Pre-commercial thinning stumps' susceptibility to *Heterobasidion* spp.

- A comparison between high and low Norway spruce and birch stumps
- A measuring of the efficacy of Phlebiopsis gigantea stump treatment

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Master Thesis no. 166
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Abstract

Each year, *Heterobasidion* spp. is a major cause of economic losses to forestry in the northern hemisphere, including Sweden. New results indicate that pre-commercial thinnings could be at risk for *Heterobasidion* spp. infections. Since no wood usually is used from pre-commercial thinnings, there would not be any timber losses from leaving high stumps instead of low stumps when carrying out this measure. It could therefore be of a great interest if the stump height, of both Norway spruce (*Picea abies* (L.) Karst.) and birch (*Betula* spp.), affects the susceptibility to *Heterobasidion* spp. after a pre-commercial thinning is performed.

The objective of this study was to investigate if there were any differences in infection frequency from airborne *Heterobasidion* spp. spores depending on whether the stump height is high (1 m) or low (15 cm) in pre-commercial thinnings in the southern Sweden. The efficacy of stump treatment by *Plebiopsis gigantea* (Fr.) Jül. and the infection frequency of birch were also investigated for the two stump heights in five different sites.

There was a significant difference in infection frequency between treated spruce, untreated spruce and birch when samples from both high and low stumps were included.

The efficacy is a measure of the reduced mean relative infected area of the infections related to a control. High untreated spruce did not show a difference in efficacy compare to low untreated spruce, nor did treated spruce and birch.

The infection frequency of birch stumps was significant higher on high stumps (17.3 %) and hence they were more susceptible to *Heterobasidion* spp. than low (12.2 %).

No significant effect of the susceptibility depending on the stump height was observed for untreated spruce nor treated spruce.

Other reasons than risk of infection could be a reason to create high treaded spruce stumps. One reason could be the more ergonomic treatment height compared to perform stump treatment on low height.

A five year long-term research study was established with the objective to investigate if a known *Heterobasidion* spp. infection spreads into surrounding trees or if it dies out in inoculated high and low spruce stumps.

It is possible that the stump height is of importance for the spreading of *Heterobasidion* spp. infections in the long-run, which is the purpose of the long-term study to find out.

Sammanfattning

Infektioner av rotticka (*Heterobasidion* spp.) orsakar årligen omfattande ekonomiska förluster för skogssektorn på norra halvklotet, inklusive Sverige. Nya resultat tyder på att även röjningar kan vara i riskområde för infektionsangrepp. Eftersom virke vanligtvis inte tas tillvara i röjningar förloras inget virke om en högre stubbe huggs för att hindra rottickan att infektera stubbar. Det är av den anledningen intressant att studera om mottagligheten för infektioner av rotticka påverkas av höjden på röjningsstubbar av gran (*Picea abies* (L.) Karst.) och björk (*Betula* spp.).

Syftet med studien är att undersöka om infektionsfrekvensen skiljer sig åt beroende på om rottickans luftburna sporer träffar en hög (1 m) eller låg (15 cm) röjningsstubbe i södra Sverige. Dessutom undersöktes effektiviteten från stubbehandling av *Plebiopsis gigantea* (Fr.) Jül. beroende på stubbhöjden som pergamentsvampen placeras på.

Skillnaden i infektionsfrekvens var signifikant beroende på om stubben var behandlad gran, obehandlad gran eller björk, oavsett stubbhöjd.

Den relativa effektiviteten är ett mått på reduktionen av den relativa infekterade arean genom medelvärdena från varje bestånd i förhållande till en kontroll. Av de olika stubbtyperna visade det sig att höga obehandlade granstubbar inte skilde sig från låga obehandlade granstubbar. Inte heller stubbehandlade granstubbar eller björkstubbar skilde sig från låga obehandlade granstubbar.

Skillnaden i infektionsfrekvensen av björkstubbar visade att låga stubbar var signifikant mindre mottagliga för rottickeinfektioner (12,2 %) än höga stubbar (17,3 %).

Inga signifikanta infektionsskillnader relaterade till stubbhöjden observerades bland obehandlad eller stubbehandlad gran.

Det kan finnas andra fördelar med att skapa höga stubbehandlade granstubbar om man väljer att stubbehandla. En fördel är att höga stubbar bidrar till en mer ergonomisk höjd att utföra manuell stubbehandling på i jämförelse med låga stubbehandlade granstubbar.

Ett femårsförsök anlades för att kunna styrka resultaten från den här studien i ett längre tidsperspektiv. Syftet var att i framtiden undersöka om rottickan sprider sig vidare till intillstående träd eller om den dör ut i höga och låga inokulerade granstubbar.

Stubbhöjden kan fortfarande visa sig ha betydelse för rottickeinfektioners fortsatta spridning inom grandominerade röjningsbestånd, vilket långtidsstudien har till uppgift att ta reda på.

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Introduction

Heterobasidion spp. is a major cause of annual economical losses to forestry in the northern hemisphere including Sweden (Bendz-Hellgren and Stenlid, 1995, Bendz-Hellgren et al., 1998, Woodward et al., 1998) and is therefore of great interest to the forestry sector. The economical losses are first and foremost caused by decreased volume growth and lowered timber quality, but also trunk breakages and windthrows at a later stage (Bendz-Hellgren and Stenlid, 1995). Another potential loss is the risk that the pathogen stays in the stand and infects the next generation of coniferous forest (Bendz-Hellgren et al., 1998, Rönnberg et al., 2007). In Sweden, economical losses are estimated to be 275 million SEK/year from timber quality losses alone (Stenlid, 1992). If the estimated growth loss is included, this amount should be 475 million SEK/year (Bendz-Hellgren and Stenlid, 1995). These estimates were taken from the early to mid 1990s and as the occurrence of *Heterobasidion* spp. seems to have increased during recent years (Thor et al., 2005), it can be assumed that the economical losses have also increased. A major problem from the forest industries' point of view is the restriction of use of chlorine when producing chemical pulp, which makes the decayed pulp logs impossible to use and as a result almost worthless (Swedjemark and Stenlid, 1997). Decayed saw logs are also transferred to lower valuable timber assortments due to wood decay in the timber, which contributes to a lower value and waste (Möykkynen and Pukkala, 2009).

Heterobasidion species

This study relates to the two *Heterobasidion* spp. that occur in Sweden, *Heterobasidion annosum* s.s. (Fr.) Bref. and *Heterobasidion parviporum* Niemelä & Korhonen, and the problems caused by these pathogens in Swedish pre-commercial thinnings. This is an area into which little research has been carried out. *H. annosum* and *H. parviporum*, behave differently. The first, *H. annosum*, attacks pine (*Pinus* spp.), spruce (*Picea* spp.) and some deciduous tree species. The second, *H. parviporum*, attacks mostly Norway spruce (*Picea abies* (L.) Karst.) (Korhonen, 1978). (Norway spruce is hereafter called spruce). These are the only two forms of *Heterobasidion* spp. present in Sweden and will therefore be called *Heterobasidion* spp. in this study.

Dispersal strategy

Heterobasidion spp. have the ability to spread and infect surrounding trees through linked tree roots. This often results in greater timber damages in the forest (Stenlid, 1987, Piri et al., 1990). The most common dispersal strategy of *Heterobasidion* spp. is by airborne basidiospores (Rishbeth, 1950, Rishbeth, 1951a, Korhonen and Stenlid, 1998). Although the spores can travel very long distances, they usually infect trees in the same area as the fruiting body. Fresh woody tissues become infected by spores (Rishbeth, 1950), for example from a wound or a new stump after a harvest operation. When the spores have found a suitable surface, the mycelium starts to colonize it (Rishbeth, 1950). The mycelium extracts energy by breaking the bindings in cellulose, starch and pectin (Asiegbu et al., 1998) and start growing down into the wood. This mycelium grows down towards the roots (Rishbeth, 1951b), especially if it colonized a stump. There is a risk in spruce dominated stands that the roots will grow together and create links between individual trees (Rishbeth, 1951b).

Heterobasidion species' natural ecosystem function

Root rot fungi are very important decomposer in forests (Eidmann and Klingström, 1990) and species such as *Heterobasidion* spp., which is a part of this umbrella term, cause a natural succession of tree species in the forest. By infecting gymnosperms and slowly killing them *Heterobasidion* spp. create gaps possible for angiosperm trees, that are not susceptible to the fungus, to colonize. This usually results in a mixed tree species forest containing dead wood and a multilayer structure (Ostry and

Laflamme, 2009). These kinds of forests are more resistance to disturbances and are not as vulnerable to large *Heterobasidion* spp. outbreaks. Instead these forests slow down the spread (Jactel et al., 2009), even if *Heterobasidion* spp. are still present in the forest.

Management suggestions to prevent the spread of Heterobasidion spp.

There are a number of suggested measures that can be performed to prevent the spreading of *Heterobasidion* spp. into spruce stands. For example, some studies show that it could be better to use natural regeneration instead of planting (Graber, 1996) or that an interspersion of deciduous tree species could cut off the root contact between spruce roots, since birch roots seldom get infected from root contacts with diseased spruce (Piri, 1996).

Phlebiopsis gigantea **stump treatment**

The product Rotstop®S contains the parchment fungi *Phlebiopsis gigantea* (Fr.) Jül. and is, in Sweden, the most common stump treatment for protection of fresh spruce stumps against *Heterobasidion* spp. infections (Berglund and Rönnberg, 2004). This treatment agent needs to cover the complete stump to give the best protection against *Heterobasidion* spp. (Berglund, 2005). In southern Sweden, the temperature is above zero degrees Celsius for most months of the year, meaning that basidiospores are produced and spread most of the year (Brandtberg et al., 1996, Bendz-Hellgren et al., 1998). This also means that it may not be possible to perform winter harvesting measures as a substitute to stump treatment with *P. gigantea* in the south. In the more northern and colder parts of the country, winter harvesting measures should be sufficient and stump treatment is not needed during most of the winter.

Management of Swedish spruce dominated stands

A common way to manage Swedish spruce dominated stands is to perform one or two pre-commercial thinnings in young stands, followed by two commercial thinnings and last a final-felling (Näslund et al., 2010).

Pre-commercial thinning

When pre-commercial thinnings are performed in spruce dominated stands, the natural regeneration of deciduous tree species and large and small spruce trees are usually removed, resulting in a more homogenous spruce stand (Pettersson et al., 2007). According to Vollbrecht et al. (1995) spruce stands which are approximately 20 years old should have enough root contact to spread *Heterobasidion* spp. further in the stand. It is known from studies by Solheim (1994) that small thinning stumps can be infected by *Heterobasidion* spp. This is not seen as a major problem, mostly based on Vollbrecht et al. (1995), who indicated that pre-commercial thinning stumps are of minor importance to the spreading of *Heterobasidion* spp. to remnant trees, independent of stand density and pre-commercial thinning strength. As a result of this, the Swedish University of Agricultural Science (SLU) started further research concerning the correlation between the stump diameter in pre-commercial thinnings and the frequency of infected remnant trees which surrounded an artificially infected mother stump. Recently the results from this research were released and show that spruce stumps in all investigated diameters, 2-14 cm, had the ability to spread *Heterobasidion* spp. infections further to surrounding trees (Gunulf, personal communication[1]). Also Berglund et al. (2007) showed results that indicated that late pre-commercial thinnings could be of importance to the development of *Heterobasidion* spp. infections, since the stumps are larger the later the measure is performed.

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[1] Gunulf, A. 2010. Personal Communication, Swedish University of Agricultural Sciences, Alnarp.

High pre-commercial thinning stumps

As an attempt to reduce the pre-commercial thinning costs and increase the timber quality a new technique of point cleaning has been researched and tested since late 1990s and the beginning of 2000s (Karlsson et al., 2002, Fällman et al., 2003, Ligné et al., 2005). Instead of cutting the pre-commercial thinning trees at a normal low height (15-20 cm (Lundh and Huisman, 2002)) the new technique whereby they are cut at a higher level (between 71-132 cm above ground (Karlsson and Albrektson, 2000, Fällman et al., 2003)) has been researched and tested (Karlsson and Albrektson, 2000). The technique does not include removal of living branches and has not yet been widely commercial used.

There are several possible advantages with this technique;

- The stems can survive and contribute to a higher quality of the remnant surrounding trees with less juvenile wood and thinner branches. This through competing with branches from below from the cut stem (Karlsson and Albrektson, 2000).
- The cut stems will provide fodder for game during winters, especially deciduous trees and pine (Karlsson and Albrektson, 2000).
- More efficient pre-commercial thinnings with a higher production and lower costs through better visibility, smaller stem diameter and fewer cuts in stones with the saw (Karlsson and Albrektson, 2000).
- The method can also be seen as a more “nature like process”, similar to self-thinnings (Karlsson and Albrektson, 2000).
- Today saws which can cut at different heights have been developed, making this method easier to carry out (Ligné et al., 2005, Anon, 2010a, Anon, 2010b).

The disadvantages on the other hand are not so well documented since the method is not widely used today, but some of them could be;

- Suits better for birch than for spruce, since spruce has a strong ability to keep competing from below while birch usually gets outcompeted in the long-run (Karlsson and Albrektson, 2000).
- If the stem is not cut low enough, the stump will continue to have a relatively high mean annual increment and compete with main stems (Karlsson and Albrektson, 2000).
- If the high stumps do not decompose quickly enough, there will be a lot of standing wood debris left in the forest that may lead to higher costs and difficulties in performing the first commercial thinning, e.g. through visibility and accessibility problems.

Hypothesis

- High stumps will live longer than low stumps and it is possible that they will have greater ability to defend themselves against *Heterobasidion* spp. infections, especially if they still have a few green branches.
- The micro climate on high stumps will be less suitable to spores, since the surface will be more exposed to wind and sun, i.e. dryer wood surface.
- High spruce stumps treated with *P. gigantea* using the product Rotstop®S are expected to be less susceptible to *Heterobasidion* spp. due to the same reasons as mentioned above.
- Birch is usually felled during pre-commercial thinnings in spruce dominated stands (Fahlvik, 2005) and could possibly lead to a point of entry for infections in spruce stands. Piri (1996) showed the opposite with a possibility for spruce stumps transferring infections to birch trees. High birch stumps will also be less susceptible to *Heterobasidion* spp. due to the same reasons as mentioned above.

A five year long-term research study was established to confirm the results from the main work of this study. The aim is to investigate if there is any difference in how a known *H. parviporum* infection continues spreading into surrounding trees, depending on if the spruce stump is high or low.

Method

Research establishment

Five spruce dominated pre-commercial thinning sites were found to remove 120 spruces and 60 birches per hectare. One site was located in the Swedish county Halland, one in Scania, and three in Småland (see coordinates in table 1). The age distribution was between 8 and 15 years old and the site index differed between G26 and G32 (see table 1).

Table 1. Site information. The average stand diameters come from the height of 1 m and are the averages from all samples within the site.

Site	Location	County	Coordinates (RT90)	Stand age	Site Index	Stems/ha	Average diam (1 m)
S1	Tönnersjöheden	Halland	6288804, 1335074	15	G32	2500	6.3
S2	Åryd 497	Småland	6298980, 1450741	6	G26	2700	4.2
S3	Åryd 200	Småland	6303559, 1449943	6	G26	4100	3.5
S4	Åryd 268	Småland	6301429, 1450354	11	G28	3120	4.3
S5	Kullaskogen 65	Scania	6241540, 1403207	11	G28	4750	5.9

A starting point for laying out the experiment was selected in each site and located ten meters from the stand border, which was located nearest to the closest situated road. The stumps were cut and distributed in blocks along a sampling path in the following order from the starting point; a high untreated spruce stump (H) (see figure 1a) followed by a low untreated spruce stump (L), a high treated spruce stump (HS) (see figure 1b), a low treated spruce stump (LS), a high birch stump (HB) and a low birch stump (LB). These six different stump types, within three different stump groups (birch, untreated and treated spruce), were kept together in the same area and then the same order was repeated 30 times at each of the five sites. Hence, of the total sample, a third of the stumps were untreated spruce, a third were treated spruce and a third were birch. Each group was then split in half containing one half high stumps and one half low stumps. The sampling path followed the shape of the stand and turned around when it reached a stand border. The reason for this sampling path was to imitate a pre-commercial thinning operation. All stumps were situated at least ten meters from all borders to avoid edge effects.

The stumps were marked with ribbons which indicated stump identity and type. The same information was also stapled with labels into the bark. The direction in which the plots were distributed along was marked with another ribbon.

The high stumps were cut with a chainsaw 1 m above ground and the low stumps 15 cm above ground. Both stump heights had green branches removed and only the smallest branches which were difficult to cut with the chainsaw were left (see figure 1). The diameter was noted for both heights on all stumps by using an average from cross-measuring with a caliper. The stump diameters at the stump surface were distributed within a range from 2 cm to 14 cm. In other words, depending on the availability at the sites, the trees were chosen in as broad a range as possible between 2 and 14 cm.

Figure 1a and b. High untreated spruce to the left (a) and high stump treated spruce to the right (b). *Photo by: Rebecka Mc Carthy*

The stump treatment of the spruce was performed using the product Rotstop®S which contains the parchment fungus *P. gigantea*. Coloration was added to the solution to make it easier to see the cover of the treatment on the stump surface. Each morning the treatment was dissolved into a solution according to the instructions from the producer; 0.025 g Rotstop®S dissolved into 1000 ml of water. The treatment was also applied on the stump surface according to the instructions using a spray bottle. A measuring glass was used and sprayed from to achieve 1 mm coverage evenly over the stump surface (see table 2). The treated stumps received treatment directly after they had been cut and the full amount of treatment was applied while waiting until the solution started to be absorbed. This way it was possible to apply the whole dosage on the stump surface.

All stumps with any discoloration were excluded since they might already have been infected by *Heterobasidi*on spp., even those with discoloration not located in the core area.

Table 2. Amount of treatment applied on the treated spruce stumps. In field the table was more specific and had accuracy for each 0.1 cm diameter. The amount of treatment was rounded off to closest 0.1 ml.

Stump diameter (cm)	**Treatment (ml)**
2	1.26
3	2.83
4	5.03
5	7.85
6	11.31
7	15.39
8	20.11
9	25.45
10	31.42
11	38.01
12	45.24
13	53.09
14	61.58

Disc collection

Two months after the stumps were created, the collection of discs started. A handsaw, disinfected with 70 % ethanol, was used during the collection of discs. The disc depth was calculated from Bendz-Hellgren et al. (1999) growth rates of naturally dispersed spores in spruce stumps. According to this model and the climate at the sites, the *Heterobasidion* spp. could roughly have grown 14 cm since the stumps were created. To make sure that the discs were not thicker than the mycelia actually had grown, the disc depth was decreased. First, the bark and saw were disinfected by spraying 70 % ethanol. A top disc, with a thickness of 1 cm, was then cut off from the stump and thrown away (see figure 2a). Next, a 5 cm thick disc was cut from the new surface and placed in a marked plastic bag (see figure 2b). All discs were marked before they were cut to indicate which side was top. The cut discs were refrigerated at 4° C within 36 hours after harvest until 7 days before analysis.

Figure 2a and b. High treated spruce stump with the first 1 cm top disc being cut off (a) and low untreated spruce stump with the 5 cm sample disc being cut off (b). A discarded top disc is shown to the left of the stump in picture b. All stumps were cut with disinfected bark and handsaw. *Photo by: Rebecka Mc Carthy*

Laboratory analysis

After the discs had been incubated in room temperature for 7 to 10 days they were analyzed under dissecting microscope. Each disc was striped with a marker and then examined for infections of *Heterobasidion* spp. between the stripes on both sides of the disc, to make sure the whole disc's surfaces were examined. *Heterobasidion* spp. were recognized by the special shape of its conidiophores. If an infection was found, it was marked, measured and rounded off to an accuracy of 0.5 cm^2 (see figure 3). If more than one infection was found, their sizes were summarized for each side and rounded off to closest 0.5 cm^2. The number of discrete infections on each side was also noted.

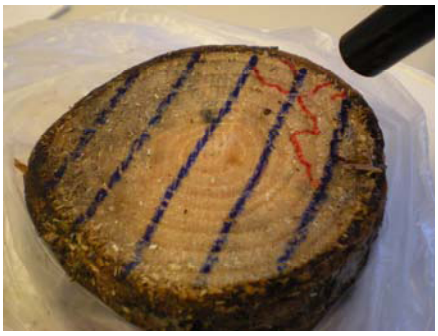

Figure 3. Analyzed spruce disc with stripes (blue) and marked infections (red). *Photo by: Rebecka Mc Carthy*

If there was any doubt as to whether the infection found was a *Heterobasidion* spp., a clean culture was prepared by dipping a sterile needle on the top of the conidiophores and placing the conidiospores on a Petri dish containing clean Hagem malt agar extract. After approximately 3 days, when mycelium could be seen, it was cut out from the initial agar and placed on a new agar. The fungus was left to grow on the final agar for approximately 7 days and then checked for conidiophores under a dissecting microscope, to see if the correct fungus had been found.

Data analyses

The data analyses were carried out with help of the software Minitab®. The statistical method used was an analysis of variance (ANOVA) through different constellations of collected data in general linear models (GLM). A Tukey's test was added in comparison to make it possible to see which parameters that differed. For all disc types, the responses (Y-parameters) used in the statistical model were infection frequency, average relative infected area, average size and average number of infections per infected disc. These parameters were also tested among all different stump types (for the two heights of untreated spruce, treated spruce and birch) to see if there were any differences in those parameters between the stump types. Additionally, a statistical analysis was carried out to compare the difference of average infection size among top and bottom sides of the discs. As X-parameters in the model, untreated spruce, treated spruce and birch (1-3) were set as factors against stump height (1-2), block (1-30) and site (1-5). Blocks and sites were set as random factors. Histograms were created and normal distribution tests performed for all residuals after running the model.

The infection frequency shows if the disc was infected or uninfected by *Heterobasidion* spp. The average number of infections was calculated per disc and represents an average of the two sides. The total relative infected area was calculated by taking the total infected area in relation to the total area of all infected discs. When analyzing the relative infected area the diameter was added as a covariate in the model to see if it could influence the results.

The efficacy was calculated for all stump types in comparison with low untreated spruce. Additionally, the efficacy of high birch stumps was compared to low birch stumps. The mean relative area infected by *Heterobasidion* spp. for each site was compared with each other. This was to calculate the reduction of infection for the five stump types compared to the low untreated control (Berglund and Rönnberg, 2004) and get a relative efficacy of the different stump types. The efficacies were analyzed in a general linear model through the averages from each site. The stump types and sites were set as the model and the stump types were also added to the comparison. Since site one seemed to give a negative efficacy for all stump types except of low birch, this site was excluded and the model was run again with only four sites.

Additionally, the diameters recorded from the cut trees were split into 6 diameter classes to make it possible to see the diameter distribution among the stump types and sites. The infection frequency depending on the diameter class was analyzed in a binary logistic regression with not infected/infected discs (0/1) as responding Y-parameter and the diameter as the model (X-parameter).

Also the recorded weather conditions were divided into classes to make it possible to compare the weather conditions (temperature, precipitation, degree of cloudiness, etc) with the infection frequency as the weather may affect the spore dispersal.

Invalid discs

After collection and analysis of the discs, one sample from site two had to be excluded due to concerns that it was not carrying a new wind spread *Heterobasidion* spp. infection. It carried a centered infection with a discoloration on the underside of the disc.

Three low stumps from site four were never found as a result of pre-commercial thinning that started at site four before all discs were collected. This means that in total four low stumps was excluded or missing among the 900 cut stumps. It also means that the study was slightly unbalanced (see table 3) and resulted in removal of these blocks when it was necessarily to have a balanced sample in the analysis.

Long-term research preparations

To investigate whether the likelihood of transference of infections differ depending on the stump height, a five year long-term research was established. The long-term study, with artificially infected spruce stumps, was established at the same sites used in the shorter study mentioned in the method section above. A known individual of *H. parviporum* was used to infect the stumps with the aim of seeing if the infection spreads into the surrounding trees or if it dies out in the inoculated stump before reaching neighboring trees.

Tilth of *Heterobasidion parviporum*

The known individual of *H. parviporum* used in this long-term study was RB 175 (Swedjemark and Stenlid, 1997). Spores from RB 175, which was stored in the department of southern Swedish forest research centre, were placed and grown for two weeks in Petri dishes containing Hagem agar. After approximately two weeks the mycelium covered the Petri dishes and carried conidiospores, which were used to grow additional Petri dishes of RB 175.

Conidiospore density per Petri dish

To find out the concentration of conidiospores in the solution that was going to be artificially applied to the stump surface the average amount of spores per Petri dish was calculated. Three Petri dishes were chosen and used to create three dilution series. Each Petri dish was rinsed with 10 ml of distilled water (2×5 ml) and poured into a can. From this can 1 ml was taken and dissolved in another can containing 9 ml of distilled water. A further 1 ml was taken from the initial can and dispersed on a new agar Petri dish by using a glass triangle. This was repeated until the solution was diluted enough and it could be assumed that the concentration was less than 5-15 spores/ml. The dilution series were left so that the spores could grow for four days, which was assumed to be enough to be able to count the colonies of each spore without using a dissecting microscope.

The desired spore density was 5-15 spores/ml and a Petri dish with this density was sought in the dilution series. Based on which number in the series the desired concentration of 5-15 spores/ml was, it was then possible to calculate the density of spores in the original Petri dish. An average was then calculated for the three original Petri dishes. This value was then used as the assumption for the number of spores that were collected from a Petri dish of RB 175 when rinsed with 10 ml (2×5 ml) distilled water.

Dilution of spores

The conclusion of the above dilution series exercise was that on average each Petri dish was generating 10 000 conidiospores when rinsed. To achieve the right spore concentration in the spore solution that was going to inoculate the stumps another dilution was performed. Each Petri dish was rinsed with 10 ml (2×5 ml) of distilled water and then diluted with 10 ml of distilled water to achieve a concentration of approximately 1000 spores/ml solution. Preferably the solution should have been 1000 spores/ml, but since a stump with a diameter of two centimeters needed to get at least one spray of 0.5 ml (one spray was 0.5 ml) the solution had to be diluted to a manageable concentration. In those stands where it was known that the stumps were of larger diameters, the spore concentration in the solution was higher and fewer sprays were given. This procedure was performed every morning the same day as the solution was used. In the beginning and the end of each day the solution was tested by spraying an agar Petri dish, which was supposed to show if the spores were alive. This method did not work very well since there were problems with contamination after spraying the Petri dishes.

Spore inoculation density

Each stump should receive the same amount of spores per surface unit (cm^2). The desired spore concentration was 50 spores/cm^2, which was based on a previous study (Gunulf, personal communication[1]) with the same *H. parviporum* individual (RB 175). This means that a stump with a diameter of 6.5 cm was going to receive three sprays of the spore solution well distributed over its surface according to the following calculations;

Average volume of one spray: 0.5 ml/spray
Area of the stump: $A = \pi \times (6.5/2)^2 \approx 33.2\ cm^2$
Aimed spore density: 50 spores/cm^2

$$\text{No. of sprays} = \frac{(A \times 50)/1000}{\text{Volume of one spray}} \qquad \text{No. of sprays} = \frac{(33.2 \times 50)/1000}{0.5} \approx 3.32$$

Field method

The study was established in the same five locations as the master thesis study (see table 1). The establishment of the research was performed in a similar way as mentioned in the thesis method section above, but some things had to be changed to suit the long-term study.

30 low and 30 high stumps of spruce were cut and distributed on the outside of the blocks where the master thesis was situated. The same direction was followed and when the edge of the site was reached the sample path turned around. Alternative stumps were cut to high and low levels. A radius of at least five meters was left between each stump, in other words there was at least ten meters between all stumps where all other trees were left untouched. Each stump was sprayed with the relevant volume of the RB 175 solution immediately after the cut. The stumps were marked with a metal label containing an identity number between 1 and 300, which was attached to the stump with a metal string. Based on previous research (Gunulf, personal communication[1]), the four largest surrounding spruce trees were marked in this case with plastic ribbons and fluorescent spray paint (orange). The inoculated stumps were placed at least 20 meters from stand borders and master thesis blocks to avoid edge effects.

To protect the stump surface and the pathogen from disturbances, the stumps were covered by a rain protector if there was any risk of precipitation in the following 24 hours according to the weather forecasts presented by the SMHI-website. If there was risk of precipitation, the fresh stump surface was first protected by a large umbrella. This was replaced by a ventilating plastic cover left on for the next following 24 hours and then removed (Redfern et al., 1997) (see figure 4).

Figure 4. A high stump artificially infected by H. parviporum (RB 175) protected from precipitation with a plastic cover. *Photo by: Rebecka Mc Carthy*

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[1] Gunulf, A. 2010. Personal Communication, Swedish University of Agricultural Sciences, Alnarp.

The trees sought were trees which had a diameter at cut height between 2 and 14 cm. The trees should also be surrounded by other spruce trees within a two meter radius, preferably at least four surrounding spruce trees. In addition to the 60 stumps in each site, 3 high and 3 low test stumps were created to make it possible to interim follow-up and ensure that the pathogen has infected the stumps.

In spring 2011, approximately 6-7 months after establishment of the research, discs from the test stumps will be cut to verify that RB 175 has been successfully established.

The plan is to come back to the sites after five years and verify if there are any differences between the spreading of the RB 175 infection into the remnant trees depending on the height of the inoculated stump. The remaining trees that are supposed to be followed up are spruce trees within a two meter radius from the inoculated stump.

Results

Infection frequency

Among all samples, the infection frequency for the three stump groups untreated spruce, treated spruce and birch differed significantly from each other (P=0.005) (see Tukey's test in table 3). The site was a factor which influenced the infection frequency (P=0.021), which also the site in combination with stump type did (P=0.000).

Differences were observed in infection frequency between low and high birch stumps. The result shows low birch stumps being less infected by *Heterobasidion* spp. than high (P=0.037). On the other hand, both untreated and treated spruce stumps showed a small difference in infection frequencies which were not great enough to be statistically significant. In these cases high stumps tended to be slightly less infected than low stumps (see table 3).

The measure of total infection frequency only indicates if the disc was infected or not and no attention was given to the amount, size or placement of visible infections. In total 52.0 % of the untreated high spruce stumps were infected compared to 58.1 % of the low (control) (P=0.416). The same differences in infection frequencies for treated spruce were 28.7 vs. 34.0 % (P=0.099) and for birches 17.3 vs. 12.2 % (P = 0.037) (see table 3).

Table 3. Three different stump groups on two different heights distributed on frequency of infected discs (%). To the right in the total infection frequency column, the results from Tukey's test are shown. Means of infection frequency that do not share a letter are significantly different.

	Top side of the disc	**Bottom side of the disc**	**Total**		
Stump type	Top Inf Freq (%)	Bottom Inf Freq (%)	Inf Freq (%)		Population Size
Untreated Spruce					
H	43.3%	44.7%	52.0%	A	150
L	47.3%	46.6%	58.1%	A	148
Treated Spruce					
HS	14.7%	26.0%	28.7%	B	150
LS	20.0%	28.7%	34.0%	B	150
Birch					
HB	5.3%	14.0%	17.3%	C	150
LB	5.4%	8.8%	12.2%	D	148
Total	22.7%	28.1%	33.7%		896

The disc's infection frequency differed among the five sites. At three of the sites (one, three and five) the ratio of infections was fairly equal at around 30 %, but at site two only 11.7 % of the discs were infected and at site four the infection frequency was as high as 63.8 %. The differences in infection frequency between the sites were significant for untreated spruce (P=0.027), treated spruce (P=0.000) and birch (P=0.020).

The top side of the discs did not differ in infection frequency, compared to the bottom side for any of untreated spruce (P=0.662), treated spruce (P=0.648) and birch (P=0.239).

Efficacy of stump treatment

In table 4, the efficacy of the Rotstop®S treatment is shown. In site one, the efficacy was negative for all stump types compared with the low untreated spruce stump (control), i.e. the mean relative infected area was smaller on the control stump in this site (see table 4). The different stump types were not equal in efficacy. Significant differences were found between the stump types, which showed that the efficacy for high untreated spruce stumps did not differ from the control but did differ from the other four stump types (HS, LS, HB and LB) (P=0.001). The control stumps, on the other hand, did not significantly differ in efficacy compared to any of the stump types (see table 4). The efficacy of high birch stump did not significantly differ to low birch stump (P=0.161).

Since site one seemed to give a negative efficacy for all stump types except low birch, this site was therefore excluded and the test was run again. When site one was excluded some significant differences were found; low treated stumps had a higher efficacy than the control (P=0.012), as did high birch stumps (P=0.001) and low birch stumps (P=0.001). The comparison of high treated stumps with the control showed differences which were not significant (P= 0.090). High untreated spruce stumps did not show any significant differences in efficacy when compared to the control (P=0.231). Neither the efficacy of high and low birch stumps differed significantly when site one was excluded (P=0.190).

Table 4. The efficacy of infected high untreated spruce (H), high treated spruce (HS), low treated spruce (LS), high birch (HB) and low birch (LB) stumps in comparison to infected low untreated spruce stumps (L). Also the efficacy of infected high birch stumps (HB) is compared to infected low birch stumps (LB) and is shown in the bottom row. The efficacy shows the reduced mean relative infected area (%) in relation to a control (L or LB). In the right column in the results from Tukey's test, when all sites were included, are shown. Means of efficacy that do not share a letter are significantly different.

Efficacy	Site 1	Site 2	Site 3	Site 4	Site 5	Mean	Tukey's test
H	-63.4%	51.1%	-237.5%	-168.2%	-34.7%	-98.2%	A
L	0%	0%	0%	0%	0%	0%	A B
HS	-62.2%	80.0%	24.0%	6.6%	86.3%	44.5%	B
LS	-13.8%	53.0%	44.5%	29.0%	72.4%	48.4%	B
HB vs. L	-32.8%	70.3%	58.5%	84.4%	78.1%	69.8%	B
LB vs. L	26.4%	67.6%	76.1%	87.0%	94.5%	82.2%	B
HB vs. LB	-80.5%	8.4%	-73.8%	-20.8%	-300.6%	-69.9%	

Average infection size, relative infected area and number of infections

The sample blocks within the sites seem to matter for the average number of infections on untreated spruce discs (P=0.045). The average number of infections per infected disc did not differ significantly between the two stump heights (P=0.230). Despite this, a connection between the stump height in association with the site was found for the relative infected area (P=0.002). This means that the high stumps in association with the site showed a larger proportion of infection in their stump surface than low stumps. The average infected area and relative infected area tended to be larger on the high untreated spruce stumps compared to the control (see table 5), but these results were not significant (P=0.357 and P=0.246).

Treated spruce stumps did not show any significant relation between the average area per infection and stump height (P=0.239). Neither the relative infected area or the average number of infections were linked to the stump height (P=0.768 and P=0.086).

Birch was the only stump type that showed differences in relative infected area depending on the stump height (P=0.036), which shows that the high stumps had a larger relative infected area than the low. Birch did not show any other relation between stump height and average infected area or number of infections (P=0.171 and P=0.546).

The relative infected area among all infected discs was 3.0 %, but the largest average was 8.7 % of the surface of high infected untreated spruce stump. The overall average infected area among all infected discs was 1.15 cm^2 and the average number of discrete infections for all infected discs was 3.0 (see table 5).

Table 5. Average area of an infection (cm^2), relative infected area (% of the surface) and average number of infections per infected side or disc. The results are shown for the top side, the bottom side and the average of the discs. The relative infected area shows the percentage of the side or disc that was infected.

	Top Side			**Bottom Side**			**Total**		
Stump type	Ave Area/Inf (cm^2)	Rel Inf Area (%)	Ave No Inf	Ave Area/Inf (cm^2)	Rel Inf Area (%)	Ave No Inf	Ave Area/Inf (cm^2)	Rel Inf Area (%)	Ave No Inf
Spruce									
H	1.75	9.2%	3.4	1.74	8.1%	2.9	1.75	8.7%	3.1
L	0.99	4.8%	3.8	1.27	5.7%	3.6	1.13	5.3%	3.7
Treated Spruce									
H	0.43	0.5%	1.9	0.77	2.0%	2.5	0.67	1.2%	2.2
L	0.60	0.9%	2.8	0.70	1.7%	3.1	0.66	1.3%	2.9
Birch									
H	0.50	0.2%	1.0	0.39	0.5%	1.6	0.41	0.4%	1.3
L	0.50	0.1%	1.1	0.29	0.2%	2.2	0.34	0.2%	1.7
Total	1.15	2.8%	3.1	1.15	3.3%	2.9	1.15	3.0%	3.0

A slight difference in average area infected by the pathogen between high and low stumps' disc sides can be seen in table 5 for all stump types, but none of them could be statistically proven (P=0.485, 0.338 and 0.121).

Diameter correlations

The infection frequency showed a tendency to depend on the stump diameter with a higher infection frequency at larger diameters (see figure 5).

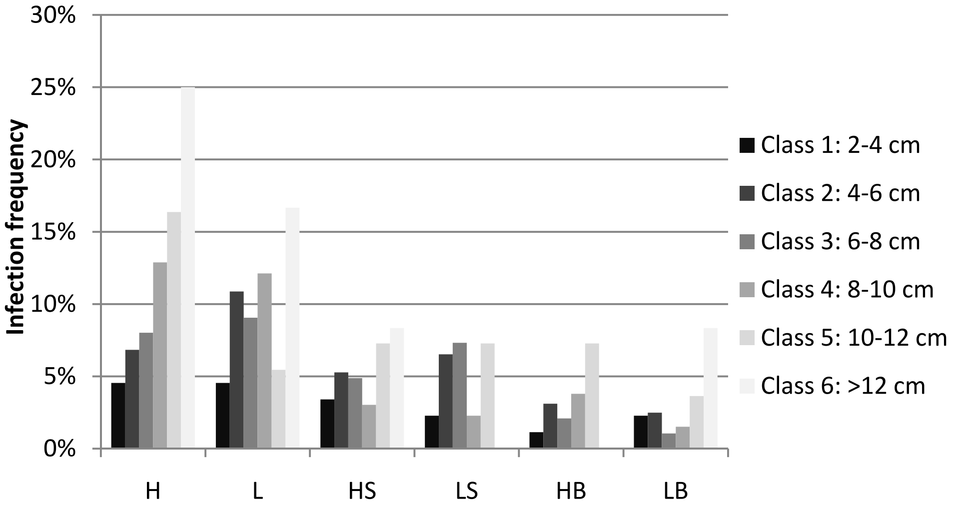

Figure 5. The infection frequency distribution of the infected discs in the study divided into diameter classes, measured at 15 cm height regardless of whether the stump was cut high or low, and stump types. The diameter distribution between high untreated spruce stump (H), low untreated spruce stump (L), high treated spruce stump (HS), low treated spruce stump (LS), high birch stump (HB), and low birch stump (LB).

Figure 6, as well as figure 8, show that the proportion of infected stumps increases with diameter, except in the case of class 2 which has a slightly higher infection frequency than class 3. Consideration should be given to the fact that as much as 36.4 % of the samples in class 1 and 83.3 % of the samples in class 6 were infected among the untreated spruce stumps. To be noted is that diameter class 6 only contained six samples, whereof five were carrying an infection (see figure 6). Diameter was found to have significance affect for the infection frequency among untreated spruce stumps at cut height (P=0.018), but not at 15 cm (P=0.098).

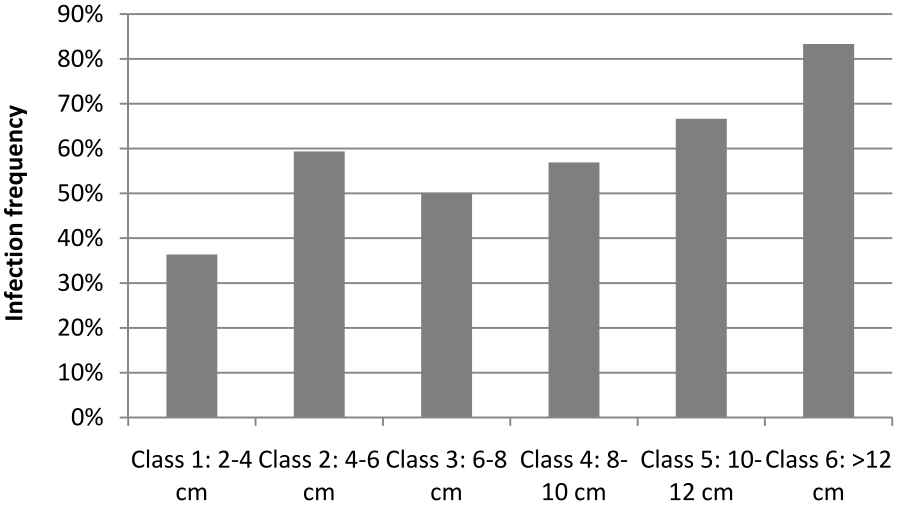

Figure 6. The infection frequency shown per diameter class, measured at 15 cm height regardless of whether the stump was cut high or low, for both high and low untreated spruce stumps.

The trend seen in figure 6 and 8 was not as obvious when looking at the infection frequency among treated spruce stumps. Also, treated spruce stumps contained relatively few samples from diameter class 6 compared to the other classes. No significance was found in the relationship between the infection frequency and the diameter of treated spruce stumps, as seen in figure 7 (P=0.357).

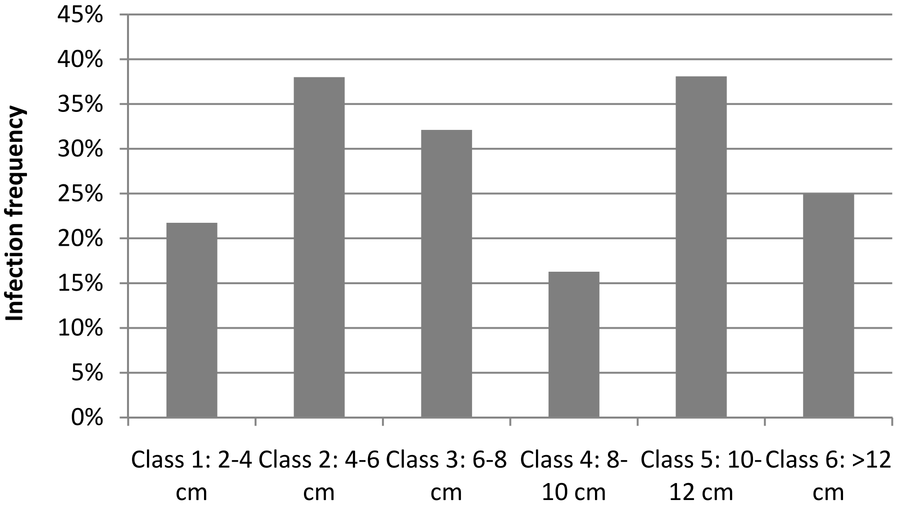

Figure 7. Infection frequency of stump treated spruce distributed on diameter classes, measured at 15 cm height regardless of whether the stump was cut high or low.

The trend noted in diameter correlation in figure 6 was also found among the birch stumps (see figure 8). Considerations should be given to the fact that this stump type only contained two samples in diameter class 6, whereof one sample was infected (50 %). The differences in infection frequency depending on diameter was significant at 15 cm (P=0.013) but not for the diameter at cut height.

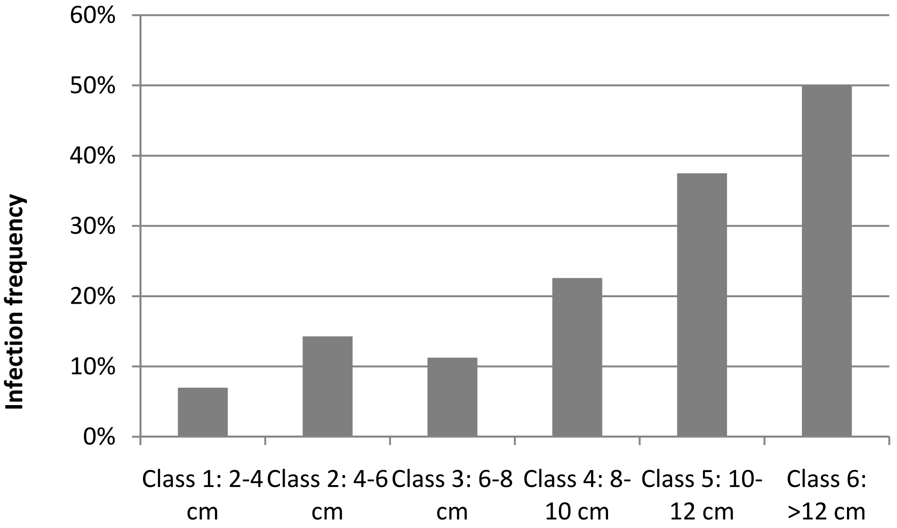

Figure 8. Infection frequency of birch stumps distributed on diameter classes measured at 15 cm height regardless of whether the stump was cut high or low.

The diameters for all trees at cut height were distributed between 2.0 and 14.5 cm, which resulted in a diameter distribution between 2.4 and 14.5 cm at 15 cm height. At the normal stump height of 15 cm, all diameter classes were represented in all stump types (see figure 9). In other words, for trees cut at 1 m the diameter given in figure 9 represents the diameter at 15 cm height, regardless of where the disc was cut. As a result of this, at cut height not all diameter classes were represented for all high stump types. In general the diameter distribution between the stump types at the common cut height was evenly distributed, which means that broadly the same number of samples from each diameter class were represented in each stump type.

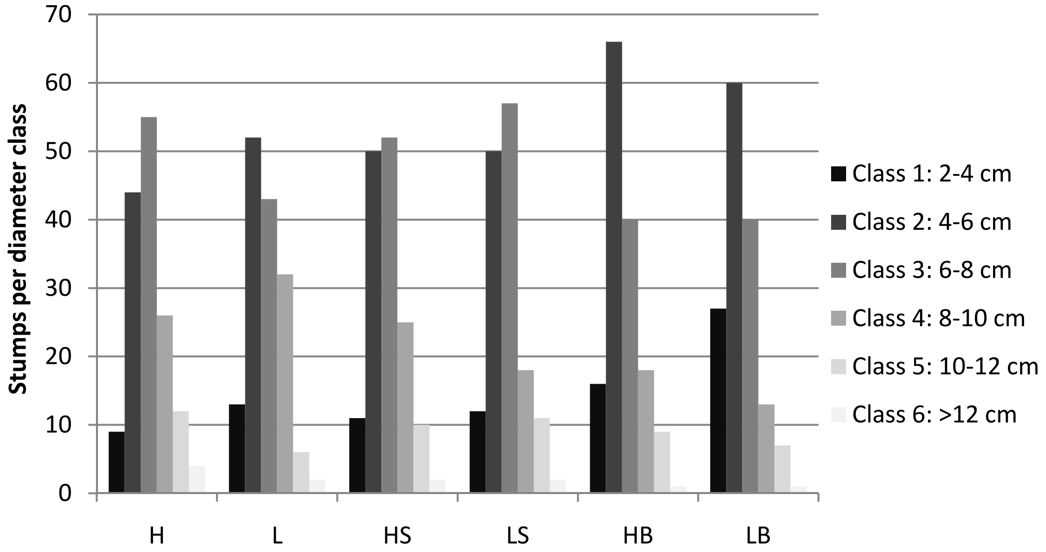

Figure 9. The stump diameter distribution divided into 6 different diameter classes, measured at 15 cm regardless of whether the stump was cut high or low. The diameter distribution between high untreated spruce stump (H), low untreated spruce stump (L), high treated spruce stump (HS), low treated spruce stump (LS), high birch stump (HB) and low birch stump (LB).

Even though all diameter classes were found among all stump types, there was a difference in diameter class distribution at 15 cm height between the sites. Class 6 was absent from site two, three and five. Due to the lack of larger diameter classes at site three, no samples from diameter class 5 were taken. Site four had samples in all diameter classes but only one sample in the highest class and three samples in class 4 and 5.

Weather correlations

Weather class 7 and 8 have higher ratio of infected stumps compared to other weather conditions. This weather occurred at sites four and both conditions have more than 60 % infected stumps (see table 6).

Table 6. Ratio infected discs per site and weather condition. The weather classes are; **Class 1:** little moist air, partly cloudy, 20-23°C, **Class 2:** partly cloudy, 23-25°C, **Class 3:** sunny, few clouds, 24-26°C, **Class 4:** partly cloudy, 25-29°C, moist (rained earlier), **Class 5:** dry, 24-30°C, partly cloudy, **Class 6:** humid, fresh, 19-30°C, partly cloudy, clear sky in the afternoon, **Class 7:** windy, sunny, partly cloudy, 21-28°C, fresh air in the morning, **Class 8:** partly thin clouds or sunny, 20°C, **Class 9:** cloudy, no wind, 18-21°C and **Class 10:** sunny, few clouds, 20-23°C, slightly windy in the afternoon.

	Weather class										
Site	**Class 1**	**Class 2**	**Class 3**	**Class 4**	**Class 5**	**Class 6**	**Class 7**	**Class 8**	**Class 9**	**Class 10**	**Total**
S1	37.5%	29.6%									32.8%
S2			24.5%	4.5%	10.5%						11.7%
S3					34.9%	25.6%					28.9%
S4							62.8%	67.5%			63.8%
S5									28.7%	34.9%	31.7%
Total	37.5%	29.6%	24.5%	4.5%	25.7%	25.6%	62.8%	67.5%	28.7%	34.9%	33.7%

Discussion

There was no significant difference regarding infection frequency depending on the stump height for untreated spruce, even though the high stumps were infected by a few percent less *Heterobasidion* spp. infections than low stumps. Therefore it seems like the expected dryer surface, if this was the case, on the higher stumps does not have any impact on the infection frequency. A similar scenario was recorded for the infection frequency of treated spruce stumps.

It had been hypothesized that the stump surfaces would dry faster on high stumps due to wind and sun exposure compared to low stumps. The hypothesis was based on the assumption that a dry surface would be less suitable for *Heterobasidion* spp. spores to start growing on. This was hypothesized despite results indicating that at least the proportion of colonized spruce sapwood decreases with increasing moisture content (Bendz-Hellgren and Stenlid, 1998). A possible explanation to the absent differences in infection frequency depending on stump height among spruce stumps could be the moisture content which ended up at a more suitable level for *Heterobasidion* spp. on high stumps than expected, while low stumps did not stay too damp. This could have led to a more even infection frequency between high and low spruce stumps than predicted.

It had also been hypothesized that the infection frequency between high and low stumps would differ because of high stumps' branches that were left, which would keep the high stumps alive for a longer time. This was based on previous observations that living trees are more resistant and have slower growth rates of *Heterobasidion* spp. infections compared to stumps (Bendz-Hellgren et al., 1999). Hence, it seems like the small branches left on the high stumps' stem were not enough to affect the infection frequency. If, on the other hand, the study had left more living branches on the stumps there could have been a risk that the stumps would not die-off over an appropriate time span. It is possible that more than two months was needed for the branches to give an effect and this study only covered the initial phase of the infections' development. The long-term study will hopefully show if the branches have a greater impact on the infections after a longer time span.

Furthermore, it was also a surprise that none of the stump types had a significant efficacy compared to the control. Some significant differences depending on the stump type were found when site one was excluded, but these differences in efficacy were still not as great as expected. Other studies have shown much higher efficacy for treated spruce stumps in commercial thinnings (Berglund, 2005), which were not found in this pre-commercial thinning study. One reason to the small differences between the control stump and other stump types could be explained by the short time span. The stumps in this study were only out in field for two months, which does not say anything about the parchment fungus competitiveness in the long run. Another reason could be that the stump treatment did not act in the same way on pre-commercial thinning stumps as on commercial-thinning stumps and hence did not have the same efficacy.

The high birch stumps were more susceptible to *Heterobasidion* spp. compared with the low ones. It may be caused by the sap flow making the surface wetter than expected and hence spores did not land on a completely dry surface. In addition, the high stump height can perhaps have made them more vulnerable to airborne spores since they are more exposed to wind containing spores. It is probable that the sprouting branches on the high birch stumps did not protect the stumps' susceptibility as predicted. The branches could also for birch have needed a longer time span to give an effect and protect the high stumps from the infections.

Most of the birch infections seemed to be bouquet shaped. This was not measured in this study, but it caught attention when analyzing the discs. It looked like the infections on spruce discs tended to be more widely spread over a larger area than the birch infections. It can therefore be interpreted that *Heterobasidion* spp. finds it more difficult to expand in birch wood in comparison to spruce wood. The fact that birches are not as susceptible to *Heterobasidion* spp. infections compared to spruce is no surprise since it is documented from other studies, for example Piri (1996), although it can still carry an infection. Piri (1996) showed that spruce stumps can transfer a *Heterobasidion* spp. infection to birch trees. This means that it is not possible to exclude the opposed situation; that birch stumps could possibility act as an entry point for *Heterobasidion* spp. infections into spruce dominated pre-commercial thinning stands.

The differences among the sites were statistically significant for many of the analyses. This shows that the desired differences between the sites could be proven and that the results are representative for a broader range of spruce dominated stands. The fact that all diameter classes were represented among all stump types also brings representativity to the study. The reasons for the differences among the sites can be many, but different site conditions were pursued and found. For example, sites with different site indices, ages and locations were found and used. Also the occurrence of basidiospores, which were not measured, can result in differences in infection frequency among the sites. Site four achieved a higher infection frequency than any other site. This might have been caused by stress due to the very high stem density at the site, which was according to subjective estimations higher than recorded in the site description. The stress could have affected the trees in such way that they became more susceptible to *Heterobasidion* spp. (Shain, 1967, Piri, 2003).

The sample blocks within the sites seemed to be a factor for the average number of infections on untreated spruce discs, which means that the blocks within the sites also covered several environments. It was not possible to include the blocks as a factor when analyzing the infection frequency, since that analysis was performed with the infection frequency from each site.

It seems likely that the sites themselves differed in infection frequency due to reasons other than the weather conditions, since the weather classes did not have a distinct variation between each other. The fact that the weather could be interpreted as constant means that it can be dismissed as a cause of differences or indifferences found in this study.

Potential sources of error

It was more difficult to find trees with only a few living branches than expected. Most trees had only living branches which resulted in removal to avoid competition from these stumps in the future. It is possible that they have not yet had enough competition to start reducing the amount of lower living branches. This means that if high spruce stumps are cut, it is necessary to remove branches in pre-commercial thinnings. If this is ignored, to enhance the tendency noted in this study, it is very likely that the spruce stumps will keep competing and cause later problems and costs at the first commercial thinning.

The handsaw was only disinfected with ethanol before the first cut during the collection of discs. This might have led to a greater risk of contamination on the bottom surface. Although, disinfection once should been enough, since the saw should not have been exposed to a great risk of contamination during the first cut. Both the bark and the saw were already disinfected at the start, which would have minimized the risk of contamination even if it would have been more appropriate to disinfected it twice. Since the top side and bottom side did not differ in infection frequency for any of the stump types the importance of this risk should be minor. There is also an opposed risk that remnants of ethanol on the saw blade could have disinfected parts of the discs' top surface.

An infection was recognized as the area where conidiophores were observed, since the mycelium is difficult to recognize and type without using a very time consuming method. This means that an area infected by a single individual could be split to more than one infection due to lack of constant conidiophore cover. Infections could also be missed because of lack of conidiophores. It may therefore be uncertain exactly how many infections a disc contained. For this study it was not important to know exactly how many infections a stump was carrying, even if it would have helped to make assumptions from this information. If the numbers and sizes of infections were known to be correct, the assumptions could have been more certain. It would, for example, be easier to presume chance of survival if some stump types were infected by larger infections than other. In this study the most important thing was to know if the stumps became infected or not, which was successfully observed. The risk that infections without conidiophores could have been missed remains, even if mycelium would have carried conidiophores after seven days of incubation.

It is difficult to determine whether the infections came from the roots or new airborne spore infections. If an infection came from the roots, it would be more probable that the infection was centered to the middle of the disc and no more than one larger visible infection. On the other hand, it is difficult to predict how *Heterobasidion* spp. act when the mycelium starts to exploit the disc surface and produce conidiophores. It is still not likely that a massive infection from the root would have been mistaken for being a new two month old infection. These doubts can also be dismissed by the fact that the top and bottom sides did not differ in the relative infected area or infection frequency, but could be seen as equal. In the unlikely case that an infection would have been misjudged as airborne, it would only be those with very small infections coming from the roots.

To enhance the quality and certainty of this study an infection assessment could have been performed during the establishment by taking an infection trial with an increment drill at the bottom of the stem. This would, on the other hand, have led to a risk that the stump would have been disturbed and that it could have had an impact on the results. Another approach could have been to take a disc from the bottom of the stem when collecting the samples to see if there were any infections further down. This method could have been a problem for the low stumps since the wind spread infection could have had time to spread that far. Probably the best solution would have been to establish the research on uninfected arable land, which had not carried a spruce stand or been harvested before. It would, on the other hand, have been difficult to find suitable sites for this within the time span of a master thesis. To further decrease the risk of contamination, the bark could have been removed before disinfection of the stump stem. None of these measures were needed, but are merely suggestions of how to possibly increase the quality of the study. Nothing in the results indicated that there were any problems with the method used, i.e. no further actions are needed to enhance the quality.

Management suggestions

The difference in observed infection frequency among birch, untreated and treated spruce was expected since the stump treatment protects the stump surface and birch is not a favored species. This means that in order to decrease the infection frequency in pre-commercial thinning stands spruce should not be cut and left untreated.

There are no reasons, according to the results found in this study, to recommend creating high stumps in pre-commercial thinnings if the purpose is decreasing the susceptibility to *Heterobasidion* spp. This may later be shown to be the case from the long-term study if there is any difference in the survival of the infections depending on spruces' stump height. Forthcoming results could mean new recommendations concerning the stump height in the purpose of stopping the spreading to surrounding trees.

Even if there were no differences in susceptibility between high and low treated spruce stumps, there could still be reasons to create high treated stumps. It will be easier, and probably faster, to manually treat the stumps at a height of 1 m instead of at 15 cm, i.e. no time-consuming bending up and down is needed. The stump diameter at 1 meter is smaller than at 15 cm, which means that less treatment is needed and hence it is cheaper to treat high stumps, but this cost reduction is marginal (see Appendix I).

To justify manual stump treatment of pre-commercial thinning stumps at an interest rate of 3 %, at least 3 m^3 sub of spruce pulp log at first commercial thinning needs to remain uninfected as a result of the treatment. The same amount of uninfected saw log at final felling should be 4 m^3 sub (see Appendix II). If these estimations seem to be reached, one should perform manual stump treatment.

Low birch stumps showed the lowest infection frequency, meaning there are no reasons according to this study to create high birch stumps, which have a higher risk to be infected.

Birch stumps, regardless of stump height, have a significantly lower infection frequency than spruces. The much lower infection frequency of birch, especially low birch, indicates that birch in admixture with spruce seems to be an efficient method of preventing the spread of *Heterobasidion* spp. through root contact in spruce dominated stands. Combining treated spruce stumps, low birch stumps and living birch trees could probably be an efficient way of preventing *Heterobasidion* spp. attacks in an early stage. A higher share of birch, combined with treated spruce stumps, will probably result in a healthier production forest and a more diverse ecosystem (Felton et al., 2010) with lower risks of *Heterobasidion* spp. infections.

I believe today’s spruce monocultures have provoked and encouraged the problems and increased the extent of *Heterobasidion* spp. infections. In most cases, the spruce monocultures are not something which is commonly found in nature. As a result, they have triggered *Heterobasidion* spp. and given the circumstances it has to become a major problem, e.g. through root contacts. There are probably no simple solutions, but if no action is taken the problem and economical losses will escalate in the future.

Heterobasidion spp. will never be extinguished from spruce forests, which is good since it has an important ecosystem function and is vital for other species in the decomposing and feeding chain. The important thing is how to control the extent of this pathogen and make sure that it does not become too dominant.

In conclusion, the recommendation for pre-commercial thinning operations with the purpose of avoiding infections caused by *Heterobasidion* spp. is to thin relatively early, when the diameters are smaller, with low birch stumps and treated spruce stumps. Lower diameters in combination with the two stump types that showed the lowest infection frequency should result in lowest infection threat to spruce dominated pre-commercial thinning stands. Low birch stumps in combination with living birches will possibly serve to break the continuity between spruce roots.

The future

Further research would be interesting with the purpose of seeing how the birch infections proceed; whether they survive, continue down to the roots and acquire the ability to transfer the infections further in the pre-commercial thinning stand.

The long-term study will hopefully show both how degraded the high spruce stumps are after five years and if the inoculated infections are still vital. In other words; if infected high stumps die-off before spreading the infection to surrounding trees or if the infection itself will die before reaching remnant trees. Hopefully we will also have an answer within the next five years as to whether the results from this study can be confirmed or if spruce stumps’ height actually affect the susceptibility to *Heterobasidion* spp. differently in the long-run.

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Appendix I

Reduced product consumption as a result of the smaller diameter at 1 m

If a 1 m high stump would be treated with Rotstop®S instead of a 15 cm high stump it should, as a result of the smaller diameter, be possible to save stump treatment. The estimations of the diameter reduction was calculated from the means of the spruce stumps' diameters in this study. Also the thinning strength was based on the site conditions in this study.

500 spruce trees	14 % removal
The reduced mean diameter	1.4 cm
Treatment agent cost	20 SEK/m^2

After this, the reduced stump treatment cost was calculated based solely on the reduced consumption of the treatment agent for manual stump treatment at 1 m instead of at 15 cm high stumps.

$\frac{1.422}{2}/100=0.00711$ m

$(0.00711^2 \times \pi) \times 500 = 0.079434$ m^2

$0.079434 \times 20 = 1.59$ SEK/ha

If stumps would be treated at 1 m, the reduced cost for solely the treatment agent would be 1.59 SEK/ha if the average diameter of 500 cut spruce stumps per hectare were 1.42 cm smaller at 1 m compared to at 15 cm.

Appendix II

To justify manual stump treatment in pre-commercial thinnings

Costs for manual treatment (14 % removal)	420 SEK/ha (Berglund et al., 2007)
Price spruce pulp log, commercial thinning	300 SEK/m^3sub
Price spruce saw log, final felling	525 SEK/m^3sub

"Normal harvest" commercial thinning	40 m^3sub (30 % removal)
"Normal harvest" final felling	300 m^3sub (95 % removal)

Time for measures

Pre-commercial thinning (10 year old stand)	0
1st commercial thinning (35 year old stand)	25
Final felling (65 year old stand)	55

Table 7. How much spruce pulp log or saw log one have to "save" from total degradation in the pulp log and saw log classification to be able to justify manual stump treatment in pre-commercial thinnings.

	Interest rates		
	2%	**3%**	**4%**
1st Commercial thinning (SEK/ha)	689.05	879.39	1119.65
Final felling (SEK/ha)	1248.13	2134.50	3631.47
Amount of pulp log (m^3sub)	2.30	2.93	3.73
Amount of saw log (m^3sub)	2.38	4.07	6.92
% of commercial thinning harvest	5.7%	7.3%	9.3%
% of final felling harvest	0.8%	1.4%	2.3%

Example

Calculations of amount of pulp logs that have to be saved from total degradation at first commercial thinning with an interest rate of 3 %:

$$420 \times 1.03^{25} = 879.39 \text{ SEK/ha}$$

$$\frac{879.39}{300} = 2.93 \text{ m}^3\text{sub}$$

This is equalized with 7.3 % of a first commercial thinning harvest, since:

$$\frac{2.93}{40} = 7.3 \text{ \%}$$

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