



# Efficacy of *Phlebiopsis gigantea* treatment on spore infections of *Heterobasidion spp.* on *Larix x eurolepis*



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Swedish University of Agricultural Sciences

Master Thesis no. 170

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Master thesis in Forest Management,  
Advanced level, 30ects(hp) SLU course code EX0629

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## Abstract

The effectiveness of *Phlebiopsis gigantea* to prevent spore infections from *Heterobasidion annosum* and *Heterobasidion parviporum* on hybrid larch stumps were investigated in five stands in southern Sweden. All sites are former forest land and the age of the trees was between 9 and 13 years.

The study was implemented in August 2010, a month where spore dispersal should be great. The five sites were located in previously un-thinned monocultures of hybrid larch. The spore load in the air was studied with the help of spore traps from three different tree species; Norway spruce, Scots pine and hybrid larch. A total of 146 spore traps, evenly distributed in all five sites were analyzed. The spore traps were exposed for three hours then brought back to the laboratory for incubation and analysis. The hybrid larch trees were randomly selected, cut down and every second stump was treated with Rotstop® S and the others were left as controls. No visible signs of infections were discovered. Roughly 60 days after felling and treatment, disc samples were collected for further analysis in lab.

The study indicates an abundant amount of basidiospores of *Heterobasidion spp.* in the air. 63,7 % of all spore traps was infected with *Heterobasidion spp.* Most infections were found in Södra Rörum II, with 83 % infected spore traps. Out of 263 colonies, 61,2 % of the isolations were infections of *H. annosum* and 38,8 % were *H. parviporum*. Scots pine and hybrid larch were most attacked by *H. annosum* whilst Norway spruce was equally attacked by *H. annosum* and *H. parviporum*. The mean percentage of infected stumps for all five sites per tree species was; larch 52 %, Norway spruce 58 % and Scots pine 72 %.

A total of 176 hybrid larch stumps were analyzed and 20 % were infected by *Heterobasidion spp.* Both *H. annosum* and *H. parviporum* were isolated from the hybrid larch stumps. 60 % of the isolations were *H. parviporum* and 40 % were *H. annosum*. Highest infection rate was found in Fulltofta and Klippan where 24 % of the stumps were infected.

There is a significant difference of infection rate and infected area between treated stumps and un-treated control stumps, where  $p \leq 0,000$  and  $p \leq 0,024$  respectively. This result makes Rotstop® S an interesting stump treatment alternative also on hybrid larch.

Key words: Root rot, hybrid larch, *Larix x eurolepis*, stump treatment *Phlebiopsis gigantea*

## Sammanfattning

Effektiviteten hos *Phlebiopsis gigantea* att förhindra sporinfektioner av *Heterobasidion annosum* och *Heterobasidion parviporum* på stubbar av hybridlärk undersöktes i fem bestånd i södra Sverige. Samtliga bestånd är tidigare skogsmark och åldern på träden var mellan 9 och 13 år.

Fältförsöket anlades 1 augusti 2010, en månad då sporspridningen bör vara hög. De fem bestånden var tidigare ogallrade monokulturer av hybridlärk. Sportrycket i luften undersöktes med hjälp av sporfällor från tre olika trädslag; gran, tall och hybridlärk. Totalt analyserades 146 sporfällor som varit jämnt fördelade i de fem bestånden. Sporfällorna exponerades i tre timmar innan de togs till laboratorium för inkubation och analys. Hybridlärkträden valdes ut slumpmässigt, sågades ner varefter varannan stubbe behandlades med Rotstop® S medan de andra lämnades som kontroller. Inga tecken på infektioner sedan tidigare upptäcktes på de utvalda träden. Ungefär 60 dagar efter trädfällning och stubbehandling samlades prover in för analys i laboratorium.

Studien visar på en hög halt av basidiosporer av *Heterobasidion spp.* i luften. 63,7 % av alla sporfällor var infekterade av *Heterobasidion spp.* Mest infektioner hittades i Södra Rörum II, där 83 % av sporfällorna var infekterade. Ut av 263 kolonier var 61,2 % av isoleringarna infektioner av *H. annosum* och 38,8 % var av *H. parviporum*. Tall och hybridlärk var mest infekterad av *H. annosum* medan gran var lika mycket infekterad av *H. annosum* och *H. parviporum*. Medel av procent infekterade stubbar för alla fem bestånd per trädslag var; lärk 52 %, gran 58 % och tall 72 %.

Totalt analyserades 176 stubbar av hybridlärk och av dessa var 20 % infekterade av *Heterobasidion spp.* Både *H. annosum* och *H. parviporum* isolerades från stubbarna. 60 % av isoleringarna var *H. parviporum* och 40 % var *H. annosum*. Mest infektioner hittades i Fulltofta och Klippan där 24 % av stubbarna var infekterade.

Det är en signifikant skillnad på infektionsgraden och den infekterade arealen mellan behandlade och obehandlade stubbar,  $p \leq 0,000$  respektive  $p \leq 0,024$ . Dessa resultat gör Rotstop® S till ett intressant alternativ för stubbehandling av hybridlärk.

## List of contents

1. Introduction.....	7
2. Materials and methods .....	10
2.1 Sites .....	10
2.2 Spore Traps.....	11
2.2.1 Preparing spore traps.....	11
2.2.2 Implementation.....	11
2.2.3 Spore trap analyze.....	12
2.3 Treatment trial .....	13
2.3.1 Phlebiopsis gigantea.....	13
2.3.2 Stump treatment.....	13
2.3.3 Collection of discs.....	14
2.3.4 Stump analyze .....	14
2.4 Calculation and statistics.....	14
2.4.1 Spore traps .....	14
2.4.2 Treatment trial .....	15
3. Results .....	16
3.1 Spore trap study .....	16
3.2 Treatment trial .....	18
3.2.1 Efficacy .....	18
3.2.2 Infection rate.....	18
3.2.3 Species.....	18
3.2.4 Colonies .....	18
3.2.5 Significance.....	18
4. Discussion .....	21
Conclusions.....	23
Acknowledgement .....	24
Literature list .....	25
Appendix 1.....	29





## 1. Introduction

*Heterobasidion spp.* and the rot it creates is according to Hodges (1969) an economically very important disease that negatively affects the conifers in Scandinavia. The economic losses due to infections of *Heterobasidion spp.* are devastating and cost the Swedish forestry sector about 1 billion SEK per year and the whole of Europe about 7 billion SEK per year (Stenlid et al. 2000). The expensive machines and the increased industrial demand for wood makes it necessary to harvest both winter and summer time in Sweden (Bendz-Hellgren et al. 1998). The risk of stump infection from *Heterobasidion spp.* is much higher when the thinning takes place during the summer instead of winter (Brandtberg et al. 1996).

The main cause for root and butt rot on Norway spruce (*Picea abies* (L.) Karst.) in Sweden is *Heterobasidion spp.* (Vollbrecht et al. 1995). There are two species of *Heterobasidion* in Sweden; *Heterobasidion annosum* s.s (Fr.) Bref. and *Heterobasidion parviporum* Niemelä & Korhonen. (Bendz-Hellgren et al. 1998). *Heterobasidion parviporum* attacks Norway spruce and Scots pine (*Pinus sylvestris* L.) saplings whereas *H. annosum* has a wider range of hosts, e.g. Scots pine, Norway spruce, Japanese larch (*Larix kaempferi* (Lamb.) Carr), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and juniper (*Juniperus communis* L.) (Korhonen 1978). *Heterobasidion annosum* is found in southern Sweden whilst *H. parviporum* is found in almost the entire country, except in the far north (Korhonen et al. 1998). Air-borne spores of *Heterobasidion spp.* spread at plus degrees (°C) (Stenlid et al. 2000) infects freshly cut stumps and wounds but it can be outcompeted by other fungi if it doesn't colonize the stump surface fast enough, within a couple of weeks (Rishbeth 1951a). *Heterobasidion annosum* can also spread via root contacts, from infected roots to roots of healthy stumps or living trees (Rishbeth 1951b).

Thinning is a major cause for heavy infections by *Heterobasidion spp.* in our forests (Rishbeth 1950). Thinning often results in a large number of freshly cut stumps with exposed wood, which really favours this fungus, it is possible to reduce the damage by delaying the first thinning according to Rishbeth (1957) or by stump treatment with biological control agents, like *Phlebiopsis gigantea* (Fr.) Jül. (Rishbeth 1959, 1963). Trees attacked by *Heterobasidion spp.* are afflicted with growth losses, greater risks for wind throw and impaired timber quality (Bendz-Hellgren et al. 1998).

Rotstop is a biological pesticide, which is used in Sweden for treating freshly cut stumps, to prevent infections from *Heterobasidion spp.* in spruce stands. The stump treatment agent contains living spores from *P. gigantea*, a native saprotrophic fungus. *Phlebiopsis gigantea* colonize spruce stumps very effectively and quickly and prevents *Heterobasidion spp.* from attacking the stump surface (Korhonen et al. 1994, Pratt et al. 2000). Rotstop® is produced in Finland by Kemira Oy and it originates from a Norway spruce that was found in Finland (Thomsen & Jacobsen 2001). In the year 2002 Berglund et al. found that a Swedish strain of *P. gigantea* was more effective against *Heterobasidion spp.* infections within Swedish conditions than the original strain from Finland. This study led to an introduction of a new stump treatment agent on the Swedish market; Rotstop® S and it has been accessible in

Sweden since 2004. Verdera Oy in Finland manufactures the new agent whilst InterAgro skog AB is responsible for marketing in Sweden.

Best protection against *Heterobasidion spp.* is achieved when 100 % of the stump surface is covered with *P. gigantea* (Berglund & Rönnerberg 2004). This is a problem due to the mechanical way of treating stumps in Sweden today, it is very hard to cover the whole stump surface on all stumps and at the same time don't waste expensive treatment agent outside the stump. Stump treatment against *Heterobasidion spp.* has been used since 1992 in southern Sweden (Stenlid et al. 2000). In Sweden approximately 35 000 hectares are treated every year and at present only *P. gigantea* is used as a control agent. Almost all treatment is carried out in thinning and it cost about 1,3 Euro per m<sup>3</sup> solid under bark. A discussion is in progress if treatment in final felling should be implemented as well in Sweden (Thor 2003).

The first hybrid larch (*Larix × eurolepis Henry*) was found at the beginning of the 20<sup>th</sup> century in Scotland. It was a spontaneous cross between a European larch (*Larix deciduas Mill.*) and a Japanese larch. Hybrid larch has some advantages compared to Norway spruce, for example; higher volume growth and older larch trees are less susceptible to windthrow (Larsson-Stern 2003). There were severe storms and a lot of *H. annosum* attacks on Norway spruce stands in the 1960's, which created an interest for new tree species (Stern 1988). This made hybrid larch an interesting alternative to Norway spruce in southern Sweden. The hybrid larch is not a native species in Sweden, which means special regulations in the usage of this species, i.e. a registration is required for plantations bigger than 0,5 hectare (Skogsstyrelsen 2009, Larsson-Stern 2003, SVL 2010). The yield of hybrid larch proves to be a little higher than Norway spruce in southern Sweden (Ekö et al. 2004). It is easy to understand the fact that forest owners in southern Sweden were willing to try a complimentary species for Norway spruce and chose to try hybrid larch with the facts they had at that time. According to the Swedish statistical yearbook of forestry (2010) the larch corresponds to only 0,1 % of the total standing volume in Sweden i.e. 1,5 million m<sup>3</sup>sk.

Later studies show that European larch and Japanese larch probably are very susceptible to *Heterobasidion spp.* This might be because of their high susceptibility for infections by *Heterobasidion spp.* transferred from old stumps (Vollbrecht et al. 1995). Rönnerberg et al. (1999) also found that Japanese larch was severely attacked by *H. annosum*. Hybrid larch is also proved to be at least as infected as Norway spruce by *H. annosum* according to Swedjemark & Stenlid (1995), Rönnerberg & Vollbrecht (1999), Vollbrecht & Stenlid (1999) and also by *H. parviporum* (Swedjemark & Stenlid 1995). Therefore, at present it would be unwise to recommend planting this species on former infected sites. Even though it was known that severe attacks by *H. annosum* damaged populations of Japanese larch and European larch, hybrid larch was often planted as substitute to Norway spruce after harvesting a site infected with *H. annosum*. The aim of planting hybrid larch was to minimize possible economical losses (Vollbrecht & Stenlid 1999). Fast growing tree species, like hybrid larch, might become more damaged by *Heterobasidion spp.* than slower growing species (Rishbeth 1957).

According to earlier studies hybrid larch is very susceptible to infections from *Heterobasidion spp.* and it affects the forest owners very negatively due to economic losses every year. Sweden has a successful approach when treating Norway spruce stumps with *P. gigantea*, why not implement this on hybrid larch as well. A study made by Thomsen and Jacobsen (2001) in laboratory conditions in Denmark show that *P. gigantea* is able to grow and prevent infections from *Heterobasidion spp.* on hybrid larch. Hence it is of great importance to further investigate this in field as well. Studies have been made where this matter has been investigated in field. Mårtensson (2007) found that *Heterobasidion spp.* attacks larch and that stump treatment with *P. gigantea* was clearly beneficial due to the losses caused by *Heterobasidion spp.* No trial of the efficiency of *P. gigantea* on larch was performed. Pålsson (2009) on the other hand investigated stump treatment with *P. gigantea* and urea on hybrid larch. The result indicate a tendency that *P. gigantea* has capacity to prevent infections from *Heterobasidion spp.* Essential facts are still lacking. Former studies haven't investigated the spore load in the air, which is an important factor when testing whether a stump treatment agent work or not. The spore load in the air during the study is analyzed in this thesis by using spore traps of different tree species. This study aims to find out if Rotstop® S is able to prevent infections from *Heterobasidion spp.* on hybrid larch stumps in southern Sweden.

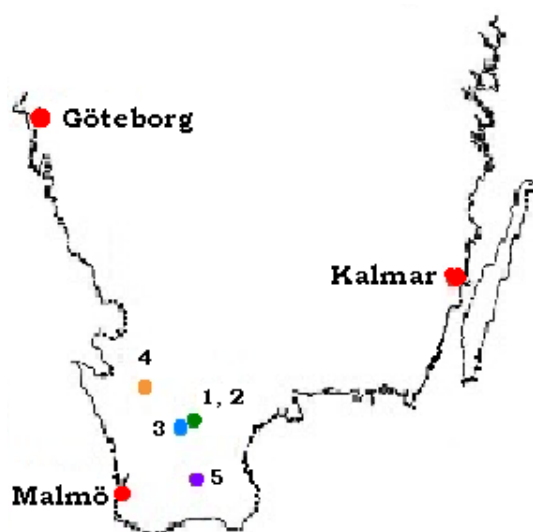
The hypotheses are:

- 🌲 Highest infection rate of *Heterobasidion spp.* on Scots pine spore traps and least infections on hybrid larch spore traps.
- 🌲 Lower infection rate of *Heterobasidion spp.* on treated stumps of hybrid larch.
- 🌲 Mainly infections of *H. annosum* on hybrid larch.

## 2. Materials and methods

### 2.1 Sites

The field study was established on former forest land in five un-thinned, hybrid larch stands in the first week of August 2010 in Scania, Sweden. The sites had to be monocultures of hybrid larch and with a diameter of approximately 10 - 20 cm at breast height (Table 1). As shown in figure 1 the sites were located to Klippan, Sjöbo, Fulltofta and two sites in Södra Rörum.



**Figure 1.** Map of Southern Sweden showing the distribution of sites used in the study.  
1, 2 Södra Rörum, 3 Fulltofta, 4 Klippan and 5 Sjöbo.

**Table 1.** Stand characteristics. Age is stated as total age, area in hectares, mean diameter is in breast height, SI H100 for spruce except site 3 where it is for birch and the coordinates are WGS 84, latitude/longitude.

Site	Size	Age (2010)	Mean Diameter (cm)	SI (m)	Coordinates	Origin
1. Södra Rörum I	2,1 ha	9	12,3	G 33	N 55° 55.742', E 13° 41.572'	Maglehem
2. Södra Rörum II	3,2 ha	9	13,2	G 30	N 55° 55.976', E 13° 41.927'	Maglehem
3. Fulltofta	6,4 ha	11	10,9	B 27	N 55° 53.889', E 13° 38.652'	Maglehem
4. Klippan	1,6 ha	13	12,2	G 30	N 56° 8.861', E 13° 11.342'	N/A
5. Sjöbo	4,2 ha	10	11,1	G 32	N 55° 37.068', E 13° 44.971'	Maglehem

Södra Rörum I, II and the site in Klippan were all pre-commercially thinned a few years ago whereas Fulltofta and Sjöbo were not managed since plantation. No visible signs of infections were discovered on the hybrid larch trees used in the study. A grid was used to distribute the plots systematically over the entire site. The distance between the plots was determined by the site area and calculated with this formula:

$$\sqrt{\frac{A}{B}} = C$$

*A is the site area stated in m<sup>2</sup>*

*B is how many plots you want to fit inside the site (10 was used consistently)*

*C is the distance between the plots*

10 plots were distributed in each site. The first plot was always randomly chosen and the plot distribution always started in the most westerly point of the site. The final grid is in a north - south and east - west direction. A compass was used to know the direction and the footsteps were counted to get the plots fairly distributed. Four trees were marked and numbered in each plot → 10\*4 = 40 trees per site. A label maker and a stapler were used to mark the trees. Two trees were chosen that were close to each other, which makes two pairs per plot. This made it easier to see differences between treated and un-treated stumps. The diameter in breast height of all 40 trees was cross-calipered.

## **2.2 Spore Traps**

### **2.2.1 Preparing spore traps**

Three different sites were visited to collect Norway spruce, Scots pine and hybrid larch trees to make spore traps. The trees were cut down and transported back to the department where they were decorticated with a debarking knife. Before the stem was cut into small discs the stem and the saw were sterilized with 99,5 % ethanol and then burned, this procedure was continued repeated during this phase. Every time one disc was finished, the fingertips were sterilized with 70 % ethanol, to avoid contaminations, before they touched the disc and put into a petri dish. The ethanol was diluted according to the volume and not to the molecular weight. All petri dishes were marked with site number, plot number and species to make it more efficient out in the field.

### **2.2.2 Implementation**

The spore traps were used when cutting the larch trees, one spore trap per species per plot gives three spore traps in each plot (Fig. 2). The spore traps were exposed three - four hours before they were collected. Three extra spore traps (controls), one of each species, were also brought to the field but were never exposed, to make sure that the discs did not get contaminated without getting exposed. A total of 165 spore traps were used in the study. When all the spore traps were collected they were put into a cooling-room, with a temperature of about - 4 °C, in the Alnarp Laboratory.



**Figure 2.** Spore traps in one of the plots in Södra Rörum II. Photo: Li Ying Wang.

### 2.2.3 Spore trap analyze

The spore traps were incubated in room temperature, 20 - 22 °C, for seven days before analyze. A microscope was used to see if there were any infections on the discs. If there was an infection, the colony of *Heterobasidion spp.* was marked with a pen and the infected area was estimated with a cm<sup>2</sup> sheet. From every colony, two isolations of conidiophores were transferred to and cultured on an agar plate using a sterile needle. Isolations were taken from maximum five colonies per disc. Only Hagem's malt extract agar plates were used throughout the whole study.

The isolations were incubated for five days in room temperature, if they hadn't grown or were contaminated after five days, they were re-isolated once more from the original spore trap. A few of the reisolated colonies never showed signs of infection from *Heterobasidion spp.* and were therefore excluded from the study as it was impossible to clean culture these samples. After five days when the isolations were satisfying, they were clean-cultured on a new agar plate. This was performed in a clean bench to minimize the risk of contamination. A clean piece of *Heterobasidion spp.* was collected from the isolation plate with a sterile needle and placed in the middle of a new agar plate. These new clean cultures were incubated for seven days before the mating test could take place.

After seven days every unknown isolate was re-cultured in five new agar plates, two together with testers of *H. annosum*, two with testers of *H. parviporum* and one with itself. The incubation time was three weeks in room temperature. To be able to determine whether it was *H. annosum* or *H. parviporum* one can see how the sample reacts to other homocaryotic known testers of *H. annosum* and *H. parviporum* and to the sample itself. The mating test was performed according to Korhonen (1978). The mating test was carried out visually at first. A dense wall between the two colonies show that the sample and the tester are different species (shown in Figure 3), whereas a sparse zone between the colonies or that the colonies are merging, illustrate that the sample and the tester are the same species.

When no morphological change in the mycelia could be found the species was determined by looking for clamps on the hyphae on the tester side in a microscope with greater magnification (Fig. 4). If a clamp was found the sample and the tester are the same species. Every 10<sup>th</sup> sample was examined through the microscope as a calibration even though the species could be determined visually.



**Figure 3.** Pairing of *Heterobasidion* after three weeks of incubation. A sample paired with two different *H. parviporum*, which is forming a distinct wall.  
Photo: Erik Ek



**Figure 4.** A sample is being analyzed and searched for clamps in a microscope with high magnification. Photo: Erik Ek

## **2.3 Treatment trial**

### **2.3.1 *Phlebiopsis gigantea***

The treatment agent, Rotstop<sup>®</sup> S was kept in a refrigerator until it was used. To make the agent ready for stump treatment a bag containing two grams of spores was used. The bag was filled with 300 millilitres of water and then shaken thoroughly. The content of the bag was poured into a bigger can with 1400 ml of water. The bag was filled again with 300 ml of water, this to make sure to use all the Rotstop<sup>®</sup> S, the liquid was shaken again and put into the bigger can, now with totally 2000 ml of water. Finally a blue marking pill (manufactured by Becker-Underwood, Inc. Iowa, USA) was added to the solution, just to make it easier to see the coverage on the stump surface in the field. The treatment agent was not tested before use.

### **2.3.2 Stump treatment**

The trees were cut down with a chainsaw, 30 centimetres above ground level. Every second stump was treated with Rotstop<sup>®</sup> S with the help of a spray bottle. There were two pairs of trees in each plot, in every pair one was treated and one was left un-treated. In every site 20 stumps were treated and 20 stumps were left as control.

A sheet showing different diameters and how much Rotstop<sup>®</sup> S needed on different stump areas was calculated as help during the treatment. The treatment agent was applied according to the manufacturer's recommendation, 2000 ml of solution / m<sup>2</sup> stump surface or a 1 mm thick layer covering the whole stump. To calibrate the amount, occasional measures were made to know how many sprays that corresponded to 10, 20 and 30 ml in a small measuring cup. To be on the safe side, the stumps were marked once more with coloured marking tape.

### **2.3.3 Collection of discs**

The larch discs were collected after approximately 60 days of exposure in the field. The stumps were sterilized by spraying roughly 70 % ethanol on the bark to prevent contamination before using the chain saw. One - two cm on top of the stump was cut to waste. The top side of the disc that was going to be collected was marked with a pen, then a five cm thick disc was cut and immediately put into a plastic bag. The bag was used as a glove and the human body was never in contact with the larch samples. Finally the stump was cut down to ground level where a one cm thick disc was taken and put into a plastic bag. If discoloration was detected it was marked on the bags. The bottom discs were collected to be able to see if the infection came from the roots.

### **2.3.4 Stump analyze**

The discoloured bottom discs were the only discs at ground level to be analyzed. The larch discs were incubated for seven to ten days in room temperature. All stumps were divided into smaller sections with a pen, to guarantee a complete analysis of the entire disc surface. First to be analyzed were the discoloured bottom discs. If *Heterobasidion spp.* was found, the tree was excluded from the study. Finally the top disc was analyzed, first the upper side, if an infection of *Heterobasidion spp.* was found it was noted as explained above and the lower side was not analyzed. If an infection couldn't be found on the upper side, the lower side was analyzed. This time only two colonies per disc were isolated for mating test, despite this all was carried out as explained above.

## **2.4 Calculation and statistics**

### **2.4.1 Spore traps**

Missing spore traps were excluded from the study. Due to rain during the field work in site 5, it was excluded in some of the calculations, to look at the difference and to prevent misleading results. Infections where the species couldn't be identified was counted as non-infected. Spore traps used as controls were not included in the calculations.



### **2.4.2 Treatment trial**

Trees with infections from below (infected bottom discs) and missing samples were excluded from the study. Mean size of colonies is calculated as total area of infections divided by number infected discs. Efficacy is calculated as how many percent you reduce the number of infections by stump treatment. It has been calculated both per site and the total reduction. To know if there were any significant difference of infection rate or infected area between treated and control stumps, a variance analysis (ANOVA) was performed to know if Rotstop<sup>®</sup> S works on hybrid larch or not. The software used for variance analysis was Minitab<sup>®</sup> (15.1.1.0 statistical software, © 2007 Minitab Inc.).

### 3. Results

#### 3.1 Spore trap study

The results from this study show a high abundance of spores of *Heterobasidion spp.* in the air during the implementation of the field trial. Most infections were found on Scots pine where the mean infection rate for all sites was 72 %, Norway spruce had 58 % respectively and hybrid larch was least infected with a mean infection rate of 52 % (Fig. 6). The highest infection rate was found in Södra Rörum II, where 83 % of the spore traps were infected. Infections from *Heterobasidion spp.* were found in all five sites. Sjöbo had very little infections, only one infected spore trap of hybrid larch was found. Both *H. annosum* and *H. parviporum* were present in four sites. Totally 61,2 % of all infections were of *H. annosum* and 38,8 % were of *H. parviporum* (Table 3). Scots pine and hybrid larch were most heavily attacked by *H. annosum* whilst Norway spruce was equally attacked by *H. annosum* and *H. parviporum* (Fig. 5).

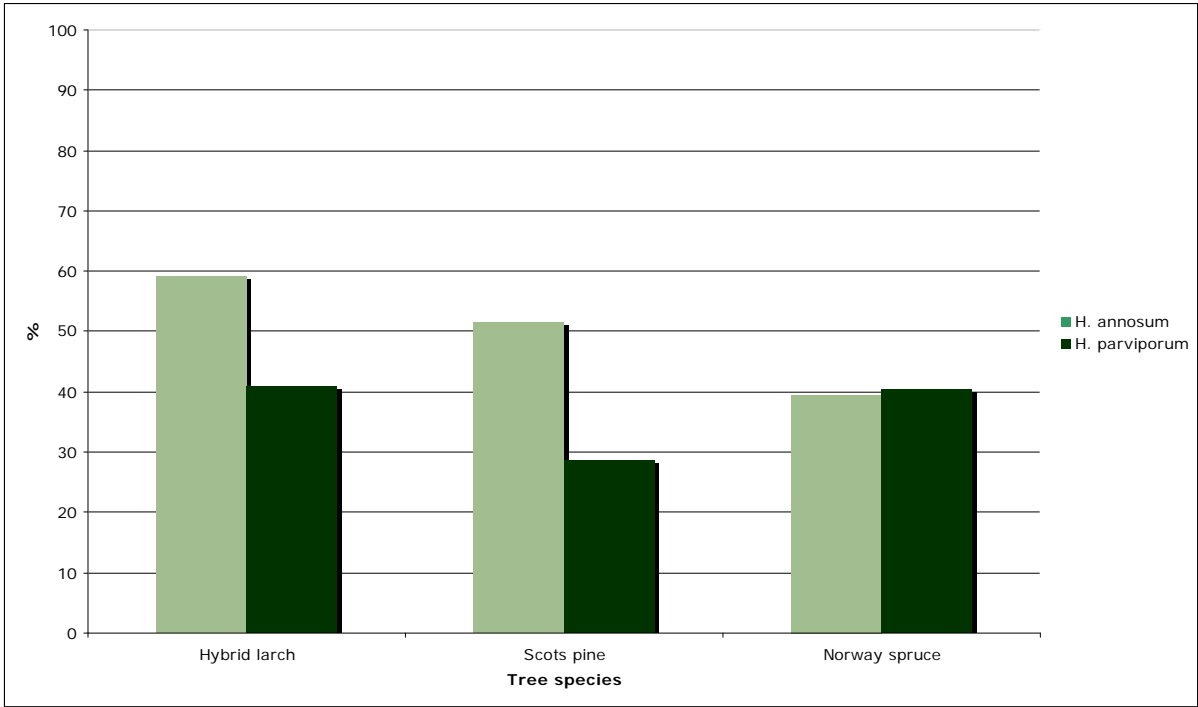
Totally 63,7 % of 146 spore traps were infected by *Heterobasidion spp.* (Table 2). 263 colonies were found with a total of 579,5 cm<sup>2</sup> of infections. *Heterobasidion annosum* have greatest infected area with 321 cm<sup>2</sup> compared to *H. parviporum* with 258,5 cm<sup>2</sup>. The mean colony size of all spore traps is 2,2 cm<sup>2</sup>.

**Table 2.** Illustration of the results from the spore trap study, average for all traps including Norway spruce, Scots pine and hybrid larch. *Heterobasidion annosum* and *H. parviporum* infections are percentage of the total infections per site.

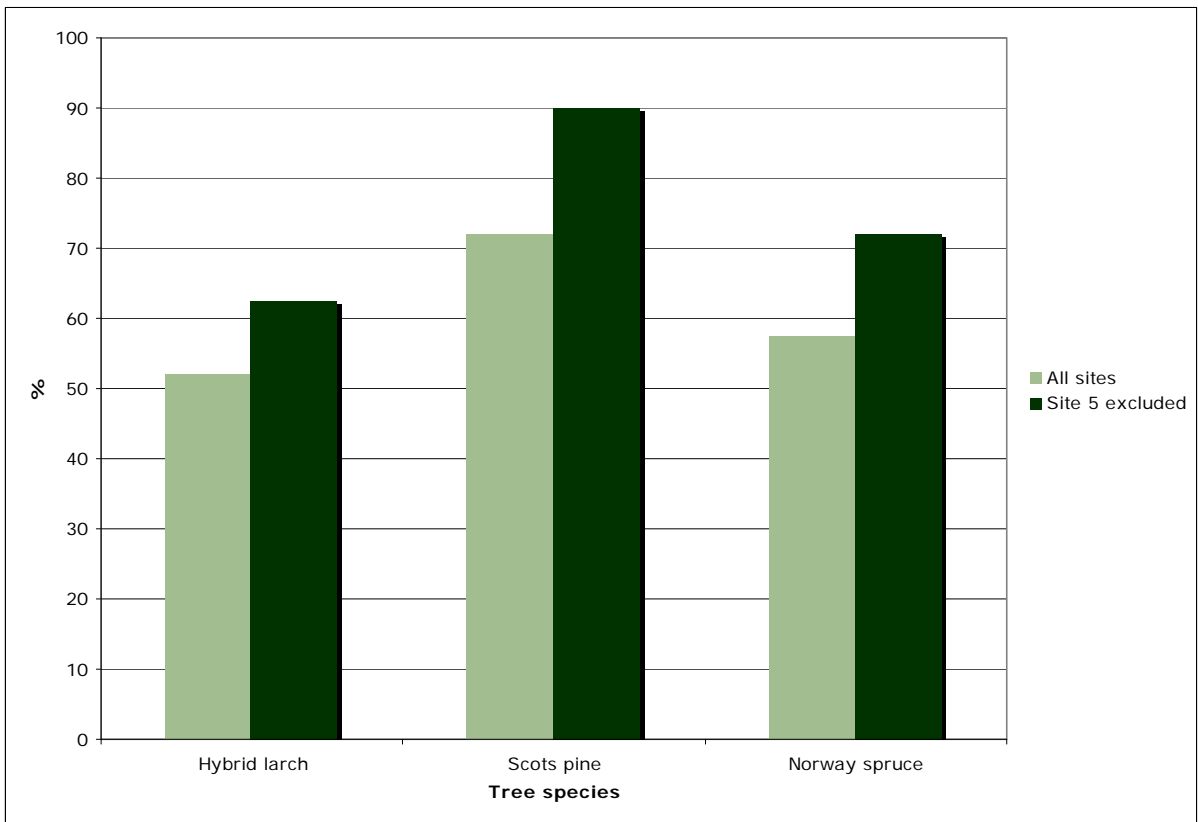
Site	Nr. of samples	Infection rate (%)	(%) H. annosum Inf.	(%) H. parviporum Inf.	Tot nr. of colonies	H. annosum (cm <sup>2</sup> )	H. parviporum (cm <sup>2</sup> )	Tot area of Inf. (cm <sup>2</sup> )
S. Rörum I	30	73	93	7	69	143	11	154
S. Rörum II	29	83	56	44	70	75,5	96,5	172
Fulltofta	29	76	30	70	69	49	125	174
Klippan	29	69	69	31	54	53,5	25,5	79
Sjöbo	29	3	0	100	1	0	0,5	0,5

**Table 3.** Total amount of colonies found in all sites. Percentage *H. annosum* and *H. parviporum* of total number of colonies.

	Colonies	%
H. annosum	161	61,2
H. parviporum	102	38,8
Total	263	100



**Figure 5.** Distribution of total spore infections from *H. annosum* and *H. parviporum* for hybrid larch, Scots pine and Norway spruce.



**Figure 6.** The mean infection rate of *Heterobasidion spp.* per site of the three tree species used as spore traps, for all sites and with site 5 excluded.

## 3.2 Treatment trial

### 3.2.1 Efficacy

By treating the stumps with *P. gigantea* the number of infected stumps will be reduced with 67 % (Table 6). The best efficacy was discovered in Södra Rörum I, where *P. gigantea* lowered the number of infected stumps with 79 %.

### 3.2.2 Infection rate

Out of 176 samples a total of 20 % of all hybrid larch stumps were infected by *Heterobasidion spp.* (Table 4). Both treated and un-treated stumps were infected. As shown in figure 8 stumps treated with *P. gigantea* have fewer infections than the un-treated control stumps. Also the area of infection was greater in un-treated stumps for all sites (Fig. 9). 5 % of the treated stumps were infected by *Heterobasidion spp.* whilst 20 % of the un-treated stumps were infected.

### 3.2.3 Species

Totally 60 % of all infections were of *H. parviporum* whilst *H. annosum* corresponded to 40 % (Table 5). On treated stumps 75 % of the infections were of *H. parviporum* whilst on un-treated stumps *H. parviporum* corresponded to 55 % (Table 7). *Heterobasidion spp.* attacked all sites and most infections were discovered in Fulltofta and Klippan, see table 4.

### 3.2.4 Colonies

Totally 50 colonies of *Heterobasidion spp.* were found which resulted in a total area of 39 cm<sup>2</sup>. The mean colony size of all infected stumps is 1,28 cm<sup>2</sup>. All infections were found in the sapwood and in the border to the heartwood. Most infections from below were found in Södra Rörum II, see figure 10.

### 3.2.5 Significance

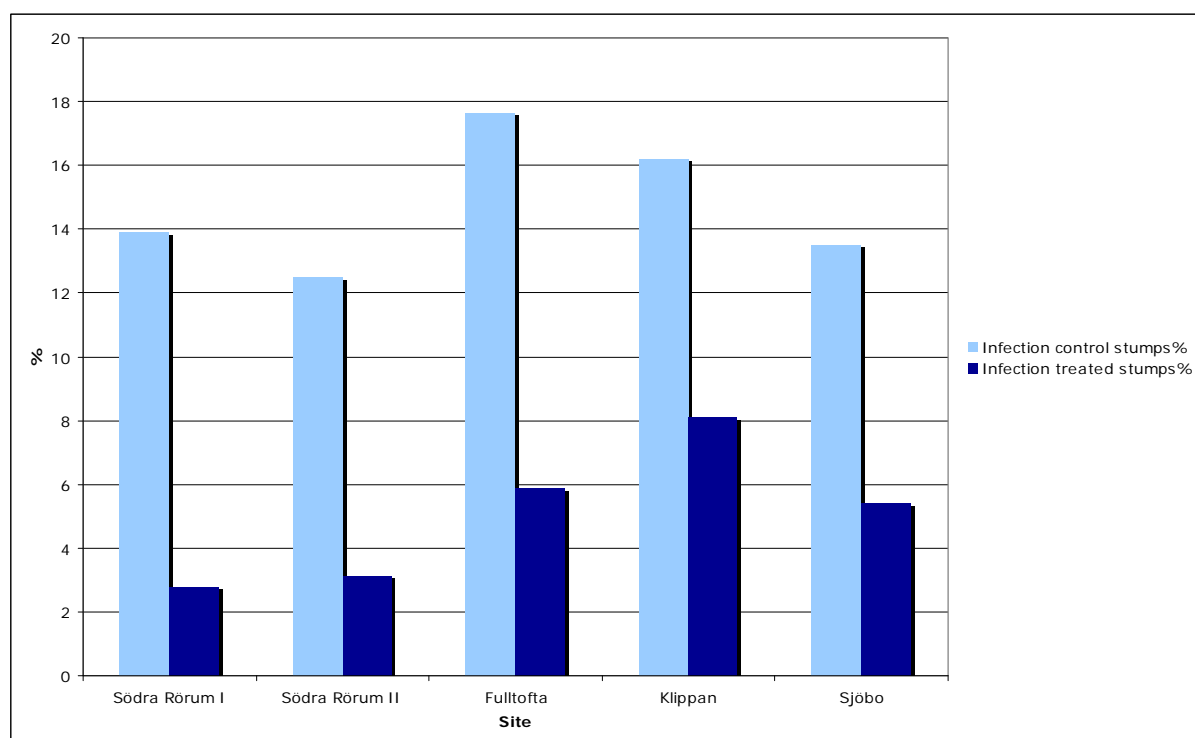
It is a statistically significant difference between treated and un-treated stumps when looking at the infection rate per site,  $p \leq 0,000$ . Furthermore comparison of infected area for treated and un-treated stumps per site is also significant where  $p \leq 0,024$ .

**Table 4.** Illustration of results approx. 60 days after implementation. *Heterobasidion annosum* and *H. parviporum* infections are percentage of the total infection per site.

Site	Nr. of samples	Infection rate (%)	(%) <i>H. annosum</i> inf.	(%) <i>H. parviporum</i> inf.	Tot. nr. of colonies	<i>H. annosum</i> (cm <sup>2</sup> )	<i>H. parviporum</i> (cm <sup>2</sup> )	Tot. Area of inf. (cm <sup>2</sup> )
S. Rörum I	36	17	75,0	25,0	8	3,0	1,0	4,0
S. Rörum II	32	16	42,9	57,1	7	1,5	2,5	4,0
Fulltofta	34	24	10,0	90,0	10	2,0	10,5	12,5
Klippan	37	24	60,0	40,0	15	5,0	3,5	8,5
Sjöbo	37	19	10,0	90,0	10	0,5	9,5	10,0

**Table 5.** Total amount of colonies found on hybrid larch stumps in all sites. Percentage *H. annoum* and *H. parviporum* of total number of colonies.

	Colonies	%
<i>H. annosum</i>	20	40
<i>H. parviporum</i>	30	60
Total infection	50	100



**Figure 8.** Difference of infections of *Heterobasidion spp.* between treated and un-treated hybrid larch stumps per site.

**Table 6.** Efficacy of stump treatment. How much you lower the percent of infected stumps per site and total by stump treatment.

Site	Nr. of samples	Infected control stumps (%)	Infected treated stumps (%)	Efficacy (%)
Södra Rörum I	36	14	3	79
Södra Rörum II	32	13	3	77
Fulltofta	34	18	6	66
Klippan	37	16	8	50
Sjöbo	37	14	5	64
Total	176	15	5	67

**Table 7.** Percentage of total number of colonies per treatment.

	<i>H. annosum</i>	<i>H. parviporum</i>
Un-treated stumps	45	55
Treated stumps	25	75

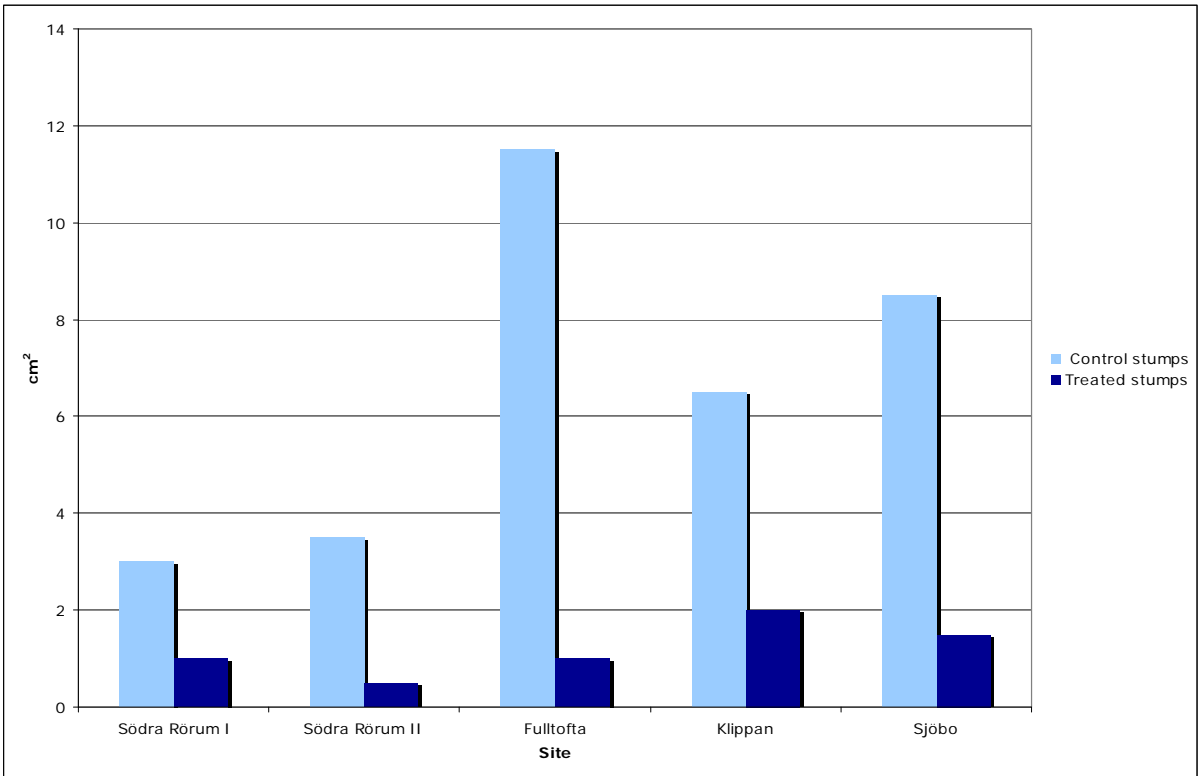


Figure 9. Distribution of infected area between treated and un-treated control stumps in each site.

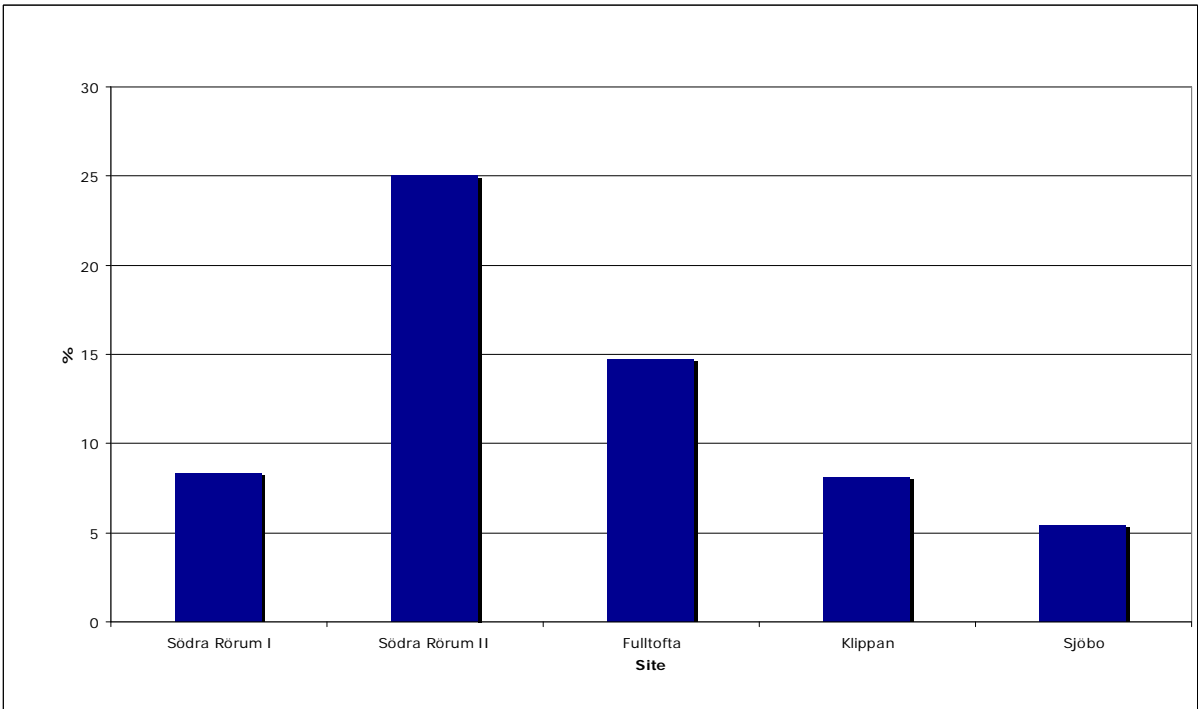


Figure 10. Infection rate from the roots in each site.

## 4. Discussion

The significant difference of spore infections of *Heterobasidion spp.* on hybrid larch stumps treated with *P. gigantea* and those that were left un-treated as controls clearly illustrates that *Phlebiopsis gigantea* reduces the rot frequency in hybrid larch stumps. This study demonstrates successful stump treatment with Rotstop® S in hybrid larch cuttings. If stump treatment on hybrid larch is implemented in Sweden a large amount of trees won't be infected by root and butt rot. It will result in a more profitable forestry because the quality will increase and it will get less degraded timber.

It is obvious that both *H. annosum* and *H. parviporum* attacks hybrid larch stumps. This is a very important matter, which needs a proper solution immediately and *P. gigantea* can be a good alternative. The results from this study are similar to earlier findings made by e.g. Vollbrecht et al. (1995), Rönnerberg & Vollbrecht (1999), Vollbrecht & Stenlid (1999), Stenlid et al. (2000), Thomsen & Jacobsen (2001) and Rönnerberg et al. (2007). Swedjemark & Stenlid (1995) did also find that hybrid larch was infected by both *H. annosum* and *H. parviporum*.

The majority of infections found on both treated and un-treated stumps were from *H. parviporum*. It was not a big difference between infections of *H. annosum* and *H. parviporum* on the un-treated control stumps. However the difference when looking at infections on the treated stumps was considerably greater, three quarters was of *H. parviporum*. According to these results it seems like *H. parviporum* might be able to resist *P. gigantea* better and establish faster and more aggressive on hybrid larch stumps than *H. annosum*.

It might be valuable to test different strains of *P. gigantea*, just like Berglund et al. (2005), to find an even more efficient treatment agent, operational on hybrid larch. It could also be of importance to investigate the number of oidia in the treatment agent, hybrid larch may need a higher concentration than Norway spruce to get better protection (Rönnerberg et al. 2006; Korhonen et al. 1994).

It would be very easy to implement stump treatment on hybrid larch in Sweden because the existing treatment agent, *P. Gigantea*, successfully reduces the infections on hybrid larch. There won't be any high expenditures for entrepreneurs and forest companies due to the fact that most harvesters in southern Sweden already are equipped with the technology and devices needed for stump treatment since it is necessary when cutting Norway spruce during growing season.

Until stump treatment on hybrid larch is implemented, a good way to prevent infections from *Heterobasidion spp.* is to harvest hybrid larch trees during wintertime when spore dispersal is negligible. Since there is an efficient way of treating stumps of Norway spruce, these trees can be cut during spore dispersal and stands of hybrid larch should be cut during wintertime.

The spore traps showed a great load of spores from *Heterobasidion spp.* in the air during the establishment of the study where more than half of the spore traps were infected. This show

that the freshly cut stumps of hybrid larch was exposed to air-borne spores from *Heterobasidion spp.* Without knowledge about how much spores that were present in the air during the study it would be impossible to say whether *P. gigantea* would be able to prevent these infections. If there weren't any spores or a very high amount of spores in the air and spore traps were not used it would be very difficult to interpret the results.

The fact that the spore traps of hybrid larch were more infected and has larger colonies than the un-treated standing stumps could be due to the younger age of the trees from where the spore traps were taken. In this case younger trees seems to be more susceptible to infections from *Heterobasidion spp.* Rönnerberg & Vollbrecht (1999) also discovered young hybrid larch trees to be very susceptible to infections through root contact. It is possible that the stump surface also is more vulnerable in early age. Even though the possible infection surface is bigger on a cut tree than a spore trap, it is not as much infected.

It is economical feasible to treat stumps of hybrid larch on former arable land according to Mårtensson (2007), Rönnerberg et al. (2007). They compared thinned stumps which have been exposed to natural spore infection and un-thinned stands. With six thinnings including the cost for stump treatment and an interest rate of 3 % they found it clearly beneficial. With facts from this thesis regarding *P. gigantea* and its efficiency of preventing infections from *Heterobasidion spp.*, it is evidently worth treating stumps of hybrid larch planted on former arable land. The question is if it also is economical feasible to treat hybrid larch stands planted on former forest land, with the possibility that it already exist infections on previous stumps or roots. Hybrid larch is very susceptible to *Heterobasidion spp.* infections in early age (Rönnerberg & Vollbrecht 1999) and this can't be prevented by stump treatment. If the stumps already contain infections before thinning age, stump treatment could be questioned whether it is economically sustainable.

A hypothetical case was invented to see if stump treatment is economically feasible in a site on former forest land according to the results from this study. A treated hybrid larch site with 5 % infected stems was compared with an un-treated hybrid larch site with 20 % infected stems, see Appendix 1. The rotation period included five thinnings and one final harvest. The percentage of infected trees was excluded from the final harvest only. All of the harvested trees were assumed to be sold as pulpwood and no stump treatment was performed in the final harvest. A comparison between the profits for the two sites show that it is clearly beneficial to treat the stumps with *P. gigantea*, as you save approximately 6115 SEK/ha during one rotation period. The calculations also show that as long as the percent of infected trees exceed 10 % is it profitable to treat the stumps with *P. gigantea*. If you instead of pulpwood sell the trees for timber production it will be even more profitable to treat the stumps because the costs for stump treatment and machines will stay the same but the profit will increase due to the higher price for timber.

In contrast to Pålsson (2009) this study shows a distinct difference between stumps treated with *P. gigantea* and un-treated stumps of hybrid larch. In that previous study no spore traps were used and the fluctuating results might be due to very low presence of air-borne spores. In this study where spore traps were part of the study one can say for sure that there were



spores of *Heterobasidion spp.* in the air during the implementation of the field trial. Therefore the results of this study are more reliable and accurate.

Infections from *Heterobasidion spp.* were in a few stumps found at the ground level but not on the top disc, which tend to be infections from the roots and from earlier stands. Hybrid larch is susceptible to infections from both air-borne spores and via root contact (Rönnerberg & Vollbrecht 1999). Once again it is clearly illustrated that it is not recommended to plant hybrid larch on sites where the possibility exist of remaining infections in the ground.

As shown in table 2, the infection rate on the spore traps is quite similar for all sites except for site five. A possible explanation can be that it started to rain when the spore traps were collected in site five and the spore load in the air and the spore dispersal could have been reduced due to the rain. When looking at infections on the hybrid larch stumps, there are no big difference between the sites in spite of the rain and the spore load seem to be normal in the long run. The spore traps in site five were only exposed for three to four hours, including rain, therefore they were more sensitive to fluctuations of the spore load in the air compared to the hybrid larch stumps that were exposed for approximately 60 days.

Infections of *Heterobasidion spp.* were also found on spore traps used as controls, they were used to make sure that no infections were present on the spore traps before the field trial. They were not supposed to be infected but must've been exposed somehow in field in two of the sites. Controls of spore traps in three sites showed no signs of infection and all spore traps from the same species came from the same tree. The spore traps were sawn off from the top end of young trees, not close to the ground and therefore it's not likely that they already were infected before they were put into the plastic containers.

The cutting was performed with a chainsaw, which can transfer infections from one stump to another or from the bark into the wood. The chainsaw is impossible to sterilize but the stem was sterilized before cutting to prevent infections on the bark to reach the wood.

## Conclusions

The result from this study clearly proves that Rotstop<sup>®</sup> S (*P. gigantea*) successfully reduces the frequency of infections of *Heterobasidion spp.* in sites where hybrid larch has been planted. According to this and earlier studies it is economically feasible to treat hybrid larch stumps with *P. gigantea* in sites where it has been both forest and arable land. If successful stump treatment of hybrid larch is implemented in the near future, private forest owners and forest companies will save a lot of timber and money. General recommendations considering reduced infections from *Heterobasidion spp.* are to plant hybrid larch on former arable land or sites where the former site consisting mostly deciduous species and to treat the stumps in all commercial thinnings with *P. gigantea*.

## **Acknowledgement**

I would like to thank my supervisor Jonas Rönnerberg for all knowledge and great support during this thesis, my co-supervisor Li Ying Wang for great patience and practical help, Linus Andersson at Skogssällskapet for helping me to find suitable sites, InterAgro skog AB and Carl-Magnus Walde and Future Forests for financial support which made this study possible. I would also like to thank Per-Magnus Ekö for very helpful advices and help with the statistics and Fredrik Nilsson at Södra Skogsägarna in Höör for providing me with prices and costs regarding harvest of hybrid larch. Finally I thank my father, Göran Ek for necessary help in field and letting me borrow chainsaw, protection gear and car. Thank you all.

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## Appendix 1

### ***Calculations of the revenue and the cost for stump treatment per hectare in one rotation period of hybrid larch***

Interest rate: 3 %

Harvest per thinning (Larsson-Stern et al. 2005)

Machine cost in thinning and final felling in southern Sweden (Brunberg 2010):

Thinning: 178 SEK/m<sup>3</sup>sub

Final felling: 84 SEK/m<sup>3</sup>sub

Price (pulpwood): 350 SEK/m<sup>3</sup>sub (Nilsson, pers. Comm. 2011)

Cost stump treatment: 1,3 euro / m<sup>3</sup>sub x 8,86 = 11,52 SEK/ m<sup>3</sup>sub (Thor 2003)

The infection rate is only noticed in final felling, in a totally healthy site the final harvest is 242,8 m<sup>3</sup>sub/ha (Larsson-Stern et al. 2005). In this calculation the final harvest is multiplied with the different infection rates.

Revenue = Harvest x 350 SEK/m<sup>3</sup>sub

Total costs = Cost stump treatment + Cost machines

Profit = Revenue (value year 40) – Total Costs (value year 40)

**Table 1.** A stump treated site with 5 % infections of *Heterobasidion spp.* TC is total costs. Value year 40 is calculated by compounding with an interest rate of 3 %.

Age	Thinning	Harvest (m <sup>3</sup> sub)	TC	TC (value year 40)	Revenues	Revenues (value year 40)	Profit
15	1	40	7486	15674	13825	28946	13272
20	2	45	8604	15540	15890	28699	13159
25	3	38	7164	11161	13230	20612	9451
30	4	36	6842	9195	12635	16980	7786
35	5	33	6216	7206	11480	13308	6102
40	Final felling	231	19375	19375	80731	80731	61356
Sum		422	55687	78152	147791	189277	<b>111126</b>

**Table 2.** An un-treated site with 10 % infections of *Heterobasidion spp.* TC is total costs. Value year 40 is calculated by compounding with an interest rate of 3 %.

Age	Thinning	Harvest (m3sub)	TC	TC (value year 40)	Revenues	Revenues (value year 40)	Profit
15	1	40	7031	14721	13825	28946	14225
20	2	45	8081	14596	15890	28699	14104
25	3	38	6728	10483	13230	20612	10129
30	4	36	6426	8636	12635	16980	8345
35	5	33	5838	6768	11480	13308	6540
40	Final felling	219	18356	18356	76482	76482	58126
Sum		410	52460	73559	143542	185028	<b>111469</b>

**Table 3.** An un-treated site with 20 % infections of *Heterobasidion spp.* TC is total costs. Value year 40 is calculated by compounding with an interest rate of 3 %.

Age	Thinning	Harvest (m3sub)	TC	TC (value year 40)	Revenues	Revenues (value year 40)	Profit
15	1	40	7031	14721	13825	28946	14225
20	2	45	8081	14596	15890	28699	14104
25	3	38	6728	10483	13230	20612	10129
30	4	36	6426	8636	12635	16980	8345
35	5	33	5838	6768	11480	13308	6540
40	Final felling	194	16316	16316	67984	67984	51668
Sum		386	50421	71520	135044	176530	<b>105011</b>

If a comparison is made of the summarized profits from a treated site with 5 % infections and an un-treated site with 20 % infections, you will discover that if you treat the stumps with *P. gigantea* you will save:  $111126 - 105011 = 6115$  SEK/ha

In a site with 10 % infections is it according to this calculation not economically feasible to treat the stumps;  $111126 - 111469 = -343$  SEK/ha





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