Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Botryosphaeriaceae fungi associated with apricot dieback and gummosis in Türkiye

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Summary. Apricot (*Prunus armeniaca* L.) is an important and widely grown fruit crop in Türkiye. In the last 15 years, symptoms of branch dieback and gummosis have been observed in commercial apricot orchards. A survey conducted in 2015 across 44 apricot orchards in the Adana and Mersin provinces revealed consistent infections caused by *Botryosphaeriaceae* fungi. From symptomatic branch samples (n = 232), a total of 128 fungal isolates with botryosphaeriaceous morphology were recovered, representing an incidence of 55.2%. Preliminary morphological identifications suggested the presence of three species. Representative isolates from each morphological group were identified as *Diplodia seriata* (n = 10), *Neofusicoccum parvum* (n = 7), and *Lasiodiplodia mediterranea* (n = 1), based on phylogenetic analyses of nucleotide sequences from the ribosomal DNA internal transcribed spacer, beta-tubulin and translation elongation factor 1-alpha genes. Mycelium growth rates were different among the three species, and the optimal growth temperatures were estimated as 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea*. Pathogenicity of the identified fungi was confirmed on 2-year-old 'Tyrinthe' apricot seedlings, with *L. mediterranea* being the most virulent, followed by *D. seriata*, and *N. parvum*. This is the first report of *D. seriata*, *N. parvum*, and *L. mediterranea* causing branch dieback and gummosis of apricot in Türkiye.

Keywords. *Diplodia*, etiology, *Lasiodiplodia*, *Neofusicoccum*, *Prunus armeniaca*

OPEN ACCESS

Citation: Kayim, M., Bustamante, M. I., Endes, A. & Eskalen, A. (2025). *Botryosphaeriaceae* fungi associated with apricot dieback and gummosis in Türkiye. *Phytopathologia Mediterranea* 64(2): 205-218. doi: 10.36253/phyto-15991

Accepted: July 20, 2025

Published: September 12, 2025

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Lizel Mostert, Faculty of AgriSciences, Stellenbosch, South Africa.

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INTRODUCTION

Apricot (*Prunus armeniaca* L.) is an economically important and widely grown fruit tree in Türkiye. Although commercial production of apricot is concentrated in the provinces of Malatya, Mersin, Elazığ, Iğdır, and Kahramanmaraş (Hasdemir, 2022), cultivation in the eastern Mediterranean region of Türkiye has expanded over the past 20 years to meet the demands for early-season fresh fruit consumption. Apricots are main-

ly consumed fresh and dried, as well as in processed forms including as jam, juice, and baby food. Türkiye accounts for 25.2% of the global apricot production area, covering 141,851 ha, and contributes 21.7% of total global yield, amounting to 803,000 metric tons annually (FAOSTAT, 2024). More than half of Türkiye's apricot production originates from the Eastern Anatolia and Mediterranean regions. In 2022, total world apricot production area was 562,000 ha, with total fruit yield of 3.7 million metric tons.

Several biotic factors may limit apricot production. Among these, diseases such as dieback and gummoses on trunks and scaffolds of trees have been identified as potential threats to apricot orchards in the East Mediterranean Region of Türkiye. The causal agents of dieback in stone fruit crops (*Prunus* spp.) have been partially attributed to be fungi within the *Botryosphaeriaceae,* which are distributed in many countries, and affect numerous tree species which potentially serve as inoculum sources for apricot orchards (Zhang *et al*., 2021). For example, *Diplodia seriata* has been reported affecting apricot in Wisconsin, United States of America (Smith and Stanosz, 2006), South Africa (Damm *et al*., 2007) and Canada (Ellouze and Ilyukhin, 2024). *Botryosphaeria dothidea* has been detected affecting Japanese apricots in Taiwan (Ko *et al*., 2010). In the Czech Republic, species of *Diplodia* and *Dothiorella* were recorded associated with apricot decline (Spetik *et al*., 2024). In Iran, apricot dieback and gummosis were determined to be caused by *B. dothidea*, *D. seriata, Lasiodiplodia theobromae*, *Neofusicoccum mangiferae*, and *N. parvum*, among other pathogens (Soltaninejad *et al*., 2017). In Türkiye, *Neoscytalidium dimidiatum* has been reported as the causative agent of shoot blight, dieback, and canker on apricot trees in Central Anatolia (Oksal *et al*., 2020).

Apricot orchards were established in southern Türkiye between 2005 and 2014, using European varieties for early table fruit production. Within a few years after planting, several young trees showed sudden dieback and branch canker, while trees more than 10 years old were also affected. Since apricot orchards were planted near other stone fruit orchards, including plums and nectarines which were known to be infected by *D. seriata*, *L. theobromae*, *L. pseudotheobromae*, and *N. parvum* (Endes *et al*., 2016; Endes and Kayim, 2022), it can be hypothesized that apricot trees were also infected by *Botryosphaeriaceae* fungi. Consequently, the objectives of the present study were: (i) to identify the fungi associated with dieback and gummosis of apricot trees in Türkiye; and (ii) to determine their pathogenicity and virulence on apricot.

MATERIALS AND METHODS

Field sampling and isolation of fungi

Field sampling was conducted in 44 apricot orchards with histories of branch dieback and gummoses. The orchards were located in the provinces of Adana (n = 25) and Mersin (n = 19). In each orchard, trees were inspected across diagonal transects, following the method described by Lazarov and Grigorov (1961). Common symptoms, including dieback, gummoses, and wood cankers were recorded. Symptomatic branches (lengths 20 to 70 cm) and sections of main trunks (lengths 5 to 20 cm) were collected and transported to the laboratory. Fungal isolations were carried out from symptomatic plant samples (n = 232), after surface disinfection using 1% sodium hypochlorite for 5 min followed by four rinses in sterile distilled water. The samples were then dried on clean filter papers, and four to five pieces (1 to 10 mm^2) were excised and placed onto Petri dishes containing potato dextrose agar amended with 0.01% tetracycline (PDA-tet) (Sigma-Aldrich). Plates were then incubated in the dark at 25±1°C for 4 to 7 d. Subcultures of isolates from fungi growing from tissue pieces were made by transferring hyphal tips onto fresh PDA–tet plates. Single conidium cultures from representative isolates of each pathogen species were obtained according to Choi *et al.* (1999), for molecular identification and morphological characterizations. Fungal colonies were sub-cultured on fresh PDA, and then stored in 30% glycerol in a −80°C deep-freeze for long term storage as fungal plugs.

Morphological characterization of isolated fungi

Fungal isolates identified to species level were further studied for their cultural and conidial characteristics as previously described (Damm *et al*., 2007, Phillips *et al*., 2013). All selected isolates (n = 16) were first grown on PDA or 3% oat meal agar (OMA) and incubated at 25°C under a 12 h daily photoperiod for 30 days, to induce conidium production (Amponsah *et al*., 2008; Wang *et al*., 2011). During the incubation period, colony morphology was observed for cultures on PDA. Conidium size (length and width) of each isolate was determined for 50 conidia, using a compound microscope camera (Olympus Bx51).

Molecular identification of isolated fungi

Total genomic DNA from 18 fungal isolates was extracted using a commercial kit following the manu-

facturer's protocol (Qiagen GmbH). Polymerase chain reactions (PCR) were carried out using the primer pairs ITS4/ITS5 (White *et al*., 1990), Bt2a/Bt2b (Glass and Donaldson, 1995), and 688F/1251R (Alves *et al*., 2008) to amplify, respectively, the ribosomal DNA internal transcribed spacer (ITS), the beta-tubulin (*tub2*), and translation elongation factor 1-alpha (*tef1*) gene regions. PCRs were run in a T100 thermocycler (Bio-Rad). Each PCR reaction had a total volume of 50 µL, that consisted of 25 µL of DNA polymerase master mix (GoTaq® Green MasterMix 2X, Promega), 18.6 µL of nuclease-free water, 1.2 µL of each primer (10 µM), and 4 µL of DNA template (approx. 100 ng). The PCR parameters included a 3 min preheating at 95°C, followed by 35 cycles each of 30 s at 95°C for denaturation, 45 s at 49°C for ITS and *tef1* annealing or 58°C for *tub2*, and 60 s at 72°C, with a final extension for 7 min at 72°C. The PCR products were visualized by electrophoresis in 1% agarose gels, and were purified for sequencing with the QIAquick PCR Purification Kit (Qiagen). Sanger sequencing was carried out by Genoks (Ankara, Türkiye). Obtained sequences were assembled using BioEdit software (version 7.2.5). Consensus sequences were compared with those deposited in the NCBI GenBank database using BLAST (version 2.0) searches. All isolates (n = 18) were first sequenced for ITS. From groups sharing identical ITS sequences, representative isolates were further sequenced for *tub2* (n = 8), and only one was sequenced for *tef1* (isolate MEKA194). Two sequence databases were created, one consisting of ITS and *tub2* sequences and the second consisting of ITS, *tub2* and *tef1* sequences, incorporating reference strains of species that showed similarity percentages greater than 97% in the BLAST search results (Tables 1 and 2). Alignments were performed by locus in MAFFT 7 (Katoh *et al*., 2019), and were manually trimmed on BioEdit 7 (Hall, 1999). Alignments were combined into one, and phylogenetic analyses were performed using maximum parsimony (MP) in MEGA 11 (Tamura *et al*., 2021) and maximum likelihood (ML) in IQ-TREE 2 (Minh *et al*., 2020), each with 1,000 bootstrap replicates. The MP phylogenies were generated using the tree-bisection-regrafting algorithm at search level 1, with initial trees obtained through the random addition of sequences across ten replicates. Tree length, consistency index (CI), retention index (RI), and composite index (CI) values were recorded for each analysis. For ML analyses, the dataset was partitioned by locus to identify the best-fit models, based on the PartitionFinder algorithm (Lanfear *et al*., 2012), resulting in two and three partitioned subsets. Settings for ML analysis were as described elsewhere (Bourret *et al*., 2018). The resulting trees were then compared, and support values were combined for nodes sharing identical topology using Inkscape 0.92 (http://inkscape.org).

Effects of temperature on isolate mycelium growth

Representative isolates of *Diplodia seriata* (AYKA244, MEYKA116, and MEYKA124), *Neofusicoccum parvum* (MEKA205) and *Lasiodiplodia mediterranea* (MEKA194) were cultured on PDA for 48 h at 25°C in continuous darkness to obtain actively growing mycelium. Mycelium plugs (each 4 mm diam.) were made around colony margins using a sterile cork borer, and were transferred to new Petri dishes each containing 20 mL of PDA. A plug was placed at the centre of each plate with the mycelium facing the medium surface. Plates were then incubated in darkness at different temperatures (5, 10, 15, 20, 25, 30, or 35°C) for 48 h. Resulting colony diameters were then measured using a caliper, with two (horizontal and vertical) measurements that were averaged for each colony. The experiment was repeated twice, with five replicate plates for each isolate. The optimal growth temperature (T_{opt}) and maximum colony growth (Y_{max}) were estimated for each replicate using the Analytis beta model, following Moral *et al*. (2012). Data obtained were subjected to analysis of variance (ANOVA) using generalized linear models in InfoStat (version 2008). Normality and homoscedasticity of data were checked and corrected when necessary, and means were compared using Fisher's LSD test (α = 5%).

Table 1. Locations, frequencies of orchards, plant samples, and *Botryosphaeriaceae* isolates recovered from apricot trees in Türkiye.

Provinces	Surveyed orchards (n)	Collected samples (n)	Frequency of isolates recovered (n)			
			D. seriata	*N. parvum*	*L. mediterranea*	Total *Botryosphaeriaceae*
Adana	25	135	41	35	0	76
Mersin	19	97	35	16	1	52
Total	44	232	76	51	1	128

Table 2. Conidium dimensions of isolates of *Botryosphaeriaceae* species obtained from samples from apricot trees affected by dieback and gummosis in Türkiye.

Isolate[a]	Conidium length (L) × width (W) (µm) [b]	Mean ± SD (µm)[c]	L/W ratio ± SD[d]
D. seriata			
AYKA374.3	(22.5–)24.6-27.5 × (11.5–)12.5-15.0	24.4 ± 0.2 × 12.6 ± 0.1	1.9 ± 0.02
AYKA374.1	(20.0–)24.1-27.0 × (10.0–)12.5-14.5	24.2 ± 0.2 × 12.4 ± 0.2	2.0 ± 0.03
AYKA374.2	(22.5–)24.5-27.0 × (10.5–)12.3-13.3	24.3 ± 0.2 × 12.2 ± 0.1	2.0 ± 0.02
MEYKA124	(17.3–)23.0-29.5 × (8.0–)10.0-12.5	23.4 ± 0.4 × 9.9 ± 0.1	2.4 ± 0.04
AYKA244	(19.0–)25.3-29.5 × (8.0–)10.8-13.3	25.3 ± 0.3 × 10.9 ± 0.2	2.3 ± 0.02
MEYKA116	(23.3–)25.4-29.8 × (10.0–)11.3-13.3	25.9 ± 0.2 × 11.4 ± 0.1	2.3 ± 0.02
MEYKA39	(18.5–)23.8-30.0 × (7.5–)10.0-13.0	23.7 ± 0.4 × 10.0 ± 0.2	2.4 ± 0.04
MEYKA117	(22.8–)25.4-29.3 × (8.8–)11.3-13.3	25.7 ± 0.2 × 11.3 ± 0.1	2.3 ± 0.02
CBS 112555[T]	(21.5–)22.0-28.0) × (11.0–)11.5-15.5	24.9 ± 1.9 × 12.9 ± 1.1	1.9
N. parvum			
MEKA205	(13.8–)18.8-23.8 × (3.8–)6.3-8.8	19.2 ± 0.4 × 6.0 ± 0.2	3.2 ± 0.05
AKKA308.2	(12.5–)18.9-29.0 × (3.8–)6.3-13.5	19.1 ± 0.6 × 6.9 ± 0.4	2.9 ± 0.07
AKKA308.6	(11.3–)18.3-23.8 × (3.8–)5.8-8.5	18.3 ± 0.5 × 5.8 ± 0.2	3.2 ± 0.05
AKKA308.4	(12.5–)20.1-23.8 × (3.8–)6.0-8.0	19.4 ± 0.4 × 5.9 ± 0.2	3.3 ± 0.05
AKKA308.3	(11.3–)18.1-24.0 × (3.8–)5.3-8.0	17.7 ± 0.5 × 5.5 ± 0.2	3.3 ± 0.06
AKKA308.5	(12.3–)18.1-24.0 × (4.0–)6.3-8.0	18.2 ± 0.5 × 6.0 ± 0.1	3.1 ± 0.08
AKKA308.1	(12.3–)19.4-24.0 × (3.8–)6.1-8.0	18.7 ± 0.4 × 5.9 ± 0.1	3.2 ± 0.07
CMW 9081[T]	(12.0–)15.0-24.0 × 4.0-6.0	16.9 × 5.4	3.1
L. mediterranea			
MEKA194	(18.5–)26.9-34.0 × (10.8–)15.0-20.5	26.4 ± 0.6 × 14.9 ± 0.3	1.8 ± 0.02
CBS 137784[T]	(26.3–)30.6(–37) × (13.5–)16.1(–18)	30.6 ± 2.8 × 16.1 ± 0.9	1.9 ± 0.20

[a] Type-material strains are noted with a superscript T. Data were obtained from Linaldeddu *et al.* (2015), Phillips *et al.* (2013), and Slippers *et al.* (2004).
[b] Minimum values are shown in parentheses followed by median and maximum values in length and width of 50 conidia from each isolate.
[c] SD = Standard deviation.
[d] Average length/width ratio.

Pathogenicity tests

To assess fulfillment of Koch's postulates, 2-year-old apricot seedlings ('Tyrinthe') were grown in a greenhouse at the Faculty of Agriculture of Cukurova University, and were inoculated with isolates of *D. seriata* (isolate MEYKA117), *N. parvum* (isolate MEKA205), or *L. mediterranea* (isolate MEKA194). Mycelium plugs were obtained with a sterile cork borer from the margin of a colony of each isolate after incubation for 5 d on PDA. The surfaces of the inoculation points on the stems of the seedlings, 10cm above the graft unions, were disinfected with 70% alcohol. The inoculations were each performed by making a 4 mm diam. wound on the disinfected stem with a cork borer, then placing the mycelium plug into the wound, and then wrapping with Parafilm to prevent secondary fungal contamination. Each isolate was inoculated into five apricot seedling replicates. Inoculation controls consisted of PDA plugs without mycelium. Inoculated and control seedlings were then incubated in a greenhouse for 3 months. Disease progression on the seedlings was observed, and lengths of streaking lesions were measured after the 3 month period. The experiment was conducted twice. Re-isolations from the margins of developed lesions were carried out onto PDA-tet. Analyses of variance (ANOVA) of the lesion length data were carried out in SPSS (IBM SPSS statistical software, v20.0), and means were compared using Fisher's LSD test at 5% of significance (Gomez and Gomez, 1984).

RESULTS

Field sampling, sample collections, and isolation of fungi

The field survey revealed branch dieback of young trees with lack of leaves, canker, and gummoses of

trunks (Figure 1 A, B and C). Sparse branching was observed in all trees exhibiting dieback. The most prevalent symptoms were wedge-shaped dark brown discolourations towards the xylem of cankered mature branches, with necroses (Figure 1 B) and cracking of the bark, often associated with gumming (Figure 1 C). Disease incidence varied from 1% to 50% among the affected orchards.

Sampled orchards (n = 44) yielded a total of 232 symptomatic branch samples, that were further inspected and analyzed in the laboratory. Isolations from margins of internal necrotic lesions of symptomatic samples gave

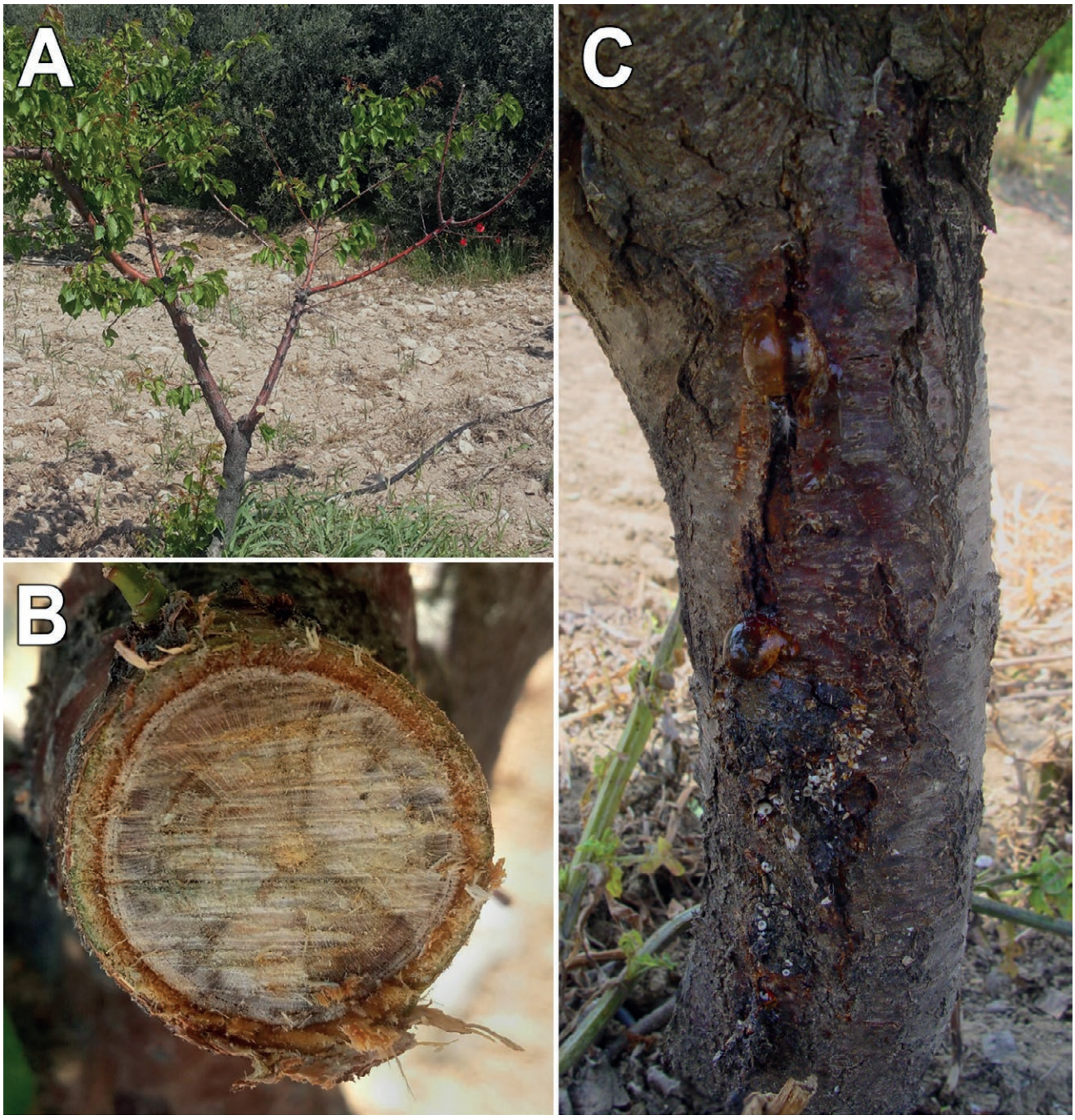

Figure 1. Symptoms of apricot dieback and gummosis in Türkiye. A, branch dieback on a juvenile tree showing lack of green leaves. B, cross section of a cankered mature branch showing wood discolouration and necroses. C, trunk gummosis on a mature tree.

consistent recovery (n = 128) of fungi exhibiting *Botryosphaeriaceae* morphology, representing 56.3% of samples from Adana and 53.6% from Mersin (Table 1). Based on colony morphology, fungal isolates were preliminarily identified up to genus level, as *Diplodia* (n = 76), *Neofusicoccum* (n = 51), or *Lasiodiplodia* (n = 1). While isolates with *Botryosphaeriaceae* morphology were consistently recovered from 55.2% of the apricot symptomatic samples, other recovered isolates morphologically identified (but not further analyzed) were *Alternaria* (n = 18), *Aspergillus* (n = 14), *Aureobasidium* (n = 8), Basidiomycetes (n = 8), *Colletotrichum* (n = 6), *Fusarium* (n = 12), *Nigrospora* (n = 2), *Penicillium* (n = 8), and *Trichoderma* (n = 8).

Morphological characterization of isolates

Isolates identified as *Diplodia* sp. (n = 10) developed white to gray colonies that became dark olivaceous gray to black with age.

One group of *Diplodia* isolates had fluffy aerial mycelium that started white gray and turned olivaceous gray to black with age, whereas the second group had flat olivaceous gray mycelium that became gray with time. All isolates produced large amounts of pycnidia on PDA and OMA at 25°C under a 12 h daily photoperiod within 25 d. Conidia were initially hyaline, aseptate, ellipsoid or cylindrical, each with a round apex and truncate base, turning brown once they matured and rarely having a single septum in some isolates. Conidium dimensions ranged from 17.3 to 30.0 μm in length (means 23.4 to 25.7μm) and from 7.5 to 15.0 μm in width (means 9.9 to 12.6 μm), with minor differences between isolates (Table 2).

The isolates preliminarily identified as *Neofusicoccum* sp. initially grew as white mycelium on PDA, turning to pale olivaceous gray and later black with age. A few isolates produced pycnidia after 30 d, and these structures were infrequent on PDA and OMA. Immature conidia were hyaline, aseptate, and fusiform with round apices, turning light brown when mature, and with one to two septa. Conidium dimensions ranged from 11.3 to 29 μm in length (means 17.7 to 19.2 μm) and from 3.8 to 13.5 μm in width (means 5.8 to 6.9 μm) (Table 2).

The isolate of *Lasiodiplodia* sp. developed abundant dense aerial mycelium on PDA that turned from white and gray to dark olivaceous gray after 15 d. Abundant pycnidia were observed after 25 d. Immature conidia were hyaline, aseptate, thick-walled, ellipsoid or cylindrical with round apices, and turned dark brown when mature, each with one septum and longitudinal striations. Conidium sizes ranged from 26.3 to 37 μm in length (mean 26.4 μm) and from 10.8 to 20.5 μm in width (mean 14.9 μm) (Table 2).

Molecular identification of fungi

Resulting sequences ranged from 505 to 582 bp for ITS, from 433 to 436 bp for *tub2*, and 377bp for *tef1*. Sequences were deposited in GenBank (Tables 3 and 4). The alignment of the first dataset (ITS-*tub2*) consisted of 37 taxa and 996 characters (including gaps). The MP analysis yielded one most parsimonious tree (tree length = 321, CI = 0.74, RI = 0.95, RC = 0.70), with an identical topology to that of the ML analysis. The MP bootstrap values were therefore combined into the ML phylogram (Figure 2). Selected isolates obtained from symptomatic apricot trees formed highly supported clades with reference strains of *Diplodia seriata* (84%/97% bootstrap values for ML/MP analyses, respectively), and *Neofusicoccum parvum* (78%/84%). The alignment of the second dataset (ITS-*tub2*-*tef1*) consisted of 22 taxa and 1,227 characters (including gaps). The maximum parsimony analysis yielded one most parsimonious tree (tree length = 420, CI = 0.75, RI = 0.77, RC = 0.58), with a comparable topology to that of the ML analysis. The bootstrap values were therefore combined into the ML phylogram (Figure 3). The *Lasiodiplodia* sp. isolate obtained from a symptomatic apricot tree formed a well-supported cluster with reference strains of *Lasiodiplodia mediterranea* (97%/95.9%).

Effects of temperature on mycelium growth

All isolates of *D. seriata* and *N. parvum* were able to grow at temperatures between 5°C and 35°C. The isolate of *L. mediterranea* grew only between 10°C and 35°C. The growth of the three tested isolates of *D. seriata* was not significantly different (P = 0.9649) at any of the temperatures; therefore, these data were averaged. The optimal growth temperatures (T_{opt}) and maximum colony growth values (Y_{max}) calculated by the model were significantly different ($P < 0.0001$) among species. The mean estimated T_{opt} were 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea* (Figure 4). Likewise, the calculated Y_{max} values were 40.5 mm for *D. seriata*, 56.2 mm for *N. parvum*, and 75.5 mm for *L. mediterranea*.

Pathogenicity tests

The three assessed isolates (*D. seriata*, *N. parvum*, *L. mediterranea*) were pathogenic on the main stems of 2-year-old 'Tyrinthe' apricot seedlings. This was evident by internal vascular lesions after peeling off the external layers of the inoculated stems and the presence of

gumming around the inoculated areas after a period of 3 months of incubation in the greenhouse. Mock inoculated plants remained symptomless. The fungal isolates used in the trial were successfully re-isolated from the margins of the lesions on the respectively inoculated seedlings, at rates that ranged from 66.7% to 100%, whereas no fungal colonies were recovered from mock inoculated plants. The mean lesion lengths caused by the fungi and the controls were significantly different ($P < 0.05$) (Figure 5). The isolate of *L. mediterranea* was the most virulent (mean lesion length = 193.4 mm), with the most extended lesions developing with gummoses around the inoculation points, followed by *D. seriata* (mean lesion length = 94.2 mm), and *N. parvum* (mean

Table 3. GenBank accession numbers of *Diplodia* and *Neofusicoccum* strains and isolates used in phylogenetic analyses.

Species[a]	Strain/isolate[b]	Host/substrate	Location	GenBank accession number	
				ITS	*tub2*
Botryosphaeria dothidea†	CBS 115476^T	*Prunus* sp.	Switzerland	AY236949	AY236927
Diplodia africana	CBS 120835^T	*Prunus persica*	South Africa	EF445343	KF766129
D. citricarpa	CBS 124715^T	*Citrus* sp.	Iran	KF890207	KX464784
D. corticola	CBS 112549^T	*Quercus suber*	Portugal	AY259100	DQ458853
D. malorum	CBS 124130^T	*Malus sylvestris*	Portugal	GQ923865	MT592507
D. mutila	CBS 136014^T	*Populus alba*	Portugal	KJ361837	MG015815
D. rosulata	CBS 116470^T	*Prunus africana*	Ethiopia	EU430265	EU673132
D. sapinea	CBS 121105	*Prunus persica*	South Africa	EF445339	KX464806
D. sapinea	CBS 393.84^T	*Pinus nigra*	Netherlands	DQ458895	DQ458863
D. scrobiculata	CBS 118110^T	*Pinus banksiana*	USA	AY253292	AY624258
D. scrobiculata	CBS 119939	*Pinus radiata*	Italy	MT587353	MT592500
D. seriata	**AYKA244**	***Prunus armeniaca***	**Adana, Türkiye**	**KX244800**	**KX259183**
D. seriata	CBS 112555^T	*Vitis vinifera*	Portugal	AY259094	DQ458856
D. seriata	CBS 121110	*Prunus armeniaca*	South Africa	EF445308	MT592554
D. seriata	CBS 124137	*Prunus domestica*	Bulgaria	MT587384	MT592559
D. seriata	**MEYKA116**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244797**	**KX259180**
D. seriata	**MEYKA117**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244798**	**KX259181**
D. seriata	**MEYKA124**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244799**	**KX259182**
Neofusicoccum australe	CMW 6837^T	*Acacia* sp.	Australia	AY339262	AY339254
N. dianense	CGMC C3.20082^T	*Eucalyptus urophylla* × *E. grandis*	China	MT028605	MT028937
N. illicii	CGMCC 3.18310^T	*Illicium verum*	China	KY350149	KY350155
N. kwambonambiense	CBS 123639^T	*Syzygium cordatum*	South Africa	EU821900	EU821840
N. luteum	CBS 562.92^T	*Actinidia deliciosa*	New Zealand	KX464170	KX464968
N. mediterraneum	CBS 121718^T	*Eucalyptus* sp.	Greece	GU251176	GU251836
N. nonquaesitum	CBS 126655^T	*Umbellularia californica*	USA	GU251163	GU251823
N. parvum	**AKKA308.1**	***Prunus armeniaca***	**Adana, Türkiye**	**MH221120**	**MH221122**
N. parvum	**AKKA308.2**	***Prunus armeniaca***	**Adana, Türkiye**	**MH221121**	**MH221123**
N. parvum	CBS 117923	*Guava* sp.	Venezuela	MT587517	MT592733
N. parvum	CMW 9071	*Ribes* sp.	Australia	EU339552	AY236909
N. parvum	CMW 9081^T	*Populus nigra*	New Zealand	AY236943	AY236917
N. parvum	**MEKA205**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244807**	**MG970283**
N. ribis	CBS 115475^T	*Ribes* sp.	USA	AY236935	AY236906
N. ribis	CBS 121.26	*Ribes rubrum*	USA	AF241177	AY236908
N. stellenboschianum	CBS 110864^T	*Vitis vinifera*	South Africa	AY343407	KX465047
N. vitifusiforme	CBS 110887^T	*Vitis vinifera*	South Africa	AY343383	KX465061
N. yunnanense	CGMC C3.20080	*Eucalyptus urophylla* × *E. grandis*	China	MT028672	MT029004
N. yunnanense	CGMC C3.20083^T	*Eucalyptus globulus*	China	MT028667	MT028999

[a] Outgroup is marked with a dagger symbol (†).
[b] Isolates obtained in this study are highlighted in bold font. Type-material strains are accompanied by a superscript T.

Table 4. GenBank accession numbers of *Lasiodiplodia* strains and isolates used in phylogenetic analyses.

Species[a]	Strain/isolate[b]	Host/substrate	Location	GenBank accession number		
				ITS	*tef1*	*tub2*
Diplodia mutila†	CBS 136014^T	*Populus alba*	Portugal	KJ361837	KJ361829	MG015815
Lasiodiplodia brasiliensis	CMM 4015^T	*Mangifera indica*	Brazil	JX464063	JX464049	n/a
L. cinnamomi	CFCC 51997^T	*Cinnamomum camphora*	China	MG866028	MH236799	MH236797
L. crassispora	CBS 118741^T	*Santalum* sp.	Australia	DQ103550	DQ103557	KU887506
L. gilanensis	CBS 124704^T	*Citrus* sp.	Iran	GU945351	GU945342	KU887511
L. gonubiensis	CBS 115812^T	*Syzygium cordatum*	South Africa	AY639595	DQ103566	DQ458860
L. hormozganensis	CBS 124709^T	*Olea* sp.	Iran	GU945355	GU945343	KU887515
L. laeliocattleyae	CBS 130992^T	*Mangifera indica*	Egypt	JN814397	JN814424	KU887508
L. lignicola	CBS 134112^T	Dead wood	Thailand	JX646797	KU887003	JX646845
L. lignicola	CGMCC 3.18061	Woody branch	China	KX499889	KX499927	KX500002
L. macrospora	CMM 3833^T	*Jatropha curcas*	Brazil	KF234557	KF226718	KF254941
L. mahajangana	CBS 124925^T	*Terminalia catappa*	Madagascar	FJ900595	FJ900641	FJ900630
L. mediterranea	CBS 137783^T	*Quercus ilex*	Italy	KJ638312	KJ638331	KU887521
L. mediterranea	CBS 137784	*Vitis vinifera*	Italy	KJ638311	KJ638330	KU887522
L. mediterranea	**MEKA194**	***Prunus armeniaca***	**Mersin, Türkiye**	**KX244816**	**PV239496**	**PV239497**
L. parva	CBS 456.78^T	Cassava-field soil	Colombia	EF622083	EF622063	KU887523
L. pseudotheobromae	CBS 116459^T	*Gmelina arborea*	Costa Rica	EF622077	EF622057	EU673111
L. pseudotheobromae	CBS 130991	*Mangifera indica*	Egypt	MT587433	MT592145	MT592629
L. subglobosa	CMM 3872^T	*Jatropha curcas*	Brazil	KF234558	KF226721	KF254942
L. theobromae	CBS 164.96^T	Fruit on coral reef coast	New Guinea	AY640255	AY640258	EU673110
L. viticola	CBS 128313^T	*Vitis vinifera*	USA	HQ288227	HQ288269	HQ288306
L. vitis	CBS 124060^T	*Vitis vinifera*	Italy	KX464148	MN938928	KX464917

[a] Outgroup is marked with a dagger symbol (†).
[b] Isolates obtained in this study are highlighted in bold font. Type-material strains are accompanied by a superscript T.

lesion length = 85.0 mm). Of the three species, *D. seriata* caused the least amount of gummosis, but caused significantly longer lesions than *N. parvum*.

DISCUSSION

In this study, three different species of *Botryosphaeriaceae*, including *Diplodia seriata*, *Lasiodiplodia mediterranea*, and *Neofusicoccum parvum*, were confirmed as causal agents of branch dieback and gummosis in commercial apricot orchards in the Adana and Mersin provinces of Türkiye. Among these pathogens, *D. seriata* and *N. parvum* have cosmopolitan distribution and broad host ranges, including apricot (Gure *et al*., 2005; Damm *et al*., 2007; Slippers *et al*., 2007; Abdollahzadeh *et al*., 2010; Linaldeddu *et al*., 2015; Soltaninejad *et al*., 2017). *Lasiodiplodia mediterranea* is known to have narrower host range and more limited geographical distribution (Linaldeddu *et al*., 2015; Wiseman *et al*., 2017).

The present study results indicated that *D. seriata* is the most abundant species affecting apricot trees in both Adana and Mersin in Türkiye. This is consistent with previous studies that reported predominance of *D. seriata* from symptomatic *Prunus* hosts in South Africa and Canada (Damm *et al*., 2007; Slippers *et al*., 2007 Ellouze and Ilyukhin, 2024). In Türkiye, *D. seriata* has previously been reported affecting commercial nectarine, plum (Endes *et al*., 2016; Endes and Kayim, 2022), and citrus orchards (Kurt *et al*., 2025) located in Adana and Mersin.

The second most frequently isolated species was *N. parvum*, one of the most common *Botryosphaeriaceae* affecting woody hosts (Sakalidis *et al*., 2013). In Türkiye, *N. parvum* has also been reported on almond and plum (Kayim *et al*. 2015; Endes and Kayim, 2022), as well as on grapevine (Akgül *et al*., 2014), pear (Kurbetli *et al*., 2020), walnut (Kara *et al*., 2021), and citrus (Kurt *et al*., 2025).

Lasiodiplodia mediterranea was the least frequently isolated species, represented by a single isolate recovered from the Mersin province. To date, *L. mediterranea* has been reported in Italy, Algeria and the Pacific Northwest of the United States of America, with a narrow host range including *Citrus sinensis*, *Quercus ilex*, *Vaccinium corymbosum*, and *Vitis vinifera* (Linaldeddu *et al*., 2015;

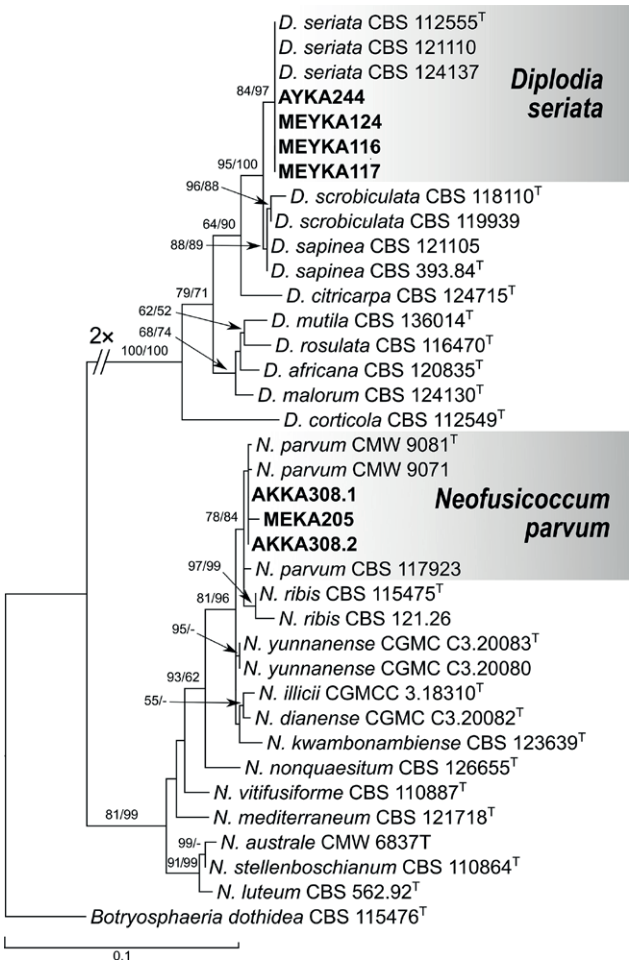

Figure 2. Maximum likelihood phylogenetic inference for isolates of *Diplodia seriata* and *Neofusicoccum parvum* obtained from apricot trees with dieback and gummosis symptoms in Türkiye, compared to reference strains of closely related species. The phylogenetic tree was inferred from a combined dataset of ITS and *tub2* sequences and rooted with *Botryosphaeria dothidea* (CBS 115476). Numbers above branches represent maximum likelihood and maximum parsimony bootstrap values from 1,000 replicates. Type-material strains are accompanied by a superscript T. Scale bar represents nucleotide substitutions per site.

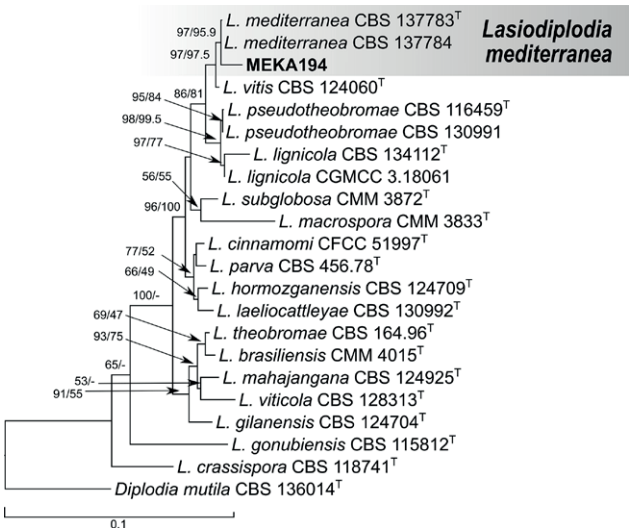

Figure 3. Maximum likelihood phylogenetic inference of a single isolate of *Lasiodiplodia mediterranea* (MEKA194) obtained from a symptomatic sample of apricot dieback in Türkiye, compared to reference strains of closely related species. The tree was inferred from a combined dataset of ITS, *tub2*, and *tef1* sequences and rooted with *Diplodia mutila* (CBS 136014). Numbers above branches represent maximum likelihood and maximum parsimony bootstrap values from 1,000 replicates. Type-material strains are accompanied by a superscript T. Scale bar represents nucleotide substitutions per site.

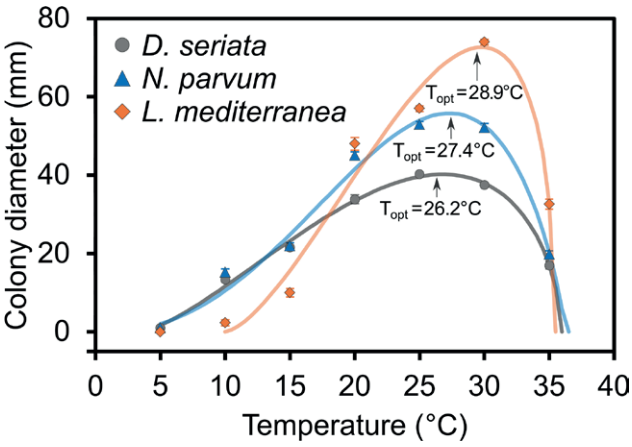

Figure 4. Mean colony diameters for representative isolates of *Diplodia seriata*, *Neofusicoccum parvum*, and *Lasiodiplodia mediterranea* causing apricot dieback and gummosis in Türkiye. Data points represent mean values of ten replicates measured after 48 h on PDA, fitted with nonlinear regression curves using the Analytis beta model. Vertical bars = standard errors. Topt = optimal growth temperature.

Wiseman *et al*., 2017). The present study provides a new geographical record and a new host record for *L. mediterranea*, in Türkiye and affecting apricot. Occurrence of this pathogen is likely due to the introduction of infected plant material.

Identification of *Botryosphaeriaceae* species currently relies on morphological observations coupled with DNA sequence analyses (Slippers *et al*., 2005; Phillips *et al*., 2013). In the present study, phylogenetic analyses using ITS, *tub2* and *tef1* sequences revealed well-supported clusters between the Turkish isolates and reference strains of *D. seriata*, *N. parvum* and *L. mediterranea* (Figures 2 and 3). This aligns with previous studies that utilized the same DNA barcodes to accurately

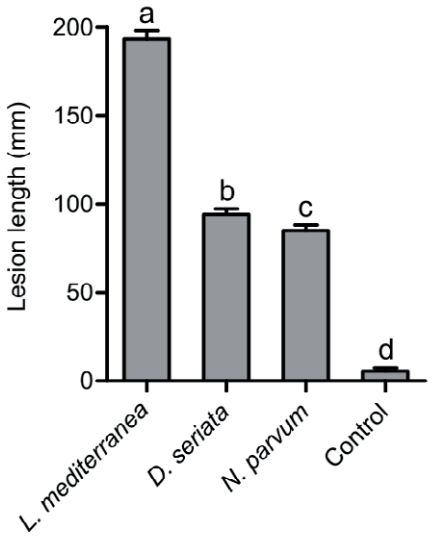

Figure 5. Mean lesion lengths (mm) caused by four *Botryosphaeriaceae* species inoculated into the main stems of 2-year-old 'Tyrinthe' apricot seedlings. Means accompanied by different letters are different according to Fisher's LSD test ($P < 0.05$).

identify *Botryosphaeriaceae* species (López-Moral *et al*., 2019; Gusella *et al*., 2022). Morphological characters were also important to confirm the species identification. The *D. seriata* isolates showed two distinct morphologies based on mycelium texture and colour when cultured at 25°C on PDA, which is consistent with previous studies (Moral *et al*., 2010). Conidium features of colour, shape and dimensions matched with the type strain of *D. seriata* (Table 2; Phillips *et al.,* 2013). The isolates identified as *N. parvum* and *L. mediterranea* had colonies that were consistent with descriptions of those species (Phillips *et al*., 2013; Linaldeddu *et al*., 2015). Conidium characteristics (size, shape, and colour) matched respective descriptions of *N. parvum* and *L. mediterranea* (Table 2; Burgess *et al*., 2006; Alves *et al*., 2008; Abdollahzadeh *et al*., 2010; Wang *et al*., 2011; Chen *et al*., 2014; Linaldeddu *et al*., 2015).

Studying effects of temperature on mycelium growth of plant pathogens assists understanding their distributions and abundance in geographical areas (Úrbez-Torres *et al*., 2006). Results of the present study showed that the three pathogens had different growth patterns across the assessed temperatures (Figure 4). At low temperatures (5, 10, and 15°C), the *D. seriata* and *N. parvum* isolates had similar growth rates, which were greater than for *L. mediterranea*. In contrast, at higher temperatures (25, 30, and 35°C), *L. mediterranea* grew more rapidly than *N. parvum,* while *D. seriata* grew more slowly than *L. mediterranea* or *N. parvum*. On the other hand, estimated optimum temperatures for mycelium growth were 26.2°C for *D. seriata*, 27.4°C for *N. parvum*, and 28.9°C for *L. mediterranea*. This suggests that the *D. seriata* and *N. parvum* isolates were better adapted to cool environments than *L. mediterranea*, which may explain the higher frequency of *D. seriata* and *N. parvum* in the surveyed apricot orchards. Similar results have been reported elsewhere, with similar growth ranges for *D. seriata* (Jacobs and Rehner 1998; Copes and Hendrix 2004) and optimum growth at 26.8°C (Úrbez-Torres *et al*., 2006). For *N. parvum*, optimum temperatures have previously been estimated to be 25°C (Espinoza *et al*., 2009; Thomidis *et al*., 2011; Ismail *et al*., 2013; Chen *et al*. 2014), 28.2°C (Úrbez-Torres *et al*., 2006), and 30°C (Puig *et al*., 2021; Martino *et al*. 2024). For *L. mediterranea*, the optimum temperatures have been determined to be between 25°C and 30°C (Linaldeddu *et al*., 2015). The sampled apricot orchards are located in regions with temperate climate, characterized by warm to hot, dry summers, occasional droughts, and mild, wet winters. These conditions resemble those of other climatic zones where *Botryosphaeriaceae* are adapted, posing significant challenges for fruit crop growers in different countries (Phillips *et al*., 2013). Therefore, the occurrence of *D. seriata*, *N. parvum* and *L. mediterranea* affecting apricot trees in Türkiye is not surprising. In addition, due to ongoing climate change, temperatures are expected to increase during the coming decades, accompanied by decreased precipitation, conditions that could create increasingly stressful environments for agriculture, and likely leading to more intense expression of diseases (Altın and Barak, 2017; Zittis *et al*., 2022). These temperature changes could alter the distribution of the *Botryosphaeriaceae* pathogens in Türkiye, with *Lasiodiplodia* spp. potentially increasing in abundance relative to *Diplodia* and *Neofusicoccum* spp. (Batista *et al*., 2021).

Results from pathogenicity tests showed that the *D. seriata*, *N. parvum* and *L. mediterranea* isolates were pathogenic to 2-year-old apricot seedlings, with *L. mediterranea* being the most virulent species, followed by *D. seriata* as intermediate, and *N. parvum* as the least aggressive species (Figure 5). These results align with previous studies that have described *Lasiodiplodia* species causing more severe symptoms than *Diplodia* and *Neofusicoccum* species (Úrbez-Torres and Gubler 2009). Conversely, other studies described *N. parvum* as a more aggressive pathogen than *D. seriata* (Úrbez-Torres and Gubler 2009; Reis *et al*., 2020). Differential levels of aggressiveness among isolates of *D. seriata* and *N. parvum* have been documented previously (Qiu *et al*., 2016; Trotel-Aziz *et al*., 2022; Fernandez *et al*., 2023), a phenomenon highly influenced by environmental factors. Therefore, it is likely that environmental conditions in southern Türkiye exacerbate

symptoms caused by *D. seriata* rather than those caused by *N. parvum* in apricot trees.

In conclusion, *D. seriata*, *N. parvum*, and *L. mediterranea* were identified as causal agents of branch dieback and gummosis of apricot in Türkiye. Detection of these pathogens highlights the need for effective management strategies to reduce their impacts on apricot production. Further research should focus on preventative management strategies for these pathogens, which could include assessments of pruning wound protection using synthetic and biological products.

ACKNOWLEDGEMENTS

This research was supported by Grant 114O048 from The Scientific and Technological Research Council of Türkiye. Part of this work was also conducted as a Ph.D. thesis, supported by TÜBİTAK.

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