Identification and Pathogenicitiy of Botryosphaeriaceae Species Causing Wood Canker on Grapevines

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ABSTRACT

Wood canker and dieback diseases, caused by Botryosphaeriaceae fungi, are among the most important fungal trunk diseases of grapevines. In recent years, the symptoms of local dead arm, shoot dieback and V-shaped discolorations in woody tissues have dramatically increased in vines of Turkey. During the 2012 growing season, symptomatic wood samples (from Ankara, Çorum, Izmir, and Manisa cities) were taken and standard mycological isolations were done to determine the fungal agents of disease. A high proportion (69.4%) of Botryosphaeriaceae fungi was isolated from these samples. In the first stage of the isolates'identification, fungal DNA was extracted and amplified in Real-Time thermocycler by using the genus spesific primers (BOT100F-BOT472R). In this way, the members of Botryosphaeriaceae species were distinguished from other similar species. In molecular identification of the isolates, ITS 1 and ITS 2 ribosomal DNA fragments were sequenced and the gene sequences were compared with those deposited in NCBI Gene Bank database.It has been determined that 4 different species *Botryosphaeria dothidea* (Anamorph; *Fusicoccum aesculi*), *Diplodia seriata*(An; *B. obtusa*) *Lasiodiplodia theobromae* (An; *B. rhodina*) and*Neofusicoccum parvum* (An; *B. parva*) were found in the sequenced 15 isolates. Colony morphology on PDA media andpicniospore shapes on woody tissues were examined in morphological / microscopic identification. To fulfill Koch's postulates, pathogenicity tests for all isolates were conducted on 1-year-old potted vines. Four months after inoculation, the lesion lengths on woody tissues were evaluated. Pathogenicity tests revealed that *Neofusicoccum parvum* species produced larger lesions on woody tissues when compared to control and other species.

Key words: Botryosphaeriaceae, Grapevine, Trunk Diseases

INTRODUCTION

Fungal trunk diseases have become a growing threat to vineyards in Turkey and throughout the world. Trunk diseases of grapevine are caused by fungal pathogens that invade through pruning wounds located on the woody parts of the plant. Botryosphaeriaceae canker (Bot canker) is one of the most common fungal trunk diseases in grape growing areas of the world. These diseases reduce yields and increase production costs. Eutypa dieback and Bot canker were estimated to cause over 260 million dollars yield loss in California US, and up to 20% yield loss in Bordeaux Region, France (Siebert, 2001; Wilcox, 2011).To date, 21 species

in the Botryosphaeriaceae family have been reported in 17 grape producing countries (Urbez-Torres, 2011) and four species of them in Turkey (Akgül *et al*. 2014).

The disease was characterized by the presence of wedge or pie-shaped perennial cankers in spurs, cordons or trunks (Figure 1a). Cankers start to develop primarily from pruning wounds, cracks as well as big cuts. In the initial stages of cankers, browning streaks appear internal woody tissues,by the time they prolong to other tissues. Due to high enzymatic activity and toxin production, affected tissues die and local or entire vine drying can occur eventually(Figure 1b). These kind of wood symptoms can also be seen in *Eutypalata, Diaporthe* and *Phomopsis* infectionson vines (Urbez-Torres, et al., 2013).

a **b**

Figure 1. Wedge-shaped discolorations on wood (a), local drying in a mature vine (b).

The Botryosphaeriaceae fungi produce a variety of toxic metabolites having an important role on their virulence and pathogenesis. The production of toxins and some exopolysaccharides (EPS) can induce phytotoxic effects on Botryosphaeriaceae-infected grapevines. Withering of the leaves, interveinalnecrotizations and discoloured spots were shown when grapevine leaves were immersed in toxin suspensions (Evidente*et al*. 2010). Environmental conditions are other factors affecting the virulence of Botryosphaeriaceae fungi. Some species are more virulent than the others in higher temperature, so these species predominate in that specific region (Rolshausen*et al*. 2008). The virulence varies between species and isolates of the same species in different countries (Urbez-Torres and Gubler, 2009).On the other hand, these differences may affect the control strategies against Bot species in that region. To date, no study has been initiated about the etiology, epidemiology and control of Bot-canker pathogens on grapevine in Turkey. Doing accurate identification and fulfilling pathogenicity tests are essential steps for any research in plant pathology.

In this study, identification of Botryosphaeriaceae species by classic and molecular biology methods and pathogenicity tests on grapevines were introduced.

MATERIALS AND METHODS

Pathogen Isolation and Colony Discrimination

Symptomatic grapevine wood samples, having typical wedge-shaped discoloration, were surface disinfested with 95% ethanol and flame sterilized and the discolored outer bark

was cut away. The internal tissues (0.5 cm^2) were excised, then they were plated onto Potato Dextrose Agar (PDA: Merck) amended with streptomycin-sulphate (150 g/L) (Sigma Aldrich). After 4 days of incubation (24°C temp, in the dark),fast growing colonies were subcultured and the pure cultures were incubated for 20-25 days in 24°C temp.- under 16 h light/8 h darkfor visual and microscopic examination.

Molecular Identification

DNA Extraction and PCR Tests

Fungal DNA was extracted by following the extraction protocol of Cenis (1992). Mycelial mats (approximately 50 mg) were directly taken from the fresh cultures of the isolates with a sterile surgical blade and they were crushed in micro-centrifuge tubes (1.5 ml) with a sterile plastic pestle by adding 550 µl DNA extraction buffer (200 mM Tris-HCl (pH:8.5), 250 mMNaCl, 25 mM EDTA and 2% Sodium Dodecyl Sulphate). After homogenization, 150 µl 3M Sodium Acetate (NaOAc) was added into tubes and they were placed at -20°C for 15 min. The homogenates were centrifuged for 10 min at 14.000 rpm and the supernatants (200 µl) were transferred to the new tubes. Equal volume of isopropanol (2-propanol) was added and mixed gently about five times, and the tubes were placed at 0°C for 10 min. After that, DNA pellet was precipitated by centrifugation at 14.000 rpm for 10 min and supernatant was discarded. DNA was washed with 1 ml of 70% ethanol for 1-2 sec and the pellet was dried for 10 min. under hood. Finally, DNA was suspended with 75 µl of TE (1M Tris-HCl, pH:8 and 0.5M EDTA) buffer and stored at -20°C.The concentration and purity of extracted DNA were measured with nano-drop device (Thermo Scientific MultiScan Go) by dropping 2 µlsample.

The genus specific primers (BOT100F – BOT 472R) were used to discriminate the Botryosphaeriaceae isolates from the other fungiin PCR tests (Table 1). 10 µl Roche FastStart Essential DNA Green Master Mix (2X conc.), 7.4 µl RNAse free water, 0.3 µl forward and reverse primers (20mM conc.) , 2 µl template DNA were included in thermocycler tubes. DNA samples were amplified with a real-time thermocycler (Roche Light Cycler® Nano) according to the amplification protocol (initial denaturation 95°C for 10 min and followed by 35 cycles at 95°C 10s., 58°C 10 s., 72°C 15 s. and 72°C 10 min final extension).PCR products were runin 1.5% agarose gel electrophoresis and stained with ethidium-bromide (EtBr) to visualize amplicons on gel documentation unit (DNR Bio Imaging Systems). To check the specificity of genus specific primer pair, DNA samples of *Alternaria alternata, Fomitiporia mediterranea*andone isolate of *Botryosphaeria dothidea* (from olive) fungi were included in PCR experiments as control and nuclease free water (instead of template) was used for the negative control.

In addition to classic PCR tests, Botryosphaeriaceae isolates were identified with both direction sequence analysis. Ribosomal DNA fragments (ITS1, 5.8S ITS2 rDNA loci) were amplified with ITS4 / ITS5 universal primers (Table 1) by using real-time thermocycler amplification protocol (initial denaturation 95°C for 10 min and followed by 35 cycles at 95°C 10s., 51°C 10 s., 72°C 15 s. and 72°C 10 min final extension).

After that, PCR products were sequencedby Macrogen Co, South Korea, and the sequences were compared with those deposited in NCBI Gene Bank database. The ITS sequences were also submitted to the NCBI GenBank and accession numbers were obtained (Table 2).

Table 1. Primer sets used to amplify two loci analyzed in this study.

Primer Pairs	PrimerSequences	Amplicon Size (bp)	Annealing Temp. (°C)	Reference
ITS 4	5 ‘ TCC TCC GCT TAT TGA TAT GC 3’	580	51	(White et al., 1990)
ITS 5	5’ GGA AGT AAA AGT CGT AAC AAG G 3’			
BOT 100 F	5’ AAA CTC CAG TCA GTR AAC 3’	372	58	(Ridgway et al., 2011)
BOT 472 R	5’ TCC GAG GTC AMC CTT GAG 3’			

Morphological and Microscopic Identification

The Botryosphaeriaceae-like fungi were grown on PDA medium in the incubator conditions (24°C constant temperature, under fluorescent lamps, 16h light/8h dark) for 20-25 days and colonies were allowed to make their typical morphology. After incubation, colony color at the bottom of petri plates, hyphal morphology and aerial mycelium constitution and picniospore morphology were examined under light microscope, if they were produced on PDA. In addition, all the isolates were inoculated onto uninfected canes to induce picnidia and picniospore formation. Twenty cm mature canes were washed under tap water and the surfaces were cleaned with 70% ethanol. These canes were woundedby removing bark with 5-mm cork borer and fresh mycelial plugs were inoculated into theholes and covered with parafilm. After inoculation, the bottom ends of canes were placed in tap water (in plastic beakers) and they were incubated in climate-room (24°C temp, 80% relative humidity). Three weeks later, picnidia and picniospores were examined under light microscope and conidia dimensions were measured.

Pathogenicity Tests

Pathogenicity tests were conducted under greenhouse conditions on 1-year-old own rooted grapevine (*Vitis vinifera* cv. Sultana Seedless) plants. These plants were inoculated (as described previously) with mycelial plugs of all isolates and they were grown in greenhouse for 12-16 weeks. After this time, outer barks of stems were carefully scrapedand the plants were examined for the extent of wood discoloration and recovery of fungal isolates. Lesion lengths were recorded and data were analyzed by performing Duncan’s multiple range test (<0.05). To complete Koch’s postulates, the isolates were re-isolated from the inoculation sites. Five plants were used for each isolate and 75 plants were in total.

RESULTS

The Botryosphaeriaceae and the others (wood colonizing saprophytic fungi: *Alternaria* spp., *Arthrinium* sp. *Chaetomium* sp. *Fusarium equiseti* and *Nectria* sp.) were isolated from the symptomatic wood samples with 69.4% and 30.1% isolation rates respectively.

PCR Tests and Sequence Analysis Results

Sequence and NCBI-BLASTn analysis of the PCR products (amplified by universal primer pair, ITS4/ITS5) revealed that four Botryosphaeriaceae species (*Botryosphaeria dothidea* (Anamorph; *Fusicoccum aesculi*), *Diplodia seriata*(An; *B.obtusa*) *Lasiodiplodia theobromae* (An; *B. rhodina*) and *Neofusicoccum parvum* (An; *B. parva*)) were obtained from the symptomatic wood samples. The DNA sequences of these species showed 98-100% similarity with those previously deposited sequences (submitted from other countries) in NCBI Gene Bank database. The species names, location, their accession number records, nucleotid sizes, maximum identity percentages and BLASTn comparisons (with the other isolates) were presented in Table 2.

Table 2. List of Botryosphaeriaceae fungi isolated from the grapevines with their corresponding GenBank accession numbers and data of BLASTn results obtained from GenBank.

Species	Isolate	Location	GenBank Accession No	Query Lenght (bp)	BLASTn Accession No	Maximum Identity (%)
Botryosphaeria dothidea	MBAi25AG	Manisa	KF182329	451	FJ790847	99
	MBAi48CL	Saruhanlı	KJ596525	558	JQ411394	99
	MBAi135AG	Manisa	KJ596531	450	KF876691	99
Diplodia seriata	MBAi23AG	Manisa	KF182328	583	KC020170	98
	MBAi30AG	Manisa	KF988138	581	KF182332	99
	MBAi32AG	Manisa	KF182332	581	KF988138	99
	MBAİ183AG	Salihli	KJ596528	597	KC020170	99
	MBAi185AG	İzmir	KJ596530	594	KC020170	99
Lasiodiplodia theobromae	MBAi28AG	Manisa	KF182331	641	JQ245975	99
	MBAi39CL	Ankara	KJ596523	554	JQ245975	99
	MBAi184AG	Alaşehir	KJ596529	548	FJ904841	99
Neofusicoccum parvum	MBAi27AG	Manisa	KF182330	581	JN135282	99
	MBAi34AG	Çorum	KF798191	583	KC706919	99
	MBAi44CL	Salihli	KJ596524	579	JN135282	99
	MBAi105AG	Turgutlu	KJ596526	585	KF182330	100
	MBAİ131AG	Turgutlu	KJ596527	581	JN135282	99

On the other hand, the genus-specific primer pair used in this study (BOT 100F / BOT 472R) amplified DNA of the 15 grapevine and 1 olive isolates.The amplified products' size (for Bot isolates) was 372 bp in agarose gel electrophoresis but DNA samples of *Fomitiporia mediterranea* and *Alternaria alternata* could not be amplified (Figure 2). Discrimination of Botryosphaeriaceae fungi from *Alternaria* or the other wood colonizers was facilitated by using these primers, so these fungi were understood to be the members of Botryosphaeriaceae.

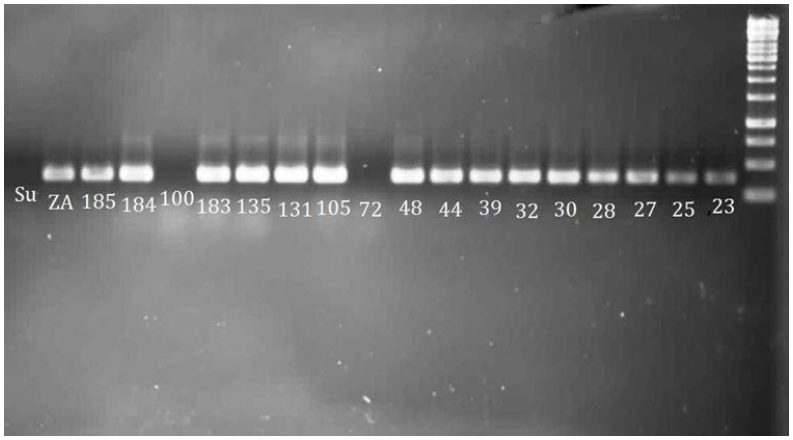


Figure 2. Electrophoretic separation of PCR amplicons of Botryosphaeriaceae isolates obtained from BOT100F and BOT472R primer pairs. DNA ladder; Fermentas Gene Ruler (1kb and each band: 250bp), 25, 48, 135: *B. dothidea*, 23, 30, 32, 183,185: *D. seriata*, 28, 39, 184: *L. theobromae*, 37, 34, 44, 105: *N. parvum*, 72: *F. mediterranea*, 100: *A. alternata*, ZA: *B. dothidea* (from olive), amplicon's size: 372 bp.

Depending on DNA concentrations of the isolates, real-time PCR thermocycler was started to detect DNA presence as early as 11.6^{th} cycle in PCR thermocyclertubes. High-quality and concentrated DNA was extracted from the extraction method that used in this study (Table 3).

Table 3.DNA extraction and real-time PCR test results (standard quantification) of the fungal isolates

Species	Isolate	DNA Conc. (ng/µl)	A260 / A280	CtValues
Alternaria alternata	MBAi100AG	176.5	1.84	-
Botryosphaeria dothidea	MBAi25AG	187.6	1.76	16.1
	MBAi48CL	198.7	1.83	14.6
	MBAi135AG	192.0	1.91	15.9
Diplodia seriata	MBAi23AG	220.3	1.87	11.9
	MBAi30AG	179.1	1.74	13.5
	MBAi32AG	196.7	1.80	12.4
	MBAİ183AG	191.4	1.82	15.7
	MBAi185AG	155.3	1.88	18.6
Fomitiporia mediterranea	MBAi72AG	173.9	1.95	-
Lasiodiplodia theobromae	MBAi28AG	225.1	1.92	11.6
	MBAi39CL	200.9	1.74	14.7
	MBAi184AG	166.5	1.87	20.1
Neofusicoccum parvum	MBAi27AG	145.3	1.90	21.0
	MBAi34AG	152.3	1.70	19.4
	MBAi44CL	204.5	1.84	12.6
	MBAi105AG	211.3	1.80	12.2

Morphological and Microscopic Identification

When infected woody tissues were placed on PDA, mycelial growth of these species were seen on PDA within 3-4 days of incubation (at 24°C, in dark), (Figure 3). On the other hand, abundant picnidia formation was observed on the inoculated canes about 3 weeks later (Figure 4). Subcultured Botryosphaeriaceae colonies were separated into four groups

according to their appearance (Figure 5). The features and some morphological characteristics of the four species were detailed below.

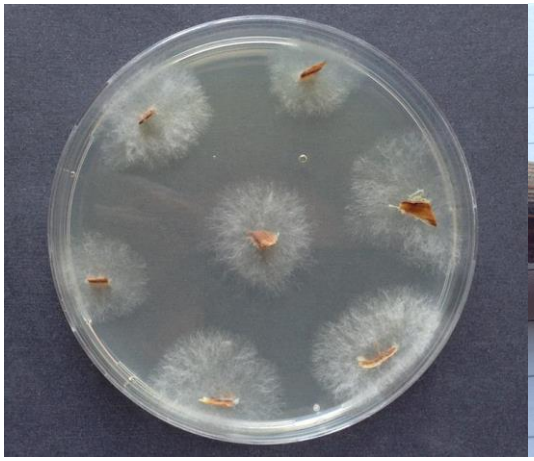


Figure 3. Mycelial growth of a Botryosphaeriaceae Fungus (*Neofusicoccum parvum*) around infected wood samples after 3-4 days incubation (at 24°C, in dark).

Figure 4. Picnidia formation on grapevine canes after 20-22 days of incubation.

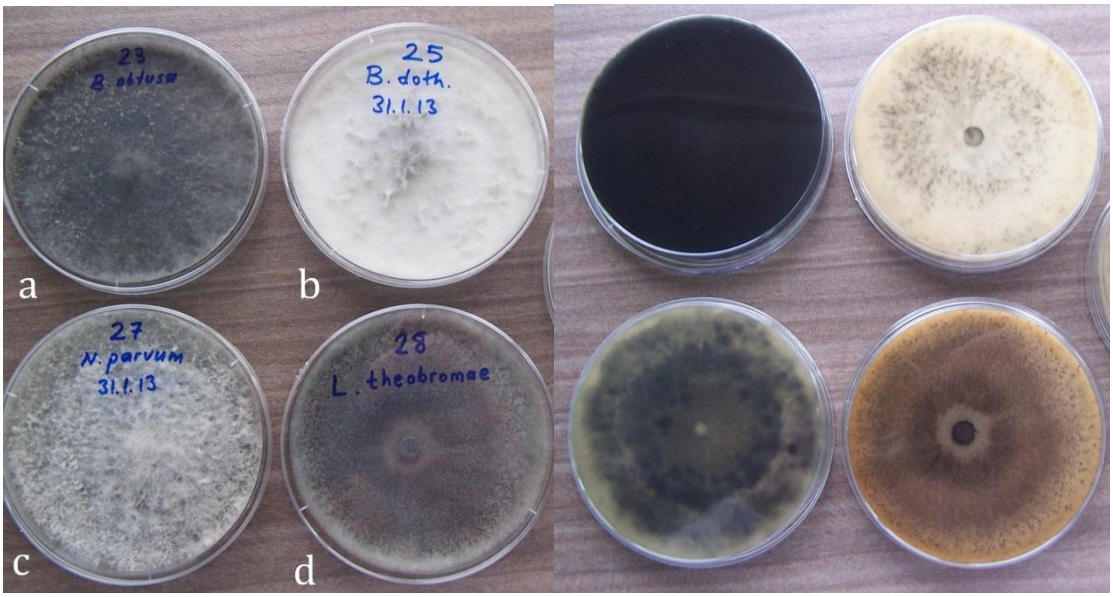


Figure 5. Colony morphology of *Diplodia seriata* (a), *Botryosphaeria dothidea* (b), *Neofusicoccum parvum* (c) and *Lasiodiplodia theobromae* (d) on PDA, after 20-25 days incubation.

Botryosphaeria dothidea (Moug.Ex Fr.) Ces.& De Not.

Colonies developing on PDA were initially colourless, turning dark olive from the centre with the dark colouration and it spread the entire colony with time. Upper view of the original (not subculted for many times) colony was generally white and it had a typical pattern (Figure 5b). The conidia were hyaline, smooth and aseptate so they were looked like a bowling lobut (Figure 6a-b).The conidia sizes were around 22.1 – 24.7 x 4.9 – 5.2 µm.

Diplodia seriata De Not.

On PDA culture, colonies appear greyish-black with dense aerial mycelium on top but it becomes black at the bottom of petri plates (Figure 5a). Growth rate is very fast, so fungus covers petri plates within 4-5 days on PDA in optimal conditions. Conidia are initially

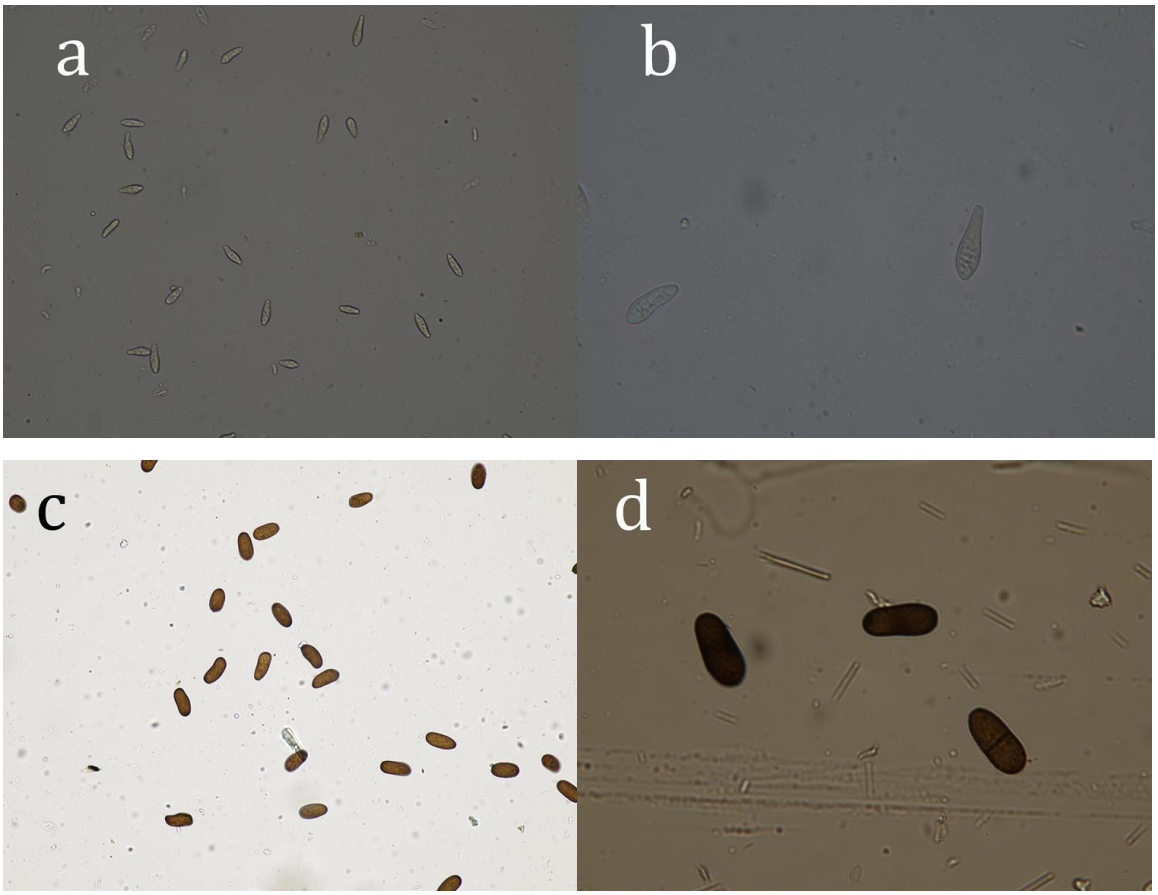
hyaline, becoming dark brown and moderately thick-walled and they are generally aseptate but rarely one-septate (Figure 6 c-d).The conidia sizes were around 22.6 – 26.5 x 11.2 – 14.3 µm.

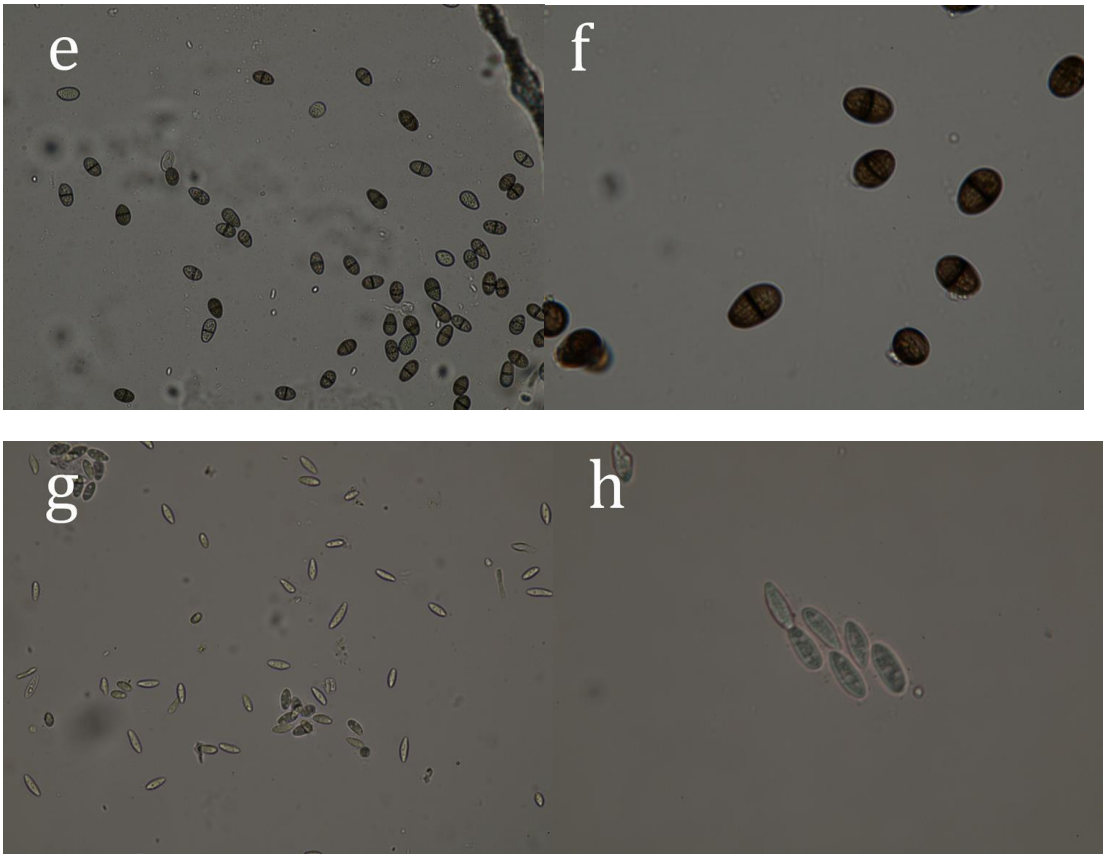
Lasiodiplodia theobromae (Pat.) Griff. & Maubl.

The colonies were grey-brown to black with dense fluffy aerial mycelia on the media (PDA), while the underside becomes black with time. The fungus produced dark brown picnidia and picniospore on PDA after 3-4 weeks of incubation (Figure 5 c). Conidia were sub-ovoid to ellipsoid, thick walled and their color was initially hyaline but turned dark brown with age (Figure 6 e-f). The most characteristic feature of conidia was that they had longitudinal strations and one-septate (when they were mature). The conidia sizes were around 23.7 – 27.1 x 12.7 – 14.1 µm.

Neofusicoccum parvum (Pennycook&Samuels) Crous, Slippers& A.J.L. Phillips

Original colony morphology of this species was white with fluffy aerial mycelium, turning pale olivaceous gray from the middle of colony after 3–4 days (Figure 5c). The aerial mycelium resembled a cloudy seeming and touched to the petri dish's lid within 8-10 days. The bottom color of colony was olivaceous grey in the first week but it turned blackish color in time. Conidia shape was generally ellipsoidal with round apices (Figure 6 g-h). Their sizes were around 14.5 – 19.2 x 6.5 – 8.0 µm.





40 X objective magnification **100 X objective magnification**

Figure 6. Conidia shapes of *Botryosphaeria dothidea* (a-b), *Diplodia seriata* (c-d), *Lasiodiplodia theobromae* (e-f) and *Neofusicoccum parvum* (g-h)

Pathogenicity Test Results

All of the Botrysphaeriaceae isolates used in pathogenicity tests were able to colonize woody tissues of the test plants. After 15-16 weeks, blackish-brown discolorations have been observed on woody tissues of young vines and the isolates could be re-isolated (by 95.6%) from necrotic tissues successfully.Lesions extended both upward and downward from the point of inoculation.Depending on virulence, each of the isolates produced moderate or severe lesions on infected plants. Mean lesion lengths were 17.2 mm for *B. dothidea*, 13.9 mm for *D. seriata*, 13.1 mm for *L. theobromae* and 83.5 mm for *N. parvum*, but it was only 6.3 mm for the control (Table 4). *N. parvum* was the most virulent one among these species and lesion length mean of this species was longer than that of other species statistically.Although slight wood discoloration was seen just around inoculation point of the control plants, no Botryosphaeriaceae isolate could be isolated from these tissues.

Table 4. Mean lesion lengths produced byBotryosphaeriaceae species on Sultana Seedless grapevine plants.

Species Name	Lesion Lengths (mm)
Botryosphaeria dothidea	17.2 b*
Diplodia seriata	13.9 b
Lasiodiplodia theobromae	13.1 b
Neofusicoccum parvum	83.5 c
Sterile agarinoculation (Control)	6.3 a

* Mean values within a column are significantly different based on Duncan (0.05) multiple range test

DISCUSSION

In this study, four Botryosphaeriaceae species were identified with classic and molecular biology methods. In classic identification, colony growth patterns, colony color, hyphal morphology and picniospore shapes were documented and these characteristics were also confirmed with the studies of Crous et al. (2006);Urbez-Torres et al. (2006); Amponsah, 2010; Pitt et al. (2010). Classic identification of Botryosphaeriaceae fungi relied on morphological features in the past. However many evaluations by several researchers of these fungi have revealed that these characteristics were very variable and often overlapped between species. Morphological characteristics can also be affected in the isolates that collected from different locations (Amponsah, 2010). Conclusively, it has been advised by many authors that DNA based molecular methods should be used for accurate identification of these fungi (Burruano et al., 2008).

The genus specific primer pairs (BOT100F/BOT472R) discriminated Botryosphaeriaceae fungi from the others successfully. Ridgway et al. (2011) found that these fungi could also be detected even in soil by using these primers.Moreover these primers may also be suitable for fast detection of these pathogens in grapevine propagation systems and in certification of young grafted plants. In addition, it may be useful for the first molecular discrimination before sequence analysis.Although the isolates were isolated from different locations, maximum identity (up to 100%) has been observed between two *Neofusicoccum parvum* isolates, MBAi27AG and MBAi105AGin sequence analysis. It can be concluded from this similarity that one of the isolates may have been transmitted from one location to another by vegetative propagation materials.

Among the identified isolates, two different virulence groups occurred in pathogenicity tests. *N. parvum* was in virulent and *B. dothidea, D. seriata* and *L. theobromae* were in moderately virulent group, so *N. parvum* produced longer lesions, when compared to *B. dothidea, D. seriata* and *L. theobromae* on woody tissues. Former studies indicated that there were three different groups in pathogenicities of Botryosphaeriaceae species. *Neofusicoccum* spp. and *Lasiodiplodia* spp. were described as highly virulent, *B. dothidea* and *Diplodia* spp. as moderately virulent and *Dothiorella* spp. and *Spencermartinsia viticola* as slightly virulent (vanNiekerket al., (2004); Luque et al., (2009); Urbez-Torres and Gubler, (2009)). Our findings corroborate these studies in some extent, although we described our *L. theobromae* isolates as moderately virulent in our pathogenicity tests. Difference in virulence was attributed to toxin production of these species during pathogen growth in plant tissues. Martos et. al., (2008) found that Botryosphaeriaceae fungi were capable of producing hydrophilic high-molecular weight compounds with phytotoxic properties, which were thought to be exopolysaccharides. Their study showed that maximum toxin production was assessed from the *Neofusicoccum*spp. inoculated grapevinesin pathogenicity tests. These findings may explain in some extent our pathogenicity results.

Botryosphaeriaceae canker is an important disease in Aegean Region Vineyards. According to the unpublished survey results, the incidence of disease has been estimated to reach 21.0% in some Sultana Seedless vineyards in Manisa and İzmir Cities (Akgül et al.). Identification is the first stage of disease management in all culture plants, and accurate identification is very important to launch correct disease control strategy.

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ÖZET

ASMADA KOL VE SÜRGÜN KURUMALARINA NEDEN OLAN BOTRYOSPHAERIACEAE TÜRLERİNİN TEŞHİSİ VE PATOJENİSİTELERİ

Botryosphaeriaceae funguslarının neden olduğu kol, sürgün kurumaları (veya kangren) ve geriye ölüm hastalıkları, asmanın en önemli fungal gövde hastalıkları arasında yer almaktadır. Son yıllarda Türkiye'deki asmalarda lokal kol kurumaları, sürgünlerde geriye ölüm ve odunsu dokularda "V" şeklindeki renk değişimi belirtileri önemli oranda artmıştır. 2012 yılı üretim sezonunda, Ankara, Çorum, Izmir ve Manisa'dan bu belirtileri taşıyan 15 odunsu doku örneği alınmış ve hastalığa neden olan fungal etmenleri saptamak için standart mikolojik izolasyonlar yapılmıştır. Bu örneklerden yüksek oranda (%69.4) Botryosphaeriaceae üyesi funguslar izole edilmiştir. İzolatların teşhisindeki ilk aşamada fungal DNA ekstrakte edilmiş ve Real-Time thermocycler'da cinse özgü primer çifti ile (BOT100F-BOT472R) çoğaltılmışlardır. Bu sayede Botryosphaeriaceae üyesi türler diğer benzer türlerden ayırt edilmişlerdir. İzolatların tür düzeyindeki moleküler tanısında ITS 1 ve ITS 2 ribosomal DNA bölgeleri diziletilmiş ve gen dizileri NCBI Gen Bankası'ndaki mevcut dizilerle karşılaştırılmıştır. Dizi analizi yapılan 15 izolat içerisinde 4 farklı türün; *Botryosphaeria dothidea* (Anamorf; *Fusicoccum aesculi*), *Diplodia seriata* (An; *B. obtusa*) *Lasiodiplodia theobromae* (An; *B. rhodina*) ve *Neofusicoccum parvum* (An; *B. parva*) bulunduğu tespit edilmiştir. Morfolojik ve mikroskobik tanıda türlerin PDA ortamı üzerindeki koloni morfolojileri ve odunsu dokulardaki pikniospor şekilleri incelenmiştir. Koch pastülatları için tüm izolatların patojenisitesi 1 yaşındaki tüplü asma fidanları üzerinde yapılmıştır. İnokulasyondan 4 ay sonra, odun dokulardaki lezyonların uzunluğu kontrole göre değerlendirilmiştir. Patojenisite testleri, *Neofusicoccum parvum* türünün kontrol ve diğer türlerle kıyaslandığında dokular üzerinde daha büyük lezyonlar meydana getirdiğini ortaya koymuştur.

Anahtar kelimeler: Botryosphaeriaceae, Asma, Gövde Hastalıkları

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