The spread of root rot fungi in mountain pine stands in the Swiss National Park
A case study of its influence on forest dynamics

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The spread of root rot fungi in mountain pine stands in the Swiss National Park: A case study of its influence on forest dynamics

A dissertation submitted to the Swiss Federal Institute of Technology Zurich for the degree of Doctor of Sciences

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Summary

Pathogenic fungi are integral elements of forest ecosystems around the world, altering forests in many ways. *Heterobasidion annosum* is a known pathogen on conifers in the northern hemisphere, while *Armillaria* species are frequent components of the mycoflora in many different forest types and, depending on the species, can attack conifers or hardwoods. Both root rot pathogens, *Heterobasidion* and *Armillaria*, can cause single tree death as well as clumped mortality of trees resulting in ‘disease centres’. By decomposing woody substrates, both pathogenic fungi cause white rot.

However, our knowledge about the role of root rot pathogens for large-scale stand dynamics is limited. Thus, the ultimate aim of this thesis was to evaluate the influence of *Annosum* and *Armillaria* root disease on forest dynamics, using the mountain pine (*Pinus mugo* ssp. *uncinata*) forests of the Swiss National Park as a case study. To address this issue, we focussed on canopy gaps (> 900 m²) and the adjacent forest. In general, the root rot fungi *Heterobasidion annosum* and *Armillaria* spp. were found to be widespread in these stands (Chapter I). *Heterobasidion* was the dominant pathogen in the canopy gaps and could be isolated from 49% of the dying or recently dead mountain pine trees ($\geq$ 12 cm dbh) and 64% of the saplings ($<130$ cm height). Most likely, management activities such as clear cuts have facilitated the spread of this pathogen in the past. *Armillaria* root disease was found on 13% of the mountain pine trees and 20% of the saplings, and was caused by three different *Armillaria* species. The dominant species, *A. ostoyae*, is a serious pathogens, while the two other species, *A. borealis* and *A. cepistipes*, are known to act primarily as saprotrophs.

*Annosum* root rot was associated with most canopy gaps studied (74%). In fewer canopy gaps, *A. ostoyae* (14%) or both pathogens, *Heterobasidion* and *Armillaria* spp. (7%) were dominant, while only in two cases (5%) other factors were presumed to be responsible for the enlargement of the canopy gaps (Chapter I). Thus, the root rot fungi *Heterobasidion* and *A. ostoyae* were the most important driving forces involved in the enlargement – and likely also in the creation – of canopy gaps, which were therefore classified as ‘disease centres’.

To describe the genetic population structure of *Armillaria* spp. at the landscape scale, additional isolates were collected from recently dead mountain pines in the forest matrix between the disease centres. Intraspecific somatic incompatibility tests were used to delineate the different genets. The results revealed that the genets of the pathogenic *A. ostoyae* were
Summary

significantly larger than those of the two saprotrophic species *A. borealis* and *A. cepistipes*. Large *Armillaria* genets encompassed several distinct disease centres associated with *Heterobasidion* and/or *A. ostoyae* (Chapter III). This suggests that the severity of disease within large *Armillaria* genets varied in space and presumably also in time. This pattern may also be observed because *Heterobasidion* might be more aggressive on mountain pine than *A. ostoyae*. The largest *A. ostoyae* genet extended over approx. 37 ha and was estimated to be 1000-2000 years old. This estimate of genet age implies that the association between *Armillaria* and the mountain pine forests studied is most probably several millennia old.

The density of regenerating mountain pine saplings (20-130 cm height) was significantly higher in disease centres than in the adjacent forest, whereas mountain pine seedlings (< 20 cm height) did not reveal any difference in their density (Chapter II). Thus, it was not the germination of mountain pine which was hampered within the forest, but the growth from the seedling into the sapling stage proved to be more successful in the disease centres compared to the adjacent forest. Mortality of regenerating trees (< 130 cm height) was low both in the disease centres (1%) and in the adjacent forest (2%). In addition, all non-symptomatic mountain pines < 130 cm tall sampled within disease centres were non-infected with *Heterobasidion* and *Armillaria*, whereas in the adjacent forest, 4% of the non-symptomatic mountain pines < 130 cm tall were infected with either or both pathogens. In contrast to mountain pine, regenerating Swiss stone pine (*Pinus cembra*) did not show any difference in its density between disease centres and the adjacent forest. Compared to the forest, a higher number of species in the ground flora and significantly greater volumes of dead wood were found within disease centres.

The symptoms and signs associated with root disease caused by *Heterobasidion* or *A. ostoyae* were investigated in dying or recently dead mountain pine trees and saplings. The best sign for the incidence of *A. ostoyae* root rot were *Armillaria* fans, and for *Annosum* root rot *Heterobasidion* mycelium (pustules and ectotrophic growth). In addition, resinosis was a good symptom for *A. ostoyae* root rot in mountain pine trees (Chapter IV).

The results of this thesis suggest that *Heterobasidion annosum* and *Armillaria* spp. play an essential role in the dynamics of the mountain pine forests of the Swiss National Park by causing and enlarging disease centres which are characterized by enhanced, non-symptomatic, and non-infected regeneration of mountain pine. As a consequence, forest turnover rate is assumed to be speeded up, while the proposed successional trajectory towards stands dominated by Swiss stone pine is decelerated.
Zusammenfassung


Die Mehrzahl der untersuchten Waldlücken war mit dem Wurzelschwamm assoziiert (74%). In 14% der Waldlücken dominierte der Dunkle Hallimasch, während in 7% der Waldlücken beide Wurzelfäulepilze (Wurzelschwamm und Hallimasch) für das Absterben der Bergföhren verantwortlich waren. In zwei untersuchten Flächen (5%) musste davon ausgegangen werden, dass hauptsächlich andere Faktoren als Wurzelfäulepilze die Vergrösserung der Waldlücke


Bei den Bergföhren war die Dichte der Verjüngung (Bäume 20-130 cm Höhe) innerhalb der Mortalitäts-Zentren signifikant grösser als im umgebenden Wald, während sich die Dichte der Sämlinge (< 20 cm Höhe) nicht unterschied (Chapter II). Die Verjüngung der Bergföhre war folglich nicht durch das Aufkommen von Sämlingen limitiert, sondern vielmehr durch das verringerte Wachstum im Wald. Die Verjüngung der Bergföhre in den Mortalitäts-Zentren war nicht nur dichter, sondern war auch dadurch charakterisiert, dass sie eine sehr geringe Mortalitätsrate aufwies und die beprobten, nicht-symptomatischen Jungbäume keinen Wurzelfäule-Befall zeigten. Im Gegensatz zur Bergföhre zeigte die Arvenverjüngung (Pinus cembra) keinen Unterschied zwischen den Mortalitäts-Zentren und dem umgebenden Wald. Die Resultate der vorliegenden Arbeit lassen darauf schliessen, dass die beiden Wurzelfäulepilze, Wurzelschwamm und Hallimasch, in den Bergföhrenwäldern des Schweizerischen Nationalparks eine bedeutende Rolle spielen, indem sie zur Entstehung und Vergrösserung von Mortalitäts-Zentren beitragen. Gerade diese Flächen, welche sich durch das geklumpten Absterben von Bergföhren auszeichneten, waren durch eine dichte, nicht-symptomatische Bergföhren-Verjüngung charakterisiert, welche keinen Wurzelfäule-Befall aufwies. Auf Grund dieser Resultate muss davon ausgegangen werden, dass durch den Einfluss der Wurzelfäule-Pilze die ,Turnover‘-Rate des Waldes beschleunigt und die Sukzession zu Arven-dominierten Beständen verlangsamt wird.
General introduction

Root rot fungi

In forest ecosystems worldwide, plant pathogens play an important role as they can strongly influence the population dynamics of the hosts, change the structure and composition of plant communities, and at the same time may help to maintain species diversity (Gilbert 2002). A widespread and important component of the mycoflora of many forests are Armillaria species, with several of them being primary pathogens (Shaw & Kile 1991). Another well-known pathogenic fungus that attacks the root systems of conifers in the temperate and boreal regions of the northern hemisphere is Heterobasidion annosum s.l. (Fr.) Bref. (Hodges 1969).

The incidence and severity of Armillaria and Annosum root disease depends on numerous factors such as host susceptibility (e.g., Delatour et al. 1998; Wargo & Harrington 1991), virulence of the fungal species involved (e.g., Omdal et al. 1995; Prospero et al. 2004), and environmental parameters such as soil properties and site history (e.g., Korhonen & Stenlid 1998). Stumps created by thinning and tree harvesting are important for the primary infection of both Armillaria and Heterobasidion (Redfern & Filip 1991; Redfern & Stenlid 1998), and it is assumed that silvicultural changes in forest composition and structure can alter and destabilize the host-pathogen relation in favour of the fungus (Morrison & Mallett 1996).

Armillaria spp.

Species of Armillaria have a very broad host range within the native vegetation where they grow (Kile et al. 1991). Pathogenicity varies considerably among the different Armillaria species (Gregory et al. 1991). Most species act as pathogens or wood decomposers, where they cause a typical white rot of infected material. Some Armillaria species behave as mycoparasites or even mycotrophs with several achlorophyllous taxa in the Orchidaceae (Kile et al. 1991).

Until the 1970s, Armillaria root rot in the northern hemisphere was assumed to be caused by one species, A. mellea s.l. There are now at least 13 clearly defined species reported from the northern hemisphere alone (Harrington et al. 1992), five of which have been found in Switzerland (A. borealis Marxmüller & Korhonen, A. cepistipes Velenovsky, A. ostoyae (Romagnesi) Herink, A. mellea (Vahl: Fr.) Kummer, and A. gallica Marxmüller & Romagnesi) (Rigling et al. 1997). Armillaria mellea s.str. and A. ostoyae are known to be
highly pathogenic, whereas *A. gallica*, *A. borealis*, and *A. cepistipes* are reported to be saprotrophs or only weak pathogens (e.g., Guillaumin et al. 1993; Prospero et al. 2004, Table 1). These five annulated *Armillaria* species, *A. mellea* s.str., *A. ostoyae*, *A. gallica*, *A. borealis*, and *A. cepistipes* are heterothallic and tetrapolar (Guillaumin et al. 1991). In addition, two exannulated species occur in Europe: *A. tabescens* (Scop.: Fr.) Emel and *A. ectypa* (Fr.) Lamoure. Both species live mostly saprotrophically. *Armillaria tabescens* occurs on hardwood, while *A. ectypa* is a rare species confined to *Sphagnum* in peat bogs of high latitudes or altitudes (Guillaumin 1973; Rishbeth 1982; Zolciak et al. 1997). *Armillaria ectypa* is the only European species described as homothallic (Guillaumin 1973).

**Table 1. Characteristics of the European annulated *Armillaria* species (Guillaumin et al. 1993).**

<table>
<thead>
<tr>
<th>Current name</th>
<th>Main hosts</th>
<th>Ecological behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. ostoyae</em> (Romagnesi) Herink</td>
<td>Conifer, mixed, and hardwood forests</td>
<td>Primary pathogen</td>
</tr>
<tr>
<td><em>A. mellea</em> (Vahl: Fr.) Kummer</td>
<td>Hardwood and mixed forests, parks, orchards, gardens</td>
<td>Occasionally aggressive primary pathogen</td>
</tr>
<tr>
<td><em>A. borealis</em> (Marxmüller &amp; Korhonen)</td>
<td>Mixed, hardwood, and coniferous forests</td>
<td>Weakly pathogenic, mainly saprotroph</td>
</tr>
<tr>
<td><em>A. cepistipes</em> (Velenovsky)</td>
<td>Hardwood, mixed, and coniferous forests</td>
<td>Mainly saprotroph</td>
</tr>
<tr>
<td><em>A. gallica</em> (Marxmüller &amp; Romagnesi)</td>
<td>Hardwood and mixed forests</td>
<td>Weakly pathogenic, mainly saprotroph</td>
</tr>
</tbody>
</table>

Primary infection of *Armillaria* is assumed to occur via basidiospores that infect freshly exposed woody tissue, such as stumps of recently cut trees (Rishbeth 1951) or wounds (e.g., Isomäki & Kallio 1974). However, the colonisation of fresh stumps seems to be an infrequent event (Rishbeth 1988). In addition, it is assumed that wounds are unlikely to increase the success of infection for the pathogenic species, whereas wounds and debilitated roots might be more important for the less pathogenic *Armillaria* species (Redfern & Filip 1991). Once *Armillaria* is established, secondary infection can proceed via mycelium growing through direct root contacts and grafts with uninfected trees, or via rhizomorphs growing through the soil to contact uninfected trees (Redfern & Filip 1991). By means of vegetative spread, *Armillaria* can produce large genets (clones) in the soil (e.g., Ferguson et al. 2003; Smith et al. 1992). A population genetics approach can be used to better understand the ecology and epidemiology of fungal tree pathogens (cf. Guillaumin et al. 1996). By assessing the size and
distribution of fungal genets, information on the frequency of new infections and the growth
and size of individual genets can be obtained. The extension of Armillaria genets can be
determined by applying different methods such as intraspecific somatic incompatibility tests
(e.g., Korhonen 1978), analyses of mating type alleles (e.g., Kile 1983), RAPD (Randomly
Amplified Polymorphic DNA, e.g. Smith et al. 1992), RFLP (Restriction Fragment Length
Polymorphism, e.g. Rizzo et al. 1995), or isozymes (e.g., Rizzo & Harrington 1993). In
general, intraspecific somatic incompatibility tests have been reported to be an efficient and
reliable technique to delineate Armillaria genets, although it has its limitations (e.g., Dettman
& van der Kamp 2001; Guillaumin et al. 1996; Rizzo & Harrington 1993; Smith et al. 1994).
In contrast to Heterobasidion annosum, of which the genes involved in somatic
incompatibility have been determined (Hansen et al. 1993), the genetic basis for somatic
incompatibility in Armillaria is still unknown (Worrall 1997).

Heterobasidion annosum

Heterobasidion annosum s.l., formerly known as Fomes annosus ((Fr.) P. Karst.), is a
polypore having perennial basidiocarps and a Spiniger anamorph (i.e., conidial stage). In the
temperate and boreal regions of the northern hemisphere, Heterobasidion is a widespread
pathogenic fungus attacking conifers (Hodges 1969). In pines, infection by Heterobasidion is
achieved by ectotrophic growth of mycelium in the outer bark scales followed by infection of
the phloem. The pathogen then causes root mortality and eventually kills the infected trees. In
pines, root and stem decay are generally less important, whereas in most non-pine species,
heart rot is of greater importance than root mortality (Stenlid & Redfern 1998, Table 2).
Norway spruce (Picea abies L.) can survive infection for extended periods of time although
decay column in trees can be several meters high (e.g., Stenlid & Wästerlund 1986). It is
assumed that before tree harvesting and the creation of managed monocultures of pines and
spruce, Annosum root rot must have been a relatively rare disease (Harrington & Wingfield
1998). The infection of freshly-cut pine stumps by Heterobasidion spores was recognized by
Rishbeth (1951), who elucidated the epidemiology of the disease in pine plantations. He also
noted that competing fungi, most notably Phlebiopsis gigantea (Fr.) Jul., could exclude
Heterobasidion from stumps if applied quickly on the stump surface after harvesting
(Rishbeth 1951). This led to one of the most effective biological control methods of a plant
disease. In addition, treatment of freshly cut stumps with borate, urea or other chemical
control agents may effectively prevent *Heterobasidion* from colonizing the stumps (e.g., Korhonen et al. 1993; Pratt 1993; Vasiliauskas et al. 2004). The principal source of inoculum for infection with *Heterobasidion* is provided by colonized stumps or by infected tree roots (Stenlid & Redfern 1998). It is reported that *Heterobasidion* can remain active in stumps for several decades (e.g., Greig & Pratt 1976; Piri 1996; Stenlid & Redfern 1998). In contrast to the colonisation of stumps via basidiospores, the direct infection of trees by spores (e.g., through wounds) is considered to be a comparatively rare event (Redfern & Stenlid 1998).

*Heterobasidion annosum* s.l. is a species complex that consists of several different species or intersterility groups. In Europe, *Heterobasidion annosum* s.l. is divided into three species (formerly referred to as ‘types’): *H. annosum* s.str., *H. parviporum* Niemelä & Korhonen, and *H. abietinum* Niemelä & Korhonen (Niemelä & Korhonen 1998). Their sexual system is heterothallic and bipolar (Niemelä & Korhonen 1998, Table 2).

### Table 2. Characteristics of the European *Heterobasidion* species (Niemelä & Korhonen 1998; Stenlid & Redfern 1998).

<table>
<thead>
<tr>
<th>Name</th>
<th>Former type</th>
<th>Type of disease</th>
<th>Main host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. annosum</em> s.str. ((Fr.) Bref.)</td>
<td>P-type</td>
<td>Root rot; root mortality often extensive</td>
<td><em>Pinus</em></td>
</tr>
<tr>
<td><em>H. parviporum</em> (Niemelä &amp; Korhonen)</td>
<td>S-type</td>
<td>Heart rot; decay more important than mortality</td>
<td><em>Picea</em></td>
</tr>
<tr>
<td><em>H. abietinum</em> (Niemelä &amp; Korhonen)</td>
<td>F-type</td>
<td>Heart rot; decay more important than mortality</td>
<td><em>Abies</em></td>
</tr>
</tbody>
</table>

**Role of canopy gaps in forest ecosystems**

The importance of canopy gaps has been described for many different forest ecosystems worldwide (e.g., Foster & Reiners 1986; Lertzman & Krebs 1991; Liu & Hytteborn 1991; Runkle 1981; Veblen 1986; Yamamoto 1995). The size of the canopy gaps largely determines the species composition of the regeneration, with light-demanding species expected to regenerate in larger canopy gaps, while more shade-tolerant species regenerate more successfully in smaller canopy gaps or within the forest. The different extensions of canopy gaps (Denslow 1980) and the internal heterogeneity within the gaps are thought to act as a mechanism of species coexistence (Orians 1982).
Canopy gaps can be caused by different disturbance agents such as wind throw, avalanches, fire or insect outbreaks (e.g., bark beetles). In addition, root rot pathogens can be important driving forces in creating and expanding canopy gaps in forests depending on various factors such as tree species composition, site characteristics, and past human management (e.g., Hansen & Goheen 2000; Worrall et al. 2005). Besides having a high incidence of root disease, gaps caused by root rot fungi are generally characterized by the presence of dead trees that have died at different times, symptomatic trees in various stages of decline, and fallen trees that show evidence of stem or root failure, typically lying with the stems pointing in different directions. Thus, pathogens can influence the spatial and temporal diversity of forests by creating canopy gaps of different size and, consequently, special habitats for plants and animals (van der Kamp 1991). If pathogens are the key agents in the creation or extension of canopy gaps, these sites are also referred to as ‘disease centres’.

Although it is generally assumed that root diseases can be major disturbance agents in shaping forest structure and composition (e.g., Hansen & Goheen 2000; McLaughlin 2001; Rizzo & Slaughter 2001), only few studies have focused on the role of root disease centres for large-scale forest dynamics. The impact of root disease centres on forest succession has been studied in Western North America, where Phellinus weirii (Murr.) Gilbertson, which causes laminated root rot leading to expanding centres of mortality, was found to alter the successional trajectories depending on the susceptibility of the tree species present (Hansen & Goheen 2000; Holah et al. 1997). It was reported from the Pyrenees and Australia that the same tree species killed by Armillaria formed patches of dense and non-symptomatic regeneration within Armillaria disease centres (Durrieu et al. 1985; Shearer et al. 1997). However, up to now, the level of infection in the regeneration within root disease centres is largely unknown. In addition, our understanding of the distribution of root disease centres in relation to the population structure of the root rot pathogen at a large scale is still limited (cf. Ferguson et al. 2003).

Study site Swiss National Park

The Swiss National Park – where direct human influences have been absent since 1914 – offers the opportunity to study the influence of natural disturbance factors such as root rot pathogens on forest dynamics at a large scale. The Park extends over an area of roughly 172 km², 50 km² of which are covered with forests. The dominant forest types are the Erica-
Pinetum mugo, Larici-Piceetum, and Laricetum (Zoller 1995). The largest mountain pine stands of Switzerland, which cover approx. 26 km², are found mainly west of the Ofen Pass in the Swiss National Park (Brunies 1906).

The study area includes the mountain pine forests between Champlönch and Buffalora in the Swiss National Park (46°39’N to 46°41’N, 10°10’E to 10°16’E). These forests are found mainly on southern slopes, and extend over an area of approx. 10 km² at an altitude between 1800 and 2200 m a.s.l. The climate is characterized 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.).

**Dominating tree species: Mountain pine**

Mountain pine (*Pinus mugo* s.l.) is known as a light-demanding species that does not exhibit particular soil preferences (CAB International 2002). It occurs from the Pyrenees throughout the Alps and the Jura, the Central Apennines in Italy, and the Dinaric Alps to the Carpathian Mountains in the east. Mountain pine shows a wide morphological variation (CAB International 2002; Dengler 1992) and can hybridize naturally with Scots pine (*Pinus sylvestris* L.) (e.g., Schmid 1951). The systematic position of *P. mugo* s.l. in relation to *P. sylvestris* is not yet sufficiently understood (e.g., Boratynska & Bobowicz 2001). In contrast to Scots pine, mountain pine has a shallow, far-reaching root system that lacks a taproot (Hegi 1981). Studies from the Alps, the Jura Mountains, and the Pyrenees have reported the oldest mountain pine trees to be 200 to approx. 300 years old (Brang 1989; Brunies 1906; Cherubini et al. 2002; Durrieu et al. 1985; Frelechoux et al. 2000; Gaussen 1923; Rathgeber & Roche 2003; Rolland et al. 1998; Schlegel 1985).

According to the growth form and the geographical distribution, mountain pine (*Pinus mugo* s.l.) can be divided into two subspecies: *Pinus mugo* ssp. *uncinata* (DC.) Domin, the up-right growth form, and *Pinus mugo* Turra s.str., the dwarf mountain pine (Binz & Heitz 1990; Lauber & Wagner 2001). While *Pinus mugo* ssp. *uncinata* has its main distribution in the western Alps and the Pyrenees, the dwarf subspecies occurs mostly in the eastern Alps. In the Swiss National Park, both subspecies are almost equally frequent (Zoller 1995).
**Past human management**

In the Swiss National Park, most of the mountain pine (*Pinus mugo* ssp. *uncinata*) forests west of the Ofen Pass in the area of Il Fuorn and Champlönch stock on sites that were clearcut in the past (Brunies 1906; Parolini 1995). It is assumed that European Larch (*Larix decidua* Miller) and Swiss stone pine (*Pinus cembra* L.) were more widespread in these stands in former times (e.g., Braun-Blanquet 1931; Brunies 1906; Leibundgut & Schlegel 1985; Risch et al. 2004). However, these reports have been challenged by a recent palynological study conducted in a small bog at Il Fuorn at the southern border of our study area where mountain pine was found to have been the dominant tree species in this area during the past approx. 6000 years (Stähli 2004).

**Mortality of mountain pines**

The first report of dying mountain pines in the area of the Swiss National Park dates back to 1932 (Gäumann & Campell 1932). Tree mortality was concentrated in disease centres and *Armillaria* was reported as the causal agent of the observed mortality (Gäumann & Campell 1932). In 2001, *Armillaria* was isolated from the roots of recently dead mountain pines in a two-hectare study plot in the Swiss National Park (Dobbertin et al. 2001). Armillaria root rot was found to be caused by *A. borealis* and *A. cepistipes* (Rigling 2001); both species are known to act as saprotrophs or weak pathogens on coniferous trees (Guillaumin et al. 1993). Apart from *Armillaria*, *Heterobasidion annosum* s.str., was recovered from the roots of recently dead mountain pines for the first time in this area (Dobbertin et al. 2001; Rigling 2001). *Heterobasidion annosum* proved to be more widespread than *Armillaria* and was thought to be the main reason for the mortality of mountain pines in the two-hectare study plot (Dobbertin et al. 2001).

**Main objectives**

To estimate the influence of the root rot fungi *Heterobasidion annosum* and *Armillaria* spp. on forest dynamics, I have focused on disease centres and the surrounding forest in the mountain pine stands of the Swiss National Park. These forests, which have not been
managed for most of the 20th century, offer the opportunity to study ecological processes in the absence of management interventions.

In particular, the objectives of this study were:

i) to determine the spatial distribution of Annosum and Armillaria root rot in mountain pine forests of the Swiss National Park and assess their role for canopy gap formation (Chapter I),

ii) to estimate the influence of disease centres on stand dynamics, particularly on the regeneration of *P. mugo* and *P. cembra* (Chapter II),

iii) to reveal the genetic population structure of *Armillaria* spp. at a landscape scale and relate it to the distribution of disease centres associated with *Heterobasidion* and/or *Armillaria* (Chapter III),

iv) to identify the most conspicuous signs of the fungi and external symptoms induced by the fungi that indicate Armillaria and Annosum root rot in dying and recently dead mountain pines (Chapter IV).

To achieve the first objective, *Heterobasidion* and *Armillaria* were isolated from the roots of dying or recently dead mountain pines found within or at the edge of 42 canopy gaps in the Swiss National Park. According to the incidence of Annosum and Armillaria root rot, every canopy gap was classified as ‘disease centre’ associated with either or both pathogens, or as ‘canopy gap’ where other factors were presumably involved in its formation (Chapter I). A sub-sample of those disease centres associated with *Heterobasidion* and/or *Armillaria* was chosen to assess the impact of the disease centres on tree regeneration, vegetation, and the volumes of dead wood. Non-symptomatic mountain pines were checked for Armillaria and Annosum root disease to estimate the incidence of infection in healthy-looking mountain pines in both the disease centres and the adjacent forest (Chapter II).

In order to relate the incidence and spatial distribution of disease centres with the population structure of *Armillaria* spp., intraspecific somatic incompatibility tests were applied to determine the size and extensions of *Armillaria* genets in the study area (Chapter III). Using logistic regression models, the most conspicuous symptoms and signs associated with Annosum and Armillaria root disease in mountain pine trees and saplings were identified and discussed in Chapter IV.
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Chapter I

Incidence and distribution of *Heterobasidion annosum* and *Armillaria* spp. and their influence on gap formation in unmanaged mountain pine forests in the Swiss Alps

Submitted as:
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Abstract

Various disturbance factors on different spatial scales can lead to the creation of canopy gaps in forest ecosystems. In this study, we investigated the role of root rot fungi in the formation of canopy gaps in the Swiss National Park in the Central Alps. Dying or recently dead mountain pine trees (n = 172) and saplings (n = 192) from 42 canopy gaps were assessed for *Armillaria* and Annosum root rot. *Heterobasidion annosum* proved to be the dominant pathogen and was isolated from 49% of the trees and 64% of the saplings. *Armillaria* was found on 13% of the trees and 20% of the saplings. Three *Armillaria* species, *A. borealis*, *A. cepistipes*, and *A. ostoyae*, were identified. *Armillaria ostoyae* was the most frequent species, accounting for 72% of all isolates. A total of 31 (74%) gaps were associated with *Heterobasidion*, and six (14%) with *A. ostoyae*. The
remaining gaps were either associated with both pathogens (7%) or with other, unknown factors (5%).

Our findings suggest that the two pathogenic fungi, *H. annosum* and *A. ostoyae*, are the main reason for the large-scale mortality of mountain pines and the creation of canopy gaps in the Swiss National Park.

**Introduction**

Canopy gaps are important structural elements in forest ecosystems that influence their dynamics and diversity (e.g., Lawton & Putz 1988; Lertzman 1992; Liu & Hytteborn 1991; Runkle 1981). Since a large proportion of the seedlings and saplings in a forest are often found in such openings, canopy gaps can play a fundamental role in tree regeneration. One pathogenic fungus that may cause canopy gaps, *A. ostoyae* (Romagn.) Herink, has even been described as a ‘rejuvenating factor’ (Durrieu et al. 1985). In general, plant pathogens such as root rot fungi can act as destructive agents that reduce plant fitness, cause mortality, and thereby change the structure and composition of plant communities. At the same time, plant pathogens facilitate successional processes or may help to maintain species diversity (Gilbert 2002; van der Kamp 1991).

*Armillaria* species are important components of the mycoflora in many forest ecosystems worldwide (Shaw & Kile 1991). These species behave as primary or secondary pathogens, causing root and butt rot on a large number of coniferous and broadleaved tree species (Guillaumin et al. 1993). All *Armillaria* species can survive saprotrophically on woody substrates (Redfern & Filip 1991), and they typically produce highly differentiated structures called rhizomorphs (Garraway et al. 1991). As a saprotroph, *Armillaria* degrades all wood components causing white rot (Morrison et al. 1991). Several *Armillaria* species act as primary pathogens and can cause significant economic losses by damaging timber (Morrison & Mallett 1996). They may also influence tree species composition (Kile et al. 1991).

In forest ecosystems, *Heterobasidion annosum* s.l. (Fr.) Bref. is another widespread pathogenic fungus (Woodward et al. 1998). This species is a serious pathogen, attacking conifers in the temperate and boreal regions of the Northern Hemisphere (Hodges 1969).

*Armillaria* predominantly spreads vegetatively via root contacts between healthy and infected roots and via rhizomorphs in the soil, while dispersal by spores is less common (Shaw & Kile...
Heterobasidion spreads by both root contact and spore infections (Woodward et al. 1998). As a consequence, Heterobasidion mostly forms numerous small genets, whereas Armillaria is found to build fewer, but larger genets (e.g., Ferguson et al. 2003; Garbelotto et al. 1999; Stenlid 1985). Through this vegetative spread, Heterobasidion and pathogenic Armillaria species can infect and kill nearby trees, which often results in expanding disease centres (Hodges 1969; Kile et al. 1991). These disease centres may be limited to a few trees or encompass several hectares (Kile 1983, 1986; Korhonen 1978; Legrand et al. 1996; Rishbeth 1991; Rizzo et al. 1995; Worrall 1994). Beside having a high incidence of root rot, gaps caused by root rot fungi are generally characterized by the presence of dead trees that have died at different times, symptomatic trees in various stages of decline, and fallen trees that show evidence of stem or root failure, typically lying with the stems pointing in different directions. The inoculum potential of root rot fungi is typically highest at the margin of these disease centres (e.g., Peet et al. 1996).

Pathogenic fungi have been reported to be the cause of some of the mortality of mountain pines (Pinus mugo subsp. uncinata (DC.) Domin) in the Swiss National Park in the Grisons. As early as 1932, Gämunn & Campell (1932) claimed that Armillaria was the reason for the dying of the mountain pines they observed. Relatively high tree mortality, often occurring in clusters, has also been reported by Brang (1988), Hauenstein (1998), and Stöckli (1996). Armillaria root rot, bark beetles, and competition have been suspected as possible agents, but the exact causes of the observed mortality were not examined in these studies. Only recently, Heterobasidion annosum has been identified as an important cause of tree mortality in this area (Cherubini et al. 2002; Dobbertin et al. 2001a). Both pathogens, Armillaria spp. and Heterobasidion, were isolated from recently dead mountain pines in a two-hectare study plot in the Swiss National Park. The Armillaria species were identified as A. cepistipes Velenovsky and A. borealis Marxmüller and Korhonen, and Heterobasidion as Heterobasidion annosum s.str. (Fr.) Bref. (Rigling 2001). A. cepistipes and A. borealis are considered to be generally weakly pathogenic (Guillaumin et al. 1993), whereas Heterobasidion annosum is a serious pathogen, particularly on pine species (Hodges 1969).

The objectives of this study were: i) to determine the large-scale occurrence of Armillaria and Annosum root rot in the mountain pine forests of the Swiss National Park, and ii) to assess the importance of these root rot fungi as driving forces in the formation of canopy gaps.
Material and Methods

Study species and site

The mountain pine (*Pinus mugo* subsp. *uncinata*) is a subalpine pioneer conifer species that occurs in the mountains of western Europe from the Pyrenees to the Central Alps (Dengler 1992). The tree typically grows on unfavourable or disturbed sites, and has a far-reaching and shallow root system (Hegi 1981).

The Swiss National Park is located in the Engadine in the Central Alps. The Park extends over an area of approx. 172 km², 50 km² of which are covered with forests. Its climate is characterized 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.). Since the foundation of the Park in 1914, management interventions have ceased.

The study area includes the mountain pine forests west of the Ofen Pass in the Swiss National Park (lat. 46°39’N, long. 10°13’E). These forests are found mainly on southern slopes, and extend over an area of approx. 10 km² at an altitude between 1800 and 2200 m a.s.l.

Field sampling

In the mountain pine forests of the Swiss National Park, canopy gaps with a minimum size of 900 m² and a maximum of 20% tree cover have previously been delineated on aerial photographs (Guthapfel 2002). In total, 95 canopy gaps have been defined, and their areas calculated in ArcMap™ 8.3 (© ESRI Inc. 1999-2002). Among the 95 gaps, 45 were selected for the present study from two sub-areas. Permanent openings, in which the rocky substrate conditions restrict tree growth, were excluded. In the field, between May and October 2003, the gap edges were defined according to Runkle (1982) by connecting the locations of the stems of living, non-symptomatic trees that had a crown transparency of less than 50%. Crown transparency was estimated according to Dobbertin et al. (2001b) by comparing the trees at the edges of each gap with local reference trees that had the maximum amount of foliage under local growing conditions. Crown transparency is expressed as the percentage reduction in local crown density. Gaps smaller than 900 m² were not considered in this study because their delineation in the open type of forest found in the Swiss National Park becomes increasingly arbitrary.
In each gap, five trees (≥ 12 cm dbh, diameter at 130 cm) and five saplings (< 110 cm height) were selected that were symptomatic but still living (≥ 50% brown needles) or recently dead (100% brown needles). For each tree, the dbh was recorded, while for the saplings their height was measured. The sampled trees and saplings had to be within or at the edge of the canopy gaps. Three main roots were excavated from each tree, 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 each root sampling, the increment borer was sterilized in 70% ethanol and dried with paper towels. Depending on the size of the saplings, either their whole root system was dug out or three main roots were sampled, as described above. Core samples were placed in sterile plastic tubes and kept cool until isolation. All wood samples were processed within four days after sampling.

**Isolation of fungi and Armillaria species identification**

Three pieces (about 1 cm long) of each root sample were surface sterilized in sodium hypochlorite (active chlorine = 7%) for 30 seconds and rinsed twice in sterile, demineralised water for ≥ 15 seconds. The pieces were dried between paper towels and placed on agar plates (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, 100 mg l⁻¹ sodic benzylpenicillin) modified from Legrand & Guillaumin (1993). 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).

The presence of *Heterobasidion* was indicated by its *Spiniger meineckellus* (Olson) Stalpers conidial stage (Worrall & Harrington 1992). The *Armillaria* isolates were identified by diploid-haploid pairings using three haploid tester strains of each *A. cepistipes*, *A. borealis*, and *A. ostoyae* (Korhonen 1978). No attempt was made to identify other decay-causing fungi growing out of the wood samples.

A tree was considered infected if one or both pathogenic fungi could be isolated from at least one root sample. The presence of rhizomorphs was not regarded as evidence of infection, since rhizomorphs can surround tree roots without infecting them (Gregory et al. 1991).
Classification of gaps according to incidence of fungi

We considered a canopy gap to be associated with a pathogen if at least 50% of the mountain pines sampled (trees and saplings combined) were infected with either *Heterobasidion* or *Armillaria* (Table 1). If neither fungus reached 50%, but together they were isolated from at least 50% of the trees, the gap was considered to be associated with both fungi. If less than 50% of the trees were infected with *Heterobasidion* or *Armillaria*, other factors were assumed to be involved in the formation and the enlargement of the canopy gaps.

**Table 1. Criteria for the classification of canopy gaps.**

<table>
<thead>
<tr>
<th>Gap associated with</th>
<th>% of trees infected with</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heterobasidion</em> (H)</td>
<td>≥ 50 &lt; 50 ≥ 50</td>
</tr>
<tr>
<td><em>Armillaria</em> (A)</td>
<td>&lt; 50 ≥ 50 ≥ 50</td>
</tr>
<tr>
<td><em>Heterobasidion &amp; Armillaria</em></td>
<td>&lt; 50 &lt; 50 ≥ 50</td>
</tr>
<tr>
<td>Other factors</td>
<td>&lt; 50 &lt; 50 &lt; 50</td>
</tr>
</tbody>
</table>

Results

**Incidence of fungal infections and species identification**

In most gaps, nine or ten mountain pine trees and saplings could be found that were symptomatic or had recently died. In total, 172 trees and 192 saplings from 42 gaps were examined for *Armillaria* and *Annosum* root rot (Table 2). Considering all sampled trees and saplings, 76% showed infection of *Armillaria* and/or *Heterobasidion*. Compared to *Armillaria*, *Heterobasidion* was more often isolated from both trees and saplings. Only a small percentage of trees were infected with both pathogens. Except for one symptomatic sapling, *Armillaria* was isolated only from dead trees and saplings, whereas *Heterobasidion* was found on more than half of the symptomatic but still living trees and saplings sampled (Table 2). Comparing infected with non-infected mountain pines, both pathogens were relatively more often isolated from saplings than from trees ($X^2$-test, $p = 0.001$; all statistical analyses were conducted on R (R Development Core Team 2004)). Infected saplings proved to be taller than non-infected saplings (Wilcoxon rank sum test, $p = 0.01$, data not shown). Trees and saplings infected with *Armillaria* were found at higher
Table 2: Incidence of *Heterobasidion annosum* and *Armillaria* spp. in symptomatic and recently dead mountain pine trees and saplings.

<table>
<thead>
<tr>
<th>Mountain pines sampled</th>
<th><em>Heterobasidion</em></th>
<th><em>Armillaria</em></th>
<th><em>Heterobasidion</em> &amp; <em>Armillaria</em></th>
<th>Other or no fungi</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trees (&gt; 12 cm dbh)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>Dead</td>
<td>74</td>
<td>23</td>
<td>7</td>
<td>52</td>
<td>156</td>
</tr>
<tr>
<td>Total (%)</td>
<td>84 (49%)</td>
<td>23 (13%)</td>
<td>7 (4%)</td>
<td>58 (34%)</td>
<td>172 (100%)</td>
</tr>
<tr>
<td>Saplings (&lt; 110 cm height)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Dead</td>
<td>117</td>
<td>38</td>
<td>2</td>
<td>26</td>
<td>183</td>
</tr>
<tr>
<td>Total (%)</td>
<td>122 (64%)</td>
<td>39 (20%)</td>
<td>2 (1%)</td>
<td>29 (15%)</td>
<td>192 (100%)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>206 (57%)</td>
<td>62 (17%)</td>
<td>9 (2%)</td>
<td>87 (24%)</td>
<td>364 (100%)</td>
</tr>
</tbody>
</table>

elevations than those infected with *Heterobasidion* (Wilcoxon rank sum test, p = 0.03). In addition, two recently dead Swiss stone pine saplings (*Pinus cembra* L., 26 and 50 cm height) were found, and both were infected with *Heterobasidion*.

Among the 71 *Armillaria* isolates, three *Armillaria* species were identified (Table 3). *Armillaria ostoyae*, known as a primary pathogen, was the dominant species and accounted for 72% of the isolates. The two mainly saprotrophic species, *A. cepistipes* and *A. borealis*, each accounted for 14% of all isolates. All trees and saplings were infected with only one *Armillaria* species.

Table 3: *Armillaria* species isolated from the roots of symptomatic or recently dead mountain pine trees and saplings.1

<table>
<thead>
<tr>
<th>Species</th>
<th>Trees</th>
<th>Saplings</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. ostoyae</em></td>
<td>18</td>
<td>33</td>
<td>51 (72%)</td>
</tr>
<tr>
<td><em>A. cepistipes</em></td>
<td>8</td>
<td>2</td>
<td>10 (14%)</td>
</tr>
<tr>
<td><em>A. borealis</em></td>
<td>4</td>
<td>6</td>
<td>10 (14%)</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>41</td>
<td>71 (100%)</td>
</tr>
</tbody>
</table>

1 trees ≥ 12 cm dbh; saplings < 110 cm height.
Fruiting bodies of *Heterobasidion* were found on 13 (10.5%) saplings and on six (5.5%) trees. One fruiting body was found on a recently dead tree, but *Heterobasidion* could not be isolated from its roots. This tree was regarded as non-infected. All fruiting bodies of *Heterobasidion* were rather small (0.5 – 3 cm) and were growing at the stem base of the trees, just below the litter surface. No fruiting bodies of *Armillaria* spp. were observed in the study area between May and October 2003.

**Causes of gaps**

A canopy gap was considered to be associated with *Heterobasidion* and/or *Armillaria* if at least 50% of the sampled mountain pines were found to be infected (see Methods). The majority of the gaps (74%) were associated with *Heterobasidion* (Table 4, Figure 1). In five of the six gaps (14%) that were associated with *Armillaria* root rot, the only *Armillaria* species that occurred was *A. ostoyae*. In one *Armillaria* gap, one tree was infected with *A. borealis*, while *A. ostoyae* was isolated from five trees. Only three gaps were associated with both *Heterobasidion* and *Armillaria* (*Heterobasidion* occurred in two gaps with *A. ostoyae* and in one gap with *A. cepistipes*). In 20 of the 31 *Heterobasidion* gaps, a low incidence (one to three trees infected) of *Armillaria* root rot was observed (Figure 2). In nine gaps it was caused by *A. cepistipes*, in six gaps by *A. borealis*, and in three gaps by *A. ostoyae*. In two *Heterobasidion* gaps, two *Armillaria* species (*A. ostoyae* and *A. borealis*, or *A. borealis* and *A. cepistipes*) were found. Individual gaps ranged from 911 m² to 10’304 m² in size with a mean of 3199 m² (median 2447 m²) (Table 4). *Armillaria* gaps were found to be significantly larger than *Heterobasidion* gaps (Wilcoxon rank sum test, p = 0.05).

**Table 4: Characteristics of canopy gaps associated with *Heterobasidion* and/or *Armillaria* in the mountain pine forests of the Swiss National Park.**

<table>
<thead>
<tr>
<th>Gap associated with</th>
<th>n</th>
<th>%</th>
<th>Mean elevation (m a.s.l. ± S.D.)</th>
<th>Mean area (m² ± S.D.)</th>
<th>Median area (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Heterobasidion</em></td>
<td>31</td>
<td>74</td>
<td>1982 ± 74</td>
<td>2898 ± 1898</td>
<td>2428</td>
</tr>
<tr>
<td><em>Armillaria ostoyae</em></td>
<td>6</td>
<td>14</td>
<td>2044 ± 101</td>
<td>5281 ± 3141</td>
<td>4695</td>
</tr>
<tr>
<td><em>Heterobasidion</em> +</td>
<td>3</td>
<td>7</td>
<td>1956 ± 72</td>
<td>1813 ± 445</td>
<td>1837</td>
</tr>
<tr>
<td><em>Armillaria spp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other factors</td>
<td>2</td>
<td>5</td>
<td>2122 ± 43</td>
<td>3705 ± 2151</td>
<td>3705</td>
</tr>
<tr>
<td>Total / mean / median</td>
<td>42</td>
<td>100</td>
<td>1996 ± 83</td>
<td>3199 ± 2195</td>
<td>2447</td>
</tr>
</tbody>
</table>
Figure 1. Size-frequency distribution of canopy gaps (n = 42) in the mountain pine forests of the Swiss National Park.

*Heterobasidion* gaps were mainly found in the western part of the study area along the former pass route, which ran from NW to SE crossing the grassland, and in the eastern part of the study area at lower elevations close to today’s pass route (Figure 3). Within the eastern part of the study area, *Armillaria* gaps were found at higher elevations than gaps associated with *Heterobasidion* (Wilcoxon rank sum test $p = 0.04$).

Figure 2. Incidence of *Heterobasidion* and *Armillaria* in canopy gaps (n = 42) associated with either or both root rot pathogens in the mountain pine forests of the Swiss National Park.
Discussion

Incidence of Heterobasidion and Armillaria spp.

In this study we found that *Heterobasidion annosum* and *Armillaria* spp. occur over a large spatial area in the mountain pine forests of the Swiss National Park and are a major cause of tree mortality. Approx. 75% of the sampled symptomatic or recently dead trees and saplings within or at the edge of canopy gaps were infected with *Heterobasidion* and/or *Armillaria* spp. *Heterobasidion* was the dominant pathogen on both trees and saplings. Our findings extend the results from a two-hectare study plot in the Swiss National Park where *Heterobasidion* was also found to be more prevalent than *Armillaria* on dead trees (Dobbertin et al. 2001a). In saplings, both pathogens were found significantly more often in larger than in smaller height classes. This suggests that the root system of a sapling needs to attain a certain size before it is likely that the roots will come in contact with *Heterobasidion* or *Armillaria*, and subsequently become infected and then die.

![Figure 3. Spatial distribution of canopy gaps associated with Armillaria ostoyae and/or Heterobasidion annosum in the mountain pine forests of the Swiss National Park (SNP).](image)

Sources: borderline, GIS-SNP, unpublished material; DHM25©1994 Bundesamt für Landestopographie.
The reason why *Heterobasidion* is so widespread in the area is not yet fully understood, but it is probably due to intense logging in the past. Mining activities in this area between the 14th and 17th century required timber and fuel and led to the first significant human-induced changes in the forests. A second important influence was the intensive logging carried out between the 17th and 19th century (Parolini 1995). *Heterobasidion* may have spread through spore infections of the fresh stumps and then become widely established in the forests. After the widespread clear-cuts, it was mainly mountain pines that were able to quickly establish on the rocky limestone soils. The trees developed to relatively even-aged and homogenous mountain pine stands, which today represent the dominant forest type in the Park (Kurth et al. 1960), with the *Erico-Pinetum montanae* being the dominant forest association (Brang 1989). The homogeneous stands further favoured the spread of root rot fungi via root contacts. The effect of such past vegetation management and the resulting incidence of *Heterobasidion* was also described by Slaughter & Rizzo (1999) for forest stands in California. These authors suggest that the numerous stumps created by management activities probably favoured the establishment of *Heterobasidion* through primary infection of the stumps with basidiospores. The pathogen then spread from the initial infection points to the surrounding forests and created numerous gaps in the forest (Slaughter & Rizzo 1999).

In our study, two recently dead Swiss stone pine (*Pinus cembra*) saplings were also infected with *Heterobasidion annosum*. Only recently has it been reported that *Heterobasidion* can infect this tree species and cause butt rot (Gonthier et al. 2003; Nicolotti et al. 1999). The isolation of *Heterobasidion* from two saplings proves the ability of the fungus to infect and kill also regenerating Swiss stone pines under natural conditions. Although *Heterobasidion* can readily produce fruiting bodies on infected host tissue (e.g., Schmitt et al. 2000), we observed them only on a limited number of trees and saplings. *Armillaria* fruiting bodies were reported to be rare in this area (Favre 1960), and during our field work, no *Armillaria* fruiting bodies could be found. Most likely, the scarcity of *Heterobasidion* conks and the lack of *Armillaria* fruiting bodies can be attributed to the low precipitation (Blumer 1946) and the cold climate of the study area.

**Gap formation**

In these mountain pine forests, most of the canopy gaps studied were associated with *Heterobasidion*. These gaps were concentrated in the western sub-area where the former pass route ran, and in the eastern sub-area at lower elevations along today’s and the former pass
route. This pattern of *Heterobasidion* gaps might be attributed to the past human activities in the area that favoured the establishment of *Heterobasidion* close to the pass route where trees were cut more frequently. Gaps associated with *Armillaria*, however, were all found in the eastern part of the study area, and were concentrated at higher elevations. All these gaps were associated with *A. ostoyae*, a well-known pathogen of conifers and cause of gap formation in forest ecosystems (e.g., Durrieu et al. 1985; Guillaumin et al. 1993). Interestingly, *A. ostoyae* was not found in a previous study conducted on a smaller scale in the area (Rigling 2001).

The other two *Armillaria* species, *A. cepistipes* and *A. borealis*, play a minor role in gap formation in our study area, and probably only cause tree mortality as secondary pathogens (Cherubini et al. 2002; Dobbertin et al. 2001a). Only two canopy gaps (5%) showed a low incidence of root rot and were probably associated with other disturbance factors, such as windthrow. For the other gaps, windthrow can be ruled out because most of the fallen trees showed evidence of root failure. Moreover, uprooting (i.e., upended root plates) or stem breakage of mountain pines was rarely observed in our study area. The majority of the downed trees showed evidence of stem or root failure or small decayed root plates that were uprooted. However, we believe that wind is an important interacting factor for the creation and the enlargement of gaps in our study area, since root rot is thought to predispose trees to wind throw (Whitney et al. 2002).

In our study, *Armillaria* gaps were significantly larger than gaps associated with *Heterobasidion*. Rizzo & Slaughter (2001) found the opposite in canopy gaps in California where the gaps associated with *Heterobasidion* were significantly larger than the *A. mellea* gaps. Most probably, the small size of *Heterobasidion* gaps in our study – similar to the large-scale incidence of *Annosum* root rot in trees and saplings – has to be attributed to past human activities. The larger *Armillaria* gaps suggest that *A. ostoyae* has been active in the area for a longer time than *Heterobasidion* and maybe was even established before the intensive logging activities in these forests. Some of the large *Armillaria* gaps may have resulted from smaller coalescing gaps (cf. Rizzo & Slaughter 2001).

The strong positive skewness of the gap-size distribution we found, with a few large and many small canopy gaps is a common phenomenon in coniferous forests (e.g., Foster & Reiners 1986; Holeksa & Cybulski 2001; Lertzman & Krebs 1991; Liu & Hytteborn 1991; Rizzo & Slaughter 2001; Yamamoto 1995). In our study, the minimum gap size mapped was 900 m², which, compared to other studies, represents a relatively large area. But the mountain pine forests in the Swiss National Park are rather open, so that the delineation of the numerous smaller canopy gaps on aerial photographs becomes increasingly subjective the
smaller the gap, and would have required very detailed maps of the areas similar to those used in Rizzo & Slaughter (2001).

Generally, assigning the causes of canopy gaps is often a rather subjective process. In other studies, various methods have been applied to determine their causes: Symptoms (e.g., resinosis or foliage chlorosis) and signs (fruiting bodies, mycelial fans or type of wood decay) of disturbance factors such as pathogens and insects were observed, and/or the pathogens were isolated from collected samples (Rizzo & Slaughter 2001; Shearer et al. 1997; Worrall & Harrington 1988). In other studies, gap causes were assessed qualitatively by a team of experts (e.g., Lundquist 1995). Defining gap causes was not always unambiguous in our study, as we only used the criterion of a high incidence of root rot. With a few exceptions, however, most gaps were also characterized by a large amount of standing or lying dead wood in different stages of decomposition. This suggests that the trees died at different times over several decades, which is what we would expect in gaps caused by root rot fungi. Only in five gaps, all of which were found in the western sub-area, comparatively low quantities of dead wood were found. Nevertheless, *Heterobasidion* was the dominant pathogen in four of these gaps. Two explanations could account for the smaller amounts of dead wood in some gaps: These gaps either represent remnants of former grazing meadows in the forest, or the dead wood was removed before the foundation of the National Park. Gäumann & Campell (1932) reported that people used to cut down the standing dead trees in this area. In two of these five gaps, seasonally wet areas could also have played a role in the preservation of the canopy gaps. Regeneration was found to be abundant in almost all canopy gaps and was not a useful criterion for classifying the gaps.

In conclusion, our data suggest that *Heterobasidion* and, to a lesser extent *A. ostoyae*, are the main reasons for the large-scale mortality of mountain pines and the enlargement of canopy gaps in the Swiss National Park. Most probably, the large-scale incidence of trees infected with *Heterobasidion* is related to past human land uses before the foundation of the National Park.

**Acknowledgements**

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References


Canopy gaps associated with root rot fungi 33


Chapter II

Evidence of acceleration of forest dynamics by root rot pathogens in unmanaged mountain pine stands

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Abstract

The root rot pathogens *Heterobasidion annosum* and *Armillaria* spp. can cause single tree death as well as clumped mortality resulting in disease centers. In the mountain pine (*Pinus mugo*) forests of the Swiss National Park in the Central Alps, disease centers associated with *Annosum* and *Armillaria* root rot are characteristic elements of the stands. To assess the impact of root rot fungi on forest dynamics, twelve transects running through disease centers into the adjacent forest were established and the tree regeneration, vegetation, and the volumes of standing and lying dead wood were recorded. Using a stratified sampling technique, healthy-looking mountain pines were checked for *Armillaria* and *Heterobasidion* infection in their roots.

Overall, mountain pine was the most abundant regenerating tree species and accounted for 83% of all seedlings (< 20 cm height) and 93% of all saplings (20-130 cm height), whereas
Swiss stone pine (*Pinus cembra*) was less frequent (seedlings: 15%, saplings: 7%). The density of mountain pine seedlings did not differ significantly between disease centers and the adjacent forest, whereas mountain pine saplings were more frequent within disease centers, indicating that the growth from the seedling to the sapling stage was favored in disease centers. All healthy-looking, regenerating mountain pines in disease centers proved to be not infected with *Heterobasidion* and/or *Armillaria*. The results suggest that root rot fungi speed up forest turnover rates in these stands by causing premature mortality of mountain pines and creating disease centers that are characterized by dense mountain pine regeneration. This is probably slowing down the succession that is assumed to be taking place towards stands with a higher proportion of *P. cembra*.

**Introduction**

Canopy gaps are considered important elements for the dynamics and diversity of forest ecosystems worldwide (e.g., Lertzman et al. 1996; Runkle 1981; Veblen 1986; White et al. 1985; Yamamoto 1995). Depending on various factors such as tree species composition, site characteristics, and past human management, plant pathogens can be an important driving force in creating canopy gaps. Such canopy gaps are also referred to as ‘disease centers’. Thus, pathogens are major agents of spatial and temporal diversity by creating disease centers of different size and, consequently, special habitats for plants and animals within the forest (e.g., Hansen & Goheen 2000; van der Kamp 1991). Tree mortality caused by pathogens occurs within and particularly at the edge of disease centers, and consequently vegetation composition may shift towards more resistant plant species. Such changes may be the direct result of the presence of pathogenic fungi, or the indirect effect of changes in e.g. light and nutrient conditions (Shearer et al. 1997b).

*Armillaria* species are important components of the mycoflora in many forests worldwide (Shaw & Kile 1991). These fungi mainly behave as primary or secondary pathogens, causing root and butt rot on a large number of coniferous and broadleaved tree species (Guillaumin et al. 1993). Those *Armillaria* species acting as primary pathogens can cause significant economic losses by causing mortality and/or stem rot (Morrison & Mallett 1996). *Armillaria* species can survive saprotrophically on woody substrates, thus causing white rot (Morrison et al. 1991; Redfern & Filip 1991). Because *Armillaria* preferentially attacks specific tree species in forests, tree species composition may be altered (Kile et al. 1991). *Heterobasidion*
annosum s.l. (Fr.) Bref. is another widespread pathogenic fungus in many forest ecosystems in the temperate and boreal regions of the northern hemisphere (Woodward et al. 1998). Both *Heterobasidion* and *Armillaria* can spread vegetatively and thereby infect and kill nearby trees, which often results in expanding disease centers (Hodges 1969; Kile et al. 1991).

Information on the influence of root disease centers on forest dynamics is limited. Since most forest stands in Central Europe have been managed for centuries, little is known about how pathogens such as root rot fungi may influence stand dynamics in the absence of management activities. The Swiss National Park offers the opportunity to study the impact of root rot pathogens on forest dynamics in a mountain area where management ceased in 1914. Gäumann & Campell (1932) claimed as long ago as 1932 that Armillaria root rot was the reason for the observed mortality of mountain pines (*Pinus mugo* ssp. *uncinata* (DC.) Domin) in the Swiss National Park. However, Dobbertin et al. (2001a) isolated mainly *Heterobasidion annosum* from the roots of mountain pines that had died recently in a two-hectare study plot in the Swiss National Park, whereas *A. borealis* Marxmüller & Korhonen and *A. cepistipes* Velenovsky were found to be less abundant (Rigling 2001). Bendel et al. (in prep.) determined the incidence of Annosum and Armillaria root rot in symptomatic and recently dead mountain pines from 42 canopy gaps in the mountain pine forests of the Swiss National Park. The majority of the canopy gaps proved to be associated with these root rot fungi and were therefore classified as root disease centers associated with *Heterobasidion* (74% of all canopy gaps), *A. ostoyae* (Romagn.) Herink (14%), or both pathogens, *Heterobasidion* and *Armillaria* spp. (7%). Only 5% of the canopy gaps studied were associated with other factors.

Ultimately, we aimed to assess the functional role of root disease centers in forest dynamics, using the mountain pine (*Pinus mugo* ssp. *uncinata*) stands in the Swiss National Park as a case study. Mountain pine is a subalpine pioneer conifer species that occurs in the mountains of western Europe from the Pyrenees to the Central Alps (Dengler 1992). This pioneer tree has anemochorous seeds and grows typically on unfavorable or disturbed sites (Hegi 1981). In the mountain pine stands of the Swiss National Park, Swiss stone pine (*Pinus cembra* L.) occurs predominantly in the sapling class. In the proximity of our study site, *P. cembra* is concentrated on northern or eastern slopes (Rikli 1909; Zoller 1995). This tree is known to be shade-tolerant and mainly occurs in high elevation sites in the inner-Alpine dry valleys (Hegi 1981). The nutcracker, *Nucifraga caryocatactes* (L.), is the most effective dispersal agent of *P. cembra* seeds (Mattes 1982). In the Swiss National Park, forest communities dominated by *P. cembra* and *Larix decidua* Mill. are thought to represent the late-successional forest
conditions of most of the *P. mugo* stands of our study area (Braun-Blanquet 1931; Kurth et al. 1960; Zoller 1995).

In this study, we hypothesize that: root disease centers accelerate forest turnover rates; favor the regeneration of the early-successional *P. mugo*; and decelerate the establishment of the late-successional *P. cembra*. In addition, we hypothesize that disease centers differ from the adjacent forest in the volumes of dead wood and the number of species they contain. These hypotheses were tested in the mountain pine forests of the Swiss National Park by comparing disease centers with the adjacent forest in terms of tree regeneration, vegetation, and volumes of dead wood.

**Material & Methods**

**Study area and species**

The Swiss National Park is located in the Engadine in the Central Alps. The Park extends over an area of approx. 172 km², 50 km² of which are covered with forests. Its climate is characterized 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.). With the foundation of the Park in 1914, management interventions in the area ceased.

The study area includes the mountain pine forests west of the Ofen Pass in the Swiss National Park (lat. 46°39'N, long. 10°13'E). The forests are found mainly on southern slopes and extend over an area of approx. 10 km² at altitudes between 1800 and 2200 m a.s.l.

**Field sampling**

Twelve disease centers (> 900 m²) were selected in the mountain pine forests of the Swiss National Park. Eight disease centers were known to be associated with *Heterobasidion*, two with *Armillaria*, and two with both pathogens (Bendel et al. in prep.) In the following, the locations of these root disease centers are called ‘sites’ (Figure 1).

In the field, the edges of disease centers were defined according to Runkle (1982) by connecting the stems of living, non-symptomatic trees that had a crown transparency of less than 50%. Crown transparency was estimated by comparing the trees at the edges of each
disease center with local reference trees that showed the maximum amount of foliage under local growing conditions (Dobbertin et al. 2001b). In each site, one transect running along the maximum diameter of the disease center was laid out. The midpoint of the disease center was defined as half the length of the diameter of the disease center. The transects were extended for half the diameter of the disease center into the adjacent forests. Within a distance of one meter on both sides of the transect, all living trees and standing dead trees of any height were recorded. In addition, all standing dead trees of any size and living trees ≥ 12 cm dbh (diameter at 130 cm) were recorded within two meters on both sides of the transect length. The variables recorded for each tree were: its species, position in the transect, height, dbh, percentage of brown and yellow needles, presence of cones, sign of maturation feeding of pine shoot beetles (*Tomicus* spp.), and incidence of brown felt blight (*Herpotrichia juniperi* (Duby) Petrak) and snow blight (*Phacidium infestans* Karst). The height of trees > 4 m was measured using a digital hypsometer (Vertex, Forestor Instruments AB, Sweden).
At regular distances along each transect, twelve vegetation relevé surveys (2x2 m quadrats) were conducted (six in the forest and six in the disease center). In the ground layer (up to a height of 100 cm), plant species were noted and their cover was estimated in percentages. Per quadrat, percentages of open ground, moss, and lichen were estimated. The nomenclature used is according to ‘Flora Helvetica’ (Lauber & Wagner 2001).

In this study, woody debris was defined as any dead twigs, branches, and stems of trees lying on the ground ≥ 0.6 cm in diameter. Woody debris was assessed using the line-intersect method (van Wagner 1968; Warren & Olsen 1964) along the transects in each site. To account for the non-random distribution of woody debris in some sites, a second transect perpendicular to the first was laid out in all sites, and woody debris was estimated accordingly.

Standing dead tree volume was calculated by applying allometric biomass equations for trees with dbh ≥ 7.5 cm (Kaufmann 2002) (Eq. 1), while for standing dead trees with dbh 1 - 7.5 cm, the equation for a paraboloid was used (Eq. 2).

\[
V_{>7.5} = 0.00978 + 0.37868 \times \text{dbh}^2 \times h - 0.09278 \times \text{dbh}^3 \times h \quad \text{(Eq. 1)}
\]

\[
V_{<7.5} = 0.5 \times \pi \times 0.25 \times \text{dbh}^2 \times h \quad \text{(Eq. 2)}
\]

dbh = diameter at 130 cm (in meters); h = height (in meters).

**Incidence of disease in the regeneration**

In six of the twelve sites (nos. 19, 25, 50, 55, 70, 86), the transect was divided into eight segments of equal length, and in each segment one non-symptomatic mountain pine of each size class (< 20 cm height; ≥ 20 & < 130 cm height; ≥ 130 cm height & < 12 cm dbh; ≥ 12 cm dbh) was selected randomly and checked for infection caused by *Heterobasidion* and *Armillaria*. From the selected trees, 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 each sampling, the increment borer was sterilized in 70% ethanol and dried with paper towels. Depending on the size of the saplings, either their whole root system was dug out or three main roots were sampled as described above. Core samples were placed in sterile plastic tubes and kept cool until isolation. Within four days after sampling, all wood samples were processed as described in Bendel et al. (in prep.).
Data analysis
The hypotheses that disease centers differ from the adjacent forest in terms of the density of regenerating trees and the volumes of dead wood (i.e. woody debris and standing dead trees) were tested using paired Wilcoxon tests. The vegetation relevés were analyzed accordingly by comparing the mean number of species and the mean cover of selected taxa between disease centers and forest. Taking the mean cover of plant species in the vegetation relevés in forest and disease centers, rank-abundance curves (also called dominance-diversity curves) were drawn for each site with the abscissa representing species rank and the ordinate representing species abundance.

The densities of regenerating mountain pines in the different size classes (< 20 cm tall; ≥ 20 cm & < 130 cm tall; ≥ 130 cm tall & < 12 cm dbh) along each transect were calculated using a Gaussian Kernel density estimator. The optimal bandwidth was determined according to Scott (1992). The median density was calculated separately for each size class and the curves were smoothed using the ‘lowess’-function (f = 0.1).

All statistical analyses were conducted using R for Windows, version 1.9.1 2004 (R Development Core Team 2004).

Results
Tree regeneration
*Pinus mugo* was the dominating tree species in all size classes (Table 1). *Pinus cembra* accounted for 15% of the seedlings (< 20 cm height), 7% of the saplings (20-130 cm height), and 1% of the trees taller than 130 cm. European larch (*Larix decidua* Mill., n = 36) was found at three sites only, and Norway spruce (*Picea abies* (L.) H. Karst., n = 3) at one site.

The overall density of *P. mugo* seedlings (< 20 cm height) did not differ significantly between disease centers and forest (p = 0.15). However, densities within disease centers varied greatly and tended to be higher within disease centers than in the adjacent forest (Figure 2). In contrast to *P. mugo* seedlings, *P. mugo* saplings (20-130 cm height) were significantly more abundant in disease centers than in the adjacent forest (p = 0.002, Figure 2).

When the densities of regenerating *P. cembra* (< 130 cm tall) between disease centers and forest were compared, no significant difference could be found (p = 0.14). Seedlings and saplings were pooled for this comparison because of the overall low number of *P. cembra*. 
Table 1. Averaged percentages of mountain pine (*Pinus mugo*), Swiss stone pine (*Pinus cembra*), European larch (*Larix decidua*), and Norway spruce (*Picea abies*) recorded in four height classes, and mean densities of mountain pine and Swiss stone pine calculated from twelve study sites in the Swiss National Park.

<table>
<thead>
<tr>
<th>Height class</th>
<th>Disease center (D) / Forest (F)</th>
<th>Percentages a / Mean densities (stems ha⁻¹) b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Pinus mugo</em></td>
</tr>
<tr>
<td>&lt; 20 cm</td>
<td>D</td>
<td>87% / 6870</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>79% / 5487</td>
</tr>
<tr>
<td>20 – 130 cm</td>
<td>D</td>
<td>95% / 9819</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>83% / 2442</td>
</tr>
<tr>
<td>≥ 130 cm &amp; &lt; 12 cm dbh</td>
<td>D</td>
<td>99% / 2249</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>98% / 1561</td>
</tr>
<tr>
<td>≥ 12 cm dbh</td>
<td>D</td>
<td>94% / 197</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>94% / 1050</td>
</tr>
</tbody>
</table>

a calculated as percentages of the total number in all sites.

b calculated as averages of the site means.

Fig. 2. Kernel density (median) of regenerating mountain pines in two-meter-wide transects through twelve disease centers into the adjacent forest in the mountain pine forests of the Swiss National Park. Vertical grey lines represent the edges of the disease centers.
**Incidence of disease in the regeneration**

Regarding all trees < 130 cm in height in the disease centers, 0.1% were symptomatic (≥ 50% & < 100% brown needles) and 1.2% dead, whereas in the adjacent forest, 0.2% were symptomatic and 1.5% dead. A few regenerating trees were infected with brown felt blight (0.8% of all living trees < 130 cm tall), while only one *P. cembra* sapling was infected with snow blight. Pine shoot beetles were found mainly on larger living trees (< 130 cm tall: 1.8%, ≥ 130 cm tall & < 12 cm dbh: 13.4%, ≥ 12 cm dbh: 72.8%).

Out of the 162 non-symptomatic mountain pines sampled, 15 trees (9%) were infected with *Heterobasidion* and/or *Armillaria* (*Heterobasidion*: 10 trees, *Armillaria*: 3; *Heterobasidion* and *Armillaria*: 2; Figure 3). Within the disease centers, all infected trees belonged to the largest size class (≥ 12 cm dbh), whereas in the adjacent forest smaller mountain pines were also infected. The smallest non-symptomatic mountain pine tree infected with *Heterobasidion* was 46 cm tall, and the smallest tree infected with *Armillaria* was 26 cm tall.

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**Fig. 3.** Incidence of non-symptomatic mountain pines infected with *Armillaria* spp. and/or *Heterobasidion annosum* in disease centers and the adjacent forest, grouped by size classes (< 20 cm; 20-130 cm; ≥ 130 cm & < 12 cm dbh; ≥ 12 cm dbh).
Table 2. Tree densities and volumes of dead wood. The table shows total transect length, tree density (dbh ≥ 12 cm), and lying woody debris (≥ 0.6 cm) in disease centers (D) and the adjacent forest (F), including mean and standard deviation (± SD) over all sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Size of disease center (m²)</th>
<th>Transect length (m)</th>
<th>Standing trees (dbh &gt; 12 cm)a</th>
<th>Woody debris (m³ ha⁻¹)</th>
<th>Total dead wood (m³ ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Living (stems ha⁻¹)</td>
<td>Dead (stems ha⁻¹)</td>
<td>(m³ ha⁻¹)b)</td>
</tr>
<tr>
<td>9</td>
<td>3671</td>
<td>117.5</td>
<td>333</td>
<td>856</td>
<td>190</td>
</tr>
<tr>
<td>17</td>
<td>1584</td>
<td>86.2</td>
<td>387</td>
<td>966</td>
<td>129</td>
</tr>
<tr>
<td>19</td>
<td>991</td>
<td>76.2</td>
<td>205</td>
<td>888</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>1423</td>
<td>106.6</td>
<td>283</td>
<td>1214</td>
<td>405</td>
</tr>
<tr>
<td>22</td>
<td>2176</td>
<td>89.6</td>
<td>333</td>
<td>856</td>
<td>419</td>
</tr>
<tr>
<td>25</td>
<td>4046</td>
<td>97.6</td>
<td>0</td>
<td>1047</td>
<td>407</td>
</tr>
<tr>
<td>50</td>
<td>2704</td>
<td>118.3</td>
<td>278</td>
<td>1071</td>
<td>556</td>
</tr>
<tr>
<td>55</td>
<td>934</td>
<td>72.5</td>
<td>190</td>
<td>1519</td>
<td>696</td>
</tr>
<tr>
<td>57</td>
<td>1837</td>
<td>110.5</td>
<td>285</td>
<td>1236</td>
<td>380</td>
</tr>
<tr>
<td>70</td>
<td>1357</td>
<td>88.7</td>
<td>0</td>
<td>1129</td>
<td>81</td>
</tr>
<tr>
<td>77</td>
<td>1378</td>
<td>110.5</td>
<td>51</td>
<td>1429</td>
<td>408</td>
</tr>
<tr>
<td>86</td>
<td>1372</td>
<td>83.6</td>
<td>175</td>
<td>698</td>
<td>0</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1956±1016</td>
<td>96.5±16</td>
<td>212±135</td>
<td>1119±248</td>
<td>306±223</td>
</tr>
</tbody>
</table>

a calculated from four-meter-wide transects.

b volume of standing dead wood calculated for trees > 1 cm dbh.
**Volumes of coarse woody debris and standing dead trees**

There were substantial differences between the twelve sites regarding coarse woody debris and standing dead wood (Table 2). In two sites (nos. 17 and 19), comparatively low volumes of total dead wood were found. Overall, there was significantly more woody debris in disease centers than in the adjacent forest ($p = 0.001$), whereas the volumes of standing dead wood did not differ ($p = 0.7$). Comparing the total volume of dead wood (sum of woody debris and standing dead wood) between the forest and disease centers, the result was significant with more total dead wood found in the disease centers ($p = 0.005$).

**Ground vegetation**

Significantly more species were found in the disease centers than in the adjacent forest ($p = 0.007$) (Table 3; Figure 4). In both disease centers and forest, *Erica carnea* L. was the most abundant species with, however, significantly higher cover in the forest than in the disease centers (Table 3). In contrast, ‘grasses’ (sum of Poaceae, Cyperaceae, and Juncaceae) were more abundant in the disease centers than in the forest (Table 3).

<table>
<thead>
<tr>
<th>Characteristics of the vegetation relevés</th>
<th>Disease center</th>
<th>Forest</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of species</td>
<td>21.7 ± 8.3</td>
<td>17.3 ± 4.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Cover (%) of <em>Erica carnea</em></td>
<td>40.0 ± 17.7</td>
<td>56.0 ± 19.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Cover (%) of ‘grasses’*</td>
<td>24.1 ± 5.8</td>
<td>19.2 ± 5.8</td>
<td>0.012</td>
</tr>
</tbody>
</table>

* Poaceae, Cyperaceae, and Juncaceae.
Regarding the percentages of open ground, cover of lichens, and cover of mosses, there was no significant difference between the disease centers and forest (data not shown). Between 20 and 60 species were found in the vegetation relevés in the disease centers and the adjacent forest (Figure 4). The rank-abundance curves reflecting species number and evenness show a negative exponential decrease. In most sites, the mean cover of the species in the

Fig. 4. Species rank-abundance curves of all sites in the mountain pine forests of the Swiss National Park.
disease center is higher than in the adjacent forest (Figure 4). This shows that the vegetation in the forest was characterized by fewer and often dominant species, whereas in the disease centers a higher number of species with a generally more even distribution (i.e. greater evenness) was found.

Discussion

Tree regeneration

*Pinus mugo* was the most abundant tree species in the regeneration both in the disease centers and the adjacent forest of the mountain pine stands in the Swiss National Park. The density of mountain pine seedlings (< 20 cm height) did not differ significantly between disease centers and forest, whereas mountain pine saplings (20-130 cm height) were found significantly more often in disease centers. This suggests that mountain pine seedlings could establish both in disease centers and forest, but the growth of the seedlings into the sapling stage was enhanced in disease centers. Since mountain pine is a light-demanding pioneer tree species (CAB International 2002; Hegg 1981), one of the most important factors that triggers the regeneration of mountain pine in root disease centers is the increased light availability.

In our study area, the species composition of tree regeneration suggests that disease centers were favorable for the regeneration of mountain pine. These results are in accordance with Shearer et al. (1997a), who reported that tree regeneration was often particularly abundant in disease centers caused by *A. luteobubalina* Watling & Kile. In mountain pine stands in the French Pyrenees, the pathogenic *A. ostoyae* (Romagnesi) Herink has been described as a ‘rejuvenating factor’ because it caused disease centers characterized by dense regeneration of mountain pine with a very low incidence of mortality (Durrieu et al. 1985). Our finding that the regeneration of mountain pine within disease centers was enhanced is also in accordance with Bosch et al. (1992), who reported that tree regeneration always started in canopy gaps in the two subalpine mountain pine stands in the Pyrenees that they studied.

In the seedling and sapling class, *P. cembra* accounted for 5-20% of all regenerating trees, whereas larger Swiss stone pines were found only rarely in our study area. It is most likely that *P. cembra* seeds were brought into the mountain pine stands by nutcrackers from outside the study area. In contrast to mountain pine, the densities of *P. cembra* did not differ
significantly between the disease centers and forest. Assuming that sapling mortality is a constant fraction of understory sapling density for all species, the current densities of the regenerating trees would lead to the prediction that *P. cembra* will increase in the mountain pine stands and become more frequent than it is today.

The occurrence of small and vigorous *P. cembra* saplings on the south-facing slopes of the mountain pine forests was noted already at the beginning of last century by Brunies (1906) and Rikli (1909). They suggested that *P. cembra* must have had a wider distribution in the area in the past. Based on the microscopic analysis of wood samples collected from large stumps found in the mountain pine forests, Schlegel (1985) concluded that *L. decidua* made up a considerable proportion of the forest in the past. In general, forest communities dominated by *P. cembra* and *L. decidua* are thought to represent the late-successional forest types of most of the mountain pine stands in our study area (Braun-Blanquet 1931; Kurth et al. 1960; Zoller 1995). However, mountain pine stands on dry, steep, southern slopes (*Erico-Pinetum mugo caricetosum humilis*) were until recently considered to be ‘climax’ communities (Braun-Blanquet 1931; Trepp 1968; Zoller 1995). By examining data series covering a period of 44 years, Risch et al. (2004; 2005) found evidence that a succession from *P. mugo* towards *P. cembra* or *P. cembra/L. decidua* dominated stands has taken place in the forests of the Swiss National Park. It was concluded that current natural disturbances in the study area do not prevent succession of the *P. mugo* stands to *P. cembra/L. decidua* communities (Risch et al. 2003b). However, these reports have been challenged by a recent palynological study conducted in a small bog at the southern border of our study area where mountain pine was found to have been the dominant tree species in this area during the past approx. 6000 years (Stahli 2004). Thus, stands dominated by mountain pine probably represent natural forest communities in this area.

It is generally recognized that root diseases are major factors in shaping forest structure and composition wherever they are found (e.g., Hansen & Goheen 2000; McLaughlin 2001). Root diseases can change successional trajectories depending on the susceptibility of the tree species present. In the Cascade Mountains in Western North America, mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) was killed by *Phellinus weirii* (Murr.) Gilbertson and the expanding disease centers were filled with early-successional tree species (mostly root rot-resistant pines) that regenerated in the light of the disease centers (Hansen & Goheen 2000). However, it was also reported that the successional development can be accelerated in early-
successional Douglas fir (\textit{Pseudotsuga menziesii} (Mirb.) Franco) forests infested with \textit{P. weirii} (Holah et al. 1997).

\textbf{Incidence of disease in the regeneration}

In general, a very low incidence of mortality and a low incidence of disease were observed in the regeneration, although mountain pine is the main host of \textit{Heterobasidion} and \textit{Armillaria} in our study area. In disease centers, all the regenerating, healthy-looking mountain pines sampled showed no evidence of infection. This could possibly be due to the change in inoculum potential, which was probably low within the root disease centers and higher at the edge of the disease centers or in the adjacent forest. Our results confirm reports (e.g., Durrieu et al. 1985) that the abundant regeneration found in disease centers is not only non-symptomatic, but also probably not infected in most cases. In disease centers, only mountain pines of the largest size class had infected roots. The actual rate of infection was, however, probably even higher in large trees where infection might have been undetected since only the three main roots and not the whole root system were sampled. In our study sites, it is likely that a proportion of mountain pines regenerating in the disease centers will become infected with \textit{Heterobasidion} and/or \textit{Armillaria} in the future. Although regeneration in infested stands can be abundant (e.g., Durrieu et al. 1985; Shearer et al. 1997a; van der Kamp 1995), it has been found that infection rates generally increase with the size and age of the advance regeneration (Piri & Korhonen 2001). Our results are in contrast to the reports of Piri & Korhonen (2001), who found that 21\% of the advance-growth spruce (\textit{Picea abies} L.) in the infested areas were infected with \textit{Heterobasidion}, whereas on healthy-looking control plots only 2.4\% of the advance regeneration was infected. We assume that the differences between our results and the reports of Piri & Korhonen (2001) are probably due to the different sampling designs used and the different tree species studied.

\textbf{Volumes of coarse woody debris and standing dead trees}

Significantly more dead wood was found in the disease centers than in the adjacent forest. The standing and fallen dead trees were most probably killed by the root rot pathogens during the expansion of the disease centers. Only in two out of twelve study sites (nos. 17 and 19), were comparatively low volumes of dead wood recorded. These canopy gaps were probably not caused directly by root rot pathogens, but rather because they had most likely been used in
the past as small pastures within the forest. Old stumps were found at both sites, which means that the stems must have been removed before the foundation of the Park. The hypothesis that these areas were formerly used as small pastures is also supported by the occurrence of plant species indicating past human management such as *Plantago alpina* L., *Polygonum viviparum* L., *Trifolium repens* L., and *Leontodon* spp. However, most of the dying or recently dead mountain pines within or at the edge of these two disease centers were infected with *Heterobasidion* (Bendel et al. in prep.), suggesting that this root rot pathogen helped to maintain the openness of these sites.

Comparable values for the total amount of dead wood in mountain pine stands in the Swiss National Park were reported by Risch et al. (2003a). In comparison to other studies that recorded volumes of dead wood in European forests, the quantities found in the Swiss National Park are relatively high (cf. Bretz Guby & Dobbertin 1996). Virgin and semi-natural forests generally tend to contain large volumes of dead wood (e.g., Albrecht 1991; Burschel 1992; Kirby et al. 1991; Leibundgut 1982). Although the mountain pine stands in the Swiss National Park are neither virgin nor semi-natural forests, the volumes of dead wood we recorded are comparable to such sites. We assume that the large quantities of dead wood in our study area were most probably caused by the widespread infestation of *Heterobasidion* and *Armillaria* spp.

**Ground vegetation in disease centers and adjacent forest**

Significantly more plant species were present in the disease centers compared to the adjacent forest. The number of species in disease centers was augmented mainly by the presence of grassland species typically found at this altitude (such as *Plantago alpina* L., *Cirsium acaule* Scop., *Carlina acaulis* L., and *Helianthemum* spp.). Thus, the root disease centers enabled grassland species to occur in small patches within the forest matrix.

The vegetation composition and species densities in the ground layer may also influence tree regeneration. Schmid et al. (1995) reported that the growth of seedlings of dwarf mountain pine (*Pinus mugo* Turra s.str.) in a bog in South Germany was enhanced in sites characterized by low densities of ericaceous shrubs, whereas their survival was reduced in sites with high densities of ericaceous shrubs. The negative impact of dense ericaceous shrubs on the regeneration of dwarf mountain pine was also mentioned by Hafenscherer & Mayer (1986) for stands in the Tyrol (Austria). Hence, apart from the increased light availability in disease
centers, the lower density of Erica might also have enhanced the regeneration of mountain pine in the root disease centers of our study area.

In conclusion, Pinus mugo was the most abundant regenerating tree species both in the disease centers and in the adjacent forest in the mountain pine stands in the Swiss National Park. In disease centers associated with root rot fungi, P. mugo regeneration was significantly more abundant than in the adjacent forest, whereas the density of regenerating P. cembra did not differ significantly between disease centers and the adjacent forest. There was a very low incidence of mortality in the regeneration in disease centers and on all non-symptomatic regenerating mountain pines sampled in disease centers there was no incidence of Anosum or Armillaria root rot. These results suggest that the spread of root diseases can speed up stand turnover rate by causing premature mortality of mountain pine trees and creating disease centers within which mountain pine regeneration is increased. At the same time, root disease centers may slow down the succession assumed to be occurring of the mountain pine forests towards stands with a higher abundance of P. cembra.

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References


Chapter III

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

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Abstract

Armillaria species are plant pathogens that cause root rot and are known to be involved in large-scale 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 km² 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. Armillaria cepistipes and A. borealis mainly occurred as genets of small spatial extent (mean of 0.2 ha and 0.6 ha, respectively), 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; 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 & 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 to 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 (DC.) Domin) 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 – besides Heterobasidion annosum – causes root rot 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 i) to identify the Armillaria species and determine the spatial extent, fragmentation, and potential age of Armillaria genets on a landscape scale in a mountain pine forests of the Swiss National Park, and ii) to 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 km², 50 km² 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 west of the Ofen Pass 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 southern slopes, and extend over an area of approx. 10 km² 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 m²), 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, 9-10 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 sterilized 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 (about 1 cm long) of each root sample were surface sterilized in sodium hypochlorite (active chlorine = 7%) for 30 seconds and rinsed twice in sterile, demineralised water for ≥ 15 seconds. 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 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-sterilized 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 observed very rarely, 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 3-5 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\(^{TM}\) 8.3 (© ESRI Inc. 1999-2002). To better visualize small genets in Fig. 1B-C, genet boundaries were drawn with a radius of approx. 20 m around the infected trees. One and the same 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). Prior to 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 II 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. Armillaria 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Only Root</th>
<th>Root &amp; Rhizom.</th>
<th>Only Rhizom.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ostoyae</td>
<td>118</td>
<td>5</td>
<td>7</td>
<td>130 (64%)</td>
</tr>
<tr>
<td>A. borealis</td>
<td>34</td>
<td>2</td>
<td>4</td>
<td>40 (20%)</td>
</tr>
<tr>
<td>A. cepistipes</td>
<td>16</td>
<td>5</td>
<td>11</td>
<td>32 (16%)</td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>12</td>
<td>22</td>
<td>202 (100%)</td>
</tr>
</tbody>
</table>

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

**Armillaria genets**

Intraspecific somatic incompatibility pairings among the Armillaria isolates revealed eleven genets of A. ostoyae, 13 of A. borealis, and 16 of A. cepistipes (Table 2). In the study area Champlönch, two 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. Armillaria ostoyae produced the largest genets with a mean size of 6.8 ha, compared to 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).
Figure 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 II 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.
Table 2. Characteristics of Armillaria populations in the mountain pine forests of the Swiss National Park.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>A. ostoyae</th>
<th>A. borealis</th>
<th>A. cepistipes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of genets</td>
<td>11</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Mean genet size (ha)</td>
<td>6.8</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Total genet area (ha)</td>
<td>75.2</td>
<td>7.9</td>
<td>3.1</td>
</tr>
<tr>
<td>Portion of study area colonized (%)</td>
<td>23%</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>Mean (median) distance from pass route (m)</td>
<td>297 (225)</td>
<td>263 (165)</td>
<td>176 (130)</td>
</tr>
</tbody>
</table>

The cumulative colonization 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 II
Table 3. Characteristics of the largest Armillaria genets \((n = 16)\) that were found in the mountain pine forests of the Swiss National Park\(^1\).

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

\(^1\) Number of genets that were recovered from one (or two) trees: A. ostoyae: 2 (2); A. borealis: 7 (1); A. cepistipes: 7 (5).

\(^2\) Measured between most distant collection points.

\(^3\) Disease centre associated with Armillaria (A), Heterobasidion (H), and both fungi (A & H).

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 described to date in Europe. Armillaria ostoyae genets of similar 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 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 conditions suitable 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, i.e. 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 under-represented in our study, and more detailed sampling most likely would have yielded a higher number of small genets. Also, although intraspecific somatic incompatibility has been reported to be in general an efficient and reliable technique to delineate *Armillaria* genets, 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 intraspecific 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). Since 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 today’s pass route (a sealed main road approx. 8 m wide with cleared strips several meters 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 colonized wood has been displaced and 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. Since the site in the current study is also characterised by low temperatures and, compared to 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 years. However, it is possible that the age of 2000 years 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.
Genetic population structure of *Armillaria* species

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 & van der Kamp (2001b). Small genets might be rather old, 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 & 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 pathogens (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* root rot 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 pathogen 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 years 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

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References


Genetic population structure of Armillaria species


Chapter IV

Symptoms and signs of Heterobasidion and Armillaria ostoyae root disease in mountain pine

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Bendel, M.1,2, and Rigling, D.1 Symptoms and signs of Heterobasidion and Armillaria ostoyae root disease in mountain pine. Forest Pathology.

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Abstract
The symptoms and signs associated with root disease caused by Heterobasidion annosum or Armillaria ostoyae in mountain pines (Pinus mugo) were investigated in the Swiss Alps. Dying or recently dead mountain pine trees (≥ 12 cm dbh) and saplings (< 130 cm height) were assessed for root disease by taking root core samples followed by isolations in the laboratory. From a sub-sample of 50 trees, an additional core was taken from the butt. A total of 157 mountain pine trees and 184 saplings whose roots were infected with either pathogen or lacked infection were analyzed using logistic regression models. The objective was to determine the most prominent symptoms induced by the fungi (resinosis, dbh/height) and signs of the fungi (mycelium, fruiting bodies, rhizomorphs) that would allow to easily and reliably determine the incidence of A. ostoyae and Anno sum root rot on mountain pines in the field.

Heterobasidion was found to cause both root and butt rot on mountain pine, whereas A. ostoyae was mostly restricted to the root systems of the trees sampled. The best sign for the
presence of Armillaria root rot were *Armillaria* fans, and for Annosum root rot *Heterobasidion* mycelium (pustules and ectotrophic growth). In addition, resinosis was a good symptom for *A. ostoyae* root rot in trees. Symptoms and signs indicating *A. ostoyae* or Annosum root rot were more reliable for saplings than for trees. *Armillaria* rhizomorphs and *Heterobasidion* fruiting bodies were generally poor signs for root rot both in mountain pine trees and saplings.

**Introduction**

Root rot fungi are important components of many forest ecosystems worldwide. In the temperate and boreal regions of the northern hemisphere, *Heterobasidion annosum* s.l. (Fr.) Bref. is known as a serious pathogen attacking conifers (Hodges 1969). Another widespread root rot pathogen is *Armillaria*, with several species acting as primary pathogens on trees and causing significant economic losses in forests (Morrison & Mallett 1996).

A considerable proportion of roots can be infected and killed by *Armillaria* or *Heterobasidion* before any signs of the fungi and external symptoms induced by the fungi are visible (Greig 1998; Maloy & Gross 1963; Whitney et al. 1988). The degree of root decay at which aboveground symptoms become apparent depends on different factors such as tree species, age of the tree, site condition, and root pathogen(s) present (Omdal et al. 2004). In conifers with Armillaria root rot, symptoms indicating disease include basal resinosis, changes in foliage characteristics, crown dieback, and the incidence of root lesions (Morrison et al. 1991). Signs typical for Armillaria root rot are mycelial fans, rhizomorphs, and *Armillaria* fruiting bodies (Morrison et al. 1991). In trees infected with *Heterobasidion*, resin exudation on the stem or roots, decreased crown density, changed crown colour, and butt swelling are symptoms that can indicate internal decay of trees (Greig 1998). *Heterobasidion* fruiting bodies may also be used as signs for Annosum root rot, whereas the thin *Heterobasidion* mycelium is thought to be an unreliable sign (Greig 1998), as *Heterobasidion*-caused wood decay can also be present in trees that do not show any external symptoms (Bradford et al. 1978; Greig 1998).

In general, studies that sought to develop relationships between root infection of trees and expression of symptoms and signs have yielded variable results (e.g., Kelsey et al. 1998; Kurkela 2002; Omdal et al. 2004; Pratt 1979; Vollbrecht & Agestam 1995; Wiensczyk et al.
Symptoms and signs of root disease

1997; Worrall et al. 2004). Bloomberg & Morrison (1989) concluded that their classification of *Pseudotsuga menziesii* based on basal resinosis had good potential for estimating the growth losses caused by *A. ostoyae* (Romagnesi) Herink. In contrast, Whitney et al. (1988) reported for symptomless saplings of *Picea mariana*, *Picea glauca*, and *Pinus resinosa* growing around *Armillaria*-killed trees variable but high levels of infection with *A. ostoyae*. And in a mixed conifer stand dominated by *P. menziesii*, *Abies grandis*, and *Pinus ponderosa*, 78% of all trees infected with *A. ostoyae*, *Heterobasidion annosum* or *Phellinus weirii* (Murr.) Gilbertson did not show any crown or root collar symptoms (Filip 1986). However, ectotrophic mycelium of *P. weirii*, mycelial fans of *Armillaria*, and dead cambium were found to be reliable indicators for root rot (Filip 1986). MacKenzie & Shaw (1977) classified *Pinus radiata* seedlings as being killed by *Armillaria* when they showed *Armillaria* signs (rhizomorphs and/or mycelial fans) and symptomatic host response (basal resinosis). However, rhizomorphs were reported to be of limited value for the identification of *Armillaria* root rot since they may be difficult to find and recognize and because they can be present without causing infection (Greig & Strouts 1977). In general, symptom expression can vary depending on the tree and pathogen species involved, the severity of disease, and the site characteristics. In addition, the expression of symptoms may also differ between climatic regions, as shown for conifers with *Armillaria* root rot (Morrison et al. 2000). As a result, there is a need to determine reliable symptoms and signs for each pathogen-tree interaction.

Both *Armillaria* and *Annosum* root rot have been found on mountain pine (e.g., Dobbertin et al. 2001; Goggioli et al. 1996; Vasiliauskas 1999). To our knowledge, symptoms and signs indicating root disease on mountain pine have not been determined yet. In addition, there is little information about the extension of *Heterobasidion* and *Armillaria* in the root systems of mountain pines (Bendz-Hellgren et al. 1998; Thomsen & Jørgensen 1997), i.e. whether the pathogens attack mainly the roots, the butt, or both.

In the mountain pine forests of the Swiss National Park in the Alps, root rot fungi have been suspected to be causal agents in the observed mortality of trees (Bendel et al. in prep.; Cherubini et al. 2002; Dobbertin et al. 2001). *Heterobasidion annosum* was found to be the most widespread root rot pathogen, while *Armillaria* spp. were less abundant (Bendel et al. in prep.). *Armillaria* root rot was mainly caused by *A. ostoyae*, whereas *A. cepistipes* Velenovsky and *A. borealis* Marxmüller and Korhonen were less common. Knowing the most important, easily detectable symptoms and signs that indicate the presence of
Heterobasidion or A. ostoyae root rot in dying or recently dead mountain pines would be useful to quickly and reliably determine the cause of tree death in the field.

The objectives of our study were i) to link the incidence of A. ostoyae and Annosum root rot in dying or recently dead mountain pines with external symptoms and signs using logistic regression models, and ii) to evaluate the frequency of Heterobasidion and A. ostoyae disease in the roots and the butt.

**Materials and Methods**

**Study site**

The Swiss National Park is located in the Engadine in the Central Alps. Its climate is characterized 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.). With the foundation of the Park in 1914, management interventions have virtually ceased.

The study area includes the mountain pine forests around Il Fuorn and Champlönch west of the Ofen Pass in the Swiss National Park (lat. 46°39'N, long. 10°13'E). These forests are found mainly on southern slopes, and extend over an area of approx. 10 km² at an altitude between 1800 and 2200 m a.s.l.

**Assessment of root rot, symptoms, and signs**

Dying and recently dead mountain pines (172 trees ≥ 12 cm dbh, 192 saplings < 130 cm height) were checked for the incidence of Armillaria spp. and Heterobasidion annosum in their roots in 2003 (Bendel et al. in prep.). Three main roots were excavated from each tree, and after having removed the bark, one wood core sample was taken from every root at a distance of approx. 20 cm from the stem using an increment borer. Between root samplings, the increment borer was sterilized in 70% ethanol and dried with paper towels. Depending on the size of the mountain pine saplings, either their whole root system was dug out or three main roots were sampled, as described above. Core samples were placed in sterile plastic
tubes and kept cool until isolation. All wood samples were processed within four days after sampling.

Three pieces (about 1 cm long) of each root core sample were surface sterilized in sodium hypochlorite (active chlorine = 7%) for 30 seconds and rinsed twice in sterile, demineralised water for ≥ 15 seconds. The pieces were dried between paper towels and placed on agar plates (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, 100 mg l⁻¹ sodic benzylpenicillin) modified from Legrand & Guillaumin (1993). The roots of the saplings were thoroughly washed with tap water and three to four discs (5-10 mm thick) were cut from the main roots. After removing the bark, one piece (10 x 10 mm) was cut from each disc and surface sterilized as described above. 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). No attempt was made to isolate Armillaria from mycelial fans.

The presence of Heterobasidion was indicated by its Spiniger meineckellus (Olson) Stalpers conidial stage (Worrall & Harrington 1992). The Armillaria isolates were identified by diploid-haploid pairings using three haploid tester strains of each A. cepistipes, A. borealis, and A. ostoyae (Korhonen 1978). A tree was considered infected with Heterobasidion and/or A. ostoyae if either or both pathogens were identified on at least one root core sample. Those mountain pines that showed Armillaria root rot caused by A. borealis or A. cepistipes were excluded in order not to confound the symptoms and signs that may be associated with Armillaria species other than A. ostoyae.

Parameters that were recorded for each tree and sapling were the presence of resinosis at the base of the stem, the presence of wounds at the base of the stem, the dbh of the trees (diameter at 130 cm), and the height of the saplings. Putative signs of Armillaria root rot recorded were the presence of rhizomorphs attached to the roots and Armillaria mycelial fans under the bark of the roots sampled. Small, white pustules and ectotrophic growth of paper-thin mycelium of Heterobasidion (combined as ‘Heterobasidion mycelium’) (see colour plates in Woodward et al. 1998) and fruiting bodies of Heterobasidion (at least 1 cm in diameter) were recorded as putative signs of Annosum root rot. A sign of root rot was considered present on a tree if it was found on at least one of the three roots assessed.

From 50 of the 172 mountain pine trees checked for the incidence of A. ostoyae and Annosum root disease, an additional core was taken from the butt just above the soil surface to
determine the incidence of the pathogens in the butt. Isolation of the fungi was carried out as described above.

**Statistics**

Four logistic regression models were developed to determine the parameters that best predicted the incidence of *A. ostoyae* or Annosum root rot in trees and saplings, respectively. Only those trees and saplings infected with either pathogen or that lacked infection were considered. In total, 157 trees (*A. ostoyae*: 15; *Heterobasidion*: 84; without infection: 58) and 184 saplings (*A. ostoyae*: 33; *Heterobasidion*: 122; without infection: 29) were included in the logistic regression models. As a rough rule of thumb, no more than m/10 predictors should be examined to fit a regression model, where m is the number of observations in the least represented category in the case of a binary response (Harrell et al. 1996). Therefore, three explanatory variables could be included in both sapling models, whereas up to six variables could have been used in the *Heterobasidion* tree regression model and two variables in the *Armillaria* tree model. In the *Armillaria* tree model, at first regression analysis was applied to reduce the number of explanatory variables from four to two. Those two variables that yielded the lowest AIC values were excluded from the *Armillaria* tree model. Resinosis was excluded from both sapling models because resinosis could hardly be observed on mountain pine saplings (Table 1). Explanatory variables that were included in the four regression models are listed in Table 1.

All statistical analyses were conducted using R for Windows, version 1.9.1 2004 (R Development Core Team 2004). For the regression analyses, ‘lrm’ from the Design library was applied (Harrell 2001). In all models, penalized maximum likelihood (PML) was used to accurately estimate the parameters of the models (Harrell 2001). Optimal penalty was evaluated over a grid of 0.1-10 using the ‘pentrace’-function. Resampling validation (‘validate.lrm’, B = 150) with backward step-down variable deletion based on the AIC-criterion was applied. The fit of the models was estimated by Nagelkerke’s $R^2$, abbreviated with $R^2_N$ (Nagelkerke 1991), which gives a measure of the variance in the dependent variable that is explained by the independent variables. In addition, the AUC, area under the ROC curve (Harrell 2001), is given as a measure of discrimination accuracy. AUC can take values between 0 and 1. Hosmer & Lemeshow (2000) suggested interpreting AUC values as follows: $0.7 \leq \text{AUC} < 0.8 = \text{acceptable}; \ 0.8 \leq \text{AUC} < 0.9 = \text{excellent}; \ 0.9 \leq \text{AUC} = \text{outstanding}.$
A $X^2$ test was conducted to compare the number of *Heterobasidion* and *A. ostoyae* trees infected only in the roots with those infected in the roots and the butt.

### Results

**Selection of explanatory variables**

Generally, symptoms and signs regarded characteristic for *A. ostoyae* and Annosum root disease were also found on trees from whose roots neither or both pathogens were isolated (Table 1). *Armillaria* fans were also detected on trees that had Annosum root rot or lacked *A. ostoyae* and/or *Heterobasidion* infection. From rhizomorphs attached to the roots sampled, three *Armillaria* species were identified. Rhizomorphs found on mountain pines that showed Annosum root rot or that lacked infection ($n = 29$) were identified as *A. cepistipes* ($n = 9$), *A. borealis* ($n = 3$), and *A. ostoyae* ($n = 6$); isolation was not successful in eleven cases. With one exception, all rhizomorph samples collected from mountain pines with *A. ostoyae* root rot were identified as *A. ostoyae* ($n = 6$), while *Armillaria* could not be isolated from three rhizomorphs.

Most *Heterobasidion* fruiting bodies were found on mountain pine saplings infected with Annosum root rot; two conks, however, were detected on mountain pine trees from whose roots *Heterobasidion* could not be recovered (Table 1, Bendel et al. in prep.).

To reduce the number of explanatory variables in the *Armillaria* tree model from four to two, a logistic regression was performed and the two variables with the lowest AIC value (i.e., dbh, the presence of rhizomorphs) were excluded. In mountain pine trees and saplings infected with *Armillaria* or *Heterobasidion*, the four logistic regression models yielded similar results (Table 2): In all models, the presence of mycelium (*Armillaria* fans or *Heterobasidion* mycelium) was highly significant. In addition, *A. ostoyae* root rot tended to be found in mountain pine trees that showed resinosis at the base of the stems ($p = 0.006$). Except for the *Heterobasidion* tree model, the explanatory variables accounted for a high proportion of the variance in all models as indicated by the $R^2_N$ and AUC values (Table 2).
Table 1. Incidence of symptoms and signs associated with root disease caused by *Armillaria ostoyae* and/or *Heterobasidion annosum* in mountain pine (*Pinus mugo*).

<table>
<thead>
<tr>
<th>Number of mountain pines with symptoms and signs</th>
<th>Type of root rot&lt;sup&gt;1&lt;/sup&gt;</th>
<th>TREES&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SAPLINGS&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterobasidion</td>
<td>A. ostoyae</td>
<td>Heterobasidion &amp; A. ostoyae</td>
</tr>
<tr>
<td>Total No. of mountain pines</td>
<td>84</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td><em>Armillaria</em> fans</td>
<td>13</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Rhizomorphs</td>
<td>13</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td><em>Heterobasidion</em> mycelium&lt;sup&gt;4&lt;/sup&gt;</td>
<td>51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Heterobasidion</em> fruiting bodies</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Resinosis (0/1)</td>
<td>7/77</td>
<td>0/15</td>
<td>0/3</td>
</tr>
<tr>
<td>Wounds</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>1</sup> Determined by isolations from three root core samples per tree.

<sup>2</sup> ≥ 12 cm dbh.

<sup>3</sup> < 130 cm in height.

<sup>4</sup> Ectotrophic growth and/or pustules of *Heterobasidion*. 
Table 2. Estimates and p-values of the four logistic regression models of mountain pine (Pinus mugo) trees (≥ 12 cmdbh) and saplings (< 130 cm height) infected with Armillaria or Heterobasidion annosum. A backward selection based on the AIC criterion was applied to reduce the number of variables. Resampling validation was applied to estimate the explained variance as expressed by Nagelkerke’s $R^2$ ($R^2_N$) and the AUC (area under the ROC-curve).

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>TREES $^1$</th>
<th>SAPLINGS $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. ostoyae</td>
<td>Heterobasidion</td>
</tr>
<tr>
<td></td>
<td>Estimate (± S.E.)</td>
<td>p-value</td>
</tr>
<tr>
<td>Dhb / height (cm)</td>
<td>3</td>
<td>ns $^4$</td>
</tr>
<tr>
<td>Heterobasidion fruiting bodies (0/1)</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Rhizomorphs (0/1)</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Resinosis (0/1)</td>
<td>1.81±0.66</td>
<td>0.006</td>
</tr>
<tr>
<td>Armillaria fan (0/1)</td>
<td>1.65±0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>H. annosum mycelium (0/1)</td>
<td>-</td>
<td>0.84±0.31</td>
</tr>
<tr>
<td>$R^2_N$</td>
<td>0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>AUC</td>
<td>0.84</td>
<td>0.61</td>
</tr>
</tbody>
</table>

$^1$ Number of trees included in the models: A. ostoyae trees: 15, Heterobasidion trees: 84, trees without infection: 58.

$^2$ Number of saplings included in the models: A. ostoyae saplings: 33, Heterobasidion saplings: 122, saplings without infection: 29.

$^3$ Explanatory variables not included in the specific models are characterized by “-”.

$^4$ Explanatory variables excluded in the backward selection are indicated with “ns”.


Table 3. Number of dead mountain pine (Pinus mugo) trees (≥ 12 cm dbh) showing infection of *Heterobasidion* or *Armillaria ostoyae* in the butt, the roots, or both (n = 29).

<table>
<thead>
<tr>
<th>Location of decay</th>
<th>Heterobasidion*</th>
<th>A. ostoyae</th>
</tr>
</thead>
<tbody>
<tr>
<td>only in butt</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>only in roots</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>in butt and roots</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>14</td>
</tr>
</tbody>
</table>

* Two mountain pines were infected with both pathogens; these trees are listed twice in the table – as trees with infection of *Heterobasidion* and *A. ostoyae*.

Comparing infection in roots and butt

A sub-sample of 50 mountain pine trees was additionally assessed for the incidence of *A. ostoyae* and *Heterobasidion* root disease in the butt. In 29 trees, infections with *A. ostoyae* and/or *Heterobasidion* were found in the roots, the butt, or both; from 21 trees neither pathogen could be recovered. In most trees infected with *Heterobasidion*, the pathogen was detected in the roots and in the butt, whereas *A. ostoyae* infections were restricted mostly to the roots (Table 3). In contrast to *Heterobasidion*, *A. ostoyae* infections were significantly more often restricted to the roots ($X^2$ test, $p = 0.04$, comparing the number of trees infected in the roots with those infected in the roots and the butt). From two trees, *Heterobasidion* or *A. ostoyae* was isolated only from the butt but not from the roots (Table 3). From two other mountain pines, both pathogens were recovered; in both, *A. ostoyae* was restricted to the roots, while *Heterobasidion* was also isolated from the butt.

Discussion

*Root disease caused by Armillaria ostoyae*

In the sapling model, *Armillaria* fans accounted for a high proportion of the variance, whereas in the tree model, a smaller proportion of the variance was explained by the presence of *Armillaria* fans and resinosis. Thus, in the field, the incidence of *A. ostoyae* root rot can be easily and reliably determined in mountain pine saplings, whereas its detection is more difficult in mountain pine trees. Therefore, the presence of *Armillaria* fans under the bark of mountain pine trees does not necessarily imply that *A. ostoyae* can be recovered from the root
core samples. In cases where mycelial fans were found but the pathogen could not be isolated from the roots, Armillaria root rot was probably in an early stage of infection where the pathogen has not penetrated into the wood yet. In addition, since no attempt was made to isolate Armillaria from mycelial fans, we cannot rule out that some mycelial fans were also formed by Armillaria species other than A. ostoyae. Armillaria fans have often been used to diagnose the incidence of root disease (e.g., Gibson 1960; Morrison et al. 1991; Rizzo & Slaughter 2001; Roth et al. 1977).

In the case of A. ostoyae, resinosis was a significant symptom indicating Armillaria root rot. This finding is in accordance with Bloomberg & Morrison (1989) who separated successfully Pseudotsuga menziesii trees infected with A. ostoyae into broad growth-reduction classes based on the extent of basal resinosis. In mixed-conifer forests with Abies concolor, Picea pungens, P. menziesii, Pinus ponderosa, and Picea engelmannii, Worrall et al. (2004) also found resinosis to be an efficient symptom indicating A. ostoyae root rot. In contrast, Omdal et al. (2004) reported that only a small proportion of P. menziesii, A. concolor, and P. ponderosa infected with A. ostoyae or Heterobasidion exhibited basal resinosis regardless of the extent of infection. And in low-vigour P. menziesii trees infected with Armillaria, resinosis was found to be only slight or almost absent (Buckland 1953). In addition, Rishbeth (1982) mentioned that resin production in inoculated Pinus nigra saplings was more vigorous in saplings inoculated with A. mellea compared to those inoculated with A. ostoyae. Thus, the extent of resinosis may not only depend on the tree species and the vigour of the trees, but also on the Armillaria species involved.

The presence of rhizomorphs was found to be a poor sign for A. ostoyae root rot in both mountain pine trees and saplings. Two thirds of all successful isolations from rhizomorphs were identified as A. borealis or A. cepistipes, indicating that these mainly saprotrophic species may be present as rhizomorphs whereas root rot at this stage was mainly caused by the pathogenic A. ostoyae and Heterobasidion.

**Root disease caused by Heterobasidion annosum**

In dying or recently dead mountain pine trees and saplings infected with Heterobasidion, the presence of Heterobasidion mycelium (i.e., ectotrophic mycelium and pustules) was the only significant explanatory variable indicating Annosum root disease. In the sapling model, a high proportion of the variance was explained by the presence of Heterobasidion mycelium. Thus, in mountain pine saplings, the presence of Annosum root rot can be reliably identified in the
field by the identification of *Heterobasidion* mycelium. This result is in contrast to reports suggesting that *Heterobasidion* mycelium is an unreliable diagnostic feature for *Annosum* root rot, since other fungi may form similar mycelia (Greig 1998). It is possible that these fungi are rare or absent in our study area and *Heterobasidion* remains the dominant species to produce this type of mycelium.

In contrast to the sapling model, only a limited proportion of the variance could be explained by the presence of *Heterobasidion* mycelium in the tree model. This low value of variance explained by the model probably occurs because i) *Heterobasidion* mycelium was destroyed when excavating the roots and could not be identified correctly, ii) mycelium was not consistently produced, or iii) isolations from core samples failed from roots that showed *Heterobasidion* mycelium. In any case, the low value of variance explained by the *Heterobasidion* tree model implies that mountain pine trees killed by *Heterobasidion* can hardly be identified in the field because of a lack of consistent symptoms and signs.

In our study, the majority of the mountain pines with *Annosum* or *Armillaria* root rot did not show any sign of wounding at the base of the stem, suggesting that wounds do not play a major role in either root disease. As reported by Rönnberg & Jørgensen (2000) for *Picea abies*, a rare incidence of visible root or stem damage may not correspond to the incidence of *Annosum* root disease in trees. The rare occurrence of *Heterobasidion* fruiting bodies, the absence of *Armillaria* fruiting bodies (personal observation), and the scarcity of rhizomorphs may be linked with the cold and comparatively dry climate of our study area in the Alps.

**Comparing infection in roots and butt**

The successful detection of stem and butt rots largely depends on the sampling method applied in the field. In our sub-sample of 50 mountain pine trees, *A. ostoyae* was significantly more often restricted to the roots than *Heterobasidion*, which was most often isolated both from the roots and the butts. This result is in accordance with Whitney (1997) who reported for *Picea glauca, Picea mariana*, and *Abies balsamea* that *A. ostoyae* was mainly recovered from the roots.

We assume that our estimation of the proportion of mountain pine trees infected with *Heterobasidion* was rather conservative, and we may have underestimated the frequency of the pathogen in our study area. The only way to determine the actual incidence of root disease would be to dig out the complete root system of the tree, which is not possible in a National...
Symptoms and signs of root disease

Park. Relative to the size of the root system, the sampling size in saplings was larger and it is therefore unlikely that infection remained undetected in mountain pine saplings.

In conclusion, Armillaria fans and Heterobasidion mycelium were reliable signs for the incidence of A. ostoyae and Anosum root rot in mountain pine saplings. In trees with Armillaria root disease, Armillaria fans and resinosis were valuable indicators, whereas in trees with Annosum root disease, the expression of external symptoms and signs were inconsistent. The presence of rhizomorphs and Heterobasidion fruiting bodies were found to be unreliable sings for root rot in mountain pines. Comparing the incidence of root and butt rot in mountain pine trees, A. ostoyae was found to be more restricted to the roots than Heterobasidion, which was often isolated both from the butt and the roots.

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Synthesis

The overall aim of this thesis was to evaluate the influence of the root rot fungi *Heterobasidion annosum* (Fr.) Bref. and *Armillaria* spp. on forest dynamics, using the mountain pine (*Pinus mugo* ssp. *uncinata* (DC.) Domin) stands in the Swiss National Park as a case study. These forests offer the opportunity to study this question in a mountain area that has not been managed for most of the 20th century.

Below, I first view the four manuscripts in hand in the context of the entire thesis with respect to ecological findings; then I discuss some issues of the methodology applied. The chapter is concluded with an outlook on additional research topics that, from my point of view, would provide valuable insights regarding the influence of *Heterobasidion* and *Armillaria* on forest dynamics.

New insights regarding the influence of root rot fungi on forest dynamics

*Importance of root rot pathogens in the dynamics of disease centres*

Up to the present, research on the incidence of Armillaria and Annosum root rot in the mountain pine forests of the Swiss National Park has been limited to a two-hectare study plot (Cherubini et al. 2002; Dobbertin et al. 2001). Gäumann & Campell (1932), who were the first to report Armillaria root disease from the area, unfortunately did not give any information on their study design and thus on the spatial scale of their observations. In order to determine the influence of *Heterobasidion* and *Armillaria* on forest dynamics at different spatial scales, I have chosen a sampling design that focused on canopy gaps (> 900 m²) and the adjacent forest within a 10 km² study area. In 95% of all canopy gaps studied, *Heterobasidion* and/or *Armillaria* could be isolated from a majority of dying or recently dead mountain pines. Overall, *Heterobasidion annosum* was the dominant root rot pathogen (Chapter 7). Regarding the different *Armillaria* species, *A. ostoyae* (Romagnesi) Herink was identified for the first time in the Swiss National Park and proved to be the most widespread *Armillaria* species. The results suggest that these two root rot pathogens, *Heterobasidion*
*annosum* and *A. ostoyae*, are important driving forces involved in the enlargement of disease centres in these forests. Most likely, the pathogens have also been key factors in the creation of new disease centres; however, this hypothesis could not be tested because neither smaller disease centres nor the temporal sequence of the processes (e.g., the forest turnover rate or the rate of canopy gap creation and enlargement) were considered in this study.

**Population structure of Armillaria spp.**

In five of six Armillaria disease centres, Armillaria root rot was caused exclusively by *A. ostoyae*, which is known to be more pathogenic than the other two Armillaria species occurring in the study area, *A. borealis* Marxmüller & Korhonen and *A. cepistipes* Velenovsky (Guillaumin et al. 1993; Prospero et al. 2004) (*Chapter I*). Intraspecific somatic incompatibility tests of Armillaria isolates sampled in disease centres and in the forest matrix between revealed the occurrence of large *A. ostoyae* genets, some of which encompassed several currently observed disease centres associated with *Heterobasidion* and/or Armillaria (*Chapter III*). This suggests that the severity of disease has probably been changing in space and time within the same *A. ostoyae* genet. This pattern may also have been observed because *Heterobasidion* might be a more aggressive pathogen on mountain pine than *A. ostoyae*.

The occurrence of large (maximum 37 ha) and likely old (approx. 1000-2000 years) *A. ostoyae* genets suggests that the association between *A. ostoyae* and the forests studied is most probably several millennia old (*Chapter III*). In addition, past management activities in the Swiss National Park (cf. Parolini 1995) have likely enhanced the incidence of Armillaria root disease, since silvicultural changes in forest composition and structure can alter the host-pathogen relation in favour of the fungus by providing stumps which become new food sources for the fungus (Morrison & Mallett 1996; Morrison et al. 1991).

**Reasons for the widespread incidence of Heterobasidion**

*Heterobasidion annosum* was found to be the dominant root rot pathogen in the study area. I assume that past management activities such as clearcuts have indirectly facilitated the spread of this pathogen. Past logging events mainly between the 14th and 19th century (cf. Parolini 1995) must have created numerous stumps that could be colonized by *Heterobasidion* basidiospores. In addition, two characteristics of the mountain pine forests studied, namely the dominance of one tree species and the quite homogeneous age structure of the stands,
which are reported to be 150-200 years old (e.g., Cherubini et al. 2002; Schlegel 1985; Brang 1988) could have favoured the extensive spread of *Heterobasidion* via root contacts. The age of the mountain pine stands studied in this thesis is unlikely to be the main cause of the observed mortality, since mountain pines can evidently attain older ages in nearby stands: Zoller (1995) mentioned mountain pine/Swiss stone pine (*Pinus cembra* L.) stands near Alp Ivraina (west of Champlönc, just outside the Park) with mountain pine trees far older than those found between Il Fuorn and Buffalora (i.e., in our study area). He suggested that these mixed *P. mugol/P. cembra* forests outside the Park were remnants of the former natural vegetation.

**Influence on forest succession**

Mountain pine was found to regenerate more successfully in disease centres than in the adjacent forest, and all regenerating, non-symptomatic mountain pines sampled within the disease centres proved to be non-infected with *Heterobasidion* or *Armillaria* (*Chapter III*). Although only a limited number of disease centres were evaluated in detail, the results underline the importance of root rot fungi in driving forest dynamics: Both pathogens were involved in the enlargement and likely also in the creation of disease centres, within which regeneration was denser than in the adjacent forest (*Chapter III*). The occurrence of disease centres mainly favoured the regeneration of mountain pine, whereas Swiss stone pine regeneration was not affected significantly. As a consequence, root rot fungi causing disease centres in these mountain pine stands decelerate the development towards stands dominated by Swiss stone pine/European larch (*Larix decidua* Miller), which was proposed to be the natural successional trajectory for most mountain pine stands in the Swiss National Park (e.g., Braun-Blanquet 1931; Kurth et al. 1960; Risch et al. 2004; Zoller 1995). Our findings are in line with the results of Hansen & Goheen (2000) who studied the structure of mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) stands in the Pacific Northwest of the United States. They reported that plant community diversity and successional trajectories were altered by the impact of the root rot pathogen *Phellinus weirii* (Murr.) Gilbertson that caused mortality centres which were filled with early successional tree species.
Methodological aspects

Symptoms and signs of Armillaria and Annosum root rot

It would be helpful and time-saving if the incidence of Armillaria and Annosum root rot could be reliably determined in the field by using signs of the fungi or symptoms induced by the fungi on dying or recently dead mountain pines. In mountain pine saplings (< 130 cm height) infected with Armillaria or Heterobasidion and in trees (≥ 12 cm dbh) infected with Armillaria, the presence of Armillaria fans and Heterobasidion mycelium (ectotrophic, paper-thin mycelium and white pustules) were found to be reliable signs for the incidence of Armillaria and Annosum root rot (Chapter IV). However, mountain pine trees (≥ 12 cm dbh) infected with Heterobasidion hardly expressed any external signs and symptoms indicating the incidence of root disease. Therefore, the isolation of the pathogen from root samples cannot be substituted by the identification of symptoms and signs in the field because particularly the incidence of Annosum root rot would be underestimated strongly (Chapter IV). The lack of typical symptoms and signs was probably also the reason why Annosum root rot has not been noticed in the Swiss National Park until recently (Dobbertin et al. 2001).

Temporal dynamics in forest ecosystem processes: How do root diseases affect mountain pine stands over time?

For the understanding of the influence of root disease on forest dynamics, it would be important to additionally assess the temporal scale of the process, such as e.g. the forest turnover rate. Valuable information on the temporal development of disease centres could be gained by i) determining the age structure of regeneration within root disease centres, ii) dating the growth release reflected by an increase in tree ring width in regenerating trees, or iii) delineating the disease centres on older aerial photographs. However, it proved difficult to reliably identify the age of recently dead and non-symptomatic saplings within disease centres (samples from Chapters I and III) because of the frequent incidence of very narrow and often wedging tree rings (personal observation). In addition, it proved impossible to reliably delineate disease centres on older aerial photographs because of the limited resolution of the photographs and the open type of forest which made a manual delineation too subjective. Thus, only future time series of high-quality aerial photographs can probably provide accurate information on the temporal dynamics of the disease centres.
**Inoculation experiment as a missing link**

Reports of Annosum and Armillaria root rot on mountain pine suggest that *Heterobasidion* and *A. ostoyae* can act as primary pathogens on this species (Dobbertin et al. 2001; Durrieu et al. 1985; Goggioli et al. 1996; Vasiliauskas 1999). However, to my knowledge, direct evidence for the pathogenicity of *Heterobasidion* and *Armillaria* spp. on mountain pine is still missing, i.e. the third of Koch’s postulates has not been fulfilled yet. In order to bridge this gap, inoculation experiments with *A. ostoyae, A. borealis, A. cepistipes*, and *Heterobasidion* were started in 2004 with potted mountain pine saplings in the forest nursery of WSL (all isolates obtained from *Chapter I*; host provenance was chosen as close to the study area as possible). *Armillaria* was inoculated on 3-year-old mountain pine saplings following the method described by Prospero et al. (2004). In addition, 3-year-old saplings of *Pinus mugo* were inoculated with *Heterobasidion* according to Swedjemark & Stenlid (1995). Until winter 2004, no mortality occurred in the saplings inoculated with *Heterobasidion*, and *Heterobasidion* inoculation was therefore repeated in spring 2005 (end of April) with the same saplings and *Heterobasidion* isolates following the method described by Garbelotto et al. (2004). However, no sapling inoculated with *Armillaria* or *Heterobasidion* has died until now (September 2005). Various explanations could account for the failure of the inoculation experiment: i) *Armillaria* inoculum may need more time to produce a sufficient quantity of rhizomorphs to infect and kill the saplings, ii) most saplings inoculated with *Heterobasidion* formed a callus in both inoculation methods, suggesting that either the size of the inoculum was too small or that inoculation should have been conducted at a different time of the year when the reaction of the saplings may be less pronounced, iii) inoculation with *Heterobasidion* should have been done on the roots and not on the stems of the saplings, or iv) specific conditions (e.g., regarding soil or climate) required for the expression of the disease were not fulfilled. Thus, the method of *Armillaria* and *Heterobasidion* inoculation first needs to be adapted before new inoculation experiments should be conducted to determine the pathogenicity of different isolates of *Armillaria* and *Heterobasidion* on mountain pine.
Overall conclusion

Root disease caused by *Heterobasidion annosum* and *Armillaria ostoyae, A. borealis, and A. cepistipes* has proven to be widespread in the mountain pine forests of the Swiss National Park. *Heterobasidion annosum* was the dominant root rot pathogen, followed by *A. ostoyae*. The widespread incidence of Annosum root disease most probably is the consequence of past logging activities in the area. In contrast, the extension of large *A. ostoyae* genets suggests that the forests that occupy the study area have developed in the presence of these *Armillaria* genets.

*Heterobasidion* and *Armillaria* were involved in the enlargement of most canopy gaps, which were therefore classified as disease centres associated with Annosum and/or Armillaria root rot. The premature mortality of mountain pines causing disease centres was presumed to speed up forest turnover rates, because within the disease centres the regeneration of mountain pine was dense, showed a very low incidence of mortality, and all non-symptomatic regenerating mountain pines sampled proved to be non-infected with *Armillaria* or *Heterobasidion*. Thus, while the mortality of trees is often intuitively associated with negative implications (e.g., loss of timber and of the protective function), the creation and enlargement of disease centres by pathogens can generate an important regeneration niche for light-demanding tree species such as mountain pine. In contrast to mountain pine, regeneration of Swiss stone pine was not influenced by the occurrence of disease centres. These results suggest that disease centres most probably decelerate the proposed succession towards stands dominated by Swiss stone pine.

Prospects

To better understand the ecology and epidemiology of fungal tree pathogens, a population genetics approach is increasingly being used (cf. Anderson & Kohn 1995; Burdon 1993; Coetzee et al. 2001; Guillaumin et al. 1996). Valuable information on the frequency of new infections and the growth and size of individual mycelia can be obtained by assessing the size and distribution of fungal genets.
In particular, the following research projects would give valuable and complementary insights into the influence of Armillaria and Annosum root rot on forest dynamics in the mountain pine stands of the Swiss National Park:

- Regarding *Armillaria* genets, DNA-based methods could be applied in order to determine the relatedness of the *Armillaria* genets and to assess the spatial and temporal stability of large genets. In addition, the question whether sibling genets were comprised within some of the large *Armillaria* genets could be answered.

- The number and spatial distribution of *Heterobasidion* genets within disease centres and in the adjacent forest could be determined. This would give indirect evidence to test the hypothesis that past management activities were essential for the spread of *Heterobasidion* (i.e., numerous small genets would support the hypothesis, while the incidence of a few and large genets would reject it).

- Ultimately, the knowledge of the establishment of new infections and of the spread of the disease could then be used to refine the modelling of forest dynamics including root rot pathogens as disturbance agents (e.g., Frankel 1998; Woodward et al. 2001). For this purpose, information of the temporal scale of the processes (e.g., the forest turnover rate or the rate of canopy gap creation and enlargement) as well as supplementary data on a smaller spatial scale (e.g., automated delineation of smaller canopy gaps on aerial photographs) would be a prerequisite.

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