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Review

## Current status of *Botryosphaeriaceae* species in Italy: Impacts on agricultural crops and forest ecosystems

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**Summary.** Many fungi belonging to *Botryosphaeriaceae* are well-known as causal agents of diseases in economically and ecologically important agricultural crops and forest trees. In Italy, the high diffusion of *Botryosphaeriaceae* infections observed over the last decade, has shown the importance of this group of fungi, which are becoming limiting factors for plant production in agricultural systems, nurseries and natural and urban landscapes. Global warming and stress factors such as occasional extreme climatic events can affect the susceptibility of host plants, as well as fungus behaviour, increasing the risk of future infections. Available reports of *Botryosphaeriaceae* in Italy have been examined, focusing on wood and fruit pathogens, resulting in a list of ten genera and 57 species. *Diplodia* is the most widespread genus in Italy with 76 records on 44 hosts, while at species level, *Neofusicoccum parvum*, *Botryosphaeria dothidea* and *Diplodia seriata* show the widest host ranges and many records. The ability of the pathogens to remain latent on asymptomatic plants, and uncontrolled trade of plant materials among countries, facilitate the dissemination and potential introduction of new *Botryosphaeriaceae* species. Preventive detection and adequate control strategies are always needed to limit the potential damage caused by *Botryosphaeriaceae*. This review had particular emphasis on host-pathogen associations, disease symptoms, geographic distribution, metabolite production, and accurate pathogen identification.

**Keywords.** Geographic distribution, host-range and disease symptoms, invasive pathogens, metabolites production, species identification.

## INTRODUCTION

*Botryosphaeriaceae* Theiss. & Syd. is one of the most investigated families of fungi (Agnoletti *et al.*, 2022). In addition to including primary plant pathogens such as *Diplodia corticola* and *Lasiodiplodia theobromae*, *Botryosphaeriaceae* includes some species that can live as endophytes in healthy plants or as saprophytes on dead host tissues (Alberti *et al.*, 2018; Aiello *et al.*, 2020, 2022). Recent interest in this family has been linked to the abilities to survive as latent endophytes and to change to pathogenic behaviour when host plants are under stress conditions. Many fungi belonging to *Botryosphaeriaceae* may cause severe diseases of woody plants in natural and urban areas, nurseries and in agricultural crops (Slippers and Wingfield, 2007; Linaldeddu *et al.*, 2016a; Mehl *et al.*, 2017; Zlatković *et al.*, 2017; Aiello *et al.*, 2020; Guarnaccia *et al.*, 2016, 2023). Some of these fungi can also be found on important agricultural non-woody crops.

The global spread of these fungi occurs through the international movement of plants and derivatives without appropriate quarantine systems, while short-distance spread is mainly due to spores carried by rain, wind and, less so, via insects (Van Niekerk *et al.*, 2006; Moyo *et al.*, 2014; Valencia *et al.*, 2015; Panzavolta *et al.*, 2018; Pinna *et al.*, 2019). Diseases caused by *Botryosphaeriaceae* can be mono- or oligo-cyclic (undergoing two to three infection cycles per season), and epidemic events may occur for subsequent years, resulting in high economic losses. These fungi can also spread from nurseries to open fields as latent infections (Moral *et al.*, 2019).

Prior to the mid-1990s, most *Botryosphaeriaceae* species were identified based on micro- and macro-morphological characters. In the recent years, research on *Botryosphaeriaceae* diseases has extended on many crops and required increasingly efficient identification tools, especially due increased recognition and awareness that these fungi are important wood and fruit rot pathogens. Advances in molecular DNA molecular methods have provided reliable tools to discriminate cryptic species, accommodate or synonymize some *taxa*, and describe new genera and species. Currently, the family includes 22 genera and 281 species and some putative hybrids such as those found in *Lasiodiplodia* (Crous *et al.*, 2006; Liu *et al.*, 2012; Phillips *et al.*, 2013; Dissanayake *et al.*, 2016a; Linaldeddu *et al.*, 2016a; Slippers *et al.*, 2017; Zhang *et al.*, 2021).

Climate is considered a major factor affecting the geographical distribution of *Botryosphaeriaceae* species. Some have a limited distribution, whereas a few species such as *Botryosphaeria dothidea*, *D. sapinea*, *D. seriata*, *Dothiorella sarmentorum*, *L. theobromae* and *Neofu-*

*sicoccum parvum*, are distributed much more widely (Batista *et al.*, 2021). It is possible to predict the occurrence of *Botryosphaeriaceae* species in space and time, and to evaluate the potential for their spread over time, using Species Distribution Models (SDMs) (Batista *et al.*, 2023). As a consequence of global warming and climate change some species could shift their ecological ranges, such as *B. dothidea* that could spread in the northern hemisphere, or *N. parvum* for which a change in its latitudinal range is expected. Otherwise, for species such as *L. theobromae*, future scenarios predict diffusion within tropical and sub-tropical regions (Batista *et al.*, 2023). In Italy, *D. sapinea* is mostly widespread in central and southern areas on pine forests. However, the future climate scenario foresees a 9 to 40% increase in its infection habitat, mainly in response rises in mean temperature of the wettest and driest areas (Bosso *et al.*, 2017).

Differences in host susceptibility, pathogen virulence and environmental factors have significant effects on disease development caused by *Botryosphaeriaceae* (Zwolinski *et al.*, 1990; Swart and Wingfield, 1991; Johnson *et al.*, 1997). Stress factors such as occasional climatic events can affect host susceptibility and pathogen behaviour, increasing the risk of infections. Drought or heat stress can negatively impact plant physiology, enhancing pathogen colonization and increasing host susceptibility (Batista *et al.*, 2021). Swart and Wingfield, (1991) reported that water stress, pruning and hail injury could promote *D. sapinea* infections on *Pinus* species. However, drought affects the disease only in aggressive pathogen strains (Blodgett and Stanosz, 1997). Pathogens such as *B. dothidea* (Ma *et al.*, 2001) and *D. mutila* (Ragazzi *et al.*, 1999) have been reported to infect water stressed hosts. These observations were also confirmed for other *Botryosphaeriaceae* pathogens, such as *N. australe*, *N. parvum*, *L. theobromae* and *D. seriata* (Van Niekerk *et al.*, 2011a, 2011b).

As for many pathogens, *Botryosphaeriaceae* use virulence factors to overcome plant defences and facilitate adhesion to hosts, as well as to facilitate colonization in the initial stage of infections (Sacristán and García-Arenal, 2008; Tan and Liang, 2013). Next-generation sequencing techniques have demonstrated that different gene classes are involved in *Botryosphaeriaceae* pathogenesis, toxins and other secondary metabolites are known to have phytotoxic effect on plants, and wood degradation enzymes may cause some disease symptoms (Belair *et al.*, 2022). Grapevine foliar symptoms caused by fungal wood pathogens are usually associated with phytotoxic metabolites produced during wood colonization (Masi *et al.*, 2018a, 2018b). Some metabolites have activity against many fungal pathogens, such as com-

pounds produced by *D. corticola* and *D. subglobosa* that could offer numerous benefits in multiple biotechnology sectors (Cimmino *et al.*, 2016; Masi *et al.*, 2022), and occurrence of typical chloro-necrotic foliar symptoms on grapevine have been associated with infections by *Botryosphaeriaceae* species (Dubos *et al.*, 2001; Abou-Mansour *et al.*, 2015).

In Italy, the increasing number of reports of *Botryosphaeriaceae* infections in different agricultural crops and urban and natural ecosystems have shown the importance of this group of fungi, which are now recognized as limiting factors for plant production. The aim of the present review is to analyze recent advances in knowledge of *Botryosphaeriaceae* that cause wood infections, decline and/or fruit damage in Italy, with particular emphasis on new host-pathogen interactions, geographic distribution, disease symptoms, production of metabolites and aspects related to accurate pathogen identification.

#### SPECIES AND GEOGRAPHIC DISTRIBUTION OF BOTRYOSPHAERIACEAE IN ITALY

Although severe diseases caused by *Botryosphaeriaceae* have been well-known for a long time in Italy, these pathosystems have only been investigated in detail during the last 20 years.

Based on available reports that include sequence data, ten genera and 57 species of *Botryosphaeriaceae* have been reported in Italy, with 271 host-pathogen associations in the agricultural, horticulture and forestry (Table 1). The distributions of pathogen species and genera among Italian regions is irregular, with most reports from intensely cultivated areas and Mediterranean forest ecosystems dominated by native shrubs and trees (Table 1). The distribution of pathogen species is not associated to a phylogeographic patterns, although some fungal genera are more common in mountain areas (e.g. *Dothiorella* and *Neofusicoccum*) and others (*Lasiodiplodia*) in the warmest areas of southern Italy. In particular, Sardinia (26 species) and Sicily (20 species) are the regions with the greatest numbers of reported species (Table 1). The few records from some regions probably reflects the limited sampling efforts, distributions of plant hosts, and levels of susceptibility.

Some polyphagous species, such as *B. dothidea*, *D. seriata* and *N. parvum*, have large/wide geographic distributions. On the other hand, other species that infect only a few host plants are restricted to small geographic areas, and these pathogens include *B. auasmontanum*, *D. insularis* and *Sardiniella urbana* (Table 1).

Overall, 45 plant host families, 94 genera and 130 species of native and exotic plants have been recorded as susceptible to *Botryosphaeriaceae* species in Italy (Table 1). Commonly affected host genera include *Fraxinus*, *Olea*, *Quercus*, and *Vitis*. On *Quercus* and *Fraxinus* spp., *D. corticola* and *D. fraxini* are reported as the main pathogens involved in the complex aetiology of, respectively, oak and ash decline (Linaldeddu *et al.*, 2014, 2020b). In addition to these two key species, many other species of *Botryosphaeriaceae*, such as *B. dothidea*, *D. seriata*, *D. subglobosa*, *Do. iberica* and *N. parvum*, have been isolated from symptomatic oak and ash tree tissues (Moricca *et al.*, 2012; Linaldeddu *et al.*, 2014; Moricca *et al.*, 2016; Linaldeddu *et al.*, 2020b).

Besides damaging natural ecosystems, *Botryosphaeriaceae* are important pathogens of many traditional and emerging agricultural crops, such as avocado, fig, grapevine, hazelnut, lemon, loquat, mango, olive, orange, pistachio, pomegranate and walnut (Lazzizzera *et al.*, 2008a; Ismail *et al.*, 2013; Carlucci *et al.*, 2015; Linaldeddu *et al.*, 2015a, 2016b, 2020a; Giambra *et al.*, 2016; Aloï *et al.*, 2021; Aiello *et al.*, 2020, 2022; Gusella *et al.*, 2021, 2022, 2023a). Several aggressive species are involved in these pathosystems, including *B. dothidea*, *D. olivarum*, *L. mediterranea* and *N. parvum*. Grapevine and olive are susceptible to a large number of *Botryosphaeriaceae* species responsible for diverse symptoms, including cankers, dieback and fruit rots. Out of 46 species of *Botryosphaeriaceae* reported on grapevines worldwide, 19 have been reported in Italy (Table 1), whereas *Macrophomina phaseolina* is the main causal agent reported on herbaceous plants (Faedda *et al.*, 2016).

#### IMPACTS OF DISEASES CAUSED BY BOTRYOSPHAERIACEAE SPECIES

This section outlines reports of host ranges and symptoms caused by *Botryosphaeriaceae* on ecologically and economically important forestry and agricultural plants.

Very few species of *Botryosphaeriaceae* are host specific. Most are polyphagous and can potentially cause infections on a broad range of crops under particular conditions (neutral host behaviour). *Botryosphaeriaceae* can also infect native hosts, and then move to other introduced hosts in the same region (Pavlic *et al.*, 2007; Luo *et al.*, 2022). *Botryosphaeriaceae* are responsible for cankers on host trunks, branches and twigs, dieback and shoot blight, bark cracking, wood discolouration, stem-end rots and fruit rots (Carlucci *et al.*, 2015; Aiello *et*

**Table 1.** Species of *Botryosphaeriaceae* and their hosts reported in Italy.

Species	Host	Symptoms	Region	Reference
<i>Botryosphaeria dothidea</i>	<i>Acer pseudoplatanus</i>	Cankers, branch	Apulia, Basilicata, Campania,	Aiello <i>et al.</i> , 2022
	<i>Ailanthus altissima</i>	dieback, wood	Emilia Romagna, Friuli Venezia	Bertetti <i>et al.</i> , 2013
	<i>Artemisia</i> sp.	necrosis, shoot	Giulia, Lazio, Lombardy,	Carlucci <i>et al.</i> , 2013, 2015
	<i>Carpinus betulus</i>	blight, fruit rot	Molise, Piedmont, Sicily,	Dardani <i>et al.</i> , 2023
	<i>Clinopodium nepeta</i>		Sardinia, Tuscany, Veneto	De Corato <i>et al.</i> , 2007
	<i>Colutea arborescens</i>			Dell'Olmo <i>et al.</i> , 2023
	<i>Colutea cilicica</i>			Dissanayake <i>et al.</i> , 2017
	<i>Cornus sanguinea</i>			Fiorenza <i>et al.</i> , 2022, 2023
	<i>Cydonia oblonga</i>			Garibaldi <i>et al.</i> , 2012
	<i>Euonymus europaeus</i>			Grasso and Granata, 2010
	<i>Ficus microcarpa</i>			Gusella <i>et al.</i> , 2021, 2022
	<i>Fraxinus excelsior</i>			Lazzizzera <i>et al.</i> , 2008b
	<i>F. ornus</i>			Li <i>et al.</i> , 2020
	<i>Galium</i> sp.			Linaldeddu <i>et al.</i> , 2009, 2014,
	<i>Juglans regia</i>			2015a, 2020a, 2020b
	<i>Laburnum</i> sp.			Marinelli <i>et al.</i> , 2012
	<i>Malus domestica</i>			Martino <i>et al.</i> , 2023
	<i>Mangifera indica</i>			Moricca <i>et al.</i> , 2008
	<i>Micromeria graeca</i>			Piskur <i>et al.</i> , 2011
	<i>Olea europaea</i>			Raimondo <i>et al.</i> , 2019
	<i>Ostrya carpinifolia</i>			Scala <i>et al.</i> , 2019
	<i>Persea americana</i>			Schlegel <i>et al.</i> , 2018
	<i>Phaseolus vulgaris</i>			Spagnolo <i>et al.</i> , 2011
	<i>Pistacia vera</i>			Turco <i>et al.</i> , 2006
	<i>Populus tremula</i>			Wijesinghe <i>et al.</i> , 2021
	<i>Prunus armeniaca</i>			Zimowska <i>et al.</i> , 2020
	<i>Pseudotsuga menziesii</i>			
	<i>Punica granatum</i>			
	<i>Pyrus communis</i>			
	<i>Quercus ilex</i>			
	<i>Quercus robur</i>			
	<i>Quercus rubra</i>			
	<i>Quercus suber</i>			
<i>Sambucus ebulus</i>				
<i>Sambucus nigra</i>				
<i>Salix</i> sp.				
<i>Torilis arvensis</i>				
<i>Urtica dioica</i>				
<i>Vitis vinifera</i>				
syn. <i>B. auasmontanum</i>	<i>Alnus cordata</i>	Shoot blight,	Emilia Romagna	Dissanayake <i>et al.</i> , 2017
	<i>Rosa canina</i>	cankers and dieback		
<i>Diplodia africana</i>	<i>Grevillea robusta</i>	Cankers, dieback	Campania, Sardinia, Sicily	Cristinzio <i>et al.</i> , 2015
	<i>Juniperus oxycedrus</i>			Giambra <i>et al.</i> , 2019
	<i>Juniperus phoenicea</i>			Linaldeddu <i>et al.</i> , 2011a, 2015a
	<i>Pinus nigra</i>			Luchi <i>et al.</i> , 2014
	<i>Pinus pinea</i>			Seddaiu <i>et al.</i> , 2019
	<i>Quercus ilex</i>			
<i>D. corticola</i>	<i>Vitis vinifera</i>			
	<i>Quercus coccifera</i>	Sunken and	Apulia, Molise, Sardinia, Sicily,	Carlucci <i>et al.</i> , 2015
	<i>Quercus ilex</i>	bleeding cankers,	Tuscany	Carlucci and Frisullo, 2009
	<i>Quercus pubescens</i>	dieback, wood		Linaldeddu <i>et al.</i> , 2011b, 2013,
<i>D. crataegicola</i>	<i>Quercus suber</i>	necrosis, V-shaped		2014
	<i>Vitis vinifera</i>	necrotic sectors		Raimondo <i>et al.</i> , 2019
	<i>Crataegus</i> sp.	Cankers and branch	Emilia Romagna	Ariyawansa <i>et al.</i> , 2015
	<i>Prunus</i> sp.	dieback		Dissanayake <i>et al.</i> , 2017
	<i>Tilia</i> sp.			

(Continued)

Table 1. (Continued).

Species	Host	Symptoms	Region	Reference
<i>D. cupressi</i>	<i>Cupressus sempervirens</i> * <i>Pinus nigra</i>	Cankers, dieback, V-shaped necrotic sectors, shoot blight	Abruzzo, Calabria, Sardinia*	Luchi <i>et al.</i> , 2014 This study*
<i>D. fraxini</i>	<i>Fraxinus angustifolia</i> <i>F. excelsior</i>	Cankers, bark discoloration, dieback, V-shaped necrotic sectors	Veneto, Friuli Venezia Giulia	Alves <i>et al.</i> , 2014 Linaldeddu <i>et al.</i> , 2020b
<i>D. pseudoseriata</i> syn. <i>D. insularis</i>	<i>Fraxinus angustifolia</i> <i>Pistacia lentiscus</i>	- Leaf chlorosis, crown thinning, V -shaped necrotic sectors branch dieback, sunken cankers	Sardinia Sardinia	Alves <i>et al.</i> , 2014 Linaldeddu <i>et al.</i> , 2016c
syn. <i>D. alatafructa</i>	<i>Picea abies</i>	Cone necrosis	Emilia Romagna	Dissanayake <i>et al.</i> , 2017
<i>D. malorum</i>	<i>Populus alba</i>	-	Sardinia	Alves <i>et al.</i> , 2014
<i>D. mutila</i>	<i>Acer negundo</i> <i>Colutea arborescens</i> <i>Fraxinus excelsior</i> <i>Olea europaea</i> <i>Phaseolus vulgare</i> <i>Populus tremula</i> <i>Vitis vinifera</i>	Cankers, wood necrosis	Apulia, Campania, Emilia Romagna, Friuli Venezia Giulia, Molise, Piedmont, Sardinia, Veneto	Carlucci <i>et al.</i> , 2013, 2015 Alves <i>et al.</i> , 2014 Dardani <i>et al.</i> , 2023 Dissanayake <i>et al.</i> , 2017 Linaldeddu <i>et al.</i> , 2015a, 2020b Liu <i>et al.</i> , 2015 Raimondo <i>et al.</i> , 2019 Dell'Olmo <i>et al.</i> , 2023
<i>D. olivarum</i>	<i>Ceratonia siliqua</i> <i>Olea oleaster</i> <i>Olea europaea</i> <i>Pistacia lentiscus</i> <i>Vitis vinifera</i>	Leaf chlorosis, crown thinning, branch dieback, sunken and bleeding cankers, fruit rot	Sardinia, Sicily, Apulia	Alves <i>et al.</i> , 2014 Granata <i>et al.</i> , 2011 Lazzizzera <i>et al.</i> , 2008a Linaldeddu <i>et al.</i> , 2015a, 2016c Manca <i>et al.</i> , 2020
<i>D. scrobiculata</i>	<i>Arbutus unedo</i> <i>Olea europaea</i> <i>Pinus radiata</i>	Cankers and branch dieback	Apulia, Sardinia	Lazzizzera <i>et al.</i> , 2008a Linaldeddu <i>et al.</i> , 2006a, 2010 Zhang <i>et al.</i> , 2020
<i>D. seriata</i>	<i>Cornus sanguinea</i> <i>Corylus avellana</i> <i>Cupressus sempervirens</i> <i>Eriobotrya japonica</i> <i>Euonymus europaeus</i> <i>Fraxinus angustifolia</i> <i>F. excelsior</i> <i>Galium</i> sp. <i>Grevillea robusta</i> <i>Magnolia grandiflora</i> <i>Malus domestica</i> <i>Olea europaea</i> <i>Pinus nigra</i> <i>Pinus sylvestris</i> <i>Populus nigra</i> <i>Quercus pubescens</i> <i>Prunus laurocerasus</i> <i>Quercus ilex</i> <i>Quercus suber</i> <i>Rosa canina</i> <i>Sambucus nigra</i> <i>Ulmus minor</i> <i>Vitis vinifera</i>	Cankers, branch dieback, wood necrosis, shoot blight, leaf necrosis, fruit rot	Apulia, Emilia Romagna, Friuli Venezia Giulia, Molise, Piedmont, Sardinia, Sicily, Tuscany, Umbria, Veneto	Alves <i>et al.</i> , 2014 Ariyawansa <i>et al.</i> , 2015 Carlucci <i>et al.</i> , 2013, 2015 Dardani <i>et al.</i> , 2023 Dissanayake <i>et al.</i> , 2017 Giambra <i>et al.</i> , 2016, 2019 Lazzizzera <i>et al.</i> , 2008a Linaldeddu <i>et al.</i> , 2006b, 2013, 2014, 2015a, 2016c, 2020b Lorenzini and Zapparoli, 2018 Luchi <i>et al.</i> , 2014 Martino <i>et al.</i> , 2023 Mondello <i>et al.</i> , 2013 Quaglia <i>et al.</i> , 2014 Raimondo <i>et al.</i> , 2019 Spagnolo <i>et al.</i> , 2011 Wijayawardene <i>et al.</i> , 2016

(Continued)

Table 1. (Continued).

Species	Host	Symptoms	Region	Reference
<i>D. sapinea</i>	<i>Cedrus deodara</i> <i>Corylus avellana</i> <i>Cupressus sempervirens</i> <i>Olea europaea</i> <i>Picea abies</i> <i>Pinus halepensis</i> <i>Pinus nigra</i> <i>Pinus pinaster</i> <i>Pinus pinea</i> <i>Pinus radiata</i> <i>Pinus sylvestris</i>	Cankers, branch dieback, Cone necrosis, needle and shoot pine blight	Apulia, Basilicata, Calabria, Campania, Emilia Romagna, Friuli Venezia Giulia, Lazio, Lombardy, Marche, Molise, Piedmont, Sicily, Sardinia, Tuscany, Trentino Alto Adige, Umbria, Veneto	Cabras <i>et al.</i> , 2006 Maresi <i>et al.</i> , 2007 Dissanayake <i>et al.</i> , 2017 Lazzizzera <i>et al.</i> , 2008a Linaldeddu <i>et al.</i> , 2016b Luchi <i>et al.</i> , 2014
syn. <i>D. rosacearum</i>	<i>Eriobotrya japonica</i>	Cankers	Sicily	Giambra <i>et al.</i> , 2016
syn. <i>D. italica</i>	<i>Crataegus</i> sp.	Canker, branch dieback	Tuscany	Wijayawardene <i>et al.</i> , 2016 Wijesinghe <i>et al.</i> , 2021
<i>D. subglobosa</i>	<i>Fraxinus excelsior</i> <i>F. ornus</i>	Cankers, bark discoloration, dieback, V-shaped necrotic sectors	Veneto, Friuli Venezia Giulia, Sicily	Alves <i>et al.</i> , 2014 Linaldeddu <i>et al.</i> , 2020b
<i>Dothiorella eriobotryae</i>	<i>Rhamnus alaternus</i> <i>Tamarix gallica</i>	Bleeding cankers, dieback	Emilia Romagna	Dissanayake <i>et al.</i> , 2017
<i>Do. franceschini</i>	<i>Rhamnus alaternus</i>	Bleeding cankers, dieback	Sardinia	Senanayake <i>et al.</i> , 2023
<i>Do. guttulata</i>	<i>Alnus</i> sp.	-		Tian <i>et al.</i> , 2018
<i>Do. iberica</i>	<i>Acer opalus</i> <i>Corylus avellana</i> <i>Pinus nigra</i> <i>Quercus cerris</i> <i>Q. suber</i> <i>Rosa canina</i> <i>Vitis vinifera</i>	Cankers and branch dieback	Apulia, Emilia Romagna, Sardinia, Tuscany, Umbria	Carlucci <i>et al.</i> , 2015 Dissanayake <i>et al.</i> , 2016b Linaldeddu <i>et al.</i> , 2011b, 2016c Luchi <i>et al.</i> , 2014 Pavlic-Zupanc <i>et al.</i> , 2015 Phillips <i>et al.</i> , 2005 Wijayawardene <i>et al.</i> , 2016
<i>Do. iranica</i>	<i>Paliurus</i> sp.	-	Emilia Romagna	Dissanayake <i>et al.</i> , 2016b
<i>Do. omnivora</i>	<i>Cornus sanguinea</i> <i>Corylus avellane</i> <i>Fraxinus excelsior</i>	Cankers, branch dieback	Emilia Romagna, Friuli Venezia Giulia, Veneto	Dissanayake <i>et al.</i> , 2017 Linaldeddu <i>et al.</i> , 2016b, 2020b
<i>Do. parva</i>	<i>Corylus avellana</i> <i>Ostrya carpinifolia</i>	Cankers and branch dieback	Friuli Venezia Giulia, Sardinia, Veneto	Linaldeddu <i>et al.</i> , 2016b, 2020b Pavlic-Zupanc <i>et al.</i> , 2015 Scala <i>et al.</i> , 2019
<i>Do. sarmentorum</i>	<i>Clematis vitalba</i> <i>Coronilla emerus</i> <i>Crataegus</i> sp. <i>Hippocrepis emerus</i> <i>Paliurus spina-christi</i> , <i>Prunus dulcis</i> <i>Olea oleaster</i> <i>Ulmus minor</i> <i>Pinus nigra</i> <i>Robinia pseudoacacia</i> <i>Ulmus minor</i> <i>Vitis vinifera</i>	Cankers, dieback, pine shoot blight	Apulia, Emilia Romagna, Sardinia	Carlucci <i>et al.</i> , 2015 Dissanayake <i>et al.</i> , 2016b, 2017 Luchi <i>et al.</i> , 2014 Manca <i>et al.</i> , 2020
syn. <i>Do. italica</i>	<i>Cupressus</i> sp. <i>Ligustrum</i> sp. <i>Melia azedarach</i> <i>Prunus</i> sp. <i>Rosa canina</i> <i>Rubus</i> sp.	Cankers	Emilia Romagna, Umbria	Dissanayake <i>et al.</i> , 2017

(Continued)

Table 1. (Continued).

Species	Host	Symptoms	Region	Reference
<i>Do. sempervirentis</i>	<i>Cytisus</i> sp. <i>Fraxinus excelsior</i>	Cankers, dieback	Umbria, Veneto	Dissanayake <i>et al.</i> , 2016b, 2017 Linaldeddu <i>et al.</i> , 2020b
<i>Do. symphoricarpicola</i>	<i>Cornus sanguinea</i> <i>Corylus avellane</i> <i>Laburnum alpinum</i> <i>L. anagyroides</i> <i>Laurus nobilis</i> <i>Symphoricarpos</i> sp. <i>Sambucus nigra</i>	Cankers and branch dieback	Emilia Romagna, Sardinia	Dissanayake <i>et al.</i> , 2016b Li <i>et al.</i> , 2014 Linaldeddu <i>et al.</i> , 2016b
<i>Do. vidmadera</i>	<i>Fraxinus ornus</i>	Dead branch	Emilia Romagna	Dissanayake <i>et al.</i> , 2016b
<i>Do. viticola</i>	<i>Citrus</i> sp. <i>Morus</i> sp.	Dieback	Sicily, Emilia Romagna	Bezerra <i>et al.</i> , 2021 Rathnayaka <i>et al.</i> , 2022
<i>Eutiarospora dactylidis</i>	<i>Arrhenatherum elatius</i> <i>Avenella flexuosa</i> <i>Dactylis glomerata</i>	Stem cankers	Emilia Romagna	Dissanayake <i>et al.</i> , 2016b Wijesinghe <i>et al.</i> , 2021
<i>Lasiodiplodia citricola</i>	<i>Acacia dealbata</i> <i>Acacia retinoides</i> <i>Persea americana</i> <i>Vitis vinifera</i>	Cankers, wood necrosis, V-shaped necrotic sectors	Apulia, Molise, Sicily	Carlucci <i>et al.</i> , 2015 Costanzo <i>et al.</i> , 2022 Fiorenza <i>et al.</i> , 2023 Raimondo <i>et al.</i> , 2019
<i>L. hormozganensis</i>	<i>Quercus cerris</i>	-	-	Kee <i>et al.</i> , 2019
<i>L. iraniensis</i>	<i>Vitis vinifera</i>	-	-	Jayawardena <i>et al.</i> , 2018
<i>L. laeliocattleyae</i>	<i>Laeliocattleya</i> sp.	-	-	Custodio <i>et al.</i> , 2018 Dissanayake <i>et al.</i> , 2016b Kee <i>et al.</i> , 2019
<i>L. mediterranea</i>	<i>Quercus ilex</i> <i>Vitis vinifera</i>	Canker with V-shaped necrotic sectors and dieback	Sardinia	Linaldeddu <i>et al.</i> , 2015a
<i>L. theobromae</i>	<i>Mangifera indica</i> <i>Olea europaea</i> <i>Persea americana</i> <i>Rosa canina</i> <i>Vitis vinifera</i>	Cankers, shoot blight, wood necrosis, V-shaped necrotic sectors	Apulia, Emilia Romagna, Molise, Piedmont, Sicily	Aiello <i>et al.</i> , 2022 Bertetti <i>et al.</i> , 2013 Burruano <i>et al.</i> , 2008 Carlucci <i>et al.</i> , 2013, 2015 Mondello <i>et al.</i> , 2013 Raimondo <i>et al.</i> , 2019 Wijayawardene <i>et al.</i> , 2016 Wijesinghe <i>et al.</i> , 2021
<i>Macrophomina phaseolina</i>	<i>Beta vulgaris</i> <i>Cicer arietinum</i> <i>Citrullus</i> sp. <i>Cucumis melo</i> <i>Fragaria × ananassa</i> <i>Glycine max</i> <i>Helianthus annuus</i> <i>Hibiscus</i> sp. <i>Opuntia humifusa</i> <i>Osteospermum</i> sp. <i>Persea americana</i> <i>Phaseolus vulgaris</i> <i>Prunus persica</i> <i>Solanum tuberosum</i>	Dry root rot, collar rot, charcoal rot and soft stem rot, dark brown discoloration	Basilicata, Calabria Campania, Sardinia	Dell'Olmo <i>et al.</i> , 2022 Faedda <i>et al.</i> , 2016 Fiorenza <i>et al.</i> , 2023 Gerin <i>et al.</i> , 2018 Infantino <i>et al.</i> , 2021 Poudel <i>et al.</i> , 2021
<i>Mucoharknessia anthoxanthi</i>	<i>Anthoxanthum odoratum</i> -		Emilia Romagna	Dissanayake <i>et al.</i> , 2016b

(Continued)

Table 1. (Continued).

Species	Host	Symptoms	Region	Reference
<i>Neofusicoccum australe</i>	<i>Eucalyptus camaldulensis</i>	Leaf chlorosis,	Apulia, Sardinia, Sicily	Deidda <i>et al.</i> , 2016
	<i>Mangifera indica</i>	crown thinning,		Ismail <i>et al.</i> , 2013
	<i>Myrtus communis</i>	shoot and branch		Lazzizzera <i>et al.</i> , 2008b
	<i>Olea europaea</i>	dieback, sunken		Linaldeddu <i>et al.</i> , 2010b, 2015a
	<i>Pinus nigra</i>	Cankers, epicormic		Luchi <i>et al.</i> , 2014
	<i>Vitis vinifera</i>	shoots gummosis, V-shaped necrotic sectors, fruit rot		Nicoletti <i>et al.</i> , 2014
<i>N. batangarum</i>	<i>Opuntia ficus-indica</i>	Cankers	Sicily	Aloi <i>et al.</i> , 2020 Masi <i>et al.</i> , 2020b Santagata <i>et al.</i> , 2022
<i>N. buxi</i>	<i>Buxus sempervirens</i>	Leaf spots	Liguria	Cecchi <i>et al.</i> , 2020
<i>N. cordaticola</i>	<i>Vitis vinifera</i>	-	-	Jayawardena <i>et al.</i> , 2018 Sakalidis <i>et al.</i> , 2013
<i>N. cryptoaustrale</i>	<i>Olea europaea</i>	Cankers, dieback,	Sardinia	Fiorenza <i>et al.</i> , 2023
	<i>Pistacia lentiscus</i>	V-shaped necrotic		Linaldeddu <i>et al.</i> , 2015a, 2016c
	<i>Persea americana</i>	sectors		Yang <i>et al.</i> , 2017
	<i>Vitis vinifera</i>			
<i>N. hellenicum</i>	<i>Pistacia vera</i>	Shoot and panicle blight	Sicily	Gusella <i>et al.</i> , 2022
<i>N. luteum</i>	<i>Cinnamomum camphora</i>	Leaf chlorosis,	Apulia, Liguria, Sardinia, Sicily	Carlucci <i>et al.</i> , 2013
	<i>E. camaldulensis</i>	crown thinning,		Deidda <i>et al.</i> , 2016
	<i>Erica arborea</i>	shoot and branch		Fiorenza <i>et al.</i> , 2023
	<i>Olea europaea</i>	and twig dieback,		Gusella <i>et al.</i> , 2023a
	<i>Persea americana</i>	sunken cankers,		Linaldeddu <i>et al.</i> , 2015b, 2016c
	<i>Pinus pinea</i>	epicormic shoots		Luchi <i>et al.</i> , 2014
	<i>Pistacia vera</i> *	gummosis, V		Zhang <i>et al.</i> , 2020
	<i>Pistacia lentiscus</i>	-shaped necrotic		
	<i>Viburnum</i> sp.	sectors, fruit rot		
<i>N. mediterraneum</i>	<i>Arbutus unedo</i> *	Leaf chlorosis,	Apulia, Lazio, Sardinia*, Sicily	Brunetti <i>et al.</i> , 2022
	<i>E. camaldulensis</i>	crown thinning,		Deidda <i>et al.</i> , 2016
	<i>Ficus microcarpa</i>	shoot and branch		Fiorenza <i>et al.</i> , 2022
	<i>Juglans regia</i>	dieback, sunken		Gusella <i>et al.</i> , 2020b, 2022
	<i>Olea europaea</i>	cankers, epicormic		Manetti <i>et al.</i> , 2023
	<i>Pistacia vera</i>	shoots gummosis, V-shaped necrotic sectors		This study*
<i>N. occulatum</i>	<i>Platanus hybrida</i>	-	-	Yang <i>et al.</i> , 2017

(Continued)



Table 1. (Continued).

Species	Host	Symptoms	Region	Reference
<i>N. parvum</i>	<i>Acer pseudoplatanus</i> <i>Acacia melanoxylon</i> <i>Brachychiton</i> spp. <i>Cannabis sativa</i> <i>Castanea sativa</i> <i>Citrus × limon</i> <i>Citrus</i> spp. <i>Cinnamomum camphora</i> <i>Corylus avellana</i> <i>Eriobotrya japonica</i> <i>E. camaldulensis</i> <i>Eupatorium cannabinum</i> <i>Ficus carica</i> <i>Ficus microcarpa</i> <i>Fraxinus excelsior</i> <i>Juglans regia</i> <i>Malus</i> sp. <i>Mangifera indica</i> <i>Meryta denhamii</i> <i>Microcitrus australasica</i> <i>Olea europaea</i> <i>Olea oleaster</i> <i>Persea americana</i> <i>Pinus pinea</i> <i>Punica granatum</i> <i>Rhododendron</i> sp. <i>Quercus ilex</i> <i>Quercus robur</i> <i>Quercus suber</i> <i>Raphiolepis indica</i> <i>Rhododendron</i> sp. <i>Rubus fruticosus</i> <i>Salix</i> sp. <i>Torilis arvensis</i> <i>Ulmus hollandica</i> <i>Vaccinium</i> sp. <i>Vitis vinifera</i>	Cankers, wedge-shaped necrotic sectors, chlorosis, leaf and shoot blight, leaf drop, fruit rot, gummosis, V-shaped necrotic sectors, twig dieback wilting shoots,	Abruzzo, Apulia, Basilicata, Emilia Romagna, Friuli Venezia Giulia, Lazio, Lombardy, Molise, Piedmont, Sardinia, Sicily, Tuscany, Veneto	Aiello <i>et al.</i> , 2020, 2022 Alberti <i>et al.</i> , 2018 Aloi <i>et al.</i> , 2021 Bezerra <i>et al.</i> , 2021 Carlucci <i>et al.</i> , 2013, 2015 Dardani <i>et al.</i> , 2023 Deidda <i>et al.</i> , 2016 Dissanayake <i>et al.</i> , 2017 Faedda <i>et al.</i> , 2018 Fiorenza <i>et al.</i> , 2022 Garibaldi <i>et al.</i> , 2011 Giambra <i>et al.</i> , 2016 Guarnaccia <i>et al.</i> , 2016, 2020a Gusella <i>et al.</i> , 2020a, 2020b, 2021, 2023a, 2023b Ismail <i>et al.</i> , 2013 Linaldeddu <i>et al.</i> , 2007, 2014, 2015a, 2020b Luchi <i>et al.</i> , 2014 Manca <i>et al.</i> , 2020 Mang <i>et al.</i> , 2022 Mondello <i>et al.</i> , 2013 Moricca <i>et al.</i> , 2012 Polizzi <i>et al.</i> , 2023 Raimondo <i>et al.</i> , 2019 Riccioni <i>et al.</i> , 2017 Seddaiu <i>et al.</i> , 2021 Sidoti, 2016 Spagnolo <i>et al.</i> , 2011 Waqas <i>et al.</i> , 2022 Wijesinghe <i>et al.</i> , 2021 Zlatkovic <i>et al.</i> , 2019
syn. <i>N. italicum</i>	<i>Vitis vinifera</i>	-	-	Marin-Felix <i>et al.</i> , 2017
<i>N. stellenboschiana</i>	<i>Olea europaea</i>	Cankers, branch and twig dieback	Apulia	Manetti <i>et al.</i> , 2023
<i>N. vitifusiforme</i>	<i>Eriobotrya japonica</i> <i>E. camaldulensis</i> <i>Mangifera indica</i> <i>Olea europaea</i> <i>Pinus nigra</i> <i>Vitis vinifera</i>	Leaf chlorosis, crown thinning, shoot and branch dieback, sunken cankers, epicormic shoots gummosis, V-shaped necrotic sectors	Sardinia, Sicily	Deidda <i>et al.</i> , 2016 Dissanayake <i>et al.</i> , 2016b Giambra <i>et al.</i> , 2016 Luchi <i>et al.</i> , 2014 Mondello <i>et al.</i> , 2013 Moral <i>et al.</i> , 2010 Zhang <i>et al.</i> , 2020
<i>Neoscytalidium dimidiatum</i>	<i>Citrus sinensis</i> <i>Meryta denhamii</i>	Shoot blight, canker, gummosis, dieback	Sicily	Gusella <i>et al.</i> , 2023b Polizzi <i>et al.</i> , 2009
<i>Sardiniella celtidis</i>	<i>Celtis australis</i>		Emilia Romagna	Hyde <i>et al.</i> , 2017
<i>S. urbana</i>	<i>Celtis australis</i>	Shoot and branch dieback, sunken cankers	Sardinia	Linaldeddu <i>et al.</i> , 2016a

\* New host-pathogen interactions reported in this study.

*al.*, 2020, 2022; Gusella *et al.*, 2020a, 2020b, 2021, 2022; Linaldeddu *et al.*, 2020a; Bezerra *et al.*, 2021; Fiorenza *et al.*, 2022, 2023), and these infections are often caused by multiple pathogen genera that may play different roles in infection processes of host plants.

*Botryosphaeriaceae* have been commonly recorded in agro-ecosystems, and in nurseries, urban landscapes and forest ecosystems including timber plantations. The plant propagation processes in nurseries are crucial for many production sectors (ornamentals, forestry and fruit crops). Fungal latency, in conjunction with intercontinental plant transport without adequate quarantine, can lead pathogen spread, which is why preventive detection and adequate control strategies are always needed to limit the destructive potential of *Botryosphaeriaceae*. As summarized in Figure 1, *Botryosphaeriaceae* inoculum (spores or mycelium) can be present during initial propagation steps in nurseries, which is why symptoms can occur and plant material is discarded before being sale, but the pathogens can also remain latent. From nurseries, infected plant material can be shipped around the world, and symptoms can appear months or years later once the plants are transplanted in the field. For this reason, careful hygiene during propagation and healthy plant material are crucial for avoiding infection establishment in nurseries. Before symptom appearance during propagation steps or in orchards, diagnostic analyses could identify latent *Botryosphaeriaceae* in the plant tissues. Traditional laboratory analyses, such as isolation on growth media, are still valuable for determining frequency of active fungal population within plant tissues. These traditional methods are usually time-consuming compared to molecular diagnostic methods. Real-time PCR has been demonstrated to be an important tool for detecting latent infections and to investigate canker pathogen epidemiology (Luo *et al.*, 2017, 2019, 2020; Romero-Cuadrado *et al.*, 2023). Once *Botryosphaeriaceae* become established in an orchard, transmission of inoculum (mycelium, pycnidia or perithecia overwintering in old cankers, fruit mummies or within the buds) (Michailides, 1991) can occur through human activities (e.g., pruning, irrigation), and animals such as birds and insects (Michailides and Morgan, 2016), and environmental factors such as rain and wind. Riparian vegetation near the orchards can also be important (Figure 1), as many wild species, bushes and forest trees can be important dissemination pathways for *Botryosphaeriaceae* (Ma *et al.*, 2001).

#### Forest ecosystems and timber plantations

Forest ecosystems (natural, seminatural and artificial) cover 36% of the Italian territory, and are impor-

tant for human services and income (Ferrara *et al.*, 2017; Agnoletti *et al.*, 2022). The Italian forest heritage includes a wide variety of ecosystems, spanning from natural Mediterranean evergreen sclerophyllous formations to Norway spruce plantations in the Alps (Gasparini *et al.*, 2022). The health status of these ecosystems is continuously threatened by several native and exotic pathogens, including species of *Botryosphaeriaceae* (Santini *et al.*, 2013; Luchi *et al.*, 2014; Moricca *et al.*, 2016; Linaldeddu *et al.*, 2020b). A meta-analysis of the literature has allowed determination of the occurrence of 37 *Botryosphaeriaceae* species and 129 host-pathogen interactions in natural ecosystems and timber plantations. This analysis showed some distribution patterns partially explained by the host preference of some species: *D. sapinea* for *Pinus* spp., *D. corticola* for *Quercus* spp., *D. cupressi* for *Cupressus* spp. and *S. urbana* for *Celtis australis*. In contrast, many species, especially those that are polyphagous, have irregular geographic distributions (Linaldeddu *et al.*, 2014, 2016a, 2020b; Luchi *et al.*, 2014; Batista *et al.*, 2021).

Different species of *Diplodia* and *Neofusicoccum* are increasing threats to forest ecosystems in Italy (Linaldeddu *et al.*, 2011a, 2014, 2020b; Deidda *et al.*, 2016; Manca *et al.*, 2020). In particular, *D. corticola*, *D. fraxini*, *D. insularis*, *D. scrobiculata*, *D. subglobosa*, *N. australe*, *N. luteum*, *N. mediterraneum* and *N. parvum* are associated with disease symptoms including leaf spot, fruit rot, shoot blight, branch dieback, sunken canker, decline and mortality on different shrubs and forest trees (Figure 2). Since 2010, an unusual decline and mortality of young and mature *Eucalyptus camaldulensis* trees has been observed in several plantations in Sardinia (Deidda *et al.*, 2016). Five species of *Neofusicoccum*, namely *N. australe*, *N. luteum*, *N. mediterraneum*, *N. parvum* and *N. vitifusiforme*, were consistently isolated from trees showing symptoms of leaf chlorosis, shoot and branch dieback, sunken cankers, epicormic shoots and exudations of kino gum. In particular, *N. australe* was the most frequently isolated fungus, and other studies conducted in grapevines, almond and olive orchards adjacent to eucalypt plantations showed that this species was isolate from sunken cankers and fruit rots, demonstrating its invasive potential in the Mediterranean region (Linaldeddu, personal communication).

Among the many symptoms caused by *Botryosphaeriaceae* on woody hosts, wedge-shaped necrotic sectors visible in stem cross section associated with the sunken cankers are frequent and typical of this group of pathogens (Figure 3).

Although several species of *Botryosphaeriaceae* have been described from different forest ecosystems and Ital-

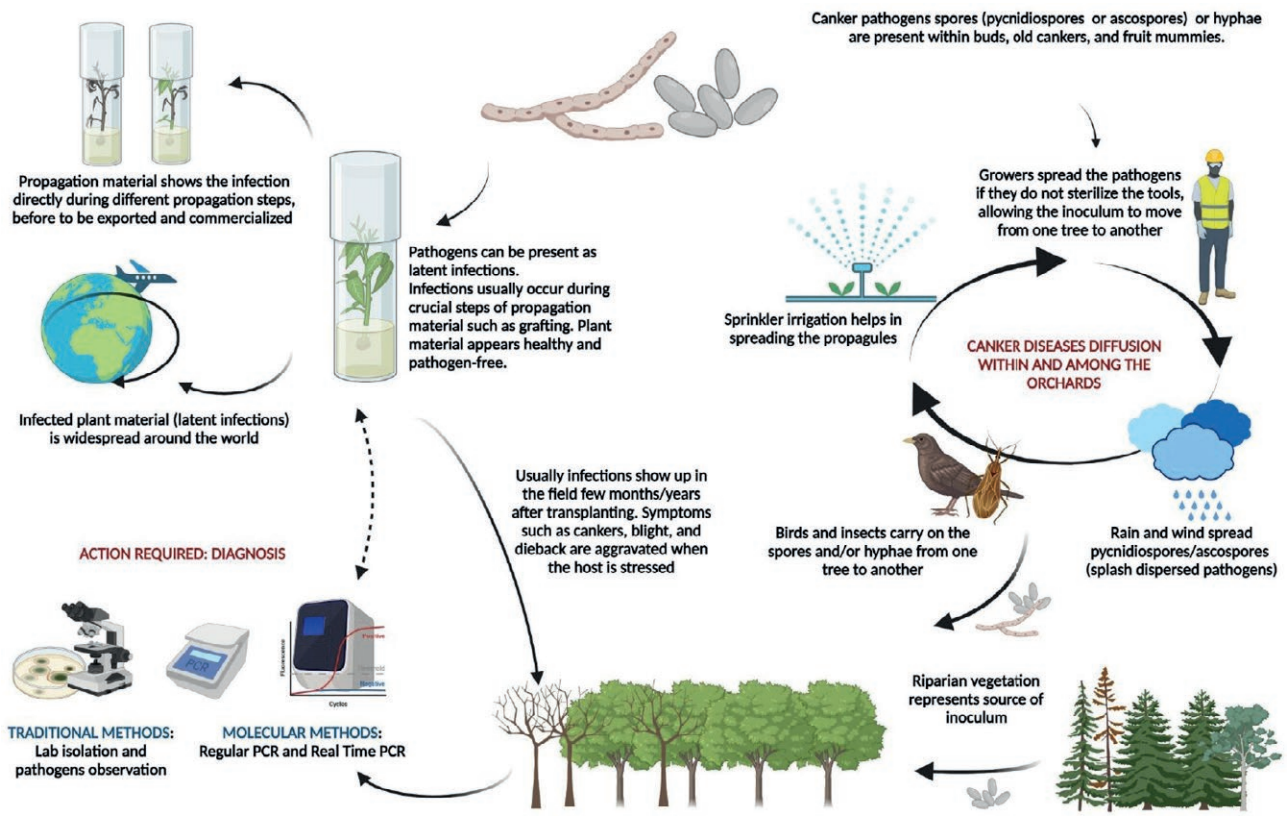


Figure 1. Cycle of *Botryosphaeriaceae* dispersion from the nurseries to the field (created with BioRender).

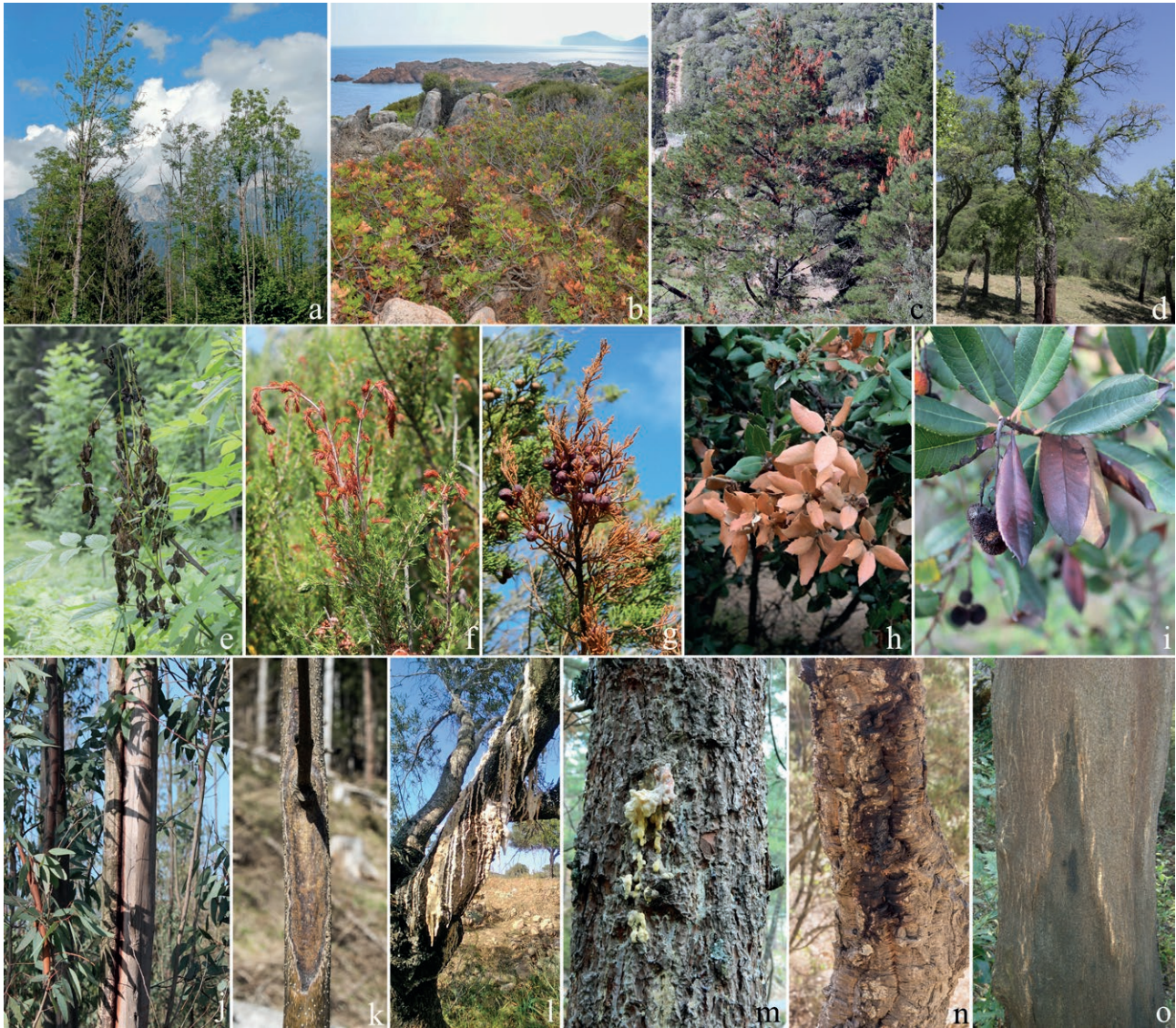
ian regions, the diversity and distribution of this group of fungi has not been widely of the 129 host-pathogen interactions known, only 35 were verified by pathogenicity tests under experimental conditions. Given the small number of sampling efforts in some regions, and current climate changes which may favour the invasiveness of some of these pathogens, further studies should target these invasive fungi to develop the basis for suitable disease management strategies.

*Ornamental and urban plants*

Proliferation of phytopathological cases ascribable to *Botryosphaeriaceae*, especially in nurseries, has occurred where infections start latently and continue once plants are transplanted in open fields (Figure 4). Most of the symptomatic and/or dead plants that have been observed in the field were probably already infected (asymptomatic) in nurseries. *Botryosphaeriaceae* infections occur easily during some propagation steps, including grafting, has been observed with *Acacia* spp. infected by *L. citricola* (Figure 4 h) (Costanzo et al., 2022). Among the *Botryosphaeriaceae* spp., *N. parvum* has been con-

sistently isolated from different hosts. Severe infections causing trunk cankers, massive gummoses and canopy dieback were observed in nurseries of the ornamental *Brachychiton* spp. (Figure 4 e) (Gusella et al., 2021), as well as wood necroses and dieback on ornamental fig (*Ficus carica*) cuttings (Figure 4 f) (Aiello et al., 2020), and on Indian hawthorn (*Raphiolepis indica*) (Figure 4 g) (Gusella et al., 2020a).

As mentioned above, ornamental crops in urban environments are important sources of *Botryosphaeriaceae* inoculum. Urban trees often grow in non-native environments, and *Botryosphaeriaceae*, being endophytes, occupy the endophytic niche left open, normally occupied in native habitats, by horizontally acquired endophytes (Slippers and Wingfield, 2007). Surveys conducted in 2019 and 2020 on *Ficus microcarpa* on several tree-lined streets, squares and public parks in Catania and Siracusa provinces (Sicily, southern Italy) revealed common presence of shoot and branch canker, canopy defoliation, internal wood necroses and dieback (Figure 4, a to d). Multi-locus phylogenetic analyses showed that *B. dothidea*, *N. mediterraneum*, and *N. parvum* were responsible for the tree decline (Fiorenza et al., 2021).



**Figure 2.** Overview of external disease symptoms caused by *Botryosphaeriaceae* on different forest trees and shrubs. (a) progressive canopy dieback caused by *Diplodia fraxini* on *Fraxinus excelsior*; (b) *Diplodia insularis* on *Pistacia lentiscus*; (c) *Diplodia sapinea* on *Pinus radiata*; (d) *Diplodia corticola* on *Quercus suber*; (e) shoot blight caused by *D. fraxini* on *F. excelsior*; (f) *Neofusicoccum luteum* on *Erica arborea*; (g) *Diplodia africana* on *Juniperus phoenicea*; (h) *D. corticola* on *Quercus ilex*; (i) *Neofusicoccum mediterraneum* on *Arbutus unedo*; (j) sunken canker with exudations caused by *Neofusicoccum australe* on *Eucalyptus camaldulensis*; (k) *D. fraxini* on *F. excelsior*; (l) *Neofusicoccum parvum* on *Olea oleaster*; (m) *D. sapinea* on *Pinus radiata*; (n and o) *D. corticola* on *Q. suber*.

Branch cankers and dieback caused by *N. parvum* and *Neoscytalidium dimidiatum* were also observed on *Meryta denhamii* in a historical botanical garden (Gusella *et al.*, 2023b).

#### Tropical crops

In recent years, increased occurrence of symptoms caused by *Botryosphaeriaceae* has been observed

on mango and avocado plants in Sicily (Southern Italy) (Figures 5 to 7). Cultivation of these plants started in the Catania province (eastern Sicily) in 1980. Thereafter, mango and avocado cultivation expanded to the other provinces of Sicily, and to Calabria and Apulia. These tropical crops are alternatives to citrus, and they are economically important in European markets. Botryosphaeriaceae infections may occur pre- and post-harvest, and these compromise plant growth and/or fruit quality



**Figure 3.** Overview of internal disease symptoms caused by *Botryosphaeriaceae* on different forest trees and shrubs: (a) cross section of branches showing the characteristic wedge-shaped necrotic sector caused by *Neofusicoccum mediterraneum* on *Arbutus unedo*; (b) *Neofusicoccum parvum* on *Olea oleaster*; (c) *Diplodia insularis* on *Pistacia lentiscus*; (d) *Dothiorella* sp. on *Rhamnus alaternus*; (e) *Sardiniella urbana* on *Celtis australis*; (f) *Diplodia cupressi* on *Cupressus sempervirens*; (g) *Diplodia seriata* on *Corylus avellana*; (h) *Neofusicoccum australe* on *Eucalyptus camaldulensis*; (i) *Neofusicoccum parvum* on *Fagus sylvatica*; (j) *Diplodia fraxini* on *Fraxinus excelsior*; (k) *Diplodia sapinea* on *Pinus mugo*; (l) *Botryosphaeria dothidea* on *Rhododendron ferrugineum*; (m) *Diplodia corticola* on *Quercus ilex*, (n) *Q. pubescens*, (o) *Q. robur* and (p) *Q. suber*.

leading to substantial yield losses and decreases in market value. After the first report of dieback caused by *N. parvum* on mango (Ismail *et al.*, 2013), surveys carried

out between 2014 and 2019 detected severe symptoms of woody canker, shoot blight, and dieback on different cultivars of young mango plants (Kent, Keitt, Sensation,



**Figure 4.** Disease symptoms caused by *Botryosphaeriaceae* on ornamental plants in urban environments and nurseries: (a and b) diseased plant of *Ficus microcarpa* with defoliation and shoot dieback all over the canopy; (c and d) bark discolored and cracked along the branch and internal tissues showing blackish V-shape lesion of *F. microcarpa*; (e) canker and gummosis of *Brachychiton* sp.; (f) internal discoloration of *Ficus carica* cutting; (g) necrosis at the bottom of the leaves of *Raphiolepis indica*, moving from petioles upward through the mid rib and blade; (h) canker at the graft union on young *Acacia dealbata* plant grafted on *A. retinodes*.

Osteen, and Kensington Pride) in north-eastern Sicily (Figure 5, a to e). Morphological and molecular characters and pathogenicity tests identified the associated pathogens, including *B. dothidea*, *L. theobromae* and *N. parvum* (Aiello *et al.*, 2022). Among these, *N. parvum* was widespread on tropical crops in the area (Guarnaccia *et al.*, 2016; Aiello *et al.*, 2022), and on citrus (Bezerra *et al.*, 2021). This species was also detected causing seedling blight of mango (*Mangifera indica*) in a nursery (Polizzi *et al.*, 2023). Mango fruit symptoms were not reported in Italy, but different authors showed the severe rot symptoms caused by *Botryosphaeriaceae* on the stem ends of fruit when infections commenced from pedicles or from fruit surfaces (Figure 7, d to f) (Ni *et al.*, 2010).

Further and future investigations in Italy will aim to assess the spread of symptoms on mango fruit, which is becoming a crop of increasing relevance.

In 2016, surveys on avocado orchards showed branch canker and fruit stem-end rot, caused by *N. parvum* in association with other *Diaporthaceae* and *Glomerellaceae* (Guarnaccia *et al.*, 2016). The same *Neofusicoccum parvum* was also reported, together with *N. stellenboschiana*, causing branch canker on avocado in Greece (Guarnaccia *et al.*, 2020b). Studies of avocado diseases in Sicily continued, and during 2020/2021, surveys were conducted in Sicily on 11 orchards, to investigate the etiology of branch canker and dieback (Figure 6, a to e). Among these orchards, four showed constant presence



**Figure 5.** Disease symptoms caused by *Botryosphaeriaceae* on field-grown mango plants: (a) shoot dieback; (b and c) external and internal canker; (d) internal necrotic tissue; (e) external canker and bark cracking.

of *Botryosphaeriaceae*. Phylogenetic analyses identified five *Botryosphaeriaceae* species: *B. dothidea*, *L. citricola*, *M. phaseolina*, *N. cryptoaustrale* and *N. luteum*. The symptoms included cankers on shoots and branches, and trunk and shoot dieback. Cankers were usually associated with reddish sap that became white/beige with age. Bark was cracked, darkly discoloured and sometimes slightly sunken. Occasionally, white sugar-like powder was present on the bark surface. Under the bark, canker wood tissues were reddish or light brown to black, and variable in shape. Characteristic wedge-shaped discoloration affecting the xylem was visible in cross sections. Under high disease pressure, wilting of shoots and leaves was also observed (Fiorenza *et al.*, 2023).

On fruit, external symptoms developed as dark brown to black rot sometimes affected the stem ends or most of the fruit epicarps. Internally, the flesh had discoloured vascular streaking (Figure 7, a to c). As the

fruit ripened, the rots progressed and resulted in general discolouration, brown flesh and fruit shriveling. Occasionally, signs of the fungus (mycelium and/or fruiting bodies) were observed on symptomatic tissues.

#### *Fruit trees*

##### Pistachio

In Sicily, investigations of agricultural and ornamental crops showed presence of *Botryosphaeriaceae*-caused diseases on pistachio that had not been previously recorded (Polizzi, personal communication). In 2019 field investigations of pistachio orchards showed the presence of *Botryosphaeriaceae* pathogens on these plants. Pistachio (*Pistacia vera*) is historically important for the Sicily economy (Barone and Marra, 2004), and is



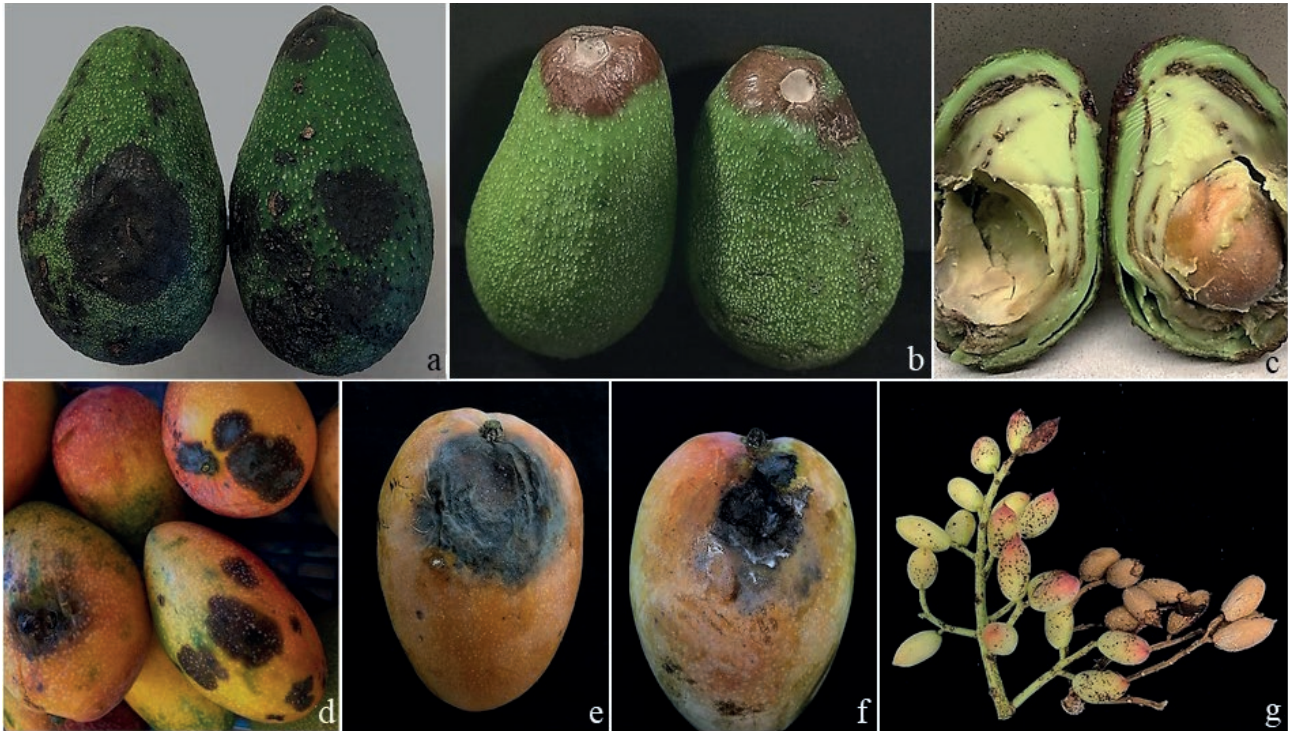
**Figure 6.** Disease symptoms caused by *Botryosphaeriaceae* on field-grown avocado plants: (a) severe shoot dieback in the canopy; (b) branch dieback; (c) infection starting from pruned wound; (d and e) external canker and discoloured internal tissues.

traditionally linked to the territory of Bronte (Catania province) where natural plantings of pistachio are present. These orchards are defined as “natural pistachio plantings” obtained by grafting *in situ* spontaneous terebinth plants (*Pistacia terebinthus*) that grow widespread in the volcanic mountain soils of the area (Barone *et al.*, 1985). More recent pistachio orchards in Agrigento and Caltanissetta provinces, named “new” orchards characterized by rational design, scheduled irrigation and fertilization, and mechanical harvest, are increasing in the territory (Marino and Marra, 2019).

In these new orchards, symptomatic fruit panicles, leaves and shoots were observed during summer of 2019. Fruit showed black rounded spots on the epicarps enlarging with time (also including rachis black discoloration) (Figure 7 g). Leaves were necrotized and shoots showed dieback, wood discolouration (i.e., necrotic, and sunken lesions on lignified tissues), and external cankers (Gusella *et al.*, 2022). Morphological and phylogenetic

analyses showed the presence of *B. dothidea*, *N. hellicum* and *N. mediterraneum* causing *Botryosphaeria* panicle and shoot blight in Italy, with *N. mediterraneum* the most prevalent species affecting pistachio in Sicily (Gusella *et al.*, 2022). In countries where pistachio cultivation is intensive (e.g. the United States of America), this disease has been well-known since the early 1990’s, when the causal agent was identified as *B. dothidea* (Michailides, 1991). Later, with progress in multi-locus phylogenetic analyses, more than one pathogenic species was shown to be involved, so the condition is better defined as a disease complex (Moral *et al.*, 2010; Chen *et al.*, 2014). In Italy, before the investigations of 2019, a report of ‘*Botryosphaeria*’ dieback on pistachio in 1938 attributed the disease to *Botryodiplodia pistaciae* (Cristinzio, 1938). In Sicilian nut crops, *B. dothidea*, *N. mediterraneum* and *N. parvum* were also reported on English walnut (*Juglans regia*), causing shoot and trunk canker, shoot blight, and necroses (Gusella *et al.*, 2020b).





**Figure 7.** Disease symptoms caused by *Botryosphaeriaceae* on fruit: (a and b) fruit rot affected most of the epicarps and the stem-ends of avocado fruit; (c) discoloured vascular streaking of avocado flesh; (d) rot of mango fruit; (e and f) stem-end rot of mango fruit; (g) fruit spot and panicle blight of pistachio.

## Grapevine

Investigations of grapevine wood diseases in Italy during the last 15 years have shown conspicuous presence of *Botryosphaeriaceae* species as causal agents of severe diseases. To date, 16 species in *Botryosphaeriaceae* have been reported and described as important vineyard pathogens. The main reports assessed presence of these fungi in Apulia, Sardinia and Sicily, the greatest Italian production regions for wine and table grapes. The grapevine symptoms caused by *Botryosphaeriaceae* species infections are of two kinds; external and internal symptoms. External symptoms include leaf wilting, fruit rots, bud necroses and perennial cankers, cordon dieback, and plant decline (Figure 8 a). Internal symptoms include wedge-shaped necroses in stem cross sections, and brown streaking below the bark in longitudinal sections (Figure 8, b and c). The first Italian tentative associations of bark cankers, dieback, and leaf chloroses with *Botryosphaeriaceae* species were reported by Cristinzio (1979), and Rovesti and Montermini (1987), who associated *D. seriata* with grapevine dieback. Burrano *et al.* (2008) and Carlucci *et al.* (2009) provided the first reports of cankers of grapevines caused by a

*Botryosphaeriaceae* species in, respectively, Sicily and in Apulia regions. In Sicily, *Lasiodiplodia theobromae* was, for the first time, considered responsible for wood cankers and mild leaf chloroses, while in Apulia, nine species (*B. dothidea*, *D. corticola*, *D. mutila*, *D. seriata*, *Do. iberica*, *Do. sarmentorum*, *N. luteum*, *N. parvum* and *L. theobromae*) were found to be responsible for grapevine dieback, often in association with Esca complex symptoms. In particular, *Do. sarmentorum* and *D. corticola* were isolated for the first time from wood streaking on grapevines by Carlucci *et al.* (2009) and Carlucci and Frisullo, (2009). Spagnolo *et al.*, 2011 isolated *N. parvum*, *D. dothidea* and *D. seriata* in Tuscany, while Mondello *et al.* (2013) reported *N. vitifusiforme* for the first time, on grapevines in western and central Sicily, and described decline symptoms similar to those observed by Burrano *et al.* (2008). Linaldeddu *et al.* (2015 a) isolated, and associated *D. africana* and *D. olivarum* with grapevines, for the first time in Sardinia and elsewhere in the world. These authors also isolated and described *L. exigua* and *L. mediterranea* as new from grapevines. Carlucci *et al.* (2015) first described the presence of *L. citricola* associated with grapevine wood cankers and dieback. That paper described symptoms on host samples collected

during 2012/2013 surveys, and the symptoms detected during pathogenicity tests carried out on green shoots and 1-year-old canes of two cultivars ('Lambrusco' and 'Sangiovese') with the nine species of *Botryosphaeriaceae* listed above. The study demonstrated that different species produced different severities, and that all the species caused wood discolourations, confirming the fungi as primary causes of dieback and decline of vineyards in southern Italy. Although *Botryosphaeriaceae* fungi have been demonstrated to be severe pathogens for grapevines, they have always been isolated together with other fungal pathogens known to be responsible for grapevine trunk diseases (GTDs), such as those associated with the Esca disease complex (Carlucci *et al.*, 2015; Raimondo *et al.*, 2019) and black foot (Carlucci *et al.*, 2017).

### Olive

The presence of *Botryosphaeriaceae* fungi associated with decline of olives in Italy is not well defined, although several studies on olive diseases have reported and described the involvement of some of these fungi. Lazzizzera *et al.*, (2008a, 2008b), during surveys carried out in southern Italy (Apulia and Basilicata regions), isolated many fungi belonging to *Botryosphaeriaceae*, from rotted olive drupes. These fungi included the new species *D. olivarum*. They also isolated, from rotted drupes, *D. seriata*, *D. pinea*, *D. scrobiculata*, *B. dothidea*, *N. australe*, *N. vitifusiforme*, *N. mediterraneum* and *N. parvum*. They reported that the most aggressive pathogens on olive drupes, among those tested, were *N. australe*, *N. vitifusiforme* and *D. olivarum*. Carlucci *et al.* (2013) showed that some of the above-mentioned fungi caused severe symptoms in olive wood tissues, and were responsible for reduced olive yields in Apulia region. These studies associated *B. dothidea*, *D. mutila*, *D. seriata*, *L. theobromae*, *N. luteum* and *N. parvum* with severe damage to wood tissues, although other fungal pathogens, including *Phaeoacremonium* spp. and *Pleurostoma richardsiae* were also severe olive pathogens. Carlucci *et al.* (2020) showed that the olive quick decline syndrome (OQDS) that occurs in southern Italy (Lecce province, Apulia region) is due mainly to *Xylella fastidiosa*, but has also been associated with several lignicolous fungi including *Botryosphaeriaceae*, such as *B. dothidea*, *D. seriata*, *N. luteum*, *N. parvum* and *N. mediterraneum*. In particular, *N. mediterraneum* was reported by Brunetti *et al.* (2022) as one of the most aggressive fungal pathogens involved in OQDS, and caused olive twig dieback in Apulia region. Their data, supported by pathogenicity tests, agree with earlier studies (Carlucci *et al.*, 2020). The symptoms observed on olive trees consisted of wood

discolourations in stem cross and longitudinal sections, cankers, dieback and general decline, often associated with leaf yellowing, wilting and/or leaf scorch (Figure 8, d to g) (Carlucci, personal communication).

### Citrus

Citrus cultivation is globally important. In Europe, Greece, Italy, Portugal, and Spain are important citrus producers (FAOSTAT, 2019). Several abiotic and biotic factors are involved in rot and gummosis of citrus trunks and primary branches. Frost damage, sun scald, or irregular water distribution affect infection by *Ascomycetes* and *Basidiomycetes* (Timmer *et al.*, 2000). Several trunk pathogens are known to cause diseases of citrus in Europe (Guarnaccia and Crous, 2017; Sandoval *et al.*, 2018; Leonardi *et al.*, 2023), and focus has been given to *Botryosphaeriaceae*. Several species of *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum*, and *Neoscytalidium* have been documented as affecting citrus hosts (Figure 8 h). For example, *Ne. dimidiatum* has been identified as the cause of citrus branch canker in California (Mayorquin *et al.*, 2016) and Italy (Polizzi *et al.*, 2009). Bezerra *et al.* (2021) demonstrated occurrence, genetic diversity, and pathogenicity of *Botryosphaeriaceae* associated with symptomatic *Citrus* spp. in Greece, Italy, Portugal, Malta and Spain. Extensive sampling was carried out, along with morphological and DNA phylogenetic analyses of potential isolated pathogens. Symptomatic plants were observed in all the investigated citrus orchards and regions, and all isolates used in pathogenicity tests caused lesions on the wood of inoculated citrus plants. Phylogenetic analyses identified four *Diplodia* species, with *D. pseudoseriata* being the most prevalent, followed by *D. seriata*, *D. olivarum*, and *D. mutila*. The only *Neofusicoccum* spp. identified were *N. parvum*, *N. luteum*, and *N. mediterraneum*. Additionally, *Do. viticola* and *L. theobromae* were also recorded, and *Diplodia* and *Neofusicoccum* spp. were the dominant genera reported. Among the inoculated species, *D. seriata*, *D. olivarum*, *L. theobromae*, *N. mediterraneum*, *N. luteum*, and *N. parvum* were highly aggressive to *C. sinensis*, *C. limon*, and *C. reticulata*, with mean lesion lengths on these hosts ranging from 5 to 7 cm. Only *Do. viticola* and *N. parvum* were found among the *Botryosphaeriaceae* in Italy. Specifically, *Do. viticola* was isolated from twig dieback of *Citrus sinensis*, while *N. parvum* was isolated from stem necroses in *C. sinensis* × *Poncirus trifoliata*, commonly used as rootstock, and from trunk cankers in *Microcitrus australasica*, a citrus-related species belonging to *Rutaceae*.



**Figure 8.** Disease symptoms caused by *Botryosphaeriaeae* on grapevine, olive and citrus in field-grown plants: (a) dieback in the host canopy; (b and c) canker and discoloured internal tissues occurred on grapevine stem; (d and e) canker and subcortical discoloured tissues on olive branches; (f) olive rotted drupes, and (g) emerging perithecia by bark from an affected olive trunk; (h) trunk canker, bark cracking and gummosis on lemon.

## PATHOGEN PRODUCTION OF METABOLITES

Interest in phytotoxic metabolites (PMs) produced by *Botryosphaeriaceae* species has increased due to severe impacts of the diseases caused by these fungi in agriculture and forestry (Masi *et al.*, 2021; Salvatore *et al.*, 2021). Phytotoxins are involved in several diseases, contributing to decline, dieback and specific foliar symptoms (Masi *et al.*, 2018a). Phytotoxic metabolites are usually characterized in two main groups: host-selective toxins (HSTs) and non-host-selective toxins (NHSTs) (Pusztahelyi *et al.*, 2015). Most PMs produced by *Botryosphaeriaceae* are NHSTs, but an HST named Fraxitoxin (isochromanone) active on ash has been isolated from the emerging pathogen *D. fraxini* (Cimmino *et al.*, 2017).

Beyond phytotoxic activity, several secondary metabolites produced by *Botryosphaeriaceae* possess other biological activities, including antifungal, anticancer, antibacterial or insecticidal activities, which make these metabolites promising for different biotechnological applications (Masi *et al.*, 2018a). Ability to produce structurally diverse bioactive secondary metabolites in liquid culture has been recognized for several *Botryosphaeriaceae* spp. in *Diplodia*, *Dothiorella*, *Lasiodiplodia*, *Neofusicoccum* and *Sardiniella* (Andolfi *et al.*, 2012, 2014a, 2014b; Cimmino *et al.*, 2019; Reveglia *et al.*, 2020). Among the most interesting metabolites produced by *Botryosphaeriaceae*, the tetracyclic pimarane diterpene Sphaeropsidin A (SphA) has broad spectrum of activity, for potential applications in agriculture and medicine. Cytotoxicity of SphA, towards apoptosis- and multidrug-resistant cancers, is of particular interest (Mathieu *et al.*, 2015; Masi and Evidente, 2021). SphA and several analogues is produced *in vitro* by different pathogenic *Diplodia* spp. and particularly by the oak pathogen *D. quercivora* (Andolfi *et al.*, 2014b). Recent advances regarding bioactive secondary metabolites produced by *Botryosphaeriaceae* spp. are reported in Table 2.

## BOTRYOSPHAERIACEAE TAXONOMY AND IDENTIFICATION

Systematics and taxonomy have been revisited and updated according to the newest molecular evidence, which has clarified the phylogenetic relationships of several cryptic species (Crous *et al.*, 2006; Alves *et al.*, 2008; Phillips *et al.*, 2013). In addition to morphological data, phylogenetic analyses based on concatenated ITS and *tef1* sequence data are usually used for the identification and description of new putative species (Alves *et al.*, 2014; Linaldeddu *et al.*, 2015a), and sequences of the LSU, ITS,

*tef1* and *tub2* regions have been used for delimitation or description of new genera (Phillips *et al.*, 2008, 2019; Linaldeddu *et al.*, 2016a). The rapid increase in the number of newly described *Botryosphaeriaceae* spp. shows that sequence data analyses for species identification in some genera such as *Diplodia* and *Neofusicoccum* is becoming increasingly difficult (Lopes *et al.*, 2017, 2018).

Incorrect analysis of nucleotide sequences or in choice of the gene region to use in phylogenetic analysis exposes risk that new names are assigned to species haplotypes, rather than to new biologically distinct species. For example, Linaldeddu *et al.* (2013), revealed the existence of two distinct haplotypes in *D. corticola*, named A and B, based on nine fixed differences in the sequences of the *tef1* region. The intraspecific variability in some housekeeping genes of *Botryosphaeriaceae* raises doubts about the limits of multilocus sequence analyses for accurate species delimitation (Lopes *et al.*, 2017, 2018). For *D. corticola*, the *tef1* locus should be used to study phylogeographic diversity among countries, but not for discrimination of closely related species (Smahi *et al.*, 2017; Lopes *et al.*, 2018). For *Neofusicoccum* and *Diplodia*, *MAT* genes have been shown to be the excellent phylogenetic markers, giving capacity to identify and delimit cryptic species (Lopes *et al.*, 2017, 2018). Using *MAT* genes together with ITS regions for the description of new *Diplodia* and *Neofusicoccum* species has considerable potential.

## CHALLENGES AND FUTURE PERSPECTIVES

In depth observations of economically and ecologically important agricultural crops and forest plantation trees in Italy have led to the discovery of the high diffusion of some destructive diseases caused by *Botryosphaeriaceae*. These plant pathogens are becoming limiting factors for plant production, reducing yields, product quality, and profitability (Carlucci *et al.*, 2015; Linaldeddu *et al.*, 2016a, 2016b, 2016c; Gusella *et al.*, 2020b; Aiello *et al.*, 2022; Guarnaccia *et al.*, 2022). Pathogen spread and infection development could occur during any of the crop cultivation steps. However, host plant propagation processes and grafting are key steps for obtaining and producing healthy plants. Disease symptoms observed during the first years after transplanting often reveal the presence of previous fungal infections which have occurred in the nurseries, especially during these propagation steps (Aiello *et al.*, 2020; Costanzo *et al.*, 2022). Use of certified propagation material and an early detection are then required to limit potential damage caused by these fungal diseases. A key challenge in

**Table 2.** Secondary metabolites produced by *Botryosphaeriaceae*, and their biological activities.

Secondary metabolite	Species	Biological activity	References
(1R,2R)-jasmonic acid	<i>Lasiodiplodia mediterranea</i>	Phytotoxic	Andolfi <i>et al.</i> , 2014a
(+)-epi-Epoformin	<i>Diplodia quercivora</i>	Antifungal, antioomycetes, phytotoxic	Andolfi <i>et al.</i> , 2014b
Afritoxinone A	<i>Diplodia africana</i>	Phytotoxic	Evidente <i>et al.</i> , 2012
Afritoxinone B	<i>Diplodia africana</i>	Phytotoxic	Evidente <i>et al.</i> , 2012
Botryosphaerone D	<i>Neofusicoccum australe</i>	Phytotoxic	Andolfi <i>et al.</i> , 2012
Cyclobotryoxide	<i>Neofusicoccum cryptoaustrale</i>	Phytotoxic	Andolfi <i>et al.</i> , 2012
Diplopyrone	<i>Diplodia corticola</i>	Phytotoxic	Evidente <i>et al.</i> , 2003a Masi <i>et al.</i> , 2016
Diplopimarane	<i>Diplodia quercivora</i> , <i>Diplodia olivarum</i>	Antifungal, antioomycetes, phytotoxic, zootoxic	Andolfi <i>et al.</i> , 2014b; Di Lecce <i>et al.</i> , 2021
Diorcinol	<i>Diplodia corticola</i>	Phytotoxic, antifungal, antioomycetes, zootoxic	Cimmino <i>et al.</i> , 2016 Masi <i>et al.</i> , 2016
Diplopyrone B	<i>Diplodia corticola</i>	Antifungal, antioomycetes, phytotoxic	Cimmino <i>et al.</i> , 2016 Masi <i>et al.</i> , 2016
Diploquinones A	<i>Diplodia mutila</i>	Phytotoxic	Reveglia <i>et al.</i> , 2018a, 2019
Diploquinones B	<i>Diplodia mutila</i>	Phytotoxic	Reveglia <i>et al.</i> , 2018a, 2019
Epi-Sphaeropsidone	<i>Diplodia africana</i> , <i>Diplodia cupressi</i> , <i>Diplodia subglobosa</i>	Antifungal, phytotoxic	Evidente <i>et al.</i> , 2012; Masi <i>et al.</i> , 2022
Fraxitoxin	<i>Diplodia fraxini</i>	Phytotoxic	Cimmino <i>et al.</i> , 2017
Luteoethanones A	<i>Neofusicoccum luteum</i>	Phytotoxic	Masi <i>et al.</i> , 2021
Luteoethanones B	<i>Neofusicoccum luteum</i>	Phytotoxic	Masi <i>et al.</i> , 2021
Neoanthraquinone	<i>Neofusicoccum luteum</i>	Phytotoxic	Masi <i>et al.</i> , 2020a
Olicleistanone	<i>Diplodia olivarum</i>	Zootoxic	Di Lecce <i>et al.</i> , 2021
Oxysporone	<i>Diplodia africana</i>	Phytotoxic	Evidente <i>et al.</i> , 2012
Pinofuranoxins A	<i>Diplodia sapinea</i>	Antifungal, phytotoxic zootoxic	Masi <i>et al.</i> , 2021
Pinofuranoxins B	<i>Diplodia sapinea</i>	Antifungal, antioomycetes, phytotoxic, zootoxic	Masi <i>et al.</i> , 2021
R(-)-Mellein	<i>Diplodia africana</i> , <i>Sardiniella urbana</i>	Antifungal, phytotoxic	Cimmino <i>et al.</i> , 2019
Resorcinol	<i>Dothiorella vidmadera</i>	Phytotoxic	Reveglia <i>et al.</i> , 2018b
Sapinofuranone A	<i>Diplodia sapinea</i>	Phytotoxic	Evidente <i>et al.</i> , 1999
Sapinofuranone B	<i>Diplodia sapinea</i>	Phytotoxic	Evidente <i>et al.</i> , 1999
Sapinopyridione	<i>Diplodia sapinea</i>	Phytotoxic, Antifungal	Evidente <i>et al.</i> , 2006
Spencertoxin	<i>Dothiorella viticola</i>	Phytotoxic	Reveglia <i>et al.</i> , 2020
Sphaeropsidin A	<i>Diplodia africana</i> , <i>Diplodia cupressi</i> , <i>Diplodia corticola</i> , <i>Diplodia olivarum</i> , <i>Diplodia quercivora</i> , <i>Diplodia subglobosa</i>	Phytotoxic, antifungal, antioomycetes, antibacterial, anticancer, insecticidal, zootoxic	Andolfi <i>et al.</i> , 2014b; Masi <i>et al.</i> , 2016, 2022; Masi and Evidente, 2021; Di Lecce, 2021; Salvatore <i>et al.</i> , 2021; Roscetto <i>et al.</i> , 2020
Sphaeropsidin B	<i>Diplodia cupressi</i>	Phytotoxic, Antifungal	Evidente <i>et al.</i> , 1997, 2011
Sphaeropsidin C	<i>Diplodia corticola</i> , <i>Diplodia cupressi</i> , <i>Diplodia olivarum</i> , <i>Diplodia quercivora</i>	Phytotoxic	Evidente <i>et al.</i> , 1997; Andolfi <i>et al.</i> , 2014b; Di Lecce, 2021; Masi <i>et al.</i> , 2016
Sphaeropsidin D	<i>Diplodia cupressi</i>	Phytotoxic	Evidente <i>et al.</i> , 2002
Sphaeropsidin F	<i>Diplodia cupressi</i>	Phytotoxic	Evidente <i>et al.</i> , 2003b
Sphaeropsidin G	<i>Diplodia corticola</i> , <i>Diplodia olivarum</i>	Zootoxic	Cimmino <i>et al.</i> , 2016; Di Lecce, 2021
Sphaeropsidone	<i>Diplodia cupressi</i>	Phytotoxic	Evidente <i>et al.</i> , 1998

the knowledge of *Botryosphaeriaceae* involves developing tools that provide rapid identification of fungi in asymptomatic plants, particularly in planting material. High

throughput sequencing (HTS) diagnostics are important advances in plant pathology, as key molecular biology contributions since the development of the PCR process

(Robert-Siegwald *et al.*, 2017). In addition, image analysis is a promising technique among non-invasive detection techniques for *Botryosphaeriaceae* spp. Hyper- or multi-spectral image analysis allows diagnosis of wood diseases of symptomatic and asymptomatic grapevine plants, even before disease symptoms appear (Pérez-Roncal *et al.*, 2022). Unmanned aerial vehicles could be used to monitor entire orchards (Di Gennaro *et al.*, 2016), and future studies should focus on these, aiming to enable early disease detection and improve plant protection management processes.

To decrease the use of synthetic fungicides on crops, cultural practices to manage *Botryosphaeriaceae* diseases (elimination of dead wood or pruning residues to reduce potential inoculum sources) must be complemented by employing biological control measures for pruning wound protection, such as microbial active ingredients or substances of botanical origin (essential oils, wood extracts) (Špetík *et al.*, 2022), or chemio-physical tools (Baaijens *et al.*, 2019). As outlined above, global warming and stress factors such as occasional climatic events or inappropriate agronomic management may further compromise plant health, and can affect susceptibility host plants and behaviour of pathogen, increasing infection risk (Batista *et al.*, 2021). The use of windbreaks, precision irrigation, anti-frost irrigation, and soil mulching, may also be useful for the pathogen management by reducing host stress.

Further research should aim to ascertain the epidemiology of these pathogens in Italian nurseries, forestry and orchards, and to evaluate the risks of fungal infections over time in different global climate change scenarios. Decision-support systems develop models to predict species occurrence in time and space (Batista *et al.*, 2023), and these can support grower choices for determining the best times for disease management intervention. This research will help to reduce the impacts of ubiquitous *Botryosphaeriaceae* as pathogens of economically and aesthetically important plants.

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