Journal of Plant Diseases and Protection
https://doi.org/10.1007/s41348-022-00591-x

SHORT COMMUNICATION

Occurrence of twig blight and branch dieback of walnut caused by Botryosphaeriaceae species in Turkey

Ayhan Yildiz[1] · **Seher Benlioglu**[1] · **Kemal Benlioglu**[1] · **Yunus Korkom**[1]

Received: 11 August 2021 / Accepted: 14 February 2022

Abstract

In the fall season 2017, forty samples showing necrotic and wilted leaves, stem cankers, shoot cankers, leaf and fruit blight were collected from walnut trees in seven commercial orchards in Aydin province of Turkey. Ten isolates were identified based on morphology of monoconidial cultures obtained from pine needles. Morphological characteristics and DNA sequence comparisons of ITS and EF-1α gene sequences of the isolates revealed the presence of *Botryosphaeria dothidea, Neofusicoccum parvum,* and *Neofusicoccum mediterraneum*. Four isolates were identified as *Neofusicoccum parvum* with 100% similarity for ITS (accession nos. MW426289 to MW426292) and EF1α (MT212730 to MT212733); two isolates were *Neofusicoccum mediterraneum* with 100% similarity for ITS (accession nos. MW426287 and MW426288) and EF1α (MT212728 and MT21729), and one isolate was *Botryosphaeria dothidea* with 100% similarity for ITS (accession nos. MW426293) and EF1α (MT212734). Pathogenicity tests were conducted on 2-year-old walnut trees cv. Chandler, and ten monoconidial isolates were pathogenic. This is the first report of *N. parvum* and *B. dothidea* causing twig blight and branch and dieback disease of walnuts in the Aegean region of Turkey and *N. mediterraneum* on walnut trees in Turkey. This emerging disease could pose a potential threat to new tree plantations of walnut in Turkey.

Keywords *Botryosphaeria dothidea* · *Neofusicoccum parvum* · *Neofusicoccum mediterraneum* · Canker · Walnut · Blight

Introduction

Turkey ranks fourth in production of walnut (*Juglans regia* L.) worldwide and new plantations are increasing to meet rising demands (TUIK 2020). Currently, walnut is grown extensively all over the Aegean Region of Turkey. Recent attention has been focused on newly emerging fungal diseases in fruit trees because of climate change and increasing new plantations of high-value nuts, such as *Ophiognomonia leptostyla* (Uzunok and Kurbetli 2017; Koç et al. 2020), *Neoscytalidium dimidiatum* (Derviş et al. 2019), *Neofusicoccum parvum* (Kara et al. 2020) on walnuts in Turkey. Botryosphaeriaceae species are very damaging to walnuts, which have been reported to cause canker and dieback in walnut trees (Chen et al 2014). *Botryosphaeriaceae* spp. are considered as endophytes and opportunistic pathogens of over 500 trees and bush species in the tropics and sub-tropics, also have been reported causing cankers and dieback of walnut trees (Trouillas et al. 2010; Chen et al. 2014; Eichmeier et al. 2020; López-Moral et al. 2020; Sohrabi et al. 2020). Species of Botryosphaeriaceae are very damaging to walnuts worldwide. Studies over the past 10 years in Turkey has reported several *Botryosphaeriaceae* spp. in different hosts, like fig (Çeliker and Michailides 2012), almond (Kayim et al. 2015; Erdem 2020), grape (Akgül et al. 2013), pear (Kurbetli and Demirci 2014), walnut (Yildiz et al. 2018) and olive (Tabaklı 2019; Korukmez et al. 2020) in Turkey. Recently, Kara et al. (2020) reported *Neofusicoccum parvum* causing dieback and canker on English walnut trees growing in Hatay Province of the Mediterranean region of Turkey. Over the past years, there have been observations of twig blight and branch dieback disease in walnut trees located in Aegean region. However, the cause of these blight and dieback has not been investigated. Therefore, this present study sought to identify *Botryosphaeriaceae* species causing twig blight and branch dieback diseases in walnut.

✉ Ayhan Yildiz
ayildiz@adu.edu.tr

[1] Faculty of Agriculture Department of Plant Protection, Aydın Adnan Menderes University, Aydin, Turkey

Published online: 09 March 2022

Materials and methods

Isolation and morphological identification

In the fall season of 2017, field surveys were conducted in 7 commercial orchards in Kuyucak (37° 54′ 47.83″–28° 27′ 38.78″), Bozdogan (37° 40′ 19.62″–28° 18′ 34.95″), Sultanhisar (37° 53′ 23.84″–28° 9′ 18.06″), and Efeler (37° 50′ 29.13″–27° 50′ 51.26″) districts of Aydin Province. A total of 40 diseased samples were collected from walnut trees (8 local and 2 Chandler cvs) exhibiting symptoms such as necrotic and wilted leaves, stem and shoot cankers (V-shaped unilateral discolored xylem tissue), and blighted fruit were collected (Fig. 1). Symptomatic twigs and branches were excised to 5 × 5 mm pieces, surface sterilized with 1% NaOCl solution for 1 min, rinsed twice in sterile water and plated onto Petri dishes containing Potato Dextrose Agar (PDA, Merck). The petri dishes were incubated at 24 °C for 7–15 days when fungal colonies initially white and then dark gray to black, developed and, produced a dark hue in the media (López-Moral et al. 2020).

For diagnostic purposes, pycnidia and conidia from ten isolates were produced on pine needles (Phillips et al. 2013). Monoconidial cultures of each isolate were obtained by plating a conidial suspension from 1 to 2 pycnidia on water agar (2%) and single germinating conidia were transferred to PDA. Conidia and pycnidia on pine needles from single spore isolates were mounted on microscope slides and examined under a standard light microscope (Leica DM/LS equipped with DFC320 camera, Germany). The morphological characteristics of fungal colonies and the features of conidia and pycnidia were compared to the morphological descriptions in Phillips et al. (2013) and Slippers et al. (2013) for identification.

Molecular identification

Genomic DNA was extracted from the mycelia of 10 monoconidial isolates 7 days-old as pure cultures on PDA. Mycelia (50 mg) of each isolate were cultivated in Czapek-Dox broth (Millipore) at 25 °C for 72 h with shaking (150 rpm) and collected for genomic DNA isolation using Cenis (1992). The purity and yield of the extracted DNA was determined using Picodrop™ spectrophotometer (Picodrop, UK), and diluted to 50 ng/µl for PCR amplification of two gene fragments targeting ITS and EF1-α. The ITS region of rDNA was amplified with primers pairs ITS1/ITS4 (White et al. 1990) and the translation elongation factor 1-α (*EF1*) gene with EF1-728F/EF1-986R (Carbone and Kohn 1999). Amplifications were performed in 40 µl reaction volumes containing 4 µl of 10 × PCR buffer, 0.8 µl of dNTP mix (10 mM of each dNTP), 0.2 µl of 100 µM of each primer, 0.4 µl of 5 U/µl Dream Taq Green DNA polymerase (Thermo Fisher Scientific), 2.4 µl of 25 mM of $MgCl_2$, 4 µl of DNA extract and 28 µl of sterile ultra-pure water in a thermal cycler (BIO-RAD C1000 Touch™). The PCR cycling conditions followed: (i) the initial denaturation 180 s at 95 °C; (ii) 35 cycles of 10 s at 55 °C, 15 s at 72 °C; and (iii) a final extension step at 72 °C for 5 min. PCR products were electrophoresed on a 1.5% TBE Agarose gel at 45 V for 30 min, stained with GelRed (Biotium) and photographed. Amplicons were commercially sequenced by Macrogen Europe in Amsterdam, the Netherlands.

The sequence quality was checked by Sequence Scanner v.1.0 software (Applied Biosystems, Foster City, CA, United States) and, the ITS and partial EF1-α sequences of seven strains submitted to GenBank database with the accession numbers illustrated in Table 1. The obtained sequences were searched against the NCBI GenBank database using Basic Local Alignment Search Tool (BLASTs) to confirm the identity of the isolates.

Fig. 1 Disease symptoms observed in walnut in Bozdoğan/Aydın. **A** Branch dieback, **B** branch canker developing from a shoot, **C** cross section of branches showing brown to dark brown discoloration of vascular tissues

Table 1 Isolates sequenced and sequences from other studies used in phylogenetic analyses for this study

Species	Isolate	Host	Location	GenBank accession number	
				ITS[a]	EF-1α[a]
Neofusicoccum mediterraneum	CB2[b]	Walnut	Turkey	MW426287	MT212728
Neofusicoccum mediterraneum	CB3[b]	Walnut	Turkey	MW426288	MT212729
Neofusicoccum mediterraneum	PD312[T]	Almond	California	GU251176	GU251308
Neofusicoccum mediterraneum	UCD719SJ	Grapevine	California	GU799451	GU799482
Neofusicoccum mediterraneum	CBS121558	Grapevine	California	GU799463	GU799462
Neofusicoccum mediterraneum	1L85	Walnut	California	KF778818	KF779008
Neofusicoccum parvum	CB4[b]	Walnut	Turkey	MW426289	MT212730
Neofusicoccum parvum	CB6[b]	Walnut	Turkey	MW426290	MT212731
Neofusicoccum parvum	CB7[b]	Walnut	Turkey	MW426291	MT212732
Neofusicoccum parvum	CB9[b]	Walnut	Turkey	MW426292	MT212733
Neofusicoccum parvum	CMW9081[T]	*Populus nigra*	New Zealand	AY236943	AY236888
Neofusicoccum parvum	CBS11030[T]	*Vitis vinifera*	Portugal	AY259098	AY573221
Neofusicoccum parvum	BOT-02	Grapevine	Spain	MG745827	MG745818
Botryosphaeria dothidea	CB10[b]	Walnut	Turkey	MW426293	MT212734
Botryosphaeria dothidea	CMW8000[T]	*Prunus* sp.	Switzerland	AY236949	AY236898
Botryosphaeria dothidea	2E55	Walnut	California	KF778783	KF778973
Botryosphaeria dothidea	2D20	Walnut	California	KF778785	KF778975
Botryosphaeria dothidea	5A02	Walnut	California	KF778787	KF778977
Botryosphaeria dothidea	ColPat-443	Walnut	Spain	MK431835	MK461889
Botryosphaeria dothidea	BDPIS1	Pistachio	Iran	JX073098	KP128065
Botryosphaeria dothidea	Karaburun 7/2-2	Olive	Turkey	MG753998	MG816211
Botryosphaeria dothidea	Kusadasi 6/1-1	Olive	Turkey	KX898358	MG816213
Guignardia philoprina	CMW7063	*Clonostachys* sp.		AY236956	AY236905

[a]ITS = internal transcribed spacer, EF1-α = translation elongation factor 1-α, gene regions

[b]Isolates sequenced in this study

[T]Type strains

Phylogenetic analysis

The multiple sequence alignments were conducted in MEGA v. 6 (Tamura et al. 2013). Sequences of the type specimen isolates or strains for closely related Botryosphaeriaceae were obtained from GenBank (http://www.ncbi.nlm.nih.gov) to compile datasets for phylogenetic analyses (Table 1). We performed phylogenetic analyses in MEGA v. 6, using maximum likelihood (ML), on the combined datasets from (ITS and EF1-α) of the test isolates.

Pathogenicity tests

Pathogenicity tests were performed on healthy 2-year-old walnut trees (cv. Chandler) grown in 10-L plastic (soil mix containing 1/3 peat, 1/3 soil and 1/3 sand) pots under net tunnel and watered as needed. Inoculations were made during the third week of May 2020 when the leaves had fully emerged, and the twigs were tender. The area of the inoculation on twigs was surface disinfected by spraying 70% ethanol solution and inoculated with 5 mm-diameter PDA plugs from a 7-day-old culture by wounding the plant with a sterile scalpel. Wounding was done by removing bark tissue. The wound was covered with parafilm to prevent desiccation. Ten isolates shown in Table 1 including negative control (PDA agar plugs) were used by inoculating each of two shoots of five walnut trees for each isolate. The length of vascular discoloration above and below inoculation point were measured with a caliper ("Electronic Digital Caliper 0–150 mm") (4 weeks after inoculation) (Chen et al. 2014). Re-isolations were made from the lesions and identified to genus level as previously described to fulfil the Koch postulates (Sohrabi et al. 2020).

Results

Botryosphaeriaceae fungi were consistently isolated from infected twigs and branches of walnut trees. Colony morphology and conidial characteristics of ten isolates resembled those described for the species within the Botryosphaeriaceae. *N. mediterraneum* were incubated at 24 °C for

5 days, and produced initially light olive green to gray aerial mycelium on potato dextrose agar (Fig. 2), becoming dark olive green with age. Conidia were unicellular, hyaline and fusiform, and measured 18.5 µm × 5.6 µm. *N. parvum* white to pale gray and rapidly growing colonies with abundant aerial mycelia were observed on potato-dextrose-agar (PDA) (Fig. 2). *C*onidia hyaline, unicellular, fusiform-ellipsoid average length × width were 15.1 µm × 6.4 µm *B.dothidea* colonies were initially white, becoming grey or dark grey by age on PDA (Fig. 2), conidia hyaline, thin-walled, unicellular, average length x width were 21.2 µm × 5.4 µm (Fig. 2). Based on morphological characteristics and BLASTn analysis of ITS and EF1-α sequences at the NCBI Gene Bank, 4 isolates were identified as *N. parvum* (CB4, CB6, CB7, CB9) with 100% similarity for ITS (accession nos. MW426289 to MW426292) and EF1-α (MT212730 to MT212733), 2 isolates as *N. mediterraneum* (CB2, CB3) with 100% similarity for ITS (accession nos. MW426287 and MW426288) and EF1-α (MT212728 and MT212729), and one isolate as *B. dothidea* (CB10) with 100% similarity for ITS (accession nos. MW426293) and EF1-α (MT212734).

The best-fit model of nucleotide evolution for ML analyses was selected, based on the ITS and EF1-α. The evolutionary tree with the highest log likelihood (− 1566.2063) is shown (Fig. 3). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. Our Walnut isolates CB2 and CB3 grouped with the representative isolates of *N. mediterraneum,* isolates CB4, CB6, CB7 and CB9 with those of *N. parvum,* and isolate CB10 with those of *Botryosphaeria dothidea* by 100% bootstrap support (Fig. 3).

Four weeks after inoculation, the shoots displayed dark brown to black discoloration of the bark and vascular tissues exhibited large black discoloration around the inoculation point (Fig. 4). The pathogen was successfully re-isolated from the margin of symptomatic tissues, thus fulfilling Koch's postulates. Five control shoots inoculated with sterile PDA plugs remained symptomless. Ten isolates tested were able to induce internal wood discoloration on the inoculated shoots. The mean lesion length of ten isolates ranked in the following order: *N. parvum* 41 ± 2.8 mm (CB1), *N. mediterraneum* 26.4 ± 1.4 mm (CB2), *N. mediterraneum* 7.2 ± 2.8 mm (CB3), *N. parvum* 15.7 ± 2 mm (CB4), *N. parvum* 29.9 ± 3 mm (CB5), *N. parvum* 39.8 ± 2.8 mm (CB6), *N. parvum* 33.8 ± 4.3 mm (CB7), *N. parvum* 18.3 ± 2 mm (CB8), *N. parvum* 18.3 ± 2 mm (CB9), *B. dothidea* 26 ± 3.5 mm (CB10).

Discussion

Based on morphological characteristic and comparisons of ITS, EF1-α sequence data, 3 species of Botryosphaeriaceae (*N. mediterraneum, N. parvum and B. dothidea*) belonging to three genera were identified from symptomatic walnut trees affected by stem canker and branch and twig dieback in the Aegean region of Turkey.

Botryosphaeriaceae fungi have been reported in top walnut producing countries such as China (Li et al. 2015), Iran (Sohrabi et al. 2020), United States (Chen et al. 2014), Turkey (Kara et al. 2020), Chile (Díaz et al. 2018), Spain (Moral et al. 2010; López-Moral et al. 2020) and Czech Republic (Eichmeier et al. 2020). Botryosphaeriaceae and related members of this family are latent pathogens causing severe diseases in host stressed plants or under other

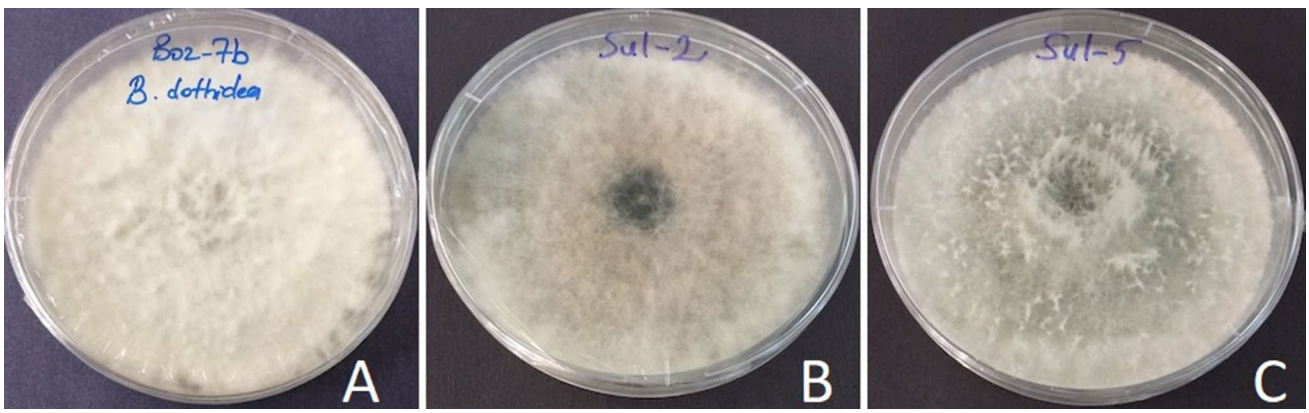

Fig. 2 Colony morphology of *Botryosphaeria dothidea* (**A**), *Neofusicoccum mediterraneum* (**B**), *Neofusicoccum parvum* (**C**) on PDA after 7 days incubation at 24 °C in continuous darkness and conidia

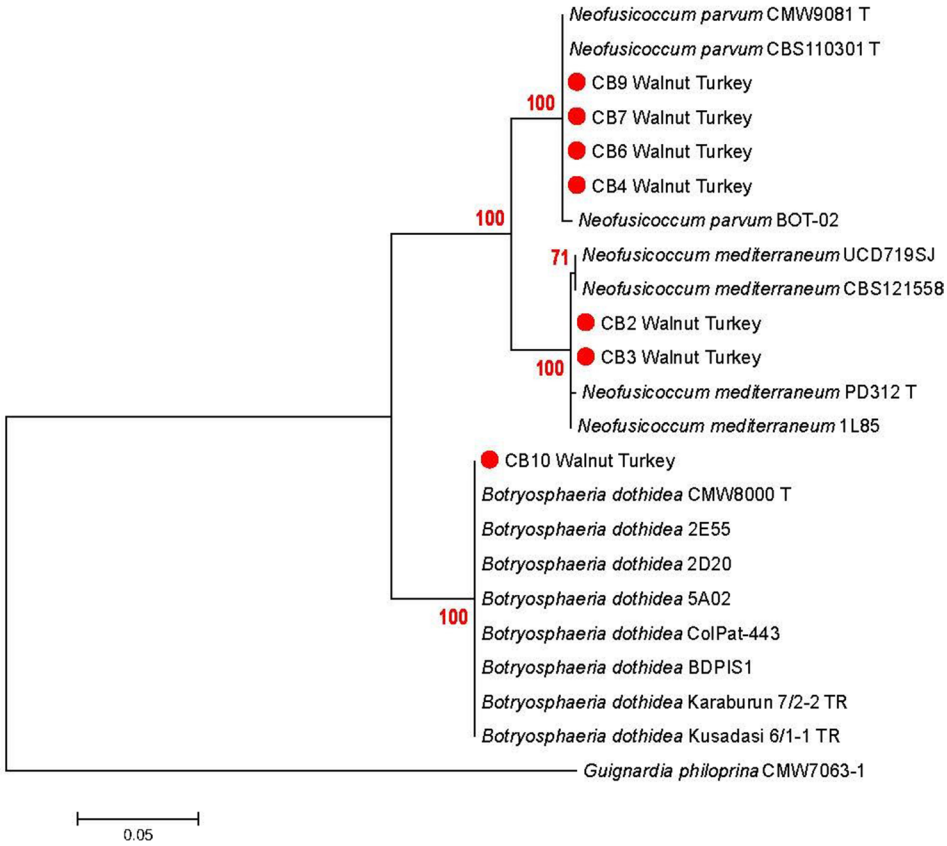

Fig. 3 Distance tree obtained from the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980) of the combined ITS and EF1-α dataset of GenBank accessions given in Table 1. Species name and culture ID are shown for each sequence. Sequences labeled with a "T" at the end correspond to the ex-type culture. Red dot circles represent sequences isolated from walnut in this study. Bootstrap values of 1000 replicates of maximum likelihood analyses are shown above and below branches, respectively. *Guignardia philopirina* was used as the outgroup taxon. Branch lengths are scaled, and scale bar is 0.05 nucleotide substitutions per site. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013)

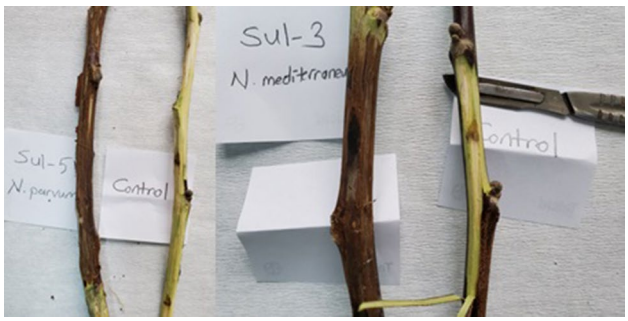

Fig. 4 Pathogenicity test: 4 weeks after inoculation symptoms on shoots; dark brown to black discoloration of the bark and vascular tissues exhibited large black discoloration around the inoculation point

favorable conditions for disease development. *Botryosphaeria dothidea* is reported as pathogen on walnut in Spain (Moral et al. 2010) in China (Li et al. 2015). Chen et al. (2014) demonstrated that *B. dothidea*, *Diplodia mutila, D. seriata*, *Dothiorella iberica, Lasiodiplodia citricola*, *N. mediterraneum, N. nonquaesitum, N. parvum N. vitifusiforme* and *Neoscytalidium dimidiatum* were recovered from infected walnut fruit and trees in California. In Spain, Botryosphaeriaceae isolates were the most aggressive fungi to walnut in all tissues evaluated (López-Moral et al. 2020). *Lasiodiplodia theobromae* was the most virulent fungal species and caused the largest lesions on walnut shoots in Iran (Sohrabi et al. 2020).

In this study, *B. dothidea*, *N. mediterraneum* and *N. parvum* were tested on 2-year-old plants that demonstrated their pathogenicity, and based on the length of the lesion, appeared to have varying virulence. In our study *N. parvum* caused the largest lesions. Similarly, *N. parvum* is one of the most pathogenic species on walnut (Chen et al. 2014; López-Moral et al. 2020). *Botryosphaeria dothidea*, were intermediately or weakly virulent to the tested walnut cultivars Chandler and Tulare. In California, pathogenicity tests showed that the virulence of *N. mediterraneum* and *B. dothidea* was intermediate (Chen et al. 2014). In contrast, *B. dothidea* was weakly in virulent in our findings.

Moral et al. (2010) successfully isolated *N. mediterraneum, B. dothidea, D. seriata,* and *N. parvum* from olive and other hosts in Spain. However *N. parvum* was isolated only from branches of walnut. Trouillas et al. (2010) showed that *N. mediterraneum* was the first report as pathogen on *J. regia* cvs. Hartley and Chandler in California. *Neofusicoccum parvum* and *B. dothidea,* were previously reported associated with walnut dieback in southeast Turkey. This is the first report of *N. parvum*, and *B. dothidea* causing twig and branch blight and dieback disease of walnuts in Aegean Region and *N. mediterraneum* on walnut trees in Turkey.

Therefore, further investigations are required to better ascertain their incidence throughout the areas of walnut cultivation, and the specific interactions amongst the various fungal species. This emerging disease could pose a potential threat to new tree plantations and to walnut production.

Acknowledgements We thank Dr. M. Erhan YILDIZ for the English review. The authors did not receive support from any organization for the submitted work.

Author contributions AY and SB designed the experiments; AY, SB, KB, and YK conducted the research AY, SB, and KB analyzed and discussed the results, and writing—review, editing, and all authors read and approved the final manuscript.

Funding No funding was received for conducting this study.

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

Declarations

Conflict of interest The authors have no conflicts of interest to declare that apply to the content of this article.

References

Akgül DS, Savaş NG, Teker T, Karahan B, Keykubat B (2013) Identification and pathogenicity of Botryosphaeriaceae species causing wood canker on grapevines. J Turk Phytopathol 42(1–3):35–45

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3):553–556. https://doi.org/10.1080/00275514.1999.12061051

Çeliker NM, Michailides TJ (2012) First report of *Lasiodiplodia theobromae* causing canker and shoot blight of fig in Turkey. New Dis Rep 25:12. https://doi.org/10.5197/j.2044-0588.2012.025.012

Cenis JL (1992) Rapid extraction df fungal DNA for PCR amplification. Nucl Acids Res 20:2380. https://doi.org/10.1093/nar/20.9.2380

Chen SF, Morgan DP, Hasey JK, Anderson K, Michailides TJ (2014) Phylogeny, morphology, distribution, and pathogenicity of Botryosphaeriaceae and Diaporthaceae from English walnut in California. Plant Dis 98:636–652. https://doi.org/10.1094/PDIS-07-13-0706-RE

Derviş S, Türkölmez Ş, Çiftçi O, Serçe ÇU, Dikilitas M (2019) First report of *Neoscytalidium dimidiatum* causing black canker and root rot of walnut in Turkey. Plant Dis 103(8):2129. https://doi.org/10.1094/PDIS-02-19-0306-PDN

Díaz GA, Latorre BA, Ferrada E, Gutiérrez M, Bravo F, Lolas M (2018) First report of *Diplodia mutila* causing branch dieback of English walnut cv. Chandler in the Maule Region, Chile. Plant Dis 102(7):1451. https://doi.org/10.1094/PDIS-11-17-1860-PDN

Eichmeier A, Pecenka J, Spetik M, Necas T, Ondrasek I, Armengol J, León M, Berlanas C, Gramaje D (2020) Fungal trunk pathogens associated with *Juglans regia* in the Czech Republic. Plant Dis 104:761–771. https://doi.org/10.1094/PDIS-06-19-1308-RE

Erdem C (2020) Determination of fungal pathogens causing twig, shoot blight and drying on almond trees in Datça town of Muğla province. Aydın Adnan Menderes University, Department of Plant Protection, M.Sc. Thesis, Aydın, p xxv+65 **(in Turkish)**

Kara M, Soylu EM, Soylu S, Uysal A, Kurt Ş (2020) First report of *Neofusicoccum parvum* causing branch dieback on *Juglans regia* in Turkey. J Plant Pathol 103:335. https://doi.org/10.1007/s42161-020-00662-8

Kayim M, Endes A, Eskalen A (2015) First report of *Neofusicoccum parvum* and *Diplodia* sp. associated with wood canker and dieback on almond in Turkey. In: XVIII. International plant protection congress (IPCC), Berlin, p798

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Koç İ, Çelik A, Durak ED, Demir İ, Bayman S, Mirtagioğlu H, Demir S (2020) Bitlis İli Ceviz Yetiştiriciliği Yapılan Tarım Alanlarında Görülen Ceviz Antraknozu (*Ophiognomonia leptostyla*) Hastalığının Morfolojik ve Moleküler Karakterizasyonu. J Inst Sci Technol 10(1):28–38. https://doi.org/10.21597/jist.594094

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

Kurbetli İ, Demirci F (2014) Outbreak of stem canker and dieback of pear trees caused by *Botryosphaeria obtusa* (anamorph *Diplodia seriata*) in Turkey. New Dis Rep 30:6. https://doi.org/10.5197/j.2044-0588.2014.030.006

Li G, Liu F, Li J, Liu Q, Chen S (2015) Characterization of *Botryosphaeria dothidea* and *Lasiodiplodia pseudotheobromae* from English walnut in China. J Phytopathol 164:348–353. https://doi.org/10.1111/jph.12422

López-Moral A, Lovera M, Raya MDC, Cortés-Cosano N, Arquero O, Trapero A, Agustí-Brisach C (2020) Etiology of branch dieback and shoot blight of English walnut caused by Botryosphaeriaceae and *Diaporthe* Species in Southern Spain. Plant Dis 104(2):533–550. https://doi.org/10.1094/PDIS-03-19-0545-RE

Moral J, Muñoz-Díez C, González N, Trapero A, Michailides TJ (2010) Characterization and pathogenicity of Botryosphaeriaceae species collected from olive and other hosts in Spain and California. Phytopathology 100:1340–1351. https://doi.org/10.1094/PHYTO-12-09-0343

Phillips AJ, 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

Slippers B, Boissin E, Phillips AJ, Groenewald JZ, Lombard L, Wingfield MJ, Postma A, Burgess T, Crous PW (2013) Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Stud Mycol 76:31–49. https://doi.org/10.3114/sim0020

Sohrabi M, Mohammadi H, León M, Armengol J, Banihashemi Z (2020) Fungal pathogens associated with branch and trunk cankers of nut crops in Iran. Eur J Plant Pathol 157:327–351. https://doi.org/10.1007/s10658-020-01996-w

Tabaklı S (2019) Occurence of olive antracnose in Aydin province and sensitivity of olive cultivars. Aydın Adnan Menderes University, Department of Plant Protection, M.Sc. Thesis, Aydın, p 89 **(in Turkish)**

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Bio Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Trouillas FP, Úrbez-Torres JR, Peduto F, Gubler WD (2010) First report of twig and branch dieback of English walnut (*Juglans regia*) caused by *Neofusicoccum mediterraneum* in California. Plant Dis 94(10):1267. https://doi.org/10.1094/PDIS-06-10-0412

TUIK (2020) Bitkisel Üretim İstatistikleri. https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr. Accessed Oct 2021

Uzunok S, Kurbetli İ (2017) Orta Anadolu koşullarında Ceviz antraknozu etmeni *Gnomonia leptostyla* (Fr.) Ces et de Not.'nın

mücadelesine yönelik biyolojik parametreler. Bitki Koruma Bülteni 57(3): 349–356. https://doi.org/10.16955/bitkorb.305885
White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322
Yildiz A, Benlioğlu S, Benlioğlu K, Korkom Y (2018) Determination of shoot and fruit blights walnuts production areas in Aydin. In: III. Forest entomology and pathology symposium, Artvin, p 59