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Plant health and food safety

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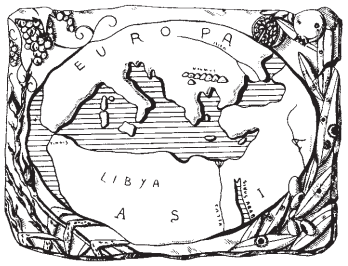

PHYTOPATHOLOGIA MEDITERRANEA

Plant health and food safety

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

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

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60th MPU Anniversary Special Section

Preface

This Special Section of *Phytopathologia Mediterranea* is dedicated to the 60th Anniversary of the foundation of the Mediterranean Phytopathological Union (MPU).

On 7 August, 1962, six Italian researchers, three from the University of Bari and Bologna (Vincenzo Grasso, Gilberto Govi and Antonio Graniti) and three from the Plant Pathology Station of the Ministry of Agriculture in Rome (Anna Saponaro, Anna Luisa Madaluni and Maria Benetti) presented before Alfredo Tassitani Farfaglia, a Notary in Rome, to sign the deed of incorporation of a new Scientific Association, the "Mediterranean Phytopathological Union". The registered location of this Association was the Plant Pathology Station at Via Casal de' Pazzi, n. 280, Rome, Italy. The first appointments in this Society, pending regular elections, were Prof. Cesare Sibilia as President, Jean Barthelet and Umberto Francisco Diaz as Vice Presidents, and Vincenzo Grasso as Secretary-Treasurer.

The MPU was founded as a regional Society of plant pathologists, in response to the invitation of Professor Israel Reichert ("On research and co-operation of Mediterranean Phytopathologists") published in the first issue of *Phytopathologia Mediterranea* (Reichert, 1960).

This journal was devoted to plant diseases in the Mediterranean region with a mission to assist the development of agriculture and agricultural research in the region. It had been founded in 1960 by Prof. A. Ciccarone and Prof. G. Goidanich, and started its work with an Editorial Board from several Mediterranean countries, including: J. Barthelet, C. Catsimbas, H. Dias, A.F. El-Helaly, G. Karel, L. Ling, G. Malençon, I. Reichert, J.R. Sardiña and M. Yossifovitch. As soon as MPU was funded Phytopathologia Mediterranea became the MPU official organ.

Paragraphs 1 and 2, of the Article 1 of the first Statute of the MPU, clearly stated the objectives of this research association, which were:

– to disseminate and increase phytopathological knowledge referring to the Mediterranean region, considered as an ecological unit;
– to establish a meeting point between plant pathologists and those in general who carry out technical activities in the region in phytopathology, so that they can make personal contacts, collaborate with each other and exchange news and information.

With these intentions, the active members of the Association, including Antonio Ciccarone, Gabriele Goidanich from Italy, Jean Barthelet and G. Viennot-Bourgin from France, I. Reichert from Israel, H. Dias and A.L. Branquiñho De Oliveira from Portugal, among many others, aimed to give the MPU high scientific and social values. Activities of the MPU commenced, and continued under guidance of multiple term Presidents including; A. Ciccarone, G. Viennot-Bourgin, A. Graniti, E.C. Tjamos, F. Lamberti, K.M. Makkouk, A. Phillips, A. Logrieco, and presently D. Tsitsigiannis. These people were assisted by Boards and Councils with members from different countries.

Congresses of the MPU have been held in Italy (Bari and Naples) in 1966; France (Montpellier and Avignon) in 1969; Portugal (Oeiras) in 1972; Yugoslavia (Zadar) in 1975; Greece (Patras) in 1980; Egypt (Cairo) in 1984; Spain (Granada) in 1987; Morocco (Agadir) in 1990; Turkey (Kusadasi) in 1994; France (Montpellier) in 1997; Portugal (Evora) in 2001; Greece (Rodos) in 2006; Italy (Rome) in 2010; Turkey (Istanbul) in 2014; in Spain (Cordoba) in 2017, and in Cyprus (Limassol) in 2022.

The MPU Statute is currently being revised to reinforce collaboration with all associations dealing with Plant Pathology. This revision aims to promote and expand the networks of plant and crop protection, and for food safety and environmental sustainability.

The 60th Anniversary of the MPU was celebrated in April 2022, during the 14th MPU Congress in Limassol, Cyprus (AA.VV., 2022). This Special Section of the journal contains four papers from the Congress, including a current topic paper and three reviews. These papers outline modern challenges to plant protection in Mediterranean crops, continuing the long-established traditions of the MPU and *Phytopathologia Mediterranea*.

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The first of these papers, by Giovani *et al*. (2022) underlines what was a major motivation for establishing *Phytopathologia Mediterranea* in 1960 (Graniti *et al*., 2010) and the MPU in 1962: the need to strengthen plant health research through coordination and collaboration in research, and to maximise outputs from limited research funding. The paper introduces and presents the relevance of the 'Plant health research priorities for the Mediterranean region' initiative.

The second paper, by Velasco-Amo *et al*. (2022),is a review focused on *X. fastidiosa*, a invasive transboundary and emerging plant pathogen, that is ranked as the first priority pest for the Europe Union. The review summarises knowledge on modern detection of *Xylella fastidiosa*, emphasising that surveillance and monitoring are essential for preventing spread of this harmful plant pathogen.

The review by Guarnaccia *et al*. (2022) outlines the spread of wood colonizing pathogens affecting fruit crop trees and other woody hosts in the Mediterranean region. After the increasing importance of grapevine trunk diseases, a topic frequently addressed in *Phytopathologia Mediterranea*, this review summarizes the situation affecting many economically important Mediterranean crops.

The third review paper, by Mellikeche *et al*. (2022), concerns the rapid and efficient diagnosis of decay-inducing pathogens using Loop-mediated isothermal amplification (LAMP) molecular assays. Early detection of these pathogens, that can be performed *in situ*, is essential for food safety and to reduce food waste. The LAMP assay provides a simple way to test products at production sites and borders, thus facilitating rapid treatment decisions to avoid the risk posed by the presence of harmful postharvest pathogens.

The efforts of members of the MPU, including all affiliated national plant pathology and protection organisations and individual researchers, will continue to promote MPU activities and *Phytopathologia Mediterranea*. These will be valuable avenues for knowledge exchange, building open, inclusive and safe environments, and contributing to food safety and security for Mediterranean countries and elsewhere.

Laura Mugnai, Richard Falloon

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60th MPU Anniversary Special Section - Current topics

Plant Health research collaboration in the Mediterranean region: case studies on citrus tristeza virus, tomato brown rugose fruit virus and *Xylella fastidiosa*

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Summary. Strengthening Plant Health research is a major challenge for Mediterranean countries. The diversity and fragmentation of the research landscape in this region have weakened the impacts of national efforts. Mediterranean countries can benefit from coordination of research activities to increase efficiency and impacts. The initiative 'Plant health research priorities for the Mediterranean region', led by the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM Bari)[1] and the Euphresco network for phytosanitary research coordination and funding[2] can promote convergence of national programmes and optimize the use of the scarce funding available to plant health, thus strengthening international cooperation and increasing the excellence and relevance of research.

Keywords. Research coordination, international collaboration, science diplomacy.

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INTRODUCTION

Approximately 25,000 plant species inhabit the Mediterranean region, and of these 13,000 are endemic. The region has been recognised as a biodiversity hotspot that is suffering unprecedented loss of habitat (Myers *et al*., 2000).

[1] CIHEAM Bari, International Center for Advanced Mediterranean Agronomic Studies, (https://www.iamb.it/), is an intergovernmental organization for high education, applied scientific research and planning of partnership actions in the field of research and international cooperation in the Mediterranean region.

[2] Euphresco, Network for phytosanitary research coordination and funding (https://www.euphresco.net/), is an international network of research programme owners, programme managers, policy makers, regulators and research and educational organizations from ca. 60 countries in 5 continents.

Mediterranean agriculture, forests and other environments are threatened by numerous quarantine and emerging pests. The negative impacts of these organisms have increased due to global trade and climate change that favour their movement over long distances and facilitate their survival in previously unfavourable environments. The EU Farm to Fork strategy, with its target to reduce by 50% the use and risk of chemical and hazardous pesticides by 2030, and the rise of biological crop products market, have also reduced the availability (if any) of control measures for some quarantine or emerging pathogens. In the face of these challenges, the Mediterranean region is particularly vulnerable.

The political and cultural diversity that are a characteristic of the Mediterranean region are its strength and its weakness. Diversity creates fragmentation, is an obstacle to collaboration, and reduces opportunities for concerted actions, which can result in individual countries having to deal alone with problems. The International Centre for Advanced Mediterranean Agronomic Studies of Bari (CIHEAM Bari) and the Euphresco network for phytosanitary research coordination and funding joined forces in 2016 to help Mediterranean countries to rethink organization of research activities and their coordination, to increase the efficiency and impacts of national efforts.

The benefits of research coordination and international collaboration are diverse. Identifying research priorities (important pests, infrastructure and capabilities) at the supra-national level will enhance convergence of national and regional programmes and will allow research funders to take advantage of the optimization of the scarce funds dedicated to plant health. Regulators will benefit from reinforced links with research funders and researchers, and from research support for policy development. Scientists will benefit from international knowledge exchange projects, from opportunities for enhancing their science capability and from increased relevance and visibility of plant health (research) activities.

THE CIHEAM BARI AND EUPHRESCO INITIATIVE

The approach followed by the CIHEAM Bari and Euphresco to identify the plant health research priorities for the Mediterranean region merged research experience and national guidance. A survey collected opinions of national experts from Mediterranean countries. The survey resulted in lists of pests, research priorities, infrastructures and research capacity that are considered important for the region. The priorities from the survey were refined and complemented by taking into account the short- and medium-term (up to 5 years) national research programmes, which provided information on the more urgent research topics planned for funding in each country. The views of national regulators provided additional guidance for selecting the most relevant priorities (Table 1).

Table 1. Priority pests for the Mediterranean region, identified through a survey.

Selected pests (in alphabetical order)
Anoplophora chinensis
Bursaphelenchus xylophilus
'*Candidatus* Liberibacter africanus'
'*Candidatus* Liberibacter americanus'
'*Candidatus* Liberibacter asiaticus'
'*Candidatus* Liberibacter solanacearum'
Citrus tristeza virus
Drosophila suzukii
Erwinia amylovora
Fusarium oxysporum f.sp. *albedinis*
Phyllosticta citricarpa
Plum pox virus
Rhynchophorus ferrugineus
Spodoptera frugiperda
Tomato brown rugose fruit virus
Xylella fastidiosa

Workshops and consultations were organised to involve and seek the endorsement of high-level representatives from Mediterranean countries, international organizations, and initiatives, that represent research funders, policy makers and research organizations in the Mediterranean region, including the Arab Society for Plant Protection (ASPP)[3] and the Mediterranean Phytopathological Union (MPU)[4] (D'Onghia *et al*., 2022; Giovani *et al*., 2022). Given the large number of priorities and the limited human and financial resources available, it was agreed that the research effort would focus on a small number of research topics. These shortlisted topics were those that received the largest number of expressions of interest from organizations in the four Mediterranean regions of Balkan-Mediterranean, Eastern Mediterranean, Maghreb, and Western Mediterranean. The three projects are outlined below.

[3] ASPP, Arab Society for Plant Protection (https://www.arabspp.org/), is an organization of scientists from public and private academic institutions and from industry that promotes research, education, and extension activities related to pests in Arab speaking countries

[4] MPU, Mediterranean Phytopathological Union (http://www.mpunion.eu/), is a regional not-for-profit organization that aims to advance and disseminate knowledge on phytopathology and closely related fields relevant to Mediterranean agro-ecological regions, and to establish and foster research relationships among scientific societies, academia, scientists and stakeholders

Rapid and efficient detection and identification of citrus tristeza virus (CTV) isolates that induce severe symptoms on Citrus

This research initiative aims to simplify the diagnosis of CTV isolates that cause severe symptoms on *Citrus*. To date, six major CTV phylogenetic groups have been described, including: T36 (Karasev *et al*., 1995), T3 (Hilf and Garnsey, 2002), VT (Mawassi *et al*., 1996), T68 (Harper, 2013), T30 (Albiach-Marti *et al*., 2000), and RB (Harper *et al*., 2010). These groups are mainly based on their genomic features. Virus genotype variation may occur after the passage through different hosts and CTV genotype populations may influence the success of virus transmission by the vector *Aphis gossypii* (Camps *et al*., 2022). Some of CTV genotypes are known to cause severe symptoms in *Citrus* orchards and have restricted distribution in the EPPO region. As molecular tests alone are of limited value for the prediction of pathogenic properties of CTV isolates (Bar-Joseph *et al*., 2010; Harper, 2010), the diagnosis of CTV isolates that induce severe symptoms on *Citrus* has been classically performed using a combination of molecular, serological and/or biological tests. This protocol is lengthy and difficult to perform. Moreover, the monoclonal antibodies MCA13 (Permar *et al*., 1990) used to diagnose CTV isolates that cause severe symptoms are no longer commercially available, which negatively impacts the diagnostic protocol currently in use. During this project, information on the diagnostic molecular tests available or in development will be collected, and plant material from the field (infected, healthy, symptomatic, asymptomatic) will be sampled, following a common methodology for monitoring. In addition, relevant plant material from reference CTV collections will also be collected. The CTV isolates will be molecularly characterized, and sequence data will be used to design the primers and the probes. A test performance study will be organized to validate the tests available or in development, and the test developed in the framework of the project. Several countries have expressed interest in participating in the project, including Australia, Austria, Croatia, Egypt, France, Greece, Israel, Italy, Morocco, Palestine, Portugal, Spain, Switzerland, and Tunisia.

Insights into the biology of tomato brown rugose fruit virus: virus survival in soil

Tomato brown rugose fruit virus has rapidly emerged from initial outbreaks in Jordan and Israel (Salem *et al*., 2016; Luria *et al*., 2017), and has now been reported from multiple countries across the Northern Hemisphere (EPPO, 2022). The virus particles are stable and can survive for months outside hosts on inert and biological surfaces as well as in nutrient film solutions and soil, without losing their virulence (Skelton *et al*., 2021). Studies on the survival of this virus have been ongoing in Israel (Dombrovsky *et al*., 2022), but additional information is required, including effects of soil type, environmental conditions, crops cultivated and the management practices. The project aims to develop new knowledge on survival of the virus in soil in different agro-ecological and pedoclimatic conditions. Survival during composting for bioremediation will also be considered within the project. Tests will be validated for the diagnosis of the Tobamovirus in soil and compost (including eDNA approaches), considering approaches to confirm infectivity of detected viral nucleic acids. These data will support development of guidelines for management of the virus. Several countries have expressed interest to participate in the project, including Australia, Austria, Chile, Germany, United Kingdom, Ireland, Israel, Italy, the Netherlands, New Zealand, Palestine, Russia, Slovenia, Switzerland, and Turkey.

Diagnosis of Xylella fastidiosa*: detection on dormant plant species which are important for Mediterranean countries*

Several research projects on *Xylella fastidiosa* have been commissioned since 2015, by national and regional funders in Europe. This has allowed development of knowledge on the bacterium, which has been useful for developing guidelines for sampling and diagnostics. Hopkins (1981) concluded that sampling should be performed during the period of active plant growth to maximize the likelihood of detection. However, recent experiments have shown that in Mediterranean countries, *X. fastidiosa* can be detected in plants (such as olive, almond and cherry) throughout the year, and especially during the asymptomatic phases or host dormancy, the period with the lowest bacterial concentrations (D'Onghia *et al*., 2022). The Euphresco project aims to evaluate the distribution dynamics of *X. fastidiosa* within dormant Mediterranean plant species and matrices that are commercially important throughout the year and during dormancy on woody host stems. During the project, samples from dormant Mediterranean plant species that are hosts of *X. fastidiosa* will be collected. An inventory will be made of tests (sampling, DNA extraction, diagnostic tests) used by different laboratories to detect *X. fastidiosa* in dormant plants. Selected tests will be validated on spiked and naturally infected host samples at low bacterial concentrations. Distribution dynamics of the pathogen within natu-

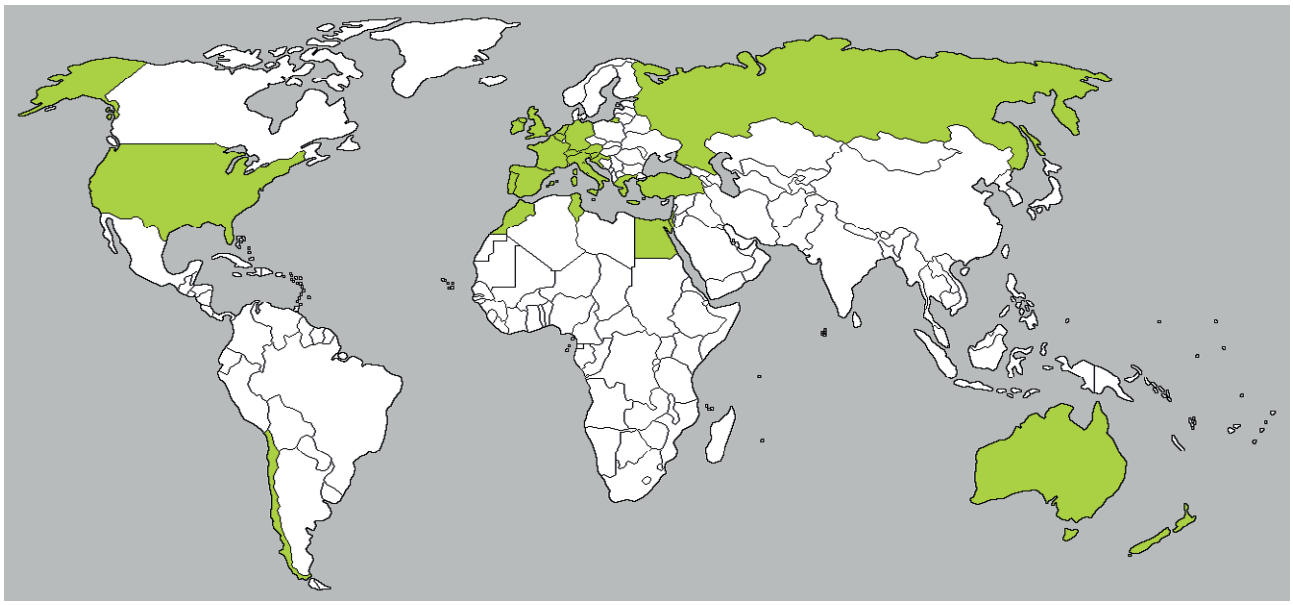

Figure 1. Countries that have expressed interest to participate in at least one of the research projects commissioned through Euphresco on research priorities for the Mediterranean region.

rally infected woody stems will be assessed throughout the year (including during dormancy), if plant material is available. Several countries have expressed interest to participate in the project, including Australia, Belgium, Egypt, France, United Kingdom, Israel, Ireland, Italy, Morocco, the Netherlands, Slovenia, Spain, Switzerland and Turkey.

GEOGRAPHIC EXTENT OF THE PROJECTS

The geographic coverage of the three research consortia is presented in Figure 1.

CONCLUSIONS

The Strategic Framework 2020–2030 of the International Plant Protection Convention (IPPC)[5], adopted at the 15th Session of the Commission on Phytosanitary Measures (CPM-15) in April 2021, includes global phytosanitary research coordination as one of the eight development agenda items to be addressed by the global Plant Health community over the current decade. Euphresco and CIHEAM Bari have concluded that the inclusive and participatory approach used to strengthen plant health research coordination and transnational collaboration for the Mediterranean region can be used for other regions, and also for global research coordination. Regional consultations may be part of processes that will allow transition from local needs (e.g., reduction of particular pests) to shared global priorities (e.g., prevention of pest spread). Opportunities will be created for scientific communities from less research-intense countries to reduce their isolation and increase their international exposure. In this context, involvement is essential of organizations and initiatives that are deeply rooted locally and that have leading regional roles. A workshop was organized by Euphresco, Better Border Biosecurity (B3; New Zealand) and the Plant Biosecurity Research Initiative (Australia), and was held on the 20th of September, 2022 in London. This workshop gathered representatives from the Australian Centre for International Agricultural Research (ACIAR), the Centre for Agriculture and Biosciences International (CABI), CIHEAM Bari, the Department for Environment, Food and Rural Affairs (Defra), the Consultative Group on International Agricultural Research (CGIAR), and the National Institute for Agricultural Research and Food Technology, National Research Council (INIA-CSIC). The workshop participants discussed the structures, operations and resources for international research coordination. This started from the models developed in the framework of ongoing Euphresco

[5] The IPPC, International Plant Protection Convention (https://www.ippc.int/en/), is an intergovernmental treaty signed by over 180 countries worldwide that aims to protect the world's plant resources from the spread and introduction of pests, and to promote safer trade.

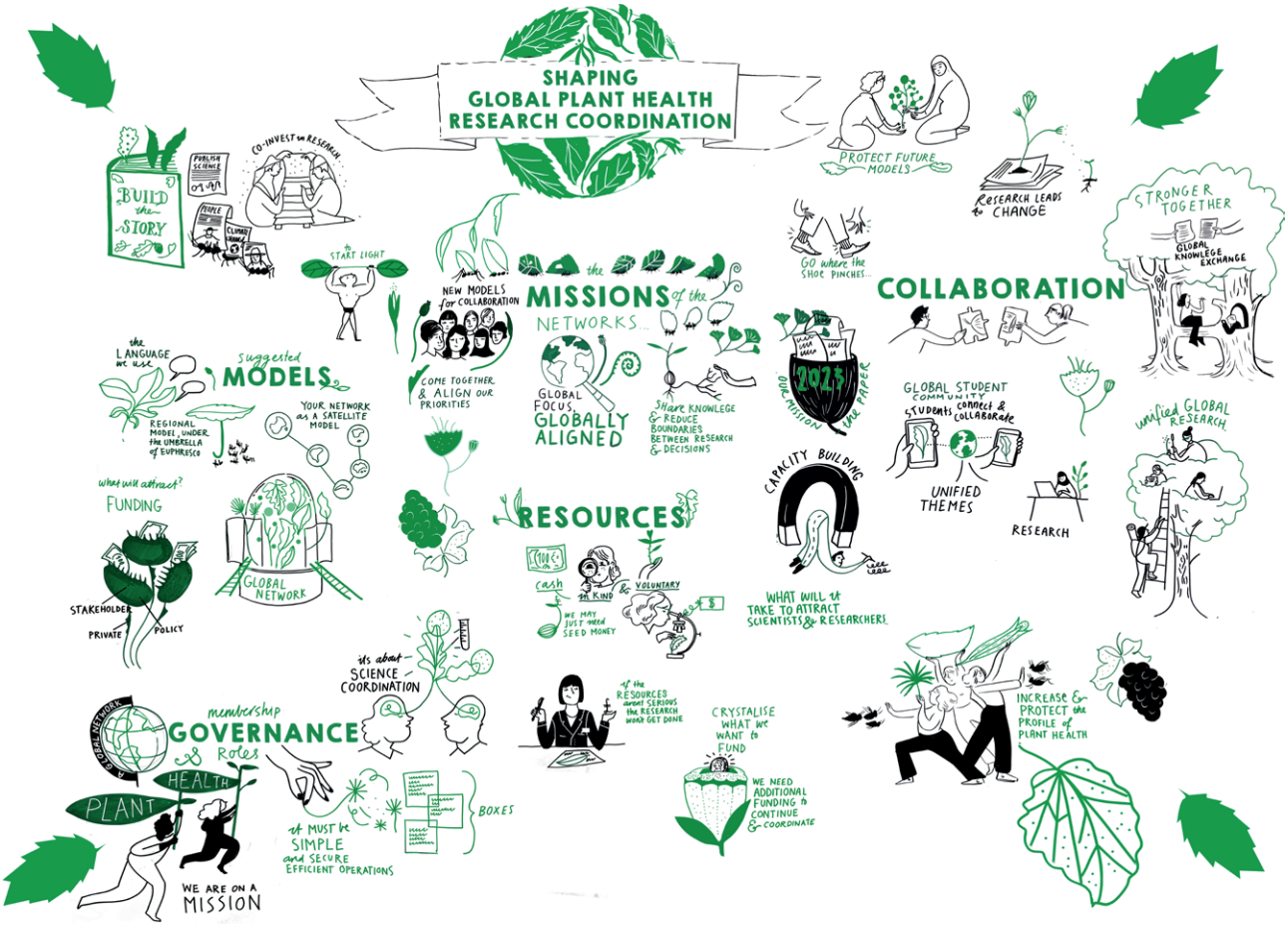

Figure 2. Transcription and illustration (by Josephine Ford) of the main topics discussed during the Euphresco B3 PBRI workshop, held in London (United Kingdom), on 20 September, 2022.

activities, taking into account the specificities of other international initiatives and organizations (Figure 2). Work will continue throughout 2023, with contributions from policy makers, research funders and research organizations that operate in plant health and are interested in joining this initiative.

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60th MPU Anniversary Special Section - Review

Detection of post-harvest pathogens by loop-mediated isothermal amplification: a review

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Summary. Postharvest losses, which occur between harvest and consumption of agricultural commodities, are major causes of food waste. Minimizing food loss helps provide nutritious food for animals and humans, and alleviate adverse environmental effects on food production. These losses are often related to the presence of postharvest pathogens, including fungi and bacteria, which typically start by infecting crops in the field as well as during postharvest chain. Control of these pathogens relies on development of tools that ensure their early and accurate detection. Among these is loop-mediated isothermal amplification (LAMP), a molecular method for pathogen detection. LAMP characteristics of rapidity, specificity and simplicity have encouraged development of a number of LAMP assays for detection of postharvest pathogens. Each LAMP assay allows to detect a specific genetic region of the target microorganism, which can be directly related to mycotoxin production, fungicide resistance and phytotoxicity. The LAMP amplicons are rapidly visualized, either at a specific timepoint, or in real-time by taking measurements throughout reaction, thereby necessitating less sophisticated facilities than those needed for PCR assays. In addition, many studies have developed simple protocols for the direct detection of pathogens on fresh produce. This paper explains the LAMP reaction, and its importance for postharvest detection of fungi and bacteria. Previous studies that have developed LAMP assays are also discussed.

Keywords. Food losses, microorganism contamination, mycotoxins, LAMP.

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INTRODUCTION

Human population growth has created major concerns about food security. By 2050, global food production will have to increase by an estimated 70% to adequately feed humans and avoid an unprecedented food crisis (Mvumi and Stathers, 2015). Although intensifying food production seems an obvious solution, this is unpractical due to the challenges of cli-

mate change and the role of intensive agriculture in their escalation (Giovani *et al.,* 2022). A good way to improve this situation while protecting the environment would be to reduce the important amounts of wasted commodities (Parfitt *et al.*, 2010). Approximately one-third of all food produced for human consumption is lost or wasted along supply chains (FAO, 2011), thereby rendering postharvest food losses a leading cause of food insecurity. These losses occur between harvest and consumption, at rates of 20% to 50% in developing countries and 5% to 25% in developed ones, depending on product type, cultivar, environmental factors, and postharvest conditions (Kader, 2003; Yahia *et al.*, 2019). Presence of postharvest pathogens on produce, whether in the field or during product handling, are major factors in product commodity deterioration. For each fruit or vegetable species, pathogenic bacteria, fungi and yeasts can cause many postharvest diseases (Antunes and Cavaco, 2010). Several bacterial pathogens such as *Bacillus, Pseudomonas, Pectobacterium* and *Xanthomonas* can cause important losses in the field and postharvest. Nevertheless, fungi are considered the most important degrading agents that affect foods during storage, making the food products unfit for human consumption by decreasing their nutritive value. Many of these pathogens are also able to produce carcinogenic mycotoxins. The health hazards posed by these compounds for humans have led most countries to issue regulations of their consumption, which target the mycotoxins or, in some cases, the toxigenic agent (Wenderoth *et al.,* 2019). In addition, agricultural exports are subjected to maximum tolerated mycotoxin levels. In some cases, these have reshaped the trade patterns of economically important crops (Bui-Klimke *et al.*, 2014).

For a long time, synthetic fungicides were the primary means of controlling postharvest decays (Spadaro and Gullino, 2005). However, their use has decreased due to their potentially hazardous effects on human health and environments, as well as the development of fungicide-resistant strains of postharvest pathogens (Baibacova *et al.*, 2019). These factors have restricted the approval of many products and motivated researchers to find alternative ways to control postharvest pathogens. Consequently, new technologies, substances and practices have emerged for fresh produce storage which target these pathogens, to preserve agricultural products and extend their shelf-lives (Tripathi and Dubey, 2004).

Most postharvest pathogens start infection processes in the field and often remain latent in fresh produce before causing serious damage during storage (Suarez *et al.*, 2005; Wenneker and Thomma, 2020). Many farmers therefore apply treatments on their crops to avoid these contaminations. However, in some cases, these treatments may be unnecessary since they are applied without accurate verification of the presence of pathogens. This random decision-making contradicts the principles of precision agriculture, and can have severe effects on the environment. Furthermore, they are often costly. Successful treatment depends on early detection and accurate identification of spoilage agents. This relies on several methods, traditionally including morphological characterization after growth on agar media (Samson *et al.*, 2007). However, these methods are time-consuming and require laboratory facilities and mycological expertise (Luo *et al.*, 2012).

Molecular methods such as PCR and real-time PCR are more rapid, sensitive and specific than culturing techniques (Schaad *et al.*, 2002; Rodríguez *et al.*, 2012). They can be used to identify mycotoxigenic strains by targeting the genes linked to toxin production (Stakheev *et al.*, 2011). However, they are costly and require suitably trained personnel and well equipped laboratories. As an alternative technology, Loop-mediated isothermal AMPlification (LAMP) reaction was described as a specific, rapid, cost-effective, and easy-to-use method by Notomi *et al.* (2000). This method uses four to six primers from the target region of each organism which is amplified at a fixed temperature. The high levels of specificity and sensitivity obtained with LAMP, coupled with its robustness to inhibition substances and its user-friendliness, have encouraged researchers to improve this method by developing real-time way to visualize the amplification products such as real-time LAMP. This method is largely used for the detection of several pathogens in preharvest among viruses (Bhat *et al.*, 2022), fungi (Abderraouf *et al.*, 2022) and bacteria (Yaseen *et al.*, 2015; Valentini *et al.*, 2022). Previous studies that developed specific LAMP primer sets for the identification of postharvest pathogens also assessed the possibility to apply LAMP-based assays to rapidly detect pathogens directly from infected commodities (Niessen *et al.*, 2018). In these protocols, simplicity is often researched throughout all the steps of the analysis, from the nucleic acid extraction to the amplification and detection of results. This paper reviews these studies and offers insights on LAMP and its potential effects on the management of postharvest pathogens.

POSTHARVEST PATHOGENS

Plant protection for economically important species is based on two essential pillars: the first is protection of plants throughout production stages to maximize yields by avoiding losses due to pest attacks; the second is pro-

tection of agricultural produce after their harvest to preserve food security and reduce waste due to postharvest pathogens. It is estimated that, in some cases, postharvest losses can be up to 50% of potential production (Kasso and Bekele, 2018; Kader, 2003; Yahia *et al.*, 2019). This can especially be the case when postharvest management lacks advanced technologies, such as continuous cold storage (Kitinoja *et al.*, 2019). Harvesting is the detachment of product from living plants, which renders products vulnerable to opportunistic spoilage agents that enter through wounds caused by agricultural practices, feeding animals, or the handling processes. These agents, such as *Ralstonia solanacearum* (Lemma *et al.*, 2014), *Erwinia carotovora* (Zhao *et al.*, 2013) and *Botrytis cinerea* (Suarez *et al.*, 2005), are often encountered in the field, and many can also cause damage at preharvest stages.

Several taxonomic groups of pathogens can cause postharvest infections. These include bacteria, yeasts and filamentous fungi. The most important filamentous fungi are species of *Alternaria, Aspergillus, Botrytis, Fusarium, Geotrichum, Gloeosporium, Monilinia, Penicillium, Mucor* and *Rhizopus* (Barkai-Golan, 2001). These are responsible for decay of agricultural commodities, they break barriers that would otherwise protect against other microorganisms such as bacteria and human pathogens, and many produce mycotoxins (Dukare *et al.*, 2019). Mycotoxins are secondary metabolites that can be highly toxic and carcinogenic, mutagenic and teratogenic to humans and animals (Omotayo *et al.*, 2019). They are mainly produced by species of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* (Table 1). Due to the hazardous effects of these fungi, many countries have issued regulations to control the importation of mycotoxin-susceptible commodities (van Egmond *et al.*, 2007). In some cases, the costs imposed by these regulations have caused important economic losses to exporting countries. For example, in 1997, the EU banned pistachio nut imports from Iran due to high aflatoxin levels. This decision shifted the trade patterns when the United States of America became the main exporter of this crop to countries with strict aflatoxin tolerance regulations (Bui-Klimke *et al.*, 2014).

DETECTION METHODS FOR POSTHARVEST PATHOGENS

Successful management of postharvest pathogens is directly related to their early and accurate detection. Therefore, many detection methods have been developed and improved. These methods can be either microbiological, serological or molecular.

Table 1. The most important postharvest pathogens, the symptoms they cause, and the main mycotoxins they produce.

Pathogen group	Main species	Symptom	Main mycotoxins	Reference
Aspergillus section *flavi*	*A. flavus* *A. parasiticus*	Green mold	Aflatoxins	Varga *et al.*, 2011
Aspergillus section *nigri*	*A. carbonarius* *A. niger*	Black mold	Ochratoxin A Fumonisin B_2	Astoreca *et al.*, 2010 Palumbo *et al.*, 2011
Penicillium	*P. verrucosum* *P. expansum* *P. italicum*	Blue mold	Ochratoxin A Patulin	Perrone and Susca, 2017
Alternaria	*A. alternata*	Black spots	Alternariol Tenuazonic acid Altertoxins I, II, III	Ostry, 2008
Fusarium	*F. verticillioides* *F. moniliforme* *F. graminearum*	Dark to brown rot	Fumonisin	Duvick *et al.*, 2001
Colletotrichum	*C. acutatum* *C. gloeosporioides* *C. boninense*	Anthracnose	—	Shi *et al.*, 2020a
Geotrichum	*G. candidum*	Sour rot	—	Talibi *et al.*, 2012
Botrytis	*B. cinerea*	Gray mold	—	
Monilinia	*M. fructicola* *M. laxa* *M. fructigena*	Brown rot on stone fruit	—	Côté *et al.*, 2004
Rhizopus	*R. microsporus* *R. stolonifer*	Rhizopus rot, Black bread mold	Rhizonin	Partida-Martinez *et al.*, 2007

Microbiological methods

Microbiological methods are the traditional ways of identifying and differentiating postharvest pathogens. They are based on pathogen cultivation on agar media followed by the observation of microorganism macro and micro-morphological characteristics (Klich and Pitt, 1988). Microbial growth manifests differently depending on the medium and environmental conditions (Cotty, 1994). In addition, some media are selective or semi-selective, encouraging growth and/or sporulation of particular fungus species while preventing development of others. For example, Samson *et al.* (2007) described the boscalid MEA medium, which only allowed the sporulation of *Aspergillus carbonarius* amongst all other black aspergilli. Other media, such as coconut cream agar (CCA) (Dyer and McCammon, 1994) and *A. flavus* and *A. parasiticus* agar (AFPA) (Pitt *et al.*, 1983) are particularly suitable for the growth of toxigenic strains.

Although these methods have played important roles in improving microbiological analyses, they are inadequate for current challenges, even though they are still needed if the pathogen is new and other kind of methods are still not available. They are time-consuming and require high levels of laboratory expertise and mycological knowledge in order to provide accurate diagnoses. Furthermore, these methods cannot be applied for every species and strain, and their results are strictly dependent on appropriate incubation conditions (Balajee *et al.*, 2007a; b). These methods also do not ensure high sensitivity due to low survival of fungal propagules under stressful conditions of selective and semi-selective media (Beuchat, 1993).

Serological methods

Serological diagnostic methods, such as Enzyme-Linked Immunosorbent Assay (ELISA), are based on detection of antibodies against pathogens and constitute a group of sensitive, rapid, specific and cost-effective tests (Clarck *et al.*, 1986). ELISA targets specific proteins based on the interaction between antigens specific to each pathogen, and their specific antibodies (Crowther, 1995). ELISA methods have been widely used to detect plant pathogens (Le and Vu, 2017), and were tested for the detection of pathogens in food products (Tsai and Cousin, 1990). Some researchers were interested in using ELISA for postharvest analyses, such as in the quantification of *B. cinerea* (Fernàndez-Baldo *et al.*, 2011) and for the detection of mycotoxins (Pei *et al.*, 2009). As field test, the lateral flow assay is applied for the rapid, equipment-free detection of different pathogens, e.g., *Phytophthora* spp. (Lane *et al.*, 2007). Nevertheless, sensitivity of these methods remains low compared to molecular methods, which are the most trusted tools for pathogen identification.

Molecular methods

Molecular methods are based on detection and amplification of target sequences from reference genes in pathogen nucleic acids. Therefore, they are used for species and strain differentiation and in phylogenic studies (Luo *et al.*, 2012). Among these methods, PCR is most commonly used. It amplifies target regions using polymerase and two specific primers throughout a series of repeated thermal cycles. PCR-based methods (PCR, real-time PCR, qPCR, multiplex qPCR) are powerful tools that provide high levels of specificity and sensitivity for detection of postharvest pathogens (Suarez *et al.*, 2005; Samson *et al.*, 2007). These methods have also been applied for amplification of genes relevant to mycotoxin biosynthesis (Shapira *et al.*, 1996) which, in some cases, can also be involved in pathogenicity (Sanzani *et al.*, 2012). However, PCR-based methods are costly due to the necessity for expensive reagents and high technology equipment such as thermocyclers. They also require advanced laboratory training and long DNA or RNA clean-up steps before amplification procedures. Therefore, these methods do not detect contaminants *in situ*. As an alternative molecular technology, LAMP was described by Notomi *et al.* (2000) as a specific, rapid, cost-effective, and easy-to-use method.

LAMP

LAMP is a molecular detection technique that amplifies DNA or RNA fragments using a strand displacing DNA polymerase (usually the Bst DNA polymerase from *Bacillus stearothermophilus*). This allows production of high amounts of DNA in a short time (Luo *et al.*, 2014). LAMP works under isothermal conditions (operating at a constant temperature), and can be highly specific since it uses four to six primers able to hybridize from six to eight regions of the target sequence. Usually, this technique requires no post-reaction processing because results can be quickly observed using indicators. Consequently, it can speed up the diagnostic process in comparison to a PCR-based method. LAMP is also highly tolerant to sample inhibitors, allowing it to be used directly on crude DNA extracted from infected or infested commodities (King *et al.*, 2019).

LAMP primer design

The most popular softwares to design LAMP primers are: Primer Explorer, a free, online tool with five versions released to date (the latest version is available at https://primerexplorer.jp/e/), OptiGene Limited (Horsham, UK) using Genie platforms, and "LAMP Designer" by PREMIER Biosoft (USA) (Le and Vu, 2017). While taking into consideration the four key factors in LAMP primer design (melting temperature, stability at the end of each primer, GC content, and secondary structure), these tools facilitate the design of the following primers (Figure 1): Forward inner primer (FIP), which consists of an F2 region complementary to the F2c region at the 3' end of the target sequence; whereas at the 5' end, it consists of an F1c region identical to the F1 region of the target sequence. Forward outer primer (F3), which is complementary to the F3c region of the target sequence. Backward inner primer (BIP), which consists of a B2 region complementary to the B2c region at 3' end of the target sequence; whereas at 5' end, it consists of the B1c region identical to the B1 region of the target sequence. Backward outer primer (B3), which is complementary to the B3c region of the template sequence. Forward loop primer (LF) which is complementary to the region between F1 and F2. Backward loop primer (LB) which is complementary to the region between B1 and B2.

The loop primers reduce the reaction time and increase the rate of amplification by binding the loops that are incorrectly oriented to bind to internal primers (Nagamine *et al.*, 2002).

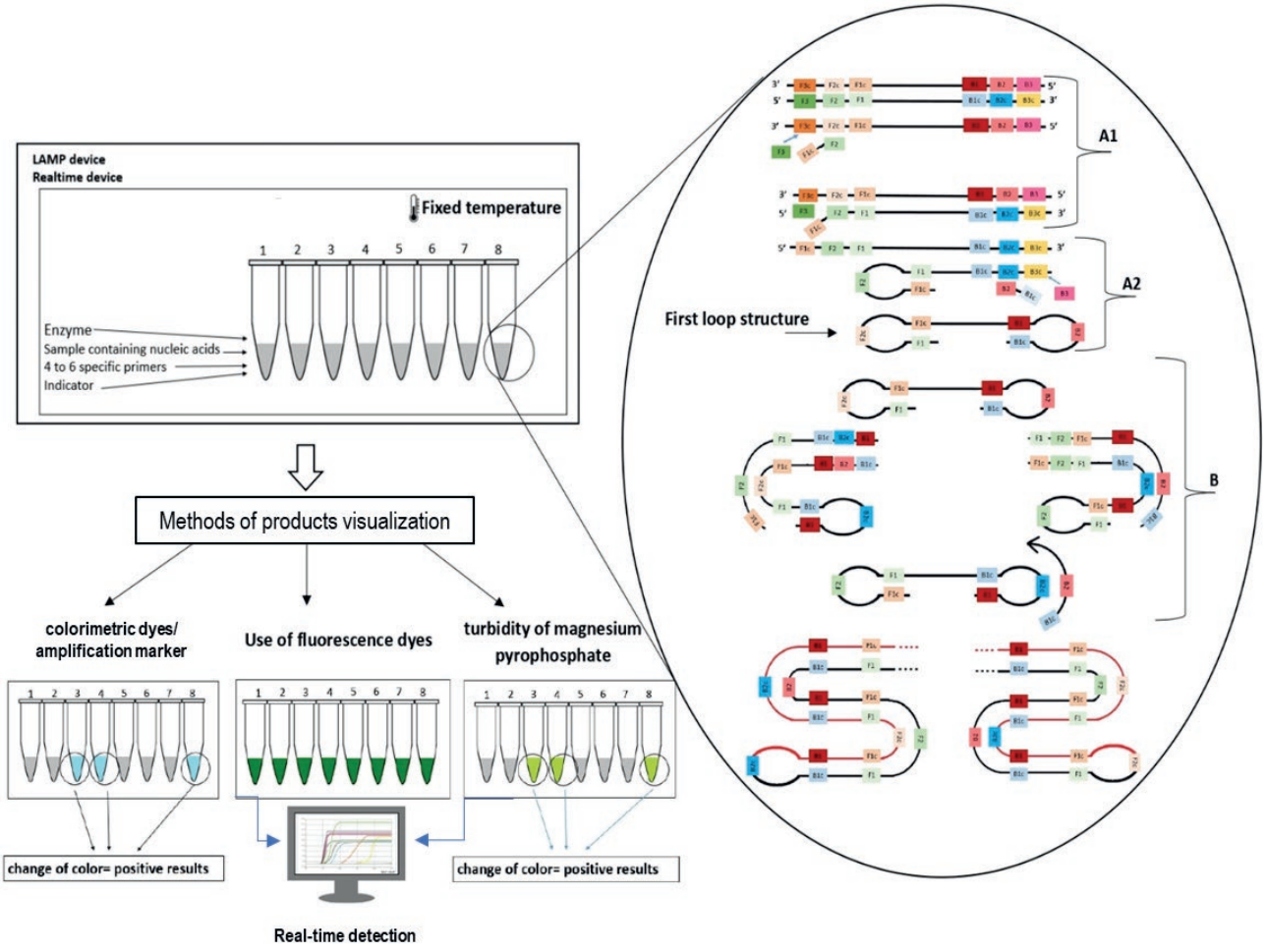

Figure 1. LAMP reaction and amplicon detection. Loop structure production: (A1) annealing and elongation of primer F2 (on primer FIP), followed by annealing and elongation of primer F3 which allows the first strand displacement and first loop formation through the annealing of F1(on primer FIP); and (A2) the annealing of primer B2 (on primer BIP), followed by annealing and elongation of primer B3 with the displacement of the polymerized strand and the formation of the second loop through B1 (on primer BIP). Target amplification (B): repetition of the annealing and elongation cycles on the produced loop and primer sites.

LAMP reaction

Two main steps can be differentiated in the LAMP reaction: loop structure production (Figure 1, A1 and A2) and amplification (Figure 1, B).

Loop structure production begins when FIP anneals the target sequence and separates the amplified sequence from the template by extending the primer, thereby forming the first product. This product is then displaced by synthesis when F3 anneals to an upstream target region (F3c), and the end of it forms a self-hybridizing loop structure due to the presence of the reverse complementary sequence F1c (Figure 1, A1). The same cycle repeats on the other end of the target sequence by the backward primers (BIP and B3) to form the first loop dumbbell structure (Figure 1, A2).

During elongation and amplification, the nucleic acid structure resulting from the previous step serves as a template for carrying on the amplification. It contains several sites from which the synthesis can initiate including the 3' end of the loop and the annealing sites of FIP and BIP. This allows the distinction of two elongation cycles: self-elongation from the loop and binding elongation of the inner region (Notomi *et al*., 2015) (Figure 1, B). The amplification step allows synthesis of complex structures with multiple loop sites that allow for exponential amplification of the sequence chosen as target.

Detection of LAMP products

Detection of LAMP amplicons can be accomplished by agarose gel electrophoresis. However, the most widely used methods are those that can ensure rapid observation of results without requiring further experimental steps (Figure 1). These methods can be either end-point tests (measured at specific timepoints) or real-time tests (measurement of amplification progress throughout the reactions). These methods can be classified into two groups (Moore *et al*., 2021). Sequence-independent methods rely on the detection of concentration changes of substrates or products produced throughout the reaction related to the amplification of the target sequences, and include changes in turbidity pH reactive dyes, intercalating fluorescent dyes, or bioluminescence. Conversely, sequence-dependent methods generate a signal directly dependent on the specific sequence targeted and allow the multiple target detection in a single tube; they include Quenching of Unincorporated Amplicon Signal Reporters (QUASR), Detection of Amplification by Releasing of Quenching (DARQ), CRISPR-Cas cleavage systems, one step strand displacement, and molecular beacons.

LAMP assays for postharvest molds

The review of Niessen (2018) identified 23 research publications describing development of LAMP assays to detect mycotoxigenic fungal pathogens on food matrices. Among these, two assays were panfungal, detecting presence of any fungal contamination in samples (Zhang *et al.,* 2017). Since then, similar studies have shown increased interest in LAMP as effective for distinguishing mycotoxigenic pathogens. However, other fungi, such as *Botrytis* spp. and *Monilinia* spp., which are unknown for mycotoxin production, can also severely damage harvested commodities. Therefore, these fungi have been the subject of several LAMP assays. In addition, some important postharvest bacterial pathogens have been subjects for development of rapid LAMP detection assays. In total, the present review lists 42 articles for fungi, and many of the studies provide simple and rapid protocols for detection of postharvest fungal contaminants directly from food. Since these pathogens are often present in preharvest as latent infections (Sanzani *et al*., 2012), the assays conducted on plant parts or seedlings have also been taken into consideration in the present review (Table 2).

Aspergillus

Among postharvest fungal pathogens, *Aspergillus* spp. are the most studied for development of rapid detection LAMP assays. Luo *et al.* (2012) were the first to aim to detect aflatoxigenic *Aspergillus* spp. directly from food samples, including Brazil nuts, peanuts and coffee beans. Their assay targeted the *acl1*-gene of *A. flavus* and *amy1*-genes of *A. nomius* and *A. parasiticus,* and positive results were detected by bright green fluorescence under UV 366 nm light. The detection limits were 2.4, 7.6 and 20 pg of pure DNA per reaction, respectively, for *A. flavus, A. nomius* and *A. parasiticus*. Specificity of the assays was also high with the *A. nomius* primer set not detecting any non-target isolate, and the other two primer sets detecting only some *Aspergillus* spp., which are very closely related to the targets.

The same primers were further tested by Luo *et al.* (2014) as parts of species-specific turbidimeter-based real-time LAMP assays, where turbidity was measured at 600 nm at intervals of 6s. These assays attempted to define contamination levels in samples of shelled Brazil nuts, maize, and peanuts. The detection limit was 10 conidia g^{-1} for *A. flavus* and *A. nomius* in Brazil nuts. The assay detection limits for *A. flavus* were 10^2 conidia g^{-1} for peanuts, and 10^4 conidia g^{-1} for maize, and for *A. parasiticus* were 10^5 conidia g^{-1} for peanuts and 10^4 conidia g^{-1} for maize.

Table 2. LAMP assays developed for the detection of postharvest fungal pathogens.

Pathogen	Target gene	Sensitivity	Food matrix	Reference
Aspergillus flavus *A. nomius* *A. parasiticus*	Alpha amylase (*amy1*) *amy1* ATP citrate lyase subunit 1	2.4 pg of pure DNA/ reaction 7.6 pg of pure DNA/ reaction 20 pg of pure DNA/ reaction	Brazil nuts, peanuts, green coffee beans	Luo *et al.*, 2012
A. flavus *A. nomius* *A. parasiticus*	a*my1* *amy1* ATP citrate lyase subunit 1	10 spores 100 spores 100 spores (sensitivity according to matrix and pathogen)	Brazil nuts, peanuts, maize	Luo *et al.*, 2014
A. flavus, *A. flavus* (toxygenic strains)	ITS1–5.8S–ITS2 rDNA region aflatoxin-encoding gene *aflP*	10 fg 1 pg of pure DNA	Peanuts, maize	Luo *et al.*, 2014
Aflatoxigenic Aspergilli	*nor1*	9.03 pg of DNA 211 conidia	Rice, nuts, raisins, dried figs	Niessen *et al.*, 2018
A. flavus, *A. parasiticus*	Aflatoxin efflux pump gene *aflT*	100-999 pg of DNA	Hazelnuts	Ortega *et al.*, 2020
A. carbonarius *A. niger* (ochratoxigenic)	polyketide synthase genes *pks*	Between 0.01 and 0.1 ng	Grapes	Storari *et al.*, 2013 Storari and Broggini, 2017
Ochratoxigenic strains of *Aspergillus* spp.	*pks*	Not mentioned	Peanuts	Al-Sheikh, 2015
A. niger *A. welwistchiae*	*fum10* (Fumonisin production)	10 conidia g^{-1} of maize	Maize	Ferrara *et al.*, 2020
A. caelatus *A. flavus* *A. nominus*	*acl1*	10^1 for *A. nomius*, 10^2 for *A. flavus*	Brazil nuts	Luo *et al.*, 2012
Botrytis cinerea	*bcos5*	10^{-3} ng μL^{-1}	Tomato and strawberry petals	Duan *et al.*, 2014a
B. cinerea	β-tubulin gene (*tub2*) mutation that causes resistance to benzimidazole	2×10^5 copies per μL of the plasmid	_____	Fan *et al.*, 2019
B. cinerea	Intergenic spacer (IGS) of nuclear ribosomal DNA	65 pg *B. cinerea* DNA	Detached rose petals, pelargonium leaves	Tomlinson *et al.*, 2010
B. cinerea	β-tubulin gene (*tub2*) mutation that causes resistance to benzimidazole	2×10^3 copies per μL of the plasmid	_____	Duan *et al.*, 2018
Penicillium expansum	*pex2_044840*	25 pg genomic DNA of *P. expansum*	Apples, grapes, apple juice, apple puree, grape juice	Frisch *et al.*, 2021
Patulin producing *Penicillium* spp.	isoepoxydon dehydrogenase *idh*	2.5 pg of purified genomic DNA	Grapes, apples	Frisch and Niessen, 2019
P. oxalicum	*pde_07106*	100 pg genomic DNA	Grapes	Vogt *et al.*, 2017
Monilinia laxa; M. fructicola	Intron in the cytochrome b, 166 associated with the *qoi* fungicides resistance	100-999 fg of DNA (*M. fructicola)*, 100-999 fg of DNA (*M. laxa)*	Nectarines	Ortega *et al.*, 2019
Fusarium graminearum	F167Y mutation of carbendazim-resistance of the b2-tubulin gene	Not mentioned	Perithecia produced on rice, infected wheat spikelets	Duan *et al.*, 2014b
Fusarium fujikuroi	*nrps31*	1 to 10 fg of DNA extracted from pure culture	Rice seeds and seedlings	Zhang *et al.*, 2019
Fumonisin-producing *Fusarium* spp.	*fum1* polyketide synthase involved in the biosynthesis of fumonisins	5 pg of genomic DNA 10^3 spores per reaction	Maize	Wigmann *et al.*, 2020

(Continued)

Table 2. (Continued).

Pathogen	Target gene	Sensitivity	Food matrix	Reference
F. graminearum	galactose oxidase gene *gaoA* of *F. austroamericanum*		Cereal	Niessen, 2013
Fusarium spp. *F. graminearum*	*hyd5*	0.74 pg of DNA	Barley	Denschlag *et al.*, 2012
Fusarium spp	hyd5	27 gene copies	Barley	Denschlag *et al.*, 2013
Fusarium spp.	*tri6*, *tri5* combination of the two sets	1.7 pg of DNA	_____	Denschlag *et al.*, 2014
A. carbonarius *A. niger* *A. awamori*	polyketide synthase genes	100 and 10 pg of DNA	Grapes	Storari *et al.*, 2013
Alternaria alternata	*actts2*	15 pg of DNA		Moghimi *et al.*, 2016
Alternaria spp.	cytochrome b (*cytb*)	15 pg	Pears	Yang *et al.*, 2019
Monilinia fructicola	*mfcyp51*	10 fg of purified target DNA	Peaches	Chen *et al.*, 2019
Phomopsis longicolla	1-α (tef1-α)	100 pg μL^{-1}	Soybeans	Dai *et al.*, 2016
Venturia carpophila	rDNA-ITS	56.6 Fg μL^{-1}	Peaches	Zhou *et al.*, 2021
A. fumigatus *P. expansum* *P. marneffei* *Histoplasma capsulatum*	rRNA-28S	20 copies of plasmid DNA for *A. fumigatus*	Mycelium culture	Tone *et al.*, 2017
F. graminearum	gaoA	2 pg of DNA	Wheat grains	Niessen and Vogel., 2010 Abd-elsalam *et al.*, 2011 Almoammar *et al.*, 2013
F. asiaticum	cyp51C	100 pg of DNA	Wheat grain	Xu *et al.*, 2017
F. culmorum	cyp51C	100 pg of DNA	Soybeans	Zeng *et al.*, 2017
F. equiseti	cypP51C	10 pg μL^{-1} 4 conidia per g of soil	Soybean roots	Lu *et al.*, 2015
Aflatoxin producers section *Flavi*	nor1	9 pg gDNA per rxn 211 conidia per rn after disruption	Rice, maize, raisins, figs, hazelnuts, almonds, paprika, ginger	Niessen *et al.*, 2018
Claviceps purpurea	cpn60	50 genome copies per rxn	Cereal grains	Comte *et al.*, 2017

Ferrara *et al.* (2020) aimed for rapid detection by targeting the *fum10* gene of *A. niger* and *A. welwitshiae,* which can produce the mycotoxin fumonisin (FB2) in maize kernels. The amplification was carried out in a portable thermal block, and the results were detected using phenol red according to colour change from red (negative) to yellow (positive). These assays were highly specific when tested using the nucleotide BLAST search tool on the NCBI sequence database. The detection limit was as low as 10 conidia per reaction. Ferrara *et al.* (2020) also developed a user-friendly "in field" analysis protocol based on extraction of crude DNA from contaminated maize kernels using a programmable, portable device with long-life battery. Since *Aspergillus* spp. have wide host ranges, matrix-specific assays must be developed, especially for species/host combinations targeted by regulations. Currently, the present authors are developing specific real-time LAMP assays for detection of *A. carbonarius, A. flavus* and aflatoxigenic aspergilli, on pistachios and almonds which are some of the most susceptible commodities to mycotoxigenic contamination. These assays are based on detection of the pathogens directly from samples, without laborious DNA purification steps, aiming to offer simple product tests for growers.

Penicillium

The study of Sun *et al.* (2010) was the first to focus on detecting *Penicillium* species by LAMP, targeting the human pathogen *P. marneffei* in archived human tissues. Later, LAMP assays were developed to detect *Penicillium* spp. in food samples (Tone *et al.*, 2017; Frisch and Niessen, 2019). Frisch and Niessen (2019) focused on rapid detection of *P. expansum,* which causes blue

mold decay, an important postharvest fruit disease. This assay targeted the *pex2_044840* gene, and was able to detect *P. expansum* DNA at high specificity and sensitivity of 25 pg per reaction. Results were visualized using neutral red as indicator. The assay was further tested on artificially contaminated food samples including apples, grapes, apple juices, apple puree and grape juice. The protocol required different DNA preparations depending on the type of sample; for grapes and apples, detectable amounts of DNA were obtained after simple steps of washing and mechanical treatment. For juices and purees, extraction of pure DNA was necessary for LAMP amplification. Despite the importance of *Penicillium* spp. as dominant food pathogens which produce hazardous mycotoxins, only a few LAMP assays targeting these fungi have been developed, compared to other important toxigenic postharvest pathogens such as *Aspergillus* and *Fusarium*. Development of rapid species-specific real-time LAMP assays would facilitate detection and treatment of these common mold fungi.

Botrytis

LAMP assays were developed both for toxigenic postharvest fungi and those such as *Botrytis cinerea*, whose damaging effects are unrelated to mycotoxin production. This pathogen causes grey mold, an important pre- and postharvest disease on many high value crops such as grapes, strawberries and kiwifruit (Droby *et al.*, 2007; Williamson *et al.*, 2007). This pathogen is often present as latent infection, and causes damage on fresh produce after periods of quiescence. For this reason, the fungus must be detected in early stages on plant material, rather than later, when the damage has occurred. The first rapid LAMP protocol for *B. cinerea* detection was published by Tomlinson *et al.* (2010), detecting the pathogen on plant material. Their study compared this protocol with the two previously used detection methods, TaqMan real time PCR and lateral flow devices. The LAMP assay targeted the intergenic spacer (IGS) of the *B. cinerea* nuclear ribosomal DNA (rDNA) sequence, which was the same sequence targeted by the Taq-Man real time PCR assay of Suarez *et al.* (2005). LAMP was carried out on DNA extracted from inoculated rose and pelargonium, and the results were visualized by electrophoresis on 1.4% agarose gels, followed by staining with ethidium bromide. The assay was further optimized and tested in real time, with sensitivity of 6.5 pg of DNA. Comparison of LAMP and TaqMan PCR showed that both methods were very specific by only detecting *B. pelargonii* among other closely related species. When tested directly on inoculated rose petals, only real-time PCR gave results 5 h after inoculation. Both methods detected the pathogen 29 h after inoculation. *Botrytis cinerea* is an important pathogen that often commences infection in the field and spreads rapidly at postharvest stages. However, few LAMP studies have been carried out with this fungus, but two have targeted the b-tubulin gene (*tub2*) mutation that causes resistance to benzimidazole (Duan *et al.,* 2014). While those studies are important for the detection of fungicide-resistant strains, further research is required to develop protocols for field detection and identification of this pathogen.

LAMP assays developed for the detection of postharvest bacteria

Postharvest bacteria often begin infections in the field and continue to cause damage on products after harvest. They mostly cause rotting which makes pathogen differentiation difficult from symptoms. Management of these pathogens is complicated and often requires extreme measures such crop eradication. Regular monitoring for presence of these organisms in susceptible crops is mandatory. In addition, these pathogens are strictly regulated by countries which classify them in quarantine lists. Development of new, rapid and sensitive tools for detection of postharvest bacteria will ease monitoring processes and border surveillance. Several studies have aimed to develop simple LAMP protocols for the detection of postharvest bacterial pathogens (Table 3).

Kubota *et al.* (2008) were the first to attempt to develop a LAMP assay on a postharvest bacterial pathogen, for *Ralstonia solanacearum*. This pathogen is important because of its wide host range (at least 200 plants, including economically important potato, tomato and peanuts). *R. solanacearum* is also of quarantine importance in several countries, so susceptible imported crops are often tested for this pathogen at borders, making it important to develop rapid and simple detection. Kubota *et al.* (2008) targeted the *fliC* sequence of the *R. solanacearum* genome to design specific LAMP primers. They also developed an assay for direct detection from edible ginger plants. The amplification results were electrophoresed at 85V for 90 min through 2% agarose gel (1× Tris-acetate-EDTA), followed by staining with ethidium bromide. Detection was by observation of white turbidity in reaction mixtures using magnesium pyrophosphate (Mori *et al.,* 2001). This assay was highly specific when tested on other soil-borne bacteria, but sensitivity varied according to the *R. solanacearum* strain. Efficacy of the same primers was also tested by Kubota *et al.* (2008) on potato tubers in a real-time LAMP assay, that

Table 3. LAMP assays for postharvest bacterial pathogens.

Pathogen	Gene	Sensitivity	Food matrices	Reference
Pectobacterium parmentieri	*petF1* gen	10 CFU mL^{-1} 100 fg of DNA	Potato plants and tubers	Domingo *et al*., 2021
P. atrosepticum	*sorA* *cfa6*	2.5×10^2 CFU mL^{-1}	Edible ginger plants	Li *et al*., 2011
P. carotovorum	(*p4h*) (α subunit)	1 ng μL^{-1} – 5 fg μL^{-1}	Potato	Yasuhara-Bell *et al*., 2016
P. atrosepticum	*gyr*B	3 CFU/reaction from pure cultures 22 CFU/reaction from samples	Potato tubers	Hu *et al*., 2016
Dykeya spp.	*mglC*	5 pg/reaction	Pineapple, Potato	Yasuhara-Bell *et al*., 2017
P. carotovorum	*pmrA*	10^4 CFU mL^{-1}	Celery	Shi *et al*., 2020b
Xanthomonas euvesicatoria	*recG*	100 fg of pure DNA 1,000 fg of DNA in samples spiked with tomato DNA	Tomato plants	Larrea-Sarmiento *et al*., 2018
X. arboricola pv. pruni	ABC transporter ATP-binding protein	1.8 ng μL^{-1} of genomic DNA	Peach orchards	Li *et al*., 2019
Ralstonia solanacearum	*fliC*	10^4 to 10^6 CFU mL^{-1}	Edible ginger plants	Kubota *et al*., 2008
R. solanacearum	*egl*	10^4 cells mL^{-1} (25 cells per LAMP reaction) for strains of phylotypes I and III 10^5–10^6 cells mL^{-1} for strains of phylotypes II	Tomato plants, potato plants and tubers	Lenarčič *et al*., 2014
R. solanacearum	*orf428*	100 fg mL^{-1} of DNA 10^3 CFU mL^{-1} of bacterial fluid	Sweet potato	Li *et al.,* 2021

gave a detection limit of 1.25×10^5 CFU g^{-1}. This is low sensitivity, compared to results previously reported from PCR, which amplified the gene at 2×10^2 CFU g^{-1} (Horita *et al*., 2004). Improvements of LAMP techniques have allowed development of more sensitive LAMP protocols, with lower detection limits. For example, Li *et al.* (2021) designed a LAMP primer set targeting the *orf428* gene, with a detection limit of 100 fg mL^{-1} of DNA and 10^3 CFU mL^{-1} of bacterial fluid.

In addition to *Ralstonia* spp., *Pectobacterium* spp. have also been subjects for development of rapid LAMP assays. Li *et al*. (2011) developed a LAMP assay for the detection of *Pectobacterium atrosepticum* (*Erwinia carotovora* subsp. *atrosepticum*), which causes potato blackleg associated with pre- and postharvest losses in potato crops. The assay targeted the gene cluster encoding a pathogenicity-related phytotoxin, specifically PKS *cfa6* and *Polyangium cellulosum* soraphen polyketide synthase A (*sorA*) genes. The assay had a specificity and a low detection limit of 2.5×10^2 CFU mL^{-1}. However, the assay described by Hu *et al.* (2016) for the same pathogen, which targeted the *gyr*B gene, had a detection limit of 3 CFU per reaction from pure cultures, and 22 CFU per reaction from samples of contaminated potato tubers. Improvement of LAMP has resulted in assays with greater specificity and simpler detection protocols. Domingo *et al.* (2021) aimed to specifically detect among *Pectobacterium* spp. and *Dickeya* spp. that can cause the soft rot of potato. This highly specific real-time LAMP assay allowed detection of the target species, and no other very closely related species. This is due to the signature region within the *petF1* gene that was not found in other *Pectobacterium* spp.. In addition to real-time measurement, results were also observed from orange to bright green colour change after adding SYBR green before the reaction. Domingo *et al.* (2021) also developed a simple and effective protocol for the detection of *P. parmentieri* from potato plants and tubers. Several LAMP assays have been developed for food-borne bacteria such as *Salmonella* spp. and *Escherichia coli*, in association with foods such as meat, milk and juice. Some studies tested LAMP on fresh agricultural produce, such as that targeting the *invA* gene of *Salmonella* spp. (Zhang *et al*., 2011). This assay had a detection limit of 2 CFU per 25 g and was tested on coriander, lettuce, parsley, spinach, tomato, jalapeno

and pepper. Yokoyama *et al.* (2010) developed a LAMP assay for *E. coli* associated with radish sprouts, broccoli sprouts, ready-to-eat salads, ground pork and beef, which targeted the *aggR* gene of this bacterium, and was able to detect 6.3 CFU per reaction.

CONCLUSIONS

Postharvest waste threatens food security, and is mainly caused by decay-inducing pathogens. Protection of harvested commodities relies heavily on the early detection of these pathogens, which often commence host infections in the field (Logrieco, 2022). LAMP is a rapid, sensitive and specific method for detecting and accurately identifying these pathogens, even in the field. Indeed, LAMP is easily performed as requires no special expertise, is less expensive than other molecular identification tools. To amplify a target sequence of target nucleic acid, LAMP uses four to six primers designed specifically according to the relevant DNA. Many assays have been developed to detect postharvest fungi using this method as reported in Table 3. These assays have targeted several mold species, particularly *Aspergillus* and *Fusarium* spp. For postharvest bacteria, however, assays have been designed for only a few agricultural product hosts, and tests for this general group of products should be more widely developed. This can be achieved utilizing the current revolution in molecular biology, specifically in gene sequencing, which provides material to design specific and functional LAMP primer sets. The continuous improvement of real-time LAMP using simple extraction methods, with crude extract instead of highly purified DNA (Kogovšek *et al.,* 2017; Yaseen *et al.,* 2015), in combination with lyophilized primers contribute to the suitability of this technique for *in situ* detection of postharvest pathogens.

Since most of these pathogens and their mycotoxins are regulated by many countries, LAMP represents an easy way of testing commodities at production sites, to facilitate future treatment decisions at borders and to alert importing countries about the presence of potentially harmful and damaging pathogens.

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Recent research accomplishments on early detection of *Xylella fastidiosa* outbreaks in the Mediterranean Basin

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Summary. *Xylella fastidiosa* is a major transboundary plant pest, causing severe socio-economic impacts. Development of preventive strategies and methods for surveillance, early detection, monitoring, and accurate diagnosis of *X. fastidiosa* and its vectors, are keys to preventing the effects of this plant pathogen, and assist timely eradication or optimisation of containment measures. This review focuses on approaches for early detection of *X. fastidiosa* in the Mediterranean Basin, including development of climatic suitability risk maps to determine areas of potential establishment, and epidemiological models to assist in outbreak management through optimized surveillance and targeted responses. The usefulness of airborne hyperspectral and thermal images from remote sensing to discriminate *X. fastidiosa* infections from other biotic- and abiotic-induced spectral signatures is also discussed. The most commonly used methods for identifying *X. fastidiosa* in infected plants and vectors, and the molecular approaches available to genetically characterize *X. fastidiosa* strains, are described. Each of these approaches has trade-offs, but stepwise or simultaneous combinations of these methods may help to contain *X. fastidiosa* epidemics in the Mediterranean Basin.

Keywords. Climatic suitability risk maps, diagnostics, molecular approaches, quarantine plant pathogens, sequential adaptive survey.

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INTRODUCTION

Xylella fastidiosa is a plant-associated bacterium that is a major transboundary plant health threat, and is a serious plant pathogen in terms of socioeconomic impacts. The bacterium causes diseases on a wide host range of plants including crops of economic importance in agriculture and forestry, and with cultural and heritage value. Diseases caused by *X. fastidiosa* include Pierce's Disease (PD) of grapevine, Citrus Variegated Chlorosis (CVC),

Almond Leaf Scorch (ALS), and Olive Quick Decline Syndrome (OQDS) (EFSA, 2022). A recent study by the Joint Research Center (JRC) and European Food Safety Authority (EFSA) (Sanchez *et al*., 2019) has identified *X. fastidiosa* as the quarantine pathogen with the greatest potential impact in the European Union (EU), in all economic, social, and environmental domains. That study estimated that *X. fastidiosa* could ultimately cost the EU over €5.5 billion per year due to production losses, and €0.7 billion per year in export losses, since the bacterium has the potential to affect 70% of EU production value of full-productive old (>30 years) olive trees, 13% of almond, 11% of citrus, and between 1 to 2% of grape production, in a full spread scenario across the EU.

Early detection of *X. fastidiosa* is important for taking timely measures for its eradication, containment or management (Almeida 2016; Zarco-Tejada *et al.* 2018). Consequently, development of efficient methods and strategies for surveillance, early detection and monitoring of *X. fastidiosa* have been the foci of several EU research projects such as XF-ACTORS (https://www.xfactorsproject.eu/) and POnTE (https://www.ponteproject.eu/), and grants by the EFSA. A major effort is currently being made in the EU to detect and assign *X. fastidiosa* strains from outbreaks to subspecies and, within them, to Sequence Types (STs). This is to infer relationships between ST and host range, and to trace back the possible origins of the introductions. Accurate diagnoses of *X. fastidiosa* at subspecies levels in the EU is essential for regulatory measures on outbreak response and management (e.g., removal of plants and replanting) (Regulation EU 2021/1688; EC, 2021). Currently, methods for *X. fastidiosa* identification are based on the European and Mediterranean Plant Protection Organization (EPPO) diagnostic protocol (EPPO, 2019).

The present review is based on an oral presentation entitled 'Current situation of *Xylella fastidiosa* impacts in Spain: ongoing research initiatives to understand and tackle this pathogen', which was presented at the 16th Congress of the Mediterranean Phytopathological Union in April 2022, Limassol, Cyprus (Landa, 2022). This review describes research outputs from the framework of the EU projects POnTE and XF-ACTORS for early detection of *X. fastidiosa*, together with current knowledge reported in *X. fastidiosa* literature and experienced gained during current EU outbreaks of the pathogen in the Mediterranean Basin. It is not intended, however, to provide a detailed state of the art summary on this topic. Topics covered include development of climatic suitability risk maps, how they can assist to determine areas of potential *X. fastidiosa* establishment, and how epidemiological models and surveillance strategies can help to track the outbreaks of *X. fastidiosa* and their potential spread. Other topics include the usefulness of remote sensing to support surveillance and monitoring of areas affected by *X. fastidiosa* outbreaks, and how to discriminate *X. fastidiosa* infections from other biotic and abiotic-induced stresses. Also provided are an extended description of the most commonly used methods to identify *X. fastidiosa* in infected plants and vectors, molecular approaches available to characterize *X. fastidiosa* strains at subspecies and ST level, and future directions for efficient molecular diagnostics and genetic characterization of *X. fastidiosa* strains.

Although the procedures for field sampling of plant material, in places of production and in consignments, as well as laboratory sample preparation, are all essential for obtaining reliable diagnostic results, description of these procedures is beyond the scope of this review. Detailed information on these aspects can be found in PM 7/24 Diagnostics for *Xylella fastidiosa* (EPPO, 2019), PM 3/81 Inspection of consignments for *Xylella fastidiosa* (EPPO, 2022a), PM 3/82 Inspection of places of production for *Xylella fastidiosa* (EPPO, 2022b), Methodologies for Sampling of Consignments ISPM 31 (IPPC, 2008), and in D'Onghia *et al.* (2022) and Loconsole *et al.* (2021).

CLIMATIC SUITABILITY RISK MAPS TO ESTIMATE REGIONS FOR POTENTIAL ESTABLISHMENT OF *XYLELLA FASTIDIOSA*

Xylella fastidiosa occurs in a variety of climatic zones, although it is particularly prevalent in the tropics and sub-tropics. The pathogen is also found in areas where climatic conditions are similar to those prevailing in Mediterranean climate zones, such as California, and in various European regions including Corsica in southern Italy, the Côte d'Azur in France, southern Portugal and the Balearic Islands and the Valencian Community in Spain. Records of diseases caused by *X. fastidiosa* also occur from much colder climates, such as New Jersey and Washington in the United States of America and the Niagara Peninsula, southern Ontario, British Columbia, Saskatchewan and Alberta in Canada (EFSA, 2015).

Different approaches have been used to infer areas with favourable climatic conditions for *X. fastidiosa*. Feil and Purcell (2001) used isotherms of winter minimum temperatures to propose the following severity levels and thermal ranges (minimum winter temperatures) for PD in grapevine: severe impact, >4.5°C; moderate, from 1.7 to 4.5°C; occasional, from 1.7 to -1.1°C; and rare, <-1.1°C. However, Anas *et al.* (2008) established areas at risk for PD at much lower temperatures, based on the number of days with minimum temperature below

-12.2ºC or -9.4ºC. Following this criterion of minimum temperatures in winter, most southern European countries have climatic conditions that would allow survival of *X. fastidiosa,* and these regions overlap with the production areas for several crops relevant to the EU economy, including olive and grapevine. All of these regions where *X. fastidiosa* has been described in Europe, including the outbreaks in Italy, France, Portugal and Spain, have climatic conditions that are considered favourable for *X. fastidiosa* survival, demonstrating the validity of this criterion. In contrast, Hoddle (2004) used the CLIMEX model to map the potential distribution of PD, and its Californian vector *Homalodisca vitripennis*, based on data from Feil and Purcell (2001), and concluded that regions with tropical, semi-tropical, temperate and moderate Mediterranean climates are suitable for both organisms. These additional criteria indicate that most wine-growing regions of southern France, central and southern Spain and Italy have climatic conditions suitable for PD. Conversely, low winter temperatures would exclude this disease from vineyard-growing areas in France, northern Spain, and Italy (Hoddle, 2004).

In 2019, the Plant Health Panel of EFSA evaluated the potential for establishment of *X. fastidiosa* in the EU. In that study, Köppen-Geiger climate matching revealed that most parts of the EU could be suitable for establishment of the bacterium, excluding only some higher altitude and northern EU regions. However, analyses using species distribution ensemble modelling identified areas in southern Europe to be at more risk, mainly in southern regions of Portugal, Spain, France, Italy, Greece, Malta, and Cyprus (EFSA, 2019), as well as coastal regions of Morocco, Algeria, Tunisia, Libya, Turkey, Syria, and Israel within the Mediterranean Basin (POnTE project, 2019). These results agree with those of Cardone *et al.* (2022), who evaluates risks of establishment and spread of *X. fastidiosa* in the EU, the Balkans and the Middle East and North Africa regions. They identified Malta, followed by Lebanon, Greece, Portugal, Algeria, Spain, Turkey, Egypt, Morocco, and Albania, as the most vulnerable countries with respect to climate suitability. The North European and Arabian Gulf countries were less vulnerable to the spread of the bacterium. When developing these models at pathogen subspecies level, it has been estimated that *X. fastidiosa* subsp. *multiplex* presents the greatest potential for establishment in the EU, compared with that predicted for subspp. *fastidiosa* and *pauca,* with subsp. *multiplex* able to establish the furthest north in the EU (EFSA, 2019).

Using species distribution ensemble modelling, Arias-Giraldo *et al.* (2022) determined relationships between sample location for *X. fastidiosa* with associated regional environmental variables for Andalusia, Southern Spain; the area with the largest olive production in the world. They analyzed ecological requirements for the three main *X. fastidiosa* subspecies, and estimated that the Eastern part of Andalusia was at the greatest relative risk.

Future directions for this research include development of modelling tools that integrate the main components of *X. fastidiosa* epidemics at different spatial and temporal scales, including the effects of environmental drivers (increased precision of weather and land use, and inclusion of insect vector distribution databases), and of climate change. These models will allow prioritization of surveillance programmes for *X. fastidiosa*, based on risk levels in areas free of the pathogen or with recent outbreaks (Arias-Giraldo *et al.*, 2022).

EPIDEMIOLOGICAL MODELS TO ASSIST OUTBREAK RESPONSE PROGRAMMES FOR *XYLELLA FASTIDIOSA*

After an outbreak of *X. fastidiosa* in a region, official surveys are implemented, initially to delimit the infested area, and then to maintain the pest-free status of a surrounding buffer zone (Commission Implementing Regulation (EU) 2020/1201; EC, 2020). Surveillance is a large proportion of the resources required in outbreak response programmes, so several methods have been developed for optimizing survey efficiency. A sequential adaptive delimiting survey for *X. fastidiosa* with increasing spatial resolution was evaluated, using occurrence data of *X. fastidiosa* in Alicante, Spain (Lázaro *et al.*, 2021). Inspection and sampling intensities were adjusted using an optimization algorithm, considering the results obtained in a previous coarse spatial resolution, with three-phase or two-phase designs. With this sequential adaptive survey strategy, it was possible to delimit the distribution of *X. fastidiosa* in the study area, with substantial reduction of the total number of samples to be collected and tested. With some adjustments, this approach could also be used to optimize delimiting surveys in other *X. fastidiosa* outbreaks in Europe and elsewhere.

Effects of climatic and spatial factors on the geographic distribution of *X. fastidiosa* in Lecce, Italy, and Alicante, Spain, were studied with Bayesian hierarchical models (Cendoya *et al.*, 2020). These two outbreaks represent different, but simple, epidemiological scenarios, one with OQDS, caused by *X. fastidiosa* subsp. *pauca* ST53, in Lecce (Morelli *et al.*, 2021; Saponari *et al.*, 2013), and other with ALS, caused by *X. fastidiosa* subsp. multiplex ST6, in Alicante (Landa *et al.*, 2020; Marco-Noales

et al., 2021). The climate covariates presented low variabilities and were not relevant in the resulting models, so they were not related with the distribution of *X. fastidiosa* in the study areas. These results indicate that climate is not likely to stop the spread of the pathogen from outbreaks to adjacent areas. Furthermore, the models were mainly driven by the spatial components, so probability of *X. fastidiosa* presence substantially increased with proximity to infested area. Overall, these results highlight the importance of implementing control measures based on reduction of inoculum and vector populations, to limit further disease spread from outbreak areas.

In epidemiological models, spatial dependence is often considered as direction-invariant and uniform (i.e., isotropic and stationary). However, these assumptions do not hold when there are elements limiting disease spread. This is the case when geographic barriers are present, or control measures are implemented to contain disease spread. Using the outbreak in Alicante, Spain, as a case study, *X. fastidiosa* occurrence data were analyzed through stationary and nonstationary models (Cendoya *et al*., 2022). The nonstationary models considered a cordon sanitaire surrounding the infested area, where host plants were removed and measures applied to impede disease spread. The mean value of the spatial range of the stationary model indicated that host plants closer than 4 km to the infested area would be at risk of *X. fastidiosa* infections. Consequently, the plant health authority increased by 10 km the minimum width of 2.5 km established by the Regulation (EU) 2020/1201 (EC, 2020) for buffer zones surrounding infested zones (Generalitat Valenciana, 2020). The nonstationary models with the cordon sanitaire resulted in a substantial reduction of the probability of *X. fastidiosa* presence outside the infested area. Nevertheless, these models assume that barriers are completely impermeable to pathogen spread, which is not realistic for those causing most plant diseases. Further methodological research is thus required to consider realistic barriers with different levels of permeability.

AIRBORNE HYPERSPECTRAL AND THERMAL IMAGES FROM REMOTE SENSING TO DETECT *XYLELLA FASTIDIOSA* INFECTED HOST PLANTS

Remote sensing studies on *X. fastidiosa* have manily focused on development of algorithms for early detection of symptoms induced by infections either using unmanned (e.g., Castrignanò *et al*., 2021) or manned (e.g., Zarco *et al*., 2018; 2021b) aerial vehicles. Zarco-Tejada *et al.* (2018) studied the Italian *X. fastidiosa* outbreak, evaluating more than 7000 olive trees using high-resolution hyperspectral and thermal imagery. This revealed that pre-visual detection of *X. fastidiosa*-infected trees was feasible using radiative transfer modelling and spectral plant-trait retrievals from imaging spectroscopy and thermal data. Their study showed that changes in specific plant functional traits detected using hyperspectral and thermal imagery could reveal *X. fastidiosa* infections occurring months before symptoms were visible. Important inputs identified for *X. fastidiosa* detection included spectral ratios in the blue region, plant traits such as leaf anthocyanin and carotenoid pigment content estimated using a radiative transfer model inversion, tree temperature, and estimates of solar-induced chlorophyll fluorescence emission. Later Poblete *et al.* (2020) used high-resolution hyperspectral and thermal imagery to assess performance of spectrally constrained machine-learning algorithms to measuring multispectral bandsets, selected from the original hyperspectral imagery, that were compatible with large-scale monitoring from unmanned platforms and a manned aircraft, as well as the contribution of solar–induced chlorophyll fluorescence (SIF) and the temperature-based Crop Water Stress Index (CWSI) retrieved, respectively, from hyperspectral and thermal imaging. This research demonstrated that large-scale *X. fastidiosa* monitoring could be supported using airborne platforms carrying multispectral and thermal cameras with limited numbers of spectral bands (e.g., six to 12 bands with 10 nm bandwidths), as long as the bands were selected for their sensitivity to distinguish *X. fastidiosa* symptoms.

Although these studies have shown that spectral screening methods can detect non-visual symptoms of early infection by *X. fastidiosa*, and can help prevent pathogen spread, the subtle pathogen-induced host physiological alterations detected by imaging spectroscopy can be entangled with dynamics of abiotic stresses. Zarco-Tejada *et al.* (2021b) used airborne spectroscopy and thermal scanning to monitor different EU areas covering more than one million trees, including different host species (olive and almond), affected by two vascular pathogens (*X. fastidiosa* and *Verticillium dahliae*), and comprising a gradient in water stress levels. This study demonstrated the existence of divergent pathogen- and host-specific spectral pathways, that could disentangle biotic-induced symptoms, and showed that uncoupling biotic and abiotic spectral dynamics diminished uncertainty in *X. fastidiosa* detection to less than 6% across different hosts (almond and olive). The study also assessed these deviating pathways against *V. dahliae,* another vascular pathogen that produces symptoms analogous to *X. fastidiosa*, and showed that the divergent

routes remained pathogen- and host-specific, with detection accuracies exceeding 92% across the pathosystems.

Recent studies have also correlated chemical compounds closely associated with *X. fastidiosa* infection in olive plants (i.e., higher contents of malic acid, formic acid, mannitol and sucrose and lower contents of oleuropein; Jlilat *et al*., 2021) with hyperspectral signals, by identifying specific wavelength packages also associated with bacterial infection. This combined spectro-metabolomic approach may represent a new paradigm for reliable detection of *X. fastidiosa* by remote sensing at the early stages of bacterial infection (Ahmed *et al*., 2021; A.M. D'Onghia, *personnal communication*).

The research described above has shown that early detection of *X. fastidiosa*-induced symptoms is feasible with high-resolution hyperspectral and thermal imagery and physically based plant trait retrievals. New research (Poblete *et al*., *in press*) has demonstrated that high resolution multispectral satellite imagery failed to detect early symptoms of infection, but was able to monitor medium and advanced severity levels at large scales. Results from these studies are essential for implementation of effective management of plant diseases, using airborne, drone and satellite based remote sensing technologies. These imaging methods could also contribute to future operational monitoring of infected crop areas at large scales, well beyond what is possible from field surveys and laboratory analyses (Zarco-Tejada *et al*., 2021a).

MOLECULAR DIAGNOSTIC TESTS FOR EARLY DETECTION AND SUBSPECIES DETERMINATION OF *XYLELLA FASTIDIOSA*

The EPPO Diagnostic protocol PM 7/24 (4) for *X. fastidiosa* is the most complete source for current screening tests available for detection of this bacterium and subspecies determination (EPPO, 2019), which includes a detailed description of each screening test with validation data. Among all the screening tests described, those based on molecular approaches are the most sensitive and rapid for *X. fastidiosa* detection and will be the focus of this review.

Most molecular tests used for *X. fastidiosa* diagnoses are based on conventional end-point PCR or real-time PCR (qPCR). However, for some of the PCR protocols, if the amplicon product is sequenced it can also provide information for subspecies assignation. The Commission Implementing Regulation (EU) 2021/1688 (EC, 2021) indicates which tests from those described in the EPPO Diagnostic protocol PM 7/24 should be used for official surveys and identification of *X. fastidiosa* and its subspecies.

There are several conventional PCR tests for *X. fastidiosa* diagnoses, and one of the most commonly used is that developed by Minsavage *et al.* (1994). This test is based on amplification of the RNA polymerase sigma-factor 70 (*rpo*D). A search on Google Scholar using 'Minsavage and *Xylella fastidiosa*' retrieved more than 800 publication records. This test can also be used to assign *X. fastidiosa* at the subspecies level, by sequencing the *rpo*D amplicon, and comparing its sequence using the Basic Local Alignment Search Tool (BLASTN; http://www.ncbi.nlm.nih.gov/) available at the National Center for Biotechnology Information, against the RefSeq Genome database of *Xylella fastidiosa* (taxid: 2371) as the search organism. This tool also allows a distance tree of results to be obtained, that clusters the target sequence with those *rpo*D sequences included in the reference genomes of different strains of *X. fastidiosa* belonging to different subspecies (Figure 1).

Other conventional PCR diagnostic tests are based on the gene encoding the β-subunit polypeptide of the DNA gyrase (*gyrB*). Although this methodology is not included in the EPPO Diagnostic protocol PM 7/24, it was used for developing a mini sequencing or single-nucleotide primer extension (SNuPE) approach for the multiplex amplification of six *X. fastidiosa gyrB* sequences targeting subspp. *fastidiosa, multiplex* and *sandyi*, and three genotypes within subspecies *pauca* present in the EU territory, and including strains from coffee and citrus from Brazil, and the type isolate infecting olive in Italy (Montes-Borrego *et al*., 2015; Saponari *et al*., 2016).

Several qPCR tests are available and have been validated at different laboratories (EPPO, 2019), including tests described by Harper *et al.* (2010), Francis *et al.* (2006), Ouyang *et al.* (2013), and Li *et al.* (2013). The target sequences for these tests are, respectively, the 16S rRNA processing RimM protein, the hypothetical protein HL gene, the cobalamin synthesis protein-coding gene, and the 16S rRNA. Because of their greater analytical sensitivity than other molecular tests, the use of qPCR is recommended for detection surveys to substantiate pest freedom in areas where *X. fastidiosa* is not known, and for asymptomatic plants (EPPO, 2019). These qPCR tests have been widely used in studies assessing distribution and host range of *X. fastidiosa* in Europe (e.g., Saponari *et al*., 2013; Jacques *et al*., 2016; Moralejo *et al*., 2020; Olmo *et al*., 2021), potential insect vectors of *X. fastidiosa* (e.g., Elbeaino *et al*., 2014; Cavalieri *et al*., 2019; Cunty *et al*., 2020; Moralejo *et al*., 2020; Marco-Noales *et al*., 2021), and in remote sensing studies (e.g., Zarco-Tejada *et al*., 2018; Poblete *et al*., 2020; Zarco-Tejada *et al*., 2021b; Camino *et al*., 2022) to outline examples of their usefulness.

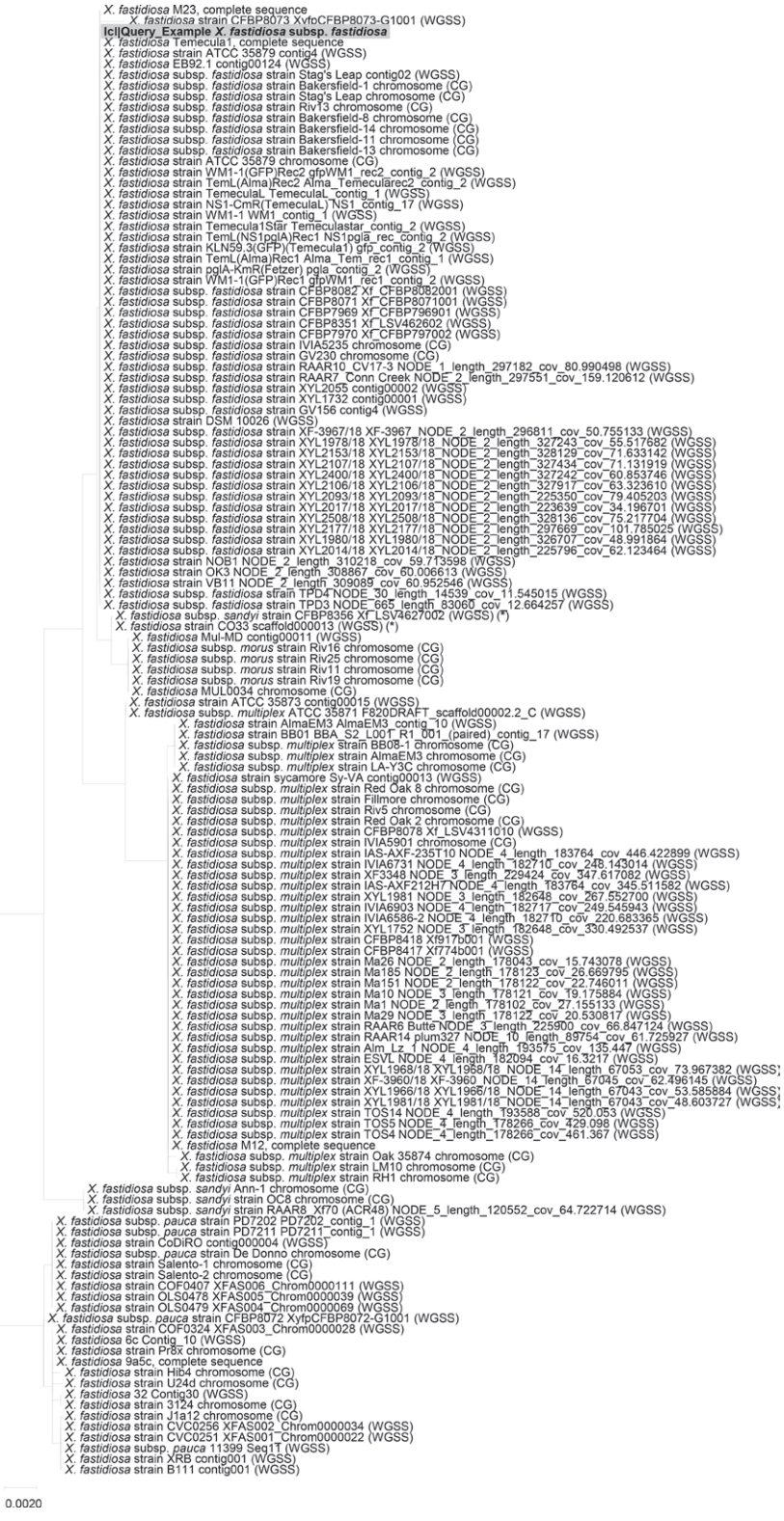

Figure 1. Phylogenetic distance tree of RNA polymerase sigma-factor 70 (*rpo*D) partial sequences obtained after BLAST analysis (http://www.ncbi.nlm.nih.gov/) of a query sequence against the RefSeq Genome database of *Xylella fastidiosa* (taxid: 2371). The different clusters correlate with main *X. fastidiosa* subspecies. lcl|Query= Represents an example of a query made for the *rpo*D sequence of a *X. fastidiosa* subsp. *fastidiosa* strain. (CG)= Complete Genome; (WGSS)= Whole Genome Shotgun Sequence. (*)= recombinant subspecies *morus/sandyi*.

Where a positive result is obtained in areas outside of demarcated areas (i.e., pest-free areas), the Commission Implementing Regulation (EU) 2020/1201 (EC, 2020) indicated that presence of *X. fastidiosa* must be confirmed by two tests targeting different parts of the bacterial genome, as recommended by EPPO (2019). To facilitate diagnosis of *X. fastidiosa* with two tests, Bonants *et al.* (2019) implemented a triplex qPCR test based on the primers and probes included in Harper qPCR and Ouyang qPCR tests, and an additional primer pair and probe for internal controls. This test facilitates two diagnostic tests simultaneously, saving time and resources, and can provide the same analytical sensitivity as each test independently (Bonants *et al.*, 2019).

Results from test performance studies (TPS) and proficiency tests (PT) performed in the frameworks of XF-ACTORS and POnTE, Euphresco PROMODE projects, have compared the performance characteristics of qPCR tests when used with plant or insect mock-inoculated matrices, and these results are available at the EPPO Database on Diagnostic Expertise (https://dc.eppo.int; EPPO, 2023). Results from these validation tests have indicated that all the qPCR diagnostic protocols were robust, and were suitable for the diagnoses of *X. fastidiosa* in plant and insect materials. However, although most qPCR protocols produced good performance values, their analytical sensitivity was slightly different when using mock-inoculated samples. A recent study has also shown greater analytic sensitivity of the Harper qPCR test compared to the Francis qPCR test, when using DNA samples extracted from naturally infected almond trees (Anguita-Maeso *et al.*, 2021).

For *X. fastidiosa* subspecies assignment, the Commission Implementing Regulation (EU) 2020/1201 (EC, 2020) determines Multilocus Sequence Type (MLST) analysis to be used (Yuan *et al.*, 2010), especially for new records (i.e., for a new outbreak or new hosts). This test is based on amplification and sequencing of seven housekeeping gene (HKG) loci (*cys*G, *glt*T, *hol*C, *leu*A, *mal*F, *nuo*L, *pet*C). Analysis of *rpo*D and *mal*F or *cys*G and *mal*F sequences have been shown to be sufficient for assignment of sample pathogens to subspecies (EPPO, 2019); whereas the sequences of the seven loci are needed to assign samples into STs. Originally, MSLT analysis was designed to be used with DNA extracted from *X. fastidiosa* pure cultures (Yuan *et al.*, 2010), and when used with plant DNA samples is partly efficient. To improve sensitivity, Cesbron *et al.* (2020) developed a direct nested-MLST assay for plant and insect extracted DNA, based on the same seven targeted HKG loci as those used in the Yuan *et al.* (2010) test. This nested-MLST assay improved detection threshold by 100 to 1000 times, compared to conventional MLST. Using this nested-MLST assay, plants that were previously not considered hosts (giving high or inconclusive Ct values in qPCR assays) tested positive, and novel alleles were revealed in France. In samples from Spanish outbreaks, the nested-MLST assay allowed to identify the *X. fastidiosa* subspecies or ST infecting new hosts in Europe at that time (Cesbron *et al.* 2020). This nested-MLST assay has been used to type *X. fastidiosa* positive samples to the subspecies and ST level in the outbreaks in Spain. These include three *X. fastidiosa* subspecies and four STs in the Balearic Islands: *X. fastidiosa* subsp. *fastidiosa* ST1 and *X. fastidiosa* subsp. *multiplex* ST7 in Mallorca, *X. fastidiosa* subsp. *pauca* ST80 in Ibiza, and *X. fastidiosa* subsp. *multiplex* ST81 in Mallorca and Menorca (Olmo *et al.*, 2021). Only a single subspecies and ST (i.e., *X. fastidiosa* subsp. *multiplex* ST6) was identified in the outbreak in Alicante (Marco-Noales *et al.*, 2021).

Some qPCR tests have been developed to specifically detect subspecies of *X. fastidiosa* (e.g., the tests of Burbank *et al.*, 2018; Dupas *et al.* 2019; Hodgetts *et al.*, 2021). These tests are based on Taqman probes, designed to specifically and simultaneously target one or several *X. fastidiosa* subspecies. The advantage of these protocols is, while the presence of the bacterium is detected, even at low concentration, the subspecies is also defined. The Burbank qPCR test was used by Moralejo *et al.* (2020) to track the *X. fastidiosa* DNA inside growth rings of infected almond trees, through dendrochronological analysis. The protocol allowed differentiation between subsp. *fastidiosa* and *multiplex* in rings of 25 trees, with nine infected by subsp. *fastidiosa*, and 19 infected by subsp. *multiplex,* and three trees had mixed infections. This qPCR test combined with the conventional and nested-MLST tests (described above) enabled dating infections back to 1998 for *X. fastidiosa* subsp. *fastidiosa* ST1, and before 2000 for *X. fastidiosa* subsp. *multiplex* ST81 (Moralejo *et al.*, 2020), indicating that the bacterium was introduced to the Balearic Islands earlier than previously thought.

Time and portability are also important factors in pathogen diagnoses, especially for quarantine plant pathogens (Aglietti *et al.*, 2019). Isothermal nucleic acid amplification tests have been developed for *X. fastidiosa* field diagnoses. These included loop-mediated isothermal amplification (LAMP) based on primers developed by Harper *et al.* (2010) that were modified by Yaseen *et al.* (2015), and the AmplifyRP® XRT+ test, using isothermal amplification based on recombinase polymerase amplification (RPA) (Kersting *et al.*, 2014), based on the protocol of Li *et al.* (2016). Both tests have kits and specific portable devices that are commercially available,

but other equipment can also be used. For instance, the AmplifyRP® XRT+ test uses the battery powered AmpliFire® Portable Fluorometer device, that is easy to transport, and does not require DNA extraction, since the test can be performed with the crude plant macerate and the amplification takes 20 min. This test is easy to use by untrained laboratory staff, and is well adapted to the field for preliminary on site screening, as it requires no particular expertise (Cesbron *et al*., 2022).

A constraint of these tests is that detection limits are greater than for qPCR tests. Nevertheless, negative or doubtful results can be verified with another, more sensitive test. During a field campaign in Mallorca, Spain, almond trees in 14 orchards under rainfed and irrigated conditions were visually scored for the presence of ALS. A total of 356 symptomatic and symptomless trees were sampled and analyzed using the AmplifyRP® XRT+ test and the AmpliFire® device by two operators in less of 5 days (Camino *et al*., 2022). When comparing results of this test with that of the Harper qPCR test using the same almond plant branch samples, 92.8% agreement for infected samples was obtained for the two methods. The samples that were negative by the AmplifyRP® XRT+ test showed cycle thresholds (Cts) >31 in the Harper qPCR test (Landa *et al*., unpublished).

Digital PCR (dPCR) is an innovative PCR tool based on partitioning of PCR reagents and DNA samples into thousands of droplets or microchambers (depending on thermocycler brand), that allows increased precision, sensitivity and absolute quantification without requirements for reference samples or standard curves. Detection of phytopathogenic bacteria by droplet PCR has provided successful results for pathogen diganoses, due to its detection efficiency at low pathogen concentrations and tolerance to PCR inhibitors (Dreo *et al*. 2014). Dupas *et al*. (2019) developed a droplet ddPCR protocol based on the Harper qPCR test. Both protocols showed the same detection limits for olive, *Polygala myrtifolia* and *Rosmarinus officinalis*, but the Harper qPCR test allowed better detection of 0.5 log for *Lavandula angustifolia*, and droplet dPCR allowed better detection of 0.5 log for *Quercus ilex*.

Investigation of pathogen strain origins in a new disease outbreaks requires whole genome sequencing (WGS) of pure bacterial cultures to resolve phylogenetic reconstruction. This is a challenge for *X. fastidiosa* due to its fastidious nature. For all the available nucleic acid based amplification methods described above for the detection of *X. fastidiosa,* the target sequence is a single locus, making the assays prone to false-positive or false-negative results (Bonants *et al*., 2019). High throughput Next Generation Sequencing (NGS) technologies allow sequencing of total DNA in samples, potentially providing detection of *X. fastidiosa* and characterization to subspecies and strain levels, without requirement for pathogen cultivation. NGS based on Illumina (Bonants *et al*., 2019; Román-Reyna *et al*., 2022) or Oxford Nanopore technologies (Johnson *et al*., 2022) have been explored for fast detection and identification of *X. fastidiosa*. The potential advantages and disadvantages of both of these technologies are beyond the scope of this review.

Using Illumina sequencing, Bonants *et al.* (2019) analyzed DNA extracts, by WGS, for presence of *X. fastidiosa* in artificially inoculated host plants and from naturally infected plants sampled or intercepted in different European countries. In all samples, even in samples with low infection levels, some DNA reads specific for *X. fastidiosa* were detected, and in several cases the pathogen could be identified to the subspecies level. Only for one sample was the whole genome assembled and the ST determined. Samples in which more *X. fastidiosa* genomic information was obtained corresponded to those with low Ct values (i.e., high *X. fastidiosa* titres). Thus, alinear relationship is found between the Log number of *X. fastidiosa* reads obtained by NGS and the Cts from Harper and Ouyang qPCR tests, and the time for positivity in a LAMP assay (Figure 2). Similarly, Anguita-Maeso *et al.* (2021) found a linear relationship between the Ct values obtained for the Harper and Francis qPCR tests and the Log of *X. fastidiosa* reads. Román-Reyna *et al.* (2021) developed a metagenomics pipeline using in-house short read Illumina sequencing to analyze samples from different plant species originating from Europe and the United States, and naturally infected by *X. fastidiosa*. They identified *X. fastidiosa* to the strain level in single and mixed infected plant samples at concentrations of 1 pg of bacterial DNA per gram of plant tissue, and in samples previously considered inconclusive when using qPCR (Ct >35), the protocol was able to confirm infection by *X. fastidiosa*. These results indicate that using the NGS approach, only in cases where DNA has been extracted from highly infected material, and where high genome coverage is used during sequencing, is possible to identify *X. fastidiosa* at the strain level.

To overcome this limitation, we have developed a Targeted Sequence Capture Enrichment (TSCE), in combination with High Throughput Sequencing (HTS). This uses an Illumina platform to provide adequate bacterial genome information for identification of *X. fastidiosa* at strain level (Velasco-Amo *et al*., 2021). Results indicated that although <0.25% of *X. fastidiosa* reads were detected by direct WGS of host DNA, this was increased by 41–73% when using the TSCE-HTS approach, for indi-

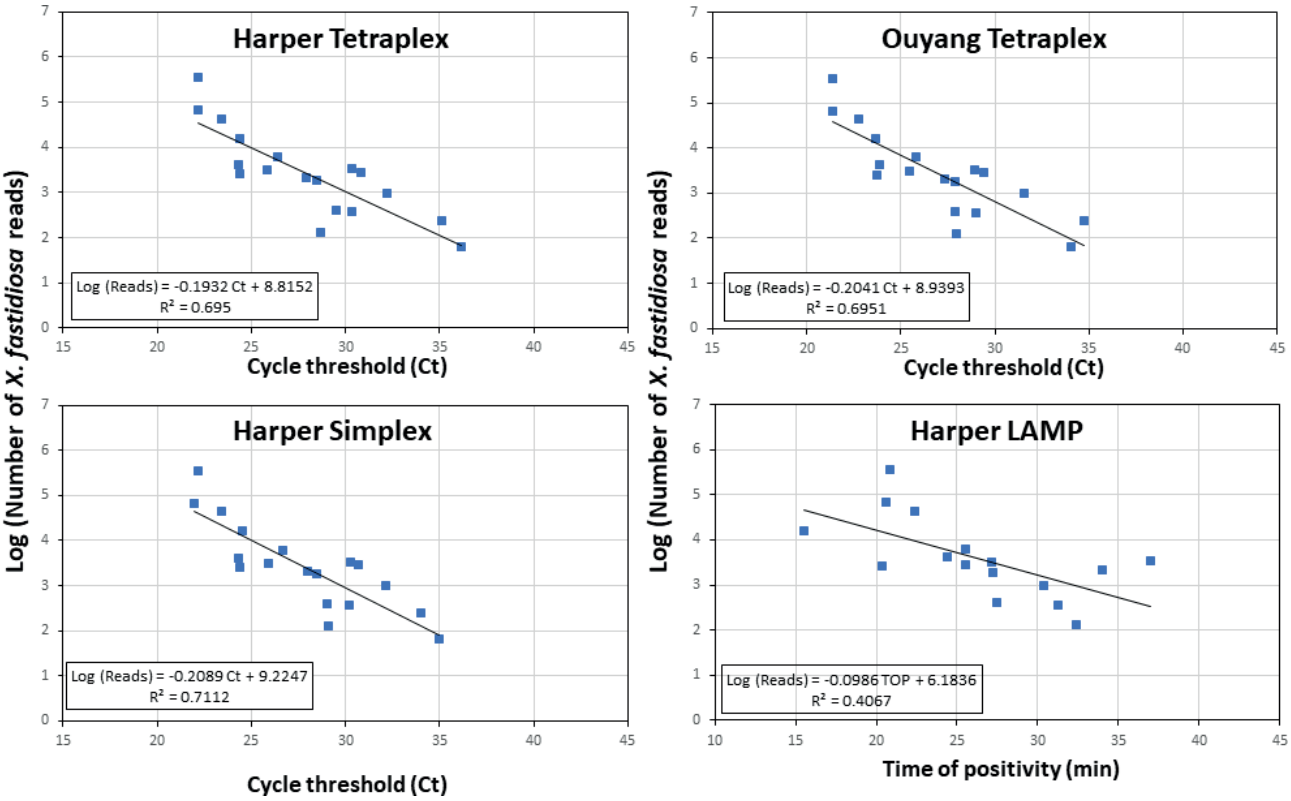

Figure 2. Linear regressions between Log [*Xylella fastidiosa* reads] obtained by next generation sequencing (NGS) and Ct values from quantitative polymerase chain reaction (qPCR) protocols of Ouyang tetraplex and Harper tetraplex (Bonants *et al.*, 2019), Harper Simplex (Harper *et al.* 2010), and LAMP test based in Harper *et al.* (2010). Data were obtained from Table 7 of Bonats *et al.* (2019), and represent the mean Ct value obtained for each sample.

vidual samples or in mixtures of up to four plant samples. The protocol was also validated using a range of insect and plant samples from different naturally infected host plants, and with levels of *X. fastidiosa* ranging from very high (CT >20) to close to the detection limit for Harper qPCR assay. More importantly, 80–100% of the 140 target sequences used to design the baits were captured, which allowed phylogenetic reconstruction of the *X. fastidiosa* strains infecting the samples, and identifying these at strain level (Landa *et al.*, 2021). This methodology may be useful for studies of *X. fastidiosa* introductions at outbreak stages, since a limited number of genetic markers do not provide sufficient phylogenetic resolution to determine dispersal paths or relationships among strains that are of biological and quarantine relevance.

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Fungal trunk diseases of fruit trees in Europe: pathogens, spread and future directions

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Summary. Production from crops of pome, stone fruit, nut, berry fruit, citrus, grapevine, and olive is increasingly threatened by fungal trunk diseases (FTD). These diseases and the consequent production losses are major problems. Many fungi (including *Botryosphaeriaceae*, *Calosphaeriaceae*, *Diaporthaceae*, *Diatrypaceae*, *Nectriaceae*, *Phaeomoniellaceae*, *Pleosporaceae*, *Togniniaceae*, *Valsaceae*) infect host wood, mainly through wounds and subsequent colonization of woody tissues, causing symptoms such as cankers, gummosis, wood rotting, blight and dieback. Propagative plant material, seedlings and fruit play a significant role in pathogen spread. Several abiotic factors (e.g. shifts in cultural practices and climate change) are involved in the disease development. This paper reviews recent literature on FTD of fruit crops, particularly focusing on the European status of pathogen occurrence. Case studies are described related to diseases of apple, citrus, grapevine, berry, nut and stone fruit, and olive trees. Aspects related to epidemiology and the increase in disease incidence along with the future perspectives on the FTD research are also discussed.

Keywords. Wood cankers, dieback, *Botryosphaeriaceae*, abiotic factors, epidemiology.

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INTRODUCTION

Trees cultivated in plantations are increasingly threatened by fungal trunk diseases (FTD) (van Niekerk *et al.,* 2004; Gramaje *et al*., 2016). There is increasing evidence that trees share pathogens with plants of forestry environments and with woody hosts that are not considered to be trees (Crous and Wingfield, 2018). Examples are pathogens in the family *Botryosphaeriaceae*, which are polyphagous, being often involved in diseases affecting diverse crops and plants of forest importance (Van Niekerk *et al.,* 2004; Guarnaccia *et al*., 2022). High incidence of FTD in fruit crops, such as pome and stone fruits, nut, berry fruit, citrus and olive, has been reported (Gramaje *et al.,* 2012; Úrbez-Torres *et al.,* 2013b; Carlucci *et al.,* 2015b; Guarnaccia and Crous, 2017), demonstrating a need for focus on this group of host plants. FTD have become major concerns for fruit industry stakeholders, and their occurrence in orchards and consequent production losses, is likely to have resulted from several causes, including shifts in cultural practices and climate change (Doll *et al.,* 2013).

Canker diseases of fruit crops are caused by a broad range of fungi that infect host wood, mainly through different kinds of wounds and subsequent colonization of vascular tissues (Crous and Wingfield, 2018). Wood pathogens cause symptoms such as cankers, gummosis, wood rotting, blight and dieback (Fig. 1; Gramaje *et al.,* 2012). Dieback of shoots, branches, and main trunks can lead to tree death in severe cases (Slippers and Wingfield 2007). Several FTD pathogens have been identified as species within *Botryosphaeriaceae*, *Calosphaeriaceae*, *Diaporthaceae*, *Diatrypaceae*, *Nectriaceae*, *Phaeomoniellaceae*, *Pleosporaceae*, *Togniniaceae*, and *Valsaceae,* as well as *Basidiomycota* (Rumbos 1988; Moral *et al.,* 2010; Kaliterna *et al.,* 2012; Carlucci *et al.,* 2013; 2015; Úrbez-Torres *et al.,* 2013a; Guarnaccia *et al.,* 2018b).

Some of these fungal pathogens live as endophytes in hosts after entering through wounds or natural openings, and pathogen spread occurs through asymptomatic plant material, seedlings and fruit, frequently circumventing country and region quarantine measures (Slippers and Wingfield, 2007).

Abiotic factors are strongly involved with FTD. High planting densities are required to maximize production and land resources, and these are combined with plant nutrient programmes, giving stressed cultivated plants. For example, almond production in Spain has increased in recent years, and new agronomic practices were adopted to increase yields from new plantations (León *et al*., 2020). However, incidence of almond associated FTD, such as twig cankers and shoot blight caused by *Diaporthe* spp., has also increased (León *et al.,* 2020). Abundant pruning wounds also promote chances of infections through possible airborne pathogen entry points (Henderson *et al.,* 2021). Similarly, wounds induced by mechanical shaking of trunks for fruit harvesting can increase host infection (Holland *et al.,* 2021a). Tree crop nurseries are important for meeting demands for plant material, and a lack of top-quality plants means greater incidence of FTD pathogens in the orchards. Global warming and climate change can increase plant stress and generate favourable conditions for the development of FTD, as for diseases caused by *Botryosphaeriaceae* (Pour *et al.,* 2020), which are serious threats to different crops (Slippers *et al.,* 2006).

The present paper aims to review recent literature on FTD, with a particular focus on the European situation of their causal agents, distribution and host associations, particularly relating to case studies on apple, citrus, grapevine, berry, nut and stone fruit, and olive trees. Moreover, epidemiology and hypotheses on the increase of FTD incidence are discussed, and future prospects and direction of trunk disease research are presented with the purpose of achieving sustainable disease management.

PATHOGEN DISTRIBUTION AND HOST RANGE IN EUROPE

In Europe, the group of fungi causing FTD is diverse and expands as new reports demonstrate (Table 1). While some of these fungi are probably host-specific, others have broad host ranges, including members of the *Botryosphaeriaceae*. This family is the most prevalent, and members can infect many different fruit crops. For example, *B. dothidea* is ubiquitous in Europe, and has been associated with FTD of walnut, hazelnut, almond, stone fruit, grapevine, olive, pistachio, apple and blueberry (Moral *et al.,* 2010; Gramaje *et al.,* 2012; Akgül *et al.,* 2014; Fischer *et al.,* 2016; Baránek *et al.,* 2018; Hilário *et al.,* 2020a; López-Moral *et al.,* 2020a,b). In contrast, *Neofusicoccum luteum* has only been isolated from grapevines in Portugal and France (van Niekerk *et al.,* 2004). *Diplodia seriata* can has also been found in different parts of Europe on many hosts, including walnut, grapevine, pear, quince, apple, apricot, plum, nectarine, almond and olive (Luque *et al.,* 2009; Gramaje *et al.,* 2012; Phillips *et al.,* 2012; Carlucci *et al.,* 2013; Kraus *et al.,* 2019; Bien and Damm 2020; Eichmeier *et al.,* 2020; López-Moral *et al.,* 2020a). *Diplodia mutila* is also common and infects grapevine, and trees of walnut, apricot, olive, plum, pear and apple (Phillips *et al.,* 2012; Carlucci *et al.,* 2013; Alves *et al.,* 2014; Gierl and Fischer,

Figure 1. FTD symptoms on different fruit trees. Natural dieback of *Vaccinium corymbosum* 'Blue Ribbon' in the field (**A**); branch canker on *Citrus sinensis* caused by *Diaporthe* spp. (**B**, from Guarnaccia *et al*., 2020); trunk canker on apple caused by *Neonectria ditissima* (**C**); internal discolouration of *Citrus reticulata* branch affected by *Botryosphaeriaceae* (**D**, from Bezerra *et al*., 2022); internal discolouration of avocado twig caused by *Neofusicoccum* spp. (**E**); cross-section showing a central white rot surrounded by black spots and sectorial necrosis of an esca infected vine (**F**), wedge-shaped canker in a Eutypa dieback affected vine (**G**), black spots and dark brown to black streaking of the xylem tissue in almond branches (**H**), sectorial necrosis of walnut affected trees (**I**), wood discolouration in pear branches (**J**).

Table 1. Distribution and host range of fungal pathogens associated with FTD on fruit crops in Europe based on literature research.

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Bionectriaceae*	*Acremonium*	*Acremonium sclerotigenum*	*Olea*, *Vitis*	Spain, Italy	Agustí-Brisach *et al.*, 2021; Lorenzini *et al.*, 2016
Ascomycota	*Xylariaceae*	*Biscogniauxia*	*Biscogniauxia nummularia*	*Prunus* (Plum), *Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Xylariaceae*	*Biscogniauxia*	*Biscogniauxia rosacearum*	*Cydonia*, *Prunus* (Plum), *Pyrus*	Italy	Raimondo *et al.*, 2016
Basidiomycota	*Phanerochaetaceae*	*Bjerkandera*	*Bjerkandera adusta*	*Prunus* (Plum), *Prunus* (Cherry), *Vitis*	Germany	Bien and Damm, 2020; Kraus *et al.*, 2018
Ascomycota	*Botryosphaeriaceae*	*Botryosphaeria*	*Botryosphaeria dothidea*	*Juglans*, *Prunus*, *Vitis*, *Prunus*, *Olea*, *Pistacia*, *Malus*, *Prunus* (Almond), *Vaccinium*, *Mangifera*	Spain, Switzerland, Portugal, Italy, Germany, Turkey, France, Czech Republic	López-Moral *et al.*, 2020a,b; van Niekerk *et al.*, 2004; Batista *et al.*, 2020; Carlucci *et al.*, 2013; Fischer *et al.*, 2016, Akgül *et al.*, 2014; Pintos *et al.*, 2018; Turkolmez *et al.*, 2016; Moral *et al.*, 2010; Baránek *et al.*, 2018; Gramaje *et al.*, 2012; Hilário *et al.*, 2019; Aiello *et al.*, 2022
Ascomycota	*Botryosphaeriaceae*	*Botryosphaeria*	*Botryosphaeria lutea* (= *Neofusicoccum luteum*)	*Vitis*	Portugal	van Niekerk *et al.*, 2004
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora fastigiata*	*Vitis*	Germany, Switzerland	Fischer *et al.*, 2016; Casieri *et al.*, 2009
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora luteo-olivacea*	*Vitis*, *Vaccinium*, *Olea*, *Prunus* (Plum)	Germany, Spain, France, Italy	Fischer *et al.*, 2016; Pintos *et al.*, 2018; Guarnaccia *et al.*, 2020; Agustí-Brisach *et al.*, 2021; Bien and Damm, 2020; Raimondo *et al.*, 2019, Casieri *et al.*, 2009
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora malorum*	*Malus*, *Actinidia*	Germany, Italy	Gierl and Fischer, 2017; Prodi *et al.*, 2008
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora melinii*	*Vitis*	Spain	Gramaje *et al.*, 2011
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora novi-eboraci*	*Juglans*, *Malus*, *Prunus* (Cherry)	Czech Republic, Germany	Eichmeier *et al.*, 2020; Gierl and Fischer, 2017; Bien and Damm, 2020
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora prunicola*	*Prunus* (Plum), *Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Ploettnerulaceae*	*Cadophora*	*Cadophora ramosa*	*Prunus* (Cherry), *Juglans*	Germany, Czech Republic	Bien and Damm, 2020; Eichmeier *et al.*, 2020
Ascomycota	*Calosphaeriaceae*	*Calosphaeriophora*	*Calosphaeria pulchella*	*Prunus* (Cherry)	Spain	Berbegal *et al.*, 2014
Ascomycota	*Glomerellaceae*	*Colletotrichum*	*Colletotrichum fruticola*	*Persea*	Italy	Guarnaccia *et al.*, 2016
Ascomycota	*Glomerellaceae*	*Colletotrichum*	*Colletotrichum gloeosporioides*	*Citrus*, *Persea*	Italy	Guarnaccia *et al.*, 2016,, 2017
Ascomycota	*Glomerellaceae*	*Colletotrichum*	*Colletotrichum karstii*	*Citrus*	Portugal	Ramos *et al.*, 2016
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina africana*	*Prunus* (Apricot), *Prunus* (Almond), *Prunus* (Plum)	Germany	Gierl and Fischer, 2017; Bien and Damm, 2020
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina badensis*	*Prunus* (Plum)	Germany	Bien and Damm, 2020
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina germanica*	*Prunus* (Cherry)	Germany	Bien and Damm, 2020

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Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina hispanica*	*Prunus* (Apricot), *Prunus* (Almond)	Germany, Spain	Gierl and Fischer, 2017; Gramaje *et al.*, 2012
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina neorubra*	*Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Tympanidaceae*	*Collophorina*	*Collophorina paarla*	*Prunus* (Cherry)	Germany	Gierl and Fischer, 2017
Ascomycota	*Diademaceae*	*Comoclathris*	*Comoclathris incompta*	*Olea*	Spain, Croatia, Italy	Moral *et al.*, 2017; Ivic *et al.*, 2010; Carlucci *et al.*, 2013
Ascomycota	*Coniochaetaceae*	*Coniochaeta*	*Coniochaeta hoffmannii*	*Vitis*	Germany	Fischer *et al.*, 2016
Ascomycota	*incertae sedis*	*Cryptovalsa*	*Cryptovalsa ampelina*	*Juglans*, *Vitis*	Czech Republic, Spain, Germany	Eichmeier *et al.*, 2020; Luque *et al.*, 2009,, 2012; Martin *et al.*, 2009; Kraus *et al.*, 2018
Ascomycota	*Nectriaceae*	*Cylindrocarpon*	*Cylindrocarpon destructans*	*Olea*, *Vitis*	Italy, Spain	Carlucci *et al.*, 2013; Gonzalez and Tello, 2011
Ascomycota	*Nectriaceae*	*Cylindrocarpon*	*Cylindrocarpon pauciseptatum*	*Vitis*, *Prunus* (Peach), *Prunus* (Plum)	Slovenia, Portugal, Italy, Spain, Bulgaria, France	Cabral *et al.*, 2012; Yaseen *et al.*, 2012; Martin *et al.*, 2011; Piperkova *et al.*, 2017; Pintos *et al.*, 2018
Ascomycota	*Nectriaceae*	*Cylindrocarpon*	*Cylindrocarpon peruviana*	*Persea*	Italy	Aiello *et al.*, 2020
Ascomycota	*Valsaceae*	*Cytospora*	*Cytospora chrysosperma*	*Prunus* (blackthorn), *Vitis*	Germany, Spain	Gierl and Fischer, 2017; Kraus *et al.*, 2018; González and Tello, 2011
Ascomycota	*Valsaceae*	*Cytospora*	*Cytospora oleina*	*Olea*	Greece, Italy	Rumbos, 1988; Carlucci *et al.*, 2013
Ascomycota	*Valsaceae*	*Cytospora*	*Cytospora pistaciae*	*Pistacia*	Italy	Aiello *et al.*, 2019
Ascomycota	*Valsaceae*	*Cytospora*	*Cytospora pruinosa*	*Olea*	Spain	López-Moral *et al.*, 2020b; Moral *et al.*, 2017
Ascomycota	*Nectriaceae*	*Dactylonectria*	*Dactylonectria hordeicola*	*Vitis*	France	Pintos *et al.*, 2018
Ascomycota	*Nectriaceae*	*Dactylonectria*	*Dactylonectria macrodidyma*	*Vitis*	France, Spain	Pintos *et al.*, 2018
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria macrodidyma*	*Vitis*	Portugal, France, Turkey, Spain	Cabral *et al.*, 2012; Augustí-Brisach and Armengol, 2013
Ascomycota	*Nectriaceae*	*Dactylonectria*	*Dactylonectria pauciseptata*	*Vitis*	France, Spain	Pintos *et al.*, 2018
Ascomycota	*Nectriaceae*	*Dactylonectria*	*Dactylonectria torresensis*	*Vitis*	France, Spain	Pintos *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe ampelina*	*Vitis*	France, Spain, Turkey, Czech Republic, Germany, UK, Croatia, Hungary	Pintos *et al.*, 2018; Akgül *et al.*, 2015; Baránek *et al.*, 2018; Kraus *et al.*, 2018; Guarnaccia *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe amygdali*	*Prunus* (Almond), *Juglans*, *Vaccinium*, *Persea*	Portugal, Spain, Hungary, Italy	López-Moral *et al.*, 2020b; Varjas *et al.*, 2017; Guarnaccia *et al.*, 2016,, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe baccae*	*Vitis*, *Vaccinium*, *Citrus*, *Mangifera*	Spain, France, Italy, Croatia	Guarnaccia *et al.*, 2017,, 2018; Aiello *et al.*, 2022
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe bohemiae*	*Vitis*	Czech Republic	Guarnaccia *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe celeris*	*Vitis*	UK	Guarnaccia *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe cinerascens*	*Ficus*	Bulgaria	López-Moral *et al.*, 2020a,b

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Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe eres*	*Malus*, *Vitis*, *Prunus* (Cherry), *Vaccinium*, *Juglans*, *Prunus* (Peach), *Pyrus*	Germany, France, Spain, Czech Republic, UK, Italy, Hungary, Croatia, Poland, the Netherlands, Lithuanian, Greece, Switzerland	Gierl and Fischer, 2017; Pintos *et al.*, 2018; Bien and Damm, 2020; Guarnaccia *et al.*, 2018; Lombard *et al.*, 2014; Eichmeier *et al.*, 2020; Thomidis *et al.*, 2009; Prencipe *et al.*, 2017; Bertetti *et al.*, 2018; Casieri *et al.*, 2009
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe foeniculina*	*Ficus*, *Vitis*, *Citrus*, *Persea*, *Pyrus*, *Vaccinium*, *Juglans*, *Prunus* (Almond), *Mangifera*, *Litchi*	Germany, France, Spain, Greece, Malta, Portugal, Italy	Gierl and Fischer, 2017; Pintos *et al.*, 2018; Vakalounakis *et al.*, 2019; Guarnaccia *et al.*, 2016,, 2017; Mathioudakis *et al.*, 2020; Santos *et al.*, 2017; Hilario *et al.*, 2020; Lopez-Moral *et al.*, 2020; Gramaje *et al.*, 2012; Aiello *et al.*, 2022
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe hispaniae*	*Vitis*	Spain	Guarnaccia *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe hungariae*	*Vitis*	Spain, Hungary	Guarnaccia *et al.*, 2018
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe limonicola*	*Citrus*	Malta	Guarnaccia *et al.*, 2017
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe mahothocarpus*	*Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe melitensis*	*Citrus*	Malta	Guarnaccia *et al.*, 2017
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe novem*	*Vitis*, *Citrus*	France, Spain, Italy	Pintos *et al.*, 2018; Guarnaccia *et al.*, 2017
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe phaseolorum*	*Vitis*	Spain, Switzerland	Pintos *et al.*, 2018; Casieri *et al.*, 2009
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe rudis*	*Vitis*, *Vaccinium*, *Prunus* (Plum), *Prunus* (Cherry), *Litchi*	France, Spain, Italy, Germany, Portugal, UK, Switzerland, the Netherlands	Pintos *et al.*, 2018, Guarnaccia *et al.*, 2018,, 2020; Bien and Damm, 2020; Casieri *et al.*, 2009; Lombard *et al.*, 2014; Aiello *et al.*, 2022
Ascomycota	*Diaporthaceae*	*Diaporthe*	*Diaporthe vaccinii*	*Vaccinium*	the Netherlands, Lithuania, Latvia	Lombard *et al.*, 2014
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia bulgarica*	*Malus*, *Pyrus*	Bulgaria, Germany, Turkey	Phillips *et al.*, 2012; Hinrichs-Berger *et al.*, 2021; Eken, 2021
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia corticola*	*Vitis*	Italy	Carlucci *et al.*, 2015
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia pseudoseriata*	*Pistacia*	Italy	Batista *et al.*, 2020
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia juglandis*	*Juglans*	France	López-Moral *et al.*, 2020
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia malorum*	*Malus*	Portugal	Phillips *et al.*, 2012; Alves *et al.*, 2014
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia mutila*	*Vitis*, *Juglans*, *Prunus* (Apricot), *Olea*, *Prunus* (Plum), *Pyrus*, *Malus*	Portugal, Germany, Italy, Spain, France, Netherlands, England	López-Moral *et al.*, 2020, Gierl and Fischer, 2017; Carlucci *et al.*, 2013; Pintos *et al.*, 2018; Bien and Damm, 2020; Batista *et al.*, 2020; Phillips *et al.*, 2013; Alves *et al.*, 2014
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia olivarum*	*Olea*, *Prunus* (Almond), *Pistacia*	Italy, Spain	Phillips *et al.*, 2012; Gramaje *et al.*, 2012; Alves *et al.*, 2014; Linaldeddu *et al.*, 2016
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia sapinea*	*Malus*, *Vitis*, *Olea*	Portugal, France, Italy	Batista *et al.*, 2020; Comont *et al.*, 2016; Alves *et al.*, 2014; Phillips *et al.*, 2012

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Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia scrobiculata*	*Olea*	Italy	Phillips *et al.*, 2012; Alves *et al.*, 2014
Ascomycota	*Botryosphaeriaceae*	*Diplodia*	*Diplodia seriata*	*Juglans*, *Vitis*, *Pyrus*, *Cydonia*, *Malus*, *Prunus (Apricot)*, *Olea*, *Prunus* (Plum), *Prunus* (Nectarine), *Prunus* (Almond), *Prunus* (Plum)	Czech Republic, Spain, Turkey, Spain, Germany, Germany, France, Italy, Bulgaria, Croatia, Portugal	Eichmeier *et al.*, 2020; López-Moral *et al.*, 2020; Kurbetli and Demirci, 2014; Moral *et al.*, 2010; Phillips *et al.*, 2012; Kaliterna *et al.*, 2012; Carlucci *et al.*, 2013; Ende *et al.*, 2016; Batista *et al.*, 2020; Kraus *et al.*, 2019; Akgül *et al.*, 2014; Luque *et al.*, 2009; Gramaje *et al.*, 2012; Bien and Damm, 2020
Ascomycota	*Botryosphaeriaceae*	*Dothiorella*	*Dothiorella iberica*	*Juglans*, *Prunus* (Apricot), *Corylus* (Hazelnut), *Malus*, *Vitis*	Spain, Germany, Italy	López-Moral *et al.*, 2020; Gierl and Fischer, 2017; Phillips *et al.*, 2005; Carlucci *et al.*, 2015
Ascomycota	*Botryosphaeriaceae*	*Dothiorella*	*Dothiorella omnivora*	*Juglans*, *Vitis*	Czech Republic, France, Hungary	Eichmeier *et al.*, 2020; Linaldeddu *et al.*, 2016; Vaczy *et al.*, 2018
Ascomycota	*Botryosphaeriaceae*	*Dothiorella*	*Dothiorella sarmentorum*	*Juglans*, *Malus*, *Prunus* (Almond), *Cydonia*, *Vitis*	Czech Republic, the Netherlands, Norway, Germany, Italy, Spain, Poland, Ukraine	López-Moral *et al.*, 2020; van Niekerk *et al.*, 2004; Gierl and Fischer, 2017; Carlucci *et al.*, 2015; Dissanayake *et al.*, 2016
Ascomycota	*Botryosphaeriaceae*	*Dothiorella*	*Dothiorella viticola*	*Vitis*	Spain, France	López-Moral *et al.*, 2020; Batista *et al.*, 2020; Comont *et al.*, 2016
Ascomycota	*Diatrypaceae*	*Eutypa*	*Eutypa lata*	*Juglans*, *Cydonia*, *Vitis*, *Pistacia*, *Prunus* (Almond), *Prunus* (Plum), *Prunus* (Cherry), *Olea*	Czech Republic, Germany, Spain, Italy, France	Eichmeier *et al.*, 2020; Gierl and Fischer, 2017; Luque *et al.*, 2009; López-Moral *et al.*, 2020b; Baránek *et al.*, 2018; Gramaje *et al.*, 2012; Bien and Damm, 2020; Tosi and Natalini, 2009; Aiello *et al.*, 2019; Baranek *et al.*, 2018; Laveau *et al.*, 2009; Kraus *et al.*, 2022
Ascomycota	*Diatrypaceae*	*Eutypella*	*Eutypella citricola*	*Vitis*	Spain	Luque *et al.*, 2012
Ascomycota	*Diatrypaceae*	*Eutypella*	*Eutypella leptoplaca*	*Vitis*	Spain	Luque *et al.*, 2009
Ascomycota	*Diatrypaceae*	*Eutypella*	*Eutypella microtheca*	*Vitis*	Spain	Luque *et al.*, 2012
Ascomycota	*Diatrypaceae*	*Eutypella*	*Eutypella vitis*	*Vitis*	Spain	Luque *et al.*, 2009
Basidiomycota	*Hymenochaetaceae*	*Fomitiporia*	*Fomitiporia mediterranea*	*Olea*, *Vitis*, *Actinidia*, *Citrus*, *Prunus* (Almond), *Pyrus*	Italy, Spain, Turkey, Czech Republic, Germany, France, Greece, Austria, Portugal, Slovenia, Switzerland	Carlucci *et al.*, 2013; Luque *et al.*, 2009; Akgül *et al.*, 2015; Baránek *et al.*, 2018; Kraus *et al.*, 2022; Laveau *et al.*, 2009; Elena and Paplomatis, 2009; Elena *et al.* , 2006; Rumbos and Rumbou, 2001; Moretti *et al.*, 2021; Olmo *et al.*, 2017; Markakis *et al.*, 2017
Basidiomycota	*Hymenochaetaceae*	*Fomitiporia*	*Fomitiporia punctata*	*Vitis*, *Actinidia*	Italy, Greece, France	Cortesi *et al.*, 2000; Elena and Paplomatas, 2007; Jamaux-Despréaux and Péros, 2003
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria europea*	*Vitis*, *Actinidia*	Portugal, France	Cabral *et al.*, 2012
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria liriodendri*	*Vitis*, *Malus*	France, Spain, Portugal, Switzerland, Turkey	Pintos *et al.*, 2018; Cabral *et al.*, 2012; Alaniz *et al.*, 2009; Casieri *et al.*, 2009; Savas *et al.*, 2015

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Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria lusitanica*	*Vitis*	Portugal	Cabral *et al.*, 2012
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria pseudodestructans*	*Vitis*	Portugal, Spain	Cabral *et al.*, 2012; Berlanas *et al.*, 2020
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria robusta*	*Vitis*	France, Portugal, Spain	Pintos *et al.*, 2018; Cabral *et al.*, 2012; Berlanas *et al.*, 2020
Ascomycota	*Nectriaceae*	*Ilyonectria*	*Ilyonectria vitis*	*Vitis*	Portugal	Cabral *et al.*, 2012
Ascomycota	*Botryosphaeriaceae*	*Lasiodiplodia*	*Lasiodiplodia citricola*	*Vitis*	Italy	Carlucci *et al.*, 2015
Ascomycota	*Botryosphaeriaceae*	*Lasiodiplodia*	*Lasiodiplodia pseudotheobromae*	*Olea*, *Prunus* (Nectarine), *Pistacia*	Italy, Turkey, Spain	Carlucci *et al.*, 2013; Endes *et al.*, 2016; Akgül *et al.*, 2014; Aroca *et al.*, 2010; López-Moral *et al.*, 2020b
Ascomycota	*Botryosphaeriaceae*	*Lasiodiplodia*	*Lasiodiplodia theobromae*	*Olea*, *Prunus* (Nectarine), *Vitis*, *Vaccinium*, *Mangifera*	Italy, Turkey, Spain	Carlucci *et al.*, 2013, 2015; Endes *et al.*, 2016; Akgül *et al.*, 2014; Aroca *et al.*, 2010; Martin *et al.*, 2009; Borrero *et al.*, 2019; Aiello *et al.*, 2022
Ascomycota	*Botryosphaeriaceae*	*Lasiodiplodia*	*Lasiodiplodia viticola*	*Vitis*	France	Comont *et al.*, 2016
Ascomycota	*Phaeomoniellaceae*	*Minutiella*	*Minutiella pruni-avium*	*Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Sclerotiniaceae*	*Monilinia*	*Monilinia laxa*	*Prunus* (Plum)	Germany	Bien and Damm, 2020
Ascomycota	*Nectriaceae*	*Neocosmospora*	*Neocosmospora perseae*	*Persea*	Italy, Greece	Guarnaccia *et al.*, 2018, 2022
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum australe*	*Mangifera*, *Prunus* (Almond), *Vitis*, *Olea*, *Vaccinium*	Italy, Spain, Portugal	Ismail *et al.*, 2013; Gramaje *et al.*, 2012; Aroca *et al.*, 2010; Lopes *et al.*, 2016; Hilário *et al.*, 2019
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum hellenicum*	*Pistacia*	Greece, Italy	Lopes *et al.*, 2016; Gusella *et al.*, 2022
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum luteum*	*Olea*, *Vitis*	Italy, Germany, Spain, France, Portugal	Carlucci *et al.*, 2013; Fischer *et al.*, 2016; Luque *et al.*, 2009; Pintos *et al.*, 2018; Lopes *et al.*, 2016
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum mediterraneum*	*Juglans*, *Vitis*, *Olea*, *Pistacia*	Spain, Italy	López-Moral *et al.*, 2020a,b; Moral *et al.*, 2010, 2017; Brunetti *et al.*, 2022
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum parvum*	*Juglans*, *Malus*, *Vitis*, *Castanea*, *Citrus*, *Ficus*, *Prunus* (Nectarine), *Prunus* (Peach), *Pistacia*, *Mangifera*, *Vaccinium*, *Prunus* (Almond), *Persea*, *Olea*	Spain, Portugal, Italy, Turkey, France, Greece, Croatia	López-Moral *et al.*, 2020a,b; Moral *et al.*, 2010; Batista *et al.*, 2020; Mondello *et al.*, 2013; Akgül *et al.*, 2014; Luque *et al.*, 2009; Pintos *et al.*, 2018; Ciordia *et al.*, 2022; Vakalounakis *et al.*, 2019; Aiello *et al.*, 2020; Thomidis *et al.*, 2011; Kaliterna *et al.*, 2013; Ismail *et al.*, 2013; Guarnaccia *et al.*, 2020; Gramaje *et al.*, 2012; Guarnaccia, 2016; Ismail *et al.*, 2013; Hilário *et al.*, 2019; Castillo *et al.*, 2013; Polizzi *et al.*, 2022
Ascomycota	*Botryosphaeriaceae*	*Neofusicoccum*	*Neofusicoccum vitifusiforme*	*Vitis*	Italy, Spain	Mondello *et al.*, 2013; Aroca *et al.*, 2010
Ascomycota	*Nectriaceae*	*Neonectria*	*Neonectria ditissima*	*Malus*	Portugal	Cabral *et al.*, 2012
Ascomycota	*Sporocadaceae*	*Neopestalotiopsis*	*Neopestalotiopsis rosae*	*Persea*	Italy	Fiorenza *et al.*, 2022
Ascomycota	*Sporocadaceae*	*Neopestalotiopsis*	*Neopestalotiopsis siciliana*	*Persea*	Italy	Fiorenza *et al.*, 2022

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Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Botryosphaeriaceae*	*Neoscytalidium*	*Neoscytalidium dimidiatum*	*Citrus*, *Juglans*, *Vitis*, *Prunus* (Cherry), *Prunus* (Almond)	Italy, Turkey	Polizzi *et al.*, 2009; Dervis *et al.*, 2019; Oksal *et al.*, 2019; Oren *et al.*, 2020, 2022
Ascomycota	*Didymellaceae*	*Nothophoma*	*Nothophoma quercina*	*Olea*	Spain	Moral *et al.*, 2017
Ascomycota	*Diatrypaceae*	*Peroneutypa*	*Peroneutypa scoparia*	*Juglans*, *Vaccinium*	Czech Republic, Italy	Eichmeier *et al.*, 2020; Guarnaccia *et al.*, 2020
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium alvesii*	*Olea*, *Vitis*	Italy, Turkey	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium amygdalinum*	*Prunus* (Almond)	Spain, Italy	Gramaje *et al.*, 2012; Raimondo *et al.*, 2021
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium angustius*	*Cydonia*, *Vitis*	Germany, Portugal, Spain	Gierl and Fischer, 2017; Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium cinereum*	*Vitis*	Spain	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium croatiense*	*Vitis*	Croatia	Essakhi *et al.*, 2008
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium fraxinopennsylvanicum*	*Vitis*, *Actinidia*	Germany, Italy, Croatia, Hungary, Spain	Fischer *et al.*, 2016; Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium griseorubrum*	*Vitis*	Italy	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium hispanicum*	*Vitis*	Spain	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium hungaricum*	*Prunus* (Plum), *Prunus* (Cherry)	Germany, Hungary	Bien and Damm, 2020; Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium infatipes*	*Vitis*	Spain	González and Tello, 2011
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium iranianum*	*Actinidia*, *Prunus* (Almond), *Prunus* (Plum), *Vitis*	Italy, Spain, Germany	Prodi *et al.*, 2008; Gramaje *et al.*, 2012; Bien and Damm, 2020
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium italicum*	*Olea*, *Vitis*	Spain, Italy	Agustí-Brisach *et al.*, 2021; Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium krajdenii*	*Vitis*	Spain	Gramaje *et al.*, 2011
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium minimum*	*Vitis*, *Olea*, *Actinidia*, *Prunus* (Cherry), *Pistacia*	Spain, France, Turkey, Italy, Austria, Greece, Germany, Portugal	Pintos *et al.*, 2018; Akgül *et al.*, 2015, Agustí-Brisach *et al.*, 2021; Carlucci *et al.*, 2013; Luque *et al.*, 2019; Prodi *et al.*, 2008; Di Marco *et al.*, 2000; Mostert *et al.*, 2006; Lopez-Moral *et al.*, 2020; Garamaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium oleae*	*Olive*	Italy	Raimondo *et al.*, 2022

(Continued)

Table 1. (Continued).

Division	Family	Genus	Species	Host	Country	References
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium parasiticum*	*Vitis*, *Olea*, *Actinidia*, *Prunus* (Cherry)	Spain, Italy, Greece	Aroca *et al.*, 2010; Agustí-Brisach *et al.*, 2021; Prodi *et al.*, 2008; Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium rubrigenum*	*Olea*, *Vitis*, *Actinidia*	Italy, Croatia	Gramaje *et al.*, 2015; Di Marco *et al.*, 2010
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium scolyti*	*Olea*, *Prunus* (Plum), *Vitis*	Spain, Germany, Italy, France, Turkey	Agustí-Brisach *et al.*, 2021; Bien and Damm, 2020; Carlucci *et al.*, 2015; Gramaje *et al.*, 2015; Ozben *et al.*, 2012
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium sicilianum*	*Olea*, *Vitis*, *Juglans*	Italy, Spain, Czech Republic	Carlucci *et al.*, 2015; Gramaje *et al.*, 2015; Eichmeier *et al.*, 2020
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium tuscanum*	*Vitis*	Italy	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium venezuelense*	*Prunus* (Apricot)	Spain	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium vibratile*	*Prunus* (Cherry)	Sweden	Gramaje *et al.*, 2015
Ascomycota	*Togniniaceae*	*Phaeoacremonium*	*Phaeoacremonium viticola*	*Vitis*, *Actinidia*, *Prunus* (Plum), *Prunus* (Cherry), *Olea*	Germany, Spain, Italy, France	Fischer *et al.*, 2016; Luque *et al.*, 2009; Prodi *et al.*, 2008; Bien and Damm, 2020; Mostert *et al.*, 2006; Gramaje *et al.*, 2015; Raimondo *et al.*, 2022
Ascomycota	*Phaeomoniellaceae*	*Phaeomoniella*	*Phaeomoniella chlamydospora*	*Vitis*, *Actinidia*	Spain, France, Turkey, Czech Republic, Portugal, Germany, Slovakia, Switzerland	Luque *et al.*, 2009; Pintos *et al.*, 2018; Akgül *et al.*, 2015; Baránek *et al.*, 2018; Agustí-Brisach *et al.*, 2021; Di Marco *et al.*, 2000; Chicau *et al.*, 2000; Fischer and Kassemeyer, 2003; Kakalikova *et al.*, 2006; Casieri *et al.*, 2009
Basidiomycota	*Hymenochaetaceae*	*Phellinus*	*Phellinus tuberculosus*	*Prunus* (Plum), *Prunus* (Cherry)	Germany	Bien and Damm, 2020
Ascomycota	*Nectriaceae*	*Pleiocarpon*	*Pleiocarpon algeriense*	*Persea*	Italy	Aiello *et al.*, 2020b
Ascomycota	*Pleurostomataceae*	*Pleurostoma*	*Pleurostoma richardsiae*	*Vitis*	Turkey, Spain	Ozben *et al.*, 2017; Pintos Varela *et al.*, 2016
Ascomycota	*Phaeomoniellaceae*	*Pseudophaeomoniella*	*Pseudophaeomoniella oleae*	*Olea*	Greece, Italy	Markakis *et al.*, 2022; Crous *et al.*, 2015
Ascomycota	*Phaeomoniellaceae*	*Pseudophaeomoniella*	*Pseudophaeomoniella oleicola*	*Olea*	Italy, Spain	Crous *et al.*, 2015; Agustí-Brisach *et al.*, 2021
Basidiomycota	*Stereaceae*	*Stereum*	*Stereum hirsutum*	*Prunus* (Almond), *Vitis*	Germany, Spain, France	Gierl and Fischer, 2017; Luque *et al.*, 2009; Larignon and Dubos, 1997; Fischer and Kassemeyer, 2003
Basidiomycota	*Polyporaceae*	*Trametes*	*Trametes versicolor*	*Prunus* (Plum), *Vitis*	Germany	Bien and Damm, 2020; Fischer and Kassemeyer, 2003

2017; Pintos *et al.,* 2018; Batista *et al.,* 2020; Bien and Damm, 2020; López-Moral *et al.,* 2020a). Other *Diplodia* spp. occur rarely and share different hosts: *Dip. corticola* (grapevines), *Dip. juglandis* (walnut), *Dip. malorum* (apple), *Dip. prunicola* (almond), *Dip. pseudoseriata* (pistachio), *Dip. scrobiculata* (olive) (Phillips *et al.,* 2012; Alves *et al.,* 2014; Carlucci *et al.,* 2015b; López-Moral *et al.,* 2020a; Batista *et al.,* 2020).

Neofusicoccum is another common member of the *Botryosphaeriaceae*, which is a predominant FTD pathogen. The host range of *N. parvum* is broad, including walnut, grapevine, apple, chestnut, citrus, fig, nectarine, peach, pistacia, mango, blueberry, almond, avocado, and olive (Moral *et al.,* 2010; Gramaje *et al.,* 2012; Ismail *et al.,* 2013; Akgül *et al.,* 2014; Guarnaccia *et al.,* 2016; Aiello *et al.,* 2020a; Guarnaccia *et al.,* 2020; Hilário *et al.,* 2020a; López-Moral *et al.,* 2020a). To date, this pathogen has only been isolated from plants in Croatia, France, Greece, Italy, Portugal, Spain, and Turkey, indicating a preference for warm climates. Further *Neofusicoccum* spp. probably restricted to the Mediterranean area and associated with FTD mainly of grapevine and olive trees, are *N. australe*, *N. hellenicum*, *N. luteum*, *N. mediterraneum* and *N. vitifusiforme* (Gramaje *et al.,* 2012; Ismail *et al*., 2013; Hilário *et al.,* 2020a; López-Moral *et al.,* 2020a; López-Moral *et al.,* 2020b; Gusella *et al.,* 2022).

Lasiodiplodia spp. were exclusively isolated from fruit crop plants in the south of Europe, including France, Italy, Spain, and Turkey. *Lasiodiplodia citricola* was isolated from grapevine, *L. pseudotheobromae* from olive, nectarine and pistachio, *L. theobromae* from olive, nectarine and grapevine, and *L. viticola* only from grapevine (Martin *et al.,* 2009; Aroca *et al.,* 2010; Carlucci *et al.,* 2013, 2015b; Akgül *et al.,* 2014; Comont *et al.,* 2016; Endes *et al.,* 2016; Borrero *et al.,* 2019; López-Moral *et al.,* 2020b). *Dothiorella sarmentorum*, however, was associated with FTD of walnut, apple, almond, pear and grapevine, mainly in areas with cool climates, such as the Czech Republic, Germany, the Netherlands, Norway, and Poland (van Niekerk *et al.,* 2004; Carlucci *et al.,* 2015b; Gierl and Fischer 2017; Dissanayake *et al.,* 2017; López-Moral *et al.,* 2020a). The closely related *Dip. omnivora* was also found in cool regions (the Czech Republic, France, Hungary) on walnut and grapevine (Linaldeddu *et al.,* 2016; Váczy *et al.,* 2018; Eichmeier *et al.,* 2020). *Dothoriella iberica* has been associated with FTD of walnut, apricot, hazelnut, apple and grapevine, in Spain, Germany and Italy (Phillips *et al.,* 2005; Carlucci *et al.,* 2015b; Gierl and Fischer 2017; López-Moral *et al.,* 2020a). *Neoscytalidium dimidiatum* has recently been related to canker and dieback of walnut, grapevine, cherry, almond and apricot in Turkey (Derviş *et al.,* 2019; Oksal *et al.,* 2019; Oksal *et al.,* 2020; Ören *et al.,* 2020; Ören *et al.,* 2022). This pathogen also caused shoot blight, canker and gummosis on citrus in Italy (Polizzi *et al.,* 2009).

Among *Diatrypaceae*, *Eutypa lata* has been commonly associated with FTD in Europe. This fungus was found on walnut, quince, grapevine, pistachio, almond, plum, cherry, blueberry, and olive, in the Czech Republic, France, Germany, Italy, and Spain (Prodorutti *et al.,* 2008; Laveau *et al.,* 2009; Luque *et al.,* 2009; Tosi and Natalini 2009; Wenneker *et al.,* 2011; Gramaje *et al.,* 2012; Gierl and Fischer, 2017; Baránek *et al.,* 2018; Aiello *et al.,* 2019; Bien and Damm 2020; Eichmeier *et al.,* 2020; Guarnaccia *et al.,* 2020; López-Moral *et al.,* 2020b; Kraus *et al.,* 2022). Other *Diatrypaceae* are less frequently associated with FTD in Europe; *Eutypella citricola*, *Eu. leptoplaca*, *Eu. microtheca* and *Eu. vitis* were found on grapevine in Spain (Luque *et al.,* 2009; Luque *et al.,* 2012). *Peroneutypa scoparia* was recently associated with dieback of walnut and blueberry in, respectively, the Czech Republic and Italy (Eichmeier *et al.,* 2020; Guarnaccia *et al.,* 2020). *Cryptovalsa ampelina* infected walnut and grapevine, in the Czech Republic, Germany, and Spain (Luque *et al.,* 2009; Martin *et al.,* 2009; Luque *et al.,* 2012; Kraus *et al.,* 2019; Eichmeier *et al.,* 2020).

Diaporthaceae is another diverse group of fungi causing FTD of fruit crops. Like some *Botryosphaeriaceae*, *Dia. eres* is ubiquitous in Europe, and affects a wide range of hosts including apple, blueberry, cherry, grapevine, peach, pear, and walnut (Casieri *et al.,* 2009; Thomidis and Michailides, 2009; Lombard *et al.,* 2014; Gierl and Fischer, 2017; Prencipe *et al.,* 2017; Bertetti *et al.,* 2018; Guarnaccia *et al.,* 2018; Pintos *et al.,* 2018; Bien and Damm, 2020; Eichmeier *et al.,* 2020). *Diaporthe foeniculina* caused FTD symptoms on almond, avocado, blueberry, chestnut, citrus, fig, grapevine, pear, and walnut, in France, Germany, Greece, Italy, Malta, Portugal, and Spain (Gramaje *et al.,* 2012; Annesi *et al.,* 2016; Guarnaccia *et al.,* 2016; Guarnaccia and Crous, 2017b; Gierl and Fischer, 2017; Santos *et al.,* 2017; Pintos *et al.,* 2018; Vakalounakis *et al.,* 2019; Mathioudakis *et al.,* 2020; Hilário *et al.,* 2020b; López-Moral *et al.,* 2020a). Other *Diaporthe* spp. have also been linked to FTD on many different crops, especially grapevine, but their occurrence is rare (Lombard *et al.,* 2014; Guarnaccia and Crous, 2017; Guarnaccia *et al.,* 2018; Pintos *et al.,* 2018), or some species such as *Dia. amygdali* are more host specific infecting mainly almond trees (León *et al*., 2020).

Species of *Phaeoacremonium* are well known for their involvement in FTD of grapevine. This group of fungi is diverse, with *P. minimum* being the most prevalent in Europe affecting grapevine, olive, blueberry,

cherry and pistachio (Di Marco *et al.,* 2004; Mostert *et al.,* 2006; Prodi *et al.,* 2008; Luque *et al.,* 2009; Carlucci *et al.,* 2013; Gramaje *et al.,* 2015; Akgül *et al.,* 2015; Pintos *et al.,* 2018; López-Moral *et al.,* 2020b; Agustí-Brisach *et al.,* 2021). *Phaeoacremonium viticola*, *Pm. iraniaum* and *Pm. parasiticum* are also common and have similar host ranges to *P. minimum,* including blueberry, grapevine, olive and *Prunus* trees (cherry, plum) (Mostert *et al.,* 2006; Prodi *et al.,* 2008; Luque *et al.,* 2009; Aroca *et al.,* 2010; Gramaje *et al.,* 2012; Gramaje *et al.,* 2015; Fischer *et al.,* 2016; Bien and Damm 2020; Agustí-Brisach *et al.,* 2021; Raimondo *et al.,* 2021). *Phaeoacremonium cinereum*, *Pm. croatiense*, *Pm. griseorubrum*, *Pm. hispanicum*, *Pm. infatipes*, *Pm. krajdenii* and *Pm. tuscanum* were only isolated from grapevine in Croatia, Italy, and Spain (Essakhi *et al.,* 2008; González and Tello, 2011; Gramaje *et al.,* 2011b, 2015). In contrast, *Pm. amygdalinum* (from almond), *Pm. hungaricum* (*Prunus* trees, plum and cherry), *Pm. olea* (olive), *Pm. venezuelense* (apricot), and *P. vibratile* (cherry) were only found on hosts other than grapevine (Gramaje *et al.,* 2012; Gramaje *et al.,* 2015; Bien and Damm, 2020; Raimondo *et al.,* 2021).

The *Nectriaceae* family includes diverse genera of FTD pathogens, currently named *Cylindrocarpon*-like asexual morphs, which are most known for their involvement in black foot of grapevine, mainly in France, Portugal and Spain (Alaniz *et al.,* 2011; González and Tello, 2011; Martin *et al.,* 2011; Cabral *et al.,* 2012; Agustí-Brisach and Armengol, 2013; Reis *et al.,* 2013; Pintos *et al.,* 2018; Berlanas *et al.,* 2020). However, these fungi have also been associated with decline of olive trees (in Italy), peach (Italy), plum (Bulgaria), apple (Portugal) and kiwifruit (Turkey) (Cabral *et al.,* 2012; Yaseen *et al.,* 2012; Carlucci *et al.,* 2013; Erper *et al.,* 2013; Piperkova *et al.,* 2017).

Investigation of wood necroses in crop trees often results in isolation of *Cadophora* spp., being *C. luteo-olivacea* the most common species in grapevine, but also occurs on olive, plum and blueberry, in Germany, Spain, France and Italy (Casieri *et al.,* 2009; Fischer *et al.,* 2016; Pintos *et al.,* 2018; Raimondo *et al.,* 2019; Bien and Damm, 2020; Guarnaccia *et al.,* 2020; Agustí-Brisach *et al.,* 2021). Furthermore, *C. fastigiata* (in Germany and Switzerland) and *C. viticola* (Spain) were exclusively found on grapevine (Casieri *et al.,* 2009; Gramaje *et al.,* 2015; Fischer *et al.,* 2016). More *Cadophora* spp., e.g. *Ca. malorum*, *Ca. viticola*, *Ca. novi-eboraci*, *Ca. prunicola* and *Ca. ramosa*, infected stone fruit trees (plum, cherry), walnut, apple and blueberry, in Germany, Italy and the Czech Republic (Prodi *et al.,* 2008; Gierl and Fischer, 2017; Eichmeier *et al.,* 2020; Bien and Damm, 2020).

Similar to *Cadophora* spp., *Collophorina* spp., including *Collop. australe*, *Collop. badensis*, *Collop. germanica*, *Collop. hispanica*, *Collop. neorubra*, and *Collop. paarla*, have been associated with wood necrosis, but mostly on stone fruit trees (apricot, almond, cherry, and plum) (Gramaje *et al.,* 2012; Gierl and Fischer 2017; Bien and Damm 2020). Only *Collop. hispanica* has also been linked to discolouration of chestnuts in Spain (Yurkewich *et al.,* 2017).

Besides the above-mentioned fungal families and genera, further species can cause FTD symptoms on different crops in Europe. For instance, *Phaeomoniella chlamydospora*, also associated with esca, appears in every European vineyard, and is probably host-specific for grapevine (Chicau *et al.,* 2000; Fischer and Kassemeyer, 2003; Kakalíková *et al.,* 2006; Casieri *et al.,* 2009; Luque *et al.,* 2009; Akgül *et al.,* 2015; Baránek *et al.,* 2018; Pintos *et al.,* 2018; Agustí-Brisach *et al.,* 2021). However, this fugus was also isolated from kiwifruit plants showing wood decay in Italy (Di Marco *et al.,* 2003). Relatives of *Pa. chlamydospora*, *Pseudophaeomoniella olea* and *Ps. oleicola*, are also host-specific, but for olive, and are linked to wood decay and shoot dieback in Greece, Italy and Spain (Crous *et al.,* 2015; Agustí-Brisach *et al.,* 2021; Markakis *et al.,* 2022). Species of *Colletotrichum*, such as *Col. fructicola*, *Col. gloeosporioides* and *Col. karstii*, have been related to canker and stem-end rot of avocado in Italy, and citrus disease in Italy and Portugal (Ramos *et al.,* 2016; Guarnaccia *et al.,* 2016, 2017). *Cytospora chrysosperma* affected grapevine and blackthorn (González and Tello, 2011; Gierl and Fischer, 2017; Kraus *et al.,* 2019). Its relatives *Cy. oleina*, *Cy. iistaciae* and *Cy. australe* were linked to dieback of olive and pistachio, in Greece, Italy and Spain (Rumbos 1988; Carlucci *et al.,* 2013; Moral *et al.,* 2017; Aiello *et al.,* 2019; López-Moral *et al.,* 2020b).

Several basidiomycetous fungi have been recorded as involved in FTD. *Fomitiporia mediterranea* is the predominant species in Europe, especially in vineyards, where it causes white rot in grapevine trunks (Rumbos and Rumbou 2001; Laveau *et al.,* 2009; Luque *et al.,* 2009; Akgül *et al.,* 2015; Baránek *et al.,* 2018; Moretti *et al.,* 2021; Kraus *et al.,* 2022). In addition, this fungus was also associated with decline symptoms on olive, kiwifruit, citrus, almond and pear (Elena *et al.,* 2006; Carlucci *et al.,* 2013; Markakis *et al.,* 2017; Olmo *et al.,* 2017). *Fomitiporia mediterranea* caused wood decay of kiwifruit, in Italy, Greece and France (Cortesi *et al.,* 2000; Elena and Paplomatas, 2002; Jamaux-Despréaux and Peros, 2003). *Stereum hirsutum*, was reported in Germany, Spain and France, where it was collected from decayed wood of grapevine, almond and chestnut

(Larignon and Dubos, 1997; Fischer and Kassemeyer, 2003; Luque *et al.,* 2009; Gierl and Fischer, 2017; Yurkewich *et al.,* 2017).

FUNGAL TRUNK DISEASES OF APPLE

Several pathogens infect trunks, branches and shoots of apple trees, causing cankers, twig blight, wood rot and, in severe cases, production losses and tree death (Sutton *et al.,* 2014). Symptoms on adult trees develop after long periods, while young plants can rapidly die (Marek *et al.,* 2013). Many Ascomycetes (approx. 36 species) have been associated with FTD of apple (Sutton *et al.,* 2014). Among these, *Neonectria ditissima* is the most serious threat as the cause of European canker, which has typical symptoms of elliptical, sunken areas of dark reddish-brown cankers. After the first year, the cankers become irregular and rough, with bark cracks and abundant production of perithecia (Sutton *et al.,* 2014; Weber and Børve, 2021). This disease has been reported in Germany, Ireland, Poland, and Portugal (Weber and Børve, 2021; Farr and Rossman, 2022).

The apple dieback syndrome is characterized by wood lesions, stunted plants, leaf chlorosis, bark discolouration and extensive cankers associated with wood decay, and progressive tree death (Sutton *et al.,* 2014; Mang *et al.,* 2022). Species in *Botryosphaeriaceae* and *Diaporthaceae* are considered the most relevant pathogens (Havenga *et al.,* 2019; Ali *et al.,* 2020; Diaz *et al.,* 2022). *Botryosphaeria dothidea* and *Dip. seriata* are the main pathogens found associated with dieback of apple trees in several countries (Havenga *et al.,* 2019; Diaz *et al.,* 2022; Ilyukhin *et al.,* 2022). Other *Diplodia* spp. recently reported as pathogenic on apple trees include *Dip. bulgarica* (Nourian *et al.,* 2021), *Dip. mutila* (Urbez-Torres *et al.,* 2016; Sessa *et al.,* 2017; Diaz *et al.,* 2019; Lodolo *et al.,* 2022), *Dip. intermedia* and *Dip. pseudoseriata* (Delgado-Cerrone *et al.,* 2016). Similarly, *Diaporthe* spp. were reported in association with dieback of apple, with *Dia. eres* dominating (Havenga *et al.,* 2019).

Considering the wide range of pathogens, apple dieback is considered as a disease complex (Mang *et al.,* 2022). However, only a few reports have been published on apple dieback in Europe. In Italy, *Dip. malorum*, *Phomopsis mali*, *Nectria* spp. and species in the *Botryosphaeriaceae* were reported as causing dieback, cracking and necrosis of trunks and from graft union sites of apple trees (Prodorutti *et al.,* 2012). Recently, Mang *et al.,* (2022) investigated apple orchards in Southern Italy, and characterized *N. parvum*, *Dia. eres*, *Dia. foeniculina*, *Pestalotiopsis australe*, *Trametes versicolor* and *Phomopsis* spp. The Basidiomycete *Inonotus hispidus* was also shown to be responsible for severe wood decay, canker and dieback symptoms on apple trees in southern Greece (Markakis *et al.,* 2017).

FUNGAL TRUNK DISEASES OF CITRUS

Twigs, branches and trunks of citrus plants affected by several FTD caused by diverse fungi have been reported in different continents (Timmer *et al.,* 2000; Mayorquin *et al.,* 2016; Bezerra *et al.,* 2021). *Colletotrichum* spp. are responsible of twig dieback in major producing areas in Asia and the USA (Huang *et al.,* 2013; Mayorquin *et al.,* 2019), and in Europe, where *Col. gloeosporioides* and *Col. karsti* were reported as dominant *Colletotrichum* spp. causing dieback of citrus twigs and shoots in Italy and Turkey (Aiello *et al.,* 2015; Uysal *et al.,* 2022).

Guarnaccia and Crous (2017) reported severe woody cankers of lemon trees caused by *Dia. limonicola* and *Dia. melitensis* in Malta, often showing gummous exudates and causing severe blight and dieback reported exclusively in Malta. Further *Diaporthe* species, including *Dia. baccae*, *Dia. foeniculina* and *Dia. novem*, are known as secondary pathogens causing wood diseases on citrus plants in Europe. *Diaporthe citri*, a key pathogen of citrus, was associated with shoot blight of *C. reticulata* in the Azores Islands, demonstrating for the first time the presence of a potential threat for citrus fruit production in Europe.

Several studies have recently revised species and genera of *Botryosphaeriaceae*, which include species largely distributed able to cause diseases of numerous plant species (Bezerra *et al.,* 2021; Zhang *et al.,* 2021). In particular, several studies have demonstrated the roles of *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum,* and *Neoscytalidium* spp. as causal agents of FTD on *Citrus* spp. (Adesemoye *et al.,* 2011; Berraf-Tebbal *et al.,* 2020). Regarding European distribution, *Neoscytalidium dimidiatum* was reported to cause citrus branch canker in Italy (Polizzi *et al.,* 2009). A broad survey conducted by Bezerra *et al.,* (2021), through Greece, Italy, Portugal, Malta, and Spain, revealed the occurrence, genetic diversity, and pathogenicity of *Botryosphaeriaceae* species associated with symptomatic citrus cultivars. This study also demonstrated pathogenicity of *Botryosphaeriaceae* spp. in citrus-producing areas of these European countries. Phylogenetic multi-locus analyses identified four *Diplodia* species, with *Dip. pseudoseriata* being the most common, followed by three *Neofusicoccum* species, dominated by *N. parvum*, *Do. viticola* and *L. theobromae*.

Canker diseases of citrus are also caused by other fungal genera such as *Fusarium* and *Neocosmospora* (Sandoval-Denis *et al.,* 2018; Guarnaccia *et al.,* 2021), *Peroneutypa* (Timmer *et al.,* 2000), and *Phaeoacremonium* (Espargham *et al.,* 2020). Several *Fusarium* and *Neocosmospora* spp. were found in association with dry root rot, crown, trunk or twig cankers, or twig dieback, of citrus trees (Sandoval-Denis *et al.,* 2018).

FUNGAL TRUNK DISEASES OF GRAPEVINE

FTD of grapevine have become major problems in all grape producing countries, causing significant economic impacts from reduced production and vineyard longevity (AA.VV., 2022). Petri disease and black foot affect young grapevines while the diseases Eutypa, Botryosphaeria and Diaporthe diebacks, Cytospora canker and esca affect mature grapevines. These diseases are caused by a wide range of fungal pathogens producing diverse symptoms, including leaf and shoot distortion and discolouration, external wood cankers, internal wood necroses and staining, poor plant growth, mortality of roots, dieback and sudden grapevine collapse.

Petri disease is mainly caused by combinations of *Pa. chlamydospora* and 28 species of *Phaeoacremonium* (Gramaje *et al.,* 2018) with *Pm. minimum* being the most prevalent (Gramaje *et al.,* 2015). Other species associated with this disease include *Cadophora* spp. (Gramaje *et al.,* 2011a) and *Pleurostoma richardsiae* (Halleen *et al.,* 2007b). Up to 29 species of *Cylindrocarpon*-like asexual morphs belonging of *Campylocarpon*, *Cylindrocladiella*, *Cylindrodendrum*, *Dactylonectria*, *Ilyonectria*, *Neonectria*, *Pleiocarpon* and *Thelonectria* genera have been associated with black-foot of grapevine (Gramaje *et al.,* 2018; Aigoun-Mouhous *et al.,* 2019; Lawrence *et al.,* 2019; Akgül *et al.,* 2022). *Dactylonectria torresensis* is the most common species associated with this disease in Europe (Reis *et al.,* 2013, Carlucci *et al.,* 2017, Berlanas *et al.,* 2020) and Algeria (Aigoun-Mouhous *et al.,* 2019), while *Da. macrodidyma* has been considered the prevalent species in South Africa (Langenhoven *et al.,* 2018) and Canada (Úrbez-Torres *et al.,* 2014).

Eutypa dieback of grapevine is caused by 24 species of *Diatrypaceae* (Trouillas and Gubler 2010a; Luque *et al.,* 2012; Pitt *et al.,* 2013b; Rolshausen *et al.,* 2013), with *E. lata* being the most common and virulent fungus associated with this disease. Other genera of *Diatrypaceae* have been isolated from symptomatic wood, including *Anthostoma*, *Cryptosphaeria*, *Cryptovalsa*, *Diatrype*, *Diatrypella*, and *Eutypella* (Trouillas and Gubler 2010; Luque *et al.,* 2012).

At least 26 different species of *Botryosphaeria*, *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum*, *Neoscytalidium*, *Phaeobotryosphaeria*, or *Spencermartinsia* have been reported as causal agents of Botryosphaeria dieback of grapevines (Úrbez-Torres and Gubler 2011; Pitt *et al.,* 2013a,c; Rolshausen *et al.,* 2013; Yang *et al.,* 2017). *Diplodia seriata*, *N. parvum* and *B. dothidea* are the most frequently isolated species (Úrbez-Torres, 2011). Several studies have indicated that most rapidly infecting wood-colonizing fungi, and therefore the most virulent, are species of *Lasiodiplodia* and *Neofusicoccum* (van Niekerk *et al.,* 2004; Úrbez-Torres *et al.,* 2008; Úrbez-Torres and Gubler, 2009).

Several species of *Diaporthe* have been associated with Diaporthe dieback of grapevines (Baumgartner *et al.,* 2013; Úrbez-Torres *et al.,* 2013a; Dissanayake *et al.,* 2015; Guarnaccia *et al.,* 2018). Among these, the disease is primarily caused by *Dia. ampelina* (formerly *Phomopsis viticola*) (Úrbez-Torres *et al.,* 2013a; Dissanayake *et al.,* 2015), which has long been known as the causal agent of the grapevine disease named Phomopsis cane and leaf spot in the United States of America, or excoriose in Europe (Phillips 2000; Úrbez-Torres *et al.,* 2013a).

Several *Cytospora* spp. have been associated with Cytospora canker in Iran (Fotouhifar *et al.,* 2010; Arzanlou and Narmani, 2015), Canada and United States of America (Lawrence *et al.,* 2017), although their pathogenicity on grapevine has only been evaluated for *Cy. viticola* and *C. vinacea*.

Regarding Basidiomycetes associated with esca disease in adult vineyards, these belong to the genera *Inocutis*, *Inonotus*, *Fomitiporella*, *Fomitiporia*, *Phellinus*, and *Stereum* (Cloete *et al*., 2015; Gramaje *et al*., 2018), being *F. mediterranea* the most frequent species in Europe.

FUNGAL TRUNK DISEASES OF BERRY FRUIT

Dieback, canker, and twig and stem blights are common diseases of berry fruit crops (e.g., blueberry, cranberry). These have been associated with diverse fungal pathogens, but most frequently with species of *Diaporthe* and several genera in the *Botryosphaeriaceae* (Lombard *et al.,* 2014; Guarnaccia *et al.,* 2020; Hilário *et al.,* 2020a; Hilário *et al.,* 2021a). Surveys of orchards have shown that dieback and blight are the most common symptoms, which affect plant longevity and fruit yields, and therefore represent threats to production (Lombard *et al.,* 2014; Cardinaals *et al.,* 2018; Guarnaccia *et al.,* 2020; Flor *et al.,* 2022). *Diaporthe vaccinii* (= *Phomopsis vaccinii*) has been regarded as the major species of *Diaporthe* occurring on *Vaccinium* spp. (until recently included in

the EPPO A2 list), causing Phomopsis canker and dieback, twig blight, leaf spots and viscid rot (fruit rot), mainly on highbush blueberry (*V. corymbosum*) and cranberry (*Vaccinium macrocarpon* and *V. oxycoccus*), and also known as upright dieback and viscid rot of cranberry (EPPO Bulletin 2009; Lombard *et al.,* 2014; Michalecka *et al.,* 2017). *Diaporthe vaccinii* is probably widespread in the United States of America and Canada, but there are only a few reports of this fungus in Europe (Germany, Latvia, Lithuania, the Netherlands, Poland, Romania, United Kingdom) (Lombard *et al.,* 2014; Michalecka *et al.,* 2017).

Several *Diaporthe* species have been reported from symptomatic blueberry plants rendering it questionable that *Dia. vaccinii* is a major pathogen of this crop. Its taxonomic status has also been the subject of debate, and Hilário *et al.,* (2021b) proposed that *Dia. vaccinii* is a synonym for *Dia. eres*. *Diaporthe eres* is emerging as the most common and widespread pathogen of highbush blueberry in Europe, being associated with dieback, twig and stem blight, and canker, in Croatia (Ivić *et al.,* 2018), Italy (Martino *et al.,* 2022), the Netherlands (Lombard *et al.,* 2014) and Portugal (as *Dia. vacuae*, a synonym of *Dia. eres*) (Hilário *et al.,* 2020b). This fungus has also been reported as causing cane blight of raspberry in Italy (Guarnaccia *et al.,* 2022b) and of blackberry in Croatia (Vrandecic *et al.,* 2011). In addition to *Dia. eres* and *Dia. vaccini* on *Vaccinium* spp. in different countries in Europe, Lombard *et al.* (2014) described three new species, *Dia. viticola* (= *Dia. rudis*) from the Netherlands, *Dia. baccae* and *Dia. sterilis* were from Italy, *Dia. rudis* has also been reported from symptomatic highbush blueberry plants in Italy (Guarnaccia *et al.,* 2020). A survey of highbush blueberry orchards in Portugal revealed the occurrence of ten *Diaporthe* species in symptomatic (dieback and twig blight) plants, namely *Dia. ambigua*, *Dia. amygdali*, *Dia. crousii*, *Dia. foeniculina*, *Dia. hybrida*, *Dia. leucospermi*, *Dia. phillipsii*, *Dia. malorum*, *Dia. rudis* and *Dia. vacuae* (= *Dia. eres*) (Hilário *et al.,* 2020b, 2021a). Inoculation trials showed that *Dia. amygdali* and *Dia. eres* were the most aggressive to blueberry plants (Hilário *et al.,* 2021a).

Species from at least four genera of *Botryosphaeriaceae*, namely *Botryosphaeria*, *Lasiodiplodia*, *Macrophomina* and *Neofusicoccum*, are known to cause disease on *Vaccinium* spp. Botryosphaeria stem canker is caused by *B. corticis*, a species considered an important pathogen of blueberry, but that has been reported only in the United States of America (Phillips *et al.,* 2006). *Botryosphaeria dothidea* is known to cause stem blight and dieback and has been reported from highbush blueberry in Portugal (Hilário *et al.,* 2020a). Of the 12 species of *Neofusicoccum* associated with stem blight and dieback of blueberry plants, *N. parvum* and *N. australe* have been reported in Portugal and Spain (Castillo *et al.,* 2013; Hilário *et al.,* 2020a), and *N. eucalyptorum* in Portugal. From these three species, *N. parvum* was the most aggressive in inoculation trials with highbush blueberry plants (Castillo *et al.,* 2013; Hilário *et al.,* 2020a).

Species of *Lasiodiplodia*, mostly *L. theobromae*, are important pathogens of blueberry in tropical and subtropical climates (e.g., Rodríguez-Gálvez *et al.,* 2020), but have been also reported in Europe associated with canker and dieback of highbush blueberry in Spain (Borrero *et al.,* 2019) and the Czech Republic (Pečenka *et al.,* 2021). *Macrophomina phaseolina*, a common soilborne fungus, has also been reported on highbush blueberry plants, causing charcoal rot in Spain (de los Santos *et al.,* 2019) and blight in Serbia (Popovič *et al.,* 2018).

Godronia cassandrae f. sp. *vaccinii* (= *Topospora myrtilli*, syn. *Fusicoccum putrefaciens*) causes Godronia canker, also known as Fusicoccum canker. This fungus may cause severe stem dieback of highbush blueberry, especially in young plants. This pathogen has been reported in North America and Europe, as a cause of severe losses in commercial highbush blueberry production (Strømeng and Stensvand, 2011). However, there are no recent reports of this fungus, so its status in Europe is uncertain.

Although not representing major pathogens, pestalotioid fungi of the *Sporocadaceae* have also been reported from blueberry plants with canker, dieback, and blight symptoms (Rodríguez-Gálvez *et al.,* 2020; Santos *et al.,* 2022). *Neopestalotiopsis clavispora* was reported from canker and twig dieback of highbush blueberry in Spain (Borrero *et al.,* 2018). Also on this host in Portugal, Santos *et al.,* (2022) reported three species of *Pestalotiopsis* (*P. australis*, *P. biseriata*, *P. chamaeropis*) and four species of *Neopestalotiopsis*, including *N. rosae* and the newly described *N. scalabiensis*, *N. vaccinii* and *N. vacciniicola*.

FUNGAL TRUNK DISEASES OF NUT TREES

Trunk pathogens of nut trees are underestimated phenomena associated with decline, even where the plants have shown obvious symptoms of trunk diseases. The first comprehensive study of fungal trunk pathogens detected in the wood of almond trees in Mallorca was published in 2012 (Gramaje *et al.,* 2012). Pathogens detected were: the *Botryosphaeriaceae*, *B. dothidea, Dip. olivarum, Dip. seriata, N. australe,* and *N. parvum*, confirmed by Olmo *et al.* (2016) and Arzanlou *et al.* (2016);

the *Diatrypaceae E. lata;* the *Togniniaceae Phaeoacremonium amygdalinum and P. iranianum*, confirmed by Raimondo *et al.* (2021); and the *Diaporthaceae Diaporthe amygdali*, later confirmed by Varjas *et al.* (2017), León *et al.* (2020), and Beluzán *et al.* (2022). Almond wood is also invaded by *Pleurostoma richardsiae* (Olmo *et al.,* 2015) and *Calosphaeria pulchella* (Arzanlou *et al.,* 2013). Holland *et al.* (2021b) described detailed symptoms on the almond wood associated with pathogens. They observed Botryosphaeriaceae canker, Ceratocystis canker, Cytospora canker, Diaporthe canker, Collophorina canker, Eutypa and Pallidophorina canker. Severe stem canker of almond trees caused by *Fusarium solani* was also reported by Markakis *et al.* (2021).

Walnut woody parts are commonly invaded by trunk pathogens, including *Diatrypaceae*, *Diaporthaceae*, *Botryosphaeriaceae* and *Togniniaceae*. In general, the spread of these fungi is similar in regions and countries such as: California (*Diaporthe*, *Neofusicoccum*) (Trouillas *et al.,* 2010; Agustí-Brisach *et al.,* 2019), Chile (*Diaporthe*, *Diplodia, Neofusicoccum*) (Díaz *et al.,* 2018; Luna *et al.,* 2022), China (*Botryoshpaeria*, *Lasiodiplodia, Neofusicoccum*) (Yu *et al.,* 2015; Li *et al.,* 2016), the Czech Republic (*Cadophora, Cryptovalsa, Diaporthe, Diplodia, Dothiorella, Eutypa, Eutypella, Peroneutypa, Phaeoacremonium*) (Eichmeier *et al.,* 2020), Italy (*Botryosphaeria, Neofusicoccum*) (Gusella *et al.,* 2021), Romania (*Diaporthe*) (Mihaescu *et al.,* 2020), Spain (*Botryosphaeria, Diaporthe, Diplodia, Dothiorella, Neofusicoccum*) (López-Moral *et al.,* 2020a; Moral *et al.,* 2010), and Turkey (*Botryosphaeria*, *Neofusicoccum*) (Kara *et al.,* 2021; Yildiz *et al.,* 2022). Walnut wood also hosts *Ca. spacidis* and *Ca. novi-eboraci* (*Incertae sedis*) and these pathogens were detected in most surveyed orchards in the Czech Republic (Eichmeier *et al.,* 2020).

Hazelnut plants can be hosts of *Diaporthe* as described for Turkey (Arciuolo *et al.,* 2020) and *Fomitiporia* in Italy (Pilotti *et al.,* 2010). Pistachio trunk pathogens are also known. Several pathogens were isolated in California, including *Col. karstii*, *Cy. californica*, *Cy. joaquinensis*, *Cy. parapistaciae*, *Cy. pistaciae*, *Dia. ambigua*, *Didymella glomerata*, *Dip. mutila*, *N. mediterraneum*, *Pm. canadense*, and *Schizophyllum commune* (Nouri *et al.,* 2019). Survey of FTD pathogens of pistachio in Iran revealed *Pm. parasiticum* as a dominant species, followed by *Pm. minimum*, *B. dothidea*, *N. parvum*, *Pm. cinereum*, *Pm. viticola* and *Do. viticola* (Mohammadi *et al.,* 2015). Chen *et al.* (2015) collected *L. americana* sp. nov. from blighted pistachio shoots in Arizona, United States of America, and *N. hellenicum* sp. nov. in Greece, while in Italy the new pathogen *Liberomyces pistaciae* sp. nov. was detected (Vitale *et al.,* 2018). Nut trees such as almonds, pistachios and walnuts were described in Iran as hosts of *Botryosphaeria, Collophorina, Cryptosphaeria, Diatrype, Diplodia, Dothiorella, Eutypella, Lasiodiplodia, Neofusicoccum, Pleurostoma* by Sohrabi *et al.* (2020).

FUNGAL TRUNK DISEASES OF STONE FRUIT TREES

Stone fruit trees are commonly affected by numerous wood-invading pathogens causing cankers and dieback. *Eutypa lata* was first reported as the causal agent of gummosis or dieback of apricot in Australia (Carter, 1957), and has since been known to affect several types of stone fruit trees (Matthee *et al.,* 1974; Carter 1982, 1995; Munkvold and Marois 1994; Rumbos, 1997). To date, at least 19 distinct species of *Togniniaceae* (i.e. *Pm. scolyti*, *Pm. minimum*, *Pm. australiense*, *Pm. alvesii*, *Pm. parasiticum*, *Pm. infatipes*, *Pm. iranianum*, *Pm. italicum*, *Pm. griseorubrum*, *Pm. junior*, *Pm. longicollarum*, *Pm. pallidum*, *Pm. prunicolum*, *Pm. subulatum*, *Pm. fuscum*, *Pm. griseorubrum*, *Togninia africana*, *T. griseo-olivacea* and *T. fraxinopennsylvanica*) have been associated with necrotic wood of stone fruit trees (Hawksworth *et al.,* 1976; Rumbos, 1986; Hausner *et al.,* 1992; Damm *et al*., 2008; Spies *et al.,* 2018). As well, four *Cytospora* species (*Cy. chrysosperma*, *Cy. longispora*, *Cy. plurivora, Cy. sorbicola*) have been identified as canker and dieback-causing pathogens in stone fruit trees including *Persica vulgaris*, *Prunus armeniaca*, *P. avium*, *P. cerasus*, *P. domestica* and *P. persica*, in several countries (Lawrence *et al.,* 2017).

Canker and branch dieback of sweet cherry trees caused by *Calosphaeriophora pulchella* has also been reported in California, Chile, South Australia and Spain (Trouillas *et al.,* 2012; Berbegal *et al.,* 2014; Auger *et al.,* 2021).

Studies conducted primarily in South Africa demonstrated the diversity of indigenous flora and the occurrence of newly identified fungi causing trunk diseases in adult stone fruit trees and propagation material (Damm *et al.,* 2007, 2010). The pathogenic potential of most of these species has been confirmed in pathogenicity trials. Damm *et al.* (2007) isolated several *Botryosphaeriaceae* species (*Dip. seriata*, *N. vitifusiforme, N. australe*, *Do. viticola, Dip. pinea*, *Dip. mutila, L. plurivora* and *Dip. africana*) from plum, peach, nectarine and apricot trees showing wood necrosis in South Africa. In another study on *Prunus* trees showing wood necrosis (Damm *et al*., 2010), identified species of *Coniochaeta* (*C. velutina* and the two new species, *C. africana* and *C. prunicola*) and *Phaeomoniellales* (*Pa. zymoides* on *Prunus salicina*, and the new species *Pa. dura*, *Pa. effusa*, *Pa. prunicola*

and *Pa. tardicola* mainly in plum wood). The new genus *Collophorina* was also proposed, comprising *Collop. africana*, *Collop. capensis*, *Collop. pallida* and *Collop. rubra* that occurred frequently in necrotic peach and nectarine wood. Also in South Africa, apricot and plum trees were inhabited by five *Diatrypaceae* species with *E. lata* the most dominant, followed by *Cryptovalsa ampelina*, *E. cremea*, *Eutypella citricola* and *Eu. microtheca*, whereas no *Diatrypaceae* were found on peach and nectarine (Moyo *et al.,* 2018).

In a similar study conducted in Germany, *Pallidophorina paarla*, *Collop. africana* and the two new species *Collop. badensis* and *Collop. germanica*, were associated with wood necroses on *Prunus* trees (Bien and Damm, 2020). Here, *Cadophora* was reported for the first time from *Prunus*, with *Ca. luteo-olivacea* and *Ca. novi-eboraci* dominating (Bien and Damm, 2020). *Cadophora prunicola*, *Ca. ramosa* and *Minutiella pruni-avium* were also described as new species.

A study in the western cape of South Africa (van der Merwe *et al.,* 2021) showed that stone fruit propagation material and nursery plants had latent infections of canker- and wood rot-associated fungi, with *Ca. luteo-olivacea* and *Dip. seriata* as the most frequently isolated. Sampled nursery trees were 22% infected, ungrafted rooted rootstock plants, 11%, dormant rootstock shoots, 6%, dormant buds, 1%, and green buds were 0.4% infected. Van der Merwe *et al.* (2021) also made 22 new records of fungal species on stone fruit, including (among others), three species of *Coniochaeta*, two of *Cadophora* and *Cytospora*, and one species of *Biscogniauxia*, *Eutypella* and *Peniophora*.

FUNGAL TRUNK DISEASES OF OLIVE TREES

FTD of olive have been considered as emerging problems for olive cultivation. Several studies have shown many wood-inhabiting fungi in diverse groups as causal agents of discrete diseases in adult and nursery olive plants. Numerous *Botryosphaeriaceae* species (i.e. *B. dothidea*, *B. obtusa*, *Dip. mutila*, *Dip. seriata, Do. iberica*, *L. theobromae*, *N. australe, N. stellenboschiana, N. luteum*, *N. mediterraneum, N. parvum* and *N. vitifusiforme*) have been shown as causing olive twig and branch dieback in California, Croatia, Italy, Tunisia, South Africa and Spain (Moral *et al.,* 2010, 2017; Carlucci *et al.,* 2013; Kaliterna *et al.,* 2013a; Úrbez-Torres *et al.,* 2013b; Spies *et al.,* 2020; van Dyk *et al.,* 2021b).

Several *Togniniaceae* (i.e. *Pm. africanum, Pm. alvesii, Pm. italicum, Pm. minimum, Pm. oleae*, *Pm. parasiticum, Pm. prunicola, Pm. rubrigenun, Pm. scolyti, Pm. sicilianum, Pm. spadicum* and *Pm. viticola*), *Phaeomoniellales* (i.e. *Neophaeomoniella niveniae*, *Pa. chlamydospora*, *Pseudophaeomoniella globose, Ps. oleae* and *Ps. oleicola)* and *Acremonium sclerotigenum, Ca. luteo-olivacea*, *Comoclathris incompta*, *Paracremonium* sp. and *Pleurostoma richardsiae* have also been indicated as causal agents of olive wood streaking, wilting, dieback and decline in California, Croatia, Greece, Italy, South Africa and Spain (Ivic *et al.,* 2010; Nigro *et al.,* 2013; Carlucci *et al.,* 2015; Markakis *et al.,* 2017, 2022; Spies *et al.,* 2020; Agustí-Brisach *et al.,* 2021; Lawrence *et al.,* 2021; van Dyk *et al.,* 2021b; Raimondo *et al.,* 2022).

The Basidiomycetes *F. mediterranea* (Fig. 2), *Fomitiporella viticola*, *Ganoderma lucidum*, *Phellinus linteus, P. robiniae, Schizophyllum commune* and *Trametes versicolor* have also been reported to be involved in olive wood rot and decay, in California, Greece and South Africa (Crous *et al.,* 2000; Úrbez-Torres *et al.,* 2013b; Markakis *et al.,* 2017, 2019; Lawrence *et al.,* 2021; van Dyk *et al.,* 2021b). Members of *Diatrypaceae* (i.e. *Diat. oregonensis*, *Diat. stigma*, *E. lata*), and species of *Cytospora* (i.e. *Cy. oleina*, *Cy. oleicola*, *Cy. olivarum*, *Cy. plurivora, Cy. pruinosa* and *Cy. sorbicola*) and *Diaporthe* (i.e. *Dia. viticola*, *Dia. rubis, Dia. foeniculina*) were associated with canker and twig and branch dieback diseases of olive trees in California, Greece, South Africa and Spain (Rumbos, 1988, 1993; Moral *et al.,* 2017; Lawrence *et. al.*, 2017; van Dyk *et al.,* 2021b).

A recent survey by van Dyk *et al.* (2021b), in the Western Cape Province, South Africa, and coupled with pathogenicity tests, showed *Ps. globosa* as a major olive trunk pathogen, and several lesser-known fungi were also associated with olive trunk disease symptoms. These included *Biscogniauxia rosacearum*, *Celerioriella umnquma*, *Coniochaeta velutina*, *Coniothyrium ferrarisianum*, *Didymocyrtis banksiae*, *Punctularia atropurpurascens*, *Vredendaliella oleae*, an undescribed *Cytospora* sp., *Geosmithia* sp., two undescribed *Neofusicoccum* spp., and four *Xenocylindrosporium* spp. Van Dyk *et al.* (2021a) also surveyed trunk pathogens in South African olive nurseries, and found several pathogens in *Nectriaceae, Diaporthaceae*, *Botryosphaeriaceae*, *Togniniaceae*, *Phaeomoniellaceae* and *Pleurostomataceae*, with *N. australe*, *Pleurostoma richardsiae* and *Pm. parasiticum* the most common fungi in propagating plant material.

EPIDEMIOLOGY OF TRUNK DISEASES

Most FTD pathogens can infect planting material during propagation processes in nurseries. Several studies have shown evidence to support non-pathogenic

Figure 2. Wood decay (Esca) symptoms in olive trees in Thrace, Northeastern Greece, infected by *Fomitiporia mediterranea*. Canker in the trunk (**A**); white rot appeared after removing a trunk sector due to incorrectly adjusted irrigation sprinklers which created conducive conditions for fungal infection (**B**); carpophore of *F. mediterranea* formed on the trunk surface (**C**); trunk cross section revealing light-coloured wood rot surrounded by a brown necrotic zone (**D**).

endophytic phases for these fungi, as they have been isolated from asymptomatic mother plants and propagation material (Halleen *et al.,* 2003; Aroca *et al.,* 2010; Berlanas *et al.,* 2020; van der Merwe *et al.,* 2021). Several studies have confirmed that plant propagation stages are potential infection points in grapevine nurseries (Edwards and Pascoe 2004; Aroca *et al.,* 2010; Halleen *et al.,* 2003; 2007a; Agustí-Brisach *et al.,* 2013), and recent studies indicate the role of infected planting material in the dissemination of fungal trunk pathogens in stone fruit trees (Marín-Terrazas *et al.,* 2016; van Dyk *et al.,* 2021a; van der Merwe *et al.,* 2021; Capote *et al.,* 2022) and apple (Havenga *et al.,* 2019).

Cylindrocarpon-like asexual morphs are soilborne. These fungi are commonly found in nursery fields and soils, so inoculum may already exist in soils before planting (Agustí-Brisach *et al.*, 2013; Berlanas *et al.,* 2017). However, most FTD pathogens are primarily spread through the dispersion of airborne spores. Depending on the fungal species, conidia or ascospores are released from pycnidia or perithecia embedded in the surfaces of dead wood and/or in bark tissues (Eskalen and Gubler, 2001; Rooney-Latham *et al.,* 2005; Úrbez-Torres *et al.,* 2014; van Niekerk *et al.,* 2010; Kraus *et al.,* 2020). This inoculum is released under favourable environmental conditions, which have been mostly associated with rain events and/or high relative humidity along with temperatures above freezing, which also favour spore germination (Úrbez-Torres *et al.,* 2010a; van Niekerk *et al.,* 2010). Spores then land on susceptible pruning wounds and/or natural openings to germinate and start colonization of xylem vessels and pith parenchyma cells (Mostert *et al.,* 2006).

Jiménez-Luna *et al.* (2022) showed the presence and diversity of air-borne spores of FTD pathogens in almond and walnut orchards in California, where incidence was influenced by host, age of the plants and precipitation. Arthropod-mediated dispersal of FTD pathogens has also been demonstrated in vineyards, indicating potential roles for arthropods in trunk diseases epidemiology (Moyo *et al.,* 2014; Kalvelage *et al.,* 2021, 2022). Agustí-Brisach *et al.* (2015) showed that pruning equipment can spread FTD pathogens under controlled conditions. They also found that high inoculum concentrations of FTD pathogens were required to produce successful infection. In grapevine, studies have shown that high risk infection periods may vary throughout each host growing season, and from year to year, but these periods can also overlap with host dormancy seasons in the Northern and Southern Hemispheres (Larignon and Dubos, 2001; Eskalen and Gubler, 2001; Amponsah *et al.,* 2009; Kuntzmann *et al.,* 2009; Quaglia *et al.,* 2009; Úrbez-Torres *et al.,* 2010; van Niekerk *et al.,* 2010; Cloete *et al.*, 2015; Valencia *et al.,* 2015; González-Domínguez *et al.,* 2020; Billones-Baaijens *et al.,* 2018). An epidemiological equation model for *Pa. chlamydospora* was developed by González-Domínguez *et al.* (2020) in Spanish vineyards. They showed that dispersal dynamics of this fungus was best explained by hydro-thermal time which takes account of effects of temperature and rainfall.

FTD fungi are cosmopolitan, and can colonize a range of hosts as saprotrophs or plant pathogens, infecting natural ecosystems and cultivated crops. Patterns of multiple host infections have been reported for *Botryosphaeriaceae* (Damm *et al.,* 2007; Slippers and Wingfield, 2007; Mojeremane *et al.*, 2020), *Diatrypaceae* (Moyo *et al.,* 2019), and *Togniniaceae* (Damm *et al.,* 2008). This is also the case for *F. mediterranea*, a lignicolous fungus that has been found as the causal agent of wood decay in several woody hosts (Markakis *et al.*, 2017). The overlap of trunk disease pathogens between agricultural systems and native plant ecosystems has also been indicated (Damm *et al.,* 2007, 2008; Moral *et al.,* 2010; Trouillas *et al.,* 2010; Markakis et al., 2017; Moyo *et al.,* 2019). The discovery of a single fungal species on multiple hosts has important epidemiological implications, giving circumstantial evidence that inocula travel between different woody hosts, so those hosts occurring in close proximity can provide inoculum to each other.

HYPOTHESES FOR INCREASED INCIDENCE OF TRUNK DISEASES

Crop intensification

Crop intensification can increase FTD incidence in the field. Intensive cropping systems provide favourable environments for infection, since microclimate resulting from high plant densities can increase wetness duration and decrease sunlight entry into tree canopies (Kraus *et al.,* 2018). Modern super-high-density systems also demand mechanization of cultural practices (e.g. pruning and harvesting), with consequential injuries that create conducive conditions to wound-penetrating fungi such as Basidiomycetes, *Botryosphaeriaceae*, *Diatrypaceae* and *Togniniaceae* (Moral *et al.,* 2010; Úrbez-Torres *et al.,* 2013b; Markakis *et al.,* 2017; Agustí-Brisach *et al.,* 2021). In viticulture, intensive pruning schemes (e.g. spur or cane pruning), can cause more frequent external FTD symptoms than minimal pruning schemes (Lecomte *et al.,* 2018, 2022; Kraus *et al.,* 2019). It was also assumed that intensive pruning causes more and larger pruning wounds on grapevine trunks, leading

to increased infections by wood degrading fungi, and consequent interruption of host vascular systems and increased symptoms (Travadon *et al.,* 2016; Kraus *et al.,* 2022).

Intensive crop production with high yields elevates plant water demand and in combination with the ongoing climate warming provokes drought stress, which enhances FTD development. For almond trees in southern Spain, water deficiency leads to increased dieback severity compared to well-watered trees (Agustí-Brisach *et al.,* 2020). In addition, under drought stress, grapevines were more susceptible to infection and colonization by FTD pathogens (Sosnowski *et al.,* 2016, 2021; Galarneau *et al.,* 2019; Hrycan *et al.,* 2020). The exact role of climate/environmental conditions (e.g. drought, rainfall, water availability) in FTD incidence is not completely clear and requires further investigation (Fischer and Peighami Ashnaei, 2019; Songy *et al.,* 2019).

Different woody crops (e.g. olive, grapevine and almonds) that are affected by the same fungal pathogens are commonly grown in neighbouring orchards in several Mediterranean countries (Fig. 3). This is likely to allow inoculum proliferation and flow among the orchards (Markakis *et al.,* 2017). Crop intensification is also likely to disturb microflora balances in agro-ecosystems, promoting transfer of trunk pathogens and their adaptation to new woody hosts. In recent years, emergence of new FTD has been shown for several woody crops (Damm *et al.,* 2010; Markakis *et al.,* 2017; van der Merwe *et al.,* 2021; van Dyk *et al.,* 2021a; b).

Figure 3. Typical landscape of adjacent vineyards and olive orchards in Crete, Southern Greece, which allows the inoculum flowing.

Planting material and nursery practices

Plant propagation in fruit crop and grapevine nurseries includes complex systems in which pathogen management is challenging. Infected nursery stock can be important long-distance vectors for FTD pathogens (Gramaje and Armengol, 2011). Studies in Europe on death of young or newly established fruit crop trees have shown that latent infections occurring during nursery propagation are important for development of cankers observed in the orchards (Brown *et al.,* 1994; Smit *et al.,* 1996; Marek *et al.,* 2013). Certified nursery trees are not commonly surveyed for latent fungal infections, which can lead to severe symptoms in newly established stone fruit orchards (Mostert *et al.,* 2016; van der Merwe *et al.,* 2021). McCracken *et al.* (2003) found that cankers caused by *N. ditissima* on scion shoots of 1-year-old commercial apple trees, developed after infections that occurred during the final stages of propagation. Marek *et al.* (2013) found that latent infections, occurring in nurseries, caused wood cankers during cold storage of propagation material or after planting. Havenga *et al.* (2019), in South Africa, showed presence of fungal pathogens causing FTD on the 65% of assessed certified apple tree nurseries. The pathogens isolated from 1-year-old diseased commercial trees were also recovered from latent infections in nursery trees, confirming the roles of rootstock wounds and bud unions as infection sites.

Van Diyk *et al.* (2021a) reported the presence of eight known FTD pathogens of olive and other woody host from plant portions which were in direct contact with perlite/soil and water. Infected grapevine propagation material is considered to have a major role for disease occurrence in the field (Halleen *et al.,* 2003; Gramaje and Armengol, 2011). Stone fruit nursery trees have also been investigated. Isolations conducted from scions and rootstocks have highlighted the importance of these materials as sources of latent infections for nursery trees, showing the presence of FTD pathogens on the 22% of 1080 trees tested (van der Merwe *et al.,* 2021).

This information emphasizes the importance of incorporating integrated disease management that combines different preventative control measures throughout the nursery propagation processes, storage, and during crop establishment, in order to reduce incidence and severity of FTD in orchards (Gramaje *et al.,* 2018). Cultural practices such as the removal of dead and affected material, which often bring fungal fruiting bodies and spores, is important in all phases of propagation and plant establishment (Van Zyl 2011). Authorized fungicides and biological control agents should be used to protect pruning wounds throughout plant propagation to reduce infections in nurseries (Fourie and Halleen 2004). For grapevine, hot water treatments of scions/

rootstocks before grafting, and of the dormant grafted nursery grapevines are recommended practices to reduce pathogen inocula (Fourie and Halleen 2004; Halleen *et al.,* 2007b; Bleach *et al.,* 2013; Eichmeier *et al.,* 2018).

Climate change

Increased incidence of FTD on woody hosts can be attributed to climate change (Chakraborty and Newton 2011; Kaliterna *et al.,* 2013a; Markakis *et al.,* 2017). Climate change prediction models foresee more frequent extreme weather conditions, along with increases in summer air temperature and drought stress for many crops in the Mediterranean region (Lung *et al.,* 2013). In California, Allen and Luptowitz (2017) predicted significant rainfall increases in response to warming climate. Alterations of wet and dry cycles, and extremely low and/or high temperature events will probably be more frequent, as consequences of the climate change. These phenomena may favour pathogen dispersal and adaptation to hosts other than their primary hosts, and this has been suggested for the extended host range of *F. mediterranea* (Elena *et al.,* 2006; Markakis *et al.,* 2017, 2019). This was also the case for the epidemic caused by *Dip. seriata* (a weak pathogen) that occurred in summer 2010 in Croatia, when severe dieback occurred in young olive trees previously exposed to low winter temperatures (Kaliterna *et al.,* 2013a). *Botryosphaeriaceae*-incited diseases are commonly more severe in years with high rainfall, when inoculum increases are followed by drought periods and host susceptibility increases (Ma *et al.,* 2001; Michailides and Morgan 2004; Marsberg *et al.,* 2017).

Microbiome balance

All plants contain microorganisms as parts of their holobionts (Berg *et al.,* 2020). These microbiota and their activities, the so-called microbiome, have been shown to change for all diseases, and as conditions alter (Bettenfeld *et al.,* 2020). There are functional core microbiota playing central roles in plant physiology and health. Increased numbers of (latent) pathogens in the core microbiota and detrimental effects on other microorganisms, which can take place when the plants are weakened by other biotic or abiotic factors, can lead to increase virulence and visible plant symptoms (Bettenfeld *et al.,* 2020). "Satellite microbiota" (i.e. not occurring in every plant) can be also be affected.

For FTD, Bruez *et al.* (2020) suggested that fungal microbiota from non-necrotic woody tissues were similar in healthy and esca diseased grapevines. In non-necrotic woody tissues, fungal and bacterial microbiota varied according to organs and seasons, but not according to disease status. *Phaeomoniella chlamydospora*, was the most abundant fungus in non-necrotic tissues from healthy plants. The only difference between healthy and diseased young grapevines was the presence of white rot necrotic tissues in cordons that were dominated by *F. mediterranea*, associated with *Pa. chlamydospora* and a few bacterial taxa. Increased numbers of pathogens, and changes in mycobiomes, were recorded in different plant tissues.

Meta barcoding studies of grapevines in Portugal (Del Frari *et al.,* 2019) and Greece (Bekris *et al.,* 2021) have assessed the mycobiomes of grapevine trunks with esca, and cultivar and biogeography-dependent patterns were identified that could be used as to distinguish between healthy and diseased grapevines. By analyzing the microbiomes of healthy and diseased grapevines, strong interactions between the bacterial and fungal wood microbiomes in asymptomatic grapevines could be used for discovery of novel biocontrol agents (Cobos *et al.,* 2022). However, specific bacteria isolated from healthy or diseased host tissues have been described as potentially increasing wood degradation by esca pathogens. This has been shown for *F. mediterranea* (Haidar *et al.,* 2021). Some bacterial strains that degrade grapevine wood components (cellulose and hemicellulose) did not inhibit *F. mediterranea* growth *in vitro*, but had synergistic interactions with *F. mediterranea* by enhancing the degradation of wood structures (Haidar *et al.,* 2021). One of these strains has been described as belonging to a new species, *Paenibacillus xylinteritus* sp. nov. (Haidar *et al*., 2022). Analyzing the microbiomes of healthy and diseased plants can pinpoint balance/imbalance that could lead to discovery of new types of interaction, such as other microorganisms that increase or decrease pathogen virulence.

Limited availability of fungicides

Chemical control using fungicides has been the main strategy for FTD management both in propagation material produced in nurseries and adult plants in orchards, for which pruning wound protection is essential to reduce FTD incidence. Nevertheless, limited products are currently registered to effectively control FTD in Europe and worldwide. Thus, future research should be focused on expanding the range of chemical and alternative options for this purpose (Gramaje *et al*., 2018). Available management strategies against FTD on grapevines have been widely investigated in the last decades and, for instance, many new biological control agents have been evaluated and registered to control FTD both in nurseries and vineyards, including fungi, bacteria and oomycet-

es, being *Trichoderma* spp. based products the most frequent (Gramaje *et al*., 2018; Mondello *et al*., 2018a,b). In the same way, it is necessary to progressively expand the range of treatments available for all types of fruit trees.

FUTURE DIRECTION OF FTD RESEARCH

Over the last years, fungal trunk pathogens incidence has globally increased in woody crops. The etiology is still complex, as many pathogens have been recently described associated with FTD symptoms. Disease management implies the adoption of precision farming technologies and alternative strategies to the use of synthetic fungicides (i.e., microbial biocontrol agents, plant defense elicitors and possible microbiome manipulations). This will provide a new scenario to understand the role of trunk pathogens in the whole cropping system. The integration of plant pathology concepts and methodologies with those of other disciplines will be needed to deliver new disease management strategies in a wider context. Here, we discuss the future direction of FTD research and identify some key issues that, we anticipate, must be faced to overcome the losses caused by these diseases.

A key challenge in this complex pathosystem is to develop tools and methodology that enable the rapid detection of FTD fungi in asymptomatic plants, especially in planting material, and the accurate diagnosis of the causal agents. High throughput sequencing (HTS) diagnostics is revolutionizing plant pathology. HTS methods are probably the most significant advances in molecular biology since the advent of the PCR process. Microbial/fungal genome sequencing or metagenomics/metabarcoding become a routine analysis and using the latest technology it is possible to generate near complete genomes (Robert-Siegwald *et al*., 2017; Eichmeier *et al*., 2022) or whole spectra of microbial/fungal communities (Eichmeier *et al*., 2018; Bruez *et al*., 2020; Gramaje et al. 2022). There are also other applications of HTS technology which can serve understanding of the plant pathogen interaction as transcriptomics (Romeo-Oliván *et al*., 2022) or small RNA sequencing (Eichmeier *et al*., 2019).

The advent of precision farming technologies coupled with remote sensing methods opens entire new fields of research, where the performance of cultural practices for FTD management can be addressed. Imaging analysis is one promising method for a non-invasive detection of FTD. For grapevine, with hyperspectral- or multispectral imaging analysis, esca symptomatic and asymptomatic plants can be diagnosed even before clear foliar-symptoms appear (Junges *et al*., 2018; Bendel *et al*., 2020; Pérez-Roncal *et al*., 2022). Even with unmanned aerial vehicles, whole vineyards could be monitored this way for FTD, which facilitates the assessment of the vineyard's health status (Di Gennaro *et al*., 2016). Furthermore, for Laurel wilt disease on avocado, it was possible to distinguish between healthy and asymptomatic trees based on hyperspectral analysis of the leaves (Hariharan *et al*., 2019, Abdulridha *et al*., 2016). In a trial with potted olive trees artificially infected with *F. mediterranea*, the uninfected population could be differentiated from the infected population with a good accuracy also by hyperspectral analysis of the leaves (Zapolska *et al*., 2020). These examples show that spectral imaging analysis of leaves are convenient diagnostic tools to detect FTD in different perennial crops. In the future, this tool could be implemented into the plant protection management process for an early detection of the disease. With that, proper curative countermeasures can be applied and, thus, preventing the plant from becoming symptomatic.

For many IPM programs, including the management of FTD, there is a crucial need of decision-support tools to determine disease risk and the best moments for any intervention (Rossi *et al*., 2012). Mathematical models that establish relationships between the amount of inoculum and disease development, integrating knowledge on the host-pathogen interactions and environmental data, are key components of any decision-support tool for plant disease management (Ojiambo *et al*., 2017), and have been consistently incorporated in decision support systems (DSSs) to assist users in operational and tactical decision-making in crop protection. Model-based forecasting has the potential to improve the timeliness, effectiveness, and foresight for managing crop diseases, while minimizing economic costs and environmental impacts (Newlands, 2018). For instance, improving our knowledge about the dispersal patterns of FTD spores and its relation to environmental conditions is essential for identifying periods with a high risk of spore dispersal and for adopting management strategies, such as pruning wound protection.

Regulatory changes are reducing the availability of fungicide options, as the health and ecological hazards of the chemicals are increasingly raising concerns. The consumer demand for residue free products have stimulated research into new tools for pest management. Alternatives to synthetic fungicides are mainly represented by a number of microbial active ingredients. Agronomic practices, i.e., reduction of the inoculum in the field, should be combined with biological control in order to reduce the input of synthetic fungicides on the crop.

Nanoparticles (NPs) in size 1–100 nm have demonstrated activity in suppressing plant diseases. These

NPs are mainly metalloids, metallic oxides, nonmetals, and carbon nanomaterials. NPs have been integrated into disease management strategies as fungicides or as nanofertilizers to enhance plant health. Although there are reports of different NPs of single element and carbon nanomaterials affecting plant pathogens. Mainly Ag, Cu, and Zn have received much attention thus far. Some NPs act directly as antimicrobial agents but others work more in altering the nutritional status of the host and they activate various defense mechanisms (Elmer *et al*., 2018). A recent study about the use of nanomaterials against selected FTD pathogens showed that AgSe nanoparticles (NPs), CuSe NPs, Ag NPs, Cu NPs and Se NPs can serve a significant inhibitory activity against *Dia. eres*, *Dip. seriata* and *E. lata* (Štůsková *et al*., 2022). Little information is still available on the use of NPs against FTD pathogens. In addition, more research is needed to evaluate the impact of these treatments on the microbiome and the environment.

Other alternatives to synthetic fungicides are the substances of botanical origin. Recently, several authors described the antimicrobial ability of wood extracts against various fungal species (Kawamura *et al*., 2011; Minova *et al*., 2015; Salem *et al*., 2015; Vek *et al*., 2021). Špetík *et al*. (2022) demonstrated that lignans extract from knotwood of Norway spruce function against *Cad. luteo-olivacea*, *Da. torresensis*, *Dia. ampelina*, *Dia. bohemiae*, *Dip. seriata*, *E. lata* and *Pm. minimum* affecting grapevine. Further research is needed to test the efficacy of these products under field conditions.

Over the last few years, the importance of the plant microbiome, the potential role of endophytes in disease control and/or development, and the interactions among microorganisms has been postulated (Blundell *et al*., 2022). The use of the most advanced technologies will contribute to the discovery of the next generation of microbial biofungicides. In recent years, designing microbial consortia (SynComs – synthetic microbial communities) has received a great deal of interest to overcome the variable results of bioncontrol agents under field conditions. SymComs are expected to be more robust to environmental changes than single-microbiome inoculants and are designed to mimic the natural microbiome function and structure. However, research is still needed to validate the consortia functionality at field scale.

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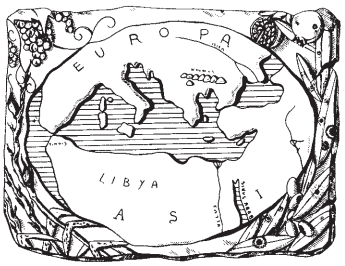

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