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Fungal Species Causing Canker and Wilt of *Ficus carica* and Evidence of Their Association by Bark Beetles in Italy

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Abstract

Field surveys conducted during 2021 and 2022 in Western Sicily, Italy, revealed the presence of common fig trees severely affected by trunk and crown root canker and bark cracking. Moreover, in conjunction with the symptomatic tissues, the same surveyed plants showed the presence of bark beetle holes and internal wood galleries. The predominant beetle *Criphalus dilutus* was previously reported attacking figs in Sicily. Phylogenetic analyses based on multilocus DNA data showed the presence of different fungal taxa associated with disease symptoms, including *Botryosphaeria dothidea*, *Ceratocystis ficicola*, *Diaporthe foeniculina*, *Neocosmospora bostrycoides*, *N. perseae*, and *Neofusicoccum luteum*. Pathogenicity tests conducted on potted fig plants showed that all the species were pathogenic to fig, with *C. ficicola* and *Neocosmospora* spp. as the most aggressive fungal species. Moreover, isolations conducted from the bodies of emerging adult insects recovered from disease samples confirmed the presence of *C. ficicola* and *Neocosmospora* spp., suggesting the potential involvement of *C. dilutus* in their dissemination.

Keywords: bark beetle, canker pathogens, *Criphalus dilutus*, *Ficus carica*, fig, fungal diseases, wilt

The genus *Ficus* (Moraceae) is one of the largest genera of angiosperms, with more than 800 flowering plants widespread in tropical and semitropical temperate areas (Vinson 1999). *Ficus* spp. have a variety of uses, from medicinal to edible, from ornamental to forage, and many others (Shi et al. 2018). *Ficus carica* L., or common fig, is the most commercially important species of the genus for its food and medicinal purposes. It is native to Southwest Asia and the Eastern Mediterranean region, where it has been cultivated for more than 11,000 years, and has spread worldwide because of its pedoclimatic adaptation (Dueñas et al. 2008; Kislev et al. 2006). According to recent statistics of the Food and Agricultural Organization of the United Nations, figs are harvested from almost 300,000 ha, with a production of more than 1.3 million tons worldwide. Mediterranean countries such as Morocco, Turkey, Algeria, and Egypt account for 50% of the production (FAOSTAT 2021). In Italy, the production of edible figs is mainly concentrated in the southern regions (1,785 ha), of which Apulia is the top producer (33,555 harvested tons), followed by Calabria (29,924) and Campania (28,745) (ISTAT 2021). In Sicily, 132 ha are considered productive, with 12,166 tons of harvested fruits per year (ISTAT 2021).

Among the fungal diseases reported to affect this crop, branch and twig cankers are widespread. Advances in molecular techniques, especially in DNA phylogeny, allowed the identification of several fungal pathogens found in association with trees showing these disease symptoms. Therefore, it is more accurate to refer to it as a disease characterized by a complex etiology. Specifically, Botryosphaeriaceae and Diaporthaceae species are well-known pathogens of perennial tree crops, including the common fig (Aiello et al. 2020; Banihashemi and Javadi 2009; Çeliker and Michailides 2012; Güney et al. 2022; Gusella et al. 2021b; Javadi and Banihashemi 2008; Nur-Shakirah et al. 2022; Ray et al. 2010; Wang et al. 2020). These species induce cankers, wood necrosis, and twig dieback. Characterized by a latent phase, these fungi can survive as endophytes (or latent pathogens), switching to pathogens when the environmental conditions are suitable, especially when the host is stressed (Slippers and Wingfield 2007). Among the Botryosphaeriaceae, relevant importance is especially attributed to the species *Neoscytalidium dimidiatum*, an emerging destructive pathogen of common fig in California (Gusella et al. 2021b, 2023) and also reported elsewhere for affecting common fig (Güney et al. 2022). An important limiting factor for common fig is the soilborne pathogen *Ceratocystis ficicola* (Ceratocystidaceae). Decline of fig orchards has been observed since the 1970s in Japan, and the disease (named fig wilt disease [FWD]) was initially attributed to *Ceratocystis fimbriata sensu lato* (Kato et al. 1982). In 2011, Kajitani and Masuya (2011) described the causal agent of FWD as *C. ficicola*, a new species distinct from *C. fimbriata.* Recently, *C. ficicola* was also reported as causing a destructive disease in Greece, leading to severe wilt and canopy defoliation (Tsopelas et al. 2021).

Moreover, two species of *Neocosmospora*, *N. caricae* and *N. metavorans*, were identified in Iran to cause stem and trunk cankers (Bolboli et al. 2022). Regarding this complex symptomatology of trunk and crown root cankers of common fig in Southern Italy, the first observations started in 2013, and preliminary data revealed the presence of fungi belonging to the genera *Alternaria*, *Botryosphaeria*, and *Fusarium* associated with the abovementioned symptoms (Di Silvestro et al. 2021). Later, *C. ficicola* was isolated from common fig trunks and from root cankers in Sicily (Southern Italy) and molecularly characterized (Crous et al. 2023). The same species was also reported to cause fig wilt and canker in the Apulia region (Southern Italy), and its pathogenicity was confirmed (Habib et al. 2023). In addition to this diversified group of pathogens affecting common fig, the role of some wood-boring insects, such as bark and ambrosia beetles (Coleoptera: Curculionidae), infesting common figs and disseminating fungal propagules complicates the

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symptomatology. As an example, infestations by the ambrosia beetle *Euwallacea interjectus* have been linked to the spread of FWD in Japan (Kajii et al. 2013) or at least to be involved in FWD as a secondary pest (Jiang et al. 2019, 2021). The simultaneous occurrence of insect infestations and symptoms related to fungal diseases complicates the etiology of the disease. During 2021 and 2022, surveys were conducted in Sicily, where common fig plants showed trunk and crown root cankers and symptoms of bark cracking. In addition, the same surveyed plants showed the simultaneous presence of bark beetle holes along the bark and excavated internal galleries. The occurrence of attacks by the bark beetles *Hypoborus ficus* and *Cryphalus dilutus* (ex *Hypocryphalus scabricollis*) and by the ambrosia beetle *Xyleborus bispinatus* to common fig trees have been previously confirmed for the same Sicilian environment (Faccoli et al. 2016). However, among these species, the bark beetle *C. dilutus* is widespread and predominant, whereas the other two beetle species are only occasionally found (Di Silvestro et al. 2021; Gugliuzzo et al. 2023a). Moreover, a crucial role of *C. dilutus* as a vector of phytopathogenic fungi infecting common fig has been suggested (Gugliuzzo et al. 2023a). Based on the diversity of fungal species described worldwide for causing cankers and wilt of common fig trees, and on the common occurrence of serious bark beetle infestations on *F. carica*, the aim of this study was to (i) characterize the fungal species associated with the trunk and crown root cankers and wilt of fig trees; (ii) test their pathogenicity on healthy, common fig plants; and (iii) identify the different fungal species occurring on emerging adult insects to evaluate the potential involvement of *C. dilutus* in their dissemination.

Materials and Methods

Sampling and fungal isolations

Surveys were carried out during spring 2021 and summer 2022 in the "Center for the autochthonous germplasm collection" of "Marianelli" in the Vendicari Nature Reserve (Noto, Syracuse, Sicily) and in a private residence in Aci Castello (Catania province), where three approximately 30-year-old common fig trees were present as part of the landscape. Symptomatic woody samples (10 sampled trees) consisting of entire trunk sections and subcortical tissues as well as branch and trunk sections showing signs of bark beetle infestation were collected in the field and brought to the laboratory of the Department of Agriculture, Food and Environment, University of Catania, for further analyses. Fungal isolation was first conducted from symptomatic woody samples as follows: small sections (0.2 to 0.3 cm^2) of symptomatic tissues (internal wood necrosis) were surface-sterilized for 1 min in 1.5% sodium hypochlorite, rinsed in sterile deionized water, dried on sterile absorbent paper under a laminar hood and placed on potato dextrose agar (PDA; Lickson, Vicari, Italy) amended with 100 mg/liter of streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, U.S.A.) (PDA-S) to prevent bacterial growth, and then incubated at 25°C for 3 to 5 days until fungal colonies were large enough to be counted (isolation frequency %), examined, and transferred to fresh PDA-S Petri plates. The isolation frequency was calculated according to the following formula: $F = (N_f/N_{Tot}) \times 100$, where F is the frequency of putative fungal pathogen; N_f is the number of wood fragments from which a fungal colony of interest emerged; and N_{Tot} is the total number of wood fragments cultured on PDA-S. Subsequently, colonies of interest were subcultured onto fresh PDA-S plates to generate pure cultures, and then single-hyphal tip/single spore cultures were obtained and maintained on PDA-S at 25°C. Representative colonies were stored in the fungal collection of the laboratory and also deposited at the Working collection Pedro Crous, Utrecht, the Netherlands. In addition, samples showing wood necrosis were examined using an Olympus SZX-ILLB2-200 dissecting microscope (Olympus, Tokyo, Japan) and the mycelium observed within insect galleries was directly transferred using a sterile needle to PDA-S plates, and fungal colonies were processed as described above. A portion of sampled branch and trunk sections infested by bark beetles were instead placed inside plastic boxes and kept at 25 ± 1°C and 65 ± 10% relative humidity for 6 weeks and checked every 2 to 3 days for beetle emergence. Adult bark beetles emerging from the infested wood were first individually collected for species identification and subsequently placed in single sterile vials to be processed for fungal isolation, as described below.

Fungal isolation from *Cryphalus dilutus*

Fungal species composing the microbial community on the bodies of adult *C. dilutus* emerging from symptomatic wood were isolated by first grinding single individuals (n = 30 from the Center for the germplasm collection in Noto; n = 15 from the ancient fig trees in Aci Castello) in a sterile phosphate-buffered saline (PBS) solution (Gugliuzzo et al. 2023b). Then, 200 µl of a 1:100 dilution (in PBS) of the obtained mixture was spread on PDA (Lickson, Vicari, Italy) amended with 100 mg/liter of streptomycin sulfate (Sigma-Aldrich, St. Louis, MO, U.S.A.) (PDA-S) to prevent bacterial growth, incubated at 25°C for 3 to 5 days, and processed as indicated above. There were three PDA-S plates for each tested beetle specimen.

Molecular characterization and phylogenetic analyses

Total genomic DNA was extracted from mycelium grown on PDA-S, using the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-Tek), according to the manufacturer's instructions. Species identification was achieved through DNA amplification and sequencing of a combined dataset of genes: the nuclear ribosomal internal transcribed spacer (ITS) region, the partial β-tubulin (*tub2*) gene, the partial region of translation elongation factor-1α (*tef1*), and RNA polymerase second largest subunit (*rpb2*) genes. The primers used for each locus are reported in Table 1. PCR mixtures and cycling conditions for the analyses of ITS, *tub2*, and *tef1* conducted on Botryosphaeriaceae and *Diaporthe* spp. were followed as described in Guarnaccia et al. (2020). For the isolates identified as *Neocosmospora* spp., the protocols were adapted according to Guarnaccia et al. (2021, 2022b). An amount of 5 µl of PCR product for each amplification reaction was examined by electrophoresis on 1% agarose (VWR Life Science AMRESCO biochemicals, U.S.A.) gels stained with GelRed to

Table 1. Primers used in this study for molecular analyses[a]

Locus	Primer name	Primer sequence 5′–3′	Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	
tub2	T1	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigelnik 1997
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Glass and Donaldson 1995
tef1	EF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn 1999
	EF1-986R	TACTTGAAGGAACCCTTACC	
rpb2	RPB2-5f2	GGGGWGAYCAGAAGAAGG C	Reeb et al. 2004
	RPB2-7cr	CCCATRGCTTGYTTRCCCAT	Liu et al. 1999
	RPB2-7cF	ATGGGYAARCAAGCYATGGG	
	RPB2-11aR	GCRTGGATCTTRTCRTCSACC	

[a] ITS = internal transcribed spacer; *rpb2* = RNA polymerase second largest subunit; *tef1* = partial region of translation elongation factor-1α; *tub2* = partial β-tubulin.

Table 2. Collection details and GenBank accession numbers of isolates included in this study[a]

Species	Code	Country	Host	GenBank accession number			
				ITS	*tef1*	*tub2*	*rpb2*
Botryosphaeria agaves	CBS 133992*	Thailand	*Agave* sp.	JX646791	JX646856	JX646841	–
Botryosphaeria corticis	ATCC 22927	U.S.A.	*Vaccinium* sp.	DQ299247	EU673291	EU673108	–
Botryosphaeria corticis	CBS 119047*	U.S.A.	*Vaccinium corymbosum*	DQ299245	EU017539	–	–
Botryosphaeria dothidea	CBS 110302	Portugal	*V. vinifera*	AY259092	AY573218	EU673106	–
Botryosphaeria dothidea	CBS 115476 = CMW 8000*	Switzerland	*Prunus* sp.	AY236949	AY236898	AY236927	–
Botryosphaeria dothidea	**BOTC10 = CPC 44191**	**Italy**	***Ficus carica***	**PP094704**	**PP105763**	**PP105774**	**–**
Botryosphaeria fabicerciana	CBS 127194 = CMW 27094*	China	*Eucaliptus* sp.	HQ332197	HQ332213	KF779068	–
Botryosphaeria fabicerciana	CERC 2948	China	*Eucaliptus* sp.	KX277983	KX278088	KX278193	–
Botryosphaeria kuwatsukai	CBS 135219 = PG2*	China	*Malus domestica*	KJ433388	KJ433410	–	–
Botryosphaeria qingyuanensis	CERC 2946 = CGMCC 3.18742*	China	*Eucaliptus* hybrid	KX278000	KX278105	KX278209	–
Botryosphaeria ramosa	CBS 122069 = CMW 26167*	Australia	*Eucalyptus camaldulensis*	EU144055	EU144070	KF766132	–
Botryosphaeria scharifii	CBS 124703 = IRAN1529C*	Iran	*Mangifera indica*	JQ772020	JQ772057	–	–
Diaporthe ampelina	CBS 114016*	France	*Vitis vinifera*	AF230751	GQ250351	JX275452	–
Diaporthe amygdali	CBS 126679*	Portugal	*Prunus dulcis*	KC343022	KC343748	KC343990	–
Diaporthe australafricana	CBS 111886	Australia	*Vits vinifera*	KC343038	KC343764	KC344006	–
Diaporthe canthii	CBS 132533*	South Africa	*Canthium inerme*	JX069864	KC843120	KC843230	–
Diaporthe cinerascens	CBS 719.96	Bulgaria	*Ficus carica*	KC343050	KC343776	KC344018	–
Diaporthe citri	CBS 135422	U.S.A.	*Citrus* sp.	KC843311	KC843071	KC843187	–
Diaporthe cytosporella	CBS 137020	Spain	*Citrus limon*	KC843307	KC843116	KC843221	–
Diaporthe eres	CBS 116953	New Zealand	*Pyrus pyrifolia*	KC343147	KC343873	KC344115	–
Diaporthe eres	CBS 138594	Germany	*Ulmus laevis*	KJ210529	KJ210550	KJ420799	–
Diaporthe eres (*alleghaniensis*)	CBS 495.72	Canada	*Betula alleghaniensis*	FJ889444	GQ250298	KC843228	–
Diaporthe foeniculina	CBS 123208*	Portugal	*Foeniculum vulgare*	KC343101	KC343827	KC344069	–
Diaporthe foeniculina	CBS 129528*	South Africa	*Rhus pendulina*	JF951146	KC843100	KC843205	–
Diaporthe foeniculina	CBS 187.27	Italy	*Camellia sinensis*	KC343107	KC343833	KC344075	–
Diaporthe foeniculina	CBS 111553*	Portugal	*Foeniculum vulgare*	KC843295	KC843104	KC843209	–
Diaporthe foeniculina (*baccae*)	CBS 136972*	Italy	*Vaccinium corymbosum*	KJ160565	KJ160597	MF418509	–
Diaporthe foeniculina (*baccae*)	CBS 136971	Italy	*Vaccinium corymbosum*	KJ160564	KJ160596	–	–
Diaporthe foeniculina (*ravennica*)	MFLUCC 15-0479	Italy	*Tamarix* sp.	–	KX365197	KX432254	–
Diaporthe foeniculina	**DIA1 = CPC 44156**	**Italy**	***F. carica***	**PP094705**	**PP105764**	**PP105775**	–
Diaporthe foeniculina	**DIAC5 = CPC 44155**	**Italy**	***F. carica***	**PP094706**	**PP105765**	**PP105776**	–
Diaporthe limonicola	CPC 28200 = CBS 142549	Malta	*Citrus limon*	MF418422	MF418501	MF418582	–
Diaporthe notophagi	BRIP54801*	Australia	*Notophagus cunninghamii*	JX862530	JX862536	KF170922	–
Diaporthe novem	CBS 127271*	Croatia	*Glycine max*	KC343156	KC343882	KC344124	–
Diaporthe pterocarpi	MFLUCC 10-0575, CBS 137021	Thailand	*Pterocarpus indicus*	JQ619901	JX275418	JX275462	–
Diaporthe pterocarpicola	MFLUCC 10-580a* = CBS 135432	Thailand	*Pterocarpus indicus*	JQ619887	JX275403	JX275441	–
Diaporthe rudis	CBS 113201	Portugal	*Vits vinifera*	KC343234	KC343960	KC344202	–
Diaporthe rudis	CBS 266.85	Netherlands	*Rosa rugosa*	KC343237	KC343963	KC344205	–
Diaporthe sojae	FAU 635	U.S.A.	*Glycine max*	KJ590719	KJ590762	KJ610875	–
Diaporthe thunbergiae	MFLUCC 10-0576a*, CBS 135769	Thailand	*Thunbergia laurifolia*	JQ619893	JX275409	JX275449	–
Diaporthella corylina	CBS 121124*	China	*Corylus*	KC343004	KC343730	KC343972	–
Geejayesia cicatricum	CBS 125552	Slovenia	Dead twig	HQ728145	HM626644	–	HQ728153
Lasiodiplodia thobromae	CBS 164.96	Papua New Guinea	Fruit along coral reef coast	AY640255	AY640258	KU887532	–
Neocosmospora acutispora	CBS 145461*	Guatemala	*Coffea arabica*	LR583700	LR583593	–	LR583814
Neocosmospora bostrycoides	CBS 392.66	Unknown	*Bertholletia excelsa*	LR583705	LR583598	–	LR583819
Neocosmospora bostrycoides	CBS 102824	Colombia	Leaf litter	LR583703	LR583596	–	LR583817

(*Continued on next page*)

[a] Ex-type, ex-neotype, and ex-epitype cultures are indicated with an asterisk (*). Isolates obtained from this study are indicated in bold. ATCC = American Type Culture Collection, Gaithersburg, MD, U.S.A.; BRIP = Plant Pathology Herbarium, Department of Primary Industries, Dutton Park, Queensland, Australia; CBS = Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CERC = Culture collection of China Eucalypt Research Center, ZhanJiang, GuangDong Province, China; CGMCC = China General Microbiological Culture Collection Center, Beijing, China; CMW = Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; CPC = Working collection Pedro Crous, Utrecht, the Netherlands; ITS = internal transcribed spacer; MFLUCC = Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NRRL = ARS Culture Collection, Peoria, IL, U.S.A.; *rpb2* = RNA polymerase second largest subunit; *tef1* = translation elongation factor 1-α gene; *tub2* = β-tubulin gene.

Table 2. (*Continued from previous page*)

Species	Code	Country	Host	GenBank accession number			
				ITS	*tef1*	*tub2*	*rpb2*
Neocosmospora bostrycoides	CBS 144.25*	Honduras	Soil	LR583704	LR583597	–	LR583818
Neocosmospora bostrycoides	CBS 239.39	Unknown	*Atta* sp. fungus garden	LR583702	LR583595	–	LR583816
Neocosmospora bostrycoides	CBS 130391	Brazil	Human eye	EU329716	HM347127	–	EU329665
Neocosmospora bostrycoides	CBS 130328	U.S.A.	Human oral wound	DQ094396	DQ246923	–	EU329564
Neocosmospora bostrycoides	NRRL 52701	Colombia	*Hypothenemus hampei*	JF740906	JF740784	–	JF741110
Neocosmospora bostrycoides	**FUS C10C = CPC 44201**	**Italy**	***F. carica***	**PP094707**	**PP105766**	–	**PP125180**
Neocosmospora bostrycoides	**FUS C11A = CPC 44202**	**Italy**	***F. carica***	**PP094708**	**PP105767**	–	**PP125181**
Neocosmospora bostrycoides	**FUS C11B = CPC 44203**	**Italy**	***F. carica***	**PP094709**	**PP105768**	–	**PP125182**
Neocosmospora brevis	CBS 144387*	Belgium	Polluted soilwater	LR583708	LR583601	–	LR583822
Neocosmospora caricae	ES216-M*	Iran	*Ficus carica*	OK422518	OK539518	–	OK415859
Neocosmospora cyanescens	CBS 518.82*	Netherlands	Human foot	AB190389	LR583605	–	LR583826
Neocosmospora hypothenemi	CBS 145464*	Benin	*Hypothenemus hampei*	LR583715	JF740850	–	JF741176
Neocosmospora hypothenemi	CBS 145466	Uganda	*Hypothenemus hampei*	LR597067	JF740851	–	JF741177
Neocosmospora liriodendri	CBS 117481*	U.S.A.	*Liriodendron tulipifera*	AF178404	AF178340	–	EU329506
Neocosmospora longissima	CBS 126407*	New Zealand	Tree bark	LR583731	LR583621	–	LR583846
Neocosmospora macrospora	CBS 142424*	Italy	*Citrus sinensis*	LT746266	LT746218	–	LT746331
Neocosmospora macrospora	CPC 28193	Italy	*Citrus sinensis*	LT746268	LT746220	–	LT746333
Neocosmospora metavorans	CBS 135789*	Greece	Human pleural effusion	LR583738	LR583627	–	LR583849
Neocosmospora parceramosa	CBS 115695*	South Africa	Soil	JX435199	JX435149	–	JX435249
Neocosmospora parceramosa	NRRL 31158	U.S.A.	Human wound	DQ094389	DQ246916	–	EU329559
Neocosmospora perseae	CBS 144142*	Italy	*Persea americana*	LT991940	LT991902	–	LT991909
Neocosmospora perseae	CBS 144143	Italy	*Persea americana*	LT991941	LT991903	–	LT991910
Neocosmospora perseae	**FUS C1D = CPC 44196**	**Italy**	***F. carica***	**PP094712**	**PP105771**		**PP125185**
Neocosmospora perseae	**FUS C8B = CPC 44199**	**Italy**	***F. carica***	**PP094710**	**PP105769**	–	**PP125183**
Neocosmospora perseae	**FUS C8C = CPC 44200**	**Italy**	***F. carica***	**PP094711**	**PP105770**	–	**PP125184**
Neocosmospora perseae	**NEC12 = CPC 44205**	**Italy**	***F. carica***	**PP094713**	**PP105772**	–	**PP125186**
Neocosmospora petroliphila	CBS 203.32	South Africa	*Pelargonium* sp.	DQ094320	DQ246835	–	LR583857
Neocosmospora petroliphila	CBS 398.66	Brazil	*Saccharum officinarum*	LR583749	LR583633	–	LR583859
Neocosmospora pseudoradicicola	CBS 145472*	Papua New Guinea	Diseased cocoa pods	JF740899	JF740757	–	JF741084
Neocosmospora pseudotonkinensis	CBS 143038	Netherlands	Human cornea	LR583758	LR583640	–	LR583867
Neocosmospora solani	CBS 101018*	Italy	Raspberry	LR583770	LR583651	–	LR583878
Neocosmospora spathulata	CBS 145474*	U.S.A.	Human synovial fluid	EU329674	DQ246882	–	EU329542
Neofusicoccum algeriense	CBS 137504*	Mexico	*Rubus idaeus*	KJ657702	KJ657715	–	–
Neofusicoccum arbuti	CBS 116131*	U.S.A.: Washington	*Arbutus menziesii*	AY819720	KF531792	KF531793	–
Neofusicoccum australe	CBS 121115	South Africa	*Prunus persica*	EF445355	EF445386	KX464948	–
Neofusicoccum australe	CBS 139662*	Australia	*Acacia* sp.	AY339262	AY339270	AY339254	–
Neofusicoccum batangarum	CBS 124924*	Cameroon	*Terminalia catappa*	FJ900607	FJ900653	FJ900634	–
Neofusicoccum cryptoaustrale	CBS 122813*	South Africa	*Eucalyptus* sp.	FJ752742	FJ752713	FJ752756	–
Neofusicoccum italicum	MFLUCC 15-0900*	Italy	*Vitis vinifera*	KY856755	KY856754	–	–
Neofusicoccum kwambonambiense	CBS 102.17*	U.S.A.: Florida	*Carya illinoensis*	KX464169	KX464686	KX464964	–
Neofusicoccum lumnitzerae	CBS 139676	South Africa	*Lumnitzera racemosa*	MT587481	MT592194	MT592686	–
Neofusicoccum luteum	CBS 562.92*	New Zealand	*Actinidia deliciosa*	KX464170	KX464690	KX464968	–
Neofusicoccum luteum	CBS 118842	South Africa	*Syzygium cordatum*	–	MT592196	MT592688	–
Neofusicoccum luteum (*mangroviorum*)	CMW 41365*	South Africa	*Avicennia marina*	NR_147360	MT592206	MT592698	–
Neofusicoccum luteum	**BOT1 = CPC 44160**	**Italy**	***F. carica***	**PP094703**	**PP105762**	**PP105773**	–
Neofusicoccum mangiferae	CBS 118532	Australia	*Mangifera indica*	AY615186	DQ093220	AY615173	–
Neofusicoccum mediterraneum	CBS 121718*	Greece	*Eucalyptus* sp.	GU251176	GU251308	–	–
Neofusicoccum parvum	CMW 9081*	New Zealand	*Populus nigra*	AY236943	AY236888	AY236917	–
Neofusicoccum pistaciarum	CBS 113083*	U.S.A.: California	*Pistacia vera*	KX464186	KX464998	KX464712	–
Neofusicoccum protearum	CBS 114176	South Africa	*L. laureolum*	AF452539	KX465006	KX464720	–
Neofusicoccum rapaneae	CBS 145973	South Africa	*Myrsine melanophloeos*	MT587511	MT592226	MT592718	–
Neofusicoccum stellenboschiana	CBS 110864*	South Africa	*Vitis vinifera*	AY343407	AY343348	KX465047	–
Neofusicoccum variabile	CMW 37742	South Africa	*Mimusops caffra*	MH558609	MH576585	MH569154	–
Neofusicoccum vitifusiforme	CBS 110887*	South Africa	*Vitis vinifera*	AY343383	AY343343	KX465061	–

check PCR amplification. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen), and the amplicons were sequenced in both directions by Macrogen (Italy). The generated DNA sequences were analyzed and consensus sequences were computed using the software Geneious v. 11.1.5 (Auckland, New Zealand). BLASTn analyses were conducted against the NCBI's GenBank nucleotide database to determine the closest relatives for a taxonomic framework of the studied isolates. Alignments of the different gene regions, including sequences obtained from this study and sequences downloaded from GenBank used as taxonomic references, were performed with the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2013) and then manually corrected in MEGA v. 7 (Kumar et al. 2016).

Phylogenetic analyses were conducted individually for each locus (data not shown) and as multilocus sequence analyses using the following locus combinations: ITS, *tef1*, and *tub2* for members of Botryosphaeriaceae and *Diaporthe* (Guarnaccia et al. 2020; Zhang et al. 2021); ITS, *tef1*, and two portions of the *rpb2* regions for isolates related to *Neocosmospora* (Guarnaccia et al. 2022b). *Lasiodiplodia theobromae* (CBS 164.96; Zhang et al. 2021) was used as the outgroup for Botryosphaeriaceae, *Diaporthella corylina* (CBS 121124; Guarnaccia et al. 2020) was selected as the outgroup for *Diaporthe* spp., and *Geejayesia cicatricum* (CBS 125552; Guarnaccia et al. 2022b) was used as the outgroup for *Neocosmospora* spp. Phylogenies for the multilocus analyses were based on Bayesian inference (BI) and maximum parsimony (MP). Considering BI, the best evolutionary model for each locus was determined through MrModeltest v. 2.3 (Nylander 2004) and incorporated into the analyses performed with MrBayes v. 3.2.5 (Ronquist et al. 2012) to generate phylogenetic trees. The Markov chain Monte Carlo analysis used four chains that run for 1,000,000 generations starting from a random tree topology. Trees were sampled every 1,000 generations and the heating parameter was set with the value of 0.2. Analyses stopped once the average SD of split frequencies was below 0.01. The MP analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony, v. 4.0b10) (Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 100 random addition sequences. Tree bisection-reconnection was used, with the branch swapping option set on "best trees" with all

Table 3. Representative isolates obtained from trunk and crown root canker, characterized and used in pathogenicity test

Isolate ID	CPC code[a]	Host	Cultivar	Location	Fungal species
BOTC10	CPC 44191	*Ficus carica*	Natalina	Noto	*Botryosphaeria dothidea*
CERA 30	CPC 44213	*Ficus carica*	–	Noto	*Ceratocystis ficicola*
DIA1	CPC 44156	*Ficus carica*	–	Noto	*Diaporthe foeniculina*
DIAC5	CPC 44155	*Ficus carica*	–	Noto	*Diaporthe foeniculina*
FUS C10C	CPC 44201	*Ficus carica*	Natalina	Noto	*Neocosmospora bostrycoides*
FUS C11A	CPC 44202	*Ficus carica*	Natalina	Noto	*Neocosmospora bostrycoides*
FUS C11B	CPC 44203	*Ficus carica*	Natalina	Noto	*Neocosmospora bostrycoides*
FUS C1D	CPC 44196	*Ficus carica*	Pacchione	Noto	*Neocosmospora perseae*
FUS C8B	CPC 44199	*Ficus carica*	Bifera Nera	Noto	*Neocosmospora perseae*
FUS C8C	CPC 44200	*Ficus carica*	Bifera Nera	Noto	*Neocosmospora perseae*
NEC12	CPC 44205	*Ficus carica*	–	Noto	*Neocosmospora perseae*
BOT1	CPC 44160	*Ficus carica*	Nera Spinagallo	Noto	*Neofusicoccum luteum*

[a] CPC = Working collection Pedro Crous, Utrecht, the Netherlands.

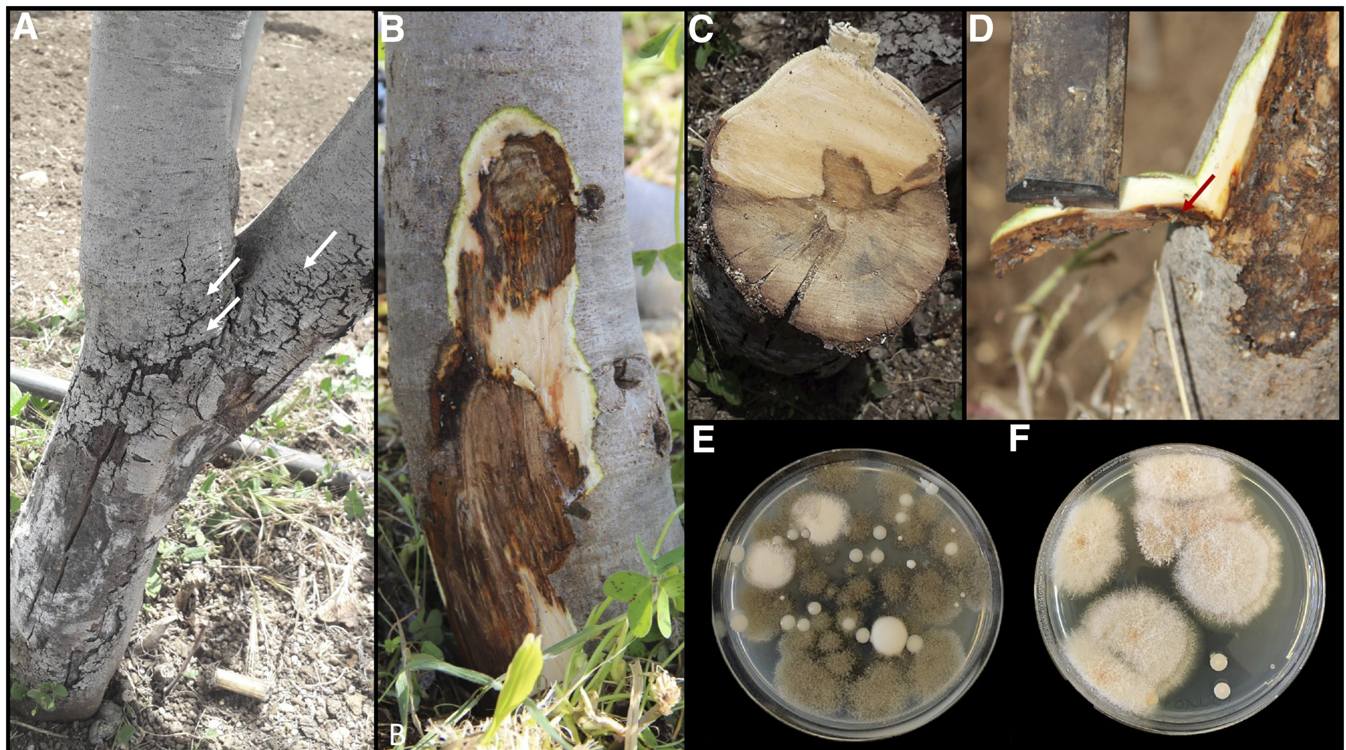

Fig. 1. Symptoms of trunk canker of common fig. **A,** Bark cracking and holes excavated by bark beetles (arrows). **B,** Symptoms of trunk and crown root canker. **C,** Internal necrosis reaching the pith. **D,** Presence of larvae in proximity of internal wood necrosis (arrow). **E and F,** Fungal colonies emerged from insect isolations showing gray colonies of *Ceratocystis ficicola* (E) and white *Neocosmospora* spp. colonies (F).

characters weighted equally and gaps treated as fifth state. Tree length, consistency index, retention index, and rescaled consistency index were calculated for parsimony, and the bootstrap analyses (Hillis and Bull 1993) were based on 1,000 replications. The obtained trees were visualized with FigTree v. 1.4.3 (Rambaut 2010). Sequences generated in this study are deposited in GenBank (Table 2) and the alignments and resulting phylogenetic trees in TreeBASE (www.treebase.org; study number: S31134).

Pathogenicity tests

To test the pathogenicity of the recovered fungal species, representative isolates for each group of fungi were selected (Table 3). A total of five 1-year-old potted fig plants were used as replicates for each tested fungal strain. The inoculation site was surface disinfected by spraying with 70% ethanol solution, and wounds were made in the center of the trunk with a sterilized 5-mm-diameter cork borer to remove the bark. A 5-mm-diameter mycelium plug from a 7- to 10-day-old culture of the selected isolates was placed upside down into the plant tissue wound. Wounds were then sealed with Parafilm to prevent desiccation. Five potted plants inoculated with sterile PDA plugs served as control. Plants were then incubated in a growth chamber with a 12-h photoperiod at 25 ± 1°C. Lesion lengths were measured 30 days after inoculation. Reisolations were conducted as described above. Results of the pathogenicity test were subjected to the analysis of variance, and mean differences were compared with the Fisher's protected least significant difference test at $\alpha = 0.05$ using Statistix 10 (Analytical Software 2013).

Results

Sampling and isolations

Fig trees surveyed in this study showed symptoms of bark cracking and trunk and crown root canker (Fig. 1A and B). Internal symptoms consisted of necrosis and discoloration, but in some cases, the necrosis reached the pith (Fig. 1C). Some plants showed stunted growth. Plants investigated in this study showed the presence of numerous holes in the bark, in correspondence of the necrotized area. The holes were identified as the entry holes of the bark beetle *C. dilutus*. Presence of galleries, larvae, and adults were found under the bark in correspondence to the necrotic area (Fig. 1D). From the isolation of the cankered area, fungal colonies were divided in four main fungal groups, according to their general morphological characters: Botryosphaeriaceae spp., *Ceratocystis* sp., Diaporthaceae spp., and *Neocosmospora* spp. From the Center for the germplasm collection, the most predominant group of fungi belonged to *Ceratocystis* sp., followed by *Neocosmospora* spp. Botryosphaeriaceae and Diaporthaceae were occasionally encountered. Mycelium directly isolated from the inner part of the insect galleries resembled *Neocosmospora* spp. From Acireale, *Neocosmospora*-like colonies were consistently isolated, and Botryosphaeriaceae colonies were occasionally encountered. A total of 111 isolates were recovered from all the symptomatic samples, including the insect's matrix, and stored in the fungal collection.

Fungal isolation from *Cryphalus dilutus*

Ceratocystis sp. and *Neocosmospora* spp. were the most prevalent groups of fungi isolated from adult beetles emerging from branch and trunk sections of common fig collected in the Center for the germplasm collection in Noto. In particular, *Ceratocystis* sp. were isolated from 47% of sampled insects, whereas *Neocosmospora* spp. were isolated from 73% of sampled insects. Compared with bark beetle individuals emerging from branch and trunk sections of the infested ancient fig trees in Aci Castello, no *Ceratocystis* was isolated, whereas *Neocosmospora* spp. were isolated from 36% of collected adults (Fig. 1E and F). Moreover, no Botryosphaeriaceae or Diaporthaceae were isolated from either sample group.

Molecular characterization and phylogenetic analyses

The combined-locus phylogeny of Botryosphaeriaceae consisted of 36 sequences, including the outgroup, and it comprised a total of 1,268 characters (*tef1*: 1 to 305, ITS: 312 to 838, and *tub2*: 845 to 1,268; six N's were added as spacer between the different data partitions). A total of 28 sequences, including the outgroup, and 1,656 characters (*tef1*: 1 to 428, ITS: 435 to 1,012, and *tub2*: 1,019 to

Table 5. Evolutionary models as determined by MrModeltest (Nylander 2004)[a]

Genus	Locus	Evolutionary model
Botryosphaeriaceae	ITS	GTR + G
	tef1	HKY + G
	tub2	HKY + G
Diaporthe	ITS	SYM + I + G
	tef1	GTR + I + G
	tub2	HKY + G
Neocosmospora	ITS	SYM + G
	tef1	GTR + G
	rpb2	SYM + G

[a] G = gamma distributed rate variation among sites; GTR = generalized time-reversible; HKY = Hasegawa-Kishino-Yano; I = proportion of invariable sites; ITS = internal transcribed spacer; *rpb2* = RNA polymerase second largest subunit; SYM = symmetrical model; *tef1* = partial region of translation elongation factor-1α; *tub2* = partial β-tubulin.

Table 4. Parsimony and Bayesian analyses characteristics obtained in this study[a]

Phylogenetic analysis	Loci	Botryosphaeriaceae ITS + *tub2* + *tef1*	*Diaporthe* ITS + *tub2* + *tef1*	*Neocosmospora* ITS + *tef1* + *rpb2*
Maximum parsimony	Total sites	1,268	1,656	2,605
	Constant sites	842	719	1,929
	Variable sites	185	391	359
	Parsimony informative sites	229	534	287
	Tree length	697	2,339	1,033
	Consistency index	0.805	0.628	0.753
	Retention index	0.929	0.700	0.735
	Rescaled consistency index	0.747	0.439	0.554
Bayesian inference	Unique site patterns of ITS	128	191	97
	Unique site patterns of *tef1*	193	308	186
	Unique site patterns of *tub2*	100	276	–
	Unique site patterns of *rpb2*	–	–	229
	Generations ran	1,225,000	1,555,000	530,000
	Generated trees	2,452	3,112	1,062
	Sampled trees	920	1,167	399

[a] ITS = internal transcribed spacer; *rpb2* = RNA polymerase second largest subunit; *tef1* = partial region of translation elongation factor-1α; *tub2* = partial β-tubulin.

1,656; six N's were added as spacer between the different data partitions) were included in the *Diaporthe* phylogenetic analyses. The analyses for the *Neocosmospora* group consisted of 36 sequences, including the outgroup, and 2,605 nucleotides (ITS: 1 to 459, *tef1*: 466 to 1,123, and *rpb2*: 1,130 to 2,605; six N's were added as spacer between the different data partitions) were included in the analysis. A maximum of 1,000 equally most parsimonious trees were saved, and characteristics of the combined gene partitions used for each session are reported in Table 4. Bootstrap support values from the MP analysis were plotted on the BI phylogenies presented in Figures 2, 3, and 4. Unique site patterns for each locus and all the parameters of the Bayesian analyses are reported in Table 4. The models recommended by MrModeltest for the Bayesian analysis are reported in Table 5. In the Botryosphaeriaceae species analysis, one isolate (BOTC10) grouped with two reference strains, including the epitype, of *Botryosphaeria dothidea*, whereas one isolate (BOT1) clustered with the ex-type and two reference strains of *Neofusicoccum luteum* (Fig. 2). Two isolates (DIA1 and DIAC5) of *Diaporthe* clustered with seven reference strains of *Di. foeniculina*, including the ex-type reference strains of *Di. rhusicola* and *Di. neotheicola*, two reference strains of *Di. baccae*, and one reference strain of *Di. ravennica* that were recently grouped with *Di. foeniculina* (Hongsanan et al. 2023; Fig. 3). The final tree generated for *Neocosmospora* species showed that three isolates clustered with the type specimen and six reference strains of *N. bostrycoides*, whereas four isolates grouped with the epitype and one reference strain of *N. perseae* (Fig. 4). Characterization of *C. ficicola* isolates was conducted in Crous et al. (2023).

Pathogenicity tests

Inoculation studies confirmed the pathogenicity of the fungal species identified. There were significant differences in lesion length on inoculated potted plants between fungal species ($P < 0.05$). Specifically, the results showed that mean lesion length of *C. ficicola* isolate CERA 30 was significantly different (13.7 cm) compared with all the other tested isolates. The mean lesion lengths of the other isolates were as follows: FUSC10C: 8.34 cm; FUSC1D: 7.4 cm; FUSC11A: 6.12 cm; FUSC8C: 5.12 cm; Bot1: 4.82 cm; FUSC11B: 4.78 cm; FUSC8B: 3.28 cm; Nec12: 2.38 cm; Dia1: 2.34 cm; DiaC5: 1.8 cm; and BotC10: 1.76 cm. Moreover, plants inoculated with

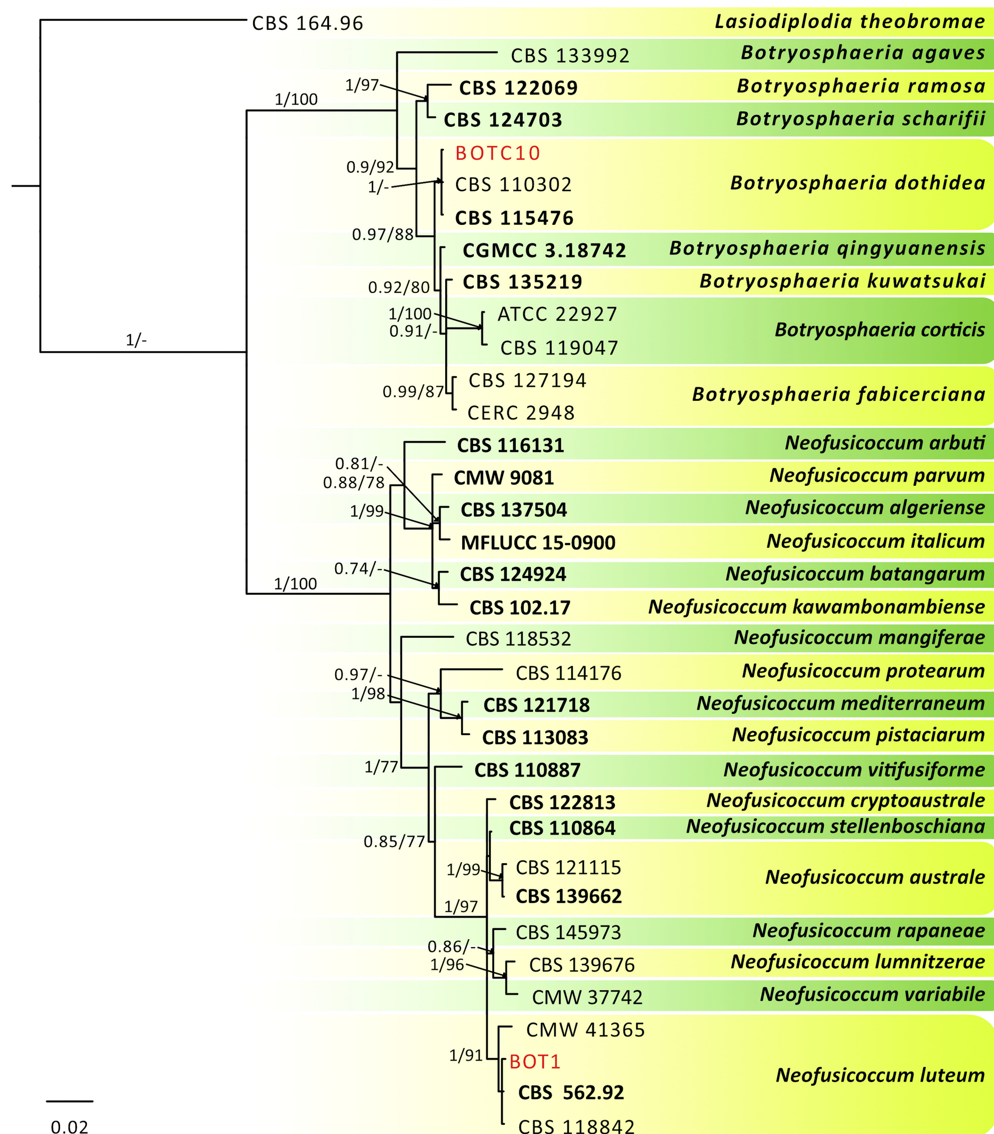

Fig. 2. Consensus phylogram of 2,452 trees resulting from a Bayesian analysis of the combined internal transcribed spacer, partial region of translation elongation factor-1α, and partial β-tubulin sequences of Botryosphaeriaceae isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Lasiodiplodia theobromae* (CBS 164.96). Type specimens are indicated in bold. Isolates from the current study include BOTC10 and BOT1.

C. ficicola isolate CERA 30 and FUSC11A and FUSC11B showed wilting in addition to wood necrosis. The control plants showed a mean of 0.7 cm of brownish lesion around the inoculation site. In general, the group of isolates FUSC8B, Nec12, Dia1, DiaC5, and BotC10 do not statistically differ from the control. The results of the pathogenicity tests are shown in Figures 5 and 6. Reisolations confirmed the identity of the inoculated pathogens via morphological observation.

Discussion

This study revealed trunk and crown root canker and wilt of common fig in Southern Italy to be associated with five fungal genera, namely, *Botryosphaeria*, *Ceratocystis*, *Diaporthe*, *Neocosmospora*, and *Neofusicoccum*. The most aggressive species according to our pathogenicity results was *C. ficicola*, followed by *Neocosmospora* spp., whereas the other identified species were less aggressive and, in the case of Botryosphaeriaceae and Diaporthaceae, less frequently encountered as well. Kajitani and Masuya (2011) reported *C. ficicola* as a devastating canker-wilt pathogen of fig in Japan in 2011. Botryosphaeriaceae and Diaporthaceae have, however, frequently been reported to attack figs worldwide (Aiello et al. 2020; Banihashemi and Javadi 2009; Çeliker and Michailides 2012; Güney et al. 2022; Gusella et al. 2021b; Javadi and Banihashemi 2008; Nur-Shakirah et al. 2022; Ray et al. 2010; Wang et al. 2020), whereas fungi belonging to the genus *Neocosmospora* have only recently started to be investigated as pathogens of figs (Bolboli et al. 2022). Regarding pathogenicity tests conducted in our study, it is important to highlight that some of the young trees inoculated with *C. ficicola* and *Neocosmospora* spp. showed a severe wilt in conjunction with the abovementioned canker symptoms. The results of *Ceratocystis* inoculation are in accordance with those of Morita et al. (2016) and Sumida et al. (2016), who inoculated mature fig trees, as well as young seedlings, revealing that xylem discolorations were correlated with xylem dysfunction and consequent leaf wilting and plant death. Originally, Ceratocystis disease in Japan was referred to as “Ceratocystis canker,” but Kajii et al. (2013) highlighted the fact that the disease displays symptoms more typical of a vascular wilt. The pathogen colonizes the roots and the main stems of host plants, inducing xylem dysfunction and wilt symptoms on infected fig trees, but also kills the cambium and bark tissues, resulting in canker symptoms (Tsopelas et al. 2021). Therefore, in light of all the studies

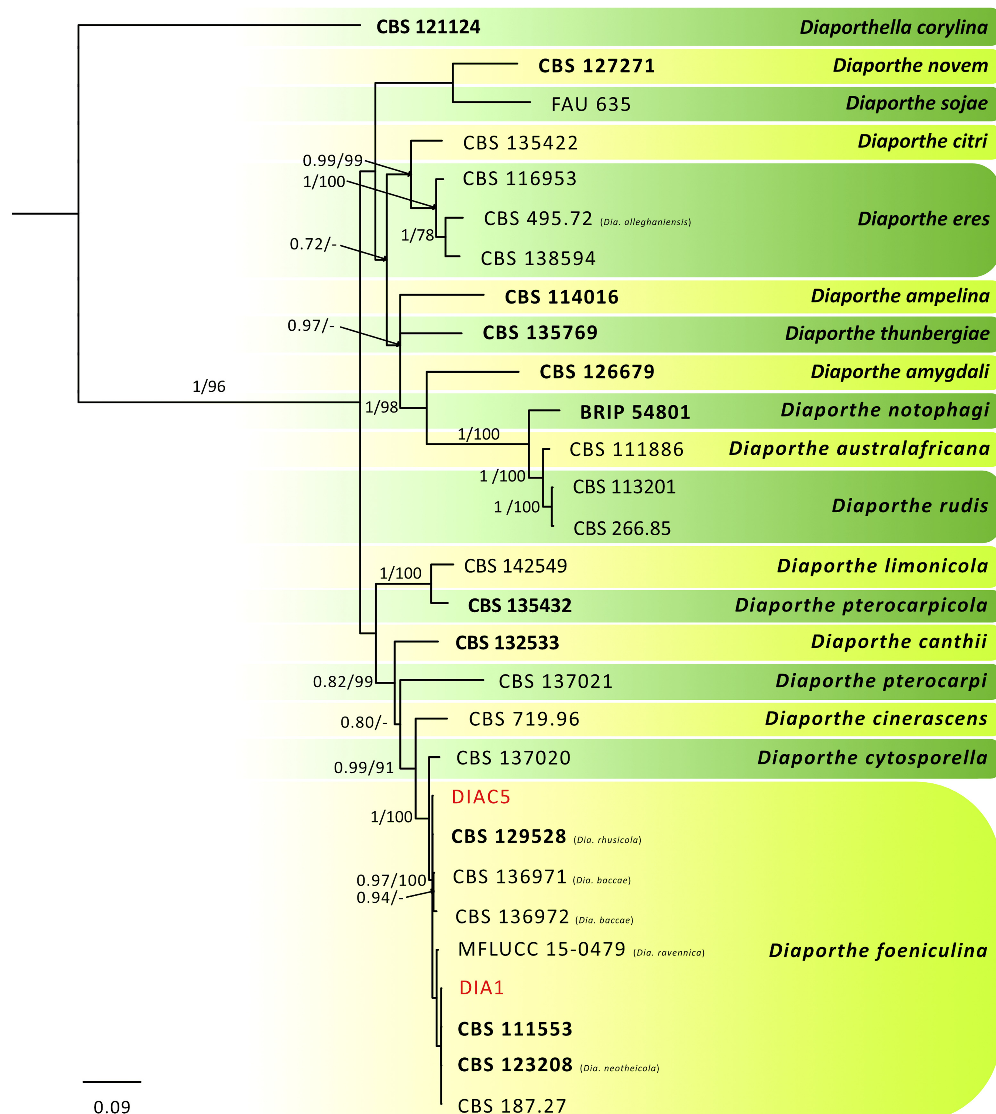

Fig. 3. Consensus phylogram of 3,112 trees resulting from a Bayesian analysis of the combined internal transcribed spacer, partial region of translation elongation factor-1α, and partial β-tubulin sequences of *Diaporthe* isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Diaporthella corylina* (CBS 121124). Type specimens are indicated in bold. Isolates from the current study include DIAC5 and DIA1.

conducted worldwide on Ceratocystis diseases, it is preferable to refer to it as "canker-wilt" disease (Habib et al. 2023; Nasution et al. 2019; Tsopelas et al. 2017, 2021).

Neocosmospora spp. (syn. *Fusarium solani*) (Crous et al. 2021, 2022; Geiser et al. 2021) represent an important group of pathogens involved in this disease. Although our pathogenicity tests showed differences among isolates belonging to the same *Neocosmospora* species, they remained an important part of disease development. A historically relevant disease of common fig caused by *Fusarium* spp. is endosepsis, known also as pink rot, brown rot, soft rot, and eye-end rot (Michailides et al. 1996). In contrast, *Neocosmospora* spp. have only recently been identified as beetle-dispersed canker pathogens of different woody host plants, including common fig (Bolboli et al. 2022). Our investigation revealed the presence of *N. bostrycoides* and *N. persae* associated with canker and being the most relevant group of fungi isolated from symptomatic samples along with *C. ficicola*. Regarding these two species, *N. bostrycoides* has been reported causing wilt on passion fruit in Brazil (Ninos et al. 2021), but it was never reported as canker pathogen thus far, whereas *N. perseae* is a well-known canker pathogen, described for the first time in 2018 in Italy as trunk canker pathogen of avocado (Guarnaccia et al. 2018) and later identified in Crete (Greece) causing branch canker on avocado (Guarnaccia et al. 2022a).

In this study, two Botryosphaeriaceae species were identified, including *B. dothidea* and *Ne. luteum*. Botryosphaeriaceae are well-known canker pathogens, and despite different aggressiveness among the species, they affect several crops as a result of their polyphagous behavior. In particular, *B. dothidea* has been reported in Sicily causing canker on nut crops such as pistachio and walnut (Gusella et al. 2021a, 2022) and on Indian laurel-leaf fig and avocado (Fiorenza et al. 2022, 2023), whereas *Ne. luteum* has been reported on avocado (Fiorenza et al. 2023). Moreover, two isolates of *D. foeniculina* were found as part of this complex etiology, although their aggressiveness, compared with the other pathogens characterized in this study, was significantly lower. However, this pathogen has been historically considered one of the main pathogens of common fig, especially in California (Ferguson et al. 1990). Even if these single fungal species are known as plant pathogens, the surveys conducted in this study highlight the importance of their synergistic role in disease development. As observed in our samples, more than one phytopathogenic genus is involved in the final symptom's expression.

All the tested strains were able to induce lesions on woody tissues, revealing that an accurate approach to canker disease diagnosis should take into account more than one or two single species in the etiology. Following these findings, the use of the term "complex disease" should be preferred to explain the difficulty in diagnosis and management of these diseases (Lamichhane and Venturi 2015). Single microbial cultures are still widely considered the etiological agents of observed diseases, but there is growing evidence of their synergism between different pathogens occurring in plant diseases (Lamichhane and Venturi 2015). Traditional approaches in pathogen identification already revealed that several plant species can frequently be infected at the same time by more than one pathogenic species (Fitt et al. 2006). Moreover, severe disease development in many cases may not result from a single pathogen if compared with

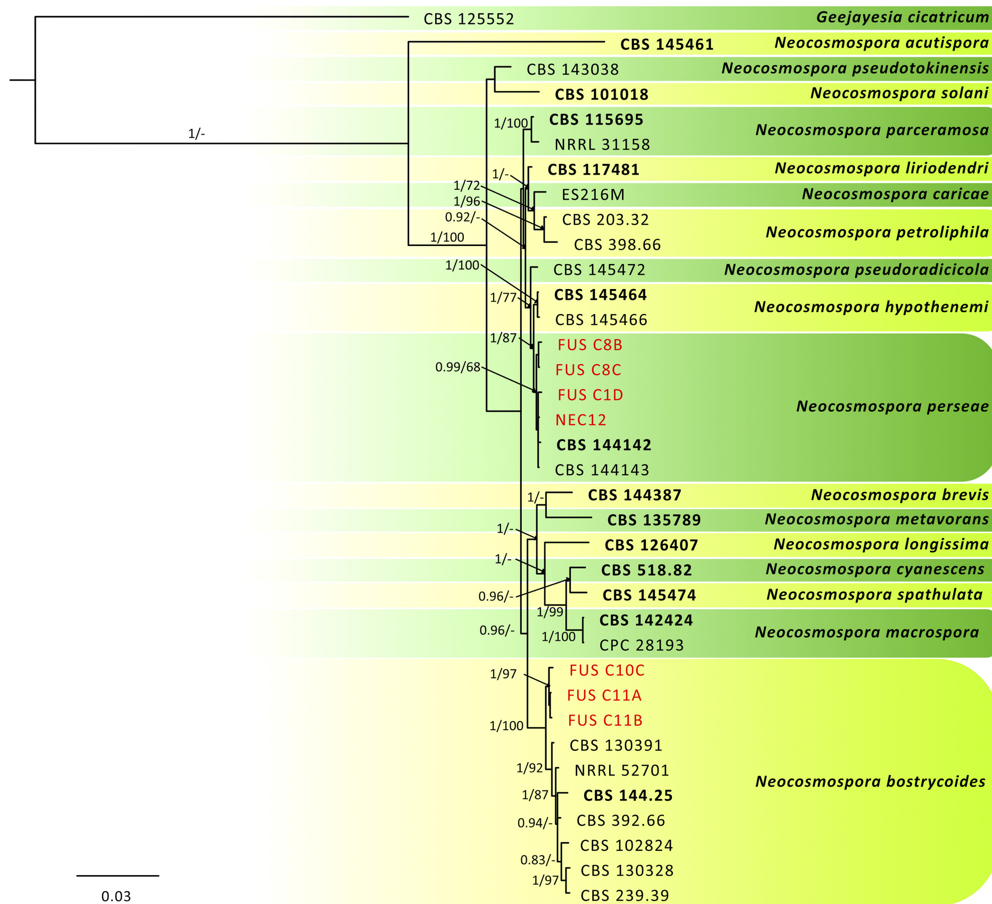

Fig. 4. Consensus phylogram of 1,062 trees resulting from a Bayesian analysis of the combined internal transcribed spacer, partial region of translation elongation factor-1α, and RNA polymerase second largest subunit sequences of *Neocosmospora* isolates. Bayesian posterior probability values and bootstrap support values are indicated at the nodes. The tree was rooted to *Geejayesia cicatricum* (CBS 125552). Type specimens are indicated in bold. Isolates from the current study include FUS C8B, FUS C8C, FUS C1D, NEC12, FUS C10C, FUS C11A, and FUS C11B.

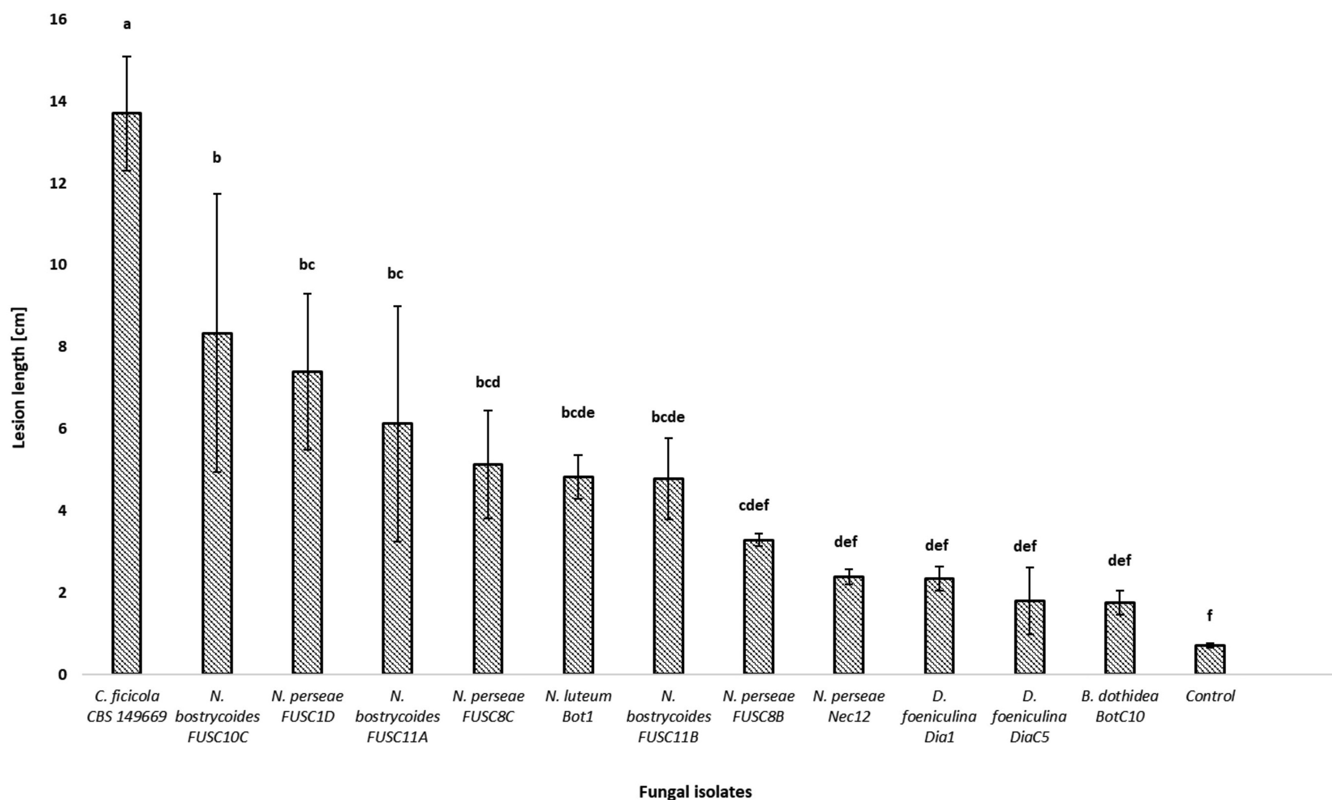

Fig. 5. Comparisons in average lesion length (centimeters) resulting from pathogenicity tests among different fungal species and strains on stems of common fig. Columns are the means of five plants for each fungal inoculation. Vertical bars represent the SE of the means. Bars topped with different letters indicate treatments that were significantly different according to Fisher's protected least significant difference test ($\alpha = 0.05$).

Fig. 6. Pathogenicity test. **A,** *Ceratocystis ficicola* CERA 30 wilt. **B,** *C. ficicola* canker. **C,** *Neocosmospora bostrycoides* FUSC10C. **D,** *N. perseae* FUSC1D. **E,** *N. bostrycoides* FUSC11A wilt. **F,** *N. bostrycoides* FUSC11A canker. **G,** *Neofusicoccum luteum* Bot1. **H,** *N. bostrycoides* FUSC11B. **I,** *N. perseae* FUSC8B. **J,** *Botryosphaeria dothidea* BotC10. **K,** *Diaporthe foeniculina* Dia1. **L,** *N. perseae* NEC12. **M,** *Dia. foeniculina* DiaC5. **N,** *N. perseae* FUSC8C. **O,** Control.

the coinfection by many different species (Lamichhane and Venturi 2015). Fungal canker diseases also reveal complex etiology, and subsequent complex management, as for example demonstrated for apple dieback (Martino et al. 2024), almond decline syndrome and canker disease (Antón Domínguez et al. 2023; Holland et al. 2021), blueberry canker (Avilés et al. 2021), and pistachio branch dieback and panicle and shoot blight (López-Moral et al. 2020; Nouri et al. 2019). Our observations are in accordance with the results shown in Greece by Tsopelas et al. (2021). In their study, the authors confirmed the etiology of vascular wilt and trunk canker caused by *C. ficicola*, observing symptoms of canker only at the base of the trunks, although no other fungi seemed to be isolated from those symptomatic orchards. On the contrary, in Greece, infestation by wood-boring insects in *C. ficicola*–infected fig trees has not been observed (Tsopelas et al. 2021). In Italy and Iran, however, different species of *Neocosmospora* are associated with beetles infesting fig trees (Bolboli et al. 2022).

Increasing research efforts in deepening new knowledge on the associations between bark beetles and co-occurring fungi have been made, and it remains of high scientific interest (Biedermann and Vega 2020; Hulcr et al. 2020; Kolařík and Hulcr 2023; Li et al. 2022; Salem et al. 2023; Six and Klepzig 2021).

In the present study, we demonstrate the common association of the bark beetle *C. dilutus* with relevant phytopathogenic fungal species and suggest its potential role in fungal dissemination. In particular, *C. ficicola* and *Neocosmospora* spp. were consistently isolated from the bodies of dispersing beetle individuals emerging from symptomatic common fig branch and trunk sections, according to Gugliuzzo et al. (2023a). The obtained evidence suggesting a certain role of this bark beetle as a vector of common fig fungal pathogens highlights the need to further investigate the degree of association of different phytopathogenic fungal species with *C. dilutus* populations in other regions where this beetle–host tree combination occurs.

Our results confirmed the emerging roles of *C. ficicola* and *Neocosmospora* spp. in causing canker and wilt and the well-known involvement of Botryosphaeriaceae and Diaporthaceae in cankers. Moreover, this investigation suggests for the first time the role of the bark beetle *C. dilutus* in the dissemination of *C. ficicola* and *Neocosmospora* spp. Further investigations are needed to (i) to elucidate the diversity of fungal species associated with canker disease on fig trees and (ii) experimentally confirm the role of *C. dilutus* as a vector of the different associated phytopathogenic fungi by fulfilling Leach's postulates (Hulcr et al. 2020).

Acknowledgments

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