Assessment of presence and distribution of Armillaria and Heterobasidion root rot fungi in the forest of Vallombrosa (Apennines Mountains, Italy) after severe windstorm damage

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One of the main problems for the management and conservation of silver fir stands has long been pathogens causing root rot, in particular Armillaria spp. and Heterobasidion annosum s.l. These opportunistic pathogens are especially threatening now that climate change related stress is increasing tree susceptibility to disease and vulnerability to windstorms. The northern Apennines Mountains (central Italy) are forecast to be one of the areas with the highest temperature increase in the next future. However, no systematic assessment exists of the risk posed by the disturbance due to secondary pathogens in the Apennine forests. In the Nature Reserve of Vallombrosa (northern Apennines), where silver fir forests have been managed and conserved for centuries since the Middle Ages, making it an ideal site for studying these parasites, the high presence of H. annosum was reported already in 1990, while only sporadic observations are available on Armillaria species. The aim of this work was to examine the occurrence of both pathogens, since detailed knowledge about their distribution may assist forest management planning and decision-making. Systematic sampling was undertaken at the intersection of 52 grid points covering the whole forest. Different fungal species from soil and fungal samples (fruiting bodies or rhizomorphs) were identified by combining morphological descriptions with molecular methods. The analyses confirmed the presence of H. abietinum in about 70% of the investigated points. The fungus was detected at two new localities above 1000 metres suggesting a possible expansion of the parasite at upward elevation, which might be associated with climate change. Armillaria was widespread: almost 90% of the samples resulted positive, and four different Armillaria species were successfully identified. The most frequent species were A. cepistipes, whose rhizomorphs were especially abundant, and A. ostoyae, which was often detected just in soil samples. At sites where A. cepistipes was found to coexist with A. gallica, these two species might specialize themselves to necrotrophic and saprotrophic lifestyle, respectively. Besides, there were unexpected findings of A. mellea, supposed to be a residual from the previous rotation of broadleaves.

Keywords: Abies alba, Armillaria spp., Butt Rot, Climate Change Disturbances, Heterobasidion annosum, Root Rot, Silver Fir, Windstorm Damage

Introduction

Heterobasidion annosum s.l. (Fr.) Bref. and Armillaria species are among the most destructive forest pathogens in many parts of the world. As facultative necrotrophs, these parasitic fungi are able to survive saprotrophically on dead wood, and the same individual can switch from one mode of lifestyle to the other (Guillaumin & Le Grand 2013). Both species complexes cause root rot and decay of the stem, which typically leads to uprooting under intense mechanical stress (Honkaniemi et al. 2017), as a consequence of the decreased stability of the tree. Such damages, which are typical in conifer plantations, took place after the windstorm of March 5th, 2015, which destroyed about 50 ha of forest at the Nature Reserve of Vallombrosa (central Italy). This event gave actuality to a complex monitoring in order to shed light on the key factors determining the susceptibility of the forest to extensive windthrows (Chirici et al. 2018). H. annosum s.l. had long been regarded as a single species until mating tests revealed the occurrence of intersterility groups (ISGs – Korhonen 1978b), all of which have later obtained formal description as species. The Eurasian groups were named H. annosum s.s., H. abietinum Niemelä & Korhonen and H. parviporum Niemelä & Korhonen (Niemelä & Korhonen 1998). H. annosum s.s. is mostly associated with pines, especially Scots pine (Pinus sylvestris L.), but attacks several other conifers and even some broadleaved tree species. H. parviporum shows a relatively strict specialization for Norway spruce (Picea abies Karst.), while H. abietinum is commonly associated with European silver fir (Abies alba Mill.) and other species of the genus Abies (Gonther & Thor 2013). Root and butt rot caused by Heterobasidion is widespread in
coniferous forests of Italy. Along the Apennines, the disease incidence is particularly high in silver fir plantations that are older than 50 years (Capretti 1998). High economic losses associated with the pathogen have been reported from this area for decades, along with the dieback of fir stands (Cantiani 1960, Moriondo & Tiberi 2000). Typically, the epidemic begins right after the first thinning, because the remaining live stumps are a favourable substrate for the vegetative spread of the parasite (Cer- tini et al. 2000). Farina et al. (1990) reported the massive presence of H. abieti- num in the forest of Vallombrosa, performing a systematic sampling of the whole area. Their findings imply that root rot is a chronic disease in fir stands, and likely among the most important factors of the species’ decline and high vulnerability to windstorms.

The genus Armilaria is distributed in all continents. In Europe, seven biological species have been distinguished based on sexual incompatibility (Korhonen 1978a, Guillaumin et al. 1993). Their geographical distribution has been detailed by Guillaumin et al. (1993). A. mellea (Vahl:Fr.) Kummer is distributed in the Atlantic and Mediterranean parts of Europe, in various deciduous forests (Shaw & Kile 1991). A. ostoyae (Romagn.) Herink is less thermophilic and predominantly linked to conifers (Shaw & Kile 1991, Guillaumin & Legrand 2013). A. borealis Marxm. & Kornonely mainly populates coniferous forests of Northern Europe (Guillaumin & Legrand 2013), but it is also common in highlands of Central Europe (Jankovsky 2005). A. gallica Marxm. & Romagn. is regarded as a low-elevation species typical of floodplain forests, while A. cepistipes Velen. occurs most frequently in the zone of European beech (Fagus syl- vatica L. – Shaw & Kile 1991, Janovsky 2003, Antonin et al. 2009). A. socialis (DC.: Fr.) Fayod, frequently mentioned as A. tabescens in the literature, is a thermophilic species of Southern Europe (Antonin et al. 2006). A. ectypa (Fr.) Lamoure is confined to wetlands and extremely rare (Sveta- sheva 2015). Identification of the various Armilaria spp. present on a site can be of great practical importance because the virulence and host range markedly differ among species. The most pathogenic European species seem to be A. ostoyae and A. mellea (Guillaumin & Legrand 2013). According to the scheme of Manion (1981), Armiliaria spp. appear sometimes as a predisposing factor, through numerous early infections of the root systems, and sometimes as a contributing factor giving the deathblow to weakened trees, as in fir stands of Vallombrosa (Intini 1988).

Under current and projected changes in climatic conditions, Armilaria species are expected to increase their activity and rhizomorphogenic capacity, and more easily defeat tree defence (Haavik et al. 2015, Kubiak et al. 2017, Holuša et al. 2018). Tree vulnerability to Heterobasidion species is also expected to rise in a warmer climate because of increased fungal growth and sporulation rate (La Porta et al. 2008, Gori et al. 2013, Müller et al. 2014). More generally, climate change might exacerbate drought stress, increasing tree susceptibility to secondary pathogens (Ghelardini et al. 2016), including Heterobasidion and Armilaria species. Considering that increased windstorm damages in the forest of Vallombrosa (Chirici et al. 2018), as in European forests in general (Gregow et al. 2017), may be exacerbated by higher occurrence and/ or virulence of root rot pathogens, the goal of present work was to provide distribu- tion maps of Armillaria and Heterobasidion species in the area to support forest man- agers in planning and decision-making. The several DNA-based diagnostic techniques, which were developed since the past studies on root rots in Vallombrosa, allowed for a more straightforward identification of wood rotting fungi in this work. Material and methods

Data acquisition

The study area is the forest of Vallom- brosa (43°44′4″ N, 1°34′34″ E), a biogeographic re- serve located about 50 km east-southeast of Florence, Tuscany, Italy. The forest cov- ers 1273 ha in the northern Apennines Mountains. The climate is characterized by a mean annual air temperature of 9.8 °C and a mean annual precipitation of 1275 mm (thermopluvimetric station of Vallom- brosa, 980 m a.s.l.). Western and north- western slopes dominate, with an average inclination of 18.8%. The altitude ranges be- tween 470 and 1440 m a.s.l. The soil units belong to Inceptisols and Alfisols (Soil Sur- vey Staff 1999), formed on Oligocene sandstone. Fragipan soil layers are present in 30% of the area, close to the surface (Bolla 2001). Silver fir has been cultivated since the 11th century and occupies more than half of the area today. The native vegetation is mainly represented by beech at higher elevations, oak-hornbeam stands (Quercus spp. mixed with Carpinus betulus L. and Ostrya carpinifolia Scop.) and chest- nut (Castanea sativa Mill.) at lower alti- tudes. Another important change is the intro- duction of European black pine (Pinus nigra Arn. spp. nigra and Pinus nigra Arn. ssp. laricio), and to a lesser extent, coast Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco var. menziesii) and Norway spruce. Field surveys were carried out in July 2015. Since summer 2015 had been especially hot and dry, conditions that could re- strict the formation of Heterobasidion fruiting bodies (Otrosina & Garbelotto 2010), the survey was repeated in May 2017 for Heterobasidion. The area, where Abies alba dominated more than half of the stands (57.7%), ahead of Pinus nigra (21.2%) and Fa- gus sylvatica (13.5%) forests, was covered by a 500 × 500 m grid which resulted in 52 sampling points (Fig. 1, Tab. S1 in Supple- mentary material). The same sampling grid has been used as in the study of Farina et al. (1990), so it was possible to compare the spatial distribution of H. annosum s.l. over the past 28 years. Points were identi- fied in the field with a handheld GPS navi- gator (Garmin™ GPSMAP® 62s, containing the forest management map of the Re- serve) and a topographic map. Soil sampling was done at all points, through hammering a steel cylinder of approximately 170 cm² (8.5 cm high × 5 cm in-
Distribution of root rot fungi after severe windstorm damage

DNA analysis
All types of samples (Heterobasidion basidiomes, Armillaria rhizomorphs, and soil) were utilized for DNA extraction. Each soil sample was homogenized by mixing; fungal samples were homogenized by grinding the tissue in liquid nitrogen using sterile mortar and pestle. Approximately 0.25 g of samples was used. DNA was extracted using the PowerSoil™ DNA Isolation Kit (MoBio, Carlsbad, USA), according to the manufacturer’s instructions. The extracted DNA solution was conserved at -25 °C. Identification of species within H. annosum s.l. as described by Mugnai & Capretti (1989). For rapid confirmation of visual identification of H. annosum s.l., the taxon-specific competitive-priming (TSCP)-PCR method was used according to Gonthier et al. (2003). For amplifying DNA, a mix of four primers (MLS, MLF, Mito 5 and Mito 7) was applied. PCR was performed in a 25 µl reaction mixture containing approximately 50 ng of template DNA, 0.5 µmol of each primer, 5 µl MyTaq® Reaction Buffer (comprising dNTPs and MgCl2, in a final concentration of 1 mM and 3 mM, respectively) and 1 µl MyTaq® DNA Polymerase (Bioline, London, UK). The PCR was amplified using a Mastercycler® ep Thermocycler (Eppendorf, Hamburg, Germany). The PCR programme was as follows: 3 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 20 s at 64 °C and 20 s at 72 °C with a final extension of 7 min at 72 °C. Identification of H. abietinum was confirmed by sequencing of the ITS region (Internal Transcribed Spacer) by the DNA Sequence Service of Macrogen Inc. (Seoul, Korea).

The ITS region was selectively amplified from soil and rhizomorph samples by nested PCR. The first reaction was carried out with external primers ITS1 and ITS4 used for amplification of ITS region of fungi (White et al. 1990). In the second reaction, the internal primers AR1 and AR2 for Armillaria ITS region were used (Lochman et al. 2004b). In both reactions, 1 µl of isolated DNA was used. Reaction conditions were as described for TSCP-PCR. Amplifications were carried out in a Mastercycler® ep Thermocycler (Eppendorf, Hamburg, Germany) with thermal cycling parameters: initial denaturation at 94 °C for 2.5 min, followed by 35 cycles of heat denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s, extension at 72 °C for 30 s and final extension at 72 °C for 5 min for ITS-PCR; initial denaturation at 94 °C for 2.5 min, followed by 35 cycles of heat denaturation at 94 °C for 30 s, annealing at 60 °C for 40 s, extension at 72 °C for 30 s and final extension at 72 °C for 7 min for AR-PCR. In some cases, due to scarce visibility on agarose gel, it was necessary to repeat the PCR with higher volume (2 µl) of extracted DNA. Negative samples were discarded after each reaction; positive ones were further processed. During restriction fragment length polymorphism (RFLP) analysis, DNA is cut into shorter strands by restriction enzymes that can be visualized after gel electrophoresis. Digestion of unpurified PCR products was carried out using restriction endonuclease Hinfl (Fermentas, Lithuania). As shown by Lochman et al. (2004a), this enzyme is able to discriminate the six main European Armillaria species. The restriction mixtures containing 19 µl of PCR product with 1 µl of buffer R and 1 µl of the enzyme Hinfl were incubated for 12 h at 37 °C (Lochman et al. 2004b).

After each reaction, PCR products were electrophoresed in agarose (Serva, Heidelberg, Germany) gel in TBE buffer at 5 V cm⁻¹ for approximately 35 min (ITS, AR) or 80 min (TSCP, RFLP). 1% gel was used for simple identification (ITS, AR) and 2% gel for identification to the species level (TSCP, RFLP). One µl of ethidium bromide or Serva DNA Stain G was added to the gel. The PCR was amplified using a Mastercycler® ep Thermocycler (Eppendorf, Hamburg, Germany). The PCR programme was as follows: 3 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 20 s at 64 °C and 20 s at 72 °C with a final extension of 7 min at 72 °C. Identification of H. abietinum was confirmed by sequencing of the ITS region (Internal Transcribed Spacer) by the DNA Sequence Service of Macrogen Inc. (Seoul, Korea).

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Data processing
The mapping of sampling points was conducted using the QGIS 3.0.0 software. Spatial data transferred from GPS was linked with the collected attribute data in the program. After georeferencing each sampling location, thematic map layers were created. The effect of environmental factors (altitude, site fertility, soil type, prevalent tree species and age of forest stand) on the presence/absence of H. annosum s.l. was tested using binomial logistic regression. The influence of the above parameters on the detection frequency of different Armillaria spp. was tested by multinomial logistic regression. Species identified from soil and rhizomorphs were pooled for this analysis. Statistical analyses were performed using the software STATISTICA®, 12.0 (StatSoft 2013).

Results
Heterobasidion annosum s.l. fruiting bodies were found on Abies alba stumps (cut or uprooted) at 71% of the sampling points. The pathogen was present in 85% of conifer stands and in only two broadleaved stands (where A. alba was growing sporadically). All samples were assigned to H. abietinum. The parasite was found in all silver fir stands, pure and mixed to other conifers (mainly European black pine and Douglas-fir) or broadleaved species. Fruiting bodies of H. abietinum were also present in about half of the stands where European black pine was the prevalent species. The findings of the present survey are summarized in Tab. 1, and mapped in Fig. 2, together with results by Farina et al. (1990). No ecological pattern was detected in the distribution of the pathogen.

Armillaria rhizomorphs were found at one third of the sampling points. However, Armillaria was widespread throughout the forest in soil (88.5% positive soil samples based on PCR results). The most common species distinguished by restriction were A. ostoyae at 21 points (29% of rhizomorphs/37% of positive soil samples) and A. cepistipes at 20 points (53/28). A. gallica was found at 16 points (18/31), while A. mellea was present in only 2 soil samples (0/4). A. cepistipes was twice more frequent in rhizomorph samples than in soil. The spatial distribution of Armillaria species identified from both sample types is shown in Fig. 3. A. mellea was found only in silver fir-Douglas-fir stands.

Tab. 1 - Number of stands with different tree species composition included in the study, and the incidence of Heterobasidion abietinum fruiting bodies in each stand type.

<table>
<thead>
<tr>
<th>Prevalent tree species</th>
<th>Number of stands</th>
<th>H. abietinum incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. alba</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td>A. alba with conifers</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>A. alba with broadleaves</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>P. nigra</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>P. nigra with conifers</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td>P. nigra with broadleaves</td>
<td>7</td>
<td>43</td>
</tr>
<tr>
<td>F. sylvatica</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Other broadleaves</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>71</td>
</tr>
</tbody>
</table>
A. cepistipes was mostly found in silver fir stands (70%), while A. ostoyae and especially A. gallica more abundantly occurred in forests dominated by other tree species, particularly beech.

Effects of tree species composition (i.e., prevalent tree species in the stand) and soil type were non-significant. Armillaria spp. were present at a wide range of elevations and various forest sites. There was no statistically significant difference found in the altitudinal distribution of Armillaria species. The difference between A. cepistipes and A. ostoyae occurrence can be explained by the effect of site fertility levels (coef.=0.737, p=0.024), the former species inhabits more productive sites. Another parameter that had a statistically significant effect on the distribution of the pathogen was the age of the stand. A. mellea was only present in pole stands. The majority of A. cepistipes were found in the 61-80 years age class, while the occurrences of A. gallica and A. ostoyae were shifted towards the oldest stands (Fig. 4). The difference between A. cepistipes and A. gallica occurrence can be explained by this pattern (coef.=0.027, p=0.028).

Discussion

The results of the study confirm and extend the knowledge about presence of Heterobasidion in the forest of Vallombrosa since the investigation by Farina et al. (1990). In that study, H. abietinum (F ISG) was found in the surrounding area of about 73% of the sampling points. The survey carried out in 2015-2017 confirmed the presence of the pathogen at all these points except for three. Nevertheless, in the present survey the fungus was recorded at two new localities at elevation of about 1100 m a.s.l. These detections indicate a possible expansion of the parasite at upward elevation, which might be associated with climate change (Yan et al. 2017).
Contrasting to the work by Farina et al. (1990), where _H. annosum_ s.s. (P ISG) was detected in Vallombrosa at a very low frequency (4% of sampling points), this species was not found in the forest in our survey. However, Farina et al. (1990) isolated the fungus also from wood samples gathered at each point, while the present survey was based solely on the apparent fruiting bodies. Indeed, basidiomes of _H. annosum_ s.s. might be more difficult to detect since they are less thick and less persistent than those produced by _H. abietinum_ (Niemelä et al. 1999).

Although the majority of _H. abietinum_ occurrences fell to fir stands, basidiomes of the fungus occasionally appeared in stands dominated by Douglas-fir, European black pine and beech, where there was admixture of silver fir. We did not identify the pathogen on other host species than Abies alba. However, in the forest of Vallombrosa _H. abietinum_ had been reported on Norway spruce, Douglas-fir, chestnut, Japanese red-cedar and pine by Farina et al. (1990).

Sedlák & Tomšovský (2014) reported _H. abietinum_ on pine and Norway spruce in the Czech Republic, always in stands where the historical presence of _A. alba_ was documented, supporting the evidence that the fungus can survive at sites with a changed tree species composition. Therefore, the presence of the fungus might represent a threat for other tree species besides _A. alba_, especially conifers, but also deciduous trees when growing in mixture with conifers, or at difficult sites (Korhonen et al. 1998), on high pH (>6) soils (Redfern & Ward 1998) or under the pressure of climate change, which is expected to be especially strong and sudden in the Mediterranean region (Giorgi 2006, Lionello et al. 2006) and which may defeat tree defences against secondary pathogens (Chelardini et al. 2016). In the case of Douglas-fir, which is only occasionally attacked by the pathogen and seldom suffers early mortality and killing even at an older age (Dela-tour et al. 1998, Korhonen et al. 1998), the highest risk is posed by _H. abietinum_ to young individuals (Sala et al. 1995).

Regarding _Armillaria_ species, the differences in their infection biology must be taken into account. Some species like _A. cepistipes_ propagate directly in soil (Prospero et al. 2006), whereas others such as _A. ostoyae_ are restricted to spreading through root contacts (Cruckshank et al. 1997). Therefore, the presence of DNA of distinct _Armillaria_ spp. in soil does not necessarily correlate with their prevalence in the ecosystem.

_Armillaria_ spp. are present in the entire area of the Nature Reserve of Vallombrosa. In many cases, the DNA of the fungus was amplified from both the rhizomorphs and the soil samples collected at the same location, and these isolates often belong to different species. This type of coexistence within the same forest stand is well documented in the literature (Prospero et al. 2006, Antonin et al. 2009), and has been explained by the different ecological strategy of the species, i.e., their specialization to saprophytic or parasitic behaviour.

In many respects, _A. cepistipes_ seems to be the most influential _Armillaria_ species in the Vallombrosa forest. The fact that more than half of the identified rhizomorph samples belong to this species is consistent with observations by many authors who describe it as highly rhizomorphogenic (Shaw & Kile 1991). Most probably, _A. cepistipes_ finds its ecological optimum in the young fir stands, because the fungus is more competitive in weakened hosts such as pines. Our results foreshadow its ability to overcome host resistance even on the most fertile sites. Given its dominance in relatively young fir stands, this species is considered potentially hazardous in Vallombrosa. _A. ostoyae_ is equally common as _A. cepistipes_, but its DNA was more often isolated from soil. Despite this species is usually associated with conifers, 38% of its occurrences in the forest of Vallombrosa were broad-leaved stands. The third important species is _A. gallica_. This facultative parasite did not show any preference to forest type, is evenly distributed in the area. In the points where both species are present, _A. cepistipes_ was found as rhizomorphs while _A. gallica_ was determined from soil. This points to the fact that _A. cepistipes_ acts as a parasite whereas _A. gallica_ is a decomposer, which is in line with the abovementioned hypothesis of Prospero et al. (2006).

Surprisingly, _A. mellea_ was detected from Douglas-fir stands. European plantations of this species are considered resistant to _Armillaria_ root rot (Guillamín & Legrand 2013). It is reasonable to assume that _A. mellea_ is a residual from the previous rotation of broadleaves in these quite young stands rather than a colonizer of conifers.

Besides root rot disease, two factors are conceived to be crucial in silver fir decline. Soil properties decisively affect fir growth; at Vallombrosa, the high bulk density of the BC horizon hinders deep rooting, thus limiting the stability of trees. Moreover, the almost impermeable layer prevents the soil from accumulating adequate amounts of water. Suffering from water stress, trees are unable to produce inhibitor metabolites for _H. abietinum_, so their susceptibility increases to the infection (Certi et al. 2000). Mediterranean climate is not very well suited to the needs of silver fir either. In addition to long dry periods in summer, particular weather events, such as heavy snowfalls and cyclones put these trees under stress (Puddu et al. 2003). The situation is getting worse with the advancing of the global climate disruption (IPCC 2014).

Conclusions

Our results confirmed the widespread presence of root decay fungi in the forest of Vallombrosa (central Italy). Reviewing the relevant literature has revealed an etiology in which soil properties and climatic circumstances are crucial elements, being able to predispose the trees to fungal infection and determine the disease outcome. When assessing the possibility of intervention to manage the forest so as to limit the damage from root rot caused by _H. annosum_ s.l. and _Armillaria_ spp., it is necessary to know the potential risk posed by the different species regarding their host spectrum, infection biology and ecological needs, considering their possible different behaviour with regard to management strategies and interventions. Abies alba and Pinus spp. are known to be the most vulnerable tree species to these pathogens, but young Douglas-fir individuals may also suffer heavy damage by _Heterobasidion_ attacks. Bearing in mind that the extent of damages at Vallombrosa has crossed a threshold where it does not merely cause large economic loss, but threatens the stability of the ecosystem (Chiriči et al. 2018), it is advisable to apply practical measures to limit the spread of parasites that have a crucial role in weakening trees and mining their resistance to mechanical stress. Since the area is protected, drastic operations like stump removal are not allowed. Instead, preventive treatment on the stumps utilizing antago-nistic fungi is recommended immediately after cutting to inhibit the airborne colonization by _H. abietinum_ and thus reduce the further extension of the rot through root contacts. Cuts should be done in summer when sporulation rate of _Heterobasidion_ is low (Garbelotto et al. 2010). In the case of silver fir, lowering the rotation period to 100-120 years is advisable. Fir stands should be planted only in the most suitable soils of the area, Ultic Hapludalfs (IU1s). Planting of Douglas-fir in areas where the presence of _Heterobasidion_ is documented should be avoided. Creating mixed stands with broadleaves is a preference; as such forests are less susceptible to _Heterobasidion_ (Puddu et al. 2003) and _Armillaria_ (Guillamín & Legrand 2013) attacks. Our results may help forest managers to decide which tree species are to be planted, based on their resistance to root rot pathogens.

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References


Bolla G (2001). Distribution, characterization and genes of the orizzonti compatti nei suoli delle foresta di Vallombrosa [Firenze]. Their influence on the upbringing of silver fir. Bachelor's thesis, Faculty of Agricultural and Forest Science, University of Florence, Italy. [In Italian]

Cantiani M (1960). Note sulla diffusione del mar-Beoee in the forest of Vallombrosa]. L'Italia Forestale e Montana 15: 122-124. [In Italian]


Chirici G, Bottalico F, Giannetti F, Del Perugia B, Cantiani M (1960). Note sulla diffusione del mar-Beoee in the forest of Vallombrosa [Firenze]. Their influence on the upbringing of silver fir. Bachelor's thesis, Faculty of Agricultural and Forest Science, University of Florence, Italy. [In Italian]

Dálya LB et al. - iForest 12: 118-124


Dálya LB et al. - iForest 12: 118-124


Dálya LB et al. - iForest 12: 118-124


Dálya LB et al. - iForest 12: 118-124


Dálya LB et al. - iForest 12: 118-124


Dálya LB et al. - iForest 12: 118-124

Distribution of root rot fungi after severe windstorm damage

UK, pp. 27-33.


Supplementary Material

Tab. S1 - GPS data of sampling points.

Link: Dalya_2929@suppl001.pdf