungal isolates, including *Talaromyces variabilis*, *T. pinophilus*, *Aspergillus terreus*, *Cladosporium omanense* and several *Trichoderma* species exhibited strong antagonistic activity against *P. aphanidermatum* in vitro and significantly improved seedling survival under greenhouse conditions. These fungi inhibited pathogen growth through multiple mechanisms, including production of cellulase and glucanase enzymes, suppression of oospore formation, and induction of morphological abnormalities in pathogen hyphae. Seedling survival rates improved by up to 70% when treated with these biocontrol agents. In addition, the endophytic bacterium *Enterobacter cloacae*, isolated from different hosts, reduced disease incidence significantly and caused structural damage to pathogen hyphae, while maintaining cucumber growth and vigor. Overall, this research demonstrates the promising potential of native microbial isolates as sustainable alternatives to chemical fungicides for managing soilborne diseases. The findings support the development of integrated, environmentally friendly disease management strategies suited to arid agricultural systems in the GCC countries.

Harnessing *Aeromonas* FONT1B for sustainable tomato cultivation: growth promotion and biocontrol under abiotic and biotic stress. C. ANTONELLI, M. NARDUZZI, A.M. VETTRAINO. *Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), University of Tuscia, Via S. Camillo de Lellis, snc I-01100, Viterbo, Italy.* E-MAIL: vettrain@unitus.it

Global climate change and the intensification of international plant trade have significantly increased crop exposure to abiotic and biotic stressors. Conventional management practices rely heavily on chemical fertilizers and pesticides, which contribute to environmental degradation, human health risks, and the emergence of resistant pathogen strains. In this context, Plant Growth-Promoting Bacteria (PGPB) offer a sustainable alternative for enhancing crop resilience and productivity. This study evaluated the multifunctional role of the *Aeromonas* strain FONT1B as both a PGPB and a biological control agent (BCA) in tomato plants subjected to drought stress and *P. infestans* infection. FONT1B demonstrated key plant- beneficial traits, including indoleacetic acid (IAA) production, phosphate and potassium solubilization, and tolerance to salinity, drought, and pH fluctuations. Whole-genome sequencing revealed genes involved in stress adaptation, phytohormone biosynthesis, and the production of antimicrobial compounds. *In vivo* assays confirmed the efficacy of this strain in promoting plant growth and reducing disease severity under both abiotic and biotic stress conditions. These findings highlight the potential of FONT1B as a bioinoculant for integrated disease and stress management in sustainable tomato cultivation.

This research was supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022, CUP: J83C22000830005).

CONCURRENT SESSION F2 – Postharvest - new control tools

SESSION KEYNOTES

Biological control of post-harvest diseases: from single microorganisms to synthetic microbial communities (SynComs). L. SCHENA[1], M.M. ACI[1], N.Z. MOHAMED[1], A. MALACRINÒ[2]. [1]*Department of Agriculture, Università degli Studi Mediterranea di Reggio Calabria, Località Feo di Vito, 89124 Reggio Calabria, Italy.* [2]*Department of Biological Sciences, Clemson University, Clemson, SC, USA.* E-MAIL: lschena@unirc.it

The idea of managing postharvest diseases of fresh fruits and vegetables using beneficial microorganisms dates back to the mid-1980s, stemming from concerns on the risk of using synthetic fungicides on postharvest products. Starting from the early 90s, the expected ban of several synthetic chemicals used in crop protection pushed the interest toward biological control in postharvest products, with hundreds of scientific papers published each year. Many potential biocontrol agents (BCAs) including bacteria, filamentous fungi, and yeasts

were enthusiastically proposed as the one-size-fits-all solution to safely manage postharvest diseases. Due to concerns about the production of antibiotics by bacterial strains, yeasts were considered more acceptable by consumers and were largely the most investigated. After more than 40 years, this huge scientific effort yielded several strains of BCAs with commercial potential, but only few commercial products have been developed, and none of them has seen a widespread commercial use. The reasons for this limited success can be identified in difficulties in managing products based on living organisms, the high registration costs, the restricted range of application of most BCAs (both in terms of target host and pathogens), and, more importantly, the lack of a consistent efficacy. Over the years, it has become increasingly clear that the simplistic model where a single antagonist is used to control a single or multiple pathogens is ineffective. Indeed, this approach does not consider the complexity of the postharvest systems and the interactions between host, its microbiome, pathogens, and the surrounding environment. Recent research is therefore gradually shifting from analyzing individual microbes to examining entire microbial communities, viewing microorganisms as a complex network of interacting organisms playing a critical role in mitigating plant diseases, including postharvest rots of fruits and vegetables. In this study, instead of focusing on individual BCAs, we engineered microbial communities capable of effectively contrast postharvest diseases. Microbial communities from different environmental sources were used to inoculate wounds on apple fruits. Microbial communities were then selected for their ability to quickly colonize wounds without causing disease through 10-cycle successive passaging. Microbial communities from the first and the last cycle were then tested to evaluate their in vivo efficacy in preventing the development of the fungal pathogens *Botrytis cinerea* and *Penicillium expansum*. Amplicon sequencing analyses revealed shifts in the structure and diversity of the microbiome across the cycles, resulting in a reduction in disease incidence by 90% (*B. cinerea*) and 70% (*P. expansum*). To further validate the robustness of our engineered communities, we conducted additional tests using three SynComs designed to mimic the microbiome we recovered in wounds after ten re-inoculation cycles. The efficacy of a SynCom proved comparable to that of the sourcing selected microbiomes, thereby reinforcing the validity of our approach for designing functional SynComs and developing sustainable and effective solutions for postharvest disease management.

ORAL PRESENTATIONS

Control efficacy of a new SIGS-based biofungicide against *Penicillium digitatum* on citrus fruits. S. TESTEMPASIS[1,4], A. DALAKOURAS[2], V. KOIDOU[2], K.K. PAPADOPOULOU[3], G.S. KARAOGLANIDIS[1]. [1]*Laboratory of Plant Pathology, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece;* [2]*Institute of Industrial and Forage Crops (IIFC), Hellenic Agricultural Organization - Demeter, 41335 Larisa, Greece;* [3]*Department of Biochemistry and Biotechnology, University of Thessaly, 41500 Larissa, Greece;* [4]*Department of Agriculture, School of Agricultural Sciences, University of Western Macedonia, 53100 Florina, Greece.* E-MAIL: testempasis@gmail.com

Penicillium digitatum (*Pd*) is considered as one of the most significant post-harvest pathogens of citrus fruits worldwide. This study aimed to develop a new biofungicide using exogenous application (Spray Induced Gene Silencing, SIGS) to address post-harvest infections caused by the fungus Pd in orange fruits. Specifically, the effectiveness of pathogen control was tested using exogenous applications of dsRNA targeting the silencing of various important genes involving in the RNA silencing mechanisms *(Pd_ds1, Pd_ds2, Pd_ds3, Pd_ds4*) and pathogens development (*Pd_ds5*). The effectiveness of dsRNA applications was tested on artificially inoculated orange fruits (cv. Salustiana) with the pathogen Pd, where the dsRNA was applied at the wound site prior to the infection. The evaluation of effectiveness was conducted by measuring the severity and incidence of the disease in the infected fruits, seven (7) days post-inoculation. Among the dsRNAs tested, the silencing of the *Pd_ds1, Pd_ds2*, and *Pd_ds3* genes, significantly reduced ($P < 0.05$) the severity and incidence of disease compared to the control treatment. Specifically, the silencing of genes *Pd_ds1, Pd_ds2*, and *Pd_ds3* significantly inhibited the disease development by 84.2%, 89.8%, and 84.2%, respectively. The innovation of this study lies in the use of SIGS technology to develop an environmentally friendly new biofungicide, offering a new approach to addressing post-harvest diseases of citrus fruits.

This research titled "Innovative Solutions for Sustainable and Environmentally Friendly Crop Protection of Greece's Horticultural Crops in the Europe of the Future" (TAEDR-0535675), was implemented within the framework of the National Recovery and Resilience Plan "Greece 2.0", with funding from the European Union – NextGenerationEU.

Electrolyzed salt solutions application as sustainable control tool against major postharvest diseases of fresh fruit and vegetables. O. INCERTI, M. ROBBE, M. PASQUALICCHIO, G. CELANO, M. DE ANGELIS, A. IPPOLITO, S.M. SANZANI. *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy.* E-MAIL: ornella.incerti@uniba.it; simonamarianna.sanzani@uniba.it

Over the last decades, the postharvest management of fresh fruits and vegetables (FFVs) has been dependent on chemicals. This resulted in the rise of resistant pathogen populations and the formation of unhealthy products, causing a progressive restriction in the allowed active substances. As such, the sustainable management of phytopathogens, microbial contaminants, and FFVs' quality is a major concern for the horticultural industry. The electrolysis of water has gained considerable interest as sanitizer and cleaning agent, due to nonthermal, nontoxic, and broad-spectrum microbial inactivation, as well as for its low cost, portability, and preservation of FFVs quality. In the present study, we tested the electrolysis in presence of different electrolytes, as sanitization process of washing water of a citrus packinghouse, and thus against *Penicillium digitatum*. A consistent reduction of the microbial population of the water was recorded after 1 h of electrolysis in presence of NaHCO3. Hints on the putative mode of action are also provided. Furthermore, electrolyzed water obtained in the absence (eW) and presence of NaCl and NaHCO3 (eNaCl and eNaHCO3) was tested against some of the most relevant postharvest pathogens and diseases of fresh fruit and on a range of commodities. Overall, eNaCl resulted the most efficient treatment for preventing spore germination, as well as for minimizing fruit rots, followed by eW and eNaHCO3. As such, electrolysed solutions seem promising as dipping treatments for preventing postharvest decay of FFVs.

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods".

Isolation and evaluation of endophytes as biological control agents to control wound and latent apple pathogens. G. REMOLIF[1], V. GUARNACCIA[1,2], M. MEZZALAMA[1,2], D. SPADARO[1,2]. [1]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* [2]*Interdepartmental Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* E-MAIL: gi.remolif@unito.it

Apple fruit diseases, caused by both wound and latent pathogens, cause significant production losses during cold storage, transportation, and marketing. Biological control using microbial antagonists is a promising alternative to synthetic fungicides for disease management. Endophytes are gaining increasing attention as biocontrol agents (BCAs), due to their symbiotic relationship with the host plant, which may enhance their competitiveness and efficacy in disease control. The aim of this work was to isolate endophytes from healthy apples and to evaluate their potential as BCAs against several apple pathogens. A protocol was developed to isolate endophytic yeasts and bacteria from mature apples of various cultivars and geographical origins. A total of 137 strains were obtained. *In vivo* assays were performed to evaluate their potential antagonistic activity, using *Botrytis cinerea* as the target pathogen. The most effective strains were molecularly identified and selected for further analyses. Yeasts belonged to the genera *Aureobasidium*, *Bullera*, *Metschnikowia,* and *Wickerhamomyces*, while bacteria to the genera *Pantoea*, *Pseudomonas*, *Rahnella*, *Orchobactrum,* and *Stenotrophomonas*. The growth of the selected strains at different temperatures was also tested to assess their ability to survive in field and storage conditions. Additionally, their efficacy against latent apple pathogens, including *Phlyctema vagabunda* and two *Colletotrichum* species, was evaluated. Most strains showed a significant reduction in disease severity compared with the inoculated control, showing promising potential as biocontrol agents. The most effective ones will be further studied to elucidate their mechanisms of action and evaluate their potential for field and postharvest applications.

This research was financially supported by Next Generation EU - National Recovery and Resilience Plan (PNRR) - Mission 4, Component 2, Investment 1.4, National Research Centre for Agricultural Technologies - AGRITECH, code CN00000022, CUP D13C22001330005.

Flow cytometry: a rapid and low-cost tool for detection and quantification of feared fungal plant pathogens. E. CHIAROMONTE[1], A. AGNUSDEI[1], E. ALTAMURA[2], D. GERIN[1], F. MAVELLI[2], S. POLLASTRO[1],

F. FARETRA[1]. [1]*Department of Soil, Plant and Food Sciences, (DiSSPA), University of Bari Aldo Moro, Bari, Italy.* [2]*Department of Chemistry, University of Bari Aldo Moro, Bari, Italy.* E-MAIL: stefania.pollastro@uniba.it

The demand for rapid, reproducible and low-cost tools for the detection and quantification of plant pathogens has increased over the last years. Flow cytometry (FCM) allows to rapid provide a multiparametric analysis of single cells in a complex matrix, through two morphological parameters, forward scattering (FCS) and side scattering (SSC) respectively, combined with fluorescence signals. *Aspergillus* spp. and *Botrytis cinerea* are responsible for mold, affecting worldwide several crops both in field and in postharvest. Furthermore, some species belonging to *Aspergillus* genera produce dangerous mycotoxins such as ochratoxin A (OTA). Here, FCM was successfully applied for a rapid identification of *A. carbonarius*, *A. niger* and *B. cinerea* conidia both in pure conidia suspension and in strawberry wash water. Conidia of each species, stained with calcofluor white, were properly characterized based on, both, morphological parameters and fluorescence signals. The availability of an albino mutant of *A. carbonarius* allowed to observe in conidia water suspension the autofluorescence of OTA. The mycotoxin was not detectable in the wild type, probably due to a quenching effect caused by the melanin. In mixed suspensions, conidia of the two *Aspergillus* species were easily distinguishable based on their morphology, which affected light scattering. Even in more complex matrices, such as strawberry wash water spiked with single-species conidial suspensions, detection was still achievable, opening new possibilities for multiplex analysis. These findings confirm the applicability of flow cytometry for the rapid detection of plant pathogens, representing a pioneering step in the agricultural field.

This research was partially financially supported by: Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

POSTER PRESENTATIONS

Microbiome dynamics of sooty fungal epiphytes on postharvest apples. F. REY[1,2], S. OETTL[1], H. SCHULER[2]. [1]*Laimburg Research Centre, Laimburg 6-Pfatten/Vadena, 39040, Auer/Ora, Italy;* [2]*Faculty of Agricultural, Environmental and Food sciences, Free University of Bozen-Bolzano, Piazza Università 1, 39100, Bolzano BZ, Italy.* E-MAIL: FiRey@unibz.it

During postharvest storage, certain fungi outcompete other microorganisms on the surface of apples and produce dark, sooty blemishes. These visual imperfections reduce the marketability of the fruit and require fungicide treatments and controlled storage conditions. To assess the dynamics of the epiphytic fungal community of the cultivar Rosy Glow/Pink Lady® during storage, a metabarcoding analysis was performed: apples were harvested from two experimental orchards, both treated in a randomized block design with Merpan® 80 WDG (Captan), Geoxe® (Fludioxonil), or Ulmasud (rock powder, bentonite and lignosulfonates). Sampling occurred at harvest and after six months of storage at 1.5°C and 96% relative humidity. Metabarcoding analysis reveals that alpha diversity (shannon and pielou) significantly decreases from healthy to symptomatic apples. Furthermore, ordination of the Bray-Curtis metric produces distinct clusters, with significant percentage of the variation explained by field, treatment, symptoms and storage. Clusters derive from universal changes from healthy to symptomatic apples, which consist in a reduction of the relative abundance of yeasts, known to have biocontrol properties (e.g. *Kalmanozyma*, *Vishniacozyma*) and an increase in wood decaying fungi (e.g. *Hypoxylon, Diaporthe, Diplodia*). To identify potential causal agents, fungal thalli from symptomatic peel plugs will be isolated using a needle under a dissecting microscope, inoculated serially on PDA and, when pure, the ITS 3-4 region sequenced. These isolates will be cross-referenced with Illumina data from peel plugs with matching symptoms. This research provides key insights into postharvest microbiome dynamics and contributes to the optimization of epiphyte management during postharvest storage.

A non-invasive approach to targeted lenticel and core inoculation of apple fruit with fungal phytopathogens. S. BHARTI[1], H. QAYYUM[1], S. BARIC[1,2]. [1]*Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bozen-Bolzano (BZ), Italy.* [2]*Competence Centre for Plant Health, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bozen-Bolzano (BZ), Italy.* E-MAIL: shbharti@unibz.it

Apple (*Malus* × *domestica*) is the third most widely cultivated pome fruit tree worldwide. However, postharvest

fungal pathogens can significantly threaten the apple quality during storage. Early detection of latent postharvest pathogens would greatly aid the sorting process before sending the produce to the market. Hyperspectral imaging has potential for the early detection of pathogens, but to develop accurate diagnostic protocols, it is crucial to use inoculation methods closer to natural infection routes. Nonetheless, injury-based inoculation techniques have been extensively studied; there remains a need for noninvasive, targeted methods of inoculation. In this study, we used *Colletotrichum godetiae* and *C. fioriniae*, both responsible for bitter rot of apple, for targeted lenticel inoculation, and *Fusarium avenaceum* for inducing core rot. Spot plasters soaked with 50 µL of conidial suspension (10^8 spores mL^{-1}) were attached to the fruit of 'Golden Delicious' and 'Gala' at 90-degree intervals across the equatorial plane to induce lenticel rot. Plasters were removed after 14 days post-inoculation (dpi), and the fruits were monitored for visual infection symptoms until 40 dpi. For core rot, 100 µL of spore suspension was injected through the calyx of 'Red Delicious' fruit using needles. Fruits were evaluated at 7, 14, and 21 dpi. The infection rates for targeted lenticel inoculation were 69% for *C. fioriniae* and 60% for *C. godetiae*, while 98% of the fruits showed infection in the core rot inoculation. Further optimization is ongoing to establish a reliable noninvasive protocol for inducing targeted lenticel and core rot infections in apple fruit.

This study is part of the research project HIPPA ("Hyperspectral imaging for the detection of physiologically and parasitically induced damages on apple fruit at harvest and during postharvest"; CUP I53C23001650007; EFRE1044) that was funded within the EFRE-FESR 2021-2027 Programme of the European Union.

Ligninolytic fungi as biocontrol agents against postharvest phytopathogens. N. SCHLOSSEROVA[1,2], M. ROBBE[3], A. MOSTACCI[3], O. INCERTI[3], A. IPPOLITO[3], S.M. SANZANI[3*]. [1]*Department of Chemistry and Biochemistry, Mendel University in Brno, 613 00 Brno, Czech Republic;* [2]*Department of Biosciences, Biotechnologies and Environment, University of Bari Aldo Moro, Via Orabona 4, 70126 Bari, Italy;* [3]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy.* E-MAIL: simonamarianna.sanzani@uniba.it

Postharvest losses of fruits and vegetables caused by phytopathogens and inadequate handling remain a significant issue in many countries. Common fungal pathogens such as *Alternaria alternata, Botrytis cinerea, Fusarium avenaceum* are known to lead to rapid fruit decay during both pre- and postharvest stages. Some of those pathogens are known to produce toxic metabolites known as mycotoxins. Control of diseases has relied heavily on the use of fungicides, both during crop cultivation and after harvest. However, in recent years, there has been an increasing interest in developing more environmentally friendly and safer alternatives to extend the shelf-life of fruits and vegetables. In the present investigation, basidiomycetes, belonging to the genera *Ganoderma*, *Laetiporus* and *Fomitopsis*, already used in traditional medicine, were morphologically and molecularly identified and used as biocontrol agents against selected postharvest fungi both as live cells and cultural filtrates on different growth media. The effect was assessed both *in vitro* and *in vivo*. The selected basidiomycetes, free from any detrimental effect on the target hosts, controlled the phytopathogen growth. In particular, the extracellular fraction of the basidiomycete culture proved to be efficient in controlling fungal viability. Moreover, *in vivo*, it reduced the ability of phytopathogens to colonise fruit tissues. Although further large-scale trials are needed results seem promising.

This study was partially carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

Mycovirus-based biocontrol potential of post-harvest fungal pathogens in Citrus fruits: current insights and future prospects. H. BENZAHRA[1,2], I. MRABTI[1,2], H. GRIJJA[1], K. SELMAOUI[2], M. AFECHTAL[1]. [1]*Laboratory of Virology, Regional Agricultural Research Center of Kenitra, National Institute of Agricultural Research, 14000 Kenitra, Morocco.* [2]*Faculty of Sciences, Laboratory of Plant, Animal, and Agro-Industry Productions, University Ibn Toufail, Kenitra, Morocco.* E-MAIL: mohamedafechtal.inra@gmail.com

Postharvest losses in citrus fruits are largely attributed to fungal pathogens, particularly *Penicillium digitatum* and *P. italicum*, the main causative agents of green and blue mold, respectively. In recent years, mycoviruses have emerged as potential modulators of fungal virulence and promising tools for biocontrol. This review explores the diversity, characteristics, and potential biocontrol applications of mycoviruses infecting *Penicillium* species associated with citrus postharvest diseases. A comprehensive literature review was conducted using databases such as PubMed, Scopus, and Web of Science,

focusing on studies published between 2000 and 2024. We compiled and analyzed reported mycovirus infections in *P. digitatum* and *P. italicum*, assessing virus families, genome characteristics, and their effects on fungal phenotype. Key findings revealed that mycoviruses from families such as *Partitiviridae*, *Chrysoviridae*, *Hypoviridae*, and *Totiviridae* have been identified in Penicillium species. Notable examples include Penicillium digitatum partitivirus-1 (PdPV-1) and Penicillium chrysogenum virus (PcV). Certain mycoviruses, such as those in the Hypoviridae family, have been linked to hypovirulence, reduced spore production, and decreased mycotoxin biosynthesis. Both in vitro and in vivo studies suggested that these mycoviruses can significantly attenuate pathogenicity, highlighting their potential as alternative biocontrol agents for managing citrus postharvest decay. While the prospects are promising, the practical application of mycoviruses in integrated postharvest disease management remains limited by gaps in our understanding of host-virus interactions, viral stability, and effective delivery systems. Future advances in RNA sequencing technologies and reverse genetics will be crucial in identifying cryptic infections and developing mycovirus-based strategies as sustainable alternatives to chemical fungicides.

Evaluation of biocontrol agent alternatives for winter management of fungi associated with canker disease in hazelnut. E. MOYA-ELIZONDO[1], J. SAN MARTÍN[1], B RUIZ[1], K. ROJAS[1], Y. VEGA[2], M.J. LISPERGUER[3]. [1]*Departmento de Producción Vegetal, Facultad de Agronomía, Universidad de Concepción, Campus Chillán 3812189, Chile.* [2]*Department of Research adn Development, Bioprotegens Innovation SpA., Arauco 360, Chillán, Chile.* [3]*Departamento Técnico, Frutícola Agrichile S.A., Lote A, Hijuela 1, La Florida del Alto, Curicó, Chile.* E-MAIL: emoya@udec.cl

Hazelnut (*Corylus avellana* L.) is the second most important nut tree in Chile, currently occupying 46,000 hectares. Various fungal species cause cankers and branch dieback, necessitating effective management options. In this study, biocontrol agents (BCAs) and BCA- based paints were evaluated to protect pruning cuts in 'Tonda Di Giffoni' hazelnut. In late winter, ten branches per tree were cut with pruning shears and treated on the same day with *Trichoderma atroviride* MUCL 45632 (Ta), a *Pseudomonas protegens* Ca6-based paint (PPp), an organic acid-based paint (POA), and a tebuconazole-chlorathalonil based paint (5 and 50 g L^{-1} [PFTC]), plus an untreated control (UTC). Five branches were inoculated with 5 mm APDA mycelial plugs of *Diaporthe ambigua* (Da), *Fusarium culmorum* (Fc), and *Diplodia mutila* (Dm). The remaining five branches were treated, covered with aluminium foil, and inoculated 7 days later with the mentioned pathogens. After eight months, branches were collected, the canker length was measured, and fungi from the necrotic tissues were reisolated. Results indicated that Ta and PPp reduced significantly the length of necrosis caused by Da. PPp and PFTC treatments had the lowest re-isolation frequency of fungal pathogens. In branches inoculated 7 days later, treatments did not differ from UTC, except for PPp, which reduced Fc necrosis by 40%. Ta, PPp, and PFTC reduced Fc re-isolation. In both inoculation scenarios, POA significantly increased canker length compared to UTC, but had the lowest re-isolation for all pathogenic fungi. Winter treatments with BCAs could be an alternative for managing canker fungi in hazelnut.

This research was financially supported by the Phytopathology Laboratory of the Facultad de Agronomía at the Universidad de Concepción.

Sweet cherry canker diseases: understanding their ecology, epidemiology and control in Chile. D. GRINBERGS, J. CHILIAN, R. ORREGO, M. ISLA. *Instituto de Investigaciones Agropecuarias INIA Quilamapu, Av. Vicente Méndez 515, Chillán, Chile.* E-MAIL: dgrinbergs@inia.cl

Sweet cherry cultivation has increased in Chile becoming the first exporter worldwide, reaching 413.979 t and USD 2.048MM FOB in 23/24, and turning into the most profitable fruit crop. Fungal diseases are the main causes of limiting productivity and fungal trunk diseases have become, especially in recent years, highly relevant affecting both productivity and longevity of sweet cherry. Determining the etiology and understanding the epidemiology of these diseases are highly relevant to design prevention and control strategies. Symptomatic woody samples (n=830) have been collected since 2019 from the Metropolitana to Los Lagos Regions. Fungi were isolated and pathogenicity tests were performed for representative isolates from most isolated species, in detached twigs and nursery cherry plants var. Lapins. The most isolated and pathogenic genera were *Cytospora* (43%), followed by *Calosphaeria* (20%), *Chondrostereum* (10%), *Nectria* (3%), and *Eutypa* (2%). To study the epidemiology of the most relevant pathogens, four commercial orchards with different climatic conditions were weekly monitored for 24momths collecting the aerial inoculum

on glass spore traps. Inoculum of each pathogen was quantified by qPCR and correlated with temperature and precipitation, which were the most significant factors affecting spore release. Knowing the periods of highest risk, it was possible to design a prevention and control strategy. Chemical (n=15), biological (n=5) and immune response inducers (n=2) treatments were evaluated *in vitro* and in orchard trials, obtaining that all the products had an effect on fungal growth, wood discoloration, canker formation, and number of dead spurs, with benomyl, tebuconazole, chlorothalonil, and two biologicals showing the most significant results.

This research was financially supported by National Agency for Research and Development (ANID), Foundation for Agricultural Innovation (FIA) and O´Higgins Regional Government.

Metabolic profiles of Crisp A and D isolates of *Erwinia amylovora*. G. SOUSA-GOMES[1], Á. QUEIROZ[1], L. MOURA[2]. [1]*Instituto Politécnico de Viana do Castelo, Escola Superior Agrária (IPVC/ESA), Refóios, 4990-706 Ponte de Lima, Portugal.* [2]*CISAS - Centre for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo 4900-367, Viana do Castelo, Portugal.* E-MAIL: guilherme.g@ipvc.pt

Fire blight, a disease caused by *Erwinia amylovora*, poses a significant threat to plants in the Rosaceae family, particularly pome fruits. In Portugal, the bacterium is widely spread across the main pear and apple production areas, especially in the Oeste Region. The 'Rocha' pear sector has experienced significant yield losses recently, resulting in substantial economic losses for Portuguese producers. This study aims to isolate and genetically and phenotypically characterize the *Erwinia amylovora* population obtained from symptomatic plant material collected in 2024, from pear and apple orchards in the Oeste Region of Portugal. Ten *Erwinia amylovora* isolates were characterized using the CRISPR-PCR technique and the Gen III Biolog MicroPlates for phenotypic analysis. CRISPR genotyping distinguished the isolates into two genotypes: genotype A and genotype D, depending on the presence or absence of the duplication of spacer 1029, respectively. Phenotypic characterization revealed heterogeneity within the population, regarding carbon source use and chemical sensitivity. The presence of both genotypes A and D, along with the observed diversity in metabolic carbon profiles and chemical sensitivity in the recently isolated *Erwinia amylovora* population from the main Portuguese pear and apple production region, could suggests different adaptive processes of this pathogen. These processes are possibly driven by the disease control methods applied and its adaptation to environmental and climatic conditions over the time. This study demonstrated that despite this species's previously known genetic homogeneity, there is phenotypic diversity within the *E. amylovora* population in Portugal. This diversity provides a better understanding of the pathogen's epidemiological behaviour under Portuguese production conditions.

This research was financially supported by the project BioFago - New strategies to control Fire Blight (PRR- C05-i03-I-000179) financed by national funds through the Agriculture and Fisheries Financing Institute, Public Institute - I.F.A.P.

Fungal diversity and pathogenicity in olive wood decline: Insights from the North Aegean Region. E.A. MARKAKIS[1,2], M.M. MATHIOUDAKIS[3], S. SOULTATOS[1,2], N. KRASAGAKIS[1,2], M. KISSANDRAKI[3], A. KARAGIANNI[3], G. LAGOUTARIS[4], E. KOUKOULI[4], G. KATSIKOGIANNIS[4], S. NTOUGIAS[5], N. KAVROULAKIS[3]. [1]*Institute of Olive Tree, Subtropical Crops and Viticulture, ELGO-DIMITRA, 71307, Heraklion, Greece.* [2]*School of Agricultural Sciences, Hellenic Mediterranean University, 71004, Heraklion, Greece.* [3]*Institute for Olive Tree, Subtropical Crops and Viticulture, ELGO-DIMITRA, 73134 Chania, Greece.* [4]*Directorates of Agricultural Economy (DAOK) of the North Aegean Region.* [5]*Department of Environmental Engineering, Democritus University of Thrace, 67100, Xanthi, Greece.* E-MAIL: kavroulakis@elgo.gr

An extensive survey was conducted in olive groves across the islands of Lesvos, Chios, Samos, and Ikaria (North Aegean Region, Greece) during 2023–2024 to investigate fungal pathogens associated with wood diseases. Wood samples were collected from symptomatic olive trees exhibiting leaf chlorosis, wilting, scorching, twig and branch dieback, wood discoloration and overall tree decline. Fungal isolations were performed on acidified PDA, and isolates were identified based on morphological characteristics and sequencing of the ITS region and additional genes as needed. Results revealed the presence of several phytopathogenic fungi associated with wood diseases, primarily from the genera *Pseudophaeomoniella, Pleurostoma, Phaeoacremonium* and *Comoclathris*. Among these, *Pseudophaeomoniella* species appeared to be the most prevalent. Additionally, non-primary pathogens such as *Cladosporium* spp., commonly linked to wood-feeding insects, were also identified. The study is ongoing, with further evaluation of the pathogenicity

of these fungi on the major regional olive cultivars, i.e., Koroneiki, Kolovi, Adramytini and Throumbolia.

The study was funded by the North Aegean Region under the programming contract "Research and information actions to enhance the preparedness of the North Aegean Region regarding the immediate eradication of the pathogen Xylella fastidiosa *in case of detection"-XyLeVA.*

At the heart of decay: Investigating the ecology and epidemiology of *Phellinus pomaceus* in California prune orchards. L. HOFFMAN. *Department of Plant Pathology, University of California, Davis, One Shields Ave, Davis, CA 95616.* E-MAIL: hoffiman@ucdavis.edu

Surveys have confirmed the widespread presence of the wood decay fungus, *Phellinus pomaceus*, in California prune orchards. This pathogen is associated with a significant decline in orchard lifespans. Infection by *P. pomaceus* leads to heart rot decay, associated with the loss of tree scaffolds and the need for tree replacement and more rapid orchard turnover. Despite its increasing prevalence, little is known about the biology, life cycle, and epidemiology of this fungal pathogen—particularly in the Western United States. This study investigated the seasonal development, sporulation dynamics, and spatial distribution of *P. pomaceus* across orchards in California through field surveys, spore trapping, whole-tree dissections, and laboratory culturing. Using monitoring of airborne spores, we are specifically tracking the timing of sporulation events to identify key environmental conditions associated with spore release. Our findings thus far indicate that sporulation is highly seasonal. Entry into healthy prune trees by *P. pomaceus* was thought to be associated with pruning wounds due to the position of fruiting bodies emerging at branch stubs. Further work in this project now supports this hypothesis, highlighting the importance of understanding pruning practices and seasonal timing considerations in disease management.

This research was financially supported by the California Prune Board, Roseville, CA 95661.

***Diaporthe* complex is responsible of Phomopsis canker on *Prunus dulcis* in Apulia.** D. SALAMONE, V. MONTILON, G. INCAMPO, D. GERIN, S. POLLASTRO, F. FARETRA. *Department of Soil, Plant and Food Sciences, University of Bari, Via G. Amendola, 165A/ 70126 Bari, Italy.* E-MAIL: giuseppe.incampo@uniba.it

The almond industry in Italy represents a high degree of structural variability where old traditional orchards coexist with new plantations, very different in age and orchard systems. Apulia and Sicily remain the most important production areas where 90% of the almonds are grown profitably under suitable climatic conditions. Self-fertile cultivars, clonal rootstocks and new planting designs optimized for mechanization were the most important technical features revealing a new way to set up almond orchards. Several wood pathogens damage almond tree and frequently bud necrosis and severe twig dieback can be observed. The disease symptoms occur widely and are cultivar-dependent with 'Ferragnes' and 'Guara/Tuono' more being more susceptible than 'Filippo Ceo'. Brown, sunken, elongated, necrotic lesions were observed around the buds and the new shoots usually wilted and died. A putative pathogenic fungus was isolated from the necrotic tissues on potato dextrose agar (PDA) producing pycnidia mainly with one-celled, hyaline, fusoid α-conidia and rarely with β- conidia. For molecular identification, DNA was extracted from the mycelium of the isolates, subjected to PCR using ITS primers and custom-sequenced. The obtained sequences let to ascertain the presence of a complex of *Diaporthe* species with *Diaporthe amygdali* (anamorph: *Phomopsis amygdali*) being the most common. Additionally, a co-culture assay was used to evaluate the intra-species variability in terms of vegetative compatibility and a high intra-species variability, suggesting the need for further detailed study. This work builds the foundation for further studies to help develop appropriate plant-pathogen disease management strategies.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022) and Almond Management Innovations: Approcci per una Mandorlicoltura biologica Innovativa (AMÌ) PSR 2014-2020 avviso pubblico approvato con D.A.G. n. 194 del 12/09/2018, pubblicata nel B.U.R.P. n. 121 del 20/09/2018. This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

CONCURRENT SESSION F3 – EUPHRESCO III: strengthening the plant health research community

SESSION KEYNOTE

Strengthening plant health in the Mediterranean region through coordinated research and international collaboration. M. D'ONGHIA[1], A. ALAWAMLEH[2], M. CARA[3], L. CRUZ[4], A. NAJAR[5], G. BALDISSERA[6]. [1]*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM Bari), Via Ceglie, 9- 70010, Valenzano (BA), Italy.* [2]*Phytosanitary Laboratories, Plant Wealth Laboratories Directorate, Ministry of Agriculture, Amman, Jordan.* [3]*Faculty of Agriculture and Environment, the Agricultural University of Tirana, Rruga "Pajsi Vodica", 1029 Tirana, Albania.* [4]*National Institute for Agricultural and Veterinarian Research, Republic Avenue, Quinta do Marquês (headquarters building) 2780-157 Oeiras, Portugal. National Institute of Agricultural Research of Tunisia, Tunis, Tunisia.* [6]*Euphresco, 21 Bd Richard Lenoir, 75011 Paris, France.* E-MAIL: donghia@iamb.it

Plant health risks—primarily driven by global trade and climate change—demand a rethinking of current research strategies to enable rapid and effective responses. Strengthening research in this field is a key challenge that countries across the Mediterranean region must confront. However, the wide variation in national priorities—regarding pests, infrastructure, and expertise—has weakened the overall impact of the research efforts. Coordinated action is therefore essential to enhance the efficiency and effectiveness of national research initiatives. The Euphresco self-sustained network provides a platform for cooperation and coordination, aiming to optimise strategies to meet these challenges and ensure a stronger link between research and policy. The network's success has laid the foundation for global discussions on research priorities, as demonstrated in the EUPHRESCO III project. Two main factors are crucial for aligning national research programmes: (i) bottom-up alignment through the involvement of research stakeholders, and (ii) top-down alignment through the engagement of research funding organisations. Building trust and achieving consensus at all levels through regular dialogue and consultation is equally important. In line with these principles, the EUPHRESCO III project has launched consultations with stakeholders active in plant health from diverse regions and disciplines relevant to the network. The main role of regional and disciplinary champions is to create or strengthen mechanisms for engaging and communicating with their respective communities on plant health research issues. For the Mediterranean region, a dedicated survey was conducted by CIHEAM-Bari—as regional champion—together with the Euphresco Network Office, to identify plant health priorities in the Mediterranean basin. Feedback was collected through a questionnaire distributed to national stakeholders. The analysis focused on key areas such as the geographical distribution of respondents, stakeholder types, priority pests and crops, capacity-building needs, research demands, and critical gaps. This initial assessment provides a valuable foundation for shaping future research and regional cooperation in plant health. The results of national surveys will support the upcoming face-to-face consultation with national and regional decision-makers. This event aims to secure formal commitments on topics such as collaborative research agendas, priorities for transnational cooperation, and institutional engagement (e.g. participation in regional and global networks). Outcomes from four participating countries—Jordan (Near East), Portugal (Southern Europe), Morocco (North Africa), and Albania (Balkans)—will be presented as case studies. Each represents a different sub-region and illustrates the outcome of the national survey within the regional consultation mechanism that have been developed and tested through the EUPHRESCO III project.

POSTER PRESENTATION

CIHEAM Bari - Former Trainees Network (FTN): an institutional tool to evaluate the impact of plant health educational programs. N. DRIOUECH, N. AL GNDI, O. ANTONELLI. *International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM Bari), Via Ceglie, 9- 70010, Valenzano (BA), Italy.* E-MAIL: driouech@iamb.it

Since 1962, CIHEAM Bari has been a leader in advanced agricultural education and training for young agronomists and experts from the Mediterranean region. In 1985, it performed a Master's program curricula in Integrated Pest Management, contributing significantly to the development of a professional community in plant health. The FTN was therefore established for strengthening such a community and linking to several CIHEAM Bari activities. To support this professional network, the CIHEAM Bari Alumni Follow-up Research Study was initiated in 2010. Conducted every five years using Ex-Ante and Ex-Post methods, the study evaluates the long-term impact of CIHEAM Bari's educational

programs while updating its alumni database. The latest study surveyed 1300 alumni from 1999 to 2021 using an online, self-administered questionnaire (available in English and French), with 366 IPM graduates answers. Respondents were predominantly male (66%) and originated mainly from the Mediterranean, followed by the Balkans, Central Africa, and the Near and Middle East. Approximately 34% pursued PhD studies, often in collaboration with Italian universities and CIHEAM Bari. Job placement data show 62% work in the public sector, 20% in the private sector, and 18% in semi-public or international institutions. Alumni hold positions such as researchers (38%), academics (14%), project managers (10%), and consultants (5%). Notably, 82% of respondents reported continued collaboration with CIHEAM Bari through joint research, scientific publications, and technical and Institutional partnerships. These findings highlight the effectiveness of CIHEAM Bari's education/training programs in advancing and promoting sustainable agriculture and regional cooperation, particularly within countries engaged in the CIHEAM Bari education mission and within FTN Network.

CONCURRENT SESSION G1 – Advances in understanding and managing canker diseases of fruit, nut, and forest trees

SESSION KEYNOTES

***Neoscytalidium dimidiatum*, a last century's fungal tree pathogen has become this century's menace to fruit and nut tree crops in California.** T. MICHAILIDES[1], G. MAKRIS[1], V. GABRI[1], G. GUSELLA[2]. [1]*University of California, Department of Plant Pathology, Kearney Agricultural Research and Extension Center, Parlier, CA 93648, United States of America.* [2]*University of Catania, Department of Agriculture, Food & Environment, Plant Pathology, Via Santa Sofia 100, 95123 Catania, Italy.* E-MAIL: tjmichailides@ucanr.edu

The tree pathogen known as *Hendersonula toruloidea* or Nattrassia mangiferae and now as *Neoscytalidium dimidiatum* has caused major diseases on fruit and nut crops in California in the last couple of decades. Walnut trees have had a chronic, but sporadic, branch wilt caused by *H. toruloidea* (reported in 1947) and the same pathogen along with *Botryosphaeria dothidea* were reported causing band canker of almond back in 1974. Similarly, a branch wilt and killing initially reported in 1964 on old fig orchards in central San Joaquin Valley (SJV), was again caused an epidemic on figs about two decades ago. The disease killed major branches and limbs of fig trees, resulting in significant yield reductions. Infection courts included wounds created by mallets used during harvest, sunburn tissues, and pruning wounds. The same pathogen was also found in causing severe hull rot of almond from infection by airborne inoculum coming off neighboring walnut and/or fig orchards where the pathogen had killed major tree branches. In one case, an old walnut orchard was removed, and the branches were ground to make the field available to a construction company. To the south of this orchard, there was a Nonpareil almond orchard at the hull split-stage, which is a susceptible stage to infection by hull rot fungi, *Rhizopus stolonifer, Monilinia fructicola,* and *Aspergillus niger.* However, in this case, *N. dimidiatum* caused high levels of hull rot, long cankers internally and unusually large, black gum galls on the surface of infected branches. In a second case, a grower shredded the prunings of his old fig orchard, severely infected by Neoscytalidium canker, which resulted in a severe case of hull rot on his nearby almonds. In another case, a severe band canker of young almond trees developed in Kern County in 2022. It is known that up to eight different species in the Botryosphaeriaceae fungal family can cause band canker of almond; however, *N. dimidiatum* was the solo pathogen isolated from the band canker of these almond trees in Kern Co., CA. In 2013, a nursery producing walnut trees lost approximately 5,000 trees due to infection at the graft union by *N. dimidiatum* (90%) and *Lasiodiplodia citricola* (10%). In 2017, orchards of 'Star Ruby' grapefruit suffered a major limb killing caused by *N. dimidiatum* at the UC Lindcove Research and Extension Center in Exeter, CA. Following two major heat waves in August 2024, a severe epidemic of a branch killing (citrus gummosis) disease appeared in a plethora of Star Ruby grapefruit orchards in central and southern SJV. A total of 15 orchards surveyed and the recovery of the pathogen in samples ranged from 90-100% in 'Star Ruby' grapefruit, 86% in a lemon, and 86% in each of two Pomelo orchards. In several of these orchards, the canker entered the trunk, resulting in tree death. The dry bark of Infected branches and trunks was naturally peeled and revealed underneath a black, powdery mass of arthrospores of *N. dimidiatum*. Disease management methods will be discussed.

This research was financially supported by the California Walnut Board, the California Fig Institute, the Almond Board of California, and the Citrus Research Board.

Fungal Trunk Diseases of fruit crops in the Mediterranean Basin: advancing knowledge to tackle a huge diversity of pathogens. V. GUARNACCIA[1,2]. [1]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* [2]*Interdepartmental Centre for Innovation in the Agro-Environmental Sector AGROINNOVA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* E-MAIL: vladimiro.guarnaccia@unito.it

Fungal trunk diseases (FTDs) are increasingly recognized as a major threat to fruit and nut crop production across the Mediterranean Basin. These diseases, caused primarily by Ascomycota fungi, compromise tree health and yield, reduce orchard longevity, and challenge sustainable agriculture. The complexity and impact of FTDs are emphasized by a wide diversity of fungal taxa involved, including species in the families *Botryosphaeriaceae*, *Cytosporaceae*, *Diaporthaceae*, *Diatrypaceae*, *Nectriaceae*, *Togniniaceae*, and others. Those species often co-occur, showing latent pathogenic behavior, and exhibit wide host ranges, contributing to diagnostic and management challenges. A comprehensive overview of the diversity of FTD-associated fungal species reported on fruit and nut crops such as apple, pear, pistachio, hazelnut, citrus and avocado in Mediterranean countries is provided. Recent advances in fungal taxonomy were highlighted, which, supported by multi-locus phylogenetic analyses, allowed the identification of numerous novel or cryptic species, as well as better delineation of known taxa. For example, genera like *Neofusicoccum*, *Lasiodiplodia*, *Diaporthe*, and *Cytospora* have been frequently associated with dieback, canker, gummosis, and wood decay symptoms across multiple hosts. In detail, case studies from Italy, Spain, Greece, Turkey, and North African countries demonstrate the regional variation in species prevalence and disease symptoms, as well as the role of environmental and agronomic factors, such as climate, cultivar susceptibility, orchard age, and stress conditions, in disease outbreaks. Molecular detection tools, including real- time PCR, are discussed as critical methods for early diagnosis and epidemiological studies. Moreover, imaging-based and hyperspectral imaging are considered an innovation. Furthermore, the current limitations in curative treatments and regulatory restrictions on synthetic fungicides highlight the urgent need for integrated and innovative strategies. Thus, emerging perspectives on disease management, including the role of cultural practices, and biological control agents, are explored. Significant progress has been made in unraveling the complex etiology and diversity of fungal trunk pathogens in Mediterranean orchards, the road ahead calls for stronger interdisciplinary collaboration. A synergistic approach that combines mycology, genomics, climatology, and sustainable agronomy is essential to design effective monitoring systems and deploy targeted interventions.

ORAL PRESENTATIONS

Hazelnut trunk diseases, ecology clarification and development of biological management strategies. V. PIATTINO[1], I. MARTINO[1], M. MASPERO[2], T. DE GREGORIO[2], D. SPADARO[1,3], V. GUARNACCIA[1,3]. [1]*Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy;* [2]*Hazelnut Company division, Ferrero Group, 16 Rue de Trèves – L – 2633 Senningerberg, Luxembourg;* [3]*Interdepartmental Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Turin, Largo Braccini 2, 10095 Grugliasco (TO), Italy.* E-MAIL: valeria.piattino@unito.it

Trunk diseases, a major threat for hazelnut, are characterized by internal necrotic lesions, cankers and production of reddish spore masses on the bark. Highly spreading in the main hazelnut growing areas, these diseases cause plant death and yield losses. Historically, the main trunk disease has been associated with *Cytospora* spp. such as *Cytospora corylicola*. However, a polyphasic approach conducted on fungal strains obtained from Italian and Spanish hazelnut plants, identified *Anthostoma decipiens* as the predominant causal agent, with reddish conidial masses serving as the main inoculum, potentially influenced by climate change. Due to the limited number of available active ingredients for hazelnut crop in Italy, the development of innovative management strategies is needed. Thus, effectiveness of commercial biological products was evaluated on detached twigs and potted plants. The twigs and plants were wounded with a scalpel, treated twice at 5-day intervals, inoculated with mycelial plugs of *A. decipiens,* and the twigs incubated in a moist chamber at 25°C with a 12 h photoperiod for 20 days, while the plants placed in a greenhouse for 4 months. Disease severity was assessed by measuring the length of the internal necrotic lesion. Data were analyzed using ANOVA followed by Tukey's post-hoc test (p-value > 0.05). The effectiveness of commercial biological products containing *Bacillus* spp., *Trichoderma* spp. and *Pythium oligandrum* was statistically comparable to that of chemical fungicides used as a positive control, revealing a reduction in lesion length between 30% and 55%.

This research was financially supported by the Hazelnut Company division of Ferrero.

Effect of wood age on the canker development in almond through multi-inoculations with *Botryosphaeria dothidea* and *Neofusicoccum parvum*. C. LUQUE-CRUZ[1], B.I. ANTÓN-DOMÍNGUEZ[1], L. ROMERO-CUADRADO[2], N. CAPOTE[2], C. AGUSTÍ- BRISACH[1]. [1]*Departamento de Agronomía (Unidad de Excelencia 'María de Maeztu' 2020-2024), ETSIAM, Universidad de Córdoba, Campus de Rabanales, Edif. C4, 14071 Córdoba, Spain.* [2]*Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Center Las Torres, Sevilla, Spain.* E-MAIL: b72lucrc@uco.es

Botryosphaeria dothidea and *Neofusicoccum parvum* are the most frequent and aggressive fungal species associated with young almond decline syndrome in southern Spain. In this study, the effect of age of almond wood on canker development was evaluated by inoculating periodically these pathogens. For this purpose, green (1-year-old) or lignified (2-3 years-old) detached shoots and potted almond plants ('Avijor' grafted on GF-677) were inoculated with conidial suspensions using the following treatments: single inoculations of each pathogen, simultaneous co-inoculation, or sequential co-inoculations with a 4-day interval between pathogens. Canker developed in lignified wood were significantly smaller than those in green wood, regardless of the inoculation treatment. There were not significant differences in aggressiveness among treatments in lignified wood. In green wood, *B. dothidea* and *N. parvum* showed significantly higher aggressiveness in single inoculations, or when *N. parvum* was inoculated first, followed by *B. dothidea* 4 days later. Due to the higher aggressiveness observed in green tissues, the inoculum density of each fungus was quantified by multiplex qPCR. The highest relative inoculum density was found in plants inoculated only with *B. dothidea*, in plants co-inoculated simultaneously with both pathogens, or in those where *B. dothidea* was inoculated 4 days before *N. parvum*. In these treatments, the highest amount of DNA corresponded to *B. dothidea*. Despite the inoculum levels recorded, these two co-inoculation treatments resulted in the smallest lesions. These findings suggest that interactions during multi- infections alter the relative aggressiveness of these pathogens.

Funding: This research was funded by the 'Junta de Andalucía' (project DECALMOND Ref. ProyExcel_00327X), co- funded by the European FEDER funds and by the State Plan for Scientific and Technical Research and Innovation 2017–2020 of the Spanish Ministry of Science and Innovation and the European Regional Development Fund (ERDF), project PID2020-115639RRI00 funded by MICIU/AEI/10.13039/501100011033. We acknowledge financial support from the MICINN, the Spanish State Research Agency, through the Severo Ochoa and María de Maeztu Program for Centers and Units of Excellence in R&D (Ref. CEX2019-000968-M).

Genetic structure of the *Cryphonectria parasitica* population in the Lake Garda area of San Zeno di Montagna (Northern Italy). D.H. TABET[1], A. ESPOSITO[2], A. BRIGHENTI[3], S. BARIC[1,4]. [1]*Faculty of Agricultural, Environmental and Food Sciences, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bozen-Bolzano (BZ), Italy.* [2]*Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134 Verona (VR), Italy.* [3]*Consorzio di Tutela del Marrone di San Zeno DOP, Via Ca' Montagna 11, 37010 San Zeno di Montagna (VR), Italy.* [4]*Competence Centre for Plant Health, Free University of Bozen-Bolzano, Università 5, 39100, Bozen-Bolzano (BZ), Italy.* E-MAIL: DaniaHanna.Tabet@unibz.it

The sweet chestnut (*Castanea sativa*) faces threats from multiple diseases, one of which is the chestnut blight caused by *Cryphonectria parasitica*. Disease attenuation in several European regions has been attributed to the presence of hypovirulent fungal strains infected by the mycovirus *Cryphonectria hypovirus* 1 (CHV-1). Hypovirulence is transmitted horizontally via hyphal anastomosis between strains of the same vegetative compatibility (vc) types, and vertically through asexual conidia. Given the importance of the effective hypovirus transmission, a comprehensive understanding of the population structure of *C. parasitica* is crucial for successful biological control of chestnut blight. This study investigated the genetic structure of *C. parasitica* isolated from 89 bark canker samples collected in San Zeno di Montagna (Province of Verona, northern Italy). DNA-based methods were employed to analyze the isolates for the variability at six vegetative compatibility (vic) loci, the mating type locus and eleven microsatellite markers, as well as the internal transcribed spacer (ITS) region. Additionally, RNA isolation, real-time RT-PCR and Sanger sequencing of the ORF- A region were conducted to investigate the presence and diversity of CHV-1. Among 91 *C. parasitica* isolates, 12 distinct vc types were identified. Both mating types were found with nearly equal ratios, and two different ITS haplotypes were revealed. The presence of CHV-1 was determined in 35.2% of isolates and sequence analysis pointed to a high degree of genetic variation. Consequently, the high genetic population variability of *C. parasitica* coupled with sexual

reproduction and the low frequency of CHV-1 may hinder the effectiveness of biological control strategies in this region.

This study was performed within the project "Esplorazione del microbioma del cancro corticale del castagno per migliorare il controllo biologico della malattia in Veneto" (Meta-Blight) funded by Fondazione Cariverona. We are grateful to the Consorzio di tutela del Marrone di San Zeno D.O.P. for the collaboration in this project.

Fungal canker pathogens and pruning wound management strategies in California apple orchards. K. ELFAR[1], M. BUSTAMANTE[1], M.T. NOURI[2], A. ESKALEN[1]. [1]*Department of Plant Pathology, University of California, Davis, CA 95616, USA.* [2]*UCANR, University of California Cooperative Extension, Stockton, CA 95206, USA.* E-MAIL: aeskalen@ucdavis.edu

Apple orchards in California—including San Joaquin, Santa Cruz, and Sonoma counties—have shown increasing incidences of branch dieback and wood canker disease, particularly in cultivars such as 'Fuji', 'Gala', and 'Granny Smith'. A 2021–2023 survey across nine commercial orchards revealed *Diplodia* spp. (including *D. seriata*, *D. mutila*, and *D. bulgarica*) as the most prevalent pathogens, followed by *Eutypa lata*. Additional fungal taxa included *Diaporthe* spp. (*Di. australafricana*, *Di. eres*, *Di. chamaeropis*, *Di. foeniculina*), *Cytospora parasitica*, *Kalmusia variispora*, and *Phaeocremonium* sp. All isolates were pathogenic on wounded apple wood in field trials, confirming their role in the canker complex. In 2024, dormant-season field trials were conducted in Sebastopol, CA to evaluate the efficacy of pruning wound protectants. Treatments included thiophanate-methyl, fluopyram + tebuconazole, *Trichoderma asperellum + Trichoderma gamsii, Aureobasidium pullulans*, and thyme oil. Branches were treated and then inoculated with *D. seriata*. Thiophanate-methyl showed complete control (0% mean percent infection, MPI), followed by fluopyram + tebuconazole (54.2% MPI). The biological products offered limited protection, with Trichoderma-based and Aureobasidium-based treatments yielding 75–91.7% MPI, and thyme oil showing the least efficacy (95.8% MPI). These results emphasize the dominance of *Diplodia* spp. in apple canker etiology in California and demonstrate the critical need for effective pruning wound protection. Synthetic fungicides remain the most reliable in protecting pruning wounds, though biologicals may play a role in integrated disease management.

Investigation of symptomatic and genetic diversity of *Xanthomonas arboricola* pv. *Juglandis* isolates in walnut orchards and biological control possibilities with bacterial antagonists. D. ERTIMURTAŞ[1], H. ÖZAKTAN[2]. [1]*Directorate of Plant Protection Research Institute Bornova, Department of Biological Control, 35040, Izmir, Türkiye.* [2]*Ege University, Faculty of Agriculture, Department of Plant Protection, 35040, Izmir, Türkiye.* E-MAIL: ertimurtasdamla@gmail.com

Walnut can be affected by walnut bacterial blight (WBB) and brown apical necrosis (BAN), which are mainly caused by *Xanthomonas arboricola* pv. *juglandis* (*Xaj*). Depending on the prevalence of these emerging diseases, an increase in yield losses is observed in the last quarter century. In this research, surveys were carried out in Western Anatolia during 2018-2019. Thirty-one bacterial strains were identified as *Xaj* by classical methods and 20 of them were confirmed to be pathogenic on immature walnut fruits. In addition, molecular identification by 16S rRNA showed a 99.83 to 100% similarity among the different *Xaj* isolates. Genetic variability among 31 *Xaj* isolates associated with WBB and BAN was best revealed by ERIC-PCR, where our set of bacteria was divided into 9 subgroups. It was found that *Xaj* strains obtained from same symptom, neighbouring regions and with similar pathogenicity were in the same group. The phylogenetic tree obtained by MLSA (Multi Locus Sequence Analysis) using 7 housekeeping genes highlighted differences due to changes in geographical variation and disease symptoms. While *Xaj* strains from WBB were categorised in 4 groups, 2 of 3 *Xaj* from BAN were in the same group. In biocontrol studies, 585 antagonist candidate bacterial isolates were obtained from healthy walnut trees. Out of three bacterial isolates showed biocontrol potential in *in vitro* and *in vivo* conditions. The most successful bacterial antagonists were identified as *Bacillus velezensis* and *Erwinia billingiae* as a result of sequence analysis.

POSTER PRESENTATIONS

Investigating the etiology, cultivar susceptibility, and fungicide efficacy against twig canker disease caused by *Diaporthe amygdali* in peach orchards of Greece. D. PAPADIMITRIOU[1], S. TESTEMPASIS[1,2], G.S. KARAOGLANIDIS[1]. [1]*Laboratory of Plant Pathology, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece;* [2]*Department of Agriculture, School of Agricultural*

Sciences, University of Western Macedonia, 53100 Florina, Greece. E-MAIL: testempasis@gmail.com

Twig canker, caused by the fungus *Diaporthe amygdali*, is a significant disease affecting almond and peach orchards, especially in Mediterranean regions of Europe. The disease targets annually emerging shoots, including fruit-bearing ones, leading to substantial yield losses. Characteristic symptoms include shoot blight, rapid drying, and gummosis of infected twigs. Despite its importance, *D. amygdali* has been insufficiently studied in Greece, so the aim of this study was: (a) the molecular identification, at species level, of twig isolates mainly of peach trees affected by fungal cankers, b) the determination of the susceptibility of 23 peach cultivars at field level along with the investigation of the comparative susceptibility between clingstone and freestone cultivars and c) the investigation of the inhibition of the fungus in GF 667 rootstocks, using 20 different commercial fungicides and a potential biological control agent. Molecular analysis of 100 isolates, using nucleotide sequencing and phylogenetic analysis of the β-tubulin and calmodulin gene regions, identified *D. amygdali* as the predominant pathogen. The highest disease incidence and severity were observed in cultivars 'Papayanni', 'Fortuna', and 'VLG', while 'Early May', 'Cal-2000', and 'Ferlot' showed the lowest severity. Regarding fungicides, fluopyram, boscalid, and copper hydroxide showed the lowest efficacy, while *Aureobasidium pullulans* (biological control) and tebuconazole demonstrated high effectiveness. The fungicide dithianon was the most effective, causing almost no necrosis. This study provided valuable insights into the etiology of twig canker, cultivar susceptibility, and effective disease management strategies, highlighting key factors in controlling *D. amygdali* in peach orchards.

Characterization of new fungal species causing pomegranate decline in Italy. S. TUNDO[1,2], D. GERIN[3], A. BOLZONELLO[1], R. CARACCIOLO[1], L. SELLA[1], F. FARETRA[3], F. FAVARON[1], S. POLLASTRO[3]. [1]*Dipartimento di Territorio e Sistemi Agro-Forestali (TESAF), Università degli Studi di Padova, Viale dell'Università 16, Legnaro, Italy;* [2]*Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente (DAFNAE), Università degli Studi di Padova, Viale dell'Università 16, Legnaro, Italy;* [3]Dipartimento *di Scienze del Suolo della Pianta e degli Alimenti - Università degli Studi di Bari Aldo Moro, via Amendola 126/A Bari, Italy.* E-MAIL: donato.gerin@uniba.it*;* angela.bolzonello@unipd.it

Pomegranate is an ancient fruit crop, with origins tracing back to at least 4,000 years ago in central Asia. The global expansion of pomegranate cultivation is due to its adaptability to adverse environmental conditions and the increasing demand for foods with improved organoleptic characteristics. With this study, we report the occurrence of pomegranate fungal pathogens isolated from canker/dieback of trees located in Basilicata, Apulia and Veneto regions (Italy). The isolates were identified based on BLASTn analysis of ITS1-5.8S-ITS2 rDNA internal transcribed spacer (*ITS*) region, translation elongation factor 1-α (*TEF1*), calmodulin (*cmdA*), β-tubulin (*TUB2*), and large subunit (*LSU*) of rDNA as *Diaporthe eres*, *D. foeniculina*, *Neopestalotiopsis rosae*, *Ophiostoma stenoceras* and *Xenoacremonium* sp. Pathogenicity of these fungi was demonstrated by artificial inoculations of 2-years-old pomegranate trees cv. Wonderful. *Coniella granati* and *Neofusicoccum parvum*, fungal species already described for their involvement on pomegranate decline, were also isolated. The colony growth of each isolate was assessed at different temperatures (from 5 to 35°C) on malt extract agar in dark, and for all species the optimum temperature was in the range 25–30°C. All isolates were grown in self- and cross-pairing with the other pathogens and the most important interactions were represented by the competition due to the different growth rates of the isolates. In some cases (e.g. *D. foeniculina*/*O. stenoceras*), a mutual intermingling between hyphae was observed and both pathogens were re-isolated from plugs collected in the contact zone. This work provides knowledge on new pomegranate pathogens, representing a threat for the cultivation of pomegranate.

This research was financially supported by SAgritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them. This work was also financed with BIRD 2021 funds, Dept. TESAF, University of Padua –Italy".

Fungal pathogen surveillance in almond orchards: A comprehensive approach through the ATENA project. M.I. DRAIS, C. MACOR, D.M. PINTO, F. PAPA, L. ROSSINI, M. CONTARINI, A. MAZZAGLIA. *Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy.* E-MAIL: drais@unitus.it

The (ATENA-*Agricoltura di precisione e Intelligenza Artificiale per l'innovazione sostenibile dell'arboricoltura*) project supports the sustainable intensification of almond production by integrating advanced technologies and data-driven approaches into the design and management of intensive and super-intensive orchard systems. Central to this initiative is the Decision Support Design Platform (DSDP), a low-code, user-oriented framework that provides agronomists and growers with tools to optimize productivity, resource efficiency, and disease control. A key module within the DSDP is dedicated to phytopathological surveillance, focusing on the detection and identification of fungal pathogens affecting almond orchards. The monitoring system integrates weekly field inspections, symptomatic tissue sampling, and spore trapping for early detection of airborne propagules. Laboratory diagnostics follow a multi-step protocol, including morphological identification based on mycelial and conidial features, molecular analysis using conserved markers (e.g., ITS, β-tubulin), and germination trials to assess spore viability and pathogenic potential. Initial results confirmed the presence of *Monilinia laxa* and *Diaporthe* spp. as major pathogens in the surveyed orchards. The integration of this surveillance data into the DSDP enhances early warning capabilities, supports site-specific phytosanitary measures, and enables adaptive management by aligning control strategies with real-time and historical field conditions. This comprehensive, integrated approach strengthens disease prevention and contributes to the ATENA project's broader goal of promoting resilient, efficient, and environmentally sustainable almond cultivation systems.

This research was financially supported by ATENA project: Agricoltura di precisione e Intelligenza Artificiale per l'innovazione sostenibile dell'arboricoltura. Regione Lazio – POR FESR LAZIO 2021/2027.

Unveiling *Diaporthe* spp. associated with almond canker in central Italy. M.I. DRAIS, I. GIUBILEI, C. MACOR, A. MAZZAGLIA. *Department of Agriculture and Forest Sciences, University of Tuscia, Viterbo, Italy.* E-MAIL: drais@unitus.it

The incidence of almond-associated fungal diseases, particularly twig cankers and shoot blight caused by *Diaporthe* spp. is increasing and poses a significant threat to crop productivity, especially in areas with high humidity and mild temperatures. Among these, constriction canker caused by *Diaporthe amygdali* is considered one of the most severe diseases affecting *Prunus dulcis* (almond) across Europe, particularly in Mediterranean countries. Field surveys conducted in Lazio Region (Italy) revealed widespread symptoms of twig canker and shoot blight in almond orchards managed under intensive cultivation systems. The highest disease incidence was recorded in orchards planted with the 'Tuono' cultivar. A total of 25 *Diaporthe*- like isolates were obtained through isolation in the laboratory. Morphological characterization and molecular identification based on sequencing and phylogenetic analyses of the ITS, *tef1-α*, and *tub2* gene regions were used to identify the isolates. The effect of various temperatures (5, 10, 15, 20, 25, 30, and 35°C) on mycelial growth was assessed on PDA medium, while lesion development was evaluated on inoculated detached almond shoots. Pathogenicity tests on detached almond shoots are ongoing to assess differences in aggressiveness among isolates. This study provides an updated assessment of *Diaporthe*- induced cankers in Central Italy, offering integrated morphological and molecular insights into the identity and behavior of these pathogens.

This research was financially supported by ATENA project: Agricoltura di precisione e Intelligenza Artificiale per l'innovazione sostenibile dell'arboricoltura. Regione Lazio – POR FESR LAZIO 2021/2027.

CONCURRENT SESSION G2 – Innovation in alternatives to synthetic fungicides

SESSION KEYNOTES

Use of basic substances as alternative to synthetic pesticides for the management of plant diseases. G. ROMANAZZI. *Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131, Ancona, Italy.* E-MAIL: g.romanazzi@univpm.it

Plant pathogens can induce up to 40% of loss and waste of the potential production of fresh fruit and vegetables. Nowadays the application of synthetic fungicides is the main strategy to prevent and manage plant diseases on fresh produces. At the same time, the consumers request products free from fungicide residues, and they are concerned about their use in the field to protect plant health, often forgetting the drugs they use for human or pet health. Retailers use (and at times induce) this fear, to promote more expensive products with zero pesticide residues or with concentrations way lower than allowed maximum residue levels (e.g. even 33% of MRL), and a cap number of active ingredient (in some cases even 3 to 5). Registration of a synthetic pesticide nowadays has

a cost of around 300 million Euro, and it can take up to 13 years. The unfavourable environment toward the synthetic pesticides leads to reduction of the research to discover new active ingredients, while a high number of compounds is disappearing from the market for banning or for expiring or registration (that for a standard pesticide lasts 10 years, while for a low risk fungicide last 15 years). While in 2014 they were registered in EU 30 new compounds and more than half were synthetic products, since 2019 no new synthetic products were registered, and overall yearly registrations were much less than 10. The risk is then to have reduced solutions for plant protection, together with the introduction of new pathogens from other areas (so called aliens) and with the increase of virulence of previously mild pathogens, that were contained by the application of wide spectrum compounds, and/or are taking advantage by the evolution of climate conditions. Therefore, the adoption of alternatives to synthetic fungicides, like biocontrol agents and natural compounds, is mandatory. Among those, the basic substances represent compounds that are already used as a food or feed and can have a secondary activity in plant protection. Then, it is not needed to test their side effects on human and environment, so registration cheaper and can be obtained in a shorter time (around 1-2 years and 50000 Euro, including registration for organic agriculture), and last forever. There are currently 28 basic substances registered for use toward plant diseases, including chitosan hydrochloride, chitosan, vinegar, lecithin, *Equisetum arvense* extract, *Urtica* extracts, *Salix* spp. cortex, magnesium hydroxide, cow milk, whey (liquid or powder), talc and sodium bicarbonate. Some of these basic substances were used in open field and were able to contribute to plant disease management, with example of partial or even total replacement of synthetic fungicides. So, new biosolutions with a reduced impact can, at least partially, replace the synthetic fungicides not anymore on the market, although they require a deeper knowledge by technicians supporting growers to optimise the application and get a protection level similar to what achieved with the synthetic fungicides, sometimes with a higher number of applications.

ORAL PRESENTATIONS

Phytotoxins: a virulence factor or an opportunity? A. EVIDENTE. *Institute of Biomolecular Chemistry, National Research Council (CNR) Viale Campi Flegrei 34, 80078 Naples Italy.* E-MAIL: evidente@unina.it

Nature is an almost inexhaustible source of a myriad of compounds with different chemical structure and biological activities. Fungal phytotoxins, that play an important role in many plant diseases induced by fungal pathogens, belong to different classes of natural compounds. They frequently have original chemical structures and diversified biological activities. Thus, could be a fundamental tool to protect crops from pathogens, avoiding the use of chemical pesticides. Phytotoxins could be used: (a) to develop rapid and specific diagnostic methods in order to detect diseases and be the basis for developing simple kits to be used directly in the field by farmers (b) to help in selecting plants naturally resistant from those susceptible to a pathogen by knowing the genes responsible of innate resistance; furthermore they could help in different research fields: (c) formulating new biopesticides (fungicides, bactericides, but also herbicides and insecticides, etc.) (d) developing new drugs with important applications in medicine due to their activity and specificity frequently associated with a new mechanism of action (anticancer, antiviral, antimalarial, etc.). Significant examples of this kind of bioactive metabolites are ophiobolin A and sphaeropsidin A, two fungal terpenes active as wilt inducing phytotoxins now also developing as promising anticancer metabolites.

TRV-assisted RNA silencing of *VdSNF1* and *VdNLP1* potentiates the development of innovative management strategies against *Verticillium dahliae* in herbaceous crops. C. TSOUKAS, P. SARIDIS, A. VENIERAKI, E.J. PAPLOMATAS. *Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece.* E-MAIL: epaplom@aua.gr

Verticillium dahliae, a devastating vascular pathogen, affects a wide range of economically important crops. Limited effective control methods are currently available. The advancement of RNA interference (RNAi) comprises promising strategy to manage this pathogen. The aim of this study was to utilize a TRV-based host-induced gene silencing (HIGS) approach to suppress pathogen's critical genes. For this, genes involved in several functions such as regulation of expression of cell wall-degrading enzymes (*SNF1*), and induction of necrosis and ethylene production (NLP genes) were selected. Initially, specific gene segments were chosen to avoid off-target effects, ensuring a pathogen-specific outcome. Each segment was cloned into the TRV2 vector and *Agrobacterium tumefaciens* AGL-1 cells were subsequently transformed with the resulting constructs. Afterwards, cotton and tomato

plants were agro-infiltrated and artificially inoculated with *V. dahliae*. The effect of TRV-assisted HIGS was evaluated against a highly defoliating (D) and a non-defoliating (ND) isolate in cotton plants, while it was also used against Race 1 (R1) and Race 2 (R2) strains in tomato. Silencing of the *VdSNF1* gene reduced the severity in the ND strain by approximately 50%, while plants expressing the partial *VdNLP1* gene demonstrated 51% less disease upon inoculation with the D strain. Similarly, at 20 dpi of the ongoing experiment, silencing of the *VdSNF1* gene resulted in approximately 40% and 56% less disease against the R1 and the R2 isolates, respectively. These findings suggest that RNA silencing is a promising approach that could lead to the development of innovative control strategies against vascular wilt pathogens.

This project is carried out within the framework of the National Recovery and Resilience Plan Greece 2.0, funded by the European Union – NextGenerationEU (Implementation body: HFRI).

Exploring plant-based protein hydrolysates as resistance inducers in wheat. A. MOSTACCI[1], C. SCAVARDA[2], G.L. SALA[3], L. DE FERRA[4], O. INCERTI[1], N. DE DIEGO[5], L. SPÍCHAL[5], A. IPPOLITO[1], S.M. SANZANI[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy.* [2]*A. Costantino & C. SpA, Via Francesco Romana, 11, 10083 Favria (TO), Italy.* [3]*Biofer S.P.A., Via Gabrio Serbelloni 7, 20122 Milan, Italy.* [4]*Sts Pharma Srl, Via Gabrio Serbelloni 7, 20122 Milan, Italy;* [5]*Czech Advanced Technologies and Research Institute, Šlechtitelů 27, 77900 Olomouc, Czech Republic.* E-MAIL: a.mostacci@phd.uniba.it*;* simonamarianna.sanzani@uniba.it

By 2030, as part of the Green Deal Farm to Fork Strategy, the European Commission intends to reach the goal of a 50% reduction in the overall chemical pesticide use. An alternative approach to meet the objective is to empower plant natural defense responses using biostimulants, which can positively act on plant biological processes including the defense against stresses. In the present study, a formulation (AgricostanD) based on a protein hydrolysate (PH) derived from soybean flour, was evaluated *in vivo* as a resistance inducer against *Fusarium culmorum*, a causal agent of fusarium head blight on durum wheat in southern Italy (cv. Svevo). Seeds, showing infection symptoms, were subjected to an additional artificial inoculation with *F. culmorum*, thus undergoing a double biotic stress. In this condition, the seeds priming with AgriD led to notable effects on germination rate, growth speed, and the plant's tolerance to both biotic stresses up to the tillering stage. An induction of plant defence might be hypothesized, as AgriD at the label dosage did not show an antifungal direct effect *in vitro* against plant pathogens. To improve PH formulation, several enzymatic hydrolyses were conducted using proteases of various origins on soybean flour and pea protein isolate. The aim is to generate original formulations, which will be evaluated through *in vivo* and *in vitro* assays to explore their potential involvement in plant responses to biotic and abiotic stresses.

Asia Mostacci was supported by a PhD fellowship funded within D.M.117/2023, PNRR, Missione 4, componente 2 "Dalla Ricerca all'Impresa" - investimento 3.3 – Cofunded by A. Costantino & C. S.p.a - Applicazioni di biostimolanti di origine vegetale per migliorare la risposta agli stress delle piante e/o loro prodotti, CUP H91I23000050007.

Overview of plant defense inducers' mode of acon against the biotrophic pathogen *Podosphaera xanthii*. T. MARGARITOPOULOU[1], K. KOTSARIDIS[2], M. SAMIOTAKI[3], D. TSIRIVA[4], E. MARKELLOU[1]. [1]*Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Stefanou Delta 8, 14561, Kifissia, Greece.* [2]*Laboratory of Virology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, Stefanou Delta 8, 14561, Kifissia, Greece.* [3]*Protein Chemistry Facility, Biomedical Sciences Research Center "Alexander Fleming", Vari, 166 72, Greece.* [4]*Research and Development Department, Phytorgan SA, Nea Kifissia, Greece.* E-MAIL: th.margaritopoulou@bpi.gr

The high adaptability of the biotrophic fungus *Podosphaera xanthii* to diverse environments, along with the increasing negative effects of excessive fungicide use, necessitates the exploration of alternative control methods. Plant Defense Inducers (PDIs) activate plant defense mechanisms similarly to pathogens and are considered eco-friendly and sustainable for disease management. However, their mode of action is still not fully understood. Using transcriptomic, proteomic, and metabolomic approaches alongside physiological studies and targeted analyses, we demonstrated that two PDIs—the extract from the knotweed plant *Reynoutria sachalinensis* and a formulation of chitosan nanoparticles loaded with salicylic acid—effectively induce plant defences against *P. xanthii*. Our results revealed a direct correlation between the production and signalling of major cell membrane phospholipids, such as phosphatidylserine, phosphatidylethanolamine, and phosphatidic acid, which

are involved in the initial stages of signal perception. This correlates with the reprogramming of the protein quality control mechanism in the endoplasmic reticulum during the later stages of signalling, which also accompanies induced defense. Moreover, the application of PDIs enhanced the expression of plant defense-related proteins, including glutathione transferases, kinases, and chitinases. Protein-protein interaction enrichment indicated the formation of an interactive network that promotes defense. Simultaneously, we found that the pathogen secretes proteins related to ubiquitination during host invasion to manipulate the plant's protein translation and quality control machinery. Notably, the application of the PDIs significantly downregulated the expression of various secreted proteins from the pathogen, resulting in reduced powdery mildew severity.

This research was financially supported by the project "Innovations in Plant Protection for sustainable and environmentally friendly pest control, InnoPP - TAEDR-0535675 that is ¨Funded by the European Union- Next Generation EU, Greece 2.0 National Recovery and Resilience plan, National Flagship Initiative "Agriculture and Food Industry", by the project "Nanoshield - T2EDK-02113", and Benaki Phytopathological Institute.

Biocontrol-mediated activation of defense mechanisms in grapevine against *Fusarium equiseti* infection. M.L. TELLO-MARISCAL[1], J.M. ALONSO DE ROBADOR[2], B. PINTOS[2], A. GOMEZ-GARAY[2]. [1]*National Institute for Agricultural and Food Research and Technology (INIA). National Research Council (CSIC), Ctra. N-VI. 28040 Madrid, Spain.* [2]*Research Group FiVe-A, Department of Genetics, Physiology and Microbiology, Faculty of Biology, Complutense University of Madrid, Ciudad Universitaria, 28040 Madrid, Spain.* E-MAIL: marisa.tello@inia.csic.es

Grapevine trunk diseases (GTDs) are among the most devastating threats to viticulture worldwide, causing significant economic losses and compromising vineyard longevity. In this study, we isolated and characterized a *Fusarium equiseti* strain from infected grapevine (*Vitis vinifera* L.) tissues, confirming its pathogenicity through Koch's postulates and assessing virulence via enzymatic and physiological assays. Given the limitations of chemical phytosanitary treatments, we evaluated the potential of *Meyerozyma guilliermondii* CECT13190, a patented yeast strain (ES-2792777 B2), as a biocontrol agent (BCA). The BCA demonstrated multiple plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore synthesis, and atmospheric nitrogen fixation. Dual-culture and resazurin assays showed strong antagonism against *F. equiseti*, supported by in planta experiments that revealed both preventive and curative effects on infected grapevine plants. Proteomic analyses revealed that *M. guilliermondii* modulates host plant defense by enhancing expression of key pathogenesis-related (PR) proteins, including β-1,3- glucanase, thaumatin, and chitinase, and activating systemic acquired resistance (SAR). Gene expression studies confirmed transcriptional regulation of these defense components under both prophylactic and therapeutic treatments. Our findings highlight the complex tripartite interaction between host plant, pathogen, and biocontrol agent, showcasing the ability of *M. guilliermondii* to induce molecular, enzymatic, and metabolic defense responses in grapevine. These results provide a strong foundation for sustainable GTD management strategies and contribute to the growing body of evidence supporting biocontrol as a viable alternative to synthetic fungicides in viticulture.

This research was supported by the Ministry of Science, Innovation and Universities, Spain.

POSTER PRESENTATIONS

Interactions between ontogenetic and acquired resistance shape grapevine metabolic responses to *Plasmopara viticola*. L. NASSI[1,2], G. FEDELE[1,2], I. RAGNOLI[3], L. ZHANG[3], L. LUCINI[3], V. ROSSI[1,2], T. CAFFI[1,2]. [1]*Department of Sustainable Crop Production, Università Cattolica dal Sacro Cuore, Piacenza, Italy.* [2]*Research Center on Plant Health Modelling (PHEM), Department of Sustainable Crop Production, Università Cattolica dal Sacro Cuore, Piacenza, Italy.* [3]*Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, Piacenza, Italy.* E-MAIL: luca.nassi@unicatt.it

Plant Resistance Inducers (PRIs) are promising alternatives to conventional plant protection products for controlling the grapevine pathogen *Plasmopara viticola*. This study aimed to investigate the metabolic effects caused by PRI-based treatments on grapevine leaves and to evaluate how these effects combine with the morpho-metabolic changes given by ontogenetic resistance. For this purpose, the effects of two well-known PRI-based plant protection products, Laminarin (LAM) and Potassium Phosphonate (PHO), were evaluated and compared with an untreated control (TNT). Ten leaves from the treated vine shoot, ranging from apical to basal, were sampled 48 h after PRIs treatment (Priming assessment) and 48 and 96 h post artificial inoculation of *P. viticola* (Defence response assessment). The effects on their

metabolism were assessed through an untargeted metabolomics approach using LC-MS. The annotated metabolites through plant-based specific databases and the list of compounds were subjected to unsupervised (PCA) and supervised (AMOPLS-DA and OPLS-DA) statistical analysis that allowed to highlight the main discrimination factors that influenced leaf metabolism. After treatments and 48 h after pathogen inoculation, the metabolic profiles were strongly affected by the leaf age and LAM showed a different modulation of metabolite profile compared to the other treatments. LAM and PHO shared a similar metabolite profile only after 96 h post inoculation Overall, LAM and PHO treatments activated significantly different metabolic profiles respect to the timing of priming and pathogen inoculation. Moreover, the results of this study confirmed a significant influence of leaf age on leaf metabolism response.

This study was supported by the PhD in Agro-Food System (Agrisystem) of the Università Cattolica del Sacro Cuore (Italy).

Salvia spp. extracts contain bioactive terpenoids to control _Plasmopara viticola_ infections on grapevine. A. SMALDONE[1,2], O. GIOVANNINI[3], M. OBERHUBER[2], P. ROBATSCHER[2], M. PERAZZOLLI[1,3]. [1]*Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy.* [2]*Laboratory for Flavours and Metabolites, Laimburg Research Centre, Laimburg 6, Pfatten (Vadena), 39040 Auer (Ora), Italy.* [3]*Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38098 San Michele all'Adige, Trento, Italy.* E-MAIL: anna.smaldone@unitn.it

Downy mildew, caused by *Plasmopara viticola*, is one of the major threats to viticulture, especially in areas with warm and humid climate conditions. Grapevine downy mildew is controlled by frequent application of copper and synthetic fungicides, with possible negative impacts on human health and the environment. Alternative products, such as extracts of medicinal plants, are highly desired to develop sustainable plant protection strategies. Alcoholic extracts of *Salvia officinalis* previously showed inhibitory activity against phytopathogens. This study aims to characterize the activity of sage extracts against grapevine downy mildew and to identify bioactive metabolites. Alcoholic extracts of sage shoots, leaves, and flowers showed disease reduction in grapevine leaf disk assays comparable to copper, while a stem extract was only partially active against *P. viticola*. Fractions of shoot extracts were obtained using preparative liquid chromatography and subjected to inhibitory activity tests on leaf disks. Active fractions were analyzed with untargeted metabolomics using liquid chromatography-high resolution mass spectrometry (LC-HRMS), and 25 putative bioactive compounds were annotated as terpenoids, such as eight abietane diterpenoids, three icetexane diterpenoids, three pentacyclic triterpenoids, two secoabietane diterpenoids, and nine other terpenoids. Five annotated compounds were identified by LC-HRMS analysis using reference standards, and their inhibitory activity was confirmed on leaf disks. The identification of novel bioactive compounds from *Salvia* spp. and the characterization of their mode of action will pave the way for the development of new sustainable alternatives for grapevine protection.

Inactive yeasts as preventive treatments against _Botrytis cinerea_ on grapevine. C. GONZALEZ-DEHENNAULT[1], A.G. ILESANMI[2], G. LION[2], S. TUNDO[2], R. MUSETTI[2], L. SELLA[2], M. LUCCHETTA[2]. [1]*Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, 35020 Legnaro, Italy.* [2]*University of Padua, Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padua, 350320 Legnaro, Italy.* E-MAIL: charlottemarilyn.gonzalezdehennault@phd.unipd.it

The demand for alternatives to synthetic fungicides has been increasing due to the rise of pathogens resistant to active principles, consumers and producers' consciousness, and EU directives on sustainable farming. *Botrytis cinerea* is a worldwide distributed pathogen of grapevines causing losses of grape production. In the last years, yeasts and their derivatives have been studied as promising alternatives to fungicides for the management of plants' pathogens. In this study, a screening of various inactive yeast suspensions from the food industry was performed. The objective of this research was to check several yeast derivates efficacy in plant stimulation and defence activation toward *B. cinerea*. Grapevine plants, cultivar Merlot grown in pots in a greenhouse, were treated twice using inactive yeast-based suspensions. Stomatal conductance was monitored 48 hours later to assess the plant metabolism. Inoculation of *B. cinerea* was performed on detached leaves at 72 hours after the second application and extension of the lesions was observed three days later. Results suggest that inactive yeast *Saccharomyces cerevisiae* grown on exhausted medium of *Salvia officinalis* efficiently reduce *B. cinerea* lesions on leaves in comparison to the untreated control. An increase in plants photosynthetic activity has

also been observed after the application of the exhausted medium of *S. officinalis*. Those extracts could offer interesting alternatives to synthetic fungicides. Further in vivo trials need to be conducted to investigate the efficiency of these compounds in protecting Grapevine production.

Testing the efficacy of cyanobacterial silver nanoparticles against fungal plant pathogens. C. AGLIETTI, M. CIANI, M. GIAMBERINI, A. ADESSI, L. GHELARDINI. *Department of Agricultural, Food, Environmental and Forest Sciences and Technologies (DAGRI), University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy.* E-MAIL: chiara.aglietti@unifi.it

Plant diseases caused by fungi and bacteria can be managed and controlled using a variety of techniques that have been implemented during time. However, traditional management often relies on the use of synthetic chemical pesticides, despite the growing need for more sustainable and eco-friendly solutions. Among these, silver nanoparticles (AgNPs) application has proven effective in inhibiting the growth of fungal and bacterial pathogens, but there is still a gap in identifying sustainable synthesis technologies and user-friendly application methods in plant health defense. This study explores the green synthesis of AgNPs using cyanobacteria, promising biofactories for the eco-friendly synthesis of metal nanoparticles (MNPs). The antifungal efficacy of AgNPs was assessed *in vitro* against *Botryosphaeriaceae* sp., *Gnomoniopsis castaneae* and *Fusarium* sp. by testing AgNPs obtained by cell-free culture medium, extracellular polysaccharides (EPS) extracted from cell-free culture medium, and cellular extract. The antifungal activity of the different cyanobacteria-derived AgNPs on the different fungal species was assessed through *in-vitro* growth tests. To verify the persistence of the antifungal effect, at the end of the treatment each fungal colony was reinoculated onto PDA petri dishes without AgNPs. The mycelial growth of two of the three fungal species tested was clearly inhibited by high concentration of AgNPs (100ppm), although the effect was not found to be persistent on reinoculated PDA dishes. Cellular extract was the most effective synthesis method, inhibiting fungal radial growth of c.a 60% (1.5 cm against 4 of control). These results underline the potential of cyanobacteria-derived AgNPs as a sustainable antifungal method for plant disease control.

This work was supported by the COMBINE project funded by the University of Florence in the frame of "Competitive call of biannual projects for Temporary Researchers, edition 2024- 2025 (D.R. n. 419 02/05/2023)".

Copper nanoparticles against *Colletotrichum gloeosporioides:* effectiveness and insights on the mode of action. A. MALANDRAKIS[1,2], N. KAVROULAKIS[2], M. MATHIOUDAKIS[2], M. KISANDRAKI[2], V. KATZOURAKIS[3], C. CHRYSIKOPOULOS[3]. [1]*School of Chemical and Environmental Engineering, Technical University of Crete, 73100 Chania, Greece.* [2]*Institute for Olive Tree, Subtropical Plants and Viticulture, Hellenic Agricultural Organization "Dimitra", Agrokipio-Souda, 73164 Chania, Greece.* [3]*Department of Civil Infrastructure and Environmental Engineering, Khalifa University of Science and Technology, Abu Dhabi 127788, UAE.* E-MAIL: amalandrakisg@tuc.gr

Anthracnose, caused by the fungal pathogen *Colletotrichum gloeosporioides*, is among the most prevalent and severe postharvest diseases affecting various fruits, including olive, citrus, and avocado. In an era where chemical control is being challenged by fungicide resistance development and strict EU legislation, causing the withdrawal of a large number of established active ingredients used for disease management, metallic nanoparticles (MNPs) represent promising, eco-friendly fungicide-alternatives for controlling pathogens. In this study, the effectiveness and potential fungitoxicity mechanisms of copper nanoparticles (Cu- NPs) was tested against *C. gloeosporioides* strains isolated from orange and avocado fruit both *in vitro* and *in vivo*. Cu-NPs outperformed their ionic counterpart Cu(OH)2 (which was used as a reference fungicide) in reducing mycelial growth and disease symptoms. Pearson correlation analysis revealed a positive cross resistance between Cu-NPs and the demethylase inhibitor (DMI) fungicide difenoconazole. A significant antagonism between Cu-NPs and detoxifying inhibitor agents DEM, PBO as well as DMI fungicides difenoconazole, prochloraz, and prothioconazole was observed as indicated by the reduced effectiveness of applied combinations against the pathogen. Furthermore, an induced C-14 alpha-demethylase overexpression was found, at least partly, to be responsible for the observed superior fungitoxic action of Cu-NPs against *C. gloeosporioides*. Overall, inhibition of ergosterol biosynthesis is proposed as a candidate fungitoxicity mechanism for the mode of action of Cu-NPs against the pathogen.

This research was financially supported by the European Union-Next Generation EU, Greece 2.0 National Recovery and Resilience plan (project code: TAEDR-0535675).

Green synthesized silver nanoparticles against *Pseudomonas savastanoi* pv. *savastanoi:* effectiveness and mode of action. N. KAVROULAKIS[1], A. MALANDRAKIS[1,2], M. KISSANDRAKI[1], V. KATZOURAKIS[3], C. CHRYSIKOPOULOS[3]. [1]*Institute of Olive Tree, Subtropical Plants and Viticulture, ELGO-DIMITRA, Agrokipio-Souda, 73164 Chania, Greece.* [2]*School of Environmental Engineering, Technical University of Crete, 73100 Chania, Greece.* [3]*Department of Civil Infrastructure and Environmental Engineering, Khalifa University of Science and Technology, Abu Dhabi 127788, UAE.* E-MAIL: kavroulakis@elgo.gr

Knot disease is an important olive tree bacterial infection caused by *Pseudomonas savastanoi* pv. *savastanoi*, leading to significant economic losses in olive production worldwide. Control measures against the pathogen include cultural methods, selection of resistant cultivars, use of biocontrol agents and the application of copper-based bactericides which are the only available chemical control option. Although copper is a multisite inhibitor, various cases of *P. savastanoi* pv. *savastanoi* resistant to this metal have been reported leading to control failures. This lack of available alternatives in combination with the loss of copper effectiveness make the need for alternative control measures an urgent matter. In this study, the effectiveness and potential antimicrobial mechanisms of green synthesised silver nanoparticles (GAg-NPs) were tested against two *P. savastanoi* strains isolated from olive orchards both *in vitro* and *in vivo*. GAg-NPs exhibited superior bactericidal action compared with chemically synthesized nano silver (Ag-NPs) and a reference Cu(OH)2–containing bactericide in reducing bacterial growth and disease symptoms. *In vitro* experiments using the strong chelating agent EDTA in combination with NPs indicated that silver ion release is not the main mechanism responsible for the antibacterial action of Gag-NPs against the pathogen. Overall, GAg-NPs have demonstrated a great potential to be used as copper alternatives against Olive knot disease and a promising tool against bactericide resistance.

This research was financially supported by the European Union-Next Generation EU, Greece 2.0 National Recovery and Resilience plan (project code: TAEDR-0535675).

Environmentally-friendly methods and tools for disease control in table grapes. E. CHALBAOUI, P. ALBANESE, F. SANTORO, S. GUALANO, F. DIGIARO, F. VALENTINI. *Centre International de Hautes Etudes Agronomiques Méditerranéennes Bari (CIHEAM Bari), Via Ceglie, 9 - 70010 Valenzano (BA), Italy.* E-MAIL: chalbaouieya@gmail.com

Powdery mildew, downy mildew, gray mould, and thrips represent major challenges in table grape cultivation, particularly in the Apulia region of southern Italy, where they can severely reduce both yield and fruit quality. Traditionally, these threats have been managed through chemical treatments. However, increasingly stringent EU regulations on pesticide use— driven by environmental concerns and consumer awareness of food residues—are prompting a shift toward more sustainable viticultural practices. This study investigates the efficacy of hydrogen peroxide, a GRAS (Generally Recognized As Safe) product, applied using a Micro 28 precision spraying system, as an alternative to conventional chemical-based Integrated Pest Management (IPM). The trial was conducted in a commercial vineyard in Acquaviva delle Fonti (Bari) from February to September. Disease and pest presence, weather conditions, and grape ripeness were monitored using a decision support system, visual inspections, and laboratory analyses. The hydrogen peroxide treatment, when applied with the Micro 28 system, showed comparable disease control to traditional IPM while significantly reducing treatment time and costs. Its effectiveness is attributed to the precision and timeliness of applications, which are critical for optimal performance of GRAS products. Importantly, this method also contributes to a lower environmental impact by reducing machinery use, thereby minimizing carbon emissions and soil compaction. These findings highlight the potential of combining GRAS products with innovative application technologies for sustainable disease management in viticulture. Further research is necessary to validate the results across different environments and growing seasons.

This research was funded by The National Operational Program of the Italian Ministry of Education, University and Research (PON-MIUR) "AGREED—Agriculture, Green & Digital" ARS01_00254, Decreto concessione prot. n.580/2020, CUP. B94I20000170005, RNA COR 1739311".

Application of plant-formulations to improve growth parameters and resistance to stresses of Mediterranean crops. A. BEROUAKEN[1], C. PITUELLO[2], A. SCHENA[1], O. INCERTI[1], A. IPPOLITO[1], S. M. SANZANI[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy.* [2]*SICIT Group S.p.A., Via Arzignano 80, 36072 Chiampo (VI), Italy.* E-MAIL: simonamarianna.sanzani@uniba.it

Moringa oleifera Lam. (Moringaceae), consumed in several tropical and subtropical countries, is a highly valued multi-purpose herbal plant with numerous medici-

nal properties and high nutritional value, being rich in polyphenols, bioactive peptides, carotenoids, and glucosinolates. Besides, a great interest is emerging towards the exploitation of Moringa as an added-value food ingredient. In a recent study the preharvest application of two lab-made aqueous extracts from *Moringa oleifera* leaves (MLEs) improved yield, quality, and storability of lettuce grown in a hydroponic system, as compared to an untreated control and other biostimulant formulations on the market. In the present investigation a Moringa-based formulation has been tested to improve the growth parameters of two relevant crops as wheat and tomato. Preliminary assays on seeds were conducted confirming a significant increase in germination rate and root and shoots length. As such the plant should withstand better stressing events. Further *in planta* assays are in progress.

Abdereak Berouaken was supported by a PhD fellowship funded within D.M.630/2024, PNRR, Missione 4, componente 2 "Dalla Ricerca all'Impresa" - investimento 3.3 – Cofunded by SICIT S.p.a - Applicazione di estratti vegetali per migliorare la risposta della pianta agli stress e la conservabilità dei suoi prodotti, CUP H91I24000260007.

Antimicrobial activity, and defense gene activation by natural extracts: toward sustainable plant health management. C. BILEN[1], L. VACCARO[1], S. LAERA[1], P.R. ROTONDO[1], S. POLLASTRO[1], M. MARASHI[1], T. MASCIA[1], R. SPANÒ[2], D. EL CHAMI[3], F. FARETRA[1], R.M. DE MICCOLIS ANGELINI[1]. [1]*Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy.* [2]*National Research Council -Institute for Sustainable Plant Protection, Via Amendola, 122/D Bari, 70126, BA, Italy.* [3]*TIMAC AGRO Italia S.p.A., 26010 Ripalta Arpina, CR, Italy.* E-MAIL: ritamilvia.demiccolisangelini@uniba.it

The growing demand for sustainable agricultural practices has accelerated research on eco- friendly plant health management strategies, focusing on natural substances like plant extracts with bioactive compounds for antimicrobial activity or plant defense-inducing properties. This study evaluates natural extracts as sustainable alternatives for managing plant diseases. A variety of substances, including essential oils, extracts from ornamental and edible plants, by-products from beer and coffee processing, *Aloe vera* extracts, and selected commercial formulations, were assessed for their antimicrobial efficacy through *in vitro* and *in vivo* assays. Several extracts demonstrated notable bioactivity: *Agapanthus africanus* showed significant antifungal effects against *Botrytis cinerea*, while essential oils from thyme, tea tree, and lavender were effective against both *B. cinerea* and *Pseudomonas syringae* pv. *tomato*. Greenhouse trials on tomato plants revealed the protective effects of *Aloe vera* polysaccharides and ornamental plant extracts, particularly *Eremophila nivea* and *Limoniastrum monopetalum*, against PVY^{c}-to, a recombinant strain of *Potato virus Y* necrogenic on tomato. Additionally, microencapsulated thyme essential oil and alginate- based formulations showed enhanced *in vivo* performance, particularly against the bacterial and viral pathogens. Gene expression analyses at 12- and 72-hours post-treatment indicated strong upregulation of the defense-related gene *PR1*, with extracts from coffee residues, *A. vera* gel, and the synthetic resistance inducer BION®. The latter used as reference, elicited the most pronounced response, while *PR4* exhibited a more moderate induction. These findings highlight the promising role of natural plant-derived and agro-industrial substances in providing both direct antimicrobial action and stimulation of plant immune responses, contributing to sustainable health management strategies.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022), and INVESTIMENTO 3.3 D.M. 352/2022 and TIMAC AGRO Italia S.p.A. and by Innovazione di prodotto e di processo nel florovivaismo biologico per il verde pubblico, healing e kitchen garden BIOGARDEN (MASAF Progetti di Ricerca in agricoltura Biologica Avviso pubblico per la concessione di contributi per la ricerca in agricoltura biologica n. 9220340 del 8 ottobre 2020 – CUP H93C24000920001).

Preliminary assessment of plant powders and aqueous extracts from different botanical species to inhibit fungal growth under *in vitro* conditions. M.G. MOREA, G. RICCIARDI, A. LIBUTTI, A. CARLUCCI. *Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Via Napoli 25, 71122, Foggia, FG, Italy.* E-MAIL: maria.morea@unifg.it

Many botanic plants are reported to be rich in antioxidant and polyphenolic compounds, resulting in effectiveness to prevent microbial contaminations (bacteria and fungi). The natural compounds might be useful in biocontrol against fungal pathogens instead of synthetic fungicides. This preliminary study was carried out to assess the ability of five botanical species to control 15

fungal soilborne pathogens by the use of their dehydrated tissues and aqueous extracts. Vegetative tissue portions of *Raphanus raphanistrum*, *Brassica nigra*, *Sinapis alba*, *Matricaria chamomilla* and *Solanum lycopersicum* were collected and left to dry at room temperature for one week and then at 30°C until constant weight, before to be finely grounded and used at 2% as plant powders (PPs) by inclusion in PDA medium. The same PPs were used to obtain aqueous extracts (AEs) by infusion in distillate water (ratio w:v; 1:10) at room temperature for 48 h. The aqueous fractions were obtained by two centrifugations at 20.000 rpm and then added to PDA medium at four different concentrations (25, 50, 75 and 100%). For both kinds of experiment (inclusion PPs and AEs), three PDA Petri dishes were inoculated with the fungal species, and every 48 h the diameter growths were measured until 21 days. The data collected were used to calculate the inhibition activity played from each PPs and AEs. The dataset was subjected to statistical analyses (multifactorial and one-way Anova, and principal components). This study highlighted that AEs were more effective to inhibit fungal growth.

This research was financially supported by Fondazione Puglia (Ricerca Scientifica e Tecnologica) – Italy through the grant "Estrazione di Biomolecole da residui vegetali di origine agro-industriale e loro impiego per la Sostenibilità delle colture Agrarie – BioSostAgr (2023-2025).

Embryo rescue of local *Vitis vinifera* varieties and F1 progenies for rethink the powdery mildew protection strategies. F. SPATARO, G. BOTTALICO, A. CAMPANALE, A. AGNUSDEI, F. DALENA, E. CHIAROMONTE, F. FARETRA, S. POLLASTRO. *Department of Soil, Plant and Food Sciences, University of Bari, Via G. Amendola, 165A/ 70126 Bari, Italy.* E-MAIL: giovanna.bottalico@uniba.it

This study explored the efficacy of *in vitro* embryo rescue as a valuable tool for the early and efficient production of F1 progeny of *Vitis vinifera* and subsequent assessment of resistance/susceptibility to powdery mildew. *In vitro* culture of grape seeds (pips) was performed starting from immature and mature grape bunches of six varieties including wine and table grapes with seeded and seedless berries, selected for different levels of tolerance/resistance to powdery mildew and their hybrids. Following surface sterilization, the seeds/embryos were cultured on medium supplemented with GA3, IAA, and activated charcoal. An equivalent number of seeds was also directly sown in soil. *In vitro* and *in vivo* germination were monitored, as well as the growth of seedlings transferred to a greenhouse, where resistance/susceptibility to powdery mildew was evaluated through natural infection. A higher germination percentage was proved for *in vitro* tests compared to direct sowing in soil, as well as significantly earlier and more uniform germination time, particularly when embryos were excised from immature seeds. For evaluations on the F1 progeny, the most promising results in terms of powdery mildew resistance were obtained for an unidentified variety. This study demonstrates that embryo rescue is an effective method to accelerate seed germination and for the early assessment of resistance/susceptibility to powdery mildew in F1 progeny, offering a significant advantage for grapevine breeding programs.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

BIOBIVE PROJECT: eco-innovative delivery systems for resilient horticulture and sustainable control of soil-borne pathogens. M. CARLUCCI, F. CONVERTINO, A. HACHEM, C. CARBOTTI, A. PACIFICO, G. VOX, F. NIGRO, E. SCHETTINI. *Department of Soil, Plant and Food Sciences (DiSSPA), Via Giovanni Amendola 165/A, University of Bari- Aldo Moro, Bari, 70126, Italy.* E-MAIL: franco.nigro@uniba.it

The BioBIVE project introduces an innovative approach aimed at developing bio-based and bioactive delivery platforms for the management of plant diseases in a variety of horticultural crops. The global agricultural sector faces significant challenges from soil-borne diseases, which result in considerable economic losses. The increasing global relevance of these diseases is evidenced by their high frequency in specific regions, exacerbated by intensive cropping systems and climatic conditions that favor pathogen proliferation. Soil-borne fungal pathogens such as *Phytophthora nicotianae*, *Fusarium oxysporum* f. sp. *lycopersici*, *Pseudopyrenochaeta lycopersici*, and *Verticillium dahliae* primarily infect roots, causing root rot, wilting, and chlorosis. These pathogens can persist in soil and plant debris for extended periods, rendering their control particularly challenging. A thorough understanding of their biology, ecology, and epidemiology is essential for developing sustainable disease management strategies that extend beyond conventional

chemical control. Effective management requires an integrated approach, incorporating advanced diagnostic tools, resistant plant varieties, and innovative agricultural practices to reduce pathogen spread and minimize crop losses. BioBIVE Project aims to incorporate natural active compounds - such as plant-derived basic substances, phlorotannins (polyphenols from marine algae), and beneficial microorganisms - into eco-friendly, biodegradable delivery systems. These systems will include mulch films, biochar, and sprayable biodegradable coatings, designed to enhance crop productivity and resilience while reducing the environmental impact of agricultural practices.

This study has received funding from the BioBIVE Project (no. 101130442), funded by the EU through the Horizon Europe Framework Programme (HORIZON-CL4-2023-RESILIENCE-01-34).

Future innovation for pesticide use reduction in agriculture – FORTUNA. D. TSITSIGIANNIS. *Department of Crop Science, Iera Odos 75, Agricultural University of Athens, Athens, 11855, Greece.* E-MAIL: dimtsi@aua.gr

FORTUNA is a collaborative research project supported by Horizon Europe. Its primary objective is to identify the forthcoming research requirements essential for realizing the EU policy objectives outlined in the Farm to Fork and Biodiversity strategies. These strategies are geared towards reducing the use and risk of chemical pesticides, as well as the use of more hazardous pesticides, by 50% by 2030. Advanced Integrated Pest Management (IPM) strategies are characterised by implementing a high degree of preventive measures to avoid establishment of pests and reduce significantly the use of chemical pesticides. However, the implementation of IPM at a multidisciplinary level is still lacking due to economic reasons and a low acceptance by farmers. A pivotal aspect of FORTUNA involves the identification and analysis of available methods, tools, and mechanisms from biological/ecological, technological, and socio-economic standpoints. These endeavors aim to facilitate a significant reduction in the usage and associated risks of chemical pesticides, ultimately fostering the adoption of innovative pesticide-free farming practices. The outcomes and strategies derived from these efforts are discussed with stakeholders across the food and supply chain. Such stakeholder engagement aims to foster a transition away from heavy reliance on chemical pesticides while concurrently supporting robust yields and ensuring food security. A critical aspect of this process involves identifying strategies that entail lower risks of productivity and profitability losses compared to other approaches, with a focus on long-term sustainability and viability.

*The presented research has received funding from the European Union's Horizon Europe research and innovation programmes under grant agreement No 101137089 (*https://horizon-fortuna.eu*/).*

Ozonized olive oil as an innovative natural fungicide: a promising alternative to synthetic agents. M.E. MERZOUKI[1], D. BOUZID[2], S. MERZOUKI[3], M.M. ZERROUG[2]. [1]*Department of Science and Technology, Faculty of Science and Technology, Optics and Precision Mechanics, Ferhat Abbas University Setif 1, 19000 Sétif, Algeria.* [2]*Laboratory of Applied Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas University Setif 1, 19000 Sétif, Algeria.* [3]*Private Medical Clinic, Sétif, Algeria.* E-MAIL: bouzid.djihane@yahoo.fr

The increasing resistance of pathogenic fungi to conventional antifungal agents necessitates the development of innovative, natural alternatives. This study investigates the antifungal potential of ozonized olive oil, obtained by bubbling a medical-grade oxygen-ozone gas mixture through cold-pressed *Olea europaea* L. oil. The antifungal activity was evaluated against five filamentous fungi (*Aspergillus niger*, *Penicillium digitatum*, *P. expansum*, *Aspergillus itaconicus*, *Fusarium solani*) and two yeasts (*Candida albicans*, *Aureobasidium pullulans*) using agar diffusion and broth dilution methods. Results demonstrated a strong inhibition of fungal growth, with *Fusarium solani* being the most sensitive (inhibition zone of 36.66 ± 4.71 mm). Minimum fungistatic and fungicidal concentrations (MFCs/MFCc) ranged from 0.38 to 18.3 mg mL^{-1}. Notably, the antifungal effect on *C. albicans* was significantly stronger than in previous reports, with an MFC of 0.38 mg mL^{-1}. The ozonation process leads to the generation of active compounds including ozonides and peroxides, which are believed to disrupt fungal membranes and cellular metabolism via oxidative stress mechanisms. These findings highlight the potential of ozonized olive oil as a safe, biodegradable, and effective natural fungicide. Its use could reduce dependency on synthetic fungicides and contribute to sustainable agriculture and infection control. Further investigation for olive oil-based formulations and their field application isneeded.

***In vitro* evaluation of natural compounds against *Verticillium* wilt of olive.** M. MOUMNI[1], S. M. MAKAU[1,2],

S. ÁLVAREZ-GARCÍA[1,3], M. TUNC[1], S. GODENA[4], G. ROMANAZZI[1]. [1]*Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 60131, Ancona, Italy.* [2]*Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield, Pretoria 0028, South Africa.* [3]*Plant Physiology Area, Engineering and Agricultural Sciences Department, Universidad de León, León, Spain.* [4]*Institute of Agriculture and Tourism, Carlo Hugues 8, 52440 Poreč, Croatia.* E-MAIL: m.moumni@staff.univpm.it; g.romanazzi@univpm.it

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, poses a significant threat to olive trees (*Olea europaea*) globally, leading to substantial economic losses. Its persistence in soil makes management challenging. In this study, two *V. dahliae* strains (AN1, AN2) were isolated from diseased olive trees in Cerignola, Italy. An *in vitro* study was conducted to evaluate antimicrobial activities in contact phase of COS (chito-oligosaccharides)-OGA (oligo- galacturonides), chitosan hydrochloride, *Urtica* spp., and *Equisetum arvense,* all at 0.2% concentration. Five essential oils (EOs) – *Rosmarinus officinalis*, *Rosmarinus officinalis* var. *verbenone*, *Lavandula hybrida*, *Origanum majorana*, and *Thymus vulgaris* - were evaluated at 0.1% concentration in the contact phase and at two distinct concentrations/EO in the vapor phase. *Urtica* spp. showed the highest mycelial growth inhibition (92.9% for AN1, 82.6% for AN2), followed by COS-OGA (74.8% and 76.0%) and chitosan hydrochloride (65.8% and 63.4%). *E. arvense* was the less effective. In contact assays, *T. vulgaris* completely inhibited mycelia growth of both isolates, and *L. hybrida* completely inhibited AN1. *O. majorana* showed 89.4% (AN1) and 63.3% (AN2) inhibition. In vapor phase, *T. vulgaris* was the most effective, achieving 99.0% and 82.5% inhibition at 90.9 µL/L and 45.4 µL L^{-1}, respectively against AN1. *In vivo* investigations are required to validate the possible application of these treatments for *Verticillium* wilt disease management.

This work was supported by the Croatian Science Foundation under the project number UIP-2020-02-7413.

Recent findings on *Sclerotinia* spp. control with a formulation based on eugenol, geraniol and thymol, on different leafy vegetables and fennel. M. PAGNANI[1], F. GUASTAMACCHIA[1], A. GUARNONE[2], D. BITONTE[2], S. CIANNAMEA[2]. [1]*Sipcam Oxon,* [2]*Sipcam Italia, R&D Department, Via Sempione 195, 20016 Pero, MI, Italy.* E-MAIL: mpagnani@sipcam.com

Leafy vegetables are highly specialized crops that require careful agronomic and phytosanitary management. Among the most damaging pathogens is crown rot, caused by *Sclerotinia sclerotiorum* and *S. minor*. Sipcam studied the application of a commercial fungicide based on Eugenol, Geraniol, and Thymol (3LOGY®), to control *Sclerotinia* in several open-field and greenhouse-grown horticultural crops such as lettuce, baby leaf, herbs, edible flowers, cabbage, and fennel. Field and greenhouse trials were conducted in 2021 and 2022 in collaboration with research centers. Trials followed EPPO guidelines, using randomized block designs with four replicates and natural infection conditions. Treatments were applied with a backpack sprayer before symptom onset. Disease incidence and severity were assessed and data were statistically analyzed using ANOVA and the Student-Newman-Keuls test ($P < 0.05$). Four trials were conducted on fennel, baby leaf lettuce, rocket, and open-field lettuce. 3LOGY® demonstrated high efficacy in all trials. In fennel, it achieved up to 91.1% efficacy on disease severity. In baby leaf lettuce and rocket, combinations with biological and chemical fungicides achieved 100% control.

The inhibitory and fungicidal effect of Saharan plants essential oils on *Fusarium* head blight species. H. HECHACHNA[1,2], L. BENFEKIH[1], N. GOURINE[2], M. YOUSFI[2]. [1,2]*Laboratory for Research on Medicinal and Aromatic Plants, Department of Biotechnology and Agroecology, Faculty of Nature and life sciences, Saad Dahlab University Blida 1, Route de Soumaa, 09000 Blida, Algeria.* [2]*Laboratory for Research on fundamental Sciences, Po Box 35G, Ammar Telidji University of Laghouat, Laghouat 03000, Algeria.* E-MAIL: leila.benfekih@univ-blida.dz

Fusarium head blight is a serious fungal disease of wheat caused by species belonging to the Fusarium genus worldwide. *F. graminearum* and *F. culmorum* have been reported as the most common causal agents for *Fusarium* head blight as well as major producers of mycotoxins leading to significant yield losses in Algerian cereal crops. Natural compounds such as essential oils (EOs) have been recognized for their efficient antifungal activity, proposed for using in agricultural fields. In this regard, the antifungal effect of three tested essential oils extracted from aerial plant parts of *Artemisia herba alba*, *Artemisia campestris* and *Teucrium polium* harvested in Laghouat region (North Central Sahara, Algeria) was determined on the isolates *F. graminaerum* (F5883) and *F. colmorum*. The EOs displayed effective antifungal action, which was most likely due to the presence of bioactive components when comparing plant harvest sea-

son. Our findings showed that *F. culmorum* isolate was more sensitive than *F. graminearum* strain to *A. campestris* essential oil from the plant harvested in autumn season than for the one collected in winter, with minimal inhibitory concentration and minimal fungicidal concentration values of 1.25 µL mL^{-1} (v/v). The *A. herba alba* EO exhibited a lower (5 µL mL^{-1} v/v) fungicidal activity against both *Fusarium* species whereas *T. polium* essential oil from the plant collected in autumn and winter did not affect these fungi (MIC and MFC >20 µL mL^{-1}).

Antifungal activity of methanolic and aqueous extracts of *Pistacia atlantica* from central Algeria: an *in vitro* study. K. CHAOUATI[1,2], L. BENFEKIH[1], F.Z. TCHAKER[3], W. GUESSAB[2]. [1,2]*Laboratory for Research on Medicinal and Aromatic Plants, Department of Biotechnology and Agroecology, Faculty of Nature and life sciences, Saad Dahlab University Blida 1, Route de Soumaa, 09000 Blida, Algeria.* [2]*University Yahia Farès, Faculty of Sciences, Department of Agronomy, Ouzera 26100 Medea, Algeria.* [3]*University Yahia Farès, Faculty of Sciences, Department of Agronomy, Laboratory of Biotechnology and Valorization of Biological Resources Ouzera 26100 Medea, Algeria.* E-MAIL: *leila.benfekih@univ-* blida.dz

Medicinal plants are a rich source of bioactive compounds that may serve as new antifungal agents. The Atlas pistachio (*Pistacia atlantica*) is widely used in traditional medicine for its antimicrobial properties. This study aimed to evaluate in vitro the antifungal activity of aqueous and methanolic extracts from the leaves and unripe fruits of *P. atlantica* against five phytopathogenic fungi: *Fusarium* sp., *F. solani*, *F. oxysporum*, *Aspergillus fumigatus*, and *A. flavus*. Aqueous and methanolic extracts were prepared by maceration of the plant material. Total polyphenol and flavonoid contents were quantified spectrophotometrically using standard methods. Major phenolic constituents were identified by LC-MS/MS. Antifungal activity was assessed on PDA agar by measuring inhibition zone diameters and calculating percentage inhibition of mycelial growth (PI %) at various extract concentrations. The methanolic leaf extracts stood out for their high phenolic content (352.10 ± 0.70 mg GAE g^{-1} extract and 163.60 ± 5.67 mg RE g^{-1} extract) and contained twenty-one phenolic compounds identified by LC-MS/MS, including rutin, gallic acid, coumaric acid (the principal phenolic), maleic acid, naringenin, and hydroxycoumaric acid. High-doses methanolic leaf extracts significantly inhibited in vitro the growth of *Fusarium*, with inhibition rates ranging from 44.44% to 75.16%, whereas no notable antifungal effect was observed against *A. fumigatus* or *A. flavus*. Methanolic leaf extracts of *P. atlantica*, exhibit particularly strong antifungal activity against *Fusarium* species and represent a promising natural source of antifungal compounds. These preliminary findings pave the way for further studies on biofungicide formulation and the assessment of their efficacy and phytotoxicity under crop production conditions.

CONCURRENT SESSION H1 – Smart innovations in plant pathology: the digital Era

SESSION KEYNOTES

Decision Support Systems for tactical management of plant diseases. V. ROSSI. *Research centre on Plant Health Modelling (PHEM), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 20100 Piacenza, Italy.* E-MAIL: vittorio.rossi@unicatt.it

The increasing demand for sustainable crop protection requires tools that combine precision, reliability, and real-time support. Decision support systems (DSSs) are informatic tools that provide farmers (or agronomists) with the information needed for addressing key questions in crop protection, e.g., is there a disease risk? If so, are plants susceptible to infection? Or, are they protected from a previous fungicide application? Then, which plant protection product (PPPs) should I use and at what dosage? And finally, are environmental conditions suitable for the PPPs? Modern DSSs are based on the following main components: i) an integrated system for monitoring the crop environment (plants, pathogens, and the physical environment) and may include multiple sensors (proximal and remote, including satellites), weather stations or algorithms estimating weather data for specific geographic coordinates, Apps for pathogen detection and disease assessments, etc.; ii) structured databases in which monitoring data are stored and checked for consistency/quality; iii) databases for plant protection products that can be used in the specific agricultural context (e.g., in a country, in conventional, IPM or organic farming, under production specifications, etc.) and provide all the key information on the product, including dosages and the risk that target pathogens develop resistance; iv) mathematical models that use monitoring data and database information as input to predict plant growth and development, the plant canopy volume, infection periods for multiple pathogens, disease onset and development, physical mode of action of fun-

gicides, etc.); v) decision algorithms that transform model output into agronomic information (e.g., the plant is highly susceptible, the infection risk is high, the residual activity of a previous fungicide application does not provide sufficient crop protection, etc.); vi) a user interface that allows the user to navigate within the DSS, interact with the DSS, and receive up-to-date information and easy-to-use decision supports; vii) a crop diary in which the users insert information on agronomic interventions, which may also be used as model inputs. Mathematical models and decision algorithms are the core of a DSS and determine its reliability; different kinds of modelling approaches exist (e.g., data-based vs process-based models) that provide different performances in terms of accuracy, robustness, versatility and scalability. Demonstration of the DSS reliability under varying agricultural environments and situations, and benefits originated by the DSS use is then a key step for DSS success. In conclusion, DSSs have the potential to become a cornerstone of precision plant protection, enabling a shift from calendar-based to data-driven, adaptive crop protection that enhance efficacy and (economic, environmental and social) sustainability in modern agriculture.

ORAL PRESENTATIONS

Early detection of *Botrytis cinerea* in horticultural crops using deep learning segmentation, transformer-based classification, and molecular quantification. E. KALOGEROPOULOU[1], P. CHRISTAKAKIS[2], D. KAPETAS[2], C. KLARIDOPOULOS[3], E.M. PECHLIVANI[2]. [1]*Laboratory of Mycology, Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, 14561 Athens, Greece.* [2]*Information Technologies Institute, Centre for Research and Technology Hellas, 57001 Thessaloniki, Greece.* [3]*iKnowHow S.A., 15451 Athens, Greece.* E-MAIL: e.kalogeropoulou@bpi.gr

Early and accurate detection of *Botrytis cinerea*, the causative agent of grey mould disease, is critical for mitigating yield losses in high-value horticultural crops such as cucumber, tomato and pepper. This study integrates advanced artificial intelligence (AI) approaches and molecular techniques to detect *B. cinerea* during early and even latent infection stages. Multi-spectral image datasets were acquired at various wavelengths ranging from 450 to 900nm, including RGB and generated vegetation indices, from both artificially and naturally infected plants under controlled conditions. Deep learning models, including U-Net++ and YOLO, were used for high-accuracy leaf segmentation. Feature descriptors were extracted using advanced Vision Transformer architectures and subsequent classification was performed using ensemble methods involving K-Nearest Neighbors (KNN), Long Short-Term Memory (LSTM) and ResNet models. The optimal configurations achieved a high overall accuracy of 92% for cucumber, with segmentation mean average precision (mAP50) scores exceeding 81% for tomato and 86% for pepper. Classification F1-scores reached 71,12% and 81,13% for tomato and pepper, respectively. Complementary RT-qPCR analyses quantified fungal biomass in plants, confirming AI-based predictions even at asymptomatic stages. This integrated approach demonstrates a robust and scalable framework for early disease detection, supporting the development of *in situ* diagnostic systems in precision agriculture.

This research was partially supported by the Green Deal Pest-Nu project, funded by European Union's Horizon 2020 research and innovation programme under the grant agreement No. 101037128, and partially supported by the E- SPFdigit project, funded by the European Union's Horizon Europe research and innovation program under the grant agreement No. 101157922.

Machine learning-based prediction of olive tree health against Vercillium wilt using rhizosphere microbiome profiles. L.F. ARIAS-GIRALDO[1], L. ANTONIELLI[2], S. COMPANT[2], J.A. NAVAS-CORTÉS[1], B.B. LANDA[1]. [1]*Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain.* [2]*Center for Health & Bioresources, Bioresources Unit, AIT Austrian Institute of Technology GmbH, Austria.* E-MAIL: lfarias@ias.csic.es

Verticillium wilt, caused by *Verticillium dahliae*, poses a major threat to Mediterranean olive production due to its severe impact and the lack of effective post-infection treatments. Modulating the soil microbiome has emerged as a promising strategy to manage this disease, given the rhizosphere's role in naturally suppressing soil-borne pathogens. In this study, we analysed the rhizosphere microbiome of 720 olive trees (359 healthy and 361 diseased) from 180 commercial orchards in Andalusia, Spain. Bacterial and fungal communities were characterized through amplicon sequencing of the 16S rRNA (V1–V2) and ITS1 regions. Environmental, climatic, soil, and agronomic data were also collected for each orchard. Using classical ecological approaches and supervised machine learning, we explored microbial patterns associated with environmental variables that predict tree health. Random Forest and Lasso regression models were tested on four datasets: bacterial ASVs (N=1251), fungal ASVs (N=360), merged ASVs (N=1611), and a set

of differentially abundant ASVs between healthy and diseased trees (N=55). Random Forest slightly outperformed Lasso, achieving the best results with the merged dataset (ROC-AUC ~ 0.85), especially in accurately identifying diseased trees. Four ASVs, *Peribacillus* spp., *Fusarium* spp., *Aspergillus* spp., and *Pseudomonas* spp., emerged as key predictors of tree disease status across both models, highlighting their potential as microbial biomarkers for olive tree health. Although the DA subset yielded competitive results, the inclusion of additional ASVs in the merged dataset suggests complex microbial interactions that enhance prediction accuracy.

Funding: Projects AGL2012-37521, AGL2016-75606-R, PID2020-114917RB-100, P18-RT-4184, QUAL2-023-IAS, KODA, EMBO.

Stem colonisation and proximal sensing-based detection of *Xylella fastidosa* infection in olive trees. M. ROMÁN-ÉCIJA, C. OLIVARES-GARCÍA, B.B. LANDA, J.A. NAVAS-CÓRTES. *Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC),14004 Córdoba, Spain*. E-MAIL: mromanecija@ias.csic.es

The emergence of *Xylella fastidiosa* (*Xf*) poses a serious threat to agricultural and natural ecosystems. *Xf* ability to adapt to varying environments raises concerns about potential host shifts and increased virulence in the Mediterranean region. Moreover, accurate and prompt detection is crucial for effective disease control. Understanding the interactions of European *Xf* strains and local olive cultivars responses is key for designing targeted management strategies. The pathogenicity and extent of stem colonization of five *Xf* strains of different subspecies were investigated and the sequence types (ST) inoculated on three olive cultivars widely grown in Spain. All tested strains were able to infect olive plants, although detection rates declined over time. Strains belonging to subspecies *pauca* (XYL1961/18 of ST80 and De Donno of ST53) exhibited higher colonization levels and bacterial loads compared to those of subspecies *multiplex*. Additionally, we assessed the physiological impact of *Xf* infection on the inoculated olive plants analyzing leaf spectral signatures using non-invasive handheld proximal sensors, allowing the estimation of 74 vegetation indices. Among these, 17 to 31 indices, mainly associated to carotenoids, flavonoids, xanthophylls and blue band related indices, proved to be reliable indicators of infection status, achieving over 90% classification accuracy across olive cultivars. Our findings emphasize the importance of understanding host-pathogen interactions between local cultivars and *Xf* strains adapted to Mediterranean conditions. Proximal sensing technologies proved to be useful tools for early detection of *Xf*-infected plants, even before symptoms appearance. Furthermore, these approaches could be integrated into breeding programs to support the development of *Xf*-resistant olive cultivars.

This research was financially supported by Projects BeXyl (Grant ID 101060593, EU-Horizon Europe), XF-ACTORS (Grant ID 727987, EU-Horizon 2020), E-RTA2017-00004-C06-02 (AEI-INIA Spain) and the Spanish Olive Oil Interprofessional, and the Projects PIE202240E067, PIE202340E021 and the PTI-SolXyl of CSIC.

Modelling multitrophic interactions between grapevine, microorganisms causing sour rot of bunches, and the *Drosophila* spp. Vectors. G. FEDELE[1,2], C. BRISCHETTO[1,2], L. CHIAPPAROLI[1,2], V. ROSSI[1,2], T. CAFFI[1,2]. [1]*Department of Sustainable Crop Production (*DI.PRO.VE.*S.), Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy;* [2]*Research Center on Plant Health Modelling (PHeM), Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy.* E-MAIL: giorgia.fedele@unicatt.it

Sour rot (SR) is one of the late-season, non-Botrytis, rots affecting grapevines, which can cause severe yield losses and relevant decay for wine making. A model framework was designed for describing the complex relationships between the following: i) development of grapevine bunches and changes in SR susceptibility; ii) occurrence, growth and pathogenicity of causal microorganisms (specifically, yeasts and acetic acid bacteria, AAB); iii) the vectoring activity of the *Drosophila* flies; and iv) the environmental conditions. The ultimate aim was to predict SR epidemics so that to inform disease management actions. Since several aspects of SR etiology, epidemiology and ecology are still not completely understood, a series of research activities were carried out for model parametrization. First, a characterization of the microbiota in both healthy and affected bunches was conducted across different bioclimates, to identify microorganisms associated with SR-infected bunches, their changes in response to bioclimatic conditions, and their relationship with the Drosophila flies. Then, nine yeasts and two AAB species were selected and characterized for their temperature requirements for growth on a liquid substrate that mimicked the chemical composition of ripened grape berries, in the range of 5 to 40°C in. Finally, the pathogenicity of the selected species was tested on grape berries through single and sequential inoculations. Further research is planned to study the seasonal micro-

biome dynamics of healthy and SR-affected berries, and of *Drosophila* spp. flies, to better parameterize the role of flies on disease development.

POSTER PRESENTATIONS

Importance of pathosystems' knowledge and process-based modelling approach in developing decision support systems for optimal crop management. S.E. LEGLER, E. GONZALEZ-DOMINGUEZ. *Horta srl, Piacenza, IT 29122, Italy.* E-MAIL: s.legler@horta-srl.com

Decision Support Systems (DSS) in agriculture are digital tools that can integrate different technologies to assist farmers, agricultural professionals, and decision-makers in taking informed decisions. These systems use data, models, and algorithms to provide insights, predictions, and recommendations that can optimize agricultural practices. The goal is to improve productivity, resource management, sustainability, and profitability in farming operations. Although several alternatives are available for agricultural stakeholders, adoption is still low, with the main obstacle being convincing farmers that a DSS can address their specific needs, even if it has been developed in a different country/context. Horta's DSSs are based on key aspects that enable them to address the challenges of demonstrating DSS robustness to farmers, to enter new contexts (countries/environments/crops) or to respond to climate change. These key aspects are: i) comprehensive agronomic and pathological knowledge, supported by continuous yearly field and controlled-environmental trials *vs*. machine learning data analysis, ii) mechanistic process-based *vs*. empirical data-based modelling, and iii) flexibility/customization *vs*. standard black-box solutions. Thanks to these features, Horta's DSS are now used by thousands of farmers and advisors, as well as agri-food industries on 16 crops in 12 countries. Two case studies are of particular interest to demonstrate how Horta's deep understanding of pathosystems knowledge and process- based models can help a DSS in territorial wide-spreading and in facing new cultivation contexts: the grape DSS used from Prosecco, to Champagne, to Napa Valley region, and the tomato DSS used by Italian processed tomato industry to Spanish greenhouse cherry tomato growers.

Postgraduate in agricultural sciences: challenges and opportunities in Egypt. A. HUSSIEN[1], Y. AHMED[2]. [1]*Undergraduates Research Scholarships Initiative, Tanta, Egypt.* [2]*Regional office for Near East and North Africa, Food and Agriculture Organization of the United Nations "FAO", Cairo, Egypt.* E-MAIL: ahmedaboulmagd@hotmail.com

Although Egypt has experienced a significant increase in tertiary education enrolment, the number of postgraduate students in agricultural sciences has seen considerable decline. This study aimed to discuss the challenges facing students in pursuing postgraduate studies. Data were collected from 206 students/graduates from 21 universities/institutes through questionnaire and in-person interviews. Less than half of the surveyed students and graduates have decided to pursue postgraduate studies. Although 86% of the participants were from agriculture and biological sciences backgrounds, medical applications emerged as the top choice for postgraduate research (65%), followed by agricultural applications (49%). The main motivation was the influence of the curricula taught during the undergraduate studies, then assumptions about higher income, better media coverage, publication in higher- impact journals, and access to grants. The students/graduates identified the main challenges they face include scientific thinking, foreign languages, writing resumes and cover letters, academic writing, and finding information online. A large portion of the sample indicated that undergraduate education has not efficiently prepared them to address those challenges. Many students/graduates sought advice from university professors, however, 54% evaluated it as partially useful, and 9% found it was not useful. Undergraduate agricultural education in Egypt needs to undergo reforms to attract higher-caliber students to pursue postgraduate studies and careers in agricultural research. Research and development (R&D) should be integrated into the undergraduate syllabus, and universities should establish specialized support units beyond the academic education. Furthermore, online platforms are essential in the era of digital information to enhance access to information and learning opportunities.

Machine Learning-Based Analysis of Reflectance Spectra in Eggplants under Biotic and Abiotic Stresses. C. TSELIOS[1,2], E. MANIATI[2], T. MPATZAKA[3], I. RAPTIS[3], A. KALOGERAS[1], D. ALEXANDROPOULOS[1,2] and D. KIZIS[4]. [1]*Industrial Systems Institute, Athena Research Center, Patras Science Park Building Platani, 26504 Patras, Greece.* [2]*Department of Materials Science, University of Patras, 26504 Rion, Greece.* [3]*ThetaMetrisis S.A., 12132 Athens, Greece.* [4]*Laboratory of Mycology,*

Scientific Directorate of Phytopathology, Benaki Phytopathological Institute, St. Delta 8, 14561 Kifissia, Greece. E-MAIL: dalexa@upatras.gr, d.kizis@bpi.gr

This study investigates the use of reflectance spectroscopy combined with machine learning to classify eggplant spectral profiles in response to different stress treatments. Four plant-treatment groups were generated: control (C), water-stressed (S), *Verticillium dahliae* infected (V), and combined stress (VS). Two experiments were conducted: the first involved groups C, S, and V, while the second included all four groups. Reflectance spectra were collected over multiple measurement days, with repeated sampling across leaf area to monitor stress progression. A machine learning pipeline was developed using Savitzky-Golay filtering for noise reduction and principal component analysis (PCA) to extract dominant spectral features. The extracted features were used to train a Random Forest classifier to distinguish plant groups. Model performance was evaluated with 80–20 train-test splits and assessed using accuracy and confusion matrix metrics. In the first experiment, high classification accuracy was observed during early-to-middle measurement days, supported by confusion matrices showing clear class separation. In the later days, classification accuracy decreased to 70–80%, likely due to overlapping spectral signatures at advanced stages; although tissue damage was visible, spectral differences between stress conditions became less distinguishable. Earlier measurements captured physiological changes prior to visible symptoms, enabling more reliable classification. In the second experiment, including the VS group, accuracy remained consistently high (81–88%), demonstrating robust spectral differentiation. These findings support the sensitivity of reflectance spectroscopy for detection of early plant responses to stress and emphasize the value of temporal monitoring for non-invasive plant diagnostics, particularly during the onset of stress when intervention remains effective.

The present paper has been partially funded by BPI and the SUSTAINABLE project, funded by the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie-RISE Grant Agreement No.101007702. https://www.projectsustainable.eu.

Integrating digital tools for plant health: use case pilots of STELLA pest surveillance system in Greece. S. DIMITROPOULOU, D. TSITSIGIANNIS. *Department of Crop Science, Laboratory of Plant Pathology Agricultural University of Athens, Greece.* E-MAIL: dimtsi@aua.gr

The EU-funded STELLA project is developing an integrated Pest Surveillance System (STELLA PSS) aimed at enabling early warning, detection, and response to quarantine and regulated non-quarantine plant pathogens (RNQPs). By combining advanced sensing technologies, Internet of Things (IoT) devices, and artificial intelligence (AI)-driven analytics, STELLA addresses key limitations of conventional pest monitoring methods, such as delayed detection, restricted accessibility in remote areas, and high resource demands. In Greece, STELLA PSS is being implemented across two Use Case Pilots targeting *Ceratocystis platani* - a quarantine fungal pathogen responsible for canker stain in *Platanus orientalis* - as well as two RNQPs: *Verticillium dahliae* and *Pseudomonas savastanoi* pv. *savastanoi,* causal agents of Verticillium wilt and Olive knot disease, respectively. Pilot deployments are situated in the Kireas riparian forest on Evia Island and selected olive groves in the Atalanti and Attiki regions, providing real-world assessment under both natural ecosystems and commercial cultivation conditions. The system leverages proximal (EDEN Viewer) and remote sensing (UAVs and satellite imagery), *in situ* IoT sensors, and spatiotemporal modelling to monitor plant host health, environmental parameters, and pest risk dynamics in real-time. This digital surveillance framework improves spatial resolution, expedites diagnosis, and lowers the cost of monitoring efforts. Furthermore, stakeholder engagement is fostered through mobile applications and citizen science initiatives, improving data collection and raising public awareness. STELLA PSS presents a scalable, science-based solution for proactive pest management, fully aligned with EU plant health policies.

*The presented research has received funding from the European Union's Horizon Europe research and innovation programmes under grant agreement No 101134750 (*https://stella-pss.eu/*).*

Remote sensing technology for grape health status monitoring under nursery conditions. F. DALENA, D. CORNACCHIA, R. CORONELLI, G. INCAMPO, F. MANNERUCCI, A. FARELLA, G. POPEO, S. PASCUZZI, S. POLLASTRO, F. FARETRA, D. GERIN. *Department of Soil, Plant and Food Sciences, University of Bari, Via G. Amendola, 165A/ 70126 Bari, Italy.* E-MAIL: stefania.pollastro@uniba.it

Remote sensing applications are becoming a valuable tool for crop production, including the viticulture sector. Different studies report the use of unmanned aerial vehicles (UAVs) for providing grapevine vigour indexes

related to the canopy shape and showing relationships with plant physiology and productivity. With this study, remote sensing UAV-based technology was applied under grape nursery condition on a mother plant field and on a rootstock production field, to evaluate possible relationships with grapevine trunk diseases. In the mother plant field, the 1103 Paulsen and Kober 5BB varieties were grown, while rootstock field was planted with Kober 5BB, 140 Ruggieri and 1103 Paulsen varieties. The NDVI data collected by DJI Mavic 3M, (DJI Sciences and Technologies Ltd, Shenzhen e Pechino, Cina) and analysed by using JMicrovision software (jmicrovision.github.io), allowed to follow the canopy development, along June-August months. In the mother plant field, the presence of weeds caused an overestimation of the NDVI values for grapevines and further AI-based analysis are currently being developed. In the rootstock production field, a lower NDVI index were observed for Kober 5BB compared to 140 Ruggieri and 1103 Paulsen. NDVI data were positively correlated r≥0.7 with the percentage of un-sprouted grapevine cuttings. These latter show internal wood browning symptoms whose fungal species of the Botryosphaeriaceae family, and belonging to the genera *Diaporthe*, *Pestalotia*, *Fusarium*, and *Dematophora* were isolated. In conclusion, the use of remote sensing represents a promising methodology for monitoring young vine health under nursery condition.

This research was financially supported by Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) –MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

New frontiers in plant pathology: innovative and cutting-edge tools to detect and manage plant pathogens. F. SAID[1], H. ALI[2], M.H. KHAN[3]. [1]*Department of Entomology, Abdul Wali Khan University, Mardan, Pakistan.* [2]*Entomology Section, Agricultural Research Institute Peshawar, Pakistan.* [3]*Plant Protection Division, Nuclear Institute for Food & Agriculture, Peshawar.* E-MAIL: dr.fazal@awkum.edu.pk

Plant pathogens have long been associated with different plant diseases across the globe and pose threats to agricultural commodities, enhancing food insecurity, crop yield both in terms of quality as well as quantity. For the sake of food security and improved crop yield, it is essential to address such issues and for that timely detection of plant pathogen and management is indispensable enabling us ensure sustainable food production. The current case study focuses on cutting-edge innovations and new frontiers in the field of plant pathology and integrated disease management. We explore the development and application of molecular diagnostics, remote sensing technologies, and machine learning algorithms for early and accurate disease detection. Additionally, we discuss advances in disease management, including biocontrol agents, resistance breeding, and precision agriculture practices. Case studies demonstrating the successful implementation of these technologies and strategies are presented, showcasing their impact on reducing disease incidence and improving crop health. Finally, we address the challenges and future directions in plant pathology research, emphasizing the need for multidisciplinary approaches and enhanced collaboration between researchers, farmers, and policymakers.

CONCURRENT SESSION H2 – Mycotoxins and climate change: management strategies to mitigate their impact on food safety

SESSION KEYNOTES

Epidemiology and Biodiversity of mycotoxigenic fungi: a constantly evolving world. A. MORETTI. *Research National Council of Italy, Institute of Sciences of Food Production, CNR-ISPA, Bari, Italy.* E-MAIL: antonio.moretti@cnr.it

Mycotoxins, secondary metabolites produced by toxigenic fungi, are natural contaminant of food and feed and represent a serious threat worldwide, because of their impact on animal, human and plant health. According to a recent report, around 25% of food is contaminated by at least one mycotoxin, with important implications for the health of humans and animals and the global trade and economy. Hot and dry weather conditions, prolonged periods of rainfall deficits, and tighter supplies and market uncertainty have contributed in the last years to reduce agrifood production and increase the risk of mycotoxin contamination worldwide. Therefore, mycotoxin management requests advanced strategies along the whole supply chain, from pre- to post-harvest stages. Among the toxigenic fungi that produce mycotoxins *in planta*, the species belonging to *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* genera are the most common, showing a great variability of their mycotoxin profiles, even in species phylogenetically closely related. In addition, such variability can be detected also at an

intraspecific level. The results of ISPA team studies, carried out in cooperation with other research institutions, for several species belonging to the above-mentioned genera, and conducted through metabolomics analyses and Whole Genome Sequencing approach, are here reported. In particular, we will report the variability of Aflatoxins, Ochratoxin A and Fumonisin B2 (FB2) production related to the occurrence of gene clusters (*afla*, *ota* and *fum*), in *Aspergillus* spp, where both the intact and deleted gene clusters co-exist, variability of FBs and Beauvericin in *Fusarium* where toxigenic potential can be related to Single Nucleotide Polymorphism occurrence , variability of trichothecenes production in the *Fusarium* where a different distribution among the TRI genes cluster has been detected. Taken together, these data show that mycotoxin gene clusters can dramatically differ within a single species or among very closely related species and the lack of a given mycotoxin production, at least *in vitro* conditions, is frequently, but not always, related to the absence of related gene clusters. Due to the examples of genetic variants in several toxigenic fungi with respect to mycotoxin gene clusters, we need continuous epidemic studies from different crops and geographic areas by using a consistent and statistically significant approach to generate data that better would reflect the current dynamics of species profile and their genetic variability, also in the perspective of the current climate change scenario. Several results of our epidemiological studies for all the four *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* genera will be provided. In addition, a further current challenge should be to use genomic approach to better understand the relationships between genotyping and phenotyping for pathogenicity, mycotoxin profile and stress related genes, in order also to better understand how the genetic plasticity of the toxigenic fungi can be a precious tool for their adaptation to the current climate changes.

Biological detoxification of mycotoxins. CIMBALO, P. VILA-DONAT, A. MORENO, C. LUZ, S. RAFAI, L. ANTELO, G. MECA. *Biotech Agrifood, Faculty of Pharmacy and Food Sciences, Universitat de València, Avda. Vicent Andrés 6 Estellés s/n, 46100, Burjassot, Spain*. E-MAIL: alessandra.cimbalo@uv.es

Nowadays, biologically based detoxification strategies are attracting great interest in replacing conventional methods for counteracting highly toxic compounds in contaminated food products. Among these compounds, Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) are two major mycotoxins known for their harmful effects on human and animal health, as well as for their persistence in the body once ingested. Following this line, the aim of this study was to evaluate the detoxification potential of bacterial strains belonging to the genera *Lactobacillus* (*n*=10) and *Bacillus* (*n*=10) on these two mycotoxins, using naturally contaminated corn (AFB1) and barley (OTA) flour as the substrate. For this purpose, the selected strains were grown in their culture medium by inoculating 1% of bacterial suspension and then incubated for 12, 24 and 48h. At each time point, culture was withdrawn, diluted, and filtered prior for chromatographic analysis. All samples were processed and analyzed in duplicate to evaluate AFB1 and OTA detoxification efficiency. Following strain selection, an *in vitro* simulated human digestion was performed to assess detoxification potential under real gastrointestinal conditions, including oral, gastric, duodenal, and colonic phases. Mycotoxin quantification and metabolite detection was performed using a Liquid Chromatography system coupled with Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) operating in targeted MS/MS mode. Based on the results, the best AFB1 detoxification rates were observed in *Lactobacillus curvatus* 14 (41.1 ± 19.3%) and *Pediococcus pentosaceus* 4 (25.4 ± 11.3%) after 48 h, and *Bacillus firmus* 6 (25.1 ± 12.9%) after 24 h. Furthermore, metabolite analysis revealed that both lactic acid bacteria and *Bacillus* strains were capable of transforming AFB1 into various degradation products, with aflatoxicol being the most consistently detected metabolite across all strains and time points, at concentrations ranging from 31.00 to 55.40 μg L^{-1}. *Lactobacillus curvatus* 14 and *Bacillus siamensis* 4 exhibited the highest aflatoxicol levels, peaking at 47.45 μg L^{-1} and 55.40 μg L^{-1}, respectively, at 48 h and 24 h. In addition, *L. curvatus* 14 and *Pediococcus pentosaceus* 4 produced aflatoxin D1, while *P. pentosaceus* 29 was the only strain to generate aflatoxin Q1. Among the *Bacillus* strains, *B. siamensis* 4, *B. firmus* 6, and *B. velezensis* 9 showed a broader metabolic profile, including aflatoxin D1, P2, B2a, and Q1, suggesting multiple biotransformation pathways. In contrast, some strains such as *B. licheniformis* 10 and *B. amyloliquefaciens* 20 primarily produced aflatoxicol, with no other detectable metabolites, possibly indicating more limited degradation mechanisms. On the other hand, the simulated digestion model showed strain-dependent AFB1 detoxification. *L. curvatus* 14 and *P. pentosaceus* 4 significantly reduced AFB1 in gastric and duodenal phases, while *firmus* 6 was less effective. In the colonic phase, AFB1 levels decreased over time even in the control, suggesting non-microbial degradation may also occur. For OTA, the most effective strains at 48 h were *P. pentosaceus* 4 (59.22 ± 6.78%), *Lactobacillus plan-*

tarum 5 (70.00 ± 7.00%), and *L. fermentum* 8 (67.02 ± 9.23%). Significant reductions were observed for after the colonic phase, reaching 72.26 ± 7.54% for *P. pentosaceus* 4 and 69.67 ± 9.70% for *L. curvatus* 14. These findings highlight the potential application of selected strains in biological detoxification strategies for reducing dietary AFB1 and OTA exposure.

This research was financially supported by MCIU /AEI / AEI /10.13039/501100011033 /FEDER, EU, project PID2022-140722OB-I00.

ORAL PRESENTATIONS

Dynamics of *Aspergillus* spp. section *Flavi* in Spanish dried figs: field assessment. Z. JANFI[1], M.T. GARCIA-LOPEZ[2], F.A. GUERRERO-PAEZ[1], M.A. ROMERO[1], C.M. DIEZ[1], J. MORAL[1]. [1]*UCOLIVO. Department of Agronomy. María de Maeztu Unit of Excellence, University of Cordoba, Cordoba, Spain.* [2]*AgroPhenoLab, Institute for Sustainable Agriculture-Spanish National Research Council (IAS-CSIC), Cordoba, Spain.* E-MAIL: tgarcial-opez@ias.csic.es

Fig cultivation (*Ficus carica*) represents a significant agricultural sector in Spain, yielding approximately 59,900 tons annually. A persistent challenge facing dried fig producers is contamination with aflatoxins—potent mycotoxins synthesized by members of *Aspergillus* section *Flavi*. Despite their economic importance, critical knowledge gaps remain regarding contamination pathways and risk factors in fig production systems. This research investigated *Aspergillus* section *Flavi* population dynamics in Spanish fig orchards during the 2022-2023 growing seasons. We systematically monitored fungal presence in orchard soils, tree-hanging fruits, and ground-fallen figs, while quantifying airborne spore concentrations using volumetric air samplers deployed in both field and processing environments. Our findings revealed significantly higher *Flavi* colonization in ground-fallen figs compared to tree-harvested fruits (10% vs. 4.2% in 2022; 35.7% vs. 14.2% in 2023). Soil inoculum densities averaged 25.7 CFU/g in 2022 (range: 5.0-140.1) and 27.9 CFU/g in 2023 (range: 5.2-395.5). Airborne *Aspergillus* spores were detected throughout the summer months (mean: 0.04 CFU/m^3), with concentration peaks in late August (maximum: 2.43 CFU/m^3). Notably, *Aspergillus* spore density in cold storage facilities remained minimal (0.001 CFU/m^3), substantially lower than other fungal genera including *Penicillium* (0.02 CFU/m^3) and *Alternaria* (0.01 CFU/m^3). These results demonstrate that soil contact represents the primary contamination pathway for aflatoxigenic fungi in fig production. Implementation of agronomic practices that minimize fruit-soil contact, such as improved harvesting techniques and orchard floor management, could significantly reduce aflatoxin risk in Spanish dried figs.

This research was financially supported by the "HEPATOCARCINOGENIC FUNGI IN DRIED FIGS" CDTI Spanish project and the "Agrupación de Cooperativas del Valle del Jerte" company. M. Teresa García López's contract is part of grant JDC2022-048362-I, from MICIU/AEI/10.13039/501100011033 and the European Union NextGenerationEU/PRTR.

Effect of ozone as an alternative treatment of *Aspergillus* contamination in pistachios. M. ABUKHMAISH[1], W. MELLIKECHE[1], A. RICELLI[2], M. GALLO[1], A.M. D'ONGHIA[1], G. COLELLI[3]. [1]*International Centre for Advanced Mediterranean Agronomic Studies, Via Ceglie, 9-70010, Valenzano (BA), Italy.* [2]*National Research Council - Institute of Molecular Biology and Pathology,* P.le *A. Moro, 5-00185, Rome, Italy.* [3]*Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Via Napoli, 25-71122, Foggia, Italy.* E-MAIL: marahabukhmaish@gmail.com

Toxigenic *Aspergillus* molds pose a major challenge to the commercialization and export of pistachio nuts. Therefore, it is essential to develop effective postharvest strategies to control these contaminations, particularly with increasing restrictions on chemical pesticides. Ozone has emerged as a promising alternative treatment due to its powerful oxidizing properties and broad-spectrum antimicrobial activity. It has been previously tested on different food commodities and proven effective in reducing fungal contamination and mycotoxin content. This study explores the efficacy of ozone in reducing *Aspergillus* contamination by applying two doses (20 ppm and 13 ppm) for six hours on in-shell and shelled pistachios. The antimicrobial effect was evaluated by monitoring *Aspergillus* spp. occurrence at fixed intervals over six months of storage. Additionally, the impact of ozone treatment on the color and peroxide content of pistachios was assessed. Results showed that ozone treatments significantly reduced fungal contamination, with the higher dose of 20 ppm resulting in up to a 90% reduction in in-shell pistachios and 91.5% reduction in shelled pistachios. This reduction remained significant throughout long storage, with consistent results obtained 3 and 6 months after the treatment. Importantly, ozone had no significant effect on the color and peroxide levels of treated pistachios. These findings show that ozone

is a highly effective and safe postharvest treatment that reduces fungal contamination without compromising the quality of pistachios, offering a valuable solution for enhancing food safety during storage.

Programma Operativo Nazionale Ricerca e Innovazione 2014–2020 (CCI 2014IT16M2OP005), Fondo Sociale Europeo, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale".

First report of the relationship between *Drosophila suzukii* and mycotoxigenic fungi on blueberries. N. BASER[1], A. BEN HASSINE[1], S. KHEMIES[1], W. MELLIKECHE[1], A. RICELLI[2], A.M. D'ONGHIA[1]. [1]*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM IAMB), Bari, Italy.* [2]*CNR-Istituto di Biologia e Patologia Molecolari, Rome, Italy.* E-MAIL: baser@iamb.it

Drosophila suzukii is a ubiquitous pest insect which attacks a wide range of soft and stone fruits including blueberries (*Vaccinium corymbosum* L.). Adult insects can lay eggs deep inside healthy fruits inducing their deterioration. In addition to its direct damage, *D. suzukii* can also cause secondary infections by post-harvest fungi. This study was carried out on blueberries produced in Italy to determine their mycobiota. It revealed a high presence of diverse genera of fungi already reported in blueberry, including *Alternaria*, *Botrytis*, *Colletotrichum*, *Aspergillus*, and *Penicillium*. Based on these results, the vectoring relationship of *D. suzukii* with toxigenic fungal contaminants was studied on blueberries. It was revealed that indeed the insect is capable of spreading fungal spores among blueberries. Sterilized fruits infested with the insect showed a high occurrence of fungi compared to non-infested sterilized fruit. The fungal species transmitted by *D. suzukii* were mainly *Alternaria alternata* and *Aspergillus niger*, both known for their production of mycotoxins. HPLC analysis, aiming to detect any mycotoxin production of isolated fungi, revealed that several *A. alternata* isolates, transmitted by *D. suzukii*, were able to produce alternariol and tenuazonic acid mycotoxins.

Impact of agroforestry systems on fungal populations in wheat crops. E. FORGUES, M. FOULONGNE-ORIOL, L. CARLES. *UR1264 Mycology and Food Safety (MycSA), INRAE. 71 Avenue Edouard Bourlaux, 33883 Villenave d'Ornon.* E-MAIL: elise.forgues@inrae.fr

As new agroecological practices like temperate agroforestry emerge, the issue of biological regulation, particularly regarding plant pathogens within these systems is poorly documented. It is essential to gain knowledge of the benefits and potential risks associated with the deployment of such a practice applied to field crops. The aim of this study is to investigate the impact of the agroforestry system associated with wheat on fungal populations and in particular on the pathogens responsible for Fusarium Head Blight (FHB). FHB is one of the major diseases affecting cereals including wheat. A cohort of several *Fusarium spp.* are associated with FHB symptoms. While *F. graminearum* is often prevalent, the spectrum of other Fusarium species is strongly influenced by the environment. In this context, the presence of hedges or trees in intra-parcel wheat fields may modify fungal populations, by acting as pathogens reservoirs, or by modifying the microclimatic conditions favorable to their development. Using metabarcoding approaches targeting either ITS or EF1alpha gene regions, we will investigate the representativeness of fungal genera and the occurrence of *Fusarium* species in samples collected in the wild compartment (leaves, wild grasses) and in wheat fields (heads) during the growing season. Preliminary results from the first year of sampling on the spatio-temporal dynamics of fungal populations will be presented. Our study will contribute to fuel scientific knowledge on FHB epidemiology in agroforestry landscapes and will also provide broader insights into potential non-intended effects relevant to disease management strategies in diverse cropping environments.

POSTER PRESENTATIONS

Temporal dynamics of *Fusarium* spp. on wheats and possible relationship with their ecological requirements. M-A. GARCIA[1,3], R. MAHMOUD[2], M-O. BANCAL[3], P. BANCAL[3], A. ROUCOU[4], R. VALADE[4], S. BERNILLON[1], F. RICHARD-FORGET[1], M. FOULONGNE-ORIOL[1]. [1]*INRAE, Mycology and Food Safety (MycSA), 33140 Villenave d'Ornon, France.* [2]*Institut Agro, Univ Rennes, CNRS, IRMAR-UMR 6625, 35042 Rennes, France.* [3]*Université Paris-Saclay, INRAE, AgroParisTech, EcoSys, 91120 Palaiseau, France.* [4]*ARVALIS Institut du Végétal, Boigneville, France.* E-MAIL: marie-anne.garcia@inrae.fr

Fusarium Head Blight (FHB) is one of the most devastating fungal diseases affecting wheat worldwide. This disease is caused by a complex of different fungal species, belonging to the *Fusarium* genus, that coexist within the same host plant. Beyond the significant impact on yield due to infection, FHB also alters grain qual-

ity, leading to economic losses, due to contamination by harmful mycotoxin produced by *Fusarium* species. Global changes, including agronomic and climatic factors, are known to affect the distribution of *Fusarium* species infecting wheat and the associated mycotoxins. Studying the evolution of *Fusarium* species in a changing environment is essential for predicting and controlling FHB, and therefore, ensuring the safety of cereals products. The purpose of this study was to understand *Fusarium* species dynamics and their adaptive potential in the context of climate change. The diversity of *Fusarium* species in French wheat fields has been studied over the last 15 years using a metabarcoding approach targeting the *EF1α* gene, together with mycotoxins profiling. A large database gathering numerous metavariables was available, enabling the study of the impact of agronomic and climatic factors on *Fusarium* diversity on wheat fields. Differences in *Fusarium* predominance were observed, suggesting distinct ecophysiological behaviour between species in response to environmental factors. Therefore, growth and mycotoxin production were *in vitro* analysed in response to combined temperature and water activity variations. Our findings will contribute to improving mycotoxin risk prediction models, thereby supporting the development of effective strategies for managing future mycotoxin threats in wheat production.

Preventing food-borne threats at the source: a one health approach to crop and animal biosecurity. F. ALI[1], M. SADIQ[2], I. AHMAD[2]. [1]*Pet Street Veterinary Clinic Abu Dhabi UAE.* [2]*University of Agriculture Peshawar Pakistan.* E-MAIL: drfarzand_2011@yahoo.com

Ensuring food safety begins long before animal production starts in the field, with healthy plants. As a veterinarian, the link between plant health and animal health is clear: contaminated crops can lead to unsafe feed, compromise animal health, and ultimately impact human food safety. Recent advances in plant pathology offer new tools for detecting and controlling plant diseases that threaten the safety and quality of crops used for animal feed and the broader food chain. By integrating veterinary and plant sciences, we can develop more effective strategies for preventing foodborne illness, reducing exposure to toxins (such as mycotoxins), and enhancing biosecurity. Strengthening education and collaboration across disciplines is essential to training professionals capable of addressing these challenges comprehensively. This approach supports a safer, more sustainable food system, one where plants, animals, and human health are protected together.

Evaluation of biocontrol agents for mitigation of aflatoxin contamination in pistachios. V. BARTZIS[1], M. VARVERI[1], S. DIMITROPOULOU[1], C. KAFTANI[2], M.D. KAMINIARIS[1], M. GEORGIADOU[2], C. PAPPAS[2], D.I. TSITSIGIANNIS[1]. [1]*Department of Crop Science, Laboratory of Plant Pathology Agricultural University of Athens, Greece,* [2]*Department of Food Science and Human Nutrition, Laboratory of Chemistry Agricultural University of Athens, Greece.* E-MAIL: dimtsi@aua.gr

Pistachios, one of the most economically significant nuts globally, are particularly susceptible to fungal infections that produce aflatoxins. Aflatoxins are a major group of mycotoxins known for their teratogenic and carcinogenic effects on humans and animals. Among various evaluated mitigation strategies, biological control methods have demonstrated the highest efficacy in reducing aflatoxin contamination. The aim of this study was to evaluate the ability of two yeast strains of *Aureobasidium pullulans* and *Meyerozyma caribbica*, one bacterial strain of *Pantoea agglomerans* and one non-toxigenic strain of *Aspergillus flavus* to reduce aflatoxin contamination levels in pistachio orchards in Greece. These microbial strains were originally isolated epiphytically from pistachios. Previous studies have demonstrated that these isolates can effectively suppress aflatoxin biosynthesis in pistachio fields on the island of Aegina. For the purpose of this study, the strains were applied to two distinct pistachio orchards: a commercial orchard located on Aegina Island and an experimental orchard at the Agricultural University of Athens. Analysis of harvested pistachio kernels demonstrated that aflatoxin formation was significantly inhibited by all four strains, with reductions ranging from 86% to 99%. These results align with earlier studies and further support the potential of these strains in developing biocontrol products for the effective management of aflatoxins in pistachio production.

The research has been funded under Action 2 "Implementation of the Operational Plan (project) of the ESK Operational Groups for the productivity and sustainability of agriculture" of Sub-measure 16.1-16.2 of Measure 16 of the AGRICULTURAL DEVELOPMENT PROGRAM 2014 - 2020 "Cooperation" in Greece (M16ΣΥΝ2-00161).

Study of *Drosophila suzukii*'s role in the transmission of postharvest fungal contaminants on blueberries and the hypothesis of their control by ozone. S. KHEMIES[1], W. MELLIKECHE[1], A. RICELLI[2], N. BASER[1]. [1]*International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM IAMB), Bari, Italy.* [2]*CNR-Istituto di*

Biologia e Patologia Molecolari, Rome, Italy. E-MAIL: sabrinakhemies00@gmail.com

Drosophila suzukii (Matsumura; *Diptera: Drosophilidae*), commonly known as the Spotted Wing Drosophila (SWD), is an invasive pest native to temperate Asia that has recently spread to North America and Europe. Unlike typical vinegar flies, *D. suzukii* females possess a characteristic serrated ovipositor, allowing them to lay eggs in ripening fruits, including blueberries (*Vaccinium corymbosum* L.), which leads to significant economic losses and reduced fruit quality. Beyond direct damage, *D. suzukii* also facilitates the transmission of postharvest fungal pathogens, increasing the risk of mycotoxin contamination and posing serious food safety concerns. In this study, we aimed to investigate the fungal postharvest pathogens associated with *D. suzukii*. Fungal isolations were performed from both wild- caught and laboratory-reared insects to explore the possibility that spore transmission occurs through physical contact between the insect and the fruit. The isolated fungi were identified using molecular tools, including PCR and LAMP. Given the predominant presence of Aspergilli spp., their possible ability to produce ochratoxin A (OTA) and aflatoxin B1 was investigated using High-Performance Thin-Layer Chromatography (HPTLC). We also conducted fungal isolations during different periods of the year to monitor potential variations in fungal associations. Furthermore, we evaluated the potential of ozone treatment as a postharvest strategy to minimize fungal contamination on blueberry. Our results showed that some of the fungal isolates are able to produce OTA, and ozone treatment at 5 ppm for 48 hours significantly reduced fungal growth and sporulation, as well as the egg hatchability of *D. suzukii* on blueberries without causing damage to the texture of the fruit.

***Aspergillus flavus*-based product for biological control of aflatoxins in corn.** V. BOSCO, F. BOVE, J. MATHIEU, O. GURKAN, S. LAMPERTI. *Corteva Agriscience Italia – Via dei Comizi Agrari 10 26100 Cremona (CR).* E-MAIL: federica.bove@corteva.com

Aflatoxins are highly toxic secondary metabolites mainly produced by *Aspergillus flavus*, contaminating diverse crops including corn, peanuts, cottonseed, pistachios and other tree nuts, as well as various cereals and legumes worldwide and posing serious health risks to humans and animals. Climate change is increasing the prevalence of aflatoxin contamination in regions like Central and Southern Europe beyond the traditionally subtropical regions. Effective control strategies are urgently needed, especially for economically important crops like corn. AF-X1 is an innovative biocontrol product developed by Università Cattolica del Sacro Cuore and Corteva Agriscience, specifically for corn protection. It utilizes an atoxigenic *A. flavus* strain (MUCL54911) isolated from Italian soils, which is genetically characterized by a large deletion in chromosome 3, resulting in the complete absence of the aflatoxin gene cluster and inability to produce cyclopiazonic acid, providing dual mycotoxin control. AF-X1 is formulated as sterilized sorghum seeds coated with MUCL54911 spores (1.0×10^5 CFU g^{-1}), applied at 25 kg ha^{-1} during corn stem elongation (BBCH 30-39). Field trials across Italy (2013– 2019) demonstrated that AF-X1 reduced aflatoxin B_1 by 86.4% and total aflatoxins by 87.3% compared to untreated controls, consistently keeping contamination below regulatory limits without affecting yield or quality. AF-X1 represents a sustainable and effective solution for managing aflatoxins in corn.

A patented bioactive formulation supporting SDG-Based food safety: hydroxytyrosol acetate as a natural antifungal agent. D. BOUZID[1], S. MERZOUKI[2], M.E. MERZOUKI[3], M.M. ZERROUG[2]. [1]*Laboratory of Applied Microbiology, Faculty of Natural and Life Sciences, Ferhat Abbas University Setif 1, 19000 Sétif, Algeria.* [2]*Private Medical Clinic, Sétif, Algeria.* [3]*Department of Science and Technology, Faculty of Science and Technology, Optics and Precision Mechanics, Ferhat Abbas University Setif 1, 19000 Sétif, Algeria.* E-MAIL: bouzid.djihane@yahoo.fr.

Mycotoxins and fungal spoilage remain major threats to food safety worldwide, especially in fruit and cereal supply chains. In response, we present a patented method to produce a natural antifungal formulation enriched in hydroxytyrosol acetate, derived from extra virgin olive oil via controlled oxidative transformation. The ozonation process leads to the emergence of bioactive phenolic esters, including hydroxytyrosol acetate, which are absent in untreated oil. This compound showed strong *in vitro* inhibition of *Fusarium solani*, *Penicillium expansum*, and *Aspergillus niger*, as well as preventive effects on spoilage development in fresh produce under experimental conditions. Unlike synthetic fungicides, this formulation is food-grade, biodegradable, and solvent-free, making it suitable for post-harvest applications. The ability to combine natural preservation, regulatory compliance, and sustainability aligns with current food safety regulations. This innovation supports the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero

Hunger) and SDG 12 (Responsible Consumption and Production), offering a scalable opportunity to reinforce food safety in storage and distribution.

CONCURRENT SESSION H3 – Certification programs counteract the introduction and spread of dangerous, harmful organisms. QVI - The Italian Experience

SESSION KEYNOTES

Certification of nursery plants to preserve the fruit tree industry and genetic resources in NENA region. T. YASEEN. *Regional Plant Protection Officer, Regional Office of the Near East and North Africa, Food and Agriculture Organization of the United Nations (FAORNE).* E-MAIL: Thaer.Yaseen@fao.org

The Near East and North Africa (NENA) region is home to a rich diversity of fruit tree species that are vital to food security, rural livelihoods, and the preservation of agricultural heritage. However, the sustainability of the fruit tree industry is increasingly threatened by the spread of transboundary plant pests and diseases, the loss of genetic diversity, and the widespread use of uncertified, low- quality nursery material. Climate change, expanded trade, and limited phytosanitary infrastructure further exacerbate these challenges. Therefore, the implementation of nursery plant certification schemes offers a sustainable solution to protect the fruit tree sector and safeguard the region's unique and vital genetic resources. Despite its importance, countries in the NENA region face significant constraints in establishing and managing effective certification systems. These include weak regulatory and institutional frameworks, limited diagnostic and inspection capacities, weak coordination among public and private sector stakeholders, and a shortage of trained personnel. Additionally, many farmers still rely on traditional propagation methods due to limited access to tested, certified materials. The situation is even more difficult in organic production systems, where certified organic propagative material may be unavailable. Furthermore, the lack of awareness and incentives for nurseries to participate in certification schemes remains a significant barrier. Nevertheless, some countries have made notable progress in developing and implementing effective models. Countries such as Morocco, Tunisia and Lebanon have demonstrated the potential of well-structured certification systems to enhance the quality and traceability of nursery plants, promoting the conservation of locally fruit tree genotypes. In this context, the Food and Agriculture Organization of the United Nations (FAO) has played a pivotal role in supporting member countries across the NENA region by advocating for the development of nursery plant certification schemes, fostering regional collaboration, and formulating technical guidelines and protocols tailored to key crops such as date palm and olive. Building on these efforts, FAO recently launched the NENA Plant Health Strategy (2025–2035), which aims to strengthen regional plant health systems with a particular focus on plant certification. This initiative promotes the adoption of harmonized standards and certification protocols, enhances regulatory and diagnostic capacity, and strengthens institutional coordination. These actions are critical for enabling sustainable fruit production, improving market access, and preserving the region's agrobiodiversity. Moving forward, coordinated regional action and targeted investment in institutional and technical infrastructure are crucial to scaling up successful models and ensuring the production and dissemination of healthy, true-to-type nursery plants.

The Italian experience in the development and adoption of the voluntary certification protocol for the vines and fruit plant propagating material. B.C. FARAGLIA[1], O. CORBO[2]. [1]*Masaf – Central Phytosanitary Service - National voluntary certification service, Rome.* [2]*CREA DC - Masaf – Central Phytosanitary Service - National voluntary certification service, Rome.* E-MAIL: b.faraglia@masaf.gov.it

In Italy the qualification of fruit plant propagation materials through the implementation of a certification protocol was started at the regional level in 1980. In consideration of the interest shown by nurseries and fruit growers, voluntary certification of fruit species was envisaged at ministerial level in 1987. In 1991, the National Voluntary Certification Service was established and by 1993, the technical protocols for citrus, stone fruit, pome fruit, strawberry, olive and walnut were defined. With the introduction of EU mandatory rules as the CAC (Conformitas Agraria Communitatis) quality standards starting in 1992, and those relating to quarantine and phytosanitary measures (the Plant Passport in 1993 and Regulation EU 2016/2031 on protective measures against plant pests), the national rules on voluntary certification have undergone numerous modifications. The implementing measures to this rule define the level for the qualification of the plant propagating material: CAC and Certified EU. They provide that individual EU member states may adopt additional measures

at the Certified EU level in order to better qualify and guarantee the health of the fruit plant propagation material. In 2021, Italy implemented and definitively adopted the national voluntary certification protocol called QVI (Qualità Vivaistica Italia), constituting a precise trade mark for this quality level. It covers all the species ruled, as well as kiwi and artichoke which are agamically propagated. The evolution of a 45-year legislative technical process for the highest qualification of plant propagation material produced by Italian nurseries is illustrated and discussed in this report.

The role of the Italian nursery association CIVI-Italia within the National Voluntary Certification Scheme. L. CATALANO, B. NOVELLI, G. CONSALVO. *CIVI-Italia, Corso Vittorio Emanuele II, 101, 00186 Rome, Italy.* E-MAIL: l.catalano@agrimeca.eu

CIVI (Interprofessional Centre for Nursery Activities) - Italia is the national consortium of nursery associations and producers' unions. Its main task is to qualify nursery production in support of the fruit, citrus, olive and vine production chains. It is the exclusive national association, officially representing the nursery sector, in promoting and implementing plant propagation certification programs in Italy. Since the introduction of voluntary certification schemes for fruit plant propagation material in Italy, a strong partnership has been established between public institutions and professional nursery associations. CIVI-Italia was officially recognized by the Italian Ministry of Agriculture through a Ministerial Decree in 1993, at the same time with the launch of the national voluntary certification system. With the implementation of the new national voluntary protocol, aligned with the reformed EU regulations and enacted by Ministerial Decree in June 2020, CIVI-Italia was appointed as the managing body of QVI – QUALITÀ VIVAISTICA ITALIA. This quality mark is used to identify and guarantee products that have been first certified as virus-free. The Italian experience demonstrates that certification of fruit plant propagating material has been carried out under the coordination and supervision of the public authorities - Ministry and Regional Plant Health Services, in close cooperation with the nursery associations. The key aspects of this collaboration between public authorities and the nursery association, including the responsibilities assigned, the roles undertaken, and the organization of activities, are presented and discussed with the overarching goal of qualifying Italian nursery production.

PLENARY CLOSING

KEYNOTE LECTURE

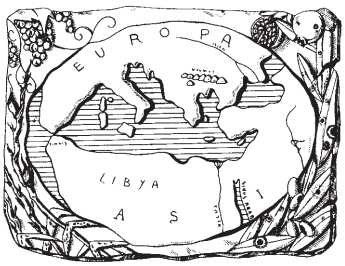
Plant Health – Food Safety – One Health. G. STANCANELLI. *Environment, Plants and Ecotoxicology (PLANTS) Unit, European Food Safety Authority (EFSA), Via Carlo Magno 1A, Parma (Italy).* E-MAIL: giuseppe.stancanelli@efsa.europa.eu

The European Food Safety Authority (EFSA) serves as the central scientific body providing risk assessments and evidence-based scientific advice on food and feed safety, animal health and plant health within the European Union (EU). EFSA's scientific outputs inform EU policy and regulatory frameworks, support national risk management authorities, and contribute to a harmonized, science-driven European food safety system. In the area of food safety, EFSA undertakes risk assessments on chemical and biological hazards across the food chain, including risk assessment for: pesticides; food additives; food flavourings; food enzymes; feed additives; food contact materials; contaminants in the food chain; genetically modified organisms; food claims; novel foods; microbiological risks in the food chain. In plant health, EFSA provides scientific advice on the risks posed by quarantine, new or emerging plant pests threatening the EU agriculture, forestry, landscape or biodiversity. EFSA plant health covers a broad range of activities: horizon scanning for new or emerging plant health threats; pest risk assessment; climate suitability assessment including climate change scenarios; modelling the introduction and spread of plant pests and the efficacy of phytosanitary measures; commodity risk assessment for High Risk Plants and derogation requests to provision of the EU Plant Health Law; support to early detection and surveillance of plant pests; databases on plant pests and plant commodities (e.g.: *Xylella* host plants database, Scolytinae database, apple pests database); research to reduce key knowledge gaps on new and emerging plant pests. EFSA communicates on the risks to food chain, animal and plant health by publishing open access its scientific outputs and by proactive risk communication strategies aiming at informing stakeholders and the general public. Various communication campaigns on food safety, plant health and animal health contribute to raising awareness among the EU citizens. EFSA, in cooperation with other EU agencies and international partners, aims to develop and apply a One Health risk assessment approach integrating human health, animal health, plant health and environmental health. This is evident in antimicrobial resistance

(AMR), where data collection on antimicrobials use and resistance need to cover human health, animal health and plant health. Other key issues addressed in a One Health risk assessment approach include zoonotic diseases and climate changes. Plant health is a major component of food security and environmental health, but it is also closely connected to food safety (in particular regarding pesticides and mycotoxins). Multidisciplinary risk assessment need to take into account these interdependencies, facilitating the integration of activities. For example, an integrated surveillance systems in border control points allow early detection of biological hazards that may affect animal health, plant health and human health. The One Health framework enables to align risk assessment with broader sustainability goals, while ensuring resilience in the face of climate change, biodiversity loss, and global trade changes.

Mediterranean Phytopathological Union

Founded by Antonio Ciccarone



The Mediterranean Phytopathological Union (MPU) is a non-profit society open to organizations and individuals involved in plant pathology with a specific interest in the aspects related to the Mediterranean area considered as an ecological region.
The MPU was created with the aim of stimulating contacts among plant pathologists and facilitating the spread of information, news and scientific material on plant diseases occurring in the area. MPU also intends to facilitate and promote studies and research on diseases of Mediterranean crops and their control.
The MPU is affiliated to the International Society for Plant Pathology.

MPU Governing Board

MPU National Society Members

Croatian Plant Protection Society
Egyptian Phytopathological Society (EPS)
French Society of Plant Pathology (SFP)
Hellenic Phytopathological Society (HPS)
Israeli Phytopathological Society (IPS)
Italian Association for Plant Protection (AIPP)
Italian Phytopathological Society (SIPAV)
Italian Nematological Society (SIN)
Palestinian Plant Production and Protection Society (PPPPS)
Plant Protection Society in Bosnia and Herzegovina
Plant Protection Society of Serbia
Plant Protection Society of Slovenia
Portuguese Phytopathological Society (PPS)
Spanish Phytopathological Society
Turkish Phytopathological Society

MPU Affiliated Members

Arab Society of Plant Pathology
Euphresco
CIHEAM-Bari
International Mycotoxicology Society
International Society for Plant Pathology

2025 Information for authors of the Open Access journal *Phytopathologia Mediterranea*

Only MPU members are eligible to publish according to MPU membership categories (see https://oajournals.fupress.net/index.php/pm/about):

- All authors belonging to an MPU National Society Member (see list above) or to an MPU Affiliated Member (international organizations or networks), that signed a Memorandum of Understanding with MPU, are entitled to publish with a contribution to publication cost (https://oajournals.fupress.net/index.php/pm/about)
- All Individual Members (not in the above categories), including members of profit or non-profit entities, and physical person.

To become an Individual Member see www.mpunion.org or contact phymed@unifi.it

To receive the paper version of the journal please contact phymed@unifi.it

For information visit the MPU web site:
www.mpunion.org
or contact us at: Phone +39 39 055 2755861/862 – E-mail: phymed@unifi.it

Phytopathologia Mediterranea

Volume 64, August, 2025

Contents

Discovery of signature peptides through proteomic approach as potential biomarkers for root wilt infection in coconut trees
Perumalla Aman Jayaker, Sundararajan Vino, Subramanian Babu 161

***Gnomoniopsis paraclavulata*, a previously unrecorded causal agent of oak decline in Italy**
Simone Mavica, Giuseppa Rosaria Leonardi, Giancarlo Polizzi, Dalia Aiello 183

First report of *Biscogniauxia mediterranea* causing cankers on almond trees (*Prunus dulcis*)
Ana Faustino, Cláudia Marinho, M. Margarida Oliveira, Maria do Rosário Félix, Liliana Marum 191

First report of cereal yellow dwarf virus (CYDV-RPS) on maize in Bosnia and Herzegovina
Amani Ben Slimen, Armin Vukojevic, Mirsad Mujkovic, Emina Krajina-Ibrulj, Toufic Elbeaino 199

***Botryosphaeriaceae* fungi associated with apricot dieback and gummosis in Türkiye**
Mukaddes Kayim, Marcelo I. Bustamante, Ali Endes, Akif Eskalen 205

Occurrence of *Grapevine fanleaf* virus in Algerian vineyards, and complete genome sequencing
Nour Elhouda Laidoudi, Osamah Alisawi, Bilal Yahiaoui, Anfel Djenaoui, Imane Mahdid, Adel Bachir, Francesca De Luca, Elena Fanelli, Angelantonio Minafra, Naima Mahfoudhi, Arezki Lehad 219

Cellulose synthase gene expression profile and physiological responses of tomato cultivars exposed to virus and salt stresses
Samra Mirzayeva, Irada Huseynova, İbrahim Özer Elibüyük, Canan Yüksel Özmen, Alİ Ergül 229

Survey of banana bunchy top virus in southern Mozambique
Sandra Carvalho I. Mussa Barros, Antonia Dos Reis Figueira, Antonia Thalyta Lopes Silveira, Edson A. Pozza 245

Survey for *Pyrenophora teres* and *Ramularia collo-cygni* in barley grain from Italy
Emilio Balducci, Francesco Tini, Laura Roehrig, Giovanni Beccari, Andrea Onofri, Massimo Montanari, Ilaria Alberti, Antonio Prodi, Neil D. Havis, Paolo Benincasa, Lorenzo Covarelli 255

Pseudomonas putida* has potential for biological control of bacterial spot of tomato, caused by *Xanthomonas euvesicatoria
Gizem Eryigit, Hatice Ozaktan, Utku Sanver 271

New opportunity for early on-site detection of *Plasmopara viticola* by qPCR assay
Roberta Coronelli, Giuseppe Incampo, Davide Cornacchia, Flora Spataro, Francesco Faretra, Stefania Pollastro, Donato Gerin 285

Abstracts of invited Keynote lectures, Keynote and Oral presentations and Poster papers presented at the 17th Congress of the Mediterranean Phytopathological Union, July 6-10, 2025, in Bari, Italy **301**

Direttore responsabile: Giuseppe Surico • Autorizzazione del Tribunale di Firenze n. 4923 del 5-1-2000 • *Printed by* Logo S.r.l. - Padova, September 2025