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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**

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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 & Legrand 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* (Gonthier & 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 50-60 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 (Certini et al. 2000). Farina et al. (1990) reported the massive presence of *H. abietinum* 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 *Armillaria* 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 fundamentally linked to conifers (Shaw & Kile 1991, Guillaumin & Legrand 2013). *A. borealis* Marxm. & Korhonen mainly populates coniferous forests of Northern Europe (Guillaumin & Legrand 2013), but it is also common in highlands of Central Europe (Jankovsky 2003). *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 sylvatica* L. – Shaw & Kile 1991, Jankovsky 2003, Antonín et al. 2009). *A. socialis* (DC.: Fr.) Fayod, frequently mentioned as *A. tabescens* in the literature, is a thermophilic species of Southern Europe (Antonín et al. 2006). *A. ectypa* (Fr.) Lamoure is confined to wetlands and extremely rare (Svetasheva 2015). Identification of the various *Armillaria* 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), *Armillaria* 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, *Armillaria* 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 *Armillaria* 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 distribution maps of *Armillaria* and *Heterobasidion* species in the area to support forest managers 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.

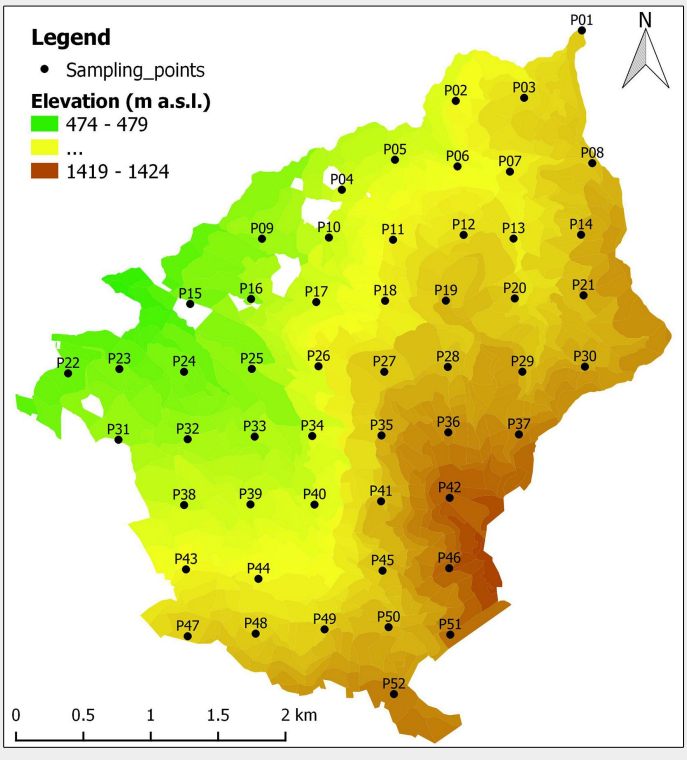

Fig. 1 - Topographic map of Vallombrosa forest with the location of sampling points. Points were unevenly distributed among age classes, with the prevalence of middle-aged stands (Source: database of the forest management plan 2006-2025 of Vallombrosa, central Italy).

Material and methods

Data acquisition

The study area is the forest of Vallombrosa (43° 44′ N, 11° 34′ E), a biogenetic reserve located about 50 km east-southeast of Florence, Tuscany, Italy. The forest covers 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 (thermopluviometric station of Vallombrosa, 980 m a.s.l.). Western and northwestern slopes dominate, with an average inclination of 18.8%. The altitude ranges between 470 and 1440 m a.s.l. The soil units belong to Inceptisols and Alfisols (Soil Survey 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 chestnut (*Castanea sativa* Mill.) at lower altitudes. Another important change is the introduction of European black pine (*Pinus nigra* Arn. ssp. *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 restrict 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 *Fagus sylvatica* (13.5%) forests, was covered by a 500 × 500 m grid which resulted in 52 sampling points (Fig. 1, Tab. S1 in Supplementary 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 identified in the field with a handheld GPS navigator (Garmin™ GPSMAP® 62s, containing the forest management map of the Reserve) and a topographic map.

Soil sampling was done at all points, through hammering a steel cylinder of approximately 170 cm^3 (8.5 cm high × 5 cm in-

ner diameter) to the topmost layer of soil, excluding the organic horizon. Fruiting bodies of *Heterobasidion* and *Armillaria* rhizomorphs were collected in an area of about 300 m² around the points. Samples were put into plastic bags or disposable plastic tubes and conserved at 4 °C until processed.

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.

Morphological traits of basidiomes differ between 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× MyTaq® Reaction Buffer (comprising dNTPs and $MgCl_2$ in a final concentration of 1 mM and 3 mM, respectively) and 1U 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 TSCR-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 *Hin*fI (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 *Hin*fI 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. Six µl of each sample and control, as well as 3 µl of 100 bp DNA Ladder (New England BioLabs, Ipswich, MA, USA), to determine the fragment size, were mixed with loading dye (New England BioLabs) and loaded into the wells. In the case of RFLP-PCR products, DNA fragment sizes were estimated using ΦX174 DNA/*Hin*fI Marker (Fermentas) as DNA molecular size marker. Amplified DNA of *H. annosum* s.s. (for TSCP) and *A. borealis* (for ITS, AR and RFLP) served as positive controls. To visualize DNA fragments, the gel was placed on an ultraviolet transilluminator and documented by digital camera (Olympus™ C8080WZ, Japan).

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 attributive 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® ver. 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-Dou-

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.

Prevalent tree species	Number of stands	*H. abietinum* incidence (%)
A. alba	21	100
A. alba with conifers	5	100
A. alba with broadleaves	4	100
P. nigra	1	0
P. nigra with conifers	3	67
P. nigra with broadleaves	7	43
F. sylvatica	7	29
Other broadleaves	4	0
Total	52	71

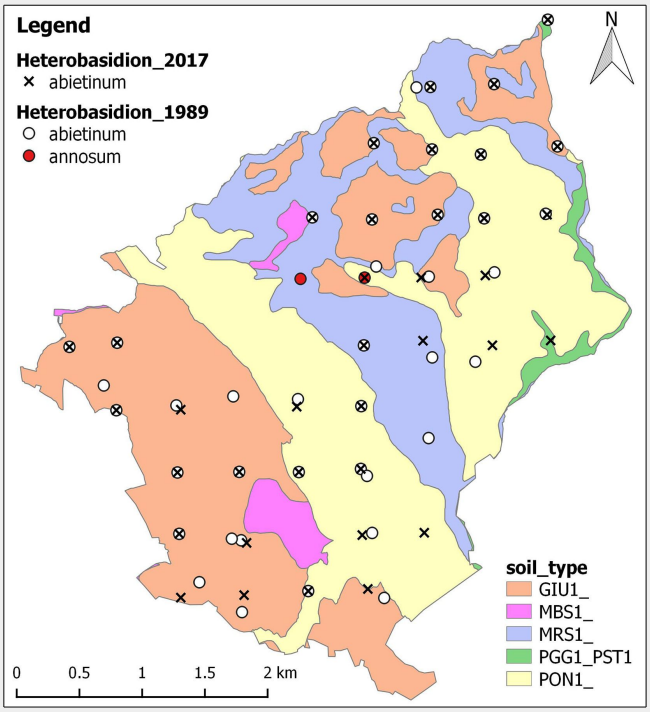

Fig. 2 - Spatial distribution map of *Heterobasidion* samples identified by Farina et al. (1990) and by present study (2015-2017). In some cases, where new host species were concerned or the damages from root rot were very evident, Farina et al. (1990) also collected material outside of the grid points. Soil types according to the Italian soil classification system and the Soil Survey Staff (1999): (GIU1) Giunchete - Ultic Hapludalfs; (MBS1) Monte Bastione - Typic Hapludalfs; (MRS1) Maresca - Humic Dystrudepts; (PGG1_PST1) Poggio di Petto - Lithic Dystrudepts; (PON1) Pontepetri - Typic Dystrudepts. (Source: http://159.213.57.101/pmapper/map.phtml).

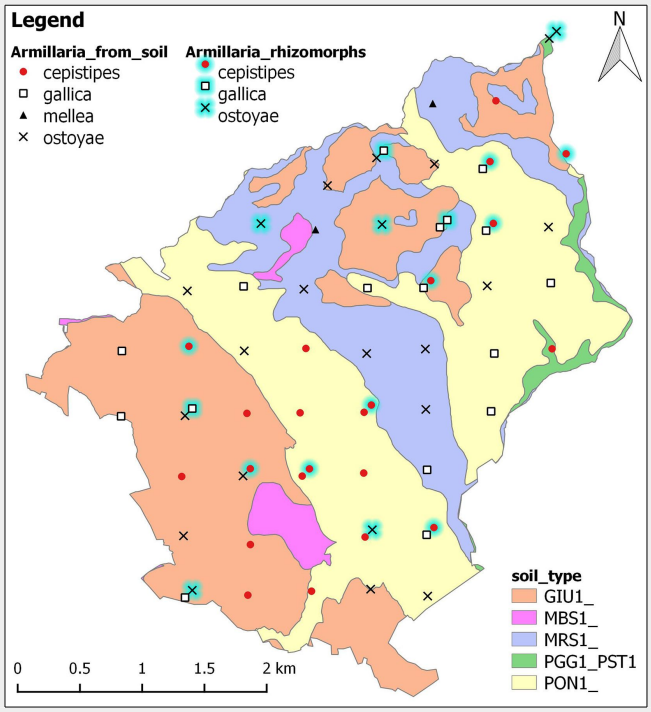

Fig. 3 - Spatial distribution map of *Armillaria* samples included in the study. Points with an outer glow indicate rhizomorph samples. Soil types according to the Italian soil classification system and the Soil Survey Staff (1999): (GIU1) Giunchete - Ultic Hapludalfs; (MBS1) Monte Bastione - Typic Hapludalfs; (MRS1) Maresca - Humic Dystrudepts; (PGG1_PST1) Poggio di Petto - Lithic Dystrudepts; (PON1) Pontepetri - Typic Dystrudepts (Source: http://159.213.57.101/pmapper/map.phtml).

glas-fir mixed stands in Humic Dystrudepts (MRS1). *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).

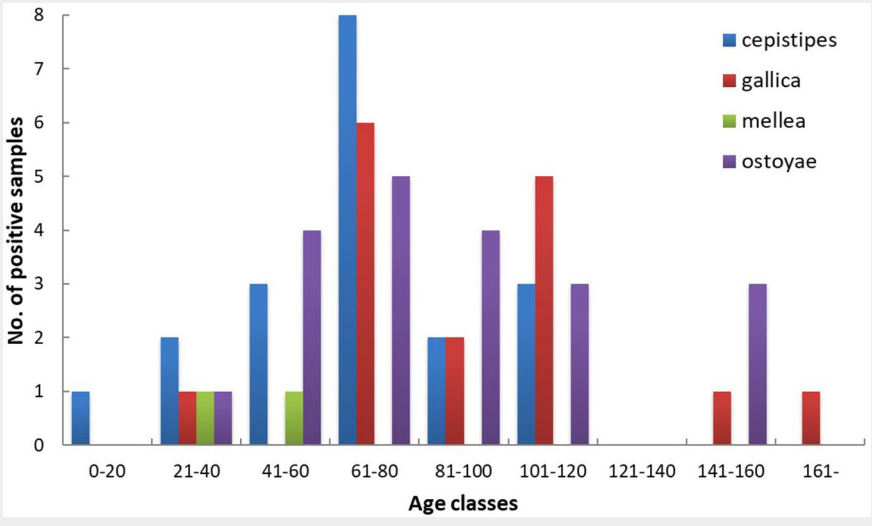

Fig. 4 - Distribution of *Armillaria* spp. according to age classes of the forest stands.

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).

Contrastingly 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 & Korhonen 1998).

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 & Tomšovsky (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 (Ghelardini 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 (Delatour 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 (Cruickshank 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, Antonín 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 area and consequently shows pathogenicity 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 broadleaved 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 (Guillaumin & 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 (Certini 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 conifers under stress (Puddu et al. 2003). The situation is getting worse with the advance of the global climate disruption (IPCC 2014).

Conclusions

Our results confirmed the widespread presence of root decaying 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 the applied 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 (Chirici 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 antagonistic 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 (GIU1). 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* (Guillaumin & 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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Supplementary Material

Tab. S1 - GPS data of sampling points.

Link: Dalya_2929@suppl001.pdf