Journal of Plant Pathology
https://doi.org/10.1007/s42161-025-01877-3

ORIGINAL ARTICLE

Molecular identification and pathogenicity of Botryosphaeriaceae species associated with citrus wood diseases in the eastern Mediterranean region of Türkiye

Şener Kurt[1,2] · Aysun Uysal[1] · Vladimiro Guarnaccia[3,4] · Ilaria Martino[3] · E. Mine Soylu[1,2] · Soner Soylu[2] · Merve Oğuz[2]

Received: 27 August 2024 / Accepted: 8 February 2025

Abstract

Citrus spp. are economically important fruit crops produced worldwide. Surveys were carried out in citrus orchards showing yellowing, wilting on twigs and decline and gummosis of trunks and branches in the Adana, Mersin and Hatay provinces of the eastern Mediterranean region from 2019–2020. The fungal isolates obtained from symptomatic plant materials were identified as 5 different species belonging to Botryosphaeriaceae: *Diplodia seriata, D. olivarum, Dothiorella viticola, Neoscytalidium dimidiatum* and *Neofusicoccum parvum*. The identification was achieved through a robust multilocus phylogeny based on three genomic loci: ITS, *tef-1* and *tub2*. All the species were pathogenic to healthy *Citrus* plants that presented 100% symptomatic twigs in all the cases. Despite the wide distribution and economic importance of this crop, serious fungal diseases are reported worldwide and, in some cases, need to be investigated. This study provides comprehensive insight into the identification of Botryosphaeriaceae species that are causal agents of trunk cankers and branch dieback in citrus in Türkiye.

Keywords Citrus · Botryosphaeriaceae · Dieback · Wood disease · Türkiye

Introduction

Citrus is widely cultivated in more than 140 countries under tropical, subtropical and Mediterranean climate conditions (Liu et al. 2012). Türkiye is among the largest producers in the world, with a production area of 172,900 hectares and a total yield of 4,512,808 tonnes (FAOSTAT 2022). Despite the wide distribution and economic importance of this crop, serious fungal diseases are reported worldwide and, in some cases, need to be investigated. *Diaporthe citri*, which induces gummosis, is known as a major fungal wood pathogen of citrus (Huang et al. 2012). Moreover, *Colletotrichum* spp. have recently been reported to cause twig blight and branch dieback (Uysal et al. 2022; Leonardi et al. 2023). Other genera commonly known as wood pathogens, including *Cytospora, Diplodia, Lasiodiplodia, Neofusicoccum,* and *Sphaeropsis* (Bezerra et al. 2021; Guarnaccia et al. 2022; Piattino et al. 2024), have also been reported to be associated with diseases affecting citrus twigs, branches and trunks. These pathogens are considered responsible for internal wood discolouration and gumming on trunks and main shoots, twigs and branches on citrus crops, causing various symptoms, such as bark rot, branch canker, shoot blight, dieback, and, in severe cases with favourable disease conditions, plant death (Batista et al. 2021; Bezerra et al. 2021; Xiao et al. 2021). Diseases such as branch canker and dieback have been reported in citrus since the late 1900s (Whiteside 1980). Recently, canker diseases affecting citrus

✉ Aysun Uysal
aysunuysal31@gmail.com; aysun.uysal@mku.edu.tr

1 Centre for Implementation and Research of Plant Health Clinic, Hatay Mustafa Kemal University, Hatay 31040, Türkiye

2 Department of Plant Protection, Faculty of Agriculture, Hatay Mustafa Kemal University, Hatay 31040, Türkiye

3 Department of Agricultural, Forest and Food Sciences (DISAFA), University of Torino, Largo Braccini 2, 10095 Grugliasco, Italy

4 Interdipartimental Centre for Innovation in the Agro-Environmental Sector, AGROINNOVA, University of Torino, Largo Braccini 2, 10095 Grugliasco, Italy

Published online: 18 February 2025

trees have been reported to be caused by fungal species such as *Eutypella, Diaporthe, Fusarium, Neocosmospora, Peroneutypa* and *Phaeoacremonium* (Mayorquin et al. 2016; Sandoval-Denis et al. 2018; Espargham et al. 2020; Guarnaccia et al. 2021a, b). Currently, diagnostic studies of fungi causing symptoms in citrus plants, along with morphological, molecular, and pathogenicity tests, are rapidly increasing (Batista et al. 2021; Bezerra et al. 2021; Xiao et al. 2021; Uysal et al. 2022).

Botryosphaeriaceae is a species-rich family with a cosmopolitan distribution and is often associated with wilting, death, twig-branch blight and cankers on woody plants, and it can be saprobic, endophytic, or plant pathogenic (Phillips et al. 2013; Yang et al. 2017; Aiello et al. 2023). Botryosphaeriales was identified on the basis of morphology and multimarker phylogenetic analyses of a large collection of isolates and was determined to include 6 families, 20 genera and 280 species (Batista et al. 2021; Bezerra et al. 2021).

Infections caused by Botryosphaeriaceae species are major problems in citrus production in California (Adesemoye et al. 2011, 2014; Mayorquin et al. 2016), Italy (Polizzi et al. 2009), Iran (Abdollahzadeh et al. 2010; Espargham et al. 2020), Tunisia (Hamrouni et al. 2018), Chile (Guajardo et al. 2018), Mexico (Bautista-Cruz et al. 2019), Algeria (Berraf-Tebbal et al. 2020), and Europe (Spain, Portugal, Malta, Italy, Greece) (Bezerra et al. 2021). In recent years, increased branch canker and dieback symptoms of Botryosphaeriaceae have been observed in the eastern Mediterranean region of Türkiye, thus posing a serious threat for producers that are demanding effective management strategies. In line with modern taxonomic approaches, identification through morphological characterization and multimarker DNA sequence data is essential for developing effective control strategies against pathogens that could affect these crops. Therefore, the purposes of the present study were to (i) conduct field surveys to monitor wood diseases of citrus plants, collect symptomatic plants, and isolate Botryosphaeriaceae isolates in the main citrus-producing areas in the eastern Mediterranean region of Türkiye, (ii) identify and characterize the Botryosphaeriaceae species isolated through the use of molecular and phylogenetic tools, and (iii) assess the pathogenicity of representative isolates on *Citrus limon, C. reticulata, and C. sinensis* under controlled conditions to fullfil Koch's postulates.

Materials and methods

Survey and fungal isolation

During 2019 and 2020, 38 commercial citrus orchards were surveyed in the Adana, Mersin and Hatay Provinces in the eastern Mediterranean region (Fig. 1). Branch, twig and trunk portions of *C. limon, C. reticulata* and *C. sinensis* showing symptoms of dieback, gummosis and canker were collected. Wood samples (5 × 5 mm) were cut from the margin between necrotic and healthy tissue, disinfected in 70% ethanol for 30 s, washed in distilled water for 30 s and then dried on sterile filter paper. Small fragments (5–10 mm) were transferred to potato dextrose agar (PDA) supplemented with 100 µg mL^{-1} amoksisilin (PDA-A) and then incubated at 25 ± 1 °C in the dark for 5 days. After incubation, mycelial plugs from the edge of the resulting colonies were transferred to new 6-cm-diameter PDA plates. Fungi growing on agar plates were purified by the collection of single hyphal tips, which were cut and placed on new PDA plates. A total of 10 isolates were maintained on both PDA slants at 4 °C in the dark and on Whatman no. 2 dry filter paper at −80 °C for storage. The isolates used in this study are maintained in the culture collection of the Centre for Implementation and Research of Plant Health Clinic, Hatay Mustafa Kemal University, Hatay, Türkiye.

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

For all fungal cultures, total genomic DNA was extracted from 0.5 mg of 7-day-old mycelium using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the standard protocol. DNA concentrations were measured via a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Witham, MA, USA). Three genomic loci were amplified: the nuclear ribosomal internal transcribed spacer (ITS) region, the partial translation elongation factor-1α (*tef1*) gene and a portion of the β-tubulin (*tub2*) gene. The primer sets ITS4/ITS5 (White et al. 1990), EF1-728F/EF2-986R (Carbone and Kohn 1999) and Bt2a/Bt2b (Glass and Donaldson 1995) were used to amplify the ITS, *tef1* and *tub2* regions, respectively. The PCR amplification mixture and protocols were followed as described in the literature (Bezerra et al. 2021; Aiello et al. 2022). The amplification products were examined using high-resolution capillary electrophoresis (QIAxcel Advanced System, QIAGEN, and Hilden, Germany). Sequencing services for both directions of the PCR products were obtained from MEDSANTEK (Istanbul, Türkiye). The sequences were computed using MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms (Kumar et al. 2018), and the BLAST function of NCBI's GenBank nucleotide database was utilized to identify the closest relatives of the studied isolates.

Phylogenetic analyses

The novel sequences obtained in this study were compared against the NCBI GenBank nucleotide database through

Fig. 1 Symptoms caused by Botryosphaeriacae species on citrus in eastern Mediterranean region of Turkiye. Trunk canker and bark cracking, gum exudate at the crown level, brown to black discoloration and necrosis of vascular tissues of *Citrus sinensis* (**A**–**E**). Twigs and shoot dieback on young *Citrus reticulata* trees

the standard nucleotide Basic Local Alignment Search Tool (BLAST) to determine the closest species of the studied isolates in the current taxonomic frame. The three genomic regions were initially aligned via the MAFFT v. 7 online server (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh and Standley 2013) and then manually adjusted via MEGA v. 7 when necessary. Phylogenies were performed individually for each locus (data not shown), followed by multilocus analysis of the three concatenated loci. The newly obtained sequences and reference sequences downloaded from GenBank were included in the analyses. Reference sequences (Table 1) were selected on the basis of recent studies on the family Botryosphaeriaceae, and *Botryosphaeria dothidea* CBS 110302 was selected as the outgroup (Xiao et al. 2021; Zhang et al. 2021). Bayesian inference (BI) and maximum parsimony (MP) analyses were used to determine the multilocus phylogeny. For BI, the best evolutionary model was evaluated through MrModeltest v. 2.3 (Nylander 2004) for each locus and included in the analysis. MrBayes v. 3.2.5 (Ronquist et al. 2012) was used to generate the best phylogenetic tree on the basis of optimal setting criteria for each locus through the Markov chain Monte Carlo (MCMC) method. The MCMC analyses started from a random tree topology and used four chains. The trees were sampled every 1000 generations, with a heating parameter of 0.2, and the analysis ended when the average standard deviation of split frequencies was less than 0.01. The MP analysis was performed with Phylogenetic Analyses Using Parsimony (PAUP) v. 4.0b10 (Swofford 2003). Phylogenetic relationships were estimated by heuristic searches with 100 random additional sequences. Tree bisection reconnection (TBR) was used with the branch swapping option on the "best trees", with all the characters weighted equally and the gaps treated as the fifth state. The tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were calculated, and bootstrap analyses were based on 1000 replications. The resulting trees were

Table 1 Information on fungal isolates used in the phylogenetic analyses and their corresponding GenBank accession numbers

Species	Culture No.[1]	Host	Locality	GenBank No.[2]		
				ITS	*tef1*	*tub2*
Botryosphaeria dothidea	CBS 110302	*V. vinifera*	Portugal	AY259092	AY573218	EU673106
Diplodia africana	CBS 120835 = CPC 5908 t	*Prunus persica*	South Africa	EF445343	EF445382	KF766129
Diplodia afrocarpi	CBS 131681	*Afrocarpus falcatus*	South Africa	MT587333	MT592035	MT592471
Diplodia agrifolia	CBS 124.30	*Ulmus sp.*	USA	KX464087	KX464557	KX464783
Diplodia allocellula	CBS 130408 = CMW 36468 t	*Acacia karroo*	South Africa	JQ239397	JQ239384	JQ239378
Diplodia bulgarica	CBS 124135	*Malus sylvestris*	Bulgaria	GQ923852	GQ923820	–
Diplodia bulgarica	CBS 124254 = CAP332 t	*Malus sylvestris*	Bulgaria	GQ923853	GQ923821	–
Diplodia citricarpa	CBS 124715 = CJA 131 = IRAN 1578C t	*Citrus sp.*	Iran	KF890207	KF890189	KX464784
Diplodia corticola	CBS 112546	*Quercus ilex*	Spain	AY259090	EU673310	EU673117
Diplodia corticola	CBS 112549 = CAP 134 t	*Quercus suber*	Portugal	AY259100	AY573227	DQ458853
Diplodia cupressi	CBS 168.87 t	*Cupressus sempervirens*	Israel	DQ458893	DQ458878	DQ458861
Diplodia cupressi	CBS 261.85	*Cupressus sempervirens*	Israel	DQ458894	DQ458879	DQ458862
Diplodia eriobotryicola	CBS 140851 = BN-21 t	*Eriobotrya japonica*	Spain	KT240355	KT240193	MG015806
Diplodia estuarina	CMW 41231	*Rhizophora mucronata*	South Africa	KP860831	KP860676	KP860754
Diplodia estuarina	CMW 41363	*Rhizophora mucronata*	South Africa	KP860829	KP860674	KP860752
Diplodia fraxini	CBS 136010 = CAD001 t	*Fraxinus angustifolia*	Portugal	KF307700	KF318747	MG015807
Diplodia gallae	CBS 212.25	*Quercus sp.*	–	KX464091	KX464565	KX464796
Diplodia malorum	CBS 124130 = CAP271 t	*Malus sylvestris*	Portugal	GQ923865	GQ923833	–
Diplodia mutila	CBS 112553 t	*Vitis vinifera*	Portugal	AY259093	AY573219	KY554743
Diplodia mutila	CBS 121862 = PD 03708099	*Pyrus sp.*	Netherlands	KX464093	KX464567	KX464799
Diplodia neojuniperi	CPC 22753 = B0031 t	*Juniperus chinensis*	Thailand	KM006431	KM006462	–
Diplodia neojuniperi	CPC 22754	*Juniperus chinensis*	Thailand	KM006432	KM006463	–
Diplodia olivarum	CAP 257	*Olea europaea*	Italy	GQ923874	GQ923842	–
Diplodia olivarum	CBS 121887 = CAP 254 t	*Olea europaea*	Italy	EU392302	EU392279	HQ660079
Diplodia olivarum	IMI 390972	*Carob tree*	Italy	HM028640	HQ660078	HQ660080
Diplodia olivarum	CBS 121886	*Olea europaea*	Italy	EU392301	EU392278	MT592517
Diplodia olivarum	**LDo1**	***Citrus limon***	**Turkey**	**MZ410762**	**MZ441086**	**MZ418114**
Diplodia pseudoseriata	CBS 124906	*Blepharocalyx salicifolius*	Uruguay	EU080927	EU863181	MG015820
Diplodia quercivora	CBS 133852 = BL8 t	*Quercus canariensis*	Tunisia	JX894205	JX894229	MG015821
Diplodia rosulata	CBS 116470 t	*Prunus africana*	Ethiopia	EU430265	EU430267	EU673132
Diplodia rosulata	CBS 116472	*Prunus africana*	Ethiopia	EU430266	EU430268	EU673131
Diplodia sapinea	CBS 124462 = CAP273	*Malus sylvestris*	Portugal	GQ923858	GQ923826	–
Diplodia sapinea	CBS 393.84 t	*Pinus nigra*	Netherlands	DQ458895	DQ458880	DQ458863
Diplodia scrobiculata	CBS 118110 = CMW 189 = BOT 1195 t	*Pinus banksiana*	USA:Wisconsin	AY253292	AY624253	AY624258
Diplodia seriata	CBS 112555 = HAP 052 = CAP 063 t	*Vitis vinifera*	Portugal	AY259094	AY573220	DQ458856
Diplodia seriata	CBS 112661	*Vitis vinifera*	South Africa	MT587378	MT592084	MT592541
Diplodia seriata	CBS 119049	*Vitis sp.*	Italy	DQ458889	DQ458874	DQ458857
Diplodia seriata	CBS 171.82	*Rubus sp.*	Italy	KX464108	KX464598	KX464834
Diplodia seriata	CBS 177.26	*Gossypium* sp.	-	KX464113	KX464604	KX464840
Diplodia seriata	CPC 28088	*Citrus reticulata*	Spain	MW413849	MW419167	MW419230
Diplodia seriata	CPC 28101	*Citrus reticulata*	Spain	MW413856	MW419174	MW419237
Diplodia seriata	**MDs4**	***Citrus reticulata***	**Turkey**	**MZ410760**	**MZ441084**	**MZ418112**
Diplodia seriata	**MDs2**	***Citrus reticulata***	**Turkey**	**MZ410761**	**MZ441085**	**MZ418113**
Diplodia subglobosa	CBS 124132 = JL375	*Fraxinus excelsior*	Spain	DQ458887	DQ458871	DQ458852
Diplodia subglobosa	CBS 124133 = JL453 t	*Lonicera nigra*	Spain	GQ923856	GQ923824	MT592576
Dothiorella acericola	KUMCC 18–0137 t	*Acer palmatum*	China	MK359449	MK361182	-

Table 1 (continued)

Species	Culture No.[1]	Host	Locality	GenBank No.[2]		
				ITS	*tef1*	*tub2*
Dothiorella alpina	CGMCC 3.18001 t	*Platycladus orientalis*	China	KX499645	KX499651	–
Dothiorella americana	CBS 128309 t	*Vitis vinifera*	USA	HQ288218	HQ288262	HQ288297
Dothiorella citricola	CBS 124729 t	*Citrus sinensis*	New Zealand	EU673323	EU673290	KX464853
Dothiorella iberica	CBS 115041 = CAP 145 t	*Quercus ilex*	Spain	AY573202	AY573222	EU673096
Dothiorella iranica	CBS 124722	*Olea europea*	Iran	KC898231	KC898214	KX464856
Dothiorella magnoliae	CFCC 51563 t	*Magnolia grandiflora*	China	KY111247	KY213686	-
Dothiorella mangifericola	CBS 124727 t	*Mangifera indica*	Iran	KC898221	KC898204	-
Dothiorella ominivora	CBS 124716 = CJA 241 = IRAN 1573C	*Juglans regia*	Iran	KC898232	KC898215	KX464864
Dothiorella parva	CBS 124720 = CJA 27 = IRAN 1579C t	*Corylus* sp.	Iran	KC898234	KC898217	KX464866
Dothiorella plurivora	CBS 124724 t	*Citrus* sp.	Iran	KC898225	KC898208	KX464874
Dothiorella prunicola	CBS 124723 t	*Prunus dulcis*	Portugal	EU673313	EU673280	-
Dothiorella sarmentorum	IMI 63581b	*Ulmus* sp.	UK	AY573212	AY573235	EU673102
Dothiorella viticola	CBS 112869	*Vitis vinifera*	South Africa	AY343373	AY343336	KX464869
Dothiorella viticola	CBS 117007	*Vitis vinifera*	Spain	AY905556	KX464623	KX464890
Dothiorella viticola	CBS 117009 t	*Vitis vinifera*	Spain	AY905554	AY905559	EU673104
Dothiorella viticola	CPC 33257	*Broussonetioa papyrifera*	Italy	MT587419	MT592131	MT592611
Dothiorella viticola	DAR 80529	*Vitis vinifera*	Australia	HM009376	HM800511	HM800519
Dothiorella viticola	**MDv6**	***Citrus reticulata***	**Turkey**	**MZ410754**	**MZ441079**	**MZ418106**
Dothiorella viticola	**ODv3**	***Citrus sinensis***	**Turkey**	**MZ410758**	**MZ441082**	**MZ418110**
Dothiorella viticola	**ODv2**	***Citrus sinensis***	**Turkey**	**MZ410759**	**MZ441083**	**MZ418111**
Dothiorella yunnana	CGMCC 3.17999 t	*Camellia* sp.	China	KX499643	KX499649	-
Neofusicoccum algeriense parvum	CBS 137504 t	*Rubus idaeus*	Mexico	KJ657702	KJ657715	_
Neofusicoccum arbuti	CBS 116131 t	*Arbutus menziesii*	USA: Washington	AY819720	KF531792	KF531793
Neofusicoccum australe	CBS 139662 t	*Acacia* sp.	Australia	AY339262	AY339270	AY339254
Neofusicoccum australe	CBS 121115	*Prunus persica*	South Africa	EF445355	EF445386	KX464948
Neofusicoccum cryptoaustrale	CBS 122813 t	*Eucalyptus* sp.	South Africa	FJ752742	FJ752713	FJ752756
Neofusicoccum hongkongense	CERC 2967	*Araucaria cunninghami*	China	KX278050	KX278155	KX278259
Neofusicoccum kwambonambiense	CBS 102.17 t	*Carya illinoensis*	USA: Florida	KX464169	KX464686	KX464964
Neofusicoccum luteum	CBS 562.92 t	*Actinidia deliciosa*	New Zealand	KX464170	KX464690	KX464968
Neofusicoccum mangiferae	CBS 118532	*Mangifera indica*	Australia	AY615186	DQ093220	AY615173
Neofusicoccum mediterraneum	CBS 121718 t	*Eucalyptus* sp.	Greece	GU251176	GU251308	-
Neofusicoccum occulatum	CBS 128008 t	*Eucalyptus grandis*	Australia	EU301030	EU339509	EU339472
Neofusicoccum parvum	CMW 9081 t	*Pinus nigra*	New Zealand	AY236943	AY236888	AY236917
Neofusicoccum parvum	CBS 112931	*Vitis vinifera*	South Africa	AY343466	AY343358	MT592644
Neofusicoccum parvum	CBS 118832	*Syzygium cordatum*	South Africa	MT587501	MT592216	MT592708
Neofusicoccum parvum	CBS 123650	*Syzygium cordatum*	South Africa	KX464182	KX464708	KX464994
Neofusicoccum parvum	CBS 133503	*Persea americana*	USA	MT587504	MT592219	MT592711
Neofusicoccum parvum	CBS 139672	*Bruguiera gymnorrhiza*	South Africa	MT587446	MT592156	MT592646
Neofusicoccum parvum	CBS 140889	*Vitis vinifera*	France	MT587479	MT592192	MT592684
Neofusicoccum parvum	**MNp4**	***Citrus reticulata***	**Turkey**	**MZ410755**	**MZ441080**	**MZ418107**
Neofusicoccum parvum	**LNp2**	***Citrus limon***	**Turkey**	**MZ410757**	**MZ441081**	**MZ418109**
Neofusicoccum parvum	**LNp3**	***Citrus limon***	**Turkey**	**MZ410763**	**MZ441087**	**MZ418115**
Neofusicoccum parvum	**MNp3**	***Citrus reticulata***	**Turkey**	**MZ410764**	**MZ441088**	**MZ418116**
Neofusicoccum parvum	**LNp9**	***Citrus limon***	**Turkey**	**MZ410765**	**MZ441089**	**MZ418117**
Neofusicoccum podocarpi	CBS 131677 t	*Podocarpus henkelii*	South Africa	MT587508	MT592223	MT592715
Neofusicoccum protearum	CBS 114176	*L. laureolum*	South Africa	AF452539	KX464720	KX465006

Table 1 (continued)

Species	Culture No.[1]	Host	Locality	GenBank No.[2]		
				ITS	*tef1*	*tub2*
Neofusicoccum ribis	CBS 115475 t	*Ribes sp.*	USA	AY236935	AY236877	AY236906
Neofusicoccum stellenboschiana	CBS 110864 t	*Vitis vinifera*	South Africa	AY343407	AY343348	KX465047
Neofusicoccum stellenboschiana	CBS 121116	*Prunus armeniaca*	South Africa	EF445356	EF445387	KX465049
Neofusicoccum terminaliae	CBS 125264	*Terminalia sericea*	South Africa	GQ471804	GQ471782	KX465053
Neofusicoccum vitifusiforme	CBS 110887 t	*Vitis vinifera*	South Africa	AY343383	AY343343	KX465061
Neoscytalidium dimidiatum	CBS 125610 t	Human	Gabon	MH863573	MT592262	MT592754
Neoscytalidium dimidiatum	CBS 125617	Human	France	MH863577	MT592264	MT592756
Neoscytalidium dimidiatum	CBS 145.78 t	Human	UK	KF531816	KF531795	KF531796
Neoscytalidium dimidiatum	CBS 122071 t	*Crotalaria medicaginea*	Australia	EF585540	EF585580	MT592760
Neoscytalidium dimidiatum	CBS 122072	*Adansonia gibbosa*	Australia	EF585535	EF585581	MT592761
Neoscytalidium dimidiatum	MFLUCC 12–0533 t	Orchid	Thailand	KU179865	-	-
Neoscytalidium dimidiatum	**MNn8**	***Citrus reticulata***	**Turkey**	**MZ410753**	**MZ441078**	**MZ418105**
Neoscytalidium dimidiatum	**MNn11**	***Citrus reticulata***	**Turkey**	**MZ410752**	**MZ441077**	**MZ418104**

Isolates from this study are indicated in bold type

t typespecimen

[1]CBS: Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Culture collection of P.W. Crous, housed at the WesterdijkInstitute; CMW: China Medical University and Weizmann Institute of Science; CAP: College of American Pathologists; IMI: Innovative Medicines Initiative; KUMCC: Kasetsart University Microbial Culture Collection; CGMCC: China General Microbiological Culture Collection Center; DAR: Department of Agricultural Research; MFLUCC: Mae Fah Luang University Culture Collection; CERN: Conseil Européen pour la Recherche Nucléaire

[2]ITS: internal transcribed spacers 1 and 2 together with 5.8S nrDNA; tef1: the partial translation elongation factor-1α gene; tub2: a portion of the β-tubulin gene

visualized with FigTree v. 1.6.6. The sequences generated in this study were deposited in GenBank (Table 1).

Pathogenicity tests

The pathogenicity of the five species identified was evaluated to fulfil Koch's postulates. Tests were conducted using the following representative isolates: MDS4 (*Diplodia seriata*), LDo1 (*D. olivarum*), ODv6 (*Dothiorella viticola*), MNp3 (*Neofusicoccum parvum*), and MNn8 (*Neoscytalidium dimidiatum*). A total of 18 potted, healthy, 2-year-old plants of *C. reticulata, C. limon* and *C. sinensis* were used for testing each selected fungal isolate. To evaluate the cross-infections caused by the pathogens, the isolates were tested on 18 plants from each different citrus species. For each inoculation, a sterile blade was used to wound the bark tissue, exposing the cambium. Each plant was wounded at five points. Mycelium plugs (5-mm diameter) were taken from 7-d-old cultures grown on PDA at 25 °C, and the mycelia were placed in contact with the internal wounded plant tissue. Plants inoculated with PDA plugs were included as controls. Each inoculation point was wrapped with Parafilm® (American National Can, Chicago, IL, USA). All the plants were placed in a greenhouse under controlled conditions with 80–90% relative humidity at 26±1 °C. After 60 days, the plants were evaluated for the presence of symptoms, and the length of the internal lesions was measured after bark removal, which represented the disease severity. Wood fragments (0.5 cm) were taken from symptomatic tissues and processed as described above to reisolate the inoculated fungal species, which were identified on the basis of colony morphology and ITS sequencing to fulfil Koch's postulates (Aiello et al. 2020; Bezerra et al. 2021). All the experiments were performed twice. The data obtained were tested for normality, homogeneity of variances, and residual patterns. The data were subjected to analysis of variance (one-way ANOVA) using the SPSS statistical program (v.17.0, SPSS Inc., Chicago, IL, USA), and the significance of differences between treatments was determined using least significant difference (LSD) tests ($P \leq 0.05$).

Results

Survey and fungal isolation

Symptomatic plants of *C. limon, C. reticulata* and *C. sinensis* were found in the Adana, Mersin and Hatay Provinces in the eastern Mediterranean region of Türkiye (Fig. 2). In these production areas, affected citrus trees exhibit yellowing and wilting on twigs and leaves, dieback of branch tips with scaffold branches and canker areas with gum exuding from the bark of trunks. Additionally, black to dark brown necrosis or irregular wood discolouration in the xylem was observed. When each

Fig. 2 Eastern Mediterranean region, where various Botryosphaeriaceae species were detected

Table 2 Distribution of fungal isolates, host species and associated plant tissues of *Bot* isolates in provinces of eastern Mediterranean region

Location	Citrus species	Number of isolates	Associated plant tissues		
			Trunk	Branch	Twig
Mersin	*Citrus limon*	3	1	2	
	C. reticulata	4	2	1	1
	C. sinensis	1	1		
Hatay	*Citrus limon*	3	1	2	
	C. reticulata	4	2	1	1
	C. sinensis	3	2		1
Adana	*Citrus limon*	3	1	1	1
	C. reticulata	6	1	2	3
	C. sinensis	3	2	1	
Total		**30**	**13**	**10**	**7**

tree trunk was cut transversely, necrotic areas of the vascular tissue were observed. Severely affected citrus trees presented dieback and decline symptoms. Disease symptoms were determined in each of the surveyed orchards. Generally, disease incidence on the basis of the number of plants showing symptoms (yellowing and wilting of leaves and twigs, dieback of branch tips, defoliation and branch deterioration, gummosis) associated with Botryosphaeriaceae was observed in Mersin (8), Hatay (10) and Adana (12) (Table 2). A total of 30 isolates morphologically similar to Botryosphaeriaceae fungi were obtained after isolation. In all the surveyed areas, the majority of the isolates were collected from discoloured trunks and dead branches. Among the collected isolates, 13 were obtained from trunk cankers, 10 from branch infections, and 7 from twig dieback (Table 2). All the isolates produced dark green to grey, fast-growing mycelia on PDA, and they were classified as belonging to the Botryosphaeriaceae family according to previous morphological descriptions by Phillips et al. (2013) and Yang et al. (2017). A total of 10 isolates were selected for molecular identification, and one isolate from each species was subsequently used for pathogenicity trials.

Phylogenetic analyses

Three alignments representing single-locus analyses of ITS, *tef1*, and *tub2* sequences and one combined alignment of the three loci were analysed for the representative isolates. The combined locus phylogeny consisted of 105 sequences, including the outgroup *Botryosphaeria dothidea* (CBS 110302). A total of 1422 characters (ITS: 1-561, *tef1*: 568-979, *tub2*: 986-1,422; six Ns were added as spacers between the different data partitions) were included in the analyses. For the Bayesian analyses, the following models were used according to MrModeltest: HKY+I+G for ITS, GTR+I+G for *tef1* and GTR+G for *tub2*. In the Bayesian analyses, the ITS had 235 unique site patterns, the *tef1* locus had 284 and the *tub2* locus had 217. The analyses ran for 3,270,000 generations, resulting in 6542 trees, of which 4908 were used to calculate the posterior probabilities. With respect to the MP analysis, 526 characters were parsimony informative, 215 variables were parsimony uninformative, and 669 were constant. A maximum of 1000 equally most parsimonious trees were saved (tree length = 1866; CI = 0.644; RI = 0.943; RC = 0.608). Considering the combined analyses, one isolate (LDo1) clustered with the epitype and three other reference strains of *D. olivarum*, whereas two isolates (MDs2 and MDs4) clustered with the type strain and six additional references of *D. seriata*. Two isolates (ODv2 and ODv3) were grouped into two types of specimens, and the other four reference strains, *Do. viticola*. Five isolates (LNp2, LNp3, LNp9, MNp3 and MNp4) clustered with eight reference strains of *N. parvum,* and two isolates (MNn8 and MNn11) grouped with six reference strains of *Ne. dimidiatum*, including two types of *Ne. dimidiatum* and the ex-type strains of *Ne. novaehollandiae* and *Ne. orchidearum* (Zhang et al. 2021). The bootstrap values obtained for the

parsimony analyses are shown on the Bayesian phylogenetic tree in Fig. 3.

Pathogenicity tests

All the isolates inoculated on wounded twigs were able to cause dieback symptoms 60 days postinoculation. Twigs presented brown to chocolate-brown lesions extending under the inoculation point. Typical symptoms of Botryosphaeriaceae gummosis were observed near the inoculation site (Fig. 4). The DI, which was based on the number of symptomatic twigs on citrus plants inoculated with all the isolates, was 100%. The greatest mean lesion length was observed on the plants inoculated with the *N. parvum* isolate, with values of 79.5 ± 2.5 mm, 73.5 ± 3.5 mm and 65.3 ± 2.0 mm on the mandarin, lemon and orange varieties, respectively. The representative isolate of *D. seriata* (75.0 ± 3.4 mm) caused severe lesions, comparable to those mentioned for *N. parvum*, when inoculated on mandarin. The isolate of *Ne. dimidiatum* was the least aggressive,

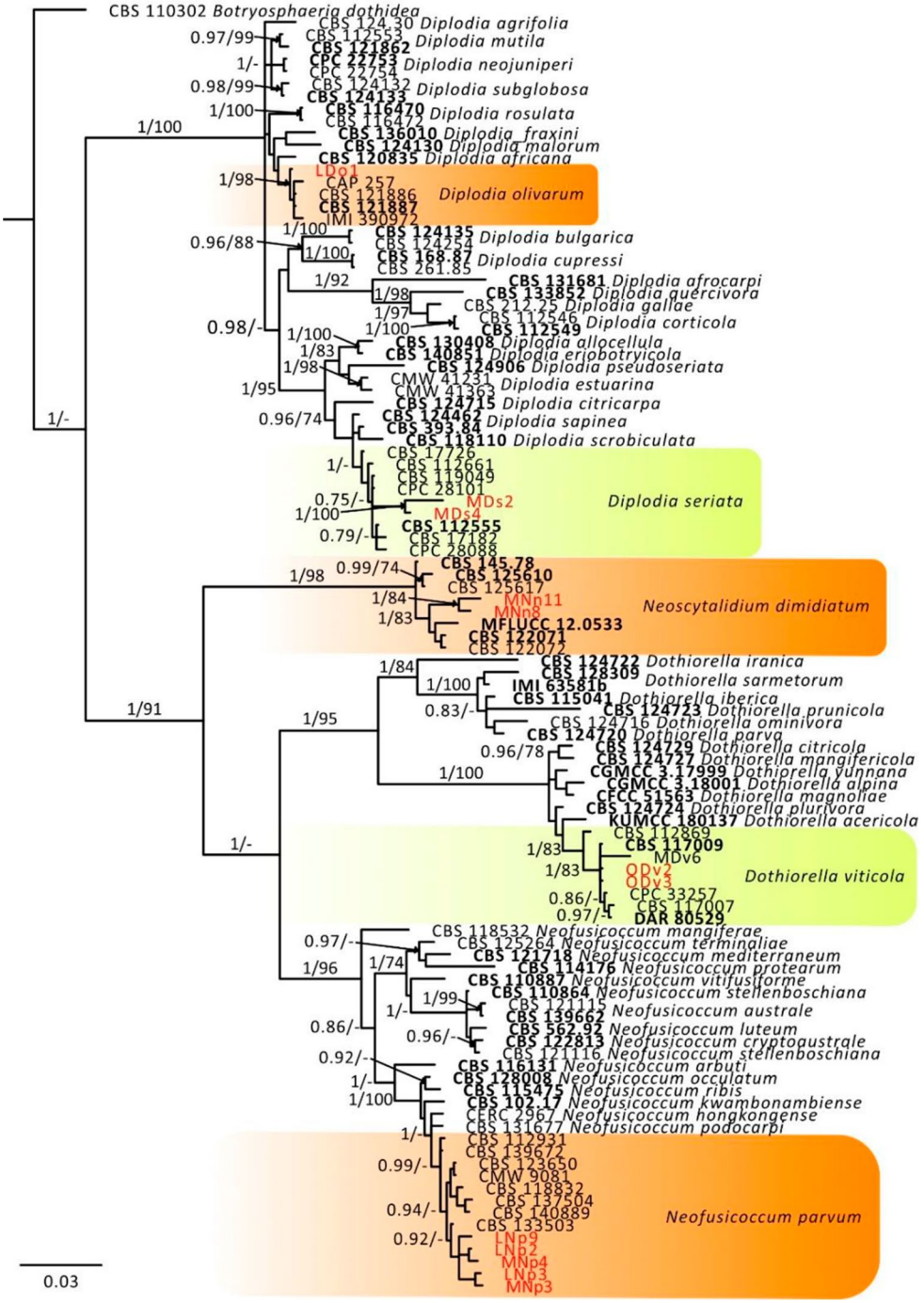

Fig. 3 Consensus phylogram of 4908 trees resulting from a Bayesian analysis of the combined ITS, tef1 and tub2 sequences of Botryosphaeriaceae isolates. Bayesian posterior probability values (BI analysis) and bootstrap support values (MP analysis) are indicated at the nodes. The tree was rooted to *Botryosphaeria dothidea* (CBS 110,302). Isolates obtained from the current study are indicated in red

Fig. 4 Pathogenicity trials of six Botryosphaeriacae isolates on *Citrus* spp. 60 d after inoculation. Abundant gummosis exudate caused by *N. parvum* and *Ne. dimidiatum* (**A, B**). Shoot blight inoculated with *N.parvum, D. olivarum* and *D. seriata* (**C, D**). Internal discoloration of shoots inoculated with all species (*Ne. dimidiatum, Do. viticola, N. parvum, D.seriata, D. olivarum*)

at 30.6±4.0 mm, on the mandarin. *Do. viticola* had the greatest lesion length (70.6±3.6 mm) on the orange. On the other hand, *D. olivarum* presented high lesion lengths (69.0±2.6 mm, 50.3±1.3 mm) on mandarin and lemon (Fig. 5). No symptoms were observed on the control plants. Reisolation of the inoculated fungal species and reidentification on the basis of morphological characteristics and tef-1 sequencing fulfilled Koch's postulates.

Discussion

The present study confirms the occurrence of Botryosphaeriaceae species affecting citrus in the main growing areas in the eastern Mediterranean region of Türkiye for the first time. The identification and characterization of the fungal pathogens associated with citrus twig and branch dieback, cankers and gummosis were performed via molecular and phylogenetic analyses. Different Botryosphaeriaceae species were isolated from symptomatic plant materials collected from the surveyed orchards. In particular, five species were identified: *D. seriata, D. olivarum, Do. viticola, N. parvum* and *Ne. dimidiatum*. These findings agree with previous studies in which these fungal species were isolated from the same type of citrus symptom elsewhere (Adesemoye et al. 2014; Hamrouni et al. 2018; Berraf-Tebbal et al. 2020; Espargham et al. 2020; Bezerra et al. 2021; Xiao et al. 2021). The Botryosphaeriaceae species that cause diseases in citrus plants are well known in European countries and worldwide. *Diplodia seriata* and *D. olivarum* have been previously reported to affect citrus trees in Algeria, California, China and Europe (Adesemoye et al. 2014; Berraf-Tebbal et al. 2020; Bezerra et al. 2021; Xiao et al. 2021). *Dothiorella viticola* has been detected as a causal agent of branch and trunk dieback and gummosis of several *Citrus* species in Algeria, Tunisia, California İran, Greece, Italy, Portugal, Malta and Spain (Adesemoye et al. 2014; Hamrouni et al. 2018; Berraf-Tebbal et al. 2020; Espargham et al. 2020; Bezerra et al. 2021). *Neofusicoccum parvum* has been described in association with yellowing of the canopy, gummy cankers on trunks and scaffold branches of mature citrus trees in California, China, and European countries (Adesemoye et al. 2014; Vakalounakis et al. 2019; Aloi et al. 2021; Bezerra et al. 2021; Xiao et al. 2021). *Neoscytalidium dimidiatum* has been detected as a

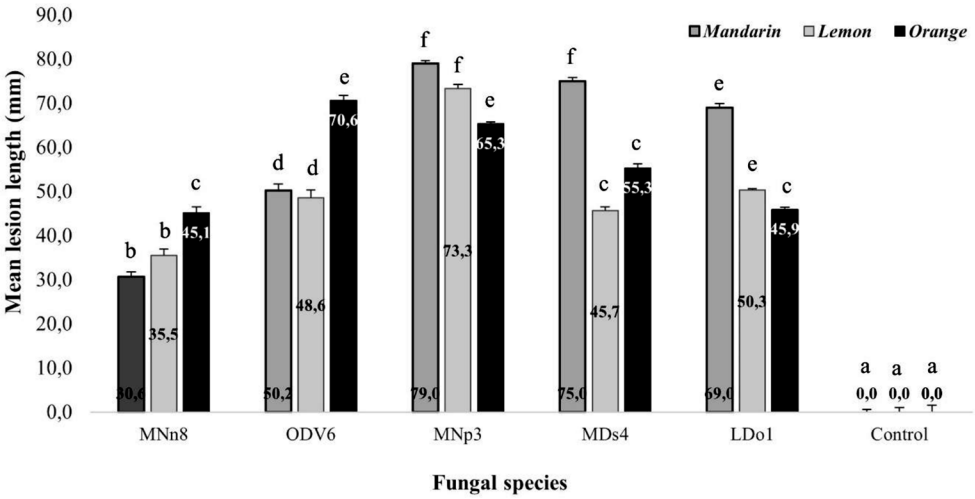

Fig. 5 Column chart indicating the mean lesion lengths (mm) from inoculation with 6 representative isolates of Botryosphaeriaceae (MNn8: *Neoscytalidium dimidiatum*, ODv6: *Dothiorella viticola*, MNp3: *Neofusicoccum parvum* MDs4: *Diplodia seriata* LDo1: *Diplodia olivarum* on *C. reticulata, C.limon, C. sinensis.* (Vertical lines represent standard error of the mean according to Tukey's honestly significant difference mean separation test at $\alpha = 0.05$). Error bars represent standard deviation

causal agent of citrus gummosis and dieback in California and Italy (Polizzi et al. 2011; Adesemoye et al. 2014).

In the surveyed region, fungal isolates were mostly obtained from mandarin, followed by lemon and orange, and *N. parvum* was the predominant isolated species, followed by *D. seriata*. These findings are in accordance with previous investigations by Adesemoye et al. (2014) and Bezerra et al. (2021), who reported different species of *Neofusicoccum* and *Diplodia* as the most frequent pathogens causing gummosis on citrus in different areas worldwide, including California, Greece, Italy, Portugal, Malta, and Spain. Moreover, other species belonging to Botryosphaeriaceae that were found as pathogens on citrus plants include *Botryosphaeria dothidea, B. fabicerciana, D. alpina, D. plurivora, D. citrimurcotticola, D. pseudoseriata, D. mutila, Lasiodiplodia citricola, L. iraniensis, L. microconidia, L. pseudotheobromae, L. theobromae, L. guilinensis, L. huangyanensis, L. linhaiensis, L. ponkanicola Neodeightonia subglobosa, N. luteum, N. mediterraneum, Ne. hyalinum, Ne. novaehollandiae* and *Sphaeropsis linhaiensis.*

In Türkiye, the presence and diversity of Botryosphaeriaceae species associated with branch, twig and wood cankers and dieback have been reported on different hosts, including olive (Korukmez et al. 2020), grapevine (Akgül et al. 2013, 2014, 2015, 2020; Akgül and Ahioğlu 2019; Oksal and Çelik 2021), pistachio (Toker Demiray and Akçalı 2020), almond (Kayım et al. 2015; Sakcı et al. 2022), and walnut (Kurt et al. 2020); however, this phenomenon has not been reported in citrus plants. The climate conditions in the surveyed area differ significantly from those in regions where grapevines and pistachios grow. The Botryosphaeriaceae species that were identified on citrus plants in the present study are important pathogens on olive, almond and nut trees established in geographically overlapping areas (Guarnaccia et al. 2022; Martino et al. 2024). Botryosphaeriaceae species can jump from citrus plants to olive, almond and nut orchards near or adjacent to citrus orchards. Conversely, Botryosphaeriaceae pathogens could represent a threat from olive, almond and walnut to citrus trees. The mechanisms of transmission and spread of these species among hosts over a geographical range, as well as the proximity to suitable hosts, need to be further explored to determine effective strategies for management. The pathogenicity tests performed here confirmed the ability of all the species to infect wounded citrus twigs. Significant differences in aggressiveness were observed among species, with *N. parvum* being the most aggressive species. Conversely, *Ne. dimidiatum* was weakly virulent. The knowledge of the aggressiveness of pathogens, coupled with precise and correct identification, provides useful information for the adoption of effective management strategies. Therefore, determining the pathogenicity of Botryosphaeriaceae species is necessary to ensure the effective management of dieback and trunk canker disease in citrus orchards. In Türkiye, where there is a constant increase in the number of citrus trees planted every year, infections caused by Botryosphaeriaceae represent a growing concern for producers. In this context, epidemiological studies should be performed to determine high-risk infection periods throughout the growing season. Currently, citrus Botryosphaeriaceae gummosis in the surveyed area is managed by pruning and removing dead limbs and twig waste from fields during rainy periods. Studies conducted on grapevines have confirmed the effectiveness of this cultural practice (Bertsch et al. 2013). However, it is necessary to conduct larger surveys for the further implementation and development of effective disease management measures for citrus plants.

Conclusion

In Türkiye, where the number of citrus trees planted each year is increasing, infections caused by Botryosphaeriaceae are becoming a growing concern for producers. On the basis of this study, epidemiological studies should be conducted to determine high-risk infection periods throughout the growing season. Management of citrus Botryosphaeriaceae gummosis in the surveyed area involves pruning and removing dead limbs and twig waste from fields during rainy periods, and studies on grapevines confirm the effectiveness of this cultural practice. However, larger surveys are necessary for further implementation and development of effective disease management measures for citrus plants.

Acknowledgements We thank all the researchers involved in this study. Additionally, all stages of this research were conducted in the Plant Health Clinic (BISAK) laboratories of Hatay Mustafa Kemal University.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Data availability Data sharing is not applicable to this article, as no new data were created or analysed in this study.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest. This research does not contain any studies with human participants or animals.

References

Abdollahzadeh J, Javadi A, Goltapeh EM, Zare R, Phillips AJ (2010) Phylogeny and morphology of four new species of Lasiodiplodia from Iran. Persoonia 25:1–10. https://doi.org/10.3767/003158510X524150

Adesemoye AO, Eskalen A, Faber B, O'Connell N (2011) Multiple *Botryosphaeria* species causing 'Dothiorella' gummosis in citrus. Citrograph 2(2):32–34

Adesemoye AO, Mayorquin JS, Wang DH, Twizeyimana M, Lynch SC, Eskalenet A (2014) Identification of species of Botryosphaeriaceae causing bot gummosis in citrus in California. Plant Dis 98:55–61. https://doi.org/10.1094/PDIS-05-13-0492-RE

Aiello D, Bregant C, Carlucci A, Guarnaccia V, Gusella G, Linaldeddu BT, Polizzi G (2023) Current status of Botryosphaeriaceae species in Italy: impacts on agricultural crops and forest ecosystems. Phytopathol Mediterr 62(3):381–412. https://doi.org/10.36253/phyto-14711

Aiello D, Giorgio G, Fiorenza A, Guarnaccia V, Polizzi G (2020) Identification of *Neofusicoccum parvum* causing canker and twig blight on *Ficus carica* in Italy. Phytopathol Mediterr 59(1):147–153. https://doi.org/10.36253/phyto-10798

Aiello D, Guarnaccia V, Costanzo MB, Leonardi GR, Epifani F, Perrone G, Polizzi G (2022) Woody canker and shoot blight caused by Botryosphaeriaceae and Diaporthaceae on mango and litchi in Italy. Horticulturae 8:330. https://doi.org/10.3390/horticulturae8040330

Akgül DS, Ahioğlu M (2019) Fungal pathogens associated with young grapevine decline in the Southern Türkiye vineyards. Viticulture 01027:6. https://doi.org/10.1051/bioconf/20191501027

Akgül DS, Güngör Savaş N, Teker T (2013) Identification and pathogenicitiy of Botryosphaeriaceae species causing wood canker on grapevines. J Turk Phytopathol 42(1-2-3):35–45

Akgül DS, Özarslandan M, Erkılıç A (2020) Türkiye'nin güneyinde asmada Botryosphaeria geriye ölüm hastalığına neden olan fungusların filogenetik ayrımı ve patojenisiteleri. Plant Prot Bull 60(2):63–72. https://doi.org/10.16955/bitkorb.646134

Akgül DS, Savas NG, Eskalen A (2014) First report of wood canker caused by *Botryosphaeria dothidea, Diplodia seriata, Neofusicoccum parvum*, and *Lasiodiplodia theobromae* on Grapevine in Türkiye. Plant Dis 98(4):568. https://doi.org/10.1094/PDIS-07-13-0726-PDN

Akgül DS, Savas NG, Teker T, Keykubat B,, Mayorquın J, Eskalen A (2015) Fungal trunk pathogens of Sultana seedless vineyards in Aegean region of Türkiye. Phytopathol Mediterr 54(2):380–393. https://doi.org/10.14601/Phytopathol_Mediterr-16138

Aloi F, Riolo M, Sanzani SM, Mincuzzi A, Ippolito A, Siciliano I, Pane A, Gullino ML, Cacciola SO (2021) Characterization of *Alternaria* species associated with heart rot of pomegranate fruit. J Fungus 7:172. https://doi.org/10.3390/jof7030172

Batista E, Lopes A, Alves A (2021) What do we know about Botryosphaeriaceae? An overview of a worldwide cured dataset. Forests 12:313. https://doi.org/10.3390/f12030313

Bautista-Cruz MA, Almaguer-Vargas G, Leyva-Mir SG et al (2019) Phylogeny, distribution, and pathogenicity of *Lasiodiplodia* species associated with cankers and dieback symptoms of Persian lime in Mexico. Plant Dis 103:1156–1165. https://doi.org/10.1094/PDIS-06-18-1036-RE

Berraf-Tebbal A, Mahamedi AE, Aigoun-Mouhous W, Špetík M, Čechová J, Pokluda R, Baránek M et al (2020) *Lasiodiplodia mitidjanasp* nov. and other Botryosphaeriaceae species causing branch canker and dieback of *Citrus sinensis* in Algeria. PLoS One 15(5):e0232448. https://doi.org/10.1371/journal.pone.0232448

Bertsch C, Ramírez-Suero M, Magnin-Robert M, Larignon P, Chong J, Abou-Mansour E et al (2013) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol 62:243–265. https://doi.org/10.1111/j.1365-3059.2012.02674.x

Bezerra JDP, Crous PW, Aiello D, Gullino ML, Polizzi G, Guarnaccia V (2021) Genetic diversity and pathogenicity of Botryosphaeriaceae species associated with symptomatic citrus plants in Europe. Plants 10:492. https://doi.org/10.3390/plants10030492

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycol 91:553–556. https://doi.org/10.1080/00275514.1999.12061051

Espargham N, Mohammadi H, Gramaje DA (2020) Survey of trunk disease pathogens within citrus trees in Iran. Plants 9:754. https://doi.org/10.3390/plants9060754

FAOSTAT (2022) Word production data. https://www.fao.org/faostat/en/#data/QCL

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61:1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Guajardo J, Riquelme N, Tapia L, Larach A, Torres C, Camps R, Besoain X (2018) First report of *Lasiodiplodia theobromae* causing bot gummosis in *Citrus limon* in Chile. Plant Dis 102:818. https://doi.org/10.1094/PDIS-09-17-1517-PDN

Guarnaccia V, Kraus C, Markakis E, Alves A, Armengol J, Eichmeier A, Gramaje D (2022) Fungal trunk diseases of fruit trees in Europe: pathogens, spread and future directions. Phytopathol Mediterr 61:563–599. https://doi.org/10.36253/phyto-14167

Guarnaccia V, Martino I, Gilardi G, Garibaldi A, Gullino ML (2021a) *Colletotrichum* spp. causing anthracnose on ornamental plants in northern Italy. Plant Pathol J 103:127–137. https://doi.org/10.1007/s42161-020-00684-2

Guarnaccia V, Van Niekerk J, Crous P, Sandoval-Denis M (2021b) *Neocosmospora* spp. associated with dry root rot of citrus in South Africa. Phytopathol Mediterr 60(1):79–100. https://doi.org/10.36253/phyto-12183

Hamrouni N, Nouri M, Trouillas F, Said A, Sadfi-Zouaoui N, Hajlaoui M (2018) Dothiorella gummosis caused by *Dothiorella viticola*, first record from citrus in Tunisia. New Dis Rep 38:10. https://doi.org/10.5197/j.2044-0588.2018.038.010

Huang F, Zhu L, Hou X, Li HY (2012) Identification of the pathogenic fungus causing brown spot on Ougan. J Zhejiang Agric Sci 9:1281–1282 (in Chinese)

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7, improvements in performance and usability. Molec Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Kayım M, Endes A, Eskalen A (2015) First report of *Neofusicoccum parvum* and *Diplodia seriata* associated with wood canker and dieback on almond in Türkiye. In: XVlll. International Plant Protection Congress

Korukmez N, Yildiz F, Yayla S, Gencer R, Akpinar O (2020) First report of fruit rot caused by *Botryosphaeria dothidea* on olive in Türkiye. J Plant Pathol 102:537. https://doi.org/10.1007/s42161-019-00429-w

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Kurt Ş, Soylu S, Uysal A, Soylu EM, Kara M (2020) Identification of walnut stem canker disease agent *Botryosphaeria dothidea* and determination of *in vitro* antifungal efficiencies of some fungicides against the disease agent. MKU Tar Bil Derg 25(1):46–56. https://doi.org/10.37908/mkutbd.686111

Leonardi GR, Aiello D, Camilleri G, Piattino V, Polizzi G, Guarnaccia V (2023) A new disease of kumquat (*Fortunella margarita*) caused by *Colletotrichum karsti*: twig and branch dieback. Phytopathol Mediterr 62(3):333–348. https://doi.org/10.36253/phyto-14544

Liu Y, Heying E, Tanumihardjo SA (2012) History, global distribution, and nutritional importance of citrus fruits. Compr Rev Food Sci Food Saf 11:530–545

Martino I, Monchiero M, Gullino ML, Guarnaccia V (2024) Characterization and pathogenicity of fungal species associated with hazelnut trunk diseases in North-western Italy. Plant Pathol J 1:19. https://doi.org/10.1007/s42161-024-01595-2

Mayorquin JS, Wang DH, Twizeyimana M, Eskalen A (2016) Identification, distribution, and pathogenicity of Diatrypaceae and Botryosphaeriaceae associated with Citrus branch canker in the southern California desert. Plant Dis 100:2402–2413. https://doi.org/10.1094/PDIS-03-16-0362-RE

Nylander JAA (2004) MrModeltest v. 2. program distributed by the author. Uppsala Evolutionary Biology Centre Uppsala University

Oksal E, Çelik Y (2021) Determination of grapevine stem fungal diseases in Malatya province. Plant Prot Bull 61(3):42–50. https://doi.org/10.16955/bitkorb.891435

Phillips AJL, Alves A, Abdollahzadeh J, Slippers B, Wingfield MJ, Groenewald JZ, Crous PW (2013) The Botryosphaeriaceae: genera and species known from culture. Stud Mycol 76:51–167. https://doi.org/10.3114/sim0021

Piattino V, Aiello D, Dardani G, Martino I, Flores M, Aćimović SG, Guarnaccia V (2024) *Lasiodiplodia iraniensis* and *Diaporthe* spp. are associated with twig dieback and fruit stem-end rot of sweet orange, *Citrus sinensis*, in Florida. Horticulture 10(4):406. https://doi.org/10.3390/horticulturae10040406

Polizzi G, Aiello D, Castello I, Vitale A, Groenewald JZ, Crous PW (2011) Occurrence, molecular characterisation, and pathogenicity of *Neoscytalidium dimidiatum* on citrus ın Italy. Acta Hortic 892. https://doi.org/10.17660/ActaHortic.2011.892.27

Polizzi G, Aiello D, Vitale A, Giuffrida F, Groenewald J, Crous PW (2009) First report of shoot blight, canker, and gummosis caused by *Neoscytalidium dimidiatum* on citrus in Italy. Plant Dis 93:1215. https://doi.org/10.1094/PDIS-93-11-1215A

Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2 efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Sakcı N, Kurt Ş, Uysal A, Soylu EM, Kara M, Soylu S (2022) Identification and pathogenicity of *Neoscytalidium novaehollandiae*, the agent of canker and dieback in almonds and in vitro activities of some fungicides. YYU J Agr Sci 32(1):132–142. https://doi.org/10.29133/yyutbd.964030

Sandoval-Denis M, Guarnaccia V, Polizzi G, Crous PW (2018) Symptomatic citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. Persoonia 40:1–25. https://doi.org/10.3767/persoonia.2018.40.01

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (*and other methods) v. 4.0b10. Sinauer Associates, Sunderland; MS, USA

Toker Demiray S, Akçalı E (2020) Pathogenic and molecular characterization of panicle and shoot blight disease (*Botryosphaeria dothidea*) in pistachio in the mediterranean and southeastern anatolia regions. YYU J AGR SCI 30(4):721–731. https://doi.org/10.29133/yyutbd.715163

Uysal A, Kurt Ş, Guarnaccia V (2022) Distribution and characterization of *Colletotrichum* species associated with Citrus anthracnose in eastern Mediterranean region of Türkiye. EurJ Plant Pathol 163:125–141. https://doi.org/10.1007/s10658-022-02462-5

Vakalounakis DJ, Ntougias S, Kavroulakis N, Protopapadakis E (2019) *Neofusicoccum parvum* and *Diaporthe foeniculina* associated with twig and shoot blight and branch canker of citrus in Greece. J Phytopathol 167(9):527–537. https://doi.org/10.1111/jph.12843

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Academic Press, San Diego, CA, USA, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Whiteside JO (1980) Dothiorella Gummosis. In: Whiteside JO, Garnsey SM, Timmer LW (eds) Compendium of citrus diseases, 1st ed. American Phytopathological Society, St. Paul, MN, pp 14

Xiao XE, Wang W, Crous PW, Wang HK, Jiao C, Huang F, Pu ZX et al (2021) Species of Botryosphaeriaceae associated with citrus branch diseases in China. Persoonia 47:106–135. https://doi.org/10.3767/persoonia.2021.47.03

Yang T, Groenewald JZ, Cheewangkoon R, Jami F, Abdollahzadeh J, Lombard L, Crous PW (2017) Families, genera, and species of Botryosphaeriales. Fungal Biol 121:322–346. https://doi.org/10.1016/j.funbio.2016.11.001

Zhang W, Groenewald JZ, Lombard L, Schumacher RK, Phillips AJL, Crous PW (2021) Evaluating species in Botryosphaeriales. Persoonia 46:63–115. https://doi.org/10.3767/persoonia.2021.46.03

Zhu L, Zhao XL, Wu RC et al (2011) Identification of the pathogen causing foot rot of *Fortunella margarita* (Lour.) Swingle. Acta Phytopathol Sinica 41:631–634. [In Chinese]