

Ecology of *Armillaria* species on silver fir (*Abies alba*) in the Spanish Pyrenees

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Abstract

- We describe the distribution and the ecology of three *Armillaria* species observed in silver fir (*Abies alba*) forests of the Pyrenees.
- We surveyed the presence and abundance of *Armillaria* above and belowground in 29 stands. Isolates were identified by the PCR-RFLP pattern of the IGS-1 region of their ribosomal DNA. We measured several ecological and management parameters of each stand in order to describe *Armillaria* infected sites.
- *Armillaria cepistipes* was the most abundant of three species observed. *Armillaria gallica* was dominant in soils with a higher pH and at lower elevations. *Armillaria ostoyae* seemed to be more frequent in stands where *A. alba* recently increased its dominance relative to other forest tree species. Thinning activities correlated with an increased abundance of *Armillaria* belowground. In 83% of the stands the same *Armillaria* species was observed above and belowground.
- It seems that in a conifer forest, *A. cepistipes* can be more frequent than *A. ostoyae*, a virulent conifer pathogen. Since logging is related to a higher abundance of *Armillaria* in the soil, the particular *Armillaria* species present in a given stand could be considered an additional site factor when making management decisions.

Résumé – Écologie des espèces d'*Armillaria* du sapin blanc (*Abies alba*) dans les Pyrénées espagnoles.

- Nous décrivons la distribution et l'écologie de trois espèces d'*Armillaria* sur le sapin blanc (*Abies alba*) dans les forêts pyrénéennes
- Nous avons recherché la présence d'*Armillaire* au dessus du sol et dans le sol dans 29 peuplements. Les isolats ont été identifiés par RFLP-PCR de la région IGS-1 de leur ADN ribosomal. Plusieurs paramètres écologiques et de gestion ont été mesurés dans chacun des peuplements, pour caractériser les sites infestés.
- *Armillaria cepistipes* était la plus abondante des trois espèces observées. *Armillaria gallica* dominait dans les sols de basse altitude et à pH élevé. *Armillaria ostoyae* a semblé plus fréquent dans les peuplements où la dominance relative d'*A. alba* avait récemment augmenté par rapport aux autres espèces forestières. L'activité d'éclaircies était corrélée à l'augmentation d'*Armillaire* dans le sol. La même espèce d'*Armillaria* a été observée au dessus du sol et dans le sol, dans 83 % des peuplements.
- Il apparaît que, en forêt de conifères, *A. cepistipes* peut être plus fréquent qu'*A. ostoyae*, pathogène virulent des conifères. Puisque les coupes forestières sont reliées à une plus grande abondance d'*Armillaire* dans le sol, la présence d'une espèce particulière d'*Armillaria* dans un peuplement donné pourrait être un paramètre stationnel supplémentaire à considérer lors de décisions de gestion.

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1. INTRODUCTION

Increased mortality has been affecting silver fir (*Abies alba* Mill.) forests of the Pyrenees since the mid 1980s (Camarero et al., 2002). Increased aridity of the Pyrenean region (Macías et al., 2006) has probably contributed to the mortality of this species (Camarero et al., 2002), whilst the management of these stands in the last decades of the 20th century could have predisposed certain trees of these stands to decline (Oliva and Colinas, 2007). *Armillaria* (Fries: Fries) Staude species are commonly observed on dead silver fir trees (Oliva and Colinas, 2007) and have been observed to cause increased mortality in fir forests of Italy (Clauser, 1980; Intini, 1988).

Armillaria species infect roots of stressed trees (Fox, 2000b) penetrating the bark with a specialized form of mycelium called rhizomorph. The infection then spreads from the infected tree to neighbouring trees through root-to-root contact or as rhizomorphs (Fox, 2000a). Root infection results in reduced tree growth and eventual death after years of gradual decline (Cherubini et al., 2002). In our context, both butt rot and girdling have been observed on *Pinus* and *A. alba* trees. Infected trees are more susceptible to other pathogenic agents. Increased damages caused by other agents reported in the Pyrenees (Camarero et al., 2003; Martín and Cobos, 1986; Oliva and Colinas, 2007) could be a result of the undetected presence of this pathogen.

There are differences in virulence amongst the species of the genus *Armillaria* (Guillaumin et al., 2003). *Armillaria mellea* (Vahl: Fries) Kummer is very destructive in broadleaf forests, fruit trees, grapevine orchards, and on ornamental trees. *Armillaria ostoyae* (Romagnesi) Herink is more damaging to conifer species. Other *Armillaria* species, such as *A. gallica* Marxmuller and Romagnesi (synonym: *A. bulbosa* (Barla) Velenovsky), *A. cepistipes* Velenovsky, *A. borealis* Marxmuller and Korhonen, *A. tabescens* (Scolpoli: Fries) and *A. ectypa* (Fries) Lamoure are generally considered secondary pathogens or saprobes. Knowledge of the abundance and distribution of the different species of the genus *Armillaria* in the Pyrenees would help us estimate the role of this inconspicuous fungus in silver fir mortality.

Armillaria has been reported as an influential factor in the dynamics of several mountain forest types in Europe (Bendel et al., 2006a; Dobbertin et al., 2001) and in North America (Worrall et al., 2005). To our knowledge, its role in the dynamics of silver fir forests has never been studied. Tree-species composition of silver fir forests in the Pyrenees is changing: silver fir and *Fagus sylvatica* L. are increasing whilst *Pinus sylvestris* L. and *Pinus uncinata* Ram. are decreasing in abundance (Oliva and Colinas, 2007). Since there are differences in susceptibility to *Armillaria* among these tree species (Morquer and Touvet, 1972), *Armillaria* could play a role in the shifting species composition of these forests. Management has had an impact on the health of silver fir forests in the Pyrenees (Oliva and Colinas, 2007) and potentially on the availability of substrates for *Armillaria* species. Yet, the relationships among forest management practices, *Armillaria* populations, and stand health in silver fir forests have not been evaluated.

Our research aims to study the presence and distribution of the different species of the genus *Armillaria* in silver fir forests of the Pyrenees, and their interaction with the ecology and management of these stands.

2. MATERIALS AND METHODS

2.1. Field sampling

We studied the silver fir population of the Spanish Pyrenees. In this region, silver fir grows on northern aspects with high productivity indices. It often appears mixed with *F. sylvatica* in central and western locations, with *P. uncinata* at higher elevations, and with *P. sylvestris* at drier locations. Silver fir habitats are relatively cool sites (average temperature range in January is between -3 °C and 0 °C, and in August between 15 °C and 18 °C) at elevations ranging from 700 m to 2000 m above sea level (Blanco et al., 1997). We used the sampling grid described in Oliva and Colinas (2007), based on the level 1 grid used by the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) (Montoya et al., 1998), which we further subdivided at 2 km \times 2 km or 1 km \times 1 km. This systematic sampling grid includes 29 circular plots of 10 m diameter with at least six *A. alba* trees in the canopy. Dependent and independent variables measured in every plot are explained in Table I.

From 2002 to 2003, we surveyed each of 29 stands for below-ground and above-ground signs of *Armillaria*. The above-ground frequency for *Armillaria* was estimated by the percentages of stumps and dead trees colonized by *Armillaria* (Tab. I). Stumps and living and dead trees were assessed for mycelial fans, rhizomorphs and fruiting bodies. Butt rot produced by *Armillaria* on living fir trees was assessed by culturing inner-wood cores. In each plot, we extracted 2 cores per tree from the base of the stem of 6 randomly selected trees. Drilling was performed downwards and towards the stem centre. We sterilized the borer with ethanol 70% v/v between extractions and cores were kept in polyethylene bags and stored at 5 °C until they were cultured. For plots where we found no signs of *Armillaria* and for those in which the number of collected *Armillaria* tissue samples was low, we assessed silver fir saplings and seedlings and the area surrounding the plot for additional tissue samples. We used these samples when comparing environmental parameters between *Armillaria* species, but they were not included in the calculations of *Armillaria* abundance variables.

The below-ground abundance was assessed by measuring rhizomorphs from soil following the methodology of Rigling et al. (1997). Soil samples were collected from four randomly selected trees per plot. Each soil sample consisted of four pooled sub-samples, collected 2 m from each selected tree, following the four cardinal directions. Each soil sub-sample was prismatic with approximate dimensions of 0.125 m \times 0.125 m \times 0.300 m depth. Once in the laboratory, we sieved soil samples with a 0.009 m sieve to facilitate the separation of the rhizomorphs. Once the rhizomorphs were washed, they were weighed and measured and the mean weight and the mean length per plot were calculated. A third variable characterizing *Armillaria* below-ground abundance was the frequency of *Armillaria* in soil, calculated as the percentage of soil samples with rhizomorphs present.

From each soil sample, tree, or stump where signs of *Armillaria* were observed we attempted to culture a single *Armillaria* isolate.

Table I. Description of belowground and aboveground dependent and independent variables determined for each of the 29 plots.

Variable	Units	Additional remarks
Belowground dependent		
<i>Armillaria</i> soil weight	g m ⁻³	Mean fresh weight of <i>Armillaria</i> rhizomorphs of 4 soil samples
<i>Armillaria</i> soil length	cm m ⁻³	Mean length of <i>Armillaria</i> rhizomorphs of 4 soil samples
<i>Armillaria</i> soil frequency	%	Percentage of soil samples with <i>Armillaria</i> rhizomorph presence
Aboveground dependent		
<i>Armillaria</i> stump colonisation	%	Percentage of stumps with <i>Armillaria</i> signs
<i>Armillaria</i> dead tree colonisation	%	Percentage of dead trees with <i>Armillaria</i> signs
Independent		
Elevation	m	Meters above sea level
Slope	%	Slope percentage, measured with inclinometer
North exposure	°	0° implies north, > 0 and < 0 values, east and west exposure respectively
Soil depth	cm	One unique observation in the centre of each plot
Shrub cover	0–4	Thresholds of 25% shrub coverage; 0, no shrub coverage
Herbaceous cover	0–4	Thresholds of 25% herbaceous coverage; 0, no herbaceous coverage
Moss cover	0–4	Thresholds of 25% moss coverage; 0, no moss coverage
Light index	No units	Mean of light indexes of the main understory plant species (Ellenberg et al., 1991)
Moisture index	No units	Mean of soil-moisture indexes of the main understory plant species (Ellenberg et al., 1991)
Nitrogen index	No units	Mean of nitrogen-presence indexes of the main understory plant species (Ellenberg et al., 1991).
pH	No units	Mean pH of 4 soil sub-samples per plot (Oliva and Colinas, 2007)
LAI	m ² m ⁻²	Leaf area index, using hemispherical photos and analysed with Gap Light Analyzer software 2.0 (Oliva and Colinas, 2007)
Dominant height	m	Mean height of the 100 largest- diameter trees per hectare
Mean height	m	Mean height of all <i>A. alba</i> trees within a plot
Density	stems ha ⁻¹	<i>A. alba</i> density
Canopy closure	%	Mean crown closure of all <i>A. alba</i> trees in plot. Crown closure calculated as the number of horizontal crown quadrants touching/overlapping other trees
Hart-Becking index	m	Hart-Becking index for <i>A. alba</i> trees. Assuming triangular tree distribution
Slenderness	m m ⁻¹	Mean ratio, between height and diameter at breast height (DBH), of all trees in plot
BA	m ² ha ⁻¹	Basal area. Accompanying species also included
Mean DBH	cm	Diameter of the mean BA tree
Type of mixture	class	Includes four classes: <i>A. alba</i> - <i>F. sylvatica</i> mixed stands, <i>A. alba</i> - <i>P. sylvestris</i> mixed stands, <i>A. alba</i> - <i>P. uncinata</i> mixed stands and pure <i>A. alba</i> stands
Silver fir dynamics index	No units	Indicator of <i>A. alba</i> increase relative to co-existing tree species (Oliva and Colinas, 2007). Values higher than 1 imply fir decrease, values lower than 1 imply increasing fir composition
Harvested BA	No units	Ratio between BA of stumps and BA of living trees
Number of interventions	No units	Visually assessed by the decay of stumps
Thinning intensity of the average intervention	m ² ha ⁻¹	Calculated as the total harvested BA divided by the number of interventions
Other sp. BA	%	Percentage of BA occupied by other species
Silver fir defoliation	%	Percentage of <i>A. alba</i> trees showing defoliation
Silver fir chlorosis	%	Percentage of <i>A. alba</i> trees showing chlorosis
Other species defoliation	%	Percentage of damaged trees of accompanying species according to their defoliation
Silver fir mortality	No units	Ratio between BA of dead firs and BA of living firs
Other species mortality	No units	Ratio between BA of dead trees of accompanying species and BA of living trees
Mortality	No units	Ratio between BA of dead trees and BA of living trees

Collected tissues and wood inner cores were sterilized 10–20 s in hydrogen-peroxide 30% v/v. Sterilization was stopped by placing tissues in sterile distilled water. Surface sterilized tissues were cultured in a modified BDS selective media (Harrington et al., 1992), which consisted of 4 ppm benomyl, 0.0001% w/v streptomycin, 1.5% w/v malt extract, 1.5% w/v agar and no dichloran. Plates were incubated in the dark at room temperature (≈ 20 °C), and once any mycelial growth was observed, we transferred the isolate to malt extract agar media consisting of 1.5% w/v malt extract and 2% w/v agar. Isolates

were assigned to the genus *Armillaria* based on their culture morphology.

2.2. Species identification by PCR-RFLP

We extracted the DNA of the isolates following the CTAB-based protocol used by Kårén et al. (1997), and we amplified a portion of the intergenic spacer region (IGS-1) of the rDNA operon using the

Table II. GenBank accession numbers for sequences of *Armillaria* isolates from rhizomorphs collected in silver fir (*Abies alba*) forests of the Spanish Pyrenees.

Origin	GenBank accession number	
	IGS-1	ITS
<i>A. alba</i> stump	FJ159574.1	–
<i>A. alba</i> stump	FJ159568.1	–
<i>A. alba</i> stump	FJ159566.1	FJ159578.1
<i>A. alba</i> stump	FJ159575.1	–
Soil	FJ159573.1	FJ159576.1
Soil	FJ159567.1	FJ159577.1
Soil	FJ159570.1	–
<i>A. alba</i> stump	FJ159572.1	–
Soil	FJ159571.1	–
<i>A. alba</i> stump	FJ159569.1	–

primers LR12R and O-1 as described by Harrington and Wingfield (1995). Amplifications were performed using puReTaq *Ready-to-go* PCR beads (GE-Healthcare, UK) in a Biometra (Goettingen, DE) T-Personal thermal cycler. We included a negative control with no DNA with every set of PCR reactions. Isolates were typed at species level comparing their RFLP pattern with those reported by Harrington and Wingfield (1995) and Pérez-Sierra et al. (1999). For isolates that could not be identified by their IGS-1 RFLP pattern, we amplified the Internal Transcribed Spacer (ITS) region including the 5.8S gene subunit of the same rDNA operon by using ITS1F (Gardes and Bruns, 1993) and ITS4 (White et al. 1990) primers, following the cycling conditions described by Gardes and Bruns (1993).

2.3. DNA sequencing

Several isolates resulted in a non-conclusive RFLP pattern and others were typed as *A. borealis*, a northern-European *Armillaria* species. IGS-1 and ITS amplification products of these isolates were sequenced. We also sequenced the IGS-1 amplification products of isolates typed as *A. cepistipes* and *A. ostoyae* collected near the unsuccessfully typed isolates. Genbank (NCBI) accession numbers of sequences and isolate description are presented in Table II. PCR products were sequenced in both directions with the corresponding primers using an ABI 3100 sequencer (Applied Biosystems) at the Genomic Service Facility of the Autonomous University of Barcelona (Bellaterra, Barcelona, Spain) and with an ABIPRISM 310 sequencer (Applied Biosystems) at the Sequencing and Fragment Analysis Service of the University of Malaga (Malaga, Spain). Sequences were edited and aligned first with ClustalW and then manually with the software Mega version 4 (Tamura et al., 2007). We confirmed the *Alu I*, *Nde I* and *Bsm I* digestion patterns observed in the electrophoresis by analysing the sequences with the software Bioedit version 7.0.0 (Hall, 1999). Isolates that could not be satisfactorily identified by their PCR-RFLP pattern were identified by performing a nucleotide BLAST search (Altschul et al., 1997), comparing their sequences with those available in NCBI database and those available in the database of the Dept. of Forest Mycology and Pathology (SLU, Sweden). In both cases, we only used the sequences of isolates collected in Europe and identified at species level by two independent methods.

2.4. Statistical analysis

We analyzed the differences in environmental and management stand characteristics amongst *Armillaria* species by one way ANOVA, and we compared species means by the protected least square differences method (LSD). We used the GLM procedure of SAS/STAT.

Rhizomorph weight and rhizomorph length relationships with independent variables were analysed by Poisson regression with logarithm as link function. The relationships of ecological and management variables with *Armillaria* frequency in soil, *Armillaria* stump colonisation and *Armillaria* dead tree colonisation were analyzed by logistic regression. Both generalized regression models were adjusted with the GENMOD procedure of SAS/STAT. Overdispersion was corrected by the ratio between the deviance and the degrees of freedom (Schabenberger and Pierce, 2001). The assumptions of the hypotheses of linearity, normality and homogeneity were met by selecting the best BOX-COX transformation of the independent variable.

The means are presented with the 95% confidence interval (CI). Variables subjected to *logit* or logarithmic transformations were back-transformed. In these cases, the median and the confidence limits of the median are presented. Exact confidence limits for percentages were calculated using a Bayesian approach (Clopper and Pearson, 1934).

3. RESULTS

3.1. *Armillaria* root rot: analyses at species level

Sampling of fungal tissues resulted in 111 samples of *Armillaria*. Most were from rhizomorphs (108), and only a few represented basidiome (1) and mycelia (2) tissue. From these samples, the total number of cultured isolates was 80 (Tab. III), since several samples did not yield any growth in culture, or resulted in contaminations. Out of 174 living trees assayed, *Armillaria* was isolated from only one individual with both inner wood cores providing positive colonisation results. Butt rot produced by *Armillaria* affected 0.6% (CI: 0.0–3.2) of living silver fir trees. No other *Armillaria* signs were observed on living trees.

Three *Armillaria* species were identified according to their RFLP digestion pattern: *A. cepistipes*, *A. gallica* and *A. ostoyae*. Two isolates showed the RFLP pattern of *A. borealis*. However, their IGS-1 and ITS sequencing showed greater correspondence with *A. ostoyae* isolates and were therefore considered *A. ostoyae*. Five isolates yielded the same non-reported pattern in the *Alu I* digestion, with fragments of 583 and 200 bp. We consider these five isolates to be *A. cepistipes* as their IGS-1 and ITS sequences showed greatest similarity with *A. cepistipes* isolates.

Armillaria cepistipes was the most frequent species (72% of isolates) in our survey. *Armillaria gallica* (15%) and *A. ostoyae* (10%) were less frequent (Tab. III). *Armillaria cepistipes* was predominant in all surveyed substrates: soil, stumps, dead trees, seedlings and saplings. The only sample isolated from the core of a living silver fir tree was also *A. cepistipes*. At the regional scale, *A. cepistipes* also appeared to be the most

Table III. Origin and frequency of *Armillaria* species observed in Pyrenean silver fir (*Abies alba*) forests.

Armillaria species	Sample origin					Frequency	
	Soil	Stumps	Dead trees	Seedlings and saplings	Area surrounding plot	Individual samples	Plots
<i>A. cepistipes</i>	63% (42–81) ¹	74% (49–91)	90% (55–100)	100% (47–100)	70% (46–88)	72% (61–82)	75% (53–90)
<i>A. gallica</i>	18% (6–38)	21% (6–46)	0% (0–26)	0% (0–53)	15% (3–38)	15% (8–25)	25% (10–47)
<i>A. ostoyae</i>	18% (6–38)	5% (0–26)	10% (0–45)	0% (0–53)	15% (3–38)	13% (6–22)	20% (7–42)
Number of isolates	27	19	10	4	20	80	24 ²

¹ Percentages expressed with respect to the total of samples isolated. Confidence intervals at 95% are shown in brackets.

² Refers to the number of plots. More than one species can be found in a single plot.

Table IV. Ecological and management factors with significant differences amongst *Armillaria* species present in Pyrenean silver fir (*Abies alba*) plots.

	<i>p</i> > <i>F</i>	<i>A. ostoyae</i>	<i>A. cepistipes</i>	<i>A. gallica</i>
Ecological				
Elevation (m)	0.002	1680 (1426–1924) a	1423 (1294–1551) b	1038 (816–1260) b
Nitrogen index ¹	0.023	3.62 (2.87–4.38) b	4.67 (4.26–5.08) ab	5.03 (4.34–5.71) a
pH	0.012	4.82 (4.12–5.50) b	5.14 (4.78–5.50) b	6.15 (5.52–6.77) a
Management				
Silver fir defoliation (%)	0.034	1.2 (0.0–32.3) b	18.9 (10.0–24.2) a	9.2 (2.6–28.8) ab
Silver fir dynamics index ²	0.066	0.28 (–0.13–0.69) b	0.73 (0.51–0.94) ab	0.93 (0.55–1.30) a

Note: Equal letter within a row implies non-significant differences between means at *p* < 0.05 (LSD). Confidence intervals at 95% are shown in brackets.

¹ Nitrogen index ranges from 0 to 9 (Ellenberg et al., 1991). Lower numbers mean lower nitrogen availability.

² Values higher than 1 mean recession of *A. alba*, values of 1 mean maintenance of species mixture, values from 0 to 1 mean *A. alba* BA increase.

widespread of the *Armillaria* species as it was present in 18 out of 29 stands sampled.

Armillaria ostoyae occurred at significantly higher mean elevations than *A. gallica* and *A. cepistipes* (Tab. IV). *Armillaria gallica* appeared more frequently under basic soil conditions than *A. cepistipes* and *A. ostoyae*. *Armillaria gallica* and *A. cepistipes* appeared on stands with higher nitrogen index than *A. ostoyae*. In *A. ostoyae* infected stands, silver fir had lower defoliation than stands in which other *Armillaria* species were present. *Armillaria ostoyae* was associated (*p* = 0.066) with stands where *A. alba* was increasing its dominance relative to other forest tree species, but the association of silver fir dynamics index with the presence of *A. ostoyae* was not significant.

In 83% (CI: 52–98) of the forest plots, we recovered *Armillaria* isolates of the same species from below- and above-ground samples of the same plot. In 17% (CI: 0–48) of the plots the aboveground isolates were identified as *A. cepistipes* while belowground *A. cepistipes* was found together with *A. gallica*.

The distribution of *Armillaria* species varied depending on the tree species and on the substrate of observation (stump or dead tree). The majority of cultures isolated from *A. alba* dead trees (100% CI: 65–100) and stumps (81% CI: 60–95) were identified as *A. cepistipes*. *Armillaria gallica* was only observed on *A. alba* stumps (18% CI: 5–40). *Armillaria cepistipes* was detected on a dead *P. uncinata* tree, on a single *Popu-*

lus tremula L. stump and on a single *P. sylvestris* stump. *Armillaria ostoyae* was isolated once from a dead *P. uncinata* tree.

3.2. Armillaria root rot: analyses at genus level

We observed significant differences (*p* = 0.021) among the tree species (stumps and dead trees) infected by *Armillaria*. Dead *A. alba* trees showed the highest incidence (53% CI: 32–73). It was significantly higher than the incidence observed on *P. uncinata* stumps and on *P. sylvestris* stumps, respectively of 12% (CI: 3–40) and 6% (CI: 1–45). Dead *P. uncinata* trees and *A. alba* stumps showed intermediate incidences with 41% (CI: 16–72) and 35% (CI: 19–55) of infection. Only one dead *F. sylvatica* tree and three stumps of *P. sylvestris* were found to be infected by *Armillaria*. *Armillaria* was detected neither on *F. sylvatica* stumps nor on dead *P. sylvestris* trees. The three belowground *Armillaria* abundance variables were positively correlated among themselves (all pairwise combinations significant at *p* < 0.0001). The aboveground abundance variables, dead tree and stump colonisation, did not relate significantly. Two significant relationships between below- and aboveground abundance variables were observed: a higher stump colonisation was associated with a greater frequency in soil (*p* = 0.040), and a greater weight in soil correlated with a higher dead tree colonisation (*p* = 0.018).

Stands with greater *Armillaria* weight in soil were those with higher slenderness values, with higher harvested BA,

Table V. Correlations between *Armillaria* abundance and frequency variables and silver fir (*Abies alba*) stand characteristics.

	<i>Armillaria</i> belowground			<i>Armillaria</i> aboveground	
	Weight	Length	Frequency	Dead tree colonisation	Stump colonisation
Elevation	ns	ns	ns	ns	--
Slope	ns	ns	ns	ns	ns
North exposition	ns	ns	ns	ns	ns
Soil depth	ns	ns	ns	ns	ns
Shrub cover	ns	ns	ns	ns	++
Herbaceous cover	ns	ns	ns	ns	+
Moss cover	ns	ns	ns	ns	ns
Light index	ns	ns	ns	ns	ns
Moisture index	ns	ns	ns	ns	--
Nitrogen index	ns	ns	ns	ns	ns
pH	ns	ns	ns	-	+
LAI	+	ns	ns	ns	ns
Dominant height	ns	ns	ns	ns	ns
Mean height	ns	ns	ns	ns	ns
Density	ns	-	ns	ns	ns
Canopy closure	ns	-	ns	ns	ns
Hart-Becking index	ns	ns	ns	ns	ns
Slenderness	+	ns	ns	ns	ns
BA	ns	-	ns	ns	ns
Mean DBH	ns	ns	ns	ns	ns
Type of mixture	*	ns	ns	ns	ns
Silver fir dynamics index	ns	ns	ns	ns	+++
Harvested BA	++	ns	+	ns	ns
Number of interventions	ns	ns	ns	ns	++
Thinning intensity of the average intervention	++	ns	ns	ns	ns
Other sp. BA	ns	ns	ns	ns	ns
Silver fir defoliation	ns	ns	ns	ns	ns
Silver fir chlorosis	ns	ns	ns	ns	ns
Other species defoliation	ns	ns	ns	ns	ns
Silver fir mortality	ns	ns	ns	ns	ns
Other sp. mortality	ns	ns	ns	ns	ns
Mortality	ns	ns	ns	ns	ns

Positive sign means a positive relationship. + $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$ and vice versa for negative relationships. Empty cells indicate non-significant at $p < 0.05$; * means significant differences at $p < 0.05$ (protected LSD) in class variables.

those that had been subjected to higher thinning intensity and those with higher LAI values (Tab. V). Higher mean lengths of *Armillaria* rhizomorphs in soil were observed in stands with lower density, lower BA and with lower percentage of canopy closure. *Armillaria* frequency was correlated with higher values of harvested BA. Dead tree colonisation was correlated with soil acidity. Stump colonisation by *Armillaria* occurred more frequently at lower elevations and in those stands with greater silver fir dynamic index values. Stump colonisation occurred more frequently on those stands subjected to a higher number of thinning interventions.

Pure silver fir stands showed higher median weights of *Armillaria* in soil (69.9 g m^{-3} CI: 44.9–108.7) than *A. alba-F. sylvatica* (16.3 g m^{-3} CI: 5–53.2) and *A. alba-P. sylvestris* mixed stands (13.3 g m^{-3} CI: 3–58.6). Median weight in pure

stands was not significantly different than mean weight in *A. alba-P. uncinata* stands (3.4 g m^{-3} CI: 0–296.9).

4. DISCUSSION

Within Pyrenean silver fir stands, *A. cepistipes* was the most frequently observed species of *Armillaria*. Many authors have found *A. ostoyae* associated with conifer forests (Blodgett and Worrall, 1992; Legrand and Guillaumin, 1993; Legrand et al., 1996; McLaughlin, 2001), while we found *A. ostoyae* in only 20% of these silver fir stands. Legrand and Guillaumin (1993) found *A. ostoyae* widespread in three silver fir forests in France, with *A. cepistipes* only in stands with a past presence of hardwood species. This same association between

A. cepistipes and the previous presence of hardwood species was noted by Rigling et al. (1997) and Tsopelas (1999) in *Picea abies* stands. As far as we know, there are no records of a past abundance of broadleaved species within the studied fir forests. At present, broadleaved species such as *F. sylvatica*, *Betula pendula* Roth. or *P. tremula* represent on average 7% of the BA of these stands, and no relation between the broadleaf component and any *Armillaria* species has been observed (results not shown). It seems that *A. cepistipes* can find suitable ecological conditions for its survival within a conifer forest, such as fir forests of Pyrenees.

The *Armillaria* species considered most virulent, *A. ostoyae*, was associated with silver fir stands on more acidic soils (mean pH = 4.82) and those located at higher elevations. *A. ostoyae* tends to occur where *A. alba* is increasing its canopy dominance (lower silver fir dynamics index), so it is not surprising that, at these sites, *A. alba* trees had lower defoliation. At present in the Pyrenees, *A. alba* is increasing its dominance due to BA losses of *P. uncinata* and *P. sylvestris* (Oliva and Colinas, 2007). The former pine species is considered very susceptible to *A. ostoyae* (Dobbertin et al., 2001; Morquer and Touvet, 1972) and root disease centres have been historically observed in the Pyrenees (Kile et al., 1991). This *Armillaria* species may have indirectly favoured *A. alba* progression within pine forests by reducing the health of pines or even by preventing regeneration. Further research may elucidate the role of *A. ostoyae* driving Pyrenean pine forests' dynamics as observed by Durrieu et al. (1985).

Armillaria gallica tended to occur in stands with relatively high soil pH (mean pH = 6.15) and at lower elevations as observed by Legrand and Guillaumin (1993), Rigling et al. (1997) and McLaughlin (2001). In the Pyrenees these two conditions are correlated ($r = -0.61$, $p = 0.0004$) (results not shown). Therefore we cannot exclusively associate *A. gallica* presence with either elevation or pH.

Management variables such as harvested BA, number of interventions and thinning intensity of the average intervention correlated with *Armillaria* below- and aboveground abundance. However, none of the variables associated with the presence of a given *Armillaria* species seems susceptible to being managed, such as elevation and pH. Therefore, the *Armillaria* species colonising a certain stand could be in itself considered as another site characteristic. The same *Armillaria* genet can be present in one site for centuries (Baumgartner and Rizzo, 2001; Bendel et al., 2006b; Ferguson et al., 2003; Rizzo et al., 1998; Smith et al., 1992), and shifts in the *Armillaria* species composition in one site are slow due to the low survival rate of new genets (Dettman and Van der Kamp, 2001). Of our silver fir stands, 83% showed the same *Armillaria* species above- and belowground. This, and the fact that we found silver fir infected by three different *Armillaria* species, suggests that the *Armillaria* species infecting a certain tree species might be more dependent on the *Armillaria* species present in the site than on the tree species.

The incidence of *Armillaria* on living trees was very low (0.6%). We did not observe connection between silver fir defoliation and the abundance of any *Armillaria* species. We observed *Armillaria* colonisations concentrated on *A. alba* dead

trees and stumps, and on *P. uncinata* dead trees. Colonisation of dead silver fir was mainly due to *A. cepistipes*. It is not clear whether *Armillaria* killed those trees or simply colonised them when they were weakened by other causes. Overall, *A. cepistipes* is generally considered a secondary pathogen and *Armillaria*-infected silver fir dead trees are typically of lower diameter than those living within the same stand (Oliva and Colinas, 2007). *Armillaria cepistipes* is probably only contributing to the self-thinning process of these forests, as suggested by Oliva and Colinas (2007) and not acting as a primary pathogen. The substantial incidence of *Armillaria* on *P. uncinata* supports the hypothesis that this pathogen is contributing to the reduction of this pine species within silver fir forests. Forest planners intending to maintain *P. uncinata* within silver fir forests in Pyrenees should consider the presence of *Armillaria* as a relevant site condition.

The abundance of *A. cepistipes* within silver fir forests does not seem to pose a problem at present since its virulence is low, but the frequency of *Armillaria* outbreaks could increase in the future. Pyrenean forests are far from being an undisturbed ecosystem since 90% of silver fir stands have been regularly managed (Oliva and Colinas, 2007). We have observed a high thinning intensity correlated with a higher biomass of *Armillaria* in soil, which in turn correlated with a higher incidence of *Armillaria* in dead trees. Increased mortality due to *Armillaria* associated to the onset of management activities has been reported for *A. ostoyae* and *A. mellea* (Baumgartner and Rizzo, 2001; Morrison et al., 2001), and the same process may be operating in the Pyrenean silver fir forests.

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