INFECTION AND GROWTH OF *HETEROBASIDION* SPP. IN *PICEA ABIES*

CONTROL BY *PHLEBIOPSIS GIGANTEA* STUMP TREATMENT

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Abstract

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In economical terms, species of *Heterobasidion* are among the most severe fungal pests in coniferous forests of the northern hemisphere. The fungi cause interior decay in the stem of trees and trees may also die as a cause of infection. Two species of *Heterobasidion* have been identified in Sweden, *Heterobasidion annosum* s.s. (Fr.) Bref. and *Heterobasidion parviporum* Niemelä & Korhonen. The former has been identified from southern to central Sweden whereas the latter is present throughout the whole country. Stump treatment, using chemical or biological treatment agents, is the most widely used silvicultural method to prevent infection by *Heterobasidion*. This thesis mainly focuses on different aspects of biological stump treatment using *Phlebiopsis gigantea* (Fr.) Jül.

The effectiveness of stump treatment against air-borne *Heterobasidion* spores with *P. gigantea*, when applied at different rates of stump coverage was investigated in southern Sweden. The results showed that, in order to achieve the best control, the aim should be to cover the complete stump surface with the treatment agent.

In another field experiment in southern Sweden the effectiveness of Finish and Swedish strains of *P. gigantea* was compared. Two formulations containing two species of *Trichoderma* were also included in the study. Two of the Swedish *P. gigantea* strains showed the best control against air-borne *Heterobasidion* spores. None of the *Trichoderma* formulations significantly reduced *Heterobasidion* infection.

The effect of stump treatment using *P. gigantea* on stumps from trees that were already infected by *H. parviporum* at the time of felling was studied in south-western Sweden. It was hypothesized that *P. gigantea* may restrict the growth of *Heterobasidion* in the root system of treated stumps. However, no such effect was observed and treatment of stumps from already infected trees can, based on this study, not be recommended as a means of restricting the spread of the pathogen.

In the last study, the ability of inoculated *H. annosum* s.s. to establish and grow in trees and stumps in northern Sweden (outside its natural range of distribution) was compared with the ability to establish and grow in southern Sweden. In the trees, *H. annosum* s.s. established in almost as many trees in the north as in the south of Sweden. However, spores of *H. annosum* s.s. showed an inability to establish on stumps, in both parts of the country. This indicates that routes of infection, other than through fresh stump surfaces may be involved in the disease cycle of *H. annosum* s.s. in Norway spruce.

Keywords: biological control, butt rot, forest management, silviculture, root rot

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To Rakel and Elsa

Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which are referred to in the text by the corresponding Roman numerals, I-IV.

I. Berglund, M. & Rönnberg, J. 2004. Effectiveness of treatment of Norway spruce stumps with *Phlebiopsis gigantea* at different rates of coverage for the control of *Heterobasidion*. Forest Pathology 34: 233-243.

II. Berglund, M., Rönnberg, J., Holmer, L. & Stenlid, J. 2005. Comparison of five strains of *Phlebiopsis gigantea* and two *Trichoderma* formulations for treatment against natural *Heterobasidion* spore infections on Norway spruce stumps. Scandinavian Journal of Forest Research 20: 12-17.

III. Pettersson, M., Rönnberg, J., Vollbrecht, G. & Gemmel, P. 2003. Effect of thinning and *Phlebiopsis gigantea* stump treatment on the growth of *Heterobasidion parviporum* inoculated in *Picea abies*. Scandinavian Journal of Forest Research 18: 362-367.

IV. Berglund, M. Comparison of the growth of *Heterobasidion annosum* and *Heterobasidion parviporum* in southern and northern Sweden following inoculation of Norway spruce trees and stumps. Manuscript.

During the course of this work I have changed my family name from Pettersson (study III) to Berglund.

Introduction

Background

Species of *Heterobasidion* are among the most severe fungal pests in coniferous forests of the northern hemisphere (Woodward et al., 1998). Other fungal species, such as *Armillaria* spp. cause similar damage, but in Scandinavia *Heterobasidion* spp. are the most important decay causing species attacking conifers (Johansson, 1980; Stenlid & Wästerlund, 1986; Rönnberg & Vollbrecht, 1999). The average incidence of root and butt rot in mature Norway spruce (*Picea abies* (L.) Karst.) trees in Sweden has been reported to be about 15 % (Euler & Johansson, 1983; Stenlid & Wästerlund, 1986).

The economic losses to Swedish forestry as a result of attack by *Heterobasidion* spp. are high. The losses mainly consist of death of trees and decay of the interior parts of the stem. The decay reduces the quality and thus the price paid for the timber. Scots pine (*Pinus sylvestris* L.) trees usually die as a result of infection (Rennerfelt, 1952) whereas infection of Norway spruce trees normally results in decay (Rennerfelt, 1945). Economic losses are also caused by growth reductions (Björkman et al., 1949; Bendz-Hellgren & Stenlid, 1995, 1997) and increased risk of wind-throw (Rostrup, 1902; Wood, Holmes & Fraser, 1959). A further factor to take into account is the need to shorten the rotation as a consequence of fungal attack (Bendz-Hellgren et al., 1998).

Definitions

Two species of *Heterobasidion* spp. have been identified in Sweden, *Heterobasidion annosum* s.s. (Fr.) Bref. (also referred to as the European P intersterility group of *Heterobasidion annosum* s.l.) and *Heterobasidion parviporum* Niemelä & Korhonen (also referred to as the S intersterility group of *Heterobasidion annosum* s.l.). In this thesis, when both species are considered only *Heterobasidion* is used. When a specific species is considered the complete scientific name is used.

Historical considerations

In the early work on root rot of conifers during the first part of the 19th century, the fungi were considered to be a symptom of disease in plants rather than the actual causal agents (Hüttermann & Woodward, 1998). This view shifted during the second half of the century when it was accepted that fungi were causing the diseases and not vice versa. The shift in thinking was largely due to the work of Moritz Willkomm and Robert Hartig (Hüttermann & Woodward, 1998). *Heterobasidion* was first described by Elias Fries (1821), and later by Robert Hartig in the late 19th century (Hartig, 1874). Robert Hartig wrote several books on the subject of fungal diseases of forest trees, and he has been called “the Father of Forest Pathology” (Hüttermann, 1987).

The research on *Heterobasidion* took a step ahead in the middle of the 20th century as a result of work conducted by the English scientist John Rishbeth. The principles behind the spread of *Heterobasidion*, which Rishbeth presented in a series of papers during the 1940s and 50s, are the ones upon which a large part of this thesis is based. Rishbeth showed that *Heterobasidion* enters a stand through primary infection of stump surfaces by basidiospores (Rishbeth, 1949, 1951a), and that the subsequent spread of the fungus from the infected stumps to neighbouring trees occurs via root contacts (Rishbeth, 1951b). This knowledge largely influenced the subsequent actions taken by foresters to prevent the spread of *Heterobasidion*, and Rishbeth was the first to start to develop the ideas behind stump treatment (Rishbeth, 1949, 1952, 1959a, b, 1963).

Another big step in the research on *Heterobasidion* came in the late 1970's when Kari Korhonen (1978) divided the species into two different intersterility groups with different host specificity, the P-group (P=pine) and the S-group (S=spruce). Later, a third intersterility group was identified, the F-group (F=fir) (Capretti et al., 1990).

In the literature *Heterobasidion* has been known by many different names, e.g. *Polyporus annosus* Fr., *Trametes radiciperda* R. Hartig, *Fomes annosus* (Fr.) Cooke, *Fomitopsis annosa* (Fr.) Bond. & Singer, *Ungulina annosa* (Fr). Pat. and *Heterobasidion annosum* (Fr.) Bref. (Niemelä & Korhonen, 1998). During the last decades the name *Heterobasidion annosum* (Fr.) Bref. has been the most common name used in the literature. However, Niemelä & Korhonen (1998) proposed that the different intersterility groups of *H. annosum* should be accepted as taxonomic species with the following names: *Heterobasidion annosum* s.s. (Fr.) Bref. (the former European P-intersterility group of *H. annosum* s.l.), *Heterobasidion parviporum* Niemelä & Korhonen (the former European S-intersterility group of *H. annosum* s.l.) and *Heterobasidion abietinum* Niemelä & Korhonen (the former European F-intersterility group of *H. annosum* s.l.).

Two intersterility groups of *Heterobasidion* (S and P) are also present in North America. They will not be considered further in this thesis.

The hosts and distribution of species of *Heterobasidion* in Europe

Although there is overlapping, the species of *Heterobasidion* largely differ in their choice of hosts. The distributions of the species are shown in figure 1.

Heterobasidion parviporum mainly attacks Norway spruce and Siberian fir (*Abies sibirica* Ledeb.) (Korhonen, 1978; Korhonen et al., 1997). It may also attack and kill Scots pine saplings that grow around old stumps infected by the fungus (Korhonen, 1978). Furthermore it has been shown to spread from stumps of a previous Norway spruce generation to silver birch (*Betula pendula* Roth.), lodgepole pine (*Pinus contorta* Dougl. Ex Loud. Var. *latifolia* Wats.) and Siberian larch (*Larix sibirica* Ledeb.) (Piri, 1996).

Figure 1. The distribution of *Heterobasidion* species in Europe (map kindly provided by K. Korhonen, Metla, Helsinki, Finland 2004).

The distribution of *H. parviporum* largely follows the distribution of Norway spruce (Korhonen et al., 1998). The northernmost record is from 68°N in Finland and the southernmost record is from 41°N in Greece and it has been found from France in the west to the Ural Mountains in the east (Korhonen et al., 1997; Korhonen et al., 1998) (figure 1). In Sweden it is present in most of the country except in the mountain areas. However, in Sweden it has hardly any practical importance north of 64°N (Korhonen et al., 1998).

The main hosts of *H. annosum* are different species of pine, but it also infects many other species of trees, both conifers and broad-leaves (Korhonen, 1978). Several introduced conifers that are planted on formerly infected sites may be infected by *H. annosum* (Vollbrecht et al., 1995; Rönnberg, Vollbrecht & Thomsen, 1999). The distribution extends over almost all of Europe (figure 1). It has been recorded from Portugal in the west to Ukraine in the east and from southern Greece in the south up to central Finland in the north (Korhonen et al., 1998). The reason why it does not follow the distribution of pine to the very northern parts of Scandinavia is unknown (Korhonen et al., 1998). However, one hypothesis considers the possible temperature requirement of the fungus, which is dealt with in study IV. In Sweden, *H. annosum* is common on Scots pine in some areas in the southern part of the country (Rennerfelt, 1952; Petrylaite, 2004). On Norway spruce it is present up to the central part of Sweden (Korhonen et al., 1998). However, in the central parts of Sweden it seems to be relatively uncommon as compared to *H. parviporum*. In a study by Swedjemark & Stenlid (1993), only 2% of the isolates from Norway spruce stumps and none of the isolates from Norway spruce trees, in central Sweden, belonged to *H. annosum*. In another study from central Sweden, none of the investigated isolates from Norway spruce trees belonged to *H. annosum* (Stenlid, 1985). In southern Sweden however, several studies have shown that *H. annosum* may be the dominant *Heterobasidion* species in Norway spruce stands (Stenlid, 1987; Rönnberg & Vollbrecht, 1999; Vollbrecht & Stenlid, 1999).

The distribution of the third species, *H. abietinum*, is connected to the distribution of *Abies* spp. in central and southern Europe. However, it has been shown not only to attack species of *Abies* but also for example Norway spruce when grown in mixtures with *Abies alba* (Korhonen et al., 1998). The known distribution extends from southern Italy in the south to southern Poland in the north and from northern Spain in the west to Bulgaria in the east (figure 1) (Korhonen et al., 1998).

Infection routes of *Heterobasidion*

Spreading by spores

Heterobasidion forms perennial basidiocarps that grow on old stumps, on the underside of old logs left in the forest and on roots and stem bases of infected trees (figure 2). The basidiocarps produce large numbers of basidiospores (Kallio, 1970; Möykkynen, von Weissenberg & Pappinen, 1997) that are released and spread by the wind. The rate of spore deposition decreases rapidly with distance from the basidiocarps (Kallio, 1970; Möykkynen, von Weissenberg & Pappinen, 1997), and a single basidiocarp influences the risk of infection within the stand but probably has little effect between stands (Stenlid, 1994). However, some spores can travel up to 500 km (Rishbeth, 1959c; Kallio, 1970). The capacity of spore production and dispersal is lower for *H. annosum* than for *H. parviporum* (Möykkynen, von Weissenberg & Pappinen, 1997). This is probably due to the presence of fewer pores per area unit of pore layer for *H. annosum* than for *H. parviporum* (Korhonen, 1978).

Heterobasidion also produces asexual spores, conidiospores. After incubation in humid and aerobic conditions (Rishbeth, 1951a) the conidia are produced in abundance on infected wood, and are therefore often used for identification and quantification of infection in scientific studies. Under natural conditions, conidia may be found on infected stumps and roots (Rishbeth, 1951a; Kallio, 1971a) and they may be liberated by airflow (Möykkynen, 1997). However, the role they play in nature is not fully understood and conidia are considered to be of minor importance for the aerial distribution of the disease compared to basidiospores (Rishbeth, 1951a; Redfern & Stenlid, 1998).

Heterobasidion is an early coloniser of fresh, exposed wood, and primary infection by spores may occur directly in injuries on the stem and in the roots of trees (Isomäki & Kallio, 1974; Roll-Hansen & Roll-Hansen, 1981; Schönhar, 1995). However, the infection of the surfaces of freshly cut stumps (figure 2) (Rishbeth, 1949, 1951a; Brandtberg, Johansson & Seeger, 1996; Bendz-Hellgren & Stenlid, 1998) is considered the most important mode of infection. Roots of stumps may also become infected from spores present in the soil (Jørgensen, 1961; Schönhar, 1978). The amount of infection is related to the number of available spores in the air, which is strongly influenced by season, and the ability of the spores to infect injuries such as stump surfaces. In Scandinavia, spore infection peaks during the summer months and few stumps are infected in the winter (Kallio & Hallaksela, 1979; Brandtberg, Johansson & Seeger, 1996). In other climates different relationships between season and stump infection exist. For example, in southern USA stump infection decreases during the summer due to high temperatures (Ross, 1973) and in less extreme climates stumps may be infected throughout the year (Meredith, 1959; Morrison & Johnson, 1970). The time after cutting during which the stumps are susceptible to infection is short but seems to vary with species (Rishbeth, 1951a; Cobb & Schmidt, 1964; Cobb & Barber, 1968). Yde-Andersen (1962) and Schönhar (1979) showed that stumps of Norway spruce are susceptible for less than one month after cutting.

When *Heterobasidion* has established on the stump surface, it rapidly grows down into the stump and then into the root system. The outcome of this infection is largely dependent on the presence of competition by other wood inhabiting fungi, such as *Phlebiopsis gigantea* (Fr.) Jül. *Phlebiopsis gigantea* is, among other fungal species, common as an early coloniser of fresh wood, and competes with *Heterobasidion* both in Scots pine and Norway spruce (Rishbeth, 1950; Meredith, 1960; Kallio, 1971b).

Spreading by mycelia

Heterobasidion grows poorly in the soil and needs woody substrate for mycelial dissemination (Rishbeth, 1950; Hodges, 1969). In pine it may grow ectotrophically on the bark of roots, especially on sites with high pH (Rishbeth, 1950, 1951b). The spread from roots of infected stumps and trees to adjacent healthy trees takes place via root contacts (figure 2) (Rishbeth, 1951b; Morrison & Redfern, 1994). Growth rates of about 7-12 cm/year have been reported for the fungus in roots of standing Norway spruce trees (Schönhar, 1978; Stenlid & Johansson, 1987; Bendz-Hellgren et al., 1999). The growth rate in stump-roots is two to three times higher than in roots of standing trees (Schönhar, 1978; Bendz-Hellgren et al., 1999).

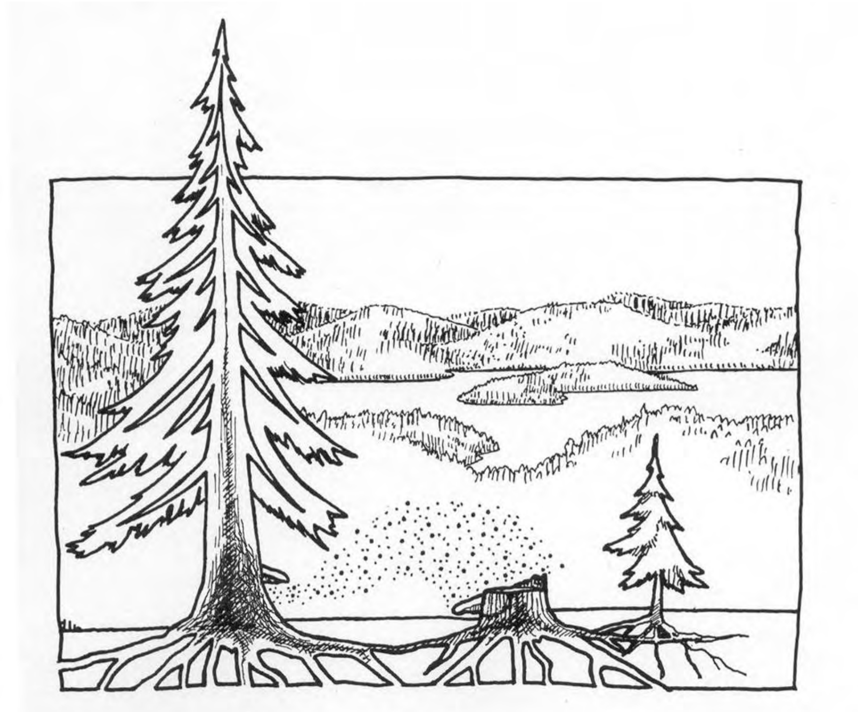

Figure 2. The main routes of spread for *Heterobasidion* (illustration by Tove Vollbrecht).

When the fungus has reached the base of the tree it starts to grow up along the stem. The average rate of growth in the stem of Norway spruce trees has been reported to be 29 to 40 cm/year (Hallaksela, 1993; Huse & Venn, 1994), although results vary. Swedjemark and Karlsson (2004) inoculated clones of Norway spruce with *H. parviporum* and found an average growth of 140 cm in nine months. There was significant variation in growth between the studied clones.

Heterobasidion may spread to considerable heights within the stems. In Norway spruce, Stenlid and Wästerlund (1986) reported a decay column of 11.8 m, but the average height is much lower. The height of decay for 573 Norway spruce trees between 20 and 68 years old from southern Sweden averaged 2.8 m (Kindstrand, 2001). For these trees the species of decay causing fungi was not investigated, and it is likely that some of the decay was caused by fungi other than *Heterobasidion*. This may have influenced the average height of the decay, since some other fungi, for example *Armillaria* spp. usually do not grow as high in the stem as *Heterobasidion* (Tamminen, 1985).

Control of *Heterobasidion* by stump treatment

It was when Rishbeth (1949, 1950, 1951a) discovered that *Heterobasidion* enters a stand mainly through the surfaces of fresh stumps that the idea of reducing spore infections by treating the stump surfaces was born. Many different stump treatment agents have been tested over the years, both chemical (Pratt, Johansson & Hüttermann, 1998) and biological (Holdenrieder & Greig, 1998). The chemicals that are used today are urea and boron (Pratt, Johansson & Hüttermann, 1998), and the only biological treatment agent used is *P. gigantea*. In Sweden, stump treatment has been practiced for about 10 years and all of the above mentioned treatment agents have been used. However, due to a desire to reduce the use of chemicals in the forestry industry *P. gigantea* is the only agent used in Swedish forestry today.

Thor (2001) has investigated the stump treatment situation in Europe, and found that *P. gigantea* is the most widely used treatment agent followed by urea. According to Thor (2001), a total of 210 000 ha. of forest are treated every year in Europe. Poland and UK treat the largest areas (about 70 000 ha. each) and Sweden comes in third place with 35 000 ha. Most of the stump treatment in Europe is carried out in spruce thinnings. However, in Poland only pine is treated and in UK, Ireland and Finland some final fellings are treated. In Ireland, UK and Denmark small areas of *Larix* spp. and other conifers are treated (Thor, 2001). According to Samuelsson & Örlander (2001) there is a need for more stump treatment in Sweden. Most of the treatments today are carried out in the southern part of the country, some in the middle part and none in the north.

The stump treatment agent may be applied either manually, using for example a spray bottle, or mechanically. In Sweden, almost all of the stump treatments are done mechanically (Thor, 2001). There are two different methods by which the treatment agent may be applied to the stump surface in mechanical stump treatment operations - through a nozzle located on the underside of the guide-bar, and through a drilled guide-bar (figure 3). Both methods are practiced in Sweden.

The development of *P. gigantea* stump treatment

Phlebiopsis gigantea is a primary colonizer of fresh wood and it is common in the boreal and temperate forests of the world (Holdenrieder & Greig, 1998). Although it may cause decay in conifer timber its ability to attack living trees is low (Asiegbu, Daniel & Johansson, 1996). Rishbeth (1950, 1951a) observed that *P. gigantea* had the ability to replace *Heterobasidion* in pine stumps and roots, which was later confirmed by Meredith (1960). Rishbeth (1952) reported good results when treating stump surfaces of Scots pine with spores of *P. gigantea*. The practical use of *P. gigantea* stump treatment however, did not start until a decade later when further research demonstrated its efficacy against *Heterobasidion* infection in pine stumps (Rishbeth, 1963).

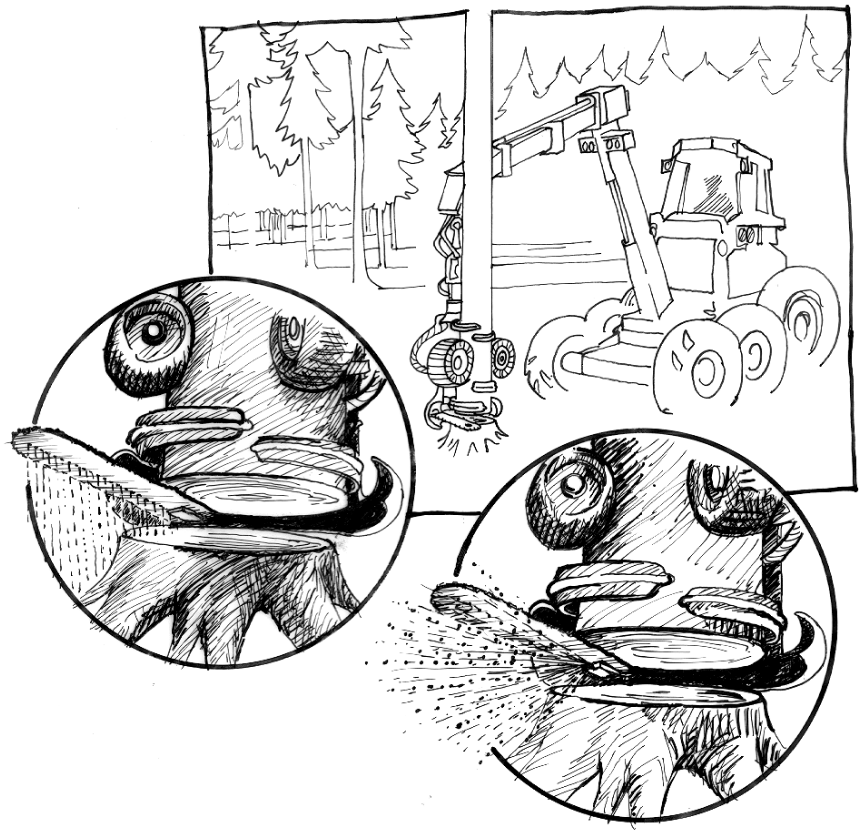

Figure 3. Application methods of treatment agent in mechanical stump treatment. In the left circle the liquid is applied to the stump surface through a drilled guide-bar. In the right circle the liquid is sprayed on to the stump surface through a nozzle on the underside of the guide-bar. In the pictures the length of the guide-bars is exaggerated to be able to illustrate the application techniques. (Illustration by Tove Vollbrecht.)

The early work on *P. gigantea* stump treatment, as described above, was mainly focused on Scots pine. However, Rishbeth also conducted some experiments on conifers other than pine (Rishbeth, 1963, 1970), the results being variable. The work on Norway spruce was continued in Finland. In the studies by Kallio (1971b) and Kallio & Hallaksela (1979), *P. gigantea* significantly reduced the infection of Norway spruce stumps by *Heterobasidion*. However, in a study by Lipponen (1991), two strains of *P. gigantea* failed in preventing *Heterobasidion* infection in Norway spruce stumps. In 1991, a Finnish strain of *P. gigantea* was formulated into a dry powder containing oidia (Korhonen et al., 1994). It was originally isolated from a spruce log in 1987, and was selected from a number of other strains in preliminary laboratory studies, as being a promising strain for control of *Heterobasidion*. Korhonen et al. (1994) reported good results from both simulated stump treatment experiments and from field studies in Finland, Norway and Sweden whereby *P. gigantea* almost completely prevented infection both on stem pieces and stumps. The preparation was registered and released onto the market under the name of Rotstop® for use on both Scots pine and Norway spruce stumps (Korhonen et al., 1994).

Rotstop has been in use in Finland and Sweden since it became available on the open market. Its effectiveness has been monitored every year in simulated stump treatment experiments (Korhonen 2003) and in occasional stump experiments in the field (K. Korhonen pers. comm.). It has also been tested in field studies throughout Europe. Thor & Stenlid (2005) compared the effect of manual versus mechanical application of Rotstop on Norway spruce in Sweden. The study also included winter thinnings without treatment. The conclusions were that there is no difference in efficacy between manual and mechanical application, and that summer thinnings with *P. gigantea* stump treatment offer the same protection as winter thinnings. In France, Soutrenon et al. (1998) tested Rotstop on Norway spruce, Sitka spruce (*Picea sitchensis* (Bong.) and Black pine (*Pinus nigra* Arn. Var. *austriaca*). On Norway spruce and Black pine the Rotstop completely prevented infection, although efficacy on Sitka spruce was slightly lower. In the Italian Alps, Rotstop was compared with local *P. gigantea* strains on Norway spruce stumps (La Porta et al., 2003). Rotstop proved to be the best strain in reducing *Heterobasidion* infection both on freshly cut stumps, and in stem pieces. In a laboratory study conducted on stem discs Thomsen & Jacobsen (2003) concluded that Rotstop may have the potential to be used for stump treatment in Larch, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Sitka spruce. However, further field studies are needed to verify these results. Another study from Denmark (Thomsen, 2003) focused on the disease expression in Norway spruce trees adjacent to treated and untreated stumps. The results showed a close relationship between the amount of *Heterobasidion* infection found in stumps, six months after treatment and the number of diseased, adjacent trees, five years after the treatment. The proportion of stumps infected by *Heterobasidion* at this time was 15% for the Rotstop treatment as compared to 88% for the untreated control stumps.

Incomplete stump coverage of *P. gigantea*

One problem in mechanical stump treatment is that it may be difficult to cover the whole stump surface without using an excess of stump treatment agent (Pratt & Thor, 2001). As *P. gigantea* is a wood degrading fungus with the ability to colonise stump wood quickly and actively compete with *Heterobasidion* (Meredith, 1960; Korhonen et al., 1994), it is not clear whether incomplete stump coverage would result in lower effectiveness of the treatment. Little work has been done, but it has been shown that incomplete coverage using chemical treatment agents (sodium nitrite and creosote) results in higher infection by *Heterobasidion* as compared with complete stump coverage in Scots pine and Sitka spruce (Phillips & Greig, 1970). To my knowledge, there have been no published results from field studies designed to investigate the effect of incomplete stump coverage using *P. gigantea*, previous to study I in this thesis. Results by Rönnberg (unpublished data) indicated that incomplete coverage of *P. gigantea* on Norway spruce stumps was less effective than complete coverage. However, these results proved not to be statistically significant . In mechanical stump treatment, Thor &

Stenlid (2005) observed that stumps that were imperfectly covered by *P. gigantea* tended to have heavier *Heterobasidion* infection than fully covered stumps. In an experiment on log pieces in a green house, Korhonen (2003) observed a clear positive correlation between the stump coverage and the efficacy of Rotstop.

Treatment of stumps from trees already infected by *Heterobasidion*

The basic aim of stump treatment is to prevent healthy stumps from becoming infected by *Heterobasidion*. Since it has been shown that *P. gigantea* may replace *Heterobasidion* in stumps (Rishbeth, 1950; Meredith, 1960), it is possible that treating stumps from trees already decayed by *Heterobasidion* could affect further colonisation of the root systems by *Heterobasidion*. If only a part of the root system is colonised by *Heterobasidion* at the time of felling, it is possible that *P. gigantea* may grow down into the still healthy roots and thus block them from becoming colonised by *Heterobasidion*. A further possibility is that *P. gigantea*, being a fast growing fungus (Korhonen et al., 1994), could check advance in roots that are only partly colonised by *Heterobasidion*. A lower proportion of stump roots colonised by *Heterobasidion* should influence the probability that the pathogen spreads from an infected stump to adjacent trees.

Stump treatment with *Trichoderma* spp.

There has been considerable interest in using *Trichoderma* for biological control of *Heterobasidion*. However, work is still at the experimental stage (Holdenrieder & Greig, 1998). *Trichoderma* species can be found in many parts of the world (Domsh, Gams & Anderson, 1980; Klein & Evaleigh, 1998) and they are a common component of the soil mycoflora. The antagonistic effect of *Trichoderma* against *Heterobasidion in vitro* has been observed in many studies (Lundborg & Unestam, 1980; Holdenrieder, 1984; Nicolotti & Varese, 1996). The effects however, largely depend on a number of factors such as temperature (Holdenrieder, 1984; Nicolotti, Anselmi & Gullino, 1992), nutrient availability (Sierota, 1977, 1982) and pH of the nutrient medium (Persson-Hyppel, 1963; Gibbs 1967). So far, most work on *Trichoderma* as a potential biological control agent against *Heterobasidion* has been done in the laboratory, and results from stump treatment experiments are scarce (Holdenrieder & Greig, 1998). However, Kallio (1971b) inoculated Norway spruce stumps with *Heterobasidion* and *T. viride* on a monthly basis over the course of one year. He found that the *T. viride* completely controlled *Heterobasidion* on stumps inoculated in May, but the effect varied on stumps inoculated in all other months. Furthermore, Kallio & Hallaksela (1979) reported good protection of *Heterobasidion* by *T. viride* on Norway spruce stumps during the summer. However, the protection failed during the cold season.

The effect of other silvicultural measures on *Heterobasidion* infection

Apart from stump treatment in thinnings, there are many other silvicultural methods of reducing *Heterobasidion* infection.

Choice of tree species

Heterobasidion can attack many different tree species (Sinclair, 1964; Greig, 1976; Laine, 1976; Korhonen, 1978; Wagn, 1987). Broad leaved trees are generally less susceptible to attack than conifers (Wagn, 1987; Swedjemark & Stenlid, 1995). In Sweden and Denmark, species of larch (*Larix kaempferi* (Lamb.) and *L. decidua* (Mill.)), sitka spruce and noble fir (*Abies procera* Rehd.) were especially susceptible to attack by *Heterobasidion* when planted on sites with previously infested forest stands (Vollbrecht et al., 1995; Rönnberg, Vollbrecht & Thomsen, 1999). Species that suffered low or moderate infections were silver fir (*Abies alba* Mill.), Caucasian fir (*Abies nordmanniana* (Stev.) Spach), Scots pine and Norway spruce (Vollbrecht et al., 1995; Rönnberg, Vollbrecht & Thomsen, 1999).

Mixed stands

The mixing of tree species to reduce the incidence of *Heterobasidion* is mainly based on the idea that the spacing between individuals of the more susceptible tree species is larger than for monocultures, and this leads to fewer root contacts and grafts through which the fungus may spread (Korhonen et al., 1998). The majority of conducted studies support this idea (Pautasso, Holdenrieder & Stenlid, 2005). In Scandinavia, Rennerfelt (1945), Enerstvedt & Venn (1979), Piri, Korhonen & Sairanen (1990), Huse, Solheim & Venn (1994) and Lindén & Vollbrecht (2002) showed that Norway spruce trees suffered relatively less infection when mixed with Scots pine or deciduous trees. Another advantage with a mixed stand is that it may allow for a delayed first thinning. This may prevent attack by *Heterobasidion* for a longer time due to lack of entry points such as fresh stumps (Lygis et al., 2004)

Re-planting

It is thought that soil preparation on sites with *Heterobasidion* present in stumps from the previous rotation may lead to a dispersal of infected root pieces on the site, possibly increasing the risk for infection of the new trees (Rönnberg & Vollbrecht 1999). However, Treschow (1958) found no difference in *Heterobasidion* infection in Norway spruce planted on ploughed and unploughed sites.

Venn & Solheim (1994) and Johansson & Pettersson (1997) found that the frequency of butt rot was lower in stands with a wider initial spacing as compared to more densely spaced stands. This difference is thought to be the result of fewer root contacts and different thinning regimes (fewer and less intense thinnings) in stands with lower stem numbers.

Precommercial thinning

The frequency of *Heterobasidion* stump infection increases with increasing stump diameter (Paludan, 1966; Morrison & Johnson, 1999) and Bendz-Hellgren & Stenlid (1998) found that stumps in precommercial thinnings were less infected than stumps from commercial thinnings and final fellings. However, even if stumps in precommercial thinnings are infected, the ability of *Heterobasidion* to spread from small stumps to adjacent trees is probably limited as shown by Vollbrecht, Gemmel & Pettersson (1995).

Commercial thinning

The commercial thinning phase is probably the most crucial regarding the introduction of *Heterobasidion* infections into the stand, due to the many stumps that are created and which may become infected. Generally, it has been concluded that the development of *Heterobasidion* infection in a stand increases with early thinnings (Rishbeth, 1957), the intensity of the thinnings (Bornebusch, 1937; Molin, 1957; Venn & Solheim, 1994; Vollbrecht & Agestam, 1995; Vollbrecht & Bilde Jørgensen, 1995) and the number of thinnings (Powers & Verrall, 1962; Vollbrecht & Agestam, 1995; Vollbrecht & Bilde Jørgensen, 1995).

Stump treatment and stump removal at final felling

Heterobasidion from old infected stumps spreads from one forest rotation to the next (Stenlid, 1987; Piri, 1996). However, little is known about the importance of spore infections in healthy clear-cutting stumps for the transfer of the disease to trees in the subsequent rotation (Korhonen et al., 1998), and the evidence is mixed. In Norway spruce it is known that stumps from clear-cuttings do become infected by *Heterobasidion* (Bendz-Hellgren & Stenlid, 1998). Yde-Andersen (1971) showed that the mortality in Douglas fir, planted after cutting of a healthy stand of mountain pine, was lower in plots where stumps had been treated with creosote than in plots with untreated stumps. In contrast Gibbs, Greig & Pratt (2002) reported no significant reduction in mortality in 30-year-old Corsican pine (*Pinus nigra* J.F. Arnold ssp. *laricio* (Poir.) Maire) as a result of stump treatment with *P. gigantea* at final felling. However, Rönnberg & Bilde Jørgensen (2000) and Rönnberg, Johansson & Pettersson (2003) showed that the incidence of butt rot in consecutive rotations of Norway spruce does not correlate with the incidence of butt rot at final felling of the previous rotation. Hence spore infection of healthy stumps in the clear-cuttings may have contributed to the spread to the next generation, thus making it important to treat stumps in final fellings with a large proportion of healthy stumps.

Removing the inoculum at final felling by excavating stumps is a drastic measure but has been shown to have a positive effect in reducing infection in the next generation, both in pine and spruce (Greig, 1984; Stenlid, 1987; Greig, Gibbs & Pratt, 2001).

Objectives

The aim of this thesis was to gain more knowledge about the use of silvicultural methods to reduce infection of *Heterobasidion* in Norway spruce. Stump treatment, being one of the most widely used silvicultural methods, was the major focus in three of the studies in the thesis. The last study dealt with the question of why *H. annosum* has not spread to the north of Sweden. This species attacks a wider range of host trees than *H. parviporum* and if it were to spread to the north of Sweden it may have an impact on future silvicultural management decisions in that part of the country, not only for Norway spruce but also for other tree species, for example Scots pine.

The objectives were to investigate:

- The influence of incomplete stump coverage of *P. gigantea*, in the treatment of Norway spruce stumps against airborne *Heterobasidion* infection.

- The effectiveness of the Rotstop strain of *P. gigantea* as compared with that of some Swedish strains of *P. gigantea* and two formulations of *Trichoderma*, in stump treatment against airborne *Heterobasidion* infection.

- The ability of *P. gigantea* to restrict the spread of *H. parviporum* in the root systems of stumps that had pre-existing infections at the time of stump treatment.

- The effect of thinning of Norway spruce trees, infected by *H. parviporum*, on the spread of *H. parviporum* in the root systems.

- The ability of *H. annosum* to grow in Norway spruce trees and stumps in the north of Sweden, outside its known distribution.

Material, methods and results

Experimental plots

The thesis was based on material from a total of 19 experimental plots. Studies I, II and III were conducted in the southern part of Sweden whereas study IV was conducted in the south and in the north of Sweden (figure 4).

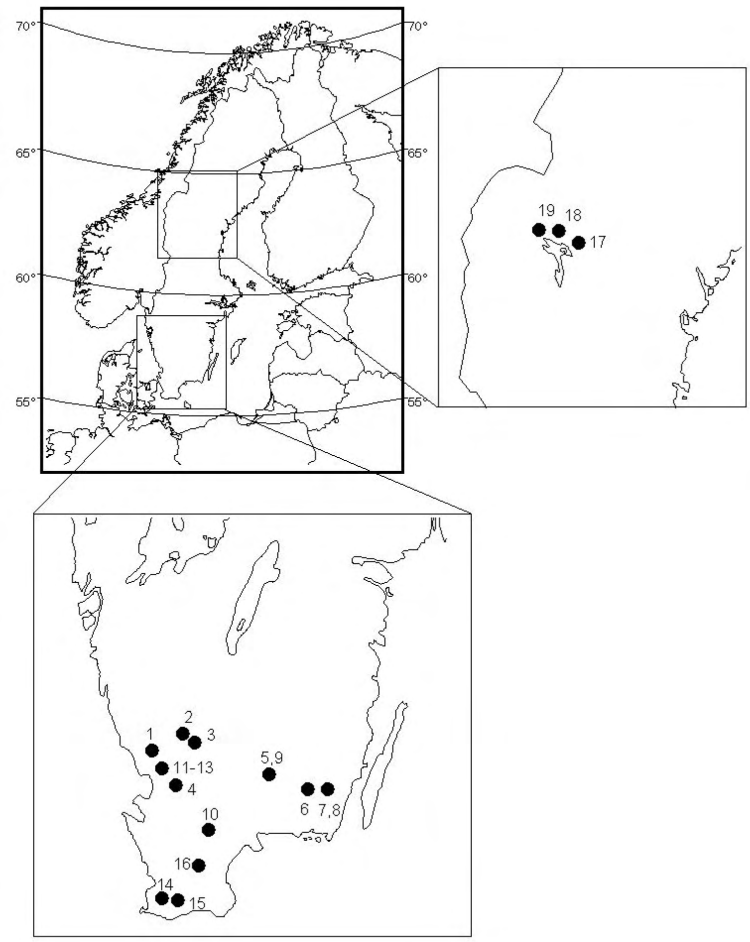

Figure 4. Geographical location of study sites. Points 1-7 show locations for study I, points 8-10 locations for study II, points 11-13 locations for study III and points 14-19 locations for study IV.

Paper I

A common problem for the operator of the machinery used in mechanical stump treatment is to achieve full coverage of the stump treatment agent on the stumps. The aim of this study was to investigate the ability of *P. gigantea* to control natural, air-borne *Heterobasidion* infection when applied at different rates of stump coverage.

Material and methods

The experiment was conducted in seven unthinned first-rotation Norway spruce stands planted on former arable land in southern Sweden (figure 4). At each site, 200 trees were cut during the summer by a single grip harvester, and the resulting stumps were treated with *P. gigantea* (Rotstop) at five different rates of coverage;

i) the whole stump surface covered (100 %), ii) three quarters of the stump surface covered (75 %), iii) one half of the stump surface covered (50 %H), iv) half of the stump surface covered in a striped pattern (50 %S), v) untreated control (0 %) (figure 5). The treatments simulated the usual patterns achieved during normal application. The 75 % and the 50 %H treatments simulate incomplete coverages often seen when applying treatments through a nozzle located on the underside of the guide-bar and the 50 %S simulates the pattern obtained after incomplete coverage using a drilled guide-bar (figure 3). To be able to sample at different depths, the stumps were made approximately 50 cm tall. The treatments were completely randomised within each site. The *P. gigantea* suspension was applied manually using a spray bottle.

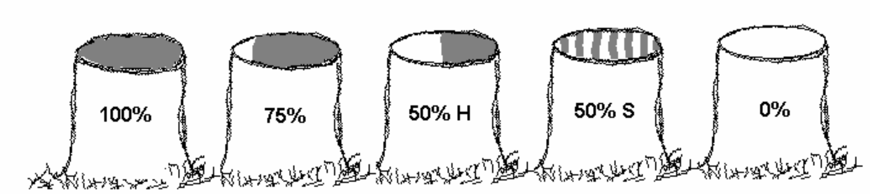

Figure 5. Stumps treated with different *P. gigantea* stump coverages in study I.

The stumps were sampled on two occasions, three and twelve months after the treatment. At three months 50% of the stumps were sampled at each location, and from each stump one disc was cut from the top of the stump and one 10 cm below the stump surface. On the second occasion 50% of the remaining stumps at each site were sampled. On this occasion, four discs were cut from each stump, from the top of the stump, and 10, 25 and 40 cm below the stump surface. All discs were analysed in the laboratory for presence and distribution of *Heterobasidion* and *P. gigantea*.

Results

In this experiment, the mean proportion of control stumps that were infected by *Heterobasidion* was 80% and 69% at the first and second sampling respectively. Taking into account all the different ways of analysing the effect of the treatments (i.e. proportion of *Heterobasidion* infected stumps, size and number of *Heterobasidion* colonies, colonization of *P. gigantea* and the "control efficacy"[1]) the results were clear. Full coverage of *P. gigantea* is clearly needed to optimise stump treatment. However, one year after the treatment, 26 % of the fully covered stumps were still infected by *Heterobasidion.* There were no significant differences between any of the partial coverage treatments, although the proportion of stumps infected by *Heterobasidion* was significantly lower in all partial coverage treatments than in the control treatment at the first sampling. At the second sampling the 75% and 50% H treatments, but not the 50% S, differed

[1] Control efficacy is the percentage of reduction in *Heterobasidion* infection compared to the untreated control, taking into account the proportion of infected stumps and the area of *Heterobasidion* infections.

significantly from the control, in terms of proportion of *Heterobasidion* infected stumps.

Since stump discs were cut at four different levels within the stumps during the second sampling period, the relative growth rates of *Heterobasidion* and *P. gigantea* could be compared in stumps where both fungi had established. The result of this comparison indicated that on average, *Heterobasidion* had grown faster down the stumps than *P. gigantea.*

Paper II

In paper II, the effect of Rotstop, which utilises a *P. gigantea* strain originally isolated from Finland was compared with that of four Swedish strains of *P. gigantea* for the control of natural *Heterobasidion* infection on Norway spruce stumps. The Swedish strains used in the study were chosen from 568 isolates on the basis of their superior antagonistic effect against *Heterobasidion* in laboratory tests (J. Stenlid, pers. comm.). Two formulations containing two species of *Trichoderma* were also included in the trial.

Material and methods

The experiment was established during the summer, in three unthinned monocultures of Norway spruce on former arable or forest land in southern Sweden (figure 4). A total of 480 stumps were created by a single-grip harvester, 160 on each site. The stumps were divided into blocks (according to their spatial distribution), each consisting of eight stumps. The surfaces of seven of the stumps within the blocks were manually treated with one of the following treatments: *P. gigantea* strain 1983, *P. gigantea* strain 1984, *P. gigantea* strain 1985, *P. gigantea* strain 1986, *P. gigantea* strain Rotstop, *Trichoderma* X, *Trichoderma* F. The remaining stump within each block was left as an untreated control. Both *Trichoderma* formulations were mixtures of the two species *T. polysporum* and *T. harzianum*, but the two mixtures contained different numbers of colony forming units (cfu). These were in the form of chlamydospores, conidiospores and mycelial fragments.

Sampling was conducted nine months after treatment. After cutting the top eight cm to waste, a five cm thick disc was cut from each stump. The discs were brought in to the laboratory, and the presence and distribution of *Heterobasidion*, *P. gigantea* and *Trichoderma* was analysed using a dissecting microscope. The discs were analysed on both sides, i.e. 8 and 13 cm below the stump surface.

Results

The mean proportion of control stumps infected by *Heterobasidion* in this study was 88%. Two of the Swedish *P. gigantea* strains (1984 and 1985) were best at reducing spore infections of *Heterobasidion,* and the proportion of infected stumps was 25% and 34% for these treatments respectively. For the Rotstop treatment, 64% of the stumps became infected by *Heterobasidion*. The “control efficacy” was 94% for strain 1984, 95% for strain 1985 and 50% for Rotstop.

None of the *Trichoderma* formulations significantly reduced *Heterobasidion* infection in this study.

Paper III

Stump treatment is primarily a measure to protect healthy stumps from spore infection by *Heterobasidion*. It has however been argued, that *P. gigantea* treatment of stumps with pre-existing *Heterobasidion* infection could have an effect on the spread of the *Heterobasidion* infection in the root systems. It is possible that the *P. gigantea* could grow down into the stumps and block the spread of *Heterobasidion* into as yet uninfected roots, and possibly also replace *Heterobasidion* in infected roots. The ability of *Heterobasidion* to spread to adjacent trees would then be reduced. The aim of this study was to investigate the further spread of *H. parviporum* infections, already existing in standing trees, into the root systems of standing trees and stumps. The effect of thinning, and thinning with subsequent stump treatment with *P. gigantea* (Rotstop) was studied.

Material and methods

The study was designed as a randomised block trial with 135 trees divided between three study sites in south-western Sweden (figure 4). The trees were inoculated with one strain of *H. parviporum* (RB 175) at stump height. One year later, 2/3 of the trees were felled and the remaining 1/3 were left standing. Of the felled trees 50% of the stumps were treated with *P. gigantea* (Rotstop) and the remaining 50% were left untreated. Three and five years after *H. parviporum* inoculation, the trees and stumps were sampled. At the first sampling, 50% of the trees and stumps were investigated, and the remaining trees and stumps were investigated at the second sampling. At both sampling periods, the root systems were excavated and samples were taken from buttress roots to investigate for the presence of *H. parviporum*. The extent of colonisation and proportion of infected roots were registered.

Results

The inoculated *H. parviporum* spread significantly faster in the root systems of the stumps than in the root systems of the standing trees. Stump treatment did not have a statistically significant effect on the spread of *H. parviporum* as compared to untreated stumps. However, at the second sampling there was a tendency for a lower proportion of buttress roots to be infected by *H. parviporum* in treated stumps than in untreated stumps.

Paper IV

In Sweden, *H. parviporum* is present across the whole country whereas *H. annosum* has been found up to approximately 61°N (figure 1). It is not known why *H. annosum* has not spread further north. Two possible hypotheses that have been put forward are: i) the temperature requirements of the fungus limit its distribution; ii) cuttings during the season when spore dissemination occurs have been uncommon in the north of Sweden. Thus, if global warming results in a rise in temperatures and a lengthening of the vegetation season, *H. annosum* may spread further north. This would consequently influence the need for silvicultural interventions, such as stump treatment, to prevent *Heterobasidion* attack in the northern part of Sweden. It is also possible that a range of tree species which are not currently threatened will become vulnerable.

The aim of this study was to investigate the ability of inoculated *H. annosum* to establish and grow in stumps and trees in the north of Sweden.

Material and methods

The experiment was carried out by inoculating Norway spruce trees and stumps with *H. annosum* and *H. parviporum*. The aim was to study *H. annosum*, and *H. parviporum* was used as reference. Two identical experiments were done, one in the very south of Sweden and one in the north (figure 4). Three different study sites were used in each part of the country and a total of 36 trees and 90 stumps were inoculated. Each tree and stump was inoculated with both species of *Heterobasidion* in order to reduce the effect of genetic differences between trees in their ability to withstand *Heterobasidion* attacks. The stump surfaces were divided into two halves, one half being inoculated with conidiospores of *H. annosum* and the other half with conidiospores of *H. parviporum*. The inoculation of the trees was done at 1.3 m above ground level by filling holes, made with an increment borer, with wood chips infected by *H. annosum* and *H. parviporum*. The two species of *Heterobasidion* were inoculated on opposite sides of the stems. Three different *H. annosum* strains and three different *H. parviporum* strains were used for the inoculations. The establishment and growth of *H. annosum* and *H. parviporum* in the south (where both are naturally present) were compared with the establishment and growth in the north (where only *H. parviporum* is naturally present).

Stump samples were taken twice, two and ten months after inoculation. Transverse discs were cut from the stumps and brought in to the laboratory. After incubation, isolations of *Heterobasidion* were made in a systematic order from the stump discs. The isolates were then tested by means of somatic incompatibility, to reveal if they belonged to the inoculated *H. annosum* or *H. parviporum* strains or if they were the result of wild infections.

The trees were sampled 14 months after inoculation. Discs were cut every 10 cm upwards from the inoculation point. When stain was no longer visible, another eight discs were cut upwards from the inoculation point. *Heterobasidion* was isolated from different sampling heights in a systematic order. The length of mycelial spread for both species was then compared.

Results

The results of establishment and growth of the fungi were based on somatic incompatibility tests of 871 isolates from the trees and stumps.

In the stumps, the rate of establishment of *H. annosum* was very low. At the first sampling, 2.3% and 3.8% of the isolates were found to be the inoculated *H. annosum*, in the south and the north respectively. Corresponding results for the inoculated *H. parviporum* isolates were 46.6% and 40.6%. At the second sampling none of the isolates from the stumps, in either the south or in the north, were found to be the inoculated *H. annosum* strains. Almost all isolates of wild origin were *H. parviporum*, in both parts of the country and at both samplings.

In the trees, *H. annosum* was detected in almost as many trees in the north as in the south. Furthermore, the relative growth rate of *H. annosum,* as compared with that of *H. parviporum*, was no different in the north than in the south.

General discussion

Treatment of healthy stumps (I, II)

Phlebiopsis gigantea

Study I and II show that stump treatment using *P. gigantea* in thinnings of Norway spruce may significantly reduce the infection of air-borne *Heterobasidion*. This is in agreement with several earlier studies (e.g. Korhonen et al., 1994; Thor & Stenlid, 2005; Soutrenon et al., 1998; Korhonen, 2003; Thomsen, 2003). However, the result of a stump treatment operation largely depends on the rate of coverage of the treatment agent and the strain of *P. gigantea* used for treatment. Study I clearly showed that the effect of the treatment was significantly reduced if *P. gigantea* did not cover the complete stump surface. In practical stump treatment operations using *P. gigantea*, the aim should thus be to cover the complete stump surface. These results are largely in accordance with results presented by Korhonen (2003) and Thor & Stenlid (2005).

Study II showed that the choice of *P. gigantea* strain for stump treatment is also important for the effectiveness of the treatment. The best control was obtained with *P. gigantea* strains 1984 and 1985. The former has recently been registered for use in practical stump treatment in Sweden and is available on the market under the name of Rotstop-S. One reason for its greater effectiveness may be that it produces a higher number of oidio spores than the original Rotstop strain (study II; K. Korhonen, pers. comm.). However, the number of spores in the preparation does not entirely explain the effectiveness of the treatment. It is possible that different strains of *P. gigantea* vary in their genetic ability to colonise Norway spruce wood, and thus control *Heterobasidion*.

A different experimental design to those outlined in study I and II would have been needed to examine fully the effect of the treatment on the disease expression in the remaining stands after thinning. Despite this, the results in both studies question the overall effectiveness of the Rotstop treatment. In several other studies (Korhonen et al., 1994; Soutrenon et al., 1998; Korhonen, 2003), the control efficacy of Rotstop has been greater than that observed in studies I and II.

Comparisons with earlier results however are complicated by the varying methodologies used. One reason for the relatively low efficacy of the Rotstop treatment in studies I and II is probably the high *Heterobasidion* spore deposition rates, as indicated by the high infection rate on untreated control stumps in both studies. Some evidence for this is provided by the fact that the two sites in study I (Hult and Mästocka), where the proportion of infected control stumps was lower than 50%, were the only sites where the 100% Rotstop treatment completely prevented *Heterobasidion* infection.

It is possible that the results in studies I and II represent a “worst case scenario”. Brandtberg, Johansson & Seeger (1996) showed that the spore deposition rate varies with season, and that it peaks during the summer months, the time during which studies I and II were established. Furthermore, the experimental sites in both studies were chosen to represent areas where the risk of spore infection was high (i.e. areas with older Norway spruce forests in the surroundings, where the probability of finding basidiocarps of *Heterobasidion* should be high). However, considering data from other studies (Brandtberg, Johansson & Seeger, 1996; Benz-Hellgren & Stenlid, 1998; M. Thor, pers. comm.; J. Rönnberg, pers. comm.), spore deposition rates of the same magnitude as in studies I and II seem to be common in thinnings during summer time in the south, but also in central Sweden. Therefore, there is a risk that Rotstop treatment of Norway spruce stumps in Sweden during the summer has relatively low effectiveness.

Both study I and II contained unexplained variation between sites in the effect of the stump treatment. The variation seemed to be largest in study I, where the effect of stump treatment on the infection of *Heterobasidion* was lower in two sites than in other sites with similar rates of *Heterobasidion* infected control stumps. In study II this was not as obvious, but it should be noted that the proportion of stumps treated with *P. gigantea* strain 1984 that became infected by *Heterobasidion* was highest at the site which had the lowest proportion of infected control stumps (i.e. the lowest *Heterobasidion* spore deposition rate) (table 1). It is possible that this variation may be explained by one or more site specific factors, for example pH of the soil or moisture conditions. Further investigations on the possible influence of site specific factors on the efficacy of *P. gigantea* stump treatment may reveal interesting results. It may well be possible to gather this information from existing data from stump treatment experiments, with the addition of some complementary measurements.

In conclusion, treatment with *P. gigantea* may significantly reduce stump infection by *Heterobasidion* in Norway spruce, although different strains of *P. gigantea* vary in their ability to reduce infection. Furthermore, it is important to cover the entire stump surface to obtain the best results. Stump treatment with Rotstop during the summer when *Heterobasidion* spore infection rates are high may result in a relatively low treatment efficacy. For private forest owners there are other options that may pose a smaller risk of introducing *Heterobasidion* infection into the stand.

Table 1. The proportion of stumps infected by *Heterobasidion* in study II.

Treatment	Frequency of stumps infected by *Heterobasidion*		
	Experimental site		
	E	J	W
Untreated control	63	100	100
P. gigantea 1983	25	80	63
P. gigantea 1984	30	20	25
P. gigantea 1985	32	25	45
P. gigantea 1986	30	70	53
Rotstop	53	84	55

One option is to thin the stands in the winter. In a study by Brandtberg, Johansson & Seeger (1996), the probability of stump infection between November and February was very low and the variation between stands was small. Thor & Stenlid (2005) found that *P. gigantea* stump treatment was as effective as winter thinning in terms of reducing the stump area colonised by *Heterobasidion*. However, the probability that stumps become infected by *Heterobasidion* was significantly higher for *P. gigantea* treated stumps than for stumps made in winter thinnings. This raised the question of which measure is the most appropriate to use to describe the efficacy of the stump treatment, area of colonisation or probability of infection? Thor & Stenlid (2005) argued that colonised stump area was the most important measure in their study, since small infections of *Heterobasidion* are less likely to develop in the stumps and subsequently spread to neighbouring trees. This will be further discussed in the next section.

Another option is to avoid thinnings. However, in dense stands thinning is needed in order to improve individual tree increment (Eriksson, 1976). A third option may be to thin the stand, and treat the stumps, during the spring or autumn when the spore deposition rate is more moderate (Brandtberg, Johansson & Seeger, 1996). It is possible that the efficacy of the treatment is greater under such conditions.

The effectiveness of stump treatment: control efficacy v. proportion of infected stumps

The results from study I and II give the status of *Heterobasidion* infection and *P. gigantea* colonisation at certain times after the treatment. From these results one can extrapolate to the probability that *Heterobasidion* will spread from stumps with different treatments to the remaining stand after thinning. The two most important ways of evaluating the effect of the stump treatment in studies I and II are the reduction in total infected stump surface area (the "control efficacy"), and the reduction in proportion of infected stumps, for treated versus untreated stumps. These measures may give different results for the same treatment. For example, in study II the effectiveness of the *P. gigantea* 1984 treatment was 94% when considering the control efficacy, but only 72% if one examines the reduction in the proportion of infected stumps. Thor & Stenlid (2005) observed a similar difference between the two measures.

Which of these two measures is the most important in describing the treatment effectiveness depends on a number of factors. Since competition between *Heterobasidion* and *P. gigantea* is a dynamic process, the time chosen after treatment to conduct sampling is important. Dimitri, Zycha & Kliefoth (1971) showed that small colonies of *Heterobasidion* are likely to be replaced by other fungi with time and should thus disappear from the stumps. Other important factors are the number of samples taken from each stump, and from what depth within the stump they are sampled. The areas colonised by *Heterobasidion* and *P. gigantea* cannot be expected to be the same at different levels within the stump. *Heterobasidion* may also be absent at one level of the stump, but present at another level, as observed during the analysis in study I.

Thor & Stenlid (2005) based their results on transverse discs from the very top of the stumps, cut 6-7 weeks after the treatment. Due to this relatively short time period, they argued that the "control efficacy" was most important, because the measure of "proportion of infected stumps" gives small *Heterobasidion* colonies, which are likely to be out-competed in the stumps, the same statistical weight as larger, more long-term colonies.

In study I, stump discs were sampled twice, and at two and four levels within the stumps, during the first and second sampling periods respectively. It was observed that some *Heterobasidion* colonies died off between the first and second sampling, which confirms the results by Dimitri, Zycha & Kliefoth (1971). However, at the second sampling many of the surviving *Heterobasidion* colonies had reached the root system. Therefore, those stumps that were still infected at that time must be regarded as potential sources of infection for the remaining stand. Thus, in study I the proportion of infected stumps is probably as important as the control efficacy in describing the effectiveness of the treatment. In study II this is not as clear, since sampling was conducted once and only at two levels within the stumps.

However, regardless of which measure is used, it is still uncertain if *Heterobasidion* infections that establish in *P. gigantea* treated stumps are as important for the future spread of the disease as infections established in untreated stumps. This is partly due to the fact that *P. gigantea* is a fast growing fungus that can quickly colonize stump wood and possibly restrict the growth of *Heterobasidion* in treated stumps (Korhonen et al., 1994). In study I, *Heterobasidion* had grown significantly faster than *P. gigantea* down the stumps. However, it is possible that *P. gigantea* occupies some of the roots, still uninfected by *Heterobasidion*. This should reduce the probability of *Heterobasidion* spreading to a neighbouring tree, as compared to a stump where a larger portion of the root system is occupied by *Heterobasidion*. Furthermore, in Scots pine stumps, *P. gigantea* has been shown to be able to replace *Heterobasidion* in the roots (Meredith, 1960), a phenomena that may also be possible in Norway spruce.

Clearly, there are many uncertainties regarding the dynamics of the competition between *Heterobasidion* and *P. gigantea* in Norway spruce stumps. There is a need for further research to quantify the importance of the size of *Heterobasidion* infections, in *P. gigantea* treated stumps, to investigate the long term development of the infections in the stumps and to look at the subsequent spread of the disease to neighbouring trees.

Trichoderma

The treatment of stump surfaces with the *Trichoderma* formulations in study II did not reduce the infection of *Heterobasidion*. The two species of *Trichoderma* included in the formulations were *T. harzianum* and *T. polysporum*. These species have both shown antagonistic effects against *Heterobasidion in vitro* (Lundborg & Unestam, 1980; Holdenrieder, 1984). They have different temperature requirements and one of the ideas behind mixing the species was to extend the range of activity across a greater range of temperatures. Earlier studies have shown that low temperatures impair the effect of *Trichoderma* (Persson-Hyppel, 1963; Nicolotti, Anselmi & Gullino, 1992). However, in study II the observed temperatures during and after treatment could not explain the poor treatment efficacy.

Research aimed at developing other biological stump treatment agents for use against *Heterobasidion* is important. Economically, it is advantageous to have more than one option of stump treatment agent to avoid there being a monopoly. Biologically, it is important since the spreading of large amounts of one organism may cause unwanted ecological side-effects (Holdenrieder & Greig, 1998). However, treatment with *Trichoderma* needs more investigation before it will be a viable alternative to *P. gigantea* in practice.

Infection of Norway spruce stumps by *H. annosum* (IV)

It is generally accepted that primary infection by *Heterobasidion* mainly arises from spore infected stump surfaces (Rishbeth, 1949, 1951a; Brandtberg, Johansson & Seeger, 1996; Bendz-Hellgren & Stenlid, 1998), and this is the rationale behind stump treatment. Therefore, the inability of the inoculated *H. annosum* strains to establish on Norway spruce stumps in southern Sweden in study IV was unexpected. In this area of Sweden it is known that *H. annosum* is often the dominating butt rot causing species in Norway spruce (Stenlid, 1987; Rönnberg & Vollbrecht, 1999; Vollbrecht & Stenlid, 1999). It could be argued that the inoculation of the three *H. annosum* strains may have failed for an unknown reason, and this would explain the poor establishment. However, even considering the 88 isolates that proved to be of wild origin at the second sampling in southern Sweden, only about 1% belonged to *H. annosum*. It was expected that in an area where *H. annosum* dominates as the butt rot causing species, it would also dominate in spore infected stumps. These results indicate that routes of infection apart from through stump surfaces may be involved in the disease cycle of *H. annosum* in Norway spruce. Since this would have serious implications for the efficacy of control methods currently in use (i.e. stump treatment) further research is needed in this area.

Thinning and stump treatment in diseased stands (III)

The discussion has so far been based on spore infections and control of *Heterobasidion* in healthy stumps. However, many Norway spruce stands are already partly infected by *Heterobasidion* at the time of thinning. Since *P. gigantea* has the potential to colonise Norway spruce stumps quickly (Korhonen et al., 1994) and possibly also replace *Heterobasidion* (Meredith, 1960) it was thought that treatment of stumps from diseased trees, may also restrict the dispersal of *Heterobasidion*. A reduced growth of *Heterobasidion* in the root system could then lower the probability that *Heterobasidion* infects neighbouring trees via root contacts. In study III, the development of pre-existing *H. parviporum* infections was compared between non-treated stumps and stumps treated with *P. gigantea*.

There was no statistically significant difference in the proportion of roots infected with *H. parviporum* in treated compared with untreated stumps during any of the sampling periods in study III. However, treated stumps tended to have a lower proportion of infected roots compared with the untreated stumps between the first and second sampling. It could thus be argued that the study period may have been too short. However, the pre-existing *H. parviporum* infection was introduced only one year before stump treatment. Thus, at the time of the stump treatment the infections had probably only colonised a small part of the root system. Therefore, because a significant effect could not be observed under those conditions, it is not likely to occur under natural conditions where a larger proportion of the root systems may already be colonised by *Heterobasidion* at the time of felling and stump treatment.

The thinning operation significantly increased the spread of *H. parviporum* in the root systems, and this result is in line with earlier studies (Schönhar, 1978; Bendz-Hellgren et al., 1999). This suggests that thinning in a stand with existing *Heterobasidion* infection may cause a more rapid build-up of infection as compared with no thinning. In any given spruce stand there is probably a critical level for incidence of infected trees, and above this critical level it should be more economical to avoid conducting the last thinning, and possibly do the final felling earlier. To be able to make such a decision in practice, the critical level and the disease incidence for a stand must be known. It should be possible to calculate the critical level using the *Heterobasidion* model by Pukkala et al. (in press). Information about the disease incidence may be collected with an increment borer. However, a more efficient way is to collect information about the disease incidence in each thinning, and save this information in the forest management plan. To obtain the present incidence of infected trees, the disease development from the last thinning until the present can then be simulated with the model by Pukkala et al. (in press).

Spread of *H. annosum* to northern Sweden (IV)

A possible future increase in temperature due to global warming will have a great impact on forest ecosystems (Sonesson, 2004). Species that currently have a more southern distribution are likely to spread further north. This may be the situation for *H. annosum* which has so far only been found up to the middle of Sweden (Korhonen et al., 1998). *Heterobasidion annosum* attacks many tree species other than Norway spruce (Korhonen, 1978). Therefore, the spread of this species to the north may result in control measures such as stump treatment being extended to tree species like pine and possibly Siberian larch in that part of the country. However, the hypothesis that *H. annosum* does not exist in northern Sweden due to a colder climate was not confirmed in study IV. Two mechanisms of fungal dissemination were studied; establishment of spores on fresh stumps, and growth in the stems of trees. In the stumps, the fungus did not establish at all. However this was the case in both southern and northern Sweden. In the trees the fungus grew equally successfully in both parts of the country. The main objective of study IV was to compare the growth of *H. annosum* with that of *H. parviporum* in both parts of the country. Norway spruce was used since it is attacked by both species of the pathogen. It may however be argued, that the study should have been conducted using Scots pine, since this is the most important host of *H. annosum* (Korhonen et al. 1998). The fact that the spores of *H. annosum* did not establish on the Norway spruce stumps (see previous discussion) favours this argument, but to follow this line of reasoning would have necessitated a different experimental design, excluding *H. parviporum*, as it does not readily attack Scots pine (Korhonen, 1978). It is also possible that some mechanism of spread (e.g. basidiocarp production and aerial spore dispersal) other than the ones studied in this investigation is influenced by climate. Alternatively, some unknown factor other than temperature, could explain why *H. annosum* does not exist in the north of Sweden.

Conclusions and practical implications

Treatment of Norway spruce thinning stumps using *P. gigantea* may reduce air-borne *Heterobasidion* infection. The best effect is obtained if the complete stump surface is covered by *P. gigantea*. *Phlebiopsis gigantea* strains 1984 and 1985 were more effective than the original Rotstop strain against *Heterobasidion* infection. Stump treatment with *T. harzianum* and *T. polysporum* did not reduce the infection of stumps by *Heterobasidion*. These agents can not yet be considered as an option for stump treatment against *Heterobasidion*.

The results of study I and II raise questions about the overall effectiveness of stump treatment with Rotstop against spore infections of *Heterobasidion*. It is possible however, that the results in those studies represent a “worst case scenario”. The experimental sites were chosen to represent areas with a high probability of spore infection, and the spore deposition rate in the studies was generally high. However, other studies (Brandtberg, Johansson & Seeger, 1996; Benz-Hellgren & Stenlid, 1998; M. Thor, pers. comm.; J. Rönnberg, pers. comm.) show that spore deposition rates of the same magnitude as those in this thesis are common in Sweden during the summer. Therefore, forest owners may wish to consider alternative management options, rather than using stump treatments on summer thinnings, for example conducting winter thinnings or possibly thinnings with stump treatment during the autumn or spring, when spore deposition rates are at a more moderate level (Brandtberg, Johansson & Seeger, 1996).

Phlebiopsis gigantea treatment of stumps from Norway spruce trees with pre-existing *H. parviporum* infection was not effective as a means of restricting the spread of the pathogen in the root systems of those stumps. Treatment of such stumps can thus not be expected to slow down the development of the disease in the remaining stand after thinning.

The actual act of thinning Norway spruce trees with pre-existing *Heterobasidion* infection may cause a more rapid build-up of infection in the remaining stand after thinning. This statement is based on the observed result that the growth of *H. parviporum* increased in the roots when a tree, already partly colonised by the pathogen, was felled. Similar results have been reported by Schönhar (1978) and Bendz-Hellgren et al. (1999). Thus, a critical level of disease incidence probably exists in Norway spruce stands, above which it is not cost-effective to conduct another thinning. It should be possible to derive this critical level of disease incidence for a Norway spruce stand using the model developed by Pukkala et al. (in press). Information about the actual disease incidence for stands must be gathered in the field to provide inputs to the model.

The question of why *H. annosum* has not spread to the north of Sweden remains to be answered. *Heterobasidion annosum* grew as well in the north of Sweden (outside its natural distribution) as in the south when inoculated into stems of Norway spruce. However, spores of *H. annosum* were poor at establishing on Norway spruce stumps, both in the north and the south of Sweden. *Heterobasidion annosum* is often the dominating butt rot causing species in Norway spruce in southern Sweden. Thus, these results suggest that routes of infection, other than through stump surfaces, may be of importance for *H. annosum* on Norway spruce in southern Sweden.

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