Pinus contorta susceptibility to Heterobasidion spp.

A study of stumps, roots and artificial spore infections of stumps

Susanne Svensson
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Sammanfattning

I denna studie undersöktes contortatallens mottaglighet för infektion av rotticka (*Heterobasidion spp.)* i tre olika delförsök. I det första försöket insamlades trissor från stubbar i nygallrade bestånd för att ta reda på om svamparna kan spridas och infektera contortatallen naturligt. I det andra försöket samlades rotprover in från contortatallar som planterats på tidigare rötinfekterad granmark för att undersöka om tallen kan infekteras via rotkontakter. I det sista försöket skapades nya stubbar som artificiellt infekterades med sporer av rottickans S-typ (*H. parviporum*) och P-typ (*H. annosum)* för att på detta sätt utreda om contortatallen är mottaglig för dessa svampar. Ingen av trissorna från de nygallrade bestånden var infekterade och det visade sig senare att dessa bestånd gallrats för ett till två år sedan vilket troligtvis påverkat resultatet. Infekterade rötter återfanns på 26 % av de träd som förväntades vara infekterade. På alla stubbar som infekterats artificiellt med P-typen och på alla utom en stubbe som infekterats artificiellt med S-typen återfanns infektioner 51 dagar efter infektionstillfället. Dessa resultat visar att contortatallen är mottaglig för båda typerna av *Heterobasidion spp.* och att de dessutom kan bli infekterade via rotkontakter vilket betyder att rottickan kan vara ett hot mot contortan. På grund av detta kan det vara nödvändigt med nya skötselåtgärder som stubbehandling vid gallring, stubbrytning och bättre ståndortsanpassning vid återbeskogning av contortatall.

Abstract

The Lodgepole pines susceptibility to *Heterobasidion spp*. was investigated in three parts of this study. In the first part, discs from freshly cut stumps were collected and analyzed to see if the fungi can spread and grow naturally in Lodgepole pine stands. In the second part, root samples were collected from Lodgepole pines planted in former infected Norway spruce stands to see if the fungi can spread through root contacts. For the third part, new stumps was created and artificially infected with spores of both the S-type (*H. parviporum*) and the P-type (*H. annosum*) of the fungi to find out if the Lodgepole pine is susceptible to them. None of the stumps from recently thinned stumps were infected and it was later discovered that these stands in fact were thinned one and two years ago which probably has had influence on the result. Infected roots were found on four out of 15 trees that were expected to be infected and totally 17 % of all sampled trees, with and without symptoms, were infected. On all stumps that were artificially infected with the P-type and all but one stump that were artificially infected with the S-type, infections were found 51 days after the time of infection. These results indicates that the Lodgepole pine is susceptible to both types of the fungi and that it can be infected through root contacts which means that *Heterobasidion spp.* may be a threat to the Lodgepole pines. Because of this, new management methods such as stump treatment during thinning, stump removal and a better habitat adaptation when re-planting stands with Lodgepole pine might be necessary.

Keywords: Lodgepoole pine, Pinus contorta, root rot, Heterobasidion spp

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1. Introduction

Heterobasidion spp. is one of the most common root and butt rot causing fungi in the northern hemisphere (Hodges 1969) and costs the Swedish forestry large amounts of money each year (Karlman 1981). *Heterobasidion spp.* was earlier divided into two intersterility groups in northern Europe, the S-type (*Heterobasidion parviporum Niemelä & Korhonen*) and the P-type (*Heterobasidion annosum*). In this study they will be called the S-type and the P-type to separate them from each other and since the expression is still commonly used in the Swedish forestry sector.

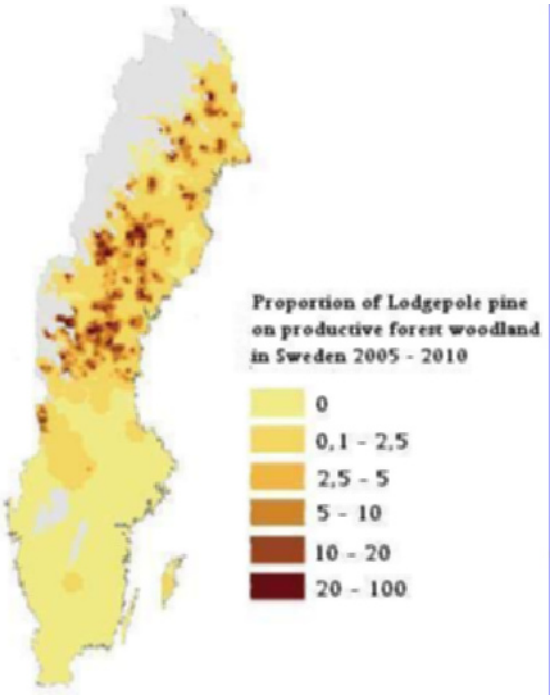
The S-type generally attacks Norway spruce (*Picea abies* (L.) Karst.) and it can also infect saplings of Scots pine (*Pinus sylvestris L.*) but it hasn't been found on mature pines. The P-type mainly infects pines in all ages but it has also been found in spruce stands and on other species like birch and alder. In Sweden, the S-type is found in almost the entire country but the P-type has only been found in the southern part (Korhonen et al. 1998). *Heterobasidion spp*. can spread in two major ways; airborne spores can infect recently cut stumps if the temperature is > 5° C (Stenlid et al. 2000) and healthy roots can become infected through root contacts between healthy and infected roots (Hodges 1969; Rishbeth 1952). Studies that took place in Britain showed that the fungus can live up to 62 years in infected stumps (Greig and Pratt 1976). Piri (1996) has also found the fungi alive in a 46 year old spruce stump in Finland. Because of this, trees that are planted in stands where the previous population was infected by *Heterobasidion spp.* can become infected and the fungi can also spread to other tree species (Piri 1996; Rönnberg et al 1999). One species that Piri (1996) found to be susceptible to *Heterobasidion spp*. was Lodgepole pine *(Pinus contorta var. latifolia).*

The Lodgepole pine origins from North America and began to be planted in Europe during the 20th century (Von Weissenberg 1975). During the 1970's the Lodgepole pine became a more and more interesting tree species in Sweden (Hagner 1983). At that time, people were afraid that there would be a shortage in timber in the Swedish forests in the future and wanted to plant a faster growing tree species than the native Scots pine to prevent this from happening (Cory 2010). According to Hagner (1983) the Lodgepole pine could have up to 70 % better production than the native alternatives and the timber could be used for both sawing and pulp production, this made the Lodgepole pine a very attractive tree species.

In 1983 Sweden was the country that had the third largest population of Lodgepole pine, only Canada and USA had more. During the same time the Swedish company SCA owned 125 000 ha woodland populated with Lodgepole pine (Hagner 1983). Today, 565 000 ha of woodland in Sweden is populated by Lodgepole pine and as shown in figure 1 most of it is situated in the northern part of the country (Engelmark et al. 2001). A lot of these plantations is now facing the first thinning period (Figure 2) (Cory 2010). When comparing the timber quality and susceptibility to different pathogens between Scots pine and Lodgepole pine, the latter is known as a tolerant conifer (Cory 2010) and is less susceptible all European rust

fungi compare to the Scots pine (Roll-Hansen 1977). On the other hand, the Lodgepole pine has more often got a crooked stem or other stem damages which gives it a lower quality (Cory 2010). Due to its shallow root system and the fact that it has longer needles that can be covered with a lot of snow and become heavy, the Lodgepole pine has also got more instability problems than the Scots pine (Martinsson 1986). It is also more susceptible to Greminiella abientina compared to the Scots pine (Hansson and Karlman 1997).

There is little knowledge about the Lodgepole pines susceptibility to *Heterobasidion spp*. in Swedish conditions. The Scots pine and the Lodgepole pine is both from the genera *Pinus* and might therefore become attacked by the same types of pathogens (Karlman 1981). In view of the fact that the P-type generally attacks the Scots Pine (Korhonen 1978) the Lodgepole pine could for that reason also be susceptible. The P-type has been found up to latitude 63°N in Finland (Korhonen 1978) and might be able to spread further north in Sweden and become a problem in the Lodgepole pine forests in the future. Stumps from the Lodgepole pine could be an entry point for the S-type when the stand is thinned, but it isn't known if the S-type is able to infect Lodgepole pine stumps. Former spruce stands has been planted with Lodgepole pine and these could also be infected by the S-type through root contacts as it has been in Finland (Piri 1996), but it isn't known if that has happened. As a consequence of global warming it cannot be excluded that the P-type will spread further north also in Sweden and therefore be an additional threat to many tree species including the Lodgepole pine.



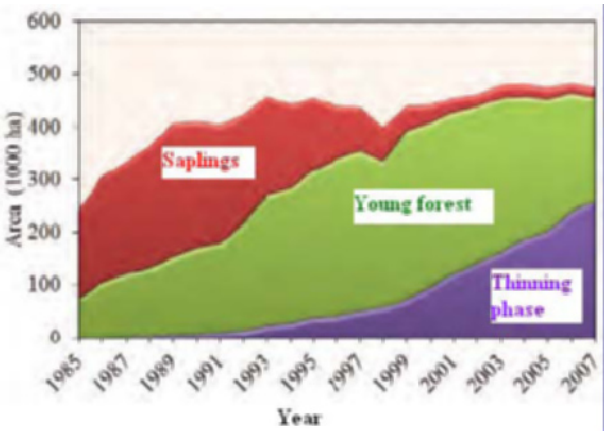


Figure 2. The area of Lodge pole pine divided into different cutting classes. Source: Riksskogstaxeringen, Skogsdata 2010

Figure 1. Distribution of Lodgepole pine in Sweden based on percentage of total productive forest woodland in Sweden 2005 – 2010 Source: Riksskogstaxeringen, Skogsdata 2010

The goal for this study is to see if *Heterobasidion spp.* can infect Lodgepole pines through recently cut stumps or by root contacts with infected stumps. Another aim is to find out if either one of the two intersterility types, or both of them, can grow on fresh wood of Lodgepole pine. If the Lodgepole pine is susceptible to *Heterobasidion spp*. it might be necessary to use a stump treatment whilst thinning in summer to prevent the spread of *Heterobasidion spp.*

The hypotheses of this study are:

- Freshly cut stumps of Lodgepole pine are being infected with spores from *Heterobasidion spp.* when thinning in summer.
- The Lodgepole pine can become infected by root contacts with old infected stumps.
- Both the S-type and the P-type can infect Lodgepole pine stumps.

2. Material and Methods

The work was divided into three parts where each part included fieldwork and analysis in laboratory. All sites included in the study were chosen together with SCA and they were all on their land, except for one stand that belonged to a private forest owner.

2.1 Part I – Recently cut stumps

Discs from stumps in recently thinned stands was collected and analyzed to see if *Heterobasidion spp*. naturally can infect and grow in fresh stumps.

2.1.1 Selection of sites

The criteria for these sites were that they should be populated by Lodgepole pine and that they had been thinned in the spring of 2010. Five sites were chosen and these were situated between Örnsköldsvik and Sollefteå in Ångermanland (Figure 3, table 1).

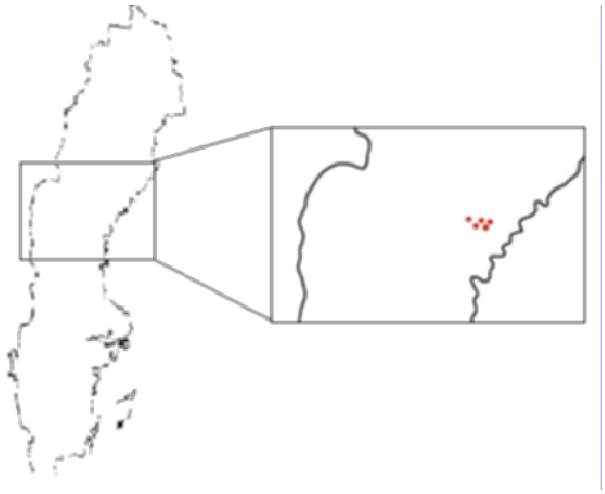


Figure 3. Location of selected sites for collection of discs in recently thinned stands.

Table 1. Location, size and age for the chosen sites in part I.

Site	Coordinates (latitude and longitude)	Size (ha)	Age (years)
1	N 63° 15.893' E 17° 47.690'	42,2	34
2	N 63° 15.945' E 17° 43.555'	29,3	29
3	N 63° 19.522' E 17° 35.655'	37,8	29
4	N 63° 14.941' E 17° 29.172'	45,7	28
5	N 63° 17.186' E 17° 14.881'	23,1	34

2.1.2 Collection of discs

It was decided that 20 discs from each site would be collected and analyzed. By using ArcGis, 20 sample plots were evenly distributed over each of the five sites. This was done to make sure that all parts of the sites were covered and that the samples would represent the whole site. A GPS was used to locate the plots in the field and when it was 10 meters from the centre of the plot it beeped and then a measuring tape was used to find the centre. The last 10 meters was always measured straight forward from the time when the GPS beeped. A colored stick with the number of the sample plot was placed in the center of each plot. The stump closest to the center was used for the collection of the sample. First the stump was decorticated and then 70 % ethanol was sprayed on the surface of the stem to reduce the risk of contamination. Afterwards, a one cm thick disc was cut off the top of the stump and then another two cm thick disc was cut of the stump again. The second disc was transferred into a plastic bag marked with the number of the site and sampling plot. All samples were put into a fridge with a temperature of 3 °C the same day as they were collected.

2.1.3 Analyzing of discs

The samples were incubated in room temperature for 7-10 days before they were analyzed in the laboratory. The discs were analyzed by looking at them in a magnification microscope (Figure 7). This makes it possible to see the conidiophores on the cross-section of the discs. Lines were painted on the discs to divide them into smaller sections which made it easier to look at them in the microscope and to make sure that the whole disc was examined (Figure 6). When a possible infection was found the infected area was examined at a higher magnification to ensure that what was found really was an infection of *Heterobasidion spp.* If an infection was found a pen was used to mark the infection and the area was then estimated by using a cm^2 sheet. Both sides of all discs were examined the same way.

2.2 Part II – Root samples

Root samples were collected from trees planted in former spruce stands that were infected by *Heterobasidion spp*. to see if the fungi can infect Lodgepole pine through root contacts with roots from old stumps.

2.2.1 Selection of sites

The criteria for these sites were that they earlier had been populated by spruce that were infected by *Heterobasidion spp*. and that they now were populated by Lodgepole pine. Another criteria was that there shouldn't have been any cuttings done in the sites since the time of planting. It was rather hard to find these sites since SCA didn't have any record of which sites that earlier had been infected by *Heterobasidion spp.* Based on information from old foresters, harvesting personnel and the knowledge from the local population three sites close to Åsarna in Jämtland were chosen (Figure 4, table 2).

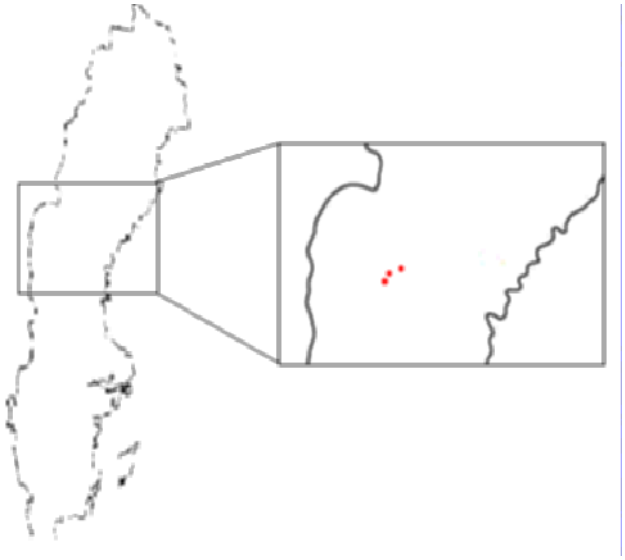


Figure 4. Location of sites for collection of root samples.

Table 2. Location, size and age for the three chosen sites in part II.

Site	Coordinates (latitude and longitude)	Size (ha)	Age (years)
Skalänget	N 62° 33.932' E 14° 18.164'	12	32
Flobergs	N 62° 41.017' E 13° 51.282'	36	25
Oviken	N 63° 0.081' E 14° 17.500'	~100	~30

2.2.2 Collection of root samples

The root samples were collected in the beginning and in the middle of September. Samples were taken from five trees that showed signs of infection by *Heterobasidion spp*. and from five trees that looked healthy in each site. Korhonen (1978) didn't find any differences in symptoms between the S-type and the P-type. To find out what kind of signs an infection can give Lodgepole pine, Tuula Piri and Kari Korhonen was contacted for help and advices. The following is a part of Tuula Piri's answer:

"In the very early stage of infection there aren't necessarily any external signs of disease that you could perceive. Sometimes changed colour of the needles (reddish or light green) and/or short needles can be signs of early infection." (Piri, T., Pers. comm., 2010)

Based on this information and from the answer from Kari Korhonen, which were similar, trees with the above mentioned symptoms and recently dead or dying trees were chosen for sampling. When a tree with one of the above named symptoms was discovered three main roots were located and a shovel was used to dig around and under them. Before the samples were taken, the roots were brushed to get rid of dirt and then the roots were sprayed with 70 % alcohol to minimize the risk of contamination. One sample was taken from each of the three roots but at different distances from the stem.

1. 0-10 cm from the stem
2. 50 cm from the stem
3. 100 cm from the stem

In the same area as the trees with symptoms were located, five healthy trees were chosen and the same amount of samples was taken from them in the same way. The samples that had a diameter >1 cm were 2-3 cm long and thinner root were 4-5 cm long to make sure that they wouldn't dry before they were analyzed. All roots were put into plastic bags marked with the number of the site, distance from the stem, diameter of the tree in breast height and noted sign of infection. The samples were then put into a fridge with a temperature of 3° C until they were going t be analyzed.

2.2.3 Analyzing of root samples

The roots were incubated in room temperature for 7 to 10 days before they were analyzed. The roots that had a diameter < 1 cm were cut in half, to expose more wood, 7 days before they were analyzed which made it easier to see if there were any infections. The root samples were analyzed in a magnification microscope on both on the cross-section on both sides and on the wood that had been exposed by the cut through them. The bigger roots were only analyzed on the cross-section on both sides. When an infection was found it was not marked, it was just noted if the root was infected or not.

2.3 Part III – Artificial infections of new stumps

New stumps with a height of ca 70 cm were created and artificial infected by the S-type and the P-type to see if the fungi can grow in the wood.

2.3.1 Selection of sites

The only criteria for these sites were that they should be populated by Lodgepole pine. Therefore, site 1, 2 and 3 from the part I of the work were chosen for this part too simply because they were the sites that were closest to drive to.

2.3.2 Cultivation of *Heterobasidion spp.*

A known individual of both the S-type (RB 175), that origins from Ramsåsa in Skåne and P-type (Mac 1558) that origins from Åmål in Dalsland, were used to cultivate spores for the artificial infection. A piece of mycelium was put on Hagem malt agar extract in petri-dishes and left for growing in two weeks. The spores from the dishes had to be diluted with water to get the right amount of spores per millilitre. First, 10 ml were put on the petri-dish covered with mycelia to get the spores in to the water (Figure 5) and then the dilution was made as follows:

- 10 ml water was put into one dish with mycelia.
- 1 ml of these 10 ml was put on malt agar extract in a petri-dish.
- 1 ml was put into 9 ml of new water.
- 1 ml out of the new mixture was put on malt agar extract in a petri-disch.
- 1 ml was put into 9 ml of new water.

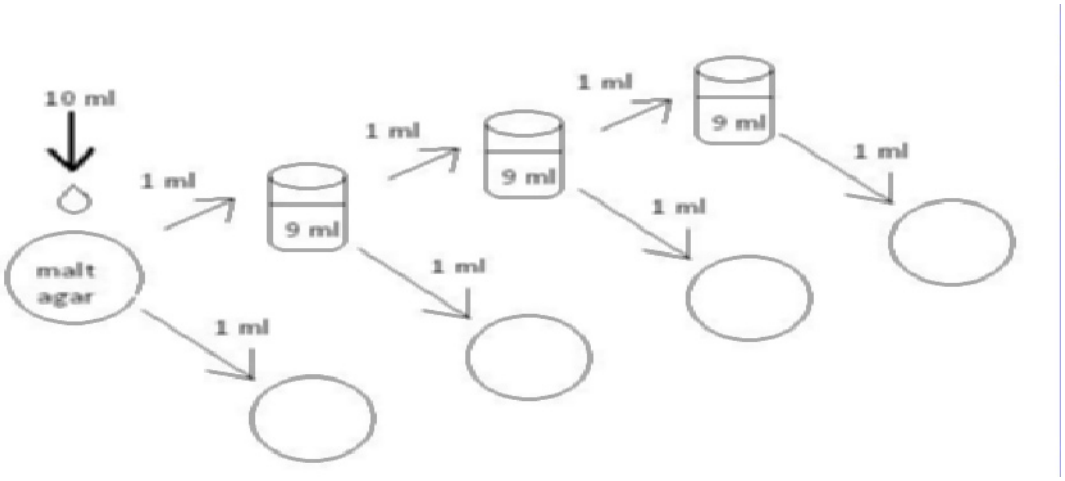


Figure 5. Process for dilution of spores.

This was done 11 times which gave 11 dishes with different dilutions on each of them. The test was done from three petri-dishes with mycelia with the S-type and from three petri-dishes of mycelia with the P-type to get a mean value of the spore concentration. The petri-dishes were put in plastic bags and kept in room temperature during four days. On the fourth day they were examined and it was possible to count the number of colonies that were growing on each petri-dish. Similar to Redfern (1972) the goal was to find the concen-

tration that gave five to ten colonies per petri-dish. When the dish with the right amount of colonies were found it was possible to calculate backwards how many spores the original dilution contained.

2.3.3 Artificial infection of *Heterobasidion spp.* on new stumps

This part was done between the 30th of August and the 1st of September. The petri-dishes with mycelia were diluted the same day as the stumps were infected to get a fresh mixture of 1000 spores per milliliter. It was mixed with de-ionized water and kept in a closed bottle until it was used. Each day, before the first stump was infected and after the last stump was infected, one ml of the mixture were put on malt agar in a petri-dish and left in room temperature to grow. This was done to see if the spores were viable during the whole day. All the stumps had to be infected with the same amount of spores per cm^2, in this case 50 spores were used. To be able to know how many ml each stump should be infected with, an excel-sheet with how many ml that should be used on stump diameters between seven cm and 20 cm was brought in field. Each stump was cross-callipered and then infected with the right amount of mixture.

Example of a stump with a diameter of 12 cm:

The area (A) of the stump = π x r^2
π x $(12/2)^2$ = 113,1

The number of spores (S) on the stump = A x 50
113,1 x 50 = 5654,9

Amount of mixture on the stump surface = S / 1000
5654,9 / 1000 = 5,7 ml

A pipette was used to measure the mixture for each stump. The mixture was put into a measuring glass and the top of a spray bottle was used to distribute the liquid evenly over the whole stump surface.

In each of the three sites 20 trees were felled to create stumps at a height of approximately 70 cm. The trees were cut with a chainsaw in two rows from a point close to the road next to the site and straight into the site. Discs were collected from all the new stumps and put into plastic bags marked with the number of the site and the number of the tree. The discs were taken for analyses in the laboratory to see if the trees were infected by *Heterobasidion spp.* before they were artificially infected. The stumps were also marked with a number so that it would be easy to relocate them by the time they were going to be collected. Since there was

no rain during the time of infection and the following 24 hours, no protection was put on the stumps.

2.3.4 Collection of discs

After 51 days, the infected stumps were located and the bark was sprayed with 70 % alcohol to reduce the risk of contamination. Afterwards a one cm thick disc was cut of the top of the stumps and then a three cm thick disc was cut and put into a plastic bag marked with the number of the site, the number of the plot and which type of *Heterobasidion spp*. they were infected with. The top of the discs were also marked with a pen. All of the stumps infected with type S were cut first and the stumps infected with type P were cut last. Rishbeth (1951b) found that *Heterobasidion spp*. can have a growth rate of 1 meter per year in pine stumps. Based on this information, a calculation of how much the fungi could have grown down in the stump was done and a little more than that was cut off. To make sure that the fungi wouldn´t be left in the sites 20 cm of each stump was cut off, brought back and destroyed.

100 / 365 = 0,274	100 cm divided with 365 days
0,274 x 51 = 13,974	The calculated growth rate for one day, 0,275, multiplied with the number of days between infection and collection, 51

2.3.5 Analyzing of discs

Both the discs collected before the artificial infection and the discs collected after the infection were analyzed in a magnification microscope the same way as in part I.

Figure 6. A disc divided into sections by a pen. Photo: Susanne Svensson

Figure 7. A disc being analyzed in a micro scope. Photo: Susanne Svensson

2.3.6 Calculations and statistics

The programme that has been used to calculate all the statistics during this study is Minitab. A paired t-test was used to find out if there was any significant difference between the discs that were infected before and after they were artificially infected. The parameters that were tested were the total area of infections on the downside of the discs before the infection and the total area of infections on the topside of the discs after infection.

3. Results

3.1 Part I – Recently cut stumps

None of the 100 discs collected from stumps in the recently thinned sites were infected by *Heterobasidion spp.*

3.2 Part II – Root samples

3.2.1 Roots from trees without signs of infection

From the trees with no sign of infection, one out of totally 45 samples was infected by *Heterobasidion spp*. That sample was taken from a root at 100 cm distance from the root collar in the site in Oviken.

3.2.2 Roots from trees with symptoms of infection

From the trees with signs of infection, totally six root samples out of 45 were infected by *Heterobasidion spp.* (Table 3). All of them were found in the sites in Oviken and Flobergs.

Table 3. Number of infected and uninfected root samples from the trees with symptoms of infection at the three different distances from the stem at ground level in all three sites

	Uninfected	Infected
10 cm	14	1
50 cm	13	2
100 cm	12	3

The six infected roots belonged to four out of the 15 sampled trees. The sites Oviken and Flobergs had two trees each with infections in the roots whilst no infections were found in Skalänget. One tree was infected in all three roots whereas the rest of the trees only had one infected root sample. The three trees with only one infected root were all dead whereas the tree where all three rots were infected was a living but small tree compared to the rest of the trees in the site (Table 4).

Table 4. The four infected trees, their symptom and the distances from the stem at ground level where the infected root samples were taken.

Site	Infected tree	Symptom	10 cm	50 cm	100 cm
Flobergs	1	Small tree	X	X	X
	2	Recently dead	-	X	-
Oviken	1	Recently dead	-	-	X
	2	Recently dead	-	-	X

3.3 Part III – Artificially infected stumps

3.3.1 Discs from new stumps before artificial infection

As shown in figure 9, the majority of the discs were uninfected but infected discs were found in all three sites. Only one disc in each site was infected on the downside of the disc only and the others had either an infection on both sides or at the topside alone.

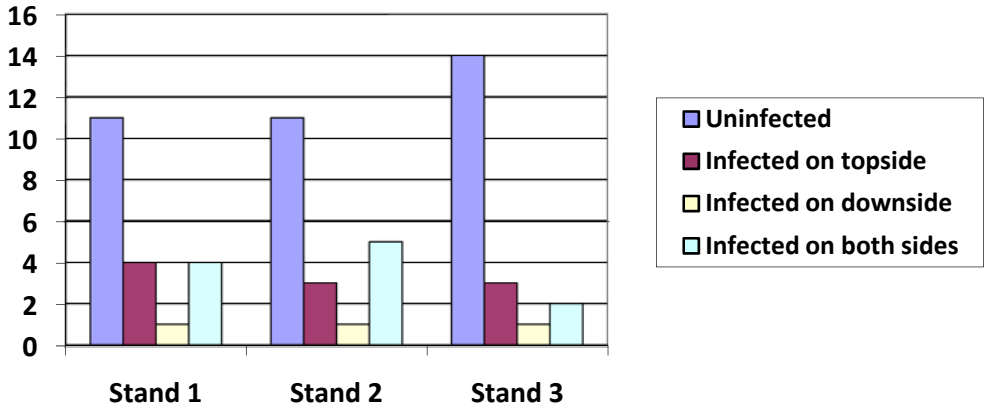


Figure 9. The number of uninfected and infected discs, on the top of the disc, the downside of the disc and on both sides of the discs in each of the three sites.

Totally 40 % of the stumps were infected with *Heterobasidion spp.* before the artificial infection. Infected stumps were found in all three sites at it were an even distribution of infections between the sites. (Table 4)

Table 4. Percent infected and uninfected stumps in the three sites before the artificial infection.

	Percent infected stumps	Percent uninfected stumps
Site 1	45	55
Site 2	45	55
Site 3	30	70
Total	40	60

3.3.2 Discs from new stumps after artificial infection

All discs, except from one in site 2, were infected by *Heterobasidion spp.* The one that weren't infected was not infected before the manual infection took place either. Most of the infections were found on both sides of the discs and no infection was found only on the downside (Figure 11).

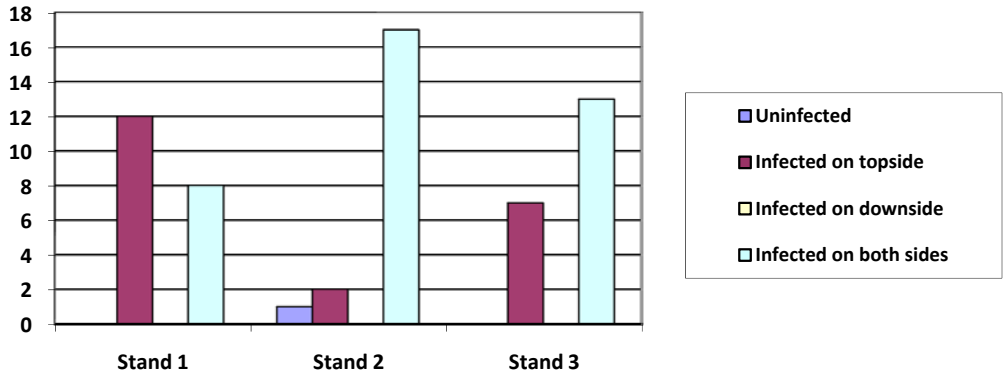


Figure 11. The number of uninfected and infected discs, on the top of the disc, the downside of the disc and on both sides of the discs in each of the three sites.

3.5 Comparison between infected discs before and after artificial infection

When comparing the downsides of the discs before artificial infection with the topsides of the discs after the infection, which bordered to each other, it was a significantly larger area ($p < 0{,}001$) of total infections on the discs after the time of infection (Figure 12, table 5).

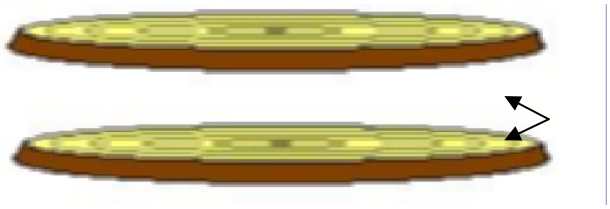


Figure 12. Compared sides of the discs.

Table 5. Comparison in total infected area on both sides of the discs before and after infection in all three sites.

		Before infection		After infection	
Disc nr. (type of artificial infection)	**Site**	**Total area infected on topside (m2)**	**Total area infected on downside (m2)**	**Total area infected on topside (m2)**	**Total area infected on downside (m2)**
1 S	1	0	7,25	0,5	0
1 P	1	0,5	1	10,5	1
4 P	1	2	6,5	4,5	0,25
5 S	1	1,5	2,25	4,25	0
7 S	1	0,5	0	0,75	0
7 P	1	1	2	16	0
8 P	1	3,5	0	4,75	0
9 P	1	1,5	0	21	1,5
10 P	1	2	0	8,75	0
1 S	2	6	5,5	13,5	0,5
1 P	2	4,5	0	3,75	0,25
4 P	2	4,5	0,75	13,25	2,25
5 P	2	6	0	9	0,5
7 S	2	2	9,75	16	0,25
8 S	2	4,5	0	13,5	2,75
8 P	2	0	2,5	2,75	4,25
9 S	2	8,25	3,75	14	2,5
10 S	2	6,75	5	8	3,5
4 S	3	0	0,5	1	1
4 P	3	1,25	0	11	0
5 P	3	3,5	0	14,25	0,5
7 P	3	2	1	20,5	0
8 P	3	5	4,5	7,75	0,25
10 S	3	2,5	0	15	2,5

4. Discussion

Artificially infected stumps of Lodgepole pine

Both the S-type and the P-type can infect and grow on freshly cut stumps of Lodgepole pine when spores are inoculated artificially. That proves that the Lodgepole pine is susceptible to both types of the fungus. The discs that were infected with *Heterobasidion spp.* before the stumps were artificially infected could have been infected in several ways. Since the bark on the trees weren't sterilized before they were cut down, the discs could have been contaminated from spores on the bark. The discs could also have been infected by spores in the air from the time when the tree were cut down to when the disc was put into a plastic bag, even if they only were exposed for a very short time. There is also a possibility that the infections came from the roots and had grown up in the stem. But since none of the cut trees showed any symptoms of infections this is hardly the case. The fact that the discs were infected without being artificially inoculated also proves that the Lodgepole pine can become infected by spores naturally. These stumps were found to be more infected after they were inoculated which proves that the artificially inoculated spores were able to infect them as well.

Infections through root contacts

Lodgepole pines that are planted in previously infected spruce stands can be infected through root contacts with other infected trees or infected, old stumps. Piri (1996) also found that the Lodgepole pine is susceptible to vegetative spread of *Heterobasidion spp.* when planted in former spruce stands infected with the fungi. In this study the roots were probably infected with the S-type since the P-type never has been found on these latitudes in Sweden (Stenlid et al. 2000). This result is of great importance because all Lodgepole pine stands planted in previously infected stands is in risk of getting infected. This is the case for a substantially large part of SCA:s Lodgepole pine stands today where approximately 50 % are planted on sites where the previously population was a mix of Scots pine and spruce (Bobik, M., pers comm., 2011).

Possible methods to prevent infections by *Heterobasidion spp.* through root contacts in the future

In the future it is a good idea to choose another tree species for the regeneration on sites that previously were populated by spruce or by spruce mixed with pine. This is even more important if it is known that the previously stand was infected by *Heterobasidion spp.* Scots pine could be an alternative when looking at the results from Piri (1996) who found that Lodgepole pine can be infected with the S-type and that the Lodgepole pine is more susceptible than the Scots pine to the S-type. Another way to reduce the risk of infections by *Heterobasidion spp.* through root contacts in future Lodgepole pine stands is to harvest the remaining stumps before the regeneration. This will be an extra cost but the stumps can also give an income as biofuel. According to Mike Bobik at SCA, it is hard to know how big that income would be since it differs a lot from one stand to another and the stump harvest should therefore be considered to be a cost of about 6 000 – 7 000 SEK/ha.

Uncertainties regarding the amount Lodgepole pines infected through root contacts

This study didn't involve any total inventory of the investigated sites and it is for that reason difficult to say how big part of the sites that could be infected and if it is a substantially part of the sites that are infected or not. There are no records on how much of the previous stand that was infected with *Heterobasidion spp.* either and it is therefore impossible to say if there is a correlation between the percent of infected spruces in the earlier stand and the percent of infected Lodgepole pines today. That could also explain why no infected roots were found in Skalänget, there might not have been any large scale infections of *Heterobasidion spp.* in the previous stand and as a result of that, none or just a few trees in the subsequent stand might be infected but not found in this study.

Possible reasons to why no infections were found in the recently thinned sites

When no infections were found on the discs from the stumps in the sites that were thinned in the spring of 2010 the weather conditions during these periods were investigated since they could be a limiting factor for the dispersal of spores. As soon as the dates for the thinning periods were found it was discovered that they hadn't been thinned this year, instead they were thinned during the following dates:

Table 7. Thinning periods and their mean temperature for the sites in part I.

Site	Thinning period	Mean temperature °C
1	091019 – 091215	0,9
2	090915 – 090925	8,9
3	081121 – 081210	-4,7
4	080605 – 080630	13,4
5	080404 – 080505	4,5

As shown in table 7, the mean temperature for site 1, 3 and 5 during the thinning periods was below 5° C which is the lower limit for when spores of *Heterobasidion spp*. can spread and infect fresh stumps (Stenlid et al. 2000). This could be an explanation to why no infections were found in those sites. During the last 13 days of the thinning period and 8 days after in site 5, the mean temperature each day was above 5° C before it got colder again (see appendix 1). During those warm days some of the stumps could have been infected but it might have been a too short period of time for the fungus to produce viable spores before the temperature declined to below 5° C again.

The mean temperatures during the thinning periods in sites 2 and 4 were above 5° C and the temperature was thereby favourable for *Heterobasidion spp.* This means that if stumps of Lodgepole pines are susceptible to *Heterobasidion spp.,* they could have been infected in these sites. The fact that only two sites out of the original five were thinned during a period with a mean temperature over 5° C makes the results for the first part of this study uncertain since there are a lot of things that can affect the result for these two sites.

First of all, the closest source of spores could be too far away and there might not have been any dispersal of spores in these sites. According to Brandtberg et al. (1996), weather conditions could also be of importance for the infections. When looking at the precipitation, site 2 had only a few millimetres during day two, four and ten for the period of the thinning whilst site 4 had 12 days of precipitation. According to Rishbeth (1951 II) spore can lose their viability in just a few days if there is low relative humidity and dry weather, the spores might therefore have been unable to colonize stumps in site 2 due to warm and dry weather. On the other hand, Sinclair (1964) discovered a lower dispersal of spores when there was rainfall during a thinning operation and he didn't find any correlation between relative humidity and spore dispersal. Based on this information, the stumps in site 2 should have been infected if there were viable spores in the air.

The thinning in site 2 was almost one year old and the thinning in site 4 over two years old. Therefore the stumps could have been infected when they were fresh but the infection could have grown down in the stump and disappeared at the top. The collected discs were only taken from the top of the stumps and an infection further down in the stump could be present but undiscovered (Morrisson and Johanson 1978)

It is also possible that stumps of Lodgepole pine can't be colonized by *Heterobasidion spp*. during normal conditions. To get a good answer to this question I would suggest that more studies needs to be done. Preferably like this study but with stands thinned in the spring the same year as they are analyzed.

Economical calculation of stump treatment on Lodgepole pine stands

If stumps from Lodgepole pine are being infected by *Heterobasidion spp.* after thinnings, it could be necessary to start performing stump treatments with for example *Phlebiopsis gigantea* (Rotstop®S) to prevent infections. Stump treatments will be an extra cost and it's not known if it can stop infections on stumps of Lodgepole pine. To find out if it would be economically to treat stumps with *Phlebiopsis gigantea* a calculation was made to compare the costs for stump treatments with the loss you get at different percents of infections in a stand. Since it's not known how much of the stands that might get infected through the stumps after thinning it was decided to compare 15 % infected trees, 5 % infected trees and a healthy stand where stump treatments are performed during each thinning operation. The harvested trees were assumed to be sold as pulpwood and the percent infected trees were subtracted from the final felling (see appendix 2).

The results showed that if the stump treatments is successful, can stop all infections through stumps and keep the stand healthy it will give a greater profit compared to untreated stands, as long as the untreated stand will be ≥ 5 % infected by *Heterobasidion spp.* (figure 13). If the remaining stumps are harvested before the regeneration the extra cost of 6 000 – 7 000 SEK has to be added to the calculation (Bobik, M., pers comm., 2011). The untreated stand, without stump harvest and stump treatments, would then have to be ≥ 12, 5 % infected by *Heterobasidion spp.* to give a profit for the treated stand.

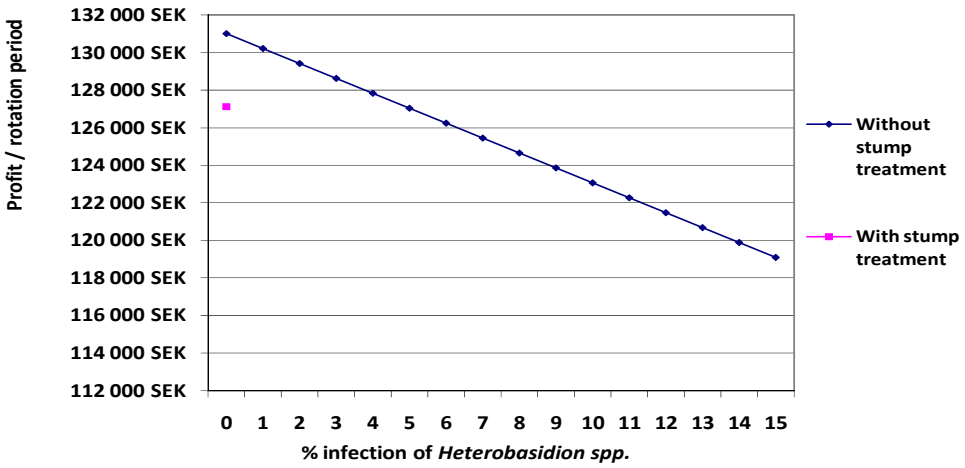


Figure 13. Comparison of the profits in a Lodgepole pine stand during one rotation period with different percent of infections by *Heterobasidion spp.* and an uninfected stand where stump treatment has been performed during the thinning operations.

Conclusions

The results in this study show that freshly cut stumps of Lodgepole pine are susceptible to both the S-type and the P-type of *Heterobasidion spp.* The pines can also become infected thorough root contacts but it isn't clear if fresh stumps get infected by spores naturally. It is necessary to do more investigations on this subject to find out how much damage *Heterobasidion spp*. causes to this species and if new silviculture methods are required to avoid infections of in Lodgepole pine stands in the future. A possible solution is to start treating the stumps during thinning operations to stop the infections since it is profitable as long as a treated stand will be free from infections and an untreated stand will get infections on ≥ 5 % of the stems.

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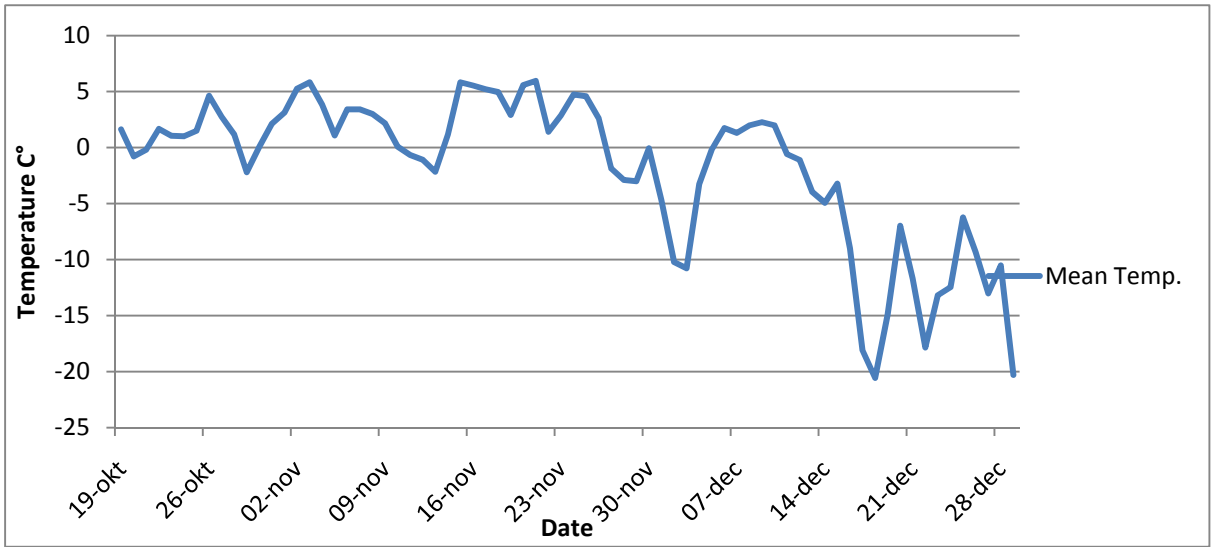
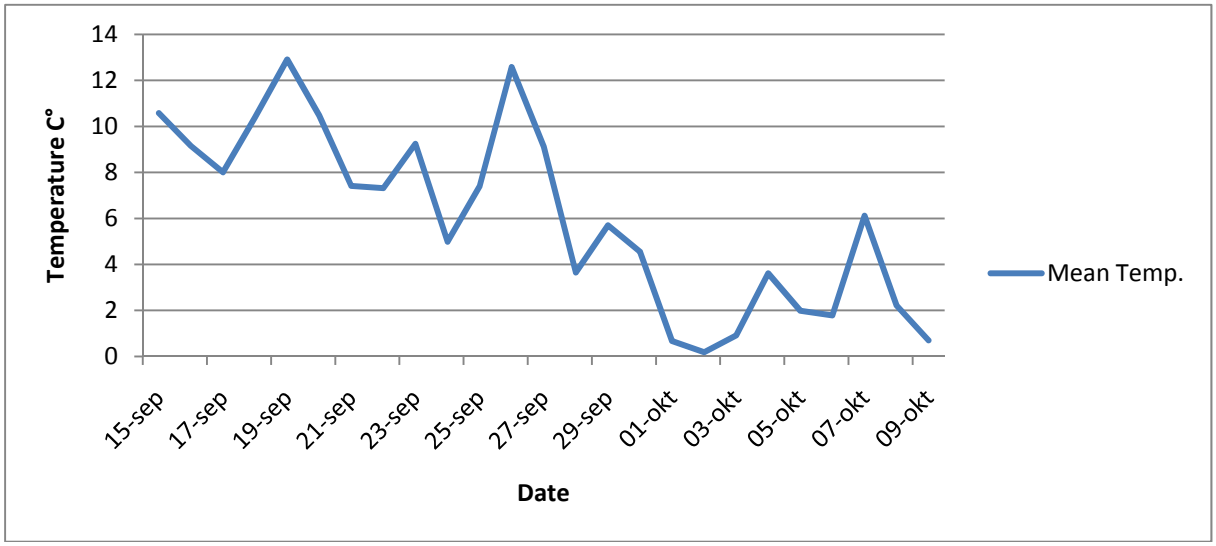
Appendix 1

The weather data is collected from one weather station in Para, close to Sollefteå (http://www.temperatur.nu/para-valj_tid.html) and one weather station at Sollefteå/Kramfors airport (http://rl.se/vadret/historik.php).

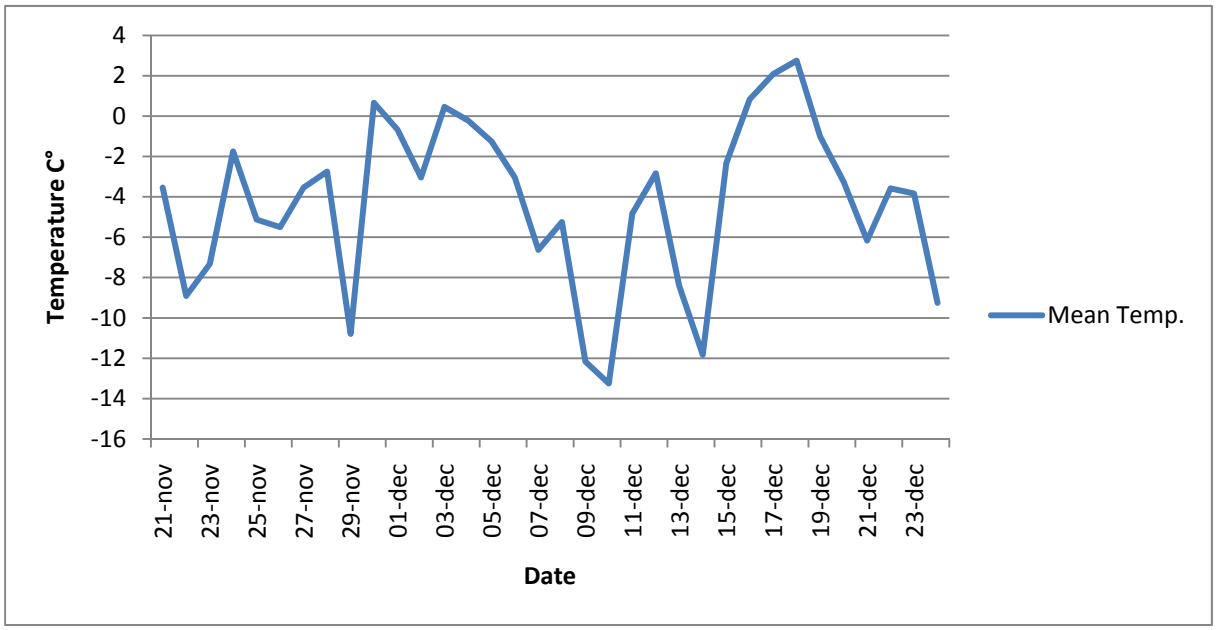
Mean temperatures during thinning period (19/10 – 15/12 2009) and two weeks after in site 1.



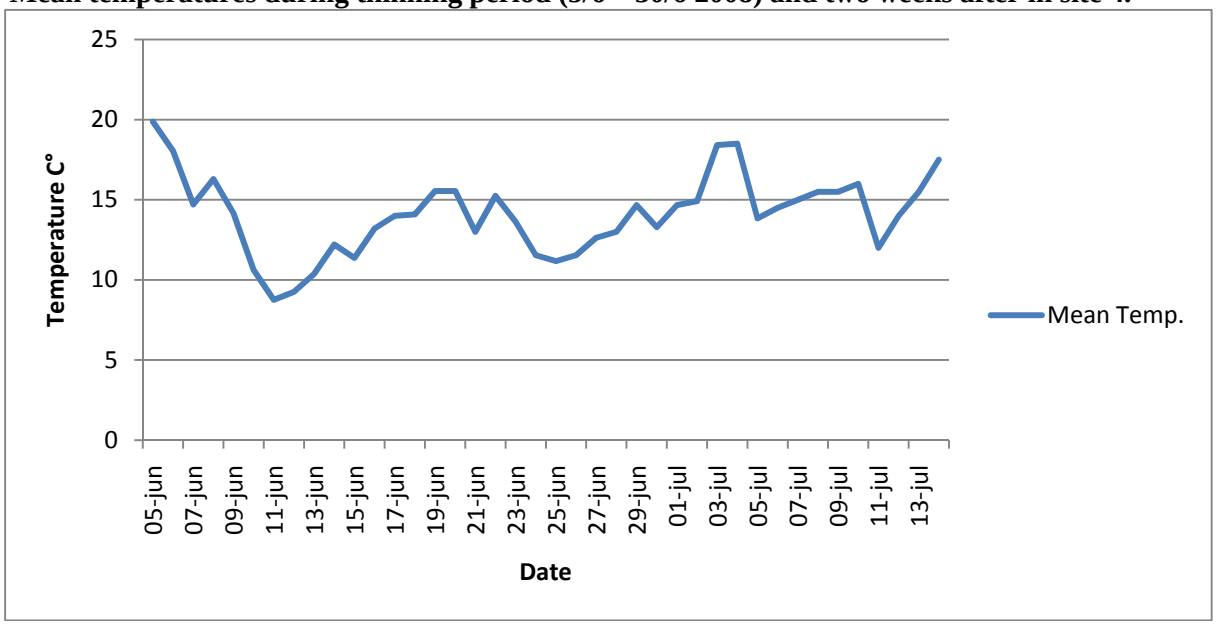
Mean temperatures during thinning period (15/9 – 25/9 2009) and two weeks after in site 2.



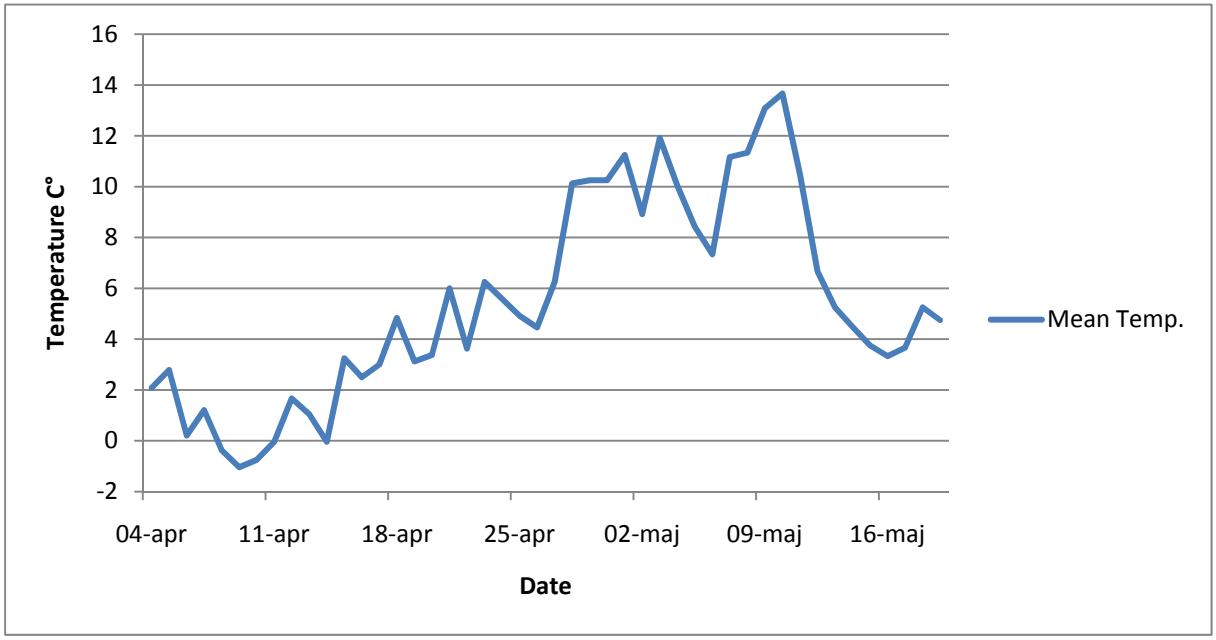
Mean temperatures during thinning period (21/11 – 10/12 2008) and two weeks after in site 3.



Mean temperatures during thinning period (5/6 – 30/6 2008) and two weeks after in site 4.



Mean temperatures during thinning period (4/4 – 5/5 2008) and two weeks after in site 5.



Appendix 2

Calculations for costs and revenues during one rotation period with stump treatment during thinning

Regeneration, stems / ha = 1700 (Bobik, M., pers comm., 2011)

Thinning % = 30 (Bobik, M., pers comm., 2011)

Thinning costs / m^3sub = 170 SEK (Brunberg 2010)

Cost for final felling: 96 SEK

Cost for stump treatment / m^3sub = 1, 3 € = 11, 52 SEK (Thor 2003)

Pulpwood price / m^3sub (mean in Sweden the fourth quarter of 2010) = 323 SEK

Interest rate = 3 %

Since none of SCAs Lodgepole pine stands are in the second thinning phase, the volumes for the second thinning operation and the final felling are assumed to be 50 m3sub and 350 m^3sub.

Table 1. Costs and revenues compounded to 80 years during one rotation period in an uninfected stand where stump treatment is performed during each thinning.

	Age	Harvest (m^3sub/ha)	Stump treatment costs (SEK/m^3sub)	Harvest cost including stump treatment (SEK/ha)	Income (SEK/ha)	Net income (SEK/ha)	Net income compounded to 80 years (SEK/ha)
1st thinning	30	35	11,52	6 353	11 305	4 952	21 709
2nd thinning	36	50	11,52	9 076	16 150	7 074	25 972
Final felling	80	350	0	33 600	113 050	79 450	79 450
Sum							**127 131**

Calculations for costs and revenues during one rotation period with 15 % infections of Heterobasidion spp.

The same numbers is used as in the calculations above.

Table 2. Costs and revenues compounded to 80 years during one rotation period in a stand with 15 % infections of *Heterobasidion spp.* and where stump treatments aren't performed.

	Age	Harvest (m^3sub/ha)	Harvest costs (SEK/ha)	Income /ha (SEK)	Net income (SEK/ha)	Net income compounded to 80 years (SEK/ha)
1st thinning	30	35	5 950	11 305	5 355	23 476
2nd thinning	36	50	8 500	16 150	7 650	28 087
Final felling	80	297, 5	28 560	96 093	67 533	67 533
Sum						**119 096**

Calculations for costs and revenues during one rotation period with 5 % infections of Heterobasidion spp.

The same numbers is used as in the calculations above.

Table 3. Costs and revenues compounded to 80 years during one rotation period in a stand with 5 % infections of *Heterobasidion spp.* and where stump treatments aren't performed.

	Age	Harvest (m^3sub/ha)	Harvest costs (SEK/ha)	Income /ha (SEK)	Net income (SEK/ha)	Net income compounded to 80 years (SEK/ha)
1st thinning	30	35	5 950	11 305	5 355	23 476
2nd thinning	36	50	8 500	16 150	7 650	28 087
Final felling	80	332,5	31 920	107 398	75 478	75 478
Sum						**127 041**

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