ROOT AND BUTT ROTS OF FOREST TREES

12th International Conference
on Root and Butt Rots

IUFRO Working Party 7.02.01

CONFERENCE PROCEEDINGS

M. Garbelotto & P. Gonthier, Editors

12th-19th August 2007
Berkeley, California - Medford, Oregon
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The University of California, Berkeley, USA
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Foreword

I would like to take the opportunity to thank everybody who made the 12th Root and Butt Rot meeting in California and Oregon, possible. In reality, this meeting amounted to three separate meetings. The idea behind the conference was to show the natural beauty of the American West to the attendees, while providing them with a flavor of the culture that characterizes this unique part of the world. I am sure the memories of dragons dancing in San Francisco's Chinatown, as well as riding in a cart in a hidden valley in the Sierra Nevada, or singing on the shores of an Oregon lake will be indelible memories for everybody. In these days of incredibly costly meetings, I hope everybody appreciated how affordable the meeting was, especially considering the quality of the lodging and food provided. I hope future organizers will keep in mind that, while it is easy to organize a meeting by charging a lot of money, it is much more of service to the scientific community to organize the same meeting at a lower cost for the attendees. Last year's meeting was also extremely rich from a scientific perspective, and these proceedings are a condensed version of the science that was presented there. One interesting novel addition to the standard format of these meetings was that of the "Short Talks"; these talks made up of a maximum of 7 slides and designed to last less than 5 minutes were a great idea and allowed for the communication of short or new projects.

The meeting would have never been possible without funding provided by the Koret Foundation of San Francisco and by the United States Forest Service, and without all of the administrative work offered for free by the Department of Environmental Science, Policy, and Management at U.C. Berkeley. Amy Smith and Doug Schmidt from U.C. Berkeley made the meeting possible through their complete dedication before and during the Conference. William Woodruff, Pete Angwin, John Pronos, David Rizzo, Garey Slaughter, Patricia Maloney, Everett Hansen, Ellen and Don Goheen were instrumental for the field trips in California and Oregon. The most heartfelt "Thank you!" to all of them both from Greg Filip and myself. Finally, thanks to Rachel Linzer, who made the publication of these proceedings possible.

A scientific conference is not made up by the studies presented, but by the people who participated. I am grateful to all the attendees for their individual contributions, and I hope to see them all at the 13th Root and Butt Rot meeting in Italy.

Your friend

Matteo Garbelotto
Organizing Chairman
12th International Conference
IUFRO Working Party 7.02.01.

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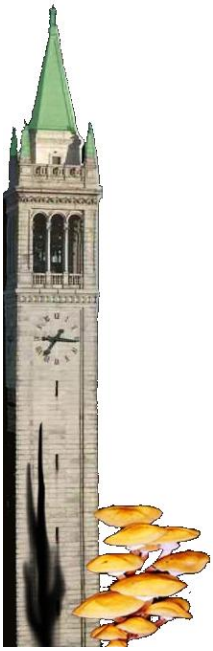
SYSTEMATICS, TAXONOMY AND PHYLOGEOGRAPHY



Morphology and ecology of three *Heterobasidion* spp. from Japan

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CONFERENCE ABSTRACT

Basidiocarp characters were examined to differentiate three Japanese *Heterobasidion* species: *H. annosum sensu lato*, *H. insulare* auct. jap., and an undetermined *Heterobasidion* sp. *Heterobasidion annosum* has perennial and effused-reflexed basidiocarps, pilei with a brown crust, small and round pores (4-5(-7) / mm). *Heterobasidion insulare* has annual and pileate to effused-reflexed basidiocarps, pilei with a reddish crust, and round to labyrinthiform pores (2-3(-4) / mm). The undetermined *Heterobasidion* sp. has annual and distinctly pileate basidiocarps, pilei with a crust limited near the base, large and round pores (1-3(4) / mm) and long tubes (up to 20 mm long). The ecological features of these species also differ in Japan. *Heterobasidion annosum* is limited to sub-alpine and boreal areas and causes root and butt rot on *Abies* spp. and *Picea* spp. *Heterobasidion insulare* is widely distributed and is thought to be a saprobe on various conifers. The undetermined *Heterobasidion* sp. is distributed in warm temperate to subtropical areas and is thought to be a saprobe on *Pinus* spp.

The polypore genus *Heterobasidion* Bref. is characterized by effused-reflexed to sessile basidiocarps, dextrinoid skeletal hyphae, generative hyphae without clamps, finely asperulate basidiospores and a *Spiniger* Stalpers anamorph (1, 3). In Japan, *H. annosum* and *H. insulare* are hitherto well known. Recently, phylogenetic studies have revealed that Japanese *H. annosum* s.l. is close to European and Chinese populations of *H. parviporum* Niemelä & Korhonen (4). Dai *et al.* (2) detected several intersterility groups within the *H. insulare* complex in China but proper names were not yet given for them. Additionally, Ota *et al.* (4) suggested that an undetermined *Heterobasidion* sp. that occurs in southern areas of Japan is a distinct phylogenetic species and is closely related to *H. araucariae* P.K. Buchanan. However, the morphological characters of these Japanese *Heterobasidion* species have not yet been well investigated. In this study, we provide detailed descriptions of the morphology and ecology of these species to give proper names for them.

Basidiocarp characters were macro- and microscopically described based on dried specimens. We examined 33 specimens of *H. annosum,* 66 of *H. insulare* auct. jap. and 8 of the undetermined *Heterobasidon* sp. (Figure 1). Microscopic characters were examined from materials mounted in Melzer's reagent. Non-dextrinoid and non-amyloid reactions were described as IKI$^-$.

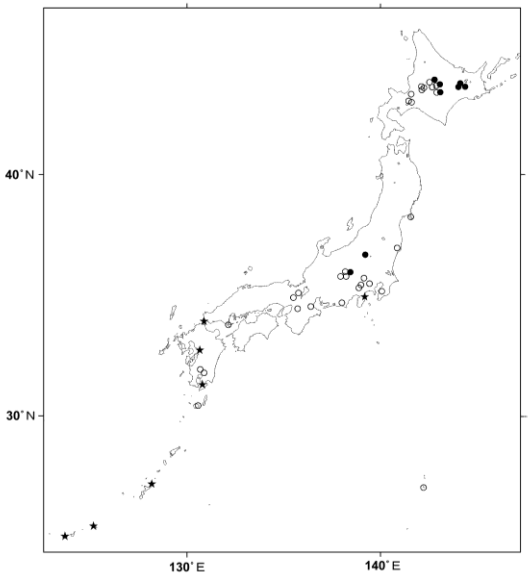

Figure 1. Map of Japan showing the locality of the basidiocarps collected. ●, *H. annosum sensu lato*; ○, *H. insulare* auct. jap.; ★, an undetermined *Heterobasidion* sp.

Heterobasidion annosum (Fr.) Bref. *sensu lato*
Habitat: On fallen trunks and roots of fallen trees of *Abies* and *Picea*. Throughout the year. Rare. Perennial. Distribution: Hokkaido Isl., Honshu Isl.
Macroscopic features: *Basidiocarp* effused-reflexed, solitary. *Pilei* irregular, 0.6-15 (30) cm, up to 3 cm thick. *Pileus surface* subtomentose to glabrous, sulcate, partly warty to rugose, brown to dark brown. *Pore surface* yellowish white. *Pores* round, 4-5 per mm. *Tubes* 0.1-0.3 cm deep in each layer, yellowish white. *Context* leathery, up to 0.5 mm thick, with a thin cuticle, same color with the tubes.
Microscopic features: *Context hyphae* dimitic, contextual generative hyphae without clamps, thin-walled, IKI$^-$, 1.5-3 µm across; contextual skeletal hyphae thick-walled, dextrinoid, 3-4 µm across. *Trama*

hyphae similar. *Basidia* clavate, 12-14 × 5-6.5 µm. *Basidiospores* finely asperulate, subglobose, hyaline, IKI$^-$, (3.3-)3.5-5(-5.6) × (2.9-)3.0-4.5(-4.8) µm.
Remarks: The hairs on the upper surfaces of the basidiocarps are shorter compared with the European *H. parviporum* materials. Although the species described here is dependent on old-growth forests in the sub-alpine areas, severe root and butt rots damage caused by this fungus in a plantation was detected by molecular determination (5).

Heterobasidion insulare (Murrill) Ryvarden, auct. jap.
Habitat: On stumps and fallen woods of various conifers. Spring-fall. Widespread. Annual. Distribution: Hokkaido Isl., Honshu Isl., Kyushu Isl., Bomin Isls., Satsunan Isls.
Macroscopic features: *Basidiocarps* usually sessile to effused-reflexed. *Pilei* semicircular to flabellate, applanate to slightly convex, imbricated or solitary, pileus 1.6-9.5 cm across. *Pileus surface* glabrous, rugose when dried, reddish brown to graysh brown when dried, yellowish white on margin. *Pore surface* pale yellow. *Pores* angular to round or labyrinthiform, 2-3(-4) per mm. *Tubes* 0.2-0.8 cm deep. pale yellow. *Context* corky, up to 2 cm thick at base, pale yellow, with a thin crust except for the marginal part.
Microscopic features: *Context hyphae* dimitic; contextual generative hyphae without clamps, thin-walled, 2-3.5 µm across; contextual skeletal hyphae thick-walled, moderately dextrinoid, 4-5 µm across. *Trama hyphae* slightly wide, otherwise as in context hyphae. *Basidia* clavate, 10.5-14 × 4-5 µm. *Basidiospores* finely asperulate, globose to subglobose, hyaline, IKI$^-$, (3.5-)4.0-5.6(-6.4) × (3.0-)3.4-4.5(-5.1) µm.
Remarks: The morphological characters of the species described here is not consistent with the features of the *H. insulare* type material but identical to those of *H. insulare* N-type from China.

Heterobasidion sp.
Habitat: On dead standing trees and stumps of *Pinaceae.* Spring-fall. Not common. Annual. Distribution: Honshu Isl., Kyushu Isl., Okinawa Isls.
Macroscopic features: *Basidiocarps* sessile. *Pilei* usually semicircular, convex, solitary or imbricated, pileus 1.5-9.0 cm in across. *Pileus surface* mostly glabrous, zonate, smooth or rugose, ivory to yellow brown, usually crusted only at the base, then dark brown. *Pore surface* smooth, cream to ivory, pores angular to round, rarely labyrinthiform, (1-)2-3(-4) per mm. *Tubes* 12-20 mm deep, pale yellow. *Context* corky, 1-2.5 mm thick, same color with the tubes.
Microscopic features: *Context hyphae* dimitic, contextual generative hyphae without clamps, thin-walled, 2-4 µm across; contextual skeletal hyphae thick-walled, moderately dextrinoid, 3-4 µm across. *Trama hyphae* similar. *Basidia* clavate, 14.5-19 × 6-7 µm.

Basidiospores finely asperulate, globose to subglobose, hyaline, IKI-, (3.7-)4.2-5.9(-6.4) × (3.1-)3.4-4.8(-5.3) µm.
Remarks: The morphological characters of the species described here is almost identical to those of *H. insulare* T-type from China.

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Armillaria species in Norway

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CONFERENCE ABSTRACT
Armillaria species in European forests have a wide distribution throughout continent. According to Roll-Hansen (1985) only three species of *Armillaria* occur in Norway. *Armillaria borealis* is the most common species and is found farther north than any other *Armillaria* species in Europe. *Armillaria cepistipes* and *A. ostoyae* are also found in Norway, while *A. gallica* is only found in the southernmost part of Sweden. Sixty five *Armillaria* isolates (previously identified as *A. borealis*, 20, *A. cepistipes*, 13, *A. ostoyae*, 5, and *Armillaria* spp., 27) were chosen from the fungal collection of NFLI. Identification was performed by PCR – RFLP according to Harrington & Wingfield (1995). Isolates which showed some specificity were tested by pairing with haploid tester strains and sequenced for comparison with data in NCBI database. Three *Armillaria* species were found among isolates: *A. borealis* (42 isolates), *A. cepistipes* (22 isolates) and only one isolate of *A. ostoyae. Armillaria gallica* was not found among studied isolates. Some isolates (1999-20/5 and 2004-276/1) have pattern (with bands 300, 200, 160 bp) for *Alu* I restriction enzyme, which was slightly different from patterns (b1 and b2) previously reported for *A. borealis* (Peréz-Sierra *et al.* 1999). We conclude that *A. borealis* is the most common and widespread *Armillaria* species in Norway. *Armillaria cepistipes* is also common, while *A. ostoyae* seems to be rare.

Armillaria species are present in most natural and exotic forests throughout the world. Seven morphological species are present in Europe and some of them are among the most significant root pathogens of trees and shrubs (6). Different methods have been developed for species identification, but the most reliable is mating test (3) and PCR-RFLP based molecular identification (7). According to Roll-Hansen (5) only *Armillaria borealis* Marxmüller & Korhonen, *A. cepistipes* Velen. and *A. ostoyae* Romagn. occur in Norway. The other four species are more termophilous and have a more southern distribution.

The goal of this work was to identify *Armillaria* species present in forest ecosystems in Norway and to determine phylogenetic relationshops between isolates. DNA based tools and somatic

compatibility tests were applied in order to obtain species identification.

Sixty five *Armillaria* isolates, from 27 localities, were chosen from NFLI fungal collection. Isolates were obtained from different parts of the infected trees and from rhizomorphs and carpophores. Isolates were subcultured on MEA (1.5% malt and 1.5% agar) with cellophane membrane and incubated at room temperature (~23-25°C) for 25 days. DNA isolation was performed with NucleoSpin Plant kit (Mecherey–Nagel GmbH & Co., Duren, Germany). IGS1 region between 3' end of the 26S gene and the 5S gene of the rDNA of *Armillaria* was amplified using the universal primers LR12R and O – 1, according to the amplification conditions of Harrington and Wingfield (2). The PCR products of IGS1 region were digested with *Alu*I, *Bsm* and *Nde*I enzymes, according to the manufacturer's protocol, and products are separated by electrophoresis on 3% agarose gels and visualized using UV light.

Isolates which could not be identified by PCR-RFLP were paired with 12 haploid testers of *A. borealis*, *A. cepistipes* and *A. ostoyae*, four of each. Prior to sequencing of IGS1 region amplicons were purified with QIAquick PCR Purification kit (Qiagen Ltd.), according to protocol. Comparison of sequences was performed with BLAST-Blastn ver 2.2.8. with the sequences from NCBI database (updated on February 2007).

PCR-RFLP based identification showed that three *Armillaria* species are present among the studied isolates. Forty two isolates were identified as *A. borealis*, 22 isolates as *A. cepistipes* and only one isolate was identified as *A. ostoyae*. Five different patterns B1, B2, C1, C2 and O, previously reported by (4), were found among tested isolates (Table 1).

Table 1. PCR-RFLP patterns found among studied isolates and identified *Armillaria* spp.

Pattern	***AluI* (bp)**	***BsmI*(bp)**	***NdeI*(bp)**	**No. of isolates**	***Armillaria* spp.**
b1	305, 200, 100	NRS	565, 380	31	*borealis*
b2	305, 200, 135	NRS	NRS	9	*borealis*
c1	400, 200, 190	NRS	NRS	14	*cepistipes*
c2	305, 200, 135	NRS	NRS	8	*cepistipes*
o	305, 200, 135	600, 300	565, 380	1	*ostoyae*
unknown	305, 200, 160	NRS	565, 380	2	*borealis*

By pairing tests two isolates with unknown pattern (Figure 1) and the isolates with patterns b2/c1/o were all identified as *A. borealis*, while four of five isolates previously identified as *A. ostoyae* were also identified as *A. borealis* and only one of them had positive reaction with *A. ostoyae* tester isolates.

Similarities of the tested isolates from NCBI database ranged from 97-99% (data not shown). Results of sequence comparison supported identification based on PCR-RFLP and mating tests.

Phylogenetic trees generated from the IGS1 region sequence data separated all isolates into three strongly supported clades (Figure 2).

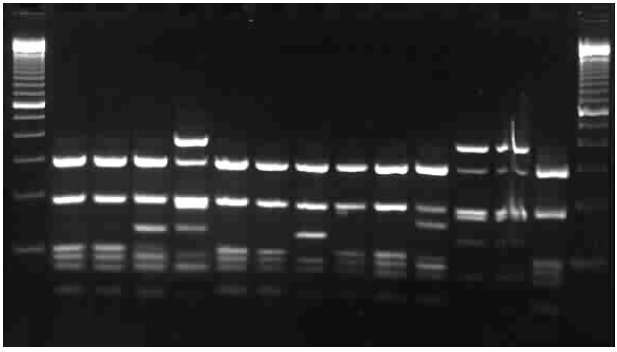

Figure 1. Fragment sizes for restriction digestion with *AluI* enzyme, based on the sequences of amplified IGS1 region. Lane 1-100 bp ladder; 2, 3, 6, 7, 9, 10, 14– *A. borealis* (b1); 4, 8- *A. borealis* (b2); lane 5, 12, 13 – *A. cepistipes*; 11 – U – for unknown pattern; 15-100 bp ladder.

One clade contained isolates of *A. ostoyae*, second clade of *A. borealis* and third included isolates of *A. cepistipes* and a tester isolate of *A. gallica.* Clades with *A. borealis*, *A. cepistipes*/*gallica* and *A. ostoyae* were supported by consensus tree analysis in 100%, 100% and 98% respectively.

Analysis of sixty-five isolates from different regions of Norway showed that three European *Armillaria* species are present in Norway. *Armillaria gallica* was not found among the studied isolates, which is in agreement with previous reports of species distribution in Europe (1). *A. borealis* was the most common species with distribution throughout the country. Earlier it has been reported as far north as Troms county, about lat. ca. 69° N, by Korhonen (3) and during this study it was found further north in Troms, in Lyngen 69° 33´ N. In both occasions

A. borealis was isolated from planted Norway spruce trees (*Picea abies*).

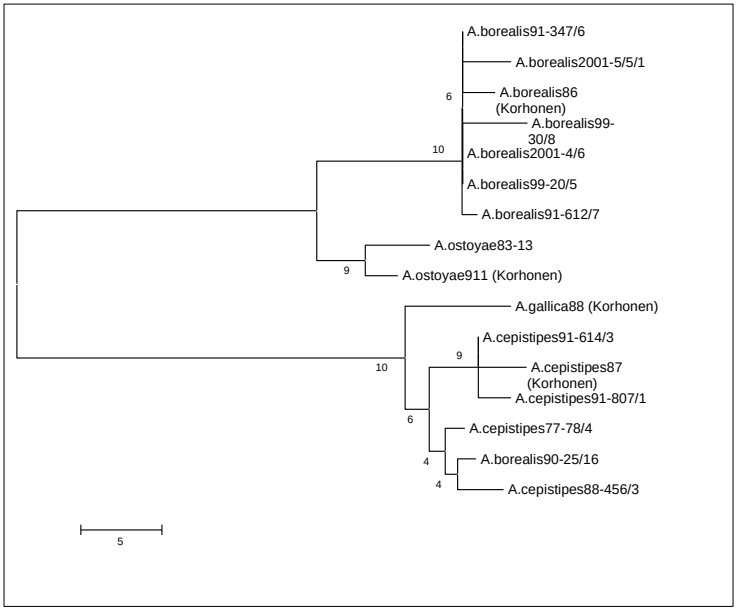

Figure 2. Phylogenetic tree based on the neighbor-joining (NJ) method of the nucleotide sequences of the IGS1 region for 12 isolates from Norway and 4 isolates from Finland. Values of percentages for bootstrap (1000 replicates) are given on each branch.

Armillaria cepistipes seems to be common in southern and central parts of Norway. In earlier work of Roll–Hansen (5) this species has been recorded in southern Norway. According to available data this species is widespread and has many coniferous and broadleaved host, but does not spread as far north as *A. borealis.*

Only one isolate has been identified as *A. ostoyae*, so this species seems to be pretty rare in Norway. However, it should be looked for in sandy pine forests which is the most common site for it in Finland (3).

AKNOWLEDGEMENTS

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The *Armillaria* species identified with the aid of mating tests in Russia

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CONFERENCE ABSTRACT

Five species belonging to the *Armillaria mellea* complex have been recorded earlier in the European part of Russia. They are *A. borealis* (from the following regions: Moscow, Voronezh, Ekaterinburg), *A. cepistipes* (Moscow, Perm', Ekaterinburg), *A. gallica* (Voronezh), *A. mellea* (Krasnodar) and *A. ostoyae* (Moscow). *Armillaria ostoyae* has been reported also from the Russian Far East. We identified with the aid of mating tests 51 specimens of *Armillaria* collected in 2003-2006 in the regions of Moscow (30 specimens), Mordovia (5) and Voronezh (16). *Armillaria borealis* and *A. cepistipes* were common in the Moscow region, and *A. gallica* was found in one locality. Most specimens collected in the Voronezh region (650 km south of Moscow) represent *A. gallica*; *A. borealis* was recorded once. The five specimens collected from Mordovia (ca. 500 km SE from Moscow) also represent *A. gallica*. In the Tellerman forest (38000 ha) at the territory of experimental forestry (2000 ha) of Voronezh region, in upland and flood-plain types of forest, the main host of *A. gallica* is common oak (*Quercus robur* L.), and rhizomorphs of the fungus are numerous in the forest litter in oak stands. However, *A. gallica* in Tellerman forest is a weak pathogen, infesting weakened and declining oaks and causing their early death.

Identification of biological species of *Armillaria* in the former USSR with the aid of mating tests was started by M.G. Radzievskaya in 1980s. Within the present Russian territory, she identified *A. borealis* and *A. cepistipes* in the Moscow region and *A. mellea s.s.* in the Krasnodar region of Russian Caucasus (6). In addition, she reported *A. borealis* from Belarus, *A. mellea* from Georgia and *A. gallica* from Ukraine. In 1990s, *A. ostoyae* was reported from the Russian Far East (2), and *A. borealis* and *A. cepistipes* were identified from the Ural Mountains, from the provinces Sverdlovsk and Perm (3, and unpublished records).

In 2002-2004 in a joint project of the RAS Forest Science Institute and the PAN Institute of Dendrology (Kurnik) was carried out for the identification of *Armillaria* species in Moscow regions. In this study (7), *A. borealis*, *A. cepistipes* and *A. ostoyae* were found in the Moscow

region while *A. gallica* and *A. borealis* were found in the same time in the southern forest-steppe of the Voronezh region, with participation of K. Korhonen.

The authors of the present report identified more *Armillaria* material in 2004 - 2007. Pure cultures obtained from fruit-body tissue or from single basidiospores were isolated from 47 specimens of *Armillaria*. Twenty-one specimens originate from Moscow forest-parks, reserves, and mixed boreal forests with spruce predominance. Eight specimens originate from Vladimir province, all of them from the same forest (Alexandrov forest, ca. 100 km NE from Moscow). Five specimens come from a broadleaved forest in Mordovia province (Saransk, ca. 500 km SE from Moscow). Thirteen specimens were collected in forest-steppe oak stands of Voronezh province (Tellerman forest, ca. 500 km from Moscow). The collection sites in Moscow and Voronezh were different from those where the collection was carried out in 2002 - 2004.

Mating tests revealed that *Armillaria borealis* and *A. cepistipes* dominated in the Moscow region as well as in the adjoining Vladimir province. *A. gallica* was recorded on one site in Moscow. This species was the only one identified in the material collected in the provinces of Mordovia and Voronezh.

Fruiting of *Armillaria* in the Moscow region has some particular features. Summer fruiting appears often in July and the fruiting species seems to be always *A. borealis*. In dry years the summer fruiting may appear later, in August, but so happened also in the unusually cool and humid summer 2006. Autumn fruiting of *Armillaria* starts in September and continues through October. In this period *A. borealis* and *A. cepistipes* may fruit in the same stand but the latter species usually a little later. *A. gallica* was found fruiting in October 2004 and 2006 at the same site in a private garden (Uspenskoye, 50 km of Moscow).

In the Tellerman Forest of Voronezh province, the common oak (*Quercus robur* L.) is the main host of *A. gallica*, and in October its basidiocarps appear around oak trees, whether living or dead. The rhizomorphs are very frequent in the forest litter and at the base of oak trees: on bark between root branches where they are less exposed for drying. However, *A. gallica* is a weak pathogen in Tellerman Forest, infesting only weakened and declining oak trees and causing early death. Sapwood decay begins at the stem base, starting from rhizomorphs that penetrate the bark, and the decay then spreads up in the stem. Root rot appears only after the tree crown dies out.

Armillaria species in Russia have been identified also on the basis of morphological characteristics of the fruit bodies, without genetic confirmation of the species identification. So, the species *A. mellea* and *A. tabescens* were reported from the Moscow region (8). However, the *A. mellea* appears to represent the species complex *A. mellea sensu lato*, and the occurrence of the thermophilic species *A. tabescens* in the rather northern Moscow region looks unlikely, particularly because this species has never been reported from southern parts of Russia and from the former Soviet Union. Four *Armillaria* species were identified in the Usmansky Forest of Voronezh region: *A. mellea*, *A. obscura, A. gallica* and *A. cepistipes* (1). A morphological study of *Armillaria* species in Krasnoyarsk region of central Siberia indicated the presence of *A. ostoyae, A. borealis* and *A. cepistipes* (4).

To summarize, the presence of five *Armillaria* species in European Russia has so far been shown with the aid of mating tests (Table 1).

Table 1. The *Armillaria* species so far identified with the aid of mating tests in Russia.

Species	Region	Host	Reference
A. borealis	Moscow region: Zvenigorod, Voskresensk and Pushkino districts	*Picea abies, Betula pendula, Pinus sylvestris*	Radzievskaya 1989
	Moscow and Zvenigorod, Borodino, Ruza, Dubna, Klin districts. Vladimir region: Alexandrov distr.	*P. abies, B. pendula, Tilia europaea, Larix europaea, Populus tremula, Corylus avellana, Quercus robur*	Selochnik et al. 2005
	Voronezh region: Tellerman forest.*	*Quercus robur*	– " –
	Ural region: Sverdlovsk district	*Populus tremula*	Korhonen 2004
A. cepistipes	Moscow and Zvenigorod, Borodino, Alexandrov districts	*P. abies, B. pendula, P. tremula*	Radzievskaya 1989 Selochnik et all. 2005
	Ural region: Sverdlovsk district, Perm district	unknown	K. Korhonen, unpubl. new records
A. gallica	Moscow region: Uspenskoye	*Prunus* sp. (felled)	new record
	Mordovia region: Saransk	unknown	new record
	Voronezh region: Tellerman forest.*	*Quercus robur*	Selochnik et al. 2005
A. ostoyae	Moscow region: Zvenigorod	*P. abies*	Selochnik et al. 2005
	Far East: Vladivostok region	*Pinus koraiensis*	Filip et al. 1995
***A. mellea* s.s.**	Krasnodar region	*Ulmus glabra*	Radzievskaya 1989

* All identifications were made by K. Korhonen

These species are *A. borealis, A. cepistipes, A. gallica, A. mellea* s.s. and *A. ostoyae*. Eastwards of Europe, the distribution area of *A. cepistipes* and *A. ostoyae* seems to extend over Siberia to eastern

Asia, and the distribution area of *A. borealis* to central Siberia at least. Besides the species mentioned above, some East Asian species of *Armillaria* probably occur in Russian Far East (5), and the occurrence of the rare species *A. ectypa* in Russia is likely although to our knowledge there are no reports of it.

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Dispersal and horizontal genetic transfer in the evolutionary history of *Heterobasidion annosum* P ISG

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CONFERENCE ABSTRACT

In the basidiomycete species complex, *Heterobasidion annosum,* the widespread P intersterility group (ISG) is an indigenous pathogen in Eurasia and North America, where it primarily attacks pines. Our earlier phylogenetic study showed three major lineages within the *H. annosum* P ISG – a Eurasian, eastern and western North American lineages, but was not sufficient to elucidate dispersal history of the fungus. Here, we report results of an expanded study including isolates from the known global distribution of the P ISG and utilizing more extensive analytical methods. We analyze DNA sequences from portions of two nuclear and two mitochondrial loci using maximum parsimony and Bayesian phylogenetic methods and median-joining networks. Notably, samples from Mexico included both eastern and western North American genotypes. This lineage intermingling is unique in the study area and suggests that Mexico is an evolutionary bridge between the North American groups. Our analyses also yielded evidence of potential inter-ISG genetic transfer in North America. After an ancient divergence, the P ISG is likely to have acquired a mitochondrial rDNA insertion from the *H. annosum* S ISG, with which it is sympatric in the western US. These results expand our current knowledge of the evolutionary history of this pathogen.

In the basidiomycete species complex, *Heterobasidion annosum,* the widespread P intersterility group (ISG) is an indigenous pathogen in Eurasia and North America, where it primarily attacks pines. Although these two taxa share many phenotypic similarities, including mode of pathogenicity, their phylogenetic relationship and evolutionary history have not previously been determined with certainty. An earlier phylogenetic study showed three major lineages within a monophyletic *H. annosum* P ISG clade – Eurasian, eastern and Western North American lineages (2). However, as areas at the limits of the pathogen's distribution were not sampled, the results were insufficient to elucidate the dispersal history of the fungus. Here, we report results from an expanded study, which included 116 P ISG isolates from throughout the known global range of this intersterility group: from the Altai region in Siberia and throughout through Europe and from southern Canada and the southeastern US through Mexico and the western US. Seventeen isolates of the related taxa *H. abietinum, H. parviporum,* North American *H. annosum* S ISG and *H. insulare* were included for comparison.

We analyzed DNA sequences from portions of two nuclear and two mitochondrial loci: parts of the nuclear coding elongation factor 1-alpha and glyceraldehyde 3-phosphate dehydrogenase genes and portions of the mitochondrial ATP synthase subunit 6 and of two non-homologous forms of an insertion into the ML5-ML6 region of the mitochondrial rDNA. Sequences were analyzed using maximum parsimony and Bayesian phylogenetic methods. As we were investigating intraspecific evolution, we also utilized median-joining network analysis, which allows for phenomena that happen below the species level such as incomplete reproductive isolation, recombination, more than two descendents of one ancestral allele, or coexistence of ancestral and descendent alleles.

Notable results of this expanded study include finding samples from Mexico had high genotypic diversity and included genotypes from both eastern and western North American lineages. The widespread intermingling of eastern and western North American lineages in Mexico is unique in the study area and suggests that Mexico represents an evolutionary bridge between the North American groups. This leads to two alternate hypotheses about the dispersal of the P ISG in North America: the P ISG may have dispersed in three stages – southward though eastern North America, then to Mexico and finally northward to western North America. Alternatively, the North American P lineage may have been confined to Mexico during a period of glaciation, later recolonizing both eastern and western North America.

Our analyses also yielded evidence of potential inter-ISG genetic transfer in North America. Phylogenetic and median-joining network

analysis revealed that in North America, both forms of the mitochondrial rDNA insertion were most closely related to the North American S ISG sequences and were separated by only a few mutational steps. This pattern is at odds with the pattern of all other sequenced regions in which the North American S ISG is most closely related to *H. parviporum* and *H. abietinum*, while it is distantly related to the North American P-type. This mitochondrial rDNA insertion is absent from all sampled Eurasian P ISG isolates. After an ancient divergence then, the P ISG is likely to have acquired a mitochondrial rDNA insertion from the *H. annosum* S ISG, with which it is sympatric in the western US. Interestingly, a stable SP hybrid has been recovered from a western US forest, indicating the two taxa have the potential to exchange genetic material in nature (1). These results expand our current knowledge of the evolutionary history of the global P ISG lineage in ancient and more recent time.

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Molecular characterization of *Fusarium* spp. and biological control of Fusarium root disease in forest nurseries

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CONFERENCE ABSTRACT

Root-rot disease caused by *Fusarium* spp. can cause severe loses in conifer nurseries. Isolates of *Fusarium* spp., morphologically indistinguishable from *F. oxysporum*, were collected from healthy and diseased conifer seedlings and nursery soils in the western USA. Over 300 isolates with *F. oxysporum*-like morphology were characterized using DNA sequences (mitochondrial small subunit and nuclear translation elongation factor 1 alpha). These isolates were characterized as non-pathogenic isolates of *Fusarium oxysporum* and pathogenic *F. commune* using laboratory/greenhouse pathogenicity tests and phylogenetic analyses. Although the morphology of *F. commune* appears indistinguishable from *F. oxysporum*, these two species are quite distinct genetically. Greenhouse studies on Douglas-fir seedlings were conducted to evaluate non-pathogenic *F. oxysporum* isolates for biological control of pathogenic *F. commune*. The potential use of non-pathogenic *F. oxysporum* as a biological control agent for managing Fusarium root disease in a forest nursery setting will be presented.

Is the UK population of *Heterobasidion annosum* different to other European populations?

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CONFERENCE ABSTRACT

Heterobasidion annosum isolates from the UK and other European areas were compared using microsatellite markers (RAMS) and multi-locus sequence typing (MLST). Average linkage of RAMS obtained with GAG and CGA grouped the 110 isolates tested according to geographical area, although some anomalies occurred. MLST, an unambiguous procedure for characterizing fungi using house-keeping genes, was used to sequence approx. 450-500 bp internal fragments of each of 10 nuclear and mitochondrial genes (both strands). For each gene, the different sequences present were assigned as distinct alleles and, for each isolate, the alleles at each of the loci used to define the allelic profile (sequence type; ST). The method allowed highly reproducible and extreme discrimination typing between the isolates. A major advantage of MLST is that sequence data are unambiguous: allelic profiles of isolates can easily be compared to those in large web-databases, simplifying inter-laboratory data exchange.

Anecdotal evidence suggests that UK isolates of *Heterobasidion annosum* may differ from other European isolates, being better adapted to oceanic conditions than isolates from more continental climates. No classical or molecular studies have previously been performed to investigate this possibility. A collection of isolates obtained from all over Europe were tested using RAMS markers (3), and further analyses are underway utilizing multi-locus sequence typing (MLST) based on 10 nuclear and mitochondrial genes.

Average linkage of RAMS obtained with GAG and CGA (2) grouped the 110 isolates tested according to geographical area of origin, although some anomalies occurred (Figure 1). UK isolates grouped into two clades, but were also distinct from isolates from other areas. A between population variance of 0.214 (phi) was recorded, indicating high levels of differentiation within the European *H. annosum* population as a whole. The population tested appeared to vary along a cline, according to a logical stepping stone model, where genetic flow is maximal between neighbouring populations, but minimal at extremes of geographical distance.

Preliminary results from MLST indicated that the method was highly discriminatory in establishing within-population variation in *H. annosum* isolates, based on the UK population (data not shown). Separate isolates shown to be from the same genetic background in previous work (1) grouped together in UPGMA analyses.

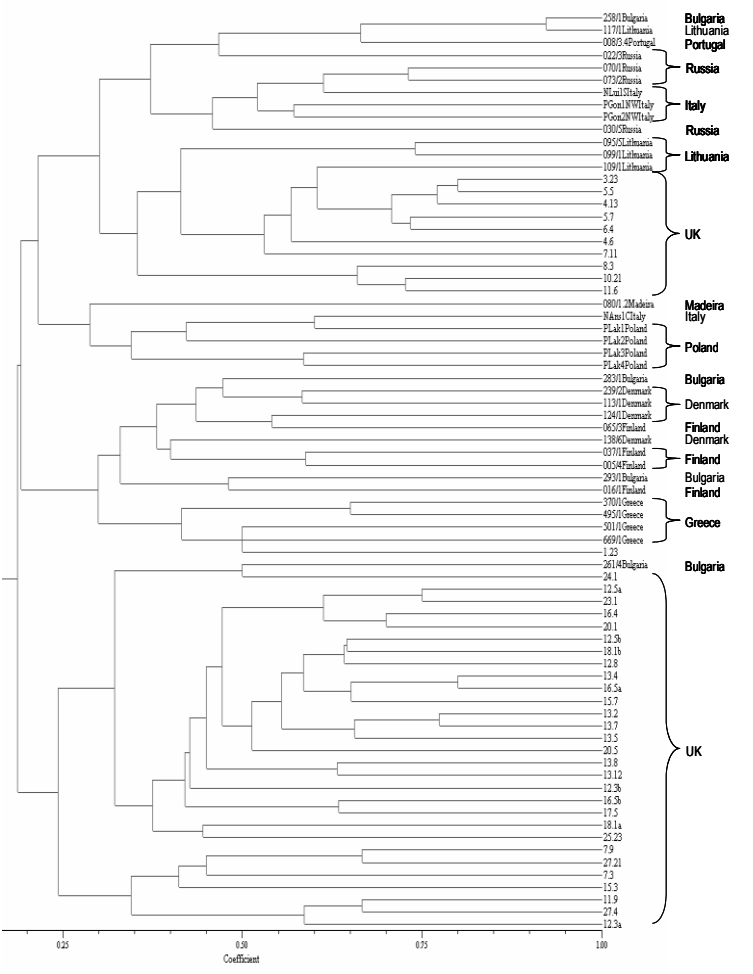

Figure 1. Phylogenetic relationships between 74 European isolates of *Heterobasidion annosum*, based on combined RAMS analyses using GAG and CGA.

Separation of the UK isolates into 2 clades by RAMS, and minimal overlap with isolates from other European areas suggests that the population is distinct. Separations between isolates obtained from different parts of Europe, however, were too small to draw definitive conclusions on the differentiation between UK and other European genotypes of *H. annosum* using RAMS alone.

These results demonstrate that both microsatellite- and MLST-based techniques are of great potential value in population studies of *Heterobasidion*.

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Relationships among Japanese and European *Laetiporus* based on phylogenetic analysis and incompatibility tests

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CONFERENCE ABSTRACT

Relationships among three Japanese *Laetiporus* taxa ('*L. sulphureus* var. *sulphureus*' auct Japan, '*L. sulphureus* var. *miniatus*' auct Japan and *L. versisporus*) and the European *L. sulphureus* were assessed with phylogenetic analysis and incompatibility tests. Phylogenetic analysis of the internal transcribed spacer region of nuclear ribosomal DNA and elongation factor 1 α gene regions suggested that Japanese *Laetiporus* divide into 4 groups: 1) the yellow pore form of *L. sulphureus* var. *miniatus*, 2) the white pore form of *L. sulphureus* var. *miniatus*, 3) *L. versisporus*/*L. sulphureus* var. *sulphureus* I, and 4) *L. versisporus*/*L. sulphureus* var. *sulphureus* II. European *L. sulphureus* divided into two clades: a hardwood type and a conifer type. The hardwood type formed a distinct clade and the conifer type shared a clade with the yellow pore form of *L. sulphureus* var. *miniatus*. Single spore isolates of the European conifer type were compatible with the yellow pore form from Japan. Single spore isolates from the three Japanese groups and the European hardwood type were incompatible with each other. Our results strongly support the new recognition of three *Laetiporus* taxa in Japan and two in Europe.

Laetiporus spp. occur worldwide from boreal to tropical zones and cause red-brown cubical heart-rot in the wood of many deciduous and coniferous trees. The *Laeitporus sulphureus* complex was divided into 6 taxa in North America (1) and into two taxa depending on the host type in Europe (2). In Japan, two species and one variety of *Laetiporus* were reclassified into three taxa: the white pore form of *L. sulphureus* var. *miniatus* (Jpn white pore form), the yellow pore form of *L. sulphureus* var. *miniatus* (Jpn yellow pore form) and the *L. sulphureus/versisporus* group (Jpn *sulphureus* group).

To establish the nomenclature of the Japanese taxa, comparative studies including phylogenetic studies, mating tests and type studies should be made. The objective of this study was to assess the relationships between Japanese and European taxa based on incompatibility tests and DNA analyses.

Nineteen isolates from Japan and 10 isolates from Europe were used in this study. DNA was extracted using a DNeasy extraction kit (Qiagen, Valencia, CA, USA). The nrDNA ITS and the EF1α genes were used as molecular markers. Some isolates were cloned into a pGEM-easy vector (Promega, Wisconsin, USA). All sequences were determined with the Applied Biosystems 3100 sequencer. Phylogenetic analyses of the aligned sequences were performed using distance and parsimony methods in PAUP ver.4.0b. Incompatibility tests were conducted by the method described by Banik *et al.* (1999).

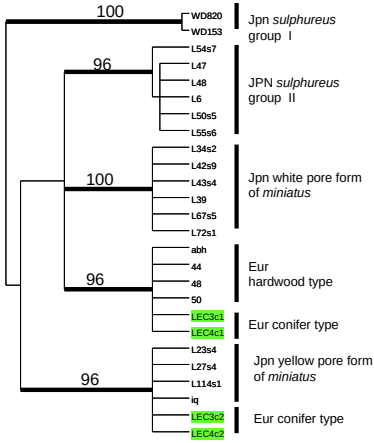

Figure 1. One of the 14 most parsimonious trees obtained from the ITS sequence data of Japanese and European *Laetiporus* isolates. Bootstrap value (>70%) is given above the branches. Tree length=59, CI=0.8644, RI=0.9697.

Phylogenetic analysis of the ITS and EF1α showed that Japanese *Laetiporus* could be divided into 4 groups: 1) Jpn yellow pore form, 2) Jpn white pore form, 3) Jpn *sulphureus* group I, and 4) Jpn *sulphureus* group II. The European *L. sulphureus* isolates from hardwood (Eur hardwood type) formed a distinct clade in the ITS and EF1α trees (Figure 1 and Figure 2). European isolates from conifers (Eur conifer type) shared a clade with the Jpn yellow pore form in the ITS tree (Figure 1), but one isolate possessed two sequence types which were assigned to the Eur hardwood type clade and the Jpn yellow form clade in the EF1α tree (Figure 2).

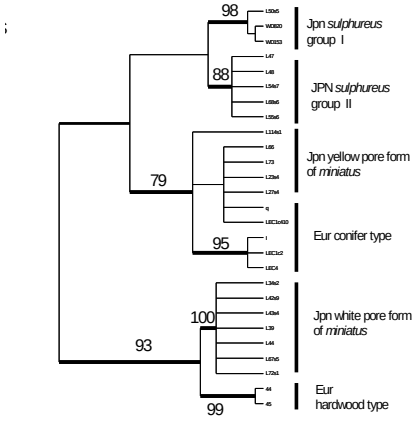

Figure 2. One of the 8 most parsimonious trees obtained from the EF sequence data of Japanese and European *Laetiporus* isolates. Bootstrap value (>70%) is given above the branches. Tree length=41, CI=0.9756, RI=0.9925.

Single spore isolates from the three Japanese taxa and the Eur hardwood type were incompatible with each other. The Eur conifer type was compatible with the Jpn yellow pore form.

These results give strong supporting evidence that three *Laetiporus* taxa in Japan and Eur hardwood type are distinct species. The Eur conifer type is considered to be closely related to the Jpn yellow pore form but it has a relationship with the Eur hardwood type. More work is needed to clarify the phylogenetic and taxonomic status of the Eur conifer type.

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Approaches to assess potential invasiveness of *Armillaria ostoyae*

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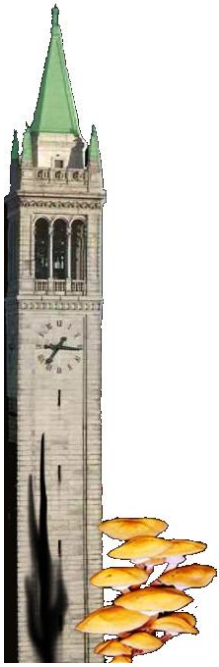
CONFERENCE ABSTRACT

Armillaria ostoyae is a root pathogen with circumboreal distribution; however, this species appears genetically variable with distinct ecological behavior in different regions. For example, *A. ostoyae* in the Colorado Plateau exists in drier habitats than *A. ostoyae* in the northwestern USA. Also, *A. ostoyae* of the Colorado Plateau appears more pathogenic on hardwoods, which are seldom affected in the northwestern USA. Previous reports indicate that *A. ostoyae* commonly causes root disease on *Larix* sp. in China; whereas, this pathogen only rarely impacts *Larix* sp. in the USA. In contrast, *A. ostoyae* is apparently absent from south-central Idaho, USA, even though suitable habitat, susceptible hosts, and other *Armillaria* species are present. These examples indicate that intercontinental and interregional movement of *A. ostoyae* could represent an invasive species risk. Furthermore, intraspecific hybridization could create pathogens with new ecological behavior and disease activity. Phylogeographic studies can provide a basis to assess potential invasive risks associated with *A. ostoyae*.

SESSION II:

GENOMICS AND PLANT-PATHOGEN INTERACTIONS



Nature and ecological implications of pathogen-induced systemic resistance in conifers: a novel hypothesis

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CONFERENCE ABSTRACT

Coniferous trees are often dominant species in both boreal and temperate forests, wherein they play critical roles in ecosystem function. In natural environments, ecosystem stability appears to be the norm, notwithstanding the co-occurrence of insect and microbial species inherently capable of killing their host trees. Adaptive plasticity of host trees involving inducible mechanisms of resistance against invading organisms is likely to play a crucial role in these interactions. We hypothesize that systemic resistance induced by pathogens represents a common and important phenomenon in coniferous trees, allowing for a balanced allocation of resources between growth and defense. This hypothesis will be discussed in the context of root disease complexes, including those incited by root and butt rot pathogens.

It is well known that host plant-mediated interactions between microbes and insects can be significant factors affecting the survival of coniferous trees, and thus the structure and function of temperate forest ecosystems, where they are often dominant species. For example, conifers visibly suffering from root disease (i.e. symptomatic trees) are more susceptible to colonization and immediate mortality caused by bark beetles. Eruptive bark beetle populations can then attack and kill virtually any host tree over extensive areas, irrespective of whether or not trees are infected with pathogens. Thus, root pathogens can directly and indirectly affect the survivability of trees to insect-caused mortality. Yet, the annual probability of the death of any particular tree caused directly by insect attack is relatively low. Many environmental factors undoubtedly contribute to tree susceptibility to insect attack, e.g. climatic extremes, soil conditions and nutrient and

water availability. However, extensive research suggests that tree survival is due, in large part, to effective defensive mechanisms against pathogen/insect colonization (2). For example, it is well known that localized defense responses make induced tissues more resistant to a subsequent insect attack (e.g. 4). But if this is true, why are trees showing symptoms of pathogenic infection usually more susceptible to pathogen and insect attack, since defenses should be induced in those trees? This apparent contradiction may have more to do with spatio-temporal relationships between attack by pathogens and insects than with species-specific host defense responses. In other words, the specific phenotype that one observes may depend on how and when a pathogenic infection alters whole-tree physiology in a way that affects subsequent insect behavior.

Coniferous trees have evolved both constitutive and inducible defense systems that deter or kill insects and inhibit or exclude pathogens physically and/or chemically. Recent fossil evidence suggests that these systems have been operating for at least the past 45 million years in the Pinaceae (4). Traditionally, defense mechanisms have been classified as constitutive (available pre-attack) and induced (expressed post-attack). Induced defense mechanisms can be manifested as localized induced resistance and/or systemic induced resistance (SIR).

SIR phenotypes against stem and branch pathogens have been observed in pine in response to pathogens. For example, Bonello *et al.* (3) demonstrated that resistance against the pitch canker pathogen, *Fusarium circinatum*, can be induced systemically in Monterey pine (*Pinus radiata*) in the field using mechanical inoculations with the same pathogen. Induced resistance was sustained and intensified with boost inoculations over the course of at least one and a half years. The natural occurrence of induced resistance to pitch canker has also been documented (5).

Blodgett *et al.* (1) showed that SIR also occurs in Austrian pine (*Pinus nigra*). When trees are inoculated with the necrogenic canker pathogen *Diplodia pinea* the whole stem becomes more resistant to subsequent inoculations with *D. pinea*. The phenomenon is bidirectional, suggesting that molecular signals move both acropetally and basipetally in the tree to elicit the SIR response (1). Work on this model pathosystem is beginning to reveal anatomical and biochemical changes that are associated with SIR in the stems. In particular, SIR may be linked to an integrated host defense response including enhanced lignin deposition, accumulation of certain soluble phenolics (1) and specific proteins (8), and induction of traumatic resin ducts and resin flow (7). Based on this evidence as well as other studies, we have proposed the systemic induced resistance hypothesis (SIRH), which predicts a dynamic interplay between trees, microbes, and

herbivores, underscored by SIR which can be sustained or transiently expressed, depending on the damage level resulting from the induction event (2) (Figure 1).

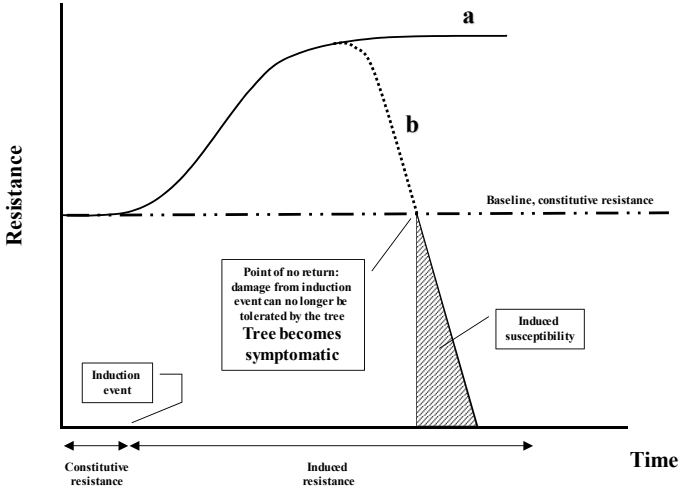

Figure 1. SIR hypothesis. Diagram illustrating the interplay between systemic induced resistance and induced susceptibility in trees against microbes and herbivores. A baseline level of constitutive resistance is present in all trees, but an induction event is predicted to induce SIR against both microbes and insects. SIR is predicted to remain sustained for extended periods of time (a), unless the induction event results in severe impairment of the tree's defensive machinery, with subsequent collapse of tree resistance (b) and expression of systemic induced susceptibility (SIS). An example of this would be pines infected with a root pathogen. Initially, i.e. in pre-symptomatic stages, the pines would be more resistant to bark beetle attack and infection by bark beetle-associated fungi. If the pine becomes symptomatic, then resistance begins to decline and results in the often observed increased susceptibility of symptomatic, root diseased pines to bark beetle infestation. Response scales are arbitrary. From Bonello *et al*. (2).

Besides providing a framework for understanding specific pathogen-tree-insect associations, the SIRH offers a foundation for expansion of current plant defense theory, specifically those hypotheses addressing environmental effects on expression of host defense such as the growth differentiation balance hypothesis (GDBH) (6).

The GDBH focuses primarily on plastic responses of constitutive secondary metabolism to variation in resource availability, and does not make predictions about expression of more or less rapidly induced

responses such as SIR to resource availability or pathogen infection. GDBH predicts a nonlinear, parabolic response of constitutive secondary metabolism across a resource gradient (Figure 2a). Rapidly growing plants in resource-rich environments are predicted to have low secondary metabolite concentrations due to a resource-based trade-off between primary and secondary metabolic pathways. However, secondary metabolism is predicted to increase under moderate water or nutrient limitation, as growth is more sensitive to resource limitation than is carbon assimilation. Consequently, substrate available for secondary metabolism may increase. However, in extremely resource-limited environments, carbon assimilation will also decrease, and secondary metabolism is predicted to be low due to energy and substrate constraints on biosynthesis. As pathogens can have a significant effect on carbon assimilation, we postulate that the time-course of pathogen infection may have a quadratic effect on the strength of SIR that is similar to spatial variation in constitutive secondary metabolism generated by resource availability (Figure 2b).

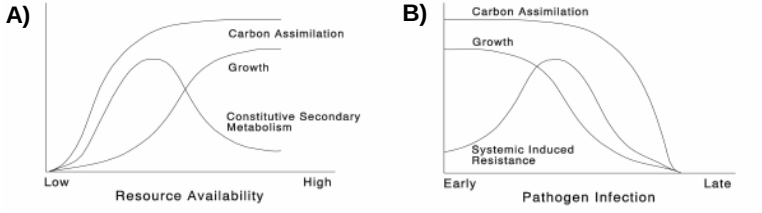

Figure 2. Postulated nonlinear effects of resource availability (A) and pathogen infection (B) on constitutive secondary metabolism and systemic induced resistance, respectively. The quadratic response for SIR in (B) is somewhat different from that shown in Figure 1 to highlight the similarities with the model described in (A).

We suggest that integration of the SIR and GDB hypotheses will provide a foundation for a better understanding of complex interactions among tree pathogens, their hosts, and associated pestiferous insects.

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Monoterpenes as potential markers for relative susceptibility of Sitka spruce to *Heterobasidion annosum*

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CONFERENCE ABSTRACT

Lesions lengths were measured on inner bark of 2 year old clones of *Picea sitchensis* 35 days after inoculation with *Heterobasidion annosum*. Monoterpene responses were compared in 2 clones with short lesions (less susceptible) and 2 with long lesions (more susceptible). Less susceptible clones had higher proportions of (+)-α-pinene, (-)-β-pinene and unknown terpene 15 in constitutive resin, whereas more susceptible clones had higher amounts of (-)-limonene, and 3 unknowns. In secondary resin produced around lesions there was a decrease in β-phellandrene and corresponding small increases in certain minor terpenes. Amounts of most monoterpenes increased in infected tissues. Inoculation with *H. annosum* resulted in an increase in unknown 19, whereas in more susceptible clones, concentrations of δ-3-carene and unknowns 13 and 16 increased. Differences in both constitutive and induced resin monoterpene profiles may provide useful markers for resistance to *H. annosum* for use in selection programmes.

Clones of spruce vary in susceptibility to *Heterobasidion* infection, but the basis for this apparent resistance is unknown. Inoculations suggest that disease development is partly determined by host tree characteristics, with strong correlations between infection progress in 4-year-old plants and 15-year-old trees. Resistance to *Heterobasidion* in spruce can be detected using inoculations and measuring lesion development, and with quantitative PCR (1, 6).

Resin components of conifers are potential defensive responses, providing barriers to invading parasites (7); secondary resin with differing terpene profiles, is induced by pathogen attack (5). These traits may be suitable markers for host genotypes showing varying resistance or susceptibility to pathogens (3).

The aim of the work reported here was to determine the potential of differential monoterpene responses in clones of *Picea sitchensis* as markers for susceptibility to *H. annosum*.

Clones of Sitka spruce were inoculated with *H. annosum* (2). Plants were harvested 35 days later and lesion lengths estimated; a cortical sample was collected from immediately around the lesion for each

ramet, avoiding healthy tissue. A second cortical sample was excised 25 cm above the wound (control: no wounding).

Cortical tissues (0.2-0.5 g) from 4 less- and 5 more susceptible clones were used in gas chromatographic analysis following established methods (4). GC-FID analysis was performed using a Perkin-Elmer Autosystem XL GC and enantiomeric monoterpenes separated on a 30 m Cyclodex-B capillary column, 0.25-mm-diam. Terpenes were identified by comparison of retention times with standards under the same conditions. Relative and total amounts of monoterpenes were calculated.

Proportions (%) of terpene components were transformed (arcsine-square root) to normalise data and subjected to ANOVA and Tukey post hoc tests. Differences between two groups were analyzed using T-tests. Data not normally distributed were analysed using Kruskal–Wallis ANOVA followed by the Mann-Whitney U Test for multiple comparisons and differences were accepted when P<5%.

Lesion lengths varied significantly with clone (Figure 1). Regardless of clone or treatment, (-)-α-pinene, (-)-β-pinene, and β-phellandrene were the major resin monoterpene constituents; minor components were (+)-α-pinene, myrcene, sabinene, δ-3-carene (+)-α-pinene, (-)-limonene, (+)-limonene, γ-terpinene, terpinolene and 7 unknown compounds (Figure 2).

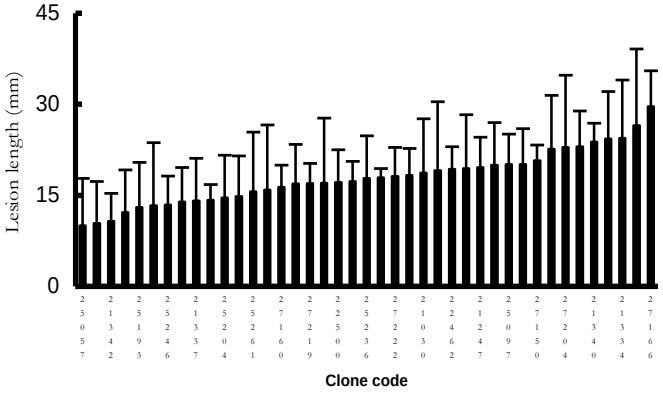

Figure 1. Lengths of lesions ±SE in the vascular cambium of Sitka spruce clones 35 days after inoculation with *Heterobasidion annosum* arranged, left to right, in order of increasing length.

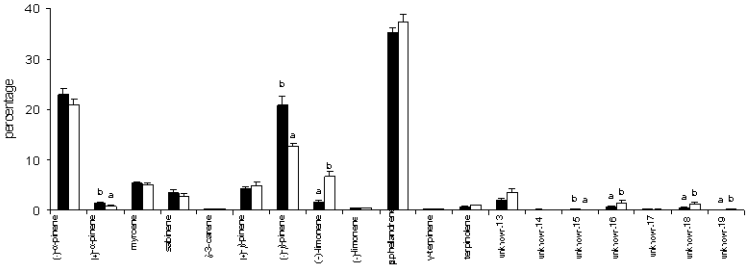

Figure 2. Comparisons of terpene profiles in cortical samples collected 25 cm above the wound of Sitka spruce clones less susceptible (■) or more susceptible (□) to *Heterobasidion annosum*. Vertical bars represent SE. Different letters above bars indicate significantly different means (Mann-Whitney test).

Relative contents of several terpenes varied significantly between 4 less- and 5 more susceptible trees (Figure 3a,b).

The availability of Sitka spruce genotypes resistant to *H annosum* is relevant for re-planting sites badly affected by the disease. The conclusions of earlier work on inoculated clones of Norway spruce were reinforced by the considerable and significant variation in lesion development observed in this study and by quantitative PCR (1). Enantiomeric monoterpene contents differed between clones; further differences emerged following wounding only or wounding plus inoculation with *H. annosum.*

These data suggest that terpenes may be useful markers for resistance/ susceptibility of different host genotypes to *H. annosum.* Further research is needed to investigate the role of monoterpenes in defenses mobilized in *P. sitchensis* in response to attack by *H. annosum* and in elucidating the possible use of enantiomeric monoterpene profiles as an aid to selection of Sitka spruce chemotypes less susceptible to infection.

Heterobasidion spp. pose serious threats to all spruce plantings and, as found with other clonal crops, lack of genetic variability could lead to pest and disease outbreaks more serious than found in heterogeneous host populations. It is important, therefore, to explore the full range of susceptibility of spruce clones to *H. annosum*, and to establish long-term trials to examine the durability of any observed resistance with time.

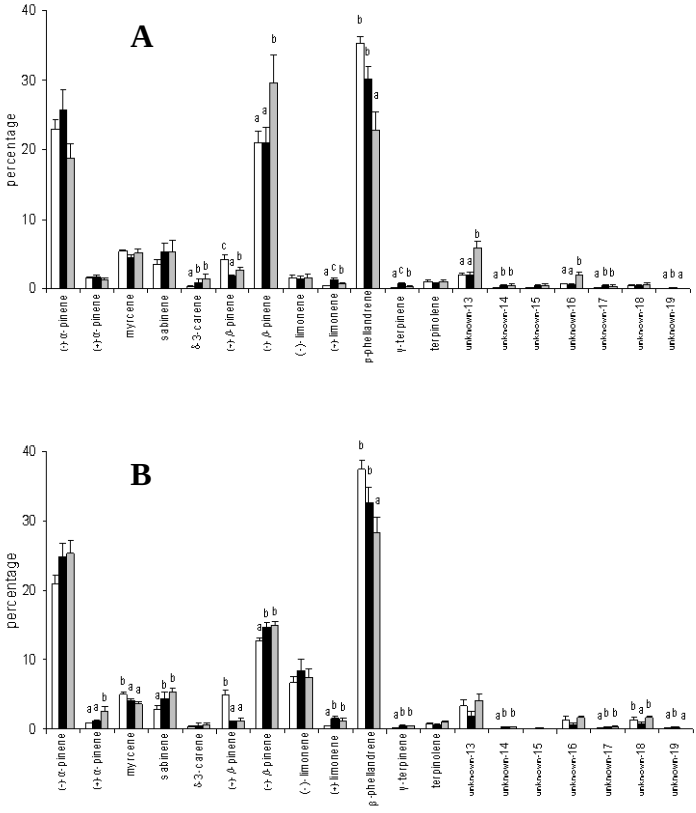

Figure 3. Changes in Sitka spruce terpene profiles in (A) less susceptible and (B) more susceptible clones in the absence of wounding (□), wounded plus inoculated with *Heterobasidion annosum* (■) or wounded only (▒). Vertical bars represent SE. Different letters above bars indicate significantly different treatment means (Mann-Whitney test).

ACKNOWLEDGEMENTS

This work was supported by the EU-funded RESROBS programme (QLRT-2000-00241). S. Bianchi, W.J.A Bodles, E. Beckett, J. Hutcheon, D.G. Moseley and L. Whiteoak assisted with inoculations and analyses, and Dr. B. Karlsson, Skogforsk, Sweden gave advice on statistical procedures.

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Are terpenes involved in cross induction of systemic susceptibility between *Heterobasidion annosum* and *Diplodia pinea* in Italian stone pine?

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CONFERENCE ABSTRACT
The recent finding of the North American P (Nam-P) type of *Heterobasidion annosum* in Italian pinewoods opens up some intriguing questions about its behaviour on the hosts and also on the possible interactions with local fungal pathogens. This study showed that infection of Italian stone pine (*Pinus pinea*) with *H. annosum*, both European P (Eur-P) and Nam-P isolates, increases susceptibility of pine to *Diplodia pinea* shoot blight. This systemic increase in susceptibility was associated with systemic alteration of terpene metabolism. The study showed that pines inoculated with the Nam-P isolate became more susceptible to *D. pinea* than hosts inoculated with the Eur-P isolate. The significance of these results will be discussed in the context of current models of conifer defense against pathogens as well as the effects of exotic pathogens on native ecosystems.

Heterobasidion annosum s.s. is an important root and butt-rot fungal pathogen of coniferous trees, especially pines (6). In the last few years, *H. annosum s.s.* North American P-group (Nam-P), has been found along the coastal Latium Region of Italy, where this species coexists, possibly since the 1940s, with the native European P-group in Italian stone pine (*Pinus pinea* L.) forests (3, 5).

The new finding of Nam-P on the Italian peninsula opens some intriguing ecological questions. Tree-mediated cross-induction of resistance and/or susceptibility between two different pathogens, and between a pathogen and an insect, has been documented in Austrian pine (*Pinus nigra* Arnold) (2, 4). Furthermore, pathogen-induced systemic resistance and susceptibility in Austrian pine are associated with changes in host biochemistry and physiology (1).

The aim of this study was to characterize the behavior of both P-groups of *H. annosum* in *P. pinea* and the possible interactions with

other endemic fungal pathogens like *Diplodia pinea*, a mitosporic fungus that causes shoot blight and canker in conifers, including *P. pinea* (8).

Three-yr-old, potted *P. pinea* seedlings were inoculated (induced) with either *H. annosum* Eur-P or Nam-P inoculations on the stem, 8-cm above ground. Three weeks later a second inoculation (challenge) was carried out with *D. pinea* on the apical shoot, 3-cm above the terminal node. Unwounded and mock-inoculated trees were used as controls.

Two weeks after the shoot challenge with *D. pinea* stem and shoot lesion lengths were measured. Fungal presence in stems and shoots was confirmed by re-isolation from the lesion margins, following (7). At the same time, small tissue samples (< 0.3 g fresh weight) were removed near the margins of the lesions on shoots and stems to determine absolute and relative concentrations of monoterpenes by gas chromatography-flame ionization detection. Lesion length and monoterpene concentrations were analyzed by ANOVA to determine the effects of basal inoculation with *H. annosum* on susceptibility to *D. pinea*, as well as the association between susceptibility and altered monoterpene concentrations in the shoots.

Results showed cross-induction of susceptibility between *H. annosum* and *D. pinea*: Seedlings inoculated with *H. annosum* (both Nam-P and Eur-P isolates) had significantly longer lesions caused by *D. pinea* in the shoots than the controls. Furthermore, *D. pinea* shoot lesions were significantly longer in seedlings inoculated with Nam-P than Eur-P.

The analysis also showed that the main monoterpenes accumulating in the tissues were (-)-limonene (~ 60.0%), (-)-β-pinene (~25.0%). Minor compounds, ranging between 1-7%, were (-)-α-pinene, p-cymene, β-caryophyllene, (+)-β-pinene, α-terpineol and (+)-α-pinene. Significant differences were found in the amounts of monoterpenes accumulating in shoots infected with *D. pinea*: Seedlings induced with *H. annosum* (both Nam-P and Eur-P) accumulated lower amounts of monoterpenes than the induction controls.

In summary, two major results were obtained in this study: (i) the Nam-P isolate of *H. annosum* increased systemic susceptibility of Italian stone pine to *D. pinea*, compared to the indigenous Eur-P; and (ii) such increased susceptibility was associated with decreased amounts of monoterpenes, suggesting that this class of compounds may be one of the resistance mechanisms used by Italian stone pine to restrict *D. pinea* in the shoots.

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Microarray analysis of conifers response to *Heterobasidion annosum* at different stages of organ development and disease resistance

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CONFERENCE ABSTRACT

A microarray profiling of juvenile *P. sylvestris* roots harvested at 1, 5 and 15 days after challenge with *Heterobasidion annosum* revealed multiple overlapping strategies employed for defence purposes. Production of pathogenesis-related enzymes and antimicrobial proteins was supplemented by a major shift in primary and secondary metabolism. A wide range of oxidative stress protecting mechanisms was documented, possibly related to the programmed cell death. Analysis of defence response of the matured woody spruce clones with varying level of resistance to *H. annosum* identified genes differentially expressed by all clones that might have a role in basic defence. On the other hand, genes expressed only by highly resistant clones might contribute to the most successful defence against the pathogen and may be useful candidates for breeding. The spruce clone with low resistance to *H. annosum* responded to the infection with down-regulation of few defence related genes which may account for its susceptibility to the pathogen.

Histochemical and proteomics approaches to study host–pathogen interactions of the Douglas-fir–*Phellinus sulphurascens* pathosystem

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CONFERENCE ABSTRACT

Laminated Root Rot, an important disease of the Pacific coastal Douglas-fir (DF, *Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco), is caused by the fungus *Phellinus sulphurascens* Pilát. Little is known about the host–pathogen interaction between Douglas-fir and *P. sulphurascens* at cellular and molecular levels. Microscopic investigations showed that *P. sulphurascens* hyphae colonize the surface of DF seedling roots within 2 days. Penetrating hyphae, which are both intra and intercellular, also form a variety of haustoria-like structures. We found variation in the cellular localization of PR proteins in infected DF roots. A thaumatin-like protein (*Pm*TLP) and an endochitinase-like protein (ECP) were highly expressed and localized in specific regions of host cell walls. A DF-PR10 protein was localized in cell walls and in the cytoplasm, while *Pm*AMP1 occurred mostly around cell walls. Proteomic analysis showed that the major proteins differentially expressed in infected seedlings included those in six functional groups, namely- disease/defense, metabolism, secondary metabolism, signal transduction, transcription factors, and energy. Our future research includes investigation of gene expression in families of DF known to be differentially susceptible to *P. sulphurascens*.

Laminated root rot (LRR), caused by *Phellinus sulphurascens* Pilát, is an important disease of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco) in northwestern North America (2). The fungus is capable of attacking and killing trees of all ages and can have a profound effect on forest productivity. Little is known about the host–pathogen interaction between DF and *P. sulphurascens*, especially at the cellular and molecular level. Previously, we identified several pathogenesis-related (PR) proteins upregulated in infected trees. These proteins include a thaumatin-like protein (*Pm*TLP), an endochitinase protein (ECP), a PR-10 protein (*Pse m* l) and a 10.6-kDa antimicrobial peptide, *Pm*AMP1 (1). Using histochemical and proteomics techniques we have gained additional information on the role of these four proteins and others, in the DF-*P. sulphurascens* interaction.

DF seedlings were inoculated with *P. sulphurascens* using an *in vitro* technique that we developed to study this pathosystem (1). Protein accumulation and localization in control and infected root tissues was evaluated using an array of techniques such as fluorescence light microscopy (we used fluorescein isothiocyanate - FITC), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) employing colloidal gold (10 nm conjugated ovomucoid- wheat germ agglutinin and 20 nm-protein A conjugated with selected antibodies).

Total proteins extracted at 7 days post inoculation (dpi) from infected and control DF roots were separated, silver-stained and analyzed using a computer interfaced Imaging Densitometer and PDQUEST® software (Bio-Rad). Selected proteins were further analyzed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis, followed by sequence homology searches against MASCOT and PEAKS databases.

Our SEM work revealed that the early stages of infection of DF roots by *P. sulphurascens* includes mycelial adhesion on the root surface at 2 dpi, the formation of appressoria-like structures, disintegration and penetration of host cell walls, and the development of haustoria inside host tissues (Figure 1). We previously reported the presence of *P. sulphurascens* mycelia inside host tissues at 3 dpi and colonization in root cortical tissues at 7 dpi. FITC-conjugated immuno-labelling of our four specific proteins showed an increasingly prominent fluorescence throughout cortical tissues from 7 dpi to 12 dpi (1).

We also found variation in cellular localization of the four PR proteins. Our TEM work showed that *Pm*TLP and ECP were highly expressed and localized in the appositions of host cell membranes. *Pse m* I was localized in cell walls and in the cytoplasm, while *Pm*AMP1 mostly occurred in cell walls (Figure 2).

Gold labelling of the four PR proteins was often associated with *P. sulphurascens* hyphae. For example, ECP-gold labelling occurred in fungal cell walls along with WGA-gold labelling. PR-10 labelling was observed in the walls and cytoplasm of fungal cells and *Pm*AMP1 labeling occurred either inside *P. sulphurascens* hyphal cells or very close to them (Figure 3).

2-DE gels resolved a total of 1303 proteins from control and 7 dpi root samples; of these, 74 upregulated and 85 downregulated proteins were statistically significant ($P < 0.05$).

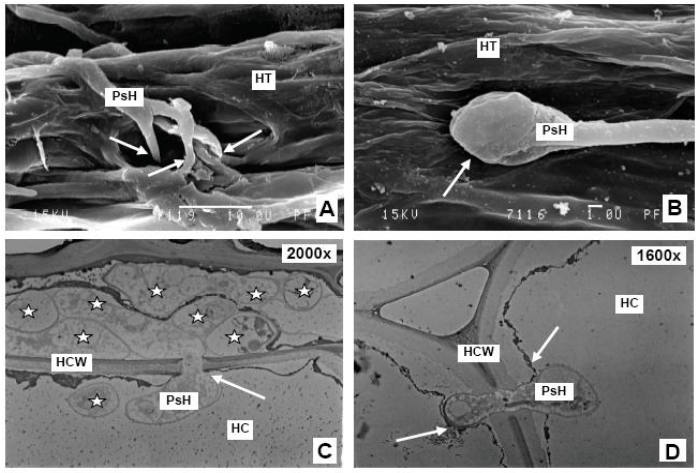

Figure 1. SEM micrographs of (A) *P. sulphurascens* hyphae (PsH) adhered to the surface of a DF root and the host tissues (HT) penetrated by hyphae, and (B) the development of an appressorium (see arrow) on DF root surface; TEM micrographs showing (C) a host cell (HC), in this case an epidermal cell, covered by *P. sulphurascens* hyphae (stars) and its cell wall (HCW) penetrated by an haustorium (see arrow), and (D) fungal-induced disintegration of one of the host's cortical cells.

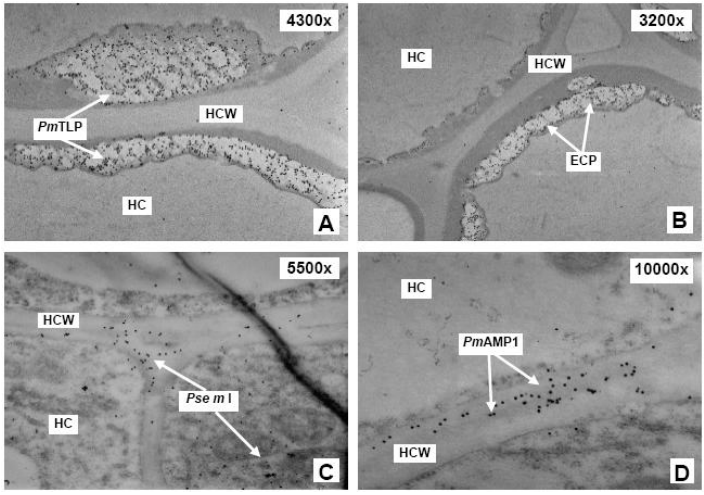

Figure 2. TEM micrographs showing the labelling of (A) *Pm*TLP- (B) ECP-, (C) *Pse m* I- and (D) *Pm*AMP1-conjugated protein A-gold particles in host cells (HC) and host cell walls (HCW) of DF infected by *P. sulphurascens*.

Identification of 47 upregulated (Figure 4) and 23 downregulated proteins using LC–MS/MS showed that the major proteins differentially expressed in infected seedlings included those in six functional groups, namely - disease/defense (27%), metabolism (16%), transcription factors (11%), signal transduction (10%), secondary metabolism (7%), and energy (4%). Overall, our results indicate that roots of DF seedlings respond quickly to *P. sulphurascens* infection at the cellular, subcellular, and molecular level.

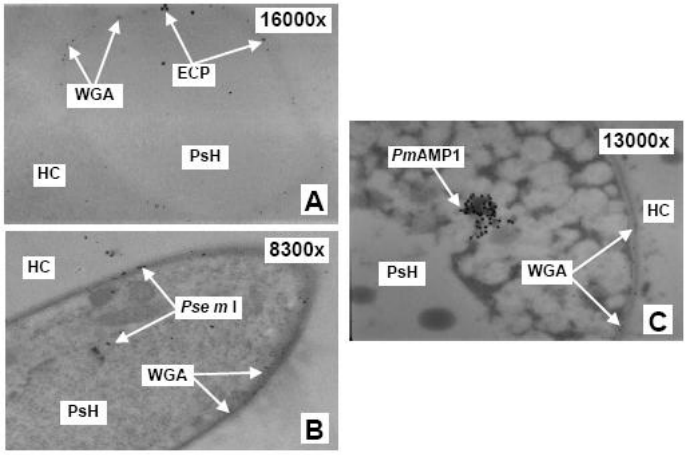

Figure 3. TEM micrographs showing the association of (A) ECP-, (B) *Pse m* I-, and (C) *Pm*AMP1- conjugated protein A-gold particles with *P. sulphurascens* hyphae (PsH) in infected host cells (HC) of DF; WGA-conjugated gold particle labelling of chitin in *P. sulphurascens* cell walls confirms their fungal status.

Our future research includes investigation of gene expression in DF families known to be differentially susceptible to *P. sulphurascens* and the identification of molecular markers for LRR resistance to be used by DF breeders.

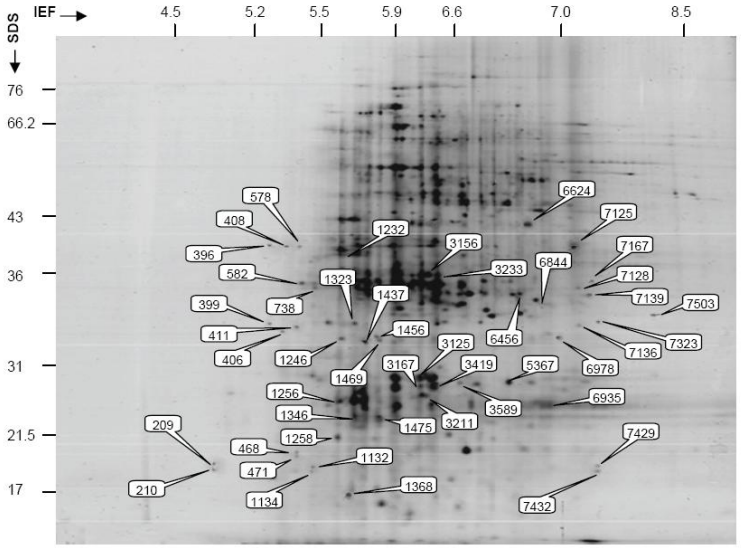

Figure 4. 2-DE silver-stained gel showing the proteins upregulated at 7 dpi in DF roots infected with *P. sulphurascens*; protein spots were excised for LC-MS/MS analysis.

ACKNOWLEDGEMENTS

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Host response to infection by *Armillaria ostoyae* in the roots of Douglas-fir and western redcedar in the southern interior of B.C.

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CONFERENCE ABSTRACT

Necrophylactic periderm formation and compartmentalization of infected tissue were examined in roots of 20-30 year-old western redcedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*) and Douglas-fir (*Pseudotsuga menziesii*) trees infected by *Armillaria ostoyae* in the southern interior of British Columbia (B.C). Microscopic investigation of abiotically wounded roots, as well as roots naturally infected and inoculated with *A. ostoyae* revealed distinct differences in the types and frequency of host responses between cedar and the other two conifers. Following invasion by *A. ostoyae*, a higher frequency of successful resistance reactions was induced in western redcedar compared to Douglas-fir and western hemlock. Breaching of non-suberized impervious tissue (NIT) and NP was common in Douglas-fir and western hemlock trees. The barrier zone in cedar formed by the uninjured cambium was comprised of axial parenchyma with pigmented deposits and provided a permanent barrier to spread by the fungus. Unique resistance mechanisms in cedar involving induced rhytidome formation impart increased resistance to the spread of *A. ostoyae* in host tissue. Results indicate that the higher degree of resistance against *A. ostoyae* in western redcedar may help alleviate long-term impacts of root disease when regenerated on sites infested with Armillaria root disease.

Armillaria root disease is a significant forest health concern in southern interior forests of British Columbia. *Armillaria ostoyae* (Romagn.) Herink is most predominant and damaging in the Interior Cedar-Hemlock (ICH) biogeoclimatic zone. Within this zone, aboveground symptoms of *Armillaria* can be detected in only one-quarter of the trees with belowground infection (2). Cumulative mortality can be as much as 20% by age 20 years (1) resulting in undesirable stocking in juvenile stands. Moreover, growth loss will occur in trees sustaining non-lethal infections and the probability of infection increases with increasing tree size (3). Ultimately, these losses can become considerable over time and create serious challenges for managing sustainable timber production on infested sites.

Few options are available to mitigate potential losses due to Armillaria root disease. Removal of stumps from the ground is a very effective means of reducing the amount of woody inoculum that would

otherwise be available to the fungus. Another less intrusive option is to plant conifer species that have a low susceptibility to killing by *A. ostoyae*.

In this study, microscopic examination of infected root tissue showed resistant mechanisms operating in western redcedar (*Thuja plicata* Donn ex D. Don) that are effective at containing infections and halting the spread of the fungus to adjacent healthy tissue. Data obtained from four inoculation trials on 20-30 year old trees showed that the frequency at which resistant reactions, including necrophylactic periderm (NP) formation and compartmentalization of infected woody tissue, are induced following invasion by the fungus was significantly higher in western redcedar trees than in Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) trees (Figure 1).

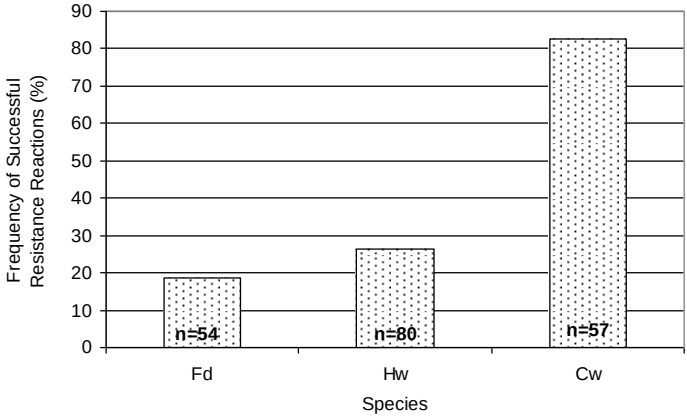

Figure 1. Successful resistance reactions as a proportion of the total number of roots showing successful penetration by the *A. ostoyae* in Douglas-fir (Fd), western hemlock (Hw) and western redcedar (Cw) trees.

In a survey of twenty juvenile mixed conifer plantations throughout the ICH zone in the southern Interior of British Columbia, cumulative mortality in Douglas-fir trees was significantly higher than in western redcedar trees ($P<0.001$) (Table 1).

Table 1. The proportion of the total number of trees by disease status category for Douglas-fir and western redcedar.

	SPECIES	
DISEASE STATUS	Douglas-fir	Western redcedar
Healthy	0.69	0.95
Infected by *A. ostoyae* (PROGRESSIVE)[a]	0.06	*tr*[b]
Infected by *A. ostoyae* (CALLUSED)[c]	0.01	0.03
Dead (KILLED by *A. ostoyae*)	0.25	0.02
Dead (unknown/other factors)	*tr*[d]	*tr*[d]
Total	1.00 (n=2396)	1.00 (n=2169)

[a] Progressive lesions are defined as infections at the root collar that lack evidence of NP formation in the bark or compartmentalization. Lesions appeared as browned tissue in advance of mycelial colonization
[b] *tr* = proportion of trees with progressive lesions at the root collar was less than 0.005
[c] Callused lesions are defined as infections at the root collar that were compartmentalized and spread of the fungus had been stopped
[d] *tr* = proportion of trees killed by unknown/other factors was less than 0.005

The probability of mortality among trees infected by *A. ostoyae* depended on both species and tree size ($P < 0.001$). The incidence of mortality was significantly greater in the smaller diameter size classes than in the larger size classes for both species and cedar mortality was consistently lower than Douglas-fir. Although the risk of mortality decreased with increasing tree size in both species, the rate of decrease was noticeably greater among cedar compared to Douglas-fir trees (Figure 2). There was an increasing trend in the proportion of infected trees showing compartmentalization and callusing with tree size for both western redcedar and Douglas-fir, but the increase was markedly greater for cedar than Douglas-fir trees and occurred much earlier even when the trees were relatively small (Figure 3).

When *Armillaria* invades the bark on roots, the host may form a NP to contain the infection. Cedar appears to form this “resistant” reaction more frequently than other conifers by initiating NP formation as well as a unique response involving induced rhytidome formation around a site of initial penetration by the fungus. Following cambial invasion on cedar roots, the fungus is rapidly compartmentalized and it is rare the fungus will escape this barrier once formed.

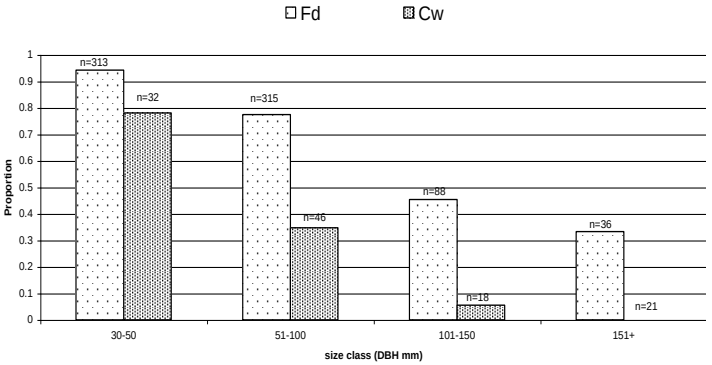

Figure 2. Mortality in Douglas-fir (Fd) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class.

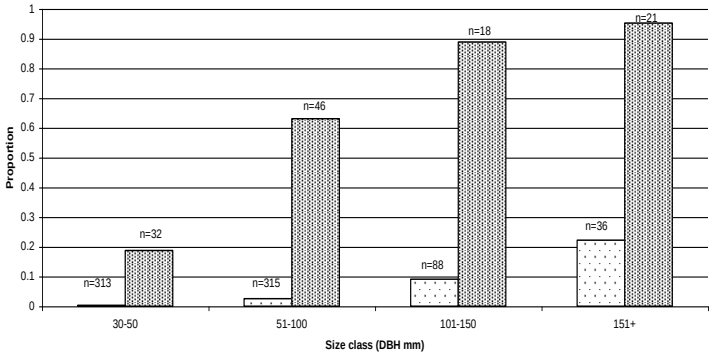

Figure 3. Callused lesions at the root collar in Douglas-fir (Fd) and western redcedar (Cw) as a proportion of the total number of trees with above-ground signs or symptoms of *A. ostoyae* by size class.

In this study, symptom development and mortality rates for cedar is in agreement with other work documenting effective root disease resistance for this species. Belowground infection on both Douglas-fir and western redcedar was almost certainly higher than what was detected in this study. However, based on our inoculation studies, it is more likely that the presence of such resistance mechanisms results in an infection being confined to tissue immediately surrounding a point of invasion, thereby limiting the extent of cambial invasion which might otherwise result in higher mortality rates in cedar.

Results of this study suggest that the higher degree of resistance against *A. ostoyae* in western redcedar may help alleviate long-term impacts of root disease when regenerated on sites infested with Armillaria root disease.

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Molecular studies of *Heterobasidion annosum* s.l. during interaction with heartwood and the reaction zone of Norway spruce

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CONFERENCE ABSTRACT

The root-rot causing fungus *Heterobasidion annosum sensu lato* is the most devastating pathogen of conifers in Europe. This pathogen enters Norway spruce through the roots and can colonize the tree from within, growing as a saprophyte when established within the dead heartwood and acting as a necrotroph when in contact with living host tissue. Despite the high incidence of damage, trees have defences against this pathogen in the bark and living wood. Furthermore, spruce has a defense against internal attack by forming a reaction zone, in this case the host defense is directed inwardly by the still living sapwood toward the central colonized heartwood. We have studied the host responses to infection in Norway spruce clones at the transcriptional level and found that the speed of recognition and that spatial defense signalling appears to be the hallmarks of trees with high degree of resistance. We strive to study both partners in this pathosystem from a molecular perspective, and are now focusing on the pathogen and what fungal gene-products are being expressed during the colonization of the heartwood compared to those expressed close to the active host defense (reaction zone) using suppressive subtractive hybridization followed by Real-Time PCR analysis. In addition the *Heterobasidion* colonization profiles were followed using quantitative Real-Time PCR on extracted gDNA.

We strive to study both partners in our pathosystem from a molecular perspective, and focus on the pathogen *H. annosum* S-type (*H. pParviporum*) gene-products being expressed during the colonization of the central heartwood compared to those expressed close to the active host defense (reaction zone) using suppressive subtractive hybridization.

The root-rot causing fungus *Heterobasidion annosum sensu lato* is the most devastating pathogen of conifers in Europe. This pathogen enters Norway spruce through the roots and can colonize the tree from within, growing as a saprophyte when established within the dead heartwood and acting as a necrotroph when in contact with living host tissue. Despite this high incidence of damage the tree has efficient defences against this pathogen and the attack is eventually fought off if present in the bark or living wood. The tree also has a defense against this internal attack by forming a reaction zone, in this

case the host defense is directed inwardly by the still living sapwood toward the central colonized heartwood. We have in the last years studied the host responses to infection in Norway spruce clones at the transcriptional level and found that the speed of recognition and that spatial defense signalling appears to be the hallmarks of trees with high degree of resistance (3, 4). We strive to study both partners in this pathosystem from a molecular perspective, and are now focusing on the pathogen *H. parviporum* and what fungal gene-products important for colonization of the heartwood.

Three mature Norway spruce trees colonized naturally by *H. parviporum* were chosen for the study. Five-centimeter-thick wood disks were cut at the height of 0.3 m above ground, and frozen immediately in liquid nitrogen to keep the fungal hyphae with their genomic DNA (gDNA) and RNA intact. *H. parviporum* gene-products being expressed during the colonization of the central heartwood (HW) compared to those expressed close to colonization front (CF) using suppressive subtractive hybridization (SSH) followed by Real-Time RT PCR (qRT PCR) analysis. In addition the *H. parviporum* colonization profiles were followed on extracted gDNA using quantitative Real-Time PCR (4).

We used the SSH method to obtain arrays of genes expressed during colonization of Norway spruce stems by *H. parviporum*. Two cDNA libraries have previously been constructed to identify genes expressed by *Heterobasidion* spp. The first was constructed from mycelia of *H. annosum* s.s. interacting with roots of two-week-old Scots pine seedlings (2), while the second library was constructed from spores of *H. parviporum* germinating on Hagem agar (1).

In natural infection of Norway spruce by *Heterobasidion annosum* s.l., the reaction zone forming in the interface between sapwood and heartwood in advance of pathogen colonization is characterized by an accumulation of antifungal host substances to border the spreading of the infection into the conductive sap wood. It can be envisioned that pathogen metabolism shows differences between a normal decay situation and a stress situation when exposed to the components of the reaction zone. Two subtracted cDNA libraries were constructed using RNA extracted from an advanced stage of decay (HW) and from colonization front (CF) in the immediate vicinity of the reaction zone. Since natural infection was now used, the subtracted cDNA libraries constructed should provide the first realistic insight into the transcriptome of *H. parviporum* operative upon wood colonization.

The obtained and sequenced SSH libraries were of high quality, and most of the sequences we obtained and annotated are likely to contain cDNA corresponding to *Heterobasidion* genes. To confirm that all the genes now monitored represent *Heterobasidion* transcriptome

we tested the primers used for transcript profiling on genomic DNA and cDNA obtained from a pure culture of the pathogen. Using cDNA we obtained clear single bands with the predicted size after gel electrophoresis for all the transcripts now monitored. For genomic DNA, a few genes produced larger PCR products than cDNA, which is most likely due to the presence of introns in the targeted areas.

The *H. parviporum* subtracted cDNA libraries we constructed contain a wide range of genes encoding proteins involved in different functions of the fungal cell such as those involved in protein synthesis, metabolism, signal transduction, transport, cell cycle and DNA processing as well as defense and detoxification. In addition, a significant amount of genes with unknown functions, hypothetical classifications or that had no homologies with sequences deposited at gene sequence databases were found. Most of the unknown genes were found in the CF library. The high representation of unknown genes in the library is not surprising as practically no molecular or genomics studies have been performed on the interaction of decay fungi with reaction zone components. Such ESTs induced during fungal-host interaction are promising candidates to be further studied.

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Differences in spread rate of *Heterobasidion* spp. from clear felled or intact decayed Norway spruce to healthy neighbouring trees over a 13 year period

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CONFERENCE ABSTRACT

Trees in a first generation 40-year-old Norway spruce stand were classified as healthy or decayed based on a bore core taken at stump height. A total of ninety pairs of one healthy and one decayed tree were chosen based on the above classification. In 42 of the pairs the decayed tree (the donor tree) was felled. In the other 48 pairs the decayed tree was left intact. The recipient trees in the pairs were checked for decay based on one bore core taken at stump height, 3 and 10 years after felling. Thirteen years after felling of donor trees, all recipient trees in the pairs were felled, decay frequency, diameter and height of the rot column were analysed. The decay frequency increased during 13 years from 0% to ca 95% in recipient trees with a decayed neighbour within 2 m distance. Mean height of the rot column was 4 m and maximum height was 10 m. Frequencies did not differ between treatments. Ca. 26% of the recipient trees in the pairs had died; frequency of mortality differed significantly between treatments. Results from this trial indicates that spread rate of *Heterobasidion* does not increase if decayed neighbours are cut but the intensity in the attack (higher frequency of dead recipient trees) may be increased when cutting the rot donor. The horizontal spread rate in a stem may reach 1 m per year.

The root-rot fungus *Heterobasidion* spp. is the most serious of the root pathogens attacking Norway spruce in Scandinavia. The fungus spreads primarily via spores that settle, germinate and form mycelia on freshly cut stumps. The mycelium then transfers from infected stumps or trees to healthy trees via root contacts (5).

The annual extension rates of *Heterobasidion* in the stem varies between 0.3 and 0.9 m x year $^{-1}$ (3, 5). In inoculated roots of Norway spruce stumps an annual extension of 25 cm x year $^{-1}$ have been reported, compared to 9 cm x year $^{-1}$ in roots of living trees (1).

The effects of removal of *Heterobasidion* infected trees have been discussed. Since the spread rate of the pathogen is faster in dead stump roots than in live tree roots, the disease problem may aggravate by cutting decayed trees. On the other hand, stumps invaded by saprophytes may block the spread of *Heterobasidion* (1).

The hypothesis tested in this study was if *Heterobasidion* spreads faster from the root system of a clear-cut decayed tree than from an intact decayed tree to a healthy neighbour.

Site. The first generation Norway spruce stand was planted in 1952 with 2 m spacing and thinned in 1974 and in 1986. In 1991 the trees were classified as healthy or decayed based on 2 bore cores taken at stump height. *Heterobasidion* spp. was found in 41.5% of the 691 trees.

Experimental design. A total of ninety pairs of one healthy and one decayed tree were chosen based on the above classification. The trees in the pairs were not more than ca 2.5 m apart. In 42 of the pairs the decayed tree (donor tree) was felled and the diameter of decay at stump height and the height of the rot column was measured (felled donor treatment). In the other 48 pairs the decayed trees were left intact (intact donor treatment).

Assessments. In 1994 and in 2001 one bore core was taken at stump height from all the recipient trees in the pairs. In 2004 all the recipient trees were felled. The tree and decay diameter was measured at stump height and the height of the rot column was measured.

After 13 years ca 95% of the recipient trees in the two treatments were decayed. Mean height of the decay column was 4 m and maximum height was 8 and 10 m, respectively in felled and intact donor. There were no significant differences in decay frequencies and mean height of discoloration between the two treatments. Totally, 25.6 % of the the recipient trees had died. In felled donors, 15 of the recipient trees had died compared to only 8 trees intact donors ($p<0.05$) (Table 1).

Previous reports on observations in *Heterobasidion* spp. infected stands show that thinning of a stand affects spread of the fungus. Fungal growth rate can be more rapid in the roots of dead or dying stumps and removal of trees infected by the pathogen does not restrict the spread but may enhance it (1, 5). The results from the present investigation do not agree with the previous results. There was no difference in spread rate between pairs with an intact donor compared to those with a cut donor. This is the first study designed to investigate the spread rate of *Heterobasdion* spp. from cut or intact donors to neighbouring trees in nature: the results are interesting but more information is needed before any definite conclusions may be drawn.

Table 1. Assessment of trees. Decay frequency in the stand was 41.5% in 1991 (age 39).

	Donor = stump		Donor = tree	
	Recipient	Donor	Recipient	Donor
Number of trees	42	42	48	48
Decay frequency (%) at age:				
39	0		0	
42	30.3		36.4	
48	84.8		75.8	
52	97.0		93.9	
Decay diameter (cm) at age 39 (stumpheight)		23		
Height (m) of decay column at age 39		3.7		
Stump diameter (cm) at age 52	37.5		40.2	
Decay diameter (cm) at age 52 (stumpheight)	21.2		21.5	
Mean height (m) of decay column at age 52	3.7		3.8	
Max height (m) of decay column at age 52	8		10	
Area of decay (cm^2) at stump height at age 52	433		478	
Number of dead trees at age 52	15		8	

After 13 years, 95% of the recipient trees were infected by *Heterobasdion* spp. The annual spread rate of the pathogen has been reported to an average of 0.22 m and maximum 0.7 m (3).

Theoretically all recipient trees should be attacked at stump height after a 13-year period. However, some trees escape infection due to, for example, genetic variation in the host tree. Genetic variation in the vertical spread and infection incidence of *Heterobasidion* spp. in

stems and stumps of Norway spruce has been detected in several inoculation experiments (2). From a breeding point of view it is interesting if resistance factors in the host may be the reason for 5% of the exposed trees escaping the infection in the present investigation.

When the donor tree was cut, twice as many of the neighboring trees died compared to when the donors were left intact. Normally mature Norway spruce trees do not die from the infection. In the present investigation the fact that more trees died when the donor was cut may indicate that the inoculum from dead donors has a higher impact compared to a live donor. The observation may be of interest for the development of future silvicultural thinning routines. However, the sample is not large enough to draw any definite conclusions.

There are not many reports on annual spread of *Heterobasidion* spp. in the stem since information of the initiation of the attack is usually lacking. In 2 previous studies of natural infection, the initiation of fungal growth was defined (3, 5). The results from the present study are in accordance with the previous: an average annual growth in the stem of ca 0.3-0.4 m and a maximum of ca 0.8-0.9 m. The information may be valuable when creating models for disease managements of attacked stands. The predicted frequency of decay corresponded to the observed frequency in the present stand using the model described in Thor *et al.* (4) if fungal growth rate in dead and live wood were doubled compared to average rates.

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Fungi inhabiting roots of trees and their stumps and their effect on growth of *Armillaria*

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CONFERENCE ABSTRACT

Fungal communities in roots, rhizosphere and soil of trees were shown to change 2 years after the trees had been felled. The changes in density and diversity of fungal communities have important ecological significance. Compared to the lived roots, (1) the increase in density of fungal communities as well as frequency of the fungi antagonistic towards *Armillaria* and *Heterobasidion*, particularly of *Trichoderma* species, on/in Scots pine stump roots may lead to the smaller susceptibility of the latter to both pathogens, (2) accumulation of fungal species stimulating a growth of *Armillaria,* and the absence of bigger population of *Trichoderma* on/in roots of the deciduous tree stumps may result in their bigger susceptibility to *Armillaria* infection. Studies on effect of metabolites of fungi stimulating and inhibiting *Armillaria* and *Heterobasidion* growth are presented.

The common association of *Armillaria* with living coniferous and dead deciduous trees (stumps) suggests the existence of factors which predispose them to infection. The diversity of saprotrophic fungi colonizing roots and the rhizosphere, the density of the fungal population at different stages of tree development and the metabolites produced by fungi can be important.

The objective of this study was to evaluate (i) microfungal communities in/on roots targeted by *Armillaria*, (ii) changes in density and diversity of microfungal communities in/on roots of stumps 2 years after trees had been felled, (iii) effects of the most common microfungi on rhizomorph growth, (iv) inoculum potential of *Armillaria* affected by microfungi in/on roots of different trees and their stumps.

Fungal communities were isolated from the rhizosphere, from 0.1 – 1 mm diam and 0.5 – 1 cm diam roots (bark and wood separately where possible) and from soil of *Quercus robur, Betula pendula* and *Pinus sylvestris*. The samples were taken from 30- and 50-year old trees and stumps of a forest in western Poland. Fungi were identified morphologically. Quantitative and qualitative changes in fungal communities were determined by comparing living trees with stumps 2

years after felling. The effects of the most common fungal species on the growth of rhizomorphs of *A. gallica*, *A. mellea* and *A. ostoyae* were studied on oak sections *in vitro*. The number of rhizomorphs, number of rhizomorph apices, and rhizomorph length and weight were determined. The inoculum potential (IP) of an individual *Armillaria* species in a single tree species was evaluated according to the formula:

$$\mathbf{IP} = \mathbf{\Sigma}\ (\mathbf{n_1 + n_2 + n_3})\ \mathrm{x}\ \mathbf{S}\ \mathrm{x}\ \mathbf{L}$$

n – number of isolates of individual fungal species in an individual habitat
1-3 – habitat, i.e. rhizosphere, roots, soil
S – increase in number of rhizomorphs affected by individual fungal species, in %, compared with control
L – increase in length of rhizomorphs affected by individual fungal species, in %, compared with control.

The most common microfungi found in the rhizosphere, roots and soil of *Q. robur, B. pendula* and *P. sylvestris* are shown (Tables 1- 2; *all tables follow references*).

The density of the whole fungal community and number of individual species usually increased, often at least doubling, particularly in/on roots of stumps of deciduous trees and more rarely in *P. sylvestris* 2 years after felling.

The greatest increases recorded in fungal populations were of *A. kanagawaensis, Cylindrocarpon* spp.*, Geomyces pannorum, Monodictis lepraria, Mortierella* spp., *Nectria grammicospora*, *Penicillium* spp., *Sesquicillium candelabrum, Sporothrix schenckii* and *Trichocladium opacum* in *Q. robur*, of *Zygorhynchus moelleri* in *B. pendula*, and of *Trichoderma viride* in *P. sylvestris.*

The majority of fungi detected in/on roots stimulated the growth of *Armillaria* rhizomorphs *in vitro* (Table 3) (1-8). Rhizomorphs were not usually formed on a sterile substrate. It is presumed that the increase in number, number of apices, length and weight of rhizomorphs by microfungi can consequently increase the *Armillaria* inoculum potential. The calculated IP of *Armillaria* was greater in stumps than in living trees of *Q. robur, B. pendula*, and 50-year old but not 30-year old *P. sylvestris* (Figure 1).

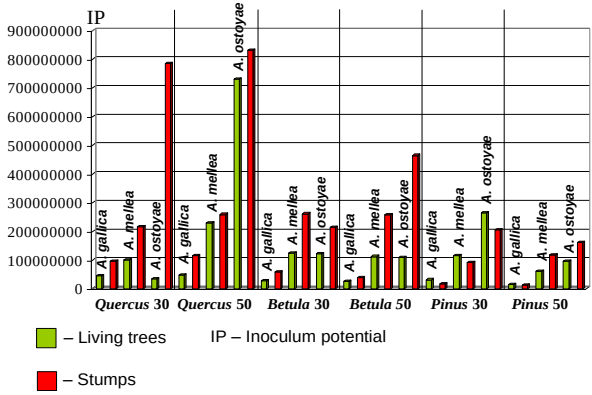

Figure 1. Inoculum potential of *A. gallica*, *A. mellea* and *A. ostoyae* in deciduous and coniferous trees.

Armillaria is, generally, held at bay or eliminated in the living host by host defense mechanisms, which are mechanical (formation of callus tissues) and chemical (resins, gums, phenols, sterols, monoterpenes, tannic acids, nitrogen compounds).

It is presumed that changes in density of particular microfungi in/on roots and soil under stumps, combined with their ability to produce metabolites affecting *Armillaria* rhizomorph growth, can contribute to increased or decreased *Armillaria* inoculum potential, i.e. the energy available for infecting potential hosts.

Different fungi are associated with the increase in *Armillaria* IP in different tree species. In stumps of *Q. robur* (particularly in a 30-year-old stand), increased densities of *Cylindrocarpon*, *M. lepraria*, *Mortierella macrocystis*, *M. parvispora* and *Penicillium* spp., which activated rhizomorph production strongly, are implicated in increased IP. In stumps of *B. pendula*, increased density of *Z. moelleri* and absence of *T. viride* are implicated. The former produces indole-3-ethanol and indole-3-acetic acid, which are growth promoting substances for rhizomorphs, and phenoloxidizing enzymes important in the degradation of wood. The moderate increase in IP in stumps of 50-year old *P. sylvestris* trees, and decrease in stumps of 30-year old trees, may relate to decreased densities of *Armillaria* stimulants (*Chrysosporium merdarium, G. pannorum*, *Hormiactis candida, Phialocephala fortinii* and *Pseudogymnoascus roseus*), moderately increased densities of *Mortierella* and *Penicillium* spp., and increased density of *Trichoderma* spp., particularly *T. viride*. *Trichoderma viride* is well-known as an antagonist of *Armillaria*, resulting from its lytic

activity and saprotrophic competitiveness. This fungus preferentially inhabits coniferous wood, where it finds the low concentration of nitrogen required.

Armillaria inoculum potential depends on the age of the stand because this affects the level of vitality and the decomposition of roots, which affect colonization by microfungi.

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Table 1. Changes in density of the most common fungal species in 2-year-old *Quercus robur* stumps (in %, compared with living trees).

Fungus	Rhizosphere from roots				Roots 0.5 – 1 cm diam				Soil	
	0.1 – 1 mm diam		0.5 – 1 cm diam		Bark		Wood			
	Age of the stand									
	30	50	30	50	30	50	30	50	30	50
A. kanagawaensis[1]	**1300**	150	**15900**	150	**4900**	400	150	150	**298**	102
C. destructans[1]	50	150	**1475**	150	**464**	**600**	90	**500**		
C. didymum[1]	**32400**	50	**1119**	**141000**	77	**10300**	**800**	**13300**		
C. merdarium	**655**	**561**	84	**30**	133	17	50	500	**300**	**577**
G. pannorum	169	**236**	**1190**	**712**	**183**	**1000**	**550**	**800**	53	116
H. candida	50			**5200**	**1000**	100	150	**1200**		
M. lepraria	150		**1720**	50	**418**	300	166		**700**	100
M. hyalina	21	200	**866**	**400**						
M. macrocystis	**43**	**900**	**486**	215	157	**5**	150		**1500**	**1200**
M. parvispora[1]	157	**718**	200	150	**800**	50	5	500		
N. grammicospora					**132**	**131**	**402**	106	**1800**	150
P. adametzii[1]	**551**	**300**	**220**	125	**211**	80	69	**400**	119	113
P. citrinum	**3**	**40**	**12**	**39**	**34**	88	**3**	83		58
P. daleae[1]	**725**	**50**	**232**	**187**	**493**	**316**	**233**	121	**231**	**54**
P. janczewskii [1]	**138**	**133**	**301**	**140**	**786**	**485**	55	200	**308**	**147**
P. lanosum					**9**	50	**13**	50		
P. spinulosum	**400**	93	**5**	**8**	150	20	**10**	**25**		
P. fortinii[1]	**500**		100	100	114	**85**	**885**	**36**		
P. roseus	**358**	**1500**	**1033**			20	150		**241**	**375**
S. candelabrum	150	**12700**	**7700**	**52200**		**900**	200	**5200**		
S. schenckii	57	**188**	**3022**	**1638**	**364**	**254**	260	**1033**	**1400**	**700**
T. niveum[1]	**345**	**34**	42	83	300	116	50	40	**900**	**400**
T. opacum						150	**1600**	**1400**	**600**	
T. polysporum	**20**	25		**2200**	110	**600**	25			
T. viride			200	250	9	**257**	66	200	150	250
U. vinacea[1]	116	**222**	119	129	**196**	110	44	134	**285**	127
Z. moelleri	**14**	50	**6**	**22**						

in **bold** = significantly different from control, average from 2-6 isolates of test fungus

Table 2. Changes in density of the most common fungal species in 2-year-old *Betula pendula* and *Pinus sylvestris* stumps (in %, compared with living trees).

Fungus	Rhizosphere				0.1– 1 mm diam roots				0.5 – 1 cm diam roots				Soil			
	Age of the stand															
	30		50		30		50		30		50		30		50	
	Betul	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*	*Betul*	*Pinus*
A.kanagawaensi		128	150	150	**392**	25							300	209	**1097**	
B. bassiana	50														150	
C. destructans[1]	150	33			67	397	**7**		**358**	**5500**	**3100**	150				
C. didymum[1]						**3**			**50**			**10**				
C. merdarium	**5700**	90	66		150								**28**	**36**	302	**25**
G. pannorum		50	**9**		50			50						**14**		50
G. candidum			150							150		**800**				
H. candida		**13**				**16**										
M. hyalina	**5810**	25			150	**16**		150	50					10		
M. macrocystis		**9**		150		**36**			150		50					
M. parvispora	**17**	**42**	**7**	**908**	50	**540**	**14**	105		**700**	150			**25**	150	**33**
N. grammicospo										**4**						
P. adametzii[1]	80	**23**	**8**	33	**600**	**6**	150	**9**	33	300	150		**10**	**1**	**14**	**51**
P. daleae[1]	**1232**	**30**	**426**	108	**879**	**192**	90	156	**800**	**1700**	**1456**	150	**51**	**14**	**25**	71
P. citrinum	**105 x**	**433**	**700**	**1775**	**1500**	150		90	200	150	**500**	66	**1015**	**250**	200	**700**
P. janczewskii[1]	**1012**	**13**	395	375	**500**	**7**	**235**	33	**500**	**400**		66	**21**	**9**	**29**	**40**
P. lanosum	150	33				20		200	150					10		**200**
P. spinulosum	150	25	**524**	**1114**			**6700**	**900**	**400**		**4400**	**5**	300	75	**1495**	**300**
P. fortinii[1]	**20**	**18**		44	**400**	**285**	69	**39**	**36**	**7**	**14**	**10**				
P. roseus	50	**1300**		**14**				25		50		50	**400**	**34**	**8**	**9**
S. candelabrum	150			200		150		200					300			
S. schenckii	**2905**	**4500**	150	**1400**		**1100**		**800**	**900**	**142 x**	700	**400**	**800**	**200**		
T. niveum	14	**25**	150			50							300	**200**		**200**
T. opacum	200				**706**				150		150		**592**		150	
T. hamatum		**800**			**1100**	**10**	**5900**	38				**400**		**20**		
T. polysporum		200		50		**550**		200	150	200		**500**		50		**200**
T. viride		71	150	**5000**		**250**	**6**	**55**		**159 x**	**1000**	**211 x**		38	**50**	**160**
U. vinacea[1]	**701**	**97**	**783**	**729**	95	**65**	120	**25**	**315**	**771**	30	**400**	**17**	**18**	**16**	**6**
Z. moelleri	**6295**	200	**233 x**	**2150**	**509**	**650**	**645**	133	**2002**	**2400**	**132 x**	200				50

[1] average from 2-6 isolates of test fungus

Table 3. Effects of the test fungi on *Armillaria* rhizomorph production (in %, compared with control).

	Number (**S**)			Number of apices			Length (**L**)			Weight		
Fungus	*A.gal*	*A.mel*[2]	*A.ost*[3]	*A.gal*	*A.mel*[2]	*A.ost*[3]	*A.gal*	*A.mel*[2]	*A.ost*[3]	*A.gal*	*A.mel*[2]	*A.ost*[3]
A. kanagawaensis[1]	65			173			**224**			276		
B. bassiana		186			193			218			**260**	
C.destructans[1]	58	193	**590**	**322**	206	**577**	**330**	**350**	**603**	**303**	**314**	**809**
C. didymum[1]	88		50	**217**		50	**296**		48	**360**		63
C. merdarium	66		325	**271**		325	**286**		550	131		325
G. pannorum	43		25	171		25	**273**		50	**241**		25
G. candidum		196			**254**			**313**			**437**	
H. candida	160			393			**827**			**1396**		
M. lepraria	80		150	266		300	**422**		**3500**	**729**		**1430**
M. hyalina	140			**736**			**1120**			**1130**		
M. macrocystis	93		175	152		**1800**	**259**		**3700**	100		**4675**
M. parvispora[1]	66		225	205		**400**	**235**		**782**	120		**300**
N. grammicospora		**251**	291		**257**	339		**278**	385		**247**	409
P. adametzii[1]	108		66	**463**		129	**483**		**300**	**359**		**425**
P. citrinum		120	200		124	200		**183**	**200**		**228**	**500**
P. daleae[3]	78	**286**	80	**399**	**290**	100	**548**	**379**	**1210**	**506**	**260**	**348**
P. janczewskii[1]	78	151	175	122	154	175	**181**	**320**	**2350**	**663**	**280**	125
P. lanosum			**674**			**595**			**765**			**754**
P. notatum			**407**			**373**			**482**			446
P. spinulosum		**362**	293		**387**	**422**		**443**	**450**		**371**	444
P. fortinii[3]	55	**231**	**414**	96	**234**	**358**	147	**345**	397	211	**295**	470
P. roseus	80		75	171		375	**26**		625	144		**700**
S. candelabrum	26		50	**10**		50	**6**		50	**3**		50
S. schenckii		**320**	50		**303**	50		**379**	500		**345**	25
T. niveum[1]		**351**	275		**390**	**275**		**500**	**4500**		**605**	**600**
T. opacum	**230**		150	**753**		**100**	70		**2500**	**2390**		500
T. hamatum			222			214			245			231
T. viride			140			137			143			141
U. vinacea[1]		**358**	100		**375**	100		**372**	70		**491**	70
Z. moelleri			**392**			**361**			**360***			**267**

[1] average from 2-6 isolates of test fungus
[2] average from 2 isolates of *A. mellea*
[3] average from 16 isolates of *A. ostoyae*

Growth of Scots pine seedlings and *Armillaria ostoyae* rhizomorphs under elevated air CO_2 conditions

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CONFERENCE ABSTRACT

Comparative cultivation experiment was carried out to verify an assumed positive dependent relationship of the rate of growth of Scots pine (*Pinus sylvestris* L.) seedlings with an elevated (750 ppm) level of CO_2, as well as a negative dependent relationship with the presence of an inoculum of *Armillaria ostoyae* (Romagn.) Herink. Eight months after germination, 5 months after the introduction of the *A. ostoyae* inoculum and 4 months after the raising of the air CO_2 concentration, most values for the analyzed biometric features of Scots pine seedlings were significantly greater than under control CO_2 conditions (ambient air at approximately 380 ppm). Significantly bigger was width at the stem base of 0.08 mm, dry mass of roots of 15.69 mg and dry mass of stem of 12.01 mg. No effects on seedling growth of the introduced pathogen inoculum were recorded at either the control or the elevated CO_2 air concentrations. In the vast majority of seedlings subjected to inoculation no root contact with rhizomorphs or infections were noted, except in a very few cases under elevated CO_2 conditions only. The production of *A. ostoyae* rhizomorphs was also found to be significantly and positively dependent on higher CO_2 concentration in the air. These very first results indicate the pathogen-host relationship in elevated air CO_2 concentrations need further studies with longer duration and older plants applied to overcome the experiment's limitations.

Air carbon dioxide concentrations is expected to reach 490-1250 ppm by the year 2100 what accompanied by climatic warming is going to change the functioning of entire forest ecosystems, especially photosynthesis and growth rates (1). In Central Europe this is expected to favor tree species of greater thermal requirements, while simultaneously bringing deterioration in growing conditions for species requiring less heat, such as Scots pine and Norway spruce (2, 3, 4, 6). In the context of *Armillaria* spp. interactions with carbon dioxide concentrations and/or climatic warming a stimulating influence of elevated CO_2 concentration on the growth of rhizomorphs has been mentioned (5), as the ability of wood-decaying fungi (*inter alia Armillaria* spp.) to fix atmospheric CO_2 (8). Extended growing season, increased precipitation and mild winters have also been thought likely to favor development of the infectious potential of *Armillaria* spp. (7). Expected climate changes are thus likely to alter host-pathogen relations, recognition of which was the goal of undertaken investigation.

The comparative cultivation experiment was undertaken to verify the assumed positive dependence of the rate of growth of Scots pine (*Pinus sylvestris* L.) seedlings with an elevated level of CO_2, (750 ppm) as well as a negative dependent relationship with the presence of an inoculum of *Armillaria ostoyae* (Romagn.) Herink. (in the form of oak branch sections colonized by the pathogen). Pine seedlings were grown in climate chambers (Mytron WB 750) under constant and controlled conditions: 20^{o}C, 75% RH, 16/8 (24hr) day/night cycle and at about 25-30% substrate moisture. The CO_2 concentration in the chamber was controlled with B-647 Controller (MKS Instruments). The measurements of biometric parameters (i.e. heights, diameters at the stem base, and dry mass of roots, needles and above-ground parts) were made prior to CO_2 treatment and after over 4 months, at the end of the experiment. The seedlings' roots were also checked for the infection symptoms and the numbers of containers with abundant (10 or more emerging rhizomorphs per inoculum), moderately-developed (less then 10) or undeveloped rhizomorphs were counted. ANOVA and contingency table analysis were applied to check the statistical significance of factors' effects on seedlings growth and rhizomorphs development.

Although all inoculum preserved alive pathogen mycelia and most of them produced rhizomorphs there was just one root of one seedling infected by *A. ostoyae* and a few seedlings whose roots were "tied up" with rhizomorphs, but no penetration was noticed. All such cases appeared under elevated air CO_2 conditions only. Neither visible nor microscopic symptoms of infection, except one mentioned above, were recorded by visual inspection of seedlings root systems at the end of the experiment. Seedlings remained healthy, with normal foliage and root system in spite of rhizomorphs development or CO_2 treatment.

Eight months after germination, 5 months after the introduction of the *A. ostoyae* inoculum and over 4 months after raising of the air CO_2 concentration up to 750 ppm, most values of the analyzed biometric features of Scots pine seedlings were significantly greater than under control CO_2 concentration. Only dry mass of needles and height increment did not differ distinctly, while dry mass of needles to dry mass of roots ratio was remarkably lower under elevated CO_2 concentration and other parameters higher.

The effects of the introduced pathogen inoculum and rhizomorphs development on seedling growth were weak (Figure 1). Under the control CO_2 concentration values of dry mass of needles to dry mass of roots ratio were significantly greater for seedlings growing in pots with moderately and abundantly developed rhizomorphs than for ones

not subjected inoculation or developing rhizomorphs. Under the doubled CO_2 level as well as for other analyzed biometric parameters in both CO_2 concentrations the differences were insignificant.

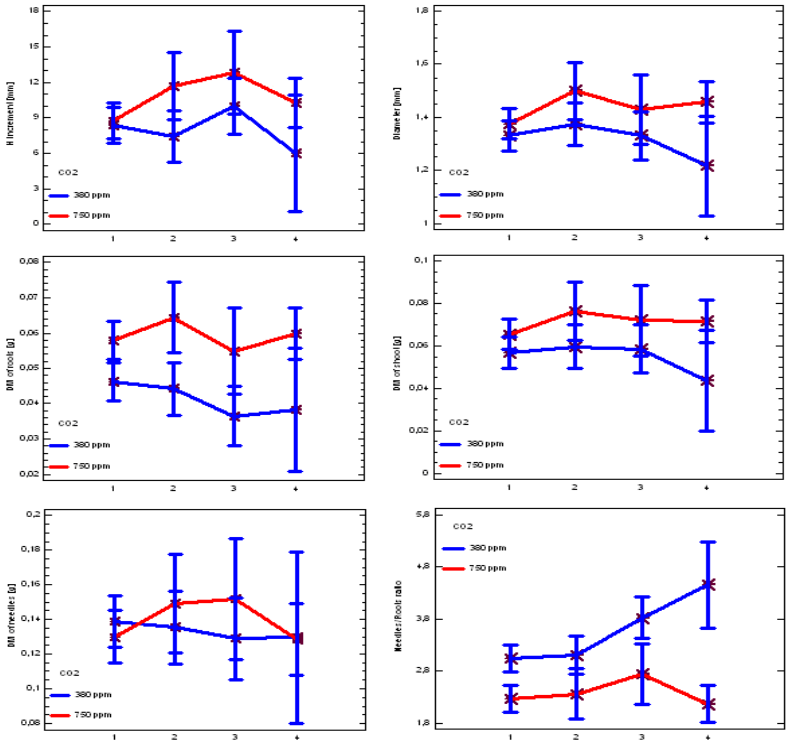

Figure 1. Effects of the introduced pathogen inoculum and rhizomorphs development on growth of seedling under control and elevated air CO_2 conditions (1 – no inoculation; 2 – lack of rhizomorphs; 3 – moderately developed rhizomorphs; 4 – rhizomorphs abundant). (See Appendix: Color Plates)

The production of *A. ostoyae* rhizomorphs was found to be positively dependent on higher CO_2 concentration. It was statistically confirmed by the contingency analysis and accompanied tests (Table 1).

The results of performed experiment indicated on the stimulating effect of elevated air CO_2 concentration up to 750 ppm on growth of both Scots pine seedlings and *A. ostoyae* rhizomorphs. This corresponds to the earlier findings (1, 5), but in the case of the fungus had not been adequately explained yet.

Table 1. Contingency table and results of accompanied tests.

CO_2 concentration	Number of seedlings	Lack of rhizomorphs		Degree of rhizomorphs development					
				Moderate		Abundant		Total	
[ppm]	[indivs.]	[indivs.]	[%]	[indivs.]	[%]	[indivs.]	[%]	[indivs.]	[%]
380	32	16	50.00	13	40.62	3	9.38	16	50.00
750	32	9	28.12	6	18.76	17	53.12	23	71.88
Total	64	25	39.06	19	29.69	20	31.25	39	60.94

Statistics	Value	P	Degree of freedom
Contingency coefficient	0.4278		
Cramer's V	0.4733		
γ (conditional Gamma)	-0.5686		
Pearson's R	-0.3930	0.0007	62
τ_B (Kendall's Tau b)	-0.3625	0.0023	
τ_C (Kendall's Tau c)	-0.4170		
χ^2 (Chi-square test)	14.34	0.0008	2

Due to insufficient infection of roots by pathogen rhizomorphs the further investigations need to be performed to look for pathogen-host interactions under the climate warming conditions. Focus on the build up of pathogen infection potential issues on one hand and host susceptibility to infection on the other might be useful in this context.

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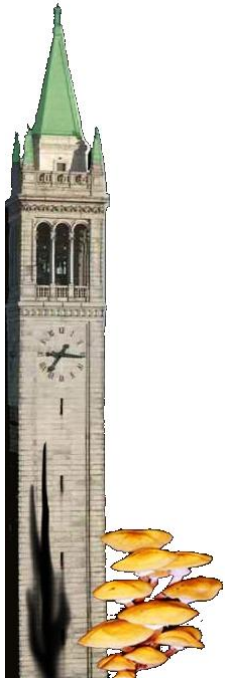
SESSION III:

GENETICS AND POPULATION GENETICS



Sequencing the genome of the forest pathogen *Heterobasidion annosum*

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CONFERENCE ABSTRACT

Heterobasidion annosum causes a devastating root rot in conifer plantations and natural forests throughout the northern hemisphere. In a collaboration with the Joint Genome Institute, the genome of *H. annosum* will be the first plant pathogenic homobasidiomycete to be sequenced allowing for new insights into plant-microbe interactions. Comparisons with plant pathogens with a gradient of taxonomic relatedness to *H. annosum* will help understanding the evolution of pathogenicity factors. Response of the model tree *Populus* to various types of trophic interactions can be studied including rust pathogen fungi and mycorrhizal mutualists. Furthermore, comparisons with the model white rotter *Phanerochaete crysosporium,* will deepening our understanding of wood degradation including ligninolytic and polysaccharide degradation pathways and several bioremediation applications. Moreover, this project will also gain insights into fungal evolutionary history and biology including development, non-self recognition, mating, and secondary metabolism.

Genetic structure of *Armillaria ostoyae* in the Landes de Gascogne forest in south-western France

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CONFERENCE ABSTRACT

The Landes de Gascogne (LdG) forest (south-western France) is a mostly artificial maritime pine (*Pinus pinaster*) monoculture of about one million hectares surface, which is intensively managed for timber and pulp production. *Armillaria ostoyae* is the most important fungal pathogen in this forest; its maximal incidence is observed in the coastal area but an increasing presence of the pathogen is reported on the interior. In the present study, we analyzed the genetic diversity of *A. ostoyae* in the LdG forest, with particular emphasis on the geographic differentiation and colonization history. A total of 541 *A. ostoyae* samples representing 28 different populations were screened at five microsatellite loci. The overall *A. ostoyae* population was composed of 98 genotypes and no genotype was shared among populations. The genetic diversity significantly decreased from the coastal area toward the interior, which suggests a colonization of the forest from the coast. The absence of correlation between genetic and geographical distances among populations indicates that colonization has probably occurred following a randomly pattern. Geographic groups of populations were lowly differentiated and genotypes were not more closely related within than among populations, suggesting important gene flow among populations.

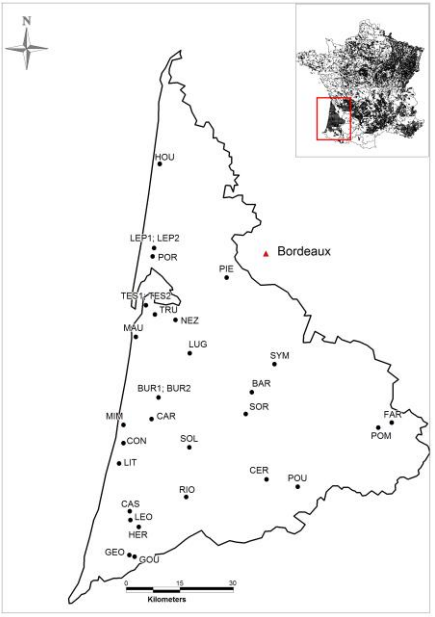
The Landes de Gascogne forest (south-western France) is a mostly artificial maritime pine (*Pinus pinaster*) monoculture of about one million hectares surface, which is intensively managed for timber production. *Armillaria ostoyae* is the most important fungal pathogen in this forest and its presence is reported since begin of the 1920s (3). The highest incidence of *A. ostoyae* is observed in the coastal area, but during the last 20 years an increasing presence of the pathogen has been reported in the eastern sector (1).

The aim of this study was to analyze the population structure of *A. ostoyae* in the Landes de Gascogne forest by using microsatellites. Specific questions were: *(i)* what is the importance of vegetative spread (i.e. root contacts) within *A. ostoyae* populations?; *(ii)* does the genetic similarity between *A. ostoyae* populations decrease as the geographic distance between them increases?; and *(iii)* is there a

genetic signal in the overall *A. ostoyae* population suggesting an eastward expansion of the pathogen?

A total of 31 *A. ostoyae* populations (i.e. disease centers) were considered in this study (Figure 1).

Figure 1. Geographic location of the 31 *A. ostoyae* populations analyzed.



At each site, 10-30 wood samples with *A. ostoyae* mycelial fans were collected from symptomatic trees/colonized fresh stumps, which were situated at a minimal distance of 10 m from another. For microsatellite genotyping, fungal DNA was extracted from the lyophilised mycelial fans using a protocol slightly modified after Gardes & Bruns (2). Each sample was then genotyped at five microsatellite loci by PCR reactions using specific primer pairs (4). The genetic diversity of the *A. ostoyae* populations was characterized by number of genotypes observed (G_{Obs}), number of genotypes expected in a sample of N = 10 (G_{10}, i.e. corresponding to the size of the smallest population being analyzed), and allelic richness averaged over loci (A_R). The significance of the correlation between G_{10} and A_R of a population and the distance of the population from the oceanic coast was tested with a Spearman's rank correlation test. The extent of population differentiation was estimated by calculating an overall F_{ST}-value

across populations and pairwise F_{ST}-values between geographic groups of populations (Table 1). The significance of pairwise differentiation was determined by randomly permuting genotypes among populations. To assess whether populations followed a pattern of isolation by distance, pairwise $F_{ST}/1\text{-}F_{ST}$ values were plotted against the logarithm of the geographical distance and the relationship was tested using the Mantel test (4).

Table 1. Genetic diversity (G_{Obs}, number of genotypes observed; G_{10}, number of genotypes expected in a population of N = 10; A_R, allelic richness averaged across loci) of the 31 *Armillaria ostoyae* populations analyzed.

Population	Code	Group	N	G_{Obs}	G_{10}	A_R
Hourtin	HOU	Coast north	20	5	3.7	2.7
Le Porge	POR	Coast north	12	8	7.1	3.3
Le Porge Canal 1	LEP1	Coast north	14	3	2.4	2.1
Le Porge Canal 2	LEP2	Coast north	11	4	3.9	2.7
Maubruc	MAU	Coast center	13	2	2.0	2.0
Truc de la Truque	TRU	Coast center	10	6	6.0	3.2
La Teste 1	TES1	Coast center	11	3	2.9	2.5
La Teste 2	TES2	Coast center	14	6	5.1	3.1
Contis	CON	Coast south	30	9	5.8	3.5
Mimizan	MIM	Coast south	28	5	4.2	2.1
Lit et Mixe	LIT	Coast south	29	3	1.7	2.3
Pierroton	PIE	Middle north	12	2	2.0	2.2
Nezèr	NEZ	Middle north	12	1	1.0	1.4
Lugos	LUG	Middle north	21	2	1.7	1.6
Caguillouse	CAR	Middle center	18	3	2.6	2.2
La Burle 1	BUR1	Middle center	15	4	4.0	2.2
La Burle 2	BUR2	Middle center	13	3	3.0	1.8
Solférino	SOL	Middle center	21	3	2.7	1.9
Rion des Landes	RIO	Middle center	13	3	2.8	2.8
Léon	LEO	Middle south	19	1	1.0	1.4
Herm	HER	Middle south	12	1	1.0	1.2
Castets	CAS	Middle south	11	1	1.0	1.4
Gourby	GOU	Middle south	28	3	2.1	2.1
St. Géours	GEO	Middle south	21	1	1.0	1.8
St. Symphorien	SYM	Interior north	14	2	1.7	1.7
Le Barthe	BAR	Interior north	25	1	1.0	1.6
Sore	SOR	Interior north	15	3	2.9	2.0
Cère	CER	Interior south	30	1	1.0	1.2
Pouydesseaux	POU	Interior south	12	2	2.0	2.0
Pompogne	POM	East	11	2	2.0	1.6
Fargues	FAR	East	16	1	1.0	1.2

The 531 isolates screened belonged to 94 multilocus genotypes and no genotype was shared among populations (Table 1). One (eight populations) to nine (CON) genotypes were identified in the single *A. ostoyae* populations. In 12 out of 23 populations composed of more than one genotype, 60-93% of the isolates belonged to a dominant genotype. The number of genotypes expected in a sample of N = 10 isolates varied between one and six (TRU). Important differences among populations were also detected in allelic richness, which

ranged from 1.2 (HER, CER, FAR) to 3.5 (CON). A highly significant negative correlation was observed between number of genotypes expected/allelic richness and the distance of the populations from the oceanic coast (Figure 2A and B).

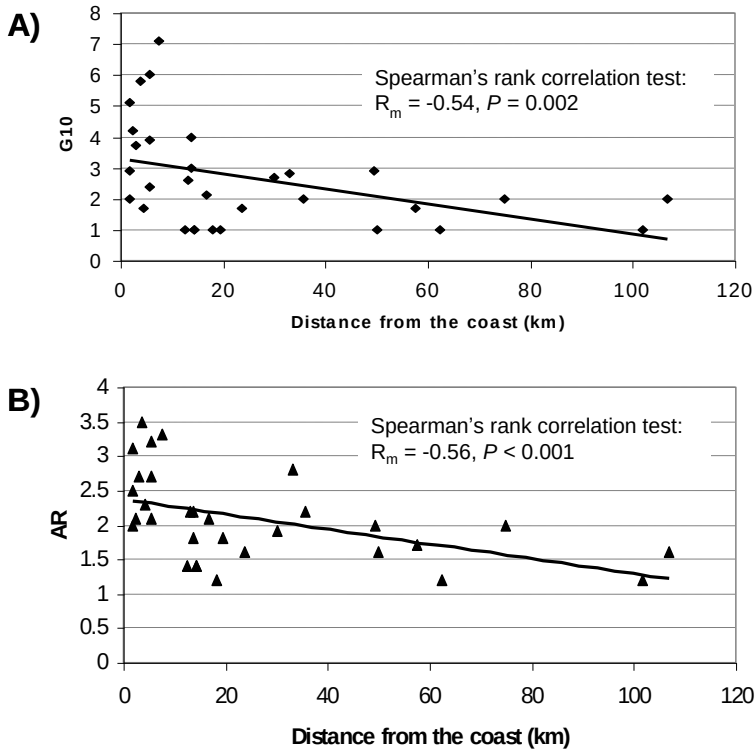


Figure 2. (A) Relationship between number of genotypes expected in a population of N = 10 (G_{10}) and the distance of populations from the oceanic coast, and (B) Relationship between allelic richness (A_R) and the distance of populations from the oceanic coast.

Global F_{ST}-value across *A. ostoyae* populations was significantly different from zero (F_{ST} = 0.43) based on non clone-corrected data sets. On the contrary, no significant population differentiation (F_{ST} = 0.04) was detected following clone-correction (i.e. only one representative of each genotypes considered). Based on permutations, all pairwise F_{ST}-values between geographic groups were significant when using non clone-corrected data sets and non significant after clone correction. Mantel test revealed a slightly significant relationship between geographical and genetic distance

among geographic groups only when clone-corrected data sets were used (r_m = 0.46, P = 0.03). The genotypic diversity of *A. ostoyae* within most disease foci is consistent with a predominant vegetative spread of the pathogen. In eight foci only one genotype was detected and in 12 other foci one genotype was dominant. Therefore, in the Landes de Gascogne forest *A. ostoyae* after establishment in a pine stand spreads most likely via root contacts since rhizomorphs in the soil are uncommon (B. Lung-Escarmant, unpublished). The co-existence at similar frequencies (i.e. no dominant genotype) of different genotypes observed in 11 out of 31 populations could be due to multiple establishments in very close proximity.

Our study indicates a low genetic differentiation among the *A. ostoyae* populations in the Landes de Gascogne forest, which suggests a strong gene flow among populations. The absence of repeated genotypes among populations shows that dispersal of genotypes through human activity (e.g. transport of colonized wood material) did not occur. Thus, dispersal of airborne basidiospores over short to long distances may be responsible for the lack of differentiation. Unfortunately, no specific studies on spore dispersal distances are available for species of *Armillaria*. The genetic diversity gradient from the oceanic coast toward the interior reflects a genetic signature of an expansion of *A. ostoyae* from the coast eastwards. The higher genetic diversity detected in the coastal area could be explained by the presence of larger *A. ostoyae* populations because of a longer presence of the pathogen in this area. Historical data indicate that in coastal area maritime pine stands have been present long before the actual Landes de Gascogne forest was created (19th century) and hence the *A. ostoyae* populations are probably older on the coast than elsewhere.

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Development of *Phlebiopsis gigantea* as a biocontrol agent for annosum root disease in the southeastern United States

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CONFERENCE ABSTRACT

In recent years, mid-rotation thinning of pine plantations has become a common practice in the southeastern United States. Associated with this change there has been an alarming rise in Annosum root disease, particularly in Georgia. In Europe, spore suspensions of *Phlebiopsis gigantea* are routinely applied to fresh stumps to control *Heterobasidion annosum*, but in the U.S. the Environmental Protection Agency (EPA) has not approved the use of *P. gigantea* for this purpose. In order to develop a *P. gigantea* biocontrol product that will meet EPA regulatory approval, we have collected 55 isolates of *P. gigantea* from 13 sites in the southeastern U.S. Twenty-one of these isolates make sufficient spores in culture for use as a biocontrol agent, and in preliminary assays, 2 out of 9 were as effective as a European commercial isolate at preventing *H. annosum* infection of loblolly pine (*Pinus taeda*). To determine how commercial application of selected *P. gigantea* strains is likely to impact local *P. gigantea* population structure, we also have developed microsatellite markers to assess *P. gigantea* genetic diversity throughout the southeastern U.S.

In recent years, mid-rotation thinning of pine plantations has become a common practice in the southeastern United States (U.S.). Associated with this change there has been an alarming rise in annosum root disease, particularly in Georgia. In Europe, spore suspensions of *Phlebiopsis gigantea* are routinely applied to fresh stumps to control *Heterobasidion annosum*, but in the U.S. the Environmental Protection Agency (EPA) has not approved the use of *P. gigantea* for this purpose. In order to develop a *P. gigantea* biocontrol product that will meet EPA regulatory approval, our project goals are to (1) collect *P. gigantea* isolates in the southeastern U.S., (2) test the biocontrol efficacy of these isolates, (3) assess the similarity among U.S. and European *P. gigantea* isolates, and (4) measure the impact of *P. gigantea* stump application on the prevalence of airborne *P. gigantea* spores. We are working closely with MeadWestvaco, the U.S. Forest Service, and Verdera Oy to meet these objectives.

During the first year of the project, we collected fifty-five *P. gigantea* isolates from thirteen sites in the southeastern U.S., primarily via spore trapping. Colleagues also sent us thirty-one *P. gigantea* isolates from elsewhere in North America and thirty-one isolates from Europe. The identity of all newly collected, southeastern isolates was confirmed by sequencing of their ribosomal DNA internal transcribed spacer region. Because *P. gigantea* treatments in the field are carried out with an aqueous suspension of spores, it is important that all potential biocontrol strains readily produce spores in culture. Thus, as a preliminary assessment of their suitability for biocontrol, the amount of spores produced in culture by each southeastern *P. gigantea* isolate was measured. Nineteen of them produced more spores in culture than a commercial biocontrol strain from Europe and six others produced nearly as many as this commercial strain. In subsequent competition assays design to estimate the biocontrol efficacy of different *P. gigantea* isolates, blocks of freshly cut loblolly pine wood (*Pinus taeda*) were inoculated with both *P. gigantea* and *H. annosum*. Eight of the eleven *P. gigantea* isolates that were tested were as effective as a European commercial isolate at preventing *H. annosum* colonization of pine wood.

As part of the product registration process, EPA has requested information on the similarity among European and U.S. *P. gigantea* isolates, as well as the similarity of U.S. isolates from different locations. This information will determine if existing animal toxicity data from European strains will be acceptable to the EPA, and whether or not more than one U.S. *P. gigantea* isolate can be covered by the product registration. Towards this end, we have compared the growth rates, and spore productivity of European and North American *P. gigantea* isolates. The growth rates of nine European, nine Canadian, and ten U.S. strains were similar between all three populations, while the average spore productivity of the Canadian strains was less than that of the European and U.S. isolates. As another method to assess similarity, we are using microsatellite markers to examine genetic diversity within and among *P. gigantea* populations. In collaboration with Jan Stenlid's lab, we have developed nine polymorphic markers. After screening a subset of *P. gigantea* isolates (16 – 27 isolates depending on the locus), the number of unique alleles for each marker ranges from three to eleven. Further screening of *P. gigantea* isolates is on-going. Ultimately we hope to examine all 117 *P. gigantea* strains in our collection. We have recently established a field trial in a thinned loblolly pine stand in order to determine if spraying stumps with *P. gigantea* affects the prevalence of airborne *P. gigantea* spores. Spore trapping at monthly intervals over the next twelve months will be used to monitor the site. Field trials designed to test the biocontrol efficacy of selected southeastern *P. gigantea* isolates are also planned for the coming year.

Identification of genets of *Armillaria tabescens* occurring on ornamental cherry trees along streets in Tsukuba City in Japan

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CONFERENCE ABSTRACT

Ornamental cherry trees along several streets in Tsukuba City have been declining for these years. Fruit bodies of *Armillaria tabescens* were frequently observed on declined trees. Isolates were obtained from mushrooms and mycelial sheets on cherry trees and genets of the fungus were identified by somatic incompatibility tests and AFLP (Amplified Fragment Length Polymorphisms) analysis. Population structure and infection method of *A. tabescens* on cherry trees will be discussed.

Ornamental cherry trees (mainly "Yoshino cherry"; *Prunus* × *yedoensis* Matsumura) along the streets in Tsukuba City, approximately 70 km north from Tokyo, have been declining since more than 10 years ago. Fruitbodies of *Armillaria tabescens* (Scop.) Emel were frequently observed on the declined and dead trees and stumps. Thus, this fungus is considered an important factor responsible for the decline of cherry trees. For disease control, it is important to determine how the pathogen infects its host in the field. This research is aimed to determine the infection method of *A. tabescens* by identifying the genets of this fungus with somatic incompatibility tests.

Sites. The research was carried out at the following 3 sites in Tsukuba City. At site 1, cherry trees were planted approximately 6 m intervals for a distance of 600 m one side of a street. At site 2, cherry trees were planted among other shade trees and shrubs for a distance of 900 m along the bicycle path that runs parallel to the walkway. At site 3, cherry trees were planted at approximately 8 m intervals for a distance of 400 m sidewalks paved with asphalt.

Isolation. In 2003, locations of *A. tabescens* fruitbody formation were recorded, and the fruitbodies were collected. Tissue isolates were established using potato dextrose agar medium (PDA; Eiken Chemical, Tokyo) that was diluted to 50% concentration, supplemented with agar (Wako Pure Chemical Industries, Osaka) in

order to obtain an agar concentration 1.5%, and amended with 0.3g/l of streptomycin sulfate (Meiji Seika, Tokyo). Single-spore isolation was also done from 3 fruitbodies using 1.5% water agar medium amended with 0.3g/l of streptomycin sulfate.

Somatic incompatibility tests. Tissue isolates were mated by inoculating 2 isolates approximately 3 mm apart on PDA (normal concentration) in a 9 cm Petri dish. For evaluating the accuracy of the somatic incompatibility test to distinguish *A. tabescens* genets, sibling isolates were prepared by fertile mating among single-spore isolates obtained from a single fruitbody. The synthesized diploids were subsequently mated. This test was carried out using isolates from 3 fruitbodies. Each mating test was duplicated.

Locations of fruitbody formation. At all the 3 sites, declined and dead trees and stumps occurred in a cluster along streets. Besides cherry trees, fruitbodies were also found on *Pinus densiflora* Sieb. et Zucc, *Quercus serrata* Thunb., *Quercus phillyraeoides* A. Gray, *Ligustrum japonicum* Thunb., *Ligustrum obtusifolium* Sieb. et Zucc., *Euonymus alatus* (Thunb.) Sieb., *Quercus myrsinaefolia* Blume, *Castanopsis cuspidata* var. *sieboldii* (Makino) Nakai and *Zelkova serrata* (Thunb.) Makino. Fruitbodies were formed on stumps, dead, declined and healthy- looking trees.

Evaluation of the somatic incompatibility tests to distinguish genets. From the 3 fruitbodies, 9, 9 and 8 sibling isolates were synthesized by inbreeding the single-spore isolates. In total, 100 mating tests were performed among these synthesized diploids, and the following 3 different results were obtained: (i) a dark pigmented line was formed between the 2 mycelia, (ii) neither a line was formed, nor the 2 mycelia had merged, (iii) the 2 mycelia had merged seamlessly. The results (i) and (ii) were observed in 84% of 32 combinations wherein the 2 siblings, when synthesized, had a common spore isolate. The same results were observed in 94% of 68 combinations wherein the 2 siblings were synthesized using completely different single-spore isolates.

Genets in the fields. Tissue isolates were established from 50 fruitbodies collected from all the 3 sites. Somatic incompatibility test among these isolates produced the same abovementioned 3 results. Based on the interpretation that results (1) and (2) indicate the 2 isolates belong to different genets, 5 genets were identified at site 1. At this site, 3 of the genets were located in 3 different areas. The rest 2 shared the same cluster of declined and dead trees. In site 2, 6 genets and 1 group of isolates were identified. The isolates of the group were distinguished from other genets, but some of the intra-group mating tests produced result (1) and (2), and others result (3), and whole results could not identify genets in the group clearly. At site

3, 1 genet and 1 group of isolates were observed, and the intra- group mating tests gave a similar result as described for site 2.

Kile (1983) evaluated somatic incompatibility test as a method for identifying genets by using synthesized inbred diploids of *A. luteobubalina* and reported an accuracy 44% (1). In the present study, the same method using *A. tabescens* exhibited an accuracy of more than 80%. This method was found to be very useful for this species.

Although somatic incompatibility test showed high accuracy to identify genets, it failed to identify distinct genets in the isolate groups at site 2 and 3. It indicates that the isolates in the groups are genetically close.

At the sites where each genet occupies cluster of dead and declined trees, *A. tabescens* infection might have occurred asexually, probably through root contacts of hosts, because rhizomorph production of the fungus in vivo has rarely been reported. Site 1 where the soil was not compartmentalized and cherry trees were planted at short distances suits infection via root contact. On the other hand, a cluster of dead and declined trees that provides habitat to a group of isolates from more than one genet suggests spore dispersal of the fungus. Such groups of isolates were found at site 2 and 3 where the soil was separated by the pavement, and it limits occurrence of fungal infection through root contact. It is concluded that *A. tabescens* can infect its hosts effectively by using both sexual and asexual methods depending on environmental conditions.

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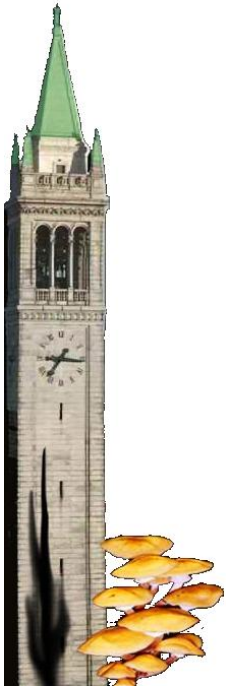
SESSION IV:

AETIOLOGY AND EPIDEMIOLOGY



Root disease in Yosemite Valley

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CONFERENCE ABSTRACT

Since Euroamerican settlement 150 years ago, fire suppression and meadow draining in Yosemite Valley have led to increased conifer densities at the expense of previously dominant meadow and oak woodland vegetation types. Mixed-conifer forests now cover over 50 % of the valley; ponderosa pine and incense cedar are the dominant species with lesser numbers of black oak, white fir and Douglas-fir. We estimate that approximately 37 % of the area of mixed-conifer forest in Yosemite Valley is in expanded gaps. Gaps had a mean area of 1085 m^2 (median 475 m^2, range 81 to 10,650 m^2). Root disease was the most common mortality agent and was associated with over 80 % of all gaps. *Heterobasidion annosum* (30 % gaps) and *Armillaria mellea* (30%) were the most common root pathogens. Gap area in Yosemite Valley has increased from 3.5 to 37 % over the last 30 years due to increases in the size of individual gaps rather than to the establishment of new gaps that tend to be small. The prevalence of root disease in Yosemite Valley is an unintended consequence of a series of management actions (fire suppression, tree felling) initiated earlier in the century. Gaining an understanding of current causes of tree mortality and how human management has influenced stand dynamics in Yosemite Valley will be important in planning restoration strategies and re-establishing the historic fire regime.

Spread of *Heterobasidion annosum* in US Pacific Northwest Christmas tree plantations

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CONFERENCE ABSTRACT

The population structure of *Heterobasidion annosum* in Pacific Northwest (PNW) Christmas tree plantations was estimated at two spatial scales to assess the relative importance of primary and secondary infection, colonization, and spread of the pathogen. Ninety-three isolates from single trees in 27 discrete mortality pockets and 104 isolates from 12 individual root systems of noble and Fraser fir trees were sampled near Mossyrock Washington. Isolates were genotyped using somatic compatibility assays and microsatellite markers to determine the spatial scale at which dispersal of single genotypes (genets) was occurring. All isolates sampled from different trees in discrete mortality pockets had distinct genotypes whereas the root systems of single trees were dominated by one or two genotypes. These results suggest that infection of PNW Christmas trees results from frequent primary infection events of adjacent stumps and localized secondary spread within root systems rather than clonal spread of the pathogen between adjacent trees. We hypothesize that mortality pockets may be due to availability of infection courts and/or variation in inoculum levels during selective harvesting of patches of mature trees.

In PNW Christmas tree plantations, non-random aggregations of diseased true fir trees (mortality pockets) infected with *Heterobasidion annosum* have been observed in increasing numbers since the 1980's which appear similar to clusters of infected trees observed in forest situations. These mortality pockets could result from clonal spread of the pathogen among adjacent trees following infection of a single stump or multiple or spatially clustered stump infections by different genotypes of the pathogen. To differentiate between these two hypotheses, we sampled *H. annosum* isolates at different ecological scales and genotyped them using microsatellite markers. Our specific objectives were to estimate genotypic diversity of the pathogen at the mortality pocket scale and the individual root system scale. These data have allowed us to determine the importance of primary infection versus secondary spread of annosus root rot disease in Christmas

tree plantations and will aid in development of improved annosus root rot management strategies.

Mortality pocket scale sampling. For genotypic diversity assessment at the mortality pocket scale, symptomatic trees in mortality pockets in 3 fields at the Mossyrock plantation were mapped and *H. annosum* sampled (2). One of the fields, designated TF3, was a monoculture of noble fir consisting of 1045 trees. The other two fields, designated TF2, and TF5, were Frasier fir monocultures consisting of 910 trees each. Within 3-6 years of replanting these fields, percent mortality due to annosus root rot had reached 10 to 40% within these fields. Ninety-three *H. annosum* isolates were obtained from single trees in 27 discrete mortality pockets of 2-8 trees spread throughout the three fields (Figure 1). Most of the sampled trees had been planted directly next to a stump left from the previous rotation, and all trees had been planted on a 1.8 by 1.8 meter spacing pattern.

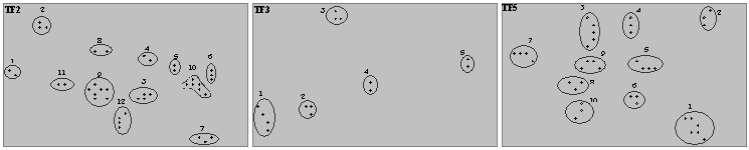

Figure 1. Locations of mortality pockets (circled) within fields TF2 (left), TF3 (middle), and TF5 (right) at the Mossyrock plantation. Each dot represents a symptomatic tree from which a single isolate of *H. annosum* was obtained.

Root system scale sampling. Root systems were excavated from within the Mossyrock plantation. These root systems were labeled RGS-1 to RGS-12. Eight of the root systems were from Frasier fir, and 4 were from noble fir. To estimate number and distribution of *H. annosum* genotypes at the root system scale, root systems of the freshly killed trees were excavated and sampled. *H. annosum* isolates were sampled from different parts of each excavated root system (Figure 2) to obtain an estimate of the spatial distribution of genotypes at this scale.

Microsatellite amplification, separation, scoring, and statistical analysis. Four microsatellite loci, designated Ha-ms1, Ha-ms2, Ha-ms6, and Ha-ms10 (1), were used to estimate genotypic diversity of *H. annosum* isolates sampled at the mortality pocket and root system scales. Fragment sizes of PCR amplicons were determined using an ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems). Alleles at each locus were visualized and scored individually to the

nearest base pair using GENESCAN and also interpreted with the aid of Genotyper version 3.7 (Applied Biosystems).

We recovered an average of 1.17 genotypes per root system as compared to an average of 3.61 genotypes per mortality pocket (Figure 3). DNA fingerprints generated for isolates from roots systems using microsatellite markers demonstrated the occurrence of a single multilocus genotype in a majority of the root systems and two genotypes in root systems RGS2, and RGS-12 (Figure 2).

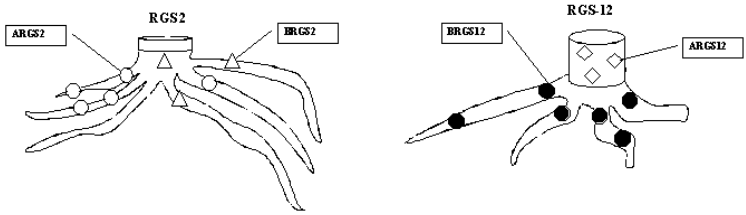

Figure 2. Distribution of *H. annosum* genotypes within single root systems in root system samples of true fir (*Abies* spp.) Christmas trees in which more than one genotype was isolated.

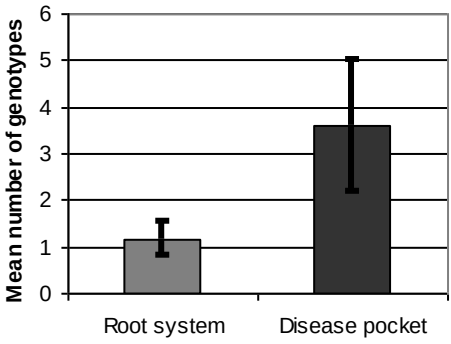

Figure 3. Comparison of the mean number of genotypes found at the root system and mortality pocket sampling scales as determined by DNA fingerprinting and somatic compatibility testing.

Microsatellite fingerprinting demonstrated that individual trees within mortality pockets at the Mossyrock plantation were each colonized by 1-2 genotype(s) of *H. annosum*. The high genotypic diversity observed may indicate high levels of primary stump infection, combined with localized clonal spread within root systems. We speculate that mortality pockets may result from availability of infection courts and/or variation in inoculum levels during selective harvesting of patches of mature trees rather than clonal spread through root-to-root contact. This hypothesis is supported by anecdotal observations in PNW Christmas tree plantations where trees are often harvested selectively in clusters as they reach merchantable size. It is thus possible that mortality pockets are the result of clusters of primarily infected stumps from which trees were harvested when high levels of inoculum were in the air, rather than the extensive spread of a single clone between trees via interconnected root systems. Basidiocarps of *H. annosum* are rarely observed in even the most severely diseased PNW Christmas tree plantations, indicating that basidiospores are likely blown in from surrounding forests or that conidia may be functioning as primary inoculum.

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Incidence of *Heterobasidion* root and butt rot in first rotation *Larix* stands and justification for stump treatment

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CONFERENCE ABSTRACT

The presence of *Heterobasidion* spp. was investigated in ten healthy looking first rotation stands of *Larix* spp. on former arable land in southern Sweden. Four of the five older stands were thinned more than once at temperatures above 5°C, and hence may have been subjected to natural spore infections by *Heterobasidion* spp. The other five younger stands were not thinned at all or only once during winter conditions. In each stand bore cores were taken from 30 randomly selected trees at stump height. In three of the younger stands samples were also taken from the roots. *Heterobasidion* spp. was found in eight stands. The older ones had an infection frequency between 20 % and 53 %. In three of the younger stands *Heterobasidion* spp. was found. The average infection frequency was 3 %. No roots of younger trees were infected. The results indicate that *Heterobasidion* spp. may infect *Larix* stands through thinning stumps. To economically justify stump treatment with *Phlebiopsis gigantea* the lack of income due to infection by *Heterobasidion* spp. was compared to the cost for treatment. At three percent interest rate stump treatment was clearly beneficial.

Two species of *Heterobasidion* cause considerable losses to conifer stands in southern Sweden, namely *H. annosum* s.s. and *H. parviporum*. *Picea abies* is severely attacked by both species while *Larix x eurolepis* is attacked only by *H. annosum* (3).

Thinning stumps created when spores are abundant provide an important infection court for *Heterobasidion* (2). In Sweden, this corresponds to the active growing season when temperatures exceed 5°C (1). The fungus then spreads vegetatively from infected stumps into roots of neighbouring living trees via root contacts and grafts. The spread of *Heterobasidion* can be controlled by treatment of fresh stump surfaces by biological or chemical means preventing spore infections (6). Stump treatment is regularly used and believed to be economically beneficial on a variety of tree species in many European countries (6). In Sweden only *P. abies* is treated.

Recent storms in southern Sweden have resulted in an interest among foresters to change tree species, to e.g. *L. x eurolepis*, on sites preferably being free of infection or where the *H. parviporum*

dominates in *P. abies*. Since *L. x eurolepis* stands have been studied after being planted on infested sites there is little information on the management of larch stands established on less infested sites.

The aim was to make preliminary investigations of the frequency of *Heterobasidion* infection in unthinned *L. eurolepis* stands compared to thinned stands established on sites originally free from infection, and if stump treatment based on these data would be feasible.

Ten first rotation stands of *Larix* spp. on former arable land in southern Sweden was studied (Table 1). Five stands, between 15 and 19 years after planting, were not thinned at all or once during winter conditions. Four of the older stands, 43 to 51 years after planting, were thinned more than once at temperatures above 5°C, and hence may have been subjected to natural spore infections by *Heterobasidion*. All stands consisted of *L. x eurolepis*, except two older stands with *L. decidua* and *L. leptolepis* respectively.

In each stand bore cores were taken at stump height from 30 randomly selected trees. Two bore cores were taken from young trees and three from old trees. On five randomly selected trees in each of three younger stands samples were also taken from lateral roots, 50 cm from the butt. The borer was sterilised between each sampling. A brush was used to clean the stem and excavated roots before boring. Bore cores were immediately put into separate plastic bags and incubated at 20°C for 7-10 days before being analysed. Cores with the presence of *Heterobasidion* conidiophores were regarded as infected.

To economically justify stump treatment with *Phlebiopsis gigantea* the decreased income due to infection by *Heterobasidion* was compared to the cost for treatment. The investigated stands served as model stands in the calculations. The stands were subjected to six thinnings with treatment in all. Three percent interest rate was used in the calculations. A rough estimate of the decreased income was derived from the price for timber and pulpwood. The cost for treatment was assumed to be the same as for *P. abies* in Sweden (5).

In three of the young stands the *Heterobasidion* infection frequency was 3 % (Table 1). Two stands were free from infection. No roots of young trees were infected. The older stands had an infection frequency between 20 % and 53 %. At the time for sampling the log volumes affected by *Heterobasidion* varied between 11.9 and 27.7 m^3 (4-12 % of total volume) in four of the older stands (Table 2). The volumes corresponding to the cost for treatment in all thinnings and at a final felling varied between 7.5 and 8.5 m^3 (Table 2).

Table 1. Stand characteristics and infection frequencies.

Stand	Tree species	Stand size (ha)	Stand age (yrs)	SI[1] (m)	Standing volume ($m^3 \cdot ha^{-1}$)	Thinning season	*Heterobasidion* infection (%)
1	*L. eurolepis*	4.3	15	G34	52	no thinning	3
2	*L. eurolepis*	3.7	16	G34	23	no thinning	0
3	*L. eurolepis*	0.5	19	G34	54	no thinning	0
4	*L. eurolepis*	0.28	19	G34	56	no thinning	3
5	*L. eurolepis*	9.79	16	G36	65	2003-02	3
6	*L. eurolepis*	1.8	54	G32	344	1996-11 2002-03	40
7	*L. eurolepis*	1.3	53	G34	229	1996-11 2002-03	53
8	*L. decidua*	1.4	51	G36	303	1994-05	20
9	*L. eurolepis*	2.1	43	G32	259	1996-03 2004-08	33
10	*L. leptolepis*	0.9	50	G30	194	unknown	47

[1]Site index measured as the height of the dominating trees at 100 years age for *P. abies*

Table 2. Volumes of infected logs in the older mature stands and the volumes corresponding to the treatment cost throughout the rotation period.

Stand	Infected volume ($m^3 \cdot ha^{-1}$)	Volume corresponding to treatment cost ($m^3 \cdot ha^{-1}$)
6	23.91	8.22
7	27.70	7.53
8	11.93	7.47
10	15.66	8.53

Larix x eurolepis may obviously become infected even if established on former non-infected sites. Since the older stands were more heavily infected than the young ones and the older stands had been thinned several times, it is tempting to conclude that thinnings made the difference. This was however not investigated. Still if compared to the young trees established on former heavily infected sites in the study by Rönnberg and Vollbrecht (3) with high frequencies of trees infected already some few years after planting, it is likely that the thinning stumps are major entry points for *Heterobasidion* as for spruce or pine. Therefore stump treatment would be recommended also in thinning of *L. x eurolepis*. According to the calculations in this study it may also be economically feasible. *Phlebiopsis gigantea* stump treatment may be effective but field experiments are lacking (4).

ACKNOWLEDGEMENTS

The study was financed by InterAgro Skog AB.

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Incidence assessment of *Heterobasidion* spp. in Italian Alps

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CONFERENCE ABSTRACT

The incidence of natural infection of *Heterobasidion* spp. was recorded in Norway spruce forests in the Eastern Italian Alps. The study interested ca. 1900 trees from 63 transects located in Trentino and Lombardy. *Picea abies* was the main species in pure and mixed stands. *Heterobasidion* species were identified in each transect by mean of dual cultures using tester isolates. The outcomes of this investigations showed that the pathogen was present in most of the 64 transects analyzed, even tough with different incidence. One or two *H. annosum* species were found per each transect and, although all three species of *H. annosum* were found in the study, the three taxa were never found in a single transect. A remarkable variability was found of infected stumps among the 65 transect with an interval ranging from 8% to 93%. The percentages of infected stump surface ranging between the transects from 5% to 60%. This study shows the recurrent and heavy presence of *Heterobasidion* species in the Alpine forests. From these preliminary data, the presence and the damages caused by this fungus appear surprisingly high. The ecological role of the *Heterobasidion* species and their economic impact are discussed on the light of the possible silvicultural actions to control the pathogen.

The fungus *Heterobasidion annosum* s.l. is considered to be the main pathogen causing severe economical damages to the conifer forest of the Boreal hemisphere (6). Currently, several methods have been developed to effectively control the pathogen. Given the costs of their use these methods are applied over a certain threshold of damage (4). The economic impact of this fungus in the Italian Alpine forests is poorly known. A survey was performed in South-Tyrol by Anselmi and Minerbi (1) where they found infections on the 24% of the investigate sites with an incidence ranging between 9.5 and over 50% and wood losses ranging between 2 and 37%.

The present study interested 1832 trees from 65 transects of Norway spruce forests in the Italian Alps. Incidence of the pathogen in the transects and extension of the infection on the stumps was recorded. The three species of *Heterobasidion* were identified by mean of tester isolates *in vitro*.

Sixty-five transect were designed in selected Norway spruce forest representative of Trentino (Eastern Italian Alps) forests. Most of the forest sites were old forest plantations, but in the last 50 years the traditional silviculture is being replaced by 'close-to-nature' silvicultural (CTNS) approaches based on scientific management practices linking biological, technical and social data to enforce ecological stability. Clear felling has not therefore been carried out in Italian Alps for several decades developing forest stands that are closer to natural ones in structure, composition and regeneration processes.

Vegetation consists in subalpine Norway spruce (*Picea abies*) stands, sometime mixed with *Pinus nigra*, *P. sylvestris*, *Abies alba* and *Fagus sylvatica* at intermediate and lower altitude, and mixed with *Larix decidua* and *Pinus* cembra at higher altitude. The average size of the transects was about 700 m^2. After delimitation of the rectangular transects clear cuttings were carried out. The following parameters were taken for every transect: the land surface, the geographical exposure, the average slope and altitude, the tree species composition.

Digital photos of all stumps were taken and analysed using software UTHSCSA *Image Tool* 2.00 for Windows (Health Science Center, University of Texas, USA). The software allowed to calculate from the stump picture the total stump surface of and the surfaces of the stump interested by rot. Number and extension of rot pockets for each stump were recorded. For each stump the X and Y transect coordinates were recorded by a portable computer through the software SURFER vers. 8 (Golden Software Inc., Golden CO, USA).

For each transect 4-5 wood samples were taken from rotted stumps to make fungal isolation. Laboratory analyses were carried out to confirm the presence of *H. annosum* s.l. and to distinguish the three close related species *H. annosum*, *H. parviporum* and *H. abietinum* as described by Korhonen and Hintikka (2). For each *Heterobasidion* species two tester strains were used.

All three species of *Heterobasidion* were found in the study. One or two *Heterobasidion* species were found per each transect. A remarkable variability was found of infected trees (IT) among the 65 transect with an interval ranging from 8% to 93%. The percentages of infected stump surface (ISS) ranging between transects from 5% to 60%. Correlation between percentage of IT and ISS in the transect showed an highly significant R^2 of 0.72 (Figure 1). This correlation suggests that are not common stands with high incidence infections and with limited spread of infection within the tree. This results may be in relation with the CTNS practices where the missing of clear felling or wide thinnings promote the infection process of few stumps by fungal spores that later may slowly spread the disease by root

connection to many other closer trees, rather than high incidence rate with smaller infections per tree.

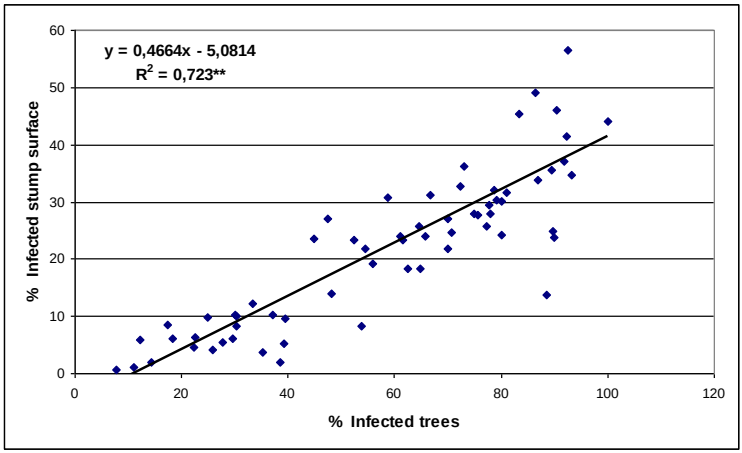

Figure 1. Correlation between % stump surface infection and % of tree infected per transect.

However, was found a descending frequency distribution of ISS classes calculated among all 1032 infected stumps out of the 1832 sampled analysed (Figure 2). That is maybe be explained with the higher susceptibility to windthrow of trees with higher infection because the loss of their root integrity. An other interesting outcome is shown in Figure 3 where the 57 % of trees shown *Heterobasidion* rot. This unexpected high incidence of the infection was mainly represented by a single pocket at the stump level. There were few stumps with 2 pockets (12%) and even less with 3 or more pockets (4%). This features may be the results of multiple infection process where several roots are colonised by the same or different genets of the fungus (5). The result of *Heterobasidion* spp. identification is shown in Figure 4. The figure outlined the association between host species composition of the transects and the presence of *Heterobasidion* species. There is a higher prevalence of the *H. parviporum* in accordance with the main host frequency, *Picea abies*, in all transect. Is interesting to point up the presence of the only *H. abietinum* in some transects were the main host is still *P. abies*, with a limited presence of other conifers including *A. alba*, but not *vice versa*. It may be related with a lower host-specificity of *H. abietinum* as it suggested by some authors (3) or to a higher virulence in Italy compared with its behaviour in central Europe.

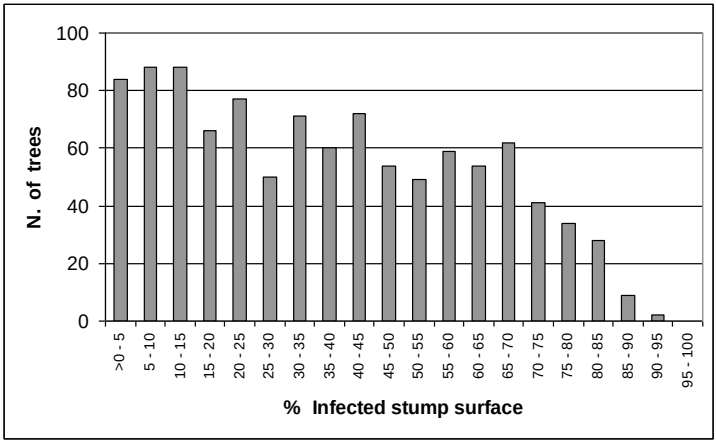

Figure 2. Frequency distribution of number of infected trees.

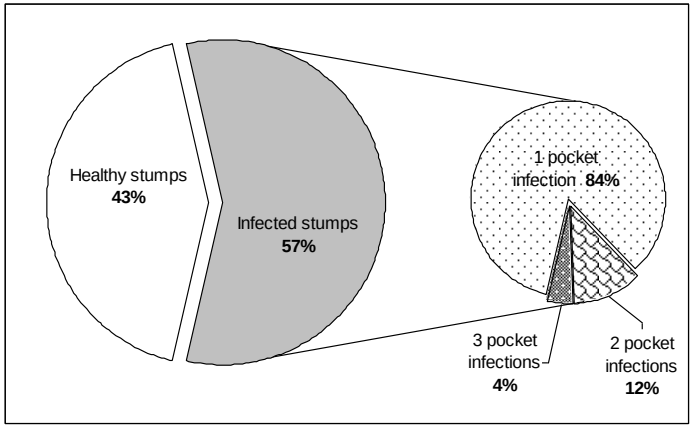

Figure 3. Percentages of healthy and infected stumps based on a total number of 1832 stumps. In the smaller pie the proportion of pocket rot numbers per stump.

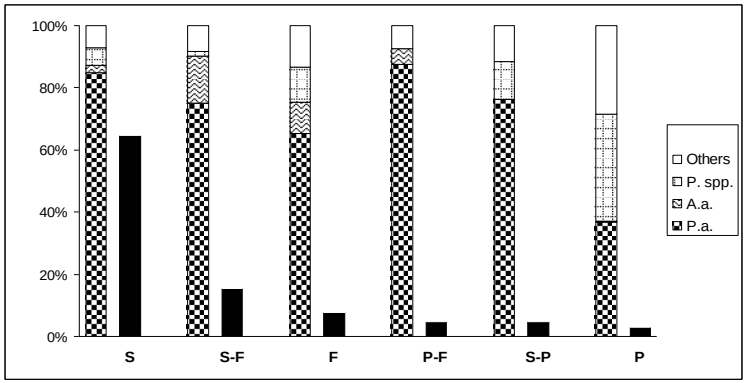

Figure 4. Black columns represent the percentage of transects with different species of *Heterobasidion*: **S** = *H. parviporum*; **F** = *H. abietinum*; **P** = *H annosum* s.s. The multilayer columns represent the average composition of different tree hosts in the transect: ***P.a.*** = *Picea abies;* ***A.a.*** = *Abies alba;* ***P.*** **spp.** = *Pinus sylvestris, P. cembra, P. nigra;* **Others** = *Larix deciduas* and *Fagus sylvatica.*

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Invasion of a North American *Heterobasidion* in central Italy and its hybridization with the European *Heterobasidion annosum sensu stricto*

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CONFERENCE ABSTRACT
Individuals of the North American P group of *Heterobasidion annosum* were recently reported in a single pine stand near Rome, in association with the movement of US troops in 1944. Here we report on some aspects of the invasion biology of this fungus in Italian coastal pinewoods, and on its interaction with the native *H. annosum sensu stricto*. Spores of *Heterobasidion* were sampled in pine stands along 280 km of coast around Rome, and characterized by nuclear and mitochondrial taxon-specific PCR primers. Spores of the exotic taxon were found over more than 100 km. The introduced taxon seems competitive with the native taxon even where the latter is well established, and has successfully invaded the dry pine stands of the region of Rome, an habitat in which *H. annosum sensu stricto* is only marginally present. Recombinant spores were found in one site, were both populations were equally represented. Hybridization was confirmed by incongruent phylogenies at three loci. This is the first report of hybridization between naturally allopatric taxa in the complex, as a consequence of anthropogenic introduction.

In 2002, it was discovered that individuals of the North American *Heterobasidion annosum* ISG P were present in a single Italian stone pine stand (*Pinus pinea* L.) in the forest of Castelporziano, near Rome (3). In the above paper, the introduction was linked to the movement of the US troops in 1944 during World War II (WWII).

In this study, we investigate some aspects of the invasion biology of this introduced fungus in Italian coastal pine woodlands on the central Tyrrhenian coast. We were particularly interested on its interaction with the native interfertile *H. annosum sensu stricto*.

The wood disk exposure method (5), as recently modified (2), was used to sample airborne spores in pure and mixed pine stands located along 280 km of coast approximately centred around Rome. In the

winter of 2005-2006, a total of 382 wood disks were exposed in 104 systematically located sampling points. Spores of *Heterobasidion* were found in 71% of the surveyed forests. 1052 single spore colonies were counted, and 582 were isolated and characterized by 3 sets of PCR primers specifically designed on the mitochondrial ribosomal operon and on the nuclear elongation factor 1-α gene to differentiate between North American (NA) and European (EU) isolates (4).

Spores of the exotic NA taxon were found in stands lying over more than 100 km of coast (Figure 1). All lines of evidence indicate the introduction occurred in the Roman area. We suggest invasion occurred at about 1.3 km yr^{-1}, through corridors provided by single trees interspaced in the landscape, and not necessarily by sizable patches of forests.

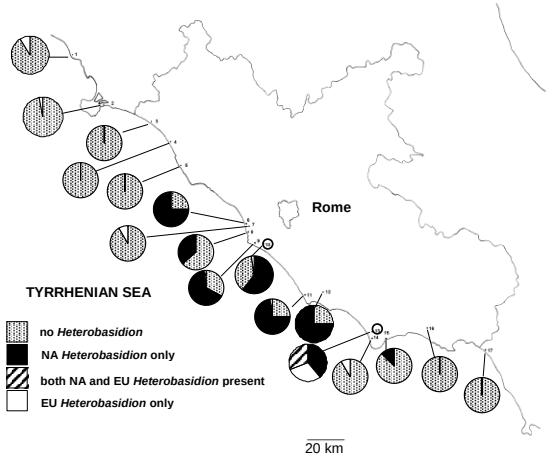

Figure 1. Spatial distribution of sampled forests. Castelporziano and Circeo National Park forests are encircled and are coded 10 and 13, respectively. Pie charts display the percentage of disks *Heterobasidion* free, or infected by the native (EU), the exotic (NA), or by both *Heterobasidion* taxa, as identified by taxon-specific PCR.

Within the current range of expansion, spore deposition rates of the NA taxon were significantly higher than those of the EU taxon in all stands, with the exception of a mixed oak-pine forest in the Circeo National Park. In this forest, located near the southernmost edge of expansion of the NA taxon, EU and NA populations were equally represented (Table 1). While deposition rate values of the native taxon differed significantly between pure dry pine stands and the mixed oak-pine forest, deposition rates of the exotic taxon were equally high in

both types of ecosystems. These results indicate that: (i) the introduced taxon is competitive with the native taxon even where the latter is well established, and (ii) the NA taxon has successfully invaded the dry pine stands of the region of Rome, an habitat in which the EU species is only marginally present.

Table 1. Mean deposition rates (spores m^2 hr^{-1}) of the native and the exotic *Heterobasidion* taxa in 17 pine stands along 280 km of Tyrrhenian coast.

site code	distance from Castelporziano (km)	Mean Depositon rates (DR)[a]	
		H. annosum sensu stricto	*H. annosum* North American P-type
1.	154 NW	0.366	0
2.	125 NW	0.122	0
3.	100 NW	0	0
4.	90 NW	0	0
5.	70 NW	0	0
6.	24 NW	0	15.171*
7.	20 NW	0.731	0†
8.	17 NW	0	3.290*
9.	7 NW	0.084	12.738*
10.	-	0.069	5.757*
11.	24 SE	0.100	13.060*
12.	32 SE	0	14.439*
13.	68 SE	21.357	42.125ns
14.	75 SE	3.290	0†
15.	79 SE	0	3.656*
16.	88 SE	0	0
17.	127 SE	0	0

[a] DR values of the native and the exotic *Heterobasidion* species differed significantly (*) or not significantly (ns) (P= 0.05; T test or χ^2 test); † not applicable, χ^2 assumptions not verified.

Discrepancy between the mitochondrial and nuclear markers occurred for about 4% of spores collected at the Circeo National Park. Most of these spores were typed as EU at the mitochondrial locus and as NA at the nuclear locus, while the opposite was true for a few individuals. These isolates were regarded as putative recombinant of the two taxa (Figure 2). In order to confirm the recombinant nature of putative recombinant isolates, three loci were sequenced for a subset of genotypes inclusive of three putative recombinants, three putative EU genotypes and three putative introduced NA genotypes. The three selected loci were the nuclear elongation factor 1-α (EFA, about 400 bp), the nuclear glyceraldehyde 3-phosphate dehydrogenase (GPD, 593 bp), and the mitochondrial ATP synthase subunit 6 (ATP, 639 bp). Phylogenetic analyses were performed on sequences from the above genotypes and a representative subset (7) of the worldwide *H. annosum* sequence dataset. Incongruent phylogenies at the three

studied loci (Figure 3) provide evidence that genetic interaction between the two taxa is occurring. Incongruent phylogenetic placement of putative recombinant genotypes was observed not only between the mitochondrial ATP and the nuclear EFA, but also between the nuclear EFA and GPD sequence datasets.

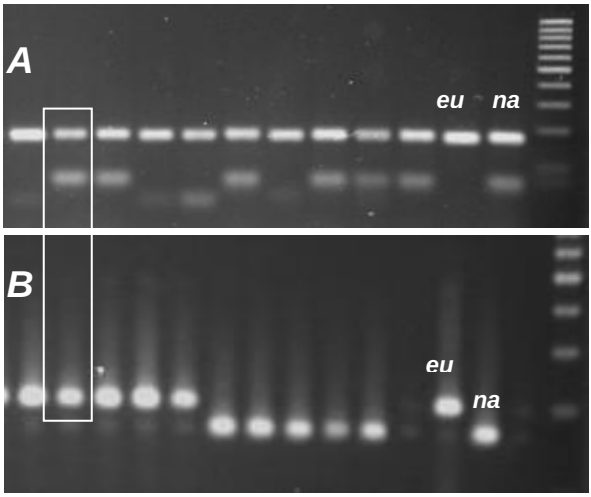

Figure 2. Example of discrepancy between identifications performed on a nuclear locus (A; elongation factor 1-α gene) and on a mitochondrial locus (B; mitochondrial ribosomal operon). Primers and PCR conditions were previously described (4). In each gel, positive controls for North American (na) and European (eu) isolates are on the right. The marked isolate (49oe) was regarded as putative recombinant between the two taxa.

Thus, the recombinant genotypes analyzed should be at least the F2 meiotic progeny of F1 NA x EU hybrids.

We believe that 4% of recombinant spores to be an underestimation of the actual hybridization rate, as, inevitably, an assay based only on two loci will miss to detect some recombinants generated by backcrossing mating events. The discovery of hybrid individuals in the only area where a seemingly recent interaction is ongoing between significant populations of both the exotic and the native taxa, may signify hybridization events may be on the rise. Considering that *in vitro* interfertility between the two taxa is almost complete (6), it is likely hybridization may be occurring in other study sites where the EU taxon is only marginally present, but at frequencies too low to be

detected at our current sampling intensity by using two or three loci only as genetic markers.

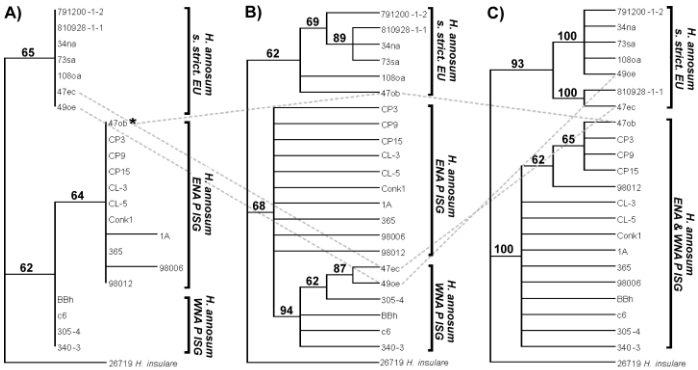

Figure 3. Maximum parsimony phylogenetic analysis of representative worldwide *H. annosum* genotypes, including putative North American (NA) x European (EU) recombinant individuals. A) mitochondrial ATP synthase subunit 6. B) elongation factor 1-α. C) glyceraldehyde 3-phosphate dehydrogenase gene. Bootstrap values are shown above branches. Dashed lines connect the same putative recombinant genotype in different trees. WNA = western North America; ENA = eastern North America; EU = Europe.

It should be noted that if the exotic organism reaches the North of Italy, where forests are significantly infested by *H. annosum sensu stricto,* hybridization may occur more frequently.

Hybrids in the complex *Heterobasidion* have been found once only, between sympatric North American taxa (1). This is the first report of hybridization between naturally allopatric taxa in the complex, as a consequence of anthropogenic introduction.

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Losses in *Quercus rubra* infected by the root pathogen *Collybia fusipes* in Southwest-Germany

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CONFERENCE ABSTRACT

North American red oak (*Quercus rubra*) has been introduced into European forestry because of its higher increment when compared with the native species *Q. robur* and *Q. petraea*. Red oak has generally quite successfully established under central European conditions. However, some premature stands of this tree species turn out to be severely infected by the root rot pathogen *Collybia fusipes* (= *Gymnopus fusipes*) and rotten roots become evident in windthrown and uprooted trees. Till now *C. fusipes* is known in Germany as a weak pathogen in old stands of the native oak species. The present study concentrates on a red oak stand in the upper Rhine valley. The following topics are investigated: a) site conditions including geology and the ground water level of infected red oak stands; b) root anatomical studies in order to follow the temporal progress of infection; c) time course of radial increment of infected and uninfected trees. The elucidation of predisposing site conditions and triggering factors like drought shall lead to recommendations for silviculture.

North American Red Oak (*Quercus rubra*) was introduced into European forestry more than a century ago because of its higher growth rate compared to the native oak species *Q. robur* and *Q. petraea*. Generally, *Q. rubra* has been successfully established under central European conditions, and it has been considered an alternative for both European oaks and beech from an economic point of view (3). Indeed this tree species covers more than 40,000 ha forest area in Germany (2).

However, there are several reports from forestry officers in Southwest Germany of decline symptoms in this tree species. A correlation of declining red oaks with carpophores of *Collybia fusipes* (syn. *Gymnopus fusipes*) was observed. Indeed previous reports from France indicate that *Q. rubra* is more sensitive to *C. fusipes* than *Q. petraea* and *Q robur* (4). Based on these observations it is important to investigate whether these findings are applicalbe for German conditions, too. In particular, the identity of the pathogen needs to be established and symptoms and the site conditions favorable for the

disease have to be investigated and described. In order to develop guidelines for forest management impact on growth rates must be considered as well.

For a detailed investigation a 45 year old red oak forest stand of ca. 40 was selected in the Upper Rhine Valley/District of Emmendingen, Baden-Wuerttemberg; altitude 170 m a.s.l. The soil profil comprises a more or less carbonate free Ah layer (pH 4.2) in the upper 15 cm and an Al-horizon down to 30 cm, and a calcareous Bt-horizon down to 70 cm. The alluvial gravel underground is strongly alcaline (pH 9.0). The ground water level oscillates at ca. 2.5 m below surface level with an absolute minimum in 1991 (-3.30 m) and a maximum in 1983 at -1.2 m. The average annual precipitation is approximately 650 mm and the mean temperature around 10°C.

In order to assess the disease incidence eight circular sample plots of 0.1 ha each were installed. The trees of each plot were investigated for disease occurrence assesed by carpophores and the specific decay symptoms in coarse roots (1). To measure the annual ring width 30 trees of defined social tree classes (from predominant to suppressed) were selected and cut. Four trees were uprooted and the loss of conducting xylem was measured in terms of portion of infected girth. In addition to its identification based on carpophors, *C. fusipes* could be regularly isolated from decaying roots displaying a typical orange color. The cultures were identified by the slow growing clamped mycelium with production of oxalate crystals and incrustation of pigment, and by an agaricoid carpophore growing on a wood specimen in sterile culture. Furthermore, the DNA-sequence of the ITS1-region proved to be identical to an isolate of *Gymnopus fusipes* in the NCBI data base.

The percentage of trees infected by *C. fusipes* in the eight sample plots fluctuated between 12.5% and 48% (mean 27%). The extent of decay in coarse roots of the uprooted trees was estimated to be 76 %, 70 %, 59 %, and 0 % respectively. The vertical root system was almost completely destroyed in the trees showing dead branches in the upper crown. When analyzing several partly destroyed roots for the first signs of infection in annual rings we traced the oldest cambial lesions to be 9 years old.

When analyzing dominant trees in terms of annual ring width at breast height it is of importance to note that growth was generally satisfying until 1995 (i.e. age 35). Afterwards the increment in trees with root rot dropped to about 0.5 mm (Figure 1). It was statistically confirmed (t-test), that the infected crop trees had a lower ring width during the last decade. The difference of the mean annual increment was 51%. Before this period these trees had shown growth rates better than the average (Figure 2).

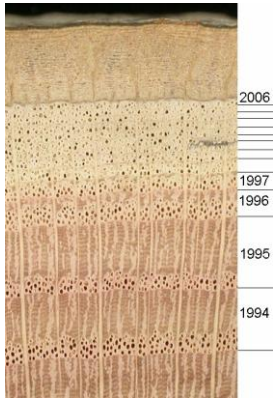

Figure 1. Cross section at breast height of a red oak infected by *Collybia fusipes*. Note the rapid decline in annual radial increment after 1995. The annual rings of the last decade before felling in 2006 are hardly discernible. (See Appendix: Color Plates)

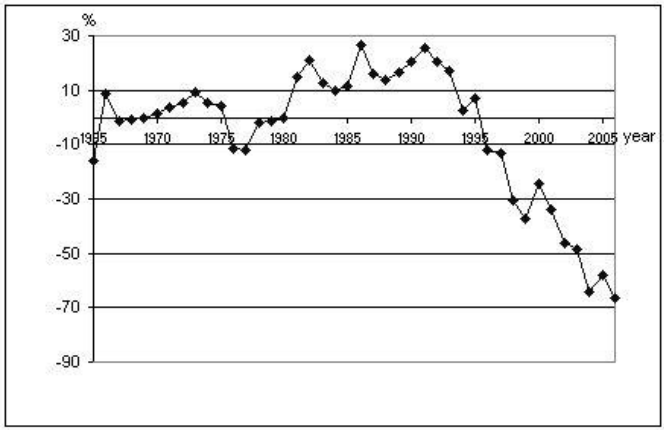

Figure 2. Annual radial increment of dominant red oaks affected by *Collybia*-root-rot compared with healthy trees (%). Note the decline of the infected trees during the last decade and their superior growth before the root system was destroyed by the infection.

It was shown that infection by *C. fusipes* in a 45 year old red oak stand was followed by a 51% decline of annual radial increment during the last decade. It must be added that many trees with rotten roots were wind-thrown before and were not considered in this study.

Remarkably, losses in growth rate of predominant trees appear later but the decline is steeper than in suppressed trees. Probably decay of a big part of the root system can be compensated under normal growth conditions.

As main stress factors which may favor the high percentage of infection and which may trigger the outbreak of the disease, we consider the following important:

i) The alkaline and calcareous soil already at 30 cm depth, which is detrimental for red oak (6).
ii) Dependency of the stand on sufficient precipitation, since the roots have no access to ground water and there is lack of ascending capillary water in the gravel. The mean annual precipitation of 650 mm may generally not be sufficient for red oak.
iii) Since the trees have reached considerable height exceeding 25 m at age 45 the damaged root system is unable to provide adequate supply of water and nutrients, especially in the dry summers of the recent past.

In summary, it can be concluded that red oak has no basis for long-term-survival as a crop tree on such forest sites given the unfavorable environmental conditions and the threat by *C. fusipes*. Red oak should be grown only in areas with higher precipitation and on non-calcareous soils.

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The importance of rhizomorphs in *Armillaria* spp.

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CONFERENCE ABSTRACT
Among the species of *Armillaria* found in lowland Europe, *Armillaria mellea* is by far the most common cause of death or decline in vigour of garden and amenity trees. However the initial symptoms of the infection are not easily detected. The above ground parts of the affected plants show signs of infection similar to those caused by other root rot disease pathogens. It is relatively easy to confirm the presence of many species of *Armillaria* because of their very distinctive network of rhizomorphs throughout the year when no fructifications are present. As well as a wide range of uninjured and apparently healthy broadleaved and coniferous trees and shrubs, an increasing list of herbaceous hosts such as iris, potato, strawberry, narcissus, bamboo, geranium and weeds such as *Rumex* species have also been found to be attacked. The study of rhizomorphs should cast more light on the epidemiology of these pathogens.

Among the species of *Armillaria* found in lowland Europe, *Armillaria mellea* is by far the most common cause of death or decline in vigour of garden and amenity trees. However the initial symptoms of the infection are not easily detected. The above ground parts of the affected plants show signs of infection similar to those caused by other root rot disease pathogens. It is relatively easy to confirm the presence of many species of *Armillaria* because of their very distinctive network of rhizomorphs throughout the year when no fructifications are present. As well as a wide range of uninjured and apparently healthy broadleaved and coniferous trees and shrubs, an increasing list of herbaceous hosts such as Iris, potato, strawberry, narcissus, bamboo, geranium and weeds such as *Rumex* species have also been found to be attacked. The study of rhizomorphs should cast more light on the epidemiology of these pathogens.

Rhizomorphs may either be flattened, associated with the sheets of white mycelium found under the bark of trees or rounded in transverse section, where they penetrate through the soil near a dead or heavily infected host. Their outer rind, cortex, is pseudosclerenchymatous, while the inner, medulla, is filled with thin-walled hyphae. The rhizomorphs develop 2.5-20 cm below the soil surface, in *Armillaria lutea* sometimes becoming more than 3 m in length growing up to

about 1 m per year, providing they are still attached to a food source. After the death of the plant the fungus may live as a saprophyte for up to thirty years before dying out.

The rhizomorphs of different species of *Armillaria* show varying degrees of branching and elasticity, so it is possible to differentiate some species by the branching patterns of their rhizomorphs (3). However the rhizomorphs used for this method of diagnosis are not dug from soil but have to be cultivated quite slowly *in vitro* in mist chambers from inoculated food base substrates. Also the mere presence of rhizomorphs is not necessarily proof of the presence of an already active *Armillaria* infection although it may indicate that one is about to be started. Even so, an extensive network of monopodial long brown/black bootlace like rhizomorphs is characteristic of the weakly virulent *A. lutea* (Figures 1 and 2), whereas the dichotomous rhizomorphs of *A. mellea* are often short, fragile and difficult to find (2).

Figure 1. (See Appendix: Color Plates)

Figure 2. (See Appendix: Color Plates)

Rhizomorphs are essentially analogous to "pipelines" (7), as the strands of white hyphae within them (Figure 3) transport nutrients from the host currently being rotted to the tips of those rhizomorphs in the network which are actively exploring their environment (6). Many become attached to and some infect fresh sites on the surfaces of root collars, roots or even leaves (Figure 4). Rhizomorphs are covered by a tough protective rind which appears to prevent other soil micro-organisms from feasting on the nutrients being transported by the complex threads of mycelium inside, or the hyphae themselves. The rind is reddish-brown when young becoming black when older or exposed to the air.

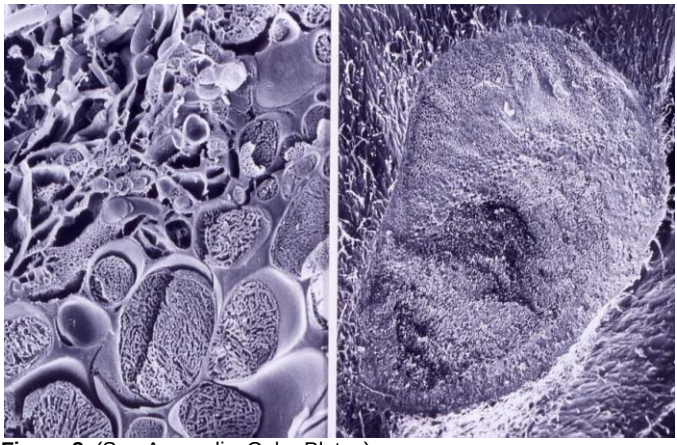

Figure 3. (See Appendix: Color Plates)

Bliss (1) reported that in culture, rhizomorphs grow best at pH 4/5, but they grow well over a wide range of type and pH of soils, from peats (pH 4) to highly alkaline sands of (pH 8). Turner (7) explained this apparent anomaly by demonstrating that now different species have been recognised, these differ in optimal temperatures and pH for mycelial growth and rhizomorph formation. Several earlier workers also observed that different *Armillaria* isolates varied in their temperature/growth relationships and other characteristics including host range.

Cultures stop growing and die soon after they are deprived of air. When carbon dioxide levels reach 30%, growth is reduced and the rhizomorphs become as flattened as those formed under the bark of infected trees. *Armillaria* cultures grow better in darkness than in light and occasionally they may glow in the same way as rotten wood packed with actively growing mycelia.

Figure 4. (See Appendix: Color Plates)

Some species of *Armillaria* rarely if ever produce as many rhizomorphs as either *A. mellea* or especially *A. lutea.* For example, *A. ostoyae* after establishing itself, effectively spreads readily from infected to healthy host roots by contact. This mode of spread can eventually result in huge patches of one genet seen in North American conifer forests (5). This is rarely the case with *A. mellea* in Europe where several genets can be found on one host. Is this likely to be the result of its reliance on dispersal by rhizomorphs. By contrast, some Australasian species (4) that attack more widely spaced hosts appear more dependant on airborne inocula.

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Distribution of *Heterobasidion annosum* inoculum in the Landes de Gascogne forest: influence of soil and silvicultural practices

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CONFERENCE ABSTRACT

In the Landes de Gascogne forest (maritime pine; one million hectares), *Heterobasidion* root disease is mainly present in the south-eastern sector. However, three surveys carried out at 10 year-intervals showed an increase of the incidence of the pathogen in the northern and south-western sectors. The disease occurs more frequently in well-drained sites than in humid and dry, sandy soils. In order to better understand the epidemiology of the disease, an experiment was carried out on the distribution of stump inoculum in the LdG forest and on the influence of soil and silvicultural practices on stump infection. *Heterobasidion annosum* was monitored on stumps of thinned trees (50/stand), which were harvested at different seasonal dates. The 48 selected stands were distributed in three different geographical areas (north, s-west, s-east) and on four different soil types. Furthermore, spore traps were placed at 20 points of a square grid in the s-west area, at different seasonal dates. *Heterobasidion annosum* was detected on stumps in the majority (87.5%) of the analyzed stands, with an incidence ranging from 2 to 48 %. Nevertheless, stump infection was less frequent when trees were harvested in autumn/winter and on dry soils. These results are discussed in relation to disease incidence and aerial inoculum trapping.

Heterobasidion annosum is among the most widespread and damaging fungal pathogens in the Landes de Gascogne (LdG) forest (south-western France), causing significant economic losses on maritime pine (*Pinus pinaster*). The LdG forest is a mostly artificial pine monoculture of about one million hectares surface, which is intensively managed for timber production. Annosum root rot is mainly present in the south-eastern sector of the forest but three surveys carried out at 10 year-intervals showed a recent increase of the incidence of the pathogen in the north and south-western sectors (1). The disease is more frequent in well-drained sites than in humid, dry, and sandy soils.

The biology of *H. annosum* is well known, especially the role of basidiospores as primary infective propagules. The freshly cut thinning

stumps are considered as the main sources of infection since the fungus usually enters a healthy stand by infecting the stump surfaces by basidiospores. In order to better understand the epidemiology of the disease, an experiment was carried out on the distribution of this first inoculum in the LdG forest and on the influence of soil and sylvicultural practices on the amount of stump infections.

The *H. annosum* colonization was assessed on 1-2-year-old stumps of thinned maritime pines (50 stumps/stand) which were harvested at different seasonal dates in 48 stands with no damage of Annosum root rot. The selected first-thinned stands were distributed in different geographical sectors of the LdG forest which showed different levels of *H. annosum* incidence, and on four different soil types (humid, well-drained, dry and sandy soils). A 5 cm-thick cross-section was cut from the top of each stump. Furthermore, spore traps consisting of fresh wood discs were placed during 24 hours at different seasonal dates at 20 points of a 4 km-square grid in a *H. annosum* free south-west sector.

Wood discs were individually wrapped in newspaper, placed in plastic bags and incubated for 14 days at 14° C before investigation for conidiophores.

H. annosum was detected in 42 out of 48 analyzed stands on a total of 11.2 % of the thinning stumps, with a stump infection rate per stand ranging from 2 to 48 %. Twenty out of 42 contaminated stands (48% of all the infected stumps) were situated in townships of the north and south-west sectors of the LdG forest where Annosum root rot had never been reported (Figure 1). In one of these stands (Vieux Boucau) the stump infection rate reached 46 %. Stump infections were significantly less frequent when trees were harvested in autumn or winter and on sandy or dry soils than when trees were cut in summer or spring and on humid or mesophyl soils (Figure 2).

These results are supported by spore trapping with wood discs. In a *H. annosum*-free area (Figure 1), 120 spores were captured by trapping during 24 h at each seasonal date, with the majority of them (66 %) found in spring.

By contrast, only 3.4 % of the spores were trapped in summer, the temperature at this date having reached 40°C. At the 20 points of the square grid, the abundance of spores on wood disks decreased from east toward West suggesting a westward spore dispersion.

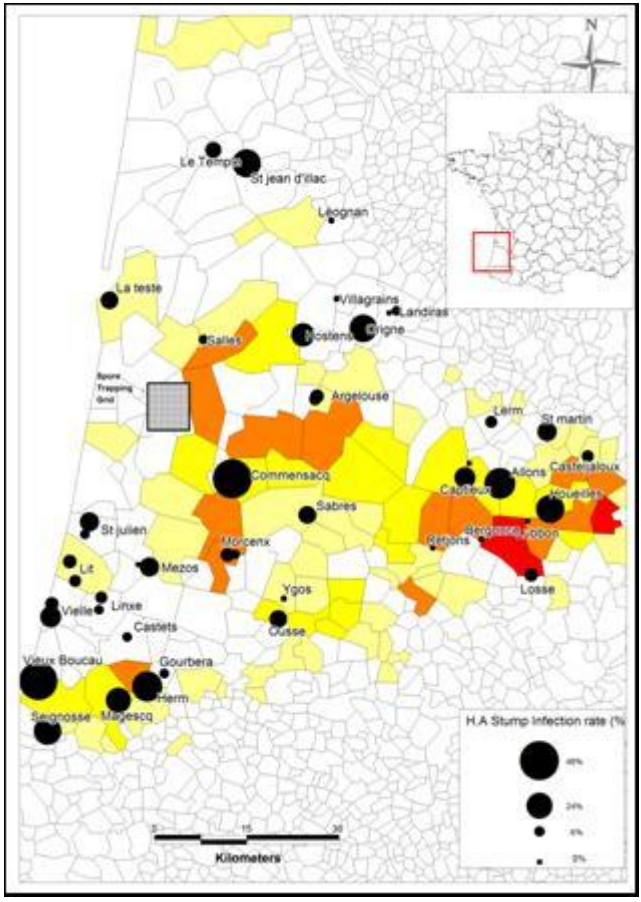

Figure 1. Localization of the 48 selected first-thinned stands with their *H. annosum* stump infection rate (size of the circles proportional to the infection rate) and distribution of Annosum root rot (A. R. R.) observed in the townships of the LdG forest (colors of the townships refer to the number of observations of A. R. R: white, no A. R. R.; pale yellow, 1 to 2; yellow, 3 to 5; orange, 6 to 9 ; red, 10 to 16). (See Appendix: Color Plates)

Our study confirms that first-thinning increases the risk of Annosum root rot in the LdG forest, even in areas where Annosum root rot is still not observed. However, this risk can be minimized by harvesting in autumn/winter, especially in humid and well drained soils.

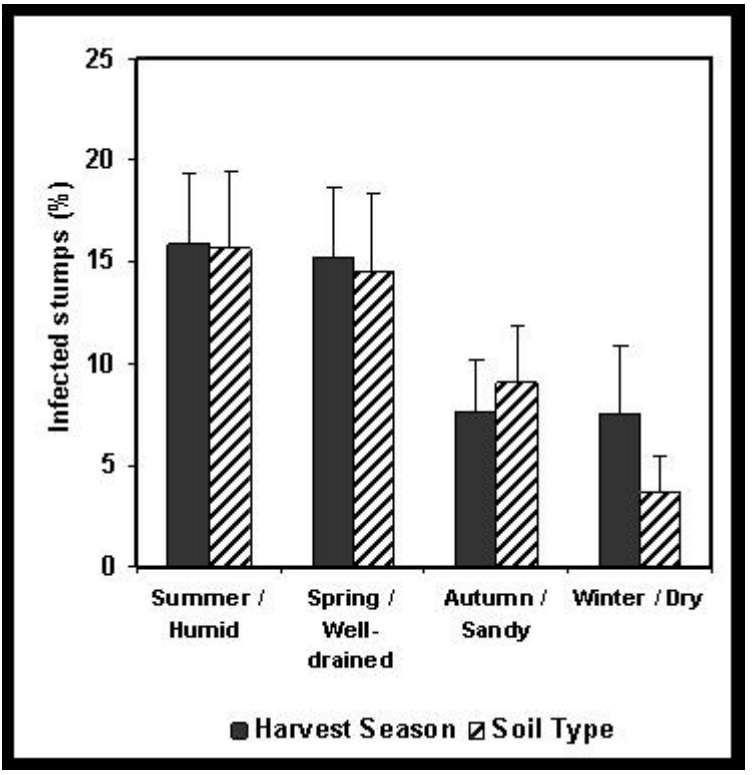

Figure 2. Percentage of stump infection by *H. annosum* after thinning at four different seasons and on four types of soil (mean and standard deviation of the 12 stands are given).

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Spread of *Heterobasidion abietinum* in natural *Abies pinsapo* ecosystems

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CONFERENCE ABSTRACT
Abies pinsapo is a protected fir species growing in natural ecosystems and suffering *Heterobasidion* root rot. In the autumn of 2005, two 50 x 50 m pure *A. pinsapo* stands (M and P) were chosen at Sierra de las Nieves Natural Park (southern Spain) to investigate spread of the pathogen. A total of 60 *A. pinsapo* trees were growing in stand M and 120 in stand P. Both stands included at least one disease focus. *Heterobasidion abietinum* was isolated from six symptomatic firs (10 %) in stand M and from 14 firs (11.7 %) in stand P. Heterokaryons obtained were confronted in all possible combinations on 1.5% MA medium and the plates examined periodically for the presence of the somatic incompatibility reaction. The low number but big size of the different genets detected, show that infection by spores may be of minor importance in disease dispersal, compared to the widening of old disease foci by direct growth of the pathogen from tree to tree through root contacts or grafts.

The objective of this work was to investigate the spread of the *H. abietinum* in *A. pinsapo* forests. Because these forests are unmanaged ecosystems, we hypothesised that the infection by spores may be of minor importance in disease dispersal, compared to the widening of old disease foci by direct growth of the pathogen from tree to tree through root contacts or grafts.

Two pure *A. pinsapo* stands (M and P) were chosen in Sierra de las Nieves Natural Park (southern Spain). The size of stand M was 50 x 50 m, with 60 *A. pinsapo* trees, and stand P was 55 x 60 m, containing 120 trees. Both stands include at least one disease focus. Before the establishment of the Natural Park in 1989, *A. pinsapo* was regularly cut for commercial use as Christmas tree, without any stump treatment. Old decayed stumps were present in both stands.

From every tree, two cores were extracted in a perpendicular way at ground level using an increment borer. Wood cores were surface sterilized, cut into small chips, plated on semi-selective medium (2) and incubated at 20º C. Each isolate obtained was checked for the presence of clamp connections. Heterokaryons obtained were

confronted in all possible combinations on 1.5% MA Petri dishes, incubated at 18-20º C for 6 – 8 weeks and examined for the presence of the somatic incompatibility reaction (3).

Somatic incompatibility reaction among isolates was generally visible as a barrier of white mycelium between paired colonies, but occasionally a zone of growth inhibition appeared.

In stand M, *H. abietinum* was isolated from six out of 60 firs sampled (10%). Each of these six trees showed disease symptoms: yellowing and wilting of needles. Three genets were identified. One of them occupied four fir trees and the largest distance between infected trees was 21.5 m. Two genets were found in one tree only.

In stand P, *H. abietinum* was isolated from 14 out of 120 firs sampled (11.7%). Four genets were detected. One genet had infected 10 trees, another two trees, and two genets were detected in one tree each. The largest distance between two infected trees was 57 m.

The size of the largest genet mapped is considerably larger than *Heterobasidion* genets recorded in fir stands earlier (1). The large size of some genets found suggests that their expansion started from aerial infections of untreated stumps generated before the Natural Park was established in 1989. More recent infections, due to later sanitary cuttings, look unlikely because the stumps were treated and wounds in standing living trees are infrequent in the stands. As these circumstances are common for the entire *A. pinsapo* forest in the Natural Park, growth of the mycelium through root contacts or grafts from tree to tree appears to be the most likely route for the spreading of *Heterobasidion* infection.

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Stem cankers of trees in dipterocarp forests: correlations with logging processes

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CONFERENCE ABSTRACT

A survey of canker incidence in dipterocarp forest was undertaken in logged and unlogged forest in Ulu Segama Forest Reserve, and on alluvial and sandstone soils in Kabili Sepilok Forest, to assess the link between mechanical damage and stem disease. Five transects were established in each forest type, and all trees of 5 – 20 cm DBH visually assessed for stem cankers and stem damage. Most sites had a low canker incidence, with a mean of 2%; the highest incidence recorded was 11%. Cankers were not consistently associated with wounds, rotten branch stubs or lianas but did appear to be associated with an absence of dead branches or scars. Cankers occurred in dipterocarps more frequently and in euphorbs less frequently than expected by chance. Incidence was similar across the forest types examined. This work suggests that canker disease is important in dipterocarp forests at a local scale, but incidence appears to be unrelated to the presence of potential infection courts.

Canker occurs on residual trees in logged dipterocarp forests of Sabah. Understanding of the impact of canker in tropical forests, however, is limited. Lack of information on incidence does not indicate disease absence in the tropics. This work examined stem canker incidence in dipterocarp forest in relation to mechanical damage, testing the hypothesis that there is no association between mechanical damage to stems and incidence or development of canker between unlogged and logged forest.

Work was conducted in March 2002 in 2 forest reserves in Sabah. Ulu Segama Forest (5°0′N, 117°30′E, 150-750 m a.s.l.) has documented histories of logging. Sites, described elsewhere (2), included conventional (CL) and reduced impact logging (RIL) areas (1). A second survey was carried out in Sepilok Kabili Forest (5°10' N, 117°56' E), a mixed dipterocarp forest on both poorly-drained, alluvial soils, and well-drained sandstone soils. Canker incidence and wounds were estimated on a min. of 100 trees, 5-20 cm DBH, in five 10 m wide, 100 m transects in each forest type, with min. of 100 m between transects. Chi square tests were used to determine if canker incidence and damage was independent of site, logging history and forest stand.

Fisher's Exact test was used to compare frequency of canker incidence.

The most common tree families were Euphorbiaceae (19%) and Dipterocarpaceae (15%); others included Anacardiaceae, Sapotaceae, Myrtaceae, Lauraceae, Ebenaceae and Meliaceae.

Median canker incidence in all forests was 2% ±0.005, with a similar incidence across sites (Table 1). The highest incidence on single a transect was 11%, in the RIL site; the most severe cankers here were on *Blumeodendron* and *Shorea multiflora*. Pooled data suggested a higher frequency of canker in dipterocarps (3.1%) than euphorbs (0.006%), compared to the full sample (Fisher's Exact tests: dipterocarps, P = 0.06; euphorbs, P = 0.05).

Table 1. Incidence (%) of cankers , wounds and scars by forest type (mean ± SE; N=5) in five study sites. Conventional logging, reduced-impact logging and unlogged forest sites in Ulu Segama Forest Reserve; alluvial and sandstone forest in Sepilok Kabili Forest Reserve. RIL = reduced impact logging site, CL = Conventional logged forest.

	Cankers	Wounds	Scars	Rotten branch stubs	Broken branches
Alluvial	2 ±0.8	15 ± 6.4[a]	50 ± 4	19 ± 2	47 ± 13
Sandstone	1 ± 0.7	6 ± 0.4[b]	34 ± 9	18 ± 5	21 ± 12
Unlogged	1 ± 0.3	8 ± 2 [b]	31 ± 10	10 ± 2	65 ± 4
RIL	4 ± 2	26 ± 4.2[a]	30 ± 11	18 ± 8	19 ± 3
CL	1 ± 0.6	21 ± 3[a]	47 ± 10	19 ± 3	54 ± 11

[a] Different letters within a column denote significant differences between sites using ANOVA followed by Tukey tests, $p \leq 0.05$. ANOVA, F = 1.83, df = 4,20, P = 0.16.

Wounded trees were less frequent in the sandstone and unlogged forest than in other sites. The proportion of trees with wounds in the alluvial site was similar to that in logged sites, possibly due to the inclusion of a transect with a high incidence of wounding. The RIL site also included one transect with high incidence of wounding.

Incidence of canker was low in dipterocarp forests, with no relationship observed between canker incidence and other variables. Mechanical injuries were common, particularly in logged sites. The complex pattern of interactions between host, pathogen and the

environment may restrict the occurrence of disease in tropical forests, due to high spatial diversity and heterogeneity.

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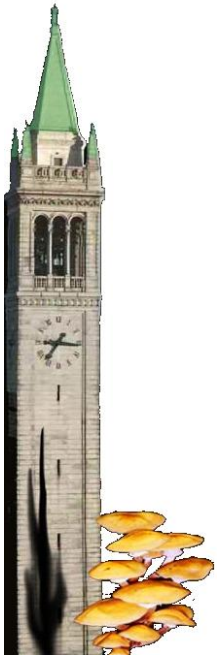
SESSION V:

ECOLOGY



An analysis of the Texas oak wilt epidemic and implications for unaffected forest ecosystems

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CONFERENCE ABSTRACT

The oak wilt fungus, *Ceratocystis fagacearum* Bretz Hunt, causes a vascular wilt of trees in the genus *Quercus*. Oak wilt was first described in 1941 as a serious disease of oaks in Wisconsin. Since then, the disease has been found only in 22 states in the U.S. Much has been learned about oak wilt, but there are still many aspects of the disease that remain obscure. Different *Quercus* spp. vary in susceptibility, symptom development, and their contributions to inoculum production for long distance spread by insects. The mechanisms for these differences have not been elucidated. The disease cycle for *C. fagacearum* has been well described, providing the basis for successful management under most conditions. The impact of the disease, however, varies throughout the range of *Quercus* in the U.S. and there are vast oak resources where the disease has yet to occur. The reasons for this variability in disease throughout the ranges of the host and pathogen can be only partially explained. Another significant gap in our knowledge is the origin of the pathogen. Limitations in our understanding of oak wilt make it difficult to predict the course of the disease into new regions. An analysis of oak wilt in the vast live oak savannahs of Texas provides a useful contrast to the disease as it occurs elsewhere, and may provide some clues to the potential behavior and impact of the pathogen in unaffected forest ecosystems.

Site conditions, fire, and root disease: *Leptographium* sp. and *Heterobasidion annosum* paradigms

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CONFERENCE ABSTRACT

Forest tree species have evolved under climatic, geological, and biological forces over eons of time. Root disease fungi, particularly root rotting Basidiomycetes are key drivers of coniferous forest ecosystems. They have co-evolved with their hosts under the pressure of these forces, and as such, are ideally in some state of equilibrium with them. Nonetheless, there are significant departures from the notion that native root disease fungi function in the same manner under many present forest conditions as they did prior to anthropogenic influences upon the forest landscape. These cases are often unpredictable and bring about significant ecological consequences. For example, fire reintroduction in fire suppressed conifer stands has unplanned pathological consequences. Issues such as decades of fire exclusion and fire reintroduction also have implications vis-à-vis *Heterobasidion annosum* root disease in *Sequoia giganteum* ecosystems. With respect to other root infecting fungi, our research showed an association of delayed mortality in longleaf pine with presence of *Leptographium terebrantis*, *L. procerum*, and other Ophiostomatoid pathogens, even after relatively cool burns. These fungal species are not considered virulent and longleaf pine is generally considered resistant to many diseases. These examples of unintended pathological consequences of management actions are a small number of many.

Forest tree species have evolved under climatic, geological, and biological forces over eons of time. Root disease fungi, particularly root rotting Basidiomycetes are key drivers of coniferous forest ecosystems. They have co-evolved with their hosts under the pressure of these forces, and as such, are ideally in some state of equilibrium with them. Nonetheless, there are significant departures from the notion that native root disease fungi function in the same manner under many present forest conditions as they did prior to anthropogenic influences upon the forest landscape. These cases are often unpredictable and bring about significant ecological consequences. For example, fire reintroduction in fire suppressed conifer stands has unplanned pathological consequences. Issues such as decades of fire exclusion and fire reintroduction also have

implications vis-à-vis *Heterobasidion annosum* root disease in *Sequoia giganteum* ecosystems. With respect to other root infecting fungi, our research showed an association of delayed mortality in longleaf pine with presence of *Leptographium terebrantis*, *Leptographium procerum*, and other Ophiostomatoid pathogens, even after relatively cool burns. These fungal species are not considered virulent and longleaf pine is generally deemed resistant to many diseases. These unintended pathological consequences arising from management actions are a small number of many.

An example of such unintended pathological consequences is found in a longleaf pine ecosystem (*Pinus palustris* Mill.). Our research on the Savannah River Site showed delayed mortality of longleaf pine following prescribed burning (Figure 1) associated with the presence of *Leptographium terebrantis*, *L. procerum*, and other Ophiostomatoid fungi in the woody roots even after relatively cool burns (3, 5). In these studies, *Leptographium* species were widespread in woody tree roots of longleaf pine in all crown symptom classes. However, when periodic annual increment (PAI) is measured with respect to symptomatic trees, a decline was statistically significant at least eight years prior to measurement in severely symptomatic trees. It is possible that decrease in PAI is an indicator of *H. annosum* impact on these trees. Why are we observing these root pathogens and associated mortality in a tree species that is adapted to fire? What are the roles of insect vectors for these fungi in this phenomenon? Notwithstanding these questions, we also found *Heterobasidion annosum* infection to be widespread on the study site, possibly indicating the Ophiostomatoid fungi are playing a secondary role in mortality.

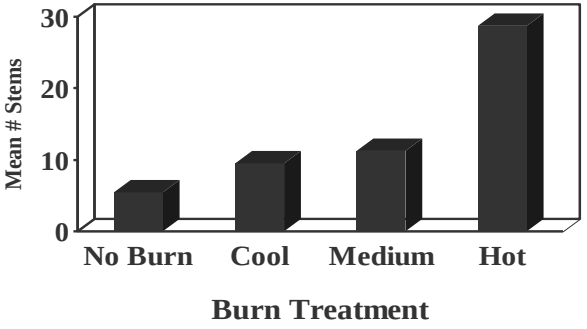

Figure 1. Longleaf pine mortality on the Savannah River Site three years after cool medium and hot intensity prescribed burn treatments.

We hypothesized many sites no longer possess specific edaphic and environmental conditions under which the species evolved because altered fire regimes, changes in soil conditions, or other factors that render trees susceptible to root pathogens. A scenario of events following prescribed burning that may explain this mortality may be the presence of fine roots in accumulated duff layers become damaged, thus weakening tree defenses. Long-standing *H. annosum* infection also continually weakens trees on such sites. This in turn predisposes roots to infection by opportunistic pathogens such as *Leptographium* sp. A slow decline syndrome is then set in motion whereby affected trees succumb to bark beetle attack or other stressors. We think opportunistic pathogens like *Leptographium* sp. are indicators of ecosystem stress or poor stand health due to various stress agents, rather than a primary cause of mortality.

On the other hand, we recently conducted (2006) root disease studies on impacted areas following the 1998 wildfires in the Osceola National Forest in Florida. We found infection by *Leptograhium* or Ophiostomatoid fungal species to be minor compared to the findings reported in studies conducted within a year following the fires. The *Leptographium* species reported by Hanula *et al.* (1) was not found in the same surviving trees. In this ecosystem, we hypothesize the slash pine and longleaf pine species are native to these sites and such pathogens, being opportunistic, do not become established in roots as they do in “off site” tree species or severely stressed ecosystems. Normal prescribed fire regimes are maintained on the Osceola National Forest sites, in contrast to less frequent fire regime in the Savannah River Site study.

Issues such as decades of fire exclusion and fire reintroduction also have implications vis-à-vis *H. annosum* root disease. The remaining stands of giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buckholz) have been subject to decades of fire exclusion in United States national parks. This allows shade tolerant true firs (*Abies* sp.) to encroach in the understory. Firs have widespread infection from the *Ha* S biological species and there is evidence for increased root rot in the sequoia through contagion of infected root contacts of sequoia with true fir (4). In a recent long-term study on fire reintroduction effects in giant sequoia stands, we found a positive correlation between white fire density in sequoia stands and incidence of *H. annosum* (Figure 2). The results await future post-treatment studies but the information gained will be valuable in preserving this tree species.

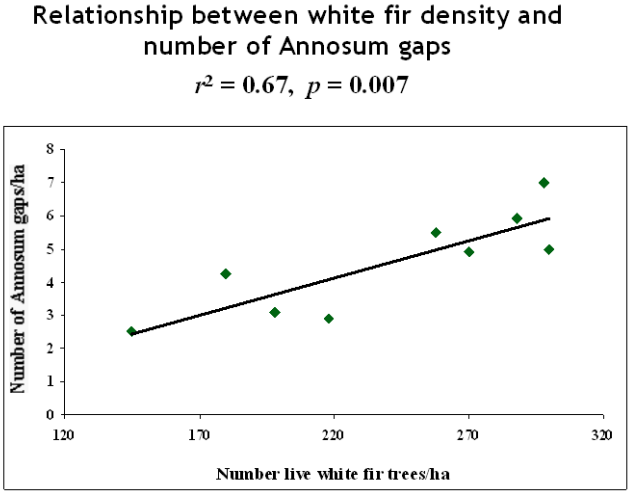

Figure 2. *H. annosum* is positively correlated with increasing white fir density in *Sequoia giganteum* stands.

Many pathological problems in forest stands arise from what we define as "exotic ecosystems", a pathologically unstable ecosystem arising from rapid edaphic and environmental changes brought about by past land use or current management practices (2). Pathological impacts or disease risk from fire, prescribed or wildfire, must be evaluated based upon a summation of factors such as tree species/site suitability, disturbances, or other stress factors. Pathological consequences can be unpredictable, but that may be a function of our incomplete knowledge of factors necessary for pathogens to affect forest stands.

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Ecology and population biology of root-rot fungi in mountain pine forests in the Alps

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CONFERENCE ABSTRACT

Annosum and Armillaria root rot are responsible for widespread mortality of mountain pine (*Pinus mugo* subsp. *uncinata*) in the Swiss National Park often creating canopy gaps in the forest. Among 42 forest gaps investigated, 31 were associated with *H. annosum* s.str. and six with *A. ostoyae.* Two other *Armillaria* species*, A. cepistipes* and *A. borealis* were also found in the study area, but seem to play only a minor role in gap formation. Somatic incompatibility tests and microsatellite analysis indicate that *A. ostoyae* formed large genets with the largest genet extending over 37 ha. In comparison, *A. borealis* and *A. cepistipes* formed significantly smaller genets. To study the impact of the root diseases on forest dynamics we assessed tree regeneration along transects running across disease centers into the adjacent forest. The mountain pine regeneration was significantly more abundant in the disease center than in the forest. In contrast, the density of Swiss stone pine (*Pinus cembra)*, a more shade-tolerant and late-successional tree species than mountain pine, did not differ between the disease centers and the adjacent forest. The incidence of root rot and mortality among the regenerating mountain pines was low, indicating that the regeneration is hardly threatened by the two pathogens. The results suggest that root-rot fungi slow down succession towards stands with a higher proportion of *P. cembra* by creating forest gaps that favor regeneration of the early-successional mountain pine.

The mountain pine (*Pinus mugo* subsp. *uncinata*) is a subalpine pioneer species that occurs in the mountains of western Europe from the Pyrenees to the Central Alps. In the Swiss National Park in the Engadine, mountain pine forests are growing at an altitude between 1800 and 2200 m a.s.l. Annosum and Armillaria root rot are responsible for widespread mortality of mountain pine in these forests often creating canopy caps in the forest (1, 2, 5). Among 42 forest gaps investigated, 31 were associated with *H. annosum* s.str. and six with *A. ostoyae*. Two other *Armillaria* species*, A. cepistipes* and *A. borealis* were also found in the study area, but seem to play only a minor role in gap formation. Somatic incompatibility tests indicate that *A. ostoyae* occurred as large genets (3). In comparison, *A. borealis* and *A. cepistipes* formed significantly smaller genets. The largest

A. ostoyae genet extended over an area of 37 ha and represents the largest *Armillaria* genet known in Europe to date. Forest gaps associated with *H. annosum* were commonly embedded within the large *Armillaria* genets. Five microsatellite markers (6) were used to genotype the *A. ostoyae* genets. All 46 isolates of the largest genet proved to have the same multilocus genotype, which was different from those of all other genets. These results confirm the delineation of the genets obtained by somatic incompatibility tests. The presence of large *A. ostoyae* genets suggest that sexual reproduction is rare, probably because of the cold and relatively dry climate in the study area.

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Comparative analyses of phenotypic and ecological traits of North American and European isolates of *Heterobasidion annosum*

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CONFERENCE ABSTRACT

Several isolates from the eastern United States, from Europe, and from the North American population introduced in Italy were inoculated on Scots pine cuttings. Significant differences in lesion size were detected amongst North American (NA) and European (EU) isolates, but not among NA isolates collected in central Italy. These results support the hypothesis that the NA population found in Italy is the result of a bottleneck associated with an introduction event during World War II. In order to compare virulence of NA and EU isolates, seedlings of loblolly pine (a US host) and of Italian stone pine (a European host) were concurrently inoculated with multiple isolates from the USA, from Europe, and of NA origin but found in central Italy. Results indicated that NA and EU isolates have comparable virulence on both hosts. Because NA isolates have been reported to be abundant in coastal pine woods where presence of the indigenous *Heterobasidion* species is limited, we have attempted to identify differences between the two provenances, other than virulence. By sampling the air for airborne basidiospores in the winter and summer months, we have found that spores of the NA taxon are present all year round, while spores of the EU species are absent in the summer season. We infer that the ability of the exotic taxon to be active all year round has given it an ecological advantage in part responsible for its spread in areas unoccupied by the native taxon.

An invasion of the North American P ISG of *Heterobasidion annosum* has been documented in coastal pine stands of Central Italy (1). An extensive sampling of approximately 280 km of coastline using spore traps indicated that the native Eurasian *Heterobasidion* species is generally rare or absent from the dry coastal pine forests (2). The rare incidence of the Eurasian species in these xeric environments was observed both inside and outside the zone of invasion, indicating the exotic species is mostly occupying a niche unoccupied by the native species. Furthermore, significant clusters of pine mortality have been observed in conjunction with the presence of the invading species,

potentially suggesting that the exotic pathogen species may be more pathogenic than the native counterpart on Italian pine trees. It is generally assumed that invasive pathogens are successful because of such increased pathogenicity in their new environment, generally explained by the lack of co-evolution between exotic pathogen and native hosts. Alternatively, the invasion may be the result of ecological traits that make the invasive species more fit than the native species in dry environments, and the observed mortality may be the result of the establishment of pathogen populations where the native pathogen has always been absent or rare. To differentiate between these two scenarios, we performed a series of experiments aimed at comparing pathogen virulence, followed by experiments aimed at determining pathogen activity by measuring sporulation potential in dry and wet periods of the year. An initial screening of 10 Eurasian, 8 North American, and 9 invasive North American isolates from Castelporziano, Italy, was performed by inoculating Scots pine cuttings, all taken from a single tree predetermined to be moderately susceptible to the pathogen. Lesion size was measured 45 days after inoculation, and results indicated that while individual isolates varied in virulence within the North American and Eurasian provenances, there was no significant difference among the three groupings. Interestingly, no differences among isolates were seen among the invasive isolates, a trait suggestive maybe of limited genetic diversity (Figure 1).

A second experiment was performed by inoculating 7 isolates (3 North American, 3 Eurasian, and 1 invasive) on 434 Italian stone pine (*Pinus pinea*) and on 443 North American loblolly pine (*P. taeda*) seedlings. After three months, lesion size (Figure 2) and frequency of successful infection indicated that North American and Eurasian genotypes had the same level of pathogenicity on both pine species, contradicting the hypothesis of higher pathogenicity levels of the exotic pathogen on the native Italian pine species. In a third experiment, spores were trapped in two forests, including the single site where both pathogen species coexist in equal numbers because of more mesic conditions, in the winter and in the summer. Results indicated that North American spores could be collected in both sites, at both times of year, without significant seasonal variation in spore loads; on the other hand, the European species could only be detected in one forest and only in winter (Figure 3). These results indicate the exotic pathogen is active throughout the year, while the native species is not active in dry conditions.

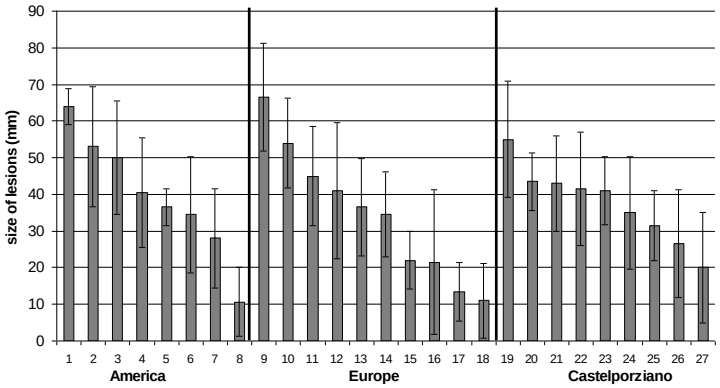

Figure 1. Lesion sizes in cuttings of a Scots pine tree 45 days after inoculation with isolates of *Heterobasidion annosum* from Europe, North America, and from the invasive North American population in Castelporziano, Italy. Each isolate was inoculated on 6 cuttings.

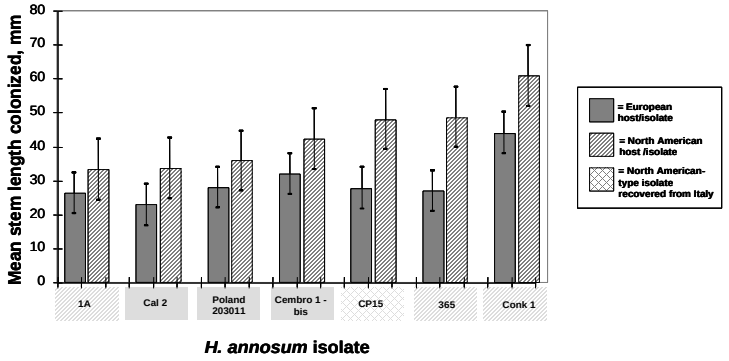

Figure 2. Lesion sizes in pine seedlings at three months after inoculation with North American (1A, Cal 2, Conk 1), European (Cembro 1-bis, Poland 203011, 365), and invasive North American isolate (CP15) in Italy. The Mediterranean pine species affected by the invasive North American species is *P. pinea*, while *P. taeda* is one of the major hosts of *Heterobasidion annosum* in North America. Isolates rank equal in their pathogenicity on both pine species, independent of provenance. Error bars are 1.96 SE.

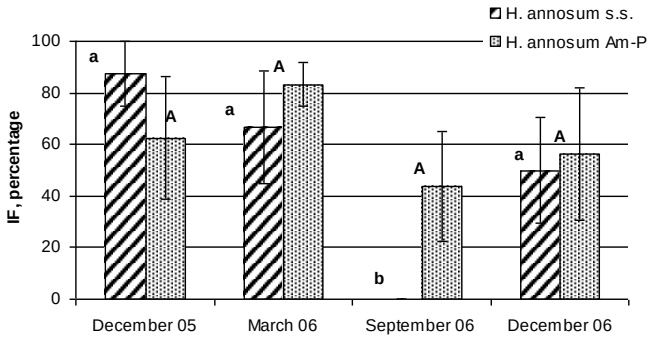

Figure 3. Frequency of spore traps (IF) in which Eurasian (*H. annosum* s.s.) and North American (*H. annosum* Am-P) basidiospores of *Heterobasidion annosum* were captured in the rainy December 2005, March and December 2006 months, and in the dry September 2006 sampling. Letters (capitalized for the North American individuals) indicate statistically significant groupings at $P<0.05$, based on chi-square tests.

We conclude that the invasion of the North American pathogen species is better explained by its ability to outcompete the native species in dry environments, than by the presence of highly susceptible hosts because of the lack of coevolution. The mortality levels observed in conjunction with the invasive species are probably due to the establishment of a root pathogen in areas that were previously devoid of a similar organism.

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Survey of potential wood decay fungi on *Eucalyptus globulus* coppice stumps in south-western Australia

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CONFERENCE ABSTRACT

In western Australia, *Eucalyptus globulus* plantations are commercially harvested and a proportion of plantations are 'coppiced' and grown as second rotation crops. Reports by managers note that coppice stumps are colonised by fungi that decay the stumps, possibly leading to frequently observed wind-throw and yield loss. A number of surveys were undertaken to determine: (1) the extent of wind-throw and/or tree losses in coppice plantations, (2) the numbers and species of fungi associated with rot in stumps of coppice stands, (3) the distribution and incidence of these fungi within the plantation estate, and (4) what environmental factors are involved in their incidence and distribution. Eighteen coppiced plantations were surveyed. Coppice plantation losses averaged 14.3 % and there were no apparent geographical trends. Fungal colonisation of stumps was high, with up to 80% of stumps colonised at one plantation. Across sites, six dominant fungal species were observed on stumps: *Trametes versicolor*, *Pycnoporus coccineus*, *Stereum hirsutum*, *S. illudens* and two as yet unidentified species (species A & species B). The incidence of the species recorded on the stumps varied depending on the plantation. Inferences and some ongoing research will be discussed.

The south-west of Western Australia (WA) has the largest area of hardwood plantations (mainly *Eucalyptus globulus*) in Australia, comprising 280,000 ha or 35% of the national total (2). *E. globulus* plantations are considered exotic to WA, as the species natural range lies in Tasmania and south-eastern Australia. Nevertheless these plantations are often surrounded by native forests dominated by trees of the same genera or family (e.g. *E. marginata*, *Corymbia callophila* and *E. diversicolor*). Plantation managers are highly aware of the potential pests and pathogens that may transfer from the natural forest onto the plantations and as a result research into potential threats has been proactive.

In the last few years, maturing first rotation plantings have been commercially harvested and between 40 to 50% of harvested sites are allowed to 'coppice'. That is, new shoots arising from 'parent' stumps are allowed to grow to produce a second rotation crop. Anecdotal reports by plantation managers have noted that some coppice plantations have suffered 'significant' losses due to wind-throw (Figure 1). The reports also noted numerous stumps being colonised by unidentified fungi that apparently decay the stumps. In other forestry environments wind-throw is often associated with internal wood decay caused by fungi. If not managed properly wood decay can cause significant economic losses (3, 4).

Figure 1. 24-month-old windthrown *E. globulus* stump, 6 main shoots were lost. Note that this tree was on the edge of the plantation. (See Appendix: Color Plates)

With this in mind, and as there is limited knowledge in Australia of the incidence, ecology and impact of wood decay fungi (1), a number of *E. globulus* coppice plantations were surveyed to determine: (a) the extent of wind-throw and tree losses in coppice plantations, (b) the numbers and species of fungi associated with stumps, (c) the distribution and incidence of these fungi within the plantation estate, and (d) what effect, if any, these fungi are having on the viability of coppice plantations.

A total of 18 coppice plantations were surveyed in five different geographic regions of south-western Australia; Boyup Brook, Collie, Manjimup, Augusta and Albany. Data collected from coppice stumps

included: height and diameter of stump, number and position of coppice shoots (living stumps only), number of dead or broken shoots, presence of fungal fruiting bodies and presence of signs of rot. Missing, dead and failed stumps were also recorded.

Stumps where windthrown shoots lead to tree loss, represented less than 1% of all stumps surveyed. Anecdotal reports of significant losses due to windthrow were possibly due to plantations managers observing losses along the edges of plantations following sporadic wind events. Overall, there was no geographical trend with regards to stump mortality. Differences in tree losses between plantations were possibly driven by particular site or harvesting conditions, rather than larger regional scale factors. First rotation stands averaged losses of 24.4%; whilst in the second rotation 14.3% of trees harvested failed to coppice successfully. However, coppice plantations were surveyed at 18-24 months after their initial harvest and further losses may be expected before the completion of the rotation.

Failed/dead stumps had a smaller average diameter (160 mm) than stumps that coppiced successfully (212 mm). Most failed stumps didn't sprout. Indicating, either host genetic, fitness or competition factors may be involved rather than fungi. Additionally, despite high fungal colonisation rates (up to 80% of stumps colonised at one plantation) an average of 85% of all stumps developed healthy coppice. From this evidence it was concluded that fungal colonization did not interfere significantly with new shoots developing. Nevertheless, in a number of cases, fungal rot was observed developing from the colonised stump into the coppice shoots (Figure 2). This has been observed in other studies (5) and could potentially threaten the productivity of the coppice rotation by causing sufficient rot to affect wood quality or lead to the breakage of coppiced stems.

Figure 2. *E. globulus* stump 24 months after harvest colonised by *S. hirsutum*. Arrows indicate where decay has entered from the stump into the coppice shoot. (See Appendix: Color Plates)

Six fungal species were commonly observed fruiting on stumps (Figure 3): *Trametes versicolor*, *Pycnoporus coccineus*, *Stereum hirsutum*, *Stereum illudens* and two, as yet, unidentified species (species A and species B). For most of the species, the incidence and frequency of fruit bodies observed varied widely, depending on the site. For example, species A was observed in only half of the 18 plantations surveyed and when present, rates of coppice stump colonisation varied from 1% in Manjimup 3 up to 70% in Manjimup 4 (Figure 4). This is suggestive of opportunistic colonisation.

Figure 3. Fungi commonly observed on *E. globulus* coppice stumps. From left to rigth and top to bottom; *Trametes versicolor*, *Stereum hirsutum*, *Stereum illudens*, *Pynoporous coccineus*, species A, species B. (See Appendix: Color Plates)

Some geographic differences were observed; *T. versicolor* was more commonly observed in the drier northern range plantations of Boyup Brook and Collie, while species A was observed more frequently in the wetter southern range plantations of Manjimup and Augusta (Figure 4). Plantations were surveyed at different times of the year, so differences may only reflect the time of survey rather than geographic or climatic differences. Alternatively, as plantations were harvested at different times of the year, differences may be due to harvest times coinciding with different fungal species dispersal times.

S. hirsutum was observed in all but one of the plantations surveyed and rates of stump colonisation (both dead and coppiced) were more consistent (Figure 4). This suggests that it can actively colonise stumps disregarding other factors such as harvest time, competition and host physiological status. Alternatively, its consistent presence may indicate that it had already colonised the trees prior to harvest.

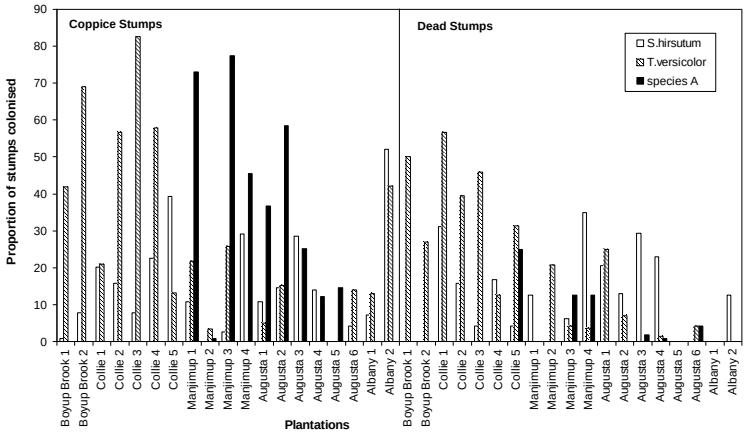

Figure 4. Proportion of stumps (coppice and dead) in second rotation *E. globulus* plantations colonised by *Stereum hirsutum, Trametes versicolor* or species A.

Most of the fungi recorded colonised both dead and coppiced stumps equally, except for species A. This species colonised a higher proportion of living stumps than dead stumps (Figure 4). Suggesting that species A is better able to colonise and decay living tissue rather than dead and therefore poses a threat to the emerging coppice shoots. Alternatively, it may only reflect the better fruiting conditions rather than preferential colonization, i.e. coppiced stumps tend to be moister than dead stumps and hence the fungus is better able to fruit on them.

Ongoing research includes: (a) testing observed fungal species for their ability to cause decay in coppice shoots, (b) examining the effect that season of harvest has on subsequent fungal colonisation of stumps, (c) monitoring stump fungi in plantations in different growing regions throughout a fruiting season.

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Distribution and ecology of *Armillaria* species in northern Spain

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CONFERENCE ABSTRACT

Armillaria root rot is one of the main root diseases affecting young forest trees in the Basque Country (northern Spain). The aim of this study was to determine the different *Armillaria* species involved and the spatial distribution of *Armillaria* in native forests in the Basque Country. During the summer and autumn months a survey was carried out for three consecutive years (2004-2006) to study the incidence of *Armillaria* on forest and grapevine plantations. A total of 228 dead and dying trees were surveyed, of which 179 presented rhizomorphs on their roots or showed typical symptoms of Armillaria root disease. A total of 78 isolates were obtained from different hosts: *Pinus* ssp., *Quercus* spp., *Fraxinus excelsior, Alnus glutinosa, Chamaecyparis lawsoniana* and *Vitis vinifera.* The isolates were identified by culture morphology, mating tests, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the intergenic spacer region (IGS) of ribosomal DNA (rDNA). Five *Armillaria* species were identified by morphological methods and the PCR-RFLP analysis detected seven restriction patterns corresponding to the same five *Armillaria* species: *A. mellea* (pattern 1 and 2), *A. ostoyae* (pattern 1 and 2), *A. cepistipes, A. gallica* and *A. tabescens.*

Armillaria root disease is one of the main root diseases affecting native and young forest trees and vineyards in the Basque Country (northern Spain). Seven *Armillaria* species have been described in Europe. Although the presence of *Armillaria* in Spain is well known, it was only recently that they were identified to species level (1). There are two bio-climatic zones that can be distinguished in the Basque Country: the Eurosiberian and the Mediterranean region. The first one, in the north area, has a meso-thermal moderate climate with high rainfall where *Pinus radiata* plantations are predominant and deciduous forests trees are present; the latter, in the south area, has a xero-phytical climate and lack of rainfall, is covered by perennial forests and is the principal vineyard area. Therefore the first objective of this study was to survey and identify the different *Armillaria* species in native forests, plantations and vineyards in the Basque Country. The second objective was to determine the geographical and spatial

distribution of the identified species and to determine any predisposing factors that encourage the development of the disease.

During the summer and autumn months surveys were carried out for three consecutive years (2004-2006) to study the incidence of *Armillaria* on native forests, plantations and grapevine plantations in the two bio-climatic areas described.

Armillaria was isolated from basidiomata, rhizomorphs or mycelium found under the bark of infected roots. All the isolates were geographically referenced, by means of GPS. Using the UTM coordinates at the environmental database supplied by one of our collaborators, IKT, S.A. (Nekazal Ikerketa eta Teknologia, S.A.), the following information was recorded for each inspected point: potential vegetation, lithology, permeability, wind exposure, slope, and micro-climate conditions.

The isolates were identified by culture morphology and diploid isolates were identified in compatibility tests with known haploid testers of six European *Armillaria* species (Figure 1).

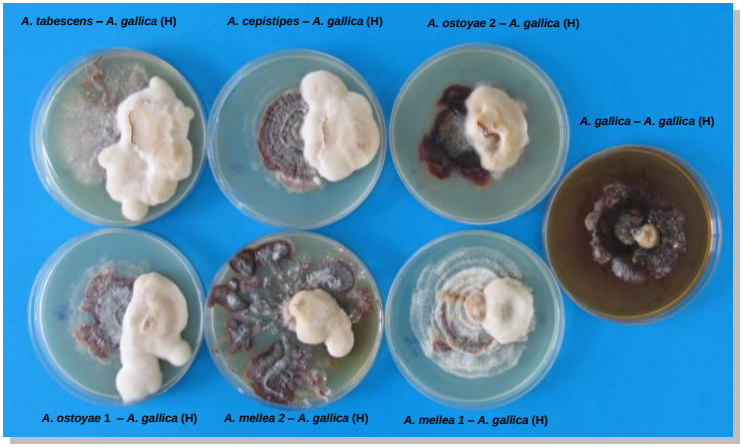

Figure 1. Pairings of a selection of diploid isolates with *A. gallica* haploid (H) (See Appendix: Color Plates).

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the intergenic spacer region (IGS) of ribosomal DNA (rDNA) was used to confirm the identification to species level (2). The restriction enzyme analysis was performed with the restriction enzymes *Alu* I, *Nde* I and *Bsm* I (3).

A total of 300 dead and dying trees were surveyed. A total of 78 isolates were obtained from different hosts: *Pinus radiata, P. nigra, P. pinaster*, *Quercus robur, Q. pirenaica, Fraxinus excelsior, Alnus glutinosa, Chamaecyparis lawsoniana* and *Vitis vinifera.* Five *Armillaria* species were identified by cultural morphology and mating tests.

The identification was confirmed by the PCR-RFLP analysis that detected seven restriction patterns corresponding to the same five *Armillaria* species: twenty-four isolates were identified as *A. mellea* (patterns 1 and 2), 45 isolates as *A. ostoyae* (patterns 1 and 2), 1 isolate as *A. cepistipes,* 7 isolates as *A. gallica* and 1 isolate as *A. tabescens.*

A. ostoyae was detected on *Pinus* spp. (*P. radiata, P. nigra* and *P. pinaster*), *A. mellea* on *P. radiata*, *Quercus* sp., *F. excelsior, C. lawsoniana* and *V. vinifera*, *A. gallica* on *A. glutinosa, P. radiata* and *Q. robur,* and the isolates of *A. cepistipes* and *A. tabescens* were found on *P. radiata* and *Q. robur* respectively (Figure 2).

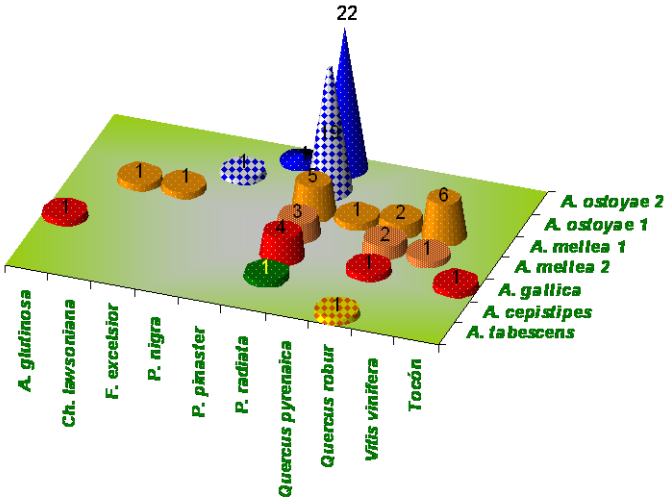

Figure 2. Frequency of species of *Armillaria* in relation to species of host. (See Appendix: Color Plates).

It has been observed, among the studied parametric variables, that rainfall and minimum temperature have stronger influence on the discrimination model among *Armillaria* species. The diversity of species of *Armillaria* decreases in the direction of the rainfall gradient

and to the area with the highest thermal oscillation where vineyard extensions are located (Figure 3).

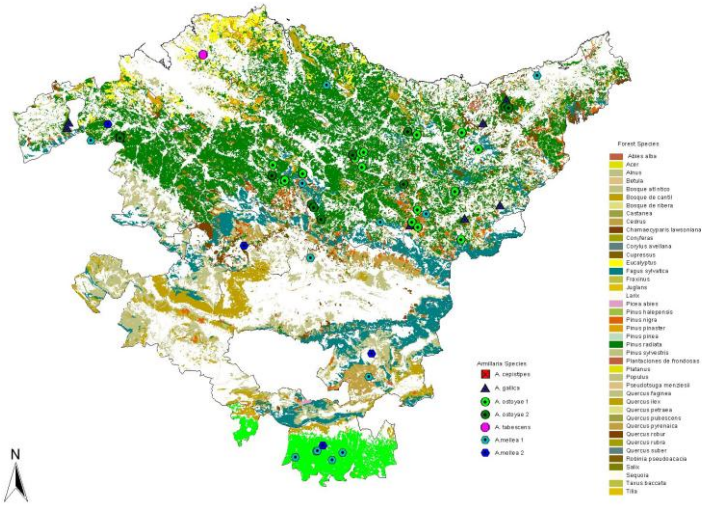

Figure 3. Distribution of forest species and detected species of *Armillaria* in the Basque Country. (See Appendix: Color Plates).

As a result of the statistical analysis of non-parametric variables, it has been observed that over 94% of the damaged areas were found in heavier soil types with low-media permeability, and wind exposure and gradient have not influence on the distribution of *Armillaria* species.

Five different *Armillaria* species have been detected in the Basque Country affecting native forest, plantations and vineyards. *A. ostoyae* is the main species causing economical losses in young *Pinus radiata* plantations and *A. mellea* on vineyards. A higher biodiversity of species has been found in the Eurosiberian region, with its Atlantic Climate, and where *Pinus radiata* has replaced native forests of acidophylous oaks. In the Mediterranean region, with its xerophytical climate and having originally been covered by alkaline oak-forests, where *Q. ilex* was predominant, only *A. mellea* was detected causing serious damages in vineyards and oaks (Figure 4).

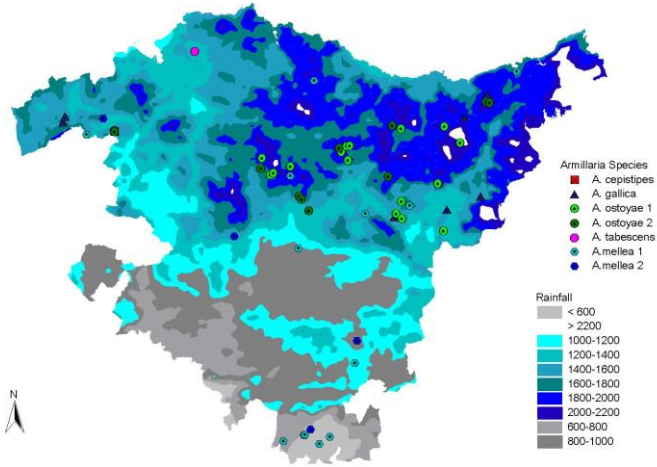

Figure 4. Distribution of rainfall levels and detected species of *Armillaria* in the Basque Country. (See Appendix: Color Plates).

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Screening a population of half-sibling interior Douglas-fir seedlings for resistance to *Armillaria ostoyae*

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CONFERENCE ABSTRACT

Interior Douglas-fir [*Pseudotsuga menziesii* var. glauca (Beissn.) Franco] seedlings from 87 maternal half-sibling families were screened in a greenhouse resistance trial to *Armillaria ostoyae* (Romagn.) Herink by inserting an inoculum source into the pots for three years. The interior Douglas-fir population is divided into four geographically distinct seed zones that represent differences in the physical and biological environment. The rate of mortality over three years and the final percent survival showed clear differences between half-sibling families (range 0-65% survival) and seed zones (range 7-25% survival). The least susceptible families originated from the drier and warmer seed zones. These trees appeared to limit the spread of the fungus especially in the first year after infection, which reduced the overall mortality at the end of the experiment. Longer-term reactions such as callus tissue occurred more frequently at lesions on tree roots from the resistant seed zones, but were not significantly different (p=0.11). Environmental temperature and moisture levels are suspected of affecting fungal inoculum and the selection of tree phenotypes able to cope with the disease in the seed zones.

Armillaria root disease causes significant growth reduction and mortality in most commercial tree species in southern British Columbia (BC) (2). Within this region, climate has been shown to strongly affect the host-pathogen relationship and disease (1, 3), while pathogen virulence has a lesser effect on disease expression (4). Little information exists concerning variation in host response to the fungus. The objective of this study was to screen an open-pollinated half-sib population of Interior Douglas-fir [*Pseudotsuga menziesii* var. glauca (Beissn.) Franco] for resistance to *Armillaria ostoyae* (Romagn.) Herink. Douglas-fir is a valuable commercial tree species used mainly for lumber, plywood and poles, and is one of the most susceptible host species to the fungus (2). The test population included families from four geographically distinct seed zones that are used in reforestation to control seed transfer. These seed zones were delineated largely on the basis of the physical and biological environment. Eighteen seedlings from each of 87 families from the four seed zones were challenged by inserting an inoculum source into

the pots next to a three-year-old seedling (Figure 1), and then monitored for three year survival.

Figure 1. An example of the Douglas-fir seedling and birch block inoculum source of *Armillaria ostoyae* used in the study. . (See Appendix: Color Plates).

The mortality after three years differed among families (range 0-56% survival) and seed zones (range 7-25% survival). The least susceptible families originated from two of the drier and warmer seed zones. Survival analysis considered seed zone, season, and time since inoculation as fixed effects and family as a random effect. All effects were significant (P<.0001), but family had a smaller effect on seedling survival than the fixed effects.

Trees from drier seed zones apparently limit fungal spread especially in the first year. Environmental temperature and moisture levels are suspected of affecting fungal inoculum and the selection of hosts that limit fungal spread at lesions. Host fitness may be more affected under drier conditions especially for slow responding hosts that cannot limit root lesions to patches instead of girdled non-functional roots. Longer-term reactions such as callus tissue occurred more frequently at lesions on tree roots from the less susceptible seed zones, but were not significantly different (*P*=0.11). Trees in all seed zones could be at increased risk to the disease if the climate warms and dries; however,

the trees in the wetter seed zones would likely be impacted to a greater degree because of the reduced host response.

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Root diseases in Forest District Wołów Scots pine stands (up to 20 years) ten years after the 1997 flood

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CONFERENCE ABSTRACT

In Wołów Forest District (south-west Poland) the results of flood in summer 1997, with water of Odra river stagnating for 3-4 weeks in forest, were disastrous. Trees were dying, weakening and overwhelmed by secondary insect pests. Scots pine plantations (under 20 yrs, established by planting), flooded and unflooded, were monitored for several years just after the flood. The flooded plantations were severely damaged, yet, *Armillaria* killed fewer trees there than in the unflooded ones. The observations were continued until 2007, in several Scots pine plantations within the same age class. Symptoms of *Armillaria* and *Heterobasidion* root rot were carefully noted and evaluated according to a scale applied previously. In the past 4 years there were no trees dying out in the flooded stands while in the unflooded ones *Heterobasidion annosum* caused considerable mortality of Scots pines. Lower intensity of root diseases in the survived flooded stands is still visible 10 years after the flood.

In summer 1997 a disastrous flood caused severe damage in Poland. The forests suffered mainly in southwest of the country. Thousands of hectares of stands were killed. Forest District Wołów, situated in the middle flow of Odra River, suffered a lot – 4.075 ha were flooded (2). Investigations of Scots pine plantations (flooded and unflooded) up to 10 years, carried out in 1998-2001 showed that *Armillaria obscura* and *Heterobasidion annosum* s.s. also suffered from the flood. *Armillaria* seemed a more aggressive pathogen and more resistant to flood but the number of trees killed was smaller in flooded stands (6). The investigations were continued in 2003-2007.

Ten plantations in Forest District Wołów were considered: 5 in stands replanted after stands damaged by flood (in 4 of them water stagnated from July 1997 till spring 1998 and in plantation 106h the water only reached the ground surface). Control plantations were found nearby, in unflooded area. All the plantations were established on suitable forest sites (Table 1). Two plots, each of 50 trees, were chosen and marked in each plantation. After finishing yearly growth, main shoot increment and number of needle sets on main and side shoots were counted in the plots (5, 6). Number of dead trees and the cause of death was evaluated in the entire plantation (8). Tukey test was applied for statistical analysis.

Table 1. Characteristics of Scots pine plantations investigated in Forest District Wołów.

Compartment	Forest site*	Age (yrs) in 2003	Area, ha	Flood status
160h	fresh coniferous mixed forest	9	0.85	flooded
134j	fresh coniferous mixed forest	9	3.1	flooded
161a	fresh coniferous mixed forest	9	1.7	flooded
161c	fresh coniferous mixed forest	9	3.94	flooded
134c	fresh coniferous mixed forest	9	3.1	flooded
166d	fresh coniferous mixed forest	11	2.71	control
222j	fresh coniferous mixed forest	12	2.14	control
224j	fresh coniferous forest	10	0.65	control
203a	fresh coniferous mixed forest	10	2.85	control
201i	fresh coniferous mixed forest	7	2.09	control

*according to the Polish forest site typology

All the trees in the investigated plantations had an increment over 30 cm (only in compartment 161c, in 2003, the average was 22 cm) (Figure 1). Average increment values of trees in all plantations increased every year. There was no significant difference between the increments in flooded and unflooded plantations (Tukey test). Average number of needle sets ranged from 2.94 to 3.4 (Figure 2) and was not significantly different, according to Tukey test, when flooded and unflooded plantations were compared.

In 4 plantations reforested after long water stagnation (134j, 161a,161c and 134c), neither dead trees nor gaps were found during the 5 years of investigation. The situation is worse in compartment 160h (water reaching the ground surface; Figure 3). In the control plantations (except for 203a, with no dead trees) root diseases were spreading slowly – no epidemics was observed. The biggest increase of dead tree number (12 pines) occurred in plantation 201i from 2006 to 2007. All the dead trees were killed by *Heterobasidion annosum* s.l.

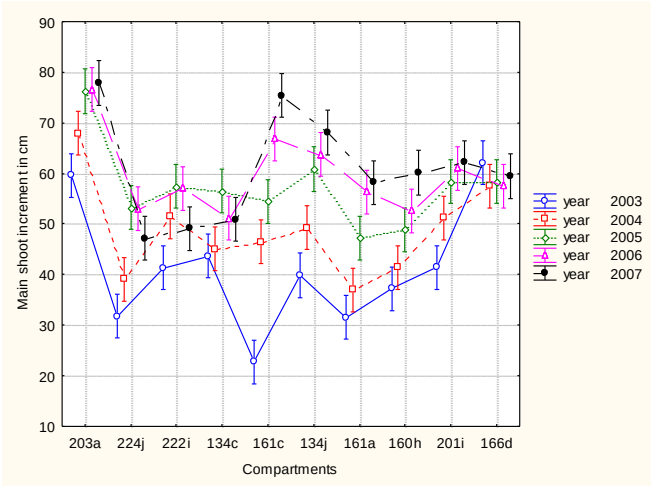

Figure 1. Average main shoot increments in Scots pine plantations (vertical rods – 0.95 confidence intervals).

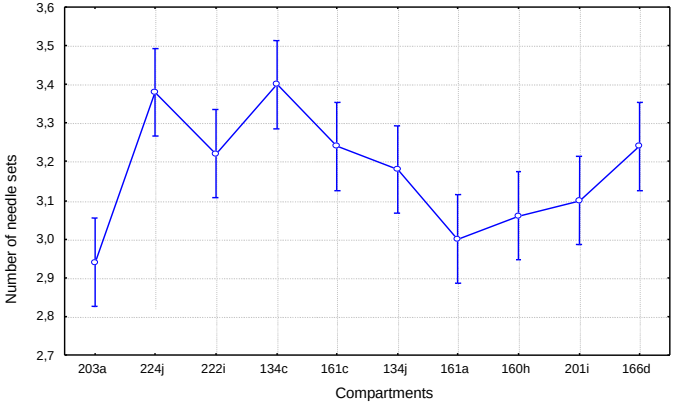

Figure 2. Average number of needle sets in Scots pine plantations (vertical rods – 0.95 confidence intervals).

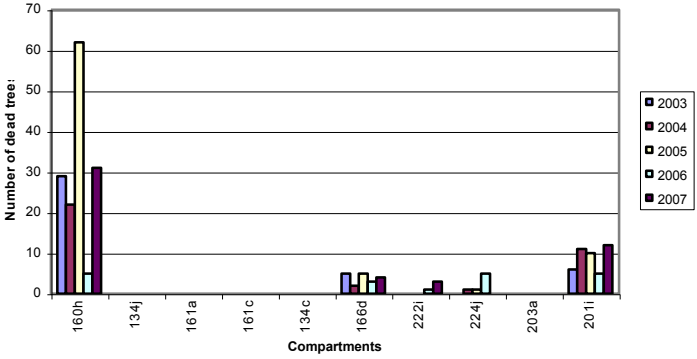

Figure 3. Number of dead trees in Scots pine plantations.

For proper image of tree health condition reliable criteria are required. Assimilation apparatus condition is a popular criterion used in Europe (3). The criterion was also applied here, in the form of needle set number assessment. No anomalies in number of needle sets were found. In all plots over 90% of pines had 3 needle sets on main shoot. In plantation 203a the average number of needle sets was 2.94 (below other average values), yet, the trees had the biggest average main shoot increment in all the years and none of them died. According to Boratyński (1) and Hanisch and Kilz (4) three sets of needles are typical of Scots pines in Poland and central Europe, yet 2 sets are most common. Smaller number of needle sets may point to some unfavourable environment changes, and quicker loss of needles can suppress physiological processes, which is of greater importance to Scots pine that to Norway spruce, as the latter is characterized by bigger number of needle sets (4).
Good main shoot increment of over 80% trees may point to their good condition, as increment decrease is considered a symptom of both *Armillaria* and *Heterobasidion* disease. It was described for *H. annosum* by Rykowski and Sierota (7).

A question to be solved is the lack of tree infestation in the 4 plantations in which water stagnated from July 1997 till spring 1998, as there is no reliable information (based on exact observations) on earlier occurrence of root pathogens there before the flood.

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Site Relationships of *Armillaria* species in Serbia

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CONFERENCE ABSTRACT

This study was performed to determine site relationships of *Armillaria* species in Serbian forests. There is not enough data, from Serbia, about behavior of *Armillaria* species in natural forests and plantations. Especially interesting is their pathogenicity and behavior of different *Armillaria* species in coniferous, broadleaf, and mixed forest stands. The study was conducted on 34 sites in 187 sampling plots. The plots were chosen to represent all forest types present in Serbia. Data about geographical distribution, hosts, forest type group and soil types, phytopathological behavior, site (altitude, aspect, slope, position) and stand conditions (diameter at dbh, number of trees, crown position) were analyzed. *Armillaria* species were found in the range between 70 and 1820 m a.s.l. Dominant species on hardwood trees were *A. gallica* and *A. mellea*, while *A. ostoyae* and *A. cepistipes* were more frequently observed on conifers. *Armillaria mellea* and *A. ostoyae* were only species found on healthy trees. Physiologically weakened or stressed trees were more attractive for *A. gallica* and butt rot was in most cases caused by *A. cepistipes*. *Armillaria mellea* caused death of trees in plantations, and *A. ostoyae* was observed more frequently in stands. Southern and western aspects were more attractive for *A. mellea* and *A. gallica*, while on sites with eastern aspect *A. ostoyae* dominated. Dominant trees were, more frequently, colonized by *A. mellea* and *A. ostoyae*.

Armillaria species can behave as primary and secondary pathogens causing root and butt rot on numerous coniferous and broadleaved trees species both in natural regenerated forests and in plantations (3). As parasites, *Armillaria* spp. can cause significant economic loss and influence the tree species composition of forests (1, 2).

This study was performed to determine site relationships of *Armillaria* species in natural forests and plantations. Influence of climate supported presence of *Armillaria* species that could be found both in central and Mediterranean part of Europe. Especially interesting is their pathogenicity and behavior of different *Armillaria* species in coniferous, broadleaf, and mixed forest stands.

The study was conducted on 34 sites in 187 sampling plots. The plots were chosen to represent all dominant forest ecosystems. Different oak associations in the plains and beech associations in mountain regions were studied. Mixed forests of broadleaved and coniferous species (beech–fir, beech–spruce, beech–fir–spruce associations)

were of special interest because of its complex host – *Armillaria* spp. interactions.

Data about forest type group and soil types, phytopathological behavior, site (altitude, aspect, slope, position) and stand conditions (diameter at dbh, number of trees, crown position) were analyzed.

Armillaria species were found in the range between 70 and 1820 m a.s.l. Dominant species on hardwood trees were *A. gallica* and *A. mellea*, while *A. ostoyae* and *A. cepistipes* were more frequently observed on conifers. *A. mellea* and *A. ostoyae* were only species found on healthy trees. Physiologically weakened or stressed trees were more attractive for *A. gallica* and butt rot was in most cases caused by *A. cepistipes*. Significant differences between presence of *Armillaria* species was found in relation with forest type groups. In spruce forest types *A. ostoyae* and *A. cepistipes* were found more frequently than expected by chance, whereas *A. mellea* was not found at all in this forest type. In spruce – fir forest types, *A. cepistipes* occurred more frequently and other *Armillaria* species much less frequently. In Hungarian oak – Turkey oak forest types, *A. gallica* and *A. mellea* were found more frequently than expected by chance.

Differences between species showed that *A. gallica* was most frequently observed in beech forest types. *A. mellea* was with *A. gallica* most abundant species in sessile oak and Hungarian oak – Turkey oak forest types.

Armillaria mellea was more frequently observed in plantation than any other species. This species was found to cause decline of trees on 61% of studied sites (Table 1). There was no significant difference in occurrence between *Armillaria* species, in natural stands (Table 2). *A. ostoyae* was more frequently found in older stands than in plantations (P =0.04).

Table 1. Relationship between presence of *Armillaria* spp. and status of cambium (alive/dead).

***Armillaria* spp.**	**Cambium**		
	live	**dead**	**p[b]**
mellea	27	17	<0.01
gallica	10	47	<0.01
cepistipes	14	9	0.06
ostoyae	9	19	0.43
Σ	60	92	
p[a]	<0.01	0.02	

p[a]-probability that there is no difference among frequencies f *Armillaria*, within columns, based on chi-squared test
p[b]- probability that there is no difference among frequencies f *Armillaria*, within rows, based on chi-squared test

Table 2. Occurence of *Armillaria* species in natural stands and plantations.

***Armillaria* spp.**	**Σ**	**stand**	**plantation**	**p^b**
mellea	44	22	22	0.12
gallica	55	42	13	0.54
cepistipes	23	21	2	0.06
ostoyae	28	24	4	0.04
Σ	150	109	41	
p^a		0.17	0.04	

p^a-probability that there is no difference among frequencies f *Armillaria*, within columns, based on chi-squared test
p^b- probability that there is no difference among frequencies f *Armillaria*, within rows, based on chi-squared test

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Occurrence of root diseases in Poland in relation to host species, forest site types, injuries of trees and crown condition

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CONFERENCE ABSTRACT

Pathogenic fungi causing root rot of forest trees were identified in Poland as most important fungal stressing agents causing forest health deterioration. Data on trees and stumps remaining in the forest provided by the specific phytopathological monitoring subprogram, developed and taken into action since 1996 were presented, accompanied by the defoliation assessment results from the period 1996-2005. Special emphasis was given to *Heterobasidion* spp. and *Armillaria* spp. appearance in relation to tree host species, forest site types and crown condition. Data sets used in the analyses contained information on over 36 thousands of trees/shrubs and almost 15 thousands of stumps of 35 species from 19 units of forest site types in stands over 40-years old across Poland. Norway spruce was found to be most severely endangered by root pathogens, which occurred most frequently on fertile and mountains forest site types. Positive and statistically significant relationship between average defoliation of trees and pathogens' abundance on plots reflected high usability of stumps as an important and complementary source of information about health status of stands.

Growing concern of defoliation assessment reliability (1, 2, 3) led in Poland in the second half of 90s to development of a concept of threat indicators of major biotic stressing agents, i.e. pathogenic fungi causing root rot of trees and insects primary defoliators. Specific phytopathological monitoring subprogram (under the biological monitoring of forests) was developed (2, 4), utilizing data both on trees and stumps remaining in the forest and taken into action since 1996. The results of this subprogram from the period 1996-2004 accompanied by the defoliation scores were analyzed to look for dependences between *Heterobasidion* and *Armillaria* root rot occurrence and tree host species, forest site types, tree injuries and crown condition parameters.

Data sets used in the analyses contained information on over 36 thousands of trees with DBH over 7 cm and almost 15 thousands of stumps of 35 species from stands over 40-years old. Data was collected during field survey performed on 820 permanent and fixed

(670 m^2) monitoring plots, located across Poland on 19 units of forest site types. The age of stands ranged between 41-164 years with the average of 72. The *Pinus sylvestris* was dominant among both trees and stumps (59% and 58% respectively), followed by *Picea abies* (12% and 17%), *Quercus* spp. (10% and 8%) and *Fagus silvatica* (8% and 6%). Injuries of trees and stump colonization by root pathogens as well as saprotrophs were identified and registered by on site visual assessment. *Heterobasidion* spp. was found on 952 stumps, while *Armillaria* spp. on 693 stumps. Injures were found on 8286 trees (over 23%). ANOVA and contingency tables analysis were applied to investigate data.

Abies alba, *Quercus* spp. and *P. abies* were found to be most susceptible species to infection by *Armillaria* (11.94%, 11.30%, 8.54% stumps colonized by *Armillaria* respectively), while *P. sylvestris* and *P. abies* by *Heterobasidion* (8.15% and 7.24%). *Armillaria* and *Heterobasidion* altogether were found most often on spruce stumps (15.78%). Norway spruce was also characterized by highest share of injured trees (40.94%), and lowest values of trees/stumps (1/40) and saprotrophs/pathogens (1/21) ratios, indicators of threat.

According to the contingency table analysis occurrence of pathogens was strongly and significantly related to species composition of stands. In the case of *Heterobasidion* spp. the share of Scots pine and Norway spruce on plots with stumps colonized by the pathogen enlarged up to 69% and 17% from 55% and 12% on the plots without this pathogen, respectively, while fir, oak, beech, birch and other species remarkably reduced their shares. Shares of spruce, fir, oak and beech raised considerably on plots with *Armillaria*, while share of pine was reduced and shares of birch and other species remained unchanged (Figure 1).

Occurrence of *Heterobasidion* spp. and *Armillaria* spp. was statistically dependent on forest site types with preferences to conifer and mixed conifer forest site types in the case of *Heterobasidion* and to much more fertile deciduous forest site types, especially in the mountain locations – as concerned *Armillaria*. It was also found that stand origin due to afforestation of farmland was found as predisposing factor of *Heterobasidion* spp. colonization of pine, spruce and oak stumps on conifer and mixed forest site types. There was no such effect of this factor on *Armillaria* spp. occurrence.

The regional variability of occurrence was high in the case of *Armillaria*, which was concentrated mostly in mountain regions in the south of the country and in areas of spruce, fir and oak appearance north-east, north and western part of Poland. However the high rates of infection by *Armillaria* spp. was accompanied by high rates of injuries of trees only in mountains on fertile sites, where spruce

admixture in the stand remarkably exceeded natural ones as well as in spruce stands outside the natural range this species in the north-west Poland. *Heterobasidion* spp. occurrence was dispersed much more evenly and was related mostly to Norway spruce in the Sudety Mountains and Scots pine in the lowland. The Carpathian Mountains were almost free from the *Heterobasidion*.

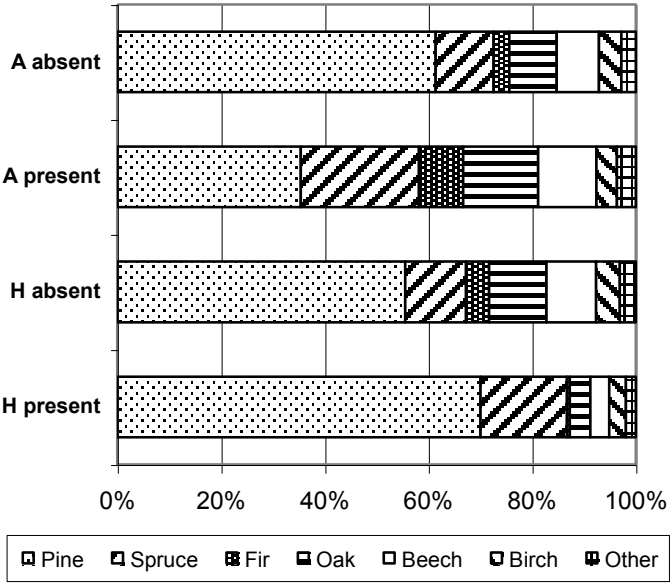

Figure 1. Species structure of stands on plots with and without stumps colonized by *Heterobasidion* spp. and *Armillaria* spp.

The dependence was also found between the intensity of root pathogens occurrence and the average defoliation score of all trees on plots (Figure 2). This corresponds to the findings that the most frequently recorded injury types concerned assimilative apparatus including lack of needles/leaves, dieback of shoots and brunches and the loss of apical dominance.

The use of stumps as a complementary source of information about threat caused to forest trees by root rotting fungi seemed very useful. It should be underline that application of stump related indicators along with tree injury data and crown condition information enables a proper assessment of fungal pathogens influence on forest health status and identification of cause-effect relationship.

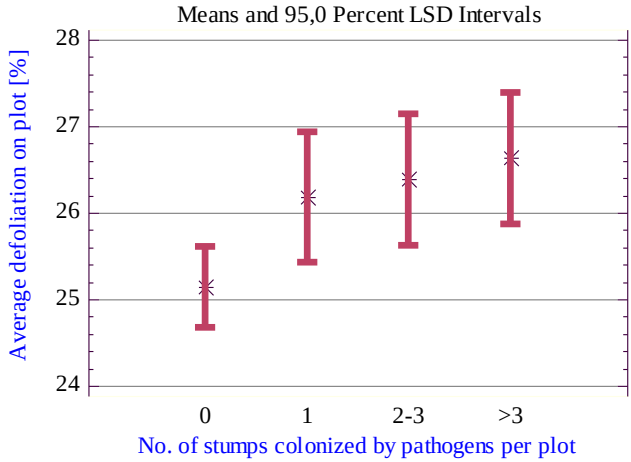

Figure 2. Relationship between abundance of root pathogens and average defoliation of trees on plots.

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Fungi associated with decayed logging wounds in *Parashorea malaanonan*

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CONFERENCE ABSTRACT

Five hymenomycete decay fungi and 24 mitosporic species were characterised from 7-yr-old wounds on *Parashorea malaanonan* in conventional (CL) or reduced impact logging (RIL) areas in Ulu Segama Forest, Sabah. Greater numbers of isolates were obtained from wounds on trees growing in CL compared to RIL areas. Fungi were most frequently isolated from basal wounds (38%) followed by mid-bole (35%) and upper-bole wounds (27%); there was no difference in number of species per wound type amongst trees sampled between the two areas. Hymenomycetes were identified as *Stereum*, *Hexagonia*, *Polyporus*, *Gleophyllum* and *Trametes* sp. Decay tests on wood blocks of *P. malaanonan*, *P. tomentella* and *Hevea brasiliensis* demonstrated that the *Polyporus* and *Gleophyllum* isolates caused greatest weight loss. *Stereum* and *Hexagonia* were the least aggressive decay species. Rate of decay appeared similar on all wood types.

Parashorea malaanonan is an important timber-producing dipterocarp in Malaysia. White rot in wounds affects up to 44% of timber in *Parashorea* within 7 years of selective logging (2), threatening sustainable harvesting, but the fungi causing decay in these forests are largely unknown. This paper compares fungal diversity in decayed wounds on *P. malaanonan* 7 years after selective logging using reduced impact (RIL) or conventional logging (CL; 1).

P. malaanonan were sampled in Ulu Segama Forest Reserve (5^{o} 0'N, 117^{o} 30'E, 150 – 750 m a.s.l.) in Sabah in January to March 2000. Ground based log extraction was carried out in 1993; some areas were logged using CL and some with RIL methods. Forty trees, DBH> 30 cm, 20 each from CL and RIL areas, were sampled along old skid trails; 5 trees were sampled in each sub-block. Sample trees had one or more visible wounds (broken top, broken branch, trunk scrape, butt-log wound or basal wounds). Wounds due to logging were distinguished from those caused by animals.

Fungi were isolated from discs taken above and below wounds. Wood chips from the healthy wood-decay interface were surface-sterilized and plated on potato dextrose agar containing 10 mg l^{-1} penicillin and

10 mg l^{-1} streptomycin. Fungi were characterized using standard methods and tested for laccase, peroxidase and ligninase activity. Fungi identified as hymenomycetes were tested for abilities to cause decay in blocks of heart- and sapwood from *P. malaanonan* and *P. tomentella*.

Five hymenomycetes (Table 1), 24 mitosporic fungi, 1 non-septate Zygomycota and 2 unknowns were isolated. Approximately 70% of isolates were from trees in RIL areas and 77% from CL; 15 (48%) were recovered from both RIL and CL areas. Isolates in RIL occurred in basal (43%), midbole (38%) and upperbole wounds (23%). In the CL area, species were more diverse in midbole (37%), compared to basal (34%) and wounds to upper parts (29%).

Table 1. Fungi isolated from wood decay originating in logging wounds on *P. malaanonan* trees sampled in reduced impact logging (RIL) and conventional logging (CL) areas in Ulu Segama Forest Reserve, Sabah, Malaysia.

Isolate Code	Fungi	Logging area/Wound position						
		RIL[1]			CL[1]			
		Basal	Mid-bole	Upper-bole	Basal	Mid-bole	Upper-bole	Enzymes[2]
RC7	*Stereum* sp.		x		x		x	Lg
RC12	*Gloeophyllum odoratum*	x		x	x	x		Lg
RC36	*Hexagonia* sp.		x			x		L Lg
RC11		x	x	x	x	x	x	L P Lg
	Polyporus sp.							
RC27	*Trametes* sp.	x	x		x	x		L P Lg

1: RIL=Reduced impact logging; CL=Conventional logging
2: Enzymes. L: laccase +ve; P: peroxidase +ve; Lg: ligninase +ve

After 48 weeks of incubation, RC11 caused the greatest weight loss in *P. malaanonan*; the least occurred with RC36 (Figure 1). For *P. tomentella,* the greatest weight loss was in blocks inoculated with isolate RC12, whilst RC7 caused the least loss.

Differences between the composition and incidence of fungi in CL and RIL areas suggest that logging methods influence wood decay development. Whether the diversity differences recorded here reflect differences in microclimate, density of damaged trees, or quantity of coarse woody debris requires further study.

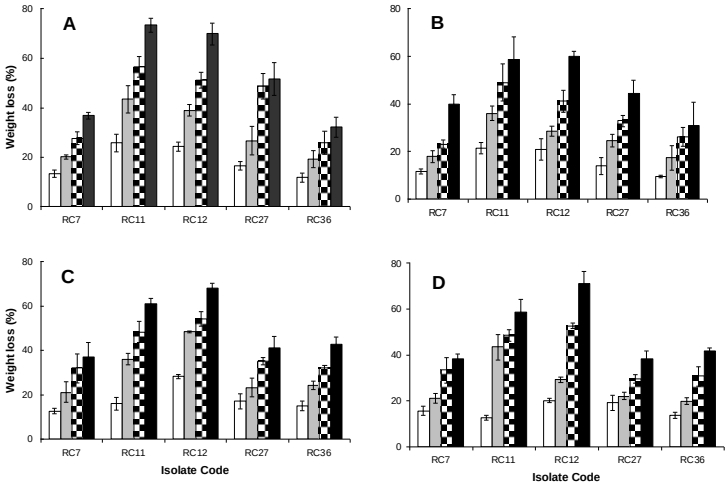

Figure 1. Weight loss in wood blocks of *P. malaanonan* and *P. tomentella* following incubation with 5 hymenomycete decay fungi isolated from decayed wounds on *P. malaanonan*. A: *P. malaanonan* sapwood; (B) *P. tomentella* sapwood; (C) *P. malaanonan* heartwood; (D) *P. tomentella* heartwood.

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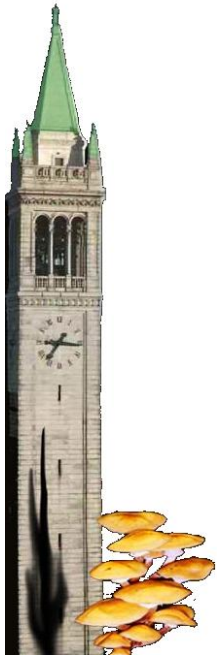
SESSION VI:

SHORT PRESENTATIONS



Specific identification of *Heterobasidion* isolates infecting *Abies pinsapo* in Spain

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CONFERENCE ABSTRACT

Heterobasidion root rot is a common disease affecting natural *Abies pinsapo* forests in southern Spain. To identify the species causing the disease, specimens of *Heterobasidion* were collected from symptomatic trees of *A. pinsapo* in seven localities in Sierra de las Nieves Natural Park and identified by mating tests. Basidiocarps were occasionally found on windthrown trees and in the internal part of decayed, broken roots or root collars. A total of 51 single-spore cultures were isolated from nine basidiocarps. The isolates were paired with homokaryotic testers belonging to the P, S and F intersterile groups of *H. annosum* s.l. When homokaryotic *Heterobasidion* isolates from *A. pinsapo* were paired with the F testers a compatible mating reaction was observed in 95% of the pairings, demonstrating that all the isolates belong to the group F of *H. annosum*, also considered as *H. abietinum* (Niemelä & Korhonen).

Abies pinsapo is a valuable and protected fir species that grows only in three Natural Parks in southern Spain. In order to assess the health status of *A. pinsapo* annually, a 1 km x 1 km grid was established in these ecosystems in 2001 (2). In field surveys carried out in *A. pinsapo* forests in 2002, a total of 81 disease foci were located. In these foci, firs were suffering from root decay causing mortality and windthrows. Fruit bodies identified as *H. annosum* s.l. were detected on windthrown trees and old stumps.

The largest *A. pinsapo* forest was investigated for the presence of basidiocarps of *Heterobasidion*. Nine basidiocarps were collected on five locations, situated 1.2 – 10 km from each other. Basidiospores were discharged on 1.5% malt extract agar in Petri dishes. A total of 51 single-spore cultures were isolated and paired with homokaryotic testers representing *H. abietinum*, *H. parviporum* and *H. annosum sensu stricto*. The Petri dishes were incubated for 4 weeks at room temperature and the occurrence of clamp connections was checked under the microscope through the bottom of the dish. Morphological changes and the formation of demarcation line between paired mycelia were noted after 6 weeks (1).

When homokaryotic isolates from *A. pinsapo* were paired with the *H. abietinum* testers a compatible mating reaction was observed in 93% of the pairings. Clamp connections appeared on the hyphae and the mycelial morphology changed, turning from the initially white colour of the colonies to beige or brown. Eventually the paired homokaryons fused into a uniform heterokaryotic colony. Isolates from *A. pinsapo* were totally incompatible with the *H. annosum* s.s. testers. They were partially compatible with the *H. parviporum* testers at low frequency (8.3 %).

According to these results, all the isolates collected from *A. pinsapo* belong to the species *H. abietinum* (3). Their degree of sexual compatibility with *H. parviporum* seems to be significantly lower than observed for other European populations of *H. abietinum*. A low degree of sexual compatibility has been observed also between *H. abietinum* and *H. annosum* s.s. (1). Our *H. annosum* testers did not react with homokaryons of *H. abietinum* from *A. pinsapo*.

Abies pinsapo forests are unique in Europe. They occupy a reduced and exceptionally wet area and have developed largely as an isolated community surrounded by the sclerophyllous Mediterranean forest typical of southern Spain. It is likely that also the local population of *H. abietinum* has been isolated from other populations of this fungus. Consequently, the relationship between *A. pinsapo* and *H. abietinum* could constitute an interesting case of study.

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Interaction effects between different species of *Armillaria* and *Heterobasidion*

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CONFERENCE ABSTRACT
Armillaria fruit bodies and rhizomorphs were collected systematically from ca. 40 forests in different parts of Trentino Province, Italian Alps. Pure cultures were isolated from 187 specimens and identified with the aid of mating tests. The most frequent species was *A. cepistipes* (Ac; 61%), followed by *A. ostoyae* (Ao; 26 %), *A. gallica* (Ag; 7.5%), *A. mellea* (Am; 4.8%) and *A. borealis* (Ab; 0.5%). Among rhizomorph samples collected from stumps and other woody material occurring in the forest the frequency of *A. cepistipes* was 85%. The 60% of all samples were collected on Norway spruce forests. The frequence of different *Armillaria* species in pure spruce forest were: *A. cepistipes* 66.2%, *A. ostoyae* 22.6%, *A. gallica* 6.8%, *A. mellea* 3.8% and *A. borealis* 0.8%. *Armillaria cepistipes* was found in 89 % of these kind of forests, *A. ostoyae* in 46 %, *A. gallica* 18 %, *A. mellea* and *A. borealis* in 4%. The interaction of *Armillaria* species Ab, Ac, Ag, Am, and Am with *H. annosum s.str., H. parviporum* and *H. abietinum* was investigated on agar medium and in small logs of Norway spruce in the laboratory. As a rule, the *Heterobasidion* species were stronger on agar medium and grew over *Armillaria*. The ability of different *Armillaria* species to resist the aggressiveness of *Heterobasidion* decreased in the order Ab and Am > Ac > Ag > Ao.

The common occurrence of butt rot reduces considerably the economical value of Norway spruce in Trentino Province of Italy. The butt rot is caused mainly by *Heterobasidion* species, but also other fungi take part in it, species of *Armillaria* in particular.

Armillaria fruit bodies and rhizomorphs were collected systematically from ca. 40 forests in different parts of Trentino Province, Italian Alps. Pure cultures were isolated and identified with the aid of mating tests. Five *Armillaria* species were identified in Trentino, in order of frequencies: *A. cepistipes*, *A. ostoyae*, *A. gallica*, *A. mellea* and *A. borealis*.

The interaction of the previous five *Armillaria* species with *Heterobasidion* species, *H. annosum s.str., H. parviporum* and *H.*

abietinum, was investigated on agar medium. Four diploid isolates from each of the five *Armillaria* species were compared with four heterokaryons of each of the three *Heterobasidion* species *H. annosum s.str., H. parviporum* and *H. abietinum*. Total number of confrontations was thus 240. The confrontations were carried out on malt extract agar containing 2% of Bacto Malt Extract (Difco). After the mycelia met each other, the dishes were observed ca. twice a week and the position of the confrontation was marked. The incubation time was 38 days from the inoculation of *Heterobasidion*. At the end of the experiment the resistance of *Armillaria* was scored by estimating the percent area of the *Armillaria* colony covered by *Heterobasidion.*

As a rule, *Heterobasidion* was distinctly the stronger partner in the confrontations and tended to grow over the *Armillaria* colony. The rate of growth of *Heterobasidion* over *Armillaria* varied from zero to total overgrowth of the *Armillaria* colony. The ability of *Armillaria* to resist the overgrowth by *Heterobasidion* was strain dependent within one species, but there were differences also between the species. *A. borealis, A. mellea* and *A. cepistipes* were relatively resistant species, whereas *A. gallica* and *A. ostoyae* were distinctly weaker (Figure 1). *A. ostoyae*, in particular, was often totally overgrown by *Heterobasidion*. As regards the species of *Heterobasidion*, *H. abietinum* seemed to be a little weaker species than *H. annosum* and *H. parviporum* except in the case of *A. ostoyae* (Table 1). From the point of view of forestry, *A. cepistipes* is perhaps the most acceptable *Armillaria* species in spruce forests. It is a weak pathogen, it produces a dense network of rhizomorphs that quickly colonises roots of stumps competing with *Heterobasidion* and restricting its spreading in the root system. *A. cepistipes* may also compete with its more pathogenic relative, *A. ostoyae* (2). As a pathogen *A. cepistipes* attacks weakened trees only. It causes some butt rot of spruce, but considering the common occurrence of the fungus the amount of damage is small (1). Moreover, *A. cepistipes* is appreciated as an edible mushroom.

Table 1. The percentage of antagonistic reactions in confrontations between the species of *Armillaria* and *Heterobasidion.*

	H. annosum	*H. parviporum*	*H. abietinum*
A. borealis	5	17	25
A. cepistipes	5	8	25
A. gallica	5	0	0
A. mellea	10	17	19
A. ostoyae	0	0	0

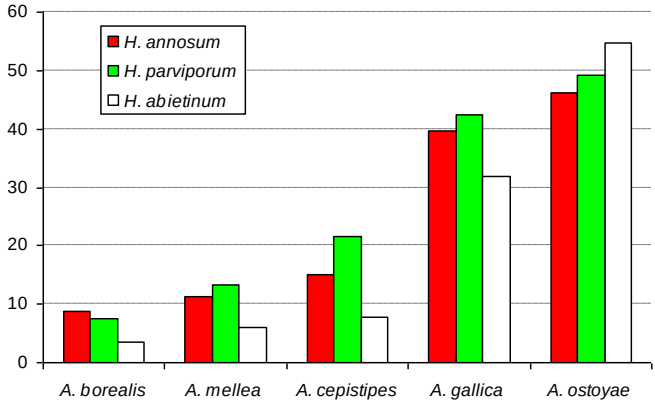

Figure 1. Resistance of five species of *Armillaria* against three species of *Heterobasidion* on malt extract agar medium. The vertical axis indicates the percentage area of *Armillaria* colony that was overgrown by three species of *Heterobasidion* at the end of the experiment.

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Early infection of *Fagus sylvatica* by *Heterobasidion annosum sensu stricto*

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CONFERENCE ABSTRACT

The common infection of *Fagus sylvatica* by *Heterobasidion annosum* has been observed in several Forest District in north-west Poland since 2005. *Fagus sylvatica* was planted as a understory or the next generation in Scots pine stands totally damaged by *Heterobasidion annosum*. Pathogen was present at least in 60% of old pine stumps in observed stands. In the most cases the first visible symptom of *H. annosum* s.s. infection was sporocarp appeared around the root collar of trees with green leaves. Other observed symptoms: leaves atrophy, chlorosis and wilting. *Heterobasidion annosum* s.s. killed from 3% to even 13% beeches per year, but the number of infected trees without symptoms could be 3%-10% bigger. The youngest beech (4-year-old) died one or two years after planting and the infection (on the base of analysis of annual increment) might be carried in the planting year. The oldest infected beech was 17-year-old. Most of dying or dead trees decreased wood increment a year before (average 51%) death. Only 24% trees decreased increment two years before death, 22% three years before and 2% four years. In contrast increment of healthy trees has increasing tendency from year to year with small fluctuations in some cases.

Heterobasidion annosum sensu stricto causes the most important damages in Scots pine stands growing on the post agricultural lands in Poland. The routine action in pine stands infested by *H. annosum*, which lost the stability, is the conversion of stand management way, which includes also the species conversion. In many cases the *Fagus sylvatica* is considered for this purpose as a resistant species for *H. annosum* infection especially in young age. Moreover there were no data of damages caused by this pathogen in *F. sylvatica* stands. This is the first notice of common *F. sylvatica* infection in a young generation.

Seven stands were investigated and assessment of *H. annosum* s.s. infection was done. In those stands seedling of *F. sylvatica* were planted as understory of severely infected *Pinus sylvestris* stands. There were improved about 6000 beech seedlings per ha.

Symptoms: in the most cases the first visible symptom of *H. annosum* s.s. infection was sporocarp (Figure 1) appeared around the root collar of trees with green leaves. Other symptoms observed were leaves chlorosis, atrophy or/ and delicate yellowish of leaves and wilting of leaves of infected trees especially during dry period. In these cases leaves became grayish.

Figure 1. *Heterobasidion annosum* s.s. sporocarp around root collar of beech with still green leaves. (See Appendix: Color Plates).

Disease: in stands, where seedlings were planted on whole area, *H. annosum* s.s. killed 17%-90% beeches in comparison to all dead trees. *Heterobasidion annosum* s.s. killed 1% - 13% beeches in whole stands. The youngest beech (4-year-old) died one or two years after planting and the infection (on the base of analysis of annual increment) might be carried in the planting year. In most cases dying or dead trees decreased wood increment a year before (average 51%) death. Only 24% trees decreased increment two years before death, 22% three years before and 2% four years. Only in one case beech died suddenly and the annual increment was bigger every year. In contrast increment of healthy trees had increasing tendency from year to year with small fluctuations in some cases (Figure 2).

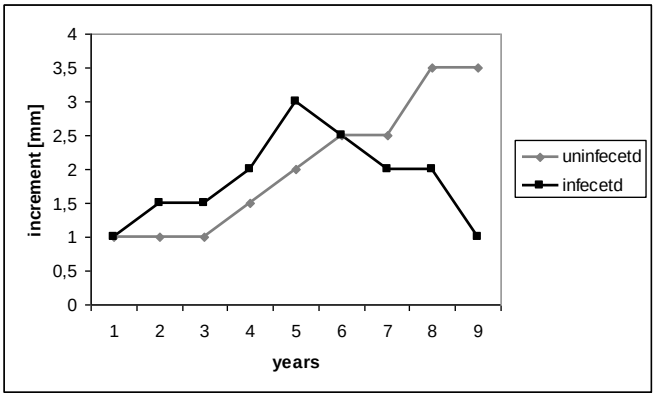

Figure 2. Increment of wood of healthy and infected beech.

This study is the first record of common infection of young *F. sylvatica* by *H. annosum* s.s. Some of earlier records of *Heterobasidion annosum sensu lato* on *F. sylvatica* originated from stumps. None has observed such infection of beeches on such a big area. Now the incidence of *H. annosum* in *F. sylvatica* plantations has been confirmed in other six Forest Districts in Poland. Common statement of the main role of mice and frost of *F. sylvatica* dying in plantation has not encouraged to confirm these facts in forests by forest service. Probably the main reason of such common infection of *Fagus sylvatica* trees could be an extremely heavily infection of pine stumps in previous stand (80-100%). The dry period, which appeared in last few years in Poland could also influenced on a low resistance of *F. sylvatica*. However the analysis of yearly increment of wood of attacked trees showed that disease development carried out in each vegetable season without increase in dry period. There is a need to continue this study and calculate the impact of *H. annosum* s.s. on beech population in conversed stands and the relation between pine stand infestation and beeches infection by pathogen.

Root deformation in young Scots pine stands threatened by root diseases

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CONFERENCE ABSTRACT

Observations referring to the discussed problems were carried out in the years 2005-2006 on the area of Experimental Forest District Zielonka, Experimental Forest District Siemianice, Forest District Złotów and Forest District Szczecinek. The main objective of the studies was to check in what degree, some faults in the planting of Scots pine (up to 20yrs) exerted an effect on pine susceptibility to root infection by pathogens on the area of Experimental Forest District Zielonka. For the sake of comparison, experimental material was collected from Experimental Forest District Siemianice (from plants originating from container nursery) and from Forest District Złotów and Forest District Szczecinek. The work was divided into field studies, laboratory analyses and cameral studies. The field work consisted in the sampling of infected roots. The laboratory work included fungi isolation from the sampled roots and the identification of the range of root deformation. Cameral work concentrated on the elaboration of results from the collected observations on the basis of a 7-degree classification scale of common pine root deformation created and elaborated by the authors to study the problem. Out of 301 sample roots 281 suffered from deformations what makes 93% of the whole material. Type 2 was represented mostly. From the sampled roots, mainly *Armillaria ostoyae* was isolated, while *Heterobasidion annosum* was identified in a lesser degree.

Deformation of root system because of their flattening, coiling or damage during planting is one of the factors contributing to their later predisposition to diseases, particularly in the case of weather abnormalities occurrence (drought, low temperatures). This defect may refer even to as many as 70% of trees which died in within 10 years after planting (1). The problem is still actual, however, the state of knowledge connected with this phenomenon is still not thoroughly investigated and there is still not enough attention devoted to this problem. In result of the stress caused by the managed tree stands, actually the most important infections diseases of root system are diseases caused by fungi *Heterobasidion annosum* (Fr.) Bref. and *Armillaria* spp. (Fr.: Fr.) Staude.

The objective of this work was determination of the deformation degree of pine roots in young Scots pine stands infected by root

pathogens. Observations referring to the discussed problem were carried out in 2005-2007 in the Experimental Forest District Zielonka, and in order to compare and verify the phenomenon, studies were also carried out in Experimental Forest District Siemianice, Forest District Szczecinek and Forest District Złotów. Observations were carried out on an especially created basis of studies using a seven-degree scale evaluating root damages (Table 1).

Table 1. Degree of root deformation.

Degree of deformation	Deformation type
1	Without a visible main root, with two lateral branches
2	Without a visible main root, with numerous lateral branches (more than two)
3	With a visible main root, vertically bent
4	With a visible main root, laterally bent
5	With a visible main root, coiled
6	With a visible main root, bent with bent lateral
7	Regularly shaped (no damages)

Among 301 root samples, 93% consisted of deformed roots. The greatest number of roots presented the second degree of deformation, 61 infected trees in Forest District Zielonka (Figure 1); 76% in the other studied Forest District, and 40% of control trees. In the remaining degrees of deformation, the number of roots did not exceed 10%, ranging from 4% in degree 1 and 3, to 8% in degrees 4, 5 and 7. In 8% of infected trees the root system was not deformed. Control trees, i.e. those which were not infected by root pathogens, were characterized by less deformed root systems. There were 40% of roots deformed in degree 2, while 22% did not show any deformations. On comparative areas in other Forest Districts from which only infected roots were taken, the situation was very similar. The greatest number of infected trees had roots deformed in degree 2, making up to over 70%.

Basing on the results obtained, one can infer that a very high percentage of trees have very deformation of root system favoring infection by root pathogens and this factor is particularly important when whether anomalies occur. The situation remains under the effect of the method of planting and the preparation of soil. In Poland, the most common method is ploughing in furrows and plantation into a hole made by planting peg.

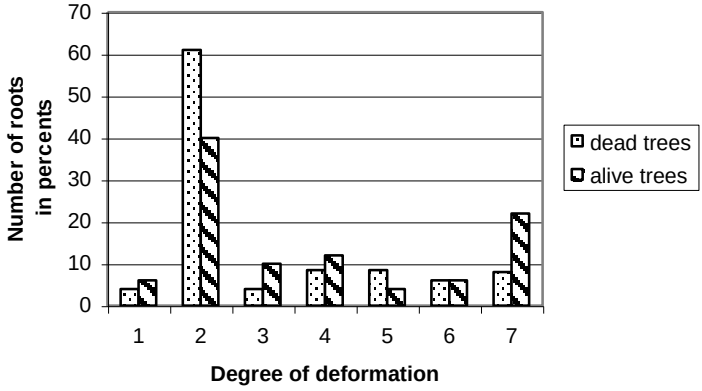

Figure 1. Degree of root deformation in Experimental Forest District Zielonka.

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Evolutionary relationships of a glutathione-S-transferase gene from different intersterility groups of the root-rot fungus *Heterobasidion annosum* s.l.

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CONFERENCE ABSTRACT

The root-rot fungus *H. annosum* (s.l.) species complex consists of several species and intersterility (IS) groups with different host preferences and overlapping geographic distribution. The IS groups are called S, F and P based on their main host specificity: spruce, fir and pine. Five putative pathogenicity genes have been partly sequenced from 100 isolates originating from the 5 populations. Two of them encode glutathione-S-transferases that are multifunctional enzymes involved in conjugating xenobiotics to a water soluble substrate. The sequences for one GST were divided into groups based on the constructed phylogenetic tree. Various calculations and statistical tests such as the McDonald-Kreitman test and Fisher's exact test were done. The highest number of non-synonymous substitutions is found within the European F, European P and North American S group and in comparison between the European P and the North American S as well as between the North American P and North American S groups. The highest number of fixed mutations is found in the comparison between the North American S and North American P groups. The results show sign of positive selection in the GST gene between the European F and North American S and between the European F and North American P groups possibly explaining their host specialisation.

Heterobasidion annosum sensu lato (s.l.) is the economically most important pathogen in the northern hemisphere where it causes root and butt rot in conifers. The basidiomycete *H. annosum* (s.l.) species complex consists of several biological species and intersterility groups with different host specialisation and overlapping geographic distribution. The intersterility groups are called S, F and P based on their main host specificity: spruce, fir and pine. In Europe, the P group infects *Pinus sylvestris*, other conifers and broad leaved trees, the S group is found on *Picea abies* and *Abies sibirica*, and the host of the F group is *Abies alba*. In North America, the P group is found on *Pinus* species and the S group on *Abies*, *Tsuga*, *Picea*, *Pseudotsuga* and *Sequoiadendron* (3).

The hypothesis is that genes responsible for the differences in host preferences between the groups will be under positive selection. The

objective of this study was to investigate if a gene encoding glutathione-S-transferase (GST) is under positive selection in *H. annosum* s.l. Positive Darwinian selection in coding genes have occurred when the number of substitutions per nonsynonymous site is significantly greater than the number of substitutions per synonymous site (the number of substitutions that lead to amino acid changes are greater than the ones that do not lead to an amino acid change).

GSTs are multifunctional enzymes involved in conjugating xenobiotics to an endogenous water soluble substrate, such as glutathione (2). Genes encoding GSTs were previously found to be upregulated during the interaction between *H. annosum* and Scots pine seedlings. The GST gene was amplified and sequenced from 94 isolates representing the five intersterility groups of the *H. annosum* species complex; *H. abietinum* (European F-type), *H. parviporum* (European S-type), *H. annosum* s.s. (European P-type), *H. annosum* (North American S-type) and *H. annosum* (North American P-type). The sequences were divided into groups based on a constructed phylogenetic tree (Figure 1). The McDonald-Kreitman test (1) and Fisher's exact test were used to test for positive selection between the different groups. The highest number of non synonymous substitutions is found within the European F, European P and North American S group and in comparison between the European P and the North American S as well as between the North American P and North American S groups (Table 1). The highest number of fixed mutations is found in the comparison between the North American S and North American P groups. The results show sign of positive selection in the glutathione-S-transferase gene between the European F and North American S and between the European F and North American P groups. This indicates that the GST gene might be an important factor that could explain the differences in host preference between the groups.

Table 1. Glutathione-S-transferase sequence characteristics from different populations of *H. annosum* s.l.

Population	Number of mutations[a]	Nucleotide diversity
European S	1 (0)	0.00031
European F	3 (2)	0.00188
European P, A	1 (0)	0.00096
European P, B	10 (2)	0.01139
North American S, A	2 (1)	0.00473
North American S, B	7 (2)	0.01655
North American P, A	5 (1)	0.00593
North American P, B	1 (0)	0.00158

[a]Number of mutations (non synonymous substitutions)

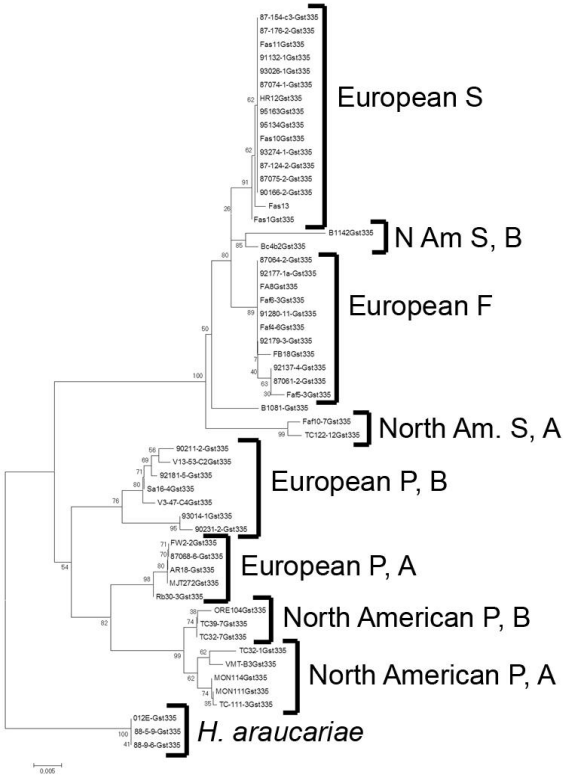

Figure 1. Neighbour Joining tree of DNA sequences of the glutathione-S-transferase gene from the different populations of *Heterobasidion annosum* s. l. The sequences from *H. araucariae* were chosen as an outgroup. Numbers indicate bootstrap values.

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Butt rot incidence and related losses in Latvian *Picea abies* (L.) Karst. stands

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CONFERENCE ABSTRACT

A total of 157 forest areas were investigated, 81 of which comprised clear-felled, and 76 thinned sites, cut 0-3 years previously. The age of clear-felled stands was 60-150 years, and of thinned stands was 30-70 years. For the investigations, cut stumps were selected either using transect method (on larger sites), or randomly. In total, 12539 stumps were examined for root rot, on average about 80 stumps per site. Stumps were classed in two categories, either as "healthy" or "containing root rot". For each "root rot" stump, we measured diameter, assessed decayed cross-section area, and classed decay into three levels of intensity. In order to correlate stump diameter, the extent and intensity of decay at stump level with the length of decay in stem, a total of 80 "model" spruce trees containing root rot were cut and the respective parameters recorded. Of all investigated stumps, 22.9% showed presence of the root rot. The incidence of the disease increased with the age of a stand: in 30-40 year-old stands the incidence was 15.7$\pm$1.4%, in 60-70 year-old – 22.2$\pm$3.5%, in 80-90 year-old – 23.7$\pm$1.9%, and in 110-150 year-old – 33.3$\pm$5.3%. Average length of decay in "model" stems was 6.9 m. Reduction of timber value in stands with a total volume of 100-400 m^3ha^{-1} was 800-4790 € ha^{-1}.

A total of 157 forest areas were investigated, 81 of which comprised clear-felled, and 76 thinned sites, cut 0 to 3 years previously. The age of clear-felled stands was 60 to 150 years, and of thinned stands – 30 to 70 years. They represented 5 forest site types. *P. abies* was the dominant tree species in all stands, its share of standing volume comprising 40 to 100% in clear-felled, and 70 to 100% and thinned stands. On the sites, stumps were investigated either using transect method (on larger areas), or randomly. In total, 12539 stumps were examined for rot, about 80 stumps per site on average. They were classed in two categories, either as "healthy" or "containing rot". For each stump, the diameter and rot diameter were measured. In order to relate stump diameter and the extent of decay at stump level with the length of decay in stem, a total of 88 "model" spruce trees containing rot were cut and analyzed. For practical reasons, all 81 clear-felled stands were gathered under 50 economical harvesting units, and from those the information on actual losses collected. Of all investigated

stumps, 23% showed presence of rot. Its incidence increased with the age of a stand: in 30 to 40 year-old stands it was about 16%, in 60 to 70 year-old – 22%, in 80 to 90 year-old – 24%, and in 110 to 150 year-old – 33% (Figure 1). The similar level of infection has been previously reported from Baltic spruce forests (1). In clear-felled areas of mature forest, the occurrence of disease was decreasing with increasing proportion of deciduous tree species, mainly *Betula*, *Populus* and *Alnus* ($r=0.71$; $P<0.05$), but not in younger thinned stands ($r=0.06$; $P>0.05$). The average diameter of rot-containing stumps did not differ from that of healthy neither on clear-felled nor on thinned sites ($P=1$). Average stump and rot diameters were larger in older stands ($r=0.47$; $P<0.05$). Average length of rot in "model" stems was 6.9 m. This is higher than the average of 3.5 to 4.5 m previously reported from butt rot attacked spruce in the Baltics (2, 3, 4), and could be explained by the fact that in the current work larger mature (80 to 100 year-old) trees were analyzed, compared with smaller and younger (40 to 60 year-old) stems in the cited studies. Length of rot correlated positively with rot diameter at stump ($r=0.56$; $P<0.05$), but not with the stump diameter ($r=0.10$; $P>0.05$). Yield loss in 50 harvesting units ranged from 3 to 25%, and strongly correlated with the frequency of infection (Figure 2). Average reduction of timber value was 800 to 4790 € per ha, and positively correlated both with standing volume ($r=0.56$; $P<0.05$) and DBH of a stand ($r=0.49$; $P<0.05$). The data obtained during this study is to be used for modeling and prognosis of production loss due to butt rot in spruce forests of Latvia.

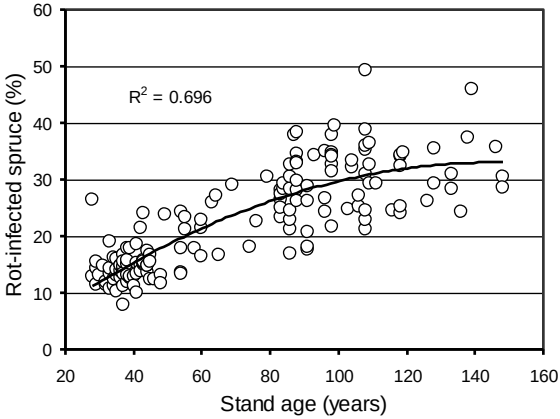

Figure 1. Butt rot incidence in Latvian *Picea abies* stands in relation to stand age.

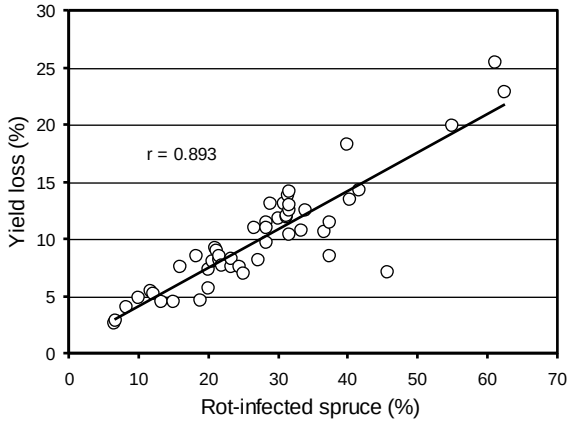

Figure 2. Yield loss in Latvian *Picea abies* stands in relation to butt rot incidence.

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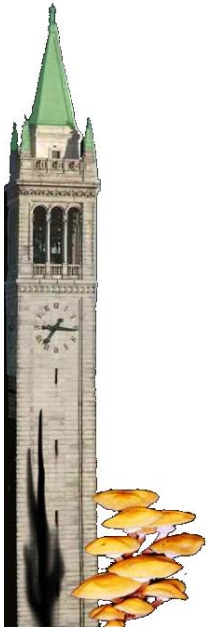
SESSION VII:

SHORT PRESENTATIONS



Timber volume losses in Scots pine stands infected by *Heterobasidion annosum sensu stricto*

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CONFERENCE ABSTRACT

Eight Scots pine stands infected by *H. annosum* were investigated. Four stands were growing on the very rich site (typical for the mixed deciduous stands) and the others on the site typical for mixed pine stands. In each stand ten trees infected and uninfected were chosen, and the sample of wood from breast height were collected with the aid of increment borer. The height and diameter of each tree were also measured. For control 40 trees were investigated in the same way in two healthy stands. Healthy pines were taller than infected trees average about 3 m. Infected trees were about average 3 cm thinner than healthy pines. Difference in wood volume between healthy and diseased trees figured out from 18% to 50%. Only in 16% infected trees decrease of wood increment was observed for at least ten years before death.

Heterobasidion annosum sensu stricto causes the most severe damages in Scots pine stands especially growing in the first and second generation on the post agricultural lands in Poland. Every year the damages caused by pathogen are estimated on area about 200000 ha. The main losses in pine stands are connected with decrease of wood increment of infected trees through years of pathogen's spread in root system.

The aim of this study was to estimate the real losses in yearly increment of wood, losses in height and in timber volume of single tree.

The study was done in 10 pine stands (38-45 years old). Eight stands were highly infected by *H. annosum* s.s. and the other two was healthy. Those stands were growing in two sites – typical for mixed coniferous-deciduous stand and richer for mixed deciduous-coniferous stand. In each infected stands 20 trees were chosen: 10 dying and 10 "uninfected". The uninfected trees (alive) were chosen on the base of crown appearance. For control in healthy stands 20 trees were chosen for the study. Each tree was drilled with the Pressler screw and the analyze of increments was done. In addition the breast diameter and height of trees was measured.

The differences in the average breast diameter between infected and uninfected trees in the same stand was 14% in mixed coniferous-deciduous site and 11% in richer site. Uninfected pines were also about 3 cm thicker that infected trees. The differences were higher, when the infected trees were compared with trees from healthy stand (respectively 18% and 21%). Pines infected by *H. annosum* s.s. were lower average about 15% (almost 3 m) than uninfected in poorer site and 13% in richer site (almost 3 m). Analyzing the timber volume of chosen trees, the average losses after long disease process reached 31% in poorer site and 28% in richer site (about 0.16 m^2 per infected tree, Figure 1). There were also differences in timber volume among uninfected trees. The volume of a largest tree was estimated on 0.84 m^3 and the smallest on 0.19 m^3 in stand growing on a richer site. The timber volume of pines on a poorer site was similar – respectively 0.85 m^3 and 0.23 m^3. We were not able to confirm that chosen uninfected pines in a severely infested stands by *H. annosum* s.s. were healthy.

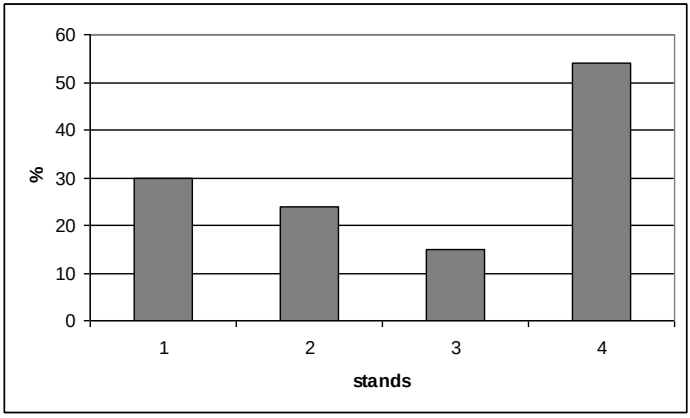

Figure 1. Average losses of timber of infected trees in stands growing on mixed coniferous-deciduous site.

The wood increments of infected pines fluctuated. Moreover there were difficult to calculate the time of disease development on the base of analyze of wood increments both infected and uninfected trees (Figure 2). The decrease of wood increment was observed for at least ten years before death only in 16% of infected trees.

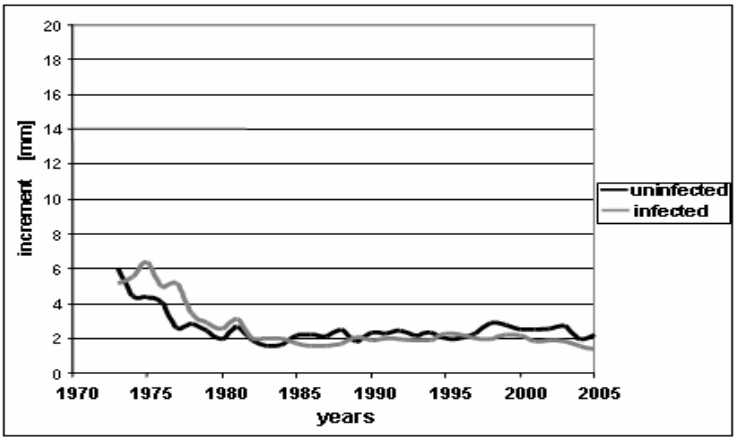

Figure 2. Average wood increment of uninfected and infected pines in stand (mixed coniferous-deciduous site).

We also calculated losses of timber value. We created a different scenario for *H. annosum* s.s. impact. We made an assumption that timber volume of individual uninfected tree was equal to average timber volume of uninfected trees measured in this study. The timber volume of infected trees was calculated in the same way. We calculated the number of trees per ha on the base of pines age and stock density. After that we tried to calculate the timber volume and value per ha in stands infected in 10%, 30% and 50%. We estimated the value of losses using the actual prices for timber. Value of losses per hectare in stand infested in 30% might be 975 $ to 1629 $ and in stand infested in 50%: 2539 $ - 4233 $.

Armillaria response tool to predict fuels management impacts on Armillaria root disease

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CONFERENCE ABSTRACT

The *Armillaria* Response Tool (*A*RT) is a web-based tool that can estimate Armillaria root disease risk in coniferous forests of the interior western USA. It uses habitat types to evaluate potential risk for developing Armillaria root disease caused by *Armillaria ostoyae*. *A*RT also indicates how some fuels management treatments may exacerbate Armillaria root disease within high-risk stands. *A*RT guides stand-level choices of appropriate fuels management treatments that should minimize future damage by Armillaria root disease. This web-based tool is part of the Fuels Planning: Science Synthesis and Integration Project, a pilot project initiated by the USDA Forest Service to provide needed tools and information for planning site-specific fuel treatment projects. More information and a functional version of this tool can be viewed at our web site: http://forest.moscowfsl.wsu.edu/fuels/art/.

Urea treatment reduces *Heterobasidion annosum* root rot in Norway spruce 15 years post-treatment

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CONFERENCE ABSTRACT

In this study we evaluate the long term effects of urea treatment of newly cut stumps against butt rot caused by *Heterobasidion annosum* in *Picea abies* stands. After the first thinning, the stumps of 16 experimental plots of *P. abies* in southern Sweden were: untreated thus subjected to natural infection, urea treated, half of the stumps urea treated and half artificially infected with *H. annosum* conidia (50% Urea + 50% conidia) and artificially infected with *H. annosum* conidia (100 % conidia). After 15 years the incidence of butt rot was observed by extracting a bore core 20 cm above ground from all stems. Cores from rot trees were stored in sterile bottles, and observed under a dissecting microscope for *H. annosum* conidiophore presence. Urea treated stands showed significantly lower butt rot incidences (2%) than the other treatments, which were of 33%, 58% and 40% respectively for natural infection, 100% conidia, 50% Urea + 50% conidia treatments. *H. annosum* was isolated from the 18 % of the cores derived from rotted trees, suggesting that it had caused the majority of rot. Urea treatment seems to be a reliable control method against *H. annosum* butt rot.

The root and butt rot fungus *Heterobasidion annosum* causes important economic losses in the coniferous forests of the northern hemisphere. One method to control the *H. annosum* infections is to apply a solution containing urea directly after cutting. The urea application on the stump leads to ammonia formation and the pH is raised to a critical level preventing basidiospore germination and the *H. annosum* mycelia is unable to survive (1). We investigated two first rotation *Picea abies* stands in southern Sweden, where the fresh stumps from the thinning in 1992 had been: 1) subjected to natural infection, 2) 100% urea treated (35% w/v), 3) 50% urea treated and 50% artificially infected with *H. annosum* conidia and 4) 100% artificially infected with *H. annosum* conidia. The incidence of butt rot was observed in 2007 by extracting inner wood cores from of all trees

at 20 cm above ground level within the experimental plots using a Pressler borer that was sterilized in 70% ethanol between each extraction. The cores from rotted trees were stored in sterile bottles and stored in the dark at 25° C. Later they were observed under a dissecting microscope for *H. annosum* conidiophore presence, and conidiophores were picked with a needle and transferred to a Petri dish containing Hagem agar. Trees were also observed for the presence of stem wounds and as two severe storm episodes affected the experiment, one in 2005 and another in 2007, we also measured whether trees were up-rooted or not. The urea treated stands showed the lowest butt rot incidence (5%). The 100% conidia-treated and the 50% conidia + 50% Urea treated stands showed the highest incidences of butt rot with almost 60% and 40% of trees rotted, respectively. Stands subjected to natural infection showed smaller incidences (35%) of butt rot than the 100% conidia treated stands. The urea treated stands showed the lowest *H. annosum* incidence amongst treatments whilst no differences were observed between 100% conidia, 50% conidia + 50% Urea and natural infection treatments. After the storm event in 2005, urea treated stands showed the smallest number of up-rooted trees (0%), whilst natural infection and 100% conidia treatments showed the highest incidences (12%) and (9%) respectively. After the storm in 2007 a similar up-rooting pattern was observed amongst treatments. We isolated *H. annosum* in 30% of the rotted extraction cores, suggesting that the majority of rot in the stands had been caused by this fungus. The urea treatment was found to be a reliable control measure for preventing *H. annosum* root rot. Stumps can be artificially infected by conidia as this treatment showed significantly higher rot incidences than the natural infection. Urea treated spruce stands also appear to be less susceptible to windthrow.

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The capacity in *Heterobasidion* spp. to resist overgrowth by the biocontrol agent *Phlebiopsis gigantea* is a heritable trait

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CONFERENCE ABSTRACT

Heterobasidion spp. are basidiomycetes that attack and kill coniferous trees throughout the northern hemisphere region. A major route of infection in managed forests is through stump surfaces where the *Heterobasidion* grows through the roots and attacks adjacent healthy trees. A biocontrol method to reduce *Heterobasidion* infection is to apply *P. gigantea* in a spore solution directly on the freshly cut stumps immediately after cutting. This treatment annually releases a large quantity of one commercialized clone into the Swedish forests. We suggest a potential risk for resistance build-up in *Heterobasidion* against the *P. gigantea* since individuals of *Heterobasidion* end up in situations of competition for substrate and space. Different strains of *Heterobasidion* have different levels of resistance or abilities to outgrow the applied *P. gigantea*. We have conducted experiments on wood blocks where the two species are forced to interact and where the overgrowth of *P. gigantea* (Rotstop®) on the *Heterobasidion* is measured at different time intervals. The *Heterobasidion* endurance against *P. gigantea* is defined as a character of resistance. A total of about 100 offspring from a QTL-mapped *Heterobasidion* hybrid strain and a total of 25 isolates collected in Fennoscandia have been tested so far. The results suggest that there is variation between *Heterobasidion* strains and that there is a heritability factor for resistance in *Heterobasidion* to *P. gigantea*.

Heterobasidion spp. are basidiomycetes that attack and kill coniferous trees throughout the northern hemisphere. In managed forests, the major route of infection is *via* stump surfaces from which the *Heterobasidion* spp. grows through the roots and attacks adjacent healthy trees. A biocontrol method to reduce *Heterobasidion* spp. infection is to apply the wood degrading fungus *Phlebiopsis gigantea* in a spore solution (Rotstop) directly on the freshly cut stumps immediately after cutting. We investigated the potential risk for a build-up in the capacity of *Heterobasidion* spp. to resist overgrowth by *P. gigantea.* The experimental population used in this study consisted of 91, previously described (2) homokaryotic *Heterobasidion* progeny strains derived from a compatible mating between North American S and P strains and the heterokaryotic hybrid. In addition, 23 strains of

Heterobasidion parviporum isolated in Fennoscandia were used to test the natural variation. Wood blocks of *Picea abies,* precolonised with the two fungal species, were juxtaposed in a Petri dish on top of water agar, and the overgrowth of the *P. gigantea* strain (Rotstop) on the *Heterobasidion* spp. was measured periodically. The estimates of individual observation broad-sense heritability $(\hat{H}^2)$ were obtained by $\hat{H}^2 = \hat{\sigma}_G^2 / \hat{\sigma}_P^2$ (1). We found a natural variation in *H. parviporum* to resist overgrowth by *P. gigantea.* There was no difference between the homo- and heterokaryotic strains. In the hybrid mapping population we were able to identify one quantitative trait locus (QTL) which controls the examined resistance capacity, and we estimated the broad sense heritability to 0.336 for the capacity to resist the *P. gigantea* overgrowth. We conclude that there exists a theoretical risk for resistance build-up in the *Heterobasidion* spp. population towards its biological control agent *P. gigantea*. However, we do not see an immediate threat on Rotstop treatments forcing the *Heterobasidion* spp. populations to become more aggressive or resistant to the biological control method, because: A) The capacity to resist overgrowth by another species is just one part among many of the inter-species interactions between fungi. B) Small individual mycelia of *Heterobasidion* spp. have a low survival rate (3) and can therefore not be expected to remain long enough to develop more resistant strains within the Rotstop treated sites. C) In Swedish forestry management thinning of spruce stands is usually performed twice during a rotation of 65-110 years and therefore the frequency of Rotstop treatments is considered very low.

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An investigation on the effect of the season of harvest on fungal community development on living *Eucalyptus globulus* coppice stumps

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CONFERENCE ABSTRACT
In Western Australia, *Eucalyptus globulus* coppice plantations vary as to the fungal species observed fruiting on stumps, with no geographic trends being apparent. As plantations are harvested at different times of the year, it was hypothesized, that differences between the plantations may be due to harvest times coinciding with different fungal fruiting and dispersal times. This hypothesis is being tested by harvesting 15 trees at each season (spring, summer, autumn, winter). Five of the trees were assigned as controls and wood samples removed and analyzed for the presence of fungi only at the commencement and at the end of the experiment (12 months after harvest). The remaining 10 trees were sampled for fungi at the time of harvest and 1 month, 3 months, 6 months and 12 months after harvest. Presence of fungi in the stumps was ascertained using molecular and morphological identification techniques. Details of the experimental design and some preliminary results arising from the morphological studies will be discussed.

In Western Australia, many second rotation *Eucalyptus globulus* plantations are established by allowing the stumps of harvested trees to coppice. Plantation managers have raised concerns about the number of stumps that are colonised by wood decay fungi, as they may potentially lead to either tree losses (through wind-throw) or decreases in the quality or volume of wood through wood decay.

In reaction to these concerns, a number of surveys of *E. globulus* coppice plantations were conducted. Plantations varied in the number of stumps colonised by fungi and in the number and the species of fungi that were observed fruiting on the stumps. As plantations were harvested at different times of the year, it was hypothesized that these differences may be due to harvest times coinciding with fruiting and dispersal of different fungal species.

To test this hypothesis, an experimental site was set up in an 8 year-old first rotation *E. globulus* plantation. A randomly selected 2 hectare plot within the plantation was chosen and trees within it were

harvested across seasons. At each harvest time (spring 2006, summer 2006-07, autumn 2007, winter 2007), a total of 15 trees were randomly chosen and felled. Five of the trees were assigned as controls and had wood samples removed at the commencement and at the end of the experiment (12 months after harvest). The remaining 10 trees were sampled for fungi at the time of harvest and at 1, 3, 6 and 12 months after harvest.

Harvest sampling was done by removing a 5 cm wood disc from the top of the stump (Figure 1). All other samples (at 1, 3, 6 and 12 months after harvest) were obtained using 12 mm hollow wood borer to extract a 4 – 7 cm long wood core from the stump. Core holes were filled with polyurethane and painted over with bitumen paint to prevent entry of fungi.

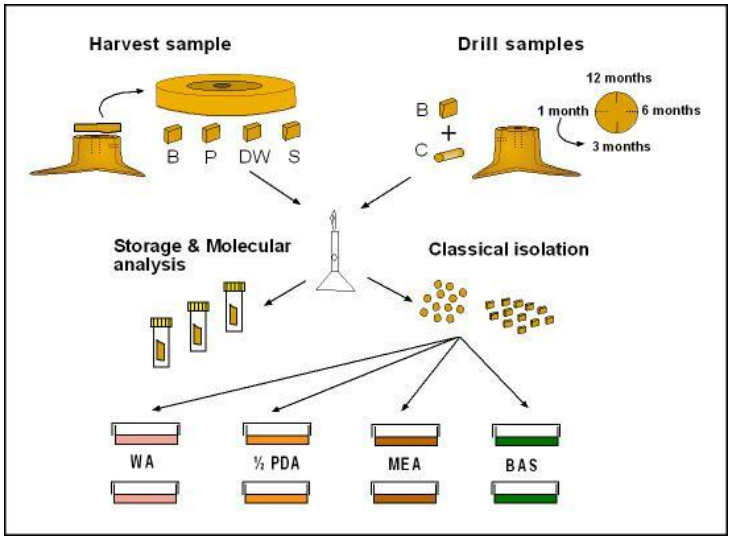

Figure 1. Methodology used in the experiment. The initial disc sample at harvest time and the subsequent drill cores (C) at 1, 3, 6 and 12 months post harvest, were surface sterilized and flamed. Sub-samples were either stored at -20^{0}C for later molecular analysis or, further subdivided and plated onto 4 different agar media; water agar (WA), half strength potato dextrose agar (½PDA), 2% malt extract agar (MEA) and a basidiomycete selective agar (BAS). Samples were obtained from the bark (B), sapwood (S), discoloured central wood (DW) and the pith (at harvest and final sample only).

In the laboratory, samples were plated onto culture media within 24 hours of collection. Sub-samples were also placed in sterile tubes and kept at -20^{o}C for molecular analysis. Samples were plated onto 2

replicates each of 4 different culture media; water agar (WA), half strength potato dextrose agar (½ PDA), 2% malt extract agar (MEA) and a basidiomycete selective agar (BAS). All media had streptomycin sulphate added to them (100 mg/L). All plates were incubated at room temperature and were checked periodically for up to 30 days. Fungi growing from the wood pieces were identified by further plating onto MEA and comparing cultural and growth characteristics (1). Fungal identification and the presence of other non-culturable wood inhabiting fungi will be further verified using RFLP analysis of rDNA.

Initial results (Figure 2) showed that potential decay fungi were already present in the pith or central core of the majority of trees harvested in spring, while sapwood was generally free of fungi. Potential problems with the methodology include: (i) inconsistent isolation of fungi, due to limited sampling and variable distribution (in space and time) of particular fungi within the stumps and, (ii) drill holes acting as fungal entry points.

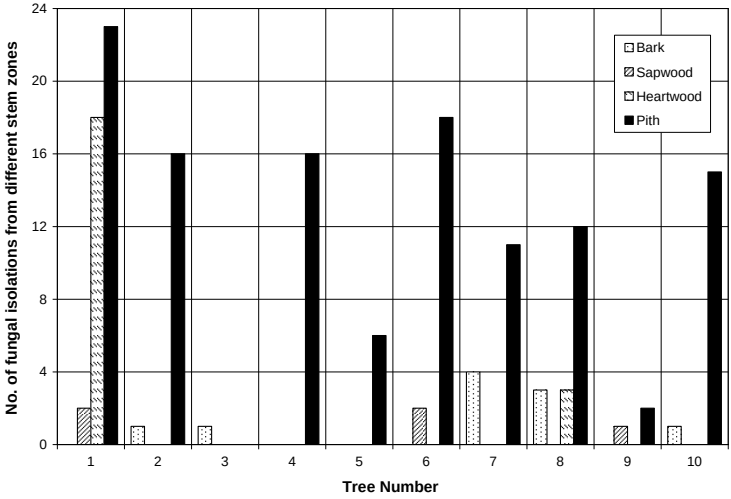

Figure 2. The number of successful isolations of fungi from bark, sapwood, discolored central core and pith from 10 *E. globulus* trees harvested in Spring 2006. There was a maximum of 24 wood slices in culture per stem zone per tree. Fungal isolates recovered may or may not be of the same species.

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Preliminary results of Norway spruce stump treatment with *Hypholoma fasciculare* and *Phlebiopsis gigantea* in an Austrian Alpine protection forest

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CONFERENCE ABSTRACT
An experiment with the fungal antagonists *Phlebiopsis gigantea* and *Hypholoma fasciculare* was conducted in 2006 in two protection forest stands of Norway spruce (*Picea abies*) in Eastern Tyrol. About 50% of the stumps showed stock-rot of *Heterobasidion parviporum*. Two isolates of *Phlebiopsis gigantea* (one from Poland and the product Rotstop®) and one strain of *Hypholoma fasciculare* (from Poland) were applied on healthy stumps. In October 2006, all stumps were examined. The *Phlebiopsis gigantea*-strain from Poland had colonized 83% to 95% of the inoculated stumps, the *Hypholoma fasciculare*-strain 87% to 95%. The variant Rotstop® resulted in only 10% to 13% colonized stumps. In more than 60% of the stumps colonized by *Hypholoma fasciculare* and *Phlebiopsis gigantea* (from Poland), large areas of the stump surface were covered by mycelium of the antagonist. In Rotstop®, only 40% showed intense colonization.

An experiment with the fungal antagonists *Phlebiopsis gigantea* and *Hypholoma fasciculare* was conducted in 2006 in two stands of Norway spruce (*Picea abies*) in Eastern Tyrol, in order to test the ability of these fungi to colonize healthy stumps under conditions typical for protection forests close to the forest limit.

Both stands consisted of over-aged spruces (170 - 200 years) at an altitude of 1600-2000 m and on a dry south exposed site. About 50% of the stumps showed stock-rot of *Heterobasidion parviporum* (identification by Dr. Paolo Gonthier, University of Torino, Italy).

One week after harvest, all stumps on the sites were mapped, diameters were measured and wounds as well as rot were assessed. 175 healthy of in total 339 stumps were selected for the experiment. Two isolates of *Phlebiopsis gigantea* (one from Poland and the product Rotstop®) and one strain of *Hypholoma fasciculare* (from

Poland) were applied in June 2006. All three compounds were suspended in water and dispersed over the stump surface with a brush. The compounds from Poland were then covered by soil to prevent the suspension from desiccation and afterwards by the wood disc, which had been cut just before the application. This was done to prevent the suspension from being washed off by the heavy rainfalls typically for this area. After one week, the discs were removed again.

In October 2006, all stumps were carefully examined in the field for colonisation by the antagonists. Discs were cut from about 20% of the stumps and incubated in the lab to analyse colonization by the antagonists or by *Heterobasidion*.

The *Phlebiopsis gigantea*-strain from Poland had colonized 83% to 95% of the inoculated stumps, the *Hypholoma fasciculare*-strain 87% to 95% (Table 1). The variant Rotstop® resulted in only 10% to 13% colonized stumps (Table 1). In more than 60% of the stumps colonized by *Hypholoma fasciculare*, large areas of the stump surface were covered by mycelium of the antagonist. The *Phlebiopsis gigantea* (from Poland) gave about the same results. In Rotstop®, only 40% showed intense colonization, whereas in most cases only punctual infections had developed. Furthermore, the compounds from Poland were able to colonize both springwood and heartwood (Figure 1).

Table 1. Number and % of stumps colonized/not colonized 3 months after application.

Stumps colonized	Plot 1				Plot 2				All			
	+	%	-	%	+	%	-	%	+	%	-	%
Rotstop®	3	***13,1***	20	***86,9***	2	***9,5***	19	***90,5***	7	***15,9***	37	***84,1***
Phlebiopsis (Poland)	19	***82,6***	4	***17,4***	20	***95,2***	1	***4,8***	39	***88,6***	5	***11,4***
Hypholoma (Poland)	20	***87,0***	3	***13,0***	20	***95,2***	1	***4,8***	39	***88,6***	5	***11,4***
untreated	0	***0***	22	***100***	0	***0***	21	***100***	0	***0,0***	43	***100***
total	42		49		42		42		85		90	

The reason for this more effective colonization by the *Phlebiopsis gigantea* and *Hypholoma fasciculare* – compounds from Poland compared to Rotstop®, is probably the more favorable microclimatic conditions on the stump surface (soil cover).

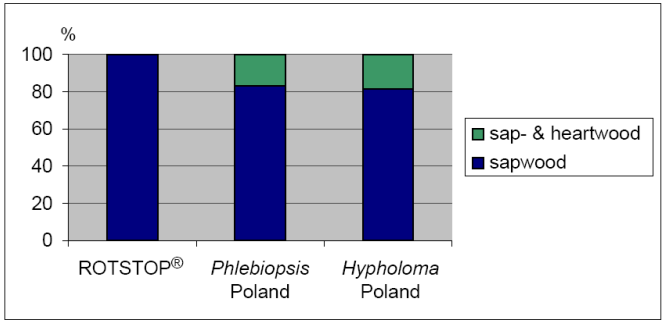
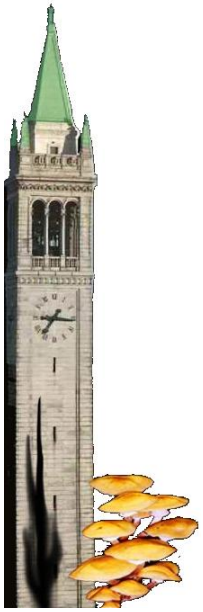


Figure 1. Percentage of stumps with sapwood or heartwood colonized 3 months after application.

These preliminary results give hope that a colonization of the sapwood of Norway spruce stumps by the tested antagonists can be effective even under alpine conditions. Further examinations of the plots after one and two winter periods will give the answer.

SESSION VIII:

NEW REPORTS, DIAGNOSTICS AND RESEARCH APPLICATION OF DIAGNOSTIC METHODS



Molecular identification of wood rotting fungi in standing trees: applications and ecological notes

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CONFERENCE ABSTRACT
Five multiplex taxon specific priming PCRs were developed to identify, from a super to a sub-generic rank, 19 wood rotting fungal taxa associated to tree failures in northern temperate regions, including *Armillaria*, *Ganoderma*, *Inonotus* and *Phellinus*. This method was validated on DNA extracted directly from wood, and proved to be efficient and specific for the diagnosis of the target taxa. This method has been employed as a device to support arborists in tree stability assessment, and forest pathologists while surveying wood rotting fungi in declining and failed trees. Overall, 233 wood samples and cores were analyzed and in 44% of them DNA of at least one of the target taxa was detected. Detection frequency of target taxa was 67% in wood samples showing visible decay. *Armillaria* spp. was detected with an unexpected high frequency on plane trees of the city of Torino. The association of target fungal taxa with the tree host species is discussed.

Wood decay increases the chance of tree failures and this may lead, especially in urban environments, to significant damage of property and/or to tragic injuries (4). Since different fungal species can affect in a different way the structural integrity of wood, their identification may be helpful for the prediction of the severity and evolution of decay processes (4). This is especially important in the case of rapidly progressing root and butt rot agents that can turn a sound tree into a hazard in a short period of time. Current diagnostic methods, mainly based on visual analysis of macro and micro morphological features of fruit bodies, can be used mostly in late stages of decay, resulting thus not suitable for timely detection of the fungal taxa involved.

In this study we describe a multiplex PCR-based method developed to early identify several fungal species frequently reported as associated to tree failures in European and North American landscapes (1, 5, 6).

Also, we report on the applications of such method and first ecological notes we have inferred from them.

By the combination of 4 fungal universal forward primers with 10 taxon-specific reverse primers designed on either nuclear or mitochondrial ribosomal DNA (2, 3), we have developed three multiplex PCRs (M1, M2 and M3) for the identification, at the super generic level, of species belonging to the group *Inonotus-Phellinus*, at the generic rank, of *Armillaria* spp., *Hericium* spp., *Ganoderma* spp., *Pleurotus* spp., *Schizophyllum* spp., *Stereum* spp., *Trametes* spp. and, at the specific level, of *Laetiporus sulphureus* and *Perenniporia fraxinea* (Table 1). Moreover, two further multiplex PCRs (Mgano and Mhyme) have been developed, by using 10 taxon-specific primers designed on nuclear ribosomal DNA (2), for the sub-generic identification of species belonging to *Ganoderma* and to the group *Inonotus-Phellinus* (Table 1).

Table 1. Multiplex PCR primers combination, diagnostic purposes and positively-tested species. * Primers are reported in (2) and (3).

Multiplex PCR	Primers combination*		Taxon-specific amplicon size/ *taxon* identified	Positively tested fungal species
	Forward	Reverse		
M1	ITS1-F	ITS4	600-850 bp/ Fungi	
		Gano2R	226-228 bp/ *Ganoderma* spp.	*G. adspersum*, *G. applanatum*, *G. lucidum*, *G. resinaceum*
	F115	Hyme2R	111 bp/ *Inonotus* spp.-*Phellinus* spp.	*I. andersonii*, *I. dryophilus*; *I. dryadeus*, *I. hispidus*, *I. radiatus*, *I. tamaricis*, *P. gilvus*, *P. igniarius*, *P. pini*, *P. punctatus*, *P. robustus*, *P. torulosus*, *P. tremulae*, *P. tuberculosus*
M2	ITS3	Armi2R	185 bp/ *Armillaria* spp.	*A. gallica*, *A. mellea*, *A. nabsnona*
	25sF	LaetR	146 bp/ *L. sulphureus*	*L. sulphureus*
		Pleu2R	158 bp/ *Pleurotus* spp.	*P. ostreatus*, *P. pulmonarius*
		Heri2R	200 bp/ *Hericium* spp.	*H. coralloides*, *H. erinaceum*
M3	ITS3	PerR	152 bp/ *P. fraxinea*	*P. fraxinea*
		Schi2R	190 bp/ *Schizophyllum* spp.	*S. commune*, *S. radiatum*
		Ste2R	231-236 bp/ *Stereum* spp.	*S. hirsutum*, *S. rugosum*, *S. sanguinolentum*
	MS1	TraR	220 bp/ *Trametes* spp.	*T. cervina*, *T. versicolor*, *T. zonatella*
Mgano	ITS1-F	GadR	211 bp/ *G. adspersum*	*G. adspersum*
		GapR	200 bp/ *G. applanatum*	*G. applanatum*
		GlR	193 bp/ European *G. lucidum*	*G. lucidum* (from Europe)
		GrR	178 bp/ *G. resinaceum*	*G. resinaceum*, *G. lucidum* (from North America)
Mhyme	25sF	FomR	258 bp/ *Fomitiporia*	*P. punctatus*, *P. robustus*
		FuscR	225 bp/ *Fuscoporia*	*P. gilvus*, *P.torulosus*
		IdryaR	254 bp/ *Pseudoinonotus*	*I. dryadeus*
		InocuR	265 bp/ *Inocutis*	*I. dryophilus*
		InssR	214 bp/ *Inonotus s.s.*	*I. andersonii*, *I. hispidus*
		PhssR	173 bp/ *Phellinus s.s.*	*P. igniarius*, *P. tremulae*, *P. tuberculosus*

After efficiency and specificity assays performed on fungal isolates belonging to the most representative species per each target taxon (Table 1), multiplex PCRs allowed to specifically prime, with an overall sensitivity of at least one pg of DNA template, the amplification of DNA fragments with size characteristic to the corresponding target taxa (Figure 1). The validation assay, performed on 118 wood samples collected from decay-affected trees, allowed to detect the target fungal taxa in 83% of samples without any aspecific amplifications (Figure 2).

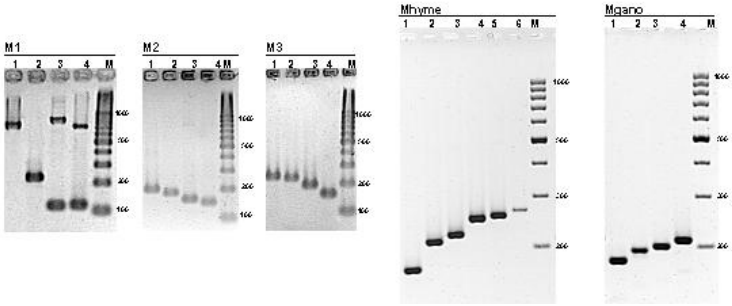

Figure 1. The results of M1, M2, M3, Mhyme and Mgano visualized on a UV-Gel documentation system after a 2 h electrophoresis at 4 V/cm on a 1% Metaphor 1% Standard agarose gel. M1: PCR products from DNA extracts of *T. versicolor* (ITS band), *G. adspersum* (228 bp), *I. hispidus* (ITS band + 111 bp) and *P. torulosus* (ITS band + 111 bp) were loaded in the lanes 1, 2, 3 and 4 respectively. M2: PCR products from DNA extracts of *H. erinaceum* (200 bp), *A. mellea* (185 bp), *P. ostreatus* (158 bp) and *L. sulphureus* (146 bp) were loaded in the lanes 1, 2, 3 and 4, respectively. M3: PCR products from DNA extracts of *S. hirsutum* (236 bp), *T. versicolor* (220 bp), *S. commune* (190 bp) and *P. fraxinea* (152 bp) were loaded in the lanes 1, 2, 3 and 4, respectively. Mhyme: PCR products from DNA extracts of *P. tuberculosus* (173 bp), *I. hispidus* (214 bp), *P. gilvus* (225 bp), *I. dryadeus* (254 bp), *P. punctatus* (258 bp) and *I. dryophilus* (265 bp) were loaded in the lanes 1, 2, 3, 4, 5 and 6, respectively. Mgano: PCR products from DNA extracts of *G. resinaceum* (178 bp), *G. lucidum* (193 bp), *G. applanatum* (200 bp) and *G. adspersum* (211 bp) were loaded in the lanes 1, 2, 3 and 4, respectively. M= Molecular weight marker 100bp DNA ladder. Molecular weights are indicated in base pairs (bp).

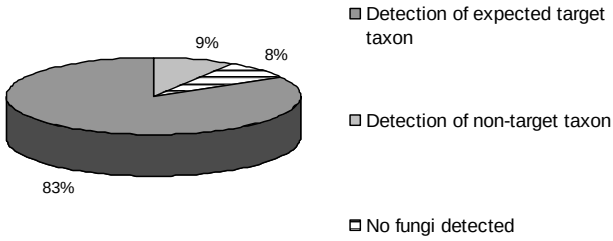

Figure 2. Pie chart displaying the diagnostic efficiency of the method on wood samples taken from decay-affected trees.

The multiplex PCR-based method has been applied for phytostatic and phytosanitary assessment of trees in urban environment, as well as for technical survey of tree failures. In the first instance, the method has been employed to complement the Visual Tree Assessment (VTA) through the identification of the decay fungi in samples collected by arborists and landscape managers. From the analysis of about 50 samples we were able to identify a target fungal taxon in 52% of samples. Also, we have applied the multiplex PCR-based method as an independent tool indicating, through the detection of decay fungi eventually present in standing trees, a probable hazardous phytostatic condition. In order to standardize a sampling method suitable to accomplish this purpose, we are testing a drilling-based technique at the tree collar for either the number of drillings or the type of mixtures necessary to earn a reliable result. Preliminary outcomes on 3 root failures have suggested the mixture of wood chips from 4 transversal drillings to be the most suitable starting sample for the detection of the root rot agent. First applications of multiplex PCRs performed on samples obtained through the tested drilling-based technique aimed at a phytosanitary survey on 18 *Celtis australis* located in a Turin avenue where a root failure occurred: although these trees didn't show any visible signs and symptoms, we have detected *Ganoderma resinaceum* and *P. fraxinea* in 9 and 2 trees, respectively.

In addition to practical purposes, applications of multiplex PCR-based method can be useful to improve the knowledge on the ecology of wood decay fungi. Among first ecological notes, we can point out the unexpected high frequency of *Armillaria* spp., whose fruit bodies are rarely visible in urban environment. Furthermore, *Armillaria* sp. has been reported as associated to a root and a collar failure of an *Ulmus* and a *Celtis australis*, respectively. *Ganoderma resinaceum* and *G. adspersum* were associated with *Platanus* sp. and *Tilia* sp. root failures. Conversely, *G. applanatum*, even if reported as one of the most widespread species on broadleaved trees in urban environment,

has never been detected. Finally, multiplex PCRs allowed to detect species belonging to *Fuscoporia*, *Inonotus* s.s. and *Stereum* as significantly associated to bole and branch failures of *Quercus agrifolia* in Californian woodlands.

First applications we have here summarized support the multiplex-PCR based method to be an effective and specific identification tool for wood decay fungi, otherwise rarely detectable.

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Gas chromatographic – mass spectrometric identification of metabolite composition in pine seedlings inoculated with *Armillaria ostoyae*

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CONFERENCE ABSTRACT

The aim of the present investigation was to find early chemical indicators of pine seedlings being affected by the pathogenic fungus *Armillaria ostoyae*, which is a cause of serious diseases of trees and degradation of forest resources. With the help of successive extraction with organic solvents of different polarity, more than 160 metabolites were extracted from the needles and roots of the 1-year seedlings and then identified by GC-MS method. The composition of the tissues extracted from control plants and those subjected to inoculation with *Armillaria ostoyae* but not infected yet were compared – the result showed that in the needles and roots of the latter (inoculated ones) increased the content of "protective" compounds (monoterpenes and flavonoids), as well as malic acid, sucrose and phytosterols. However, the best chemical indicators of infection appeared to be the compounds present only in the tissues of inoculated plants – carbohydrates raffinose and trehalose, glucosamines and free amino acids. Possible reasons of some of these compounds appearing in the roots of saplings infected with the fungus are also discussed in the paper.

Recent investigations allowed on determination of numerous biochemical changes in the plants caused by the infection of pathogens or pest attack, particularly the production of counteracting chemical compounds within affected tissues. Among those compounds are: phytoalexins, phenols, stilbenes and pathogenesis related proteins. However there was no much attention brought to the early phase of plant-pathogen relationship, when no any visible symptoms of infection were noted. The aim of this pilot investigation was to look for the biochemical changes appearing in the *Pinus sylvestris* L. seedlings due to *Armillaria ostoyae* (Romagn.) Herink inoculation during the recognition phase of plant pathogen relationship. The contrastive analysis was carried out of metabolites composition in the needles and roots of pine seedlings subjected inoculation and not treated with pathogen.

Scots pine seedlings were grown from seeds in the plastic pots (rootrainers) with the mixture (1:1 in volume) of subacid peat (pH 5.5) and agra-vermiculite. Pots were placed in the climatic chambers Mytron WB 750 in constant and controllable conditions (25^{o} C, 75% RH) at 16-hour light period. Two-month old seedlings were subjected to the artificial inoculation with *A. ostoyae* in the form of pathogen colonized 6-8 cm in length and 1-1,5 cm in diameter section of oak shoots. Seven months after inoculation seedlings were taken out of the medium, the needles were detached, and roots were thoroughly washed with water, separated from rhizomorphs and checked from being infected. 20 seedlings grown in the pots with abundantly developed rhizomorphs (as treated) and 24 not subjected inoculation (as control) served for making collective samples of roots and needles.

Three-stage extraction of roots and needles samples with organic solvents of different polarity was used to prepare the plant material (3). The first stage was the extraction of terpens and lipids (oils and cuticular waxes) with hexane, the second – aliphatic and aromatic acids and phenols compounds (carboxylic, hydroxycarboxylic and diterpene resin acids) with diethyl ether and the third – sugars and polyols (carbohydrate, carbohydrate acids and other compounds which contain several active –OH, –COOH, –NH_2 groups) with methanol. Then the gas chromatographic – mass spectrometric (GC-MS) analysis was used to identify extracts' metabolite composition. The procedure of extraction and CG-MS analysis was repeated three times.

Visible infection symptoms (rhizomorphs anchored to roots, lesions and necrosis on roots) were not found on roots of all inoculated seedlings. Alive mycelium was preserved on all 64 applied inocula. Rhizomorphs developed on 39 of them, but abundantly on 20 only.

Several hundreds of compounds were registered and more that 160 were semi-quantitatively determined by GC-MS method. The differences between inoculated and not treated seedlings were distinct just for some of them (Table 1).

Among the hexane extracts only the amount of 3-carene was found 24-35% higher in the treated pines than in the control ones. Also in roots of control seedlings, only traces of sabinene were registered whereas in roots of plants subjected inoculation its presence was distinct. Monoterpenes belong to defense chemicals and 3-carene is often considered to be the most toxic (1, 4). It was fount in the *in vitro* experiments that monoterpenes hindered the development of *A. ostoyae* and *A. gallica* (2).

Table 1. Relative composition of potential chemical indicators of stress induced to Scots pine seedlings by *A. ostoyae* during pre-infection phase of plant-pathogen relationship.

Compounds and group of compounds	Relative composition, %			
	Needles		Roots	
	Control	Inoculated	Control	Inoculated
Hexane extracts				
Monoterpene hydrocarbons including:	64.9	77.7	74.9	85.4
3-carene	40.8	54.9	36.0	44.8
sabinene	1.0	1.7	trace	1.7
Diethyl ether extracts				
Catechins	n.r.	1.4	trace	2.5
Phytosterols	3.8	8.7	8.8	15.1
Methanol extracts				
Catechins	trace	1.4	trace	5.1
Malic acid	0.2	0.2	0.2	2.7
Sucrose	trace	2.6	10.2	20.7
Raffinose	n.r.	0.2	n.r	2.3
Trehalose	n.r.	n.r	n.r	1.8
Glucosamines	n.r.	n.r.	n.r.	0.5
Amino acids including:	n.r.	n.r.	n.r.	4.4
5-oxo-L-proline	n.r.	n.r.	n.r.	3.3

In ether extracts the most significant differences were observed in the composition of catechines and phytosterols. Isomeric 2,3-catechins were the only phenol compounds present in the needles and roots of inoculated seedlings in a considerable higher amount than in the control ones. Phytosterols, among which β-sitosterol prevailed, were detected in the needles and roots of both inoculated and control seedlings, but in control plants their content was approximately two times lower. The inhibitory function of phenols to the development of *Armillaria* species was documented (1, 2, 5, 8), while the reason of elevated level of sterols under the influence of A. ostoyae is still unclear. It is possibly connected with their main biological function as regulators of the fluidity and the permeability of phospholipids bilayers.

Among the substances identified in methanol extracts, the biggest differences in their relative amounts were observed in the case of malic acid, disaccharides sucrose and trehalose, trisaccharide raffinose, as well as amino acids and glucosamines. Malic acid was present in all the extracts, however its content in the roots of treated plants was more than ten times higher than in control ones. Roots and needles of inoculated seedlings were also characterized by increased content of sucrose – one of the most widespread disaccharides, as well as by the appearance of trisaccharide raffinose. A possible

reason is the fungus originated enzymes catalyzing the hydrolysis of the polysaccharides forming the roots cell walls. It should be also considered that increased biosynthesis of these carbohydrates might be, to some extant, the plant defensive reaction to fungal infection. Unlike sucrose, trehalose, was found only in the roots of inoculated pines. Its appearance can be considered as indicator of host - pathogen relationship, because of the most vascular plants lack the ability to produce this disaccharide and obtain it from exogenous sources, such as pathogenic or non-pathogenic fungi involved in mycorrhizal symbiosis (6). Amino sugars were also found in small amounts in the roots of treated seedlings. Its formation is uncommon for plants and was found to be an enzymatic hydrolysis of chitin, fungi cell walls constituent, under the influence of β-1,3-glucanase and chitinases (7). Yield of the process is glucosamine, what indicate that the presence of 2-amino-2-deoxy-D-glucose and D-glucosamine in the roots can be considered as a reaction of plants to fungal infection. It was found chitinase-like protein in the roots of Douglas fir infected with *A. ostoyae* and *Phellinus weirii* (7). Another group of compounds present only in methanol extracts from the roots of seedlings subjected inoculation were amino acids, among which only the 5-oxo-L-proline was found in the higher concentrations. It forms a part of end links of peptides. Therefore, its presence in free state in the roots might be connected with plant peptides partial hydrolysis by pathogen emitted enzymes.

Although the presented investigation was of pilot character, its results allow making some preliminary remarks on pre-infection molecular indicators of Scots pine – *A. ostoyae* interaction. Many compounds such as monoterpenes, phytosterols, malic acid, catechines and some carbohydrates, the content of which in needles and/or roots changes under the influence of pathogen can be potential chemical indicators of that interaction. However, the substances present in bigger or smaller amounts in the tissues of infected plants only can be the most precise indicators. Among these are: trehalose, raffinose, glucosamines and free amino acids.

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Evidence of fungi in spruce roots from which fungi could not be cultured

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CONFERENCE ABSTRACT

During the course of root disease studies, many roots are encountered with small stain columns or prolific branching. Pathogens are cultured with variable but usually very low frequency. *Phaeolus schweinitzii, Inonotus tomentosus*, and *Heterobasidion annosum* have been identified from such roots. We used scanning electron microscopy to examine Douglas-fir roots from which no fungi grew when cultured on malt extract agar. Strands of mycelium are occasionally found. Most commonly observed, however, are cells filled with structures which appear yeast-like. We have not explored their role or identify further. Culturing a fungus is often considered evidence of the presence of a fungus. Failure to isolate a pathogen should not be considered evidence of the absence of a pathogen.

Large proportions of the root systems of many western North American conifers exhibit small columns of stain, often reddish in color (1). Although *Heteroabsidion annosum, Inonotus tomentosus*, and *Phaeolus schweinitzii* are commonly cultured from these symptoms, less than 10% of culture attempts from stained tissues yield fungi. Associated with the staining is a significant reduction in root hydraulic conductivity. The prevalence of staining in infested portions of stands, when taken with their effects on hydraulic conductivity suggested that these symptoms are biologically important. Yet, our limited ability to culture fungi from these roots only weakly implicates root disease fungi. This study was done to determine if fungi were present in these roots, and to better understand the nature of the disruption, and follows up on a study previously reported at this conference in 2001 (2).

We sampled coarse roots of Engelmann spruce (*Picea engelmanni*) in a stand with *I. tomentosus* (on spruce) and *H. annosum* (predominately on subalpine fir (*Abies lasiocarpa*), but occasionally on spruce.) In an area with no subalpine firs within 10 m, roots 0.4-1.0 cm in diameter were excavated, stored in a cooler, and refrigerated until processing. At 10-20 cm intervals, roots were sampled for culturing and for electron microscopy. For culturing, root sections were

debarked, surface sterilized by flaming in ethanol, and placed on malt extract agar. Samples for microscopy were fixed with gluteraldehyde and formaldehyde, rinsed in buffer, then water, dehydrated with dimethyl propane, and critical point dried in CO_2. Samples were then mounted on a carbon sticky pad, sputter coated with gold/palladium and examined with a scanning electron microscope. Here we report results only from roots which yielded no cultures of root pathogens from any of the root segments.

Fungal mycelia were sparse except in sections with resinosus and decay (Figure 1). Many tracheids were blocked by resin, tyloses, and spore-like bodies (Figure 2). In some areas of the root, the compound middle lamella appeared to have been digested. Although difficult to culture, fungi are present in these symptomatic roots.

Culturing may not be effective for confirming fungal presence. Molecular probes will be a better tool to confirm the identity and extent of the fungi, and to examine the relationship between fungal presence and root conductivity.

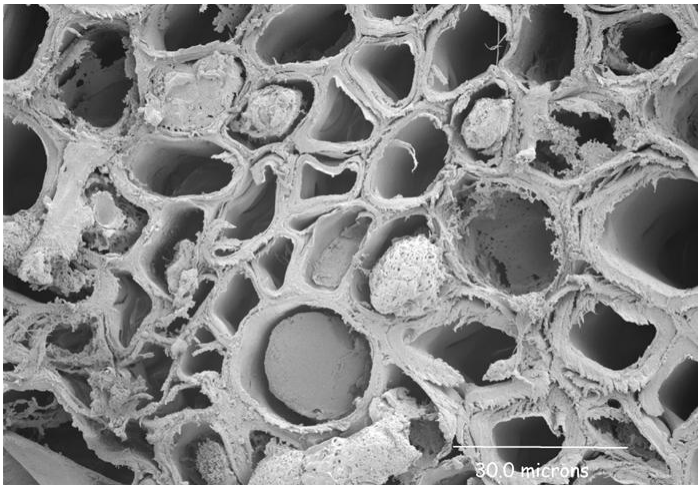

Figure 1. Sparse mycelium and cell wall degradation near center of stained root.

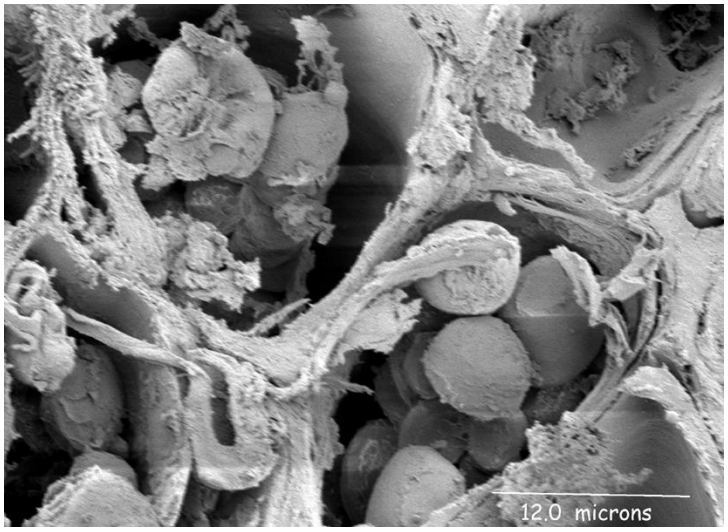

Figure 2. Mycelium and spores within stain column.

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Detection of *Armillaria tabescens* by the bait method using oak logs and cherry seedlings in hardwood stands

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CONFERENCE ABSTRACT

Armillaria tabescens is an important root rot pathogen of ornamental trees in Japan. We conducted three experiments using bait methods to investigate the distribution of *A. tabescens* in hardwood stands damaged by *A. tabescens* root rot. First, a total of 591 oak logs were placed 2 m apart in ten plots in a damaged stand. All 62 isolates originating from oak logs were identified as *A. gallica* by PCR-RFLP and mating tests. Secondly, a total of 34 oak logs were placed in contact with infected hardwood stumps and roots. All 9 isolates originating from logs were identified as *A. gallica* by PCR-RFLP. Thirdly, five cherry seedlings were planted in contact with an infected cherry stump and four seedlings were planted 3 m apart from the stump. Only one isolate originated from a living cherry seedling in contact with an infected stump was identified as *A. tabescens*. In these three experiments, *A. tabescens* was detected by the bait method using living seedlings in contact with the infected stamp. This suggests *A. tabescens* is distributed only in infected stumps.

Armillaria tabescens (Scop.) Emel is a serious root rot pathogen of ornamental trees in Japan. Recently, the decline and death of the trees by *A. tebescens* have been increasing in many hardwood stands (cherry stands, arboretums, etc.) in Gunma Prefecture, central Honshu island, Japan. One of the damaged cherry stands had ever been designated as a natural monument, so controlling *Armillaria* disease is an urgent matter. As the first step, we conducted four experiments using bait methods to investigate the distribution of *A. tabescens* in hardwood stands damaged by *A. tabescens* root rot.

The study was conducted in a cherry stand designated as a natural monument, and an arboretum damaged by *A. tabescens* in Gunma Prefecture. To detect *A. tabescens*, oak logs of 70 cm in length and 3cm in diameter were sunk about 30 cm into the ground, and 4year-old cherry seedlings of 120 cm height were planted, with cutting root. Following four experiments were conducted: 1) a total of 198 oak logs were placed 2 m apart (2×2 m grid) in three plots in a damaged cherry stand; 2) a total of 34 oak logs were placed in contact with infected

hardwood stumps and roots in a damaged arboretum; 3) four cherry seedlings were planted apart from the infected cherry stumps in the damaged cherry stand; 4) five cherry seedlings were planted in contact with the infected cherry stumps and roots in the damaged cherry stand.

After 3 to 32 months, the logs and seedlings were dug up and examined for the presence of rhizomorphs and mycelial fans of *Armillaria* spp. Pure cultures were obtained from rhyzomorphs and/or mycelial fans. The biological species were determined by mating test, RCR-RFLP analysis and sequence analysis of the intergenic spacer 1 region (IGS1) of the rDNA.

Results of all experiments are summarized in Table 1. In experiment 1, 21 isolates originating from oak logs were identified as *A. gallica*. Numerous rhizomorphs and white mycelial fans were observed on the log surface and under the bark. In experiment 2, one of 7 isolates was identified as *A. tabescens* and the other 6 isolates were *A. gallica.* The logs that generated *A. gallica* had some rhizomorphs and mycelial fans, but the log that generated *A. tabescens* had only mycelial fans. In experiment 3, neither rhizomorphs nor mycelial fans were observed on the four seedlings planted apart the infected stumps and roots. In experiment 4, only one isolate was identified as *A. tabescens,* and it originated from a mycelial fan of a living cherry seedling in contact with an infected stump and root.

Table 1. Detection of *Armillaria* spp. by the bait methods and identification of *Armillaria* isolate.

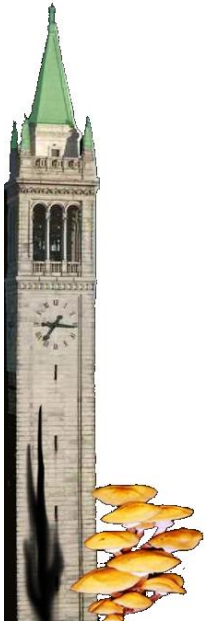
Experiment	Bait	Placement of bait	No. of baits	No. of *Armillaria* isolates	*A. tabescens*	*A. gallica*
1	oak logs	no contact	198	21	0	21
2	oak logs	contact	34	7	1	6
3	cherry seedlings	no contact	4	0	0	0
4	cherry seedlings	contact	5	1	1	0

From the results, *A. tabescens* was detected in the oak logs and cherry seedlings placed in contact with infected stumps and roots. *A. tabescens* is believed to occur only in infected stumps and roots.

On the other hand, *A. gallica* was detected in the oak logs placed over a wide area (experiments 1 & 2), but not on the living seedlings. This indicates that *A. gallica* was distributed widely in the soil.

SESSION IX:

DISEASE MANAGEMENT AND CONTROL



Planning and management of stump treatment trials

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CONFERENCE ABSTRACT

Systems for undertaking stump treatment trials using mechanised or motor-manual felling are described. The methods were developed in Scotland over 15 years of field work. Reference is made to equipment developed in Scotland to improve trial application of stump-treatment materials, and to reduce the cost of sampling.

Testing stump-treatment agents for their efficacy controlling infection of stumps by spores of *Heterobasidion annosum* (Fr.) Bref. is scientifically challenging, logistically awkward and expensive. The problem is to replicate an infection process which is highly variable, on a substratum (a stump) of huge size, complex physiology and indeterminable shape, most of which is underground. Since Rishbeth's pioneering trials in the 1950's, systems have been developed in Britain which have, broadly, kept pace with both the mechanisation of harvesting. This paper reviews those used in field trials in Britain during the past 20 years which rely heavily on techniques developed by Derek Redfern of the UK Forestry Commission for studying the infection process of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (1, 4).

The objectives of stump treatment trials as described below are assumed to be simple comparable tests of the prophylactic efficacy of different substances in reducing the incidence and severity of infection of freshly-cut stumps from airborne spores of *H. annosum.* Although large numbers of these trials have been conducted over the past 60 years, the factors that affect the success of infection and the ability of substances to prevent it are complex and little understood. Pathologists are perhaps fortunate that most of the trial methods are relatively simple: inoculum is readily available and easy to prepare, and fresh-cut stump tops represent a ready target for treatment. Accurate assessments of the distribution of the pathogen in woody samples from infected stumps can be made using simple ocular techniques. However, the processes of selecting sites and trees, creating stumps, applying the treatments and (finally) sampling the stumps after appropriate incubation need to be organised in cost-

efficient, safe and dependable ways. These are some of the practical issues.

Site selection

- Medium/high hazard soils
- First rotation
- Unthinned
- Free from *H. annosum*
- Planted in rows
- Semi-mature (heartwood present)
- Low risk of windblow

Tree selection

- Line thinning
- Undamaged stems or roots
- Limited size range of stumps

Replications

- Randomised plots or repeated sequences
- ≥25 stumps per treatment minimum
- Repeated seasonally

Labelling and marking

- Coloured labels identify trees to fell, treatment, felling and sampling height, and stump azimuth
- Label type: embossed metal/plastic (long-term trials), Tyvek loop-lock computer-printed (short trials)
- Pre-printing randomised sequences avoids transcription errors
- Fix with stainless-steel staples or nails

Felling: harvesting machine

- Faster, cheaper and safer than motor-manual
- Thorough pre-planning with machine operator needed
- Risk of damage to labels and stumps
- Mark felling cut height on each tree
- Space to accommodate machine needed

Felling: motor-manual

- Safe working and disposal of lop-and-top needs careful planning, which takes account of slope, weather and wind direction
- Good site preparation essential
- Mark felling-cut height on each tree

Recutting (of high stumps)

- Re-cutting provides a clean, level surface for accurate and even application of fluid (provide spirit level)
- Select and mark cutting height to avoid buttress roots, felling damage etc.

Stump treatment

- Objective is to apply known quantities of fluid evenly over a fresh stump
- Point of run-off ≈ 1mm thick (1litre/sq m)
- Apply manually (harvester application too variable)
    - Dropwise by syringe
        - Accurate record of volumes, with no contamination of adjacent stumps
        - Time-consuming, uneven distribution, impaired by surface resin
    - Low-pressure manually-operated drench gun
        - calibrated doses, evenly-distributed, minimal spray drift
    - Pneumatic/electric sprayer
        - set at constant height above stump can apply very low doses evenly, and replicate incomplete coverage
        - equipment is heavy and expensive, and unreliable
- Where possible, include a number of dose rates/concentrations as separate treatments in each trial
- Coordinate dyes in treatment fluids and label colours to identify treatments

Stump covers

- Cover stumps to prevent dilution of treatment fluid if rainfall is expected
- Covers to allow air movement over the stump surface
- Colour-coded for each treatment

Inoculation

- Inoculation needed where *H.annosum* spores are not plentiful
- Choice between conidiospores or basidiospores outside scope of this paper
- Basidiospores were collected, air-dried and stored on boiled cellophane for several months
- For use, cellophanes are suspended in distilled water, which is shaken to release the spores, diluted to working strength and applied to stumps

- Viability determined post-trial by serial dilution

Incubation and sampling

- Stump incubation period should allow full expression of infection in control stumps (UK ≈ 1 year)
- Sampling is by cutting transverse discs, and assessing the surface distribution of conidiophores following controlled incubation at $10^{o}C$ - $15^{o}C$
- Discs of regular thickness (5 mm – 40 mm) cut using specialist tool saves time and money
- Set sampling depth for each trial by cutting sequential, thin discs from control stumps to find depth where the pathogen is at its maximum.
- Label discs before cutting, fixing labels at a constant azimuth

These techniques provide practical solutions for trials of sufficient size to discriminate between treatment agents of varying efficacy. Because the method is based largely on line thinning, and because each stump is carefully labelled with a permanent marker placed at a regular azimuth, it is possible to re-visit stumps many years after the experiment was originally laid down, and make further observations on them. The information thus gathered has had a profound impact on national policy of stump treatment in Britain (2, 3), by demonstrating the fate of colonies of *H.annosum* in the long term.

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Ten-year effects of silvicultural treatments on tree growth and mortality caused by *Armillaria ostoyae* in south-central Oregon, USA

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CONFERENCE ABSTRACT

The 10-year effects of four silvicultural treatments on tree growth loss and mortality caused by *Armillaria ostoyae* were determined in a 80-ha mixed-conifer forest in south-central Oregon, USA. In 1995, immediately after treatment, leave-tree wounding was greatest in the commercially thinned unit (67%), moderate in shelterwood units (5 and 17%), and least in group-selection units (0 and 11%). In 2005, 10 years after harvesting, leave-tree mortality was greater and diameter growth was lower in unharvested vs. harvested units, but differences were not statistically significant. As prior to treatment in 1990, *Armillaria*-caused mortality was greatest in *Abies concolor* and *A. magnifica*, less in *Pinus ponderosa*, and least in *Pseudotsuga menziesii*. Ten years after harvesting, leave-tree mortality caused by Armillaria root disease appears, at least, not to be exacerbated by harvesting, and the stands have benefited from reducing dead fuels that could lead to wildfire losses. By 2005, about 10-15 years after pine planting in two infested clearcut units, frequency of mortality, mostly from *A. ostoyae*, was 0% for *Pinus lambertiana*, <1% for *A. concolor*, 5% for *Pinus ponderosa*, and 20% for *P. contorta*, but more time probably is needed to adequately assess the long-term success or failure of the species tested.

Armillaria root disease caused by *Armillaria ostoyae* is the most common and damaging root disease of conifers in western North America (1, 4, 5, 7). Several measures have been proposed to manage Armillaria root disease in the Pacific Northwest, USA, including biological, chemical, and silvicultural control (3, 6, 8). In a 80-ha predominantly *Abies concolor* forest affected by Armillaria root disease on the Winema National Forest in southern Oregon, USA, the objectives of our study were to determine if significant differences occur between treated and untreated areas in the amount of 10-year leave-tree mortality and diameter growth with commercial thinning, shelterwood harvests, or group-selection harvests. Frequency of mortality among three *Armillaria*-tolerant species: *Pinus ponderosa*, *P. contorta*, and *P. lambertiana* that were planted 10-15 years earlier in clearcuts with prior root disease, and naturally regenerated *A. concolor* was also evaluated.

The entire 80-ha study area is infected with *Armillaria ostoyae* (NABS I) with mortality patches abundant and varying in size from a few scattered trees to one gap of at least a hectare. Live-infected groups of trees occur among the mortality patches. The pattern and dynamics of mortality are similar to an infected area about 40 km north (2). Damage is particularly acute in areas of pure *Abies. Pinus ponderosa* and *Pseudostuga menziesii* var. *menziesii* appear to show some resistance to mortality in the area. Mortality caused by other root pathogens common to the area (i.e. *Heterobasidion annosum* and *Phellinus weirii*) is not evident. Before harvesting, tree mortality for about the last 10-15 years was 40% of the trees/ha and 33% of the basal area/ha, most of which was caused by *A. ostoyae*.

The management goal for the area is to maintain the present forest character (visual quality and wildlife habitat) while at the same time creating a forest that is sustainable and resilient to the effects of Armillaria root disease. The study area is a mixed-conifer forest of mostly *A. concolor* with a dense *Abies* understory. Large relic *Pinus ponderosa* and *Pseudotsuga menziesii* are scattered throughout the area with many trees over 500 years old. Because most of the forest is *A. concolor*, we attempted to retain and manage more of the *Abies* component than traditionally recommended in root-diseased areas. Our objectives were to test four silvicultural treatments on the 80-ha infested area: commercial thinning, shelterwood harvesting, group-selection harvesting, and clearcutting. Treatments were applied in 1995 to one plot within a pair with the other plot serving as a control (unharvested). The amount of tree mortality and *A. ostoyae* inoculum was similar before treatment between the plot pairs (Table 1) as was site productivity, stand structure, and tree-species composition.

In 1995, immediately after treatment, leave-tree wounding was greatest in the commercially thinned plot (67%), moderate in shelterwood plots (5 and 17%), and least in group-selection plots (0 and 11%, Table 2). In 2005, 10-years after group-selection harvests, leave-tree mortality appeared to be slightly greater in unharvested plots (Figure 1a). Ten-year leave-tree-diameter growth was slightly higher in the harvested plots (Figure 1b).

In shelterwood plots, leave-tree mortality was much greater in one of the unharvested plots. Ten-year leave-tree-diameter growth was similar for harvested and unharvested plots. In commercially thinned plots, leave-tree mortality appeared to be slightly greater in the unharvested plot.

Table 1. Pre-treatment (1990) Armillaria root disease, inoculum, and mortality ratings for treatment plots in south-central Oregon, USA.

Treatment/Plot no.	Plot rating[1]	Inoculum index[2]	Estimated mortality[3]	Actual mortality (%)	
Group Selection			(%)	Trees/ha	Basal area/ha
Harvested/831	5.5	6.8	67	52	28
Unharvested/830	7.1	51.3	76	45	47
Harvested/833	3.8	18.3	50	23	21
Unharvested/832	6.4	8.1	64	32	29
Shelterwood					
Harvested/837	8.0	29.3	100	83	68
Unharvested/836	6.9	52.1	77	41	37
Harvested/835	8.0	62.8	60	63	40
Unharvested/834	6.4	16.4	80	44	37
Commercial Thin					
Harvested/829	4.6	18.9	44	15	15
Unharvested/828	4.4	17.2	63	31	23
Mean, all plots					
Harvested	6.0	27.2	64	44	35
Unharvested	6.2	29.0	72	39	28
Clearcut					
Unit 4	-	60.5	-	-	-
Unit 13	-	10.7	-	-	-

[1] Mean plot severity rating for root disease where 0 = no root disease within 15m and 9 = no live *Abies* spp. in plot (all dead)
[2] Mean plot root-disease-inoculum level where inoculum index = dbh^3 (inches) * years dead/1000
[3] Estimated mortality is an ocular estimation of cumulative mortality for the past 10-15 years

Ten-year leave-tree-diameter growth was slightly higher in the harvested plot. Most differences were not statistically significant. As prior to treatment in 1990, *Armillaria*-caused mortality was greatest in *A. concolor* and *A. magnifica,* much less in *Pseudotsuga menziesii*, and least in *Pinus ponderosa*. By 2005, about 10-15 years after pine planting in two infested clearcut units, frequency of mortality, mostly from *A. ostoyae*, was 0% for *P. lambertiana*, <1% for *A. concolor*, 5% for *P. ponderosa* and 20% for *P. contorta*, but more time probably is needed to adequately assess the long-term success or failure of the species tested.

Table 2. Frequency and severity of leave-tree wounds $\geq$0.01m^2 (all species) after harvesting in 1995 in south-central Oregon, USA.

Treatment/ Plot	Trees with wounds	Wounds per tree	Wound severity[1]			Wound size (m^2)			Wound height (m)[2]		
Group Selection	(%)		1	2	3	No	Mean	Range	0-0.3	0.3-1.4	>1.4
Plot 831	11	0.2	-	-	-	5	0.16	0.02-0.04	3	1	1
Plot 833	0	0	-	-	-	-	-	-	-	-	-
Shelterwood											
Plot 837	17	0.4	0	0	5	5	0.02	0.01-0.05	1	0	1
Plot 835	5	<0.1	-	-	-	3	0.24	0.07-0.56	3	0	0
Com. Thin											
Plot 829	67	1.4	25	3	29	57	0.47	0.01-4.46	42	9	6

[1] Wound severity: 1= tree contact, but no broken bark; 2 = broken bark but no exposed wood; 3 = exposed wood. Values are number of wounds in each class. Some wound-severity data were not taken

[2] Wound height above ground; values are the number of wounds in each height class

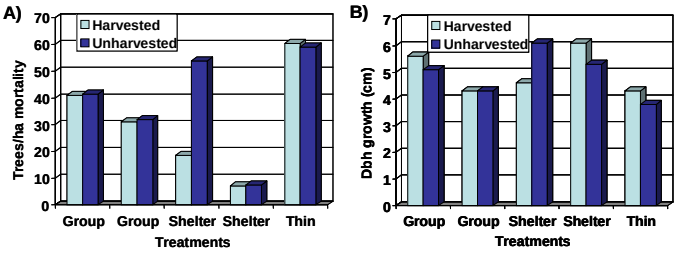

Figure 1. A) Ten-year leave-tree mortality caused by *Armillaria ostoyae* by silvicultural treatment in south-central Oregon, USA. B) Ten-year leave-tree dbh growth by silvicultural treatment in south-central Oregon, USA. Treatments are group-selection harvest, shelterwood harvest, and commercial thinning.

We conclude that:

- Commercial thinning of primarily *A. concolor* may not be effective in extending longevity or vigor of leave trees in Armillaria-root-diseased areas. While mortality caused by *A. ostoyae* appears, at least, not to be exacerbated by harvesting, stem wounding associated with improper thinning may result in future decay. Thinning, however, may reduce wildfire severity and protect relic trees if the forest burns or is attacked by bark beetles.
- *Armillaria*-tolerant and early seral species such as *P. ponderosa* and *P. menziesii* can be successfully favored or planted in root-

disease patches, shelterwood openings, or other gaps.

- Although harvest levels may be higher than normal in root-diseased areas, this may be necessary if the intent is to salvage as many dying trees as possible, significantly reduce the amount of down and standing dead fuel wood, and create sufficient openings for the successful regeneration of *Armillaria*-tolerant tree species.

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Infection of *Heterobasidion* spp. in late pre-commercial thinnings of *Picea abies* in southern Sweden

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CONFERENCE ABSTRACT

Stump treatment to reduce spore infection by *Heterobasidion* spp. has become a common silvicultural measure during commercial thinnings of Norway spruce in southern Sweden. Treatment during pre-commercial thinnings of young stands is regarded not to be necessary since the stumps are too small to spread the disease. In recent years however, it has become more and more common to do late pre-commercial thinnings with the aim to increase the net income of the first commercial thinning. The stumps from these late pre-commercial thinnings are bigger than those from traditional pre-commercial thinnings. It is likely that *Heterobasidion* spp. infect stumps from these cuttings and spread to the trees in the remaining stand. In this study, the number of stumps created in late pre-commercial thinnings, and the frequency of *Heterobasidion* spp. infection in such stumps were investigated. A questionnaire was distributed to representatives of the forest industry in southern Sweden to investigate how common this kind of thinning is and how the *Heterobasidion* threat is handled. The results show that late pre-commercial thinning is a common silvicultural measure in southern Sweden. Results also indicate that the risk of *Heterobasidion* spp. infection is not considered during late pre-commercial thinnings. The results were used to simulate the profitability of stump treatment in late pre-commercial thinnings.

Stump treatment against air-borne *Heterobasidion* infections is widely used in Sweden. Treatment is mainly applied in commercial thinnings of *Picea abies*. In pre-commercial thinnings, treatment is thought to be unnecessary since the stumps are too small to spread the disease in the remaining stand (4). Lately however, late pre-commercial thinnings, just prior to first commercial thinning, have become a common part of the silviculture of *P. abies* in Sweden. Olsson (2) and Fröberg (1) showed that, in southern Sweden, as much as 50 % of the *P. abies* stands are subjected to late pre-commercial thinnings. Late pre-commercial thinnings make it possible for forest harvesters to enter stands otherwise being too dense, and improve the economy in the first commercial thinning by increased mean diameter of the trees.

Many of the trees removed in late pre-commercial thinnings are bigger than in normal pre-commercial thinnings of young stands. Consequently, there is a risk for *Heterobasidion* spore infection of

stumps from late pre-commercial thinnings and a subsequent vegetative spread to trees in the remaining stand.

The aims of this study were: i) to investigate how the south Swedish forestry sector handles the risk of *Heterobasidion* infection in late pre-commercial thinnings, ii) measure the number of stumps created and how frequently they were infected by *Heterobasidion* in *P. abies* stands subjected to late pre-commercial thinnings, iii) to use the simulation model Rotstand to investigate the economy of stump treatment in late pre-commercial thinnings.

A questionnaire was distributed to 22 representatives at three forest companies in southern Sweden during the spring 2006. The main question were how much late pre-commercial thinnings are done, what are the instructions to the forest workers and is the risk for *Heterobasidion* spore infections considered?

In each of eight stands the number of stumps after late pre-commercial thinnings was counted in ten 16 m wide plots per stand. Only stumps with a diameter >5 cm were counted.

The frequency of *Heterobasidion* stump infection in late pre-commercial thinnings was investigated in four stands. Discs were sampled from stumps in different diameter classes, starting from 5 cm. After incubation for 7-10 days the discs were scanned for *Heterobasidion* conidia using a dissecting microscope.

The effect of stump treatment in late pre-commercial thinnings of *P. abies* was simulated using the Rotstand model (3). Stand data and thinning schedule were typical for southern Sweden, i.e. a late pre-commercial thinning at a stand age of 34 years, commercial thinnings at 36, 46 and 58 years and harvest at 75 years. Stump treatment was applied in the commercial thinnings. Results from simulations with and without stump treatment in the late pre-commercial thinning were compared. Three removal percentages in the late pre-commercial thinning were tested, 4%, 9% and 14% (of the stem number). For each removal percentage comparisons were made for stands with and without initial decay in the original stand.

The cost for manual treatment in late pre-commercial thinning was estimated to 420, 270 and 120 SEK/ha for 14, 9 and 4% stem removal respectively. Output data from Rotstand were used to calculate income differences between treated and untreated scenarios. All values were discounted, using an interest rate of 3%, to year 34 and compared with the cost for treatment. Prices of timber and pulp wood were based on price lists for southern Sweden.

Sixteen persons answered the questionnaire. It was estimated that approximately 65% of the *P. abies* stands in southern Sweden are subjected to late pre-commercial thinnings. Commonly it is recommended to remove trees up to trees giving one piece of pulp wood. The risk for *Heterobasidion* spore infections in late pre-commercial thinnings is not considered.

The number of stumps after late pre-commercial thinnings varied between 169 and 512/ha and stumps from the smaller diameter classes dominated (Figure 1).

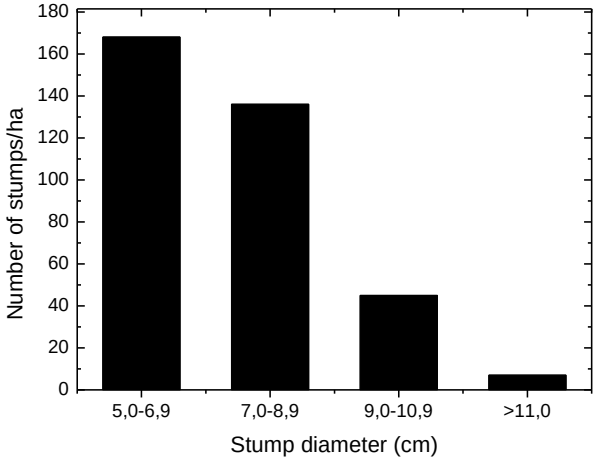

Figure 1. The mean number of *P. abies* stumps in different diameter classes created in late pre-commercial thinnings in southern Sweden.

Stumps from all diameter classes were infected by *Heterobasidion* although the infection frequency for the smallest stumps were generally lower than for bigger ones (Figure 2).

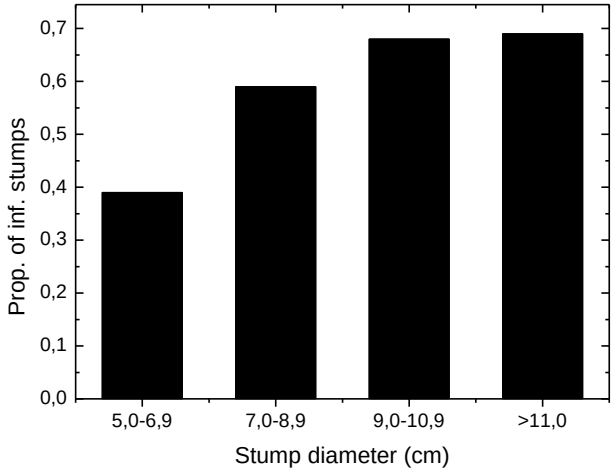

Figure 2. Proportion of *P. abies* stumps from different diameter classes created in late pre-commercial thinnings that were infected by airborne *Heterobasidion* spores.

The simulated development of *Heterobasidion* infections is shown in Table 1. The difference in incomes between alternatives with and without stump treatment was bigger than the cost for stump treatment in all cases (Table 2).

Table 1. Simulated development of *Heterobasidion* infection in *P. abies* stands with and without stump treatment in the late pre-commercial thinning year 34.

	Proportion of trees infected by *Heterobasidion* (%)											
	14 % removal				9 % removal				4 % removal			
	Prev. decay		No prev. decay		Prev. decay		No prev. decay		Prev. decay		No prev. decay	
Stand age	Treat	No treat	Treat	No treat	Treat	No treat	Treat	No treat	Treat	No treat	Treat	No treat
34	6,3	5,9	0	0	6,0	6,0	0	0	6,9	6,2	0	0
36	5,2	5,1	0	0	5,3	5,0	0	0	5,7	5,4	0	0
41	6,5	6,4	0	0	6,4	6,4	0	0	7,3	6,7	0	0
46	6,8	6,7	0	0	7,1	6,7	0	0	7,9	7,3	0	0
51	9,3	16,7	1,2	7	9,0	13,3	0,6	6,1	12,1	12,1	0,4	2,3
56	13,4	27,9	3,9	16,4	12,2	20,9	3,0	13,0	15,3	16,9	1,4	6,3
58	14,6	28,4	3,9	16,4	13,0	21,5	3,0	13,0	16,4	17,6	1,4	6,3
63	18	32,4	5,7	19,3	16,4	25,4	5,4	15,8	21,5	23,5	2,8	9,0
68	21,3	38,5	7,4	25,5	20,3	32,4	7,3	21,7	25,7	28,4	3,9	11,5
73	28,8	50,1	12,3	36,9	25,9	43	12,4	29,7	33,1	37,3	8,3	17,6
75	31,3	54,2	14,2	39,7	28,1	45,3	14,3	31,8	34,4	40,0	9,8	19,8

Table 2. The discounted differences in incomes between the alternatives with and without stump treatment in late pre-commercial thinning.

	Difference in incomes for alternatives with and without stump treatment in late pre-commercial thinning (SEK).		
	14 % removal	9 % removal	4 % removal
Prev. decay	4568	3958	557
No prev. decay	4625	3977	1948

Late pre-commercial thinnings in *P. abies* seem to be common and important for the development of *Heterobasidion* root and butt rot. It is likely that many of the stumps are big enough to spread the disease to trees in the remaining stand after thinning. Late pre-commercial thinnings are also commonly carried out during the spore spreading season, without applying stump treatment.

Simulating the development of *Heterobasidion* decay using a computer model surely involves many uncertainties and is based on many assumptions. However, the money that, according to our calculations, may be saved by treating the stumps in the late pre-commercial thinning was at least 10 times higher than the cost for the treatment (except in the 4 % removal alternative with previous decay). Therefore, it seems reasonable to conclude that stump treatment in late pre-commercial thinnings is economically justified.

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Interaction between *Heterobasidion parviporum* and *Laetiporus sulphureus*

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CONFERENCE ABSTRACT

The basidiomycete *Laetiporus sulphureus* occupies a similiar niche as *Heterobasidion parviporum* but occurs very rarely on spruce and vegetative spread between trees has never been observed. We tested *L. sulphureus* as a potential biocontrol agent for *H. parviporum* using stump inoculations and *in vitro* experiments. Norway spruce stumps (*Picea abies*) were treated in April 2006 with conidial-mycelial suspensions. The following treatments were made: 1) *L. sulphureus* strain A and *H. parviporum*, 2) *L. sulphureus* strain B and *H. parviporum*, 3) *H. parviporum* and 4) uninoculated control. After 6 months, the cross sectional area colonized by *Heterobasidion* was recorded. Compared to inoculations with *H. parviporum* alone, inoculation of mixtures of *L. sulphureus* and *H. parviporum* showed a significant efficacy of 73-85% in terms of colonized cross-sectional area but no significant effect in terms of incidence (frequency of infected stumps). The inoculated strain of *H. parviporum* was reisolated, whereas *L. sulphureus* could not be detected after 6 months. *In vitro,* the conidia of *L. sulphureus* remained dormant despite application of various treatments (temperature, nutrients). In dual cultures on MEA deadlock occurred or *L. sulphureus* was partly overgrown by *H. parviporum*, on nutrient-poor media (cellophane sheets on water agar), *L. sulphureus* was parasitized by *H. parviporum*. We conclude that *L. sulphureus* is probably not suitable as an antagonist of *H. parviporum* on Norway spruce. Nevertheless, the significant effect of the *L. sulphureus* suspension deserves further study.

The basidiomycete *Laetiporus sulphureus* occupies a similar niche as *Heterobasidion parviporum*, but it occurs very rarely on spruce and vegetative spread between trees has never been observed (1). Therefore, the potential of *L. sulphureus* was tested to control *H. parviporum*. Fresh stumps of Norway spruce (*Picea abies*, diam. 14-28 cm) were treated in April 2006 with heterocaryotic conidial-mycelial suspensions (*L. sulphureus*: approx. $3 \cdot 10^6$ propagules ml^{-1}; *H. parviporum*: approx. $1.2 \cdot 10^6$ propagules ml^{-1}). Ten stumps each received one of the following six treatments (100 ml suspension per strain and stump): (i) *L. sulphureus* strain A (no. 940523.1 from *Salix* sp.) alone, (ii) *L. sulphureus* A in combination with *H. parviporum* (no. 060122.1 from *Picea abies*), (iii) *L. sulphureus* strain B (no. 920820.2, from *Larix decidua*) alone, (iv) *L. sulphureus* B in combination with *H. parviporum*, (v) *H. parviporum* alone and (vi) 100 ml of sterilized

water. The treated stumps were covered with plastic sheets (kept at a distance of approx. 2 cm by wooden blocks) for 5 weeks. After 6 months, the size of the cross sectional area colonized by *Heterobasidion* ca. 2-3 cm below the stump surface was recorded. Additionally, the interaction was studied using light microscopy in dual cultures on malt extract agar (MEA, 20 g l^{-1} malt, 15 g l^{-1} agar) and on cellophane sheets on water (WA, 15 g l^{-1} agar) or malt extract agar at 22°C.

Compared to inoculations with *H. parviporum* alone, inoculation of mixtures of *L. sulphureus* and *H. parviporum* showed a significant efficacy of 73-85 % ($P<0.05$) in terms of colonized cross-sectional area, but control depended on the *L. sulphureus* strain in terms of the frequency of infected stumps (Table 1). The inoculated *H. parviporum* strain but not *L. sulphureus* could be reisolated after 6 months. *In vitro,* the conidia of *L. sulphureus* remained dormant despite various treatments (temperature, drying, nutrients), and on the stumps probably only mycelial fragments could grow. *H. parviporum* overgrew *L. sulphureus* after temporary deadlock in dual cultures on MEA. Growth of *L. sulphureus* hyphae ceased, their cytoplasm became coagulated and disappeared. Surface and aerial hyphae of *L. sulphureus* were parasitized by *H. parviporum* on MEA but not on WA (Figure 1a). Hyphae and chlamydospores of *L. sulphureus* submerged in MEA showed cytoplasmatic disintegration without any signs of mycoparasitism whereas the hyphae of *H. parviporum* appeared to be intact (Figure 1b) and only *H. parviporum* could be reisolated.

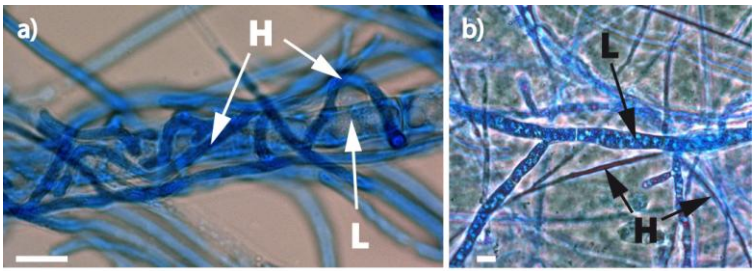

Figure 1. Interaction between *H. parviporum* (H) and *L. sulphureus* (L) in a two week old dual culture on MEA: (a) Aerial hyphae of *H. parviporum* coiling around a hypha of *L. sulphureus* (Size bar = 10 µm); (b) submerged viable hyphae of *H. parviporum* and submerged vacuolated, nonviable hyphae of *L. sulphureus* (Size bar = 10 µm). Mounting medium: Lactophenol blue solution (Merck no. 13741). (See Appendix: Color Plates).

Table 1. Presence of *H. parviporum* in stumps after various treatments.

Treatment	Mean proportion of the cut surface area (%) colonized by *Heterobasidion* (n=10)[1]	Number of stumps colonized by *Heterobasidion* (n=10)[2]
H. parviporum	11.55 a	10 a
L. sulphureus A + *H. parviporum*	1.75 b c	7 b
L. sulphureus B + *H. parviporum*	3.09 b	9 a
L. sulphureus A	0.45 c	4 b
L. sulphureus B	0.50 c	4 b
water	1.24 c	6 b

[1] identical letters indicate statistically non-significant differences (Wilcoxon's two-sample test; $\alpha = 0.05$)
[2] identical letters indicate statistically non-significant differences (z-test assuming binomial distribution and setting the probability of successful colonization by *H. parviporum* of stumps treated with this fungus to 95%; $\alpha = 0.05$)

We conclude that *L. sulphureus* is probably not suitable for biocontrol of *H. parviporum* on Norway spruce, but metabolites of *H. parviporum* might be interesting for control of *L. sulphureus*. Nevertheless, there was a significant effect (similar to the efficacy of *Phlebiopsis gigantea*) despite the very high inoculum concentration of *H. parviporum* and the protection of the stump surface with plastic screens during the first five weeks after inoculation. The explanation of this phenomenon deserves further study.

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Colonization of Norway spruce stumps by *Phlebiopsis gigantea*

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CONFERENCE ABSTRACT

Initial field experiment with application of Finnish biological preparation of *Phlebiopsis gigantea* (Fr.: Fr.) Jülich (in form of a dry powder containing 10^7 oidia/g) was performed. The study has been carried out in Norway spruce [*Picea abies* (L.) Karst.] stand (35 year old) affected by *Armillaria* root rot (Górowo Iławeckie Forest District's, Poland, N=54°17′, E=20°29′). In June 2003, 43 spruce stumps – result of a thinning - were sprayed with preparation of *P. gigantea*. 24 months after treatment 10 stumps were rooted out and cut on ca. 10 cm sections. Samples of sections' wood were than used (pieces of wood incubation on malt-agar medium) to check the presence of *P. gigantea* mycelium. The deepest colonization of stump's wood with this fungus was present in 48 cm deep (from the surface of stump).

Armillaria root rot is the very important disease affecting mainly young Scots pine (*Pinus sylvestris* L.) as well as Norway spruce *(Picea abies* Karst.) stands in Poland.

The treatment with *Phlebiopsis gigantea* (Fr.) Jül. [*Phanerochaete gigantea* (Fr.: Fr.) Rattan et al.] is effective in the case *Heterobasidion annosum* (Fr.) Bref. *sensu lato* control on pine stumps. Oppositely the spruce wood is not extensively colonized by *P. gigantea*. However, the number of studies dealing with this problem is not sufficient.

The aim of the study was to report the colonization of Norway spruce stumps by *P. gigantea*.

The experiment was laid out in early thinning of Norway spruce, in the 35-year-old stand infested with *Armillaria* spp., in Poland. The stand was established on post agriculture land, on fresh mixed broadleaved forest (according to Polish forest site typology) (Górowo Iławeckie Forest District's, N=54°17´14´´, E=20°29´21´´).

Forty three stumps (diameter approx. 13-24cm) were sprayed using the Finnish *P. gigantea* preparation (in form of a dry powder containing ca. 10^7 oidia/g; in dose 1g preparation/1l water), in June 2003. Twenty four months after treatment 10 stumps were rooted out and cut on ca. 5-10 cm sections. From each wood section sample, 5

pieces of wood (inoculum) were taken and plated onto 2% malt extract agar medium. Following incubation at 24°C for two weeks, cultures were examined for oidiospores of *P. gigantea*.

P. gigantea was reisolated from 7 stumps - numbers: 2, 4, 5, 7-10 (Table 1). *Armillaria* was isolated from roots of 4 stumps, but in one case also from lower part of the stump number 1.

Table 1. Fungi species isolated from Norway spruce stumps.

Stump no.	Wood disc/ roots	Number of inocula	Identified fungal species from mycelium growing from inoculum
1	I	5	5x*Trichoderma* sp.
	II, III	10	2x non-sporulating white mycelium, 8x *Candida* sp.
	IV	5	5x *Armillaria* sp.
	root	5	2x *Armillaria* sp., 3x no growth
2	I-III	15	15x *P. gigantea*
3	I	5	5x *Trichoderma* sp.
	II, III	10	4x non-sporul. white mycelium, 6x *Trichoderma* sp.
	root	5	5x *Armillaria* sp.
4	I-IV	20	20 *P. gigantea*
	root	5	5x *Armillaria* sp.
5	I-IV	20	20 *P. gigantea*
	root	5	2x *Armillaria* sp., 3x non-sporul. white mycelium
6	I	5	1x *Mucor* sp., 4x non-sporul. white mycelium
	II-IV	15	15x non-sporul. white mycelium
7	I, II	10	10x *P. gigantea*
	3 roots	15	15x *P. gigantea*
8	I-III	15	15x *P. gigantea*
9	I, II	10	10x *P. gigantea*
	III	5	5x non-sporul. white mycelium
10	I-III, root	20	20x *P. gigantea*
		Σ =205	*P. gigantea* - 125, *Armillaria* sp. - 17, cultures of non-sporul. white mycelium – 33, *Mucor* sp. - 1, *Trichoderma* sp. - 16, *Candida* sp.- 8, no growth – 3

It was obtained cultures of *P. gigantea* and *Armillaria* from the same stumps. It was isolated non-sporulating white mycelium. After macroscopic observation kind of the rot, it is possible that in the case of stumps numbers 6 and 9 obtained mycelium can belong to *Psilocybe fascicularis* (Huds.: Fr.) Noordel.

It was found that *P. gigantea* colonized sapwood. The same results obtained Korhonen *et al.* (2) and Anselmi and Nicolotti (1). After 24 months the deepest colonization of stump's wood with this fungus was present in 48 cm deep (from the surface of stump) (Table 2). Wood penetration by fungus was from 4 cm to 11 cm deep (from the stump cut surface), after 12 months from treatment using Finnish preparation in case of spruce stumps in diameter approx. 3 – 10 cm (3). Korhonen *et al.* (2) noticed in some cases where the stump was sampled at a lower level, the fungus had spread at least 20 cm down into the wood within 3 months.

Table 2. Depth of *P. gigantea* mycelium penetration of Norway spruce stumps and roots wood after 24 months.

Number of stump	Stump diameter (cm)	Stump height (depth of *P. gigantea* penetration) (cm)
1	16	34(--)*
2	27	36(28)
3	23	39(--)
4	16	35(28)
5	16	35(30)
6	15	32(--)
7	21	48(48)
8	22	33(30)
9	19	30(25)
10	17	34(34)

*(-) no *P. gigantea* mycelium

ACKNOWLEDGEMENTS

The author would like to thank Dr. Marina Niemi from Verdera Oy for sending the samples of Finnish *P. gigantea* preparation "Rotstop".

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Influence of different soil preparation and wood debris utilization on *Armillaria ostoyae* root rot development in Scots pine plantations

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CONFERENCE ABSTRACT
The observations of *Armillaria ostoyae* root rot development were done every year in Scots pine plantation, where the soil was prepared with deep plough (70 cm of depth), middle plough (0), mill-plough and forest mill before planting. On each plot wood debris remained after clear cutting were utilized in different way: removed from the plot, burnt, leaved on soil, split up and leaved on the soil, split up and mixed with soil. The most severe damage caused by *A. ostoyae* was observed in area after deep ploughing. On the part of stand where the soil was prepared by deep plough, *A. ostoyae* killed 3.5 times more pines then on the part prepared by active plough and 6.5 times more than on part prepared by plough-mill. *Armillaria ostoyae* infected also almost 4 times more pines on area prepared by deep plough than on area prepared by forest mill (shallow preparing). There were no influence of wood debris utilization on *Armillaria* disease development. We observed the wood debris colonization by rhizomorphs both buried and leaved on the soil. In addition *A. ostoyae* produced basidiomes the most frequently on the area where the wood debris were leaved on the soil.

It is well known that the soil preparation is the one of the most important factors which increase the *Armillaria* root rot disease development in Scots pine plantations, especially growing in a hazard sites. There are some devices using in soil preparations. Some of them cut the soil deeply and others remove only litter.

There is also some possibility of wood debris (after harvesting – branches, twigs and needles) utilization. From the ecological point of view wood debris should be left in forest to sustain the carbon or nitrogen cycle in environment. These debris, besides stumps, could be also the food base for *Armillaria* species in future stands.

The aim of the study was to check the influence of different soil preparation and wood debris utilization on *Armillaria ostoyae* root rot development in Scots pine plantations.

The study was conducted in 5 pine plantations in 2003-2006. In this area the soil were prepared with different devices: plough, active-plough, mill-plough and forest mill. Two devices penetrated soil deeply (plough and mill-plough – the soil was deeply turned and pines were planted between lines on the top) and the others remove litter and cut the soil shallowly. On each area the wood debris were utilized in five ways: removed from the plot, burnt, leaved on the soil, split up and leaved on the soil or split up and mixed with the soil. On each area (stand) the combinations of soil preparation and debris utilization were multiple at least three times and localized randomly. Individual operation area had from 600 – 2500 m^2. On four plots pines were planted in 2000 and on one in 2003. The observation of *Armillaria ostoyae* root rot development was done every year.

The disease development has been observed since the first observation (even two years after planting). The mot severe damage caused by *A. ostoyae* was observed on area after deep ploughing. There pathogen killed three times more pines than on area after active plough and even 6.5 times more after mill-plough. On the plot establish in 2003 *A. ostoyae* attacked four times more pines on area prepared by plough than forest mill. This situation was similar in every year of observation (Figure 1).

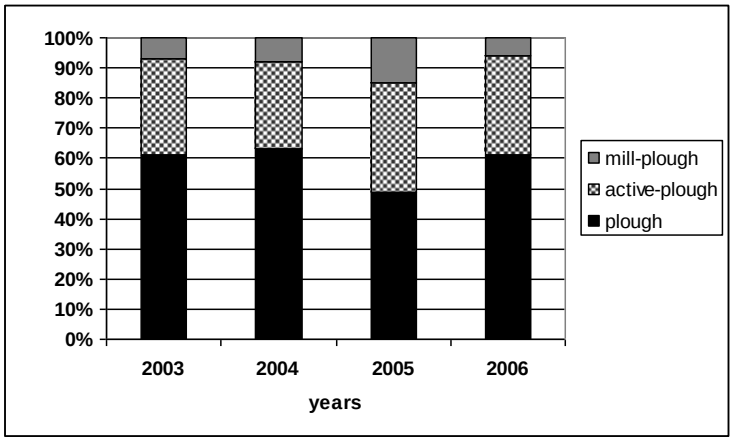

Figure 1. Share of pines killed by *A. ostoyae* on areas prepared in different ways before planting in 2000.

There was no influence (p>0.05) of wood debris utilization on *Armillaria* disease development. The mortality of pines killed by pathogen was similar on plots where debris had been removed or split up and mixed with the soil (Figure 2).

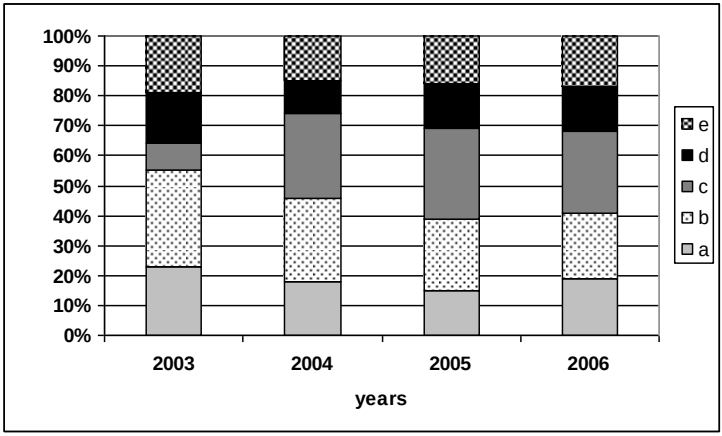

Figure 2. Share of killed pines by *A. ostoyae* on two plantations prepared by plough before planting in 2000. Combination of wood debris treatment: a - leaved on the soil, b - split up and leaved on the soil, c - removed from the plot, d - split up and mixed with the soil, e - burnt.

We found that occasionally wood debris were colonized by *A. ostoyae* both buried and left on the soil. In addition *A. ostoyae* produced basidiomes the most frequently on plots where the wood debris were left on the soil.

The use of forest mill or eventually active plough should be recommended for soil preparation before planting on *Armillaria* hazard sites. In fact that wood debris are colonized by *A. ostoyae* could increase the pathogen activity in the soil. Probably four years of observation is too short period to display the effect of wood debris on disease development.

Studies on black stain root disease on ponderosa pine

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CONFERENCE ABSTRACT
Black stain root disease of ponderosa pine, caused by *Leptographium wageneri* var. *ponderosum* (Harrington & Cobb) Harrington & Cobb, is increasing on many eastside pine stands in northeastern California. The disease is spread from tree to tree via root contacts and grafts but new infections are likely vectored by root feeding bark beetles (Coleoptera:Scolytidae). Soil and site relations along with disturbance are factors in the etiology of the disease. Thinning and prescribed burning are important silvicultural tools in maintaining forest health in eastside pine stands. Because soil compaction is a concern in many sites, skid trails are treated by subsoiling equipment to alleviate compaction where this might be an issue. However, little is known of the effects of these silvicultural treatments on incidence of black stain root disease on sites with high disease risk. These studies were initiated to address these concerns.

Black stain root disease of ponderosa pine, caused by *Leptographium wageneri* var. *ponderosum* (Harrington & Cobb) Harrington & Cobb, is increasing on many eastside pine stands in northeastern California. The disease is spread from tree to tree via root contacts and grafts but new infections are likely vectored by root feeding bark beetles (Coleoptera:Scolytidae). Soil and site relations along with disturbance are factors in the etiology of the disease. Thinning and prescribed burning are important silvicultural tools in maintaining forest health in eastside pine stands. Because soil compaction is a concern in many sites, skid trails are treated by subsoiling equipment to alleviate compaction where this might be an issue. However, little is known of the effects of these silvicultural treatments on incidence of black stain root disease on sites with high disease risk. This study was initiated in 2000 to address these concerns.

A site near Poison Lake on the Lassen National Forest in California was selected for this study. This predominantly ponderosa pine site has numerous black stain root disease centers and was thinned during the fall of 1999. Prior to thinning, the average basal area was 263 ft^2 / acre (60 m^2 / hectare) and the average QMD (quadratic mean diameter) was 7.9 inches (20 cm). Post thinning stand density was

121 ft^2 / acre (28 m^2 / hectare) with an average QMD of 14.8 inches (38 cm). Treatments consisting of underburning, subsoiling, underburning and subsoiling, and untreated control were completed during the 2000 season. The study design is a randomized complete block replicated four times. Each treatment plot is approximately 2.5 hectares in size. An unthinned area near the experimental plots was maintained as a comparison, but not included in the overall experimental design.

Pretreatment surveys were conducted and pre-existing mortality and symptomatic trees identified and tagged. Post treatment, during the spring of 2001 and 2002, Lindgren flight traps were deployed and trapped insects were counted and identified. Sub-samples of these species from the trap catches were subjected to DNA analyses to determine presence of *L. wageneri*. We conducted the final post-treatment survey during June 2005 and recorded data on mortality, black stain root disease, and crown symptoms.

Experiments involving large woody root inoculations were conducted to provide information on the minimum amount of spores carried by insects that are necessary to start root infection. In June 2005 and again in August, spore suspensions containing 50, 500, and 5,000, spores were injected in artificial wounds created by coring to 2 cm depth in the xylem with a 4 mm diameter increment hammer. The spore suspensions were placed into roots of randomly selected trees in the burn only and control plots. Lesions, including sterile control wounds were measured after 9 weeks. Two trees selected from each burn only and control plot were inoculated with either 5,000 spores (August 2004) or an *L. wageneri* infested wood core (June 2004). These roots were excavated in June 2005. We obtained data on stem cambial sucrose synthase (SCSS) activity, a surrogate for determining stress and carbohydrate status of the trees, during the 2002, 2003, and 2004 season.

While the study is long-term, intermediate results are interesting. Several root feeding insect species of interest, suspected to be potential vectors of *L. wageneri*, were caught during the two seasons. Among the more common species were *Hylastes macer*, *Hylurgops subcostulatus*, and *Hylurgops porosus*. Treatment differences in total insect trap catches are not obvious, although the underburn only plots tended to have slightly higher catches during the latter half of the flight season. In 2002, this trend appeared to be more marked, with greater catch numbers later in the season. Recently, DNA evidence indicates the insect species mentioned above, among others trapped on the study plots are carrying *L. wageneri,* presumably as spores (3). Such insect species have been suspected but heretofore have not been confirmed to be carrying *L. wageneri* in ponderosa pine stands. This confirms the long held notion that root feeding Scolytids serve as

potential vectors of the fungus, critical for spread of the disease over longer distances.

Between 2001 and 2005, the burn only treatment had the highest mortality. Scorching was evident on most of the mortality trees, which succumbed within two years following treatments. It has been approximately a century since fire last occurred in these stands. The subsoiling and burn treatment had considerably less mortality than the burn only treatment. The subsoiled skid trails may have served to mitigate at least partially fire severity or intensity in these plots. Thus, caution should be exercised when reintroducing fire to stands that have not been burned for a considerable time.

In 2005, 100% surveys of each plot showed symptomatic trees, based upon crown characteristics, were distributed evenly among treatments, and few confirmed black stain root diseased trees were found. This is to be expected due to the longer time interval we anticipate from treatment initiation to infection, colonization, and symptom expression in the trees. Thus, further long-term monitoring of these study plots is necessary and planned.

Sucrose synthase activity, a measure of tree physiological status, shows a seasonal trend between the sampled months in 2003, and 2004. Peak activity is attained during July and August and drops rapidly during September. This is consistent with other data reported earlier for ponderosa pine (2). These and future data are used to determine relationships between physiological status and infection.

Findings in 2004 inoculation experiments using the three dosages of a local isolate of *L. wageneri* spores (1) are summarized below.

1) The June inoculations produced larger lesions in roots after 9 weeks than the August inoculation.
2) The lowest spore dose, 50 spores, produced lesions that were significantly larger than controls (June inoculations).
3) This is noteworthy because it is consistent with the range of spore numbers found on potential insect vectors as determined by DNA analyses.
4) Underburned plots had generally smaller lesions than control plots.
5) Lesions from August inoculations were significantly smaller than June inoculations.
6) We recovered *L. wageneri* from lesions approximately one year (June 2005) after inoculation (June and August 2004) with either 5,000 spores or mycelial inoculum.

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Phlebiopsis gigantea survival after treatment and its natural stump colonization

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CONFERENCE ABSTRACT

The study was carried out in 16 Scots pine stands. 12 stands were chosen for the investigation of *P. gigantea* survival in stumps after treatment (one, 2, 3, 5 and 6 years after treatment). Each stump was described on the presence of saprotrophs and pathogens or other fungi presence. In addition from 20% of stumps, randomly chosen, were cut the discs and carried to laboratory for analyzing the colonization of fungi. In the other 4 stands (treated one, two, three and four years ago) was cut 20 trees in a crossing of the net 20 x 20 m and leaved for natural *P. gigantea* infection. The plots were established in May and after half year were investigated for stumps natural colonization. Six years after treatment *P. gigantea* survived in 12% of stumps, but many stumps disappeared or were totally decayed. Four years after treatment live mycelium of saprotroph was found in 33% stumps, and the natural colonization of fresh stumps in such stand occurred in 55% stumps. The highest natural colonization was found in stands treated a year and three years before experiment. The success of natural colonization of fresh stumps by *P. gigantea* depends on frequency of saprotroph in stumps, weather suitable for production spores and for colonization and the treatment quality.

Biological control of *Heterobasidion* spp. is a routine action in Scots pine stands growing in a first and second generation in the post agricultural lands irrespective of the degree of pathogen infestation. In Poland this method was improved to forest in early 70s. Up to now the commercial formula is based on alive and active mycelium overgrowing the sawdust. The application of *P. gigantea* formula on stumps is done manually.

The aim of this study was to investigate the possibility of survival of *P. gigantea* in stumps in time and saprotroph activity in natural colonization of appeared fresh stumps.

The study was carried out in 16 Scots pine stands. Twelve stands were chosen for investigation of *P. gigantea* in stumps after treatments. In those stands the Polish commercial formulation Pg-IBL was applied on the stumps surface and cover by litter. Pine stumps were treated six, five, four, two and one year before investigations.

The first estimation was done in the stands on the base of presence of *P. gigantea* mycelium on stumps or under bark and *Heterobasidion annosum* basidiocarps, while the final analyze was done in laboratory. Whereas in four stands the natural colonization of stumps by saprotroph was analyzed. In May, in each stand twenty trees were cut localized on the crossing of the net 20 x 20 m. Stumps were analyzed six month after cutting.

Survival. On the base of investigation in forest, mycelium of *P. gigantea* was observed the most frequently in stands, where treatment were carried out a year before investigation (63%). Six years after inoculation the saprotroph mycelium was recognized only on 29% of stumps, however five years after treatment the mycelium was found only on 8% of stumps In each stand treated a year, four and five years before the laboratory analysis of stumps showed that more stumps (10%-20%) were colonized by alive saprotroph in comparison to forest observations. The opposite situation was noted in stands two and six years before investigation (Figure 1).

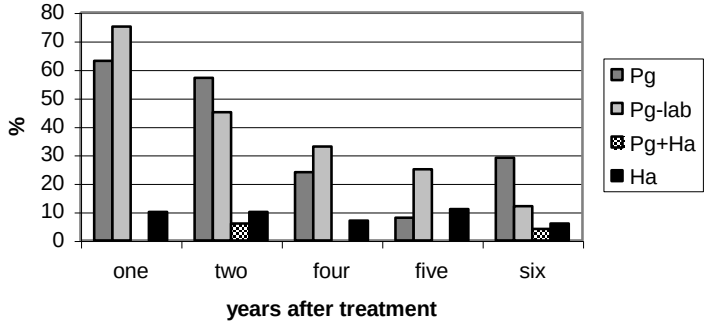

Figure 1. *Phlebiopsis gigantea* and *Heterobasidion annosum* stump colonization on the base of forest (Pg, Pg+Ha, Ha) and laboratory analyze (Pg-lab).

Natural colonization. Six months after cutting *P. gigantea* mycelium was found in 82,5% of stumps. Only in 16% of colonized stumps the mycelium occupied restricted and small portion of wood (2-3 cm in diameter on the surface). The surface of the rest stumps was colonized almost in the whole. In stands where the treatment was done a year and three years before investigation all stumps were colonized by saprotroph. Even after four years *P. gigantea* is able to effective stump colonization (55% of fresh stumps, Figure 2).

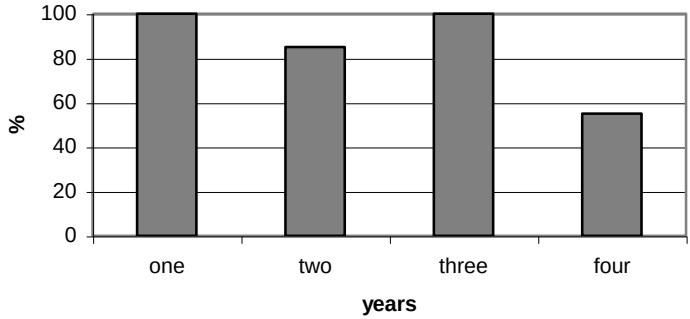

Figure 2. Natural stumps colonization of *P. gigantea* in dependence on treatment time.

Phlebiopsis gigantea could survive in stumps wood for a long time. Moreover the mycelium could be active, produce spores, spread through roots and colonized new stumps in Scots pine stands. Survival time of saprotroph could be even longer, because Sierota (1) found that 12 years after *P. gigantea* inoculation, saprotroph had been still effective in stumps colonization. The *P. gigantea* survival in stumps depends on quality of both preparation and treatment.

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Comparing biological stump treatments used against *Heterobasidion* root rot on pine and spruce in the UK

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CONFERENCE ABSTRACT

Two stump treatments are currently approved in the UK for use against the root and butt rot pathogen *Heterobasidion annosum:* the biological control agent, PG Suspension (active ingredient *Phlebiopsis gigantea*) and the commodity chemical, urea. Another *P. gigantea*-based biological control agent (Rotstop) is approved for use in Scandinavia on *Pinus* spp. and *Picea abies.* UK government policy strongly encourages the use of non-chemical protection wherever possible. However, PG Suspension is currently only licensed for use on *Pinus* spp., and the main tree species grown commercially in the UK is a North American spruce species, *Picea sitchensis,* which is very susceptible to root and butt rot. Therefore control of *H. annosum* on *P. sitchensis* is achieved through applications of urea, although this is not always effective. A potentially more effective option would be to use Rotstop on spruce species, although there is no approval for use of this product in the UK. This paper outlines some of the laboratory and field studies which are currently underway at Forest Research to evaluate whether Rotstop is able to combat *H. annosum* on *P. abies* and *P. sitchensis* in the UK. The test data could be used to support the registration of Rotstop for use in the UK through Mutual Recognition procedures under EU directive 91/414.

Two stump treatments are used in the UK to combat infection by the decay fungus *Heterobasidion annosum*. Urea is used on all conifers, whilst the biological agent, PG Suspension, based on spores of the saprotroph, *Phlebiopsis gigantea*, is restricted to *Pinus*. This fungus out-competes *H. annosum* at the stump surface if applied immediately after cutting.

Spruce comprises 50% of the UK conifer crop, the main species being the North American Sitka spruce [SS] (*Picea sitchensis*), and pine *circa* 30%. Government policy aims to reduce chemical usage so our objective is to extent biological treatment to spruce as well as pine. This paper reviews two field studies conducted to examine the efficacy of PG Suspension and the Scandinavian equivalent, Rotstop, on Corsican pine [CP] (*Pinus nigra* ssp. *laricio*), and SS.

In 2004 and 2005 respectively, a 28 year old SS stand and 24 year old CP pine stand were thinned. Using a drench gun stumps were

treated with PG suspension, Rotstop, urea (all at manufacturers' recommended dose) or water. The next day SS stumps were inoculated with *H. annosum* conidia (3 x 10^3 l^{-1}), or basidiospores (8.5 x 10^4 l^{-1}), or left open to natural infection. CP stumps were treated with conidia (1.5 x 10^4 l^{-1}), or again left untreated. After 12 months, discs were cut from all stumps, damp-chamber incubated for 7 days and examined for the presence of *H. annosum* conidiophores.

There were significant treatment differences with pine (P<0.001; Figure 1); PG Suspension and Rotstop completely excluded the pathogen from stumps whilst urea was much less effective (31% of stump surface colonised by *H. annosum*), although better than the water treatment (37% colonisation). None of the treatments significantly reduced colonisation on SS (p=0.862) and *H. annosum* was found on 54% of stumps, colonising 9% of the surface.

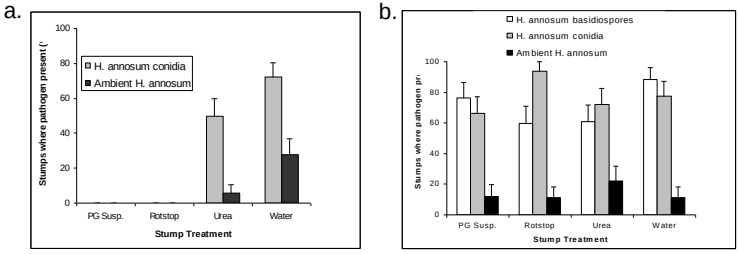

Figure 1: The proportion of **a.** CP and **b.** SS stumps colonised by the pathogen following stump treatment (errors bars show 1 SE).

Low efficacy of urea is not uncommon on SS, but it was unexpectedly poor on pine. Both biological control agents are used routinely on pine species, and they performed well. Although the isolate of *P. gigantea* in PG Suspension was originally isolated from lodgepole pine, LP (*P. contorta* var. *latifolia*), the Rotstop isolate derives from Norway spruce (*P. abies*). It was thought this might confer greater efficacy on SS, but it appeared not to be the case. Although *P. gigantea* colonised SS, growth was markedly slower than on CP (2), and European *P. gigantea* isolates seemed unable to compete with *H. annosum* on SS in the field.

The intractability of SS to colonisation also affected *H. annosum*, with spore germination reported lower on SS than LP (1). Here, *H. annosum* colonised smaller areas of unprotected SS than CP stumps, despite the higher spore concentrations used on the former. Further work on SS is recommended to establish the reasons for its

intractability, and field trials are underway to establish if increased *P. gigantea* spore concentrations aid efficacy.

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Appendix

Color Plates

Pg. 66

Growth of Scots pine seedlings and *Armillaria ostoyae* rhizomorphs under elevated air CO_2 conditions, P. LECH AND A. ŻÓŁCIAK

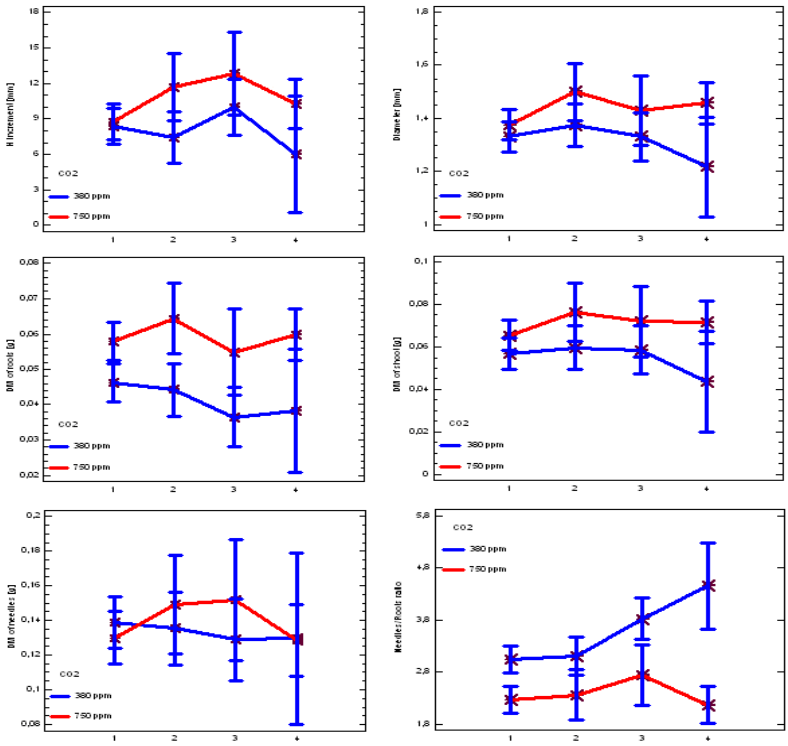

Figure 1. Pg 266. Effects of the introduced pathogen inoculum and rhizomorphs development on growth of seedling under control and elevated air CO_2 conditions (1 – no inoculation; 2 – lack of rhizomorphs; 3 – moderately developed rhizomorphs; 4 – rhizomorphs abundant).

Pg. 104
Losses in *Quercus rubra* infected by the root pathogen *Collybia fusipes* in Southwest-Germany, M. HALSDORF AND B. METZLER

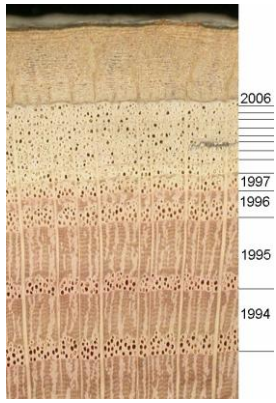

Figure 1. Cross section at breast height of a red oak infected by *Collybia fusipes*. Note the rapid decline in annual radial increment after 1995. The annual rings of the last decade before felling in 2006 are hardly discernible.

Pgs. 107 & 108 & 109.
The importance of rhizomorphs in *Armillaria* spp., J.A. TURNER AND R.T.V. FOX

Figure 1. Pg 107

Figure 2. Pg. 107

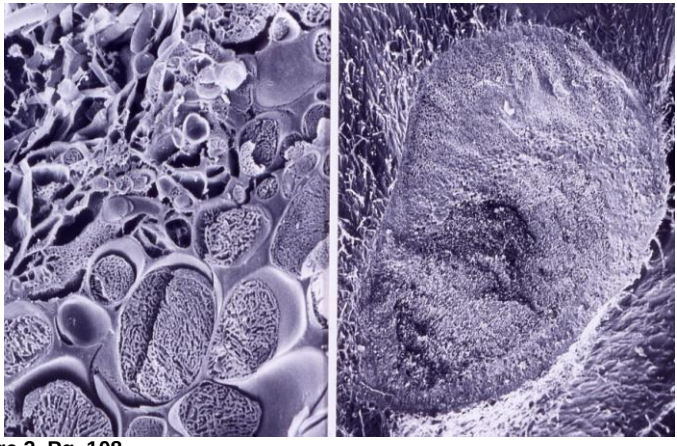

Figure 3. Pg. 108

Figure 4. Pg. 109

Pg. 113

Distribution of *Heterobasidion annosum* inoculum in the Landes de Gascogne forest: influence of soil and silvicultural practices, B. LUNG-ESCARMANT, J. LEMOINE, T.AUMONIER AND F. MAUGARD

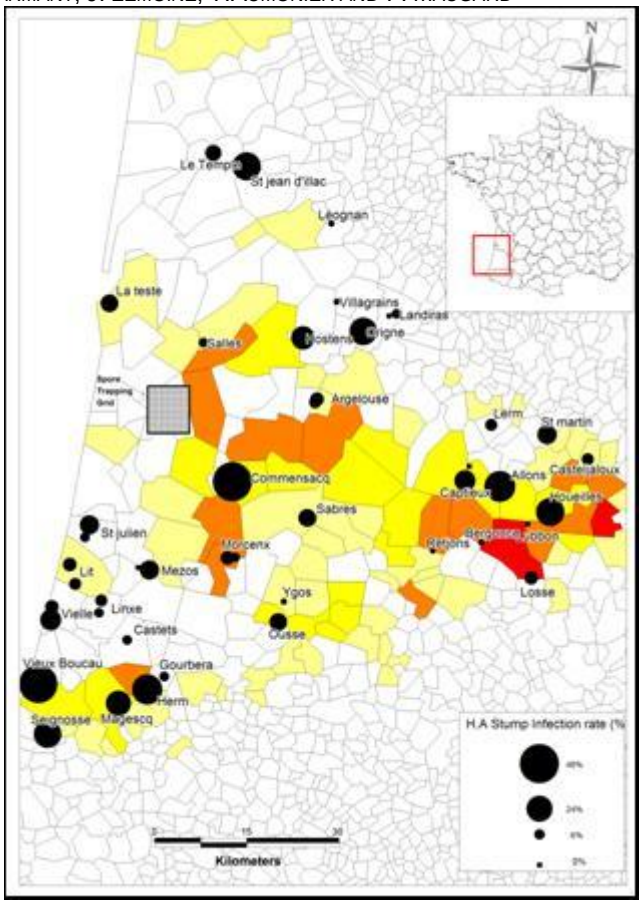

Figure 1. Pg. 113. Localization of the 48 selected first-thinned stands with their *H. annosum* stump infection rate (size of the circles proportional to the infection rate) and distribution of Annosum root rot (A. R. R.) observed in the townships of the LdG forest (colors of the townships refer to the number of observations of A. R. R: white, no A. R. R.; pale yellow, 1 to 2; yellow, 3 to 5; orange, 6 to 9 ; red, 10 to 16).

Pg. 134, 135, & 136
Survey of potential wood decay fungi on *Eucalyptus globulus* coppice stumps in south-western Australia, F.J. TOVAR, T. BURGESS, G.E. HARDY AND R.M. ROBINSON

Figure 1. Pg. 134. 24-month-old windthrown *E. globulus* stump, 6 main shoots were lost. Note that this tree was on the edge of the plantation.

Figure 2. Pg135. *E. globulus* stump 24 months after harvest colonised by *S. hirsutum*. Arrows indicate where decay has entered from the stump into the coppice shoot.

Figure 3. Pg 136. Fungi commonly observed on *E. globulus* coppice stumps. From left to rigth and top to bottom; *Trametes versicolor*, *Stereum hirsutum*, *Stereum illudens*, *Pynoporous coccineus*, species A, species B.

Pg. 140, 141, 142, & 143

Distribution and ecology of *Armillaria* species in northern Spain, E. ITURRITXA, M. LEÓN, I. GARCÍA-SERNA, E. HEPPE, J. GARCIA-JIMENEZ AND A. PEREZ-SIERRA

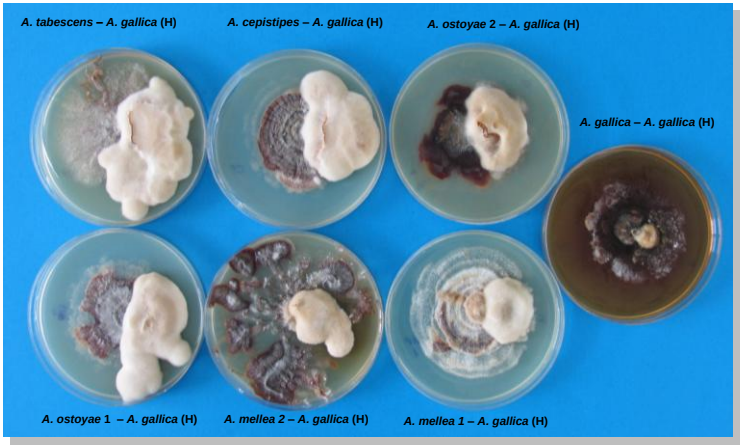

Figure 1. Pg. 140. Pairings of a selection of diploid isolates with *A. gallica* haploid (H)

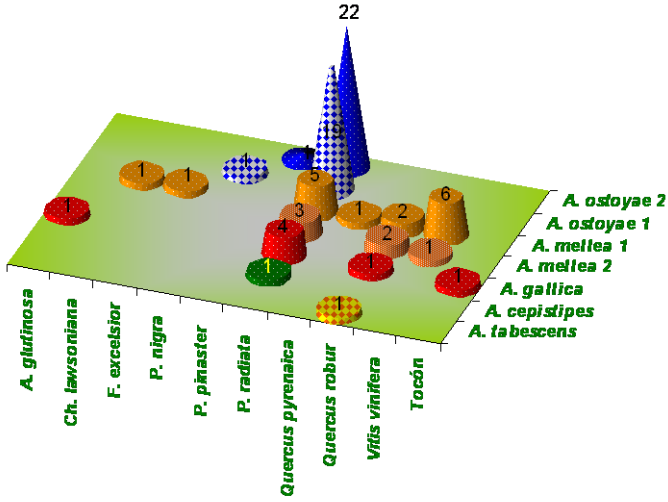

Figure 2. Pg. 141. Frequency of species of *Armillaria* in relation to species of host.

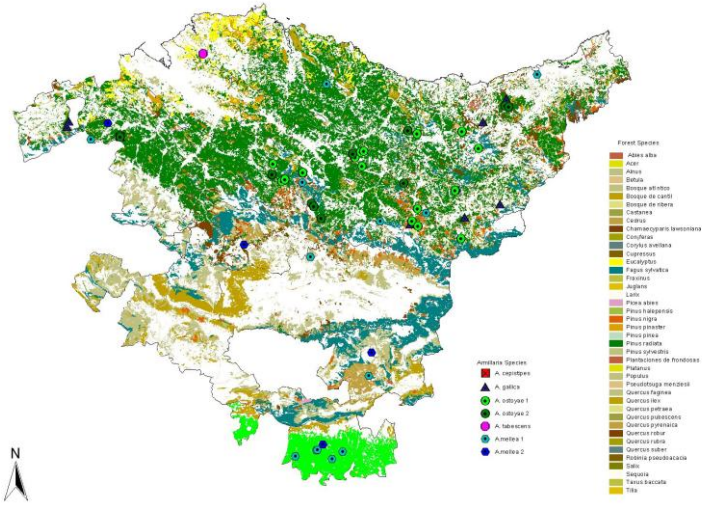

Figure 3. Pg. 142. Distribution of forest species and detected species of *Armillaria* in the Basque Country.

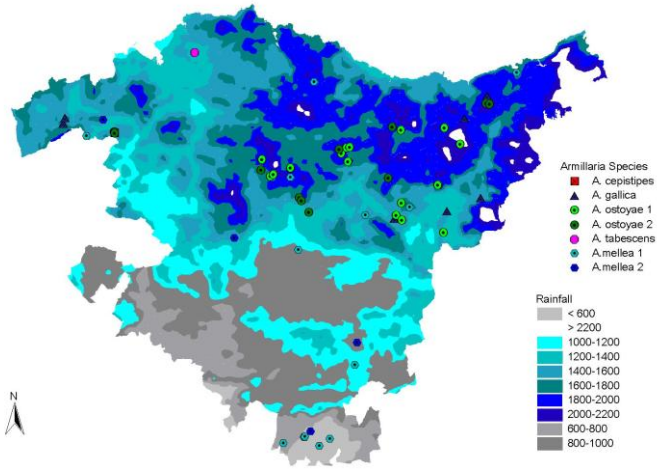

Figure 4. Pg. 143. Distribution of rainfall levels and detected species of *Armillaria* in the Basque Country.

Pg. 145
Screening a population of half-sibling interior Douglas-fir seedlings for resistance to *Armillaria ostoyae,* M.G. CRUICKSHANK AND B. JAQUISH

Figure 1. Pg. 145. An example of the Douglas-fir seedling and birch block inoculum source of *Armillaria ostoyae* used in the study.

Pg. 169
Early infection of *Fagus sylvatica* by *Heterobasidion annosum sensu strict*, P. ŁAKOMY, R. CIEŚLAK, T. ZAKRZEWSKI[1] AND M. DALKE

Figure 1. Pg. 169.. *Heterobasidion annosum* s.s. sporocarp around root collar of beech with still green leaves.

Pg. 227
Interaction between *Heterobasidion parviporum* and *Laetiporus sulphureus*

U. HETTICH, T.N. SIEBER AND O. HOLDENRIEDER

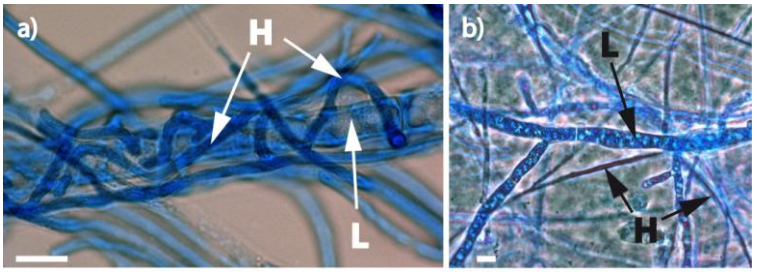

Figure 1. Pg. 227. Interaction between *H. parviporum* (H) and *L. sulphureus* (L) in a two week old dual culture on MEA: (a) Aerial hyphae of *H. parviporum* coiling around a hypha of *L. sulphureus* (Size bar = 10 µm); (b) submerged viable hyphae of *H. parviporum* and submerged vacuolated, nonviable hyphae of *L. sulphureus* (Size bar = 10 µm). Mounting medium: Lactophenol blue solution (Merck no. 13741).

Memories

Yosemite National Park

Yosemite valley

Mammoth Lakes area

Oregon field trip