Comparison of Phlebiopsis and urea and their effect when treating young spruces against *Heterobasidion spp*

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Master Thesis no. 244
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Abstract

Control of the economically important forest pathogen Heterobasidion spp. can be done by control with chemical and biological agents such as urea and *Phlebiopsis*. Today in Europe this action is mainly done during the mid stages of the rotation, thinning foremost and to a small extent in final felling. Studies have shown that the fungus is spreading not only in the late stage but also during the early pre commercial thinning stage, preventive actions should be taken also at this stage. In this thesis focus has been on investigating the differences in effectiveness of *Phlebiopsis* and urea. The aim was to compare the agents when used on small Norway spruce stumps in Sweden. The study was conducted at 18 sites distributed in southern and the central parts of Sweden. 1080 stumps were created and treated with *Phlebiopsis* and urea, 1/3 was left untreated as control. Results from the study show that stumps treated with urea wasn't infected by *Heterobasidion sp.* Control stumps and *Phlebiopsis* treated stumps were infected by the fungus at the rate of 20,9 % and 8,1%. The effect of using *Phlebiopsis* decreases the infection frequency but doesn't rule it out. This thesis also further consolidates the fact that small stumps of Norway spruce need protection against infection of *Heterobasidion spp,* where the treatment agent urea could be seen as more effective. Even though urea was more effective in this thesis, *Phlebiopsis* is an agent that also decreases the infection rate and it is significantly better to treat stumps after pre commercial thinning than to leave the stumps untreated. Urea is an agent that, with tax legislation today, is expensive to use and of this reason *Phlebiopsis* might still be the economically best agent to use.

Key words: Heterobasidion Phlebiopsis Urea Young spruce

Sammanfattning

Behandling av den ekonomiskt viktiga trrädsjukdomen *Heterobasidion spp.* kan göras på kemisk väg med urea och biologiskt med *Phlebiopsis*. Idag i Europa behandlas i huvudsak bestånd i mellanstadiet, då i huvudsak i gallring och i viss utsträckning slutavverkning. Studier har visat att svampen kan spridas också i det tidigare stadiet röjning, behandling mot rotröta bör göras också här. Fokus för denna studie var att undersöka skillnaden i effektivitet för *Phlebiopsis* och urea. Målet var att studera medlen vid behandling av små unga granstubbar i röjningsstadiet i Sverige. Studien utfördes på 18 ytor fördelat i södra Sverige samt i mellersta Sverige. 1080 stubbar skapades och 1/3 behandlades med *Phlebiopsis*, 1/3 med urea samt lämnades 1/3 obehandlade som referens. Resultatet från studien visar att stubbar behandlade med urea ej blev infekterade av rottickan *Heterobasidion spp.* För kontrollstubbarna och *Phlebiopsis*behandlade stubbar var infektionsfrekvensen 20,9% och 8,1 %. *Phlebiopsis* minskade infektionsfrekvensen men tog inte bort den helt. Denna studie styrker också ytterligare det faktum att granstubbar behöver ett skydd mot rotticka, här verkar urea vara mycket effektiv som behandlingsmedel. Även om urea var mer effektiv i denna studie, är *Phlebiopsis* också ett medel som minskar infektionsfrekvensen och är signifikant bättre än att inte behandla stubbarna efter röjning. Urea är idag ett medel som är dyrt att använda, då framförallt på grund av skatter, och detta kan göra att *Phlebiopsis* då är det ekonomiskt bästa medlet att använda.

Nyckelord: ***Heterobasidion Phlebiopsis Urea Ung gran***

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Introduction

The most important production tree species in Sweden is Norway spruce, a species very susceptible to root rot caused by *Heterobasidion*. In Sweden one of the most important pathogens in forests is the root and butt rot caused by *Heterobasidion* spp., primarily in spruce dominated areas. The losses due to this pathogen are extensive and have different appearances, from decreased volume growth (Bendz-Hellgren and Stenlid 1997), secondary losses from wind throw due to decreased stability in the stem, quality degradation and tree death. Losses between 500 – 1000 MSek every year have been estimated due to decreased stem quality and growth loss (Bendz-Hellgren et al 1998, Bendz-Hellgren and Stenlid 1995).

Heterobasidion spp

There are two primary kinds of *Heterobasidion* fungus causing damage to conifer trees and stands in Sweden. These are *Heterobasidion annosum* s.s. (Fr.) Bref. primarily affecting *Pinus sylvestris, Picea abies* and some deciduous tree species. The other species is *Heterobasidion parviporum* affecting primarily *Picea abies* (Niemelä and Korhonen, 1998). From now on different species of *Heterobasidion* are referred to as *Heterobasidion* spp. in this thesis. *Heterobasidion* is a fungal species spreading mainly through wind dispersed basidiospores created in basidiocarps growing on old trees or stumps (Redfern and Stenlid 1998). The spores colonize fresh wood of trees either newly cut, which is the most common way, through wounds on the bark or via damaged roots in contact with surface. Another from forestry point of view very important way the fungi spread is through its mycelia which can spread from the roots of an already infected stump to a healthy standing tree (Redfern and Stenlid 1998). The main period for the spreading of the fungus is the summer months, a period of the year which is very favorable for spore spreading. The temperature is of importance and spread of spores is possible when temperature reaches above 0° C. During winter time very few stumps are being infected but the temperature is the most important factor. After infection of a new host the *Heterobasidion* can grow as much as about 29 – 40 cm/year within the stem and roots, after time causing decreased volume growth or tree death (Bendz-Hellgren and Stenlid 1997).

Control of *Heterobasidion* spp.

In intensively managed forests, a part of the Scandinavian forests, the spread and risk of *Heterobasidion* infections is big (Thor 2005). In natural forests with an admixture of less susceptible tree species and, as an example, spruce the risk of spreading infection can be smaller. The positive effect with this kind of mixture can be that the spacing between susceptible trees are longer and less root contact occurs where the fungus can spread (Korhonen et al 1998). Natural like forests are more diverse in tree species mixture which probably decreases risk of infection (Lindén and Vollbrecht 2002). Establishing nature like forests is one way to control the occurrence of decay spread. Cutting during winter time is also a very effective silvicultural measure as the fungus spread less at this time of year (Redfern and Stenlid 1998), but due to industry demands harvest has to be done all year (Bendz-Hellgren et al. 1998). As mentioned earlier the most used control of the pathogen is the treatment either with *Phlebiopsis* or urea. In this thesis *Phlebiopsis* and urea are in focus.

Treatment against root rot: urea and Phlebiopsis gigantea

Urea is a chemical agent, 32% aqueous solution was used in this thesis, more used in other European countries as for example Finland than in Sweden. Urea has been registered, under the name "PS Stubbskydd", as a pesticide in Sweden since 2008 (Kemikalieinspektionen 2015). The treatment has been proven effective in decreasing growth of *Heterobasidion* spp. as it raises pH of the stump. At pH level > 7 the fungus inactivates growth (Johansson et al 2002). Oliva et al 2007 investigated the long term effect of treatment with urea. Oliva claim that the urea treatment reduced the occurrence of *Heterobasidion annosum* s.l. root rot in *Picea abies* after 15 years. Chang and Chang (1999) found that urea after hydrolysis into Ammonia by urease enzyme decreased the growth of several funguses among these was *Heterobasidion*.

Rotstop S Gel is a biological agent used to prevent the root rot infection on coniferous trees. The gel contains spores and mycelia from *Phlebiopsis gigantea (Fr.)* (Interagro Skog AB 2015), from now on in this thesis *Phlebiopsis gigantean* will be referred to as *Phlebiopsis*. *Phlebiopsis* is a common colonizer of fresh wood throughout the boreal and temperate forests in the world. Treatment with the agent is one of few biological controls used to prevent infection from *Heterobasidion* spp. Risbeth 1963 claimed that *P. gigantea* is a biological agent suitable for protection against the *Heterobasidion* spp. The efficiency of the agent was also supported by Berglund and Rönnberg 2004. The agent is sprayed on the stump surface immediately after the tree is cut and the fungus establishes on the stump out competing the pathogen *Heterobasidion* spp. The main idea of the Phlebiopsis is that the fungus is out-competing the *Heterobasidion*, leaving no room for the pathogen to grow.

Today to prevent the issues caused by root rot biological control with the *Phlebiopsis* (Rotstop S) is common in Sweden. The agent is generally applied simultaneously as the harvest head cut the tree during mechanical harvest (Thor 2001). This is a service provided and often offered by many of the big forest companies in Sweden (Sydved 2015, Södra 2015). This treatment is mainly done during the summer months, when the occurrence and risk of primary infections are high (Redfern and Stenlid 1998) and commercial thinning is conducted. In other parts of Europe the most used treatment is urea, an agent which raises the pH on the stump making it unsuitable for the

Heterobasidion (Johansson et al. 2002). The different agents are being used on more than 200000 ha in Europe every year (Thor 2001) where *Phlebiopsis* makes up for 56% and urea on about 42 %. Many studies are showing that both *Phlebiopsis* (Berglund and Rönnberg 2004) and urea (Thor 2005) is good agents against *Heterobasidion* caused rot, (Oliva et al. 2008).

Background and hypothesis

While treatment against Heterobasidion spore infection is fairly common in thinnings it has not been considered in pre commercial thinning. Earlier studies have shown that in small diameter Norway spruce stands, the risk of infection is small (Vollbrecht et al 1995) due to small surface on which *Heterobasidion* spores can land on. In addition beliefs have been that small stumps of Norway spruce are unable to transfer the rot to adjacent trees (Vollbrecht et al.1995). More recent studies have been made stating that this might not always be the case; small stumps are being infected by the *Heterobasidion* (Gunulf et al. 2013) and are able to transfer the disease to adjacent trees. This could be reduced with treatment of stumps with Phlebiopsis or urea.

Gunulf et al. 2013 has seen indications that *Phlebiopsis* might be affected by the size of the stump; smaller stumps might decrease the efficacy of the treatment of reasons unknown. Vasiliauskas et al. 2004 studied the impact from *Phlebiopsis* and urea on fungal community on commercially thinned stumps with results indicating that urea prevents colonization of *Heterobasidion.* Further studies are needed to compare and ensure if the stump size has a significant impact on the efficacy of *Phlebiopsis* and urea as treatment agents on small pre commercially thinned Norway spruce stumps. Johansson et al 2002 claimed that urea might be more effective in young stands as the vigor and temperature of the stump might affect the urea hydrolysis and pH-rise.

Hypothesis:

Environmental conditions on the stump might have an effect on the colonization by *Phlebiopsis*, for example the moisture content (Bendz-Hellgren and Stenlid 1998). This could mean that in small stumps with a higher relative amount of sapwood urea could be better as a control against *Heterobasidion*. Johansson et al. 2002 tested the pH levels after treatment with urea and the changes depending on wood characteristics, younger wood gained in pH faster and to higher levels than in old wood rendering in better protection the younger the wood was in that experiment. There might be a significant difference in efficacy between *Phlebiopsis* and urea as control of *Heterobasidion spp* since urea might be benefited by conditions on the young small Norway spruce stumps. This difference hasn't been tested for young spruce stumps in pre commercial thinning stage. Gunulf et al. 2013 claimed that there was a need for more research on the matter of which of the two treatments are the most effective. This thesis has the aim to investigate whether there is a difference in efficacy between *Phlebiopsis* and urea in small Norway spruce stumps.

Methodology

Establishment

The experiment was established in two different regions of Sweden to be representative of a greater part of the country. The area of Värmland in central Sweden is representing northern and central Sweden while the area of Skåne/Småland represents the southern parts. The target stand was one that was considered to be healthy, with no old infections in the ground. The stands were dominated by spruce and with many trees/ha with a variety in size from 2- 15 cm in stump diameter. 18 different stands were chosen according to above mentioned preferences. Different ages, management stages and site indices were represented. In every stand 60 stumps were created, the trees were chosen according to usual pre commercial thinning practice where competition and spatial properties are determining as well as quality properties of the tree. Quality properties as stem crookedness and branch angle as examples. Within a block, three randomly treated stumps, 1/3 of the stumps were treated with urea, 1/3 with *Phlebiopsis* and 1/3 were left as control stumps not being treated. The treatment on each stump was randomly decided in advance. The diameter was recorded for every stump and the goal was to ensure that there would be a variety of stump diameters. Every stump was cut and treated in blocks of three trees along a sampling path where the trees in the blocks were treated at a different order from block to block. The treatment of the stumps was *Phlebiopsis* (R), urea (U) and Control (C).

Table 1. Site information. Only sites with infection are presented.

Site	Location	Coordinates	Age (at stump height)	Site Index	Mean stump diameter (mm)
S1	Tönnersjö	56.698111, 13.116444	20	G 30	89
S2	Tönnersjö	56.674444, 13.083861	20	G32	70
S3	Asa	57.149139, 14.759083	13	G24	46
S4	Asa	57.149028, 14.780278	21	G28	72
S5	Asa	57.124583, 14.761556	7	G28	37
S7	Vidöstern	57.016056, 13.937250	22	G34	95
S8	Tönnersjö	56.679917, 13.096528	12	G32	75

The suspension of Rotstop was mixed according to the description by the retailer and applied on the surface with the same quantity on each square cm. Urea was already mixed when delivered from the retailer and applied equally on the urea treated stumps. The instructions were that the surface of the stump has to be covered by a 1 mm thick layer of the agents. The volume needed to cover the surface of the stump was calculated using the diameter.

Every sampling path started close to nearest road and followed the stand border approximately 10 meters in from border and turning when reaching a vertical border.

At every tree following actions were taken:

Practical method

1. Chose tree according to practical pre commercial thinning
2. Cut down tree at 25 cm above ground
3. Check for infections
4. Mark the stump with ribbon
5. Caliper diameter
6. Treat according to list
7. Mark block on map

Collection of sample discs

The treated stumps were left in the field for 8 weeks and one disc were collected from each stump in the same order they were established. The collection was made with hand saw, disinfected with 70% alcohol between every cut. Every sample was collected in plastic bags marked with stump number and site number. The top 1 - 2 cm of the stump was removed and the next disc was collected for examination in laboratory. Because of practical reasons the stumps couldn't all be put in cooling rooms immediately after collection but the aim was to have them cooled within 48 hours.

Practical method for collection:

1. Disinfect bark of stump and hand saw
2. Remove top part of stump
3. Mark plastic bag and mark top of disc
4. Disinfect hand saw again and collect disc

Analyzes in laboratory

The discs were stored in fridge with a temperature of approximately 5°C. At this temperature the infections can survive but doesn't grow. After storage the discs were incubated for 10 days +- 1 and checked for infection. To minimize the risk of failing to notice infection the disc was marked with stripes along which it was checked for infections of *Heterobasidion* spp. The marking makes the analysis of each disc equal. Infections were marked and the infected area measured to the closest 0, 5 cm^2. Number of distinguished infections was noted, the infection was measured as a new if there was no visible mycelial connection to any other infection.

Samples of infections were taken and cultivated on agar plate containing Hagem malt agar extract. A maximum of 10 cultivations/site and treatment were done. After mycelium from *Heterobasidion* spp had started to grow, after approximately 3-5 days, a clean sample was taken and stored in fridge for further analysis later.

To investigate what species had infected the discs a somatic incompability test was done. The plates were examined and tested against a reference of assured species, i.e. *H. annosum* or *H. parviporum*, to ensure what species was on the disc. Samples from stump were grown with the reference on different plates. Each stump sample was grown on two plates with *parviporum*, two plates with *annosum* and one plate as a control with two isolates from the same stump sample. The plates were left for two weeks and then examined to ensure species of the stump sample. The growth patterns between the tester and the sample from stump was defining if the species was the same or different. If the stump sample and the tester were able to grow together and form clamps they were of the same species. The formations of clamps were checked under microscope at magnitude 100*.

The efficacy was based on the ability of the different treatment to reduce infection frequency and infected area relative to the control, e.g. the proportion of infected area of control compared to the proportion of infected area of treated stumps within diameter classes (2-10 cm and 10-16 cm). Following formulas was used:

$$\text{Infection frequency} = \frac{(PIC - PIT)}{PIC}$$

PIC = Proportion infected area for control,
PIT= Proportion infected area for treatment

$$\text{Infected area in relation to control} = \frac{(PIFC - PIFT)}{PIFC}$$

PIFC = Proportion infection frequency control,
PIFT = Proportion infection frequency treatment

Statistical analyzes

The statistical analyzes was conducted with the help of the statistical software Minitab 16.

Since the urea treated stumps had no infections on any site these “observations” were not used in the analysis, and for this data the urea may be interpreted as more effective than *Phlebiopsis* or control/no treatment.

To calculate the effect of *Phlebiopsis* treatment, infected stump diameters were compared to uninfected stumps. Both groups were treated with *Phlebiopsis*, and the method used to analyze this was a 2-sample t-test. The different groups consisted of the diameters of infected versus uninfected stumps; a more effective agent should result in only bigger stumps being infected. The same test was used for control stumps to see whether size had an impact on the infection frequency.

When calculating efficacy only sites with three or more infected stumps were used.

Another way to compare the efficacy of *Phlebiopsis* treatment and control was done by using a sign- test where different scenarios within each block were determined.

Result

Infection frequency

Due to low infection frequency several sites could not be used. Out of 18 sites only 7 sites had more than one infected stump. To increase significance of the statistical analysis these sites had to be ignored.

The frequency of infection on the different sites and the different stumps treatments is for *Phlebiopsis* 0% - 16%, urea 0% and control 5% - 55% (figure 1). As can be seen, urea treated stumps are not infected by *Heterobasidion spp.* in any site. The control stumps are being infected at a higher frequency than the *Phlebiopsis* treated stumps at all sites with exception in site 5, equal infection frequency, and site 8 where *Phlebiopsis* treated stumps have a higher infection frequency, the difference in infection frequency is not significant (P = 0,094) (table 3). Average infection rate for the different treatments is for *Phlebiopsis* 8, 05%, urea0% and Control 20, 9 % for all stumps in the 7 sites with infections.

Site indices for all sites have been checked for influence on the infection ratio on the data set. Correlation between site indices and infection frequency for *Phlebiopsis* treated stumps (P=0,528) and untreated Control stumps (P=0,234) on the 7 sites are not significant. The site index is not having an effect on the abundance of infection.

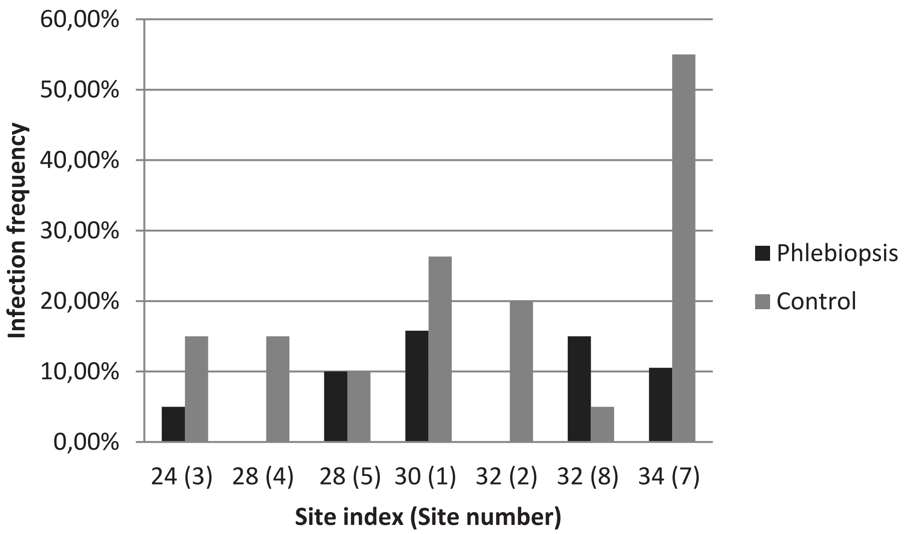

Figure 1. Infection frequency for the treatments *Phlebiopsis*, urea Control on the different sites ordered after raising site index. Urea treated stumps have 0% infection frequency.

The infection frequency for different diameter classes (figure 2) is increasing with the size of the stump for both *Phlebiopsis* treated stumps and control stumps with exception for control stumps class 2 being higher than class 3. Exception can also be found in *Phlebiopsis* treated stumps where class 2 has higher infection frequency than class 3. When doing a 2-sample t-test with infection frequency for every class and treatment *Phlebiopsis* and Control as different groups there is no significant difference between the two groups (P=0,235).

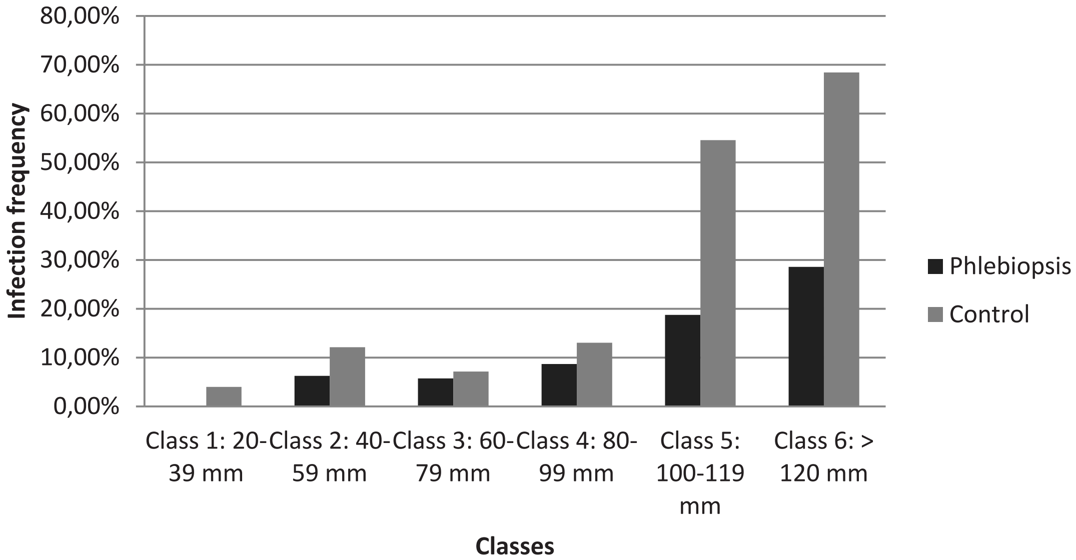

Figure 2. Infection frequency for the treatments *Phlebiopsis*, urea and Control divided in diameter classes. Urea treated stumps have 0% infection frequency.

When comparing the diameters of infected *Phlebiopsis* treated stumps with uninfected *Phlebiopsis* treated stumps there was a significant difference in diameter (P=0,000). The mean diameter of infected stumps was 92,1 mm compared to 60,1 mm for uninfected stumps (table 2).For control stumps the pattern is the same, bigger stumps are more prone to get infection than small (P=0,000). Mean diameter for the infected control stumps are 104, 5 mm versus 56, 7 mm for not infected. This implies that the bigger the stump is the higher the risk of infection for *Phlebiopsis* treated stumps. Small stumps are less likely to get infected.

Table 2. 2-sample t-test comparing difference in diameter for infected versus uninfected diameter for *Phlebiopsis* and Control separately.

Treatment	Mean diameter infected/uninfected stumps	P-value
Phlebiopsis	92,1/60,1	0,009
Control	104,5/56,7	0,000

When testing the difference between infected *Phlebiopsis* stump diameter as one group and infected Control stump diameter as the other, there is no significant difference between size of the two groups (P=0,321). The relative infected area of each disc was calculated and compared between the two groups *Phlebiopsis* treated stumps and the Control stumps. The mean for *Phlebiopsis* was 2, 78 % of the discs and for Control the corresponding relative area was 6, 69 %. The difference between the two groups is not significant with P-value 0,077(table 3).

Table 3. 2-sample t-test comparing difference with *Phlebiopsis* and Control as groups.

Treatment	Relative infected Area (%)		Infection frequency Sites(%)		Diameter of infected stumps (mm)	
	Mean	P-value	Mean	P-value	Mean	P-value
Phlebiopsis	2,78	0,077	8,05	0,094	92,1	0,321
Control	6,69		20,9		104,5	

With a binary logistic regression with infected/uninfected (1/0) as response variable and diameter class as predictor for either *Phlebiopsis* or Control the result is significant. The diameter class has a significant impact on occurrence of infection for both *Phlebiopsis* treated stumps (P= 0,006) and for control (P= 0,000).

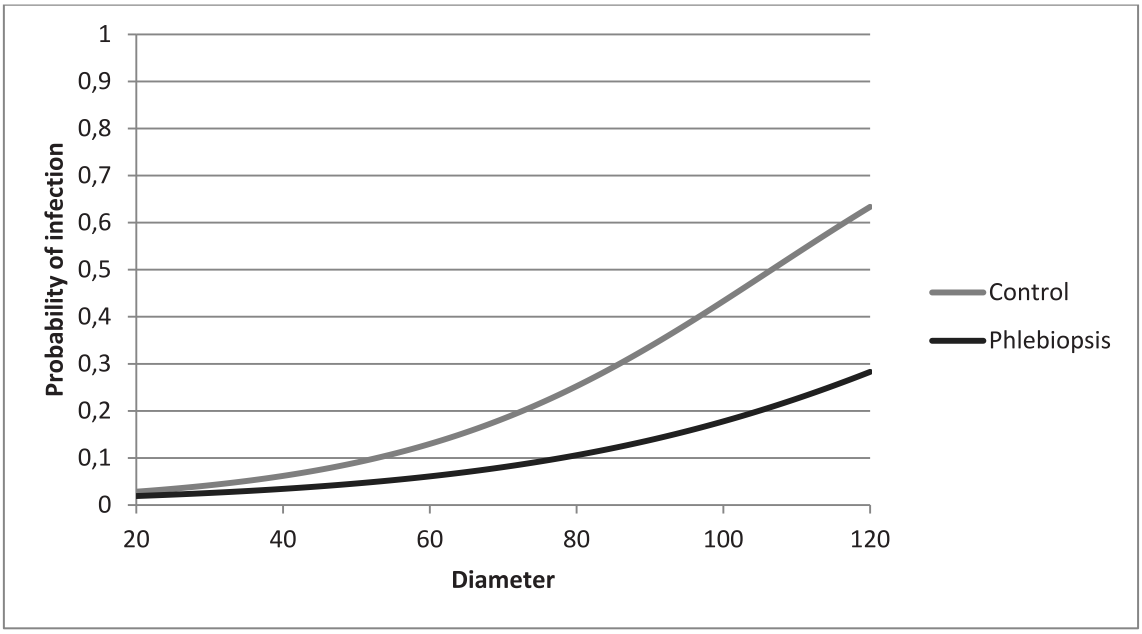

Figure 3. Fitted line for binary logistic regression

Sign-test

To compare if there is a difference in infection between *Phlebiopsis* treated stumps and control stumps a sign test was conducted. Within each block three different treatment are done, *Phlebiopsis* (R), urea (U) and Control (C). To determine which of these are the most effective different possible scenarios were determined. Since urea treated stumps never have any infections these stumps can be ignored when constructing the scenarios.

Table 4. Result from sign test

Scenario	Sign	Occasions	P-value
Infection on both C and R	0	108	0,0021
No infection on any stump	0		
Infection on C but not on R	1	25	
Infection on R but not on C	− 1	7	

The result from the sign test show that there is a significant difference (P = 0, 0021) between the different scenarios. Infection according to scenario "-1" are less frequent (7 occasions) than scenario "1" (25 occasions). On 25 occasions the *Phlebiopsis* treatment is more effective than not treating, only on seven blocks the *Phlebiopsis* treated stumps are infected when control is not infected.

Somatic incompability test

The result from the somatic incompability test was that 10 % of the stumps were infected by *H. annosum*, 85% *H. parviporum* and 5% of the infections could not be defined.

Efficacy

The ability of *Phlebiopsis* to decrease infection frequency was on average 39% for stumps 2-10 cm in diameter, for urea the same number was 100%, no infections. For stumps 10-16 cm *Phlebiopsis* decreased infection frequency with on average 72% and urea 100%.

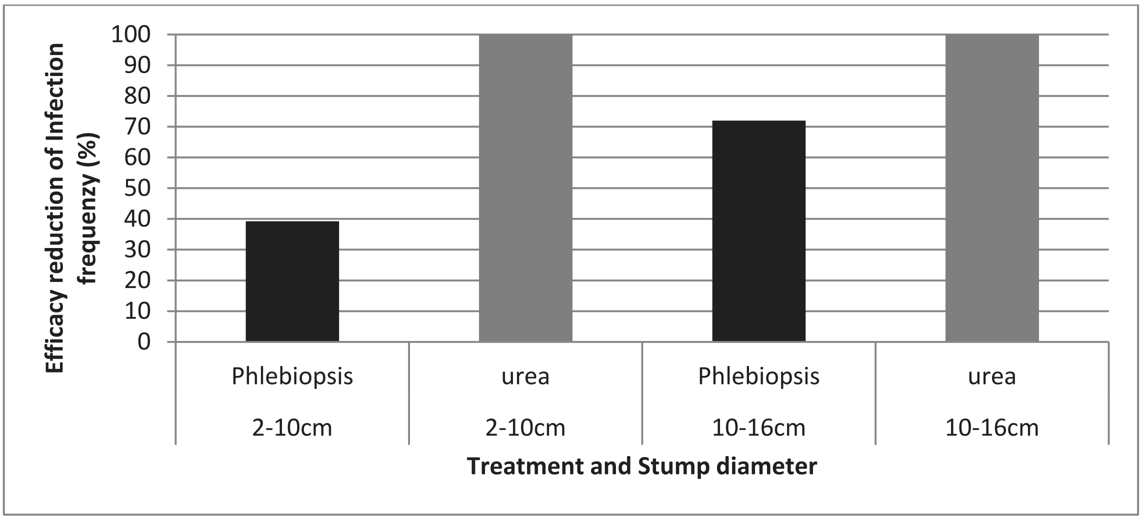

Figure 4 Efficacy reduction on infection frequency % for two different diameter classes.

The ability of *Phlebiopsis* to decrease the infected area on stumps was 73% for stumps 2-10 cm in diameter and for urea 100 %. For stumps 10-16 cm *Phlebiopsis* decreased the infected area with 93% for stumps 10-16 cm, urea decreased infected area 100% for the same stump diameter.

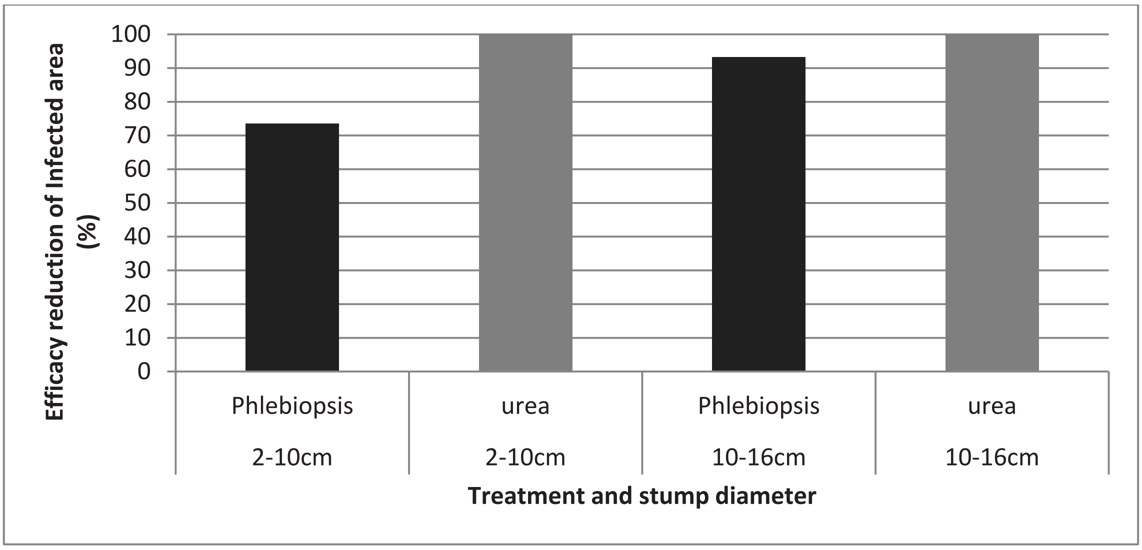

Figure 5 Efficacy reduction on infected area % for two different diameter classes (2-10 cm and 10-16 cm)

Discussion

The result indicates that the urea treatment is more effective, at least this can be claimed for with the data set used in this thesis. Urea seems to decrease, even eliminate, the occurrence of infection at a greater level than *Phlebiopsis*. That there is a significant difference in efficacy between urea and *Phlebiopsis* can be stated. With zero observations of infected urea treated stumps in comparison to 11 observations for *Phlebiopsis* treated stumps the result can be considered significant (pers.comm Englund). According to Englund at least six observations are needed in order to reach significance.The result can be supported by Vasiliaukas et al. 2004 at least for stumps at a bigger diameter. Vasiliaukas et al. 2004 didn't actually study the efficacy of urea as control per se but the result, e.i. no *Heterobasidion* growth post urea treatment, indicates that urea is effective also in that study. In the study presented here *Phlebiopsis* treated stumps are infected by *Heterobasidion* at a rate of 8, 05 %, and it might be insufficient to use this method on small diameter stumps. The long term effect from neither *Phlebiopsis* nor urea has been studied. The result might change over time since the *Phlebiopsis* could potentially decrease the infection and over grow *Heterobasidion* spp. The *Phlebiopsis* hasn't significantly decreased the relative infected area compared to control stumps for this data set but other studies have shown *Phlebiopsis* to be effective at least on commercially thinned stumps (Rönnberg et al. 2004).

Other factors that were tested were the impact of site index and diameter on the effectiveness of the *Phlebiopsis* treatment. Site index seems to have no significant impact on the infection frequency, not for *Phlebiopsis* treated stumps and not for the untreated Control stumps. The diameter is having an effect on if the stumps are being infected or not, with bigger diameter the infection frequency raises for both *Phlebiopsis* and Control. Control of *Heterobasidion* spp with *Phlebiopsis* is preferable rather than no treatment at all. This was also supported when creating the sign test where the different scenarios differed significantly. *Phlebiopsis* treatment is more effective than not treating stumps at all. The urea treated stumps could not be used in the sign-test but is of course more successful than any other option tried in this thesis.

When comparing efficacy on the two treatments urea and *Phlebiopsis* the result indicate that urea is decreasing both infection frequency and infected area at a higher level than *Phlebiopsis.* There are tendencies that urea might be the more effective agent, at least for this data set but *Phlebiopsis* is reducing both infected area and infection frequency compared to control. The result is also indicating that *Phlebiopsis* is more effective when treating bigger stumps than small stumps (efficacy increasing with stump diameter). It is possible that the size of the stump is having an impact on the efficacy of *Phlebiopsis* but not on the efficacy of urea.

The fundamental result of this thesis is that urea is more effective in preventing the infection of *Heterobasidion* than *Phlebiopsis* in pre commercial thinning in this thesis, where the stump diameter range from 20 – 150 mm. Urea creates an unsuitable environment where the fungus can't thrive (Johansson et al 2002). However future studies could be done comparing the two agents on both big (>150mm) and, as in this thesis, small (<150mm) diameter stumps to ensure if a difference can be found also here. Possibly the difference in efficacy differs between small stump and bigger stumps.

Low infection frequency

With a high number of samples, as in the experiment in this thesis, it is possible to exclude some sites in order to increase statistical significance. This was done for all of the northern sites which had fewer occurrences of infections than one per site. The northern sites might have fewer infections due to fewer spores in the air or lower survival rate on the sites. The experiment took place during the summer of 2014. This summer was very warm and dry during the time of the experiment (SMHI 2015). Startup had to be conducted anyway due to practical logistical reasons. Restrictions taken were not to establish any experimental sites during heavy rain fall. Heavy rainfall would affect the efficacy of the *Phlebiopsis* and urea sprayed on the surface of stumps by diluting the agent sprayed on the stump. Notes were taken about weather conditions to be able to follow up if needed. Since the weather might not have been optimal for the *Heterobasidion* spp. to spread this could be a risk and might possibly affect the result of this experiment. With little or no spores in the air, infection frequency and with that the success of the experiment might have been affected

A subjective observation, though not recorded, during lab analysis was that many discs had no growing fungus of any kind at all. No matter treatment fungal growth of some kind and of some species should exist i.e. the *Phlebiopsis* treated stumps should have got some Phlebiopsis infections and the discs should have been infected also with other e.g. mould fungi. Vasiliaukas et al 2004 studied the occurrence of fungi on spruce stumps in thinning stage post treatment with both *Phlebiopsis* and urea. The result showed that fungal growth of both Basidiomycetes and Ascomycetes decreased after treatment, but growth did take place. Reasons to low amounts of fungi in this data can be many, all from too little oxygen when the discs were kept in room temperature to too much alcohol when disinfecting the saw blade. Some sites were checked once again to see if infection rate had change after the samples had been exposed to oxygen, the result hadn't changed. During the collection of the sample discs hand saw was used. The hand saw was disinfected before each disc was collected to decrease risk of cross contamination from one disc to another. To decrease the risk of having too much alcohol on the blade when starting sawing the blade was kept in mid air for a short while for the alcohol to evaporate. There is a risk that this time was too short and alcohol on the blade came on the disc. The alcohol might affect the result killing infections. This stage was hard to alter with since waiting too long would take a lot of time and contamination could occur from spores in the air when holding it in mid air. Possible measure to decrease this risk could be to decrease how many times the blade was disinfected. Instead of disinfecting between every cut, it could be done only once per stump. The risk of contamination would increase but this would only be from the same stump and possibly from the outside of the stump, even though this part also was disinfected. It is hard to believe that the alcohol alone is the biggest reason to the lack of infections, in the bigger discs with diameters up to 150 mm the alcohol should not spread over the whole surface of every disc.

A way to make the experiment much more effective and decrease risk of low infection frequency would be to artificially infect stumps in field as done by Bendz-Hellgren and Stenlid 1998 and Oliva 2007. This method seems fine and generates results with possibly less risk of lacking infections. Bendz-Hellgren and Stenlid 1998 claim that the infection rate is somewhat similar under similar conditions. Also Gunulf 2013 used artificial inoculation which created a good result. The negative side of this is that the experiment won’t be as practical like as the method used in this report. This was the main reason for deciding to use natural infection of the stumps; under average weather conditions in Sweden the wind dispersed *Heterobasidion* spores should be sufficient to get a significant result. Natural infection was used in the experiment conducted by Berglund et al 2004, generating satisfying occurrence of infections. It is possible that some sites not were surrounded by enough spore sources, even though spores can travel far with the wind.

Practical implication

Practical conclusion that can be drawn from this thesis is that stump treatment or other control of *Heterobasidion* should be done also in the pre commercial stage of a rotation of spruce. In pre commercial thinning the amount of cut stumps in total is very high and labor cost due to this might be seen as potentially high. However the high amount of trees in the stand is often due other tree species e.g birch. If you focus the treatment only to spruce, number of stumps to treat decreases and with that the cost. On the other hand, if treatment is only done on spruce stumps there could potentially be the risk of birch getting the infection and in the extension transfer the infection to standing stock. This risk can’t be neglected even though it is not proven. Urea seems like a very effective protection against Heterobasidion, this could be due to the high percentage of sapwood in young spruces. The pH level in sapwood, according to Johansson et al 2002, rises to a higher level and faster than in heartwood when treated with urea. If urea would be considered for this kind of treatment the economical parts have to be taken into account. Walde (pers comm.) claims that urea is today an expensive agent to use due to amount of active compounds in the mix. In Sweden taxes has to be paid for each kilo active ingredient e.i. urea in PS Stubbskydd. The price for urea is higher than that of *Phlebiopsis* due to taxes. *Phlebiopsis* seem to decrease the amount of infected stumps when used as control of Heterobasidion and could be used when treating young spruce stumps. But due to reasons not studied here the stumps treated with *Phlebiopsis gigantea* are being infected at a higher rate than urea treated stumps. When looking at efficacy urea might be the more effective agent to use, but *Phlebiopsis* is reducing infection frequency and infected area on stump. There are indications that the stump size is having an effect on the efficacy of *Phlebiopsis* while the size is not affecting urea. *Phlebiopsis* being less effective in small diameter stumps could be a possible reason to promote other agent when treating stumps small diameter stumps, e.g. urea.

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References

Bendz-Hellgren, M., Lipponen, K., Solheim, K. and Thomsen, I. M. 1998. The Nordic countries. In: Woodward, S., Stenlid, J., Karjalainen, R and Hüttermann, A. (eds.). *Heterobasidion annosum*: Biology, ecology, impact and control. CAB International, Wallingford,333–345

Bendz-Hellgren, M. and Stenlid J 1995. Long-term reduction in the diameter growth of butt rot affected Norway spruce, Picea abies. Forest Ecology and Management 74, 239-243

Bendz-Hellgren, M. and Stenlid, J. 1997. Decreased volume growth of *Picea abies* in response to *Heterobasidion annosum* infection. Can. J. For. Res. **27**: 1519.1524

Bendz-Hellgren, M. and Stenlid, J. 1998. Effects of clear-cutting, thinning, and wood moisture content on the susceptibility of Norway spruce stumps to Heterobasidion annosum. Can. J. Forest Res. 28, 759–765.

Berglund, M. and Rönnberg, J. 2004. Effectiveness of treatment of Norway spruce stumps with Phlebiopsis gigantea at different rates of coverage for the control of Heterobasidion. For. Pathol. 34, 233–243

Chang, T.T. and Chang, R.J. 1999. Generation of volatile ammonia from urea fungicidal to Phellinus noxius in infested wood in soil under controlled conditions. Plant Pathol. 48, 337–344.

Fahlvik, N. 2005. Aspects of pre commercial thinning in heterogeneous forests in southern Sweden. Ph.D. Thesis, Swedish University of Agricultural Sciences, Sweden.

Gunulf, A., Wang, LY., Englund, J.-E. and Rönnberg, J. 2013. Secondary spread of Heterobasidion parviporum from small Norway spruce stumps to adjacent trees. Forest Ecology and Management 287: 1–8

Interagro Skog AB. 2015. http://interagroskog.se/rotroeta-rotstop/ 2015-08-31

Johansson, S.M., Pratt, J.E. and Asiegbu, F.O. 2002. Treatment of Norway spruce and Scots pine stumps with urea against the root and butt rot fungus Heterobasidion annosum—possible modes of action. Forest Ecology and Management 157, 87–100

Kemikalieinspektionen. 2015. Kommissionens beslutför verksamma ämnen i växtskyddsmedel och ämnenas status i Sverige. http://www.kemi.se/Documents/Bekampningsmedel/Vaxtskyddsmedel/EU-lista_vaxtskyddsmedel.pdf 2015-05-28

Korhonen, K., Delatour, C., Greig, B.J.W. and Schönar, S. 1998. Silvicultural control. In: Woodward, S., Stenlid, J., Karjalainen, R and Hüttermann, A. (eds.). *Heterobasidion annosum*: Biology, ecology, impact and control. CAB International, Wallingford, 283-313.

Lindén, M. and Vollbrecht, G. 2002. Sensitivity of *Picea abies* to butt rot in pure stands and in mixed stands with *Pinus sylvestris* in southern Sweden. Silva Fennica 36(4), 767–778.

Niemelä, T. and Korhonen, K. 1998. Taxonomy of the genus Heterobasidion. In: Woodward, S., Stenlid, J., Karjalainen, R. and Hüttermann, A. (Eds.), *Heterobasidion annosum*: Biology, Ecology, Impact and Control, CAB International, Wallingford, 27 - 33

Oliva J., Samils, N., Johansson, U., Bendz-Hellgren, M. and Stenlid J. 2008. Urea treatment reduced *Heterobasidion annosum* s.l. root rot in Picea abies after 15 years. Forest Ecology and Management 255, 2876–2882

Redfern, D.B. and Stenlid, J. 1998. Spore dispersal and infection. In: Woodward, S., Stenlid, J., Karjalainen, R.and Hüttermann, A. (Eds.), Heterobasidion annosum: Biology, Ecology, Impact and Control. CAB International, Wallingford, 105-124

Rishbeth, J., 1963: Stump protection against Fomes annosus. III. Inoculation with Peniophora gigantea. Ann. Appl. Biol. 52, 63–77.

Sveriges meteorologiska och hydrologiska institut – Månadens väder i Sverige. http://www.smhi.se/klimatdata/manadens-vader-och-vatten/sverige/manadens-vader-i-sverige/juli-2014-rekordvarm-manad-i-nordvast-1.76471 2015-08-31

Sydved AB. Kvalitetssäkrad gallring. https://www.sydved.se/skogsbruk/gallring/kvalitetssakrad-gallring 2015-03-06

Södra skog AB. Sälja Virke Beställ gallring av Södra. http://skog.sodra.com/sv/Salja-Virke/Bestall-gallring-av-Sodra/ 2015-03-06

Thor, M. 2001. Sverige trea i Europa på stubbehandling mot rotröta. Resultat Nr 15 2001. Skogforsk

Thor, M. 2005. *Heterobasidion* Root Rot in Norway Spruce Modelling Incidence, Control Efficacy and Economic Consequences in Swedish Forestry. Ph.D. Thesis, Swedish University of Agricultural Sciences, Sweden.

Thor, M., Arlinger, J.D. and Stenlid, J. 2006. *Heterobasidion annosum* root rot in Picea abies: Modelling economic outcomes of stump treatment in Scandinavian coniferous forests. Scand. J. Forest Res. 21, 414–423.

Vasiliauskas, R., Lygis V., Thor M. and Stenlid J.2004. Impact of biological (*Phlebiopsis gigantea*) and chemical (urea) treatments on fungal community structure in freshly cut Picea abies stumps. Biological Control 31, 405–413

Vollbrecht, G., Gemmel, P. and Pettersson, N. 1995. The effect of pre-commercial thinning on the incidence of *Heterobasidion annosum* in planted *Picea abies*. *Scandinavian journal of forest research 10*, 37–41

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Appendix 1

Table 5. Site information all sites

Site	Location	Coordinates	Age (at stump height)	Site Index	Mean stump diameter (mm)	Date of establishment
1	Tönnersjö	56.698111, 13.116444	20	G 30	89	08-jul
2	Tönnersjö	56.674444, 13.083861	20	G32	70	09-jul
3	Asa	57.149139, 14.759083	13	G24	46	14-jul
4	Asa	57.149028, 14.780278	21	G28	72	14-jul
5	Asa	57.124583, 14.761556	7	G28	37	15-jul
6	Asa	57.133194, 14.765917	7	G27	36	16-jul
7	Vidöstern	57.016056, 13.937250	22	G34	95	17-jul
8	Tönnersjö	56.679917, 13.096528	12	G32	75	18-jul
9	Klippan	56.154639, 13.169944	15	G32	44	21-aug
10	Rattsjö	60.366056, 12.911944	19	G26	55	30-jul
11	Rattsjö	60.366944, 12.888167	28	G22	52	31-jul
12	Vitsand	60.397750, 12.952806	11	G24	43	01-aug
13	Stensgård	60.158528, 13.076167	11	G28	39	02-aug
14	Slättene	60.752417, 12.817194	15	G24	47	03-aug
15	Siljansfors	60.909833, 14.381500	40	G28	108	04-aug
16	Siljansfors	60.889917, 14.406667	35	G26	88	05-aug
17	Siljansfors	60.868083, 14.368639	19	G28	72	06-aug
18	Rattsjö	60.341889, 12.927083	15	G28	47	29-jul

Appendix 2

Table 6. Weather data on the day of establishment as well as average five days before and infection frequency on the different sites. Local variation has to be taken into account, weather station not on site. Data from (SMHI 2015)

Site	Location	Date of establishment	Temperature (°C)	Average temperature (°C) 5 days before establishment	Precipitation (mm)	Average precipitation (mm) 5 days before establishment	Infection frequency (%) Control stumps	Infection frequency (%) *Phlebiopsis gigantea* treated stump
1	Tönnersjö	08-jul	22,3	21	8,5	3,26 (Göteborg)	26,3	16,0
2	Tönnersjö	09-jul	25,9	23	0	3,26 (Göteborg)	20,0	0
3	Asa	14-jul	15,9	17	0	6,68 (Växjö)	15,0	5,0
4	Asa	14-jul	15,9	17	0	6,68 (Växjö)	15,0	0
5	Asa	15-jul	17,5	16	0	6,68 (Växjö)	10,0	10,0
6	Asa	16-jul	16,9	16	0	6,68 (Växjö)	0	0
7	Vidöstern	17-jul	17,4	16	0	6,68 (Växjö)	55,0	10,5
8	Tönnersjö	18-jul	19,7	19	0	5,76 (Växjö)	5,0	15
9	Klippan	21-aug	14,9	16	0	4,28 (Lund)	0	0
10	Rattsjö	30-jul	19,4	21	0	0,22 (Karlstad)	0	0
11	Rattsjö	31-jul	18,7	20	0	0 (Karlstad)	0	0
12	Vitsand	01-aug	18,6	20	0	0 (Karlstad)	0	0
13	Stensgård	02-aug	20,4	21	1,5	0,3 (Karlstad)	0	0
14	Slättene	03-aug	19	20	37,1	7,72 (Karlstad)	0	0
15	Siljansfors	04-aug	25,9	22	0	0 (Falun)	0	0
16	Siljansfors	05-aug	22,4	23	14,2	2,84 (Falun)	0	0
17	Siljansfors	06-aug	19,8	22	11,8	5,2 (Falun)	0	0
18	Rattsjö	29-jul	21,2	21,8	0	0 (Karlstad)	0	0