Chapter 11
Olive Anthracnose and Its Management by Fungal Endophytes: An Overview

Fátima Martins, José Alberto Pereira, and Paula Baptista

Abstract Anthracnose caused by diverse species of fungi belonging to the genus *Colletotrichum* is an important disease of the olive tree, leading to high productivity losses. Their control is very difficult and none of the available control measures are effective enough. Indeed, fungicides are most commonly applied to control this disease, but they are not totally effective, and their use possesses some environmental concerns. In the last decades, the implementation of sustainable production methods has been encouraged with emphasis on the use of living organisms as control agents against plant pests, diseases, and weeds. These control agents comprise a variety of predators, parasitoids, and microorganisms, including fungal endophytes. This review highlights the importance of endophytic fungi for the management of olive anthracnose. These fungi can be effectively used to improve plant performance and plant protection against biotic and abiotic stresses. They produce a wide variety of secondary metabolites that directly or indirectly induced defense responses in the host plant against pathogens. Thus, in this review emphasis is given for the exploitation of fungal endophytes associated to olive tree in the development of new tools/approaches to manage olive anthracnose.

11.1 Olive Anthracnose: A General Overview

The European olive, *Olea europaea* subsp. *europaea* L., is one of the major cultivated species in countries surrounding the Mediterranean Sea. In 2016, approximately 9.2 million ha of land in this region were planted with olive trees (FAOSTAT 2018). Several insect pest and diseases attack the olive crop, reducing its yield both in terms of quantity and quality. Among diseases, anthracnose is the major causes of olive crop damage worldwide (Talhinhas et al. 2018). It was first described in Portugal in 1899 by J.V. d'Almeida (1899) and rapidly expanded to all continents (Cacciola et al. 2012) becoming a serious economic constraints to olive crop production (Mosca et al. 2014; Iliadi et al. 2018). This disease affects different

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Fig. 11.1 Characteristic symptoms of anthracnose on olive tree fruits of cv. Madural (**a**) production of orange/pink sticky masses of conidia in olive surface; (**b**) rot, mummification, and dehydration of fruits; (**c**) symptoms appear mostly in mature fruits, but, in favorable environmental conditions, green fruits may also be infected; (**d**) affected fruits fall prematurely to the ground (photos: Fátima Martins)

organs of the olive tree, including flowers, buds, shoots, leaves, and twigs, being the fruits the most severely affected (Cacciola et al. 2012). Thus, characteristic anthracnose symptoms arise mostly on the fruits, especially when they are nearly ripened. The first symptoms of infected olives are small and brown-colored spots in the epicarp that become later sunken. As the fruits ripe, the center of these sunken spots becomes covered with pink/orange gelatinous masses of conidia that are often produced in a concentric ring pattern (Talhinhas et al. 2011). This causes mummification, rotting, and premature drop of fruits, leading to significant crop losses (Fig. 11.1). The attacks can occur on any part of the fruit, but they are more frequent at the apex, because it stays wet in longer time (Cacciola et al. 2012). In some cases, infected fruits may persist on the tree, becoming an inoculum reservoir of olive anthracnose (Sergeeva 2011a). In the vegetative parts, the symptoms include leaf chlorosis, defoliation, and dieback of shoot and twigs (Cacciola et al. 2012). These effects are due to production of toxins by the pathogen (Cacciola et al. 2012). The infected flowers display blossom blight, dry out, and drop quickly (Moral et al. 2008; Sergeeva et al. 2008). Infections are usually most severe on the lower branches, inside the canopy on the north side, where moisture tends to remain for longer periods of time (de Cantero 1997).

The disease can be devastating, depending on the level of susceptibility of the cultivars, the environmental conditions, the inoculum pressure, and the virulence of the pathogenic strains (Talhinhas et al. 2018). Under favorable conditions, all production can be destroyed. For instance, in some olive-growing countries, the olive anthracnose was described to cause yield losses above 80% (Cacciola et al.

2012). In addition, this disease can reduce the quality of olive oil. The oils' peroxide content and acidity value from anthracnose-infected fruits sometimes can be higher than the maximum legal limit to be considered as virgin olive oil (da Silva 2016). Most of these olive oils show negative sensory and organoleptic characteristics, being classified as lampante (da Silva 2016).

11.2 Anthracnose is Caused by a Complex of *Colletotrichum* Species

Anthracnose in olive tree is associated with at least eight *Colletotrichum* species, belonging to two heterogeneous fungal species complexes, namely, *C. acutatum* sensu lato (s.l.) and *C. gloeosporioides* s.l. (Damm et al. 2012). Of these two complexes, *C. acutatum* s.l. is the most predominant, causing epidemic explosions of anthracnose in most olive-growing countries (Talhinhas et al. 2005). Multilocus molecular phylogenetic analysis revealed that there are six species in the *C. acutatum* complex considered to be causal agents of olive anthracnose, namely, *C. fioriniae*, *C. simmondsii*, *C. nymphaeae*, *C. acutatum* sensu stricto (s.s.), *C. godetiae* (syn. *C. clavatum*), and *C. rhombiforme* (Talhinhas et al. 2018). The same study also revealed that there are two species belonging to the *gloeosporioides* complex, *C. gloeosporioides* s.s. and *C. theobromicola* (Talhinhas et al. 2018). *Colletotrichum boninense* (syn. *C. karstii*) is a third species complex that was recently related with olive anthracnose (Schena et al. 2014). However, this complex does not appear to threaten olive production due to their weakly pathogenicity (Schena et al. 2014). Similarly, other fungal species belonging to *C. gloeosporioides* complex (i.e., *C. aenigma*, *C. queenslandicum*, *C. siamense*, and *C. kahawae* ssp. *cigarro*) were isolated from symptomatic fruits, but their pathogenicity in olives has not yet been confirmed (Schena et al. 2014).

Among all these fungal species identified, *C. acutatum* s.s., *C. godetiae*, and *C. nymphaeae* have been recognized as major causative agents of olive anthracnose in most olive-growing countries (Mosca et al. 2014). For instance, the majority of strains examined from South Africa, Australia, and Tunisia belonged to *C. acutatum* s.s. (Cacciola et al. 2012). In other studies performed in Montenegro, Greece, Italy, and Spain, *C. godetiae* was identified as the most prevalent species (Moral et al. 2008, 2009, 2014). In Portugal, primarily three important species have been related to the olive anthracnose, with *C. godetiae* causing major damage in the northern region, whereas *C. nymphaeae* and *C. acutatum* s.s. have been identified as the most prevalent species in the southern regions (Talhinhas et al. 2009).

Species of *Colletotrichum* have a teleomorph or sexual stage, i.e., *Glomerella* sp. (Wharton and Diéguez-Uribeondo 2004). Nevertheless, in olive crops, the teleomorph of the pathogen has not yet been detected in field conditions (Cacciola et al. 1996), suggesting the imperfect stage, i.e., *Colletotrichum* sp., as the main responsible of olive anthracnose.

11.3 Epidemiology and Life Cycle

Epidemiology and life cycle of olive anthracnose are still poorly understood, especially in what concerns propagation and inoculum maintenance in the olive groves, which required more studies (Moral et al. 2008; Cacciola et al. 2012). In Mediterranean regions, it has been reported that infections begin during the spring in flowers and in young fruits (primary infection; Fig. 11.2) (Moral et al. 2009). The mode of survival and the source of this primary inoculum have yet to be determined (Moral et al. 2009). It is thought that the major primary inoculum reservoirs are mummified fruits that remain on the tree or on the ground, from one season to the next (Moral et al. 2009). It is also plausible that the source of inoculum in spring may originate from fungi that overwinter in woody material and leaves of the tree (Talhinhas et al. 2018). After primary infection, the fungus stops growing and remains dormant until fruit begins to ripen (Moral et al. 2009). At that time, with favorable environmental conditions, sticky masses of spores are produced in acervuli. These spores are then spread by rain splash to newly fruits and other tree parts, giving rise to secondary infections (Moral et al. 2009). The spread of the pathogen and infection of olive tree depend heavily on the climatic conditions (Talhinhas et al. 2015). Olive anthracnose reaches highest disease incidence and severity in areas where relative humidity is highest (over 93%) and the air temperature is warm (ranging from 10 to 30 °C) (Cacciola et al. 2012). The occurrence of precipitation is also crucial for the conidia separation from the gelatinous mass of the acervuli and for their dispersion (Cacciola et al. 2012). Also, the infection of fruits depends on the extent of peel ripeness. Olives at later stages of ripening are more prone to fungal infection than green fruits (da Silva 2016). The severity of symptoms varies widely with the cultivar (i.e., their susceptibility to anthracnose) and the virulence of the strain (Talhinhas et al. 2015). Recent studies showed that, in several olive-growing countries, the pathogen populations are particularly adapted to both environmental conditions and the host, but severe infections occur when only virulent populations of the pathogen are present (Moral et al. 2017).

Usually, penetration and colonization of plant tissues by *Colletotrichum* species comprises a sequential set of stages. Generally, it starts with the fixation and germination of the conidia on the host surface, followed by appressorium development, which facilitates entry through the host epidermis (Wharton and Diéguez-Uribeondo 2004). A detailed study of *C. acutatum* infection on olives showed that after spores' germination, a germ tube is produced and differentiated in an appressorium, which facilitated the penetration of the fungus into the host cells (Gomes et al. 2009). This process occurs within a few hours (48–72 h), and consequently, the infections can occur rapidly under favorable conditions (Gomes et al. 2009). Fungal penetration is also believed to occur through stomas or lenticels as well as wounds caused by insect (e.g., *Bactrocera oleae*) attack (Cacciola et al. 2012).

After penetration on fruit, *Colletotrichum* sp. can follow different infection strategies. These strategies can be range from intracellular hemibiotrophic mode (colonizes living plant tissue and obtains nutrients from living host cells) to the

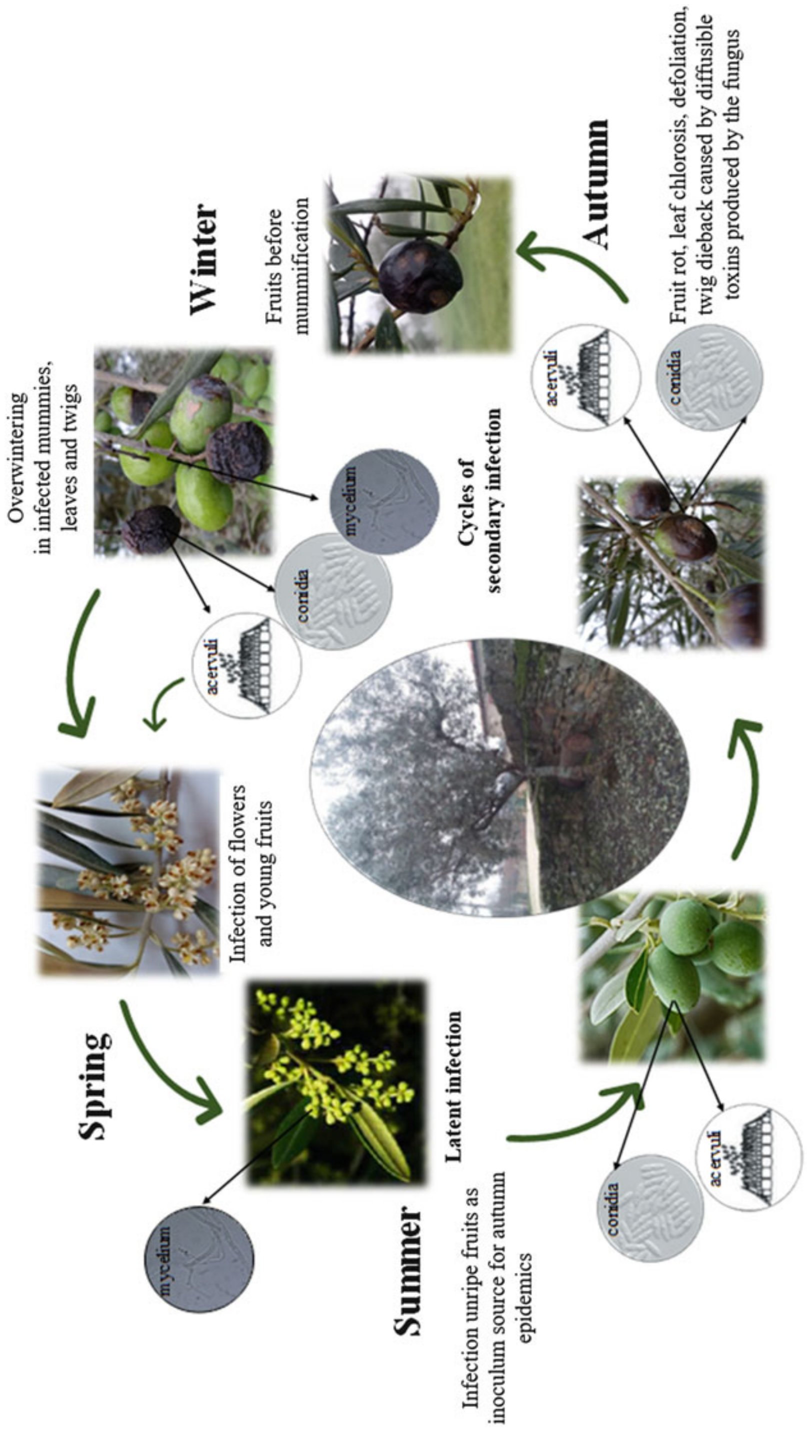

Fig. 11.2 Diagrammatic representation of disease cycle of olive anthracnose in the Mediterranean region (photos: Fátima Martins)

subcuticular intramural necrotrophic (infects and kills host tissue and extracts nutrients from the dead host cells) mode of nutrition (Gomes et al. 2009), being hemibiotrophic the most common (De Silva et al. 2017). The infection and colonization strategy of *C. acutatum* sp. on olive fruits of both susceptible (cv. *Galega Vulgar*) and tolerant (cv. *Picual*) cultivars was identified as intracellular hemibiotrophic, followed by a necrotrophic phase (Gomes et al. 2009).

11.4 Management Strategies for Olive Anthracnose

Management of olive anthracnose is very difficult, because its spreading and development relies greatly on the climatic conditions. Thus, no effective control measures have been proposed so far for its management. Generally, those measures rely on an integrated approach that combines several means and tools, either to prevent (indirect method) or to protect (direct method) olive crop against anthracnose (Cacciola et al. 2012; Moral et al. 2018).

Indirect or preventive measures of olive anthracnose rely mostly on practices aiming either to reduce the initial levels of inoculum or reduce the rate of spread of the established pathogen. These practices include agronomic techniques such as pruning, drainage and irrigation, fertilization, use of varieties tolerant/resistant to anthracnose, and control of insects that potentially may spread the pathogen, among others. Pruning of olive trees can be an effective way to eliminate sources of fungal inoculum, by removing diseased twigs of infected olive trees. After pruning, the plant material should be removed from the grove and destroyed. Olive pruning also promotes aeration and light penetration in the canopy, helping to reduce the severity of the disease (Sergeeva 2011a). Irrigation management has a strong impact on the olive anthracnose disease severity and epidemic progress rates, since *Colletotrichum* sp. are greatly dependent not only on high humidity levels for all stages of their life cycle but also on available free water for conidia dispersion, which is a process of great epidemiological consequence (Cacciola et al. 2012). Thus, overwatering should be avoided in the grove where anthracnose is present in order to prevent the outbreak of the disease (Sergeeva 2011a). Due to the dependence of *Colletotrichum* sp. to water splash for dispersion, the choice of irrigation method could be extremely important to avoid infections of epidemic-like proportions. Adequate nutrition may also have an important role in reducing the severity of olive anthracnose. Previous studies performed in strawberry showed that the source and level of nitrogen in fertilizers had a great effect on severity of anthracnose (Smith 2009). As far as we know, no studies have been carried out to evaluate the influence of nitrogen fertilization on incidence and development of olive anthracnose. However, a balanced fertilization is frequently recommended for management of olive anthracnose (Sergeeva 2011b). In general, a balanced fertilizer with fairly low nitrogen content will be ideal, since overapplication of nitrogen fertilizers has been reported to increase the incidence of diseases on olive tree canopy (Roca et al. 2018). Use of olive cultivars resistant to the anthracnose pathogens is one of the most successful approaches to the control of this disease (Moral and Trapero 2009). Numerous studies, carried out in several olive-growing countries, have already identified olive tree varieties with different levels of susceptibility to anthracnose, ranging from highly susceptible (e.g., cv. Galega Vulgar) to highly resistant (e.g., cv. Frantoio) (e.g., Talhinhas et al. 2015; Moral et al. 2017). However, response to anthracnose of olive tree cultivars under field conditions has been showed to be dependent on the *Colletotrichum* species (Talhinhas et al. 2015) and on the climatic conditions, in particular of relative humidity (Moral et al. 2014, 2017). Thus, in certain humid olive-growing areas, anthracnose-resistant cultivars can still get infected (Moral et al. 2014, 2017). Control of olive fruit fly attacks, which provides entry points for *Colletotrichum* sp., will limit the surface damage of the fruit and may also be useful to reduce the severity of anthracnose (Malacrinò et al. 2017).

Methods and tools for direct control of olive anthracnose include the use of fungicides and more recently of natural products and biocontrol agents. The fungicides generally recommended for controlling olive anthracnose are protective fungicides based on copper compounds, such as copper oxychloride, copper sulfate, and copper hydroxide (Cacciola et al. 2012). Newer chemicals, such as strobilurins, have also been showed to increase copper-based fungicides effectiveness against olive anthracnose in orchards when used in combination (Moral et al. 2018). Similarly, natural products, like botanicals (i.e., plant extracts) and products of mineral origin (i.e., calcium-rich compounds), have been recently explored in the control of olive anthracnose (Moral et al. 2018). Calcium-rich compounds have been showed to inhibit *Colletotrichum* sp. appressorial formation under in vitro tests, but their field application was not always effective in the control of olive anthracnose (Xaviér 2014). Extract obtained from the peel of pomegranate (*Punica granatum* L.) has proven to be effective against *Colletotrichum* sp. under laboratory conditions and to control olive anthracnose under in field trays (Pangallo et al. 2017). Biological control (BC) is another alternative for olive anthracnose management, although this approach has not been as effective as the chemical control (Holt et al. 2009). The possibilities of using biocontrol agents (BCAs) for controlling the pathogen of olive anthracnose were firstly illustrated by Segura (2003). In artificial inoculations of olives, the microorganisms *Aureobasidium pullulans*, *Curtobacterium flaccumfaciens*, and *Paenibacillus polymyxa* were shown to decrease the severity of the symptoms produced by *C. acutatum* in 76.4, 53.7, and 51.6%, respectively (Segura 2003). Since then, few studies have been done on the BC of olive anthracnose and this strategy has not been used against this disease in field conditions.

Although the several efforts made to better understand the epidemiology and population genetics of the different pathogenic species, the olive anthracnose still remains a “complex disease” to decipher. Indeed, it remains unclear how the pathogen interact with the host plant, which is the variability of *Colletotrichum* species in some olive-growing regions, and which are the best control strategies against this disease. In this regard, the use of fungal endophytes to control olive anthracnose could be a promising approach (Landum et al. 2016; Preto et al. 2017). These microorganisms are able to inhabit the same niche in the same environment that of *Colletotrichum* spp., favoring them as potential biocontrol agents against olive anthracnose.

11.4.1 Fungal Endophytes and Their Potential as Biocontrol Agents Against **Colletotrichum** *spp.*

Fungal endophytes are microorganisms that inhabit the inner tissue of the plant, at some part or whole of its life cycle, without causing any apparent damage to the hosts (Busby et al. 2016). According to the mechanisms used to colonize the host plant, the fungal endophytes were classified as "obligate" or "facultative" (Andreote et al. 2014). Obligate endophytes are transmitted to other plants by vertical colonization or by vectors and are strictly dependent on host cell metabolism for their survival and replication (Andreote et al. 2014). Facultative endophytes have a free life, living outside of host plant, and during a certain stage of their life cycle, they colonize the plant internally (Andreote et al. 2014).

Overall, most endophytic fungi within plant tissues belong to Ascomycota and Basidiomycota phyla (Arnold and Lutzoni 2007; Selosse et al. 2009). In particular, the composition of fungal endophytic community of olive tree has been only recently analyzed (Martins et al. 2016; Landum et al. 2016; Preto et al. 2017; Gomes et al. 2018). Overall, these studies showed that there is great diversity and abundance of fungal endophytes in several organs of olive tree, including leaves, twigs, fruits, and roots. More than 65 genera from 33 families and 2 phyla of fungal species have been reported to be associated with olive tree (Fig. 11.3). Most of the fungal isolates belong to the phyla *Ascomycota*, accounting to 93% of the total number of fungal isolates, followed by *Basidiomycota* (Martins et al. 2016; Landum et al. 2016; Preto et al. 2017; Gomes et al. 2018). The most abundant fungal families are *Pleosporaceae* (17.1% of the total fungal isolates), *Incertae sedis* (13.7%), and *Nectriaceae* (8.5%). *Alternaria*, *Penicillium*, *Epicoccum*, and *Phomopsis* were identified as the most abundant genera, accounting together 25.5% of total fungal isolates. The various olive tree organs surveyed displayed differences on endophytic fungal composition. Members of *Pleosporaceae* and *Incertae sedis* were the most abundant in leaves and twigs of olive tree, accounting together 90.3% of the total isolates, whereas *Trichocomaceae* and *Nectriaceae* were the most abundant in roots and fruits, respectively (Fig. 11.3) (Martins et al. 2016; Landum et al. 2016; Preto et al. 2017; Gomes et al. 2018). Besides plant organ, host plant geographic location, host genetics (at cultivar level), and season and climatic conditions, such as rainfall and temperature, were also shown to contribute to the shaping of fungal communities in olive tree (Martins et al. 2016; Preto et al. 2017; Gomes et al. 2018). In general, the diversity of fungal endophytes in olive tree leaves and twigs is higher in spring than in autumn (Gomes et al. 2018). The same study also identified differences on fungal composition between spring and autumn. These seasonal shifts were found to be related to climatic factors, especially to rainfall and mean temperature (Gomes et al. 2018). Geographic distance was also found to affect the structure of fungal endophytic communities especially of roots but also of leaves and twigs (Martins et al. 2016). An inverse relationship was noticed between the similarity of endophytic assemblages and their geographic distance (Martins et al. 2016).

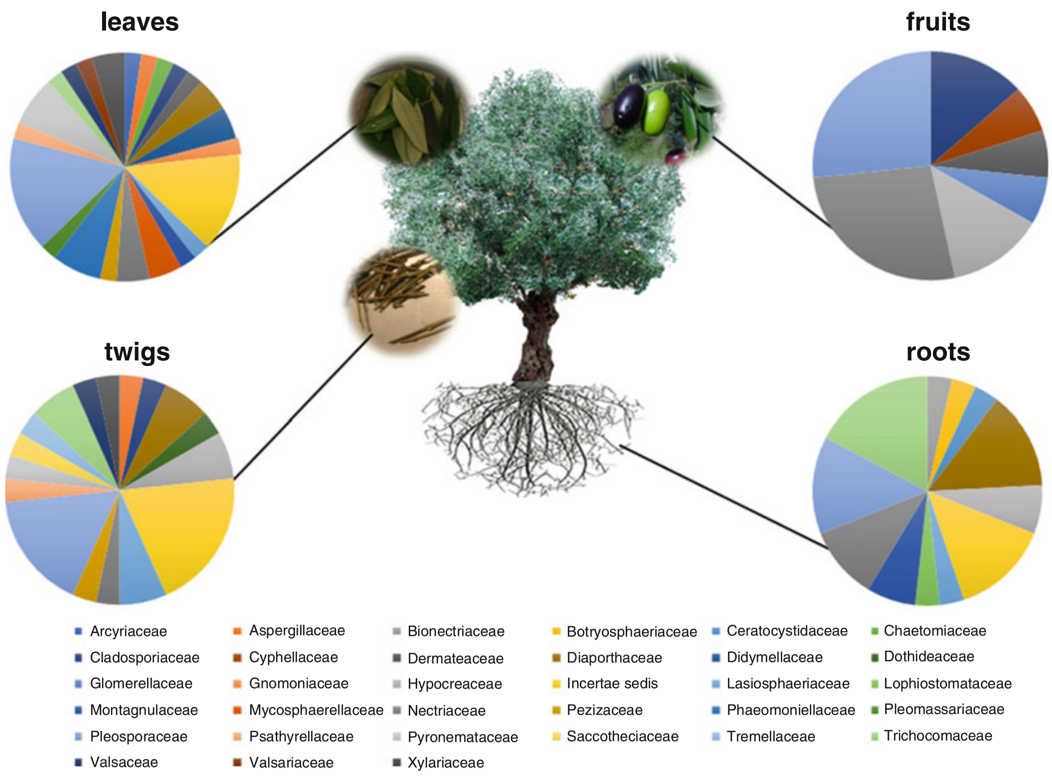

Fig. 11.3 Abundance (number of isolates) of fungal endophytes, at family level, present in leaves, twigs, fruits, and roots of olive tree (*Olea europaea* L.)

There is growing evidence that these endophytic fungi fulfill important functions for plant health and productivity (Khare et al. 2018). Endophytes can, for instance, promote plant nutrition and protection against abiotic (e.g., drought and extreme temperatures) and biotic stresses, such as plant pathogens (Bacon and White Jr 2016). In particular, the mechanisms used by endophytic fungi to protect host plant against pathogens mostly rely on the production of secondary metabolites, such as alkaloids, peptides, steroids, terpenoids, phenols, quinines, flavonoids, siderophores, and volatile organic compounds (Gao et al. 2010; Ownley et al. 2010; Speckbacher and Zeilinger 2018). Most of these classes of compounds comprise phytohormones, mycotoxins, antimicrobial molecules, as well as antibiotics that may reduce pathogen infection directly, through antibiosis, mycoparasitism, and competition, and indirectly by induction of plant resistance response (Lacava and Azevedo 2014). Endophytic fungi are also known to produce cell wall-degrading enzymes (e.g., chitinases, proteases, and glucanases) with the ability to destroy pathogens' cell wall (Lorito et al. 2010; Katoch et al. 2014). The above mechanisms regularly operated simultaneously.

Till date, few studies were conducted to explore the biocontrol activities of endophytes against anthracnose disease caused by *Colletotrichum* species under in vivo conditions (i.e., in detached fruits, field, and/or greenhouse) (Table 11.1). The results obtained up to date appear to be very promising being the level of disease suppression achieved by application of fungal endophytes ranging from 2.5 to 83%, depending on the fungal species (Table 11.1). According to the results shown in Table 11.1, the most promising fungal endophytes to control anthracnose diseases are *Trichoderma* spp., *Nodulisporium* sp., and *Cordana* sp. and also some yeasts belonging to the genera *Debaryomyces* and *Cryptococcus*. These strains were shown to be effective in reducing *Colletotrichum* growth and disease severity in several hosts like papaya (Valenzuela et al. 2015; Hernandez-Montiela et al. 2018), mango (Bautista-Rosales et al. 2014), and wild banana (Nuangmek et al. 2008). Competition for nutrients and space, antibiosis, and mycoparasitism and production of cell wall-degrading enzymes, antibiotics, and volatile organic compounds were the most important modes of action of fungal endophytes for anthracnose disease control (Table 11.1).

11.4.2 Fungal Endophytes on the Control of Olive Anthracnose

Despite the ability of fungal endophytes to control anthracnose disease, there are only limited studies on the use of these fungi against olive anthracnose. In addition, most of these studies were performed under controlled conditions, by using in vitro experiments, being field assays much more limited. Among the various endophytic fungal species tested in in vitro laboratory assays, *Alternaria* sp., *Diaporthe* sp., and *Nigrospora oryzae* isolated from olive tree leaves have been shown to inhibit up to

Table 11.1 Fungal endophytes that have been tested in vivo to control anthracnose disease caused by *Colletotrichum* spp., their possible mechanisms of action, and their efficacy

Antagonistic fungal isolates	Host plant	Assays	Disease agent	Mechanism of action	Efficacy	References
Aureobasidium pullulans	Olive (*Olea europaea* L.)	Field assay	*Colletotrichum* spp.	NA	Reduced both latent infection (14%) and disease severity (40%)	Nigro et al. (2018)
Pichia kudriavzevii *Wickerhamomyces anomalus*	Olive (*Olea europaea* L.)	In vivo (ripe olive fruit)	*C. gloeosporioides*	Competition Antibiotic production Invasive growth	Reduced disease severity (6.99–22.05%)	Pesce et al. (2018)
Debaryomyces hansenii	Papaya (*Carica papaya* L.) var. Maradol	In vivo (fruit)	*C. gloeosporioides*	Volatile organic compounds	Reduced pathogen growth (36%) and disease severity (83%)	Hernandez-Montiela et al. (2018)
Trichoderma spp.	Papaya (*Carica papaya* L.) var. Maradol	In vivo (fruit)	*C. gloeosporioides*	Invasive growth Mycoparasitism	Reduced pathogen growth (50–60%), and disease severity (77.40%)	Valenzuela et al. (2015)
Cryptococcus laurentii	Mango (*Mangifera indica* L.)	In vivo (mango fruit)	*C. gloeosporioides*	Antibiosis Nutrient competition Hydrolytic enzymes	Reduced disease severity (75.88%)	Bautista-Rosales et al. (2014)
Trichoderma viride	Bean (*Phaseolus vulgaris* L.)	In vivo (seeds)	*C. lindemuthianum*	Mycoparasitism Antibiosis	Reduced the growth (59.48%) and the germination (73.60%) of the pathogen, as well as disease severity (32.02%)	Padder and Sharma (2011)
Cordana abramovii *Nodulisporium* sp.	Wild banana (*Musa acuminata* Colla)	In vivo (detached banana)	*C. musae*	Competition Antibiotic production	Reduced the growth (90%) and the germination (91%) of the pathogen, as well as disease severity (53%)	Nuangmek et al. (2008)
Trichoderma viride	Cowpea (*Vigna unguiculata* L.)	In vivo (seedling)	*C. lindemuthianum*	Mycoparasitism Antibiosis	Reduced disease severity (2.5%)	Adebanjo and Bankole (2004)

NA not applicable

26.8% the growth of *C. acutatum* (Landum et al. 2016). This inhibitory effect was ascribed to the production of volatile compounds by the endophyte, in particular of phenylethyl alcohol, 4-methylquinazoline, benzothiazole, benzyl alcohol, lilial, and galaxolide (Landum et al. 2016). Similarly, the endophytic fungal species *Chondrostereum purpureum*, *Chaetomium globosum*, *Aspergillus westerdijkiae*, *Aspergillus* sp. 1, *Quambalaria cyanescens*, *Epicoccum nigrum*, and *Aspergillus brasiliensis*, isolated from olive fruits, have been shown to inhibit *C. acutatum* growth under in vitro conditions, reaching inhibition values of 30.9–71.3% (Preto et al. 2017). Some of these endophytic fungal strains were also shown to induce morphological alterations on pathogen hyphae and to reduce both the production (up to 46%) and germination (up to 21%) of *C. acutatum* spores (Preto et al. 2017). Although the exact mechanism of antagonism displayed by these fungi is not clear, it is hypothesized the involvement of antimicrobial compounds and lytic enzymes, secreted by endophytic isolates, which may act synergistically against the fungal pathogen (Preto et al. 2017). The degree to which fungal endophyte regulates *C. acutatum* infection is dependent on both host plant and the order of arrival of the pathogen and endophyte (Martins et al. 2013). In vitro confrontation assays between the endophyte *Penicillium commune* and *C. acutatum* in the presence of olive leaf (+leaf) revealed a greater inhibitory effect of the endophyte over the pathogen when compared to −leaf treatment (Martins et al. 2013). This result suggests that the plant-endophyte interaction is critical for the biocontrol of the pathogen. The observed inhibitory effect on *C. acutatum* sporulation and germination was strong (around 50 and 60%, respectively) when the endophyte colonized the leaf before the pathogen (Martins et al. 2013).

In olive fruit inoculation assays, the endophytic fungi *Trichoderma koningii* have been shown to reduce significantly ($p < 0.05$) both incidence (AUDPCi) and severity (AUDPCs) of olive anthracnose when compared to control (i.e., in the absence of *T. koningii*), either at 14 or 21 days postinoculation (Fig. 11.4). The effectiveness of this endophyte as a biological control agent against olive anthracnose was most notorious on fruits that start to change skin color (maturation index 2) than on purple or black olives (maturation index 3). The endophyte *T. koningii* also showed the capacity to inhibited significantly the production and germination of spores produced by the pathogen *C. godetiae* in olives, either at maturation index 2 (up to 1.6- and 6.1-fold, respectively) or 3 (up to 2.1- and 5.7-fold, respectively) when compared to control (Martins et al. 2017).

Few studies have determined the efficacy of fungal endophytes against olive anthracnose under field conditions. Only recently, it was reported that the treatment of olive tree with the endophyte *Aureobasidium pullulans* in field trays significantly reduced anthracnose severity by 40% and latent infection by 14% (Nigro et al. 2018).

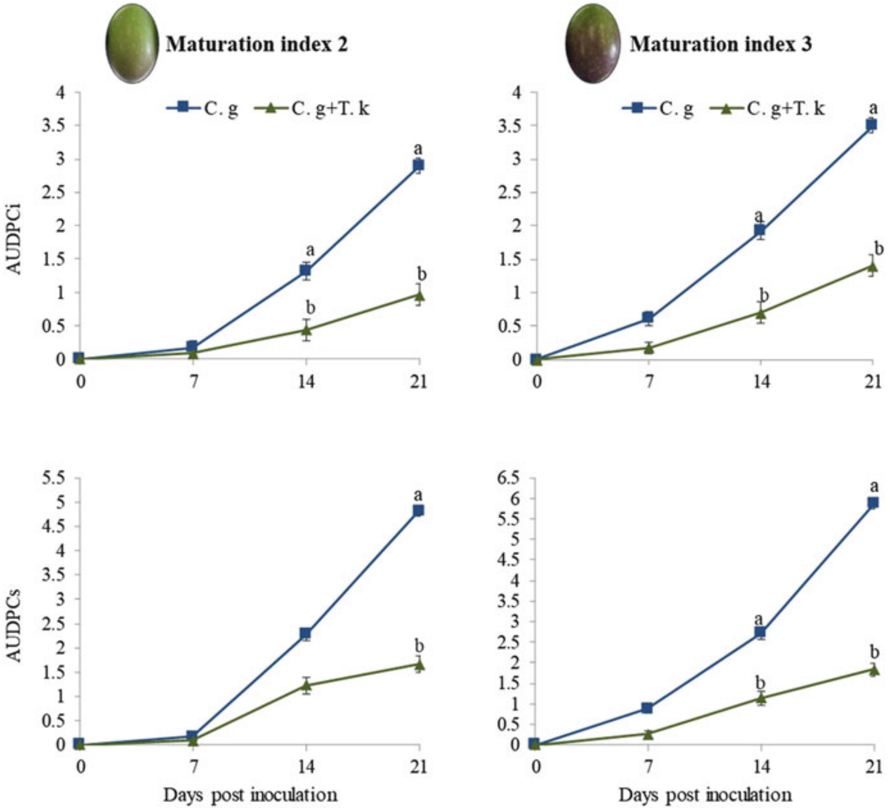

Fig. 11.4 Area under the disease progress curve of incidence (AUDPCi) and severity (AUDPCs) in olive fruits from cv. *Madural*, at maturation index 2 and 3, after 7, 14, and 21 days of inoculation only with *C. godetiae* (C.g) or in combination with the endophyte *T. koningii* (C.g + T.k). In each day, mean values followed by different letters are significantly different ($p < 0.05$)

11.5 Conclusion

The use of endophytic fungi for the biological control of olive anthracnose could be a sustainable alternative to olive crop production (Lugtenberg et al. 2016). Despite no effective biocontrol agents are still available against olive anthracnose, some authors have already described promising results in this area. However, most of these studies have detected the biocontrol activity of the fungal endophyte by using in vitro and in vivo tests on detached fruits, under controlled conditions. They therefore do not replicate the environment in which the biocontrol agent must function. More studies aiming the selection of fungal endophytes as biological control agents against olive anthracnose by using *in planta* assays, either in the field or greenhouses conditions, are required. Similarly, we still have incomplete knowledge on the various relationships that fungal endophytes can establish with their host and with other members of plant-associated microbial community, under natural conditions. Such studies will certainly contribute to enhance the chances to obtain competent endophytic biocontrol agent and therefore develop new successful and sustainable integrated crop protection against olive anthracnose.

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