Received: 2 May 2022 | Accepted: 31 August 2022

DOI: 10.1111/ppa.13652

Plant Pathology WILEY

ORIGINAL ARTICLE

Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose

Mario Riolo[1,2,3] | Antonella Pane[2] | Elena Santilli[1] | Salvatore Moricca[4] | Santa Olga Cacciola[2]

[1]Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus crops (CREA- OFA), Cosenza, Italy

[2]Department of Agriculture, Food and Environment, University of Catania, Catania, Italy

[3]Department of Agricultural Science, Mediterranean University of Reggio Calabria, Reggio Calabria, Italy

[4]Department of Agricultural, Food, Environmental and Forestry Science and Technology, University of Florence, Florence, Italy

Correspondence
Santa Olga Cacciola, Department of Agriculture, Food and Environment, University of Catania, Catania, Italy.
Email: olga.cacciola@unict.it

Abstract

Fruit anthracnose caused by *Colletotrichum* species is a major disease of olive (*Olea europaea*) worldwide. In this study, we tested in vitro the susceptibility of eight widely grown Italian olive cultivars and one Spanish cultivar to five *Colletotrichum* species. The Italian cultivars were Carolea, Cassanese, Coratina, Dolce Agogia, Frantoio, Leccino, Ottobratica and Sant'Agostino. The Spanish cultivar, included as a reference, was Picual. The five *Colletotrichum* species, included in pathogenicity tests, were *C. acutatum*, *C. gloeosporioides*, *C. godetiae*, *C. karsti* and *C. nymphaeae*. Olive drupes at comparable ripening stage were wound-inoculated with a conidial suspension and the severity of infections was rated at various time intervals after inoculation using a scale of 0 to 6. The results were expressed in terms of relative area under disease progress curve (rAUDPC). *C. acutatum* was the most aggressive while *C. karsti* was the least aggressive among the *Colletotrichum* species tested. Frantoio and Leccino were the least susceptible cultivars while Ottobratica, Coratina and Carolea were the most susceptible to all *Colletotrichum* species. Separate experiments aimed to evaluate the effect of both inoculation method and drupe ripening stage on the interaction between *Colletotrichum* species and olive cultivars. Only *C. acutatum* and *C. nymphaeae* induced symptoms in nonwounded drupes. In general, the disease severity in green drupes was significantly lower than in mature drupes; however, the rankings of olive cultivars for their susceptibility to *Colletotrichum* species on both green and mature drupes showed similar trends.

KEYWORDS
area under disease progress curve (AUDPC), *Colletotrichum acutatum*, ITS and TUB2, *Olea europaea*, phylogenetic analysis

1 | INTRODUCTION

Olive anthracnose (OA), caused by *Colletotrichum* species, is the most damaging disease of olive fruit worldwide and, together with Mediterranean olive fruit fly (*Bactrocera oleae*) and olive leaf spot (*Venturia oleaginea*), is a major phytopathological concern for olive crops in the Mediterranean region (Cacciola et al., 2012; Kolainis et al., 2020; Moral et al., 2018; Scibetta et al., 2020; Talhinhas et al., 2011, 2015). It causes a substantial deterioration of oil quality, the severity of which depends on the proportion of infected fruits,

Elena Santilli, Salvatore Moricca and Santa Olga Cacciola contributed equally.

the *Colletotrichum* species inciting the disease and the olive cultivar (Gouvinhas et al., 2019; Moral et al., 2018; Peres et al., 2021). In some areas of southern Italy, where environmental conditions, such as a warm, rainy and humid climate during fruit ripening, are disease conducive, OA is endemic, with severe epidemic outbreaks occurring frequently. Besides environmental conditions, the susceptibility of olive cultivars, the ripening stage of drupes and the differences in virulence of *Colletotrichum* species responsible for the disease are major driving factors triggering the onset and conditioning the severity of epidemics. A direct correlation was observed between the severity of OA outbreaks and attacks by the Mediterranean olive fly or the Queensland fruit fly (*B. tryoni*), as wounds caused by these insect pests on olive drupes facilitate pathogen entry (Graniti et al., 1993; Sergeeva & Spooner-Hart, 2011; Talhinhas et al., 2018). So far, around 18 *Colletotrichum* species, most within the *C. acutatum*, *C. gloeosporioides* and *C. boninense* species complexes, have been reported to be associated with OA worldwide, but not all species are equally aggressive or are responsible for disease outbreaks (Antelmi et al., 2018; Cacciola et al., 2012; Faedda et al., 2011; Moral et al., 2021; Moreira et al., 2021; Schena et al., 2014; Talhinhas et al., 2005, 2015, 2018). The *Colletotrichum* species associated most frequently with olive anthracnose outbreaks in the Mediterranean macroregion are *C. acutatum*, *C. godetiae* (syn. *C. clavatum*) and *C. nymphaeae*, all of which are in the *C. acutatum* species complex. Species in the *C. gloeosporioides* species complex, such as *C. gloeosporioides* sensu stricto (subsequently referred to as *C. gloeosporioides*), and those in the *C. boninense* species complex, such as *C. karsti*, occur sporadically and in a lower proportion. Although the role of *C. gloeosporioides* in OA outbreaks is controversial, some isolates of this species were virulent in tests on detached olive drupes; in contrast, in the same experiments, isolates of *C. karsti* were only weakly pathogenic (Schena et al., 2014). However, both species are common pathogens of citrus in the Mediterranean macroregion (Ramos et al., 2016; Riolo et al., 2021). The distribution and prevalence of *Colletotrichum* species may vary in different olive-growing countries or areas within the same country, and even on different organs of the tree (Cacciola et al., 2012; Chattaoui et al., 2016; Kolainis et al., 2020; Materatski et al., 2018; Moreira et al., 2021; Mosca et al., 2014; Peres et al., 2021; Talhinhas et al., 2009, 2018). Seasonal fluctuations of *Colletotrichum* populations and the presence of different species have also been observed (Schena et al., 2017). Until recently, *C. godetiae*, originally described as *C. clavatum*, was the prevalent species associated with OA outbreaks in Calabria (southern Italy) (Faedda et al., 2011; Moral et al., 2021). However, in the last decade, *C. acutatum* has prevailed over *C. godetiae* and is presently the most frequent species associated with the disease in this region (Pangallo et al., 2022; Schena et al., 2017). Interestingly, *C. acutatum* has recently been reported for the first time as causal agent of olive anthracnose in Albania and Greece (Cara et al., 2021; Iliadi et al., 2018); these were the countries from which it is very probable *C. godetiae*, the species responsible for the first epidemic outbreaks of OA in the 1950s, was inadvertently introduced into southern regions of Italy (Cacciola et al., 2012). Moreover, *C. acutatum*, which is supposed to be a species native to the southern hemisphere, is already widespread in some olive-growing regions of Portugal and Tunisia (Chattaoui et al., 2016; Talhinhas et al., 2015). A similar shift in *Colletotrichum* population associated with OA is probably occurring in olive orchards in Portugal. *C. godetiae*, which is presently the prevalent species in the north-eastern part of the country, is being replaced by the more aggressive *C. nymphaeae*, which has been already dominant in olive orchards of the south-western part of the country for many years (Materatski et al., 2018; Talhinhas et al., 2011, 2015, 2018). Also, in several olive-growing countries where OA is present, differences in susceptibility among olive cultivars were observed and genetic resistance to the disease was considered as part of an integrated strategy to manage OA (Cacciola et al., 2012; Moral et al., 2008, 2015, 2017, 2018; Talhinhas et al., 2018). Differences in field susceptibility have been reported among different olive cultivars in Italy, Spain and Portugal (Cacciola et al., 2012; Moral et al., 2017; Talhinhas et al., 2018). Talhinhas et al. (2015) evaluated the susceptibility of eight olive cultivars commonly grown in Portugal to the infections of diverse *Colletotrichum* species in the *C. acutatum* and *C. gloeosporioides* species complexes, including *C. acutatum*, *C. godetiae*, *C. nymphaeae*, *C. fioriniae*, *C. rhombiforme* and *C. gloeosporioides*. They found a high variability in virulence among *Colletotrichum* species as well as a significant cultivar × *Colletotrichum* isolate interaction. Moral et al. (2017) screened a large collection of olive cultivars of worldwide origin for susceptibility to natural OA infections in a major olive-growing area in Spain, where the largely prevalent *Colletotrichum* species was recently demonstrated to be *C. godetiae* (Moral et al., 2021). These authors selected a representative cultivar for each susceptibility class (highly susceptible, susceptible, moderately susceptible, resistant and highly resistant), using Picual as representative of resistant cultivars and Frantoio as representative of highly resistant cultivars. Inoculation of detached olive drupes under controlled environmental conditions has been used by many researchers as a rapid test to evaluate the pathogenicity of *Colletotrichum* isolates and the susceptibility of olive cultivars to anthracnose (Moral et al., 2008; Moral & Trapero, 2009; Talhinhas et al., 2015; Schena et al., 2014). With a few exceptions, the results of artificial inoculations on detached drupes were consistent with the rating of olive cultivars in field trials for susceptibility to OA infections (Moral & Trapero, 2009). As in the case of natural OA infections, the susceptibility of olive drupes artificially inoculated with *Colletotrichum* increased with maturity. No standard protocol has been universally accepted for in vitro artificial inoculations of olive drupes to test their susceptibility to *Colletotrichum* infections. Moral et al. (2008) evaluated the effect of wounding on the susceptibility of detached drupes to artificial inoculation with *Colletotrichum* and concluded that although wounding was not necessary for fruit infection, it enhanced the severity of symptom expression. This is consistent with the observation that in the field, susceptibility to OA increases when fruit are wounded by olive fly (Graniti et al., 1993; Mateo-Sagasta, 1968; Sergeeva & Spooner-Hart, 2011; Talhinhas et al., 2018). According to Moral et al. (2008), in assays on detached olive drupes, differences in

resistance between olive cultivars were more easily detected when a high concentration of inoculum (10^5–10^6 conidia/ml) and less mature (green) drupes were used. Talhinhas et al. (2015) inoculated unwounded mature olive drupes with a drop of conidial suspension (10^6 conidia/ml) to test the virulence of isolates of diverse species of *Colletotrichum* on a set of olive cultivars commonly grown in Portugal. Conversely, Schena et al. (2014) used wounded mature drupes and a mycelium plug as inoculum to evaluate the differences in pathogenicity of *Colletotrichum* species of the *C. gloeosporioides* and *C. boninense* species complexes.

In this study, we aimed to investigate the interaction between popular Italian olive cultivars and the Spanish cultivar Picual with five different species of *Colletotrichum*, (*C. acutatum*, *C. gloeosporioides*, *C. godetiae*, *C. karsti* and *C. nymphaeae*); the olive cultivar Picual was included as a reference due to its known resistance to OA in field (Gouvinhas et al., 2019; Moral et al., 2017; Moral & Trapero, 2009; Talhinhas et al., 2015). In addition, the effect of the inoculation method (with or without wounding) and the ripening stage (green and mature) of olive fruits on the *Colletotrichum* species x olive cultivar interaction was evaluated.

2 | MATERIALS AND METHODS

2.1 | Fungal isolates and production of inoculum

This study included 10 fungal isolates of five different *Colletotrichum* species, *C. acutatum*, *C. gloeosporioides*, *C. godetiae*, *C. karsti* and *C. nymphaeae*. All isolates, except C9D2C, RD9B and C12D1A, were identified in previous studies based on morphological characteristics and phylogenetic sequence analysis of the internal transcribed spacer regions of rDNA (ITS), intervening 5.8S nrDNA, as well as part of the β-tubulin gene (*TUB2*) regions as barcode markers (Aloi et al., 2021; Cacciola et al., 2020). The following isolates were used in pathogenicity tests: isolate C9D2C and UWS149 of *C. acutatum* s.s.; isolates RD9B and AC24 of *C. gloeosporioides*; isolates OLP12 and OLP16 of *C. godetiae*; isolates RB012 and RB428 of *C. nymphaeae*; isolates CAM, C12D1A and ALL2I of *C. karsti* (Table 1). The isolates C12D1A and ALL21 of *C. karsti* were selected in the preliminary screening on olive drupes; they were the most virulent in a set of 10 *C. karsti* isolates, including five isolates from olive collected in southern Italy. All isolates included in this study were sourced from the culture collection of the Molecular Plant Pathology Laboratory of the Department of Agriculture, Food and Environment of the University of Catania, Italy.

A conidial suspension of each isolate at a concentration of 10^6 conidia/ml in sterile distilled water (SDW) was prepared from 10-day-old cultures grown on potato dextrose agar (PDA; Oxoid Ltd) at 25°C and used as inoculum. To produce the inoculum, single-conidium isolates were cultured in Petri dishes containing PDA. Dishes were incubated at 23 ± 2°C with a 12-h photoperiod of fluorescent light (40 μmol·m^{-2}·s^{-1}). After 1 week incubation, dishes were flooded with SDW and mycelium was scraped with a spatula. The suspension was filtered through four layers of sterile gauze, and the concentration of inoculum was measured with a haemocytometer and then adjusted to obtain 10^6 conidia/ml.

2.2 | Molecular characterization of fungal isolates

The RD9B, C9D2C and C12D1A isolates, which had not been previously identified, were grown on PDA for 7 days at 25 ± 1°C. Mycelium of each isolate was harvested with a sterile scalpel, and the genomic DNA was extracted using a PowerPlant Pro DNA isolation Kit (MO BIO Laboratories, Inc.), following the manufacturer's protocol. The DNA was preserved at −20°C. The ITS1–5.8S–ITS2 region and the fragment of the β-tubulin gene (*TUB2*) between exons 2 and 6 were amplified with primers ITS5 and ITS4 and primers βt2a and βt2b (Glass & Donaldson, 1995; White et al., 1990), respectively, and sequenced to confirm the isolate species. PCR amplifications were performed on a GeneAmp PCR System 9700 (Applied Biosystems). All PCRs were carried out using *Taq* DNA polymerase recombinant

TABLE 1 GenBank accession numbers and sources of isolates of *Colletotrichum* used in this study

Isolate code	Species	Host species	Organ	Geographical origin	GenBank accession	
					ITS-rDNA	*T*
UWS149	*C. acutatum*	*Olea europaea*	Fruit	Australia	MT997785	MW001517
C9D2C	*C. acutatum*	*O. europaea*	Fruit	Calabria	MZ502315	MZ508448
AC24	*C. gloeosporioides*	*Citrus sinensis*	Leaf	Sicily	MT997808	MW001540
RD9B	*C. gloeosporioides*	*O. europaea*	Fruit	Calabria	MZ502314	MZ508447
OLP 12	*C. godetiae*	*O. europaea*	Fruit	Calabria	JN121131	JN121218
OLP 16	*C. godetiae*	*O. europaea*	Fruit	Calabria	JN121137	JN121224
ALL2I	*C. karsti*	*C. sinensis*	Twig	Sicily	MT997861	MW001546
CAM	*C. karsti*	*Camellia* sp.	Leaf	Sicily	KC425664	KC425716
C12D1A	*C. karsti*	*O. europaea*	Fruit	Sicily	ON231821	ON246203
RB012	*C. nymphaeae*	*O. europaea*	Fruit	Spain	JQ948201	JQ949852
RB428	*C. nymphaeae*	*Jugla* sp.	Bud	France	MG58986	MG666308

(Invitrogen) in a total volume of 25 µl containing PCR buffer (1×), dNTP mix (0.2 mM), $MgCl_2$ (1.5 mM), forward and reverse primers (0.5 µM each), *Taq* DNA polymerase (1 U) and 1 µl of genomic DNA. Reaction conditions were 94°C for 3 min; followed by 35 cycles of 94°C for 30 s, 58°C (ITS region)/60°C (*TUB2*) for 30 s, and 72°C for 30 s; followed by an additional 10 min extension at 72°C.

Amplified products were analysed by agarose gel electrophoresis and single bands of the expected size were purified with the QIAquick PCR Purification Kit (Qiagen) and sequenced with both forward and reverse primers by Macrogen Europe (Amsterdam, Netherlands). The ChromasPro v. 1.5 software was used to evaluate the reliability of sequences and to create consensus sequences. Unreliable sequences in which both forward and reverse sequences, or one or the other, were not successful or contained doubtful bases were resequenced. The ITS and *TUB2* sequences obtained in the present study were deposited in GenBank and the accession numbers are presented in Table 1. Validated sequences representative of all species identified within the *C. acutatum*, *C. boninense* and *C. gloeosporioides* species complexes were phylogenetically analysed to determine the relationship between different isolates and define their taxonomic status. Sequences from ex-type or authentic culture were included in the analysis as reference (Table 2).

2.3 | Morphological characteristics and optimum growth temperature of fungal isolates

Agar plugs (5 mm) were taken from the edge of actively growing cultures on PDA and transferred onto the centre of 9-cm diameter Petri dishes containing PDA. Dishes were incubated at 25°C either in the dark for 10 days, to determine both the colony morphology and radial growth rate, or with continuous fluorescent light, to observe microscopic morphological traits. Conidial and mycelial suspensions were prepared in SDW from 10-day-old cultures and examined microscopically.

Optimum temperatures for radial growth were determined for all isolates used in this study. Isolates of *C. acutatum* (UWS149), *C. gloeosporioides* (AC 24) and *C. karsti* (ALL 2I), characterized in previous studies, were used as reference (Riolo et al., 2021). Agar plugs (5 mm) were taken from the edge of actively growing cultures on PDA and transferred onto the centre of 9-cm diameter Petri dishes containing PDA. Dishes were incubated at 5, 7, 10, 12, 15, 20, 25, 30 and 35°C in the dark with three replicates per temperature and per isolate. Two orthogonal diameters were measured per colony after 3, 5 and 7 days' incubation. The experiment was conducted twice with similar results, so, the results of only one experiment are reported.

2.4 | Plant material for pathogenicity tests

The following nine olive (*Olea europaea*) cultivars were included in this study to evaluate their susceptibility to infection by diverse *Colletotrichum* species: Carolea, Cassanese, Coratina, Dolce Agogia, Frantoio, Leccino, Ottobratica, Picual and Sant'Agostino. All the cultivars were of Italian origin, with the only exception of Picual, which was included as a reference.

The ripening stage of olive drupes was determined by their colour according to the maturation index (MI) of Guzmán et al. (2015), with value 0 corresponding to >50% bright green and value 4 to 100% blackish-purple or black. In tests aimed at evaluating only the *Colletotrichum* species × olive cultivar interaction, drupes with MI values between 3 and 4 were used. However, in comparative tests aimed at evaluating the effect of ripening stage and wounding on the *Colletotrichum* species × olive cultivar interaction, we used both mature (MI 4) and green (MI 2) drupes of Coratina collected from the same trees during the véraison (onset of ripening). Olive fruits were collected from 15-year-old olive trees from two distinct experimental orchards in Calabria (southern Italy): one in the municipality of Mirto-Crosia, province of Cosenza (DATUM WGS 84, 39°36′54.5″ N 16°46′11.7″ E) and another in the municipality of Rende, province of Cosenza, (DATUM WGS 84, 39°21′59.4″ N 16°13′44.4″ E). Drupes of the same cultivar from the two orchards, and from three distinct trees per orchard, were pooled together. Drupes were stored for 3 h inside a refrigerated bag, before being transported to the laboratory and were inoculated the day after collection. Before the inoculation, they were surface disinfected by immersion in a 0.5% NaOCl solution for 30 s, rinsed in sterile distilled water, blotted dry and placed in incubation trays.

2.5 | Inoculation and rating of disease severity

Drupes were punctured with a sterile needle in an equatorial position and a 20 µl droplet of the conidial suspension (10^6 conidia/ml), prepared as described above, was pipetted onto the surface of the wound. Control drupes received 20 µl of SDW.

In the first set of experiments, four fungal isolates (one isolate per *Colletotrichum* species) and nine olive cultivars were tested. The experimental design was a complete randomized block with four replications. In each block, 25 drupes were inoculated per isolate × cultivar combination, that is, a total of 500 drupes per cultivar were used in each experiment, including the noninoculated control. After inoculation, drupes were incubated in a humid chamber at 23 ± 1°C, with 80% relative humidity and a photoperiod of 16 h of light and 8 h of dark. Health conditions of inoculated drupes were assessed at 3, 5 and 7 days postinoculation (dpi).

In the second set of experiments, the *Colletotrichum* species × olive cultivar interaction was evaluated using different isolates of the *Colletotrichum* species tested, and also including *C. nymphaeae*, which was the species responsible for severe epidemics in south-western part of Portugal. Ten isolates (two for each of the five *Colletotrichum* species tested, *C. acutatum*, *C. gloeosporioides*, *C. godetiae*, *C. karsti* and *C. nymphaeae*) and four olive cultivars (Coratina, Frantoio, Leccino and Ottobratica) were included. All isolates were from olive except for the isolates CAM and ALL2I of *C. karsti* and isolate AC24 of *C. gloeosporioides*.

TABLE 2 GenBank accession numbers of sequences of the Colletotrichum isolates of worldwide origin used as references in phylogenetic analyses

Species	Isolate	Clade	Origin	Host	Source	GenBank accession no.	
						ITS-rDNA	*β-tubulin*
*C. acutatum***	IMI 117620	acutatum	Australia	*Carica papaya*	Shivas and Tan (2009)	FJ788417	FJ788419
C. acutatum	CBS 127598	acutatum	South Africa	*Olea europaea*	Damm et al. (2012a)	JQ948363	JQ950014
C. acutatum	CBS 144.29	acutatum	Sri Lanka	*Capsicum annuum*	Damm et al. (2012a)	JQ948401	JQ950052
*C. aenigma**	ICMP 18608	gloeosporioides	United States	*Persea americana*	Weir et al. (2012)	JX010244	JX010389
*C. aeschynomenes**	ICMP 17673	gloeosporioides	United States	*Aeschynomene virginica*	Weir et al. (2012)	JX010176	JX010392
*C. alatae**	CBS 304.67	gloeosporioides	India	*Dioscorea alata*	Weir et al. (2012)	JX010190	JX010383
*C. alienum**	ICMP 12071	gloeosporioides	New Zealand	*Malus domestica*	Weir et al. (2012)	JX010251	JX010411
*C. annellatum**	CBS 129826	boninense	Colombia	*Hevea brasiliensis*	Damm et al. (2012b)	JQ005222	JQ005656
*C. aotearoa**	ICMP 18537	gloeosporioides	New Zealand	*Coprosma* sp.	Weir et al. (2012)	JX010205	JX010420
*C. beeveri**	CBS 128527	boninense	New Zealand	*Brachyglottis repanda*	Damm et al. (2012b)	JQ005171	JQ005605
*C. boninense**	CBS 123755	boninense	Japan	*Crinum asiaticum* var. *sinicum*	Damm et al. (2012b)	JQ005153	JQ005588
*C. brasiliense**	CBS 128501	boninense	Brazil	*Passiflora edulis*	Damm et al. (2012b)	JQ005235	JQ005669
*C. brisbaniense**	CBS 292.67	acutatum	Australia	*C. annuum*	Damm et al. (2012a)	JQ948291	JQ949942
*C. carthami***	SAPA100011	acutatum	Japan	*Carthamus tinctorius*	Uematsu et al. (2012)	AB696998	AB696992
*C. clidemiae**	ICMP 18658	gloeosporioides	United States	*Clidemia hirta*	Weir et al. (2012)	JX010265	JX010438
*C. colombiense**	CBS 129818	boninense	Colombia	*Passiflora edulis*	Damm et al. (2012b)	JQ005174	JQ005608
*C. fioriniae**	CBS 128517	acutatum	United States	*Fiorinia externa*	Damm et al. (2012a)	JQ948292	JQ949943
*C. gloeosporioides***	CBS 112999	gloeosporioides	Italy	*Citrus sinensis*	Damm et al. (2012b)	JQ005152	JQ005587
*C. gloeosporioides**	STE-U4295	gloeosporioides	Italy	*Citrus* sp.	Lubbe et al. (2004)	AY376532	AY376580
*C. godetiae**	CBS 133.44	gloeosporioides	Denmark	*Clarkia hybrida*	Damm et al. (2012b)	JQ948402	JQ950053
C. godetiae	CBS 160.50	gloeosporioides	–	*Citrus aurantium*	Damm et al. (2012a)	JQ948406	JQ950057
C. godetiae	CBS 796.72	gloeosporioides	United States	*Aeschynomene virginica*	Damm et al. (2012a)	JQ948407	JQ950058
*C. karsti**	CBS 132134/ CORCG6	boninense	China	*Vanda* sp.	Yang et al. (2011)	HM585409	HM585428
C. karsti	CBS 106.91	boninense	Brazil	*C. papaya*	Schena et al. (2014)	JQ005220	JQ005654
*C. nymphaeae**	CBS 515.78	acutatum	–	*Nymphaea alba*	Moral et al. (2017)	JQ948197	JQ949848
C. nymphaeae	CBS 231.49	acutatum	–	*O. europaea*	Moral et al. (2017)	JQ948202	JQ949853
*C. paspali**	MAFF 305403	graminicola	Japan	*Paspalum notatum*	Cannon et al. (2012)	EU554100	JX519244
Monilochaetes infuscans	CBS 869.96	–	South Africa	*Ipomoea batatas*	Liu et al. (2014)	JQ005780	JQ949805

*Ex-holotype; **Epitype.

Olive drupes were inoculated by wounding, as described previously. After inoculation, drupes were incubated in plastic boxes at 23 ± 1°C, with 80% relative humidity and a photoperiod of 16 h of light and 8 h of dark. The severity of symptoms on inoculated drupes was rated at 3, 5 and 7 dpi. The experimental design was a complete randomized block with four replicates and six drupes per replicate.

The third set of experiments evaluated the effect of both wounding and maturity stage on the response of drupes to inoculation with isolates of diverse *Colletotrichum* species. Both mature (MI 4) and green (MI 2) drupes of Coratina were inoculated singly with four *Colletotrichum* species (two diverse isolates of each species). The following fungal isolates were included: isolates C9D2C and UWS149 of *C. acutatum*, isolates RB012 and RB428 of *C. nymphaeae*, isolates OLP12 and OLP16 of *C. godetiae*, and isolates RD9B and AC24 of *C. gloeosporioides*. Both mature and green drupes were split into two subgroups encompassing drupes inoculated by wounding and drupes inoculated without wounding. A 20 µl drop of a conidial suspension (10^6 conidia/ml) in 0.5% water agar was pipetted on unwounded drupes in the equatorial position. Wounded drupes received the same amount of inoculum and were inoculated with the method described previously. After inoculation, both wounded and unwounded drupes were incubated in plastic boxes at 23 ± 1°C, with 80% relative humidity and a photoperiod of 16 h of light and 8 h of dark. The experimental design was a complete randomized block with four replicates and six drupes per replicate. The severity of symptoms on wounded and unwounded drupes was monitored up to 7 and 14 dpi, respectively.

The disease severity index (DSI) on olive fruits was scored on an empirical scale in accordance with Talhinhas et al. (2015): 0, no symptoms; 1, mycelium only; 2, small necrosis (<5 mm diameter) and absence of sporulation; 3, large necrosis (>5 mm diameter) and absence of sporulation; 4, few spore masses on the inoculation point; 5, abundant spore masses expanding away from the inoculation point; 6, spore masses entirely covering the fruit (Figure 1).

The symptoms were recorded daily after inoculation and the absolute area under the disease progress curve (AUDPC) was calculated conforming with Talhinhas et al. (2015). Then, the relative area under disease progress curve (rAUDPC) was estimated and is shown in the figures.

Each set of experiments was performed for two consecutive years, with similar results, so the results of only the second year are reported (2020 for the first set and 2021 for the second and third sets).

2.6 | Statistical analysis

All the data were normalized by square root transformation and then subjected to analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test as a post hoc test (R software). Differences at $p \leq 0.05$ were considered significant.

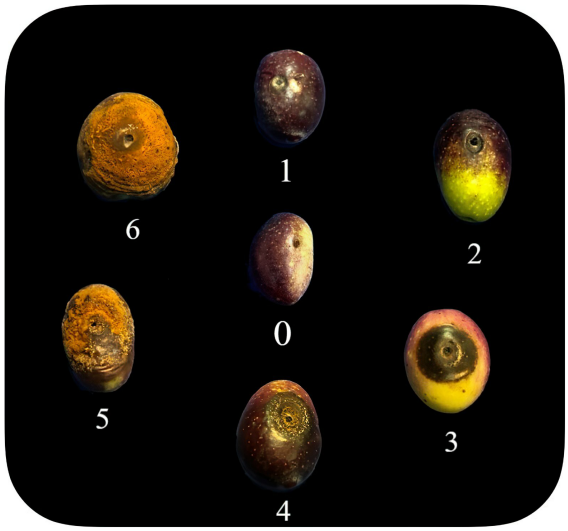

FIGURE 1 Rating scale used in this study to assess the disease severity index of olive anthracnose on olive drupes. 0, no symptoms; 1, mycelium only; 2, small necrosis (<5 mm diameter) and absence of sporulation; 3, large necrosis (>5 mm diameter) and absence of sporulation; 4, few spore masses on the inoculation point; 5, abundant spore masses expanding around the inoculation point; 6, spore masses entirely covering the fruit [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Phylogenetic analysis

Phylogenetic analysis was carried out on the combined data set of sequences from ITS and *TUB2* regions of all single-conidium *Colletotrichum* isolates tested in this study (Table 1), along with sequences of the reference isolates of *C. acutatum* (UWS 149), *C. gloeosporioides* (AC24) and *C. karsti* (CAM) and the reference sequences of *Colletotrichum* species separated within the *C. gloeosporioides*, *C. boninense* and *C. acutatum* species complexes. The phylogenetic tree (Figure 2) produced had similar topology and a high concordance with those reported by the authors who revised the systematics of these species complexes using multigene sequence analysis (Damm et al., 2012a, 2012b; Weir et al., 2012).

3.2 | Growth temperature assays

Between temperatures of 12 and 35°C, the *C. gloeosporioides* isolates grew faster than the isolates of the *C. acutatum* species complex, as expected. All tested isolates of both *C. gloeosporioides* and *C. karsti* as well as the isolates of *C. acutatum* and *C. godetiae* showed an optimum temperature for radial growth at 25°C and grew faster at 20 than at 30 or 35°C (Figure 3). None of the isolates grew at 5°C and growth was not resumed when the Petri dishes were transferred to 25°C after 7 days of incubation at 5°C. However, at 30 and 35°C, *C. gloeosporioides* isolates were less inhibited than isolates of the other species. In particular, the radial growth of the two tested

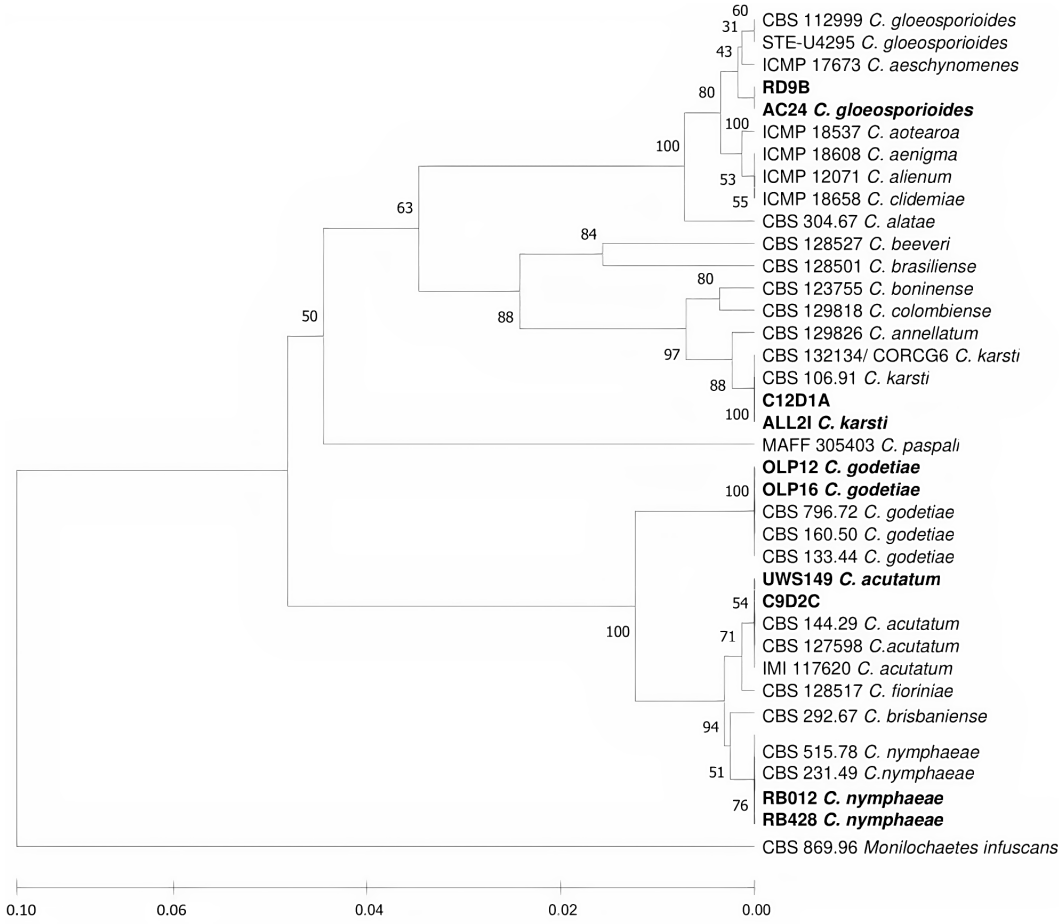

FIGURE 2 Phylogenetic tree obtained using combined internal transcribed spacers (ITS) and β-tubulin (*TUB2*) sequences of isolates of *Colletotrichum* species used in the present study (in bold) along with reference isolates of *C. karsti*, *C. gloeosporioides* and other representative species in the *C. boninense*, *C. gloeosporioides* and *C. acutatum* complexes. The evolutionary history was inferred using the maximum-likelihood method based on the Tamura–Nei model and the tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches

C. gloeosporioides isolates at 30° C was reduced only by 8.7% and 4.5%, respectively, compared with the growth at 25°C, and by 25% and 18%, respectively, at 35°C. In contrast, the growth of the two *C. karsti* isolates at 30°C was reduced by 30% and 37.5% compared with growth at 25°C, while at 35°C it was inhibited by 55% and 42.5%, respectively. The growth of the two isolates of *C. acutatum* was also dramatically reduced at 30°C, by 59% and 61.5%, respectively. Similarly, the radial growth of *C. godetiae* isolates at 30°C was reduced by 60.5% and 62%, respectively, compared with growth at 25°C, and by 60% for both isolates, compared with their growth at 20°C. Conversely, at 10°C, the *C. godetiae* and *C. karsti* isolates grew significantly faster than *C. gloeosporioides* and *C. acutatum* isolates.

3.3 | Pathogenicity assays

In the first set of pathogenicity assays, isolates of all *Colletotrichum* species were pathogenic to wound-inoculated drupes of olive cultivars while no disease symptoms were recorded in control fruits. However, isolates differed markedly in virulence. Also, olive cultivars showed substantial differences in susceptibility to the infection by diverse *Colletotrichum* species. Statistical analysis of values of disease intensity over time (rAUDPC) revealed significant differences in both virulence among *Colletotrichum* isolates and susceptibility among olive cultivars and showed a significant isolate × cultivar interaction ($p < 0.05$) (Figure 4).

The most virulent among the isolates tested was the *C. acutatum* isolate, while the most resistant to this aggressive *Colletotrichum* species among the olive cultivars tested was Leccino, although the response of this cultivar did not differ significantly from that of Frantoio. Moreover, Leccino was the cultivar that segregated most clearly from the others for its low susceptibility to *C. gloeosporioides* infections. The mean rAUDPC values in this cultivar were 0.38 for *C. acutatum*, 0.04 for *C. gloeosporioides*, 0.11 for *C. godetiae* and 0.05 for *C. karsti*. Only Frantoio showed resistance to artificial infections of *C. acutatum*, and it was comparable with that of Leccino. The susceptibility of Picual to this *Colletotrichum* species did not differ significantly ($p > 0.05$) from the susceptibility of cultivars commonly regarded as very susceptible to OA infections in field, such as Carolea, Coratina and Ottobratica. The least virulent among the

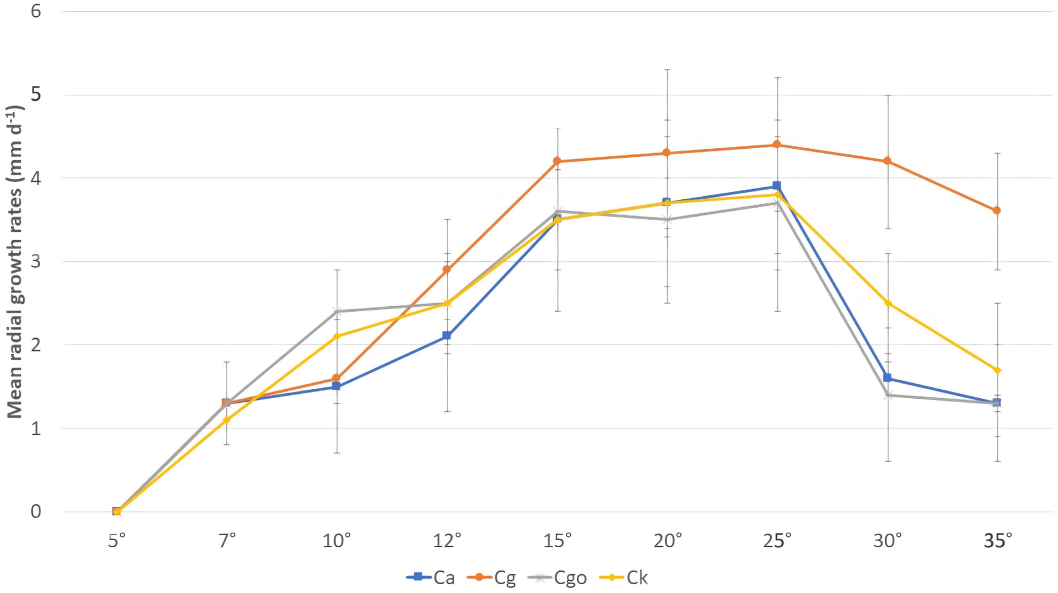

FIGURE 3 Mean radial growth rates of isolates of *Colletotrichum acutatum* (Ca), *C. gloeosporioides* (Cg), *C. godetiae* (Cgo) and *C. karsti* (Ck) on potato dextrose agar at different temperatures [Colour figure can be viewed at wileyonlinelibrary.com]

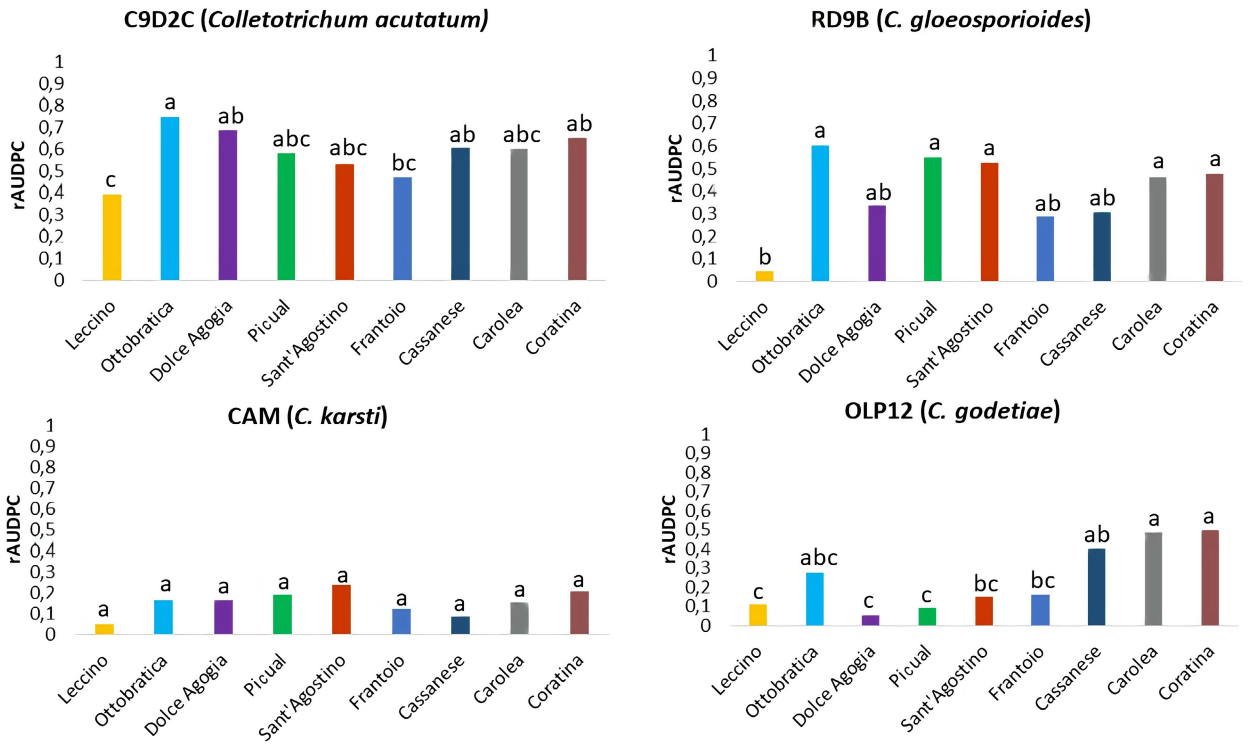

FIGURE 4 Mean olive anthracnose intensity over time (relative area under the disease progress curve [rAUDPC]) on drupes of nine olive cultivars inoculated with isolates of *Colletotrichum acutatum*, *C. gloeosporioides*, *C. godetiae* or *C. karsti* (one isolate per species). Severity of symptoms on inoculated olive drupes was assessed daily for 7 days and rated on a 0 to 6 scale according to the size of necrotic lesion and the abundance of fungal sporulation. Values sharing a common letter are not statistically different according to Tukey's honestly significant difference (HSD) test ($p \leq 0.05$). Analysis of variance F (8,81) value of C9D2C: 9.6, $p \leq 0.05$; RD9B: 9.4, $p \leq 0.05$; CAM: $p > 0.05$; OLP 12: 5.3, $p \leq 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]

isolates tested was the *C. karsti* isolate, which was weakly pathogenic on all olive cultivars. No significant difference ($p < 0.05$) in susceptibility to this *Colletotrichum* species was detectable among the olive cultivars tested, probably due to a low level of virulence (Figure 4). Conversely, more clear differences in susceptibility were observed among olive cultivars inoculated with the *C. godetiae* isolate. Dolce Agogia, Frantoio, Leccino and Picual were the least susceptible while Carolea and Coratina were the most susceptible

to this *Colletotrichum* species. The last two cultivars, together with Ottobratica, were also among the most susceptible to *C. acutatum* and *C. gloeosporioides* with mean rAUDPC values of 0.6 and 0.46, respectively, for Carolea, 0.65 and 0.47, respectively, for Coratina, and 0.74 and 0.6, respectively, for Ottobratica. Both the Picual and Dolce Agogia cultivars that were relatively resistant to *C. godetiae* (mean rAUDPC values 0.09 and 0.05, respectively), proved to be highly susceptible to *C. acutatum* (mean rAUDPC values 0.57 and 0.68, respectively).

The results of the second set of experiments confirmed the olive cultivars tested differed in susceptibility to *Colletotrichum* species (Figure 5). Coratina and Ottobratica were shown to be susceptible while Frantoio and Leccino were relatively resistant to all the *Colletotrichum* species tested. *C. acutatum* was confirmed to be the most virulent among the *Colletotrichum* species tested, followed by *C. nymphaeae*. Conversely, *C. karsti* was again the least aggressive. No significant difference in virulence was observed between isolates of the same *Colletotrichum* species.

In the third set of experiments, only the isolates of *C. acutatum* and *C. nymphaeae* induced symptoms on unwounded drupes, and exclusively on mature ones, although in the parallel test on wounded drupes, Coratina was confirmed to be very susceptible to infections by these two *Colletotrichum* species (Figure 6). On wounded drupes, isolates of both *C. acutatum* and *C. nymphaeae* proved to be very aggressive with mean rAUDPC values for UWS149 and C9D2C *C. acutatum* isolates on ripe drupes of 0.72 and 0.6, respectively, and mean rAUDPC values for RB012 and RB428 *C. nymphaeae* isolates of 0.52 and 0.46, respectively. Conversely, isolates of *C. godetiae* were the least aggressive, with mean rAUDPC values for OLP12 and OLP16 isolates on ripe olives of 0.14 and 0.16, respectively. *C. gloeosporioides* isolates, with mean rAUDPC values for RD9B and AC24 isolates on ripe olives of 0.32 and 0.21, respectively, were more aggressive than isolates of *C. godetiae*, but less aggressive than isolates of both *C. acutatum* and *C. nymphaeae*. In general, symptoms were more severe on mature than on green drupes (Figure 6). However, the susceptibility of green drupes to diverse *Colletotrichum* species correlated with the susceptibility of mature drupes. No significant difference in virulence was observed between isolates of the same *Colletotrichum* species (Figure 6).

4 | DISCUSSION

The introduction of molecular taxonomy contributed greatly to the accurate identification and characterization of cryptic species previously merged in the *C. acutatum*, *C. boninense* and *C. gloeosporioides* species complexes. This, in turn, promoted a notable advancement in both the study and knowledge of the structure of *Colletotrichum* populations associated with OA and the disease epidemiology in different geographical areas (Cacciola et al., 2012; Kolainis et al., 2020; Moral et al., 2021; Moral & Trapero, 2009;

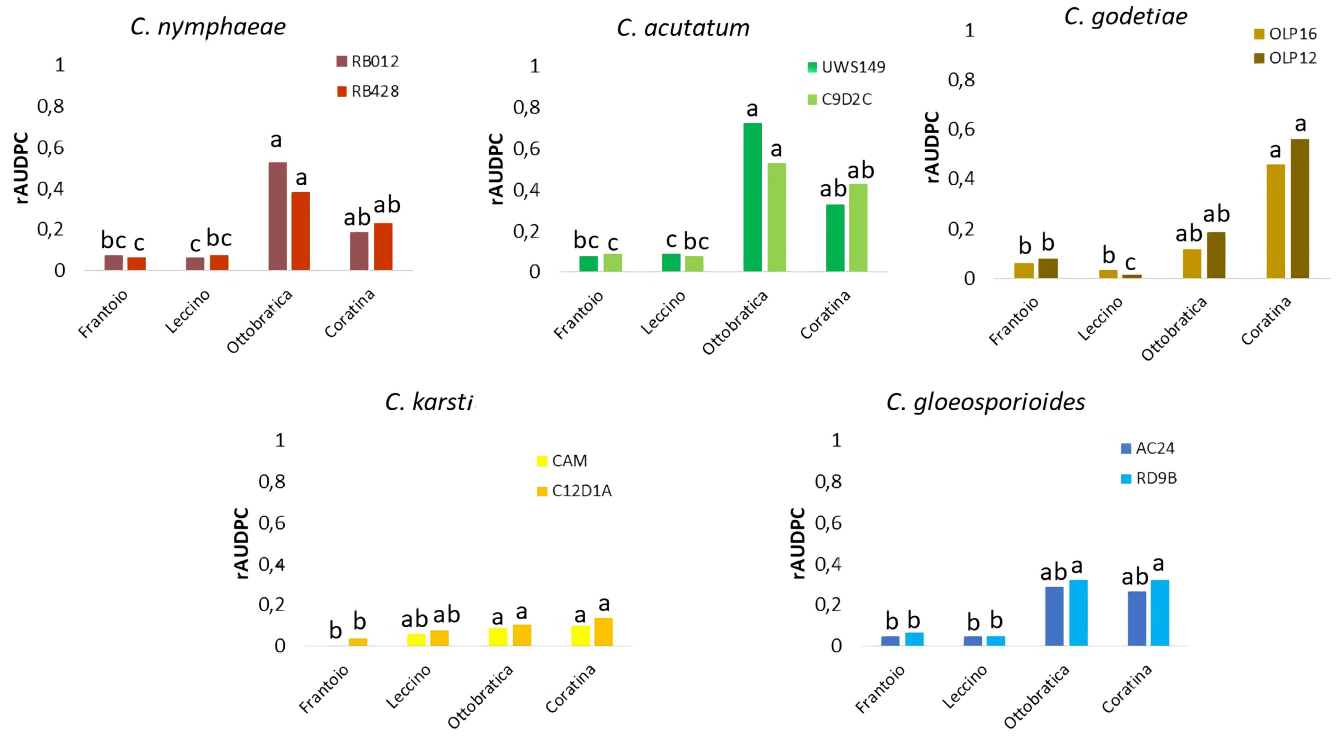

FIGURE 5 Olive anthracnose intensity over time (relative area under the disease progress curve [rAUDPC]) on drupes of four olive cultivars inoculated with isolates of *Colletotrichum acutatum*, *C. gloeosporioides*, *C. godetiae*, *C. karsti* or *C. nymphaeae* (two isolates per species). Severity of symptoms on inoculated olive drupes was assessed daily for 7 days and rated on a 0 to 6 scale according to the size of necrotic lesion and the abundance of fungal sporulation. Values sharing a common letter are not statistically different according to Tukey's honestly significant difference (HSD) test ($p \leq 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

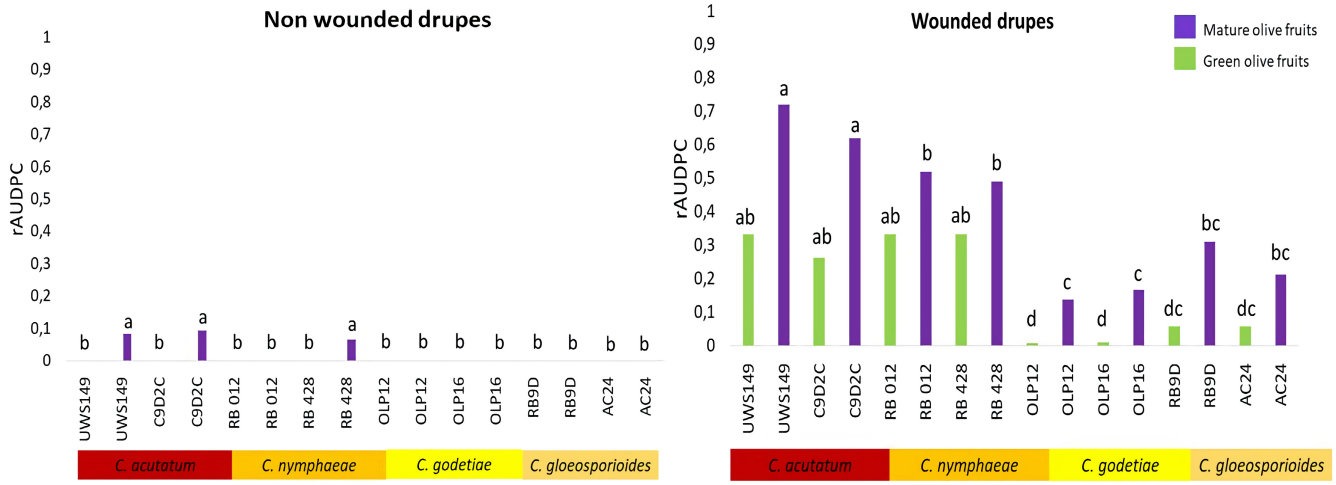

FIGURE 6 Olive anthracnose intensity over time (relative area under the disease progress curve [rAUDPC]) on ripe or green drupes of olive cultivar Coratina, which were either wounded or nonwounded and inoculated with isolates of *Colletotrichum acutatum*, *C. gloeosporioides*, *C. godetiae* or *C. nymphaeae* (two isolates per species). Severity of symptoms on inoculated olive drupes was assessed daily for 7 days for ripe olives and for 14 days for green drupes. Severity of symptoms was rated on a 0 to 6 scale according to the size of necrotic lesion and the abundance of fungal sporulation. Values sharing a common letter are not statistically different according to Tukey's honestly significant difference (HSD) test ($p \leq 0.05$) [Colour figure can be viewed at wileyonlinelibrary.com]

Moreira et al., 2021; Talhinhas et al., 2011, 2018). The present study revealed differences in temperature requirements for mycelium growth of diverse *Colletotrichum* species associated with OA. Similar results were obtained by Moral et al. (2012) in experiments aimed at evaluating the effect of temperature on conidial germination of diverse *Colletotrichum* species. These differences between species might have epidemiological implications; however, they are not sufficient to explain why some species prevail over others in different olive-growing areas. Beside climatic conditions, a major driving factor of OA epidemics is the susceptibility of olive cultivars to the disease. Different levels of in-field susceptibility to OA among Italian olive cultivars were previously reported (Cacciola et al., 2012; Graniti et al., 1993). In a field screen of the susceptibility to OA of olive cultivars of worldwide origin, Frantoio, a cultivar of Italian origin, was selected as representative of the "highly resistant" category, with a higher resistance level than Picual, a common cultivar in Portugal and Spain, which was classified as "resistant" (Moral et al., 2017). However, to the best of our knowledge, the present study is the first time that a set of olive cultivars of Italian origin has been screened in comparable conditions for their susceptibility to diverse species of *Colletotrichum* associated with OA. Both Frantoio and Picual were included in this study as references.

In the pathogenicity assays on detached olive fruits, Frantoio was confirmed to be resistant to all the species of *Colletotrichum* tested, while Picual behaved as resistant to *C. godetiae* but was found to be susceptible to the more virulent *C. acutatum* and *C. gloeosporioides*. Interestingly, so far, *C. godetiae* has been the prevalent *Colletotrichum* species associated with olive anthracnose in Spain. Carolea, Coratina and Ottobratica were found to be very susceptible, irrespective of the *Colletotrichum* species tested. Coratina, originating from the Apulia region, is the most common Italian cultivar and is grown in around 90,000 ha, most of which are in the province of Bari (Lombardo, 2003). Ottobratica is mainly grown in the alluvial plain of Gioia Tauro, in the province of Reggio Calabria (southern Italy), a vast olive-growing area of around 20,000 ha where OA is endemic. Indeed, consistent with the in vitro tests, in the Gioia Tauro Plain, Ottobratica is considered one of the local cultivars most susceptible to OA (Cacciola et al., 2012; Graniti et al., 1993). Leccino, a popular cultivar originally restricted to Tuscany and Umbria regions and presently grown also in numerous other regions of central and southern Italy (Lombardo, 2003), was shown to be as resistant as, and in some tests more resistant than, Frantoio, even to the most aggressive *Colletotrichum* species. This olive cultivar has also been proven to be tolerant of the quick decline syndrome caused by the bacterium *Xylella fastidiosa* (Boscia et al., 2017; Giampetruzzi et al., 2020). The resistance of Leccino to OA might be interesting not only in view of its widespread diffusion in commercial olive orchards, but also as a genetic source of resistance in breeding programmes of olive cultivars.

In agreement with previous results of Talhinhas et al. (2015) and Moral et al. (2021), *C. acutatum* and *C. nymphaeae* were more virulent than *C. godetiae*. Both *C. acutatum* and *C. nymphaeae* were able to sporulate on infected drupes of susceptible olive cultivars, which contributed to the high scores they attained in the pathogenicity tests (DSI values 5 and 6 in the rating scale) of our study. The production of a large amount of conidia might increase the fitness of these two species and give them an epidemiological advantage over other *Colletotrichum* species infecting olive drupes. Conversely, in agreement with a previous study by Schena et al. (2014), *C. karsti* was scarcely aggressive on olive fruits. Despite its polyphagy and widespread diffusion this *Colletotrichum* species was also shown to be a weak pathogen on other host plants, such as apple and citrus (Riolo et al., 2021). This

would confirm that the presence of *C. karsti* on olive fruits with symptoms is incidental and its role in OA epidemic outbreaks is marginal. Conversely, our study did not help to clarify the controversial role of *C. gloeosporioides* as causal agent of OA; we found that on detached olive drupes this *Colletotrichum* species was even more virulent than *C. godetiae*, although *C. godetiae* has long been, or still is, the dominant *Colletotrichum* species associated with OA in several olive-growing areas of the Mediterranean region, including Andalusia (Spain), Greece, southern Italy and north-eastern provinces of Portugal (Cacciola et al., 2012; Moral et al., 2021; Talhinhas et al., 2018). However, in contrast to *C. acutatum* and *C. nymphaeae*, we found that neither *C. gloeosporioides* nor *C. godetiae* were able to infect unwounded olive drupes. This is in agreement with Schena et al. (2014), who also found that wounding was a prerequisite for successful infections, particularly with less virulent *Colletotrichum* species, and for repeatability of results. However, Moral et al. (2008) and Talhinhas et al. (2015) reported that wounding is not necessary to have infections by *Colletotrichum* species on detached olives. A possible explanation is that green olive drupes used in our study were less mature than those used by Moral et al. (2008) and Talhinhas et al. (2015), and neither of those studies used Coratina in their tests on detached olive drupes. Nevertheless, our study confirmed the findings of Moral et al. (2008) and Talhinhas et al. (2015) that the susceptibility of detached olive drupes to artificial inoculations with *Colletotrichum* species correlated with in-field susceptibility to OA.

Another important aspect of our pathogenicity study was the evaluation of the symptoms early after inoculation (3, 5 and 7 dpi). This could have led to an underestimation of the severity of symptoms in green olives, but an early rating of symptoms on wounded ripening drupes provides a more reliable assessment of disease severity, especially in comparative studies. The rating of the severity of symptoms from 14 up to 35 dpi could result in an overestimation as well as a bias of interpretation of the severity of symptoms, as even less aggressive *Colletotrichum* strains can colonize senescent tissues.

Our evaluation of olive cultivars was in agreement with the findings of Talhinhas et al. (2015), who examined the susceptibility of eight olive cultivars commonly grown in Portugal to diverse *Colletotrichum* species. They also revealed considerable variability in both the susceptibility of olive cultivars and the virulence of *Colletotrichum* species tested and a significant interaction between olive cultivar and *Colletotrichum* species. In our study, this interaction was clearly exemplified by the cultivars Picual and Dolce Agogia, which were resistant to *C. godetiae* but susceptible to *C. acutatum*. It can be inferred that cultivars considered resistant in one geographical area where a weakly pathogenic *Colletotrichum* species prevails, may behave as susceptible in areas where a more aggressive *Colletotrichum* species is dominant, provided that environmental conditions are conducive to the disease.

A better understanding of both the population structure of *Colletotrichum* in an olive-growing area and the susceptibility of olive cultivars to the infections by different *Colletotrichum* species are prerequisites for developing an effective management strategy for OA. However, there is evidence that the structure of a *Colletotrichum* population in a given area may change, possibly rapidly, over time, depending on several factors including climate, disease management strategies, the introduction of more aggressive exotic *Colletotrichum* species from other olive-growing areas, a host jump of a *Colletotrichum* species already present on other plants, or the replacement of local olive cultivars with new, more susceptible cultivars. With regard to Italy, *C. nymphaeae*, the most common causal agent of strawberry anthracnose in this country and originally misidentified as *C. acutatum* and later as *C. simmondsii* (Agosteo et al., 2002; Faedda et al., 2011), has only recently been reported as causal agent of OA (Antelmi et al., 2018). Moreover, recent findings show an ever-mounting presence of *C. acutatum*, which is outcompeting the less aggressive *C. godetiae* and becoming the prevalent *Colletotrichum* species associated with OA in the Gioia Tauro Plain (southern Italy) (Schena et al., 2017). An additional explanation of the displacement of *C. godetiae* by *C. acutatum* in southern Italy could be the faster growth, especially at high temperatures, of *C. acutatum* compared with *C. godetiae*. It can be envisaged that the severity of OA outbreaks in olive-growing areas of Italy will increase in the coming years as a consequence of the emergence of these two aggressive *Colletotrichum* species. Perhaps it is not accidental that Leccino, Frantoio, Dolce Agogia and Sant'Agostino, all relatively resistant to *C. godetiae*, are widespread in central Italy where only this *Colletotrichum* species was reported to be associated with occasional OA outbreaks (Cacciola et al., 2012). Another *Colletotrichum* species in the *C. gloeosporioides* species complex, *C. theobromicola*, which was found to be associated with anthracnose outbreaks in Australia and was demonstrated to be very aggressive in artificial inoculations on detached olive drupes (Schena et al., 2014), has been recently reported to be responsible for anthracnose outbreaks in Uruguay in association with *C. acutatum* (Moreira et al., 2021). *C. theobromicola* has not be reported so far in olive-growing regions of the Northern hemisphere, but its introduction into the Mediterranean region might have phytosanitary implications.

Overall, the results of this study reinforced that *Colletotrichum* species in a given olive-growing area need to be identified precisely and that local olive cultivars need to be screened for OA susceptibility accordingly. However, the genetic resistance of cultivars might become ineffective if there is a shift in the *Colletotrichum* population, which seems likely according to previously mentioned literature. This implies there is a need for continuous monitoring of *Colletotrichum* populations in olive orchards and a more integrated and flexible OA management strategy.

ACKNOWLEDGEMENTS

We are grateful to Sabrina Sarrocco and Riccardo Baroncelli for providing *Colletotrichum nymphaeae* isolates and to Ann Davies for the English revision of the text. Open Access Funding provided by Universita degli Studi di Catania within the CRUI-CARE Agreement.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Salvatore Moricca https://orcid.org/0000-0003-3097-559X
Santa Olga Cacciola https://orcid.org/0000-0001-7926-3601

REFERENCES

Agosteo, G.E., di San, M., Lio, G., Frisullo, S. & Cacciola, S.O. (2002) Characterisation of the causal agent of olive anthracnose in southern Italy. *Acta Horticulturae*, 586, 713–716.

Aloi, F., Riolo, M., Parlascino, R., Pane, A. & Cacciola, S.O. (2021) Bot gummosis of lemon (*Citrus* × *limon*) caused by *Neofusicoccum parvum*. *Journal of Fungi*, 7, 294.

Antelmi, I., Sion, V. & Nigro, F. (2018) First report of *Colletotrichum nymphaeae* on olive in Italy. *Plant Disease*, 103, 765.

Boscia, D., Altamura, G., Ciniero, A., di Carolo, M., Dongiovanni, C., Fumarola, G. et al. (2017) Resistenza a *Xylella fastidiosa* in diverse cultivar di olivo [Resistance to *Xylella fastidiosa* in different olive cultivars]. *L'Informatore Agrario*, 11, 59–63.

Cacciola, S.O., Faedda, R., Sinatra, F., Agosteo, G.E., Schena, L., Frisullo, S. et al. (2012) Olive anthracnose. *Journal of Plant Pathology*, 94, 29–44.

Cacciola, S.O., Gilardi, G., Faedda, R., Schena, L., Pane, A., Garibaldi, A. et al. (2020) Characterization of *Colletotrichum ocimi* population associated with black spot of sweet basil (*Ocimum basilicum*) in northern Italy. *Plants*, 9, 654.

Cannon, P.F., Damm, U., Johnston, P.R. & Weir, B.S. (2012) *Colletotrichum* – current status and future directions. *Studies in Mycology*, 73, 181–213.

Cara, M., Iliadi, M.K., Lagogianni, C.S., Paplomatas, E.J., Merkuri, J. & Tsitsigiannis, D.I. (2021) First report of *Colletotrichum acutatum* causing anthracnose on olives in Albania. *Plant Disease*, 105, 495.

Chattaoui, M., Raya, M.C., Bouri, M., Moral, J., Perez-Rodriguez, M., Trapero, A. et al. (2016) Characterization of a *Colletotrichum* population causing anthracnose disease on olive in northern Tunisia. *Journal of Applied Microbiology*, 120, 1368–1381.

Damm, U., Cannon, P.F., Woudenberg, J.H.C. & Crous, P.W. (2012a) The *Colletotrichum acutatum* species complex. *Studies in Mycology*, 73, 37–113.

Damm, U., Cannon, P.F., Woudenberg, J.H.C., Johnston, P.R., Weir, B.S., Tan, Y.P. et al. (2012b) The *Colletotrichum boninense* species complex. *Studies in Mycology*, 73, 1136.

Faedda, R., Agosteo, G.E., Schena, L., Mosca, S., Frisullo, S., di San, M. et al. (2011) *Colletotrichum clavatum* sp. nov. identified as the causal agent of olive anthracnose in Italy. *Phytopathologia Mediterranea*, 50, 283–302.

Giampetruzzi, A., Baptista, P., Morelli, M., Cameirão, C., Lino Neto, T., Costa, D. et al. (2020) Differences in the endophytic microbiome of olive cultivars infected by *Xylella fastidiosa* across seasons. *Pathogens*, 9, 723.

Glass, N.L. & Donaldson, G. (1995) Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology*, 61, 1323–1330.

Gouvinhas, I., Martins-Lopes, P., Carvalho, T., Barros, A. & Gomes, S. (2019) Impact of *Colletotrichum acutatum* pathogen on olive phenylpropanoid metabolism. *Agriculture*, 9, 173.

Graniti, A., Frisullo, S., Pennisi, A.M., di San, M. & Lio, G. (1993) Infections of *Glomerella cingulata* on olive in Italy. *EPPO Bulletin*, 23, 457–465.

Guzmán, E., Baeten, V., Pierna, J.A.F. & García-Mesa, J.A. (2015) Determination of the olive maturity index of intact fruits using image analysis. *Journal of Food Science and Technology*, 52, 1462–1470.

Iliadi, M.K., Tjamos, E.C., Antoniou, P.P. & Tsitsigiannis, D.I. (2018) First report of *Colletotrichum acutatum* causing anthracnose on olives in Greece. *Plant Disease*, 102, 820.

Kolainis, S., Koletti, A., Lykogianni, M., Karamanou, D., Gkizi, D., Tjamos, S.E. et al. (2020) An integrated approach to improve plant protection against olive anthracnose caused by the *Colletotrichum acutatum* species complex. *PLoS One*, 15, e0233916.

Liu, F., Cai, L., Crous, P.W. & Damm, U. (2014) The *Colletotrichum gigasporum* species complex. *Persoonia*, 33, 83–97.

Lombardo, N. (2003) Descrizione delle principali cultivar di olivo da olio e da tavola italiane [Description of the main Italian olive oil and table cultivars]. In: Fiorino, P. (Ed.) *Olea – Trattato di olivicoltura*. Milan: Edagricol, pp. 169–193.

Lubbe, C.M., Denman, S., Cannon, P.F., Groenewald, J.Z., Lamprecht, S.C. & Crous, P.W. (2004) Characterization of *Colletotrichum* species associated with diseases of Proteaceae. *Mycologia*, 96, 1268–1279.

Mateo-Sagasta, E. (1968) Estudios básicos sobre *Gloeosporium olivarum* Alm. (Deuteromiceto Melanconial) [Basic studies on *Gloeosporium olivarum* Alm. (melanconial Deuteromycete)]. *Boletín de Patología Vegetal y Entomología Agrícola*, 30, 31–135.

Materatski, P., Varanda, C., Carvalho, T., Dias, A.B., Campos, M.D., Rei, F. et al. (2018) Diversity of *Colletotrichum* species associated with olive anthracnose and new perspectives on controlling the disease in Portugal. *Agronomy*, 8, 301.

Moral, J., Agustí-Brisach, C., Agalliu, G., Oliveira, R., Pérez-Rodríguez, M., Roca, L.F. et al. (2018) Preliminary selection and evaluation of fungicides and natural compounds to control olive anthracnose caused by *Colletotrichum* species. *Crop Protection*, 114, 167–176.

Moral, J., Agustí-Brisach, C., Raya, M.C., Jurado-Bello, J., López-Moral, A., Roca, L.F. et al. (2021) Diversity of *Colletotrichum* Species associated with olive anthracnose worldwide. *Journal of Fungi*, 7, 741.

Moral, J., Alsalimiya, M., Roca, L.F., Díez, C.M., León, L., de la Rosa, R. et al. (2015) Relative susceptibility of new olive cultivars to *Spilocaea oleagina*, *Colletotrichum acutatum*, and *Pseudocercospora cladosporioides*. *Plant Disease*, 99, 58–64.

Moral, J., Bouhmidi, K. & Trapero, A. (2008) Influence of fruit maturity, cultivar susceptibility, and inoculation method on infection of olive fruit by *Colletotrichum acutatum*. *Plant Disease*, 92, 1421–1426.

Moral, J., Jurado-Bello, J., Sánchez, M.I., Oliveira, R. & Trapero, A. (2012) Effect of temperature, wetness duration, and planting density on olive anthracnose caused by *Colletotrichum* spp. *Phytopathology*, 102, 974–981.

Moral, J. & Trapero, A. (2009) Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. *Plant Disease*, 93, 1028–1036.

Moral, J., Xaviér, C.J., Viruega, J.R., Roca, L.F., Caballero, J. & Trapero, A. (2017) Variability in susceptibility to anthracnose in the world collection of olive cultivars of Cordoba (Spain). *Frontiers in Plant Science*, 8, 1892.

Moreira, V., Mondino, P. & Alaniz, S. (2021) Olive anthracnose caused by *Colletotrichum* in Uruguay: symptoms, species diversity and pathogenicity on flowers and fruits. *European Journal of Plant Pathology*, 160, 663–681.

Mosca, S., Li Destri Nicosia, M.G., Cacciola, S.O. & Schena, L. (2014) Molecular analysis of *Colletotrichum* species in the carposphere and phyllosphere of olive. *PLoS One*, 9, e114031.

Pangallo, S., Nicosia, M.G.L.D., Agosteo, G.E. & Schena, L. (2022) Control of olive anthracnose and leaf spot disease by bloom treatments with a pomegranate peel extract. *Journal of the Saudi Society of Agricultural Sciences*, 21, 248–254.

Peres, F., Talhinhas, P., Afonso, H., Alegre, H., Oliveira, H. & Ferreira-Dias, S. (2021) Olive oils from fruits infected with different anthracnose pathogens show sensory defects earlier than chemical degradation. *Agronomy*, 11, 1041.

Ramos, A.P., Talhinhas, P., Sreenivasaprasad, S. & Oliveira, H. (2016) Characterization of *Colletotrichum gloeosporioides*, as the main

causal agent of citrus anthracnose, and *C. karsti* as species preferentially associated with lemon twig dieback in Portugal. *Phytoparasitica*, 44, 549–561.

Riolo, M., Aloi, F., Pane, A., Cara, M. & Cacciola, S.O. (2021) Twig and shoot dieback of citrus, a new disease caused by *Colletotrichum* species. *Cell*, 10, 449.

Schena, L., Abdelfattah, A., Mosca, S., Li Destri Nicosia, M.G., Agosteo, G.E. & Cacciola, S.O. (2017) Quantitative detection of *Colletotrichum godetiae* and *C. acutatum sensu stricto* in the phyllosphere and carposphere of olive during four phenological phases. *European Journal of Plant Pathology*, 149, 337–347.

Schena, L., Mosca, S., Cacciola, S.O., Faedda, R., Sanzani, S.M., Agosteo, G.E. et al. (2014) Species of the *Colletotrichum gloeosporioides* and *C. boninense* complexes associated with olive anthracnose. *Plant Pathology*, 63, 437–446.

Scibetta, S., Agosteo, G.E., Abdelfattah, A., Li Destri Nicosia, M.G., Cacciola, S.O. & Schena, L. (2020) Development and application of a quantitative PCR detection method to quantify *Venturia oleaginea* in asymptomatic olive (*Olea europaea*) leaves. *Phytopathology*, 110, 547–555.

Sergeeva, V. & Spooner-Hart, R. (2011) Diseases and disorders associated with environmental stress in sustainable olive orchards in Australia. *Acta Horticulturae*, 924, 145–150.

Shivas, R.G. & Tan, Y.P. (2009) A taxonomic re-assesment of *Colletotrichum acutatum*, introducing *C. fioriniae* comb. et stat. nov. and *C. simmondsii* sp. nov. *Fungal Diversity*, 39, 111–112.

Talhinhas, P., Gonçalves, E., Sreenivasaprasad, S. & Oliveira, H. (2015) Virulence diversity of anthracnose pathogens (*Colletotrichum acutatum* and *C. gloeosporioides* species complexes) on eight olive cultivars commonly grown in Portugal. *European Journal of Plant Pathology*, 142, 73–83.

Talhinhas, P., Loureiro, A. & Oliveira, H. (2018) Olive anthracnose: a yield- and oil quality-degrading disease caused by several species of *Colletotrichum* that differ in virulence, host preference and geographical distribution. *Molecular Plant Pathology*, 19, 1797–1807.

Talhinhas, P., Mota-Capitao, C., Martins, S., Ramos, A.P., Neves-Martins, J., Guerra-Guimarães, L. et al. (2011) Epidemiology, histopathology and aetiology of olive anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. *Plant Pathology*, 60, 483–495.

Talhinhas, P., Neves-Martins, J., Oliveira, H. & Sreenivasaprasad, S. (2009) The distinctive population structure of *Colletotrichum* species associated with olive anthracnose in the Algarve region of Portugal reflects a host-pathogen diversity hot spot. *FEMS Microbiology*, 296, 31–38.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J. & Oliveira, H. (2005) Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology*, 71, 2987–2998.

Uematsu, S., Kageyama, K., Moriwaki, J. & Sato, T. (2012) *Colletotrichum carthami* comb. nov., an anthracnose pathogen of safflower, garland chrysanthemum and pot marigold, revived by molecular phylogeny with authentic herbarium specimens. *Journal of General Plant Pathology*, 78, 316–330.

Weir, B.S., Johnston, P.R. & Damm, U. (2012) The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, 73, 115–180.

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungi ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. San Diego, CA: Elsevier Academic Press, pp. 315–322.

Yang, Y., Cal, L., Yu, Z., Liu, Z. & Hyde, K.D. (2011) *Colletotrichum* species on *Orchidaceae* in Southwest China. *Cryptogamie Mycologie*, 32, 229–253.

How to cite this article: Riolo, M., Pane, A., Santilli, E., Moricca, S. & Cacciola, S.O. (2023) Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose. *Plant Pathology*, 72, 255–267. Available from: https://doi.org/10.1111/ppa.13652