Host specificity of co-infecting Botryosphaeriaceae on ornamental and forest trees in the Western Balkans

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Summary

The Botryosphaeriaceae is a diverse family of endophytes and fungal pathogens of mainly woody plants. We considered the host range and distribution of these fungi by sampling diseased ornamental and forest trees and shrubs in Serbia, Montenegro, Bosnia and Herzegovina, spanning a Mediterranean and a Continental climatic region. In total, ten Botryosphaeriaceae species were identified in the Western Balkans and with the exception of *Sphaeropsis visci* and *Phaeobotryon cupressi,* which occurred on one host, all the species had a broader host range. *Phaeobotryon cupressi* was found only in the Mediterranean region and *S. visci*, *Dothiorella* sp., *Dothiorella sarmentorum* and *Diplodia seriata* were present only in the Continental region. Pathogenicity tests were conducted on a variety of hosts from which the Botryosphaeriaceae species were isolated. These included leaves and/or stems of seedlings of 21 hosts, and cut leaves and/or branches of six hosts. Moreover stems of seedlings of *Chamaecyparis lawsoniana*, *Cedrus deodara*, *Picea omorika*, *Pinus patula* and *Eucalyptus grandis* were inoculated as hosts from which some or all of the Botryosphaeriaceae species used for inoculation were not isolated. Inoculations showed that the majority of these fungi could also co-infect hosts other than those from which they were isolated. The results suggest that most of the species have broad host ranges and can potentially cause disease on a broad range of tree species under certain conditions.

1 Introduction

Species of the Botryosphaeriaceae (Ascomycota: Botryosphaeriales) have a broad host range and occur in temperate, mediterranean and tropical climates worldwide (Phillips et al., 2013; Mehl et al., 2013). They can exist as endophytes in asymptomatic plant tissues, but some are also pathogens of agricultural and forestry crops. The shift from an endophytic to a pathogenic lifestyle often occurs after hosts have been subjected to stress caused by environmental factors such as drought, flooding or high temperatures (Slippers and Wingfield, 2007; Mehl et al., 2013). The recent increased pathogenic activity and geographic range expansion of the Botryosphaeriaceae in Europe has been linked to warmer climate and extreme weather (Fabre et al., 2011; Piškur et al., 2011; Adamson et al., 2015; Zlatković et al., 2016a).

A number of Botryosphaeriaceae infect multiple hosts, including some that are distantly related. Other Botryosphaeriaceae species appear to have a narrow host range. Examples include *Botryosphaeria scharifii*, *Diplodia quercivora*, *Dothiorella brevicollis*, *Phaeobotryon mamane* and *Sphaeropsis visci,* which have been found associated with *Mangifera indica*, *Quercus canariensis*, *Acacia karroo*, *Sophora chrysophylla* and *Viscum album*, respectively (Phillips et al., 2013; Jami et al., 2015). However, patterns of host preference in the Botryosphaeriaceae are considered questionable because sampling in most studies has focused on a particular tree species or geographical area (Jami et al., 2014). Moreover, multiple Botryosphaeriaceae often occur on the same host, and within individual host trees, tissues or sites (Slippers and Wingfield, 2007; Taylor et al., 2009; Sakalidis et al., 2013; Jami et al., 2014, 2015; Linaldeddu et al., 2014; Slippers et al., 2017).

Botryosphaeriaceae have mostly been reported from angiosperms, which appear to be the major group of plants on which these fungi have diversified (De Wet et al., 2008; Phillips et al., 2013; Slippers et al., 2013). However, some Botryosphaeriaceae predominantly infect conifers; i.e. *Diplodia sapinea* (syn. *Diplodia pinea*, *Sphaeropsis sapinea*), which shows a host preference mainly on *Pinus*, but is also found on *Abies*, *Picea* and *Pseudotsuga* (Pinales: Pinaceae); *Diplodia scrobiculata* associated with *Pinus banksiana*, *P. resinosa*, and *P. greggii* (Pinales: Pinaceae); *Diplodia cupressi* isolated from *Cupressus* and *Juniperus* spp. (Pinales: Cupressaceae); *Diplodia tsugae* isolated only from *Tsuga heterophylla* (Pinales: Pinaceae); and *Phaeobotryon cupressi* isolated from *Cupressus sempervirens* and *Juniperus scopulorum* (Pinales: Cupressaceae) (De Wet et al., 2008; Phillips et al., 2013; Slippers et al., 2013; Zlatković et al., 2017).

There are apparently few barriers to the Botryosphaeriaceae moving from native to non-native species and *vice versa* where hosts occur in the same geographic regions. For example, *Diplodia seriata* has been isolated from native *Vitis vinifera* and introduced *Chamaecyparis lawsoniana* and *Thuja plicata* in Portugal (Alves et al., 2013); *D. sapinea* has been found on various native *Pinus* spp. and introduced *P. radiata* in Italy (Luchi et al., 2014); *Neofusicoccum mediterraneum* has been found associated with native *Sequoiadendron giganteum* and introduced *Pistacia vera* in the USA (Inderbitzin et al., 2010); *Neofusicoccum parvum* has been found colonizing native

Syzygium cordatum and introduced *Eucalyptus grandis* in South Africa (Pavlic et al., 2007; Pillay et al., 2013). Moreover, when they are isolated from native hosts of a region, Botryosphaeriaceae have also been able to infect and cause disease symptoms on introduced hosts existing in the same region in greenhouse trials (Pavlic et al., 2007).

Uncertainty regarding the taxonomy and overlapping morphological characters of the Botryosphaeriaceae have made these fungi difficult to identify (Phillips et al., 2013; Slippers et al., 2013). For this reason, the ecological role of Botryosphaeriaceae species has been poorly addressed in many ecosystems, including urban environments in which ecological interactions and evolution of species are still poorly understood (Shochat et al., 2006; Slippers and Wingfield, 2007; Begoude et al., 2010; Slippers et al., 2013; Zlatković et al., 2016a). In contrast to a large number of studies investigating the Botryosphaeriaceae diversity and impact in the Mediterranean region (MR) and in the tropics (e.g. Burgess et al., 2005; Rodas et al., 2009; Alves et al., 2014; Jami et al., 2014), less attention has been given to the Botryosphaeriaceae occuring in the Continental climate-type region (CR). Apart from some recent studies from the Balkans (e.g. Piškur et al., 2011; Zlatković et al., 2016a, b, 2017), most research on the Botryosphaeriaceae conducted in the CR has been focused on the pine pathogen, *D. sapinea* (e.g. Jankovský and Palovćiková, 2003; Karadžić and Milijašević, 2008; Fabre et al., 2011; Adamson et al., 2015).

In the Western Balkans, *N. parvum*, *Botryosphaeria dothidea* and *D. seriata* have been found to cause disease on *V. vinifera*, *Malus domestica* and *Olea europaea* (Latinović et al., 2013; Vasić et al., 2013; Kaliterna et al., 2012, 2013). Furthermore, a large number of Botryosphaeriaceae species has recently been found on various diseased trees in this region, including *B. dothidea*, *N. parvum*, *D. sapinea*, *Diplodia mutila*, *D. seriata*, *Dothiorella sarmentorum*, *Dothiorella* sp., *Dothiorella omnivora*, *P. cupressi* and *S. visci* (Zlatković et al., 2016a, b, 2017). These species have been found associated with both angiosperms and gymnosperms belonging to various families, i.e. Rosaceae, Fagaceae, Cupressaceae, Pinaceae, Oleaceae, Sapindaceae, Santalaceae, Pittosporaceae and Myrtaceae.

The aim of this study was to investigate the host range of the Botryosphaeriaceae in the Western Balkans. This was achieved by identifying species of the Botryosphaeriaceae from a broad collection of Botryosphaeriaceae-like isolates using the DNA sequence data for four gene regions. A selection of these isolates and isolates of the Botryosphaeriaceae identified in the previous study (Zlatković et al. 2016a) was then used to conduct reciprocal inoculations on the hosts from which the Botryosphaeriaceae had been isolated, as well as on *C. lawsoniana*, *Cedrus deodara*, *Picea omorika*, *E. grandis* and *Pinus patula* from which some or all of the Botryosphaeriaceae species were not isolated. The overlapping patterns of all Botryosphaeriaceae species identified in the Western Balkans (Zlatković et al. 2016a, b, 2017) was assessed at an individual plant, host species and geographic level.

2 Materials and methods

2.1 Sample collection and Botryosphaeriaceae isolation

Samples were collected from 84 trees (representing 28 species), ten shrubs (representing four species) and four seedlings (representing three species) between 2009 and 2014 in 12 cities, three villages, three forest stands and two ornamental nurseries in Serbia, Montenegro and Bosnia and Herzegovina (Tables S1, S2). Samples were collected from trees exhibiting various disease symptoms including cankers, die-back, and tissues displaying abundant resin flow (Table S3). The Botryosphaeriaceae were isolated from healthy and diseased tissues, as well as from fungal fruiting bodies on 2% malt extract agar (MEA) plates acidified with lactic acid (AMEA) as described in Zlatković et al. (2016a). Fungal colonies were purified using hyphal tip transfers and Botryosphaeriaceae-like isolates (mycelium mostly fast-growing, white to greyish olive in color, often with a rosette appearance) were transferred to new Petri dishes. Representative isolates from each host have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

Sampling was conducted in regions of Serbia, Montenegro and Bosnia and Herzegovina having both continental and mediterranean climates. According to the Köppen-Geiger system (Köppen 1936) continental climate with hot summers (Dwa) occurs in northern, eastern and central parts of Serbia and continental climate with dry summers (Dwb) occurs in western and southern parts of Serbia, Bosnia, Herzegovina and northern parts of Montenegro. Mediterranean climate (Csa) is found in the coastal area of Montenegro.

2.2 DNA sequence analyses

DNA was extracted from the mycelium of one-week-old cultures using PrepMan Ultra reagent (Applied Biosystems, Foster City, California) following the manufacturer’s protocol. The ITS region of the rDNA operon was amplified using primer pairs ITS-1 and ITS-4 or ITS1F and ITS-4 (White et al., 1990; Gardes and Bruns, 1993); part of the TEF-1-α gene was amplified using primer pairs EF1-728F and EF1-986R (Carbone and Kohn, 1999) or EF1-F and EF2-R (Jacobs et al., 2004); the BT2 gene was amplified using primers Bt2a and Bt2b (Glass and Donaldson, 1995) and RPB2 gene was amplified using primers RPB2bot6F and RPB2bot7R (Sakalidis, 2004). The conditions and procedures for PCR amplification, PCR sequencing and sequence alignments were as those described in Zlatković et al. (2016a).

The phylogenetic analyses were performed using Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses. The MP analyses were performed in PAUP version 4.0b10 (Swofford, 2003). The ML analyses were run using an online version of PhyML 3.0 (Guindon et al., 2010) and confidence levels were determined with 1,000 bootstrap replications (Felsenstein, 1985). For ML analyses the best nucleotide substitution model was found using jModelTest v.0.1 (Posada, 2008). Phylogenetic trees were created with MEGA v.6. The DNA sequences were deposited in GenBank (Table S2). A diagram that outlines materials and methods used in this study is presented in Figure S1.

2.3 Botryosphaeriaceae species diversity

Alpha diversity was estimated in terms of species richness S (number of species in the region/isolated from the host), the abundance (number of isolates) and evenness J (dominance of species in the region/isolated from the host). Shannon Wiener's index (H) was calculated with $H=\sum Pi \ln Pi$ (Shannon and Weaver, 1949) and normalized to correct for differences in isolate numbers with $H' = H/\ln N$, where Pi is the proportion of individuals in the i^{th} species and N is the number of isolates associated with each host. Values for H' range from 0 (single species present) to 1 (each isolate associated with the particular host represent a different species).

Beta diversity was measured using the Jaccard's similarity index with $JI=a/(a+b+c)$, where a represents the number of species occurring in both regions/gymnosperms-angiosperms, b represents the number of species restricted to region 1/gymnosperms, and c represents the number of species restricted to region 2/angiosperms. The JI values range from 0 (no species shared) to 1 (all species shared) (Kumar and Hyde, 2004). Diversity indexes were calculated in R (R Core Team, 2015), using statistical package Vegan v. 2.2.1 (Oksanen et al., 2015). Botryosphaeriaceae species diversity in the Western Balkans was assesed using the whole collection of Botryosphaeriaceae isolates from the Western Balkans region, including isolates identified in this and in previous studies (Fig. S1; Zlatković et al. 2016a, b, 2017).

2.4 Inoculations of stems and leaves of seedlings in the field

The inoculation tests were conducted during the 2014, 2015 and 2016 growing seasons, from April to October and from April to June, in an open-air nursery of the Faculty of Forestry in Belgrade, Serbia. Some of the seedlings (two or three-year-old potted plants) were obtained from the nursery of the Faculty of Forestry and others were purchased from a commercial ornamental nursery located in Novi Sad, Serbia. Seedlings were arranged in a completely randomized design and when rainfall was insufficient they were irrigated on a daily basis (Table S4).

Inoculations were done on stems of seedlings of 21 hosts from which Botryosphaeriaceae were isolated in this and in the previous study (Zlatković et al. 2016a; Tables S5,S6). When possible, inoculations were done using two isolates of both continental and Mediterranean origin of each species isolated from the given host (Tables S5, S7; Fig. S1). *C. deodara* and *P. omorika* were also inoculated with *N. parvum* isolate CMW 39327, which was not isolated from *C. deodara* and *P. omorika* neither in this nor in the previous study, but was shown to be very aggressive when inoculated on its natural hosts (Tables S5, S8; Fig. S5; Zlatković et al. 2016a). Because *C. lawsoniana* is one of the most frequently propagated ornamental tree in Serbian nurseries and a large number of seedlings of this host was available, to test for host specificity it was inoculated with nine Botryosphaeriaceae. Among them six Botryosphaeriaceae species were isolated from *C. lawsoniana* in this and in the previous study and three other Botryospaheriaceae used for inoculation were not isolated from *C. lawsoniana* (Table S5, Figs. S1, 5; Zlatković et al., 2016a). Inoculation experiments were repeated once.

Ten seedlings per species per isolate were inoculated 3-9 cm above the soil level of the stems by placing the mycelium in a wound made with a 3 or 6 mm diameter cork borer, as described in Zlatković et al. (2017). The same number of control seedlings of each species was inoculated with plugs of sterile water agar (WA). Seedlings were inspected for disease symptoms and mortality each week.

Leaves of *P. laurocerasus* seedlings were lightly wounded with a sterile needle and inoculated using isolates of *N. parvum* and *D. mutila* (Table S5) as described in Zlatković et al. (2016b). Ten leaves per *P. laurocerasus* seedling and ten seedlings per isolate were inoculated, giving a total of 40 seedlings and 400 leaves.

For seedlings of *C. lawsoniana*, *Populus nigra* var. *italica*, *Prunus laurocerasus*, *P. abies, Q. robur*, *C. arizonica* and *Liriodendron tulipifera* a score of 0-2, 1-3 or 1-2 was assigned to each Botryosphaeriaceae isolate used in the inoculation trial (Table1; Fig. S1).In the case of *Q. robur* and *P. nigra* var. *italica* the results were observed after six weeks when more then half of the plants inoculated with one isolate were girdled and dead. In the case of *P. abies* the results were observed after eight weeks when one of the isolates killed all the seedlings inoculated with that isolate. Since *C. lawsoniana*, *C. arizonica* and *L. tulipifera* did not experience mortality during inoculation trials the test lasted for the whole vegetation season (six months) for these tree species. Inoculation tests conducted on *P. lauracerasus* leaves lasted for three weeks and until all lesions formed by *N. parvum* isolate 39327 droped out of the leaves. Disease assessment for seedlings of *S. giganteum*, *T. occidentalis*, *Abies concolor*, *C. sempervirens* and *P. omorika* was based on the calculation of the Area Under Disease Progress Curve (AUDPC) and the test lasted until all of the inoculated plants were killed. The AUDPC was measured using the trapezoidal integration method (Madden et al., 2007):

$$\text{AUDPC} = \sum_{i=1}^{-1}[(y_i + y_{i+1})/2]*(t_{i+1} - t_i)$$

Where 'n' is the total number of observations, 'y_i' the severity of disease at the i^{th} observation and 't_i' time (week) at the i^{th} observation. Seedlings of *Pittosporum tobira, C. deodara, C. atlantica*, *Ligustrum vulgare*, *Juniperus horizontalis*, *C. arizonica*, *P. laurocerasus*, *Magnolia grandifolia*, *Pseudotsuga menziesii*, *L. tulipifera*, *Taxus baccata*, *P. cerasus*, *Q. rubra*, *Platanus acerifolia* and *Forsythia europaea* were sectioned longitudinally and the extent of resin accumulation/vascular discoloration was measured above and below the point of inoculation six weeks (for *P. tobira*, *C. deodara*, *T. baccata*, *P. cerasus*, *Q. rubra*, *P. acerifolia* and *F. europaea*) and six months (for all other species) after the beginning of the experiment. Botryosphaeriaceae were re-isolated from the margin between necrotic and apparently healthy tissue of inoculated plants. Fungal pycnidia were collected from the surface of the dead tissues and examined, as described in Zlatković et al. (2016b, 2017). Botryosphaeriaceae identity was verified based on the morphology of cultures and conidia in order to fulfil Koch's Postulates (Zlatković et al. 2016a).

Table 1. Disease assessment for a variety of tree species inoculated with Botryosphaeriaceae isolates. Inoculations were conducted on leaves of *Prunus laurocerasus* seedlings in the field, on cut branches of *Aesculus hippocastanum* in laboratory conditions and on stems of seedlings of all other tree species in the filed.

Inoculated species	Disease assessment
Chamaecyparis lawsoniana	**0** (**not pathogenic**)= plants looking healthy, only resinous changes in the woody tissue were observed (*B. dothidea* CMW 39301, *N. parvum*, *S. visci*, *Do. sarmentorum*, *D. mutila*, *D. seriata*, *Dothiorella* sp.) **1** (**moderately aggressive**)= >50 % of plants with browning of the leaves from the inoculation point upwards and downwards, elliptical cankers (measuring 1.4-2.1cm) formed on the stems, pycnidia formed in the cankered tissue (*B. dothidea* CMW 39315) **2** (**most aggressive**)= all plants with browning of the leaves, elongated, girdling, sunken cankers with vertical cracks within the canker and along the canker margins formed on the stems (measuring > 3 cm), *P. cupressi* pycnidia formed in cankers (*P. cupressi*)
***Populus nigra* var. italica**	**0** (**not pathogenic**)= plants looking healthy, no cankers were observed on the inoculated stems (*B. dothidea*) **1** (**less aggressive**)= < 50% of plants girdled and dead, necrotic lesions (measuring <3 cm) formed on stems of all remaining plants (*N. parvum* CMW 39317) **2** (**more aggressive**)= > 50% of plants girdled and dead; sunken, girdling cankers with cracks within the canker (measuring >3 cm) and *N. parvum* pycnidia formed on stems of all remaining plants (*N. parvum* CMW 39327)
Prunus laurocerasus	**0** (**not pathogenic**)= lesions didn't appear on inoculated leaves (*D. mutila* CMW 39385) **1** (**least aggressive**)= small (3-5 mm) lesions appeared around inoculation point but dropped out of the leaves (*D. mutila* CMW 39348) **2** (**moderately aggressive**)= reddish brown necrotic lesions (measuring 1-2.5 cm) with concentric rings surrounded by reddish borer or light green halo appeared on leaves, *N. parvum* pycnidia formed in lesions, all lesions dropped out (*N. parvum* CMW 39327) **3** (**most aggressive**)= reddish brown necrotic lesions (measuring 2.5-4 cm) with concentric rings surrounded by reddish border or light green halo appeared on leaves, <10% of lesions dropped out, >50% of leaves were completely necrotic and detached and , *N. parvum* pycnidia formed in dead leaf tissue, 20% of plants experienced disease progress towards shoots which showed die-back and *N. parvum* pycnidia formed in dead shoot tissue (*N. parvum* CMW 39317)
Picea abies	**1** (**least aggressive**)= elongated, girdling, necrotic, resin-soaked lesions (measuring 2.5-6.5 cm) formed on stems, some plants with shoot die-back (*N. parvum* CMW 39317) **2** (**moderate aggressive**)= <50 % of plants girdled and dead, remaining plants with elongated, resinous, girdling necrotic lesions (measuring 2.5-7.5 cm), resin flow observed from the infected needles and stems (*B. dothidea*) **3** (**most aggressive**)= all plants girdled and dead, resin flow observed from the infected needles and stems (*D. sapinea* [1], *N. parvum* CMW 39327)
Quercus robur	**1** (**less aggressive**)= plants with elongated, girdling cankers (measuring 1.9-7.5 cm), pycnidia formed in cankered tissue (*D. seriata* CMW 39382) **2** (**more aggressive**) = >50 % plants girdled and dead, *D. seriata* pycnidia formed in dead plant parts, elongated, girdling cankers (measuring > 2 cm) formed on all remaining plants, *D. seriata* pycnidia produced in the cankered tissue (*D. seriata* CMW 39376)
Cupressus arizonica	**1** (**less aggressive**)= plants with elliptical necrotic lesions (measuring <2 cm) (*B. dothidea* CMW 39301, *D. mutila*) **2** (**more aggressive**)= plants with elongated, girdling, sunken cankers with vertical cracks within the canker and along the canker margins (measuring >4 cm), *B. dothidea* pycnidia formed in cankered tissue (*B. dothidea* CMW 39315)

Table 1. Continued.

Inoculated species	Disease assessment
Liriodendron tulipifera	**1** (**less aggressive**)= plants with lesions measuring 5.4-8.3 cm (*B. dothidea* CMW 39315) **2** (**more aggressive**)= plants with lesions measuring 6-30 cm, >50 % of plants with stem die-back, *B. dothidea* pycnidia formed in dead stem tissue (*B. dothidea* CMW 39301)
Aesculus hippocastanum	**1** (**least aggressive**)= branches with girdling lesions measuring 1.5-4 mm, *Do. sarmentorum* pycnidia formed in lesions (*Do. sarmentorum* CMW 39365) **2** (**moderately aggressive**)= >50 % of branches dried, girdled, with dark staining of the vascular tissue, *D. mutila* pycnidia formed in the dead branch tissue (*D. mutila* CMW 39385) **3** (**most aggressive**)= all branches dried, girdled, with dark staining of the vascular tissue, pycnidia formed in the dead branch tissue (*B. dothidea*, *D. mutila* CMW 39348, *Do. sarmentorum* CMW 39364, *N. parvum*)

[1] Data taken from Zlatković et al. 2017.

2.5 Inoculations of cut branches and leaves of cut branches

When seedlings were not available for the experimental trials, inoculations were done on cut branches. Cut branches, 30-40 cm in length and 1.3-1.5 cm thick, were collected from healthy mature *Aesculus hippocastanum* trees growing in the experimental forest of the Faculty of Forestry in Belgrade, Serbia in May 2015. Cut branches of the same length and width were also collected from healthy *P. tremula*, *Fraxinus excelsior* and *Q. cerris* trees growing in urban forests "Ada" and "Košutnjak", in Belgrade during May 2016. *V. album* shrubs showing no symptoms of disease were collected in October 2014 from *Abies alba* trees in "Košutnjak", Belgrade. Isolates used for inoculations of cut branches were selected according to the same principle as for inoculations of seedlings (Tables S5, S7; Fig. S1).

Ten branches per isolate were inoculated as described in Zlatković et al. (2017) and the same number of control branches was inoculated with sterile WA plugs. For branches of *Aesculus hippocastanum* a score of 1–3 was assigned to each of the Botryosphaeriaceae isolates used in the inoculation trial (Table1; Fig. S1). Ten *V. album* branches were wounded using a 3 mm cork borer and inoculated with a plug of 10-day-old *S. visci* mycelium, covered with moist cotton wool and wrapped with Parafilm (Pechiney, Chicago, USA). Ten leaves of ten *V. album* branches were lightly wounded with a sterile needle and inoculated as described previously. The same number of branches and leaves were inoculated with sterile WA plugs as controls.

After two weeks (*V. album*) and three weeks (all other species), the extent of vascular discoloration on sectioned branches or lesions on leaves was measured and re-isolations were made as described in Zlatković et al. (2017). Experiments were repeated once.

2.6 Inoculations of stems of seedlings in the greenhouse

To test for host specificity, inoculations were also conducted on twenty potted 5-year-old *P. patula* and 3-year-old *E. grandis* seedlings as hosts from which Botryosphaeriaceae strains from this study were not isolated. Seedlings were inoculated with the two most commonly found Botryosphaeriaceae species in this study, namely *B. dothidea* and *N. parvum*. Isolates used for inoculation were selected as described above (Tables S5-S7; Fig. S1). Seedlings were inoculated in the lower parts of the stems as described in Zlatković et al. (2017). Twenty control seedlings were inoculated with sterile WA plugs. Seedlings were watered daily, maintained at 25°C under natural day/night cycles and the lesion lengths were measured after six weeks. *B. dothidea* and *N. parvum* were re-isolated as described in Zlatković et al. (2017).

2.7 Statistical analyses

Statistical analyses were conducted using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA) and IBM SPSS Statistics 20.0 (New York, USA). The normality of the datasets was checked using Kolmogorov-Smirnov test and homogeneity of variances using Leven's test. The results of the two subsequent pathogenicity tests were analysed using Student's t-test at $\alpha = 0.05$ (data not shown). Since no significant differences were found between the two repeats of the trials, a single dataset was used in further analyses. The log10 transformation was used to improve normality of the data set in order to analyse the effect of *B. dothidea* (CMW 39315) on vascular discoloration of *L. tulipifera* seedlings. The analyses were further assessed using either one way analyses of variance (ANOVA) followed by post-hock Fisher's Least Significant Difference (LSD) test, z-test (Pocock, 2006), Student's t-test or Kruskall-Wallis H test at $\alpha = 0.05$.

3 Results

3.1 Botryosphaeriaceae isolation

A total of 188 Botryosphaeriaceae isolates were obtained in this study and 120 isolates obtained in the previous studies (Zlatković et al., 2016a, b, 2017) were included in the subsequent analyses. (Tables S1, S2; Fig. S1).

Among all samples collected in the Western Balkans, including those collected in this and in previous studies (Zlatković et al. 2016a, b, 2017), Botryosphaeriaceae were most frequently isolated from necrotic lesions and cankers (45.4 % of the total samples), followed by resinous lesions and tissues (22 %). Botryosphaeriaceae were

Table 2. Botryosphaeriaceae species isolated from different tree parts in this and in previous studies in the Western Balkans (Zlatković et al. 2016a, b, 2017). Values represent the number and percentage of samples from which Botryosphaeriaceae were isolated.

Botryosphaeriaceae species	**Resinous**						**Necrotic/Cankered**				**Top dieback**	**Wood discoloration**	**Fruit bodies**	**Dead**	**Healthy**	Total	%
	Needles/ Leaves	Shoot	Branch	Stem	Bark	Cone	Needles/ Leaves	Shoot	Branch	Stem							
Diplodia sapinea	3	1	13	17	0	5	0	4	6	12	4	1	13	6	2	87	**28.2**
D. mutila	0	0	1	1	0	1	0	4	1	5	0	1	3	1	0	18	**5.8**
D. seriata	0	1	2	0	0	0	3	1	4	4	0	2	4	3	0	24	**7.8**
***Dothiorella* sp.**	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	5	**1.6**
Do. omnivora	0	0	0	0	0	0	2	0	2	1	0	1	1	0	0	6	**1.9**
Do. sarmentorum	0	0	1	0	0	0	2	3	0	2	0	0	2	1	0	11	**3.6**
Botryosphaeria dothidea	2	0	4	3	2	0	20	9	11	13	10	5	11	8	1	99	**32.1**
Neofusicoccum parvum	0	1	6	2	0	0	11	8	2	11	2	6	2	4	1	56	**18.2**
Sphaeropsis visci	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	**0.3**
Phaeobotryon cupressi	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	**0.3**
Total	5	3	29	23	2	6	38	28	26	48	16	15	38	26	4	308	**100**
%	1.6	1	9.4	7.5	0.6	1.9	12.3	9.1	8.4	15.6	5.2	4.9	12.3	8.4	1.3	100	

only occasionally isolated from healthy tissues (1.3 %), discolored wood (4.9 %) and margins between apparently healthy and dead stem wood of trees with top die-back (5.2 %) (Table2).

3.2 DNA sequence analyses

Preliminary identification of the isolates obtained in this study was made using MP analyses of the ITS sequences (tree not shown). Isolates of *N. parvum* were further subjected to analyses of the combined ITS, TEF-1-*α*, BT2 and RPB2 gene regions (trees not shown). Based on these analyses, isolates considered in this study were identified as *D. seriata*, *D. mutila*, *B. dothidea*, *Do. sarmentorum*, *Do. omnivora* and *N. parvum*. Representative isolates from each host of each identified fungal species including four that were previously identified, namely *Dothiorella* sp., *S. visci*, *P. cupressi* and *D. sapinea* (Zlatković et al., 2016a, 2017) were further subjected to MP and ML analyses of the ITS sequence data (Figs. 1, S1).

The ITS data set contained 145 sequences and was rooted to *Pseudofusicoccum stromaticum* (CBS 117448, CBS 117449). The sequence data set contained 528 characters among which 150 were parsimony informative, 378 were parsimony uninformative, with CI= 0.8, RI= 0.9 and TL= 247. The model GTR+G was chosen for the ML analyses (G= 0.2050). The topologies of the trees emerging from MP and ML analyses were similar and therefore, only ML tree is presented (Fig. 1).

3.3 Botryosphaeriaceae species diversity

Among the whole collection of isolates of Botryosphaeriaceae species from the Western Balkans, including isolates from this study and from previous studies (Zlatković et al. 2016a, b, 2017) the most commonly isolated species was *B. dothidea* (32.1 %), followed by *D. sapinea* (28.2 %) and *N. parvum* (18.2 %). *Do. omnivora* (1.9 %), *Dothiorella* sp. (1.6 %), *S. visci* (0.3 %) and *P. cupressi* (0.3 %) were isolated only occasionally. The majority of the Botryosphaeriaceae were most commonly isolated from necrotic lesions and cankers. Exceptions were *D. sapinea* that was most frequently isolated from resinous lesions and tissues (44.8 %), *S. visci* isolated from pycnidia and *P. cupressi* isolated only from resinous branch lesion (Table 2).

There were significant differences (F= 7.85, df= 9, p= 0) in host association patterns among Botryosphaeriaceae species from the Western Balkans from this and from previous studies (Zlatković et al. 2016a, b, 2017). *P. cupressi*, *S. visci* and *Dothiorella* sp. showed the strongest host association patterns being isolated from a

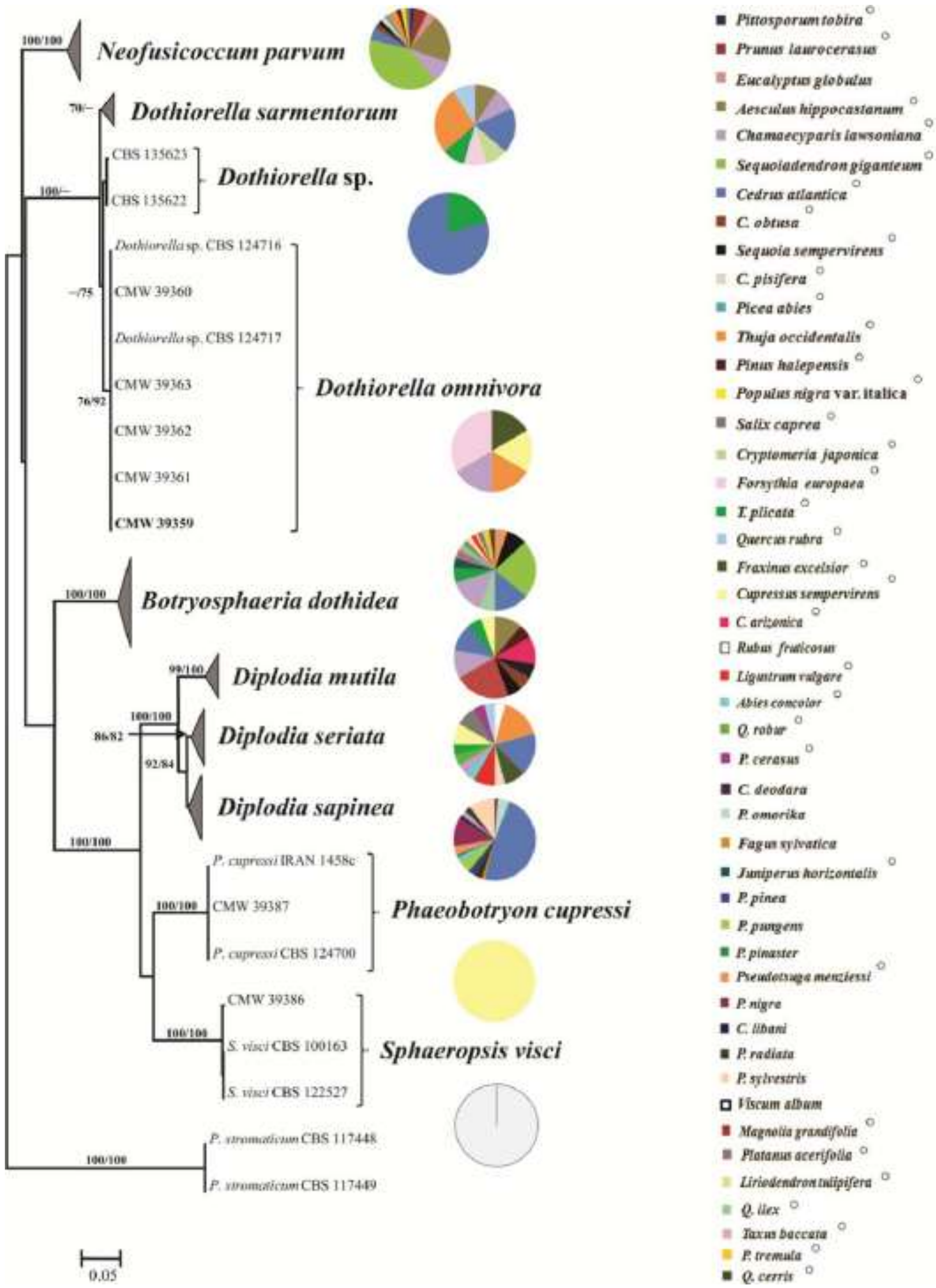

Figure 1. Maximum likelihood (ML) tree of the ITS sequence data. The bootstrap support values (MP/ML ≥ 70%) are indicated at the nodes and the scale bar represents the number of changes. The tree was rooted to *P. stromaticum* CBS 117448 and CBS 117449. Isolates sequenced in this study are presented in bold. Branches corresponding to Botryosphaeriaceae species that were isolated from more than five hosts are collapsed. Collapsed branches are displayed as gray triangles and the size of a triangle is proportional to the number of hosts. Pie charts indicate the hosts from which species were isolated. Hosts from this study are indicated with the circles.

single host or two hosts, respectively. Each of the remaining seven Botryosphaeriaceae species showed unique host association patterns (Fig. 1When considering only isolates from this study, new host associations were found for *Do. sarmentorum*, , *Do*. *omnivora*, *B. dothidea*, *D. mutila*, *D. seriata* and *N. parvum* (Table S9). Shannon Weiner's diversity index (H') showed greatest Botryosphaeriaceae diversity associated with *T. occidentalis* (H'= 0.61), followed by *T. plicata* (H'= 0.53) and 27 hosts harbored a single species. High values of Evenness (J= 0.58-0.91) indicated even communities of species for most hosts (Table3). There were significant differences (F= 1.61, df= 46, p= 0.009) in the composition and diversity of Botryosphaeriaceae among different hosts (Table3, Fig. S2).

There were no significant differences (H= 0.37, df= 1, p= 0.54) in the diversity of Botryosphaeriaceae species associated with angiosperms (H'= 0.42) compared to gymnosperms (H'= 0.29). High values of Evenness (0.76 and 0.8) showed that the communities are relatively similar. Jaccard's index showed relatively high overlap of species isolated from gymnosperms and angiosperms (J'= 0.7, Table S10). The species composition differed in such a way that *S. visci* was found only on angiosperms, *Dothiorella* sp. and *P. cupressi* were isolated only from gymnosperms, *B. dothidea* and *D. seriata* were isolated from gymnosperms and angiosperms with almost equal frequency and all the rest of the Botryosphaeriaceae species were most commonly isolated from gymnosperms (Table S10).

There were no significant differences (H= 2.28, df= 1, p= 0.13) of Botryosphaeriaceae species diversity in the Continental region (CR) (H'= 0.30) compared to the Mediterranean region (MR) (H'= 0.37). High values of Evenness indicated almost even distribution of species (0.77 and 0.8) and Jaccard's index showed moderate overlap of species (J'= 0.5). *D. seriata*, *Do. sarmentorum*, *Dothiorella* sp. and *S. visci* were found only in the CR; *P. cupressi* was found only in the MR and five species were found in both climatic regions (Table S11, Figure S3).

Individual trees, tree parts and lesions were often colonized by several Botryosphaeriaceae species. Up to five species were found co-habiting the same diseased host tree and up to three species were isolated from the same diseased tree parts (Table S12). *B. dothidea* and *N. parvum* were most commonly found co-habiting the same host trees (16 %); *B. dothidea* was found most frequently (30.6 %) in the same host tree and in same tree part (24.5 %) in combination with other species (Table S13) and multiple Botryosphaeriaceae species were most commonly isolated from *C. atlantica* (22.2 %, Table S14).

Table 3. Diversity and overlap of Botryosphaeriaceae species isolated from different hosts in this and in previous studies in the Western Balkans (Zlatković et al. 2016a, b, 2017)

Host	Species richness (S)	Abundance	Shannon index (H')	Evenness (J)	LSD
		Gymnosperms			
Chamaecyparis lawsoniana	7	26	0.44	0.73	A
Cedrus atlantica	7	71	0.30	0.66	A
Cupressus sempervirens	5	6			B
Thuja plicata	5	10	0.53	0.76	B
Thuja occidentalis	5	11	0.61	0.91	B
Abies concolor	3	5			D
Sequoiadendron giganteum	3	47	0.22	0.76	D
Pinus halepensis	3	4			D
Sequoia sempervirens	3	10	0.28	0.58	D
Chamaecyparis pisifera	2	2			D
Chamaecyparis obtusa	2	2			D
Cupressus arizonica	2	4			D
Pseudotsuga menziesii	2	8			D
Juniperus horizontalis	2	5			D
Picea abies	2	2			D
Pinus nigra	1	10	0	0	E
Pinus sylvestris	1	9			E
Cedrus deodara	1	1			E
Picea pungens	1	4			E
Picea omorika	1	4			E
Cedrus libani	1	1			E
Pinus radiata	1	2			E
Pinus pinea	1	1			E
Pinus pinaster	1	1			E
Cryptomeria japonica	1	1			E
Taxus baccata	1	1			E
		Angiosperms			
Aesculus hippocastanum	4	16	0.34	0.69	C
Salix caprea	2	4			D
Prunus laurocerasus	2	7			D
Fraxinus excelsior	2	3			D
Pittosporum tobira	2	2			D
Quercus rubra	3	3			D
Quercus ilex	1	6			E
Ligustrum vulgare	1	2			E
Populus tremula	1	2			E
Eucalyptus globulus	1	2			E
Platanus acerifolia	1	2			E
Viscum album	1	1			E
Forsythia europaea	1	1			E
Magnolia grandiflora	1	1			E
Liriodendron tulipifera	1	1			E
Fagus sylvatica	1	1			E
Quercus robur	1	1			E
Rubus fruticosus	1	1			E
Populus nigra var. italica	2	2			E
Prunus cerasus	1	1			E
Quercus cerris	1	2			E

[1] Diversity indexes were calculated only for hosts from which at least ten isolates were obtained.
[2] Hosts with the same letter did not differ significantly in terms of Botryosphaeriaceae species composition at the α= 0.05 significance level using LSD test.

3.4 Inoculations of stems and leaves of seedlings in the field

Botryosphaeria dothidea and *P. cupressi* formed cankers when inoculated onto *C. lawsoniana* seedlings, and *P. cupressi* was more aggressive. In contrast, *C. lawsoniana* seedlings inoculated with *B. dothidea* isolate from *C. lawsoniana* from the CR and five other Botryosphaeriaceae showed only resinous lesions that were not significantly different to that observed in the controls (Tables S5, S8, S15; Fig. S4). *D. seriata* produced girdling cankers with pycnidia of the fungus obvious on the canker surfaces when inoculated onto *Q. robur*, and the isolate from *C. pisifera* was more aggressive compared to that from *Q. robur*. *N. parvum*and *B. dothidea* produced resin-soaked girdling necrotic lesions when inoculated onto *P. abies*. Other disease symptoms were similar to those described by Zlatković et al. (2017). *N. parvum* isolated from *C. lawsoniana* was the most aggressive and it eventually killed the inoculated *P. abies* plants. *B. dothidea* and *D. mutila* produced girdling cankers and necrotic lesions when inoculated onto *C. arizonica* and *B. dothidea* isolated from *C. lawsoniana* grown in the MR was the most aggressive to this host. *N. parvum* was able to produce lesions and eventually kill inoculated *P. nigra* var. *italica* seedlings. The *N. parvum* isolate from *S. giganteum* was more aggressive to this host compared to the isolate from *E. globulus.* In contrast, *B. dothidea* did not produce lesions when inoculated into stems of *P. nigra* var. *italica* seedlings (Tables1, 4, S3, S8, S156; Figs. S4-S8).

Botryosphaeria dothidea, *Do. omnivora* and *N. parvum* killed *S. giganteum* plants 13 weeks after inoculation. Seedlings showed initial symptoms of shoot die-back one week after inoculation. Other symptoms included wilting and girdling stem lesions. Control plants remained asymptomatic. The *B. dothidea* isolate from *C. lawsoniana* grown in the CR and *Do. omnivora* had the highest AUDPC values and were shown to be the most aggressive. *N. parvum* isolated from *E. globulus* was the least aggressive. *D. seriata* and *B. dothidea* killed *A. concolor* seedlings 12-20 weeks post inoculation. Plants displayed symptoms and signs of yellowing and browning of the needles, stem die-back and needle cast followed by the appearance of *D. seriata* and *B. dothidea* pycnidia in the dead bark. *B. dothidea* was the most aggressive and *D. seriata* isolate from *Q. robur* was the least aggressive (Tables4, S3, S15; Figs. 2, 3, S9, S10).

Seedlings of *T. occidentalis* displayed initial symptoms of yellowing, browning, reddening of the leaves and shoot flagging seven weeks after inoculation with four Botryosphaeriaceae species. Other symptoms included dry, black and necrotic leaves, girdling lesions and fungal pycnidia in the dead tissues. Control plants remained asymptomatic. *D. seriata*, *N. parvum* isolated from *E. globulus* and *B. dothidea* isolated from *C. lawsoniana* grown in the CR were the most aggressive species. *D. mutila*, *D. seriata* and *B. dothidea* were able to kill *C. sempervirens*.

Table 4. Pathogenicity of Botryosphaeriaceae species on various hosts in this and in previous studies in the Western Bakans (Zlatković et al. 2016b, 2017).

Host	Botryosphaeriaceae species									
	Botryosphaeria dothidea	***Neofusicoccum parvum***	***Diplodia seriata***	***D. mutila***	***D. sapinea*** [13]	***Dothiorella sarmentorum***	***Do. omnivora***	***Dothiorella* sp.**	***Phaeobotryon cupressi***	***Sphaeropsis visci***
Chamaecyparis lawsoniana	− [5]/+ [6]	−	−	−	−	−	−	−	+	−
Cedrus atlantica	−	+ [7] /− [8]	−	−	−	−		−		
C. deodara		− [7]			−					
Prunus laurocerasus		+ [1,7,8] /+ [7,8]		− [1,11] / + [1,12]/+ [7,8,11]	−					
Sequoiadendron giganteum	+	+					+			
Thuja occidentalis	+	+	+		−	+				
Pseudotsuga menziesii	−				+					
Abies concolor	+		+		+					
Juniperus horizontalis	+				+					
Cupressus arizonica	+			+						
Liriodendron tulipifera	+									
Magnolia grandifolia	+									
Ligustrum vulgare			+							
C. sempervirens	+		+	+			−		−	
Picea omorika		+ [7]								
Quercus robur			+							
Pittosporum tobira		+		−						
Aesculus hippocastanum [2]	+	+		+		+				
Pinus patula [3]	+	+			+					
Eucalyptus grandis [3]	+	+			−					
P. abies	+	+			+					
P. pungens					+					

Table 4. Continued.

Host	Botryosphaeriaceae species									
	Botryosphaeria dothidea	***Neofusicoccum parvum***	***Diplodia seriata***	***D. mutila***	***D. sapinea***	***Dothiorella sarmentorum***	***Do. omnivora***	***Dothiorella* sp.**	***Phaeobotryon cupressi***	***Sphaeropsis visci***
P. nigra					+					
P. sylvestris					+					
F. sylvatica [2]					+					
Viscum album [2, 4]										+
Populus tremula [2]	+									
Fraxinus excelsior [2]			+ [9]/– [10]				–			
Forsythia europaea						–				
Quercus cerris [2]	+									
Taxus baccata	–									
Prunus cerasus			–							
P. nigra var. italica	–	+								
Quercus rubra		–	–			–				
Platanus acerifolia	–									

[1] Inoculations conducted on leaves of seedlings in the field, [2] cut branch inoculations in laboratory conditions, [3] stem inoculations in the greenhouse, [4] leaf inoculations on cut branches in laboratory conditions. All other inoculations were conducted on stems of seedlings in the field. Empty spaces indicate that the species was not isolated from the host and/or inoculated into host , + The species was pathogenic, − The species was not pathogenic, [5] CMW 39301, [6] CMW 39315, [7] CMW 39327, [8] CMW 39317, [9] CMW 39376, [10] CMW 39382, [11] CMW 39348, [12] CMW 39358, [13] Zlatković et al. (2017).

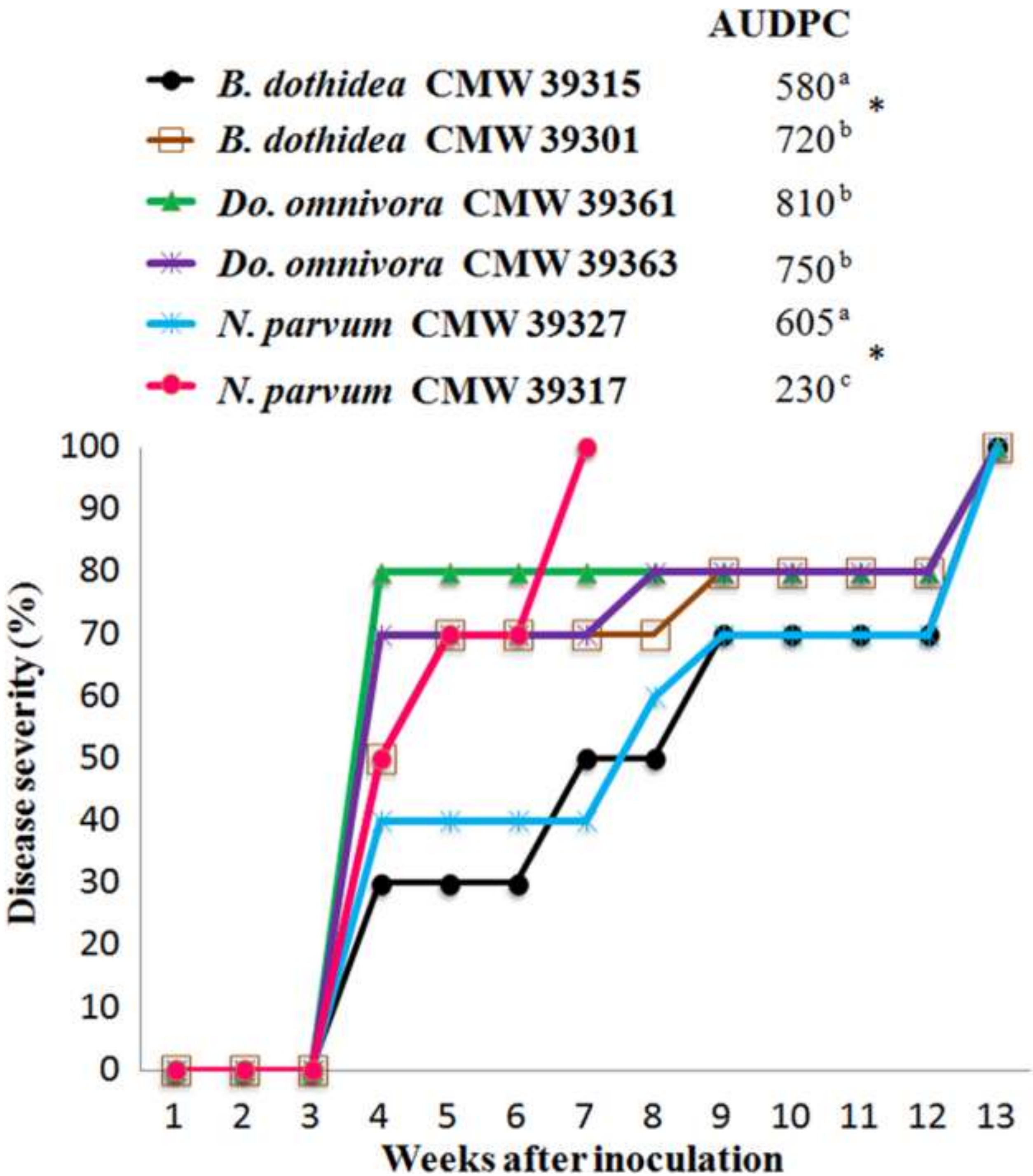

Figure 2. Disease progress curves and Area Under the Disease Progress Curve (AUDPC) of seedlings of *Sequoiadendron giganteum* inoculated with *Botryosphaeria dothidea*, *Dothiorella omnivora* and *Neofusicoccum parvum*. The ANOVA test indicated that there were significant differences in AUDPC values of seedlings of *S. giganteum* at $\alpha = 0.05$ level ($F = 70.57$, $df = 2$, $p = 0.03$). Means with the same letter did not differ significantly, as determined by the LSD test at $\alpha = 0.05$ level. "*" indicates that AUDPC values of isolates of the same species were significantly different (z-test, *B. dothidea*: $z = 3.89$, $p = 0.0001$; *N. parvum*: $z = 12.98$, $p < 0.00001$).

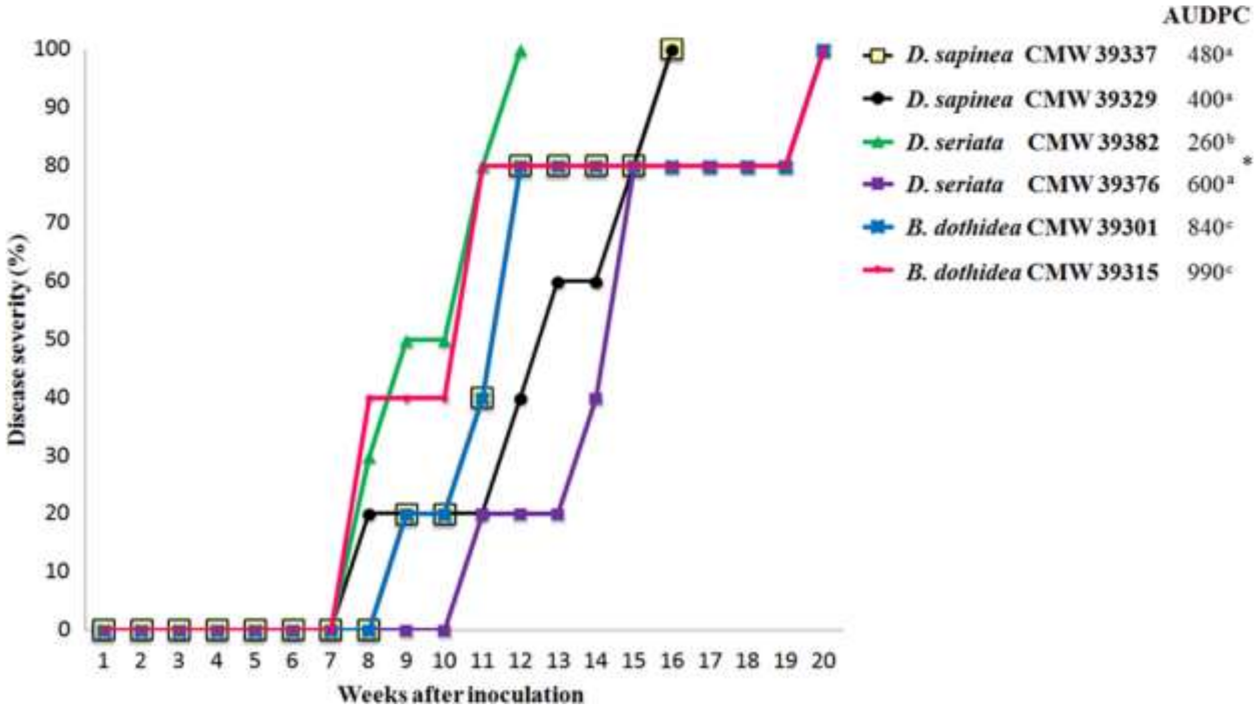

Figure 3. Disease progress curves and Area Under the Disease Progress Curve (AUDPC) of seedlings of *Abies concolor* inoculated with *Diplodia sapinea*, *D. seriata* and *Botryosphaeria dothidea*. The ANOVA test indicated that there were significant differences in AUDPC values of seedlings of *A. concolor* at $\alpha = 0.05$ level ($F = 19.9$, $df = 2$, $p = 0.02$). Means with the same letter did not differ significantly, as determined by the LSD test at $\alpha = 0.05$ level. "*" indicates that AUDPC values of isolates of the same species were significantly different (z-test, *D. seriata*: $z = 11.58$, $p < 0.0001$). Data for *Diplodia sapinea* were retrieved from Zlatković et al. 2017 and serve for comparison with other Botryosphaeriaceae inoculated onto *A. concolor*.

Seedlings displayed the first symptoms of shoot flagging two weeks after inoculation and later exhibited die-back of stems, yellowing of the needles and needle drying. Disease symptoms were not observed on seedlings inoculated with *P. cupressi*, *Do. omnivora* or on the controls. *N. parvum* was able to kill *P. omorika*. Disease symptoms were similar to those described in Zlatković et al. (2017) and controls remained healthy (Tables1,4; S3, Figs. 4-6, S11-S13).

Diplodia seriata was shown to produce vascular discoloration when inoculated onto *L. vulgare* and *B. dothidea* produced vascular discoloration on *M. grandifolia*. *B. dothidea* produced necrotic lesions when inoculated onto *L. tulipifera* and an isolate from *C. lawsoniana* grown in the CR was more aggressive compared to an isolate from the same host grown in the MR. In contrast, *B. dothidea* did not cause lesions on seedlings of *P. menziesii* and *N. parvum* did not cause lesions on seedlings of *C. deodara*. *N. parvum* isolated from *S. giganteum* produced sunken, girdling cankers on stems of *C. atlantica*. Other Botryosphaeriaceae and the controls produced only small resinous lesions. *B. dothidea* produced necrotic lesions on stems of *J. horizontalis*. *Do. sarmentorum* did not

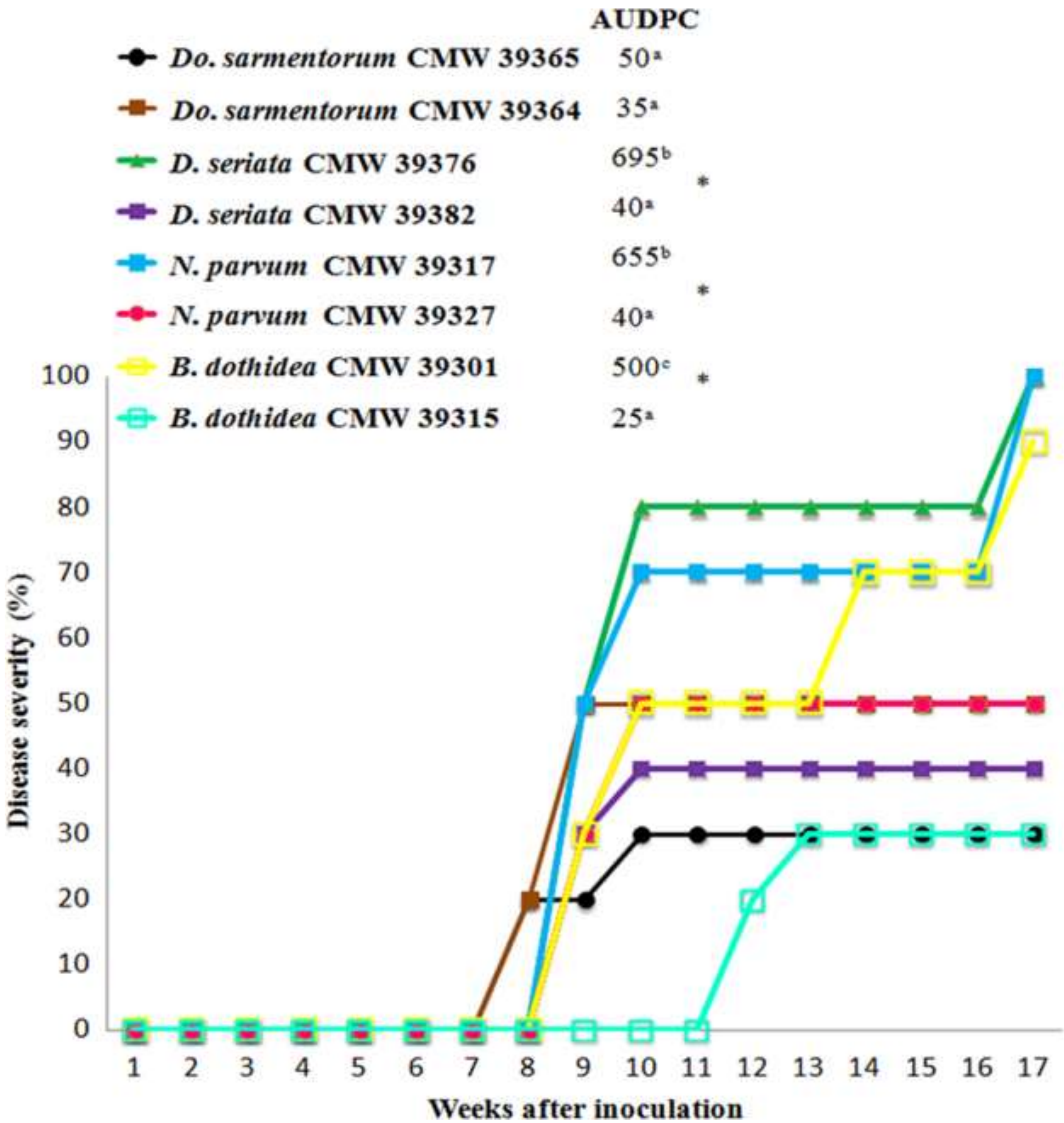

Figure 4. Disease progress curves and Area Under the Disease Progress Curve (AUDPC) of seedlings of *Thuja occidentalis* inoculated with *Diplodia seriata*, *Neofusicoccum parvum* and *Botryosphaeria dothidea*. The ANOVA test indicated that there were significant differences in AUDPC values of seedlings of *T. occidentalis* at α= 0.05 level (F= 913.15, df= 3, p= 0.000004). Means with the same letter did not differ significantly, as determined by the LSD test at α= 0.05 level. "*" indicates that AUDPC values of isolates of the same species were significantly different (z-test, *D. seriata*: z= 24.16, p< 0.00001; *N. parvum*: z= 23.33, p< 0.00001; *B. dothidea*: z= 20.73, p< 0.00001).

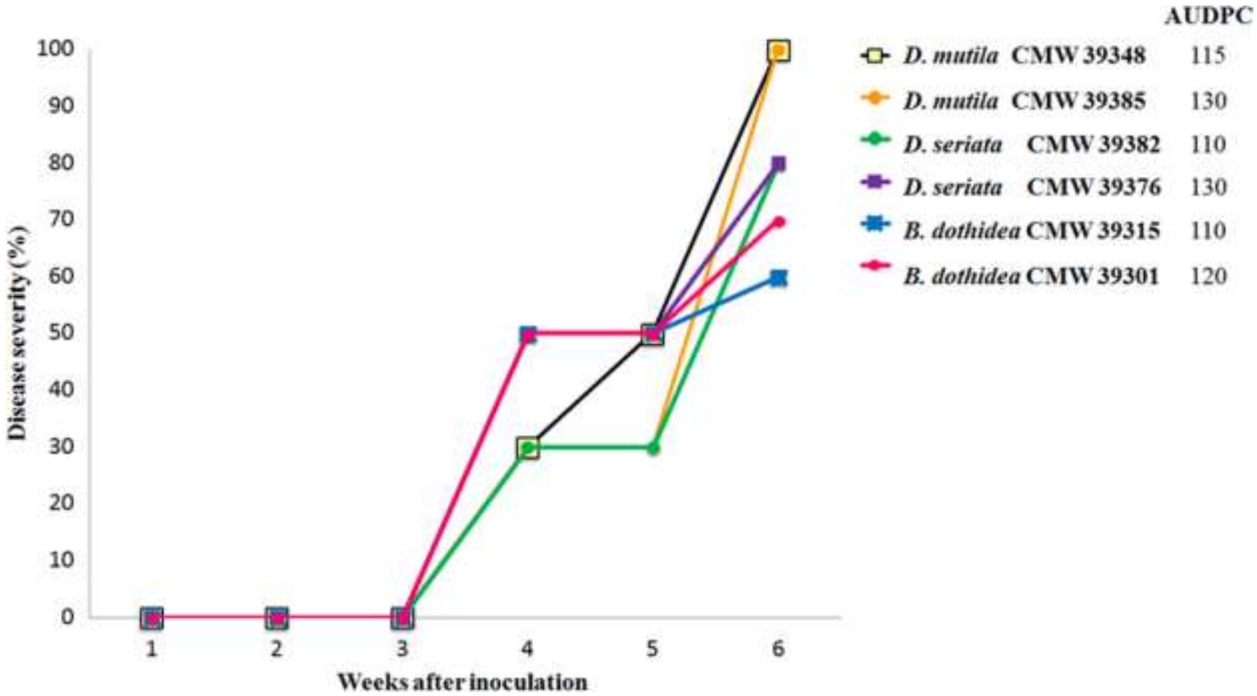

Figure 5. Disease progress curves and Area Under the Disease Progress Curve (AUDPC) of seedlings of *Cupressus sempervirens* inoculated with *Diplodia mutila*, *D. seriata* and *Botryosphaeria dothidea*. The ANOVA test indicated that there were no significant differences in AUDPC values of seedlings of *C. sempervirens* at α= 0.05 level (F= 5.97 , df= 1, p= 0.07).

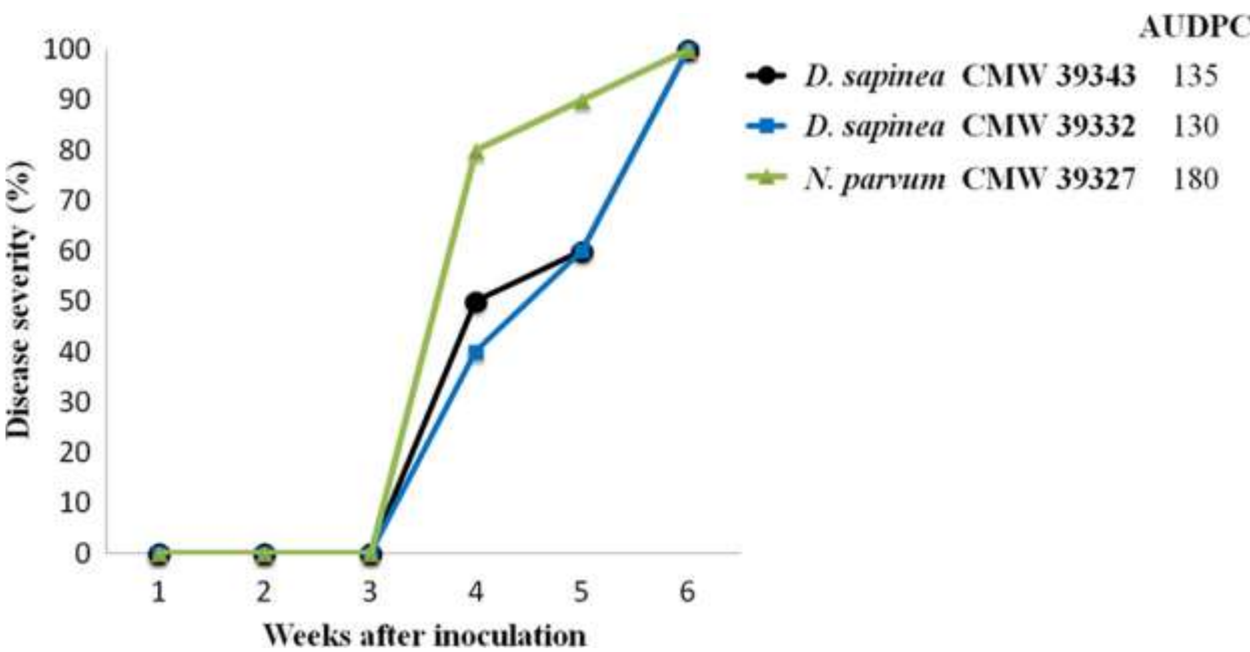

Figure 6. Disease progress curves and Area Under the Disease Progress Curve (AUDPC) of seedlings of *Picea omorika* inoculated with *Diplodia sapinea* and *Neofusicoccum parvum*. The t-test indicated that there were no significant differences in AUDPC values of seedlings of *P. omorika* at α= 0.05 level (t= -10.97, df= 1 , p= 0.06). Data for *Diplodia sapinea* were retrieved from Zlatković et al. 2017 and serve for comparison with other Botryosphaeriaceae inoculated onto *P. omorika*.

produce lesions on seedlings of *F. europaea*; *D. seriata*, *Do. sarmentorum* and *N. parvum* did not produce lesions on *Q. rubra*; *B. dothidea* did not produce lesions on *P. acerifolia* and *T. baccata,* and *D. seriata* did not produce lesions on *P. cerasus*. *N. parvum* produced dark brown elongated lesions on stems of *P. tobira*. Isolates of *D. mutila* and control plants displayed no lesions. *N. parvum* and *D. mutila* isolated from *C. sempervirens* produced reddish-brown elliptical cankers with cracks inside the canker and along the canker margin on stems of *P. laurocerasus* seedlings. In contrast, *P. laurocerasus* seedelings inoculated with *D. mutila* isolated from *T. plicata* showed only dark brown lesions visible after the outer bark was removed and these lesions were not significantly larger from those produced on the controls.

Leaves of *P. laurocerasus* showed necrotic lesions five days after inoculation. *N. parvum* isolated from *E. globulus* was the most aggressive fungus. No lesions appeared on leaves of the control plants and plants inoculated with *D. mutila* isolated from *T. plicata* (Zlatković et al. 2016b; Tables 1, S3, S8; Fig. S22). Botryosphaeriaceae were re-isolated and identified from symptomatic tissues of all inoculated stems and leaves of seedlings. Exceptions were resinous lesions formed on *C. lawsoniana* and *C. atlantica*, brown lesions fomed on *P. laurocerasus* after inoculation with *D. mutila* isolate from *T. plicata*, seedlings which were inoculated with Botryosphaeriaceae isolates but did not show lesions, and controls (Tables4, S3, S8, S15; Figs. S14-S182).

3.5 Inoculations of cut branches and leaves of cut branches

Botryosphaeria dothidea, *D. mutila* isolated from *C. sempervirens*, *Do. sarmentorum* isolated from *C. lawsoniana* and *N. parvum* were the most aggressive species when inoculated onto *A. hippocastanum* branches. *Do. sarmentorum* isolate from *T. occidentalis* was the least aggressive. *B. dothidea* produced brown, girdling lesions when inoculated onto *P. tremula* branches and brown, elongated lesions when inoculated onto *Q. cerris* branches. Control branches produced no lesions. *D. seriata* isolated from *C. pisifera* produced brown, elliptical lesions with pycnidia formed in lesions when inoculated onto *F. excelsior* branches. Control branches, branches inoculated with *D. seriata* from *Q. robur* and with *Do. omnivora* showed no lesions. *V. album* shrubs showed necrotic lesions on branches and leaves five days after inoculation. Two weeks post inoculation, branches and leaves appeared chlorotic; branches were girdled and *S. visci* pycnidia were found embedded in the dead tissues. Control branches and leaves showed only minimal discoloration around the wounds (Tables1, S3, S8, S15; Figs. S19-S21). Re-isolations from fungal pycnidia and discoloured tissues were successful and Koch's postulates were fulfilled in both pathogenicity tests conducted on cut branches and leaves of cut branches.

3.6 Inoculations of stems of seedlings in the greenhouse

B. dothidea was less aggressive compared to *N. parvum* when inoculated onto *P. patula*. *N. parvum* isolated from *E. globulus* was the most aggressive and the *B. dothidea* from CR was the least aggressive when inoculated onto *E. grandis*. In contrast, seedlings of *E. grandis* inoculated with *B. dothidea* isolate from MR showed lesions that did not differ obviously from those produced on the controls. Lesions formed on control seedlings were significantly smaller compared to those produced by Botryosphaeriaceae species (Tables4, S3; S8, S15, Figs. S23-24). Botryosphaeriaceae were successfully re-isolated and identified from all seedlings other than from the controls.

4 Discussion

In this study, six Botryosphaeriaceae species (*B. dothidea*, *N. parvum*, *D. mutila*, *D. seriata*, *Do. sarmentorum*, *Do. omnivora*,) were identified from 30 tree species and five shrubby species growing in urban areas, forests and ornamental nurseries. An additional four Botryosphaeriaceae species (*D. sapinea*, *Dothiorella* sp., *P. cupressi* and *S. visci*) were obtained in previous studies (Zlatković et al., 2016a, b, 2017), giving a total of ten Botryosphaeriaceae isolated from 47 tree and shrubby species in urban areas, forest plantations, forests and ornamental nurseries in the Western Balkans region. *P. cupressi* and *S. visci* occurred on only a single host, but *Dothiorella* sp. was found on two hosts and the remaining Botryosphaeriaceae were associated with more than four hosts. Botryosphaeriaceae were mostly pathogenic to the hosts from which they were isolated, but were also able to infect other tree species. The results of this study, based on a high number of hosts and the ability of Botryosphaeriaceae to infect both host and non-host species, illustrate the generalist nature of most of the species.

Very few Botryosphaeriaceae species appear to be host specific. The association of *S. visci* with only hemi-parasitic *V. album* shrubs could be interpreted as host specificity. Other than this study, *S. visci* has been isolated only from *V. album* (Phillips et al., 2013; Zlatković et al., 2016a). Furthermore, Varga et al. (2011) showed that *S. visci* causes disease in *V. album*. *P. cupressi* was found only on *C. sempervirens* in this study, but has previously also been isolated from *J. scopulorum*, both of which are Cupressaceae (Phillips et al., 2013). In our inoculation experiments, *P. cupressi* was able to infect *C. lawsoniana*, and it gave rise to large lesions. In contrast, *P. cupressi* did not cause lesions on inoculated *C. sempervirens.* This is possibly because *P. cupressi* was isolated as an endophyte from healthy tissues of *C. sempervirens* in the current study and previously by Abdollahzadeh et al. (2009).. *Dothiorella* sp. was also isolated from a limited number of hosts in this study, namely from *T. plicata* and *C. atlantica*. This fungus appears to display some patterns of host preference as it was not able to cause lesions on *C. lawsoniana* despite its having been found on *C. atlantica*, on which it occurs as an endophyte.

Botryosphaeria dothidea and *N. parvum* are known to have a broad host range (Sakalidis et al., 2013; Marsberg et al., 2017). In the present study, *B. dothidea* occurred on the greatest number (22) of hosts and was shown to cause lesions on many of them. Isolations and inoculation experiments showed that this fungus is a significant pathogen of ornamental trees and shrubs. Apart from producing cankers on the inoculated seedlings and lesions on the inoculated branches, the pathogen was able to kill *S. giganteum*, *T. occidentalis*, *A. concolor*, *P. abies* plants and to girdle branches of *A. hippocastanum*. *B. dothidea* was also able to infect *P. patula* and *E. grandis* from which it was not isolated and lesions produced on *P. patula* were significanly smaller than those caused by *N. parvum*. Similarly, in a study by Pavlic et al. (2007), *B. dothidea* isolated from native *Syzigium cordatum* produced lesions on *E. grandis* from which it has not been isolated and it was less aggressive compared to *N. parvum*. *Neofusicoccum parvum* is an important pathogen of *Eucalyptus* spp. (Slippers et al., 2009) and its aggressiveness on this tree in the present study is thus not surprising. *N. parvum* is also a pathogen of ornamental trees and shrubs (e.g. Begoude et al., 2010; Varela et al., 2011) and in the present study was shown to infect eight ornamental tree species. *N. parvum* was isolated from 16 hosts and these included ten gymnosperms and six angiosperms. Similarly, Sakalidis et al. (2013) showed a lack of host specifity for this fungus and argued that the ability of *N. parvum* to exist as an endophyte in asymptomatic plants and to colonize a wide range of hosts could explain its wide distribution in many countries and continents. This is also consistent with the view that the endophytic nature of fungi such as the Botryosphaeriaceae implies that they are easily overlooked by quarantine systems (Wingfiled et al., 2015; Burgess and Wingfield, 2017; Crous et al., 2016).

The majority of the Botryosphaeriaceae species (seven of 10) from the Western Balkans identified in this and in previous studies (Zlatković et al. 2016a, b, 2017) were isolated from multiple tree species and numerous new host associations have been established. This is not surprising for Botryosphaeriaceae species such as the previously mentioned *B. dothidea* and *N. parvum,* which are known to occur on a large number of different tree species (Sakalidis et al., 2013; Marsberg et al., 2017). However, the previous study showed that the host range of the pine pathogen *D. sapinea* was unexpectedly broad and the species was isolated from 16 tree and shrub species (Zlatković et al. 2017).

In this study, Botryosphaeriaceae species were shown to infect tree species having important ecological and cultural value. This adds to the threat many of these trees face already. For example, it was shown that *B. dothidea*, *D. seriata* and *D. mutila* contribute to the die-back of *C. sempervirens*, a valuable ornamental tree that is regarded as key element of the Mediterranean scenery (Xenopoulos et al., 1990). The "cypress canker" disease caused by *Seiridium* spp. is known as one of the most serious threats to the survival of *C. sempervirens* (Graniti, 1998; Danti et al., 2014) and this study adds to the understanding that there are other fungi involved in the die-back of this tree. Similarly, *N. parvum* wasable to infect and kill *P. omorika*, an endemic tree that is in danger of extinction due to its limited population distribution, loss of genetic diversity and the effects of climate change (Alberto et al., 2013). Likewise, multiple Botryosphaeriaceae species were shown to infect *A. hippocastanum*, an endemic tree threatened by the leaf-mining moth *Cameraria ohridella* (Stojanović and Marković, 2004; Valade et al., 2009).

Various Botryosphaeriaceae found in this study were able to kill tree species that are economically valuable. For example, *D. seriata* was able to infect and eventually kill *Q. robur*, which is an economically valuable tree that is threatened by a change of flood regime and climate in northern Serbia (Stojanović et al., 2015). *D. seriata* was able to produce lesions on *F. excelsior* branches. *F. excelsior* has been devastated by the fungal pathogen *Hymenoscyphus fraxineus* in many parts of Europe, including parts of the Western Balkans (Pautasso et al., 2013; Stanivuković et al., 2014; Keča et al., 2017; Milenković et al., 2017) and this study shows that *D. seriata* might be also contributing to the impact of ash die-back disease.

The results from this study showed that an individual isolate of the Botryosphaeriaceae species has the potential for a wide range of aggressiveness to various often phylogenetically unrelated hosts. However, inoculations were carried out under partially controlled conditions on plants in the field and on cut branches; the level of plant stress was not measured. Botryosphaeriaceae are known as opportunistic pathogens capable of causing disease in a stressed host (Slippers and Wingfield 2007). Thus the aggressiveness of these fungi might have been higher than it would have been under completely controlled conditions or using living plants instead of plant parts. Moreover, a large amount of inoculum supplied with nutrients in the agar plug was placed on a wound, and in contact with vascular tissues. Although this method has been considered to represent the natural mode of infection of these fungi, it would be likely that under such conditions even an opportunistic pathogen could cause the disease (Manawashinge et al. 2016) .

Multiple Botryosphaeriaceae species were commonly isolated from the same host species, trees or even the same lesions. Similarly, in a study by Pavlic et al. (2008) eight Botryosphaeriaceae were isolated from asymptomatic tissues of the native *S. cordatum* plants and many species were obtained from the same plant. Luchi et al. (2013) reported a complex process of colonization of eight conifer species in Italy, where eleven Botryosphaeriaceae were found in symptomatic and asymptomatic tissues. Likewise, Jami et al. (2014) found no evidence that Botryosphaeriaceae species were tissue specific. Results of the pathogenicity tests in the present study showed that Botryosphaeriaceae isolated from the same host might play different roles in the process of tree die-back. For example, seven species were isolated from *C. atlantica* and *C. lawsoniana*, but of these only a single strain of *N. parvum* and *B. dothidea* was able to cause disease symptoms on these hosts. However, given the opportunistic nature of Botryosphaeriaceae species and predicted more frequent extreme weather events in the Western Balkans (www.hidmet.gov.rs, accessed October 2017) the possibility of currently non-pathogenic Botryospaheriaceae becoming pathogenic to the particular host should not be neglected.

The results of this study confirm that the distribution of Botryosphaeriaceae is influenced by the host (Burgess et al., 2005; Slippers and Wingfield, 2007; Fabre et al., 2011; Jami et al., 2014). Host factors might have influenced the distribution of *S. visci* because shrubs of *V. album* grow only in the CR. In contrast, *Do. sarmentorum* and *Dothiorella* sp. were not isolated from the MR even though its hosts, *C. lawsoniana* and *C. atlantica*, are widely planted in both areas. Similarly, Burgess et al. (2006) showed that *N. australe* is prevalent in Western Australia, but can rarely be found in Eastern Australia, even though its *Eucalyptus* host is present in both areas. *P. cupressi* was found only in the MR where its host, *C. sempervirens* is native although this tree is also occasionally planted in the CR. Differences in host genetics and presence of both native and introduced trees could explain the different Botryosphaeriaceae community structures on this tree.

Diversity analyses from this study showed no significant differences in the Botryosphaeriaceae species diversity in the CR compared to the MR. This was surprising as previous studies showed that Botryosphaeriaceae species diversity and occurrences are also influenced by the environment in which the given host occurs (Slippers and Wingfield, 2007; Fabre et al., 2011). However, no general conclusions can be made based on the current data regarding the differences in Botryosphaeriaceae species distributions because the number of samples from the MR was unduly limited.

The die-back of trees sampled in this study could be associated with some form of stress to trees linked to either recent extreme weather patterns, a “heat island effect”, other stresses affecting trees in the cities, or planting of species on suboptimal sites (Allen et al., 2010; Zlatković et al., 2016a, 2017). *P. abies* sampled in this study also occur at the edge of its southern range, which might imply stress symptoms. Furthermore, the shallow-rooted *P. abies* is vulnerable to drought, which is already causing mortality of this tree in Central and Northern Europe. Similarly, *C. atlantica* is experiencing mass mortality in its native range in North Africa linked to climate change (Allen et al., 2010). In addition, a number of forest management practices might have contributed stress on these trees. For example, ornamental trees in Serbia are frequently propagated from locally collected seeds in urban areas of unknown provenance (www.minpolj.gov.rs), and that have not been adequately tested for planting in given environments. Also, *P. laurocerasus* is a shade demanding species, but in the cities of Serbia these shrubs are frequently planted on open spaces.

In urban areas, exotic tree species are planted close to native species that could provide a source of inoculum for cross infection to occur. The results of the inoculation tests confirmed that Botryosphaeriaceae isolated from introduced trees can infect native trees and *vice versa*. It also shows that Botryosphaeriaceae can move from ornamental to forest trees and *vice versa*. For example, isolates of *B. dothidea* from *C. lawsoniana* were able to infect seedlings of native *P. abies*. Similarly, close proximity of native and exotic species was hypothesized to be the reason for the occurrence of *N. eucalyptorum*, a pathogen of *Eucalyptus* spp. on native myrtaceous hosts in Uruguay (Perez et al., 2010) and for the infection of *Eucalyptus* plantations established close to the native vegetation in South Africa (Burgess and Wingfield, 2001; Pavlic et al., 2007). *D. seriata* is a well-known pathogen of fruit trees (Slippers et al., 2007), however, in this study, isolates of *D. seriata* from *C. pisifera* and *Q. robur* were not able to produce lesions on *P. cerasus*, but could produce lesions or infect and eventually kill four ornamental and two forest tree species. Population genetic analyses are needed to test the gene flow between populations of *D. seriata* on fruit, ornamental and forest trees as has recently been shown by Mehl et al. (2017).

The Botryosphaeriaceae were most diverse on gymnosperms in this study. With the exception of *A. hippocastanum* and *S. caprea*, which harbored four and three Botryosphaeriaceae respectively, all other angiosperms harbored one or two species. This is surprising and in contrast to the results of most previous studies. For example, previous authors (De Wet et al., 2008; Sakalidis et al., 2013; Slippers et al., 2013), the Botryosphaeriaceae are most common and diverse on angiosperms. In contrast, Alves et al. (2013) reported great diversity of these species associated with conifers in Portugal, but conifers were the only trees sampled in the study and no comparison with the diversity on angiosperms was made. It is difficult to explain these patterns because sampling, host diversity, climate and other factors are not consistent across these studies. Overall, however, the results show that both gymnosperms and angiosperms can harbor substantial Botryosphaeriaceae diversity in different environments and circumstances.

This study has demonstrated that various trees and shrubs in the Western Balkans harbor a wide diversity of Botryosphaeriaceae, and with the exception of *S. visci* on *V. album*, most have broad host ranges. However, some Botryosphaeriaceae species appeared to occur predominantly on certain hosts and this suggests that those tree species posses some characteristics favorable for these fungi. The fact that some Botryosphaeriaceae were able to cross-infect taxonomically unrelated trees and eventually kill the plants emphasizes the importance of this fungal group. There is clearly a need for further research considering the pathways of introduction and spread of these fungi (Slippers et al., 2017) as well as methods (Crous et al., 2016) to understand and manage the diseases with which they are associated.

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Supplementary material

Figure S1. A diagram outlining the materials and methods used in this study.

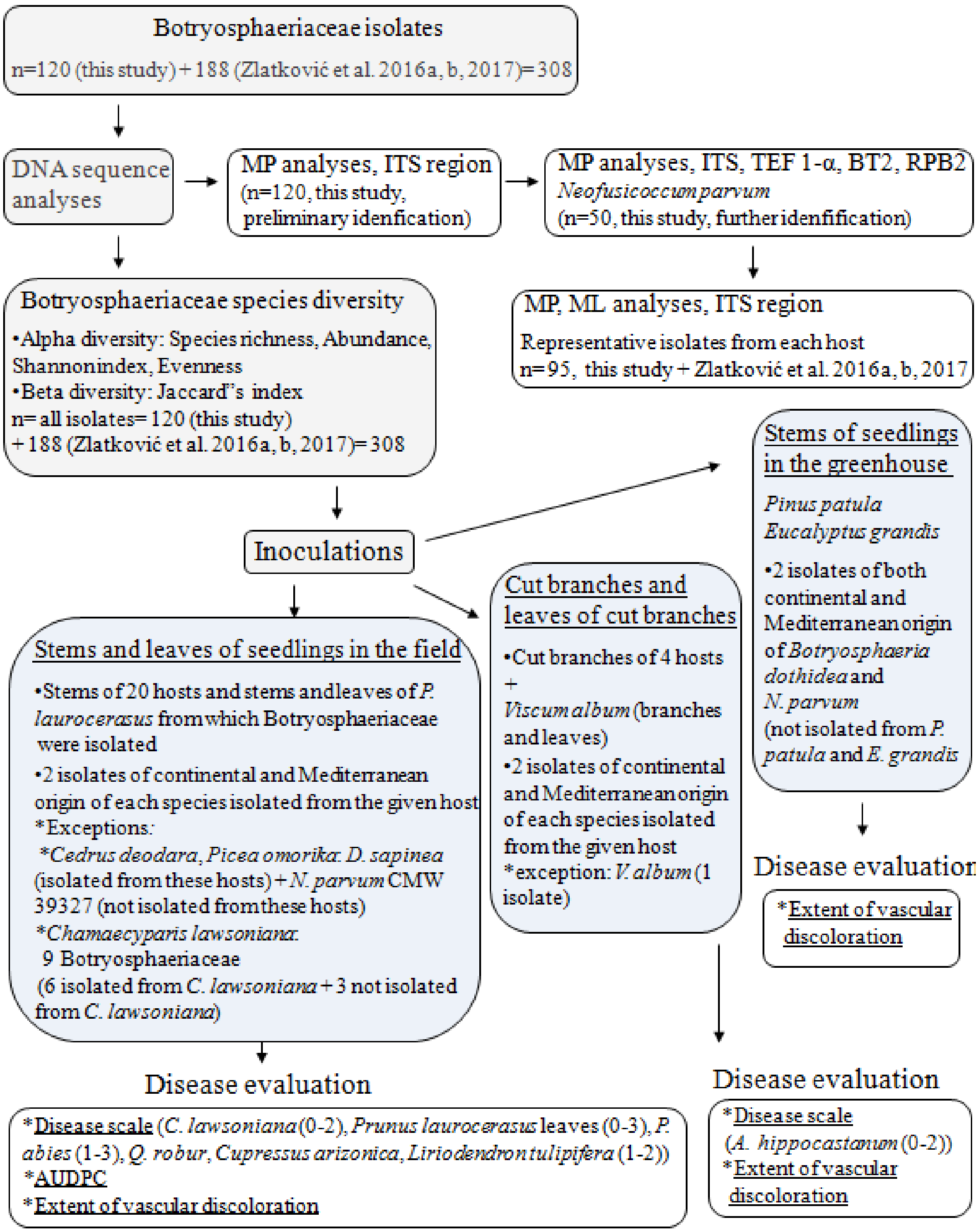

Figure S2. Botryosphaeriaceae species associated with: a- **gymnosperms**, b- **angiosperms**. Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

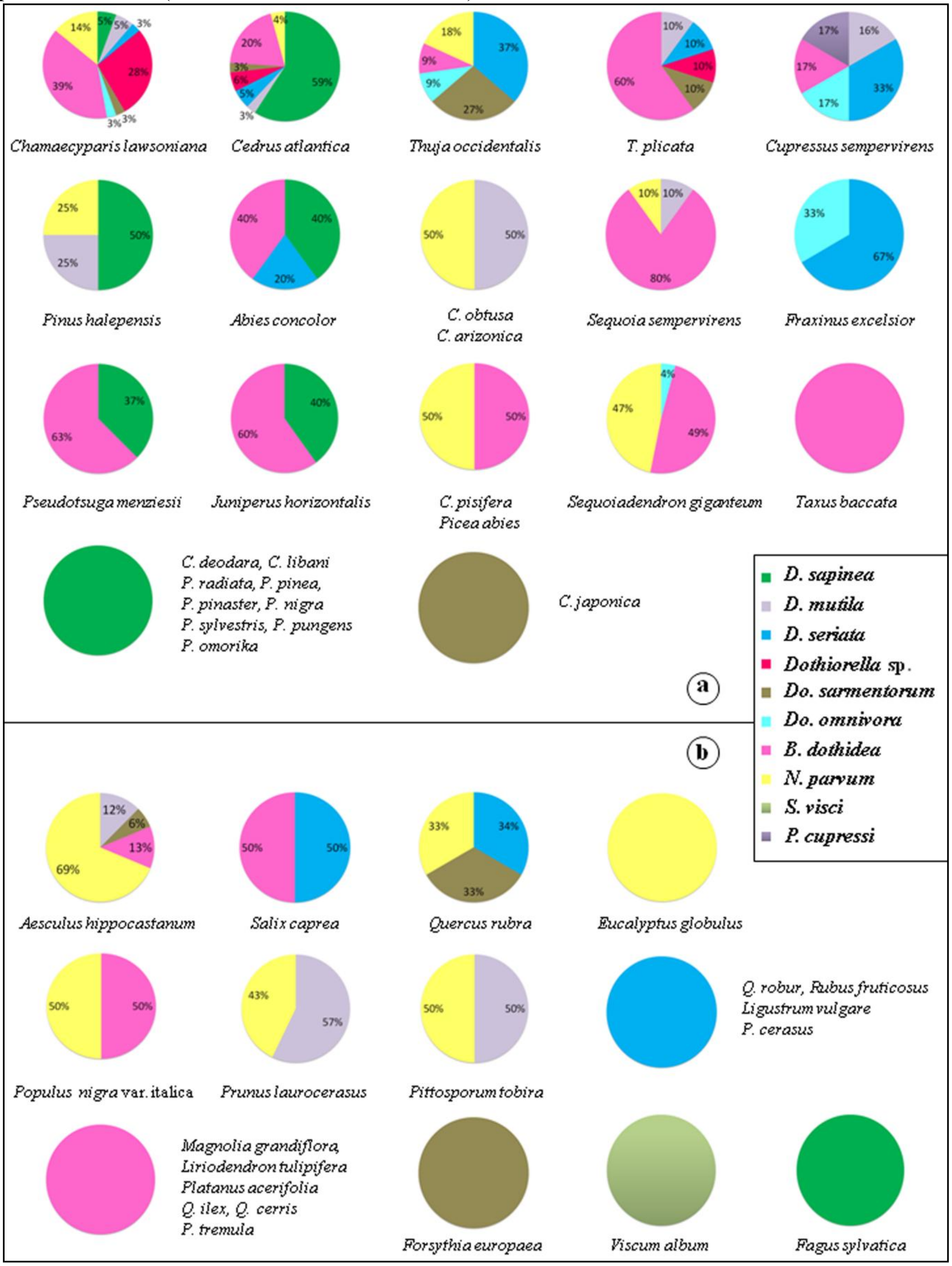

Figure S3. Botryosphaeriaceae species isolated from hosts from the Mediterranean and Continental climate-type regions. Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

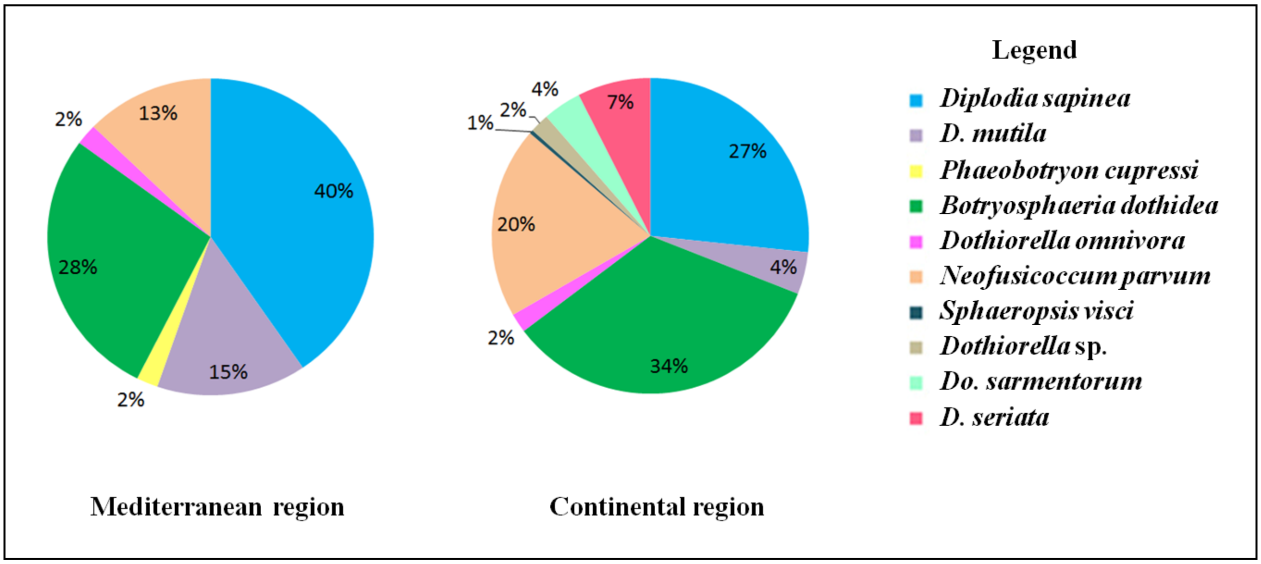

Figure S4. Signs and symptoms associated with *Phaeobotryon cupressi* and *Botryosphaeria dothidea* on inoculated *Chamaecyparis lawsoniana* seedlings. a. Browning of the leaves from the inoculation point upwards and downwards; b. Elongated, girdling, sunken cankers with cracks along the canker margin and inside the canker formed after inoculation with *P. cupressi*; c. Elliptical canker formed after inoculation with *B. dothidea* CMW 39315 (c); d, e. Pycnidia of *P. cupressi* formed in the dead stem tissue; f, g. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bars: d= 1 cm, e= 0.5 cm.

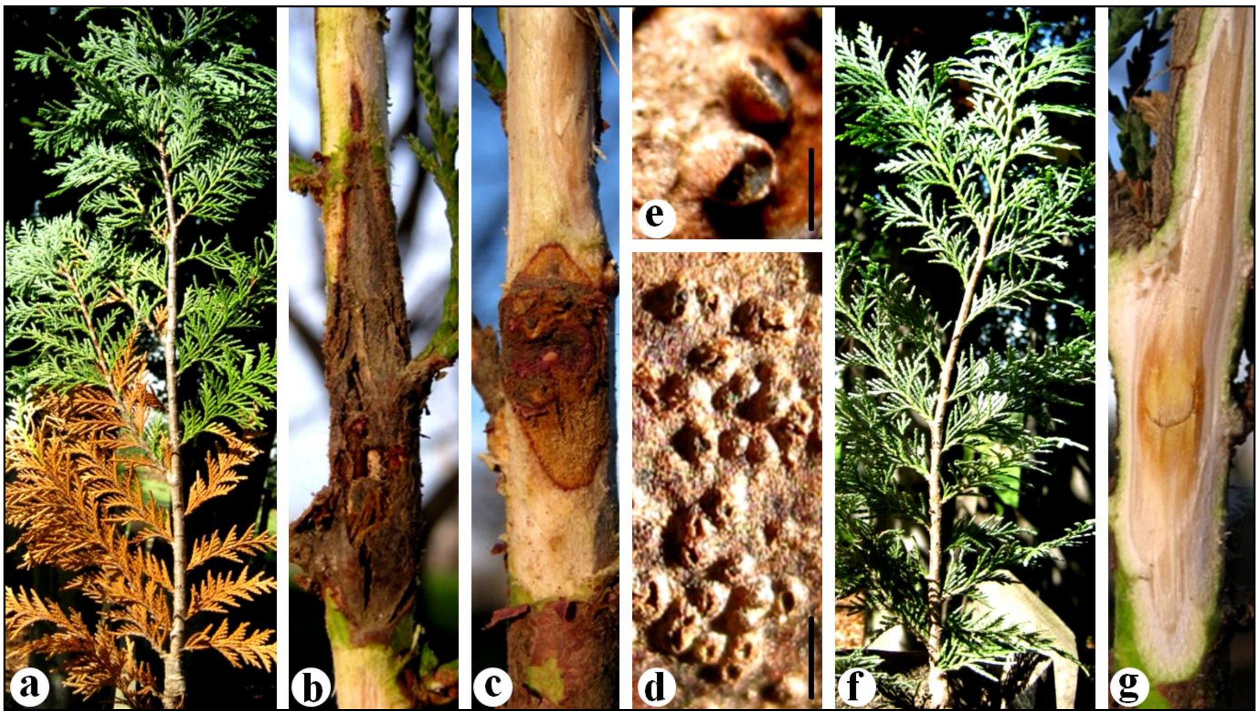

Figure S5. Elongated, girdling cankers with *D. seriata* pycnidia produced on canker surfaces of *Quercus robur* seedlings after inoculation with *D. seriata*: CMW 39382 (a, b), CMW 39376 (c-e). Bars: a, c-e= 1 cm, b= 0.5 cm.

Figure S6. Signs and symptoms associated with *Diplodia sapinea*, *Neofusicoccum parvum* and *Botryosphaeria dothidea* on inoculated *Picea abies* seedlings. a-c. Elongated, girdling, resin-soaked necrotic lesions formed after inoculation with: *B. dothidea* CMW 39301 (a), CMW 39315 (b), *N. parvum* CMW 39317 (c). d. Wilting of shoots and needle cast of seedlings inoculated with *N. parvum* CMW 39317. e. Control plant inoculated with sterile WA plug showing no disease symptoms.

Figure S7. Signs and symptoms associated with *Botryosphaeria dothidea* and *Diplodia mutila* on inoculated *Cupressus arizonica* seedlings. a, b. Elliptical necrotic lesions formed after inoculation with *D. mutila* CMW 39348. c-f. Elongated, girdling, sunken cankers with vertical cracks within the canker and along the canker margins formed after inoculation with *B. dothidea* CMW 39315. g. Pycnidia of *B. dothidea* formed in the cankered tissue after inoculation with *B. dothidea* CMW 39315.

Figure S8. Signs and symptoms associated with *Neofusicoccum parvum* on inoculated *Populus nigra* var. *italica* seedlings. a. Necrotic lesion formed after inoculation with *N. parvum* CMW 39317. b-e. Girdling, sunken cankers with cracks within the canker and *N. parvum* pycnidia produced after inoculation with *N. parvum* CMW 39327. f. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bars: c-e= 1 cm.

Figure S9. Signs and symptoms associated with *Botryosphaeria dothidea*, *Neofusicoccum parvum* and *Dothiorella omnivora* on inoculated *Sequoiadendron giganteum* seedlings. a. Shoot die-back from the inoculation point upwards; b. Girdling stem lesions; c. Control seedling inoculated with sterile WA plug showing no disease symptoms.

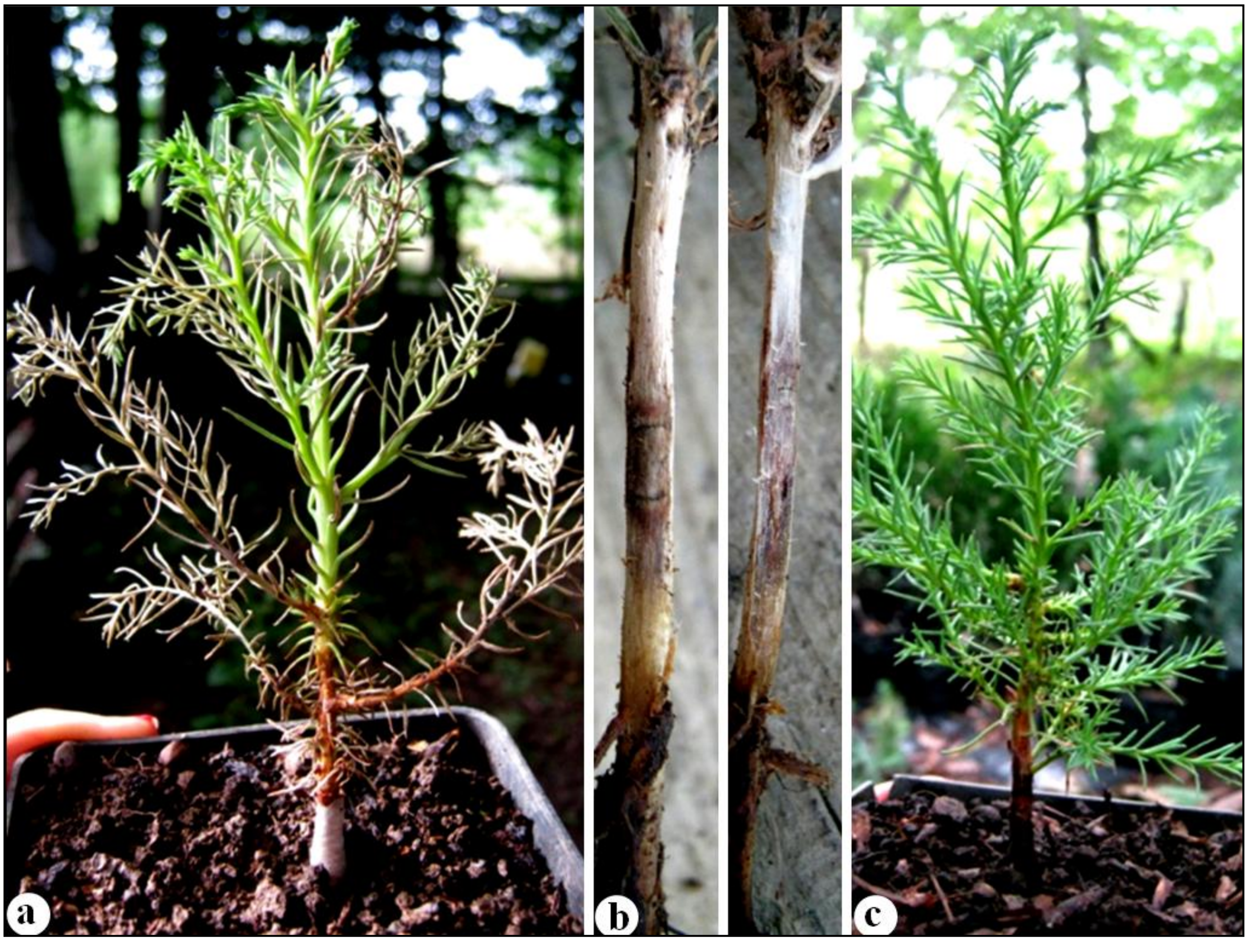

Figure S10. Signs and symptoms associated with *Diplodia seriata* and *Botryosphaeria dothidea* on inoculated *Abies concolor* seedlings. a. Stem die-back; b. Pycnidia of *D. seriata* formed on the dead bark after inoculation with *D. seriata* CMW 39376; c. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bar: b= 0.5cm.

Figure S11. Signs and symptoms associated with *Diplodia seriata*, *Neofusicoccum parvum* and *Botryosphaeria dothidea* on inoculated *Thuja occidentalis* seedlings. a. Yellowing, browning and reddening of the leaves; b. Black and necrotic leaves; c. Dry leaves; d. Control seedling inoculated with sterile WA plug showing no no disease symptoms.

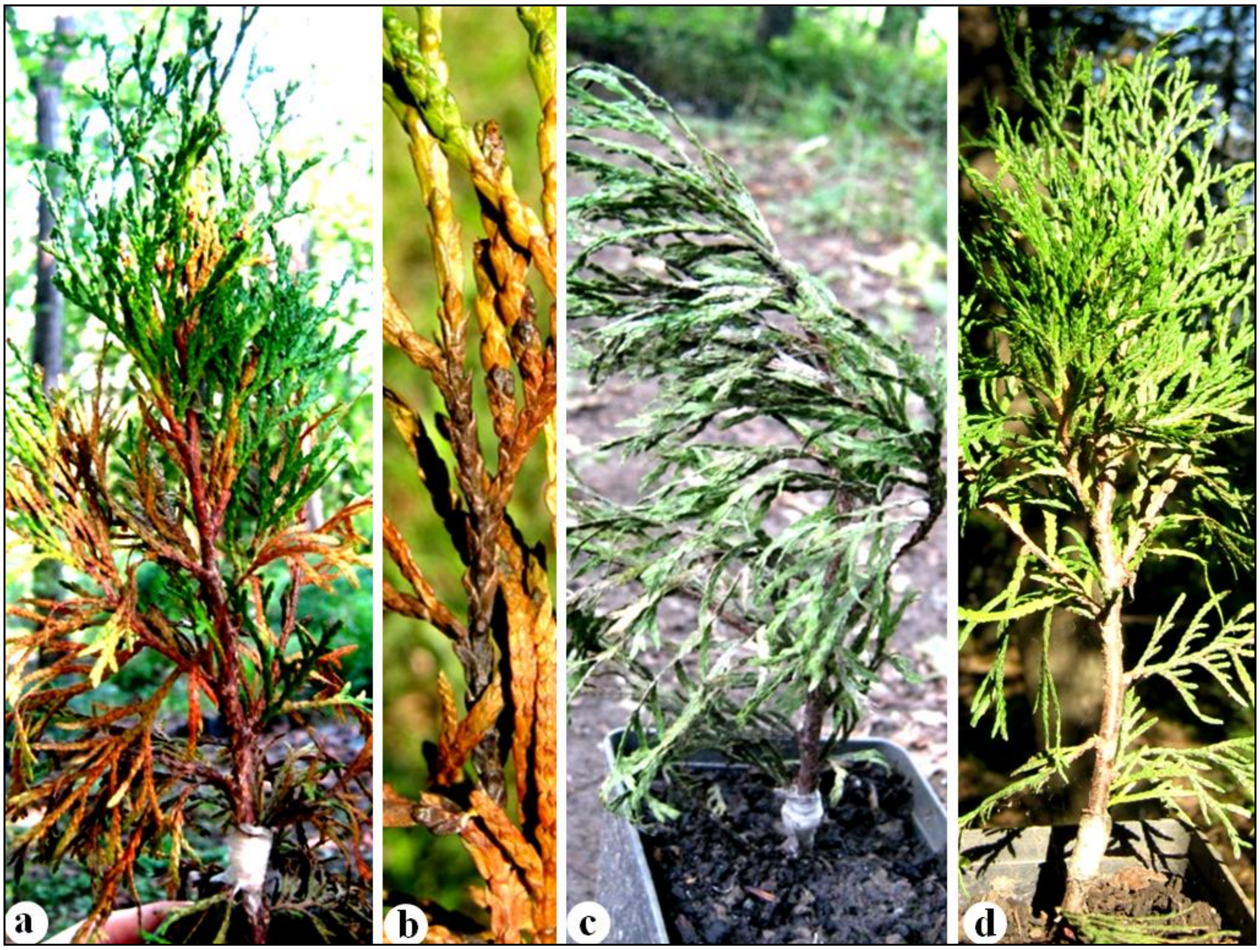

Figure S12. Signs and symptoms associated with *Diplodia mutila, D. seriata* and *Botryosphaeria dothidea* on inoculated *Cupressus sempervirens* seedlings. a. Yellowing of the needles and shoot die-back from the inoculation point upwards; b. Pycnidia of *D. seriata* produced in the dead stem tissue; c. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bar: b= 0.5cm.

Figure S13. Signs and symptoms associated with *Diplodia sapinea* and *Neofusicoccum parvum* on inoculated *Picea omorika* seedlings. a.Wilting of shoots and needle cast; b. Girdling stem lesions; c. Pycnidia produced in the dead stem tissue. d. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bar: c= 0.5cm. Data for *D. sapinea* were retreived from Zlatković et al. 2017.

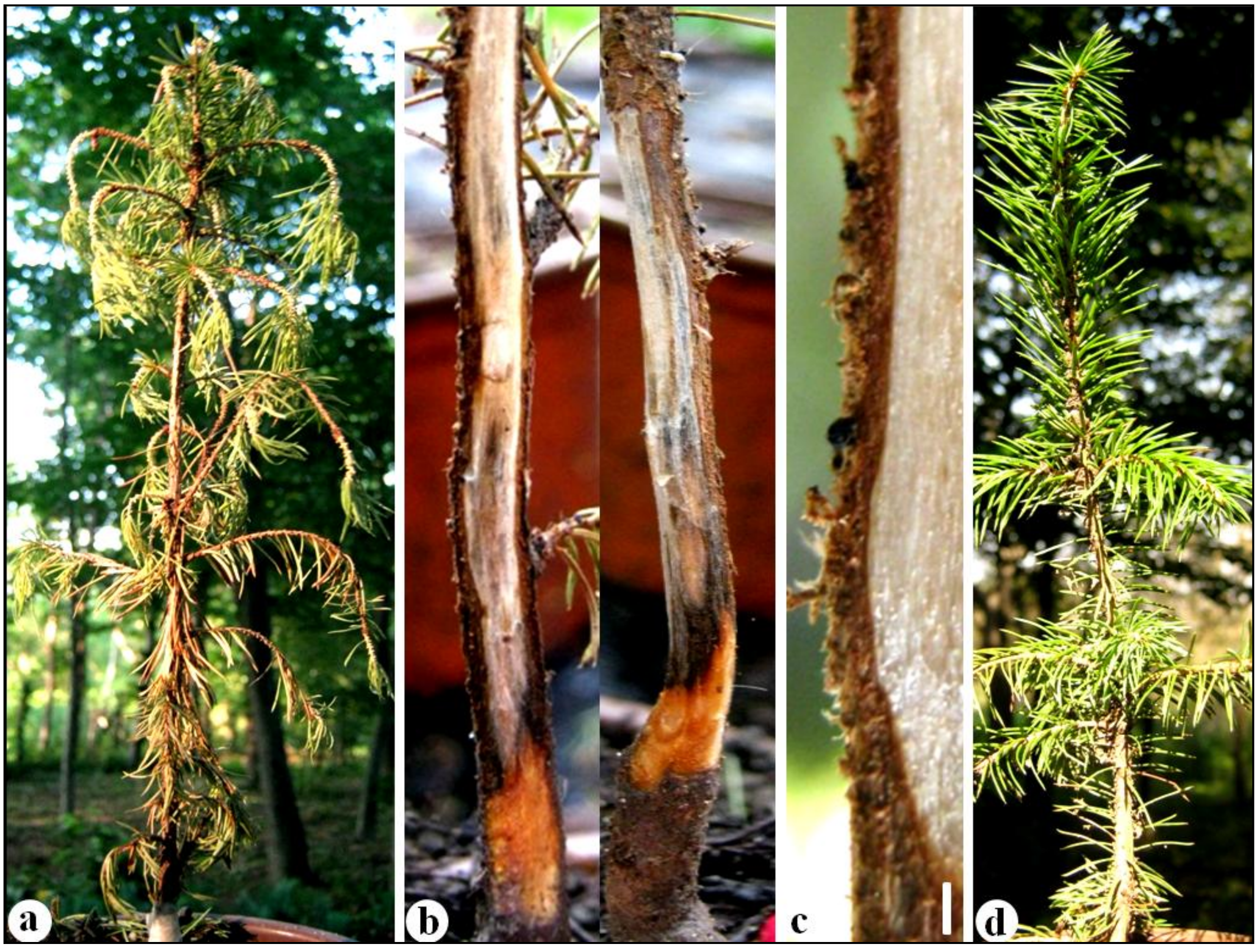

Figure S14. Signs and symptoms associated with *Diplodia seriata* and *Botryosphaeria dothidea* on inoculated *Ligustrum vulgare* and *Magnolia grandifolia* seedlings. a.Vascular discoloration of *L. vulgare* seedling inoculated with *D. seriata* CMW 39382; b. Control *L. vulgare* seedling inoculated with sterile WA plug showing no disease symptoms;c. Vascular discoloration of *M. grandifolia* seedling inoculated with *B. dothidea* CMW 39315; d. Pycnidia of *B. dothidea* produced on *M. grandifolia* seedling inoculated with *B. dothidea* CMW 39301. Control *M. grandifolia* seedling inoculated with sterile WA plug. Bar: d= 0.5cm.

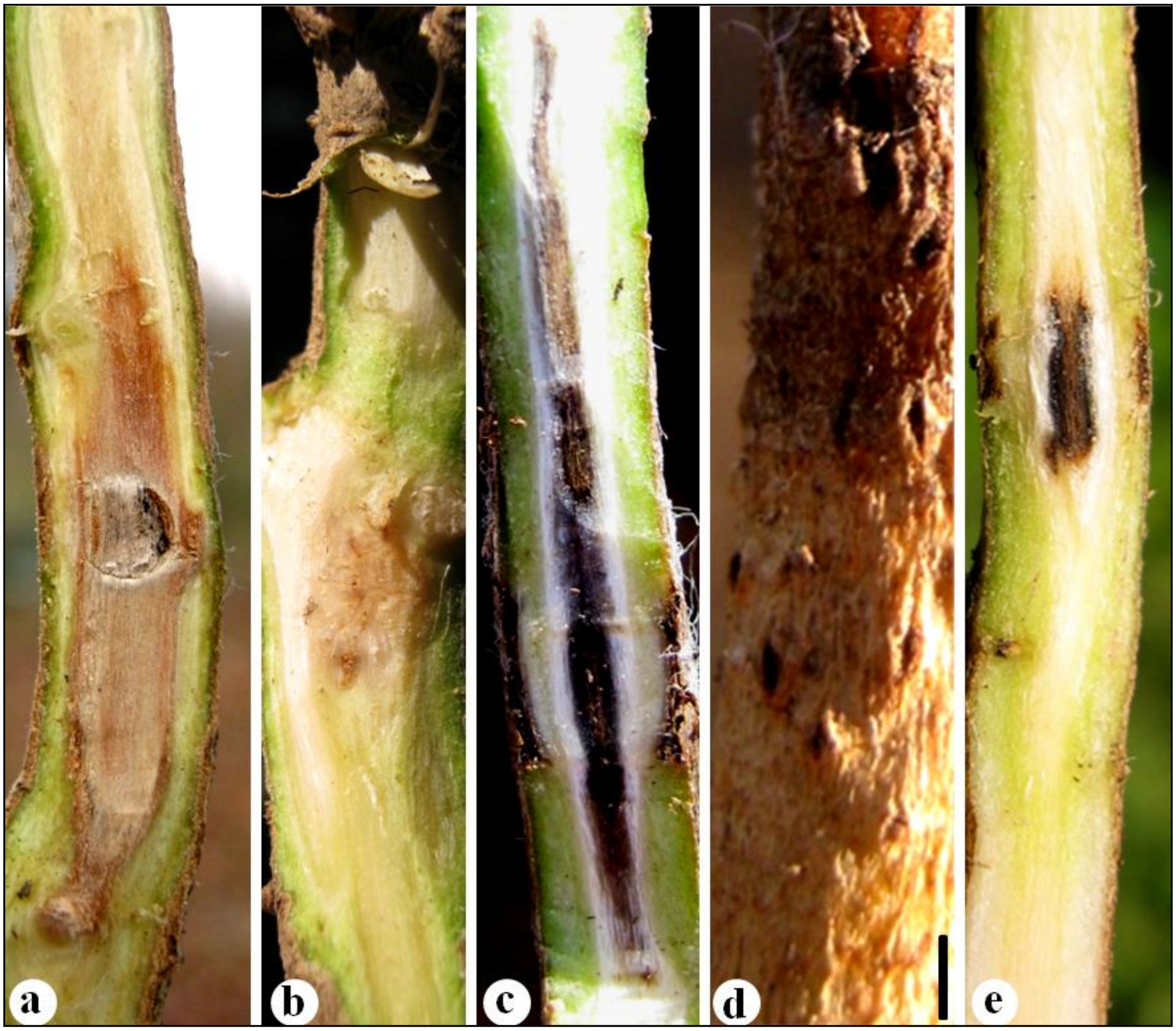

Figure S15. Signs and symptoms associated with *Botryosphaeria dothidea* on inoculated *Liriodendron tulipifera* seedlings. a. Lesion associated with *B. dothidea* CMW 39315; b. Stem die-back of seedling inoculated with *B. dothidea* CMW 39301; c, d. Pycnidia of *B. dothidea* produced in the dead stem tissue of seedlings inoculated with *B. dothidea* CMW 39301. e. Control seedling inoculated with sterile WA plug. Bars: c, d= 0.5cm.

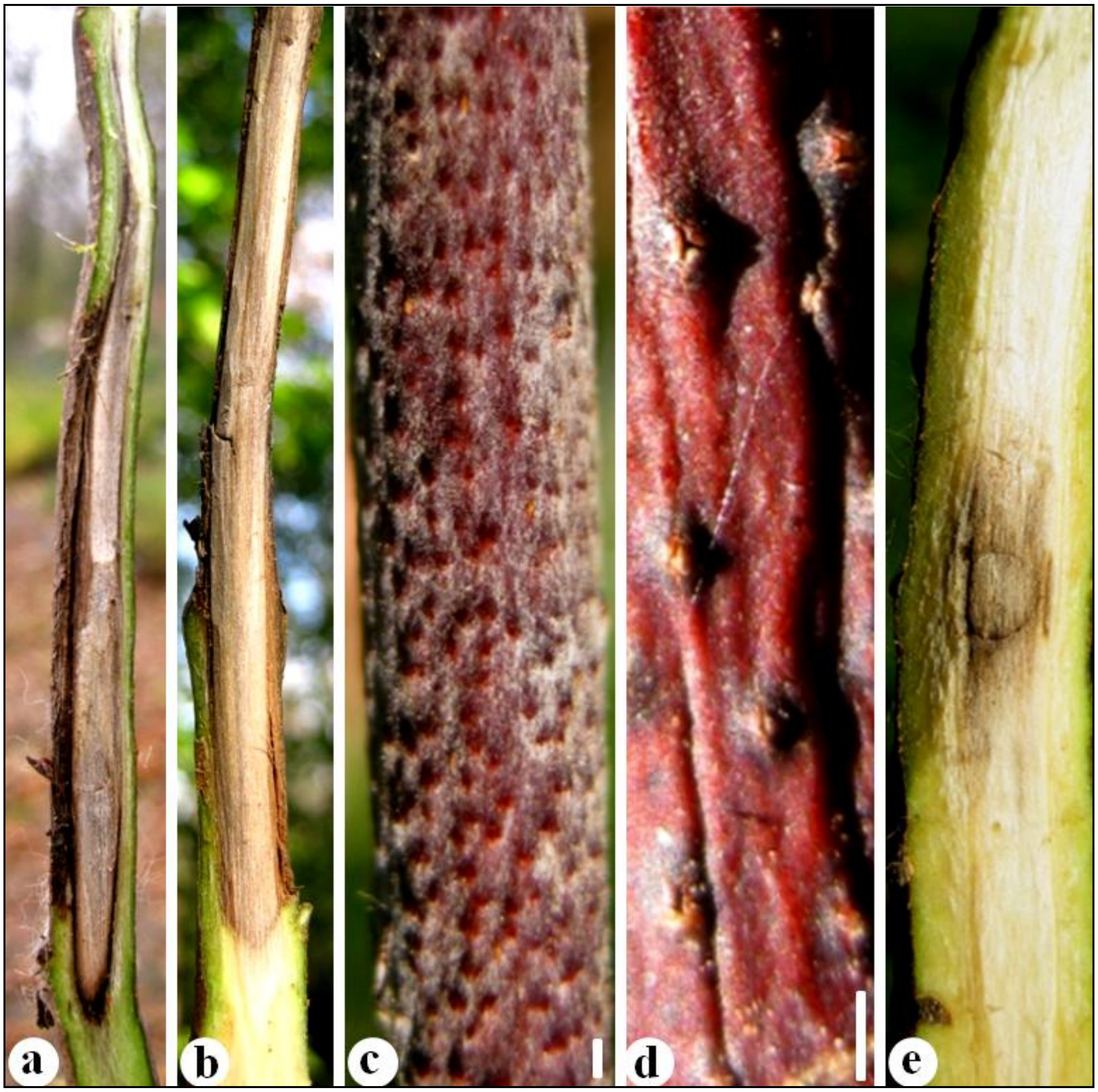

Figure S16. Signs and symptoms associated with *Diplodia sapinea* and *Botryosphaeria dothidea* on inoculated *Pseudotsuga menziesii* seedlings. a. Wilting of shoots, yellowing of the needles and needle desiccation of seedling inoculated with *D. sapinea*; b. Pycnidia of *D. sapinea* produced in the dead stem tissue of seedling inoculated with *D. sapinea* CMW 39329; c. Resinous lesion formed after inoculation with *B. dothidea* CMW 39301. d, e. Control seedling inoculated with sterile WA plug showing no disease symptoms. Bars: b= 0.5cm. Data for *D. sapinea* were retreived from Zlatković et al. 2017 and serve for comparison with inoculations conducted with *B. dothidea*.

Figure S17. Signs and symptoms associated with *Neofusicoccum parvum*, *Diplodia sapinea* and *Botryosphaeria dothidea* on inoculated *Cedrus atlantica* and *Juniperus horizontalis* seedlings. a.Girdling lesions formed on *C. atlantica* after inoculation with *N. parvum* CMW 39327; b. Control *C. atlantica* seedling inoculated with sterile WA plug; c. Necrotic lesion formed on *J. horizontalis* after inoculation with *B. dothidea* CMW 39315. d. Necrotic lesion formed on *J. horizontalis* after inoculation with *D. sapinea* CMW 39329. e. Control *J. horizontalis* seedling inoculated with sterile WA plug. Data for *D. sapinea* were retreived from Zlatković et al. 2017.

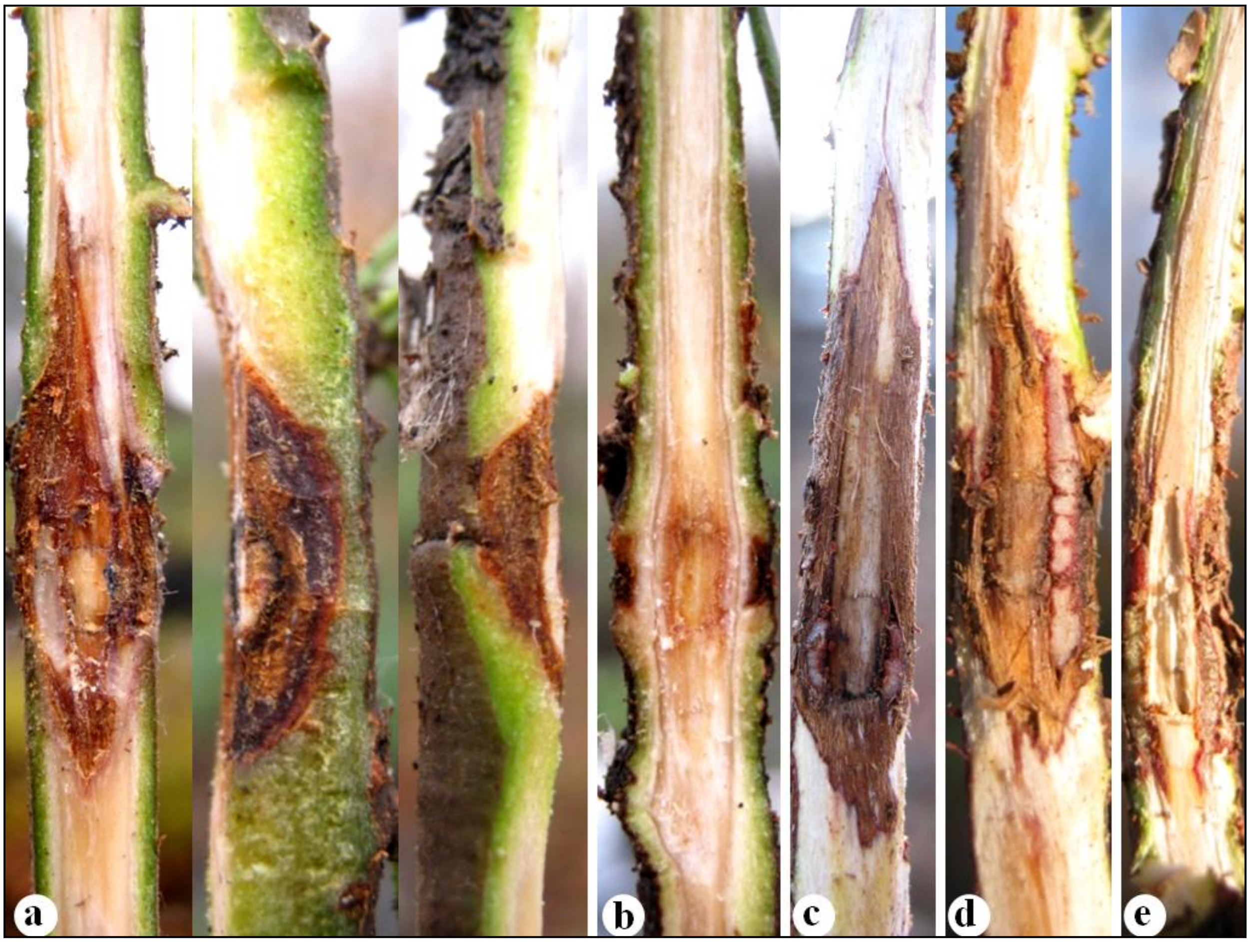

Figure S18. Signs and symptoms associated with *Neofusicoccum parvum* and *Diplodia mutil*a on inoculated *Pittosporum tobira* and *Prunus laurocerasu*s seedlings. a.Elongated necrotic lesion formed on *P. tobira* after inoculation with *N. parvum* CMW 39327; b. Control *P. tobira* seedling inoculated with sterile WA plug; c. Reddish-brown elliptical cankers with cracks inside the canker and along the canker margin formed on *P. laurocerasus* after inoculation with *N. parvum* CMW 39327; d. Dark brown lesions formed on *P. laurocerasus* after inoculation with *D. mutila* CMW 39348; e Dark brown lesions formed on a control *P. laurocerasus* seedling after inoculation with sterile WA.

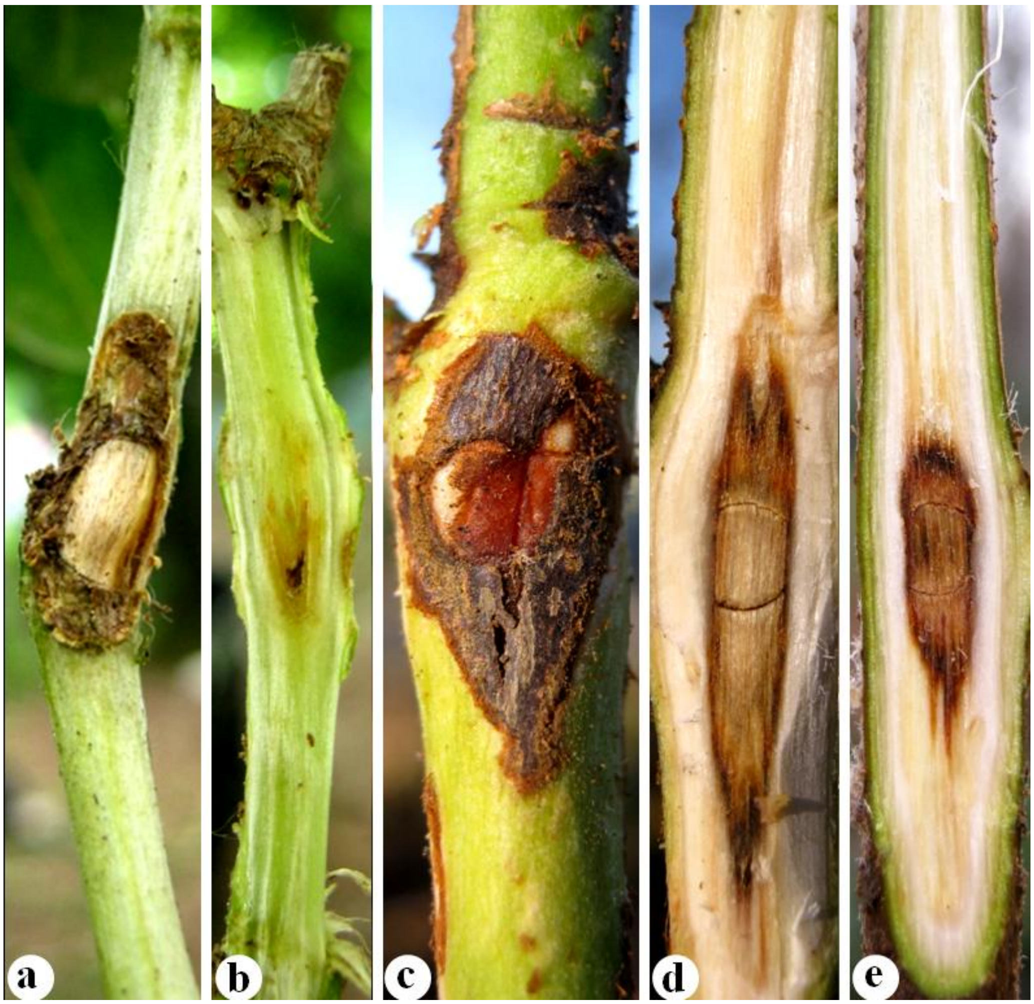

Figure S19. Signs and symptoms associated with *Botryosphaeria dothidea*, *Neofusicoccum parvum*, *Diplodia mutila* and *Dothiorella sarmentorum* on inoculated *Aesculus hippocastanum* branches. a, b. Pycnidia of *Neofusicoccum parvum* formed in the dead branch tissue after inoculation with *N. parvum* CMW 39327; c. Darkening of the vascular tissue associated with *B. dothidea* CMW 39301; Girdling lesion formed after inoculation with *Do. sarmentorum* CMW 39365.

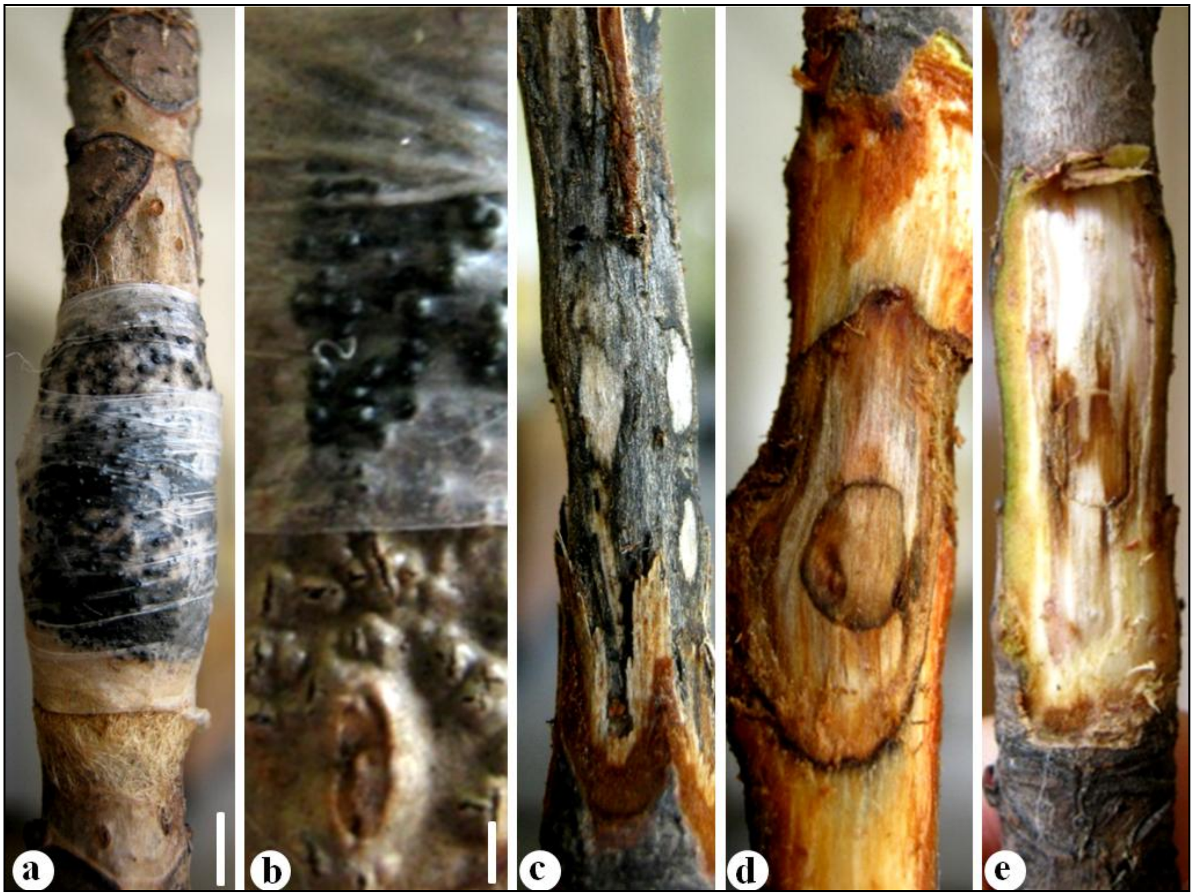

Figure S20. Signs and symptoms associated with *Botryosphaeria dothidea* and *Diplodia seriata* on inoculated *Populus tremula*, *Quercus cerris* and *Fraxinus excelsior* branches. a. Lesion associated with *B. dothidea* CMW 39315 on inoculated *P. tremula* branch; b. Girdling lesion associated with *B. dothidea* CMW 39301 on inoculated *P. tremula* branch; c. Control *P. tremula* branch inoculated with sterile WA plug; d. Lesion associated with *B. dothidea* CMW 39315 on inoculated *Q. cerris* branch; e. Control *Q. cerris* branch inoculated with sterile WA plug; f. Lesion associated with *D. seriata* CMW 39376 on inoculated *F. excelsior* branch; g. Pycnidia of *D. seriata* produced on *F. excelsior* branch after inoculation with *D. seriata* CMW 39376; h. Control *F. excelsior* branch inoculated with sterile WA plug.

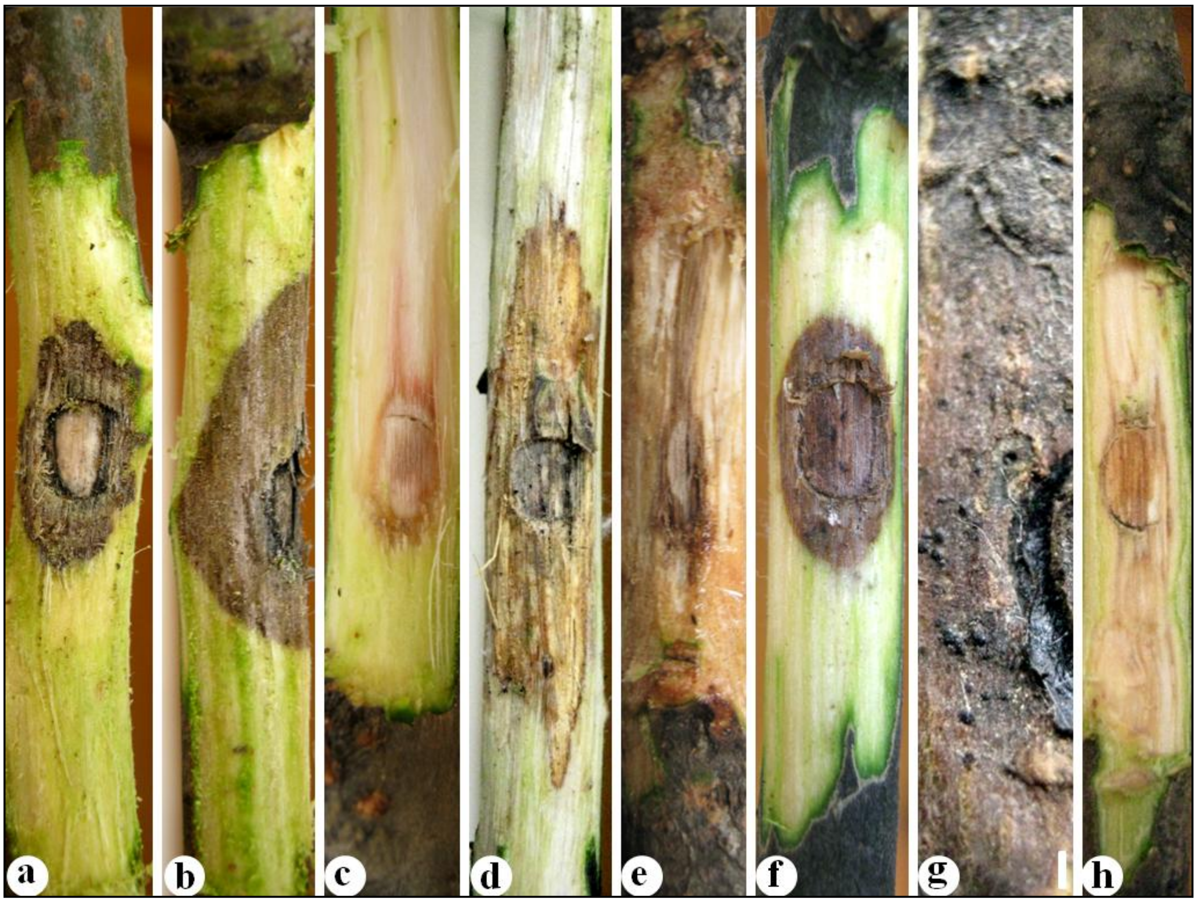

Figure S21. Signs and symptoms associated with *Sphaeropsis visci* on inoculated *Viscum album* branches. a. Necrotic lesion ; b. Pycnidia of *S. visci* formed in the dead branch tissue, exuding spores in white cirrhi; c. Pycnidia of *S. visci* formed in the dead leaf tissue; Control leaf inoculated with sterile WA plug; e. Control branch inoculated with sterile WA plug. Bars: b= 0.5cm, c= 1cm.

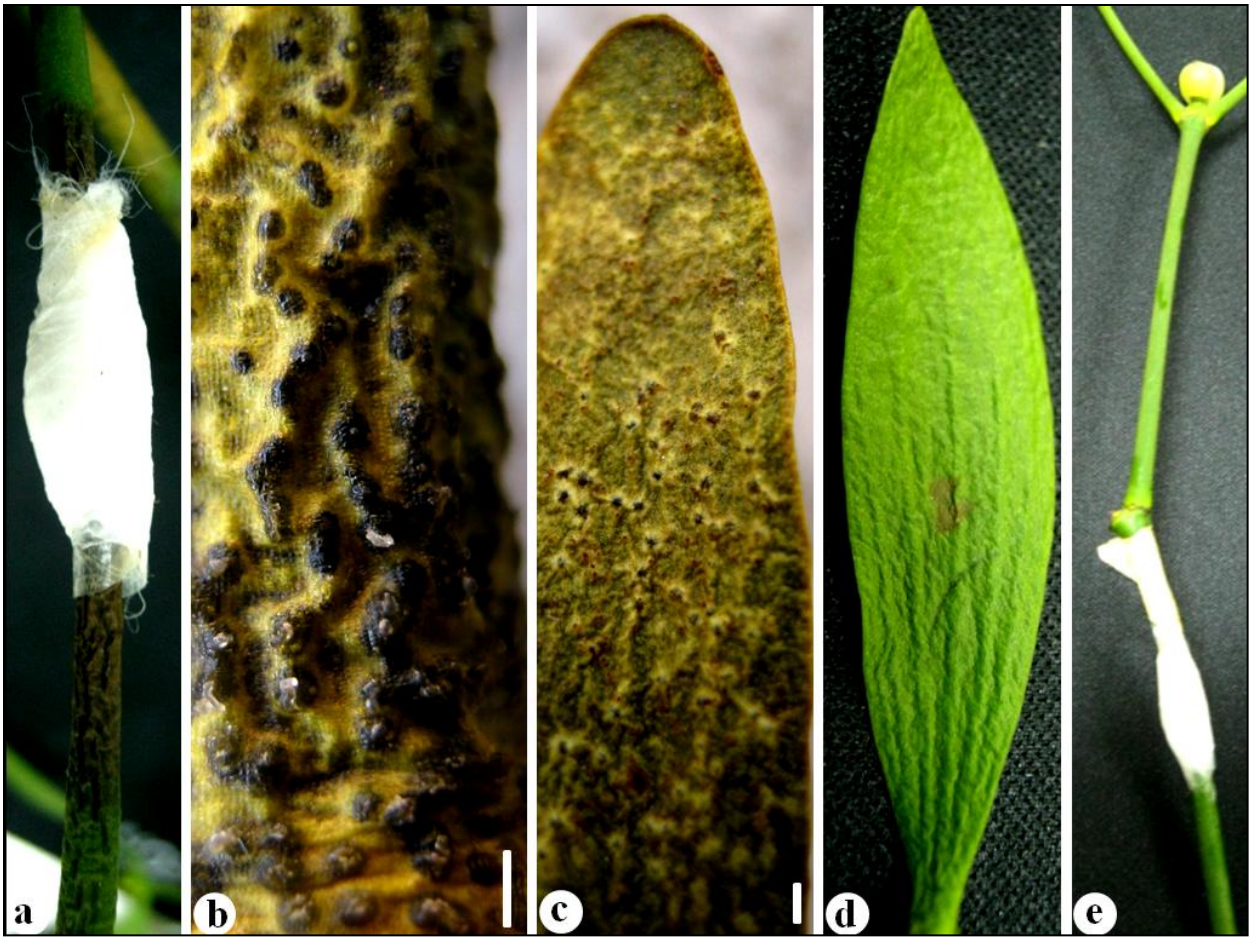

Figure S22. Signs and symptoms associated with *Neofusicoccum parvum* CMW 39317 on inoculated *Prunus laurocerasus* leaves.a. A hole in the leaf and necrotic lesion extending to cells beyond the hole, b. Necrotic lesion progressing from the inoculation point upwards and downwards, c. Necrotic lesion showing concentric rings and *N. parvum* pycnidia embedded within the lesion; d. Necrotic leaf with *N. parvum* pycnidia in the dead leaf tissue; e. Shoot die-back; f. Pycnidia of *N. parvum* produced in the dead shoot tissue. Bars: c, d, e= 1cm.

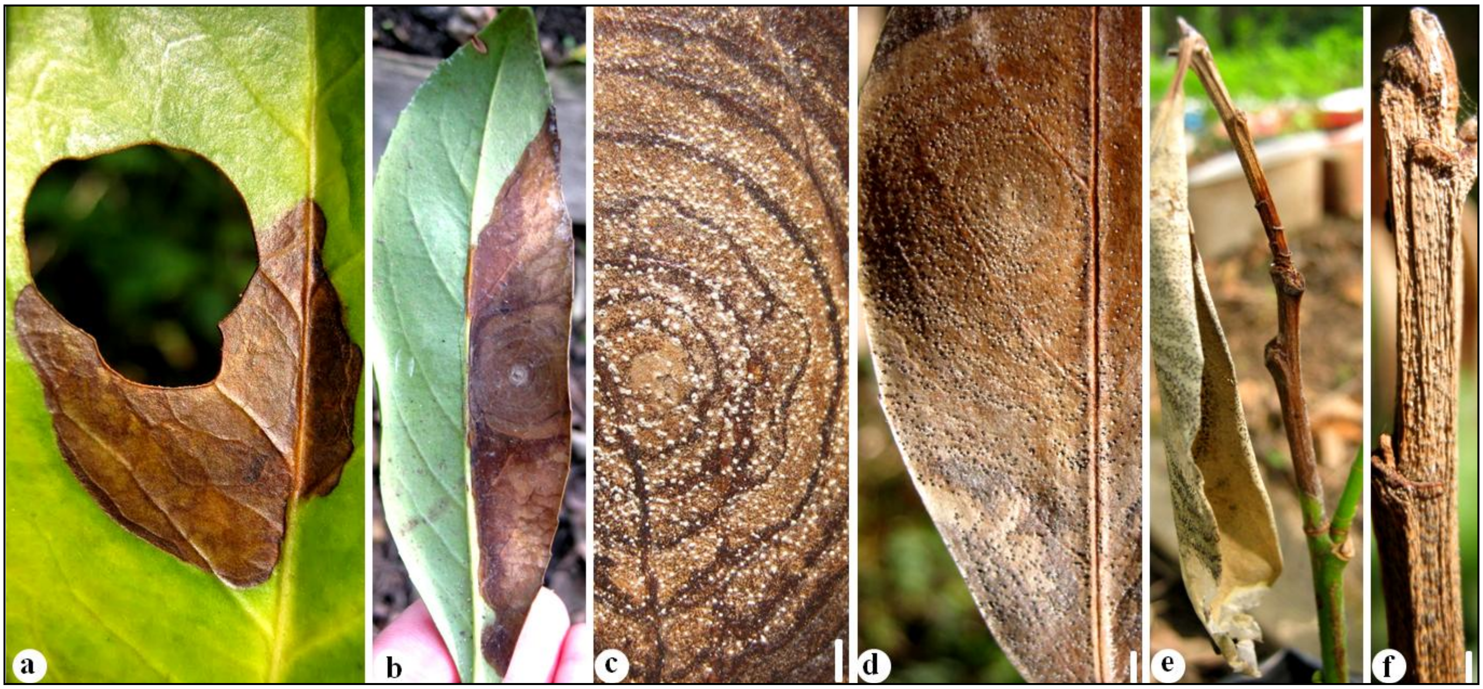

Figure S23. Signs and symptoms associated with *Diplodia sapinea*, *Neofusicoccum parvum* and *Botryosphaeria dothidea* on inoculated *Pinus patula* seedlings.a. Resin-soaked lesion associated with *D. sapinea* CMW 39329; b. resin bleeding associated with *D. sapinea* CMW 39329; c. Resinous lesion associated with *N. parvum* CMW 39317; d. Pycnidia of *N. parvum* produced after inoculation of *P. patula* with *N. parvum* CMW 39317; e. Resinous lesion associated with *B. dothidea* CMW 39301; f. Control seedling inoculated with sterile WA plug.
Bar: d= 1cm. Data for *D. sapinea* were retreived from Zlatković et al. 2017 and serve for comparison with inoculations conducted with *N. parvum* and *B. dothidea*.

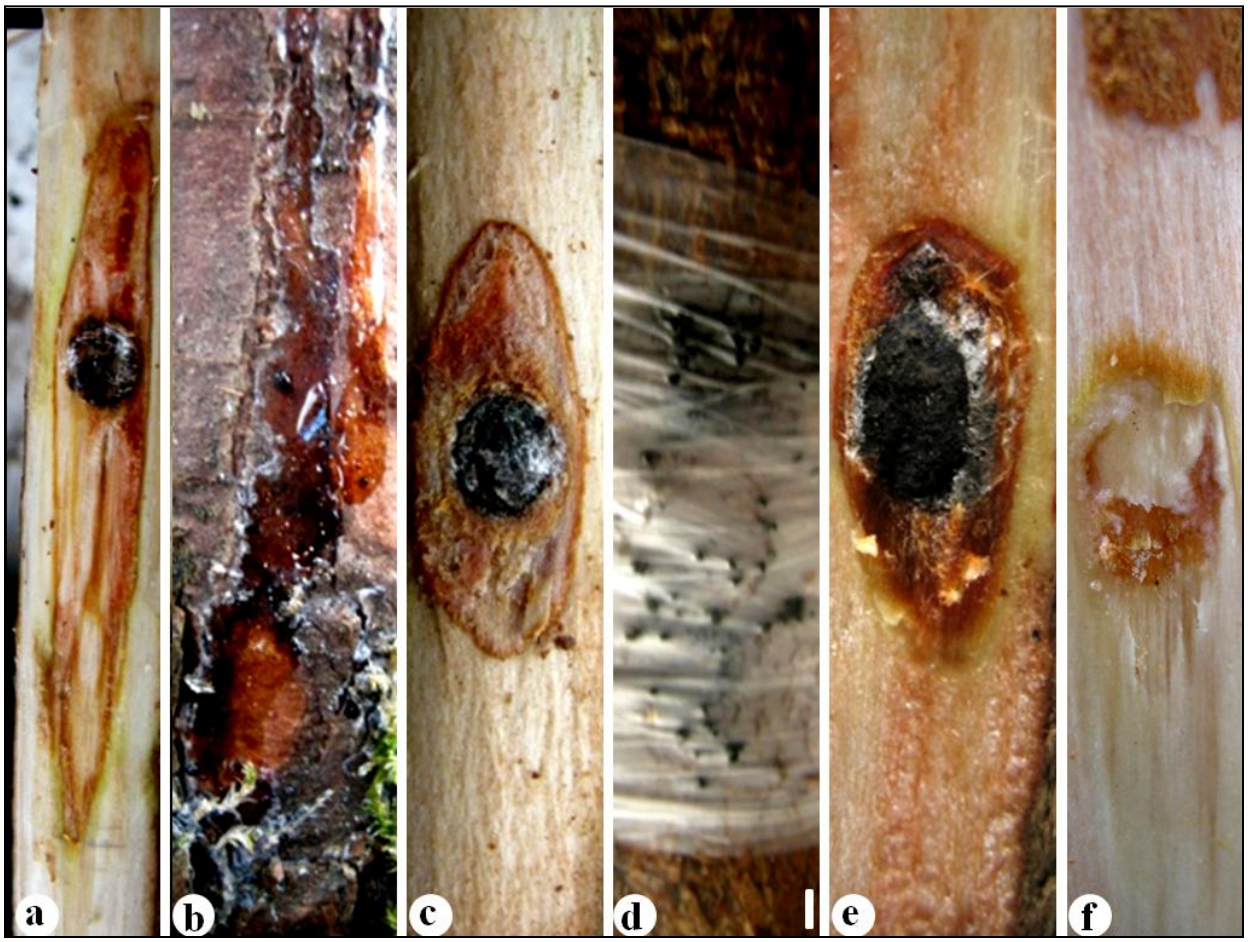

Figure S24. Signs and symptoms associated with *Diplodia sapinea*, *Neofusicoccum parvum* and *Botryosphaeria dothidea* on inoculated *Eucalyptus grandis* seedlings. a. Lesions associated with: a. *N. parvum* CMW 39317; b. *N. parvum* CMW 39327; c. *B. dothidea* CMW 39315; d. *B. dothidea* CMW 39301; e. *D. sapinea* CMW 39337; f. *D. sapinea* CMW 39329. c. Control seedling inoculated with sterile WA plug. Data for *D. sapinea* were retreived from Zlatković et al. 2017 and serve for comparison with inoculations conducted with *N. parvum* and *B. dothidea*.

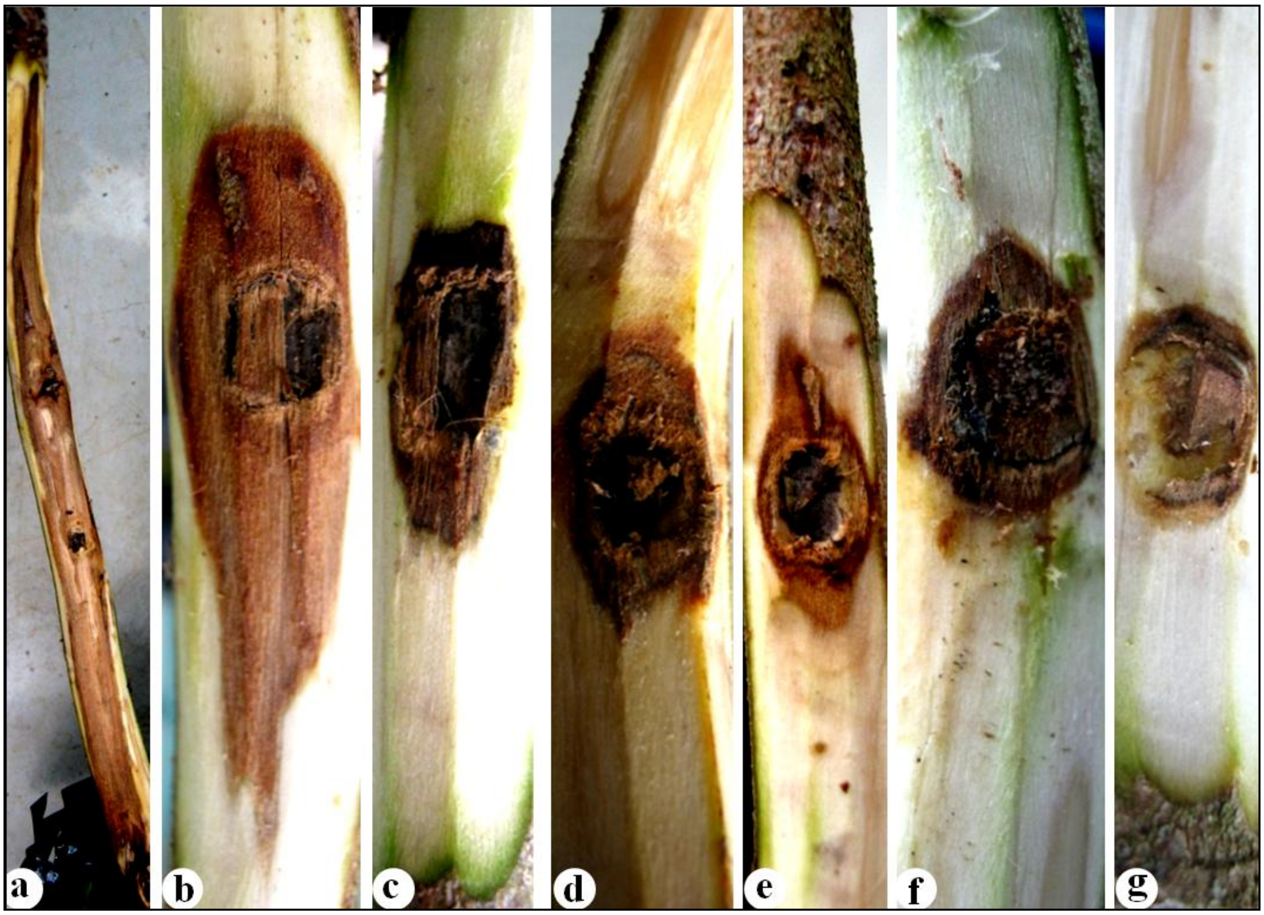

Table S1. Trees and shrubs from which Botryosphaeriaceae were isolated in this study. Shrubby species are given in bold.

	Trees/shrubs sampled in cities	**Trees/shrubs sampled in forests**	**Nursery seedlings**
Species (tree/shrub)	*Thuja occidentalis*	*Quercus robur*	*Magnolia grandifolia*
	Thuja plicata	*P. abies*	*Chamaecyparis pisifera*
	Fraxinus excelsior	*Quercus cerris*	*T. occidentalis*
	Cedrus atlantica		
	Abies concolor		
	Chamaecyparis lawsoniana		
	Chamaecyparis obtusa		
	Cupressus sempervirens		
	Cupressus arizonica		
	Pinus halepensis		
	Picea abies		
	Pseudotsuga menziesii		
	Aesculus hippocastanum		
	Sequoia sempervirens		
	Sequoiadendron giganteum		
	Quercus ilex		
	Platanus acerifolia		
	Liriodendron tulipifera		
	Cryptomeria japonica		
	Forsythia europaea		
	Populus nigra var. *italica*		
	Salix caprea		
	Quercus rubra		
	Taxus baccata		
	Populus tremula		
	Prunus cerasus		
	Juniperus horizontalis		
	Pittosporum tobira		
	Prunus laurocerasus		
	Ligustrum vulgare		
No. of sampled trees/shrubs	85	4	4

Table S2. Isolates used in the phylogenetic analyses.

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CBS 112555	*Diplodia seriata*	*Vitis vinifera*	Portugal	A. J. L. Phillips	AY259094	AY573220	DQ458856	-
CMW 39385	*D. seriata*	*Rubus fruticosus*	Bosnia and Herzegovina [1]	D. Karadžić	KF574991	KF575023	KF575087	-
CMW 39384	*D. seriata*	*Thuja occidentalis*	Belgrade, Serbia	M. Zlatković	KF574992	KF575024	KF575088	-
CMW 39378	*D. seriata*	*Cedrus atlantica*	Belgrade, Serbia	M. Zlatković	KF574994	KF575031	KF575090	-
CMW 39374	*D. seriata*	*Fraxinus excelsior*	Bosnia and Herzegovina	D. Karadžić	KF574995	KF575026	KF575091	-
CMW 39376	*D. seriata*	*Chamaecyparis pisifera*	Belgrade, Serbia [2]	M. Zlatković	KF574996	KF575027	KF575092	-
CMW 39379	*D. seriata*	*Ligustrum vulgare*	Belgrade, Serbia	M. Zlatković	KF574997	KF575032	KF575093	-
BOT 221	***D. seriata***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729250	-	-	-
CMW 39380	***D. seriata***	*Abies concolor*	Pirot, Serbia	M. Zlatković	KF729251	KT987409	-	-
CMW 39381	***D. seriata***	*Chamaecyparis lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729252	-	-	-
BOT 247	***D. seriata***	*L. vulgare*	Belgrade, Serbia	M. Zlatković	KF729253	-	-	-
BOT 158	***D. seriata***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729254	-	-	-
BOT 231	***D. seriata***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729255	-	-	-
CMW 39382	***D. seriata***	*Quercus robur*	Morović, Serbia [1]	M. Zlatković	KF729256	KT987410	-	-
CMW 39383	***D. seriata***	*Thuja pilcata*	Belgrade, Serbia	M. Zlatković	KF729257	KT987411	-	-
BOT 252	***D. seriata***	*T. occidentalis*	Pirot, Serbia	M. Zlatković	KF729258	-	-	-
CMW 39375	***D. seriata***	*Cupressus sempervirens*	Bijeljina, Bosnia and Herzegovina	D. Karadžić	KF729259	KT987412	-	-
BOT 193	***D. seriata***	*F. excelsior*	Bosnia and Herzegovina	D. Karadžić	KF729260	-	-	-
BOT 219	***D. seriata***	*T. occidentalis*	Belgrade, Serbia	M. Zlatković	KF729261	-	-	-
CMW 45092	***D. seriata***	*Salix caprea*	Pirot, Serbia	M. Zlatković	KT964308	-	-	-
BOT 284	***D. seriata***	*S. caprea*	Pirot, Serbia	M. Zlatković	KT964309	-	-	-
CMW 45094	***D. seriata***	*Quercus rubra*	Belgrade, Serbia	M. Zlatković	KT964311	-	-	-
CMW 45099	***D. seriata***	*Prunus cerasus*	Pirot, Serbia	M. Zlatković	KT964310	-	-	-
BOT 304	***D. seriata***	*C. sempervirens*	Belgrade, Serbia	M. Zlatković	KT964312	-	-	-
CBS 119049	*D. seriata*	*Vitis sp.*	Italy	L. Mugnai	DQ458889	DQ458874	DQ458857	-
CBS 393.84	*Diplodia sapinea*	*Pinus nigra*	Netherlands	H. A. van der Aa	DQ458895	DQ458880	DQ458863	-
CMW 39341	*D. sapinea*	*Cedrus deodara*	Podgorica, Montenegro	M. Zlatković	KF574998	KF575028	KF575094	-
CMW 39346	*D. sapinea*	*Picea omorika*	Belgrade, Serbia	M. Zlatković	KF575000	KF575030	KF575096	-
CMW 39338	*D. sapinea*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF574999	KF575029	KF575095	-
CMW 39336	*D. sapinea*	*Fagus sylvatica*	Mt. Rudnik, Serbia [1]	N. Keča	KF729247	KF729481	-	-
CMW 39335	*D. sapinea*	*Pinus halepensis*	Belgrade, Serbia	M. Zlatković	KF729170	KF729404	-	-
CMW 39330	*D. sapinea*	*Juniperus horizontalis*	Belgrade, Serbia	M. Zlatković	KF729173	KF729407	-	-
CMW 39343	*D. sapinea*	*P. sylvestris*	Niš, Serbia	M. Zlatković	KF729195	KF729429	-	-
CMW 39345	*D. sapinea*	*Pinus pinea*	Budva , Montenegro	M. Zlatković	KF729188	KF729422	-	-
CMW 39344	*D. sapinea*	*Picea pungens*	Niš, Serbia	M. Zlatković	KF729190	KF729424	-	-
CMW 39342	*D. sapinea*	*A. concolor*	Belgrade, Serbia	M. Zlatković	KF729199	KF729433	-	-
CMW 39339	*D. sapinea*	*Pinus pinaster*	Miločer, Montenegro	M. Zlatković	KF729205	KF729439	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CMW 39334	*D. sapinea*	*Pseudotsuga menziesii*	Belgrade, Serbia	M. Zlatković	KF729233	KF729467	-	-
CMW 39332	*D. sapinea*	*P. nigra*	Pirot, Serbia	M. Zlatković	KF729235	KF729469	-	-
CMW 39331	*D. sapinea*	*Cedrus libani*	Podgorica, Montenegro	M. Zlatković	KF729236	KF729470	-	-
CMW 39333	*D. sapinea*	*C. lawsoniana*	Herceg Novi, Montenegro	M. Zlatković	KF729239	KF729473	-	-
CMW 39347	*D. sapinea*	*Pinus radiata*	Mt. Athos, Greece [3]	D. Karadžić	KT749856	KT749858	-	-
CBS 109725	*D. sapinea*	*Pinus patula*	South Africa	M. J. Wingfield	DQ458896	DQ458881	DQ458864	-
CBS 112556	*Diplodia intermedia*	*Malus domestica*	Aveiro, Portugal	A. Alves	GQ923857	GQ923850	-	-
CBS 124462	*D. intermedia*	*Malus sylvestris*	Portugal	A. J. L. Phillips	GQ923858	GQ923826	-	-
CBS 118110	*Diplodia scrobiculata*	*Pinus banksiana*	USA	M. A. Palmer	KF766160	AY624258	AY624258	-
CBS 113423	*D. scrobiculata*	*Pinus greggii*	Mexico	M. J. Wingfield	DQ458900	DQ458885	DQ458868	-
CBS 136014	*Diplodia mutila*	*Populus alba*	Portugal	A. Alves	KJ361837	KJ361829	-	-
CMW 39356	*D. mutila*	*Aesculus hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF575001	KF575033	KF575097	-
CMW 39354	*D. mutila*	*P. halepensis*	Herceg Novi, Montenegro	M. Zlatković	KF575002	KF575034	KF575098	-
CMW 39353	*D. mutila*	*Cupressus arizonica*	Herceg Novi, Montenegro	M. Zlatković	KF575003	KF575035	KF575099	-
CMW 39349	***D. mutila***	*Pittosporum tobira*	Herceg Novi, Montenegro	M. Zlatković	KF729262	-	-	-
CMW 39351	***D. mutila***	*Chamaecyparis obtusa*	Kanjiža, Serbia	N. Keča	KF729263	-	-	-
CMW 39357	***D. mutila***	*Sequoia sempervirens*	Belgrade, Serbia	M. Zlatković	KF729264	-	-	-
BOT 129	***D. mutila***	*C. arizonica*	Belgrade, Serbia	M. Zlatković	KF729265	-	-	-
BOT 105	***D. mutila***	*Prunus laurocerasus*	Budva, Montenegro	M. Zlatković	KF729266	-	-	-
CMW 39352	***D. mutila***	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729267	-	-	-
CMW 39350	***D. mutila***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729268	-	-	-
BOT 116	***D. mutila***	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729269	-	-	-
BOT 161	***D. mutila***	*C. lawsoniana*	Herceg Novi, Montenegro	M. Zlatković	KF729270	-	-	-
CMW 39355	***D. mutila***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729271	-	-	-
BOT 151	***D. mutila***	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729272	-	-	-
CMW 39358	***D. mutila***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729273	-	-	-
CMW 39348	***D. mutila***	*C. sempervirens*	Herceg Novi, Montenegro	M. Zlatković	KF729274	-	-	-
BOT 218	***D. mutila***	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729275	-	-	-
BOT 217	***D. mutila***	*C. atlantica*	Herceg Novi, Montenegro	M. Zlatković	KF729276	-	-	-
CBS 112553	*D. mutila*	*V. vinifera*	Portugal	A. J. L. Phillips	AY259093	AY573219	DQ458850	-
CBS 124133	*Diplodia subglobosa*	*Lonicera nigra*	Spain	J. Luque	GQ923856	GQ923824	-	-
CBS 124132	*D. subglobosa*	*F. excelsior*	Spain	J. Luque	DQ458887	DQ458871	-	-
CBS 122527	*Sphaeropsis visci*	*Viscum album*	Ukraine	Á. Akulov	EU673327	-	-	-
CMW 39386	*S. visci*	*V. album*	Mt Goč, Serbia [1]	N. Keča	KF575004	KF575036	KF575100	-
CBS 100163	*S. visci*	*V. album*	Luxemburg	H.A. van der Aa	EU673324	EU673292	EU673127	-
CBS 110496	*Sphaeropsis porosa*	*V. vinifera*	South Africa	J. M. van Niekerk	AY343378	AY343339	EU673130	-
CBS 110574	*S. porosa*	*V. vinifera*	South Africa	J. M. van Niekerk	AY343378	AY343339	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
IRAN 1458c	*Phaeobotryon cupressi*	*Cupressus sempervirens*	Gorgan, Iran	M. A. Aghajani	FJ919671	FJ919660	-	-
CMW 39387	*P. cupressi*	*C. sempervirens*	Podgorica, Montenegro	M. Zlatković/ J. Lazarević	KF575005	KF575037	KF575101	-
CBS 124700	*P. cupressi*	*C. sempervirens*	Gorgan, Iran	M. A. Aghajani	FJ919672	FJ919661	-	-
CBS 122980	*Phaeobotryon mamane*	*Sophora chrysophylla*	Hawaii	W. Gams	EU673332	EU673298	EU673121	-
CPC 12445	*P. mamane*	*S. chrysophylla*	Hawaii	W. Gams	EU673336	EU673302	EU673122	-
CMW 8000	*Botryosphaeria dothidea*	*Prunus sp.*	Switzerland	B. Slippers	AY236949	AY236898	AY23692	-
CMW 39302	*B. dothidea*	*Pseudotsuga menziesii*	Belgrade, Serbia	M. Zlatković	KF575006	KF575038	KF575102	-
CMW 39304	*B. dothidea*	*Sequoia sempervirens*	Belgrade, Serbia	M. Zlatković	KF575007	KF575039	KF575103	-
CMW 39308	*B. dothidea*	*Sequoiadendron giganteum*	Valjevo, Serbia	N. Keča	KF575008	KF575040	KF575104	-
BOT 45	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729086	-	-	-
BOT 67	***B. dothidea***	*Quercus ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729087	-	-	-
BOT 77	***B. dothidea***	*Q. ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729088	-	-	-
BOT 48	***B. dothidea***	*Q. ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729089	-	-	-
BOT 19	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković/ N. Keča	KF729090	-	-	-
BOT 46	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729091	-	-	-
BOT 83	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729092	-	-	-
CMW 39313	***B. dothidea***	*J. horizontalis*	Budva, Montenegro	M. Zlatković	KF729093	-	-	-
BOT 278	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729094	-	-	-
CMW 39310	***B. dothidea***	*Magnolia grandifolia*	Belgrade, Serbia [2]	N. Keča	KF729095	-	-	-
BOT 35	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729096	-	-	-
BOT88	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729097	-	-	-
BOT 37	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729098	-	-	-
CMW 39314	***B. dothidea***	*Picea abies*	Mt. Goč, Serbia [1]	D. Karadžić	KF729099	-	-	-
BOT 74	***B. dothidea***	*P. menziesii*	Belgrade, Serbia	M. Zlatković	KF729100	-	-	-
BOT 134	***B. dothidea***	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729101	-	-	-
BOT 4	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729102	-	-	-
BOT 24	***B. dothidea***	*Platanus acerifolia*	Bosnia and Herzegovina	D. Karadžić	KF729103	-	-	-
BOT 21	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729104	-	-	-
CMW 39311	***B. dothidea***	*T. occidentalis*	Belgrade, Serbia	M. Zlatković	KF729105	-	-	-
BOT 34	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković/ I. Milenković	KF729106	-	-	-
BOT 18	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729107	-	-	-
BOT 86	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729108	-	-	-
BOT 40	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729109	-	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
BOT 85	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729110	-	-	-
CMW 39312	***B. dothidea***	*P. acerifolia*	Bosnia and Herzegovina	D. Karadžić	KF729111	-	-	-
BOT 32	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729485	-	-	-
BOT 52	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729112	-	-	-
BOT 124	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729113	-	-	-
BOT 58	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729114	-	-	-
BOT 61	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729115	-	-	-
BOT 224	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729116	-	-	-
BOT 120	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729117	-	-	-
CMW 39309	***B. dothidea***	*A. concolor*	Belgrade, Serbia	M. Zlatković	KF729118	-	-	-
BOT 80	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729119	-	-	-
CMW 39305	***B. dothidea***	*Q. ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729120	-	-	-
BOT 91	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729121	-	-	-
BOT 168	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729122	-	-	-
BOT 99	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729123	-	-	-
BOT 122	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729124	-	-	-
BOT 156	***B. dothidea***	*P. menziesii*	Belgrade, Serbia	M. Zlatković	KF729125	-	-	-
CMW 39307	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković/ I. Milenković	KF729126	-	-	-
BOT 178	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729127	-	-	-
BOT 2	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729128	-	-	-
CMW 39315	***B. dothidea***	*C. lawsoniana*	Bar, Montenegro	M. Zlatković	KF729129	-	-	-
BOT 110	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729130	-	-	-
BOT 66	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729131	-	-	-
BOT 176	***B. dothidea***	*C. lawsoniana*	Morović, Serbia	M. Zlatković	KF729132	-	-	-
BOT 68	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729133	-	-	-
BOT 81	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729134	-	-	-
BOT 175	***B. dothidea***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729135	-	-	-
BOT 190	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729136	-	-	-
BOT 44	***B. dothidea***	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729137	-	-	-
BOT 70	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729138	-	-	-
BOT 121	***B. dothidea***	*Q. ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729139	-	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
BOT 118	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	N. Keča/ M. Zlatković	KF729140	-	-	-
BOT 106	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729141	-	-	-
BOT 92	***B. dothidea***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729142	-	-	-
BOT 108	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729143	-	-	-
BOT 111	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729144	-	-	-
BOT 263	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729145	-	-	-
BOT 53	***B. dothidea***	*A. concolor*	Belgrade, Serbia	M. Zlatković	KF729146	-	-	-
CMW 39301	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729147	-	-	-
BOT 213	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	N. Keča	KF729148	-	-	-
BOT 73	***B. dothidea***	*P. menziesii*	Belgrade, Serbia	M. Zlatković	KF729149	-	-	-
BOT 41	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729150	-	-	-
BOT 72	***B. dothidea***	*P. menziesii*	Belgrade, Serbia	M. Zlatković	KF729151	-	-	-
BOT 47	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729152	-	-	-
BOT 93	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729153	-	-	-
BOT 75	***B. dothidea***	*Q. ilex*	Podgorica, Montenegro	J. Lazarević/ M. Zlatković	KF729154	-	-	-
CMW 39306	***B. dothidea***	*Liriodendron tulipifera*	Belgrade, Serbia	D. Karadžić/ I. Milenković	KF729155	-	-	-
BOT 78	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729156	-	-	-
CMW 39298	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729157	-	-	-
BOT 215	***B. dothidea***	*C. atlantica*	Bar, Montenegro	M. Zlatković	KF729158	-	-	-
BOT 181	***B. dothidea***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729159	-	-	-
BOT 94	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729160	-	-	-
CMW 39300	***B. dothidea***	*C. sempervirens*	Podgorica, Montenegro	M. Zlatković/ J. Lazarević	KF729161	-	-	-
BOT 166	***B. dothidea***	*C. lawsoniana*	Bar, Montenegro	M. Zlatković	KF729162	-	-	-
BOT 76	***B. dothidea***	*C. arizonica*	Herceg Novi, Montenegro	M. Zlatković	KF729163	-	-	-
CMW 39303	***B. dothidea***	*C. arizonica*	Belgrade, Serbia	M. Zlatković	KF729164	-	-	-
BOT 216	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729165	-	-	-
CMW 39299	***B. dothidea***	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729166	-	-	-
BOT 165	***B. dothidea***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729167	-	-	-
BOT 9	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729168	-	-	-
BOT 262	***B. dothidea***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729169	-	-	-
CMW 45089	***B. dothidea***	*Populus nigra* var. *italica*	Vršac, Serbia	N. Keča	KT964313	-	-	-
CMW 45091	***B. dothidea***	*Salix caprea*	Pirot, Serbia	M. Zlatković	KT964320	-	-	-
BOT 283	***B. dothidea***	*S. caprea*	Pirot, Serbia	M. Zlatković	KT964314	-	-	-
CMW 45096	***B. dothidea***	*Taxus baccata*	Belgrade, Serbia	M. Zlatković	KT964315	-	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CMW 45097	***B. dothidea***	*Populus tremula*	Belgrade, Serbia	M. Zlatković	KT964322	-	-	-
BOT 298	***B. dothidea***	*P. tremula*	Belgrade, Serbia	M. Zlatković	KT964323	-	-	-
CMW 45098	***B. dothidea***	*Quercus cerris*	Mt. Suva, Serbia [1]	N. Keča	KT964316	-	-	-
BOT 300	***B. dothidea***	*Q. cerris*	Mt. Suva, Serbia [1]	N. Keča	KT964317	-	-	-
BOT 302	***B. dothidea***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KT964318	-	-	-
BOT 305	***B. dothidea***	*J. horizontalis*	Pirot, Serbia	M. Zlatković	KT964321	-	-	-
BOT 306	***B. dothidea***	*J. horizontalis*	Pirot, Serbia	M. Zlatković	KT964319	-	-	-
MUCC 501	*B. dothidea*	*Eucalyptus marginata*	Yalgorup, Australia	K. M. Taylor	EF591916	EF591969	EF591952	-
ATCC 22927	*Botryosphaeria corticis*	*Vaccinium sp.*	USA	R. D. Millholland	DQ299247	EU673291	EU673108	-
CBS 119047	*B. corticis*	*Vaccinium corymbosum*	New Jersey, USA	P. V. Oudemans	DQ299245	EU017539	EU673107	-
IMI 63581b	*Dothiorella sarmentorum*	*Ulmus sp.*	England	E. A. Ellis	AY573212	AY573235	EU673102	-
CMW 39366	*Do. sarmentorum*	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF575009	KF575047	KF575105	-
CMW 39364	*Do. sarmentorum*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF575010	KF575048	KF575106	-
CMW 39370	*Do. sarmentorum*	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF575011	KF575049	KF575107	-
CMW 39369	***Do. sarmentorum***	*Cryptomeria japonica*	Belgrade, Serbia	M. Zlatković	KF729075	-	-	-
CMW 39368	***Do. sarmentorum***	*Forsythia europaea*	Belgrade, Serbia	M. Zlatković	KF729076	-	-	-
CMW 39367	***Do. sarmentorum***	*T. plicata*	Belgrade, Serbia	M. Zlatković	KF729077	-	-	-
BOT 144	***Do. sarmentorum***	*T. occidentalis*	Belgrade, Serbia	M. Zlatković	KF729078	-	-	-
BOT 195	***Do. sarmentorum***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729080	-	-	-
BOT 173	***Do. sarmentorum***	*T. occidentalis*	Pirot, Serbia	M. Zlatković	KF729081	-	-	-
CMW 39365	***Do. sarmentorum***	*T. occidentalis*	Pirot, Serbia	M. Zlatković	KF729079	-	-	-
CMW 45095	***Do. sarmentorum***	*Q. rubra*	Belgrade, Serbia	S. Milanović		-	-	-
CBS 115038	*Do. sarmentorum*	*Malus pumila*	The Netherlands	A. J. L. Phillips	AY573206	AY573223	EU673101	-
CBS 128309	*Dothiorella americana*	*V. vinifera*	Missouri, USA	K. Striegler/ G. M. Leavitt	HQ288218	HQ288262	HQ288297	-
CBS 128310	*Do. americana*	*V. vinifera*	Missouri, USA	K. Striegler/ G. M. Leavitt	HQ288219	HQ288263	HQ288298	-
CBS 124716	*Dothiorella omnivora*	*Juglans regia*	Iran	J. Abdollahzadeh/ A. Javadi	KC898232	KC898215	-	-
CBS 124717	*Do. omnivora*	*J. regia*	Iran	J. Abdollahzadeh/ A. Javadi	KC898233	KC898216	-	-
CMW 39360	*Do. omnivora*	*Fraxinus excelsior*	Bosnia and Herzegovina	D. Karadžić	KF575012	KF575052	KF575108	-
CMW 39361	*Do. omnivora*	*C. sempervirens*	Podgorica, Montenegro	M. Zlatković	KF729083	KT253576	KT253570	-
CMW 39362	*Do. omnivora*	*Thuja occidentalis*	Belgrade, Serbia	M. Zlatković	KF575013	KF575053	KF575109	-
CMW 39363	*Do. omnivora*	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF575014	KF575054	KF575110	-
BOT 177	***Do. omnivora***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729082	-	-	-
CMW 39359	***Do. omnivora***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729084	-	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CBS 135622	*Dothiorella* sp.	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF261725	KF261729	KF261731	-
MFLUCC13-0497	*Dothiorella* sp.	*Symphoricarpos* sp.	Italy	Erio Camporesi	KJ742378	KJ742381	-	-
MFLUCC13-0498	*Dothiorella* sp.	*Symphoricarpos* sp.	Italy	Erio Camporesi	KJ742379	KJ742382	-	-
DAR 78992	*Dothiorella vidmadera*	*V. vinifera*	Eden Valley, Australia	W. M. Pitt/ A. Loschiavo	EU768874	EU768881	HM800522	-
DAR 78993	*Do. vidmadera*	*V. vinifera*	Loxton, Australia	W. M. Pitt/ A. Loschiavo	EU768876	EU768882	HM800523	-
CBS 115041	*Dothiorella iberica*	*Q. ilex*	Aragón, Spain	J. Luque	AY573202	AY573222	EU673096	-
CBS 113188	*Do. iberica*	*Quercus suber*	Andalucia, Spain	M. E. Sánchez	AY573198	EU673278	EU673097	-
JL 599	*Dothiorella parva*	*Corylus avelana*	Spain	J. Luque	EU673314	EU673281	EU673099	-
CBS124720	*Do. parva*	*C. avelana*	Iran, Ardabil	J. Abdollahzadeh / A. Javadi	KC898234	KC898217	-	-
CBS 124719	*Dothiorella sempervirentis*	*C. sempervirens*	Iran, Golestan	M. A. Aghajani	KC898237	KC898220	-	-
CBS 124718	*Do. sempervirentis*	*C. sempervirens*	Iran, Golestan	M. A. Aghajani	KC898236	KC898219	-	-
MUCC 507	*Dothiorella moneti*	*Acacia rostellifera*	Yalgorup, Australia	K. M. Taylor	EF591922	EF591973	EF591956	-
MUCC 505	*Do. moneti*	*A.rostellifera*	Yalgorup, Australia	K. M. Taylor	EF591920	EF591971	EF591954	-
CMW 9081	*Neofusicoccum parvum*	*Populus nigra*	New Zealand	G. J. Samuels	AY236943	AY236888	AY236917	-
CMW 39328	*N. parvum*	*Pittosporum tobira*	Herceg Novi, Montenegro	M. Zlatković	KF575017	KF575041	KF575113	KF729318
CMW 39321	*N. parvum*	*Prunus laurocerasus*	Budva, Montenegro	M. Zlatković	KF575018	KF575042	KF575114	KF729319
CMW 39326	*N. parvum*	*Eucalyptus globulus*	Herceg Novi, Montenegro	M. Zlatković	KF575019	KF575043	KF575115	KF729320
CMW 39325	*N. parvum*	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF575021	KF575045	KF575117	KF729322
CMW 39318	*N. parvum*	*C. lawsoniana*	Belgrade, Serbia	N. Keča/ M. Zlatković	KF575022	KF575046	KF575118	KF729323
BOT 107	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729034	KF729364	KF729324	KF729277
BOT 3	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729035	KF729365	KF729325	KF729278
BOT 15	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729036	KF729366	KF729326	KF729279
BOT 49	***N. parvum***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729037	KF729367	KF729327	KF729280
BOT 79	***N. parvum***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729038	KF729368	KF729328	KF729281
BOT 6	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	I. Milenković	KF729039	KF729369	KF729329	KF729282
BOT 275	***N. parvum***	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729040	KF729370	KF729330	KF729283
CMW 39323	***N. parvum***	*C. obtusa*	Kanjiža, Serbia	N. Keča	KF729041	KF729371	KF729331	KF729284
BOT 267	***N. parvum***	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729042	KF729372	KF729332	KF729285
CMW 39320	***N. parvum***	*S. sempervirens*	Belgrade, Serbia	M. Zlatković	KF729043	KF729373	KF729333	KF729286
BOT 87	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729044	KF729374	KF729334	KF729287
CMW 39324	***N. parvum***	*C. pisifera*	Belgrade, Serbia [2]	M. Zlatković	KF729045	KF729375	KF729335	KF729288
BOT 90	***N. parvum***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729046	KF729376	KF729336	KF729289

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CMW 39322	***N. parvum***	*P. abies*	Niš, Serbia	M. Zlatković	KF729047	KF729377	KF729337	KF729290
BOT 43	***N. parvum***	*C. atlantica*	Belgrade, Serbia	M. Zlatković	KF729048	KF729378	KF729338	KF729291
BOT 82	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729049	KF729379	KF729339	KF729292
CMW 39327	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729050	KF729380	KF729340	KF729293
BOT 42	***N. parvum***	*T. occidentalis*	Belgrade, Serbia [2]	M. Zlatković	KF729051	KF729381	KF729341	KF729294
BOT 59	***N. parvum***	*S. giganteum*	Belgrade , Serbia	M. Zlatković	KF729052	KF729382	KF729342	KF729295
BOT 136	***N. parvum***	*C. atlantica*	Herceg Novi, Montenegro	M. Zlatković	KF729053	KF729383	KF729343	KF729296
CMW 39319	***N. parvum***	*T. occidentalis*	Belgrade, Serbia [2]	M. Zlatković	KF729054	KF729384	KF729344	KF729297
BOT 281	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KF729055	KF729385	KF729345	KF729298
BOT 64	***N. parvum***	*S. giganteum*	Kumane, Serbia	N. Keča	KF729056	KF729386	KF729346	KF729299
BOT 112	***N. parvum***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729057	KF729387	KF729347	KF729300
BOT 113	***N. parvum***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729058	KF729388	KF729348	KF729301
BOT 214	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729059	KF729389	KF729349	KF729302
BOT 17	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729060	KF729390	KF729350	KF729303
BOT 16	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729062	KF729392	KF729352	KF729305
CMW 39316	***N. parvum***	*P. halepensis*	Herceg Novi, Montenegro	M. Zlatković	KF729061	KF729391	KF729351	KF729304
BOT 160	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729063	KF729393	KF729353	KF729306
BOT 26	***N. parvum***	*A. hippocastanum*	Obrenovac, Serbia	D. Karadžić	KF729064	KF729394	KF729354	KF729307
BOT 1	***N. parvum***	*S. giganteum*	Kumane, Serbia	N. Keča	KF729065	KF729395	KF729355	KF729308
BOT 39	***N. parvum***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729066	KF729396	KF729356	KF729309
BOT 27	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729067	KF729397	KF729357	KF729310
BOT 131	***N. parvum***	*P. laurocerasus*	Belgrade, Serbia	M. Zlatković	KF729068	KF729398	KF729358	KF729311
BOT 13	***N. parvum***	*S. giganteum*	Kumane, Serbia	N. Keča	KF729069	KF767529	KF729484	KF729312
BOT 30	***N. parvum***	*S. giganteum*	Belgrade, Serbia	M. Zlatković	KF729070	KF729399	KF729359	KF729313
BOT 8	***N. parvum***	*S. giganteum*	Kumane, Serbia	N. Keča	KF729071	KF729400	KF729360	KF729314
BOT 11	***N. parvum***	*S. giganteum*	Valjevo, Serbia	N. Keča	KF729072	KF729401	KF729361	KF729315
BOT 286	***N. parvum***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KF729073	KF729402	KF729362	KF729316
BOT 14	***N. parvum***	*S. giganteum*	Kumane, Serbia	N. Keča	KF729074	KF729403	KF729363	KF729317
CMW 45090	***N. parvum***	*P. nigra* var. *italica*	Vršac, Serbia	N. Keča	KT964325	-	-	-
CMW 45093	***N. parvum***	*Q. rubra*	Belgrade, Serbia	S. Milanović	KT964326	-	-	-
BOT 288	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964327	-	-	-
BOT 289	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964328	-	-	-
BOT 290	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964329	-	-	-
BOT 291	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964332	-	-	-
BOT 292	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964330	-	-	-
BOT 293	***N. parvum***	*A. hippocastanum*	Belgrade, Serbia	M. Zlatković	KT964333	-	-	-
BOT 301	***N. parvum***	*C. lawsoniana*	Belgrade, Serbia	M. Zlatković	KT964331	-	-	-

Isolate	Identity	Host	Location [1, 2, 3]	Collector	GenBank Accession			
					ITS	EF1-α	β-tubulin	RPB2
CMW 9071	*N. parvum*	*Ribes sp.*	Australia	M. J. Wingfield	EU339552	AY236880	AY236909	-
CBS 123639	*Neofusicoccum kwambonambiense*	*S. cordatum*	South Africa	D. Pavlic	EU821900	EU821870	EU821840	-
CBS 123641	*N. kwambonambiense*	*S. cordatum*	South Africa	D. Pavlic	EU821919	EU821889	EU821859	-
CMW 14054	*Neofusicoccum cordaticola*	*S. cordatum*	South Africa	D. Pavlic	EU821906	EU821876	EU821846	-
CMW 13992	*N. cordaticola*	*S. cordatum*	South Africa	D. Pavlic	EU821898	EU821868	EU821838	-
CBS 117448	*Pseudofusicoccum stromaticum*	*Eucalyptus* hybrid	Venezuela	S. Mohali	AY693974	AY693975	EU673094	-
CBS 117449	*P. stromaticum*	*Eucalyptus* hybrid	Venezuela	S. Mohali	DQ436935	DQ436936	EU673093	-

Culture collections: CMW: FABI, University of Pretoria, South Africa; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CPC: Collection of Pedro Crous housed at CBS; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; ATCC, American Type Culture Collection; MUCC: Murdoch University Culture Collection; DAR: Plant Pathology Herbarium, Orange Agricultural Institute, DPI, Orange, NSW, Australia; IMI: CABI Genetic Resource Collection Bioscience, Egham, Surrey, United Kingdom; JL: J. Luque, IRTA, Barcelona, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand. Isolates sequenced in this study are given in bold. Other sequences were retrieved from GenBank. Isolate accession numbers in italics signify cultures linked morphologically to the type material. [1] Forest stand, [2] Nursery, [3] Forest plantation.

Table S3. Symptoms observed on various tree species and Botryosphaeriaceae species involved in the development of symptoms.

Host	Resin bleeding	Necrosis/Canker			Die-back		Bark cracking	Wood discoloration	Fruit bodies				Botryosphaeriaceae species [2]
		Needles /Leaves	Branch	Stem	Shoot	Stem			Cone	Stem	Branch	Needles/ Leaves	
Cedrus atlantica	+	+	+	+	+	+	+				+		*N. parvum*
C. deodara [1]	+			+		+	+						− (*D. sapinea*)
C. libani	+			+		+	+						/
Chamaecyparis lawsoniana	+	+	+	+	+	+	+	+	+	+	+		*B. dothidea*
C. obtusa		+			+	+							/
C. pisifera		+				+							/
Thuja occidentalis	+	+	+	+	+	+	+	+		+	+		*B. dothidea*, *N. parvum*, *D. seriata*, *Do. sarmentorum*
T. plicata	+	+	+	+	+	+		+			+		/
Sequoiadendron giganteum	+	+			+	+	+	+					*B. dothidea*, *N. parvum*, *Do. omnivora*
Sequoia sempervirens		+			+								/
Pseudotsuga menziesii [1]	+	+	+	+			+						*D. sapinea*
Pinus spp. [1]	+	+	+	+	+	+		+	+			+	*D. sapinea*
Abies concolor [1]	+	+	+	+	+	+	+	+					*B. dothidea*, *D. seriata*, *D. sapinea*
Picea abies	+	+	+		+	+							*B. dothidea*, *N. parvum*
P. pungens [1]	+	+	+		+	+							*D. sapinea*
P. omorika [1]	+	+	+		+	+		+					*D. sapinea*
C. sempervirens	+	+	+	+	+	+	+	+	+				*B. dothidea*, *D. seriata*, *D. mutila*
C. arizonica	+	+	+		+		+	+					*B. dothidea*, *D. mutila*
Cryptomeria japonica		+			+								/
Juniperus horizontalis [1]		+			+	+							*B. dothidea*, *D. sapinea*

Host	Resin bleeding	Necrosis/Canker			Die-back		Bark cracking	Wood discoloration	Fruit bodies				Botryosphaeriaceae species
		Needles/ Leaves	Branch	Stem	Shoot	Stem			Cone	Stem	Branch	Needles/ Leaves	
Prunus laurocerasus	gum exudation	+	+	+	+	+	+	+			+	+	*D. mutila*, *N. parvum*
P. cerasus	gum exudation		+	+	+	+	+	+					− (*D. seriata*)
Ligustrum vulgare			+	+	+	+				+	+		*D. seriata*
Forsythia europaea					+								/
Magnolia grandiflora					+	+							*B. dothidea*
Liriodendron tulipifera					+	+							*B. dothidea*
Aesculus hippocastanum	black sap exudation		+	+									*B. dothidea*, *N. parvum*, *D. mutila*, *Do. sarmentorum*
Platanus acerifolia				+									− (*B. dothidea*)
Quercus robur				+									*D. seriata*
Q. ilex					+	+				+	+		/
Q. rubra					+	+							− (*N. parvum*, *D. seriata*, *Do. sarmentorum*)
Q. cerris				+			+						*B. dothidea*
Pittosporum tobira					+	+							*N. parvum*
Rubus fruticosus					+	+							/
Fagus sylvatica [1]				+				+					*D. sapinea*
Viscum album										+		+	*S. visci*
Populus nigra var. italica					+						+		*N. parvum*
Salix caprea					+	+		+		+	+		/
Taxus baccata			+										− (*B. dothidea*)
Fraxinus excelsior			+										*D. seriata*
Eucalyptus globulus	black kino exudation			+			+						/
P. tremula				+			+						*B. dothidea*

[1] Data retreived from Zlatković et al. 2017, [2] Botryosphaeriaceae species involved in the development of symptoms: / Pathogenicity tests were not conducted, − Botryosphaeriaceae species produced lesions which were not significantly larger from those observed on control plants and thus it is assumed that Botryosphaeriaceae were not involved in the development of symptoms. Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans region, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

Table S4. Average temperatures and precipitation in Belgrade during pathogenicity evaluation (www.hidmet.gov.rs).

Month/Year	Average Temperature (°C)	Average Precipitation (mm)
April 2014	13.7 [2, *]	85.3 [7, *]
May 2014	17.2 [1, *]	280.4 [8, *]
June 2014	21.4 [2, *]	60.3 [5, *]
July 2014	23 [2, *]	250.6 [8, *]
August 2014	22.5 [2, *]	63.5 [6, *]
September 2014	18.3 [1, *]	126 [8, *]
October 2014	14.1 [2, *]	61.2 [7, *]
April 2015	13.5 [2, *]	30.7 [4, *]
May 2015	19 [2, *]	80.7 [6, *]
June 2015	21.9 [2, *]	38.6 [5, *]
April 2016	15.5 [3, #]	58.7 [6, #]
May 2016	17.5 [1, #]	82.9 [7, #]
June 2016	22.5 [2, #]	134.2 [7, #]

Average temperatures: [1] Normal, [2] Warm, [3] Very warm; Average precipitation: [4] Very dry, [5] Dry, [6] Normal, [7] Rainy, [8] Extremely rainy, [*] Compared to the reference period 1961-1990, [#] Compared to the reference period 1981-2010.

Table S5. Tree species and Botryosphaeriaceae isolates used for seedling and branch inoculations.

Inoculated species	*B. dothidea*		*N. parvum*		*D. mutila*		*D. seriata*		*Do. sarmentorum*		*Dothiorella* sp.		*Do. omnivora*		*S. visci*	*P. cupressi*
	CMW 39301	CMW 39315	CMW 39327	CMW 39317	CMW 39348	CMW 39385	CMW 39376	CMW 39382	CMW 39364	CMW 39365	CBS 135622	CBS 135623	CMW 39361	CMW 39363	CMW 39386	CMW 39387
Cedrus atlantica	+	+	+	+	+	+	+	+	+	+	+	+				
Cedrus deodara			+													
Cupressus arizonica	+	+			+	+										
Picea omorika			+													
Picea abies	+	+	+	+												
Abies concolor	+	+					+	+								
Pseudotsuga menziesii	+	+														
Liriodendron tulipifera	+	+														
Ligustrum vulgare							+	+								
Juniperus horizontalis	+	+														
Chamaecyparis lawsoniana	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thuja occidentalis	+	+	+	+			+	+	+	+						
Prunus laurocerasus [1]			+	+	+	+										
Quercus robur							+	+								
Pittosporum tobira			+	+	+	+										
Sequoiadendron giganteum	+	+	+	+									+	+		
Magnolia grandifolia	+	+														
Cupressus sempervirens	+	+			+	+	+	+					+	+		+
Forsythia europaea									+	+						

Table S5. Continued.

Inoculated species	*B. dothidea*		*N. parvum*		*D. mutila*		*D. seriata*		*Do. sarmentorum*		*Dothiorella* sp.		*Do. omnivora*		*S.visci*	*P. cupressi*
	CMW 39301	CMW 39315	CMW 39327	CMW 39317	CMW 39348	CMW 39385	CMW 39376	CMW 39382	CMW 39364	CMW 39365	CBS 135622	CBS 135623	CMW 39361	CMW 39363	CMW 39386	CMW 39387
Taxus baccata	+	+														
Prunus cerasus							+	+								
Populus nigra	+	+	+	+												
Quercus rubra			+	+			+	+	+	+						
Platanus acerifolia	+	+														
Pinus patula [2]	+	+	+	+												
Eucalyptus[2] *grandis*	+	+	+	+												
Aesculus hippocastanum [3]	+	+	+	+	+	+			+	+						
Populus tremula [3]	+	+														
Quercus cerris [3]	+	+														
Fraxinus excelsior [3]							+	+					+	+		
Viscum album [3]															+	

[1] Stem and leaf inoculations, [2] Greenhouse trial, [3] Branch inoculations, '+' indicates that the isolate was used for inoculation. '–' indicates that the isolate was not used for inoculation.

Table S6. Details of the tree species inoculated in the field trial and in the greenhouse.

Species	Age (years)	Mean stem diameter (cm)	Mean height (cm)	Cork borer (mm) [2]
Cedrus atlantica	2	0.9	17	3
Cedrus deodara	2	0.9	17	3
Cupressus arizonica	3	1.3	39	6
Picea omorika	2	0.9	18	3
Picea abies	3	1.4	29	6
Abies concolor	2	1.2	20	6
Pseudotsuga menziesii	2	0.9	18	3
Liriodendron tulipifera	3	1.5	32	6
Ligustrum vulgare	3	1.2	32	6
Juniperus horizontalis	2	0.9	14	3
Chamaecyparis lawsoniana	3	1.5	36	6
Thuja occidentalis	3	1.5	35	6
Prunus laurocerasus	2	1.3	22	6
Quercus robur	3	1.2	29	6
Pittosporum tobira	3	1.2	25	6
Sequoiadendron giganteum	2	0.9	18	3
Magnolia grandifolia	2	1.2	16	3
Cupressus sempervirens	2	0.9	19	3
Forsythia europaea	3	1.4	39	6
Taxus baccata	2	1.3	29	6
Prunus cerasus	2	1.5	40	6
Populus nigra var. *italica*	2	1.2	18	3
Quercus rubra	2	1.2	21	3
Platanus acerifolia	2	1.2	20	3
Pinus patula [1]	5	2.3	190	9
Eucalyptus grandis [1]	3	1.9	170	9

[1] Greenhouse trial, [2] Diameter of cork borer used for seedling inoculation.

Table S7. Isolates of Botryosphaeriaceae species used in inoculation trials.

Isolate	Botryosphaeriaceae species	Host	Location	Substrate
CMW 39301	*Botryosphaeria dothidea*	*Chamaecyparis lawsoniana*	Belgrade, Serbia	Pycnidia on branch
CMW 39315	*B. dothidea*	*C. lawsoniana*	Bar, Montenegro	Branch canker
CMW 39327	*Neofusicoccum parvum*	*Sequoiadendron giganteum*	Belgrade, Serbia	Necrotic needles
CMW 39317	*N. parvum*	*Eucalyptus globulus*	Bar, Montenegro	Stem canker
CMW 39348	*Diplodia mutila*	*Cupressus sempervirens*	Herceg Novi, Montenegro	Resinous branch canker
CMW 39385	*D. mutila*	*Thuja plicata*	Belgrade, Serbia	Pycnidia on shoot
CMW 39376	*Diplodia seriata*	*Chamaecyparis pisifera*	Belgrade, Serbia	Necrotic needles
CMW 39382	*D. seriata*	*Quercus robur*	Morović, Serbia	Necrotic stem lesion
CMW 39364	*Dothiorella sarmentorum*	*C. lawsoniana*	Belgrade, Serbia	Resinous branch lesion
CMW 39365	*Do. sarmentorum*	*Thuja occidentalis*	Pirot, Serbia	Pycnidia on branch
CBS 135622	*Dothiorella* sp.	*C. atlantica*	Belgrade, Serbia	Resinous branch lesion
CBS 135623	*Dothiorella* sp.	*T. plicata*	Belgrade, Serbia	Dead resinous needles
CMW 39361	*Dothiorella omnivora*	*Cupressus sempervirens*	Podgorica, Montenegro	Pycnidia on branch
CMW 39363	*Do. omnivora*	*C. lawsoniana*	Belgrade, Serbia	Blue stained wood
CMW 39386	*Sphaeropsis visci*	*Viscum album*	Mt Goč, Serbia	Pycnidia on leaves
CMW 39387	*Phaeobotryon cupressi*	*C. sempervirens*	Podgorica, Montenegro	Resinous branch lesion

Table S8. Lesion/canker lengths (lengths of the necrotic/ discolored/resinous area at the site of inoculation) of seedlings and shoots inoculated with Botryosphaeriaceae species.

Host	Botryosphaeriaceae species									
	Botryosphaeria dothidea	***Neofusicoccum parvum***	***Diplodia seriata***	***D. mutila***	***Dothiorella sarmentorum***	***Do. omnivora***	***Dothiorella* sp.**	***Phaeobotryon cupressi***	***Sphaeropsis visci***	**Control**
Chamaecyparis lawsoniana	0.9-1.2 (1) [3] / **1.4-2.1 (1.9)** [4]	0.9-1.1 (0.9)	0.9-1.2 (1)	0.9-1.1 (0.9)	0.9-1.2 (1)	0.9-1.3 (1)	0.9-1.4 (1)	**2.7-8.3 (4.7)**	0.9-1.3 (1)	0.8-1 (0.9)
Cedrus atlantica	0.4-0.6 (0.5)	**1.9-2.6 (2.4)** [5] / 0.4-0.6 (0.5) [6]	0.4-0.6 (0.5)	0.4-0.6 (0.5)	0.4-0.6 (0.5)		0.4-0.6 (0.5)			0.4-0.5 (0.5)
C. deodara		0.4-0.6 (0.5) [5]								0.4-0.5 (0.5)
Prunus laurocerasus		**1.6-2 (1.8)** [5]/ **1.8-2.2 (2)** [6]/ **1-2.5 (2.1)** [5, 7]/ **2.9-3.5 (3.3)** [6, 7]		0.9-1.7 (1.4) [10]/ **1.5-2.2 (1.9)** [11]/ – [10, 7]/ **0.6-0.9 (0.8)** [11, 7]						0.6-0.8 (0.7)/ –
Pseudotsuga menziesii	0.4-0.6 (0.5)									0.4-0.5 (0.5)
Juniperus horizontalis	(1.2-2.7) 2 [3]/ (1.3-2.6) 2 [4]									–
Cupressus arizonica	**(1.3-1.7) 1.6** [3]/ **(4.2-9.8) 7.6** [4]			**(1.3-1.7) 1.5**						–
Liriodendron tulipifera	***(6-30) 16.3*** [3]/ **(5.4-8.3) 6.5** [4]									0.9-1.1 (1)
Magnolia grandifolia	**(1.3-2.5) 1.8** [3]/ **(1.7-1.9) 1.8** [4]									0.4-0.5 (0.4)
Ligustrum vulgare			(1.4-2) 1.7 [8]/ (1.7-2) 1.9 [9]							–
Quercus robur			**(1.9-7.5) 4.7** [8]/ **(2.4-8.7) 5.3** [9]							–
Pittosporum tobira		(1.2-1.5) 1.4 [5]/ (1.7-2.1) 2 [6]		–						–
Aesculus hippocastanum [1]	+	+		+	**(1.5-3.5) 2.3** [12]					(0.6-0.9) 0.7
Pinus patula [2]	**(10-25) 16.5** [3]/ **(13-33) 20.3** [4]	**(18-90) 36.8** [5]/ ***(32-80) 52.4*** [6]								6 (6-7)
Eucalyptus grandis [2]	***(11-27) 15.4*** [3]/ (10-17) 12.3 [4]	**27.3 (12-48)** [5]/ ***80.5 (37-140)*** [6]								6 (6-7)

Table S8. Continued.

Host	Botryosphaeriaceae species									
	Botryosphaeria dothidea	***Neofusicoccum parvum***	***Diplodia seriata***	***D. mutila***	***Dothiorella sarmentorum***	***Do. omnivora***	***Dothiorella* sp.**	***Phaeobotryon cupressi***	***Sphaeropsis visci***	**Control**
Picea abies	(4-7.5) 5.8 [3]/ (2.5-4.3) 3.4 [4]	(2.5-6.5) 3 [5]								–
Populus nigra	–	**<u>(3.3-5.6) 4.2</u>** [5]/ (1.7-2.6) 2.3 [6]								–
Quercus rubra		0.4-0.7 (0.5)	0.4-0.6 (0.5)		0.4-0.8 (0.5)					0.4-0.5 (0.5)
Taxus baccata	(0.7-1) 0.8									(0.6-0.8) 0.7
Prunus cerasus	(0.7-0.9) 0.8									(0.6-0.9) 0.7
Populus tremula [1]	(1.5-2.3) 2 [3]/ (1-2.2) 1.9 [4]									–
Quercus cerris	(1.7-2.6) 2 [3]/ (1-2.3) 1.9 [4]									– –
Fraxinus excelsior			(1.2-2.3) 1.7 [8]/– [9]			–				–

Mean lesion lengths (cm) are given in brackets;"–" indicate that lesions/cankers were not formed; "+" indicate that all inoculated shoots were girdeled; Lesions statistically larger compared to controls are given in bold (α= 0.05). Cankers are given in bold and underlined. Lesions statistically larger compared to those produced by the other isolate of the same species are given in italics (α= 0.05). [1] Branch inoculations, [2] Greenhouse trial, [3] CMW 39301, [4] CMW 39315, [5] CMW 39327, [6] CMW 39317, [7] Leaf inoculations, [8] CMW 39376, [9] CMW 39382, [10] CMW 39348, [11] CMW 39385, [12] CMW 39365.

Table S9. New hosts reported for Botryosphaeriaceae species identified in the present study.

Botryosphaeriaceae species	New host-fungus association	Botryosphaeriaceae species	New host-fungus association
Do. sarmentorum	*Quercus rubra* *Cryptomeria japonica* *Forsythia europaea* *Thuja plicata* *Thuja occidentalis*	***D. mutila***	*Pittosporum tobira* *Prunus laurocerasus* *C. sempervirens* *Chamaecyparis obtusa* *C. atlantica* *T. plicata*
Do. omnivora	*Sequoiadendron giganteum*	***D. seriata***	*Quercus robur* *A. concolor* *S. caprea* *Prunus cerasus* *Q. rubra*
B. dothidea	*C. atlantica* *Chamaecyparis lawsoniana* *T. occidentalis* *T. plicata* *Cupressus arizonica* *Abies concolor* *Picea abies* *Juniperus horizontalis* *Magnolia grandiflora* *Liriodendron tulipifera* *Platanus acerifolia* *Aesculus hippocastanum* *Salix caprea* *C. sempervirens* *Taxus baccata* *Quercus cerris* *Populus tremula*	***N. parvum***	*C. obtusa* *Chamaecyparis pisifera* *T. occidentalis* *Sequoia sempervirens* *Pinus halepensis* *P. abies* *S. caprea*

Table S10. Percentages of isolates of Botryosphaeriaceae species associated with gymnosperms and angiosperms.

Botryosphaeriaceae species	Gymnosperms (%)	Angiosperms (%)
Botryosphaeria dothidea	54.5	45.5
Neofusicoccum parvum	60	40 [1]
Diplodia sapinea	93.8	6.2 [1]
D. mutila	72.7	27.3 [1]
D. seriata	50	50
Dothiorella sp.	100	/
Do. sarmentorum	62.5	37.5 [1]
Do. omnivora	80	20 [1]
Phaeobotryon cupressi	100	/
Sphaeropsis visci	/	100

[1] Percentage of isolates obtained from angiosperms differed significantly from percentage of isolates obtained from gymnosperms as determined by the z test at $z>1.96$, $p<0.05$.
Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans region, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

Table S11. Diversity of Botryosphaeriaceae species isolated from hosts in the Continental and Mediterranean climate regions and from gymnosperms and angiosperms.

Climate region/ Host group	Species richness (S)	Abundance	Shannon index (H')	Evenness (J)	Jaccard's index (J')
Continental	9	262	0.30	0.77	0.5
Mediterranean	6	47	0.37	0.8	
Gymnosperms	9	254	0.29	0.76	0.7
Angiosperms	8	55	0.42	0.8	
Total	10	309	0.29	0.74	–

Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

Table S12. Botryosphaeriaceae species isolated from the same tree and same host tissues.

Botryosphaeriaceae species composition (one tree)	Host
Botryosphaeria dothidea / Diplodia seriata	*Chamaecyparis lawsoniana* *Salix caprea*
B. dothidea / Diplodia mutila	*Cupressus arizonica*
B. dothidea / Diplodia sapinea	*Cedrus atlantica* *Pseudotsuga menziesii*
B. dothidea / Neofusicoccum parvum	*Sequoiadendron giganteum* *C. lawsoniana* *Aesculus hippocastanum* *Populus nigra* var. *italica*
***B. dothidea / Dothiorella* sp.**	*C. atlantica*
B. dothidea / N. parvum / Dothiorella omnivora	*S. giganteum*
B. dothidea / N. parvum / D. mutila	*Sequoia sempervirens*
B. dothidea / D. mutila/ Dothiorella sarmentorum	*Thuja plicata*
B. dothidea / D. sapinea / D. mutila / D. seriata / Do. sarmentorum	*C. atlantica*
N. parvum / D. sapinea	*C. atlantica*
N. parvum / D. seriata	*Chamaecyparis pisifera*
N. parvum / D. mutila	*C. atlantica* *Chamaecyparis obtusa* *Pittosporum tobira* *Prunus laurocerasus*
N. parvum / Do. sarmentorum	*A. hippocastanum*
N. parvum / D. seriata / Do. sarmentorum	*Quercus rubra*
***Do. sarmentorum / Dothiorella* sp.**	*C. atlantica*
Do. sarmentorum / D. mutila	*C. lawsoniana*
Do. sarmentorum / D. seriata	*Thuja occidentalis*
Do. omnivora / Phaeobotryon cupressi	*Cupressus sempervirens*
Do. omnivora / D. seriata	*Fraxinus excelsior*

Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

Table S13. Frequency of occurrence of multiple Botryosphaeriaceae species cohabiting in diseased host trees (A) and within diseased tree parts (B, see Table 2) in this and in previous studies in the Western Balkans (Zlatković et al. 2016a, b, 2017).

Cohabiting Botryosphaeriaceae species (A)	Frequency of occurrence (%)	Cohabiting Botryosphaeriaceae species (B)	Frequency of occurrence (%)	Botryosphaeriaceae species	Frequency of occurrence (%)
				(A)	
Botryosphaeria dothidea / Diplodia seriata	6	***B. dothidea / D. mutila***	**13**	***B. dothidea***	**30.6**
B. dothidea / Diplodia mutila	12	***B. dothidea / D. sapinea***	**13**	*N. parvum*	22.4
B. dothidea / Diplodia sapinea	10	*B. dothidea / N. parvum*	6	*D. mutila*	17.6
B. dothidea / Neofusicoccum parvum	**16**	*B. dothidea / Dothiorella* sp.	6	*D. seriata*	12.9
B. dothidea / Dothiorella omnivora	2	*B. dothidea / Do. sarmentorum*	6	*D. sapinea*	10.6
B. dothidea / Dothiorella sp.	2	*N. parvum / D. sapinea*	6	*Dothiorella* sp.	2.4
B. dothidea / Dothiorella sarmentorum	4	*N. parvum / D. mutila*	6	*Do. omnivora*	2.4
N. parvum / D. sapinea	2	*N. parvum / Do. sarmentorum*	6	*P. cupressi*	1.2
N. parvum / D. seriata	4	*D. sapinea/ D. seriata*	6	(B)	
N. parvum / D. mutila	10	***Do. sarmentorum / D. mutila***	**13**	***B. dothidea***	**24.5**
N. parvum / Do. sarmentorum	4	*Do. sarmentorum / Dothiorella* sp.	6	*N. parvum*	22.4
N. parvum/ Do. omnivora	2	*Dothiorella* sp. */ D. seriata*	6	*D. sapinea*	10.2
D. sapinea/ D. mutila	2			*Do. sarmentorum*	10.2
D. sapinea/ D. seriata	2			*D. seriata*	6.1
D. mutila/ D. seriata	2			*Dothiorella* sp.	4.1
Do. sarmentorum/ D. sapinea	2			*D. mutila*	2
Do. sarmentorum / Dothiorella sp.	2			*Do. omnivora*	2
Do. sarmentorum / D. mutila	6				
Do. sarmentorum / D. seriata	6				
Do. omnivora / Phaeobotryon cupressi	2				
Do. omnivora/ D. seriata	2				

Cohabiting Botryosphaeriaceae species with the highest frequency in diseased host trees/within diseased tree parts are given in bold and shaded.

Table S14. Frequency of isolation of multiple Botryosphaeriaceae species from the same tree of a variety of tree species.

Host	Frequency (%)
Cedrus atlantica	**22.2**
Chamaecyparis lawsoniana	11.1
Sequoiadendron giganteum	7.4
Aesculus hippocastanum	7.4
Cupressus arizonica	3.7
Pseudotsuga menziesii	3.7
Pinus nigra	3.7
Sequoia sempervirens	3.7
Thuja plicata	3.7
Chamaecyparis pisifera	3.7
Chamaecyparis obtusa	3.7
Pittosporum tobira	3.7
Prunus laurocerasus	3.7
Quercus rubra	3.7
Thuja occidentalis	3.7
Cupressus sempervirens	3.7
Fraxinus excelsior	3.7

Data refer to the whole set of Botryosphaeriaceae isolates from the Western Balkans, including isolates from this and from previous studies (Zlatković et al. 2016a, b, 2017).

Table S15. Analyses of variance (ANOVA) and Kruskal-Wallis H test results for the effect of *Botryosphaeriaceae* isolates on the extent of vascular discoloration/resinous lesions associated with a variety of tree species in this study.

	Chamaecyparis lawsoniana	
Botryosphaeria dothidea	CMW 39301 × C	
	H= 1.94, df= 1, p= 0.16	
Neofusicoccum parvum	CMW 39327 × C / CMW 39317 × C	
	H= 1.94, df= 1, p= 1.16	
Diplodia seriata	CMW 39376 × C / CMW 39382 × C	
D. mutila	CMW 39348 × C / CMW 39385 × C	
Dothiorella sarmentorum	CMW 39364 × C / CMW 39365 × C	
Do. omnivora	CMW 39361 × C / CMW 39363 × C	
Dothiorella sp.	CBS 135622 × C / CBS 135623 × C	
	H= 1.06, df= 1, p= 0.3	
Sphaeropsis visci	CMW 39386 × C	
	H= 1.17, df= 1, p= 0.28	
	Magnolia grandifolia	
B. dothidea	CMW 39301 × C	CMW 39315 × C
	F= 116.98, df= 1, p= 0	H= 15.14, df= 1, p= 0.0001
	CMW 39301 × CMW 39315	
	F= 0.29, df= 1, p= 0.6	
	Liriodendron tulipifera	
B. dothidea	CMW 39301 × C	CMW 39315 × C
	F= 35.7, df= 1, p=0.000012	F= 1225, df= 1, p= 0
	CMW 39301 × CMW 39315	
	F= 14.38, df= 1, p=0.001	
	Pseudotsuga menziesii	
B. dothidea	CMW 39301 × C / CMW 39315 × C	
	F= 3.08, df= 1, p= 0.1	
	Cedrus deodara	
N. parvum	CMW 39327 × C	
	F= 3.86, df= 1, p= 0.06	

Table S15. Continued.

Cedrus atlantica	
Botryosphaeria dothidea	CMW 39301 × C / CMW 39315 × C
	F= 3.08, df= 1, p= 0.1
Neofusicoccum parvum	CMW 39327 × C
	F= 0.95, df= 1, p= 0.34
Diplodia seriata	CMW 39327 × C
	F= 0.95, df= 1, p= 0.34
D. mutila	CMW 39358 × C / CMW 39348 × C
	F= 1.8, df= 1, p= 0.2
Dothiorella sarmentorum	CMW 39364 × C / CMW 39365 × C
Dothiorella sp.	CBS 135622 × C / CBS 135623 × C
	F= 0.53, df= 1, p= 0.48
Quercus rubra	
N. parvum	CMW 39327 × C / CMW 39317 × C
	F= 0.11, df= 1, p= 0.75
D. seriata	CMW 39382 × C / CMW 39376 × C
	F= 1.53, df= 1, p= 0.23
Do. sarmentorum	CMW 39364 × C / CMW 39365 × C
	F= 0.06, df= 1, p= 0.81
Taxus baccata	
B. dothidea	CMW 39301 × C / CMW 39315 × C
	H= 2.08, df= 1, p= 0.15
Prunus cerasus	
D. seriata	CMW 39382 × C / CMW 39376 × C
	H= 1.34, df= 1, p= 0.25
Aesculus hippocastanum	
Do. sarmentorum	CMW 39365 × C
	F= 48.03, df= 1, p= 0.000002
Populus tremula	
B. dothidea	CMW 39301 × CMW 39315
	F= 0.42, df= 1, p= 0.52

Table S15. Continued.

	Quercus cerris
Botryosphaeria dothidea	CMW 39301 × CMW 39315
	F= 0.5, df= 1, p= 0.47
	Pinus patula
Neofusicoccum parvum	CMW 39327 × C
	F= 21.5, df= 1, p= 0.0002
	CMW 39317 × C
	F= 50.5, df= 1, p= 0.000001
	CMW 39327 × CMW 39317
	F= 2.2, df= 1, p= 0.15
B. dothidea	CMW 39301 × C
	F= 53.2, df= 1, p= 0.000001
	CMW 39315 × C
	F= 47.4, df= 1, p= 0.000002
	CMW 39301 × CMW 39315
	F= 2.9, df= 1, p= 0.14
	CMW 39329 × CMW 39337 × CMW 39327 × CMW 39317 × CMW 39301 × CMW 39315
	H= 40.22, df= 5, p= 0
	Eucalyptus grandis
N. parvum	CMW 39327 × C
	F= 35.78, df= 1, p= 0.000012
	CMW 39317 × C
	F= 34, df= 1, p= 0.000016
	CMW 39327 × CMW 39317
	F= 16.19, df= 1, p= 0.0008
B. dothidea	CMW 39301 × C
	F= 36.27, df= 1, p= 0.00001
	CMW 39317 × C
	F= 73.28, df= 1, p= 0
	CMW 39301 × CMW 39315
	F= 3.43, df= 1, p= 0.08

Table S15. Continued.

Eucalyptus grandis	
Botryosphaeria dothidea	CMW 39329 ˣ CMW 39337 ˣ CMW 39327 ˣ CMW 39317 ˣ CMW 39301 ˣ CMW 39315
	H= 39.64, df= 5, p= 0

- Cankers/necrotic lesions were not detected on the control plants. The log10 transformation was used to improve normality of the data set in order to analyse the effect of *B. dothidea* isolate CMW 39315 on vascular discoloration of *L. tulipifera* seedlings.