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Research Papers – 12th Special issue on Grapevine Trunk Diseases

Current status of grapevine trunk disease pathogens on asymptomatic nursery-produced grapevines in Türkiye

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Summary. Good health of grapevine plants is important for productivity and sustainability of newly established vineyards, and accurate detection of bacterial and fungal pathogens is a prerequisite for managing the diseases they cause in nurseries. This study screened marketable, bare-rooted grapevine plants, obtained from different geographical regions of Türkiye, for fungal pathogens associated with grapevine trunk diseases (GTDs). In 2021, 43 grapevine nurseries located in eight provinces were surveyed to reveal the status of GTD pathogens on asymptomatic marketable plants. Fungal pathogens isolated from the roots and basal parts of asymptomatic dormant grapevines were identified using with morphological characteristics and molecular markers, and were subjected to pathogenicity tests. Six species; *Cytospora viticola, Diaporthe ampelina, Diplodia seriata, Lasiodiplodia brasiliensis, Neofusicoccum parvum,* and *Truncatella angustata* (associated with dieback), and six species; *Cadophora ferruginea, Cadophora luteo-olivacea, Cadophora malorum, Phaeoacremonium minimum, Phaeoacremonium tuscanicum* and *Phaeomoniella chlamydospora* (associated with Petri disease) were identified based on DNA sequencing of ITS and TEF1-α genes. GTD pathogens were detected in 12 and 14 of the 43 nurseries, respectively. Pathogenicity tests on 1103P vines revealed that all species were pathogenic (*N. parvum* and *C. luteo-olivacea* being the most virulent), and caused significant wood necroses when compared to non-inoculated experimental controls. This is the first report of *C. ferruginea, C. malorum, L. brasiliensis,* and *P. tuscanicum* associated with GTDs in Türkiye.

Keywords. *Cadophora, Cytospora, Lasiodiplodia, Phaeoacremonium,* trunk pathogens, *Vitis vinifera*.

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INTRODUCTION

Grapevine nurseries became important early in the 20 Century, when grafted vines began to be planted in many regions. According to data from

the Turkish Ministry of Agriculture and Forestry, more than 60 establishments currently produce grapevine saplings, and over three million standard grafted grapevine plants (registered) are produced annually (Anonymous, 2019). Grafted vines are often in demand for establishment of each new vineyard, for planting to replace dying grapevines, or for small scale retail sales. Although vineyard areas in Türkiye decreased by 9.8% between 2012 and 2017, demand remains high for grafted grapevine saplings (Söylemezoğlu *et al.,* 2020).

In newly established vineyards, plants may die due to factors related to physiological issues and cultivation techniques, such as unfavourable climatic conditions, poor planting practices, nutritional disorders, and the quality of propagation material. Nematodes, insects, soil-borne fungi, and grapevine trunk pathogens also cause serious plant losses in nurseries (Gramaje and Armengol, 2011).

Among fungal pathogens, *Cylindrocarpon*-like fungi are associated with black foot (Agusti-Brisach and Armengol, 2013); *Cadophora*, *Pleurostoma, Phaeoacremonium* spp., and *P. chlamydospora* are associated with Petri Disease (PD) (Travadon *et al*., 2015); *Botryosphaeriaceae, Cytospora, Diaporthe, Eutypa*, and *Truncatella* spp. are associated with other GTDs (Essakhi *et al*., 2008; Arzanlou *et al*., 2013; Billones-Baaijens *et al*., 2013); and *Fusarium* spp. are associated with root rots (Halleen *et al*., 2003). Young plants infected by these fungi may show various symptoms, such as reduced vigour or growth, delayed sprouting, chlorotic foliage, failure of grafting, reduced root biomass, necrotic roots, and dieback (Pintos *et al*., 2018). Fungal grapevine trunk pathogens can affect grapevine quality, reduce marketable-seedling yields and eventually cause plant death in grapevine nurseries. Most of these fungi can spread latently over large areas with propagation material, dormant plants, or through their reproductive structures such as pycnidia or asexual conidia. Some pathogens (*Cylindrocarpon*-like fungi, *P. chlamydospora*, and *Fusarium* spp.) can also survive in soil for many years as chlamydospores (Retief *et al*., 2006). The members of *Botryosphaeriaceae, Diatrypaceae*, *Cytospora, Diaporthe*, and Pestalotioid fungi (*Neopestalotiopsis, Pestalotiopsis, Seimatosporium,* and *Truncatella*) have opportunistic natures, strong saprophytic abilities and broad host ranges. These organisms can survive in plant residues for many years and threaten many host species in nurseries (Úrbez-Torres, 2011; Lawrence and Travadon, 2018).

Botryosphaeria dieback, Petri disease, and black foot have been shown as the most common diseases in grapevine nurseries in many countries, including South Africa (Fourie and Halleen, 2004), Italy (Pollastro *et al.,* 2009), Portugal (Rego *et al.,* 2009), Spain (Aroca *et al.,* 2010), Australia (Whitelaw-Weckert *et al.,* 2013), France (Lecomte *et al.,* 2018; Pintos *et al.,* 2018), Canada (Hrycan *et al.,* 2022), California (Todd *et al.,* 2022), and Uruguay (Carbone *et al.,* 2022).

Most of the previously described GTD pathogens were also identified in Türkiye in mature vineyards, regional grapevine nurseries and young vineyards. Poyraz and Onoğur (2013) carried out a survey targeting just Petri Disease and Esca pathogens in the nurseries and mature vineyards in the Aegean Region, where Akgül *et al.,* (2015) identified fungal trunk pathogens in 10-30-year-old Sultana Seedless plants. Akgül and Ahioğlu (2019) screened GTD pathogens associated with young grapevine decline in 1-3-year-old vineyards in Southern Türkiye. A survey 3 years later revealed occurrence and diversity of black foot pathogens in nurseries (Akgül *et al.,* 2022). However, no substantial information is available on the current status of other GTD pathogens in marketable dormant plants in Turkish grapevine nurseries. Determining the latent fungal pathogens in each nursery may help to develop appropriate plant protection technology for producing healthy plants.

The objectives of the present study were: (i) to assess the presence of GTD pathogens on asymptomatic marketable plants produced in Turkish grapevine nurseries; (ii) to identify the associated fungal species based on molecular characterization; and (iii) to determine the virulence of representative isolates using pathogenicity tests on dormant grapevine cuttings.

MATERIALS AND METHODS

Survey and isolation of dieback and Petri Disease pathogens

During January 2021, a total of 450 dormant grapevine plants were sampled from the nurseries (10–12 plants from each nursery), located in eight provinces Türkiye (Adıyaman, Bursa, Denizli, Manisa, Mersin, Şanlıurfa, Tekirdağ, and Tokat) (Figure 1). Roots and rootstocks were washed under running tap water, and were then superficially disinfected with sodium hypochlorite solution (>5% active chlorine, and diluted in sterile distilled water (1:1 v/v)) for 3 min. The tissues were then rinsed with sterile distilled water, briefly blotted on sterile paper towels, and allowed to dry under a sterile cabinet. Root hairs and inner tissues of the rootstocks (3–4 mm long) were then placed (six to seven fragments per Petri dish) onto PDA (Potato Dextrose Agar, Conda Lab) amended with streptomycin sulfate

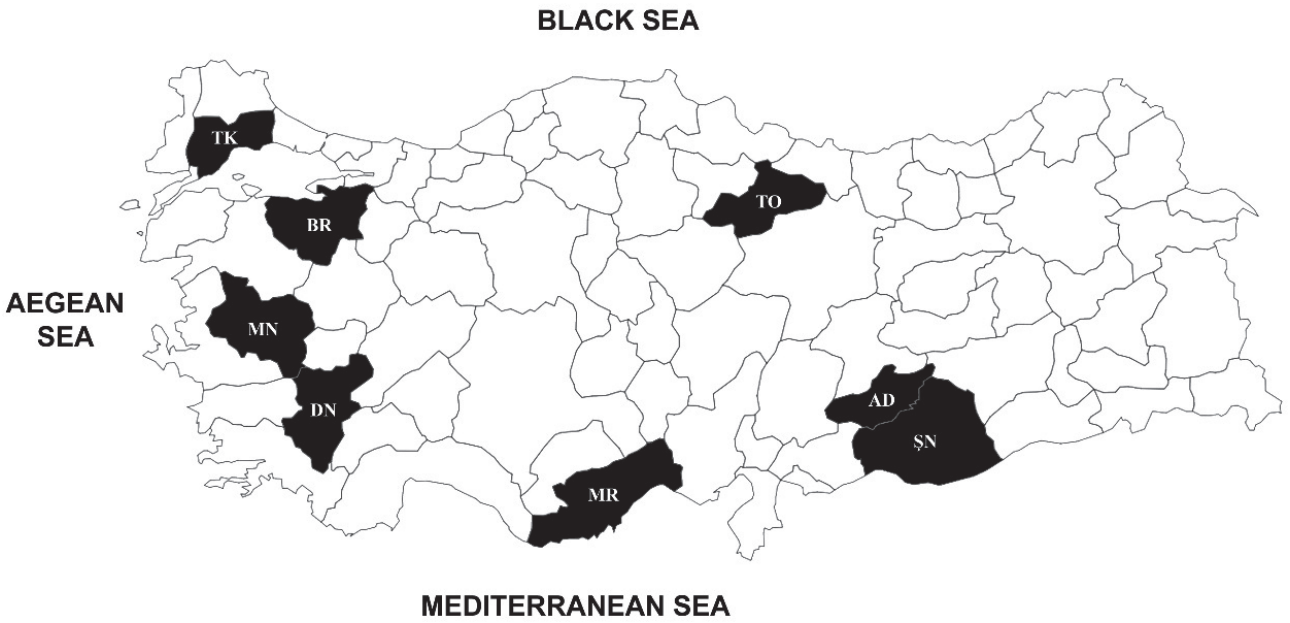

Figure 1. Provinces in Türkiye from which grapevine nurseries were sampled in this study. AD: Adıyaman, BR: Bursa, DN: Denizli, MN: Manisa, MR: Mersin, ŞN: Şanlıurfa, TK: Tekirdağ, TO: Tokat.

(250 mg×L^{-1}). Petri dishes (10–12 dishes per nursery) were incubated at 24°C in the dark for 7–10 d. Resulting fungal colonies were examined under a light microscope (Olympus BX51), and were sub-cultured to fresh PDA plates for further studies. Altogether, 285 fungal colonies were isolated from the samples. Based on microscopic features and colony morphologies specified in relevant publications (Barnett and Hunter, 2003; Essakhi *et al*., 2008; Trouillas and Gubler, 2010; Phillips *et al*., 2013; Lawrence *et al*., 2017; Maciá-Vicente *et al*., 2020), *Botryosphaeriaceae, Cadophora, Cytospora, Diaporthe, Phaeomoniella, Phaeoacremonium,* and *Truncatella* spp. were considered as probable GTDs pathogens. As well, *Acremonium, Alternaria, Aspergillus, Aureobasidium, Cladosporium, Clonostachys, Entoleuca, Epicoccum, Fusarium, Mortierella, Petriella, Penicillium* and *Trichoderma* spp. were also determined, but they were considered as endophytic species. Isolation frequencies (%) of Petri disease and other trunk pathogen fungi was calculated as proportions of 70 tissue fragments from 10–12 vines in each nursery (Table 1). The overall isolation frequency (%) of each fungus was calculated from isolation frequencies in each nursery. The prevalence of each species (%) was calculated as the proportion of the nursery numbers (pathogen detected) to the total nursery number. The overall disease prevalence (%) was calculated as the proportion of nursery numbers (pathogen detected) to the total nursery number.

MOLECULAR IDENTIFICATION OF FUNGI

To obtain pure cultures and provide genetic purity, single spores or hyphal tips of the fungi were isolated under a light microscope. Twenty-six representative isolates were used for identification with molecular markers. Approx. 50 mg of aerial mycelia was harvested from each pure culture (grown on PDA, incubated at 25°C, in the dark, for 7–10 d). Total DNA was extracted following the CTAB (2%) protocol (O'Donnell *et al*., 1998). Genomic DNA from each isolate was diluted with 80 µL of PCR grade water, then stored at -18°C for further use. For sequencing, ITS1, 5.8S, and ITS2 regions of the rDNA, and partial TEF 1-α (translation elongation factor) genes, were amplified with PCR reactions using the ITS4/ITS5 and EF688F-EF1251R primers (White *et al.,* 1990; Alves *et al.,* 2008). The PCR reaction mixtures each contained 5 µL of buffer (10× Green Buffer, DreamTaq Green DNA Polymerase; Thermo Scientific), 2 µL of the dNTPs mixture (10 mM each, Thermo Scientific), 1 µL of forward and reverse primers (stock concentration, 10 pmol·$µL^{-1}$), 0.25 µL of Taq polymerase (DreamTaq Green DNA Polymerase; Thermo Scientific), 39.75 µL PCR grade water, and 1 µL genomic DNA (approx. 100 ng·$µL^{-1}$). PCR amplifications were using a Simpli-Amp A24811™ Thermal Cycler (Applied Biosystems), with the following conditions; 95°C for 3 min. (initial denaturation), followed by 35 cycles each of denaturation at 95°C for 1 min, annealing at 52°C (for ITS) or 53°C (for TEF1-α) for

Table 1. Locations of surveyed grapevine nurseries, cultivars, isolation frequency (%) and prevalence (%) of Petri disease and other grapevine trunk disease (GTD) fungi.

Nursery	Location	Rootstock / Cultivar	Isolation Frequency (%)					
			Trunk Pathogen Fungi				Petri Disease Fungi	
			Botryosphaeriaceae	*Cytospora*	*Diaporthe*	*Truncatella*	*Cadophora*	*Phaeomoniella chlamydospora* *Phaeoacremonium* spp.
1	Bursa	1103P-Trakya İlkeren	-	-	-	-	-	-
2	Mersin	1103P- Victoria	15.7	-	-	-	-	-
3	Salihli, Manisa	Thompson Seedless	-	2.9	11.4	-	-	-
4	Salihli, Manisa	Sultana Seedless	-	-	-	-	-	-
5	Salihli, Manisa	Sultana Seedless	-	-	-	-	-	7.1
6	Salihli, Manisa	Sultana Seedless	-	-	-	-	-	1.4
7	Salihli, Manisa	1103P / Sultana Seedless	-	-	7.1	-	11.4	1.4
8	Alaşehir, Manisa	Sultana Seedless	-	-	-	-	-	-
9	Alaşehir, Manisa	Sultana Seedless	-	-	-	-	-	-
10	Alaşehir, Manisa	Sultana Seedless	-	-	-	-	-	-
11	Sarıgöl, Manisa	Sultana Seedless	7.1	-	-	-	-	-
12	Salihli, Manisa	Sultana Seedless	-	-	-	-	-	-
13	Tekirdağ	Kober 5BB / Sultan 1	1.4	-	-	-	7.1	15.7
14	Tekirdağ	Kober 5BB / Bozbey	-	-	-	-	-	1.4
15	Tekirdağ	1103P-Tekirdağ Seedless	11.4	-	-	-	-	-
16	Tekirdağ	110R-Yapıncak	-	-	-	-	-	-
17	Denizli	41B / Sultana Seedless	-	-	-	-	-	-
18	Denizli	41B / Sultana Seedless	-	-	-	-	-	-
19	Denizli	41B / Sultana Seedless	-	-	-	-	-	-
20	Denizli	41B / Sultana Seedless	-	-	-	-	-	-
21	Denizli	41B / Michele Palieri	-	-	-	-	-	-
22	Şanlıurfa	1103P - Ergin Seedless	-	-	-	21.8	-	-
23	Şanlıurfa	110R - Horozkarası	-	-	-	4.2	-	-
24	Şanlıurfa	99R - Çiloreş	-	-	-	-	-	-
25	Şanlıurfa	1103P - Victoria	-	-	-	-	-	21.4
26	Manisa	41B / Red Globe	-	-	-	-	-	2.9
27	Manisa	Kober 5BB / Royal	-	-	-	-	-	-
28	Manisa	1103P - Sultana Seedless	-	-	-	-	3.8	-
29	Manisa	Kober 5BB - Sultana Seedless	-	-	-	-	-	1.4
30	Manisa	1103P - Crimson Seedless	-	-	-	-	-	2.9
31	Manisa	110R / Alicante Bouschet	-	-	-	-	-	-
32	Alaşehir, Manisa	1103P - Thompson Seedless	-	-	-	-	-	-
33	Manisa	Kober 5BB / Ata Sarısı	-	-	-	-	-	-
34	Turgutlu, Manisa	Kober 5BB /Sultana Seedless	-	-	-	-	-	-
35	Manisa	Kober 5BB / Trakya İlkeren	-	-	-	-	-	-
36	Tokat	1103P - Narince	4.3	-	-	-	-	-
37	Tokat	1103P/Narince	-	-	-	-	-	-
38	Tokat	1103P/Narince	-	-	-	-	1.4	-
39	Tokat	1103P/Sultan7	28.6	-	-	-	-	-
40	Tokat	1103P/Narince	-	-	-	-	6.7	-
41	Tokat	Du Lot / Narince	-	-	-	-	-	12.8
42	Adıyaman	Kober 5BB / Hatun Parmağı	-	-	-	14.3	-	0.9
43	Mersin	1103P / Victoria	-	-	1.8	-	-	-
		Average (%)	11.4	2.9	6.8	13.4	6.1	6.3
		Prevalence of each species (%)	13.9	2.3	7.0	7.0	11.6	25.9
		Prevalence of all species (%)			27.9			32.5

1 min, and extension at 72°C for 1 min, and final extension at 72°C for 10 min. PCR products were sequenced by Macrogen Co. The electronic sequence files of the isolates were extracted with Chromas Lite (Technelsiyum™) software, and sequences were compared with those deposited in the National Center for Biotechnology Information (NCBI) USA National Institute of Health database, using nucleotide BLAST® (Basic Local Alignment Search Tool) software. The ITS and TEF1-α sequences were submitted to the NCBI GenBank, and accession numbers were obtained (Table 2).

Pathogenicity tests

All the 26 isolates were subjected to pathogenicity tests (two replicates in a year) on potted 1103 Paulsen rootstock plants in a controlled climate room (26°C, 80% relative humidity, and 12 h illumination). Dormant cuttings (each 30 cm long, with three buds) were superficially disinfected with sodium hypochlorite solution (2.5%) for 3 min. They were then rinsed once with sterile distilled water and blotted on sterile paper towels. The ends of the cuttings were then cut with a sterile pruning shear, and their bases were dipped in gibberellic acid solution (2000 $\mu g \cdot mL^{-1}$) for 10 sec, to induce root formation. The apical ends were inoculated with mycelium agar discs (5 mm diam.) of fungal isolates and wrapped with parafilm (Curwood®) to allow colonization. For non-inoculated controls, sterile agar discs were placed on the apical ends of the cuttings (Ayres *et al.,* 2011). After inoculation, the cuttings were planted in plastic bags (one cutting per bag) each containing 1 L of the potting mix (peat moss, perlite, and sawdust in equal volumes) and watered. Twelve plants per isolate (one plant per pot and four replicates with three plants

Table 2. Petri disease and other grapevine trunk disease pathogens identified in this study, their locations, hosts, and GenBank accession numbers.

Nursery	Isolate code	Fungal species of trunk pathogens	Location	Rootstock / Cultivar	GenBank Accession Numbers	
					ITS	TEF1
1	AFP21	*Cytospora viticola*	Mezitli, Mersin	1103P - Victoria	OP412792	OP508220
2	AFP26	*Diaporthe ampelina*	Salihli, Manisa	Sultana Seedless	OP412793	OP508221
3	AFP121	*Diaporthe ampelina*	Mezitli, Mersin	1103P - Victoria	OP412794	OP508222
4	AFP282	*Diaporthe ampelina*	Salihli, Manisa	1103P - Sultana Seedless	OP412795	OP508223
5	AFP11	*Diplodia seriata*	Mezitli, Mersin	1103P - Victoria	OP412796	OP508224
6	AFP301	*Lasiodiplodia brasiliensis*	Sarıgöl, Manisa	Sultana Seedless	OP412797	OP508225
7	AFP312	*Lasiodiplodia brasiliensis*	Tekirdağ	5BB - Sultan 1	OP412798	OP508226
8	AFP315	*Lasiodiplodia brasiliensis*	Tekirdağ	5BB - Bozbey 1	OP412799	OP508227
9	AFP317	*Lasiodiplodia brasiliensis*	Tekirdağ	1103P - Yapıncak	OP412800	OP508228
10	AFP22	*Neofusicoccum parvum*	Tekirdağ	1103P - Tekirdağ Seedless	OP412801	OP508229
11	AFP91	*Neofusicoccum parvum*	Tokat	1103P - Narince	OP412802	OP508230
12	AFP92	*Neofusicoccum parvum*	Tokat	1103P - Sultan 7	OP412803	OP508231
13	AFP145	*Neofusicoccum parvum*	Tokat	1103P - Narince	OP412804	OP508232
14	AFP152	*Neofusicoccum parvum*	Tokat	1103P - Sultan 7	OP412805	OP508233
15	AFP83	*Truncatella angustata*	Şanlıurfa	1103P - Ergin Seedless	OP412806	OP550034
16	AFP134	*Truncatella angustata*	Adıyaman	5BB - Hatun Parmağı	OP412807	OP550035
17	AFP217	*Truncatella angustata*	Şanlıurfa	99R - Çiloreş	OP412808	OP550036
	Petri disease					
18	AFP159	*Cadophora ferruginea*	Tokat	1103P - Narince	OP412809	OP961938
19	AFP23	*Cadophora luteo-olivacea*	Salihli, Manisa	1103P - Sultana Seedless	OP412810	OP550037
20	AFP24	*Cadophora luteo-olivacea*	Tekirdağ	5BB - Sultan 1	OP412811	OP550038
21	AFP53	*Cadophora luteo-olivacea*	Şanlıurfa	Victoria	OP412812	OP550039
22	AFP119	*Cadophora luteo-olivacea*	Manisa	110R - Sultan 7	OP412813	OP550040
23	AFP143	*Cadophora malorum*	Tokat	1103P - Narince	OP412814	OP550041
24	AFP57	*Phaeoacremonium minimum*	Şanlıurfa	1103P - Victoria	OP412815	OP550042
25	AFP56	*Phaeoacremonium tuscanicum*	Şanlıurfa	1103P - Ergin Seedless	OP412816	OP550043
26	AFP203	*Phaeomoniella chlamydospora*	Adıyaman	5BB - Hatun Parmağı	OP412817	OP550044

per replicate) were inoculated with each of the isolates. The plants were supplied with Hoagland solution twice each month for 4 months to provide balanced nutrition.

At the end of the period, the plants were uprooted, and their shoots and roots were removed with a pruning shear. The inoculation points were split with a knife, and the lengths of necrotic wood tissues were measured with a caliper and recorded. The pathogenicity of the isolates was confirmed by following Koch's postulates. The inoculated pathogens were re-isolated from the symptomatic wood chips but not from the non-inoculated controls. Mean lesion lengths were analyzed separately as Petri diseases or other trunk pathogens, because the growth rates of the fungi causing the diseases were not the same either in PDA cultures or in wood tissues.

Statistical analyses

Analysis of variance (ANOVA) was carried out on data of lengths of wood necrosis (mean lengths of two experiments), and the data were checked for normality. Means were compared using Fisher's least significant difference (LSD) test (at $P = 0.05$) (Gomez and Gomez, 1984).

RESULTS

Isolation of the fungi involved in disease prevalence in the nurseries

According to morphological/microscopic examination, some of the fungi related to Petri diseases and other GTD pathogens, referred to here as trunk pathogens, were detected in marketable plants in the surveyed grapevine nurseries (Table 1). These pathogens were isolated from 23 (54%) of the 43 surveyed nurseries. No pathogens were detected in 20 nurseries. One or more fungal species associated with both diseases were isolated from the roots and rootstocks of asymptomatic dormant vines. When the fungal pathogens were grouped separately, the isolation frequencies of Petri diseases were 27.9%, and other trunk pathogens 32.5%. *Phaeoacremonium* spp. and *P. chlamydospora* were the most prevalent fungi isolated (25.9%), followed by *Botryosphaeriaceae* (13.9%), *Cadophora* (11.6%), *Diaporthe* and *Truncatella* spp. (7.0%).

Molecular identification of the isolates

Identification of 26 isolates was performed using partial sequencing of ITS and TEF 1-α genes, and nucleotide sequences were deposited in the GenBank with accession numbers OP412792 to OP412817, OP508220 to OP508233, and OP550034 to OP550044 (Table 2). According to nucleotide BLAST searches (with ≥99 similarities), nine isolates were associated with Petri disease, and 17 isolates were detected as trunk pathogens. *Botryosphaeriaceae* species (*D. seriata* De Not, *L. brasiliensis* M.S.B. Netto, M.W. Marques & A.J.L. Phillips, and *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips) constituted 58.8% (ten isolates) of all the dieback-related fungi, followed by *D. ampelina* (Berk. & M.A. Curtis) R.R. Gomes, Glienke & Crous (three isolates; 17.6%), *Truncatella angustata* (Pers.) S. Hughes (three isolates; 17.6%), and *Cytospora viticola* D.P. Lawr., Travadon & Pouzoulet (one isolate; 5.9%). *Cadophora* spp. (*C. ferruginea* Koukol & Maciá-Vicente, *C. luteo-olivacea* (J.F.H. Beyma) T.C. Harr. & McNew, and *C. malorum* (Kidd & Beaumont) W. Gams)) predominated (six isolates; 66.7%) the fungi related to Petri disease. Only one isolate each (5.9%) of *Phaeoacremonium minimum, P. tuscanicum,* and *P. chlamydospora* belonged to *Togniniaceae* and *Phaeomoniellaceae*. Among these, *C. ferruginea, C. malorum, L. brasiliensis,* and *P. tuscanicum* had not been previously recorded in vineyards in Türkiye.

Pathogenicity tests

After 4 months in of 1103 Paulsen cuttings, all isolates caused blackish-brown vascular discolourations (of mean lengths from 8.2 to 40.0 mm) below inoculation points (Figure 2). No visible symptoms were observed on the green shoots or leaves of the plants. When the inoculated fungal groups (Petri disease pathogens and other GTD pathogens) are examined separately, wood lesions caused by Petri disease pathogens were less extended than those caused by other GTD pathogens. Among the dieback fungi, *N. parvum* produced the most extended lesions (from 36.8 to 40.0 mm), and was the most virulent pathogen (Table 3). It was followed by *L. brasiliensis* (mean lesion length 24.7 to 32.6 mm), *T. angustata* (22.5 to 29.9 mm), and *D. ampelina* (9.7 to 13.2 mm) (Table 3). Average lesion lengths produced by *C. viticola* were slightly longer (8.2 mm) than non-inoculated control (6.2 mm). Similarly, the isolates associated with Petri disease did not cause shoot or leaf symptoms, but their mean internal lesion lengths were longer ($P \leq 0.05$) than that for the control. *Cadophora* isolates were more virulent (mean lesion length 9.4 to 9.7 mm), compared to *Phaeoacremonium* spp. and *P. chlamydospora* (8.5 to 8.9 mm), but no statistical difference was found between mean lesion lengths of *Cadophora* spp. (Table 4). The re-isolation experiment also confirmed severe colonization of the plant wood tissues. Both dieback and Petri disease fungi were recovered, with isolation

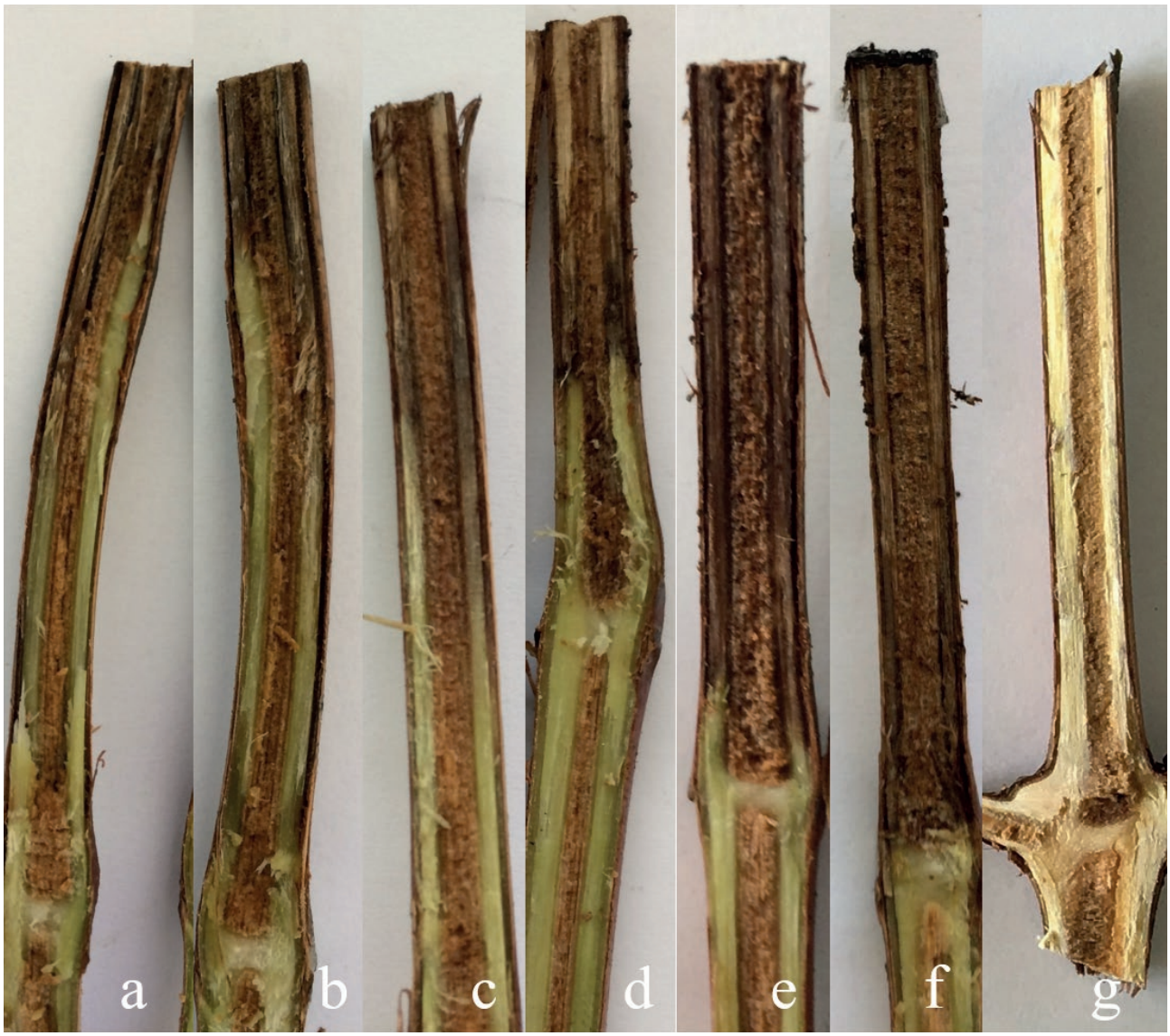

Figure 2. Internal wood symptoms caused by grapevine trunk disease pathogens on potted 1103P rootstock plants, 4 months inoculations with: a, *Cadophora luteo-olivacea*; b, *C. malorum*; c, *Diaporthe ampelina*; d, *Truncatella angustata*; e, *Lasiodiplodia brasiliensis*; f, *Neofusicoccum parvum*; and g, non-inoculated control.

rates from 18.6 to 72.4%. The virulence variability between the species was found by averaging the lesion lengths produced by isolates within each species (Figures 3 and 4). According to this evaluation, *Cadophora luteo-olivacea* was the most virulent fungus causing Petri disease and *N. parvum* was the most virulent fungus causing other GTDs.

DISCUSSION

Grafted grapevine production is a critical sector of Turkish viticulture. Screening the plants for health and determining related pathogens are important steps for preventing the distribution of diseases. The present study was conducted in Turkish grapevine nurseries to determine prevalence of GTD pathogens on asymptomatic marketable plants. The results showed that dieback (in 30% of surveyed nurseries) and Petri disease pathogens (33% of nurseries) were moderately prevalent. Previously, black foot pathogens were found to be very common in most of surveyed nurseries (Akgül *et al.*, 2022). Nine of 43 nurseries producing non-grafted Sultana Seedless grapevines were examined in this survey, and dieback and Petri disease pathogens were identi-

Table 3. Mean lesion lengths and proportions (%) of re-isolations for species of fungi associated with grapevine trunk diseases (except Petri disease) on 1103 Paulsen rootstock plants.

Fungal species	Isolate	Lesion length	Re-isolation
		(mm)	(%)
Neofusicoccum parvum	AFP91	40.0 ± 0.3 l*	72.4
N. parvum	AFP152	39.3 ± 1.0 kl	65.6
N. parvum	AFP145	38.8 ± 0.7 k	68.1
N. parvum	AFP92	37.1 ± 0.3 j	59.4
N. parvum	AFP22	36.8 ± 0.6 j	60.2
Lasiodiplodia brasiliensis	AFP315	32.6 ± 0.3 i	63.9
L. brasiliensis	AFP301	30.8 ± 0.5 h	55.3
L. brasiliensis	AFP312	30.5 ± 0.1 h	70.1
Truncatella angustata	AFP217	29.9 ± 0.9 h	65.9
L. brasiliensis	AFP317	24.7 ± 0.6 g	50.6
T. angustata	AFP134	23.7 ± 0.3 f	52.8
T. angustata	AFP83	22.5 ± 0.5 e	54.7
Diaporthe ampelina	AFP282	13.2 ± 0.6 d	38.6
Diplodia seriata	AFP11	10.4 ± 0.4 c	63.4
D. ampelina	AFP121	9.8 ± 0.3 c	44.1
D. ampelina	AFP26	9.7 ± 0.2 c	50.3
Cytospora viticola	AFP21	8.2 ± 0.2 b	36.1
Non-inoculated control	-	6.2 ± 0.1 a	-

*Means accompanied by the same letter are not significantly different (P = 0.05), according to LSD tests. Each mean is the average for 24 cuttings (12 per experiment).

Table 4. Mean lesion lengths resulting from inoculations of grapevine plants with different fungal species associated with Petri disease on 1103 Paulsen grapevine rootstock plants.

Fungal species	Isolate	Lesion length	Re-isolation
		(mm)	(%)
Cadophora luteo-olivacea	AFP23	9.7 ± 0.4 d*	28.6
C. luteo-olivacea	AFP24	9.7 ± 0.4 d	22.9
C. luteo-olivacea	AFP119	9.6 ± 0.1 d	30.5
C. luteo-olivacea	AFP53	9.4 ± 0.7 d	38.9
C. ferruginea	AFP159	9.4 ± 1.0 d	22.1
C. malorum	AFP143	9.4 ± 0.4 d	45.1
Phaeoacremonium minimum	AFP57	8.9 ± 0.2 c	18.6
Phaeoacremonium tuscanicum	AFP56	8.6 ± 0.1 bc	30.4
Phaeomoniella chlamydospora	AFP203	8.5 ± 0.3 b	27.6
Non-inoculated control	-	6.2 ± 0.1 a	-

*Means accompanied by same letter are not significantly different (P = 0.05) according to LSD tests. Each mean is the average for 24 grapevine cuttings (12 per experiment).

fied only in three nurseries, from asymptomatic plants. These results indicate that most of the rootstock mother

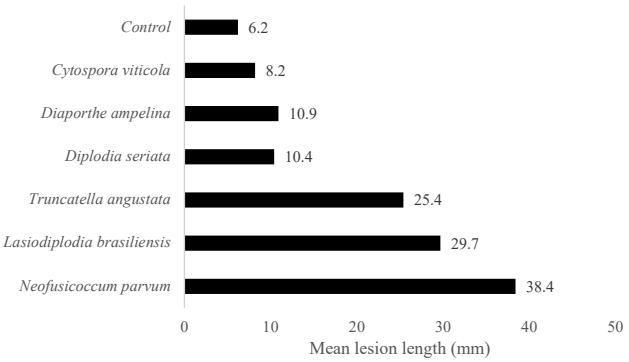

Figure 3. Mean lesion lengths (mm) from inoculations with different fungi associated with grapevine trunk diseases (except Petri disease), in a pathogenicity test.

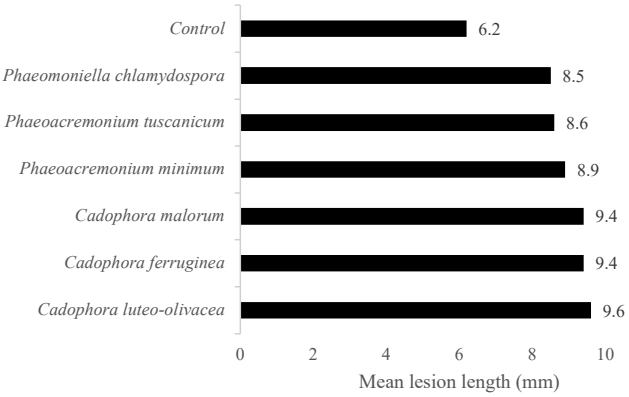

Figure 4. Mean lesion lengths (mm) from inoculations with different fungi associated with Petri disease, in pathogenicity test.

plant plots obtained latent infections during vegetative or propagation stages in the infested nurseries. Almost all grapevine rootstock mother fields in Türkiye are irrigated by flooding or furrow irrigation. Plants are also not grown on trellises, so the shoots of rootstock mother grapevines are sprawled on the soil surfaces. Soil-borne inocula of *Cadophora, Phaeomoniella,* and *Phaeoacremonium* may have caused shoot infections of the rootstock mother vines, resulting in greater prevalence of Petri disease than GTDs in the surveyed nurseries.

Rootstock/scion mother plants used for grapevine propagation may harbour trunk pathogens without showing disease symptoms. Therefore, these pathogens can be disseminated over large areas within young infected vines, and disease symptoms may appear 8-10 years later (Gramaje and Armengol, 2011). This has been confirmed in several studies using conventional fungal isolation and next-generation sequencing methods. Aroca *et al.* (2010) evaluated plant health in grapevine nurs-

ery propagation processes in five commercial nurseries in Spain. They found that apparently healthy dormant cuttings were contaminated with *Botryosphaeria dothidea, Lasiodiplodia theobromae, Neofusicoccum australe, N. mediterraneum, N. parvum, N. vitifusiforme P. minimum, P. parasiticum, P. chlamydospora,* and *D. ampelina*. Hofstetter *et al*. (2012) compared the fungal microbiota in adult grapevines with or without Esca symptoms, using conventional isolation methods, and found 158 fungal species. Along with many endophytic and saprobic fungi, *B. dothidea*, *Cadophora luteo-olivacea, P. minimum, P. chlamydospora*, *D. ampelina,* and *T. angustata* were also detected in symptomless and symptomed plants. Eichmeier *et al*. (2018) identified 189 fungal genera (including *C. luteo-olivacea, C. malorum, D. ampelina, Diplodia seriata, Pm. chlamydospora, Ph. minimum, T. angustata* species) from dormant marketable grapevines, using conventional isolation and high-throughput amplicon sequencing methods in Spanish and Czech grapevine nurseries. Billones-Baaijens *et al.* (2013) determined the presence of *Botryosphaeriaceae* fungi in marketable young plants in New Zealand grapevine nurseries. In Türkiye, we have also detected many of the species listed above in some of the surveyed nurseries, and these fungi were moderately prevalent. Incidence of Botryosphaeriaceous fungi was calculated as 19% in rootstocks and 17% in scion cuttings. These rates are greater than those (11.4%) determined in the present study.

Cadophora ferruginea, *C. malorum, L. brasiliensis* and *P. tuscanicum* were detected for the first time in Türkiye. Some *Cadophora* spp. (including *C. luteo-olivacea, C. malorum, C. melinii, C. orientoamericana, C. novi-eboraci*, and *C. spadicis*) have been previously detected in grapevine nurseries, in California, Canada, Germany, South Africa, and Uruguay (Rooney-Latham, 2005; Halleen *et al.,* 2007; Navarrete *et al.,* 2011; Gramaje *et al.,* 2011; Úrbez-Torres *et al.,* 2014; Gierl and Fischer, 2017; Travadon *et al.,* 2015). These fungi have been suggested as having important roles in the occurrence of Petri disease in young grapevines. The thesis of Özben (2020) reported *C. luteo-olivacea* and *T. angustata* (identified morphologically and microscopically) in 12 grapevine nurseries in Türkiye, but no pathogenicity or molecular identification data were reported. *Cadophora ferruginea* has been reported on grapevine in the United States of America (Travadon *et al*., 2022), so our isolate (AFP159) would be the second of this fungus. *Lasodiplodia brasiliensis* was first reported on table grapes in Brazil (Correia *et al*., 2016), then in Mexico (Rangel-Montoya *et al*., 2021). *Phaeoacremonium tuscanicum* was first reported in Spain (Essakhi *et al*., 2008), and then in the other countries, including New Zealand (Graham *et al*., 2009), Algeria (Berraf-Tebbal, 2011), Iran (Mohammadi, 2012), and Canada (Úrbez-Torres *et al*., 2014). We carried out an additional search to find the occurrence of these fungi in scientific journals and the Mycobank databases, but no record was found aside from the above countries.

Regarding to virulence of the isolates, fungal pathogens associated with Petri disease were found to be more virulent than the other GTD pathogens on 1103P rootstock plants (4 months after incubations) in pathogenicity tests. In previous studies, *Lasiodiplodia* and *Neofusicoccum* were shown to be highly virulent on grapevines when compared to other Botryosphaeriaceous fungi (Luque *et al*., 2009; Úrbez-Torres and Gubler, 2011). *Neofusicoccum parvum* was the most virulent species among 17 dieback-related isolates evaluated, followed by *L. brasiliensis, T. angustata,* and *D. ampelina* in the present study. Correia *et al*. (2016) studied the phylogeny, distribution, and pathogenicity of *Lasiodiplodia* species on table grapes in Brazil, and *L. brasiliensis* was the most virulent species, followed by *L. theobromae, L. pseudotheobromae* and *L. hormozganensis* on detached-green shoots of the Isabel grape cultivar, after 10 d incubation. Similarly, Rangel-Montoya *et al.* (2021) tested the pathogenicity of *L. brasiliensis, L. gilanensis, L. exigua,* and *L. crassispora*, and *L. brasiliensis* was the most virulent, causing average lesion lengths of 5.1-5.5 mm on Cabernet Sauvignon cuttings after 2 months incubation. Akgül *et al*. (2015) tested pathogenicity of several grapevine trunk disease pathogens, obtained in the Aegean Region of Türkiye. These tests were for approx. 4 months on potted 1-year old Sultana Seedless plants. Among these fungi, *N. parvum* was the most virulent, producing lesions of average length 79.1 mm, followed by *L. theobromae* (59.8 mm), *D. ampelina* (34.6 mm), *Phaeomoniella chlamydospora* (27.5 mm), *Togninia minima* (25.5 mm), *B. dothidea* (24.8 mm) and *D. seriata* (21.4 mm).

The pathogenicity results from some studies were different, however, from the those outlined above. Mondello *et al*. (2020) indicated that Sicilian isolates of *P. chlamydospora* produced larger lesions than *N. parvum* on excised grapevine canes (cv. Grecanio) 15 days after inoculation. Raimondo *et al*., (2019) investigated the current status of newly detected fungal GTD pathogens (*C. luteo-olivacea, Colletotrichum fioriniae, Seimatosporium vitis-vinifera* and, *T. angustata*) from Apulia and Molise regions of Italy. These fungi produced characteristic wood necroses (for *C. luteo-olivacea*: 12.9-14.1 cm, *C. fioriniae*: 8.4-8.8 cm, *S. vitis-vinifera*: 16.9-23.6 cm, *T. angustata*: 15.7-18.0 cm), when compared to non-inoculated controls (0.6 cm), after 8 months on two different grapevine cultivars in field conditions. Halleen *et al.* (2007) in a study in South Africa, showed lesion lengths

caused by *C. luteo-olivacea* were 17.1 cm, by *P. chlamydospora* were 9.6 cm, and by *P. minimum* were 8.9 mm, on 101-14 Mgt/Shiraz plants after 3 months incubation in a greenhouse.

The pathogenicity test results from the present study were similar to the previous studies but not to all. Some inconsistencies can also be seen among previous pathogenicity test results, probably due to differences in cultivar susceptibility, incubation conditions, the virulence of fungal isolates, and the inoculation methods used.

This research has demonstrated the current status of GTD pathogens on asymptomatic, marketable, nursery-produced grapevines in Türkiye. Although these pathogens were rare, the results indicate that appropriate disease control methods, such as screening of pathogens, hot water treatments, and use of biological pesticides, should be adopted in rootstock plots of grapevine nurseries.

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AUTHORS CONTRIBUTIONS

Davut Soner AKGÜL designed this study, identified-archived the fungal isolates, performed statistical analyses, molecular studies and pathogenicity tests, wrote the paper. Nurdan GÜNGÖR SAVAŞ and Murat YILDIZ surveyed the grapevine nurseries, obtained-archived the fungal isolates. İzzet BÜLBÜL surveyed the grapevine nurseries, obtained-archived the fungal isolates, did molecular identification studies. Mümine ÖZARSLANDAN surveyed the grapevine nurseries, obtained-archived the fungal isolates.

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