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ABSTRACTS

Abstracts of oral and poster presentations given at the 12th International Workshop on Grapevine Trunk Diseases, Mikulov, Czech Republic, 10–14 July 2022

The 12th International Workshop on Grapevine Trunk Diseases (12th IWGTD) was held in Mikulov, Czech Republic, from 10 to 14 July, 2022. The Workshop was chaired by Dr Aleš Eichmeier and organized by Mendel University in Brno and Svaz Vinařů České Republiky/Union of Winemakers of the Czech Republic. The ICGTD Council meeting took place on 10 July, and that evening the welcome reception was opened by Rostislav Koštial (Mayor of Mikulov, Senator and viticulturist), Dr Martin Chlad (President of Svaz Vinařů České Republiky/Union of Winemakers of the Czech Republic), and Dr Svatopluk Kapounek (Vice-Rector, Mendel University, Brno).

The scientific programme was opened by Dr Alena Salašová (Dean, Faculty of Horticulture, Mendel University, Brno). The Workshop was attended by 133 researchers from 25 countries, presenting 61 oral and 49 poster papers. These presentations were in five sessions, including; Pathogen Characterization and Identification, Epidemiology, Plant-pathogen Interactions, Microbial Ecology, and Disease Management in Nurseries and Vineyards. The Disease Management session aimed to provide grape growers with an overview of practical options for trunk disease control. This session included contributions from researchers who have completed field trials on trunk disease management. David Gramaje (Instituto de Ciencias de la Vid y el Vino (ICVV), Logroño, La Rioja, Spain, outlined an evaluation of treatments for protection of grapevine pruning wounds from natural pathogen infections; Francois Halleen (University of Stellenbosch, South Africa) presented detailed results of efficiency of hot water treatments in nurseries; and Josè Úrbez-Torres (Agriculture and Agri-Food Canada SuRDC) outlined research on biological control of Botryosphaeria dieback in grapevines.

A field trip (13 July) visited vineyards in the South Moravia. Delegates were presented with an overview of grapevine production in South Moravia, followed by discussions on the main diseases (including GTDs) affecting grapevines in the Czech Republic. Vineyards affected by GTD pathogens were also visited, where the growers outlined their viewpoints. The field trip focused on presenting the current situation of GTDs in South Moravia, and the management strategies being adopted.

Student competitions for best oral and poster presentations included papers presented by 29 postgraduate students.

Delegates attending the 12th International Workshop on Grapevine Trunk Diseases, in Mikulov, Czech Republic.

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For best posters:
1st place went to Catarina Leal (University of Reims Champagne-Ardenne, France, and Polytechnic University of Valencia, Spain), "Evaluation of *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 combination against grapevine trunk diseases pathogens in nursery propagation process";
2nd place went to Marcelo Bustamante (University of California, Davis, United States of America), "Investigating the role of *Fusarium* spp. in young vine decline in California";
3rd place went to María Julia Carbone (Universidad de la República, Montevideo, Uruguay), "Interactive effects of *Dactylonectria macrodidyma* inoculation on the rhizosphere and root microbiome of grapevine".

For best oral presentations:
1st place went to Catarina Leal, (University of Reims Champagne-Ardenne, France, and Polytechnic University of Valencia, Spain), "Beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 against the Botryosphaeria-dieback pathogen *Neofusicoccum parvum* may vary with grapevine cultivar";
2nd place went to Isidora Silva-Valderama (University of British Columbia, Vancouver, Canada), "Predicting pathogens virulence: linking host breadth and pathogenicity of the *Botryosphaeriaceae* fungal family in wine grapes (*Vitis vinifera*)";
3rd place went to Colin Todd (University of California, Riverside, United States of America), "Tracking the fungal pathobiome associated with young grapevine decline in California nurseries".

The 13th IWGTD will be held in Ensenada, Mexico, in 2025.

ORAL PRESENTATIONS

Investigating the causal agents of Sudden Vine Collapse in California. A. ESKALEN[1], E. HARDY[1], K. ELFAR[1], M. BUSTAMANTE[1], M. AL-RWAHNIH[1], C. STARR[2], S. BOLTON[3], N. MCROBERTS[1], M. BATTANY[4], L. BETTIGA[5], K. ARNOLD[6] and G. ZHUANG[7]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616, USA.. [2]Pest Control Advisor. [3]Lodi Winegrape Commission. [4]UCCE Farm Advisor in San Luis Obispo/Santa Barbara counties. [5]UCCE Farm Advisor in Monterey, San Benito, and Santa Cruz Counties. [6]UCCE Farm Advisor in Stanislaus County. [7]UCCE Farm Advisor in Fresno County. E-mail: aeskalen@ucdavis.edu*

Since 2011, grape growers in the San Joaquin Delta, Central Valley, and Coastal counties of California have reported Sudden Vine Collapse (SVC), in which patches of vines within each vineyard, especially vines grafted on virus-sensitive rootstocks (e.g. Freedom, 039-16 and 101-14), quickly die with no apparent cause. In some cases, dying patches are so large that they can be seen in satellite images, with levels of loss that have caused growers to remove whole vineyards. In 2018, the disease reached an economic threshold of destruction that was affecting increasing numbers of growers, therefore gaining greater attention. In this study, four vineyards exhibiting SVC in the San Joaquin Delta were sampled during July 2019. Samples were collected from roots, rootstocks, scions, cordons, spurs, and leaves of selected vines. Samples from each tissue type were plated and cultured on PDA amended with tetracycline. Isolated fungi were identified using molecular techniques. Additionally, leaf and rootstock samples were analyzed using high-throughput sequencing to characterize the microorganisms, including viruses. Iodine tests were also carried out to evaluate the starch content and estimate the extent of girdling at the graft unions. On each vine with severe symptoms, *Grapevine leafroll-associated virus 3* and a vitivirus, *Grapevine virus A* or *Grapevine Virus F*, were present. Many Grapevine Trunk Disease (GTD) pathogens were isolated from healthy-looking and symptomatic tissues. These pathogens included *Botryosphaeriaceae*, *Fusarium* sp., and *Diaporthe* sp., among others. However, no single fungal pathogen was consistently found in affected grapevines. Graft union girdling possibly indicates rootstock reject of scions, following infections, and this prevents flow of water and nutrients throughout affected vines. Therefore, the inability of plants to transport carbohydrates leads to starch depletion in roots and subsequent lack of feeder roots, further preventing nutrient uptake. These factors all contribute to quick collapse of affected vines. We hypothesize that SVC is not caused by a single pathogen, but is a disease complex, in which vines are predisposed to root stress due to co-infections by a leafroll virus (*GLRaV-3*), vitiviruses (*GVA, GVF*) and possibly others. Infected vines rapidly die by additional infestations by fungi associated with grapevine trunk diseases.

Aspergillus vine canker: an overlooked disease of grapevine in California. M. I. BUSTAMANTE[1], K. ELFAR[1], M. ARREGUIN[1], G. ZHUANG[2] and A. ESKALEN[1]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616, USA. [2]University of California, Cooperative Extension Tulare County, Tulare, CA 93274, USA. E-mail: aeskalen@ucdavis.edu*

Grapevine cankers are commonly associated with fungal pathogens of the *Botryosphaeriaceae*, *Diatrypaceae*, and *Diaporthaceae*, and these pathogens have been found and described in many cultivars and many countries. Symptoms include internal wood necroses, stunted or poor shoot development after budbreak, and dieback of cordons or entire vines. Cankered tissues may also exhibit dark fruiting bodies (pycnidia and perithecia) on the surfaces, which are responsible for releasing the spores that will lead to further vineyard infections. A different wood canker disease was first detected in the San Joaquin Valley in 1989, affecting excessively vigorous young 'Red Globe' grapevines. Since then, the disease has been observed on different cultivars, including Chardonnay, Grenache, and Crimson Seedless. The pathogen was morphologically identified *Aspergillus niger,* in the *Aspergillus* section *Nigri*, and the disease was named as Aspergillus vine canker. From 2003 to 2010, this disease was detected and monitored in Italy, affecting a number of table grape cultivars in different production regions. Recently, through a collaboration with UC Cooperative Extension farm advisors, we identified Aspergillus vine canker on Grenache and Malbec cultivars in, respectively, Fresno and Sonoma counties of California, Symptomatic vines were negative for viral infections. Affected vines are distinguishable by their prematurely senescent leaves during the fall, while healthy vines remain green. Our lab has focused on identification of the causal agent of Aspergillus vine canker in California, using molecular tools, particularly by constructing phylogenetic trees using DNA sequences of the calmodulin- gene. Preliminary data suggest that our isolates are *Aspergillus tubingensis*, which is closely related to the previously identified *A. niger.* Phylogenetic relationships between *Aspergillus* isolates from wood cankers and sour rot berries are currently being studied, to understand the etiology and epidemiology of both diseases.

Development of loop mediated isothermal amplification (LAMP) assays for rapid detection of Eutypa and Botryosphaeria dieback pathogens. R. BILLONES-BAAIJENS[1], M. LIU[1,2], M. R. SOSNOWSKI[3,4] and S. SAVOCCHIA[1,2]. *[1]Gulbali Institute, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [2]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [3]South Australian Research and Development Institute, Adelaide SA 5001, Australia. [4]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: rbaaijens@csu.edu.au*

Eutypa dieback (ED) and Botryosphaeria dieback (BD) pathogens are prevalent in Australian vineyards, causing yield reductions and threatening vineyard sustainability. Current diagnostics for ED and BD rely primarily on fungal isolations and PCR-based techniques, which are time-consuming, labour intensive or require expensive equipment and skilled staff to perform analyses. Rapid and simple DNA-based methods designed for on-site diagnostics of infected grapevine plant materials allows testing for ED and BD pathogens in low resource environments. We have developed real-time LAMP assays using the Genie II instrument to detect ED and BD pathogens in infected plant material. Five species-specific LAMP assays were developed for ED pathogens, and two genus-specific assays were developed for BD pathogens. A low cost and simple DNA extraction protocol was also developed for LAMP, and was suitable for rapid DNA extraction from infected wood. All seven real-time were highly specific and suitable for detecting and discriminating different ED and BD pathogens, using gBlocks, genomic DNA and crude DNA extracted from infected plant material. All assays can detect 1-2 pg of fungal DNA, alone or in combination with plant DNA. The LAMP assays were at least two times more sensitive than conventional fungal isolation, but were less sensitive than existing qPCR assays. Development of these LAMP assays was rapid and sensitive for detecting ED and BD pathogens from infected plant material. LAMP assays are cost effective, simple, and robust diagnostic tools for assessment of infected plant material in the field or in low resource environments.

Species diversity of Diatrypaceous airborne spores in Australian vineyards. R. BILLONES-BAAIJENS[1], M. LIU[1,2], M. R. AYRES[3], M. R. SOSNOWSKI[3,4] and S. SAVOCCHIA[1,2]. *[1]Gulbali Institute, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [2]School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [3]South Australian Research and Development Institute, Adelaide SA 5001,*

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Eutypa dieback (ED) is an important grapevine trunk disease in Australia, causing significant yield reductions, and threatening vineyard sustainability. *Eutypa lata* and other Diatrypaceous fungi produce ascospores that infect through pruning wounds, resulting in cankers, dieback and eventually vine death. Understanding the prevalence and distribution of airborne spores of these fungi in vineyards will assist in elucidating their importance in disease spread, and in developing subsequent disease management strategies. The present study analysed more than 4800 DNA samples collected using Burkard spore traps from eight wine growing regions over 8 years, using multi-faceted molecular tools, to investigate diversity and abundance of these pathogens in Australian vineyards. A multi-target quantitative PCR assay using SYBR green detected Diatrypaceous spores from 21% of the samples analysed, with spore numbers and frequency of detection varying between regions. Subsequent Sanger sequencing of amplified Diatrypaceous DNA identified seven Diatrypaceous species, with *E. lata* being the most prevalent in South Australia, while *Eutypella citricola* and *Eu. microtheca* were frequently detected only in New South Wales. Two new species, *Diatrype stigma* and *Cryptosphaeria multicontinentalis* were also identified at high frequency, with *D. stigma* being detected in several regions. The data generated to date showed high species diversity of Diatrypaceous spores trapped from different wine growing regions in Australia. Data continue to be generated from spore traps deployed across Australian wine regions, so this study will further elucidate the critical times of the year when ED spores are abundant in vineyards. This will provide regionally localised data that will assist growers in making decisions for optimal timing of pruning and wound protection in their vineyards.

Implementation of droplet digital PCR to determine grapevine health status and abundance of trunk disease fungi in ready-to-plant nursery material imported into Canada. J. HRYCAN[1,2], J. THEILMANN[1], A. MAHOVLIC[1,2], J. BOULÉ[1] and J. R. ÚRBEZ-TORRES[1]. *[1]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC V0H 1Z0, Canada. [2]Department of Biology, The University of British Columbia Okanagan, 3187 University Way, Kelowna, BC V1V 1V7, Canada. E-mail: jared.hrycan@agr.gc.ca*

Fungi associated with Botryosphaeria dieback, black foot, and Petri disease are prevalent in asymptomatic and symptomatic inner tissues of nursery grapevine plants and in young vineyards. Canada is reliant on the import of ready-to-plant grapevines for industry growth, but there have been no investigations of the health status of nursery material imported for use in this country. Five nurseries, which import ready-to-plant grapevine material, were selected to identify the most prevalent grapevine trunk disease (GTD) fungi. Fifteen plants each of 'Chardonnay', 'Merlot' and 'Pinot Noir', grafted on '3309C' rootstock, were analyzed from four nurseries. The same number of self-rooted 'Chardonnay', 'Merlot' and 'Pinot Noir' plants were analyzed from one nursery. Tissue samples were collected from roots, bases, graft-unions (in grafted plants), and scions from the plants. Primers were either adapted or newly developed for use in droplet digital PCR (ddPCR), to determine the quantities of *Botryosphaeriaceae* spp., *Cadophora luteo-olivacea*, *Ilyonectria* spp., *Dactylonectria torrensensis*, *Phaeoacremonium minimum*, and *Phaeomoniella chlamydospora* from each host tissue type. Traditional re-isolations were conducted from each tissue type, and isolated fungi were identified using morphological and molecular techniques. Internal necrosis was measured at the base of each plant to further understand how levels of infection may correlate with symptom expression. All plants used in this study were infected by at least one of the above listed GTD fungi. Differences were found for pathogen concentration among plants within each host cultivar, and between cultivars from the same nursery. Differences among nurseries were also observed for the GTD fungi present. These fungi and their concentrations varied among the different parts of host plants. Overall, plant bases and graft-unions contained the highest quantities of GTD fungi.

Rapid detection of *Neofusicoccum parvum* using Loop mediated isothermal amplification (LAMP). S. ECHEVERRÍA[1,2,3], J. PÉREZ[2], R. POLANCO[1] and F. GAÍNZA-CORTÉS[3]. *[1]Universidad Andrés Bello, Centro de Biotecnología Vegetal, Laboratorio de Hongos Fitopatógenos, República 330, Santiago, Chile. [2]Universidad Andrés Bello, Centro de Bioinformática y Biología Integrativa, Laboratorio de Bionanotecnología, República 330, Santiago, Chile. [3]Centro de Investigación e Innovación, Viña Concha y Toro, k-650 km 10, Pencahue, Maule, Chile. E-mail: s.echeverraaraya@uandresbello.edu*

Neofusicoccum parvum one of the fungi associated with grapevine trunk diseases (GTDs), which are important

economic problems for viticulture in Chile. Sanitary measures, such as early detection of these pathogens, are the most efficient way to mitigate and control infections caused by *N. parvum*. qPCR is the most widely used tool for detection of this pathogen, due to its sensitivity. However, this approach is of high cost when intensifying and increasing systems for early detection of *N. parvum*. LAMP is a low-cost technique that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions, and is a useful tool for the quantifying and intensifying detection of grapevine trunk fungi in greenhouse grown plants. we developed a set of LAMP primers for early detection of *N. parvum* based on the MAT locus. The analysis of sequencing products from the MAT 1-2-1 gene of the MAT 1-2 idiomorph of Chilean isolates allowed identification of new distinctive regions of the species. Visualization of the products obtained after LAMP amplification by colorimetry and electrophoresis showed that *N. parvum* was detected after 35 min of reaction at 65°C. This promising technique could be implemented in sanitary evaluations for the early detection of plant pathogens in high intensity production systems.

Botryosphaeria dieback is a major component of the grapevine trunk disease complex in Oregon vineyards. A. N. KC and M. HERNANDEZ. *Oregon State University, Southern Oregon Research and Extension Center, Central Point, OR, USA. E-mail: achala.kc@oregonstate.edu*

Western states of the United States of America, including California, Oregon, and Washington, are the major wine grape-producing regions of this country. While there are reports of grapevine trunk diseases (GTDs) in neighbouring states, there is limited information available on GTD prevalence in Oregon vineyards. We characterized the GTD pathogens in Oregon vineyards, and *Botryosphaeriaceae* spp. spore release in two geographically distinct regions. Northern Oregon has wet weather (1050 mm average annual rainfall), whereas, southern Oregon has dry weather (500 mm). Trunk tissue samples were collected from 29 vineyards in both regions during the fall of 2019 and 2020. Fungi were identified through culture and PCR based methods. The GTD pathogens included *Botryosphaeriaceae* spp. and *Phaeoacremonium* spp. from, respectively, 72% and 21% of the surveyed vineyards. *Phaeomoniella chlamydospora*, *Cryptovalsa ampelina*, *Truncatella angustata*, *Seimatosporium lichenicola*, *Hormonema viticola* were detected from 7% of the vineyards; and *Dactylonectria macrodidyma*, and *Pestaloptiopsis* sp. from 3 % of the vineyards. Pathogens were detected in both rainfall regions, and in young and mature vines. The presence of GTD pathogens in the Esca disease complex was affected ($P = 0.02$) by vineyard age, with pathogens being more abundant in old than in young vineyards. *Botryosphaeriaceae* species were the most commonly detected group, and their spore releases were affected by the region. In northern Oregon, the spore detection occurred between December and February. In southern Oregon, detection was between November and January. This study provides insights on common GTD pathogens in Oregon vineyards, and their spore release during the critical months of vineyard pruning, which is important knowledge for implementing disease management practices to prevent and mitigate Botryosphaeria dieback.

Carbohydrate-active enzymes and secondary metabolite production by the grapevine pathogen *Neofusicoccum parvum* Bt-67 grown on two lignocellulosic biomasses. J. D. RESTREPO-LEAL[1,2], M. BELAIR[1], J. FISCHER[3], N. RICHET[2], F. FONTAINE[2], C. RÉMOND[1], O. FERNANDEZ[2] and L. BESAURY[1]. *[1]UMR FARE 614 INRAE-URCA – Fractionnement des Agroressources et environnement, Chaire AFERE, Université de Reims Champagne-Ardenne, 51097, Reims, France. [2]RIBP USC INRAE 1488 – Résistance Induite et BioProtection des Plantes – EA 4707, Chaire MALDIVE, Université de Reims Champagne-Ardenne, 51100, Reims, France. [3]Institut für Biotechnologie und Wirkstoff-Forschung (IBWF), 55128, Mainz, Germany. E-mail: olivier.fernandez@univ-reims.fr/ludovic.besaury@univ-reims.fr*

Neofusicoccum parvum is an aggressive *Botryosphaeriaceae* pathogen associated with grapevine trunk diseases. This fungus may secrete enzymes capable of overcoming plant barriers, leading to wood colonization. In addition to roles in pathogenicity, carbohydrate-active enzymes (CAZymes) may be involved in plant cell wall degradation, and be useful for lignocellulose biorefining. *Neofusicoccum parvum* also produces toxic secondary metabolites that may contribute to its virulence. The mechanisms underlying pathogenicity and virulence of this pathogen, and its metabolism and CAZymes were examined for potential in lignocellulose biorefining. The potential of *N. parvum* Bt-67 to produce lignocellulolytic enzymes and phytotoxic secondary metabolites was examined *in vitro* on two biomasses, grapevine canes and wheat straw, in a multiphasic study combining enzymology, transcriptomic and metabolomic analyses. Enzyme assays showed greater xylanase, xylosidase, arabinosidase, and glucosidase activities when the fun-

gus was grown on wheat straw than on grapevine canes. Fourier Transform Infrared (FTIR) spectroscopy confirmed the lignocellulosic biomass degradation caused by the secreted enzymes. Transcriptomics indicated that the *N. parvum* Bt-67 gene expression in the presence of both biomass types was similar. In total, 134 genes coding CAZymes were up-regulated, and 94 of these expressed in both biomass growth conditions. Lytic polysaccharide monooxygenases, glucosidases, and endoglucanases were the most represented CAZymes, and correlated with the enzymatic activities obtained. Secondary metabolite diversity, analyzed by HPLC-UV-Vis-MS, was variable, depending on the carbon source. Preliminary results suggest that, contrary to enzymatic activities, secondary metabolite contents was greater when *N. parvum* Bt-67 was grown with canes than on wheat straw. These results provide insights into the influence of lignocellulosic biomass on virulence factor expression. This research also opens the possibility of optimizing enzyme production from *N. parvum* for lignocellulose biorefining.

Exploration of a non-enzymatic wood degradation pathway in *Fomitiporia mediterranea*, the main white rot pathogen in European Esca-affected vineyards. S. MORETTI[1], M. L. GODDARD[1,2], J. LALEVÉE[3], S. DI MARCO[4], L. MUGNAI[5], C. BERTSCH[1] and S. FARINE[1]. *[1]Laboratoire Vigne Biotechnologies et Environnement UPR-3991, Université de Haute Alsace, 33 rue de Herrlisheim, 68000 Colmar, France. [2]Laboratoire d'Innovation Moléculaire et Applications, Université de Haute-Alsace, Université de Strasbourg, CNRS, LIMA, UMR 7042, 68093 Mulhouse cedex, France. [3]Université de Haute-Alsace, CNRS, IS2M UMR 7361, F-68100 Mulhouse, France. [4]Institute of BioEconomy, National Research Council, Bologna, Italy. [5]Plant Pathology and Entomology Section, Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), University of Florence, Florence, Italy. E-mail: samuele.moretti@uha.fr*

Fomitiporia mediterranea (Fmed) is identified as a white rot pathogen that has been associated with Esca in European vineyards. This fungus has biomolecular wood degradation mechanisms that are not yet fully understood. White rot has an enzymatic pool (laccases, Class II-peroxidases, carbohydrate-active enzymes) that can degrade lignocellulosic biomass (cellulose, hemicellulose and lignin), leaving grapevine wood as fibrous bleached residues. Comparative genomics and previously reported observations of wood cell microvoid size allowed us to formulate the hypothesis that Fmed could adopt non-enzymatic and enzymatic mechanisms to degrade grapevine wood. This hypothesis is based on the Chelator Mediated Fenton (CMF) model, proposed in the late 1990s by American researchers for brown rot fungi. According to our results, under appropriate experimental conditions as close as possible to the physiological conditions of grapevine wood, Fmed demonstrated *in vitro* ability to: *i*) produce low molecular weight (LMW) iron-chelating compounds, which can *ii*) reduce ferric iron to ferrous iron, and *iii*) initiate the redox cycle to produce hydroxyl radicals, thus satisfying all the conditions for a CMF-like non-enzymatic wood degradation mechanism. Carbohydrate oxidative experiments also showed that reducing sugars are liberated when cellulose and hemicellulose are treated with the CMF reaction induced by Fmed LMW extracts. Variations in the CMF reaction were observed among different Fmed strains. Research is ongoing to study effectiveness of the non-enzymatic pathway *in lignum* and *in planta*.

Analyzing the presence of trunk disease fungi in heritage grapevines of Baja California. C. S. DELGADO-RAMÍREZ[1], E. SEPÚLVEDA[2], C. VALENZUELA-SOLANO[3] and R. HERNÁNDEZ-MARTÍNEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Departamento de Microbiología, Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, Baja California, Mexico. [2]CONACYT-CICESE. Departamento de Microbiología, Carretera TijuanaEnsenada 3918, Zona Playitas, 22860, Ensenada, Baja California, Mexico. [3]INIFAP, sitio experimental Costa de Ensenada, Mexico. E-mail: cdelgado@cicese.edu.mx*

In Baja California, Mexico, vineyards occur where plants over 50 years old are cultured without irrigation. These plants derive from "heritage vines", first acquired by Dominican missionaries in approx. 1697. The cultivar Mission stands out among them. It has vigorous foliage, high resistance to drought, and vigorous and long shoots. There are less than 38 ha of heritage vines in Baja California. These plants are established in dry conditions, with rainfall of less than 250 mm p. a. and with grown little or no pesticide and fertilizer applications. Climate change scenarios predict less rain and increased in area, so these vines are being re-evaluated by some viticulturists. It is important to determine the pathogens present that could limit their establishment. The present study analyzed trunk disease fungi in heritage vines. Wood samples were collected from eight vineyards, from plants with symptoms of dieback, dead arms, or cankers. Using PDA and water agar media, 78 fungal isolates

were recovered, including 18 with morphological characteristics similar to trunk disease pathogens. Isolate characterization was achieved using microscopy and molecular identification of ITS and EF1-α fragments. The isolates belonged to *Cytospora*, *Diaporthe*, and *Diplodia*. *Diplodia seriata* was the most abundant species, followed by *Diplodia pinea, Diaporthe ampelina* and *Cytospora parasitica*. Other identified fungi were: *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Trichoderma asperellum* and *Sordaria fimicola*. Assessment of Koch's postulates for these fungi is currently underway.

The most important fungi responsible for grapevine trunk diseases in southern Italy. A. CARLUCCI, M. L. RAIMONDO and F. LOPS. *University of Foggia, Department of Agriculture, Food, Natural resources and Engineering (DAFNE), Via Napoli 25, 71122 Foggia, Italy. E-mail: antonia.carlucci@unifg.it*

Grapevine cultivation in Italy has always been affected by biotic diseases affecting roots, trunks, cordons, leaves and berries. During the last 50 years, several studies on these diseases have highlighted the fungi that are responsible for vine and yield decline. Studies in Apulia region, of southern Italy during the last 30 years have reported the following fungi as grapevine pathogens: *Phaeoacremonium* spp., *Phaeomoniella chlamydospora*, *Botryosphaeriaceae* spp., *Pleurostoma richardsiae*, some black foot pathogens (*Dactylonetrica*, *Ilyonectria*, *Thelonectria* spp.), and other minor (less-isolated) fungi including *Seimatosporium*, *Truncatella*, *Cadophora* and *Colletotrichum* spp. Isolation frequencies of these fungi have been variable, associated with different external and internal host root, trunk, and branch symptoms, and with different vineyard cultivars and ages. Fungal pathogens were isolated from grapevine wood with different symptoms including asymptomatic samples. Forty-five to 50%, of wood tissue pieces were infected, mainly with *Botryosphaeriaceae* species; 8-15% of tissue pieces with *Phaeoacremonium* species; 3-10% with *Phaeomoniella chlamydospora*; 8–10% with black foot fungi, and the remaining 15% with other fungi. The same fungal species were also isolated from asymptomatic tissue pieces although with lower isolation frequencies, indicating that they were hemibiotrofic fungi during early years of grapevine growth. Pathogenicity tests confirmed the ability of all isolated fungi to infect grapevines. Further studies are necessary to understand which environmental, geographic, and host cultivar conditions could switch the behaviour of these fungi from hemibiotrofic to pathogenetic.

4D imaging of growing grapevines: towards non-destructive diagnosis of trunk diseases. R. FERNANDEZ[1,2,10], L. LE CUNFF[1,10], S. MERIGEAUD[3], J. L. VERDEIL[4,10], J. PERRY[5], P. LARIGNON[6], A. S. SPILMONT[7], P. CHATELET[10], M. CARDOSO[8], C. GOZE-BAC[9] and C. MOISY[1,10]. [1]IFV, French Institute of Vine and Wine, UMT *Géno-Vigne, F-34398 Montpellier, France.* [2]*CIRAD, UMR AGAP Institut, F-34398 Montpellier, France.* [3]*TRIDILOGY SARL, Groupe CRP - VIDI, Montpellier, France.* [4]*CIRAD, Phiv, Campus Lavalette, 389 Avenue Agropolis, Montferrier-sur-Lez, France.* [5]*CIVC Comité Champagne, 5 rue Henri Martin, 51200 Epernay, France.* [6]*IFV Nîmes. Pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France.* [7]*IFV Pôle Matériel Végétal, Domaine de l'Espiguette, 30240 Le Grau du Roi, France.* [8]*BNIF University of Montpellier, Place Eugène Bataillon, Montpellier, France.* [9]*Laboratoire Charles Coulomb, University of Montpellier and CNRS, 34095 Montpellier, France.* [10]*UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France. E-mail: cedric.moisy@vignevin.com*

Vineyards sustainability is threatened by grapevine trunk diseases (GTDs), which irreversibly degrade internal trunk tissues and ultimately cause vine death. Foliar symptoms can appear erratically, but sanitary status of vines cannot be ascertained without injuring the plants. A novel approach was developed for GTD detection based on medical multimodal 4D imaging and Artificial Intelligence (AI)-based image processing. This allowed non-invasive GTD diagnosis. Each imaging modality contributed differently to tissue discrimination, and quantitative structural and physiological markers characterizing wood degradation were identified. Combining information on anatomical distribution of degraded tissues and vine foliar symptom history in Champagne, France, it was shown that white rot and intact tissue contents were key measurements. A model was proposed for accurate GTD diagnosis. This research opens new routes for precision agriculture, by permitting field monitoring of GTD and surveying plant health *in situ*.

Managing Esca in Bordeaux: an economic analysis of curative practices under appellation yield risk and uncertain disease severity. M. Y. E. KONAN[1,2], A. ALONSO UGAGLIA[1,3] and J. KAPLAN[4]. [1]*UMR INRAE 1065 Santé et Agroécologie du Vignoble (SAVE), F-33140 Villenave d'Ornon, France.* [2]*Bordeaux School of Economics (BSE), University of Bordeaux, Maison de l'Economie, Avenue Léon Duguit, 33608 Pessac Cedex, France.* [3]*Bordeaux Sciences Agro, 1 Cr du Général de Gaulle, 33170*

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Increased incidence of grapevine trunk diseases (GTDs) over the last 10-15 years has been reported in many grape producing countries. In France, yield losses are estimated at 4.6 hL ha^{-1} $year^{-1}$ due to Esca, the main GTD affecting grapevines in France. Despite this threat, many grape producers are reluctant to adopt field-tested preventive and curative practices. We combined an epidemiological model, developed at INRAE, for Esca infection, with an economic decision-making model, for representative vineyards in AOC Entre-Deux-Mers, AOC Pauillac, and AOC Cognac, to analyze two curative practices (curettage and complantation). Within this combined nature-human model, we analyzed how risk preferences influenced grower decisions to implement a curative practice to reduce the incidence of Esca and achieve appellation yield requirements. Different decision criteria were considered, based on risk preferences and practice efficiency, and outcomes were compared across practices, risk preferences, and efficiency to identify preferred strategies and the incremental value of high practice efficiency over low efficiency. The simulations showed that the decision of growers to implement a practice mostly favoured curettage over complantation and doing nothing, and was dependent on risk preference. In the AOC Pauillac, adopting curettage generated maximum cumulative economic loss over 40 years of 239,257 € ha^{-1}, instead of 514,615 € ha^{-1} with the decision of inaction (when practice efficiency was high). High practice efficiency also generated an additional gain of 67,147 € ha^{-1} over 40 years. These results indicate that non-economic factors may be discouraging growers from adopting curative practices, or that knowledge of the economic gains may be lacking. Providing growers with evidence of the economic benefits could lead to greater adoption of effective disease management, increased profitability, and a more sustainable wine industry in France. Investments in practice efficiency can also have substantial benefits, especially in high-valued wine grape producing regions.

Variation of mycorrhizal colonization in plants with canopy symptoms of Esca. L. LANDI, S. MUROLO and G. ROMANAZZI. *Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, I-60131 Ancona, Italy. E-mail: g.romanazzi@univpm.it*

Esca is an important disease of grapevines, associated with several fungal pathogens, of which *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Fomitiporia mediterranea* are the most important. The onset of Esca can be affected by several factors, including host cultivar and rootstock, weather conditions, the trellising system and phytosanitary management. Effects of plant stress may also affect disease onset, which has yet to be fully clarified. Intensive investigations have been carried out on the microorganisms affecting the grapevine canopies, in particular on trunks and branches, while relatively little information is available on the colonization of root systems by pathogens and putatively beneficial microorganisms. The present study used molecular tools to identify microorganisms associated with roots of symptomatic plants, compared to apparently symptomless vines, in three vineyards of the Marche region of central-eastern Italy. Arbuscular mycorrhizal fungi (AMF) colonization intensity was greater in grapevines with symptoms of Esca (24.6 to 61.3%) than in neighbouring asymptomatic plants (17.4 to 57.6%). Specific primer pairs for native *Funneliformis mosseae* and *Rhizophagus irregularis* AMF showed more DNA copies (respectively, 67 vs 47 and 292 vs 201, in 20 µL) of both species in Esca symptomatic vines. This study indicates a possible relationship between Esca and native AMF in grapevines.

Diversity and pathogenicity of fungi causing grapevine trunk diseases in Chilean patrimonial vineyards. D. GRINBERGS, J. CHILIAN and M. REYES. *Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Chillán, Chile. E-mail: dgrinbergs@inia.cl*

Trunk diseases are a major problem in Chilean commercial vineyards, reducing their productivity, longevity and grape quality. However, in ancient patrimonial vineyards, mostly containing the cultivars País, Moscatel, Carignan, País, Torontel and Moscatel, which are being rescued to make historical wines, these diseases are beginning to be studied. Preliminary identifications were performed for 235 fungal isolates from affected plants collected in Cauquenes and Itata valley, to determine the identity of the most frequently isolated species and the pathogenicity on grapevine of representative isolates. The isolates were macro- and microscopically studied after 14 d on PDA at 25°C. Isolate identity was confirmed by extracting DNA from pure PDA cultures, and genes were amplified depending on the morphological identifications. Grapevine plants were produced rooting 1-year old healthy canes ('Petit Syrah') in tap water amended with 500 ppm of indole butyric acid. Pathogenicity was assessed by inoculating fresh cuts with 0.5

cm diam. mycelium plugs of actively growing PDA colonies of *Arambarria destruens*, *Seimatosporium vitifusiforme*, *Diplodia seriata*, *D. mutila* and *Neofusicoccum parvum*. Plants (n = 7) were then incubated at 25 ± 3°C for 60 d in flowing tap water. Bark was then removed, and necrotic lesions were measured and compared (Tukey tests, $P < 0.05$). Virulence levels were detected among species, and among isolates from the same species. *Botryosphaeriaceae* species *N. parvum, D. seriata* and *D. mutila* were the most virulent, followed by *S. vitifusiforme* and *A. destruens*.

Establishment of a cell model to study molecular interactions between grapevine and Esca-associated pathogens. F. RAKOTONIAINA[1,2], C. BRETON[1], L. TESSAROTTO[2], C. CHERVIN[2], A. JACQUES[1] and O. RODRIGUES[1]. *[1]Unité de Recherche Physiologie, Pathologie et Génétique Végétales, Université Fédérale Toulouse Midi-Pyrénées, INP-PURPAN, F-31076 Toulouse, France. [2]Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, 24, chemin de Borde-Rouge, Auzeville, 31320, Castanet-Tolosan, France. E-mail: olivier.rodrigues@purpan.fr*

Esca is poorly understood trunk disease complex, which causes important economic losses in vineyards. Genetic solutions or biostimulation are the main strategies being considered for vineyard protection from these diseases. Characterization of molecular events occurring during interactions between grapevine cells and Esca-associated pathogens is a prerequisite for developing these strategies, but this is impossible to perform directly in vine trunks. Different cell strategies were developed for this purpose, including assessments of calli and leaf stomata. Guard cells, a model for the characterization of cell signalling, perceive stimuli, and respond by inducing stomatal opening/closing, which can be quantified. Assays on epidermal peels show that guard cells of *Arabidopsis thaliana* and *Vitis vinifera* can detect and respond to Esca-associated pathogens. Using pharmacological and genetic approaches, involvement of ethylene signalling in the cell responses to pathogens was assessed. Guard cell and stomatal responses are efficient and promising tools to establish a cell pathosystem model to characterize and explore molecular events that occur during grapevine reactions to Esca-associated pathogens.

Diversity of *Neofusicoccum parvum* for the production of the phytotoxic metabolites (-)-terremutin and (R)-mellein. P. TROTEL-AZIZ[1], G. ROBERT-SIEGWALD[2], O. FERNANDEZ[1], C. LEAL[1], S. VILLAUME[1], J. F. GUISE[1], E. ABOU-MANSOUR[3], M. H. LEBRUN[4] and F. FONTAINE[1]. *[1]Research Unit Résistance Induite et Bioprotection des Plantes, Université de Reims Champagne-Ardenne, RIBP EA 4707, INRAE USC 1488, SFR Condorcet FR CNRS 3417, 51100 Reims, France. [2]Independent Researcher, 49000 Angers, France. [3]Département de biologie végétale, Université de Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland. [4]Research Group Genomics of Plant-Pathogen Interactions, Research Unit Biologie et Gestion des Risques en Agriculture, UR 1290 BIOGER, Université Paris Saclay, INRAE, Avenue Lucien Brétignières, 78850 Thiverval-Grignon, France. E-mail: patricia.trotel-aziz@univ-reims.fr; florence.fontaine@univ-reims.fr*

Plant pathogens have evolved various strategies to enter hosts and cause diseases. *Neofusicoccum parvum*, an aggressive member of the Botryosphaeria dieback consortium, can secrete the phytotoxins (-)-terremutin and (R)-mellein during grapevine colonization. Although an exogenous supply of purified phytotoxins to plantlets was shown to weaken grapevine immunity, their contributions to Botryosphaeria dieback symptoms in lignified plants remains unknown. Two *Neofusicoccum parvum* isolates and a UV induced mutant were characterized for their phytotoxin production *in vitro*, pathogenicity to grapevine, and their genome sequences. Isolate Np-Bt67 produced a high level of (-)-terremutin, but almost no (R)-mellein, and it was the most aggressive on grapevine, triggering apoplexy. Similar symptoms were not induced by purified (-)-terremutin. Isolate Bourgogne S-116 (Np-B) produced 3-fold less (-)-terremutin and large amounts of (R)-mellein, but was less aggressive on grapevine than Np-Bt67. The UV9 mutant obtained from Np-B (NpB-UV9) did not produce (-)-terremutin but overproduced (R)-mellein by 2.5-fold, and was as pathogenic as its parent. NpB-UV9 differed from its parent by simple mutations in two genes (transcription factor UCR-NP2_6692, regulatory protein UCR-NP2_9007), not located near (R)-mellein nor (-)-terremutin biosynthesis genes, but likely involved in the control of (-)-terremutin biosynthesis. Grapevine immunity was disturbed after challenge with these pathogens or purified phytotoxins, leading to upregulation of SA-dependent defences, while (-)-terremutin interfered with host JA/ET-dependent defences. These results indicate that neither (-)-terremutin nor (R)-mellein alone are essential for the pathogenicity of *N. parvum* on grapevine, since a (-)-terremutin non-producing mutant isolate which overproduced (R)-mellein *in vitro* was as pathogenic as the parent isolate. However, these phyto-

toxins could play quantitative roles in the infection processes.

Beneficial effects of *Bacillus subtilis* PTA-271 and *Trichoderma atroviride* SC1 against the Botryosphaeria-dieback pathogen *Neofusicoccum parvum* may vary with grapevine cultivar. C. LEAL[1,3], N. RICHET[1], J. GUISE[1], D. GRAMAJE[2], J. ARMENGOL[3], F. FONTAINE[1] and P. TROTEL-AZIZ[1]. *[1]University of Reims Champagne-Ardenne, Résistance Induite et Bioprotection des Plantes Research Unit, EA 4707, INRAE USC 1488, SFR Condorcet FR CNRS 3417, Reims, France.* [2]Instituto de *Ciencias de la Vid y del Vino, Consejo Superior de Investigaciones Científicas, Universidad de la Rioja, Gobierno de La Rioja, Logroño, Spain.* [3]*Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain. E-mail: catarinaleal09gmail.com*

Grapevine trunk diseases (GTDs) threaten viticulture, and biocontrol strategies may be alternatives to cope with environmental concerns over pesticide use for managing these diseases. Combinations of biological control agents (BCAs) may be more effective than one BCA alone. The combination of *Bacillus subtilis* (Bs) PTA-271 and *Trichoderma atroviride* (Ta) SC1 was evaluated for the protection of *Vitis vinifera* 'Chardonnay' and 'Tempranillo' rootlings against *Neofusicoccum parvum* Bt67, an aggressive fungus that causes Botryosphaeria dieback. Indirect effects on the host of each BCA alone and in combination were assessed *in planta*, as well as their direct effects on the pathogen *in vitro*. Results indicated that: (1) the host cultivar contributes to the beneficial effects of Bs PTA-271 and Ta SC1 against *N. parvum*; and (2) the *in vitro* mutual BCA antagonism switched to the strongest fungistatic effect toward *N. parvum* in a three-way confrontation. The beneficial potential of a combination of BCAs against *N. parvum* was demonstrated, especially in 'Tempranillo'. These results highlight a common feature in both cultivars. Salicylic acid (SA)-dependent defences were low in plants protected by the BCA, in contrast to symptomatic plants. The high level of expression of SA-dependent defences in 'Tempranillo' is likely to be associated with its susceptibility to *N. parvum*, although cultivar-specific responses to the BCAs require further elucidation.

Susceptibility of pruning wounds to grapevine trunk disease pathogens in different Australian climates. M. R. SOSNOWSKI[1,2], M. R. AYRES[1], R. BILLONES-BAAIJENS[3] and S. SAVOCCHIA[3,4]. *[1]South Australian Research and Development Institute, Adelaide, SA 5001, Australia.* [2]*School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia.* [3]*Gulbali Institute, Charles Sturt University, Wagga Wagga, NSW 2678, Australia.* [4]*School of Agricultural, Environmental and Veterinary Sciences, Wagga Wagga, NSW 2678, Australia. E-mail: mark.sosnowski@sa.gov.au*

Grapevine trunk diseases (GTDs), caused by fungi that infect pruning wounds, pose major threats to vineyard sustainability. Reports from different international regions indicate that there is variation in the duration of susceptibility of pruning wounds to infections at different times during pruning seasons. A series of wound susceptibility trials were conducted from 2013 to 2019 in three climatically different regions; McLaren Vale (MV) (warm-dry) and Adelaide Hills (AH) (warm-wet) in South Australia, and Big Rivers (BR) (hot-dry), New South Wales, using 'Shiraz' (MV and AH) and 'Cabernet Sauvignon' (BR). One-year-old canes were pruned in early (June), mid (July) and late (August) winter, followed by inoculations at weekly intervals for up to 16 weeks with the GTD pathogens *Eutypa lata* (Eutypa dieback) and *Diplodia seriata* (Botryosphaeria dieback). Wounds were very susceptible to infection by both pathogens immediately following pruning, after which susceptibility decreased rapidly over the following 14 d. From 21 d post-pruning, susceptibility was generally negligible, similar to non-inoculated controls, apart from *D. seriata* inoculations in BR, where susceptibility remained high, including in non-inoculated controls. For *E. lata*, wounds were less susceptible to infection when pruned in late winter in AH, but susceptibility was similar across the pruning times in MV. Wounds were generally more susceptible to infection by *D. seriata* in BR than in AH. These results indicate that, in AH, delaying pruning to late in the host dormant season could reduce the risk of *E. lata* infections. The greater susceptibility to *D. seriata* in BR was likely due to the pathogens prevalence in the hot-dry region, and the high inoculum doses used, compared to that of the warm-wet region of AH, where *E. lata* is prevalent. These results also highlight the importance of wound protection for the 2-week post-pruning period of greatest susceptibility.

Characterisation of the endomicrobiome of grapevine nursery plants in Australia. R. BILLONES-BAAIJENS[1,2], M. LIU[1,2], B. STODART[1,2], M. R. SOSNOWSKI[3,4] and S. SAVOCCHIA[1,2]. *[1]Gulbali Institute, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.* [2]*School of Agricultural, Environmental*

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Plant microbiomes are the communities of microorganisms that may form symbiotic, commensal, or pathogenic relationships with their hosts. Well-balanced and highly diverse microbiomes are important for increasing plant fitness by facilitating adaptation to changing environmental conditions. The endophytic microorganisms present in grapevine nursery plants were characterised, focusing on grapevine trunk disease pathogens, to understand the dynamics associated with health and fitness of planting materials and young vines in vineyards. Total DNA was extracted from inner tissues and roots of dormant grafted and own-rooted vines collected from four grapevine nurseries. All DNA samples were analysed utilising high-throughput amplicon sequencing, targeting the 16S rRNA gene and ITS sequences. Preliminary results indicated that microbial communities varied greatly between nurseries, grape varieties, and host tissue types. One nursery had greater overall microbial populations compared to the other three nurseries. 'Ramsey' had the greatest microbial population among rootstocks, while populations were similar for Shiraz and Chardonnay. Microbial populations were also greater in the roots than in above ground tissues. For the bacteria, the most predominant families were *Pseudomonadaceae* (30%) and *Enterobacteriaceae* (22%), some of which are considered harmless or symbionts, although some are also enteric microorganisms. The most abundant fungal taxa were common environmental species and beneficials, including *Trichoderma* spp. and mycorrhizal fungi. Of the grapevine trunk disease (GTD) pathogens, *Phaeoacremonium, Cadophora* and *Eutypella* were most abundant, followed by *Ilyonectria*, *Phaeomoniella*, *Fomitiporia* and *Cryptovalsa*. Further investigations are required to understand the importance of these GTD pathogens in grapevine nursery plants, and their potential impacts on vineyard establishment and longevity in Australia.

Deciphering early transcriptomic and metabolomic host responses in grapevine wood to the Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. A. ROMEO-OLIVÁN[1,*], J. CHERVIN[2,3,4,*], S. FOURNIER[2,3,4], V. PUECH-PAGES[2,3,4], C. BRETON[1], T. LAGRAVÈRE[1], J. DAYDÉ[1], B. DUMAS[2], G. MARTI[2,3,4] and A. JACQUES[1]. *[1]Unité de Recherche Physiologie, Pathologie, et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, Toulouse, France. [2]Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, Toulouse, France. [3]Metatoul-AgromiX platform, MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, LRSV, Université de Toulouse, CNRS, UPS, Toulouse, INP, France. [4]MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse, France. *These authors contributed equally to this work. E-mail: ana.romeo-olivan@purpan.fr*

Early host responses to the Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora* were compared, based on transcriptomic and metabolomic analyses, to characterise wood responses to either pathogen alone. Transcriptomic analysis identified specific sets of differentially expressed genes associated with each pathogen. Functional analysis of these genes revealed differences, mainly in "Signalling", "Hormonal signalling" and the "Biotic stress response". Metabolomic analysis highlighted a group of flavonoids and stilbenoids that were overproduced in inoculated plants, compared to non-inoculated plants. Metabolomic analysis identified specific metabolites associated with each pathogen. For *P. minimum*, inoculation was associated with the accumulation of a lipophilic flavonoid cluster. The results showed different transcriptomic and metabolomic responses, depending on the pathogen. These observations suggest that grapevine may differently 'perceive' and respond with different sets of defences to wood colonization by *P. minimum* and *P. chlamydospora*.

Comparative transcriptomic and metabolomic responses of grapevine to the biocontrol agent *Trichoderma atroviride* suggest early modifications before infections by the Esca pathogens *Phaeomoniella* and *Phaeoacremonium minimum*. A. ROMEO-OLIVÁN[1,*], J. CHERVIN[2,3,4,*], S. FOURNIER[2,3,4], V. PUECH-PAGES[2,3,4], C. BRETON[1], T. LAGRAVÈRE[1], J. DAYDÉ[1], B. DUMAS[2], G. MARTI[2,3,4] and A. JACQUES[1]. *[1]Unité de Recherche Physiologie, Pathologie, et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, Toulouse, France. [2]Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, UPS, Toulouse INP, Toulouse, France. [3]Metatoul-AgromiX platform, MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, LRSV, Université de Toulouse, CNRS, UPS, Toulouse, INP, France. [4]MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse,*

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The biocontrol agent (BCA) Vintec® is a commercial strain of *Trichoderma atroviride*, labelled for management of Esca in grapevines. Several *Trichoderma* species and strains have been shown to stimulate plant defence mechanisms against different phytopathogens. Early molecular responses were assessed in grapevine wood pre-inoculated with Vintec®, to later infection by the Esca pathogens *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. Transcriptional analysis identified clusters of genes differently regulated in the presence of Vintec®, compared to non-treated plants. Phenylpropanoid metabolism and stilbene biosynthesis-related genes were represented among the genes differently expressed in the presence of Vintec®. Metabolomic analysis identified clusters of plant compounds (mainly stilbenes) synthesized in response to Vintec® and to the pathogens. Five relevant 'biomarkers' were chosen for *in vitro* evaluation of their antifungal activity against *P. chlamydospora*. These compounds may play roles in limiting *in planta* development of the pathogens. The efficacy of Vintec® may be associated with early or enhanced biochemical responses to limit host colonization by the pathogens.

Deciphering susceptibility of Greek grapevine cultivars to trunk disease pathogens through measurements of defence-related gene expression. S. TESTEMPASIS, P. NTASIOU, T. TSOUVALAS, E. MPILA and G. S. KARAOGLANIDIS. *Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Plant Pathology Laboratory, Thessaloniki, Greece. E-mail: testempa@agro.auth.gr*

Grapevine Trunk Diseases (GTDs), caused by several fungi, are major threats to viticultural production. Management of GTDs is influenced by biotic and abiotic factors including pathogen, geographic region, climate, cultivar, and cultural practices. The susceptibility was evaluated of the 13 most important Greek grape wine cultivars to three GTD pathogens (*Phaemoniella chlamydospora, Phaeoacremonium minimum* and *Diplodia seriata*). Plants of each cultivar (n = 30) were artificially inoculated and incubated in a greenhouse for 9 months. Disease severity on each cultivar was evaluated by assessing necrotic wood tissue using a 5-scale disease index. Four cultivars ('Limnio', 'Xinomavro', 'Robola', 'Kotsifali') were very tolerant to all three pathogens, and four others ('Vidiano', 'Roditis', 'Savatiano', 'Malagouzia') were very susceptible. To investigate possible mechanisms of resistance to GTDs, transcriptomic analyses were conducted on one tolerant ('Limnio') and one susceptible ('Vidiano') cultivar, by measuring expression levels of 12 defence-related genes at 12, 24, 48, 72 and 168 h post-inoculation (hpi). Early gene accumulation (12 hpi) was observed in 'Limnio' compared to 'Vidiano', in which most of the genes were overexpressed at 72 and 168 hpi. For the most tolerant 'Limnio', the pathogenesis proteins PR6, PR10, PR10.1, and chitinase were triggered. . At the same time, the biotic stress response genes Glutathione S-transferase and lipoxygenase accumulated, along with genes encoding enzymes involved in phytoalexins synthesis (stilbene synthase, phenylalanine ammonia-lyase). This is the first study deciphering susceptibility of Greek grape wine cultivars to GTDs, and enhancing understanding of the molecular basis of grapevine tolerance to the GTD complex.

Tracking the fungal pathobiome associated with young grapevine decline in California nurseries. C. TODD[1], J. F. GARCIA[2], A. JACQUES[3], D. CANTU[2] and P. ROLSHAUSEN[4]. *[1]Department of Microbiology and Plant Pathology, University of California, Riverside, United States. [2]Department of Viticulture and Enology, University of California, Davis, CA, United States. [3]Unité de Recherche Physiologie, Pathologie, et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, Toulouse, France. [4]Department of Botany and Plant Sciences, University of California, Riverside, CA, United States. E-mail: philrols@ucr.edu*

Young grapevine decline can impact longevity and productivity of new field plantings, leading to decreased economic returns. Understanding the critical steps during plant propagation that are conducive to the spread of fungal pathogens will allow nurserymen to develop cost effective disease management practices to limit disease incidence in nursery stocks. A survey of grapevine cuttings has been conducted throughout the grapevine production process to determine how different cultural practices affect the fungal pathobiome. Identical Chardonnay and Cabernet Sauvignon clones grafted on 1103P rootstocks were collected across several nurseries at different steps in the propagation pipelines (mother field, callusing, green vines, dormant vines) for 2 consecutive years. Endophytic fungal community composition was assessed with culture-dependent and independent (high-throughput sequencing, HTS) methods, in three different trunk plant compartments (root-rootstocks, below or above graft unions). Culturable fungi were profiled using standard

isolation techniques from wood samples coupled with ITS PCR and Sanger sequencing. Fungal community composition was then profiled using metabarcoding primers designed *in silico* to target Grapevine Trunk Associated Ascomycetes (GTAA). Many known grapevine pathogens were found, including *Phaeoacremonium*, *Phaeomoniella*, *Cadophora*, *Neofusicoccum* and *Diaporthe*. HTS was more sensitive for pathogen detection than culturing. The differences in disease-associated fungal community composition were affected by nurseries, and at the different steps of the production pipeline. These results highlight several possible infection routes during plant propagation.

Esca leaf symptoms: histological alteration and relationship to physiological and nutritional status. M. V. ALARCÓN[1], L. MÉNDEZ-GRANO DE ORO[2], A. FLORES-ROCO[1], A. DE SANTIAGO[2], J. RODRÍGUEZ-PRIETO[1] and L. MARTÍN[2]. *[1]CICYTEX, Agronomy of Woody and Horticultural crops Department. Cell Biology and Microscopy Lab (LBCM). Spain. [2]CICYTEX, Plant Protection Department, Spain. E-mail: laura.martin@juntaex.es*

Manifestation of the Esca in leaves is known as "tiger stripes", and also Grapevine Leaf Stripe Diseases (GLSD). Esca is a plurennial disease, but symptoms are not regular throughout consecutive years, as a vine may show GLSD symptoms one year but be asymptomatic the next. Esca leaf manifestation was monitored from 2018 to 2021 in a vineyard of 'Tempranillo' in Extremadura (Spain). Grapevine leaves were compared under four conditions: (A) green leaves from asymptomatic vines, (S) symptomatic leaves entirely affected by the disease, (SA) green leaves from vines partially affected by Esca, and (SS) symptomatic leaves from vines partially affected by Esca. Histological, physiological, chemical, and nutritional characterizations of leaves were carried out at the time of GLSD manifestation in 2021. Morphometric measurements showed that GLSD reduced leaf areas, and damage was greatest on S and SS leaves. At the histological level, leaf cross-sections showed reduced thickness and area for S and SS than for A leaves. Similarly, decreased upper and lower epidermis tissues, and reduced thickness of palisade and spongy parenchyma tissues were observed. Using a Dualex® leaf clip sensor, decreased chlorophyll and NBI index were found on S leave, while anthocyanins indices increased. No changes were found in the flavonoid contents. Physiological activity (net photosynthesis, stomatal conductance, transpiration, water use efficiency) was low in S leaves. No significant nutrient composition deficiencies were found among the different leaf symptom conditions.

The physiology and drivers of Esca leaf symptom development in grapevine. G. BORTOLAMI[1], T. FREJAVILLE[1], J. POUZOULET[1], N. DELL'ACQUA[1], N. FERRER[1], L. GUERIN DUBRANA[1], P. LARIGNON[2], P. LECOMTE[1], G. GAMBETTA[3] and C. DELMAS[1]. *[1]INRAE, UMR 1065 Santé et Agroécologie du Vignoble, ISVV, F-33140 Villenave d'Ornon, France. [2]Institut Français de la Vigne et du Vin, Pôle Rhône-Méditerranée, 7 avenue Cazeaux, 30230 Rodilhan, France. [3]Bordeaux Science Agro, Institut des Sciences de la Vigne et du Vin, Ecophysiologie et Génomique Fonctionelle de la Vigne, INRAE, 33140 Villenave d'Ornon, France. E-mail: chloe.delmas@inrae.fr*

The development of Esca leaf symptoms is an erratic and misunderstood phenomenon, which varies among grape varieties and growing regions. Esca development is likely driven by multiple factors, such as pathogen populations, vine physiology, and climatic and viticultural conditions. Grapevine hydraulic functioning is at the core of interactions between biotic and abiotic factors during vascular pathogenesis. Host xylem tissues are colonized by many microbes, including the Esca pathogens. The ascent of sap is affected by soil water availability and climatic conditions. The present study focused on exploring (*i*) the impacts of Esca leaf symptom development on xylem hydraulic integrity, and (*ii*) the interactions between drought, climatic conditions, and leaf symptom incidence under controlled conditions and in the field. Leaf symptom development was associated with hydraulic failure caused specifically by xylem occlusion, and subsequent loss of hydraulic conductivity from trunks to leaf laminae. Xylem occlusion by tyloses was specific to Esca leaf symptoms, compared to other types of premature or autumnal leaf senescence, and varied among grape varieties. Using mature potted plants uprooted from a vineyard and naturally infected by Esca pathogens, a long drought (-1MPa of predawn water potential over 3 months) was shown to inhibit Esca leaf symptom development, suggesting that vine water status plays a key role in Esca pathogenesis. Water relations and carbon dynamics were compared during the combined effects of Esca and drought stress. Using bi-monthly leaf symptom monitoring in 50 vineyards in southern France (over several years) and statistical modelling, it was shown that soil humidity, evapotranspiration, and temperature were the key drivers of Esca incidence and phenology. These results provide new insights on the role of plant physiology and microbial communities, and their interactions with climate, as key drivers in Esca pathogenesis.

Influence of morphological traits of xylem vessels on the responses of table- and wine-grape and rootstocks to *Phaeomoniella chlamydospora*. D. GERIN, N. CHIMIENTI, A. AGNUSDEI, R. M. DE MICCOLIS ANGELINI, P. ROTONDO, F. FARETRA and S. POLLASTRO. *University of Bari "Aldo Moro", Department of Soil, Plant and food Sciences, via Amendola 165/A 70126 Bari, Italy. E-mail: stefania.pollastro@uniba.it*

Phaeomoniella chlamydospora causes severe economic losses on grapevines under field and nursery conditions. Xylem structural features could be key determinants of sensitivity or resistance of host plants. The relationships were investigated between *P. chlamydospora* infection and xylem vessel features for ten table grape, 17 wine grape varieties or clones, and three rootstocks. Observations were based on image analysis of cross sections and fungal isolation from host cuttings artificially inoculated with conidial suspension (10^6 conidia mL^{-1}) of the benomyl resistant-marked strain PCHCIA43.2 of *P. chlamydospora* at 30-90 d after inoculation (DAI). Mean vessel diameters of the table grape cultivars were from 58.8 ('Allison') to 70.5 µm ('Red Globe'). 'Allison', 'Sable' and 'Timco' had greater vessel densities than 'Italia', 'Sugar Crisp' and 'Sugraone' in the diameter classes 151–180 µm and >180 µm. Mean vessel diameters of the wine-grape varieties were from 44.8 ('Nero di Troia') to 72.3 µm ('Merlot'), and vessel densities were from 20.3 ('Nero di Troia') to 29.7 vessels mm^{-2} ('Sangiovese'). In the rootstocks, mean vessel diameters were 50.8, 54.0 and 60.9 µm for 34 E.M., 140 'Ruggieri' and 1103, and mean vessel density was always less than 25.5 mm^{-2}. The *in planta* bioassay showed that, at 90 DAI, PCHCIA43.2 was isolated from 93.3% of 'Timco' cuttings and 13.3% of 'Sugar Crisp' and 'Sugraone'. These data were positively correlated with frequencies of large vessels. With few exceptions, on wine-grape varieties, the proportions of re-isolation were related to the frequency of large vessels or vessel density. The pathogen was isolated from 33–51 % of rootstock cuttings, and infection data were more related to vessel densities than vessel size. These results highlight the relationships between susceptibility to *P. chlamydospora* and the size and density of host xylem vessels, which are candidates for further research.

Effects of tissue age on Botryosphaeria dieback caused by *Diplodia seriata* in 'Cabernet Sauvignon' plants. A. LARACH[1,2], E. SALGADO[1], P. SANHUEZA[1], A. SALINAS[1], F. CADIZ[1], M. SEEGER[2] and X. BESOAIN[1]. [1]Escuela de *Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota 2260000, Chile.* [2]*Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Chemistry Department & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2340000, Chile. E-mail: ximena.besoain@pucv.cl*

Symptoms associated with Botryosphaeria dieback (BD) can occur in young or mature vines (>7 years old). In vineyards, prevalence and severity of the disease has been observed to increase with the grapevine age. In Chile, *Diplodia seriata* is the most prevalent species in 'Cabernet Sauvignon' vines. Pathogenicity tests have been carried out in 1- to 2-year-old shoots, but no information is available on susceptibility of mature tissues to pathogen infections. Two strains of *D. seriata* (50 µL of 1.104 conidia mL^{-1}) were inoculated into wounds of tissues of 1, 2, or 10-year-old Cabernet Sauvignon plants under vineyard conditions. Lesions were compared with previous inoculations made in pieces of canes. *D. seriata* was more aggressive in 10-year-old tissues (doubling its aggressiveness) than in younger tissues, and the inoculations gave symptoms that were representative of damage observed in the field. This aspect must be considered when evaluating the importance of each pathogen causing BD, especially because aggressiveness increased when the grapevines were more than 7-years-old.

Wood degradation by *Fomitiporia mediterranea* M. Fischer: physiologic, proteomic and metabolomic approaches. M. SCHILLING[1], A. MAIA-GRONDARD[2], E. ROBERT[3], P. HUGUENEY[2], C. BERTSCH[3], S. FARINE[4] and E. GELHAYE[1]. [1]*Université de Lorraine, INRAE, IAM, Nancy, France.* [2]*INRAE, SVQV UMR-A1131, Université de Strasbourg, Colmar, France.* [3]*Université de Lorraine, INRAE, A2F, Nancy, France ISVV, F-33140 Villenave d'Ornon, France.* [4]*Laboratoire Vigne, Biotechnologies et Environnement (LVBE), Université de Haute-Alsace, Colmar, France. E-mail: marion.schilling@univ-lorraine.fr*

Fomitiporia mediterranea (Fmed) is the main fungus found in grapevine wood rot ("amadou"), one of the most typical symptoms of the grapevine trunk disease Esca. This fungus is functionally classified as a white-rot pathogen, able to degrade all wood structure polymers, including hemicelluloses, cellulose, and lignin, the most recalcitrant component. Specific carbohydrate active enzymes are secreted by fungi to degrade hemicelluloses and cellulose, which can be highly specific to the nature of polysaccharides, and peroxidases, which enable Fmed

to degrade lignin, with specificities also related to lignin composition. Besides polymers, a diverse set of metabolites often associated with antifungal activities, is found in wood, this set differing among wood species. Wood decomposers detoxify these specific compounds, and this ability may indicate adaptation of these fungi to specific environments. The present study aimed to better understand the molecular mechanisms used by Fmed to degrade wood structure, particularly its potential adaptation to grapevine wood. Fmed was cultivated on sawdust from grapevine, beech, and spruce. Measurements were made of carbon mineralization rates, mass losses, and contents of wood structure polymers, targeted metabolites (extractives) and secreted proteins. *Trametes versicolor*, a ubiquitous white-rot model organism, was used for comparison. No significant degradation was observed on spruce, but greater mass loss was measured on Fmed grapevine cultures compared to beech cultures. On both substrates, simultaneous degradation patterns were demonstrated, and proteomic analysis revealed only minor differences in secreted enzymes responsible for wood degradation. These results are a first step in the understanding the adaptation of Fmed to its ecological niche, and indicate potential for further research.

Occurrence of *Phaeomoniella chlamydospora* at different stages of grapevine propagation, and pathogen biomass fluctuation from the nursery to the field in association with the endophytic microbiome. C. TSOUKAS[1], P. ATHANASIADI[1], F. BEKRIS[2], S. VASILEIADIS[2], E. PAPADOPOULOU[2], D. KARPOUZAS[2], A. K. TZIMA[1] and E. J. PAPLOMATAS[1]. *[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, 11855, Athens, Greece. [2]Laboratory of Plant and Environmental Biotechnology, Viopolis, Department of Biochemistry and Biotechnology, University of Thessaly,41500 Larissa, Greece*

Phaeomoniella chlamydospora (*Pch*), associated with young grapevine decline, has been widely reported in vine nurseries and young vines. The present study investigated the fluctuation of *Pa. chlamydospora* biomass at three stages of propagation material, in order to correlate pathogen biomass with the endophytic microbiome. Dormant cuttings (stage 1) from two Greek varieties and two rootstocks and different rootstock-scion combinations of grafted unrooted (stage 2) and rooted vines planted for 4 months in the field (stage 3), were sampled. TaqMan qPCR assays were carried out to quantify pathogen biomass within the collected samples. Mean biomass of *Pch* for all dormant cuttings (1) was 3.94 pg per 100 ng of plant DNA (3.94×10^{-5} g *Pch* DNA g^{-1} plant DNA). Mean *Pch* biomass for all rootstock-scion combinations was 1.21 pg in 100 ng plant DNA for grafted unrooted plants (2) and 62.14 pg per 100 ng of plant DNA for rooted vines (3), indicating a 50-fold increase of *Pch* biomass from the nursery to the field. To investigate correlations between *Pa. chlamydospora* biomass within the different types of propagation material and the endophytic microbiome, DNA samples with the smallest and greatest biomass values were pooled for Next Generation Sequencing analysis. Based on these results and also according to the literature, *Pa. chlamydospora* is detected in dormant cuttings derived from nursery mother vines. The large increase of pathogen biomass from the grafted unrooted vines to the grafted rooted vines confirms that the propagation process plays a crucial role in dissemination and development of Petri disease. Knowledge of the endophytic microbiome during grapevine propagation will improve understanding of the mutual interactions, and contribute to management of this important disease.

Responses of different grapevine cultivars to infections by *Lasiodiplodia theobromae* and *Lasiodiplodia mediterranea*. P. REIS[1], A. GASPAR[1], A. ALVES[2], F. FONTAINE[3] and C. REGO[1]. *[1]LEAF-Linking Landscape, Environment, Agriculture and Food, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]CESAM—Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal. [3]Université de Reims Champagne-Ardenne, Unité Résistance Induite et Bioprotection des Plantes EA 4707 USC INRAE 1488, SFR Condorcet FR CNRS 3417, 51687 Reims, France. E-mail: pedroreis@isa.ulisboa.pt*

Botryosphaeria dieback is a grapevine trunk disease that affects all viticulture regions of the world. Species of *Lasiodiplodia* have been reported as pathogens of grapevine in several growing regions, including in Portuguese vineyards. *Lasiodiplodia* spp. particularly *Lasiodiplodia theobromae*, have been reported to be more aggressive than other *Botryosphaeriaceae* associated with Botryosphaeria dieback. The present study assessed responses of the most representative cultivars planted in Portugal, 'Touriga Nacional', 'Touriga Franca', 'Alvarinho', 'Aragonez' (= 'Tempranillo') and 'Cabernet Sauvignon', by performing artificial inoculations with *Lasiodiplodia* spp. collected from different international locations. Two experiments, each repeated twice, were performed using: (1) 2-year-old grapevines kept under controlled green-

house conditions and inoculated with six isolates of *L. theobromae* and one isolate of *L. mediterranea*; and (2) 7-year-old field-grown grapevines inoculated with two isolates of *L. theobromae*. Responses of the cultivars were assessed by evaluating the lesion length caused by the isolates, 5 months after inoculation. All the isolates were able to infect host annual shoots, since they were always re-isolated and produced internal wood discolouration. Differences were found for all isolate/cultivar combinations. For both experiments, 'Touriga Nacional' developed the largest lesions, while 'Aragonez' developed the smallest lesions following *Lasiodiplodia* spp. inoculations. Portuguese isolates were more aggressive than those from Peru which were mildly aggressive. These results are a first insight into responses of selected Portuguese cultivars to *Lasiodiplodia* species present in Portugal, but not commonly associated with Botryosphaeria dieback. This contributes to knowledge of the impacts that Botryosphaeria dieback causal agents have on important national cultivars, which will assist winegrowers to manage current cultural practices, and optimize decision making when planning establishment of new vineyards.

Development of an analytical approach using magic-angle spinning NMR for the study of molecular markers of dieback in French vineyards. C. ROBERT[1], A. GRÉLARD[1], A. SAAD[1], M. GADRAT[1], P. REY[2], C. DELMAS[2] and A. LOQUET[1]. *[1]Univ. Bordeaux, CNRS, CBMN UMR 5248, IECB, Pessac, France. [2]Univ. Pau et des Pays de l'Adour, IPREM UMR 5254, Pau, France. E-mail: c.robert@iecb.u-bordeaux.fr*

Grapevine trunk diseases (GTDs) such as Esca or Black Dead Arm have severe consequences for viticulture. The economic costs of vine replacement are high. To date, neither chemical nor biological treatments have been able to eradicate GTDs since the prohibition in Europe of sodium arsenite, and diagnostic methods for detecting GTDs are in early stages of development. Dieback of vine wood tissues is associated with necrosis processes that affect chemical structure and molecular architecture of wood. A new analytical approach is presented how, at the atomic scale, wood tissues are affected by the dieback. Magic-angle spinning nuclear magnetic resonance (MAS NMR) was used to characterize the main polymer components of vine wood extracted at different dieback stages. Apparently healthy and diseased wood tissues were compared for detection and identification of possible molecular markers of dieback. This approach allows for the identification of molecular markers such as the cellulose cristallinity, degradation of specific carbohydrate linkages and apparition of acidic degradation products, which were used to investigate wood dieback of French vineyards. This approach will open an avenue to investigate other wood tissues and vineyards impacted by GTDs.

Drought stress results in a compartment-specific restructuring of the grapevine root-associated fungal microbiome. M. J. CARBONE[1], S. ALANIZ[1], P. MONDINO[1], M. GELABERT[1], A. EICHMEIER[2], D. TEKIELSKA[2], R. BUJANDA[3] and D. GRAMAJE[3]. [1]Universidad de la *República, Facultad de Agronomía, Departamento de Protección Vegetal, Avenue Garzón 780, 12900 Montevideo, Uruguay.* [2]Mendel University in Brno, Faculty of Horticulture, *Mendeleum-Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic.* [3]Instituto de *Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas-Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail: mjcarbone@fagro.edu.uy*

Physiological and molecular responses of grapevine to water stress have been extensively studied. However, little is known about the effects of drought on the grapevine-associated belowground fungal microbiome. Plant roots support complex microbial communities that can influence plant nutrition, growth and health. Drought-induced shifts in fungal composition in root endospheres, rhizospheres and bulk soil were assessed by ITS high-throughput amplicon sequencing (HTAS). Three irrigation regimes were imposed (100%, 50% and 25% of field capacity) to 1-year-old grapevine rootstock plants ('SO4'), when the plants had developed two to three roots. Root endosphere, rhizosphere and bulk soil samples were collected at 6 and 12 months post-plantation. Severe water deficit (25 %) modified the overall fungal composition of all three soil compartments, with root endosphere having the greatest divergence from the well-watered control (100%). Enrichment in several fungal genera was detected, including the arbuscular mycorrhizal fungus *Funneliformis* within roots at severe water deficit. The overall response of the fungal microbiota associated with black-foot (*Dactylonectria* and "*Cylindrocarpon*" genera) and the potential biocontrol agent *Trichoderma* to drought stress was consistent across compartments, with relative abundances being greater at 50–100% than at the 25% irrigation regime. These results indicate that drought stress, in addition to its well-characterized effects on plant physiology, also affect restructuring of grapevine root microbial communities.

This suggests the possibility that members of the altered grapevine microbiota could contribute to plant survival under extreme environmental conditions.

***Aureobasidium pullulans* may promote the development of foliar symptoms on Esca-diseased grapevines**. Z. KARÁCSONY[1], V. MONDELLO[2], A. SONGY[2], F. FONTAINE[2] and K. Z. VÁCZY[1]. *[1]Food and Wine Research Institute, Eszterházy Károly Catholic University, H-3300 Eger, Hungary. [2]University of Reims Champagne-Ardenne, Unité Résistance Induite et Bio-protection des Plantes (RIBP), USC INRAE 1488, Moulin de la Housse-Bat, 18-BP1039, 51687 Reims, France. E-mail: karacsony.zoltan@uni-eszterhazy.hu*

Esca is one of the most important fungal diseases infections of grapevine. Besides the main pathogens *Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Pmin*) and *Fomitiporia mediterranea*, several fungi are associated with this syndrome. While the complex nature of Esca is well known, only the pathogenic interactions between the main pathogens have been studied, and little is known about the possible roles of non-pathogenic fungi. The abundance of *Aureobasidium pullulans* (*Apu*) in the healthy wood of Esca-symptomatic 'Cabernet sauvignon' grapevines was assessed by isolation-based methods and qPCR measurements. A positive correlation was shown between the abundance of *Apu* and severity of foliar symptoms (relative area of necrosis). The *in vitro* investigation of the interactions between *Apu* and *Pch* or *Pmin* showed that *Pmin* strongly inhibited *Apu*, while there was a mutual antibiosis between *Pch* and *Apu* without toxic effects. Further studies of the *Apu-Pch* interactions suggested mutual induction of sporulation and inhibition of growth, and these effects were mediated by secreted molecules. Artificial infections of grapevine shoot sections with three isolates each of *Pch* and *Apu*, were carried out individually and in combinations. *Apu* enhanced development of foliar symptoms on *Pch*-infected plants in a strain-dependent manner. Phytotoxicity tests with water extracts from leaves severely damaged by *Apu* + *Pch* inoculations indicated that increased symptom severity was the result of the accumulation of toxic metabolites in the co-inoculated shoots. Protein and polysaccharide secretion of the examined *Pch* and *Apu* isolates was also investigated, and showed some differences. These may explain the positive effects of some *Apu-Pch* co-infections on the development of foliar Esca symptoms.

A study to characterise diversity of fungal endophytes in young nursery grapevine plants. M. AL SARRAJ[1], E. DEYETT[2], A. ROMEO OLIVAN[1], T. LAGRAVERE[1], P. ROLSHAUSEN[2] and A. JACQUES[1]. **[1]***Unité de Recherche Physiologie, Pathologie et Génétique Végétales, Université Fédérale Toulouse Midi-Pyrénées, INP-PURPAN, F-31076 Toulouse, France. [2]Department of Botany and Plant Sciences, University of California, Riverside, USA. E-mail: alban.jacques@purpan.fr*

In sharp upsurge for three decades, Esca threatens grape production from all viticulture regions. This is caused by a complex of fungi that attack perennial organs of grapevines. Disease symptoms vary according to plant age, severity of infections, and pedoclimatic contexts. Our research aims to understand the microbiome assemblage of nursery-grown plants, and its influence on physiology of the Esca pathosystem. The working hypothesis postulates that variabilities of vine microbiomes affect the early host defence responses against Esca causal agents (*P. minimum* and *P. chlamydospora*). The first step was to determine if there were differences in microbiome profiles of rooted grafts from different grapevine nurseries. In 2020, characterization of the microbiomes of grafted vines from the United States of America (California) and France (South-West). Plants were the same genetic material, Cabernet-Sauvignon grafted on Richter 110, and from two nurseries within each region. Results presented were obtained from North American plants (results from French plants are still to be analyzed). Differences between the two nurseries were apparent, in both alpha and beta diversity, thus validating the possibility of rapidly prospecting the influence of this microbiome on the responses of host wood to pathogenic agents associated with Esca. Diversity of the observed microbiomes will allow investigation of effects of these variabilities on disease outcomes.

Comparisons of grapevine-associated plant pathogenic fungal communities among different microhabitats, host cultivars and between healthy and Esca-diseased plants. A. GEIGER[1,2], C. M. LEAL[2,3], Z. KARÁCSONY[1], R. GOLEN[1], K. Z. VÁCZY[1] and J. GEML[1,2,3]. *[1]Food and Wine Research Centre, Eszterhazy Karoly Catholic University, Eger, H-3300, Hungary. [2]Doctoral School of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, H-2100, Hungary. [3]ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterhazy Karoly Catholic University, Eger, H-3300, Hungary. E-mail: geiger.adrienn@uni-eszterhazy.hu*

Grapevine trunk diseases (GTDs) are major threats to wine industries, causing yield losses and dieback of grapevines. While the increasing damage caused by GTDs in recent decades have spurred several studies on grapevine-associated pathogenic fungi, key questions of the emergence and severity of GTDs remain unanswered, including whether differences in richness, abundance, and composition of plant pathogenic fungi exist among below- and aboveground microhabitats, among cultivars, and among asymptomatic and symptomatic Esca-affected grapevines. DNA metabarcoding was used for soil, bark, and perennial wood samples from asymptomatic and symptomatic grapevines of two global cultivars (Chardonnay and Cabernet sauvignon) and two local cultivars (Leányka and Kékfrankos). Greater compositional differences were detected in plant pathogenic fungi within grapevine plants than among them. This was due to dominance of GTD-associated fungi in perennial wood and to a lesser extent in bark, and the dominance of non-GTD pathogens in soil, as well as the lack of differences among cultivars and among asymptomatic and Esca symptomatic grapevines. These results indicate that fungi generally associated with Esca belong to the core grapevine microbiome, and are likely to be commensal endophytes and/or latent saprotrophs, some of which can act as opportunistic pathogens of stressed plants. Environmental factors may be particularly important for development of Esca, and studies are required to investigate effects of abiotic conditions on fungal compositional dynamics in Esca-affected plants.

Predicting pathogen virulence: linking host diversity and pathogenicity of the *Botryosphaeriaceae* fungi in wine grapes (*Vitis vinifera*). I. SILVA-VALDERRAMA[1], O. SILVA[2], J. BOULE[3], J. R. ÚRBEZ-TORRES[3] and T. J. DAVIES[1]. *[1]University of British Columbia, Botany Department, Vancouver, BC, Canada. [2]La Huarara, Santiago, Chile. [3]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada. E-mail: ibsilva26@gmail.com*

Plant diseases can have important negative effects on crops and human food sources. Previous research has linked host plant diversity and pathogen emergence, illustrating important connections with phylogeny of plant hosts. However, the importance of the pathogen phylogeny in shaping pathogen-host associations has been under-explored, yet this may be useful for describing pathogens likely to shift to novel hosts, and to predict potential for disease emergence following host jumps. The phylogenetic signals in host use is described for *Botryosphaeriaceae*, a widely distributed family that infects most woody perennial plants. The links between host diversity, phylogenetic relatedness, and pathogen virulence are outlined. The phylogeny of *Botryosphaeriaceae* spp. infecting grapevines (*Vitis vinifera*) was reconstructed, and whether closely related pathogens infect similar host species was examined. Virulence of the *Botryosphaeriaceae* known to infect *V. vinifera* was quantified using a high-throughput detached cane assay. A machine learning algorithm was trained to differentiate between asymptomatic and necrotic tissues, and lesion size was quantified as a proxy for virulence. The relationship between host diversity and lesion size was then modelled. Preliminary results show large differences in host diversity within the *Botryosphaeriaceae*, and a positive relationship between the phylogenetic host diversity and pathogen virulence in *V. vinifera*. This research provides a first step towards predicting virulence of a known pathogen on a novel host following a host jump. This approach could be used for coordinated global monitoring of high-risk species within *Botryosphaeriaceae*.

Exploring the microbial terroir: communities of fungi associated with grapevine trunk diseases differ among terroirs and seasons. C. M. LEAL[1,3], A. GEIGER[2,3], K. Z. VÁCZY[2] and J. GEML[1,2,3]. *[1]ELKH – EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, Leányka u. 6, Eger 3300, Hungary. [2]Food and Wine Research Centre, Eszterházy Károly Catholic University, Leányka u. 6, Eger 3300, Hungary. [3]Doctoral School of Environmental Sciences, Hungarian University of Agricultural and Life Sciences, Páter K. u. 1, Gödöllő 2100, Hungary. E-mail: lmota.carla@gmail.com*

In viticulture and oenology, the terroir concept is widely used to explain differences in sensory and chemical characteristics of grapes and wines. The concept is partly based on known or presumed spatial differences in edaphic and mesoclimatic factors. These environmental differences also likely affect plant-associated microbes, with possible implications for plant health and crop yield and quality. The population compositional dynamics of fungi in woody tissue of grapevines was compared, with emphasis on fungi associated with grapevine trunk diseases (GTDs). Living woody tissues were sampled from grapevine plants of one cultivar (Furmint; local white grape variety), in late winter and late summer, in three different terroirs in Tokaj, Hungary. DNA sequence data were generated by Illumina NovaSeq at

BaseClear (Leiden, the Netherlands), with fungus-specific primers targeting the rDNA internal transcribed spacer (ITS) region. Of the fifteen different GTD-associated fungus genera found, *Phaeomoniella*, *Diplodia* and *Phoma* had the greatest richness, with some variation among terroirs. Compositional differences among terroirs and sampling months were also detected, with terroir explaining 11.5% and season 6% of the variance in the community composition of GTD-associated fungi. These observed differences may be due to the inherent influence of different edaphic and mesoclimatic factors associated with different terroirs and seasonality. They affect the mycobiome composition and diversity possibly by secondary effects, since living conditions in trunk woody tissues are likely to be stable. This new information on the compositional dynamics of GTDs in wood tissues among terroirs and different seasons, has implications for plant health research.

Drought influences fungal community structure and diversity inhabiting grapevine vascular systems, and enhances *Phaeomoniella chlamydospora* abundance. M. M. MALDONADO-GONZÁLEZ[1], M. J. CARBONE[2], A. EICHMEIER[3], T. KISS[3], R. BUJANDA[1] and D. GRAMAJE[1]. [1]*Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain.* [2]*Departmento de Protección Vegetal, Facultad de Agronomía, Universidad de la República, Avenida Garzón 780, Montevideo 12900, Uruguay.* [3]*Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic. E-mail: david.gramaje@icvv.es*

Grapevine productivity in the Mediterranean regions could be affected by global warming, resulting in increased competitiveness for water resources. Recent studies showed that water deficit can alter grapevine root-associated microbiota, particularly organisms able to alleviate effects of abiotic and biotic stress factors. Drought influences on fungal community structure and diversity inhabiting grapevine vascular systems were investigated, with special attention to the Petri and Esca disease pathogen *Phaeomoniella chlamydospora*. One-year-old grapevine rootlings grown under greenhouse conditions were exposed to three water regimes (WRs): 100%, 50% or 25% of field capacity. Wood samples were taken before planting (t0), and 1 year (t1) and two years (t2) after planting, from the base, medium and apical parts of the rootstock plants, sing a non-destructive sampling method. Fungal composition and *P. chlamydospora* abundance were assessed, respectively, using ITS high-throughput amplicon sequencing (HTAS) and droplet-digital PCR (ddPCR). Drought altered the overall fungal compositions in the vascular systems. Diversity greater at 100% WR than at the other WRs, at t1 and t2. Several fungal taxa associated with grapevine trunk diseases (GTDs) were predominant, and determined the dissimilarities among WRs. this applied for *P. chlamydospora* at 25% WR and at t1 and t2, for *Cadophora* at 50% WR at t1, and for *Cadophora* and *Ilyonectria* at 100% WR at t2. HTAS and ddPCR methods both showed increases in *P. chlamydospora* OTUs and abundance at 25% WR. Correlation analyses showed positive (*Cadophora*/*Ilyonectria*) and negative (*Cadophora*/*Phaeoacremonium*) interactions among several genera associated with GTDs. Water deficit reduced the complexity of the co-occurrence networks among taxa, resulting in the greatest interactions with dense and compact networks at 100% WR.

AMF community diversity identification and their effects on grapevine growth parameters under black foot pressure. R. MOUKARZEL[1], H. J. RIDGWAY[1,2], J. LIU[1], A. GUERIN-LAGUETTE[3] and E. E. JONES[1]. [1]*Lincoln University, Lincoln, Canterbury, New Zealand.* [2]*The New Zealand institute for Plant and Food Research Ltd, Private bag 4704, Christchurch, New Zealand.* [3]*Mycotree C/-Southern Woods Nursery, Christchurch, Canterbury, New Zealand. E-mail: romy.moukarzel@lincoln.ac.nz*

Arbuscular mycorrhizal fungi (AMF) have potential applications for sustainable agricultural ecosystems, and have been shown to reduce infections and mitigate effects of black foot on grapevine rootstocks. However, limited information is available on AMF-grapevine interactions, and especially in New Zealand. Most studies have assessed effects of one, two or a combination of only small numbers of AMF species on fungal pathogens associated with grapevines. The present study aimed to: (*i*) characterise the AMF community diversity associated with different commercial grapevine rootstocks sampled from New Zealand vineyards; (*ii*) investigate effects of AMF communities on grapevine growth parameters; and (*iii*) evaluate how young grapevine rootstocks inoculated with their 'home' and 'away' AMF communities would respond to challenges with a black foot pathogen species mixture. The AMF communities identified from these rootstocks were *Ambispora* spp., *Claroideoglomus* spp., *Funneliformis* spp. and *Glomus* spp. Community analyses demonstrated that

rootstock influenced the AMF community composition at all sites. The AMF communities had direct effects by increasing plant biomass and nutrient uptake, and indirectly by influencing chlorophyll content in grapevine leaves through the increase of specific nutrients such as K, Mn, and Zn. High disease incidence and severity did not reduce growth in vines inoculated with AMF, compared to vines inoculated with the pathogen only, and high levels of disease in rootstocks limited the effect of the AMF community, with only little evidence that AMF treatments reduced disease incidence and severity in vines. Further research is required to understand the mechanistic effects of AMF colonisation on plant growth parameters, especially under high disease pressure.

Culturome versus DNA metabarcoding: diversity of grapevine endophytic mycobiomes in old and young vines of different health status in New Zealand. N. BESSELMA[1], H. J. RIDGWAY[1,2], D. C. MUNDY[3], B. R. VANGA[2], P. PANDA[2] and E. E. JONES[1]. *[1]Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, New Zealand. [2]The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand. [3]The New Zealand Institute for Plant & Food Research Limited, Marlborough Wine Research Centre, 85 Budge Street, Blenheim 7201, New Zealand. E-mail: Noureddine.Besselma@lincolnuni.ac.nz*

Grapevines harbour diverse communities of fungi in the woody trunk tissues, the "endophytic mycota". These communities can have profound effects on host physiology, health, growth, and ability to adapt to stress. Some of these include pathogenic fungi as causal agents of grapevine trunk diseases (GTDs), with many considered latent pathogens. For GTDs, understanding the factors affecting latency is limited. This study aimed to compare the fungal endophyte community in young and old Sauvignon blanc vines, which were symptomatic or asymptomatic for GTDs, using culture-dependent and culture-independent approaches. Nine vineyards were sampled, with 60 mature vines (>10 years old) and 30 young vines (<9 years old) sampled. Each age group consisted of equal numbers of apparently healthy and symptomatic vines. Trunk cores were taken from each vine using a sterilised 4 mm drill bit, after removing the bark with a knife. Fungal communities were characterized by isolation and metabarcoding of the ITS1 region. For the culturome, a collection of 2116 endophytic fungi were recovered, representing 42 genera. Trunk microbiota were dominated by species of *Alternaria*, *Aureobasidium*, *Diplodia*, *Epicoccum*, *Phaeomoniella*, *Eutypa*, *Botrytis*, *Cladosporium*, and *Diaporthe*. Differences in the taxa recovered into culture were observed between vines of different ages and symptomology. In the metabarcoding, 1892 OTUs were obtained, and the same fungal genera were identified as the most abundant. Alpha diversity analysis revealed that greater diversity was detected in old compared to young vines, and in asymptomatic compared to symptomatic trunks. Beta diversity analysis demonstrated differentiation in the fungal communities structure for both age and health status. This study has produced new baseline information on Sauvignon blanc endophytic mycota, and further research will determine the impacts of these microbial communities on the latency of GTDs.

Can the microbiome drive suppression of grapevine trunk diseases? D. ADEJORO[1], E. E. JONES[1], H. RIDGWAY[1,2], D. C. MUNDY[3], B. VANGA[2] and S. BULMAN[2,4]. *[1]Department of Pest-management and Conservation, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, Canterbury, New Zealand. [2]The New Zealand Institute for Plant & Food Research Limited, Lincoln 7608, Canterbury, New Zealand. [3]The New Zealand Institute for Plant and Food Research Limited, Marlborough Wine Research Centre, Blenheim, New Zealand. [4]Better Border Biosecurity (B3), Lincoln, New Zealand. E-mail: Damola.Adejoro@lincolnuni.ac.nz*

Grapevine trunk diseases (GTDs), caused by several fungi, are important diseases in New Zealand and other grape-growing countries. Control of the diseases is difficult, and there are no approved fungicides for their eradication. This has necessitated seeking alternative disease management strategies, including sustainable biological control. This study aimed to identify taxa in the grapevine microbiome that contribute to plant health. In some New Zealand vineyards, observations have revealed vines that remain healthy within backgrounds of trunk diseases. These grapevines were termed 'disease-escape' to represent their apparent health under heavy disease pressure. Recent research on grapevine microbiomes has shown that microorganisms from these 'disease-escape' plants could contribute to disease suppression. Putative disease escape vines were identified in vineyards in two grape-growing regions in New Zealand: Hawke's Bay and Canterbury. The vines were selected based on their presence in diseased areas, maturity, and absence of trunk disease symptoms. Trunk core samples were taken from the disease-escape vines and neighbouring symptomatic vines. Subsequently, the

samples' total fungal and bacterial communities were identified and compared using culture-independent DNA metabarcoding and culture-dependent approaches. After analysing the metabarcoding and culturing results, microbial taxa were identified that were more abundant in disease-escape grapevines, or that correlated negatively with GTD pathogens. The next stage of the study is to design a synthetic community using members of the taxa of interest from the disease-escape grapevines. This synthetic community (SynCom) will be introduced into young grapevines and monitored for their ability to suppress the development and severity of GTDs. The research results will provide information on the roles (if any) that the grapevine trunk microbiome plays in suppressing GTDs.

Implications of abiotic and biotic stress on *Phaeomoniella chlamydospora* colonization of young 'Merlot' grapevines. J. HRYCAN[1,2], P. BOWEN[1], T. FORGE[1], M. HART[2] and J. R. ÚRBEZ-TORRES[1]. *[1]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC V0H 1Z0, Canada. [2]Department of Biology, The University of British Columbia Okanagan, 3187 University Way, Kelowna, BC V1V 1V7, Canada. E-mail: jared.hrycan@agr.gc.ca*

Growing evidence has led to the hypothesis that some grapevine trunk disease (GTD) fungi may behave as latent pathogens, transitioning from endophytic to pathogenic lifestyles, triggered by plant stress. Two greenhouse experiments were conducted over two growing seasons, to determine the impacts of drought stress and ring nematode (*Mesocriconema xenoplax*) infestations on *Phaeomoniella chlamydospora* growth and disease development in young 'Merlot' grapevines. Research into the potential roles of arbuscular mycorrhizal (AM) fungi as stress reducers and biocontrols has also increased in recent years. The AM fungus *Rhizophagus intraradices* was inoculated into soil to investigate its potential in mitigating plant stress and reduce its impact on fungal growth. *Phaeomoniella chlamydospora* was vacuum-inoculated into the bases of dormant 'Merlot' canes at a high (25,000 spores), medium (5,000 spores), and low (1000 spores) inoculum, to investigate whether a threshold required for disease to occur would be reached more rapidly in highly than in less infected plants. Fungus quantity was determined at time of inoculation, time of planting, and upon conclusion of the experiment, using droplet digital PCR to determine the effect of stress on fungal growth. Pruning weights were measured throughout the experiment, and internal necroses were measured at the base of each grapevine at conclusion of the experiment, to monitor symptom expression. Preliminary results showed no phenotypical differences (root dry weight and pruning weights) between stressed and non-stressed plants, no matter their infection status. Percent necroses at trunk bases were greatest in water stressed grapevines, but no difference was observed in nematode infested plants. ddPCR analyses revealed increased fungal growth in water stressed plants, but not in nematode infested plants, indicating that water stress may play a role in Petri disease development in young grapevines.

Evaluating treatments for the protection of grapevine pruning wounds from natural infections by trunk disease fungi. R. BUJANDA[1], B. LÓPEZ-MANZANARES[1], S. OJEDA[1], O. ONEKA[2], L. G. SANTESTEBAN[2], J. PALACIOS[3] and D. GRAMAJE[1]. *[1]Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. [2]Dpto de Agronomía, Biotecnología y Alimentación, Universidad Pública de Navarra (UPNA), Campus Arrosadia, 31006 Pamplona, Spain. [3]Viticultura Viva S.L., Cabmesado 4, 31390 Olite, Spain. E-mail: david.gramaje@icvv.es*

Infection of grapevines by grapevine trunk disease (GTD) fungi primarily occurs through annual pruning wounds made during the host plant dormant seasons. This study evaluated and compared the efficacy of liquid formulation fungicide (pyraclostrobin + boscalid) and paste treatments, and biological control agents (*Trichoderma atroviride* SC1, *T. atroviride* I-1237, and *T. asperellum* ICC012 + *T. gamsii* ICC080), for their potential to prevent natural infections of grapevine pruning wounds by trunk disease fungi in two field trials over two growing seasons. Vineyards were located in Samaniego, Northern Spain (19-years-old; 'Tempranillo') and 'Madiran', Southern France (24-years-old; 'Cabernet Franc'). Wound treatments were applied immediately after pruning in February. Untreated controls were mock treated with sterile distilled water. One year after pruning, canes were harvested from vines and brought to the laboratory for assessment of *Trichoderma* spp. and fungal trunk pathogens. More than 1,000 fungal isolates associated with five GTDs (Esca, Botryosphaeria, Diaporthe and Eutypa diebacks, and Cytospora canker) were collected from the two vineyards each growing season. The efficacy of each product varied according to the

GTD fungi and the grape-growing region, although, in some cases, low incidence of some GTDs in untreated controls did not allow determining differences between treatments. In general, *T. atroviride* I-1237 was the most effective treatment, followed by pyraclostrobin + boscalid. *Trichoderma* recovery proportions ranged from 16.7 to 93.3% in Samaniego, and from 32.5 to 94.2% in Madiran. The experiment will be undertaken for a third season during 2022–2023.

Hot water treatment in grapevine nurseries: the dilemma of heat tolerant GTD pathogens. F. HALLEEN[1,2], M. WEBBER[1] and L. MOSTERT[2]. *[1]ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7599, South Africa. [2]Department of Plant Pathology, University of Stellenbosch, Matieland, 7602, South Africa. E-mail: halleenf@arc.agric.za*

The benefits of hot water treatments (HWT; 50°C for 30 min) in grapevine nurseries is well known, although it is also known that these do not completely eradicate all infections. A HWT protocol of 50°C for 45 min has been recommended for control of Aster Yellows in South Africa. The aim of this study was to determine effects of HWT (50°C for 45 min) on GTD pathogens in South African nurseries, firstly *in vitro*, followed by artificially inoculating rootstock cuttings of Ramsey, Richter 110, US 8-7, Paulsen 1103 and 143B Mgt. Pathogens included *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Pm. parasiticum*, *Cadophora luteo-olivacea*, *Pleurostoma richardsiae* (Petri disease), *Campylocarpon fasciculare*, *Camp. pseudofasciculare*, *Ilyonectria liriodendri*, *Dactylonectria macrodidyma* (Black foot), *Neofusicoccum australe*, *N. parvum* (Botryosphaeria canker and dieback), and *Diaporthe ampelina* (Phomopsis dieback). *In vitro* results showed that HWT completely inhibited conidium germination and mycelium growth of all pathogens associated with Black foot, Botryosphaeria canker and dieback and Phomopsis dieback. Pathogens associated with Petri disease were more heat tolerant, with *Pl. richardsiae* being the most tolerant species and *Pa. chlamydospora* the most sensitive, followed by *Ca. luteo-olivacea*. The effects of HWT temperatures greater than 50°C were also investigated. *Pleurostoma richardsiae* showed the greatest tolerance, with temperatures of up to 60°C not achieving complete control. In the *in vivo* experiments, HWT was very effective for eradicating *Pa. chlamydospora* and *Ca. luteo-olivacea*, and reduced *Pm. minimum* and *Pm. parasiticum*-associated disease incidence and severity, based on isolation studies. The effects of HWT on *Pl. richardsiae* were less consistent. Incidence of this pathogen was not reduced, but severity of the infections was reduced, although inconsistently. Although HWT may not eradicate all infections, it is recommended for use in integrated management of GTDs.

In vitro* effects of selected phenolic compounds against *Diplodia seriata, Eutypa lata, Fomitiporia mediterranea* and *Neofusicoccum parvum. K. ŠTŮSKOVÁ[1], F. FONTAINE[2], V. MONDELLO[2], E. HAKALOVÁ[1], J. WOHLMUTH[1], A. VAVŘINÍK[3], T. HELMOVÁ[3], Š. FRANKOVÁ[4] and A. EICHMEIER[1]. *[1]Mendeleum – Institute of Genetics, Mendel University in Brno, Lednice, Czech Republic. [2]Unité Résistance Induite et Bioprotection des Plantes EA4707 USC INRAE 1488, Université de Reims Champagne-Ardenne, SFR Condorcet FR CNRS 3417, Reims, France. [3]Department of viticulture and enology, Mendel University in Brno, Lednice, Czech Republic. [4]Department of Vegetable Growing and Floriculture, Mendel University in Brno, Lednice, Czech Republic. E-mail: xstusko1@mendelu.cz*

Grapevine trunk disease (GTD) pathogens cause serious damage and have a significant economic impact for cultivation of grapevines. There is no direct and effective protection against GTDs in nurseries and established vineyards. The inhibitory effects of eugenol, epigallocatechin-3-o-gallate (EGCG) and thymol against the GTD pathogens *Diplodia seriata*, *Eutypa lata*, *Fomitiporia mediterranea* and *Neofusicoccum parvum* were monitored using *in vitro* tests. Most inhibition of fungal growth was observed from eugenol, which showed the lowest EC_{50} value. For eugenol at 1.5 µL mL^{-1}, complete inhibition of growth was observed for both *F. mediterranea* and *N. parvum*, with *E. lata* and *D. seriata* also showing high inhibition values, of respectively, 99 and 98%. For eugenol, EC_{50} values of 0.94, 0.95, 0.96, and 1.00 µL mL^{-1} were obtained for, respectively, *F. mediterranea*, *E. lata*, *D. seriata*, and *N. parvum*. Thymol at the highest concentration used (45 µL mL^{-1}), inhibited all four pathogens. *D. seriata* showed 90% inhibition, *N. parvum* 88%, *F. mediterranea* 74%, and *E. lata* 67%. The thymol EC_{50} values were 25.37 µL mL^{-1} for *N. parvum*, 25.45 µL mL^{-1} for *D. seriata*, 29.02 µL mL^{-1} for *F. mediterranea*, and 34.28 µL mL^{-1} for *Eutypa lata*. To compare, EGCG did not show any statistically significant inhibitory effects on the selected GTD pathogens. The next step will be to evaluate the potential use of eugenol for control GTDs in *in planta* tests.

Minimal versus intensive: How pruning intensity affects occurrence of grapevine leaf stripe disease

and trunk wood integrity. C. KRAUS, C. RAUCH, E. M. KALVELAGE, F. H. BEHRENS and D. D´AGUIAR. *Julius Kühn-Institute, Federal Research Centre of Cultivated Plants, Plant Protection in Fruit Crops and Viticulture, 76833 Siebeldingen, Germany. E-mail: Christian.kraus@julius-kuehn.de*

Previous studies of managing grapevine trunk diseases (GTDs) have indicated that non- or minimal-pruning schemes can reduce the risk of Esca. Nevertheless, knowledge of the mechanisms behind these observations is limited. The present study investigated the effect of pruning intensity on the occurrence of grapevine leaf stripe and grapevine trunk integrity. Two German vineyards ('Dornfelder' and 'Müller-Thurgau'), partially maintained with intensive- and minimal-pruning schemes were chosen due to the accessibility of multi-annual Esca monitoring data (respectively, 5 and 6 years). Incidence of external symptoms and proportions of white rot and necrosis in trunks of Esca positive and negative vines were analysed and compared between the two pruning intensities. Only in the 'Dornfelder' vineyard, incidence of external Esca symptoms was reduced by up to 73.7% over 5 years (2017–2021) by minimal pruning compared to intensive pruning. In both vineyards, trunks of intensive-pruned vines had more pruning wounds (respectively, 86.0% and 72.9% more) than minimal-pruned vines, but also exhibited a greater trunk head circumferences (respectively 19.3% and 14.7%). Only in the 'Dornfelder' vineyard, the proportion of necrosis was greater for intensive-pruned vines (23.0%) than for minimal-pruned vines (11.5%).

Effects of pruning on desiccation cone formation of three grapevine cultivars in France. E. BRUEZ[1*], C. CHOLET[1*], T. MARTIGNON[2], M. GIUDICI[2], M. BOISSEAU[3], P. REY[4] and L. GENY[1]. *[1]UMR Oenologie, EA 4577, Université de Bordeaux, INRAE, BSA, Institut des Sciences de la Vigne et du Vin, 210 Chemin de Leysotte - CS 50008, 33882 Villenave d'Ornon, France. [2]Simonit&Sirch, Maîtres Tailleurs de Vigne, 1 Rue Porte des Benauges, 33410 Cadillac, France. [3]Hennessy Jas et Cie, 1 rue Richonne 16100 Cognac, France. [4]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, IBEAS Avenue de l'Université 64013, Pau, France Pau et Bordeaux. *: equal contribution E-mail: emilie.bruez@u-bordeaux.fr*

Since sodium arsenite was banned, researchers have sought alternative solutions to deal with Grapevine Trunk Diseases (GTDs). Several methods, including pruning, are currently being used in attempts to prolong grapevine life. Different effects of high or short pruning in grapevine. High pruning leaves a chicot and preserves the diaphragm, unlike short pruning, which damages the diaphragm. An experiment focused on necrosis formation, and examined the relationship between spur diameter and necrosis length, and also measured the evolution of desiccation cones according to pruning type. The grapevines were pruned in February and, 4 or 8 months later, five vines per modality (short or high pruning) were sampled. There was no correlation between spur diameter and necrosis length for Cabernet sauvignon, Sauvignon blanc and Ugni blanc. However, there was a correlation between necrosis length and quality of pruning wound length for Cabernet Sauvignon and Ugni blanc. Necrosis length also varied with the vintage, particularly so for Sauvignon blanc. Overall, high pruning was effective for preventing desiccation cone development in the chicot. Keeping the diaphragm safe allowed the sap flow path to function fully.

Can spray coverage of wounds and Eutypa dieback control be improved by the addition of adjuvants to fungicides? M. R. AYRES[1] and M. R. SOSNOWSKI[1,2]. *[1]South Australian Research and Development Institute, Adelaide, South Australia 5001, Australia. [2]School of Agriculture, Food and Wine, The University of Adelaide, South Australia 5005, Australia. E-mail: matthew.ayres@sa.gov.au*

Infection of grapevine pruning wounds by *Eutypa lata*, which causes Eutypa dieback, can be efficiently and effectively controlled by the spray applications of fungicides. The level of disease control is related to wound coverage. However, as tractor-driven sprayers are designed to target leaves, they require adjustment to achieve adequate coverage of pruning wounds on dormant vines. A vineyard trial was established in winter 2017, and repeated in winter 2018, in McLaren Vale, South Australia, to evaluate whether the addition of spray adjuvants (di-1-p-menthene or trisiloxane ethoxylate) to fungicide treatments (tebuconazole or fluazinam) could improve wound coverage and protection against infection by *E. lata*. Optimal spray output to achieve adequate coverage of pruning wounds is at least 600 L ha^{-1}, but to ascertain any benefits from addition of adjuvants, a recycle sprayer applied treatments at 200 L ha^{-1}, prior to inoculation of wounds with *E. lata*. A novel technique for evaluating wound coverage was developed, using fluorescent pigment added to treatments, and was

compared with the use of water-sensitive papers (WSPs). Digital images of fluorescent pigment on wounds, captured under UV light, and from WSPs, were assessed using Image J image analysis software. These assessment showed little effect of addition of adjuvants on coverage. However, when compared directly, WSPs indicated twice the coverage than the fluorescent pigment deposited directly on pruning wounds, from the same treatment. Treated canes were removed 11 months after inoculation and assessed for pathogen recovery. Overall, there was little difference in recovery between treatments, indicating that the adjuvants did not improve efficacy of fungicides. Disease control was minimal with the application of the fungicides, reiterating the importance of applying the recommended minimum of 600 L ha^{-1} to achieve sufficient wound coverage.

Effects of biocontrol agents on *Fomitiporia mediterranea*. M. RIEDLE-BAUER[1], M. GORFER[2] and M. MADERCIC[1]. *[1]Federal College and Research Institute for Viticulture and Pomology Klosterneuburg, Wienerstraße 74, 3400 Klosterneuburg, Austria. [2]Austrian Institute of Technology, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria. E-mail: monika.riedle-bauer@weinobst.at*

White rot pathogens such as *Fomitiporia mediterranea* (Fmed) and other *Fomitiporia* species are, among other fungal species, the main pathogens associated with the ESCA syndrome. Effects of the potential biocontrol agents (BCAs) *Trichoderma simmonsii* 804 and 1056, *T. citrinoviridae* 232, *Bacillus subtilis* 224, *B. subtilis* 230, *B. amyloliquefaciens/velezensis* 624, *B. amyloliquefaciens/velezensis* 2277, *B. amyloliquefaciens/velezensis* 2143, *Pseudomonas koreensis* (all isolated from grapevines) and *T. atroviridae* (Vintec, Belchim) on growth of Fmed were assessed in dual culture and wood disc assays. Mycelium disks (1 cm diam.) of Fmed cultures were placed in the centres of MEA plates. Similar disks excised from BCA cultures, were placed at the edges of the plates. The diameter of each Fmed colony was measured after 14 d. The wood disk models included either fresh or autoclaved cross sections of grapevine trunks (from dormant 10-15 y old asymptomatic vines 'Rotburger' ('Zweigelt'), approx. 4 mm thick) placed on water agar. Plates were inoculated with Fmed at either 7 d before or 7 d after treatment with the BCAs). For pathogen inoculation, mycelium disks were placed in the middle of the wood pieces, and for BCA treatment, wood pieces were briefly immersed in inoculum suspensions (bacterial isolates: OD600 0.2-0.3 in PBS; *Trichoderma* spp. 108 cfu mL^1 in tap water). Growth of Fmed on the wood disks was visually evaluated after 3-5 weeks. In the dual cultures, all BCAs reduced growth of Fmed compared to experimental controls, the *Trichoderma* species being most effective. Fmed successfully colonized the inoculated wood disks. All the *Trichoderma* species reduced pathogen growth, both on fresh and autoclaved wood, and for both inoculation time schedules. Inhibitory effects of bacterial BCAs were also recorded.

Biological control of *Phaeomoniella chlamydospora* in young grapevines with *Bacillus velezensis* K165 and *Fusarium oxysporum* F2. F. I. GKIKAS[1], A. TAKO[1], D. GKIZI[2], C. LAGOGIANNI[1], E. A. MARKAKIS[3] and S. E. TJAMOS[1]. *[1]Department of Crop Science, Agricultural University of Athens, 75 Iera Odos str., 11855 Athens, Greece. [2]Department of Wine, Vine and Beverage Sciences, University of West Attica, Ag. Spyridonos 28, 12243 Athens, Greece. [3]Department of Viticulture, Vegetable Crops, Floriculture and Plant Protection, Hellenic Agricultural Organization Demeter, Mesa Katsampas 71003, Iraklio Crete, Greece. E-mail: dgkizi@uniwa.gr*

Grapevine trunk diseases (GTDs) are major problems for viticulture, endangering sustainability of grape production. *Phaeomoniella chlamydospora* (*Pch*) is a predominant GTD-associated species in Petri disease, causing decline of young grapevines. There are no effective strategies to reduce *Pch* infections except prevention in nurseries, so there is urgent need for eco-friendly approaches to control Petri disease. The effectiveness of *Bacillus velezensis* K165 (formerly *Paenibacillus alvei* K165) and *Fusarium oxysporum* F2 against *Pch* was assessed in dual culture assays and in young grapevines. In dual culture assays, in a growth medium simulating the xylem environment, F2 decreased *Pch* growth and sporulation, whereas K165 did not have any effect on *Pch*. In rooted grapevine cuttings, K165 was applied through root drenching while F2 was applied by stem injection. K165 reduced wood discolouration, the typical symptom of *Pch* infection, whereas application of F2 by stem injection did not. Both K165 and F2 reduced the endophytic DNA amount of *Pch* compared to controls by, respectively, 90% and 82%, revealed by qPCR analysis. K165- and F2-treated grapevines harboured higher lignin levels compared to non-inoculated controls. These results indicate that F2 and K165 have potential as biocontrol agents against *Pch* in grapevines.

Lignan extract from knotwood of Norway spruce as a possible novel bioprotectant agent against grape-

vine trunk diseases. M. ŠPETÍK[1], J. BALÍK[2], P. HÍC[2], E. HAKALOVÁ[1], K. ŠTŮSKOVÁ[1], L. FREJLICHOVÁ[1], J. TŘÍSKA[3] and A. EICHMEIER[1]. *[1]Mendeleum—Institute of Genetics, Faculty of Horticulture, Mendel University in Brno, Valtická 334, 691 44 Lednice na Moravě, Czech Republic. [2]Department of Post-Harvest Technology of Horticultural Products, Faculty of Horticulture, Mendel University in Brno, Valtická 334, 691 44 Lednice na Moravě, Czech Republic. [3]Global Change Research Institute CAS, Bělidla 986/4a, 603 00 Brno, Czech Republic. E-mail: milan.spetik@mendelu.cz*

Grapevine trunk diseases (GTDs) are major threats to wine industries, causing significant economic loses. With no effective treatment against these diseases, there is urgent need for efficacious control disease management. An extract from the knotwood of spruce trees was assessed as an antifungal agent against GTDs. In an *in vitro* trial, the antifungal effects of the extract were assessed against strains of the most common GTD pathogens. Complete inhibition of growth was observed against *Cadophora luteo-olivacea*, *Dactylonectria torresensis*, and *Phaeoacremonium minimum*. Partial inhibition was observed against *Diaporthe ampelina* (62.5% growth reduction), *Diaporthe bohemiae* (58.4%), *Diplodia seriata* (30%) and *Eutypa lata* (79%) using 1 mg mL^{-1} of extract. A subsequent *in planta* experiment was carried out. Commercial grafts of grapevine were treated with the extract and then planted. The total genomic DNA of grapevines was extracted 10 and 180 d after the treatment. Fungal diversities of the treated or untreated plants were compared using high-throughput amplicon sequencing. Treated plants showed 76.9% lower relative abundance of *Diaporthe* and 70% lower relative abundance of *Phaeoacremonium* 10 d after treatment. A similar scenario was observed for *Cadophora* 180 d after treatment, where treated grapevines had 76% lower relative abundance of this genus compared with untreated grapevines.

Biological and chemical pruning wound protectants reduce infection of grapevine trunk disease pathogens in California. R. BLUNDELL and A. ESKALEN. *Department of Plant Pathology, University of California, Davis, California, USA. Email: aeskalen@ucdavis.edu*

Grapevine trunk diseases (GTDs) are important challenges for viticulture, curtailing vineyard longevity and productivity in most raisin, table, and wine grape production region. Vine pruning wounds are the main entry points for fungal pathogens responsible for these diseases, and pathogens enter the wounds following precipitation events. The present study evaluated the efficacy of selected chemical and experimental biological fungicides for protection of pruning wounds against GTD pathogens, *Eutypa lata* and *Neofusicoccum parvum*. The study was conducted on Chenin blanc grapevines at the UC Davis Department of Plant Pathology Field Station for 6 months. Several chemical or biological fungicides, notably fluopyram/trifloxystrobin, *Trichoderma atroviride*, *Trichoderma asperellum* + *T. gamsii*, and a blend of crab and lobster shell powder, provided protection against at least two of the canker pathogens used in this study. However, the majority of products tested did not provide simultaneous control of *E. lata* and *N. parvum*, highlighting the continuing challenge for controlling GTDs.

Impacts of a copper-hydroxyapatite formulation on the grapevine physiology, microbiome, and metabolome, for potential use against grapevine trunk diseases. V. MONDELLO[1], O. FERNANDEZ[1], J. F. GUISE[1], C. LEMAÎTRE-GUILLIER[2], R. GOUGEON[3], A. ACEDO[4], P. SCHMITT-KOPPLIN[5], M. ADRIAN[2], C. PINTO[6], P. TROTEL-AZIZ[1] and F. FONTAINE[1]. *[1]Unité Résistance Induite et Bioprotection des Plantes EA 4707 USC INRAE 1488 Université de Reims Champagne-Ardenne, SFR Condorcet FR CNRS 3417 Reims. [2]Agroécologie, Institut Agro Dijon, CNRS, Université de Bourgogne, INRAE, Université de Bourgogne Franche-Comté, F-21000 Dijon, France. [3]Institut Universitaire de la Vigne et du Vin, UMR PAM Université de Bourgogne, Institut Agro Dijon, Dijon, France. [4]Biome Makers, CA, USA. [5]Analytical BioGeoChemistry, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany. [6]Associação SFCOLAB—Laboratório Colaborativo para a Inovação Digital na Agricultura, Torres Vedras, Portugal. E-mail: florence.fontaine@univ-reims.fr; vincenzo.mondello@univ-reims.fr*

Concerns for human and environment health are driving development of innovative eco-sustainable plant protection products (PPPs). Copper (Cu) is a PPP active ingredient subjected to regulation, especially in Europe, where it is considered a "candidate for substitution". Copper is recognized as an effective fungicide, used for more than 130 years against mildew, and is the only authorized fungicide in organic viticulture. Previous studies using the new Cu-based formulation HA + Cu(II), containing 3.5% Cu, that is transported to plants by hydroxyapatite, have shown its efficiency against *Plasmopara viticola* and *Phaeoacremonium minimum* under

greenhouse conditions. These effects were related to induction of plant defence responses. The impacts of HA + Cu(II) on plant physiology were studied in greenhouse grown 'Chardonnay' and 'Cabernet sauvignon' grapevines, which were either healthy or infected by *Diplodia seriata* and *Neofusicoccum parvum*. The effects of HA + Cu(II) against GTDs were also evaluated in a vineyard ('Chardonnay', in the Champagne region, France) that was affected by Esca. Th vineyard assessment aimed to determine the impacts of HA + Cu(II) on: (*i*) incidence of Esca, (*ii*) the vine microbiome, (*iii*) vine physiology, and (*iv*) oenological parameters of grape juice. In the greenhouse assays, HA + Cu(II) application induced several defence genes without adversely affecting plant growth or photosynthetic activity, with induction levels comparable to those of a commercial product. The fungistatic effects on the two *Botryosphaeriaceae* reported *in vitro* were confirmed *in planta*. In the field, a decreasing trend was observed in the incidence of Esca cumulate over years, and no deleterious effects were detected on vine microbiota or physiology, or on the oenological properties of the grape juice. Some similarities of HA + Cu(II) treatment responses to those from sodium arsenite were observed. These results support the potential of HA + Cu(II) as a promising PPP for GTD management.

***In vitro* evaluation of endophytic and rhizospheric bacteria as potential biocontrol agents of grapevine trunk diseases**. M. I. BUSTAMANTE, K. ELFAR and A. ESKALEN. *Department of Plant Pathology, University of California, Davis, CA 95616, United States. E-mail: mibustamante@ucdavis.edu*

Management of grapevine trunk diseases (GTDs) is an ongoing challenge for viticulture, and there is a need for effective and long term sustainable disease management strategies. A total of 20 commercial vineyards throughout California were sampled over the summer of 2019 for isolating endophytic and rhizospheric bacteria from vine cordons, trunks, and roots. A collection of 1,344 bacterium isolates (1,344) were obtained and tested *in vitro* against *Neofusicoccum parvum* and *Diplodia seriata*. From these, a subset of 172 isolates exerted mycelium growth inhibition levels >40%. The majority of these isolates (154) corresponded to an undescribed *Bacillus* sp., closely related to *B. velezensis*, and the remaining were *Pseudomonas* (12 isolates), *Serratia* (two),and other genera, and were excluded from this study. Representative isolates of *Bacillus* sp., *Pseudomonas chlororaphis* and *Serratia plymuthica* were assessed in dual antagonism assays against *N. parvum, D. seriata, Lasiodiplodia theobromae, Eutypa lata*, *Diaporthe ampelina, Phaeoacremonium minimum, Fomitiporia polymorpha* and *Ilyonectria liriodendri*. Mycelium inhibition was consistent across the bacterium species, being slightly greater against slow growing fungi than against *Botryosphaeriaceae*. The volatile and agar-diffusible metabolites produced by these bacteria were also tested against mycelium growth of *N. parvum* and *E. lata, Bacillus* sp. isolates strongly inhibited the growth of both pathogens, by their diffusible metabolites at all tested concentrations (1, 15, or 30% v/v), but not by their volatile compounds. The isolates of *P. chlororaphis* and *S. plymuthica*, however, caused less inhibition of both pathogens, but a combination of volatile and diffusible metabolites was probably involved in the antifungal activity. These isolates are being assessed in field experiments for effectiveness against trunk disease pathogens.

Biological control of Botryosphaeria dieback on grapevines. J. R. ÚRBEZ-TORRES[1], J. POLLARD-FLAMAND[1,2], J. BOULÉ[1] and M. HART[2]. *[1]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, 4200 Highway 97, Summerland, BC V0H 1Z0, Canada. [2]Department of Biology, The University of British Columbia Okanagan, 3187 University Way, Kelowna, BC V1V 1V7, Canada. E-mail: joseramon.urbeztorres@agr.gc.ca*

Botryosphaeria dieback (BD) is a grapevine trunk disease (GTD) that causes important grape yield losses and limits vineyard lifespans. *Botryosphaeriaceae* spp. causing BD infect grapevines through pruning wounds. Therefore, pruning wound protection is the most effective and available management strategy for this disease. Demand has increased for alternatives to chemical products and sustainable control methods to manage GTDs. With no control products currently registered in Canada against GTDs, the present research aimed to identify local *Trichoderma* biological control agents (BCAs), and evaluate their potential against the BD pathogens *Diplodia seriata* and *Neofusicoccum parvum*. A total of 29 *Trichoderma* isolates were obtained from vineyards in British Columbia. Morphological studies and phylogenetic analyses of the ITS1-5.8S-ITS2 gene and TEF-1α partial gene identified seven species (*T. asperelloides, T. atroviride, T. harzianum, T. koningii, T. tomentosum*), and two novel species, *T. canadense* and *T. viticola*. *In vitro* dual culture antagonism assays showed that several isolates inhibit pathogen mycelium growth, by up to 75%. *In planta* detached cane assays under controlled greenhouse conditions identified *T.*

asperelloides, T. atroviride and *T. canadense* isolates gave 70% to 100% pruning wound protection against *D. seriata* and *N. parvum* for up to 21 d after treatment. Field trials conducted in 'Merlot' vines in 2019 and 2020 showed that mixed-species inoculum of *T. asperelloides, T. atroviride* and *T. canadense* had high biocontrol activity against BD fungi for up to 60 d after treatment. Field results also showed that the *Trichoderma* spp. provided similar or greater pruning wound protection when compared with commercial chemical or biocontrol products. This study provides data supporting development and registration of the first control products against GTDs in Canada.

Combining a HA + Cu(II) site-targeted copper-based product with a pruning wound protection programme to prevent grapevine infection by *Lasiodiplodia* spp. P. REIS[1], A. GASPAR[1], A. ALVES[2], F. FONTAINE[3] and C. REGO[1]. *[1]LEAF - Linking Landscape, Environment, Agriculture and Food, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. [2]CESAM—Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, 3810-193 Aveiro, Portugal. [3]Université de Reims Champagne-Ardenne, Unité Résistance Induite et Bioprotection des Plantes EA 4707 USC INRAE 1488, SFR Condorcet FR CNRS 3417, 51687 Reims, France. E-mail: pedroreis@isa.ulisboa.pt*

Lasiodiplodia has been reported from several grape growing regions, and is one of the most rapid grapevine wood colonizers, causing Botryosphaeria dieback. These fungi can infect grapevines through wounds, and those caused by pruning are the principal sites of infection. It is therefore important to develop effective integrated disease management, which includes cultural practices, organic products, BCAs, responsible use of chemical pesticides, and appropriate management strategies, which may combine chemical and biological controls. The present study aimed to: *i*) evaluate the efficacy of Esquive®, a biocontrol agent, as grapevine pruning wound protection applied alone or in combination with a new site-targeted copper-based treatment (LC2017); and *ii*) compare their efficacy with that of the commercially available product, Tessior®. For two growing seasons, protectants were applied onto pruning wounds, while LC2017 was applied throughout the season according to the manufacturer's instructions. Pruning wounds of two different cultivars were inoculated with three isolates of *Lasiodiplodia* spp. Efficacy of the wound protectants varied between the 2 years. However, according to cultivar, the treatments controlled the pathogen to some extent. Application of LC2017 did not show clear evidence of improving control obtained by individual applications of the other products tested. However, LC2017 was fungistatic against *Lasiodiplodia* spp. *in vitro*, and has previously been shown as an elicitor against grapevine trunk diseases. This combination of two protection strategies may be a promising long-term approach to mitigate the impacts of Botryosphaeria dieback.

Relationship between *Trichoderma* recovery from pruning wounds treated with biocontrol formulations and the control of *Diplodia seriata* in Chilean vineyards. E. DONOSO, L. ROMERO, W. HETTICH, D. BASCUÑAN, C. GARCIA and J. FIGARI. *Bio Insumos Nativa SPA, Pc Antilhue Lote 4b2, Maule, Chile. E-mail: edonoso@bionativa.cl*

The use of sprayed *Trichoderma* based products has raised doubts about the relationships between recovery of *Trichoderma* from treated cuts and control efficacy against grapevine wood decay fungi. The present study aimed to assess the relationship between recovery of *Trichoderma* decreases in damage by wood fungi in vineyards. vineyards. Two experiments were established in San Felipe (Aconcagua Region) and Talca (Maule Region), using, respectively, the grapevine 'Red Globe' and 'Cabernet Sauvignon'. Twenty-four hours after pruning 100 g/hL^{-1} of the commercial product Mamull (Bio Insumos Nativa SPA) was sprayed using conventional equipment in five 0.5 ha replicates, alternated with experimental controls without treatment, under a randomized block design. At 180 d after application, samples (n = 50) were evaluated for *Trichoderma* and pathogen recovery (natural infection), and incidence and severity of lesions. At 15 d after field application, *Trichoderma* was recovered from the samples by isolating on malt extract agar. A subsample of detached canes (n = 20) was inoculated in the laboratory with *Diplodia seriata* conidium suspension and incubated in glass flasks for 30 d, for evaluation of symptoms. In the field, mean dieback incidence (mm of lesion X pathogen recovery) in the controls was 7.5% compared with 0.2 % when treated with *Trichoderma*. ($P < 0.05$ for this reduction). Average recovery of *Trichoderma* from the treated canes (52.3%), was greater ($P < 0.05$) than the control canes (11.3%). Recovery of *D. seriata* was greater ($P < 0.05$) in the control canes (16.4%) compared to those treated with *Trichoderma* (5.4%). The detached canes inoculated in the laboratory had longer ($P < 0.01$) lesions in the control (mean = 1.8 cm) compared with those from the

Trichoderma treatment (0.2 mm). Low correlation was found between the presence of *Trichoderma* and field damage (R = 0.45; $P < 0.05$). Recovery of *Trichoderma* from treated cuts was less reliable than host damage measurements for samples treated in the laboratory. The biocontrol formulation gave significant control of wood disease, and natural infection in the field, and for artificial inoculation 15 d after spray application.

Hot water treatment as a tool to produce high-quality grapevine propagation material. D. SIMON[1,2], P. WINTERHAGEN[1], R. WALTER[1], T. WETZEL[1], A. KORTEKAMP[1], A. VON TIEDEMANN[2] and J. EDER[1]. *[1]Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Breitenweg 71, D-67435 Neustadt a.d. Weinstraße, Germany. [2]Georg-August University of Göttingen, Plant Pathology and Crop Protection Section, Grisebachstraße 6, D-37077 Göttingen, Germany. E-mail: DorottyaAgnes.Simon@dlr.rlp.de*

Hot water treatment (HWT) of dormant woody plant material has been shown to reduce infections by Grapevine Trunk Disease (GTD) pathogens. In this study, HWT on individual developmental stages of three pathogens [*Phaeomoniella chlamydospora* (*Pch*), *Phaeoacremonium minimum* (*Pmi*), *Botryosphaeriaceae* (*Bot*) species] was assessed at different combinations of temperature and exposure time. Conidia and mycelium suspensions of *Pch*, *Pmi* or *Bot* were subjected to different HWTs of 30 to 45 min at 40 to 55°C, and then incubated on malt extract agar in a moist chamber to assess subsequent colony formation. In addition, field experiments were carried out to evaluate effects of HWT on the targeted fungal pathogens, and on a commercially available biological control agent (BCA) [*Trichoderma atroviride* strain SC1 (TASC1) (Vintec®, Belchim Crop Protection Deutschland GmbH). Inoculated scion cuttings grafted with healthy rootstocks following HWT (50°C, 45 min) under practical conditions, were then planted in nurseries and analysed at different times for pathogen development. The *in vitro* results indicated that *Pmi* had reduced sensitivity to HWT at the ungerminated spore stage, while *Pch* and *Bot* conidia were more sensitive to HWT. Isolations from the inoculated cuttings confirmed that infestations caused by *Pmi* and *Pch* were reduced, whereas *Bot* was completely eliminated, through HWT. No negative impacts of HWT on the commercial BCA product were detected. Therefore the antagonistic ability of the BCA against the fungal trunk pathogens should not be affected by HWTs.

Removal of trunk disease pathogens in mature grapevines using remedial surgery. E. VAN ZIJLL DE JONG[1], H. TERNENT[2], S. ST GEORGE[2], R. KALLAS[2] and M. SOSNOWSKI[3,4]. *[1]Linnaeus Limited, PO Box 1199, Gisborne 4040, New Zealand. [2]Villa Maria Estate Ltd, 118 Montgomerie Road, PO Box 43046, Mangere, Auckland, New Zealand. [3]South Australian Research and Development Institute, Adelaide, SA 5001, Australia. [4]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: eline@linnaeus.co.nz*

Remedial surgery has been used to improve productivity and extend life of grapevines infected by pathogens causing Botryosphaeria dieback and Eutypa dieback. Efficacy is dependent on removal of infected host wood and growth of new shoots to re-establish trunks. Remedial surgery was carried out during 3 years in mature (>18 years) commercial vineyard blocks of 'Sauvignon blanc', 'Cabernet Sauvignon' and 'Merlot'. Each year, the trunks were removed at standard distances above graft unions (200 mm for 'Sauvignon blanc', 150 mm for the other varieties). Incidence of dieback in the canopies was >90 % in 'Sauvignon blanc' and 'Cabernet Sauvignon', and increased over this time from 64 to 91% in 'Merlot'. Internal trunk staining associated with dieback was frequently detected at the top of the trunks below the heads in 'Sauvignon blanc' (98%) and 'Merlot' (84%), and at the remedial cut sites (respectively, 55 and 59%). These symptoms were not as frequent in the trunks of 'Cabernet Sauvignon' (62% of top and 10% of cut sites). Staining symptoms were also observed from spur and watershoot wounds on the trunks. Over the three growing seasons, distance of staining from remedial cut sites decreased in all three cultivars, and in 'Sauvignon blanc' and 'Merlot', incidence of staining increased at the remedial cut sites. *Botryosphaeriaceae* species were frequently detected in the trunks in advance of staining in all three cultivars. Trunks were shown to be infected with multiple species of *Botryosphaeriaceae*. When present, *Eutypa lata* was usually found together with these pathogens. *Botryosphaeriaceae* species, and occasionally *E. lata*, occurred over distances >200 mm in advance of staining. Growers are now advised to intervene early, and cut as low as practical, to improve the efficacy of remedial surgery.

Trellis systems of rootstock mother grapevines affect the wood microbiome. E. BATTISTON[1], L. BORRUSO[2], S. FALSINI[3], C. PINTO[4], T. MIMMO[2], S. CESCO[2], S. DI MARCO[5] and L. MUGNAI[1]. *[1]Department of Agri-*

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Management of rootstock mother vines is the first stage for bench-grafted grapevine production in nurseries. In source mother blocks, rootstock shoots are usually sprawled on the ground, which may favour infections by trunk disease pathogens, and compromise the quality of resulting propagation material. Trellis systems could be applied to rootstock mother vines to improve the functional leaf area and exposure to sunlight. Different trellis systems influence canopy microclimates, and the lack of contact with soil from trellising rootstock mother vines may affect epiphytic and endophytic microbial communities of resulting propagation material. DNA metabarcoding was used to investigate effects of two trellising methods (vertical trellis or transpiring fabric for protecting sprawled rootstocks from the soil), compared to traditional sprawled rootstocks, assessing on resident fungal and bacterial communities focussing on wood pathogens. The rootstock cultivars Kober 5 BB and 110 Richter were assessed. Bacteria and fungi beta-diversities, including epiphytic and endophytic communities, were affected by the temporal distribution of the rootstock. Diversity was greater when comparing a trellised rootstock ('Kober 5 BB') with the same plant material sprawled on the ground. These results demonstrated that sprawling shoots, compared to vertically-positioned shoots, were more exposed to soilborne microorganisms and pathogens, due to contact with soil inoculum, and probably due to the increased temperatures and humidity in sprawling shoots.

Biological control agents for Botryosphaeria dieback of grapevine. C. S. DELGADO-RAMÍREZ[1], E. SEPÚLVEDA[1,2], C. VALENZUELA-SOLANO[3], R. HERNÁNDEZ-MARTÍNEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Departamento de Microbiología, Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, Baja California. [2]CONACYT-CICESE. Departamento de Microbiología. [3]INIFAP, sitio experimental Costa de Ensenada, Mexico. Calle del Puerto núm. 375-23, Fracc. Playa Eda.22880 Ensenada, Baja California, Mexico. E-mail: cdelgado@cicese.edu.mx*

Botryosphaeriacea cause Botryosphaeria dieback, which is responsible for damage to grapevines. In Mexico, *Lasiodiplodia brasiliensis* is a highly virulent causal agent of this disease, There is no effective control available, so integrated management includes eliminating diseased plants, and use of fungicides, wound protection, and biological control agents. The present study aimed to identify and evaluate microorganisms with biocontrol activity against Botryosphaeria dieback fungi, to provide growers with environmentally-friendly strategies for disease control. One hundred thirty-seven endophytic bacteria (137 isolates) were obtained from old grapevines, and 37 *Trichoderma* isolates were recovered from our collection. All isolates were evaluated for *in vitro* antagonistic activity against *L. brasiliensis*. Eleven *Bacillus* and four *Trichoderma* isolates were selected to assess their plant growth promotion characteristics abilities to produce volatile and non-volatile compounds with antifungal activity. Thirteen isolates were then selected for greenhouse assays. These isolates were applied directly to soil as preventive treatments, while *L. brasiliensis* was inoculated into hole made in the grapevine plants. Only the plants inoculated with *Bacillus subtilis* BEVP26 developed smaller lesions than experimental controls. Nine of the tested isolates were applied preventively in pruning wounds made on branches of grapevines established in a commercial vineyard. One hour later *L. brasiliensis* was applied at the top of each vine branch. Plants inoculated with eight of the isolates developed smaller lesions than plants inoculated only with *L. brasiliensis*. These potential biological control agents were molecularly identified as *B. subtilis*, *B. velezensis*, *T. asperellum*, or *T. longibrachiatum*.

POSTER PRESENTATIONS

Diversity of *Dactylonectria* and *Ilyonectria* species causing black foot disease in grapevine nursery stock in Uruguay. M. J. CARBONE, M. GELABERT, P. MONDINO and S. ALANIZ. *Universidad de la República, Facultad de Agronomía, Departamento de Protección Vegetal, Avenue Garzón 780, 12900 Montevideo, Uruguay. E-mail: salaniz@fagro.edu.uy*

Black foot, caused by "*Cylindrocarpon*"-like asexual morph fungi, is an important disease affecting young grapevines. Several studies have shown that grapevines are primarily infected by black foot pathogens in nurs-

eries, highlighting the role of infected nursery plants in spread of the disease. A previous study in Uruguay, recorded observation of typical black foot symptoms in grapevine planting material. These symptoms were brown to dark streaks that developed from the bases of rootstocks, wood necroses at trunk bases, sunken necrotic root lesions, and reduced root biomass. To determine the diversity of "*Cylindrocarpon*"-like fungi associated with black foot, grapevines of different cultivars grafted onto Gravesac, 1103P, SO4, 101-14 and 3309C rootstocks and with black foot symptoms, and ready to be sold to growers, were evaluated from 2017 to 2019. A total of 77 "*Cylindrocarpon*"-like strains were isolated and identified by DNA sequence analysis of the partial histone H3 gene. The BLAST search was conducted against type specimens in GenBank, and analysed phylogenetically by the Maximum Likelihood method. Five species of *Dactylonectria*, and three of *Ilyonectria*, were found. The most prevalent species was *D. macrodidyma* (32 isolates), followed by *D. novozelandica* (15), *D. torresensis* (ten), *I. liriodendri* (nine), *D. pauciseptata* (five), *D. valentina* (one), *I. robusta* (one) and *Ilyonectria* sp. (four). This study has increased knowledge of the etiology of black foot disease affecting Uruguayan nursery grapevines.

***Aspergillus* spp. causing Aspergillus vine canker on grapevines in Mexico**. E. A. RANGEL-MONTOYA[1], C. VALENZUELA-SOLANO[2] and R. HERNÁNDEZ-MARTÍNEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Departamento de Microbiología, Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, B.C. Mexico. [2]INIFAP, sitio experimental Costa de Ensenada. Calle del Puerto 375-23, Playa Ensenada, 22880, Ensenada, Baja California, Mexico. E-mail: ruhernan@cicese.mx*

Aspergillus comprises a diverse group of species with high economic and social impacts. Aspergillus vine canker is rare; the fungus infects vigorous new grapevine shoots and canes when the vines are being trained, entering through wounds. Recently, grapevines with symptoms similar to Aspergillus vine canker have been observed in Mexico. Wood samples from symptomatic plants were obtained from vineyards in Sonora, Baja California, and Guanajuato. The samples were sterilized with alcohol and flame and placed onto Potato Dextrose Agar. Seventeen isolates were obtained that showed morphology similar to *Aspergillus*. colony and microscopic characteristics were observed from cultures on Czapek Yeast Extract Agar and Malt Extract Agar. Phylogenetic analysis using calmodulin (CMD) and β-tubulin (BenA) genes revealed three species of *Aspergillus*. Nine isolates were identified as *A. niger*, seven as *A. tubingensis,* and one as *A. welwitschiae*. Pathogenicity studies were carried out using 'Merlot' grapevine plants. Eleven isolates were inoculated into host through wounds and onto leaves. *Aspergillus niger* A10BCMX, *A. niger* A8SMX, and *A. tubingensis* A13SMX were the most virulent, causing lesions of up to 2 cm in length after 50 d. On the inner sides of wounds, inside host cambium, powdery black conidia were found. On the leaves, necrotic lesions, mycelia, and black conidia formed. This is the first report of *Aspergillus* species associated with Aspergillus vine canker in vineyards of Mexico.

Molecular methods for detection and quantification of *Diplodia seriata* and *Phaeomoniella chlamydospora* in grapevine plants. M. ACUÑA[1,2], R. ROA[2], P. RODRIGUEZ[2], P. ARRAÑO[2], S. VARGAS[2], F. GAÍNZA-CORTÉS[2], G. DÍAZ[1] and M. LOLAS[1]. *[1]Universidad de Talca, Facultad de Ciencias Agrarias, Laboratorio de Patología Frutal, Casilla 747, Talca, Chile. [2]Viña Concha y Toro S.A, Centro de Investigación e Innovación, Fundo Pocoa s/n, Km10 Ruta K-650, Región del Maule, Pencahue, Chile. E-mail: felipe.gainza@conchaytoro.cl*

Grapevine trunk diseases (GTDs) are important phytosanitary problems affecting vineyards. to date, 133 fungal species, in nine families, have been associated with GTDs. In Chile, *Phaeomoniella chlamydospora*, *Diplodia seriata* and *Inocutis* sp. are the fungi most frequently isolated from adult plants affected by GTDs. Diagnosis of these fungi using classical methods is laborious and time-consuming. In addition, slow growth rates of fungi in semi-selective media compared with other microorganisms can generate false negatives due to overgrowth of these fungi, resulting in underestimation of incidence levels. Methods based on molecular detection can be complementary and effective tools for detecting these wood fungi, giving rapid and precise results. Fifty-four grapevine plants (Cabernet Sauvignon clone #337, on rootstock 110-14) were inoculated with *P. chlamydospora* or *D. seriata*. Ten months after inoculation, both phytopathogenic fungi were detected and quantified by qPCR, from four zones, 5 and 15 cm above or below the inoculated zones. Both fungi were detected in the distal zones. 27 plants were infected by *P. chlamydospora*, and 24 of 27 plants were infected by *D. seriata*. These results validate the complementary use of this molecular tool along classical methods, for diagnosis and detection of *P. chlamydospora* and *D. seriata* infec-

tions, enabling their detection in asymptomatic wood in the grapevine propagation material.

Investigating the role of *Fusarium* spp. on young vine decline in California. M. I. BUSTAMANTE[1], K. ELFAR[1], M. ARREGUIN[1], R. J. SMITH[2], L. J. BETTIGA[3], T. TIAN[4], G. A. TORRES[5], G. ZHUANG[6] and A. ESKALEN[1]. *[1]Department of Plant Pathology, University of California, Davis, CA 95616, USA. [2]University of California, Cooperative Extension Sonoma County, Santa Rosa, CA 95403, USA. [3]University of California, Cooperative Extension Monterey County, Salinas, CA 93901, USA. [4]University of California, Cooperative Extension Kern County, Bakersfield, CA 93307, USA. [5]University of California, Cooperative Extension Tulare County, Tulare, CA 93274, USA. [6]University of California, Cooperative Extension Fresno County, Fresno, CA 93710, USA. E-mail: mibustamante@ucdavis.edu*

From 2018 to 2021, young wine and table grape vineyards have shown decline symptoms in several Californian counties, including Fresno, Kern, Monterey, Napa, San Joaquin, Sonoma, Tulare, Yolo, and Yuba. Symptoms were diverse and characterized by poor or no growth during the season, dieback, sap exudation, and discoloration of vascular tissues around graft unions. Absence of feeder roots and graft failure have also been observed. In ten vineyards, the estimated decline incidence ranged from 5 to 50%. Isolations were carried out at the margins of vascular discolouration of affected vines, by placing wood sections (1 × 1 mm) onto acidified potato dextrose agar, with incubation for 7 d at 25°C in the dark. Although different fungal pathogens were obtained, including *Botryosphaeriaceae*, and *Phaeoacremonium* and *Cylindrocarpon*-like species, colonies of *Fusarium* were present in isolations from all the plant samples. Pure cultures of the *Fusarium* isolates were obtained from single hyphal tips, and were further identified using a phylogenetic approach. After DNA extraction, the translation elongation factor 1-alpha (*tef1*) and the RNA polymerase II second largest subunit (*rpb2*) partial gene regions were amplified and sequenced using, respectively, the primers EF1/EF2, 5F2/7cR and 7cF/11aR. Consensus nucleotide sequences were used to search the closest species in the NCBI database using BLAST. Phylogenetic trees revealed 13 *Fusarium* species, including members of the *F. fujikuroi*, *F. oxysporum*, *F. solani*, *F. sambucinum* and *F. incarnatum-equiseti* species complexes. The most frequent species (47.4%) was *F. annulatum* (*F. fujikuroi* species complex), and pathogenicity was confirmed for this fungus and the remaining 12 species by completing Koch's postulates in one-year-old 'Chardonnay' and '1103 Paulsen' vines.

***Diaporthe* spp. associated with dieback in Baja California vineyards**. C. A. DELGADO-RAMÍREZ[1], E. A. RANGEL ONTOYA[1], J. C. LEE-CONTRERAS[2], C. VALENZUELA-SOLANO[3] and R. HERNANDEZ-MARTINEZ[1]. *[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Departamento de Microbiología, Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, B.C. Mexico. [2]Universidad Autónoma de Nuevo León. Departamento de Fitopatología, Mexico. [3]INIFAP, sitio experimental Costa de Ensenada. Calle del Puerto 375-23, Playa Ensenada, 22880, Ensenada, Baja California, Mexico. E-mail: ruhernan@cicese.mx*

Diaporthe dieback, previously known as Phomopsis dieback of grapevine, is a trunk disease caused by several species of the *Diaporthe* genus. The main symptoms in affected plants are leaf spots, growth retardation, small bunches, fruit rot, and plant dieback. Some of the species associated with grapevines are *D. eres*, *D. viticola* and *Diaporthe ampelina,* with *D. ampelina* the most virulent species. In Mexico, cultivation of grapevines is of socio-economic importance, mainly in Sonora and Baja California. There are reports of species associated with Botryosphaeria dieback and Eutypa dieback, but there have been no reports of *Diaporthe* species in Mexico wine-producing regions. The present research isolated and characterized *Diaporthe* associated with grapevines. Isolates with morphological features similar to *Diaporthe* spp. were obtained from grapevine plants with dieback symptoms in different vineyards of Baja California. Identification of isolates was made using by morphological characterization, and molecular analyses using the ITS and EF1-α markers. Phylogenetic analysis allowed identification of strains of *D. ampelina, D. eres*, and *D. foeniculina*. Pathogenicity tests for these pathogens are being carried out. Although these species have been identified in vineyards in other countries, this is the first report of them on grapevines in Mexico.

The Hymenochaetaceous fungus *Arambarria destruens* associated with grapevine trunk diseases in Chilean patrimonial vineyards. J. CHILIAN, D. GRINBERGS and M. REYES. *Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Chillán, Chile. E-mail: jchilian@inia.cl*

Grapevine is an important fruit crop in Chile, with 141,000 ha for wine production. Grapevine Trunk Diseases (GTDs) are well studied in commercial cultivars such as Cabernet Sauvignon, Sauvignon Blanc, Merlot and Chardonnay, but there is lack of information regarding these diseases in Patrimonial Vineyards, mostly of País, Moscatel, Cinsault and Carignan cultivars. A frequent symptom observed in Patrimonial Vineyards was trunks with yellowish, spongy wood cankers. The objective of the present study was to identify the fungi associated with these symptoms. A survey was conducted from 2019 to 2021, on Patrimonial Vineyards in the south of Chile. Fungi were isolated on quarter strength potato dextrose agar (PDA) amended with antibiotics, and then purified on PDA. Yellow cottony colonies with irregular margins and dark areas were isolated from cankers (18%), and were preliminarily identified as Basidiomycetes based on microscopic structures including basidia and basidiospores. Mycelia was collected from the edges of 20 pure PDA cultures, Genomic DNA was extracted and used to amplify the internal transcribed spacer (ITS) region and the ribosomal large subunit fragments (LSU). ITS and LSU consensus sequences were compared to reported sequences, and combined to perform a multigenic analysis. The alignments were individually edited, and then concatenated, and phylogenetic trees were created using Maximum Parsimony and Maximum Likelihood algorithms. Based on these analyses, the Basidiomycetes species associated with the described symptoms was identified as the *Hymenochaetaceae* fungus *Arambarria destruens*.

Fungal species associated with grapevine decline in China. Y. Y. ZHOU[1,2], X. H. LI[1], L. N. WU[1], H. M. ZHANG[1], W. ZHANG[1] and J. Y. YAN[1]. *[1]Beijing Key Laboratory of Environment Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. [2]Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand. E-mail: zhwei1125@163.com*

Grapevine is an important fruit crop in China, with 765,018 ha of cultivation area, so ranking as the third in the world. To characterize fungi within the roots associated with grapevine decline, field surveys were conducted in Beijing, Hebei, Ningxia and Yunnan Provinces of China in 2021. Root samples were collected from symptomatic plants showing stunted growth, small leaves with yellow lesions, uneven sized grapes, and necrotic vascular or xylem tissues, as well as from neighbouring symptomless plants. Isolations were conducted, and isolates were identified based on morphological characteristics and multi-gene phylogenetic analyses. Ninety-three isolates were obtained from symptomatic plant samples, among which *Clonostachys* was the greatest proportion (41% of isolates), followed by *Neocosmospora* (26%), *Fusarium* (16%) and *Dactylonectria* (5%). *Rhizoctonia*, *Botrytis* and *Meyerozyma* were also isolated. From asymptomatic samples, 119 isolates were obtained. The four most frequent genera were the same as from symptomatic plants, while the proportion of *Fusarium* isolates was the greatest (28%). More genera were isolated from asymptomatic plants, including *Robillarda*, *Cylindrocladiella*, *Lasiodiplodia*, *Lophiostoma*, *Sacrocladium*, *Acrocalymma*, *Sporothrix*, *Stagonospora* and *Acremonium*. According to the phylogenetic results, the *Fusarium* isolates were clustered with three species, *F. oxysporum*, *F. commune* and *F. clavum*. *Neocosmospora* was identified as *N. solani*, *N. falciformis* and *N. pisi*. *Clonostachys* isolates were all identified as *C. rosea*. The main fungi isolated in this study were *Nectriaceae,* including *Neocosmospora* spp., *Fusarium* spp., *Dactylonectria* spp. and *Cylindrocladiella* spp., among which five species were first reported on grapevine. Pathogenicity of these fungi will be assessed, and relationships between fungal species of symptomatic and symptomless plants will be explored.

Presence and distribution of grapevine trunk diseases in the vineyards of Quebec, Canada. C. PROVOST[1], P. CONSTANT[2] and A.-A. DURAND[2]. *[1]CENTRE DE RECHERCHE AGROALIMENTAIRE DE MIRABEL, 9850 Belle-Rivière, Mirabel, Québec, Canada, J7N2X8. [2]INRS-ARMAND- FRAPPIER SANTÉ BIOTECHNOLOGIE, 531 Boul des Prairies, Laval, Québec, Canada, H7V 1B7. E-mail: cprovost@cram-mirabel.com*

Grapevine trunk diseases (GTDs) are very damaging for the sustainability of the vineyards heritage in all major wine-producing regions. GTDs can affect young plantations and aging vineyards. In both cases, risks from these diseases are increasing in Quebec vineyards, as many are more than 20 years old, and many producers plan to expand production. However, presence and distribution of GTDs in Quebec is unknown. This project characterized the distribution of GTDs in Quebec vineyards using to different criteria, including region, grapevine variety, and vineyard age. Five GTDs were assessed, including: Esca (Petri disease) (caused by *Phaeomoniella chlamydospora and Phaeoacremonium*

minimum), Eutypa dieback (*Eutypa lata*), Botryosphaeria dieback (*Botryosphaeriaceae* spp.), excoriosis (*Diaporthe ampelina*), and Black foot (*Cylindrocarpon*-like asexual morphs). Sampling was done in several vineyards, and qPCR analyses were performed to detect the GTD pathogens. Results showed the presence of all these diseases in Quebec vineyards, but mainly Botryosphaeria dieback. This information will allow identification of the scope of GTDs in Quebec vineyards, as background for study of the epidemiology of these diseases, and evaluation of cultural practices to limit their spread.

Identification and quantification of grapevine trunk disease and black-foot pathogens in soil, using real-time *PCR coupled with HRM*. S. TESTEMPASIS[1], E. STAVRIDOU[2], P. MADESIS[2] and G. S. KARAOGLANIDIS[1]. *[1]Aristotle University of Thessaloniki, Faculty of Agriculture, Forestry and Natural Environment, Plant Pathology Laboratory, Thessaloniki, Greece. [2]Institute of Applied Biosciences, Centre for Research and Technology Hellas, GR-57001 Thessaloniki, Greece. E-mail: testempa@agro.auth.gr*

Identification of plant pathogens and inoculum quantification in soil samples using conventional methods is labour-intensive and time-consuming. Therefore, development of rapid, and simple to perform PCR-based identification methods that use pathogen-specific primers, is necessary. A real-time quantitative PCR approach coupled with high-resolution melting (HRM) analysis, was developed with one primer set to identify and distinguish several fungal species associated with grapevine trunk and black-foot diseases. The developed method targeted several *Cylindrocarpon*-like asexual morphs of *Ilyonectria* or *Dactylonectria*, and *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Diplodia seriata*. The technique's reliability was first assessed on DNA extracted from pure fungal cultures. Melting curve analyses of the amplicons allowed distinction of all the target species with confidence levels > 99 %. HRM curve profiles were generated for each targeted genus/species. Identification of the target pathogens in fortified soil samples was achieved in a range confidence between 60 and 75%. The quantification of the detected pathogen DNA in the soil was assessed with quantitative PCR and sensitivity was evaluated using standard curves. These were constructed using serial dilution of plasmid calibrators containing the produced amplicons of qPCR. High PCR efficiency was reached (almost 100%), and results were reproducible throughout sample storage and calibration ranges for each specific plasmid calibrator. The reaction was linear over a large dynamic range ($R2 > 0.99$) of 5 $\log_{10}$ concentrations tested. The method was validated in soil samples from commercial grapevine nurseries, and the concentrations of detected DNA ranged from two to 10^5 copies. This study has developed a new molecular tool for detection and quantification of several GTD or Black foot pathogens in soil samples from grapevine nurseries, and may contribute to the optimization of production of multiplication material in grapevine nurseries.

Real-time PCR quantification of airborne inoculum of *Eutypa lata* and *Botryosphaeriaceae* spp. in California. J. CLERKIN, S. DUBROVSKY and A. L. FABRITIUS. *AL&L Crop Solutions, Inc., 7769 N. Meridian Rd., Vacaville, CA, U.S.A. E-mail: info@allcropsolutions.com*

Annually pruned vineyards and orchards suffer significant losses each year due to diseases caused by *Eutypa* and *Botryosphaeriaceae* fungi. Airborne spores of these fungi gain access to vines through pruning wounds, from which they grow into the wood, causing cankers, dieback and reduced growth. In California, diseases caused by *Eutypa lata* and *Botryosphaeriaceae* spp. are commonly initiated during winter, when seasonal rains trigger spore release by these fungi. The present study aimed to determine Rotorod- type spore traps could be used for capturing spores of *Eutypa lata* and *Botryosphaeriaceae* spp. from vineyard air. Real-time PCR was used to detect and quantify numbers of the spores in spore traps on weekly bases, from December till April in 2020, 2021 and 2022. Results were comparable with those obtained either by using glass slides or volumetric spore traps, and showed that *Eutypa* and *Botryosphaeriaceae* spp. spore release correlated with rain events in California. However, differences were found between release of the spores by these two groups of fungi. While *Botryosphaeriaceae* spores were present in the traps throughout each winter, *Eutypa* spores were detected sporadically. Data also suggested that at some locations, the spores were released without the presence of rain, possibly due to high humidity. Differences were also found in spore release patterns between old and newly established vineyards. In young vineyards, lower counts of *Botryosphaeriaceae* and *Eutypa* spores were detected compared to the older, established vineyards. The study also indicated that disease pressure could differ by location of the vineyard and the spore trap. Release of spores did not always correlate with the weather data, suggesting that the predictions of the spore release cannot be based only on weather forecasts.

Effect of cover crops on the dispersal of *Phaeomoniella chlamydospora* inoculum in vineyards. M. BERBEGAL[1], D. PINNA[1], E. GONZÁLEZ-DOMINGUEZ[2], G. HASANALIYEVA[3], T. CAFFI[3], V. ROSSI[3] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022-Valencia, Spain. [2]Horta srl., Via Egidio Gorra 55, 29122 Piacenza, Italy. [3]Department of Sustainable Crop Production (DIPROVES), Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29122 Piacenza, Italy. E-mail: jarmengo@eaf.upv.es*

Effect of different cover crops on the dispersal of *Phaeomoniella chlamydospora* inoculum was evaluated in an experimental plot in Piacenza, Italy, during two consecutive growing seasons. We hypothesized that cover crops may act as physical barriers to reduce dispersal of *P. chlamydospora* airborne and rain-splash inoculum. Spore traps (microscope slides) were placed in plots with natural grass, bare soil, or four or one cover crops grown in, respectively, the first and second seasons. Grapevine cuttings artificially inoculated with *P. chlamydospora* were placed on the soil in the middle of every two subplots per treatment; in each subplot two spore traps were located near, or 40 cm above, the inoculum source. The traps were replaced weekly from December 2018 to June 2019, and from December 2019 to May 2020. Quantitative PCR, using specific primers for *P. chlamydospora*, was used to determine DNA concentrations in the spore trap samples, and the effects of the experiment treatments on inoculum dispersal. Rainfall and average temperature data were obtained from a weather station placed in the experimental site. Soil coverage was also evaluated according to cover cropping development. Dispersal of *P. chlamydospora* was associated with rainfall events in both seasons. Results from the first season showed no reduction in *P. chlamydospora* DNA detected in grass and cover crop plots relative to the bare soil. This is most likely explained by non-uniform cover and plant development due to dry weather conditions. The second season results showed lower DNA concentrations in natural grass and cover crop plots, especially during weeks with high levels of detection, which accounted for most of the spore dispersal. Analysis for the traps placed 40 cm above the inoculum sources confirmed the reductions in spore dispersal in grass and cover crop plots, relative to the bare soil plots.

Environmental conditions influencing survival and development of reproductive structures of *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*. M. BERBEGAL[1], E. GONZÁLEZ-DOMÍNGUEZ[2] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022-Valencia, Spain. [2]Horta srl., Via Egidio Gorra 55, 29122 Piacenza, Italy. E-mail: jarmengo@eaf.upv.es*

An *in vitro* experiment was carried out to determine effects of temperature on development of perithecia of *Phaeoacremonium minimum* and pycnidia of *Phaeomoniella chlamydospora* on grapevine cuttings. Pieces of 1-year-old grapevine cuttings [(rootstock 110 Richter (110 R)] were inoculated with four isolates of *Pm. minimum* (representing complementary mating groups) or two isolates of *Pa. chlamydospora*, and were incubated at 5, 10, 15, 20, 25 or 30°C under continuous white light. After 6 weeks, the cuttings were examined under a stereoscope to evaluate formation of mature reproductive structures of the fungi. Both species produced abundant fruiting bodies at temperatures of 15 to 25°C, but *Pm. minimum* produced more perithecia at 25°C and *Pa. chlamydospora* produced more at 20°C. At 30°C, only very few reproductive structures were observed. A field experiment was conducted in two vineyards in Spain, located in Villar del Arzobispo (Valencia) and Villena (Alicante), in which 1-year-old grapevine cuttings (rootstock 110 R), inoculated with the above-mentioned isolates, were placed into perforated aluminium trays each covered with a plastic grid, and exposed to environmental conditions from December 2019 until June 2020. Cuttings were randomly collected every 15 d, and were examined under a stereoscope to determine the presence/absence of fruiting structures. Fungus isolations were also carried out to verify survival of the inoculated fungi. No fruiting bodies were observed during the experiment, but both species were recovered from the cuttings. A GLM analysis showed survival differences between species and localities with time. Differences observed between *in vitro* and field experiments indicated that development of the pathogen reproductive structures was infrequent in vineyards.

Comparing grapevine trunk disease incidence at different sites in the Tokaj wine region of Hungary. P. BALLING[1,2], T. KOVÁCS[1,2], A. KNEIP[1,2] and P. MOLNÁR[2]. *[1]Research Institute for Viticulture and Enology, Tokaj, Könyves Kálmán u. 54., H-3915 Tarcal, Hungary. [2]University of Tokaj Hegyalja, Faculty of Lorántffy, Department of Viticulture and Enology, Eötvös u.7., H-3950 Sárospatak, Hungary. E-mail: balling.peter@tarcalkutato.hu*

Grapevine Trunk Diseases (GTDs) have severe impacts in all grape-producing countries. The presence of a GTD pathogen in vines does not usually result in the immediate appearance of disease symptoms. Moreover, there is still limited information on the importance of environmental factors on disease incidence. The present study estimated occurrence of GTD in the Tokaj wine region, to gain knowledge of the biotic and abiotic factors affecting disease incidence. Four vineyards within 15 km were studied between 2016 and 2019, with different topologies, soil types, varieties, and ages. In the same period, 50 random sites across the Tokaj region were selected, to monitor GTD symptoms in different vineyards. Topology, slope characteristics, and soil type were the most important abiotic factors affecting the incidence of GTD symptoms. Vineyard age was the biotic factor with greatest effect, with disease incidence increasing with age. Neither disease incidence nor any biotic or abiotic factor was correlated with infection incidence, but there were differences among the sites. slope characteristics also influenced symptom incidence. August was the best month to detect infected vines, as symptoms were observed more frequently. Removing dead vine parts from the sites also affected disease incidence. These results indicate that infected grapevine cuttings can act as primary infection sources of healthy vines.

Spring shoot thinning wounds are susceptible to grapevine trunk disease pathogens. M. R. SOSNOWSKI[1,2] and M. R. AYRES[1]. *[1]South Australian Research and Development Institute, Adelaide SA 5001, Australia. [2]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: mark.sosnowski@sa.gov.au*

The grapevine trunk diseases (GTDs) Eutypa (ED) and Botryosphaeria dieback (BD) are primarily caused by infection of winter pruning wounds by spores of species of the *Diatrypaceae* (for ED) and *Botryosphaeriaceae* (for BD). Shoot thinning is undertaken during the spring to improve airflow and reduce foliar disease, increase leaf exposure to sunlight for increased photosynthesis, maintaining crop yield and quality, and reduce the number of pruning wounds in the following winter. Spore trapping in Australian vineyards has detected GTD pathogens throughout spring and summer, in association with rainfall. BD pathogens can infect green grapevine tissues, but there is no evidence of green shoot infection by ED pathogen spores. A preliminary trial was established on Shiraz vines, grown in pots in a shade house. In November 2020, green shoots were each either cut from 1 cm above the second or third nodes leaving a smooth pruning wound, or whole green shoots were each torn off at the joint between lignified cane and the shoot base, leaving a rough socket wound. Wounds were artificially inoculated with 200 spores of *Eutypa lata* (ED) or *Diplodia seriata* (BD). Spurs were removed 9 months later and assessed for presence or absence of the pathogens. *Eutypa lata* was recovered from 62% of pruning wounds and 69% of socket wounds, and isolation frequencies for *D. seriata* were 96% from pruning wounds and 91% from socket wounds. No pathogens were isolated from uninoculated controls. These results, and reports of detection of ED and BD pathogen spores throughout spring and summer, show that it is now important to determine vineyard infection risks under natural conditions following shoot thinning activities.

Investigating how *Lasiodiplodia brasiliensis* colonizes grapevine tissues. E. A. RANGEL-MONTOYA and R. HERNÁNDEZ-MARTÍNEZ. *Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Departamento de Microbiología, Carretera Tijuana-Ensenada 3918, Zona Playitas, 22860, Ensenada, B.C. Mexico. E-mail: ruhernan@cicese.mx*

Lasiodiplodia brasiliensis is a virulent pathogen among *Botryosphaeriaceae*, and this fungus was recently reported in Sonora and Baja California, Mexico. Little is known about how *Botryosphaeriaceae* interact with grapevines during infection processes. Grapevine colonization by *L. brasiliensis* was assessed using histological techniques. One-year-old rooted cuttings of 'Cabernet Sauvignon' were inoculated by mechanical wounding, and then maintained in greenhouse conditions for 2 months. Transverse and longitudinal sections (70 µm thick) were then obtained and stained with 0.1% Toluidine B for phenolic compounds; 5% iodine and 10% potassium iodide for starch; 0.001% Sudan black IV for suberin deposits; 0.1% phloroglucinol-HCl and Mäule stain for lignin, or 0.02% Calcofluor M2R White + 0.5% + Congo Red for cellulose and hemicellulose. Hyphae were observed using the Fontana-Masson stain. Observations were carried our using a Nikon Eclipse E200 microscope with a camera AxioCam HRc camera, and with epifluorescence microscopy using an Axio- Vert200 microscope with a HBO100 100W Mercury Lamp with ebq100 power. Cellulose and suberin were observed using a DAPI filter (excitation at 330–380 nm, emission at 420 nm), and a TEXAS RED filter (excitation at 542–595 nm, emission at 644 nm). Infected plants lacked starch in ray parenchyma cells; and cellulose, hemicellu-

lose, and lignin in the lesions. Phenolic compounds and suberin were observed in the cork and vascular cambium tissues, vascular bundles, and pith. The fungus colonized vascular cambium, vascular bundles, occlusions, and pith. Melanized hyphae were observed mainly in the pith. These observation indicate that *L. brasiliensis* overcame the defence mechanisms of host plants, and modified cell walls, mainly degrading hemicellulose and using starch as a carbon source. With time, the pathogen degraded lignin and suberin, colonizing the rays and inducing the formation of the typical Botryosphaeria canker.

Endophytic mycobiome and anthocyanins, two key components in grapevine leaves affected by 'tiger stripes'. G. DEL FRARI[1], C. INGRÀ[2], A. GOBBI[3], M. RØNNE AGGERBECK[4], T. NASCIMENTO[1], A. CABRAL[1], H. OLIVEIRA[1], A. FERRANDINO[2], L. HESTBJERG HANSEN[3] and R. BOAVIDA FERREIRA[1]. *[1]LEAF—Linking Landscape, Environment, Agriculture and Food— Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisbon, Portugal. [2]Department of Agricultural, Forestry, Food Sciences (DISAFA), University of Turin, Largo P. Braccini, 2, Grugliasco, Torino 10095, Italy. [3]Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark. [4]Department of Environmental Science, Aarhus University, 4000 Roskilde, Denmark. E-mail: gdelfrari@isa.ulisboa.pt*

'Tiger stripe' patterns manifest in grapevine leaves affected by grapevine leaf stripe disease (GLSD) and Esca proper. In red grape varieties, affected leaves display interveinal necrotic lesions, followed by development of red lamina portions, chlorotic tissues, and green tissues, with green tissues closely associated to the main leaf veins. Two other colouring patterns are occasionally found in symptomatic leaves. One follows the sequence: necrotic-purple-green tissue, which has been correlated to the black dead arm syndrome (BDA). The other pattern follows the sequence necrotic-green tissue, and is found in pre-apoplectic shoots. Despite these differences, it is still unclear the identity of the triggering factor(s) that lead to different coloring patterns, the anthocyanins profile of symptomatic leaves, and the involvement of the endophytic mycobiome. We investigated the endophytic mycobiome composition and anthocyanins profiles of grapevine leaves with GLSD and BDA-associated symptoms. Plants of 'Cabernet Sauvignon' and 'Touriga Nacional' were used, sampling symptomatic and asymptomatic leaves in June 2020 and July 2021, in a vineyard in Lisbon. Mycobiome profiles were characterised using DNA metabarcoding (Illumina® NGS), targeting the ITS1 region, employing primer set ITS1F2-ITS2, and anthocyanins profiles were analyzed using high-performance liquid chromatography. The leaf mycobiomes analysis revealed 125 taxa with relative abundances greater than 0.1%. The most common taxa were *Plasmopara viticola* (Oomycete); *Cladosporium* spp., *Aureobasidium* spp., and *Stemphylium* spp. (Ascomycetes); and *Sporobolomyces* spp. and *Filobasidium* spp. (Basidiomycetes). Differences in alpha and beta diversity and taxa over- and under-representation were detected when examining sampling year, cultivar, and symptom type. Similarly, the anthocyanins profiles, dominated by peonidin 3-O-glucoside and cyanidin 3-O-glucoside, differed quantitatively and qualitatively for cultivar and symptom type. These data indicate strong links among symptom colouring patterns, plant responses and mycobiome profiles.

High diversity of fungal grapevine trunk pathogens isolated from young canes, and the report of *Neofabraea kienholzii* in 'Albariño' grapevines in Spain. V. REDONDO-FERNÁNDEZ[1], A. ALONSO-NÚÑEZ[1,2], N. CAMBRA-GONZÁLEZ[1], S. RODRÍGUEZ-GARCÍA[1], L. AREAL-HERMIDA[2] and C. SIEIRO[2]. *[1]ProPlantae Sanidad Vegetal SL, Rúa das Pontes Nº6 36350 Nigrán, Spain. [2]Biomedical Research Center (CINBIO), Department of Functional Biology and Health Sciences. Microbiology Unit. Universidade de Vigo, 36310 Vigo, Spain. E-mail: info@proplantae.es*

Albariño is a grapevine cultivar grown in NW Spain and northern Portugal, which produces excellent white wines with increasing export amounts. Reducing productivity has been observed in a mature Albariño vineyard near the Miño river between Galicia and Portugal. In 2021, 90 grapevine plants from three different plots were labelled and monitored. After pruning in January 2022, 1-year canes were analyzed for grapevine trunk disease (GTDs) symptoms. A total of 450 pure fungus cultures of approx. 70 morphotypes were obtained. One representative isolate for each morphotype was selected. Genomic DNA was extracted, and the Internal Transcribed Spacer (ITS) region was amplified and sequenced by Sanger dideoxy sequencing technology. Blast searches classified the isolates potentially involved in GTDs complex into at least 18 species in nine genera: *Botryosphaeria*, *Cadophora*, *Diaporthe*, *Diplodia*, *Neofusicoccum, Neopestalotiopsis, Pestalotiopsis, Phaeoacremonium* and *Phaeomoniel-*

la. An isolate of *Neofabraea* was also detected, which could also be involved in GTDs. Research is in progress to confirm the pathogen identifications. Approx. 20 morphotypes with potential activity against GTDs were also found including: *Alternaria*, *Arthrinium*, *Aureobasidium*, *Clonostachys*, *Cladosporium*, *Epicoccum* and *Trichoderma*. Next-generation sequencing provides information about microbial diversity and dynamics, while traditional culturing methods allow evaluation of potential antagonism by endophytes. This study also identified a large cultivable mycobiota associated with young grapevine shoots, that was obtained through non-destructive trunk sampling.

The BIOBESTicide project: action of *Pythium oligandrum* on grapevine trunk diseases, and its impact on microbial communities. S. LOPEZ, A. CHATAIGNER and M. C. DUFOUR. *INRAE, Bordeaux Sciences Agro, ISVV, SAVE, 33140 Villenave d'Ornon, France. E-mail: severine.lopez@inrae.fr*

Grapevine trunk diseases (GTDs) have become important in viticulture (15% yield losses). Since the 2001 ban on the use of sodium arsenate, development of alternative control strategies, including biocontrol, has become increasingly important. *Pythium oligandrum* is a promising biocontrol agent, which can improve plant health by increasing natural host defences, reduce GTDs by at least 40%. The BIOBESTicide project (BIO-Based pESTicides production for sustainable agriculture management plan) aims to commercialise production of a biopesticide solution for GTDs management. Efficiency of a *P. oligandrum*-based formulated product will be evaluated in nurseries and vineyards, and the environmental impacts of the product will be assessed to ensure its safety. An objective supported by INRAE is to increase understanding of the role of plant microbiota in plant health, and the effectiveness of biocontrol treatments. This objective is in two parts: 1) assessment of the impacts of the biopesticide on grapevine microbial communities using a microbial community diversity approach; and 2) understanding the factors contributing to success or failure of the biopesticide. An experiment on 240 grafted vines (Merlot on rootstock SO4) in semi-controlled conditions was carried out in a greenhouse. Vines were treated with a *P. oligandrum* formulation or left untreated, and were then inoculated with either *Neofusicoccum parvum* or *Phaeomoniella chlamydospora*. At different times during the following 3 months, plants were harvested and leaves, wood and rhizospheres were sampled. Potential changes in the microbial communities associated with GTD pathogen or *P. oligandrum* inoculation were assessed using Illumina high-throughput sequencing. The results will extend the approval dossier for submission in all European countries to ensure environmental safety of *P. oligandrum* as a biocontrol product.

Will it be possible to predict Esca symptom manifestation based on the grapevine wood microbiome? B. GARCÍA-GARCÍA[1], M. M. ALGUACIL[2], A. ACEDO[3] and L. MARTÍN[1]. *[1]Plant Protection Department, Extremadura Scientific and Technological Research Center (CICYTEX), Badajoz, Spain. [2]Center for Edafology and Applied Biology of Segura (CEBAS-CSIC) Murcia, Spain. [3]Biome Makers Inc., West Sacramento, California, USA. E-mail: laura.martin@juntaex.es*

Esca is an important grapevine disease, and several fungi have been described as causal agents of the syndrome because they have been isolated from degraded wood of leaf-esca symptomatic vines. The present study compared culture dependent and -independent methods for identifying and quantifying fungal communities. Twenty four wood samples from six vines (three leaf-esca symptomatic and three asymptomatic) were analysed in three ways: *i*) culture-dependent microbiological analysis; *ii*) non-culture dependent microbiome analysis using Illumina Technology; and *iii*) quantification of *Phaeoacremonium minimum* (Pm) and *Phaeomoniella chlamydospora* (Pch) by TaqMan q-PCR. Four different within vine sample points were compared to determine the most informative point for pathogen detection. These were: above the graft (A), at the strain cross (B), or arms (C and D). Fungi detected included: Pm, Pch, *Diplodia seriata*, *Diaporthe ampelina* and Basidiomycete species associated with Esca, as well as other genera including *Penicillium*, *Acremonium*, *Alternaria* and *Epicoccum*. The number of OTUs found in culture (11) represented only 0.73% of the 1512 OTUs found using Illumina. The methodology affected α-diversity indices, but sampling point did not affect the wood microbiomes. Pch was detected in 22 samples using Illumina, in nine using TaqMan q-PCR, and in four using culture dependent methods. Pm was detected in, respectively, six, five, and ten samples, indicating greater detection of this pathogen using culture dependent analysis. A non-metric multidimensional scaling (NMDS) analysis of β-diversity showed discrimination of fungal communities from Esca affected vines (with manifestation of "tiger stripe"/apoplexy in canopies). Some fungi have been selected as indicators of apoplexy. When modelling relationships between network properties and disease

phenotypes, samples without Esca symptoms tended to have lower proportions of co-exclusions.

Effects of temperature on *in vitro* biocontrol of *Diplodia seriata* by psychrotolerant *Pseudomonas* strains. A. LARACH[1,2], P. VEGA-CELEDÓN[2], P. SANHUEZA[1], N. RIQUELME[1], M. SEEGER[2] and X. BESOAIN[1]. *[1]Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota 2260000, Chile. [2]Laboratorio de Microbiología Molecular y Biotecnología Ambiental, Chemistry Department & Centro de Biotecnología Daniel Alkalay Lowitt, Universidad Técnica Federico Santa María, Avenida España 1680, Valparaíso 2340000, Chile. E-mail: ximena.besoain@pucv.cl*

Timely preventive control for Botryosphaeria dieback is important for disease management, and biocontrol agents have this protective effect. Conversely, from an epidemiological viewpoint, it is important to consider that the infections can occur at different temperatures. Knowledge of biocontrol capabilities of microorganisms at different temperatures will allow improvement of selection of biocontrol agents. The *in vitro* capabilities of four psychrotolerant *Pseudomonas* strains (CpR2b, GcR15a, TmR1b, TmR8) to reduce mycelium development of three *Diplodia seriata* isolates (2120, 2142 and 2183) were assessed using the agar diffusion method at low (8°C), medium (20°C), or high temperatures (35°C). At 7, 14, and 21 d after microbe confrontations, *D. seriata* colony radii were measured. The three *D. seriata* strains grew at 8°C, 20°C, and 35°C. two strains of *Pseudomonas* showed consistent inhibition of the mycelium growth of all three *D. seriata* isolates at 8°C and 20°C. However, at 35°C variation in inhibition was observed amongst the isolates.

Phylogenetic host range in the Botryosphaeriaceae. I. SILVA-VALDERRAMA[1], J. R. ÚRBEZ-TORRES[2] and T. J. DAVIES[1]. *[1]University of British Columbia, Botany Department, Vancouver, BC, Canada. [2]Summerland Research and Development Centre, Agriculture and Agri-Food Canada, Summerland, BC, Canada. E-mail: ibsilva26@gmail.com*

Botryosphaeriaceae is a diverse family composed of endophytes, pathogens, and saprobes, and is ubiquitous in terrestrial plant biomes. Anthropogenic movement of some fungi, for example, on infected host material, is a major concern, as the family includes species known to be pathogens in endophyte communities. In grapevines (*Vitis vinifera* L.), Botryosphaeria dieback is an important grapevine trunk disease, causing severe economic losses. Prevention of new infections remains the most effective disease management tool, but it is difficult to identify highly virulent pathogen isolates before they emerge. Phylogenetics are useful tools for identifying pathogens likely to shift to new hosts, and the potential for disease emergence following host jumps. However, the taxonomy of *Botryosphaeriaceae* is in flux, and there is little phylogenetic information on the species infecting grapevines. The phylogeny of *Botryosphaeriaceae* species infecting *V. vinifera* was constructed, using molecular sequence data, and the link between host breadth and pathogen phylogenetic relatedness was explored. The distribution of *Botryosphaeriaceae* hosts across the megaphylogeny of vascular plants was outlined. Variation in host breadth was large among *Botryosphaeriaceae* pathogens, revealing the complex associations linking host and fungal phylogenies. This research provides a first step towards predicting emergence of known pathogens on new hosts. This approach will be useful for coordinated global monitoring of high-risk species within *Botryosphaeriaceae*.

Analysis of below-ground grapevine microbiomes identifies *Fusarium* spp. aggravating severity of grapevine trunk diseases. Y. H. LI[1], X.H. LI[1], W. ZHANG[1], J. ZHANG[1,2], H. WANG[1], J. B. PENG[1], X. C. WANG[1] and J. Y. YAN[1]. *[1]Beijing Key Laboratory of Environment Friendly Management on Fruit Diseases and Pests in North China, Institute of Plant Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China. [2]College of Plant Protection, Hebei Agricultural University, Baoding 071000, China. E-mail: jiyeyan@vip.163.com*

Grapevine trunk diseases (GTDs) are a disease complex which is a major threat for viticulture. The microbiota colonizing the belowground parts of plants can form complex associations and promote plant productivity and health in natural environments. This present study investigated below-ground fungal communities in symptomatic or asymptomatic grapevines. Spatial dynamics of the fungal communities associated with three soil-plant compartments (bulk soils, rhizospheres, roots) were characterized using ITS high-throughput amplicon sequencing across 2 years. The diversity and composition of fungal communities were largely affected by soil-plant compartments ($P < 0.001$; 12.04% of variation explained) and sampling year ($P < 0.001$; 8.83 %), where-

as GTD symptomatology exhibited weaker, but statistically significant, association with these characteristics ($P < 0.001$; 1.29 %). Effects of GTDs symptomatology were particularly prominent in root and rhizosphere community comparisons. Some confirmed GTD-associated pathogens were detected in the communities, but their relative abundances were not related to symptomatology. Further analyses revealed that *Fusarium* spp. were enriched in symptomatic roots and rhizospheres compared to asymptomatic counterparts, suggesting that their abundances were positively correlated with symptomatic vines. Inoculation tests showed that *Fusarium* isolates, similar to *Dactylonectria macrodidyma* which is a pathogen associated with grapevine black foot. caused dark brown necrotic spots on grapevine stems and rots that blackened lateral roots. Disease indices were greater in the co-inoculation treatment than due to single inoculations with either a *Fusarium* isolate or *Dactylonectria macrodidyma*, suggesting that *Fusarium* spp. can exacerbate disease severity when inoculated with other known GTD-associated pathogens. These results demonstrate the effects of fungal root and rhizosphere microbiota on GTDs severity, and provide new insights into GTDs and potential control strategies.

Enhancing the regrowth of vines following remedial surgery. M. R. SOSNOWSKI[1,2] and M. R. AYRES[1]. *[1]South Australian Research and Development Institute, Adelaide, SA 5001, Australia. [2]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: mark.sosnowski@sa.gov.au*

Grapevine trunk diseases (GTDs) reduce yields, and cause vine decline and death. Remedial surgery has long been used to rejuvenate grapevines, and has more recently been demonstrated to control GTDs in Australia. Current recommendations are to cut out all visibly affected wood, and train watershoots to replace affected vines. However, as little as 42–55% of 30-year-old trunks have been reported to produce shoots following surgery of Shiraz and Cabernet Sauvignon vines. A trial was established in four rows of a 22-year-old Cabernet Sauvignon vineyard in the Clare Valley, South Australia, with 100% incidence of GTD, to evaluate methods reported anecdotally to induce watershoots on trunks following remedial surgery. Treatments included hitting with a hammer, making an X-shaped cut with a tomahawk, rubbing bark with a wire brush, application of cyanamide (Dormex) plant growth regulator, or grafting with a chip bud, and surgery was carried out in winter or spring for comparison. Over each of the following 3 years, a further four vineyard rows were subjected to remedial surgery, and stumps without watershoots were chip bud grafted. In the final year, watershoots were retained from the previous year. Natural watershoot production was 83–85%, with no difference between the seasons the vines were cut, and there was also no influence of most of the treatments applied in the first year. The exception was for grafting, which increased shoot production by 11%. In subsequent years, natural watershoot production varied from 70–78%, and grafting trunks without watershoots increased shoot growth by 6–19%. In the final year, retention of watershoots from the previous season resulted in 87–88% shoot growth, similar to that of grafting in previous years. These results indicate that regrowth of vines subjected to remedial surgery for control of trunk diseases can be enhanced by grafting or retaining shoots from the previous year.

Management of grapevine trunk diseases using remedial surgery. E. VAN ZIJLL DE JONG[1], H. TERNENT[2], S. ST GEORGE[2], R. KALLAS[2] and M. SOSNOWSKI[3,4]. *[1]Linnaeus Limited, PO Box 1199, Gisborne 4040, New Zealand. [2]Villa Maria Estate Ltd, 118 Montgomerie Road, PO Box 43046, Mangere, Auckland, New Zealand. [3]South Australian Research and Development Institute, Adelaide, SA 5001, Australia. [4]School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, SA 5005, Australia. E-mail: eline@linnaeus.co.nz*

Remedial surgery is increasingly used in New Zealand to improve grape yields and extend lifespans of grapevines infected with trunk diseases. Infected vines are each "renewed" by removing the diseased trunk above the graft union, and growing a new shoot to replace the old trunk. Remedial surgery was carried out in winter and spring over 3 years in mature (>18 years) commercial vineyard blocks of 'Sauvignon blanc', 'Cabernet Sauvignon' or 'Merlot' vines. This was to study disease progression and efficacy of remedial surgery, and determine the optimal time to carry out this practice. Dieback was widespread in the canopies in 'Sauvignon blanc' and 'Cabernet Sauvignon' (>90%), and only in 'Merlot' incidence of dieback increased from 64 to 91% over the three growing seasons. During this time, severity of dieback increased in 'Cabernet Sauvignon' but not in 'Sauvignon blanc' or 'Merlot'. Vine recoveries after remedial surgery were high in Merlot. In 'Sauvignon blanc' and 'Cabernet Sauvignon', some of the vines with more severe disease symptoms did not recover from remedial

surgery. For all three cultivars, there were no differences in recoveries between vines cut in winter or spring. The vines were out of production for the first growing season following remedial surgery, but by the third year there were no significant differences in yields between these vines and the untreated control vines in 'Cabernet Sauvignon' and 'Merlot'. In 'Sauvignon blanc', a change in pruning strategies affected the yield in the reworked vines. There have been no obvious signs of trunk disease in the reworked vines since the trunk surgery was carried out.

GTD prevention: A practical application of *Trichoderma* formulations under field conditions. J. J. GUERANOGALES, L. MÉNDEZ-GRANO DE ORO, M. J. DORADO-RICO and L. MARTÍN. *Plant Protection Department, Extremadura Scientific and Technological Research Centre (CICYTEX), Spain. E-mail: laura.martin@juntaex.es*

Grapevine Trunk Diseases (GTDs) are a major concern for grapevine growers all over the world, but there are no curative fungicides for these diseases. Protection of pruning wounds has been shown to be effective for avoiding wood infection by pathogens. Biological control agents (BCA) to protect pruning wounds have recently been authorized by the Spanish Ministry of Agriculture for management of GTDs. Three BCA products based on different strains of *Trichoderma* were evaluated in two commercial vineyards. In the first trial, three products were compared under uniform field conditions, in a 'Tempranillo' vineyard (head training system), 3% of Esca incidence. . In the second trial, one product were evaluated in three different years in a ' Macabeo' vineyard (transformed from double cordon to double Guyot system), with no foliar GTD symptoms. Evaluations of the disease control treatments were made by microbiological analyses of wood samples (n = 20) to isolate GTD fungal pathogens and *Trichoderma* spp. Assessment were carried out at 30 and 90 d after treatment applications. *Trichoderma* was well-established (in 50–100% of pruning wounds, and there was little difference between the three formulations. Mean Percentage of Infection (MPI: number of GTD infected samples/number of total samples × 100) showed low recovery of *Phaeomoniella chlamydospora* and *Botryosphaeriaceae* species. Efficacy of the treatments was recorded by the mean percent disease control (MPDC), calculated as 100 × (1 - (MPI treatment/MPI non-treated control). These results indicate that *Trichoderma* treatments could be effective in vineyards with head training systems, and that their efficacy is correlated with BCA implementation and also the disease pressure in vineyards.

Biological control of *Diplodia seriata* inoculum in grapevine pruning debris in Chilean orchards. E. DONOSO, L. ROMERO, W. HETTICH, D. BASCUÑAN, C. GARCIA and J. FIGARI. *Bio Insumos Nativa SPA, Pc Antilhue Lote 4b2, Maule, Chile. E-mail: edonoso@bionativa.cl*

Increased understanding of the use of biological control agents, with mechanisms other than those of chemical fungicides, has opened new opportunities for pathogen control, enabling new biocide strategies that consider periods of host tissue susceptibility and the residual efficacy times for these products. Incorporation in control strategies of biological agents active against resistant and reproductive pathogen structures (e.g. sclerotia or pycnidia) will allow to control the pathogens in new places and times, providing different tools for disease management. A field trial was carried out where a commercial formulation containing *Trichoderma* spp. and *Bionectria ochreoleca*, Mamull® (Bio Insumos Nativa SPA) was applied to Cabernet Sauvignon vine prunings, which had pycnidia of *Diplodia seriata*. Selected cane samples (n = 30), were observed under a magnifying glass, determining numbers of pycnidia cm^{-2}, and marking and coding five sectors each of 1 × 3 cm on each sample. The samples were then placed in the field, where they were sprayed with Mamull®, at 100 g hL^{-1}, using a herbicide bar and 300 L ha^{-1}. At 30 and 180 d, levels of pycnidium parasitism were assessed, and viability of released conidia was determined after addition of sterile water. Both treatments reduced viable pycnidia ($P < 0.05$; LSD tests), by 25.3% at 30 d and 72.3% at 180 days. Levels of parasitism from both treatments were 34.5% at 30 d and 59% at 180 d, but only 12.3% from the controls ($P < 0.01$; LSD tests). This trial has demonstrated a promising additional application of biocontrol agents to for control of grapevine wood pathogens.

Effects of combined treatments with LC2017 and *Trichoderma atroviride* strain I-1237 on disease development and host defence responses in grapevines infected by *Lasiodiplodia theobromae*. P. REIS[1†], V. MONDELLO[2†], I. DINIZ[1,3], A. ALVES[4], C. REGO[1] and F. FONTAINE[2]. [1]*LEAF - Linking Landscape, Environment, Agriculture and Food, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade*

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Grapevine trunk diseases (GTDs) cause major problems for viticulture, and Botryosphaeria dieback is an important GTD. Increasing restrictions on effective chemical fungicides for mitigation of GTDs have led to expansion of research on biocontrol agents (BCAs), and new and improved fungicides with more efficient and innovative methods for pathogen control. Incorporation of these new strategies as part of integrated disease management has only recently generated interest. The present study aimed to; (*i*) evaluate effect of the combination of Esquive® (a *Trichoderma*-based product) and LC2017 (a low copper product), for control of *Lasiodiplodia theobromae*, on grapevines 'Cabernet Sauvignon' and 'Touriga Nacional' in greenhouse experiments; and (*ii*) investigate their elicitor effects on plant defence responses, through analyses of expression of a set of genes from inoculated grapevines. The pathogen was always re-isolated from the infected tissues, and able to cause wood discolouration. Touriga Nacional developed longer lesions than Cabernet Sauvignon, and applications of both products did not reduce lesion lengths when compared to LC2017 applied alone. The elicitor effect of LC2017 on grapevine defence was confirmed by gene expression analyses, and no significant differences were found between plants treated with LC2017 or with both products. A specific response related to cultivar was verified, but this apparently unique interaction between product, cultivar, and pathogen remains to be further investigated.

Will forestry waste save grapevines and help control GTDs? L. MERLEN[1,2], L. GALIMAND[1], C. TARNUS[1], C. GERARDIN[3], P. GERARDIN[3] and M. GELLON[1]. *[1]Laboratoire Vigne Biotechnologie et Environnement EA 3391, Université de Haute-Alsace, 33 rue de Herrlisheim, F-68000 Colmar, France. [2]Laboratoire de Photochimie et d'Ingénierie Macromoléculaires UR 4567, Université de Haute-Alsace, 3 bis rue Alfred Werner, F-68093 Mulhouse cedex, France. [3]Laboratoire d'Etudes et de Recherche sur le MAtériau Bois UR 4370, Université de Lorraine, Inrae, Boulevard des Aiguillettes, BP 70239, F-54506 Vandoeuvre-les-Nancy cedex. E-mail: melanie.gellon@uha.fr*

Grapevine trunk diseases are serious challenge for viticulture, causing significant economic losses on national and international scales. The three main diseases – Esca, Botryosphaeria and Eutypa diebacks – are without reliable and effective control solutions. Sodium arsenite, the only existing effective pesticide, has been banned since 2001 in France, and since 2003 in Europe. Numerous alternatives are being studied, including substances from natural origins. The wood of tree knots is an important source of extractives that meet modern pesticide acceptance criteria, and some of these compounds are being studied in human health and cancer research. Wood knots are co-products of the timber and paper industries. They can be removed from processes for which they are prejudicial, and can be valuable bio-based co-products from processing. The present study evaluated the antifungal potential of an extract from knots of Douglas fir (*Pseudotsuga menziesii*), against the GTD pathogens, *Neofusicoccum parvum* and *Fomitiporia mediterranea*. *In vitro* tests confirmed fungus growth inhibition of EC_{50}s of 0.5 to 1.0 mg mL^{-1} for both pathogens. Douglas fir knot extract therefore has potential for treatment of GTDs. Knot extracts were not toxic to callus of *V. vinifera* 'Gewurztraminer'. Protective effects of these extracts on detached canes were observed, when applied after inoculation by the pathogens. Additional greenhouse *in planta* trials, and then in vineyards, will allow evaluation of the potential of these natural substances for preventive control of GTDs.

Native isolates of *Trichoderma* spp. can protect pruning wounds against *Lasiodiplodia theobrome* in Argentinian vineyards, reducing the incidence of the disease Hoja de Malvón. V. LONGONE[1], A. PIERONI[2] and G. ESCORIAZA[1]. *[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Mendoza, San Martin 3853 (5507), Luján de Cuyo, Mendoza, Argentina. [2]Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, Luján de Cuyo, Mendoza, Argentina. E-mail: longone.maria@inta.gob.ar*

Preventing opportunistic pathogen entry through grapevine pruning woods is an important strategy for managing trunk diseases. Applications of biocontrol organisms on pruning wounds could reduce infections from

pathogens that cause "Hoja de Malvón" (HdM) a common disease in Argentina. This name recognises the symptom where host leaves resemble those of geranium. The present study aimed to assess protective effects of native *Trichoderma* isolates with *in vitro* inhibitory potential against *Lasiodiplodia theobromae* (Lt) on pruning wounds. Lt is commonly associated with HdM at different pruning times. A vineyard trial (completely randomized design) was carried out with treatments of three pruning times (early, in June; medium, July, or late, August) × five treatments (four native *Trichoderma* spp., strain ACB 3, ACB 4, ACB 20, ACB 25, and a control). After pruning, the wounds were sprayed with each biocontrol agent (BA) and the control and 24 h later, each with 50 µL (10^5 conidia mL^{-1}) of Lt inoculum. Sixty canes per treatment were collected 28 d later, and prepared for re-isolation. Effectiveness was assessed as mean percent disease control (MPDC). Pruning time × treatment interactions ($P < 0.03$) for MPDC were detected. All the BAs were re-isolated from pruning wounds at 28 d after treatment application. The most effective treatments were: for early pruning ACB 3 (81%), ACB 25 (69%), and for medium pruning ACB 3 (62%). In early and medium pruning times June and July), also ACB 4 (59%), ACB 20 (48%), ACB 25 (43%) reduced significantly pathogen infections of the pruning wounds. MPDC from all the BAs at late pruning were below 40%. Native isolates of *Trichoderma* spp. provided control against Lt mainly at early and medium pruning times, in the regional climatic conditions.

Impacts of different grapevine bench grafting methods on the xylem anatomy, hydraulic traits and wood necroses associated with young decline of grafted vines. E. BATTISTON[1], S. FALSINI[2], A. GIOVANNELLI[3], S. SCHIFF[2], C. TANI[2], R. PANAIIA[2], S. DI MARCO[4] and L. MUGNAI[1]. *[1]Department of Agricultural, Food, Environmental and Forestry Science and Technology, Plant pathology and Entomology section, University of Florence, P.le delle Cascine 28, 50144 Firenze, Italy. [2]Department of Biology, Biomorphology Laboratory, University of Florence, Via Micheli 3, 50121 Firenze, Italy. [3]Research Institute on Terrestrial Ecosystems, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino, Italy. [4]Institute of BioEconomy, National Research Council, Via P. Gobetti 101, 40129 Bologna, Italy. E-mail: enrico.battiston@unifi.it*

Grapevine grafting is essential in viticulture, and bench grafting techniques have been developed to mechanize nursery processes and increase yields of viable cuttings. Bench grafting can affect the quality of propagation material and may have a role in young vine decline associated with grapevine trunk diseases. Some authors report that grafting can also influence leaf symptom development in Esca complex of diseases. This study assessed whether three bench grafting methods: omega graft, mechanical technique; whip and tongue graft, manual technique; or full cleft graft, semi-mechanical technique, affected these phenomena. The different methods were compared for their effects on anatomical development of grafting points and xylem function, considering two factors: cultivar (Cabernet Sauvignon, Glera or Teroldego) and scion/rootstock diameter (thin or large). Light microscopy observations of anatomical evolution were correlated with the grafting methods and the investigated varieties. Differences between cultivars and/or grafting methods were detected for areas of necroses on the grafted tissues. Cultivar differences in xylem parameters were also detected, while grafting type had no significant effects. However, graft type affected the intrinsic growth rate. These results confirm the potential for lesions and dysfunctionalities related to grafting method, which could induce decline of grafted vines in vineyards, due to the necrotic area detected on the grafted tissues.

Bio-products partially protect grapevine pruning wounds against infection by the trunk pathogen *Lasiodiplodia theobromae*. G. ESCORIAZA[1], V. LONGONE[1], I. FUNES PINTER[1], M. ULIARTE[1] and A. NOLI[2]. *[1]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Mendoza, San Martin 3853 (5507), Luján de Cuyo, Mendoza, Argentina. [2]Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Almirante Brown 500, Luján de Cuyo, Mendoza, Argentina. E-mail: escoriaza.maria@inta.gob.ar*

One of the challenges related with grapevine trunk diseases ("Hoja de Malvón"; HdM) in Argentina is implementation of efficient, safe and organic control strategies. Bio-products derived from organic wastes that contain beneficial microorganisms could be alternative disease management options. Aerated (AT), non-aerated (NAT) compost teas and bio-slurry (B) were evaluated to protect pruning wounds against *Lasiodiplodia theobromae*, an important fungus associated with HdM. Sodium bicarbonate (SB), thiophanate methyl (TM) and an untreated control were also included in the field trials. The compost used to obtain AT and NAT was from exhausted grape marc, goat manure, leaves from garden

raking and alfalfa. B was brewed anaerobically from fresh material. Twelve treatment combinations [two pruning times (July or August), five products, control] were applied on 'Malbec' grapevines, in a completely randomized experimental design. After pruning, the wounds were sprayed, and 24 h later inoculated with 50 µL (10^5 conidia mL^{-1}) of conidium suspension of *L. theobromae*. Sixty canes per treatment were removed after 28 d and prepared for pathogen re-isolation. Treatment effectiveness was assessed as mean percent disease control (MPDC). TM gave more than 75% control in July and August. Although the effectiveness of AT (33%), NAT (23%) and B (24%) were less compared to TM, these treatments were more effective than SB (12%). All products showed a greater effectiveness in July than in August, where MPDC did not exceed 20%. These results indicate that beneficial microorganisms need adequate conditions, long periods, and repeated applications to become established on pruning wounds, and before challenge from the pathogen. This study is the first to assess agro-industrial residues as sustainable control alternatives against *L. theobroamae*.

Effects of hot-water treatment on grapevine viability and fungal trunk disease pathogens diversity, assessed using RNA high-throughput amplicon sequencing. M. ANDRÉS-SODUPE[1], A. EICHMEIER[2] and D. GRAMAJE[3]. *[1]Viveros Villanueva, 31251 Larraga, Spain. [2]Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic. [3]Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail: laboratorio@viverosvillanueva.es*

Effects of hot-water treatment (HWT) on grapevine fungal communities have been mostly studied using culture dependent methods. Changes in potentially active fungal communities of grapevine wood were assessed after application of two HWT protocols (50°C for 45 min, or 53°C for 30 min), using RNA high throughput amplicon sequencing (HTAS) for several cultivar/rootstock combinations during two years. Fungal diversity was only reduced by both HWT protocols during the second year of study. The effects of HWT on grapevine trunk disease (GTD) pathogen abundance varied, according to the fungal genera and the HWT protocol. In some cases, HWT increased the abundance of GTD fungi (i.e. *Diaporthe*), but in other cases, GTD abundances decreased after HWT (i.e. *Dothiorella*). To evaluate the viability of planting material, treated plants were planted in a vineyard immediately after HWT, or 1, 2 or 3 months after treatments. Grapevine viability was high 1 month after treatment, but decreased as time from HWT to vineyard establishment increased. Plants established 3 months after treatments were less than 90% viable over 30% of combinations in the first year, and over more than 50% of combinations were affected in the second year.

Sensitivity of fungal grapevine trunk pathogens to treatments with electrolyzed water. A. FRINKLER[1], M. BERBEGAL[1], F. BEN ATIA[1], J. V. ROS-LIS[2], G. GAUME[3] and J. ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera S/N, 46022-Valencia, Spain. [2]REDOLí Research Group. Universitat de València. Doctor Moliner, 50. 46100 Burjassot, Spain. [3]Aquactiva Solutions SL, Av. Blasco Ibañez 73, 46136-Valencia, Spain. E-mail: jarmengo@eaf.upv.es*

Effects were evaluated of treatments with different electrolyzed water (EW) based products on *in vitro* mycelium growth of grapevine trunk pathogens. The pathogens studied were: *Botryosphaeria dothidea* (*Bd*), *Dactylonectria torresensis* (*Dt*), *Eutypa lata* (*El*), *Ilyonectria liriodendri* (*Il*), *Lasiodiplodia theobromae* (*lt*), *Neofusicoccum parvum* (*Np*), *Phaeoacremonium minimum* (*Pmin*) and *Phaeomoniella chlamydospora* (*Pch*). Agar discs with mycelium of each fungus were treated by immersion in each of the products. The treatments lasted 30 s, or 1, 5, 15 or 30 min. Mycelium survival (%) of each fungus was determined from the different product-time combinations. Effect on conidium germination were also evaluated for *Cadophora luteo-olivacea* (*Clo*), *Dt*, *Il*, *Pm* and *Pch*. Conidium suspensions (2×10^7 mL^{-1}) of each fungus were mixed with 950 µL of different EW products for 0, 15, 30, 60 or 300 s. Exposure was ceased by adding 9 mL of neutralizing buffer (pH 7.2). Drops (20 µL) of spore suspensions were plated on water agar and incubated at 25°C for 24 h. After incubation, the drops were observed for quantification of conidium germination. All experiments were repeated once. Effects of the different products on mycelium growth and conidium germination were variable, depending on the products, treatment times and fungus species. In general, the long treatment times were more effective for reducing mycelium growth and conidium germination. *Dt*, *El* and *Np* mycelium survival was reduced after 5 min treatments with one of the products. All product-time combinations resulted in greater than 93% germination inhibition relative to the untreated controls. Electrolyzed water treatments showed promising *in vitro* results, and further

research will evaluate their effectiveness under grapevine nursery conditions.

Impacts on phenolic metabolism in 'Cabernet Sauvignon' grafts when disenfectants are used on grapevine trunk disease compromised scions. D. RUSJAN[1], D. KJUDER[1], A. ŠKVARČ[2] and M. MIKULIC-PETKOVSEK[1]. *[1]University of Ljubljana, Biotechnical faculty, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. [2]Chamber of Agriculture and Forestry of Slovenia, Agriculture and Forestry Institute Nova Gorica, 5000 Nova Gorica, Slovenia. E-mail: denis.rusjan@bf.uni-lj.si*

Phenolic metabolism responses were assessed in different parts (scion, rootstock cane, roots) of grapevine grafts, after callusing and rooting to scions with different grapevine trunk disease (GTD) sanitary status. 'Cabernet sauvignon' plants were grafted, and different disinfectants were assessed for effects against GTD. Scions were from healthy mother vines: (*i*) healthy (HLT); or from GTD affected mother vines: (*ii*) infected but asymptomatic (ASYM, without any visual symptoms), or (*iii*) symptomatic (SYM, visual necroses on scions). Before grafting, the scions were treated with Beltanol, Serenade, Remedier, Bioaction, sodium bicarbonate or a combination of Beltanol and thermotherapy OR YOU MEAN HWT??? (Beltanol + TT). After callusing, the wood of SYM scions treated with Beltanol or Remedier had the greatest flavanol contents. After rooting, the graft classification showed the greatest yield (79%) of the first-quality grafts from Serenade, while the lowest (55%) from Remedier treatment. The rootstock canes of rooted grafts with HLT scions had 2.2 to 2.4-fold greater phenolic contents than the rootstock canes of grafts with SYM and ASYM scions. At rooted grafts, greatest flavanol contents were detected in SYM scions treated with Beltanol, Beltanol + TT, Bioaction or Serenade, and in ASYM scions treated with Beltanol or sodium bicarbonate. The least content of total phenolics (2.05 ± 0.08 mg g^{-1}) was measured in roots of grafts with HLT scions, but the greatest (3.99 ± 0.25 mg g^{-1}) was in roots of grafts with SYM scions. These results showed that sanitary status of scions affected phenolic metabolism of the entire graft, while the assessed biological disinfectants did not show positive impacts against GTD pathogens. This research indicates that Beltanol + TT, Remedier and Serenade treatments gave the most promising results as disinfectants against GTDs.

Evaluation of *Talaromyces pinophilus* as an antagonist of *Botryosphaeriaceae* spp. in grapevine. P. RODRÍGUEZ-HERRERA and F. GAÍNZA-CORTÉS. *Viña Concha y Toro SA, Centro de Investigación e Innovación, Fundo Pocoa s/n, Km10 Ruta K-650, Región del Maule, Pencahue, Chile. E-mail: felipe.gainza@conchaytoro.cl*

Climate change and intensive viticulture are generating early decline in grapevine plants. Grapevine trunk diseases (GTDs) have increased following the banning of fungicides (e.g. sodium arsenite) used to control these diseases, leading to decreased vineyard productivity and longevity. This has encouraged the search for GTD control alternatives, focusing on products based on beneficial endophytic microorganisms. Two isolates of *Talaromyces pinophilus* obtained from different regions of Chile were morphologically characterized and identified with molecular techniques. They were then evaluated as biocontrol agents of the main *Botryosphaeriaceae* present in Chile; *Diplodia seriata*, *Diplodia mutila* and *Neofusicoccum parvum*. Antagonistic capacity of *T. pinophilus* was evaluated in dual cultures, with each pathogen. An extract of *T. pinophilus* was also evaluated as an *in-vitro* growth inhibitor of the pathogens. A suspension of *T. pinophilus* conidia was then evaluated in unrooted vine cuttings, a reduction of damage caused by the pathogens was detected. These results showed that *T. pinophilus* good activity as a preventive biocontrol agent against the different *Botryosphaeriaceae* species, inhibiting growth of the pathogens *in-vitro* and in unrooted vine cuttings. The growth inhibition *in-vitro* ranged from 7.7 to 14.4%, while damage reduction in unrooted vine cuttings was from 68 to 92%. Additional studies should be carried out with *T. pinophilus* to investigate its potential as an alternative for GTD control.

Moss extracts affect the pathogen causing Phomopsis cane and leaf spot of grapevine. N. LATINOVIC[1], M. SABOVLJEVIC[2], M. VUJICIC[2], A. SABOVLJEVIC[2] and J. LATINOVIC[1]. *[1]University of Montenegro, Biotechnical Faculty, Mihaila Lalica 15, 81000 Podgorica, Montenegro. University of Belgrade, Institute of Botany and Botanical Garden, Faculty of Biology, Takovska 43, 11000 Belgrade, Serbia. E-mail: nlatin@ucg.ac.me*

Recent research has focused on replacing synthetic fungicides due to their potentially negative effects on human health and the environment. An option is applications of biologically active substances. Bryophytes are known to be little damaged by pathogens due to their natural chemical components, suggesting that bryophyte extracts could be assessed against fungal pathogens of crop plants. Ten ethanol extracts from mosses

were assessed for effects on colony growth of *Diaporthe ampelina*, which causes Phomopsis cane and leaf spot of grapevine. Extracts were assessed from the mosses *Abietinella abietina* (two accessions), *Dicranum polysetum, Fontinalis antipyretica, Homalothecium sericeum, Isothecium alopecuroides*, *Pseudoscleropodium purum, Racomitrium elongatum, Thuidium delicatulum* and *T. tamariscinum*. Three doses of each extract (5, 10 or 15 µL) were applied to assess *D. ampelina* colony growth inhibition on Petri dishes of potato dextrose agar. All the applied extracts inhibited the fungus compared to nil extract experimental controls. All three doses of the extracts gave inhibition, except *F. antipyretica* extract, for which only the 15 µL dose gave inhibition. There were no differences ($P > 0.05$) among the moss different extracts. However, extracts of *A. abietina* (accession II) and *T. delicatulum* gave the greatest inhibition of *D. ampelina*. These results have demonstrated the potential of mosses as environmentally-friendly treatments against fungal pathogens.

In vitro* evaluation of native *Trichoderma* strains as potential biological control agents against *Phaeoacremonium minimum. G. CARRO-HUERGA[1], S. MAYO-PRIETO[1], A. RODRÍGUEZ-GONZÁLEZ[1], O. GONZÁLEZ-LÓPEZ[2], S. GUTIERREZ[3] and P. A. CASQUERO[1]. *[1]Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Instituto de Medio Ambiente, Recursos Naturales y Biodiversidad, Universidad de León, Avenida. Portugal 41, 24071 León, Spain. [2]Departamento de Agricultura y Alimentación, Área de Producción vegetal, Universidad de la Rioja, 26006 Logroño, Spain. [3]Grupo Universitario de Investigación en Ingeniería y Agricultura Sostenible (GUIIAS), Área de Microbiología, Escuela de Ingeniería Agraria y Forestal, Universidad de León, Campus de Ponferrada, Av. Astorga s/n, 24401 Ponferrada, Spain. E-mail: gcarh@unileon.es*

Biological control agents (BCAs) could be important options for minimizing effects of Grapevine Trunk Diseases (GTDs). These BCAs can be used in integrated pest management to increase vine health. *Trichoderma* BCAs are effective because they grow and colonize many substrates. However, recent studies have shown irregular performance of commercial products based on this fungus, so native *Trichoderma* strains isolated from vineyards could provide increased disease management efficacy. Twenty-five *Trichoderma* isolates from bark of grapevine plants in Castilla y León region (Spain) were evaluated for *in vitro* inhibition of a strain of *Phaeoacremonium minimum* (Y038-05-03a) from the same region. Dual confrontation assays of *Trichoderma* species against the pathogen were carried out, evaluating percentage of sporulation inhibition, and yellow pigment production by *Trichoderma* in an agar medium, as indications of production of diffusible antibiotic compounds (DACs). Twelve *Trichoderma* strains (T72, T74, T75, T77, T78, T105, T79, T80, T82, T84, T85 and T154) produced greatest growth inhibition. Among these, strains T75, T79, T84 and T154 sporulated over the pathogen and plates . The *Trichoderma* isolate T106 produced a yellow pigment in agar that could be of interest in the evaluation in antibiosis assays. This experiment demonstrated the importance of identifying native *Trichoderma* strains as potential biocontrol agents, and their potential for mass spore production, and for identification of other biocontrol modes of action.

Effectiveness of Mamull® (*Trichoderma* spp. and *Bionectria* spp.) for control of trunk wood diseases of table grape and blueberry. L. A. ALVAREZ[1], E. DONOSO[2] and C. TORRES[3]. *[1]Escuela Profesional de Agronomía. Universidad Nacional de Cañete, Cañete, Lima, Perù. [2]Fitonova SPA. [3]Bio Insumos Nativa SPA, Chile. E-mail: lalvarez@undc.edu.pe*

Grapevine and blueberry trunk diseases cause severe plants losses in Peru. *Botryosphaeriaceae* anamorphs are major causal agents that infect pruning cuttings. *Lasiodiplodia theobromae* dominates over other *Botryosphaereaceae* anamorphs in Peru. Chemical sprays lack prolonged protection, and use of paste formulations is expensive, has logistic complexity and is time-consuming. These factors have driven development of sprayed biological control formulations for trunk disease management. The present study evaluated effectiveness of a commercial multi-strain formulation of *Trichoderma* spp. and *Bionectria* spp. (Mamull®, Chile) in comparison with a chemical alternative. Greenhouse trials were established for each crop evaluating effectiveness of preventive and retroactive treatments. Results from preventive treatments demonstrated similar efficacy between biological and chemical products, which were better ($P < 0.01$) than the control treatment. Protection time was also extended up to 45 d. The retroactive trials showed that the biological product controlled pathogen infections with retroactive effects up to 48 h, being statistically different from the chemical and control treatments These results indicate feasibility of using biological control agents, such as Mamull®, for preventive and retroactive control of trunk diseases, with better retroactive

control than from chemical pesticides, and avoiding the logistical problems with use of paste formulations.

Efficacy of Tachigaren® SL (hymexazol 360 g L^{-1}) for suppression of grapevine branch lesions caused by *Lasiodiplodia theobromae*. L. A. ALVAREZ[1] and G. ESPINO[2]. *Escuela Profesional de Agronomía. Universidad Nacional de Cañete. Cañete, Lima, Perù. [2]Summit Agro South America SpA, Sucursal Perú. E-mail: lalvarez@undc.edu.pe*

Peru is the greatest exporter of fresh table grapes. *Lasiodiplodia theobromae* causes severe plant losses in the main grapevine production areas, and in other fruit crops. A field study assessed the efficacy of hymexazol for control of this pathogen. Two isolates of *L. theobromae* recovered from affected grapevine plants were used in field experiments. Hymexazol was applied in three doses: T1 (2.0 L ha^{-1}), T2 (2.5 L ha^{-1}), or T3 (3.0 L ha^{-1}), applied by drip irrigation with post-plant *L. theobromae* inoculation. For inoculation, a 5 mm diam. plug of the bark of each branch was removed with a cork borer, and a PDA plug (5 mm diam.) colonized by *L. theobromae* was placed onto the exposed cambium. Each inoculated area was covered to prevent wound desiccation. Non-treated plants, inoculated with each isolate, were used as experimental controls. Results were evaluated 45 d after fungicide application. Areas of developed lesions in inoculated branches were measured. Lesions from both isolates, in plants treated with hymexazol, were smaller than for non-treated controls. The factor "fungicide treatment" was statistically significant ($P < 0.05$). The average reduction in lesion size for the treatments that displayed significant differences from the nontreated controls was 64% for T1, 77% for T2, and 82% for T3. Use of hymexazol may be a worthwhile component of integrated management of *L. theobromae* in grapevine plants.

Epidemiological survey of grapevine trunk diseases in the Eger Wine Region of Hungary. A. CSÓTÓ[1,2], D. BARANYI[1], G. SZAKADÁT[3] and E. SÁNDOR[3]. *[1]Institute of Plant Protection, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. [2]Kálmán Kerpely Doctoral School, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. [3]Institute of Food Science, Faculty of Agricultural and Food Science and Environmetal Management, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. E-mail: csoto.andras@agr.unideb.hu*

Grapevine trunk diseases (GTDs) cause significant losses in vineyards, and several fungi, including polyphagous pathogens of forest trees, have been recognized as GTDs pathogens. There is no completely effective disease management method for GTDs, so prevention has high importance. The present study identified favourable environmental conditions associated with disease incidence (DI) to be considered before planting. Objectives were to confirm relationships between DI and (1) cultivar susceptibility, (2) proximity of forest vegetation, and (3) effects of position on terrain slopes. Twelve vineyards older than 8 years were surveyed in two season in the Eger Vine Region, North-Eastern Hungary. Approx. 14,500 plants were monitored, including 16 cultivars. DI was surveyed separately, for (1) surveying "tiger stripe" leaf symptoms or diebacks, and for (2) surveying 100 plants randomly selected in four non-marginal rows, in each surveyed cultivar of each vineyard. Season 2020 had very high precipitation in June and October, while season 2021 was more balanced. DI ranged from 17 to 66%. Lowest DI was detected in 'Merlot, and highest DI was detected in the white grape 'Sauvignon blanc' and 'Olaszrizling', and the red grape 'Alibernet' and 'Syrah'. Ten samples were randomly collected for identification from each surveyed vineyard. Based on morphological characteristics, species of *Botryosphaeriaceae* were mainly detected from symptomatic plants. DI was greatest in vineyards close to forests or located at lower parts of slopes.

Potential GTD antagonist microfungi isolated from grapevines. A. CSÓTÓ[1,2], M. FILE[1], A. N. PITI[1], B. ELLMANN[1], K. PÁL[3], G. SZAKADÁT[3] and E. SÁNDOR[3]. *[1]Institute of Plant Protection, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. [2]Kálmán Kerpely Doctoral School, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. [3]Institute of Food Science, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, Böszörményi út 138, H-4032 Debrecen, Hungary. E-mail: csoto.andras@agr.unideb.hu*

Antagonistic microorganisms have potential as alternative controls for grapevine trunk diseases. However, there can be efficacy, safety, and production cost differences between known and newly discovered antagonists. Marketed and recently isolated Ascomycete species, with potential biocontrol activity, were studied. One *Fusarium* isolate, two of *Trichoderma* (*T. afroharzianum* and *T. simmonsii*), and two of *Clonostachys rosea*, were assessed, all of which were isolated from wood tissues

of grapevine plants from commercial Hungarian vineyards. The isolates were identified based on morphological characters and molecular sequences (ITS). *Trichoderma* species were identified based on tef1 similarities. Their growth and spore production were studied *in vitro*, and phytotoxic effects were assessed on detached leaves soaking in filtered broth, following 1 week of growth in potato dextrose (PD) broth. Biocontrol potential was assessed in PD agar plate confrontation assays against the GTD pathogens *Eutypa lata*, *Neofusicoccum parvum*, *Phaeoacremonium* sp., or *Fomitiporia* sp.). The two *Trichoderma* isolates gave 100% inhibition of each GTD pathogen in the plate confrontation tests. The *Fusarium* sp. strain had weak biocontrol potential, its metabolites were moreover phytotoxic in detached leaf tests. All of the tested strains grew well on solid media and in batch cultures, producing minimum 10^8 to 10^9 spores mL^{-1} after 4 d of growth.

Interactive effects of *Dactylonectria macrodidyma* inoculation on grapevine rhizosphere and root microbiomes. M. J. CARBONE[1], A. EICHMEIER[2], T. KISS[2], D. TEKIELSKA[2], R. BUJANDA[3], B. LÓPEZ-MANZANARES[3], S. OJEDA[3] and D. GRAMAJE[3]. *[1]Department of Plant Protection, Faculty of Agronomy, University of the Republic, Avenue Garzón 780, Montevideo 12900, Uruguay. [2]Mendel University in Brno, Faculty of Horticulture, Mendeleum - Institute of Genetics, Valticka 334, 69144, Lednice, Czech Republic. [3]Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail: david.gramaje@icvv.es*

Black-foot is a soil-borne disease caused by several "*Cylindrocarpon*"-like asexual fungi, including the widely distributed *Dactylonectria macrodidyma*. Composition and interactions of rhizosphere and endosphere of fungal microbiomes of grafted grapevine plants inoculated with *D. macrodidyma* were characterized using ITS high-throughput amplicon sequencing. Root tissues and associated rhizosphere soil from plants were sampled at 0, 3, 9 or 16 months after *D. macrodidyma* inoculation. Pathogen inoculation did not affect microbiome species richness, diversity or community structure, but induced changes in the relative abundance of several microbial taxa. Inoculated plants increased the proportion of *Diaporthe*, *Cadophora* and *Glomus* in the roots, but reduced that of *Trichoderma*. In the rhizosphere, SparCC network maps showed almost equal numbers of positive (n = 122) and negative (n = 118) correlations with *D. macrodidyma*. The black foot pathogen *Ilyonectria* had positive correlation with *D. macrodidyma*, while arbuscular mycorrhizal *Glomus* was negative correlated with the pathogen. In the grapevine roots, most of the major OTUs (n = 49) were positively correlated with *D. macrodidyma* and *Ilyonectria*, but the biocontrol genus *Trichoderma* was negatively correlated. These results have important implications for further studies on grapevine/pathogen/microbiome interactions.

Evaluation of *Trichoderma atroviride* SC1 and *Bacillus subtilis* PTA-271 combination against grapevine trunk disease pathogens in nursery propagation. CATARINA LEAL[1,2], DAVID GRAMAJE[3], FLORENCE FONTAINE[2], PATRICIA TROTEL-AZIZ[2] and JOSEP ARMENGOL[1]. *[1]Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Valencia, Spain. [2]University of Reims Champagne-Ardenne, Résistance Induite et Bioprotection des Plantes Research Unit, EA 4707, INRAE USC 1488, SFR Condorcet FR CNRS 3417, Reims, France. [3]Instituto de Ciencias de la Vid y del Vino, Consejo Superior de Investigaciones Científicas, Universidad de la Rioja, Gobierno de La Rioja, Logroño, Spain. E-mail: catarinaleal09@gmail.com*

Grapevine trunk diseases (GTDs) are threats to viticulture, leading to important economic losses. In nurseries, grapevine planting material is susceptible to infection by GTD pathogens, through cuts and wounds made during the different steps in propagation processes. Without effective chemical treatments, combinations of biological control agents (BCAs) could improve propagation material protection against GTDs pathogens. Single or combined treatments with *Bacillus subtilis* (*Bs*) PTA-271 and *Trichoderma atroviride* (*Ta*) SC1 were assessed for effects on GTDs pathogen infections in grapevine material during propagation. Botryosphaeria dieback (BD) incidence and severity were reduced in propagation material treated with *Ta* SC1 and *Ta* SC1 + *Bs* PTA-271, and Black foot (BF) incidence and severity were reduced from treatments of *Ta* SC1, *Bs* PTA-271, and. The *Ta* SC1 + *Bs* PTA-271 treatment showed potential for reducing infections caused by some GTD pathogens in the nursery propagation. This combination could be an asset for integrated disease management, where multiple strategies are combined to reduce GTD infections during nursery plant production.

Incidence of newly described mycoviruses in *Diaporthe* sp. M. KOCANOVA[1], L. BOTELLA[2], M. RIEDLE-

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Diaporthe species are considered causal agents of Phomopsis dieback, one of the fungal groups causing grapevine trunk diseases (GTDs). Some types of mycoviruses reduce the virulence of fungal hosts, which provides a tool for development of biocontrol strategies. The present study focused on detection and description of mycoviruses present in *Diaporthe* isolates mainly from *Vitis vinifera* L. two RNA viruses were detected, using dsRNA purification and high-throughput sequencing (Small and Total RNA Seq). Diaporthe mitovirus 1 (DrMV1; *Mitoviridae*) has a (+)ssRNA genome (approx. 2455 nt) containing one ORF encoding the RNA-dependent RNA polymerase (RdRp). The second virus, Diaporthe negative-stranded RNA virus 1, (DNSRV1; probably *Mymonaviridae*) has (-)ssRNA genome of approx. 9372 nt, with five ORFs. The largest encodes the RdRp, and the other four have unknown function. Based on both viral RdRp sequences, specific primers were designed to screen occurrence of DrMV1 and DNSRV1 by RT-PCR in a collection of 35 *Diaporthe* isolates from different countries and hosts ((24 *Vitis* sp., seven *Junglans* sp., four Prunus sp.) Virus co-infections were detected in one *Diaporthe* isolate from Czech *V. vinifera*; one from Austrian *V. vinifera*, one from Czech *Juglans regia*, and four isolates from Slovakia *Prunus domestica* L. were positive for DNSRV1. Two isolates from Czech *V. vinifera* and *J. regia* were positive for DrMV1. Twenty-nine percent of surveyed *Diaporthe* isolates contain virus, and DNSRV1 was more abundant than DrMV1.

Biological and physical protection of grapevine propagation material from trunk disease pathogens. E. ABARQUERO[1], M. P. MARTÍNEZ-DIZ[1], A. DÍAZ-FERNÁNDEZ[1], Y. BOUZAS[1], R. BUJANDA[2], D. GRAMAJE[2] and E. DÍAZ-LOSADA[1]. *[1]Estación de Viticultura y Enología de Galicia (AGACAL-EVEGA), Ponte San Clodio s/n 32428, Leiro, Ourense. [2]Instituto de Ciencias de la Vid y del Vino (ICVV), Consejo Superior de Investigaciones Científicas - Universidad de la Rioja - Gobierno de La Rioja, Ctra. LO-20 Salida 13, Finca La Grajera, 26071 Logroño, Spain. E-mail: emilia.diaz.losada@xunta.gal*

Control of fungal trunk pathogens in grapevine nurseries is based on application of integrated disease management, which includes chemical, biological and cultural strategies. Hot water treatments (HWTs) and/or biological control agents have been recommended due to restrictions and difficulties with pesticide chemicals in many countries. The present study evaluated HWT duration and *Trichoderma atroviride* SC1 (TCH), for disease control and their effects on trunk pathogen infections. Two trials were established: (*i*) HWT, TCH or HWT + TCH applications to cuttings in hydration tanks before grafting, and (*ii*) HWT, TCH or HWT + TCH applications to grafted plants after rooting in field nurseries. In both trials, treated plants were planted in a commercial vineyard. Pathogen isolations were carried out from rootstock bases and roots at two different times: after rooting in field nurseries and after one season in a vineyard. Black foot control was low in both trials. HWT or HWT + TCH reduced Petri disease infections, mainly in grafted plants uprooted from field nurseries.

Compositions of phytopathogenic fungal communities in grapevine leaves differ for sampling months, but not between organic and conventional vineyard management. C. M. LEAL[1,3], A. GEIGER[2,3] and J. GEML[1,2,3]. *[1]ELKH – EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, Leányka u. 6, Eger 3300, Hungary. [2]Food and Wine Research Centre, Eszterházy Károly Catholic University, Leányka u. 6, Eger 3300, Hungary. [3]Doctoral School of Environmental Sciences, Hungarian University of Agricultural and Life Sciences, Páter K. u. 1, Gödöllő 2100, Hungary. E-mail: lmota.carla@gmail.com*

Plant health depends on plant-associated microbes, but systematic overview, and knowledge of how grapevine microbiomes are influenced by cultivation methods, are still lacking. Diversity and composition of plant pathogenic fungal communities in grapevine leaves were assessed under organic or conventional management, to assess whether differences in the fungicides used affected the leaf-associated fungal communities. DNA meta -barcoding data were generated from leaf samples ('Bianca') collected throughout a growing season at the experimental vineyard Eszterházy Károly Catholic University, Hungary. Quality-filtered sequences were

grouped into Amplicon Sequence Variants (ASVs), that were assigned to taxonomic and functional groups using In the rarefied dataset, 911 ASVs of plant pathogenic fungi represented 88 genera, of which 15 are known to be associated with grapevine trunk diseases (GTDs). *Phaemoniella*, *Diplodia*, *Stereum*, *Trametes*, and *Botryosphaeria* had the greatest ASV richness. Among non-GTD pathogens, *Cladosporium*, *Aureobasidium*, *Alternaria*, *Vishniacozyma*, and *Epicoccum* were the most diverse. The detected richness and abundance of GTD-associated fungi in leaves was noteworthy, and confirms recent results showing that many of these fungi are part of the core grapevine mycobiome. Richness and composition of plant pathogenic fungi did not differ between organic and conventionally managed grapevines, but changes were apparent among months, explaining the greatest compositional variation. This strong temporal variation in leaf-associated pathogens was probably caused by application times of fungicides, differences in sensitivity among fungal species, particularly in mid-summer, and by seasonality, i.e. leaf maturation and gradual senescence onset by September.

Bismuth subsalicylate, a fungistatic compound and plant defence stimulator with potential for management of grapevine trunk diseases. L. MERLEN[1,2], C. TARNUS[1], C. DELAITE[2] and M. GELLON[1]. [1]*Laboratoire Vigne Biotechnologie et Environnement EA 3391, Université de Haute-Alsace, 33 rue de Herrlisheim, F-68000 Colmar, France.* [2]*Laboratoire de Photochimie et d'Ingénierie Macromoléculaires UR 4567, Université de Haute-Alsace, 3 bis rue Alfred Werner, F-68093 Mulhouse cedex, France. E-mail: melanie.gellon@uha.fr*

Since use of sodium arsenite was banned in 2001, increasing numbers of grapevines affected by grapevine trunk diseases (GTDs) have been observed, and lack of highly effective control products has led research on bismuth subsalicylate (BSS). This compound has been used in pharmaceuticals (e.g. PeptoBismol®) for decades. The antifungal capacity of BSS (which contains salicylic acid) was assessed against GTD pathogens, and for ability to stimulate plant defence genes. An objective was to design an appropriate formulation for BSS which had water solubility. A suitable formulation based on a liquid polymer was developed, with small particle size which increased the bioavaibility of the compound, an extremely important feature for eventual developments. Antifungal potency of the formulated BSS against GTD pathogens was confirmed, through growth inhibition of *Neofusicoccum parvum* (isolates Bt 67 and Bourgogne), *Diplodia seriata* (98.1) and *Fomitiporia mediterranea* (PHCO36). Stimulation of defence genes was analysed by RT-qPCR on grapevine callus (VvPAL, VvEDS1, VvHSR1 overexpressed), and the non-toxicity of BSS on grapevine cells was confirmed by fluorescence microscopy. BSS was then evaluated *in planta* using vertical plant endotherapy laboratory technique, where BSS was injected directly into grapevine rotten wood where mycelium complexes are concentrated. Symptomatic grapevines (n = 100) were treated for 2 years in Alsace, Preliminary observations will be presented, taking into account the complexity of GTD symptom expression.

Trunk BioCode; a metagenomic study of grapevine trunk diseases in Portuguese vineyards, and biosensor adaptation. F. AZEVEDO-NOGUEIRA[1,2,*], A. GASPAR[3], C. REGO[3], H. M. R. GONÇALVES[4], A. M. FORTES[2], D. GRAMAJE[5] and P. MARTINS-LOPES[1,2]. [1]*University of Trás-os-Montes and Alto Douro, School of Life Science and Environment, Department of Genetics and Biotechnology, Vila Real, Portugal.* [2]*BioISI – Instituto de Biosistemas e Ciências Integrativas, Faculdade de Ciências, Universidade de Lisboa, 1749-016, Lisboa, Portugal.* [3]*LEAF - Linking Landscape, Environment, Agriculture and Food-Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.* [4]*REQUIMTE, Instituto Superior de Engenharia do Porto, 4200-072 Porto, Portugal.* [5]*Institute of Grapevine and Wine Sciences (ICVV), Spanish National Research Council (CSIC), University of La Rioja and Government of La Rioja, 26007 Logroño, Spain. E-mail: plopes@utad.pt; filipem.a.nogueira@gmail.com*

Increasing knowledge of grapevine trunk disease (GTD) pathogens, and their environmental interactions, may give new insights for vineyard protection. The present study investigated whether grapevine cultivars influenced the prevalence of fungi in symptomatic grapevine wood. Two grapevine cultivars ('Touriga Nacional', 'Aragonez'), were assessed in Portuguese vineyards, from different wine Appellations. Sampling was of symptomatic wood from 'Touriga Nacional' (n = 36) and 'Aragonez' (n = 61). Necrotic woody tissues were cultured onto PDA amended with chloramphenicol, and presence of GTD pathogen colonies was assessed, based on morpho- cultural characters. A distinct pattern was found among the two cultivars: *Botryosphaeriaceae* isolates were most prevalent in 'Touriga Nacional' symptomatic wood, whereas in 'Aragonez', the most abundant are fungi were related to Esca disease. These results indi-

cated that cultivars contain specific GTD fungi, related to the presence of host resistance/tolerance genes. Further studies are required to confirm this conclusion. Metagenomic analyses are being applied to the same samples to validate the data obtained by traditional means, and provide a basis for biosensor development. This will be achieved by identifying the most relevant fungus within the GTD complex affecting Portuguese vineyards. This research is within the TrunkBioCode project, which aims to develop molecular tools for early identification of GTDs in grapevine. One strategy is based on a Biosensor platform, for detection and identification of pathogenic taxa in field conditions.

***Sporocadaceae* associated with grapevine trunk diseases in Cyprus**. G. MAKRIS, M. CHRISTOFOROU and L. KANETIS. *Department of Agricultural Sciences, Biotechnology, and Food Science, Cyprus University of Technology, Limassol, 3036, Cyprus. E-mail: loukas.kanetis@cut.ac.cy*

Grapevine trunk diseases (GTDs) are biotic factors that threaten economic sustainability of grape industries. Besides well-known diseases, there is increased interest in wood-colonizing fungi that infect grapevine woody tissues, and new species are being described as GTD causal agents. More than 140 fungal species have been reported in association with GTDs, but the degree of involvement for many GTD-related species remains to be elucidated. In 2017, during a survey conducted in Cyprus, wood samples were collected from vines exhibiting typical GTD symptoms, including decline, and dead cordons and spurs. Based on morphological and multilocus phylogenetic analyses (ITS, LSU, TEF1, and TUB2), four species of *Sporocadaceae* were found in association with GTDs, including *Seimatosporium marivanicum* and *Se. vitis-viniferae*, and *Sporocadus kurdistanicus* and *Sp. rosigena*. Pathogenicity trials with seven selected isolates, representative of each species, were conducted on woody stems of 2-year-old potted grapevines ('Xynisteri'). All the isolates were pathogenic, causing dark brown to black vascular discolouration of wood tissue below the bark, extending upward and downward from the points of inoculation. The *Sporocadus* isolates (mean lesion length 6.9 to 9.2 cm) were more aggressive than *Seimatosporium* isolates (4.0 to 4.1 cm). Re-isolation percentages of the inoculated pathogens were 40% for *Se. marivanicum*, 35–67% for *Se. vitis-viniferae*, 28 - 44% for *Sp. Kurdistanicus*, and 25–32 % for *Sp. rosigena*. This is the first report of *Se. marivanicum*, *Sp. kurdistanicus* and *Sp. rosigena* causing symptoms on *Vitis vinifera* in Europe, and suggesting a potential role for *Sporocadaceae* in the GTD complex.

Grapevine trunk diseases of cold-hardy grapevine varieties in northern midwest USA coincide with wounds and winter injury. D. H. DeKREY[1], A. E. KLODD[2], M. D. CLARK[3] and R. A. BLANCHETTE[1]. *[1]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, United States of America. [2]University of Minnesota Extension, Farmington, Minnesota, United States of America. [3]Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota, United States of America. E-mail: dekre004@umn.edu*

Surveys to characterize grapevine trunk diseases have been conducted for most major grape growing regions. Many of these share mild Mediterranean-like climates, and mainly grow traditional *Vitis vinifera* cultivars. Vineyards in Northern Midwest United States of America contrast to other growing regions, with an atypical cold climate (as low as -35°C), snowy winters, wet springs, humid summers, wet autumns, and short growing seasons. A survey was carried out to identify the most prominent fungal pathogens associated with this unique climate, and the cold-hardy interspecific hybrid grapevine varieties grown in the region. From 172 samples collected, 640 isolates were obtained by culturing, and these were identified using ITS sequencing. A total of 420 sample-unique taxa were identified. Of these, opportunistic fungi of *Diaporthales, Cytospora* and *Diaporthe* spp., were most frequently identified. Species of *Phaeoacremonium*, *Paraconiothyrium*, and *Cadophora* were also prevalent. Species in *Xylariales* and *Botryosphaeriales*, which are frequently isolated in many other regions, where only isolated in small numbers. No taxa in the *Phaeomoniellales* were isolated. The compounding effects of winter injury, pathogens, and management strategies will be discussed. Difficulties for studying, understanding, and communicating knowledge on grapevine trunk disease will also be addressed.

Effects of dual inoculation (*Seimatosporium* species with/without GTD fungi) on lesion length (symptom expression) in Sauvignon blanc vines. N. BESSELMA[1], H. J. RIDGWAY[1,2] and E. E. JONES[1]. *[1]Faculty of Agriculture and Life Sciences, Lincoln University, PO Box 84, Lincoln 7647, New Zealand. [2]The New Zealand Institute for Plant & Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand. E-mail: Noureddine.Besselma@lincolnuni.ac.nz*

In a survey of the endophytic fungal diversity associated with grapevines symptomatic or asymptomatic for grapevine trunk diseases (GTDs), carried out in Marlborough, New Zealand in 2018, several pathogens were isolated. Among these, members of *Botryosphaeriaceae – Neofusicoccum parvum* (from symptomatic vines) and *Diplodia seriata* (from symptomatic and asymptomatic vines) – were recovered. These pathogens can be latent and virulent GTDs. Additionally, *Seimatosporium vitis* and *S. lichenicola* were recovered for the first time associated with GTDs New Zealand vines. Both species were isolated from symptomatic and asymptomatic tissues, but their roles as pathogens and interaction within GTD complexes is unclear. Interaction between these *Seimatosporium* spp. and *N. parvum* or *D. seriata* in the GTD complex, and effect on symptom expression, were examined. *In planta* dual inoculations with *Seimatosporium* spp. and *N. parvum* or *D. seriata* isolated from the same wood cankers were evaluated. Detached Sauvignon blanc green shoots and 2-year-old woody stems of potted plants were wounded and co-inoculated with mycelium colonised agar discs of *S. vitis* or *S. lichenicola* and *N. parvum* or *D. seriata*. Controls consisted of each fungal species inoculated alone. After 2 weeks for detached shoots and 4 months for attached shoots, lesion lengths and colonisation distances (by re-isolation) were assessed. In both assays, differences in the lesion lengths and pathogen movement were detected from co-inoculations of both *Seimatosporium* spp. with *N. parvum*. In contrast, co-inoculation of either *Seimatosporium* spp. with *D. seriata* did not develop lesions, although *D. seriata* were recovered at 5 cm upward and downward from inoculation points. No lesions developed with from *D. seriata, S. vitis,* or *S. lichenicola* inoculations. These results confirm that *Seimatosporium* spp. are involved in the GTD complex.