available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/mycres

Genetic population structure of three *Armillaria* species at the landscape scale: a case study from Swiss *Pinus mugo* forests

Muriel BENDEL[a,b], *Felix KIENAST*[a], *Daniel RIGLING*[a,*]

[a]*Swiss Federal Institute for Forest, Snow and Landscape Research WSL, CH-8903 Birmensdorf, Switzerland*
[b]*Forest Ecology, Dept. of Environmental Sciences, Swiss Federal Institute of Technology ETH, CH-8092 Zurich, Switzerland*

ARTICLE INFO

Article history:
Received 22 November 2005
Accepted 11 February 2006
Published online 17 April 2006
Corresponding Editor:
Brenda Wingfield

Keywords:
Armillaria root disease
Basidiomycota
Landscape pathology
Mountain pine
Population biology
Somatic incompatibility

ABSTRACT

Armillaria species are plant pathogens that cause Armillaria root rot and are known to cause mortality of mountain pines (*Pinus mugo*) in the Swiss National Park in the Central Alps. The identity of isolates and the spatially explicit population structure of the *Armillaria* species were investigated in a 3.3 km^2 study area in the Swiss National Park. In total, 242 *Armillaria* isolates, 205 from wood samples and 37 from epiphytic rhizomorphs, were collected. Species were identified using haploid–diploid pairings and genets were determined using intraspecific somatic incompatibility tests. The population structure differed markedly among the *Armillaria* species. *A. cepistipes* and *A. borealis* mainly occurred as genets of small spatial extent (mean 0.2 ha, and 0.6 ha), whereas *A. ostoyae* formed significantly larger genets (mean 6.8 ha). The largest *A. ostoyae* genet extended over approx. 37 ha. Several disease centres associated with *Heterobasidion annosum* were found to be embedded within large *Armillaria* genets. The extension of large *A. ostoyae* genets suggests that forests that occupy the study area have developed in the presence of these *Armillaria* genets. The finding of large *Armillaria* genets supports the assumption that large genets occur in areas with cold climate and little precipitation.

Introduction

Landscape pathology, the interdisciplinary field that links forest pathology and landscape ecology, is attracting increasing interest (Holdenrieder *et al.* 2004; Lundquist & Klopfenstein 2001). To understand the structure and the evolutionary pattern of vegetatively growing organisms, it is important to identify genetically identical units in a population. Numerous studies have been conducted to investigate the genetic population structure of vegetatively growing plants (e.g. Cook 1983) or fungi (e.g. Anderson & Kohn 1995) such as *Armillaria* (e.g. Ferguson *et al.* 2003; Kile 1983; Smith *et al.* 1992; Worrall 1994).

Pathogens can reduce plant fitness, cause mortality, and thereby change the structure and composition of plant communities and landscape patterns. At the same time, plant pathogens can facilitate successional processes or help to maintain species diversity (Gilbert 2002). In forest ecosystems, *Armillaria* species are important components both as pathogens and saprotrophs. By means of somatic spread via root contacts or rhizomorphs, *Armillaria* can occupy large areas with genetically identical mycelium (Kile *et al.* 1991). These *Armillaria* genets are known to be inherently territorial, stable, and potentially long-lived, and many represent a relatively stable mosaic in the forest landscape (Bruhn *et al.* 2000;

* Corresponding author.
E-mail address: daniel.rigling@wsl.ch
0953-7562/$ – see front matter
doi:10.1016/j.mycres.2006.02.002

Guillaumin & Legrand 2001; Hodnett & Anderson 2000). The influence of *Armillaria* genets on forest dynamics can extend over centuries and even millennia (Ferguson *et al.* 2003; Smith *et al.* 1992). Bruhn *et al.* (2000) called the spatial patterns of *Armillaria* genets the genetic 'memory' of a forest landscape.

Genetically identical units in a population of vegetatively growing organisms are most often referred to as clones or genets. The concept of the genet goes back to Kays and Harper (1974) and was originally applied to plants, and later also to fungi (e.g. Rayner 1991). A fungal genet has been defined as the mycelium that is produced by somatic spread of the fungus following an initial sexual mating event (Anderson & Kohn 1995; Dettman & van der Kamp 2001a; Smith *et al.* 1994). The mycelium needs to be genetically identical, whereas spatial continuity is not a prerequisite for a genet. A genet may be fragmented into several discontinuous patches, so-called ramets (Dettman & van der Kamp 2001a).

Our knowledge about spatial processes in plant pathogens is still limited. One of the major shortcomings is that most earlier studies have described the genetic population structure of pathogens such as *Armillaria* in areas several hectares in size, whereas only a few focused on larger spatial scales (e.g. Dettman & van der Kamp 2001a; Ferguson *et al.* 2003; Smith *et al.* 1992). In the southern Swiss Alps, Prospero *et al.* (2003) studied the genetic population structure of *Armillaria* species in three one-hectare plots in managed Norway spruce stands. Seven to nine genets were found per hectare, which is comparable with the average density of *Armillaria* genets observed in other forest stands in Switzerland (D. Rigling, unpublished). On a two-hectare permanent plot in the mountain pine (*Pinus mugo* subsp. *uncinata*) forests of the Swiss National Park, Rigling (2001) distinguished five genets of *Armillaria*, two belonging to *A. borealis* and three to *A. cepistipes*. *Armillaria* is widespread in these forests and beside *Heterobasidion annosum* causes root disease and mortality of mountain pines (Cherubini *et al.* 2002; Dobbertin *et al.* 2001). Tree mortality often occurs in clusters and is associated with slowly expanding disease centres.

The objectives of our study were to: (1) identify the *Armillaria* species and determine the spatial extent, fragmentation, and potential age of *Armillaria* genets on a landscape scale in the mountain pine forests of the Swiss National Park; and (2) investigate the relationship between the population structure of *Armillaria* and the occurrence of disease centres associated with *Heterobasidion* and/or *Armillaria*. Data are discussed in the context of the history of the study site in the Swiss National Park, where direct human influences have ceased since the foundation of the Park in 1914.

Material and methods

Study area

The Swiss National Park is located in the Engadine in the Central Alps. The Park extends over an area of roughly 172 km^2, 50 km^2 of which are covered with forests. Its climate is characterised by an annual mean precipitation of 902 mm and an annual mean temperature of −0.1 °C (MeteoSchweiz; measured at the weather station Buffalora situated on the edge of the Park at 1970 m a.s.l.). During the vegetation period from May to September, the mean precipitation reaches 484 mm and the mean temperature 7.2 °C. Since the foundation of the Swiss National Park in 1914, traditional management activities such as logging, hunting, and livestock grazing have been excluded from the area.

This study was conducted in the mountain pine forests in the Ofen Pass Valley in the Swiss National Park (46°39′N to 46°41′N, 10°10′E to 10°16′E). The mountain pine forests are found mainly on the south exposed slopes, and extend over an area of approx. 10 km^2 at an altitude between 1800 and 2200 m a.s.l. Two study areas approx. 1.5 km apart were established (Fig 1A), Champlönch in the west (Fig 1B), and Il Fuorn in the east (Fig 1C). The study area Champlönch extends over 123 ha, and Il Fuorn encompasses 207 ha.

Sampling design

In 2003 and 2004, a nested sampling design was applied to determine the occurrence and spatial distribution of *Armillaria* species and genets within infection centres (>900 m^2), among infection centres, and within and among the two study areas. Disease centres were previously delineated on aerial photographs and characterised by less than 20 % tree cover and high incidence of mountain pine mortality (M. Bendel, unpublished). In the majority of the 40 disease centres inspected, nine to ten symptomatic or recently dead mountain pines that were found within or at the edge of the disease centre were assessed for root rot fungi (M. Bendel, unpublished). In addition, the forest matrix between the disease centres was searched for *Armillaria*. For this purpose, the two study areas were divided into a total of 15 sub-areas of similar size (approx. 20 ha). In every sub-area, as many as possible symptomatic or recently dead mountain pines of any height were checked for *Armillaria* fans during one day. Those trees that showed mycelial fans below the bark at the root collars were sampled. The positions of all trees in disease centres and the forest in-between were determined with GPS (eTrex Summit, Garmin) with an accuracy of <10 m. Of the trees sampled, three main roots were excavated, and one wood core sample was taken from every root at a distance of approx. 20 cm from the stem using an increment borer. Between every root sample, the increment borer was sterilised in 70 % ethanol. Depending on the size of the smaller trees or saplings, either their whole root system was dug out or three main roots were sampled as described above. The sample cores were placed in sterile plastic tubes and kept cool until isolation. All wood samples were processed within four days after sampling. When present on the examined roots, epiphytic rhizomorphs were also collected.

Isolations

Three pieces (approx. 1 cm long) of each root sample were surface sterilised in sodium hypochlorite (7 % active chlorine) for 30 s and rinsed twice in sterile, demineralised water for ≥15 s. The pieces were dried between paper towels and placed on agar plates (90 mm in diameter) containing 20 $g\,l^{-1}$ malt extract, 15 $g\,l^{-1}$ Bacto Agar, 230 $mg\,l^{-1}$ thiabendazole (added in 1 ml concentrated lactic acid, 85–90 %), 100 $mg\,l^{-1}$ streptomycin, 50 $mg\,l^{-1}$ polymyxin sulphate, and 100 $mg\,l^{-1}$ sodic

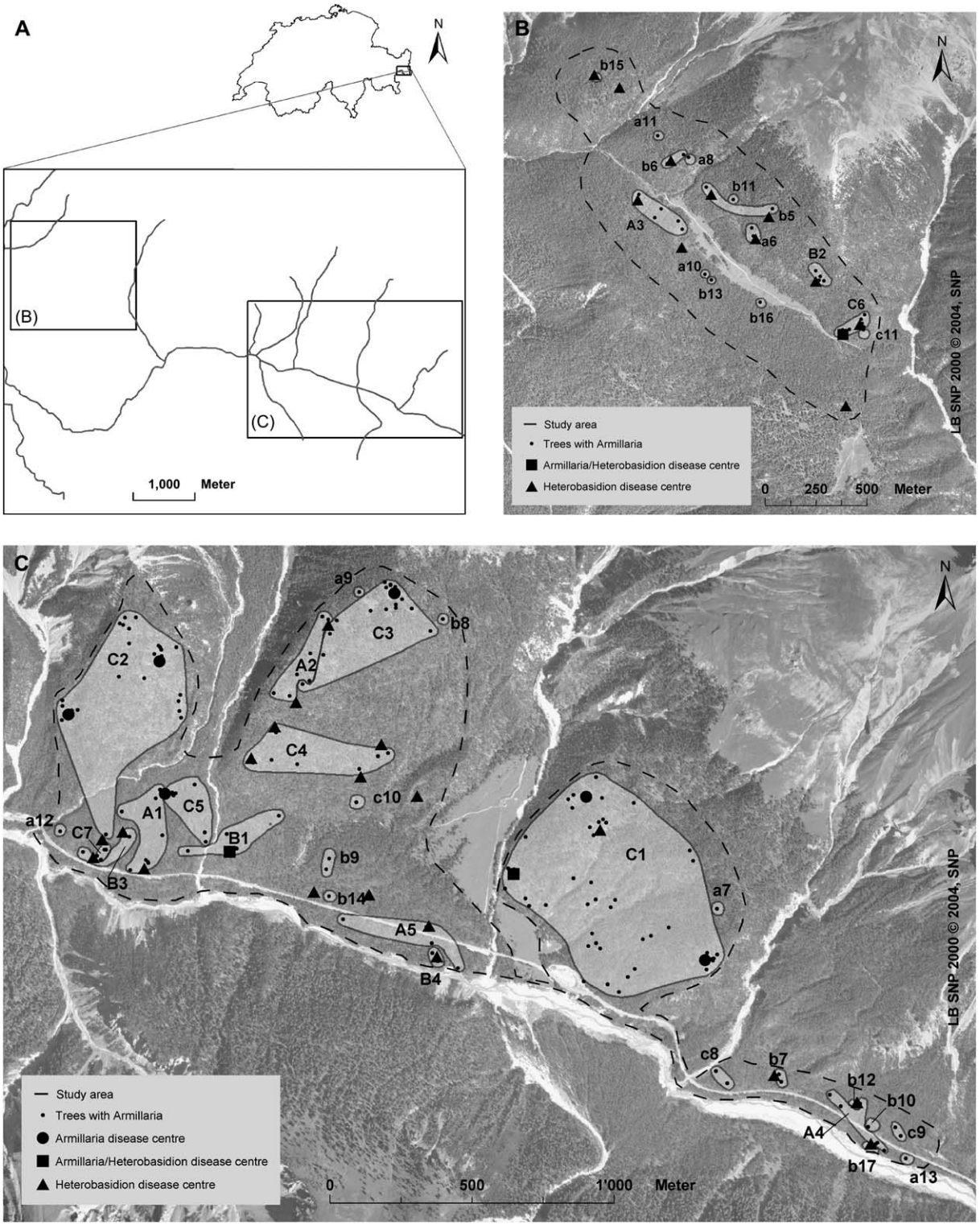

Fig 1 – Location of the study area in Switzerland (A), and spatial distribution of genets of *Armillaria ostoyae* (c), *A. borealis* (a), and *A. cepistipes* (b) in Champlönch (B) and Il Fuorn (C). Putative boundaries are drawn around the genets; all genets are numbered and those marked with capital letters are listed in Table 3. The study area that was inspected for *Armillaria* is encircled by a dashed line. Location of disease centres (M. Bendel, unpublished) associated with *Armillaria*, *Heterobasidion*, or both pathogens are indicated with symbols.

benzylpenicillin (modified from Legrand & Guillaumin 1993). *Armillaria* was isolated from rhizomorphs as described above for the pieces of wood, except that they were only surface-sterilised for 15–30 seconds. All plates were incubated in the dark at room temperature. After two to four weeks, pure cultures were transferred to malt extract agar (20 g l^{-1} Bacto Agar; 20 g l^{-1} Diamalt).

Delineation of genets and species identification

Following the procedure described by Prospero *et al.* (2003), *Armillaria* isolates were assigned to genets using intraspecific somatic incompatibility, the self–nonself recognition system in basidiomycetes (Worrall 1997). Three to four pairings were done per plate (90 mm in diameter) on Shaw and Roth's agar medium (Harrington *et al.* 1992), and every pairing was repeated once. Positive controls consisted of self-pairings that were repeated once. Two isolates were considered compatible when they merged to a single culture. A line of demarcation between the cultures indicated somatic incompatibility. Ambiguous pairings, which were very rarely observed, were repeated.

To keep the number of somatic incompatibility pairings manageable, a hierarchical design of pairings was adopted. First, if more than one isolate was available from one tree, the isolates were paired among each other. Then, one *Armillaria* isolate from each genet per tree was selected, and paired among each other within the same disease centre. In a third step, one isolate from each genet(s) in a disease centre was randomly chosen and paired with all other genets of the same species within the study area. *Armillaria* isolates collected between the disease centres were first paired with a randomly chosen isolate from each genet in the same study area. In a next step, those isolates that could not be assigned to an already defined genet were paired with each other.

One isolate from each genet was identified to the species level by pairing with haploid tester strains of *A. cepistipes*, *A. borealis*, and *A. ostoyae* (Korhonen 1978). A previous study has shown that the low altitude *Armillaria* species, *A. gallica* and *A. mellea*, do not occur in the mountain pine forest of the Swiss National Park (Rigling 2001). The isolates were paired with three tester strains of each *Armillaria* species on malt extract agar (20 g l^{-1} Bacto Agar; 20 g l^{-1} diamalt) and the change of the cottonous mycelial type of the tester strains into the crustose mycelial type (Buller phenomenon) was recorded after three to five weeks.

An isolate recovered from a single mountain pine was considered to occupy a circle area of 100 m^2 (radius of 5.6 m) around the tree assuming that the isolate extending via rhizomorphs a little beyond the root system of the infected tree. Polygons were drawn around mountain pines infected with the same genet, using a radius of 5.6 m around the trees. These putative genet boundaries and genet sizes were drawn and calculated in ArcMap™ 8.3 (© ESRI Inc. 1999–2002). To better visualise small genets in Fig 1B–C, genet boundaries were drawn with a radius of approx. 20 m around the infected trees. A genet is regarded as discontinuous or fragmented if its area is separated by intersecting landscape elements such as creeks or roads. Isolates separated by a great distance were not regarded to represent fragmented genets because our sampling intensity at a smaller scale was too low to support this assumption.

Statistical analysis

Differences in genet size were tested using one-way analysis of variance (ANOVA). Before analysis, genet size was $\log_{10}$-transformed. Statistical data analysis was performed on R, version 1.9.1 (R Development Core Team 2004).

Results

Armillaria *species*

We collected 137 *Armillaria* isolates from 96 trees in 33 disease centres. Seven disease centres did not yield any *Armillaria* isolates. In the forest matrix between the disease centres, 143 symptomatic or dead mountain pines that showed mycelial fans were found. Isolates of *Armillaria* were obtained from 105 (72 %) of them. From the total of 242 *Armillaria* isolates collected (37 isolates from Champlönch; 205 from Il Fuorn), 205 isolates were obtained from roots and 37 from epiphytic rhizomorphs. Of the 201 mountain pines from which *Armillaria* spp. were isolated, eight were symptomatic but still alive, and 193 were recently dead.

Most of the isolates were recovered from root samples, with *A. ostoyae* being the most frequent *Armillaria* species followed by *A. cepistipes* and *A. borealis*. *A. cepistipes* dominated the isolates recovered from rhizomorphs (Table 1). A total of 26 trees yielded more than one isolate, either from different roots or from roots and epiphytic rhizomorphs. On one tree, two different *Armillaria* species were found: the epiphytic rhizomorphs were from *A. cepistipes*, while the roots were colonised by *A. ostoyae*. All other trees yielded isolates of the same *Armillaria* species and genet.

Armillaria *genets*

Somatic incompatibility pairings among the *Armillaria* isolates revealed 11 genets of *A. ostoyae*, 13 of *A. borealis*, and 16 of *A. cepistipes* (Table 2). In the study area of Champlönch, two

Table 1 – Number of trees from which *Armillaria ostoyae*, *A. borealis*, or *A. cepistipes* was isolated from root samples and/or epiphytic rhizomorphs

Species	Only root	Root and rhizomorphs	Only rhizomorphs	Total
Armillaria ostoyae	118	5	7	130 (64 %)
Armillaria borealis	34	2	4	40 (20 %)
Armillaria cepistipes	16	5	11	32 (16 %)
Total	168	12	22	202 (100 %)[a]

a From one tree, *A. ostoyae* was isolated from the roots, and *A. cepistipes* from the rhizomorphs. This tree was counted for both species.

Table 2 – Characteristics of *Armillaria* populations in the mountain pine forests of the Swiss National Park

Characteristics	*Armillaria ostoyae*	*Armillaria borealis*	*Armillaria cepistipes*
Number of genets	11	13	16
Mean genet size (ha)	6.8	0.6	0.2
Total genet area (ha)	75.2	7.9	3.1
Portion of study area colonised (%)	23 %	2 %	1 %
Mean (median) distance from pass route (m)	297 (225)	263 (165)	176 (130)

genets of *A. ostoyae*, five of *A. borealis*, and seven of *A. cepistipes* were found, whereas in Il Fuorn, nine genets of *A. ostoyae*, eight of *A. borealis*, and nine of *A. cepistipes* were recovered. *A. ostoyae* produced the largest genets with a mean size of 6.8 ha, compared with 0.6 ha for *A. borealis* and 0.2 ha for *A. cepistipes*. Two *A. cepistipes* genets were found only as epiphytic rhizomorphs from one tree each (b15 in Fig 1B, b12 in Fig 1C). All other genets were recovered from at least one root sample. The difference in genet size between *A. ostoyae* and the other two species was statistically significant (one-way ANOVA, testing *A. ostoyae* against *A. borealis* and *A. cepistipes* combined, $P = 0.01$). The cumulative colonisation area by *A. ostoyae*, *A. borealis*, and *A. cepistipes* was estimated as 23, 2, and 1 % in the 330-ha study area (Table 2). Most of the *A. ostoyae* trees were infected with a few large genets with sizes larger than 4 ha (Table 3). The largest *A. ostoyae* genet comprised 48 infected mountain pines and extended over a maximum distance of 800 m; it covered an estimated area of 37 ha. In comparison, the size of the largest genet of *A. borealis* was 2.4 ha, and of *A. cepistipes* 1.4 ha (Table 3). With very few exceptions, intraspecific somatic incompatibility pairings yielded unambiguous results. The few ambiguous pairings were repeated and could all be clearly determined.

In five Armillaria disease centres, only one *A. ostoyae* genet or ramet thereof was found. In one Armillaria disease centre, two genets, one of *A. ostoyae* (C3) and one of *A. borealis* (A2), were recovered (the *A. ostoyae* genet was isolated from five trees, and the *A. borealis* genet from one tree). The largest *Armillaria* genets encompassed several disease centres, many of which were predominantly occupied by *Heterobasidion* (Table 3, Fig 1B–C).

Discussion

Spatial distribution of genets

Our study of the *Armillaria* population on a large spatial scale (330 ha) in the mountain pine forests of the Swiss National Park yielded a mosaic of genets involving three *Armillaria* species. Relatively small genets prevail in Champlönch and in the lower elevation sites in Il Fuorn, whereas larger genets occur in Il Fuorn on the slopes. The largest genet is formed by *A. ostoyae*, and extends over approx. 37 ha. To our knowledge, this is the largest *Armillaria* genet known in Europe. *A. ostoyae* genets of approximate size have been reported, e.g. by Dettman & van der Kamp (2001a) from Canada (1–16 ha). Larger *A. ostoyae* genets (95–965 ha) have been found in the Western United States (Ferguson *et al.* 2003). However, depending on the study site and the management intensity, *A. ostoyae* may also form smaller genets (e.g. Guillaumin & Legrand 2001; Legrand *et al.* 1996; Prospero *et al.* 2003; Rishbeth 1991; Rizzo *et al.* 1995).

In our study area, genet sizes varied considerably between *A. ostoyae* and the two other *Armillaria* species. Assuming

Table 3 – Characteristics of the largest *Armillaria* genets ($n = 16$) that were found in the mountain pine forests of the Swiss National Park[a]

Genet	*Armillaria* species	No. of trees	Max. extent (m)[b]	Size (ha)	Number of disease centres within genet[c]		
					A	H	A & H
C1	*A. ostoyae*	48	800	37.25	2	1	1
C2	*A. ostoyae*	24	770	17.49	2	1	-
C3	*A. ostoyae*	19	480	7.77	1	1	-
C4	*A. ostoyae*	11	490	4.24	-	3	-
C5	*A. ostoyae*	10	215	1.69	1	-	-
C6	*A. ostoyae*	8	150	6.51	-	1	1
C7	*A. ostoyae*	4	40	0.08	-	1	-
A1	*A. borealis*	8	310	2.41	1	1	-
A2	*A. borealis*	6	320	1.78	-	2	-
A3	*A. borealis*	6	275	1.64	-	1	-
A4	*A. borealis*	6	275	0.66	-	2	-
A5	*A. borealis*	4	445	1.29	-	1	-
B1	*A. cepistipes*	6	370	1.41	-	-	1
B2	*A. cepistipes*	4	65	0.16	-	1	-
B3	*A. cepistipes*	3	175	0.35	-	3	-
B4	*A. cepistipes*	3	35	0.05	-	1	-

a Number of genets that were recovered from one (or two) trees: *A. ostoyae*: 2 (2); *A. borealis*: 7 (1); *A. cepistipes*: 7 (5).
b Measured between most distant collection points.
c Disease centre associated with *Armillaria* (A), *Heterobasidion* (H), and both fungi (A & H).

a similar rate of genet expansion, this would imply that the large *A. ostoyae* genets have been established a long time before most of the genets of the other two species. Differences among the *Armillaria* species in the lifespan or the rate of genet expansion could also explain the different sizes of the genets. Fruiting bodies of *Armillaria* were rarely observed in our study area (Favre 1960). Thus, both limited fruiting body production and unfavourable conditions for basidiospore establishment could account for the presence of large *Armillaria* genets in our study area. Our finding of large genets supports the hypothesis that in dry and/or cold areas mainly large *Armillaria* genets can develop because fruiting bodies are rarely produced, while small *Armillaria* genets predominate in moist and warm areas where fruiting is more frequent (e.g. Anderson *et al.* 1979; Rizzo & Harrington 1993; Worrall 1994).

Generally, new *Armillaria* genets arise through sexual reproduction after formation of fruiting bodies, i.e. when two haploid basidiospores with compatible mating-type alleles mate to form a secondary, diploid mycelium or genet (Guillaumin *et al.* 1991). However, little is known about the suitable conditions and the substrate required for basidiospore and genet establishment (Rishbeth 1988). It is often assumed that the appearance of new *Armillaria* genets is favoured if the forest is disturbed, e.g. by management activities that create fresh stumps where new *Armillaria* genets can establish (Guillaumin & Legrand 2001). In our study area, felling of trees is forbidden since the foundation of the Park in 1914. However, according to historical records dating back to the 14th century, the forests in this area have intensively been used for fuel and timber before that time (Parolini 1995). This was probably particularly high in the areas that were best accessible close to the pass routes. The felling of trees until the early 1900s most likely has favoured *Armillaria* species to establish new, still relatively small genets in the forest.

In this study, the number of trees from which *Armillaria* was isolated appears to be adequate to determine the genetic population structure of *Armillaria* species on the landscape scale. Nevertheless, small genets were certainly underrepresented in our study, and more detailed sampling most likely would have yielded a higher number of small genets. Also, somatic incompatibility has been reported to be in general an efficient and reliable technique to delineate *Armillaria* genets, but it has its limitations (Dettman & van der Kamp 2001a; Guillaumin *et al.* 1996; Rizzo & Harrington 1993; Smith *et al.* 1994). It was reported that approx. half of all pairings with sib-related isolates do not form a line of demarcation using somatic incompatibility tests (Kile 1983). Thus, with the method applied in our study, it cannot definitively be ruled out that some large *Armillaria* genets comprise sib-related smaller genets.

Fragmentation of genets

In Il Fuorn, two genets, one of *A. ostoyae* (C2) and one of *A. cepistipes* (B1), are discontinuous and extend over creek beds (Fig 1C). As both creeks are part of an alluvial fan, we assume that the genets expanded in times when the areas have not been separated by the creeks yet. After the creeks changed their directions, the genets were cut into discontinuous ramets. Along the present pass route (an asphalted main road approx. 8 m wide with cleared strip several metres wide on both sides of the road), three genets (A4, A5, and b10) are also dissected by the road into ramets, indicating that the genets have probably occupied the area before the road was built. However, we cannot rule out that colonised wood has been displaced and the genets became established on the other side of the road or creek.

Age of Armillaria *genets*

The age of large *Armillaria* genets can be estimated only roughly using known rates of spread. In north temperate countries, the rates of spread for different pathogenic *Armillaria* species are comparable, while in warmer climates the rates are often several times greater (Peet *et al.* 1996). In north temperate areas, different rates of spread of *Armillaria* have been reported, varying between 0.2 m y^{-1} and 1.3 m y^{-1} (Peet *et al.* 1996; Shaw & Roth 1976; Smith *et al.* 1992; van der Kamp 1993). A relatively low rate of spread (0.22 m y^{-1}) was described by van der Kamp (1993) for an *A. ostoyae* genet in the central interior of British Columbia. The author suggested that the relatively cold climate accounted for this low rate of spread. As the site in the present study is also characterised by low temperatures and, compared with most sites in the Alps at this altitude, little precipitation, we assume that the rate of spread of *Armillaria* at this site is relatively low. Assuming a radial spread from the centre of genets of 0.2 m y^{-1}, the age of the largest *A. ostoyae* genet would be about 2000 y. However, it is possible that the age of 2000 y is even underestimated because this particular *A. ostoyae* genet is limited on three sides by deep creek beds and on one side by the timberline. The estimates of the genet age imply the association between *Armillaria* and the mountain pine forests studied being at least several hundred years but probably several millennia old.

The smaller dimensions of *A. borealis* and *A. cepistipes* genets in this study do not necessarily imply that they are younger than the larger *A. ostoyae* genets. Generally, the assumption that large genets are older than small ones does not have to be true, as outlined by Worrall (1994) and Dettman and van der Kamp (2001b). Old genets may be small either because their further spread was inhibited or because they represent remnants of old, formerly large genets.

Spatial distribution of Armillaria *genets in relation to disease centres*

All Armillaria disease centres (M. Bendel, unpublished) were located within *A. ostoyae* genets (except for one tree in an Armillaria disease centre that was infected with *A. borealis*). Shaw and Roth (1976) reported that distinct *Armillaria* genets can encompass several disease centres or small patches of dead trees. However, the forest between the disease centres was not sampled in their study. Our results showed that the same *Armillaria* genet that is active in different distinct disease centres is also found in the forest between these centres. This indicates that the inoculum potential of *Armillaria* genets varies in space and most likely in time.

Most *Armillaria* genets also encompass disease centres predominantly occupied by *Heterobasidion annosum*. The occurrence

of Heterobasidion disease centres within *A. borealis* and *A. cepistipes* genets supports the notion of these *Armillaria* species to act mainly as saprotrophs or weak parasites (Guillaumin et al. 1993), whereas *Heterobasidion* is known as a serious pathogen that can infect and kill healthy conifers (Hodges 1969). The occurrence of Heterobasidion disease centres within *A. ostoyae* genets may indicate that Armillaria disease varies in space and time allowing *Heterobasidion* to establish and spread within large *A. ostoyae* genets. This pattern may also be observed because *Heterobasidion* might be a more aggressive parasite on *Pinus mugo* than *A. ostoyae*.

In conclusion, a mosaic of *Armillaria* genets, with the pathogenic *A. ostoyae* forming the largest genets, were found to occur in these relatively dry and cold mountain pine forests in the Alps. One *A. ostoyae* genet is at least 2000-y-old. The occurrence of Heterobasidion disease centres within large *Armillaria* genets suggests a dynamic interaction between these two root rot pathogens in the study area.

Acknowledgements

The assistance of Jean-Marc Jeckelmann and Franziska Kaiser in field work and the comments of Harald Bugmann to the manuscript are gratefully acknowledged. We are also grateful to the Swiss National Park Service for supporting our research. We thank two anonymous reviewers for valuable comments on the manuscript. This research project is funded by the Swiss National Science Foundation (grant Nr. 3100-066798.01/1).

REFERENCES

Anderson JB, Kohn LM, 1995. Clonality in soilborne, plant–pathogenic fungi. *Annual Review of Phytopathology* **33**: 369–391.

Anderson JB, Ullrich RC, Roth LF, Filip GM, 1979. Genetic identification of clones of *Armillaria mellea* in coniferous forests in Washington. *Phytopathology* **69**: 1109–1111.

Bruhn JN, Wetteroff JJ, Mihail JD, Kabrick JM, Pickens JB, 2000. Distribution of *Armillaria* species in upland Ozark Mountain forests with respect to site, overstory species composition and oak decline. *Forest Pathology* **30**: 43–60.

Cherubini P, Fontana G, Rigling D, Dobbertin M, Brang P, Innes JL, 2002. Tree-life history prior to death: two fungal root pathogens affect tree-ring growth differently. *Journal of Ecology* **90**: 839–850.

Cook RE, 1983. Clonal plant populations. *American Scientist* **71**: 244–253.

Dettman JR, van der Kamp BJ, 2001a. The population structure of *Armillaria ostoyae* and *Armillaria sinapina* in the central interior of British Columbia. *Canadian Journal of Botany* **79**: 600–611.

Dettman JR, van der Kamp BJ, 2001b. The population structure of *Armillaria ostoyae* in the southern interior of British Columbia. *Canadian Journal of Botany* **79**: 612–620.

Dobbertin M, Baltensweiler A, Rigling D, 2001. Tree mortality in an unmanaged mountain pine (*Pinus mugo* var. *uncinata*) stand in the Swiss National Park impacted by root rot fungi. *Forest Ecology and Management* **145**: 79–89.

Favre J, 1960. Catalogue descriptif des champignons supérieurs de la zone subalpine du Parc National Suisse. *Nationalparkforschung in der Schweiz* **6**: 1–589.

Ferguson BA, Dreisbach TA, Parks CG, Filip GM, Schmitt CL, 2003. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Canadian Journal of Forest Research* **33**: 612–623.

Gilbert GS, 2002. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Guillaumin J-J, Anderson JB, Korhonen K, 1991. Life cycle, interfertility, and biological species. In: Shaw CG, Kile GA (eds), Armillaria *Root Diseases*. [Forest Service Agriculture Handbook No. 691]. Forest Service, United States Department of Agriculture, Washington, D.C., pp. 10–20.

Guillaumin J-J, Anderson JB, Legrand P, Ghahari S, Berthelay S, 1996. A comparison of different methods for the identification of genets of *Armillaria* spp. *New Phytologist* **133**: 333–343.

Guillaumin J-J, Legrand P, 2001. Evolution of *Armillaria* genets over eight years (1992–2000). In: Laflamme G, Bérubé JA, Bussières G (eds), *Proceedings of the 10th International Conference on Root and Butt Rots*. Québec City, Canada, pp. 267–275.

Guillaumin J-J, Mohammed C, Anselmi N, Courtecuisse R, Gregory SC, Holdenrieder O, Intini M, Lung B, Marxmüller H, Morrison D, Rishbeth J, Termorshuizen AJ, Tirro A, Vandam B, 1993. Geographical distribution and ecology of the *Armillaria* species in Western Europe. *European Journal of Forest Pathology* **23**: 321–341.

Harrington TC, Worrall JJ, Baker FA, 1992. *Armillaria*. In: Singleton LL, Mihail JD, Rush CM (eds), *Methods for Research on Soilborne Pathogenic Fungi*. American Phytopathological Society Press, St. Paul, Minnesota, pp. 81–85.

Hodges CS, 1969. Modes of infection and spread of *Fomes annosus*. *Annual Review of Phytopathology* **7**: 247–266.

Hodnett B, Anderson JB, 2000. Genomic stability of two individuals of *Armillaria gallica*. *Mycologia* **92**: 894–899.

Holdenrieder O, Pautasso M, Weisberg PJ, Lonsdale D, 2004. Tree diseases and landscape processes: the challenge of landscape pathology. *Trends in Ecology & Evolution* **19**: 446–452.

Kays S, Harper JL, 1974. Regulation of plant and tiller density in a grass sward. *Journal of Ecology* **62**: 97–105.

Kile GA, 1983. Identification of genotypes and the clonal development of *Armillaria luteobubalina* Watling and Kile in eucalypt forests. *Australian Journal of Botany* **31**: 657–671.

Kile GA, McDonald GI, Byler JW, 1991. Ecology and disease in natural forests. In: Shaw CG, Kile GA (eds), Armillaria *Root Diseases*. Forest Service Agriculture Handbook No. 691. Forest Service, United States Department of Agriculture, Washington, D.C., pp. 102–121.

Korhonen K, 1978. Interfertility and clonal size in the *Armillariella mellea* complex. *Karstenia* **18**: 31–42.

Legrand P, Ghahari S, Guillaumin J-J, 1996. Occurrence of genets of *Armillaria* spp. in four mountain forests in central France: the colonization strategy of *Armillaria ostoyae*. *New Phytologist* **133**: 321–332.

Legrand P, Guillaumin J-J, 1993. *Armillaria* species in the forest ecosystems of the Auvergne (Central France). *Acta Oecologica* **14**: 389–403.

Lundquist JE, Klopfenstein NB, 2001. Integrating concepts of landscape ecology with the molecular biology of forest pathogens. *Forest Ecology and Management* **150**: 213–222.

Parolini JD, 1995. *Zur Geschichte der Waldnutzung im Gebiet des heutigen Schweizerischen Nationalparks*. PhD thesis No. 11187, Swiss Federal Institute of Technology ETH, Zurich.

Peet FG, Morrison DJ, Pellow KW, 1996. Rate of spread of *Armillaria ostoyae* in two Douglas fir plantations in the southern interior of British Columbia. *Canadian Journal of Forest Research* **26**: 148–151.

Prospero S, Rigling D, Holdenrieder O, 2003. Population structure of *Armillaria* species in managed Norway spruce stands in the Alps. *New Phytologist* **158**: 365–373.

R Development Core Team, 2004. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rayner ADM, 1991. The phytopathological significance of mycelial individualism. *Annual Review of Phytopathology* **29**: 305–323.

Rigling D, 2001. *Armillaria* and *Annosum* root diseases in a mountain pine (*Pinus mugo* var. *uncinata*) stand in the Alps. In: Laflamme G, Bérubé JA, Bussières G (eds), *Proceedings of the 10th International Conference on Root and Butt Rots*. Québec City, Canada, pp. 35–39.

Rishbeth J, 1988. Stump infection by *Armillaria* in first-rotation conifers. *European Journal of Forest Pathology* **18**: 401–408.

Rishbeth J, 1991. *Armillaria* in an ancient broadleaved woodland. *European Journal of Forest Pathology* **21**: 239–249.

Rizzo DM, Blanchette RA, May G, 1995. Distribution of *Armillaria ostoyae* genets in a *Pinus resinosa–Pinus banksiana* forest. *Canadian Journal of Botany* **73**: 776–787.

Rizzo DM, Harrington TC, 1993. Delineation and biology of clones of *Armillaria ostoyae*, *A. gemina* and *A. calvescens*. *Mycologia* **85**: 164–174.

Shaw CG, Roth LF, 1976. Persistence and distribution of a clone of *Armillaria mellea* in a ponderosa pine forest. *Phytopathology* **66**: 1210–1213.

Smith ML, Bruhn JN, Anderson JB, 1992. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* **356**: 428–431.

Smith ML, Bruhn JN, Anderson JB, 1994. Relatedness and spatial-distribution of *Armillaria* genets infecting red pine seedlings. *Phytopathology* **84**: 822–829.

van der Kamp BJ, 1993. Rate of spread of *Armillaria ostoyae* in the central interior of British Columbia. *Canadian Journal of Forest Research* **23**: 1239–1241.

Worrall JJ, 1994. Population structure of *Armillaria* species in several forest types. *Mycologia* **86**: 401–407.

Worrall JJ, 1997. Somatic incompatibility in basidiomycetes. *Mycologia* **89**: 24–36.