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Swedish University of Agricultural Sciences

Faculty of Forest Science
Department of Southern Sweden Forest Research Centre

Stump treatment against *Heterobasidion* root rot in practical forestry - How well does it work?

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Abstract

The effect of *Phlebiopsis gigantea* on root and butt rot causing fungus *Heterobasidion* spp. was investigated in fifteen stands of Norway spruce (*Picea abies*) in southern Sweden. The stumps were mechanically treated immediately after felling and only stumps with no sign of decay were sampled for the study. A pictorial sampling of 396 stumps was conducted one to six hours after felling to analyze the treatment coverage. Six to seven weeks later, discs of the photographed stumps were cut and analyzed for the presence of *Heterobasidion*. The effect of *P. gigantea* on *Heterobasidion* was calculated using four measurements: treatment coverage of Rotstop®S Gel, relative infected area, number of *Heterobasidion* colonies per disc and infection frequency (proportion of infected stumps). Overall, treatment with Rotstop®S Gel did not protect against spore infection. Larger stumps were more susceptible and suffered from more severe infections, regarding size and number of *Heterobasidion* colonies. However, the relative infected area decreased with an increasing coverage of *P. gigantea*, highlighting the importance of having a 100% treatment coverage on all stumps. By contrast, treatment coverage failed to reach minimum coverage (85-95%), commonly required by stakeholders, on a large proportion of the stumps, pointing to the need of improving the technology calibration. Further studies on stump treatment against root and butt rot in practice are necessary, as the results from this study are inferior to earlier studies with controlled measurements.

Keywords: *Heterobasidion, Phlebiopsis gigantea*, stump treatment efficacy, Rotstop®S Gel

Sammanfattning

Effekten av *Phlebiopsis gigantea* på rotrötesvampen *Heterobasidion* spp. undersöktes i femton granbestånd (*Picea abies*) i södra Sverige. Stubbarna behandlades mekaniskt omedelbart efter fällning och endast friska stubbar utan tecken på röta valdes ut för studien. En bildsamling av 396 stubbar utfördes en till sex timmar efter fällning för att analysera täckningsgraden av behandlingen. Sex till sju veckor senare kapades diskar från de tidigare fotograferade stubbarna för att analysera förekomsten av *Heterobasidion.* Effekten av *P. gigantea* på *Heterobasidion* beräknades m.h.a. fyra mått: täckningsgraden av Rotstop®S Gel, den relativa infekterade arean, antal kolonier av *Heterobasidion* per disk och infektionsfrekvensen (proportionen av infekterade stubbar). Resultaten visade att Rotstop®S Gel inte skyddade mot sporinfektioner. Större stubbar hade högre infektionsbenägenhet och led av svårare infektioner, gällande storlek och antal kolonier av *Heterobasidion*. Däremot minskade den relativa infekterade arean med en ökande täckningsgrad av *P. gigantea,* vilket påvisar vikten av att behandla alla stubbar med 100% täckningsgrad. Däremot uppnådde endast liten andel av stubbarna minimumkraven (85–95%), som ofta är begärda av skogsföretagen, vilket pekar på att kalibrering av teknologin behöver förbättras. Fler studier på rot- och stubbröta i praktiken är nödvändiga, eftersom resultaten från denna studie är sämre än tidigare studier med kontrollerade mått.

Nyckelord: *Heterobasidion, Phlebiopsis gigantea*, Stubbehandling, Rotstop®S Gel

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1. Introduction

Swedish forestry is highly dependent on vital Norway spruce (*Picea abies*) trees, representing 41% of the tree composition in Sweden (https://www.skogssverige.se/gran). Science and technology are incessantly working to find and improve methods optimizing economic return. This include treatments to combat root and butt rot on Norway spruce, caused by *Heterobasidion parviporum* Niemelä and Korhonen and *Heterobasidion annosum* (Fr.) Bref. s.s., in this study referred to as *Heterobasidion*. Approximately 14% of Norway spruce trees are infected by *Heterobasidion* (Thor et al., 2005), causing an annual economic loss estimated to €0,5-1 billion in Scandinavia (Bendz-Hellgren and Stenlid, 1995; Bendz-Hellgren et al., 1998). At temperatures above 0°C, airborne basidiospores of *Heterobasidion* disperse and germinate fresh wounds and freshly cut stumps of Norway spruce (Rishbeth, 1951a; Isomäki and Kallio 1974). Secondary infections on healthy trees occur when fungal mycelia spread from infected stumps through interconnected roots (Rishbeth, 1951; Stenlid and Redfern, 1998), over time causing reduced growth and wood decay, leading to an increased risk of windthrow (Garbelotto and Gonthier, 2013).

Phlebiopsis gigantea (Fr.) Jül, the active component of the treatment agent Rotstop®, have proven to be an effective competitor against *Heterobasidion* on freshly cut stumps (Rishbeth 1963; Thor et al., 2005). However, this considers uninfected stumps as *P. gigantea* might not be able to compete with *Heterobasidion* if it already has established in the stump (Korhonen et al., 1994).

In Sweden, approximately 35’000 ha of commercially thinned forests are treated with Rotstop® (Thor, 2003). The original formula was developed in 1991, with *P. gigantea* oidiospores deriving from Finland (Korhonen et al., 1994). However, in 2004 Verdera Oy launched a new biocontrol, Rotstop®S Gel, containing Swedish isolates of *P. gigantea*, proven to be more effective as stump treatment against *Heterobasidion* infections in Sweden (Berglund and Rönnberg, 2005). Today, Rotstop®S Gel is the primary biocontrol commercially utilized in Sweden (https://www.interagroskog.se/rotroeta-rotstop/).

P. gigantea is highly sensitive to temperatures above 40°C and requires attentive management to sustain vital. Thus, the manufacturer’s instructions must be strictly followed in order to obtain full effect of the treatment, e.g. storing the solution in the fridge after reception from the distributor, or utilizing pre-mixed solutions within 36 hours (https://www.interagroskog.se/rotroeta-rotstop/).
Skepticism towards treatment efficacy have been emphasized by forest entrepreneurs. This partly derives from the additional work and perception of unpaid time managing Rotstop®S Gel which, depending on latitude, is utilized 4-8 months of the year in accordance with the manufacturer’s guidelines (https://www.interagroskog.se/rotroeta-rotstop/) to apply

treatment when average temperature exceeds +5°C (Bendz-Hellgren et al., 1998).

What seems to be lacking is a comprehensive study on the true efficacy from using stump treatment in regular forest operations considering the normal problems one may encounter during such operations; e.g. product handling (from its delivery to its actual application on stumps), issues with technical aspects in the machinery causing a faulty mixture of the agent, too high pressure in the hydraulic system killing *P. gigantea* spores, dilution of the substance on the stump due to heavy rainfall after felling, or just simple ignorance and purposely avoiding adding the agent to newly created stumps.

The purpose of this study was to investigate the correlation between the level of treatment coverage and infection frequency, number of *Heterobasidion* colonies and relative infected area on stumps. The intention was to investigate and audit a large number of forest stands that had been recently thinned and putatively treated with Rotstop®S Gel to provide "proof-of-concept" from a practical perspective.
Our hypothesis is that stump treatment is not performed properly, but when properly performed it has a significant effect on reducing and protecting stumps from infections by *Heterobasidion*.
The results are hence aimed to provide concrete evidence of treatment efficacy to mitigate the concerns of stakeholders, encourage continuous use of stump treatment in practice and consequently promote sustainable forestry in Sweden.

2. Material and method

2.1 Study sites and experimental designs

This experiment was established in 15 Norway spruce commercially thinned sites across southern Sweden (Fig. 1), between Sep-Oct 2017 and May-Aug 2018 (Tab. 1). Each stand was thinned by a single grip harvester in locations where infections from *Heterobasidion* are endemic. All stumps were immediately treated with a suspension of *P. gigantea* oidiospores at felling by an integrated system for dispersing Rotstop®S Gel in the harvester heads. On each site, 24-30 stumps were randomly selected for pictorial and wooden sampling to identify the presence of *P. gigantea*.

The presence of *P. gigantea* would evidence whether Rotstop®S Gel was added in the pre-mixed solution, containing a blue dye, water and Rotstop®S Gel, as suspicions have been stressed on machine drivers only adding blue dye to the solution, creating a visual deception. Findings of vital *P. gigantea* would certify whether Rotstop®S Gel was managed correctly before treatment application.

Stumps with any sign of decay was discarded. Six to seven weeks after treatment, discs of the previously marked stumps were collected to analyze the presence *Heterobasidion.* No data was collected on previous land use, stump height, spore abundance or climate conditions.

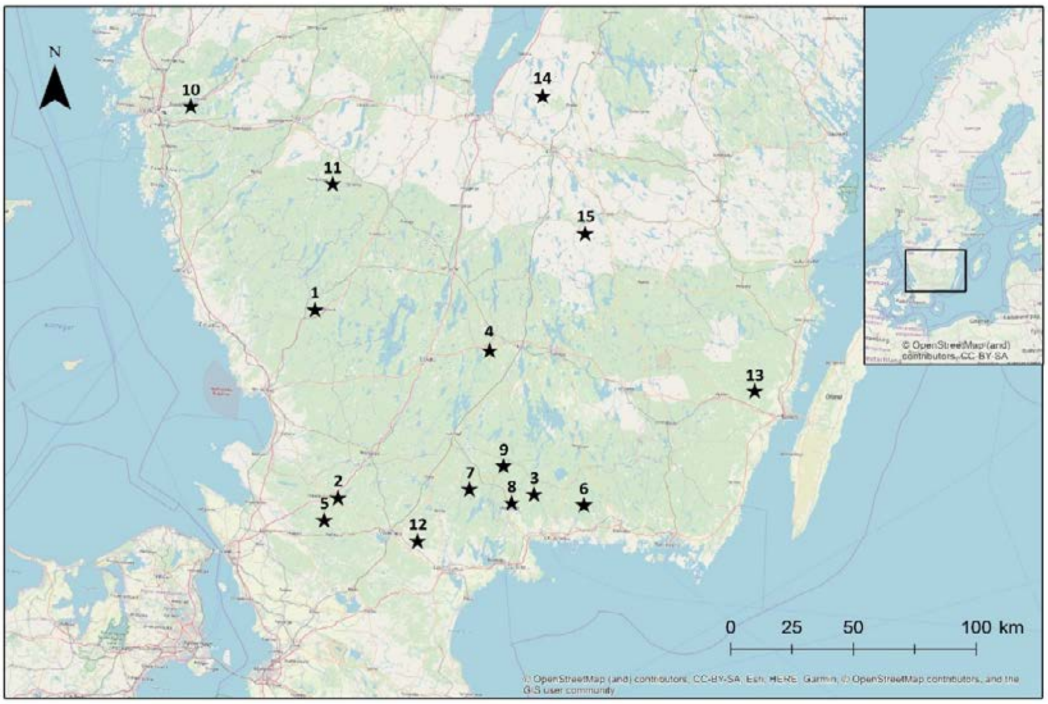

Fig. 1. Study site locations

Table 1. Name, location, and date of disc collection of the study sites.

Site	Name of area	Latitude	Longitude	Disc collection
1	Segelsbo	56.995408	13.160695	2017-11-15
2	Örkelljunga	56.280233	13.404425	2017-11-22
3	Hemsjö	56.336516	14.690919	2017-11-23
4	Lyngsåsa	56.879983	14.343667	2017-11-28
5	Toarp	56.191663	13.323821	2017-12-05
6	Högahult	56.304557	15.021981	2017-12-07
7	Vesslarp	56.343798	14.26264	2018-06-25
8	Biskopsmåla	56.299572	14.545804	2018-06-26
9	Rumpeboda	56.439878	14.479006	2018-06-27
10	Gullringsbo	57.740018	12.208672	2018-07-24
11	Uddebo	57.48337	13.216534	2018-07-25
12	Smålanden	56.133491	13.943524	2018-08-01
13	Ingelsryd	56.76458	16.122857	2018-08-02
14	Breafall	57.866973	14.607942	2018-08-22
15	Rosenholm	57.345922	14.944948	2018-08-23

2.2 Sampling and laboratory analysis

In total 396 stumps were cross calipered, photographed, marked with a GPS-point, a label marker, ribbon and spray. The pictures were taken one to six hours after felling, to ensure the color would stay intact. On every third stump three wooden cubes were extracted by using a chisel, to analyze the presence of *P. gigantea*. The cubes were wrapped in aluminum foil and stored in room temperature (20-23°C) before being freeze-dried for at least ten days. Afterwards, the cubes were pulverized in a ball mill and applied in PCR amplification and sequencing to extract DNA of *P. gigantea*. The presence of *P. gigantea* is still a work in progress due to difficulties with extracting DNA from the wooden samples. Thus, it will not be displayed in this thesis. However, once the DNA of *P. gigantea* have been successfully extracted and analyzed it will add valuable information to the results.

Six to seven weeks after thinning, discs were cut from the 396 stumps. To prevent contamination from spores on the bark, the sides of each stump were sprayed with 70% ethanol. The first two cm of the stumps were cut off and discarded. The following three cm were cut into discs and immediately transferred into plastic bags, each sealed in a separate bag. The discs were incubated in darkness for seven to ten days in room temperature (20-23°C), before being analyzed for the presence of *Heterobasidion* on the upper side of the discs, using a dissecting microscope.

The treatment covered area was identified in two ways; by manually drawing around areas and by automation, using “image J”, an image processing software, to then calculate the areas. Some discs had a light blue color that was not visible on pictures. Consequently, a visual estimation of the coverage was made directly on site in addition to the pictures. The estimation was made by covering one half of the disc at a time, without touching the disc, estimating the color percentage on each side to thereafter summarize the two percentages. Discs that looked fully covered on site or in pictures were identified as 100% covered and were not further analyzed.

The pictures were initially processed in Gimp, where everything but the area of the stump was removed from the picture (*Fig. 2 & 4*). Afterwards, the cropped pictures were transferred into “Image J” where the area of the discs were modified into pixels. The colored area was then calculated by counting the total number of blue pixels in relative to the total number of pixels (*Fig. 2 & 3*), yielding a percentage to multiply with the cross calipered based area of the stump. In some pictures “Image J” failed to detect colored areas, due to a light blue color that was only visual to the eye (*Fig. 4 & 5*). In these occasions, adding manual drawing was necessary to complete assessing the covered area. The area of *Heterobasidion* was calculated with the same procedure as for treatment coverage, once analyzed for its presence under microscope.

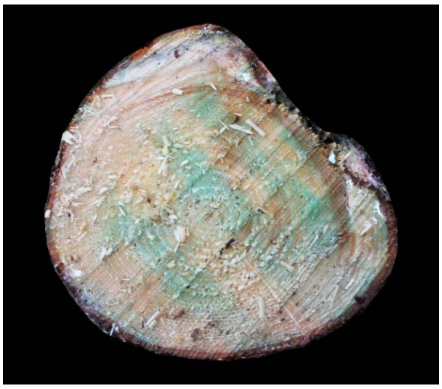

Fig. 2. Picture of a stumps surface with clear visibility of areas colored by treatment agent Rotstop®S Gel.

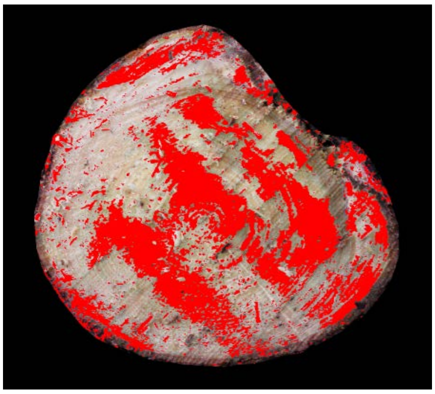

Fig. 3. Full detection of colored areas by “Image J”.

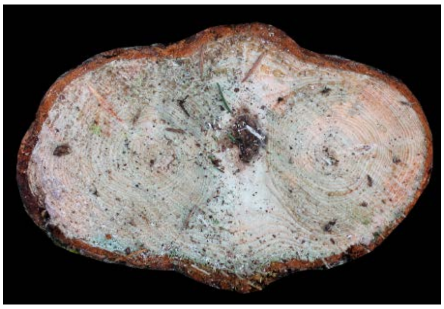

Fig. 4. Picture of a stumps surface with low visibility of areas colored by treatment agent Rotstop®S Gel.

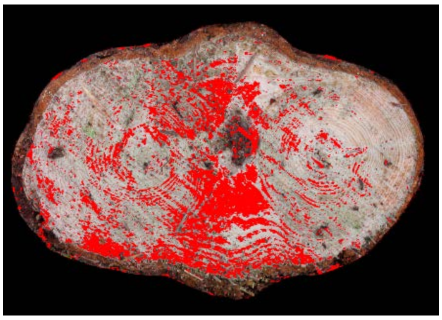

Fig. 5. Imperfect detection of colored areas by “Image J”.

2.3 Calculations and statistics

The effect of *P. gigantea* on *Heterobasidion* was measured and evaluated based on the following parameters:

1. Treatment coverage of Rotstop®S Gel (proportion of stump surface)
2. Relative infected area (proportion of stump surface)
3. Number of *Heterobasidion* colonies per disc and
4. Infection frequency (proportion of infected stumps)

In addition, the sample pool of stumps was divided into two groups: infected and non-infected stumps. The mean treatment coverage and mean stump size were tested for the groups.

To simplify calculations, treatment coverage was divided into classes of ten, from 0-100, e.g. all stumps with treatment coverage 80-89,99% were included in coverage class 80. Only stumps with 100% coverage were included in coverage class 100. Hence, onward all statistical analysis regarding treatment coverage will be referring to coverage classes of the treatment coverage. Areas of the stumps were calculated based on the cross calipered diameter of the stump. Treatment coverage was based on the colored area in relative to the total area of the disc. Frequency of infection was based on the number of discs in which *Heterobasidion* was identified under a dissecting microscope. The relative infected area was calculated as the aggregated area of infection on a disc in relative to the total area of the disc.

All statistical analysis and visualizations were made using Microsoft Excel (2017). Identifying existing correlations was performed using the regression tool, providing p-values. A p-value below 0,05 would evidence significance, indicating a strong probability for correlation between the two given variables, and vice versa. All tests for correlations only included two parameters at a time.

Treatment coverage was analyzed for correlation with infection frequency. Finding a negative correlation would evidence a decreasing infection frequency with an increasing treatment coverage, while a missing correlation would indicate that treatment coverage, regardless of coverage class, does not affect the incidence of spore infection.

Treatment coverage was analyzed for correlation with the relative infected area. A negative correlation between treatment coverage and relative infected area indicates, the relative infected area would decrease with an increasing treatment coverage.

Treatment coverage was analyzed for a correlation with number of *Heterobasidion* colonies. A negative correlation would evidence whether treatment coverage has an effect on decreasing the number of *Heterobasidion* colonies with an increasing treatment coverage.

Treatment coverage was analyzed for a correlation with stump size. A positive or negative correlation between these parameters would evidence whether treatment coverage increases or decreases with an increasing or decreasing stump size, while a missing correlation would indicate that treatment coverage, regardless of coverage class, does not depend on stump size. This could clarify the precision and selectiveness of the mechanical dispersion.

All correlations tested with treatment coverage, except for stump size, were to examine whether an increased treatment coverage could decrease the probability and severity of infection. Thus, it would verify the importance of precision when treating stumps with Rotstop®S Gel.

Stump size was analyzed for correlation with infection frequency, area of infection, relative infected area and number of colonies, individually. Finding a positive or negative correlation would indicate whether small or large stumps are more susceptible to infections, regarding areal size of the infection, proportion to stump size and number of colonies.

Lastly, number of colonies and area of infection was analyzed for a correlation. A positive correlation would evidence an increment in area of infection with an increasing number of colonies.

Means of treatment area and stump size on infected and uninfected stumps were calculated using an independent two sample t-test. The t-test compared the means of treatment area and stump size, individually, on infected and uninfected stumps which determined whether there was a statistical evidence that the means a had significant difference. Thus, a p-value lower than 0,05 would indicate a significant difference between the means of stump treatment or stump size on infected and uninfected stumps, and vice versa.

For treatment coverage, a difference between means of infected and uninfected stumps would indicate a correlation between infection frequency and coverage class, while an equal mean would indicate that spore infection does not depend on coverage class. A difference in means of stump size between infected and uninfected stumps would evidence whether infections are more frequent in small or large stumps.

Table 2. Description of measurements assembled for correlation analysis. Each x represents an individual test for a correlation between the measurements in the given column and row.

	Treatment coverage	Number of colonies	Stump size
Infection frequency	x		x
Area of infection		x	x
Relative infect. area	x		x
Number of colonies	x		x
Stump size	x		

3. Results

Infections of *Heterobasidion* were found in 45% (n = 178) of the stumps. Three of the sites exceeded an infection frequency of 70%, number 3 (72%), 11 (86%) and 13 (79%) respectively, while one of the sites, number 9, had an exceptionally low infection frequency (10%) with only two infected stumps (*Fig. 6*). All sites suffered from *Heterobasidion* infections (*Table 3).*

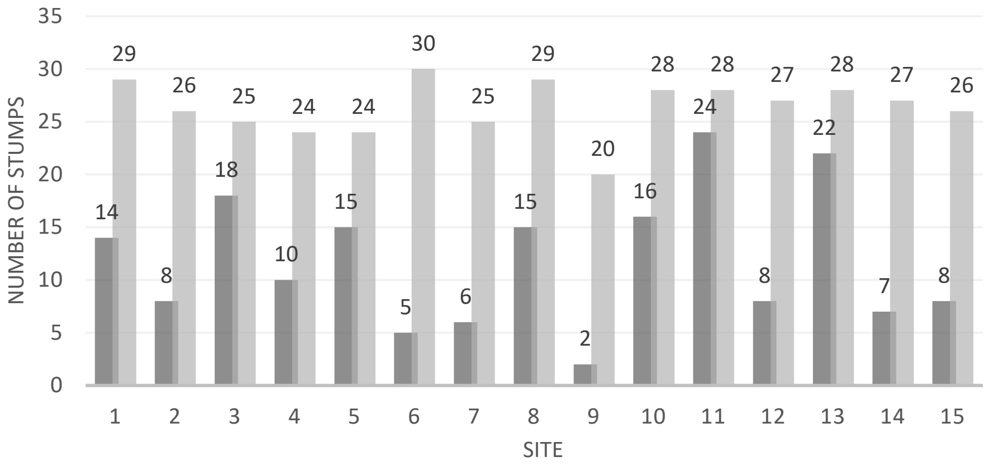

Fig. 6. The number of infected stumps (■) and total number of stumps (□) on each site.

Table 3. Parameter means for each site.

Site	Treatment coverage	Stump diameter	Stump area	Colony size	Area of infection (cm2)	Relative infected area
1	77,4	13,7	156,0	1,7	1,1	1,0%
2	81,8	15,8	204,1	1,0	0,4	0,2%
3	68,3	25,8	574,9	5,5	3,4	0,8%
4	75,0	13,8	155,7	0,8	0,3	0,2%
5	90,8	13,3	142,6	1,1	0,3	0,3%
6	88,7	11,6	108,4	0,2	0,1	0,1%
7	87,6	16,1	225,2	0,4	0,1	0,0%
8	90,2	19,7	323,3	1,7	0,4	0,1%
9	98,5	11,3	105,3	0,3	0,1	0,2%
10	87,1	11,9	118,9	2,2	0,7	0,8%
11	87,0	14,4	179,6	6,3	1,5	1,2%
12	82,9	18,1	281,9	1,0	0,3	0,2%
13	93,2	21,5	391,4	6,3	2,4	0,6%
14	84,5	15,6	217,2	0,9	0,5	0,4%
15	77,7	17,3	280,8	0,6	0,9	0,3%

3.1 Probability of infection and treatment coverage

Treatment coverage varied between stumps, 89% (n = 353) had a treatment coverage of 70% or more, 78% (n = 308) had a treatment coverage of 80% or more and 19% of the stumps had 100% coverage (*Fig. 7*).

No correlation was found between treatment coverage and infection frequency on the stumps (*p* = 0,855) (Fig. 8). This means the treatment coverage, regardless of coverage class, did not protect stumps from getting infected. However, an increased treatment coverage lowered the relative infected area on infected stumps (*p* < 0,001) (*Fig. 9*), while an individual analysis of each site showed that only three of the sites had a correlation between treatment coverage and relative infected area. The number of *Heterobasidion* colonies was not affected by the treatment coverage (*p* = 0,118) (*Fig. 10*). By contrast, the number of colonies, regardless of treatment coverage, increased with an increased area of infection (*p* < 0,001).

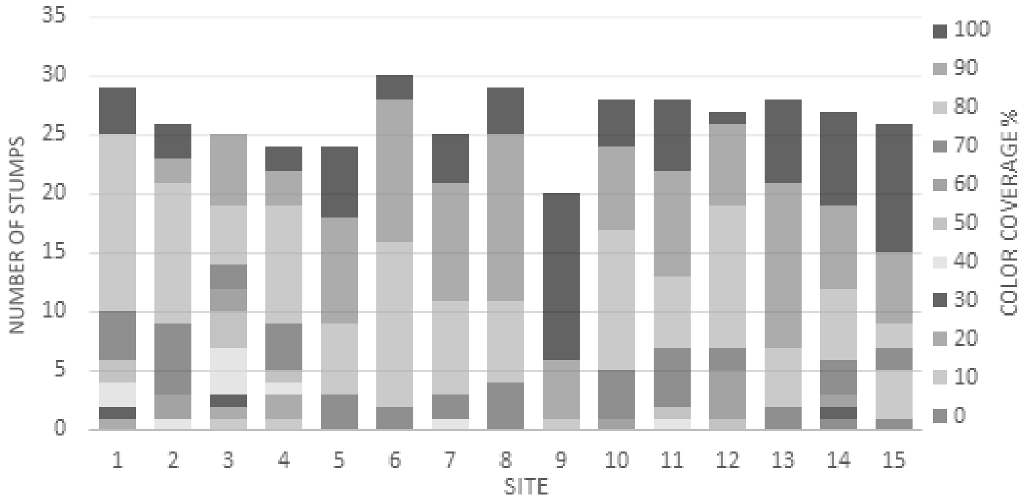

Fig. 7. The number of stumps for each coverage class per site.

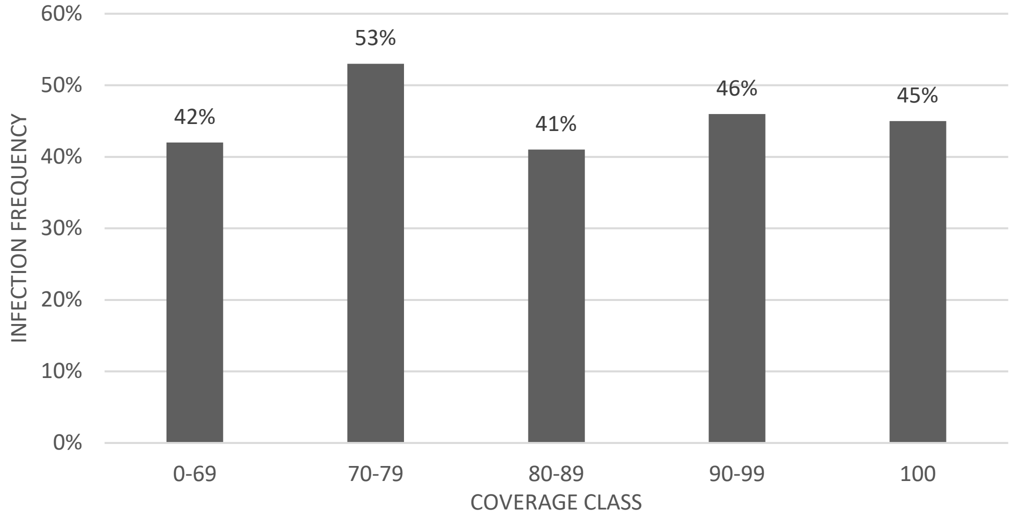

Fig. 8. The frequency of infected stumps per coverage class.

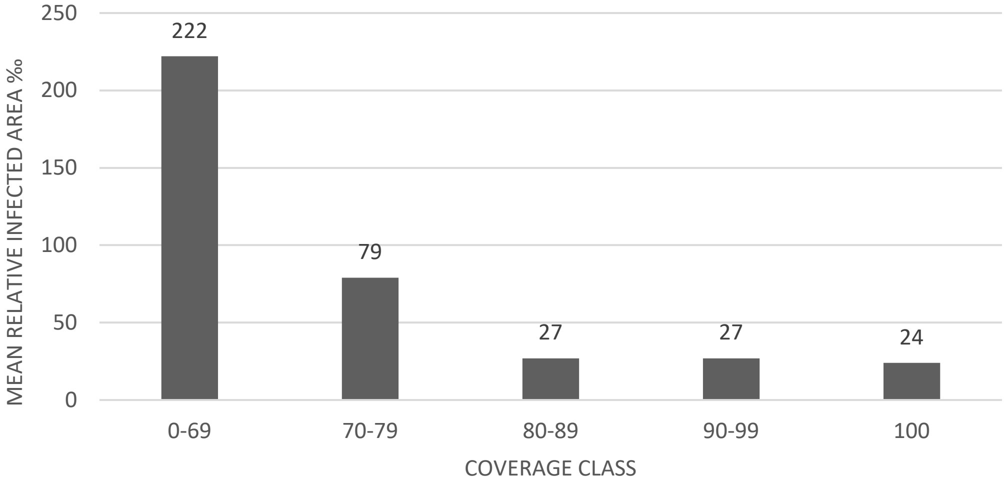

Fig. 9. The mean relative infected area (‰) per coverage class.

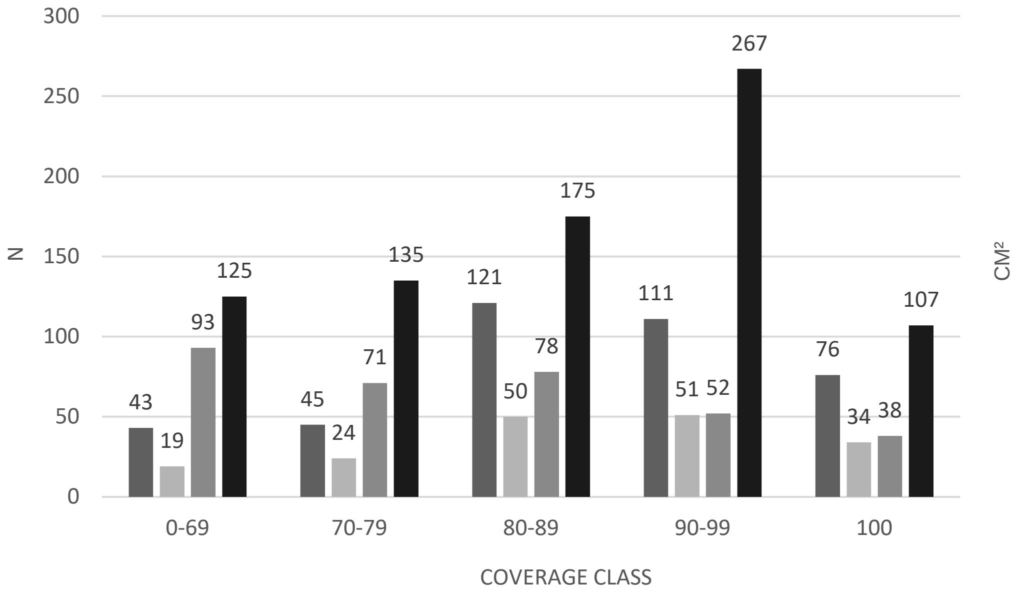

Fig. 10. The total number of stumps (■), infected stumps (■), area of infection (cm2) (■) and number of *Heterobasidion* colonies (■) per coverage class.

3.2 Probability of infection and stump size

The stump size varied between 8 and 44 cm diameter, corresponding to 48 and 1521 cm^2. The treatment coverage did not differ with stump size (p = 0,466). Thus, you can expect a similar result, regardless if the dispersal of treatment is performed well or not, on all stump sizes.

Excluding the effect from treatment and treatment coverage on the stumps, the probability of infection increased with an increasing stump size (p < 0,001). Thus, larger stumps had a higher infection frequency than smaller stumps.

No correlation was found between stump size and relative infected area (p = 0,239). Thus, the relative infected area did not differ with stump size. However, the area of infection, as well as the number of *Heterobasidion* colonies, increased with an increasing stump size (p < 0,001). Hence, larger stumps suffered from more severe infections, regarding areal size and number of colonies.

3.3 Additional observations

The mean colored area did not differ between infected (85%) and non-infected (84%) stumps (*Fig. 11*), supporting that frequency of infection did not depend on the treatment coverage. The mean diameter and diameter span were higher in infected (286cm2) than non-infected (187cm2) stumps (*Fig. 11*), meaning larger stumps were more likely to get infected, regardless of the greater diameter span.

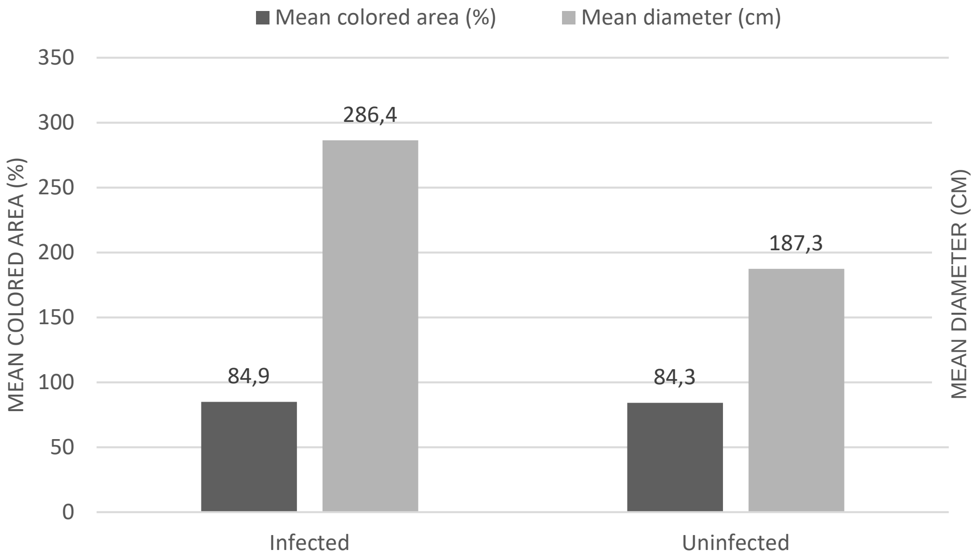

Fig. 11. The mean colored area and mean diameter on infected and uninfected stumps.

Stakeholders' commonly require a minimum treatment coverage of 85% or 95%. In this study, 65% of the stumps had a treatment coverage of 85% while only 26% had a coverage of 95% (*Fig. 12*).

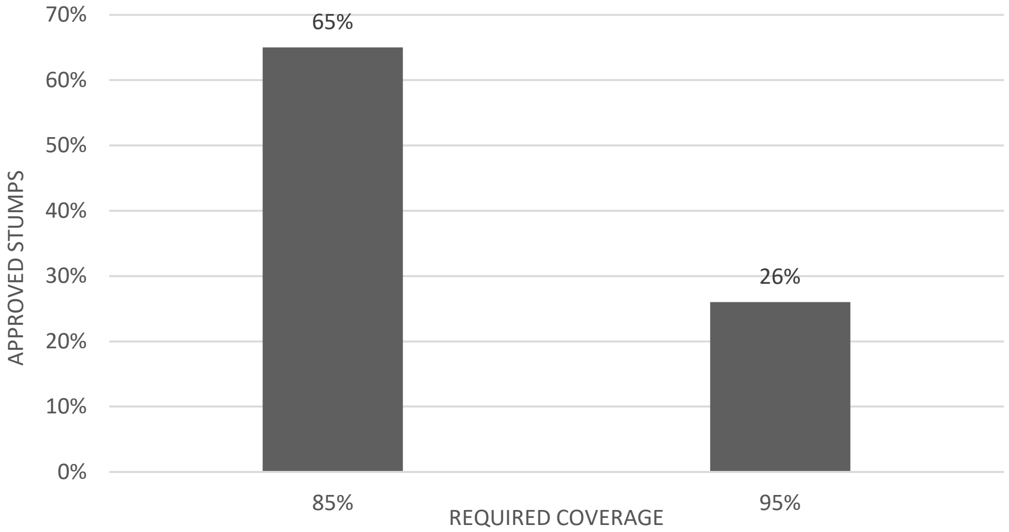

Fig. 12. Minimum required coverage and the percentage of approved stumps for the given requirement.

5. Discussion

Our discoveries and what could potentially affect the results

Stump treatment with *P. gigantea* lowered the relative infected area of *Heterobasidion* on Norway spruce stumps with an increasing treatment coverage. Larger stumps were more susceptible to infections and likely to suffer from severe infections, regarding size and number of

Heterobasidion colonies. However, only 26% of the stumps had a treatment coverage of 95% or more, while 65% of the stumps had a treatment coverage 85% or more. As stakeholders commonly require a treatment coverage of 85% or 95%, these results signal a need for an improved calibration of the technology. Stump treatment of *P. gigantea* was not able to provide full protection against infection by *Heterobasidion* on Norway spruce stumps, in accordance with Rönnberg & Cleary (2012), and did not protect stumps from spore infection. However, our results showed that we to a good extent can control the areal increase of the disease by having a 100% treatment coverage.

Site-specific factors, such as climate and previous land use, were not collected for this study. For example, spore abundance is influenced by temperature and humidity (Redfern and Stenlid, 1998). Our samples were collected in Sep-Oct 2017, characterized by high precipitations, and May-Aug 2018, an unusually dry summer with high temperatures (https://www.smhi.se/data/meteorologi/kartor/). Both conditions may have influenced spore abundance of *Heterobasidion*, this was however not considered in the analysis of the study.

Another element that could potentially have influenced our results is the delay of pictorial sampling, of up to six hours, after felling. Depending on season, the color could potentially oxidize and disappear, leading to a miscalculation of the treatment coverage and pointing to the importance of adding the correct amount of dye to the pre-mixed solution.

Treatment coverage and frequency of infection

In our study *P. gigantea* was not able to provide full protection against spore infection by *Heterobasidion* on Norway spruce stumps. Stumps with 100% treatment coverage had an infection frequency of 45%; the average treatment coverage was near equal in infected and non-infected stumps. These results indicate we cannot predict which stumps will or will not get infected. For instance, some untreated stumps will evade getting infected while others with impeccable treatment coverage will suffer from infection. However, in this study control stumps were not used, meaning there were no data on untreated stumps to compare with the treated stumps. However, in a study by Thor and Stenlid (2005) on mechanized stump treatment in 11 stands of Norway spruce, the frequency of *Heterobasidion* was significantly higher in untreated stumps than in treated stumps, six to seven weeks after summer thinnings. Thirteen years later, Oliva et al. (2010) restudied the frequency of infection in the same stands of Thor and Stenlid (2005) and found that stump treatment had a decreasing effect on *H. annosum* on former agricultural land. On former forest land, stump treatment prevented *H. annosum* from establishing new genets, lowering the diversity of *H. annosum* genotypes, leading to potential reduction of rot incidence in the future. For this reason, we cannot draw any conclusion on the missing correlation between treatment coverage and infection frequency, as numerous factors affect the frequency of infection, of some we did not consider for this study. However, on infected stumps, *P.*

gigantea reduced the relative infected area with an increasing treatment coverage, meaning we to a good extent can control the severity of infection by optimizing the coverage of Rotstop®S Gel on the stumps surface.

Treatment coverage and relative infected area per site

Twelve sites showed no correlation between the relative infected area and treatment coverage, conflicting the result from all sites aggregated. This is due to a shortage of infected stumps for a given coverage class on each site, e.g. site 9 had only two infected stumps, which regardless of coverage class will not be sufficient for a dependable analysis.

Stump size and frequency of infection

Our study found a positive correlation between frequency of infection and stump size, this is in accordance with results from Paludan (1966). Dimitri (1994) argued that young Norway spruce stumps, generally smaller in size, have relatively good resilience against *Heterobasidion* infections. It is assumed that younger stumps have a disadvantageous moisture content for the disease, due to a lack of heartwood. Simultaneously, large stumps have a greater exposed area, increasing the probability for spores to attach to (Vollbrecht et al., 1995). However, 25-55% of stumps in precommercial thinning can get infected (Gunulf et al., 2012), and infections that emerge in early rotation have more time to evolve and might therefore cause greater damage and economic loss (Vollbrecht and Agestam 1995; Cleary et al. 2012). Thus, while large stumps tend to suffer from more frequent and severe infections, it is of high importance to treat all stumps with full coverage.

In addition to stump size, Gunulf et al. (2013) found that age had an impact on resilience against infection. Older stumps were less likely to get infected than younger stumps with a corresponding stump size. Thus, stumps with narrow year rings have a lower probability of getting infected.

Colony- and stump size

Heterobasidion colonies were more likely to be abundant in larger stumps, our study found. Dimitri et al. (1971), Morrison and Jonson (1978) and Berglund and Rönnberg (2004) argued that smaller infections, regarding size and number of colonies, have a lower viability long term, while larger colonies are more likely to survive. Hence, if we treat all stumps with a full treatment coverage the probability of infections surviving and spreading to other healthy trees decreases, in particular from large stumps.

Technical issues

This study was aimed to analyze the efficacy of stump treatment in practice, which in difference from controlled studies did not consider concentration and coverage of *P. gigantea* on stumps, climate conditions, stump height, previous land use or other elements that could have influenced the probability of infection. The prospect of some infections deriving from the root is another possibility we cannot know.

Initially, three spore traps were placed in each stand to collect and measure the abundance of *Heterobasidion* spores. However, no spores were found in the traps. Due to an unfavorable concentration of alcohol they were likely unable to attach and grow on the traps. However, the number, size and location of conidiospore colonies found on discs from stumps suggests that infections were not solely deriving from old infections in the roots. Infections were primarily found in what would have been presumed to be in the sapwood. Stumps with visible decay at establishment of this study were excluded.

Abundance of viable *Heterobasidion* spores, as well as competing fungi, have been argued by Rishbeth (1951a) and Meredith (1959) to highly influence the frequency of infection. Generally, spores of *Heterobasidion* are more abundant during summertime (Rishbeth, 1951a; Isomäki and Kallio 1974), explaining the recommendation of treating stumps with Rotstop®S Gel when average temperature exceeds 5°C. A higher abundance of *Heterobasidion* spores would generate a greater probability for spores to attach to the stumps surface, while other fungal species, such as *P. gigantea*, might outcompete *Heterobasidion*, preventing it from further evolving in the stump (Rishbeth, 1951a; Meredith, 1959). However, for a given spore load, Berglund and Rönnberg (2004; 2005) suggested that the efficacy of Rotstop® would be influenced by site-specific factors, unstudied in the present study, such as humidity and temperature. It is likely that the high precipitations of autumn 2017 and extreme weather conditions in summer of 2018 (https://www.smhi.se/data/meteorologi/kartor/) may have affected the spore abundance of *Heterobasidion*. However, the conclusions from this study will remain.

Stump height was not measured in this study, but we know from anecdotal data that stump height vary depending on machine driver, season, and structure of the sites. Gunulf et al. (2012) studied the effect of *P. gigantea* in an experiment of cutting Norway spruce stumps higher up in the stem, about 60 cm, in precommercial thinnings. The data was compared to stumps cut closer to the ground. No difference was found in treatment efficacy regarding infection frequency, actual infected area, relative infected area, number of colonies or control efficacy on the reduction of infection frequency. This indicates data of stump height would likely not have modified our results.

A margin of error occur for stumps with a delay of pictorial sampling, due to a potential oxidation and fading of color, e.g. stumps in coverage class 90 might have been categorized in coverage class 70. From a practical point of view, it is important to add the recommended amount of dye to the pre-mixed solution. However, it is important not to add more than the recommended amount, as the stronger color might create a delusion of a better treatment coverage than it actually is.

Further study and potential change of results

Extracting DNA of *P. gigantea* from the sampled wooden cubes is still a work in progress and could potentially change the results of this study. *P. gigantea* requires attentive management to endure the long way from the distributor's freezer to its final destination and purpose of protecting, the stump. Any mismanagement, e.g. exposing the solution to higher temperatures than recommended, could potentially exterminate *P. gigantea* and diminish or completely eliminate the effect of treatment. However, due to a potentially low oidiospore abundance and complications with extracting DNA of *P. gigantea* from the wooden samples, the analysis has been delayed, and finding RNA, which would evidence whether *P. gigantea* was vital when applied, is at this stage not a possibility. Finding DNA would verify the existence of *P. gigantea* on the stumps but would not evidence whether it was vital when applied. This means we could demonstrate whether machine drivers added Rotstop®S Gel to the pre-mixed solution, or if they solely added the dye to make a visual deception, while we cannot know if the solution was managed correctly before treating the stumps. However, because of the correlation between relative infected area and treatment coverage, and awaiting the lab results, we must assume the entrepreneurs were applying active Rotstop®S Gel.

6. Practical implications

Conclusively, larger stumps are more likely to suffer from severe infections, regarding size and number of colonies (Gunulf et al.,2013), while infected stumps in early rotations might cause greater damage and economic loss as they have longer time to evolve before final felling (Vollbrecht and Agestam 1995; Cleary et al. 2012). Thus, our findings point to the importance of treating stumps with 100% coverage of biocontrol Rotstop®S Gel. To do so, an improved maintenance and calibration of the technology is necessary, as treatment coverage failed to reach minimum requirement on a large proportion of the stumps. In order to know when technology needs calibration, the treatment coverage needs to be clearly visual for the possibility of machine drivers to make correct estimations of the coverage. Therefore, adding the correct amount of dye to the pre-mixed solution is necessary. Increasing the treatment coverage will not protect the stumps from spore infection but will reduce the relative infected area once infected. Long term, this could potentially eliminate the infection, as smaller colonies have lower chance of survival (Rönnberg and Berglund, 2004). However, further studies are necessary on mechanical stump treatment against root rot in practice, as the results from this study are inferior to earlier studies with controlled measurements, i.e. stump treatment works but seems to be suffering from technical problems when done in regular operations in the field. There is a need to identify the problem(s). Is it about information to contractors on how to do, or is there an issue with the logistical chain for the treatment agent so that once it leaves the retailer it is not handled correctly until it is applied, are the spores of *P. gigantea* viable once sprayed on the stump? These questions would need to be addressed in separate studies.

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