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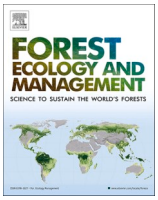

The potential of biological control against *Heterobasidion* root rot is not realized in practical forestry

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ABSTRACT

For about 25 years, forest managers in Sweden have been treating stumps following harvesting with *Phlebiopsis gigantea*, retailed as Rotstop®S gel, against spore infections of *Heterobasidion*, which cause root rot in order to minimize losses in timber production. However, not all forest managers trust the efficacy of stump treatment and this fact has hindered widespread adoption of stump treatment using *P. gigantea*. In this study, we evaluated stump treatment in the field during commercial thinning operations across 15 sites, by assessing the degree of stump coverage and subsequent infection levels in stump discs. In total, 45 % of all stumps were infected with *Heterobasidion* spp.. Nineteen percent of all stumps were considered to have full (100 %) coverage by Rotstop®S but contractors failed to achieve the manufacturers stipulated minimum coverage (85 %) in approximately 1/3 of all stumps. Using PCR, we could only detect the presence of *P. gigantea* in 73 % of the tested stumps. Large variation occurred between stump coverage and the recovery of *P. gigantea* in wood chip samples collected from stump discs across sites. In the worst case, we detected *P. gigantea* in only three out of ten treated stumps at one site. Despite this discrepancy we saw a clear reduction of the size of *Heterobasidion* infections on stumps where stump treatment coverage was more than 85 % of the stump surface.

Our results suggest that forest operators in Sweden repeatedly fail to either apply a spore solution of *P. gigantea* or cover enough of the stumps to provide the desired protection. The outcome of such sub-standard application, could further undermine the usage of biological control agents in forestry and limit any potential control against *Heterobasidion* spp..

1. Introduction

The forest sector in Sweden has long been an integrated and essential part of the national economy. However, several persistent forest health threats are currently jeopardizing sustainable wood production from Swedish forests. Norway spruce (*Picea abies* (L.) Karst.) is one of the most commercially important tree species in Sweden, but about 25 % of its stands are affected by root rot and butt rot, mainly caused by *Heterobasidion* spp.. Infection by *Heterobasidion* spp. leads to downgrading of timber, increased risk of windthrow and growth loss, which can result in severe financial losses (Bendz-Hellgren et al., 1998). Spores of *Heterobasidion* spp. enter stands mainly through the stumps created at thinnings (Rishbeth, 1951a) during the growing season (Brandtberg et al., 1996). The fungus spreads subsequently by mycelial growth to adjacent trees via root grafts and contacts (Rishbeth, 1951b). Spread of *Heterobasidion* spp. can be reduced if a control treatment is applied to surfaces of freshly cut stumps (Rishbeth, 1952, 1957). The saprophytic fungus *Phlebiopsis gigantea* (Fr.), and other chemicals such as urea or borate have been shown to be efficient stump treatments (Kallio, 1971; Korhonen et al., 1994; Rishbeth, 1963).

For the last two decades in Sweden, *P. gigantea* has been the most frequently used agent for stump treatment (Thor, 2003), and from 2017 it is the only control agent approved for application against *Heterobasidion* spp. (Kvakkestad et al., 2020). Multiple studies have

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demonstrated the effectiveness of *P. gigantea* as a stump treatment agent against *Heterobasidion* spp. (Blomquist et al., 2020; Oliva et al., 2010; Pellicciaro et al., 2021; Rönnberg et al., 2006). Several studies have stressed the importance of 100 % coverage of Rotstop on the stump surface (Berglund and Rönnberg, 2004; Oliva et al., 2017; Rönnberg et al., 2006), and the manufacturer of Rotstop®S Gel in Sweden recommends at least 85 % coverage (Rotröta 2019). In spite of overwhelming support of the efficacy of *P. gigantea* shown in research, many contractors are hesitant about stump treatment, perceive it as an annoyance and costly, and/or question its efficacy in practice (Jonas Rönnberg pers. comm).

To our knowledge, there are no previous studies on the efficacy of stump treatments when applied by contractors during regular forest thinnings. Practical issues may arise with improper handling of the product from storage to its actual application on stumps. There might be technical issues with the machinery causing improper application of the agent or temperatures high enough to kill *P. gigantea* spores rendering the treatment inefficacious. Also abiotic factors might affect stump inoculation such as dilution of the agent on the stump surface due to heavy rainfall during or after felling. The aim of this project was to investigate infection levels of *Heterobasidion* spp., the coverage and the presence of the biological control agent *P. gigantea* in several forest stands across southern Sweden that were recently thinned during normal forest practices.

2. Material and method

2.1. Study sites and sampling

The experiment was carried out in thinnings performed by contractors in commercial forest operations, i.e. thinnings and stump treatment were not designed to include controls, and contractors responsible for treatment were not informed about the scope of the study to avoid any biases. Fifteen commercially thinned stands of Norway spruce were selected in southern Sweden where *Heterobasidion* spp. are known to be endemic (Table S1).

Thinnings were conducted between September and October 2017 and between May and August 2018, with single-grip harvesters equipped with a mechanized head for treatment. Immediately after cutting, the stumps were treated with a suspension of *P. gigantea* spores prepared from Rotstop®S gel (Inter Agro Skog AB, Eslöv, Sweden) according to the manufacturer's instructions. A blue indicator dye added to the Rotstop®S formula colored the stumps upon application. In order to assess treatment coverage, 30 treated stumps in each stand were randomly selected for analyses. Any of the selected stumps showing visible evidence of existing decay was excluded from further analyses, rendering a sample of 20–30 stumps in each site (Table 1). Stumps were marked in the field with a label marker and ribbon, and the GPS point was recorded for each location. Each stump was calipered to obtain a diameter measurement and then photographed soon after cutting (within one to six hours after felling to ensure the indicator dye's color was still visible). Some discs had only a faint blue color where they were treated, so in addition we used imaging software to make a visual estimation of the coverage on site. The estimation was made by covering one-half of the disc at a time and estimating the color percentage on each side and summing the coverage for the whole disc. Discs that appeared to be fully covered on site or in photographs were considered to have 100 % coverage and were not analyzed further using imaging software. To verify the presence of *P. gigantea* on treated stumps, wood samples (1 cm × 1 cm × 0.5 cm) were taken from the surface of the stumps immediately after Rotstop treatment. A sterile chisel was used to collect each sample. Ten stumps were sampled for each site for a total of 150 samples. Wood samples were wrapped in aluminum foil and transported to the laboratory for further processing.

Six to seven weeks after thinning, sites were revisited and discs from the marked stumps were collected to analyze the presence of *Heterobasidion* spp. To prevent contamination from spores on the bark, the side of each stump was sprayed with 70 % ethanol. The top two centimeters of each stump was cut off with a chainsaw and discarded, before cutting a 3 cm disc, which was immediately transferred into a plastic bag and sealed. At the lab, discs were incubated in the dark for seven to ten days at room temperature (approximate 20–23 °C), and then analyzed for the presence of *Heterobasidion* spp. conidia on the disc surface using a stereo microscope. The edges of the infections were marked on the disc. Infected area was measured using a transparent 1-cm grid paper and the number of colonies was noted. Infection frequency of *Heterobasidion* spp. and the relative infected area was determined for each site.

2.2. Image processing of treatment coverage on stump surface

For stumps that were not considered to have 100 % treatment coverage, we used the image analysis software ImageJ (version 1.52) (Schneider et al., 2012) that utilized the blue dye. To isolate areas with blue pigment, the pictures were processed in GNU Image Manipulation Program (GIMP) (version 2.10), and everything but the treated area was removed. The image was then cropped and transferred into ImageJ which transformed the treated area into pixels. The colored area was then calculated as a proportion of the number of blue pixels relative to the total number of pixels, yielding a percentage to multiply with the cross calipered based area of the stump. Where ImageJ failed to detect

Table 1
Number of sampled stumps, stump area, frequency of *Heterobasidion* infected stumps, stump treatment coverage, positive *P. gigantea* PCR, mean number of *Heterobasidion* colonies and relative infected area for infected stumps for each site.

Site	Number of stumps	Mean stump size (cm^2)	Infected (%)	Mean coverage (%)	Positive PCR (%)	Mean number of colonies/ dm^2	Mean relative infected area (%)
1	29	156 ± 15	48	77.4 ± 3.82	80	2.76 ± 0.95	2.05 ± 1.07
2	26	204 ± 17	31	81.82 ± 2.2	60	2.15 ± 0.87	0.67 ± 0.24
3	25	575 ± 74	72	68.25 ± 4.87	60	1.65 ± 0.41	1.1 ± 0.44
4*	24	156 ± 13	42	74.96 ± 5.25	89	1.08 ± 0.12	0.48 ± 0.26
5	24	143 ± 11	62	90.8 ± 1.74	80	1.56 ± 0.27	0.46 ± 0.15
6	30	108 ± 6	17	88.71 ± 1.3	70	1.54 ± 0.42	0.77 ± 0.35
7	25	225 ± 34	24	87.65 ± 2.4	90	1.08 ± 0.35	0.18 ± 0.08
8	27	327 ± 33	56	90.28 ± 1.53	100	1.12 ± 0.28	0.27 ± 0.09
9	20	105 ± 10	10	98.54 ± 0.6	60	5.37 ± 4.1	1.58 ± 1.26
10	28	119 ± 12	57	87.11 ± 1.67	70	3.71 ± 0.56	1.33 ± 0.34
11	28	180 ± 22	86	86.98 ± 2.7	70	4.55 ± 0.88	1.36 ± 0.33
12	27	282 ± 35	30	82.93 ± 2.14	70	1.61 ± 0.73	0.54 ± 0.25
13	28	391 ± 41	79	93.16 ± 1.28	80	2.27 ± 0.46	0.76 ± 0.34
14	27	217 ± 32	26	84.51 ± 4.24	30	1.86 ± 0.76	1.39 ± 1.24
15	26	281 ± 57	31	77.66 ± 7.06	90	0.93 ± 0.34	0.84 ± 0.57

*One stump in site four could not be used in PCR due to insufficient DNA.

colored areas due to the presence of only a faint blue color, the visual estimations of coverage from the field were used to provide a more accurate estimate of the coverage.

2.3. Optimization of protocol for PCR

To optimize molecular detection of *P. gigantea* from the wood samples collected from stumps post-thinning and treatment, a controlled laboratory study was conducted that simulated treatment on fresh woody stem billets. For this, a 21-year-old Norway spruce tree was cut down, the branches and outer bark were manually removed with a knife (sterilized with 70 % ethanol), and 20 discs (about 1.5 cm thick) were cut with an electric reciprocating saw. The saw blade was sterilized with 70 % ethanol after each cut. Discs were immediately transferred to a sterile hood in the lab and phloem tissue was peeled off the discs and discarded. Ten discs were treated with sterile water and ten were treated with Rotstop®S diluted in sterile water to the manufacturer's recommended concentration of 1 g l^{-1}. For both treatments, the liquid was applied with a sterile syringe such that it totally covered the disc surface with a 1 mm thick layer, estimated visually. Discs were left in the hoods for 2 h after which the treated top layer of wood (1 cm × 1 cm × 0.5 cm) was excised with a scalpel.

Excised wood tissue was lyophilized using SCANVAC CoolSafe™ during a week and ground to a fine powder in a Retsch MM 400 ball mill. DNA was extracted from the milled tissue using (Inglis et al., 2018) CTAB method with the following minor modifications: 40 mg of tissue was used, and 0.5 µl of 5 mg ml^{-1} glycogen (RNA grade) was added to each sample during the isopropanol precipitation step. To serve as a positive control, DNA from the RotStop®S suspension, not the concentrate, used to treat the discs in the controlled study was extracted by using the above method on pelleted material following centrifugation of the solution. DNA concentrations were determined using the Qubit high sensitivity dsDNA kit.

The presence or absence of *P. gigantea* was ascertained for all wood samples using nested PCR targeting the GH28 gene (GenBank KN840450.1) described in (Hori et al., 2014) using outer primer pair GH28 and nested pair GH28nest (appendix B). For reactions using GH28 primers, 4 ng of template DNA was used in a 50 µl reaction with an initial denaturation step at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, and annealing at 53 °C for 1 min and 72 °C for 1 min, followed by a final extension step at 72 °C for 6 min. Resulting products were diluted 1:250 with nuclease-free water. For reactions using GH28nest primers, 1.5 µl of the diluted product was used in a 25 µl reaction with an initial denaturation step at 95 °C for 5 min followed by 25 cycles of 95 °C for 1 min, and annealing at 55 °C for 1 min and 72 °C for 1 min, followed by a final extension step at 72 °C for 6 min. All reactions were conducted using Platinum™ II Hot-Start PCR Master Mix (2X) on an Eppendorf Mastercycler 5333. Products were visualized on 1.5 % agarose gel in Tris-acetic acid-EDTA buffer (TAE) with GelRed (see appendix B). If no products were visible in the nested round for a sample, both PCRs were repeated to confirm it as negative.

2.4. Calculations and statistics

For each site the following descriptive information was calculated: the percentage of samples where *P. gigantea* could be reisolated using PCR, the mean stump size (cm^2), infected stumps (%), the mean coverage (%), mean number of colonies per dm^2 and mean relative infected area. The relative infected area for each stump was calculated as the infected area divided by the total area. For each stand data was grouped into three categories: stumps with less than 85 % coverage, stumps with a coverage of *P. gigantea* ranging from 85 to 99 %, and stumps with 100 % coverage.

Statistical analysis was performed in the same way for the relative infected area and the number of colonies per dm^2. Due to uneven infection pressure, uneven application of treatment across sites, and no difference in number of infected trees regardless of treatment coverage, stumps with no colonies were removed before statistical analysis. The removal of non infected trees was not done when testing if the PCR results were affected by coverage. Across all models, the site was used as a random factor in a linear mixed-effects model from package lme4 (Bates et al., 2015). Pairwise comparisons were made using least-squares means from package emmeans (Lenth et al., 2018), and was done between the aforementioned three groups with different treatment coverage. All data handling and visualization was done using tidyverse (Wickham et al., 2019) in R (R Core Team, 2021).

3. Results

Of the total 450 stumps sampled across 15 sites, the average area of the stumps ranged from 105 to 575 cm^2, with the smallest being 24 cm^2 and the largest being 1521 cm^2. Of the 450 stumps, 56 were determined to be rotten on site and were hence excluded from the experiment, leaving 394 stumps for image analysis (see Table 1). The image analysis revealed that the blue colorant mixed in with *P. gigantea* failed to reach the minimum stipulated stump cover rate of 85 % in all but site nine. This site was also the only site that had more than half of all stumps entirely covered by treatment (Fig. 1, Fig. 2). Across all sites, 19 % of all stumps were fully covered, while 46 % of stumps had coverage between 85 and 99 %, and 35 % of treated stumps had less than 85 % coverage, although coverage varied considerably across sites (Fig. 2).

3.1. Heterobasidion infections and treatment coverage

Heterobasidion infections were detected on stumps at all sites. Of the 394 stumps that were analyzed, 178 (45 %) were infected with *Heterobasidion* spp.. Three sites (sites 3, 11, and 13) had high infection frequency (72–86 %), and one site (site 9) had low infection frequency (10 %) with only two infected stumps (see Table 1). The percentage of infected stumps did not differ between treatments (less than 85 % coverage; 45 % infected, greater than 85 % covered; 46 % infected, full coverage; 45 % infected). Neither was there a significant effect of the application on the number of colonies (Fig. 3A). There was a significant difference when considering the percentage of infected area between the treatment under 85 % coverage and either of the two treatments above 85 % coverage (Fig. 3B).

3.2. PCR detection of P. gigantea from controlled pilot study

In the pilot study aiming at protocol optimization to test presence of *P. gigantea* in wood samples, all Rotstop®S-treated discs had visible products corresponding to the positive control following the GH28 PCR, while no such products were observed in the water-treated controls (see appendix B). Several bands of different sizes were visible in both water and Rotstop®S-treated discs indicating that the GH28 primers are likely amplifying Norway spruce DNA and are not specific to *P. gigantea.* However, these non-specific bands also verify that any lack of *P. gigantea* corresponding products cannot be because of unsatisfactory PCR conditions and that 4 ng of template DNA is sufficient for visible GH28 product formation. Nested PCR using primer pair GH28nest yielded no visible products for the water-treated controls, while all Rotstop®S-treated samples had a band corresponding to the positive control.

3.3. Presence of P. gigantea as determined by PCR

Of the 396 stumps that were assessed in the field study, 150 were sampled for DNA analysis to determine the presence of *P. gigantea*. No DNA could be extracted from tissue collected from one of the stumps in site four despite repeated and varied extraction methods, and it was not included in subsequent analyses. In total, 109 of the 149 stumps (73 %) were positive for *P. gigantea* (Table 1), with all sites having at least three positive stump samples and all but site 14 having ≥ 60 % of the stumps

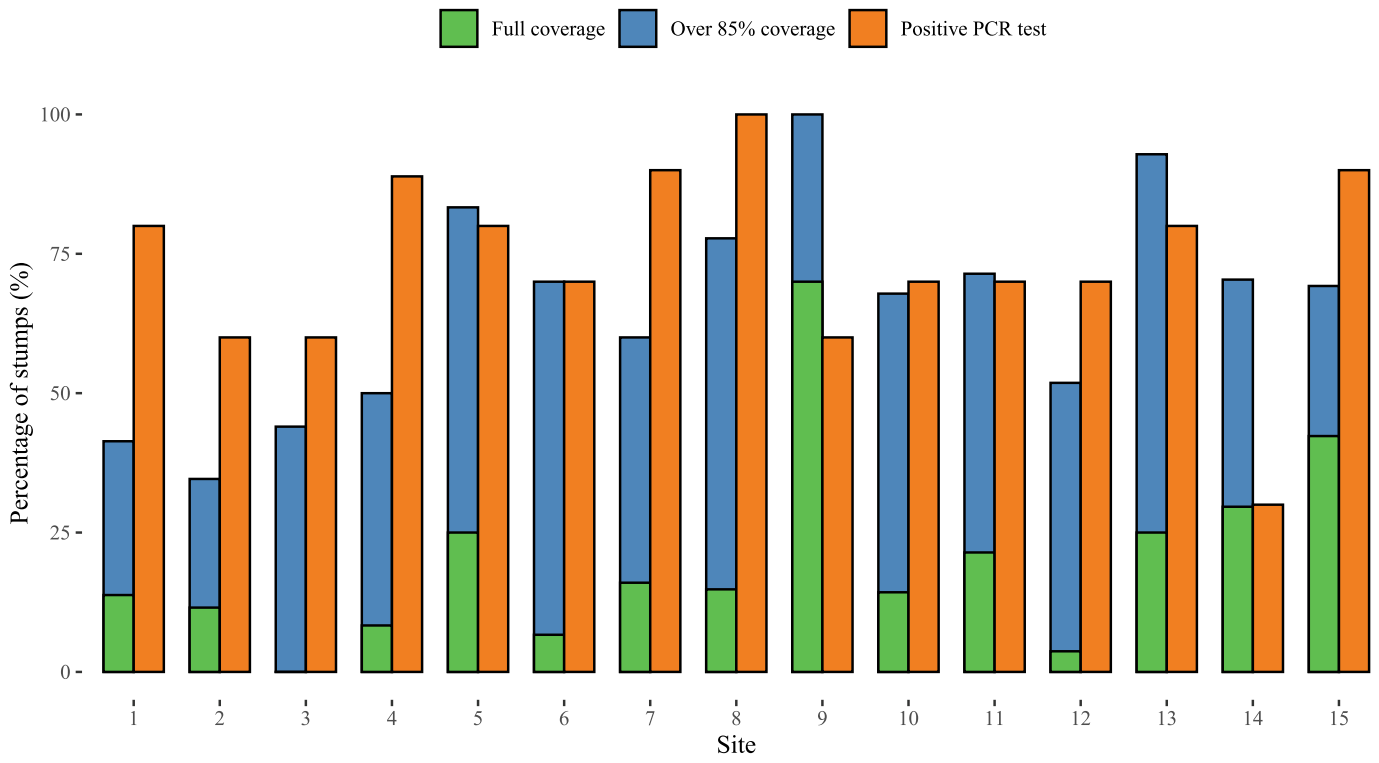

Fig. 1. Differences in stump coverage by *P. gigantea* and the presence of *P. gigantea* in wood samples after Rotstop® S treatment among different locations. On the x-axis are the different sites tested, the y axis shows the percentage of stumps that in the first stacked bar are either fully covered by the treatment containing *P. gigantea* in green or have over 85% coverage in blue. The orange bar shows the percentage of stumps where *P. gigantea* could be detected using PCR. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

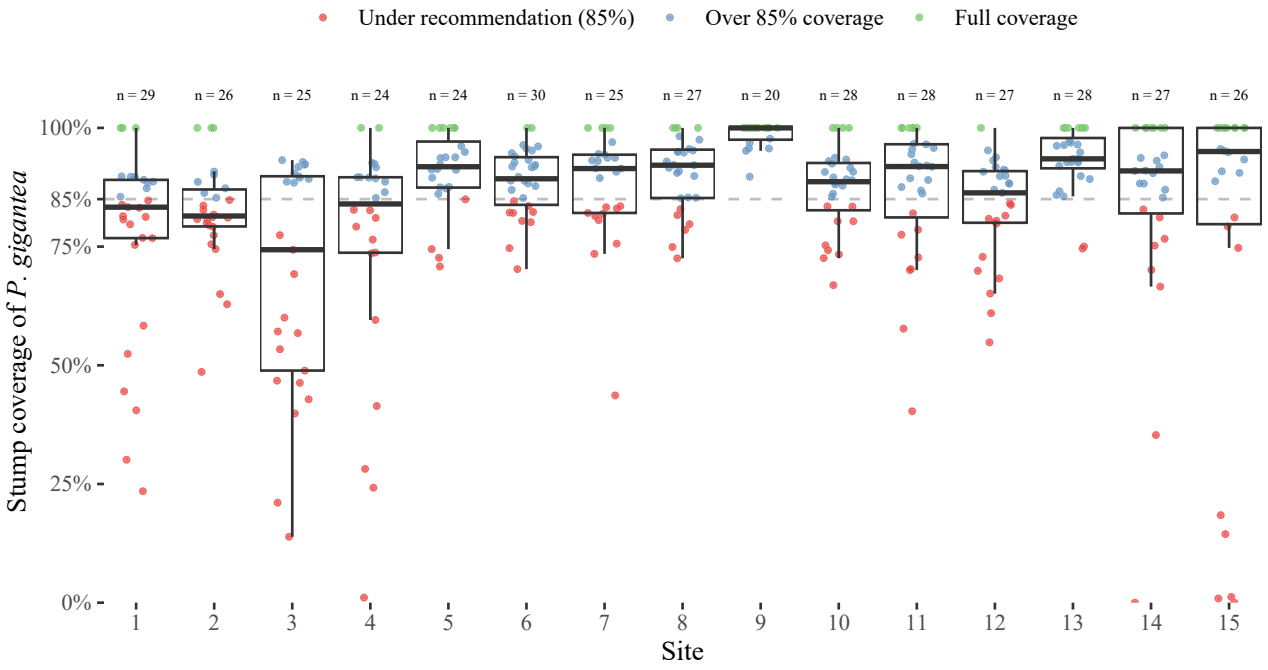

Fig. 2. Coverage of stumps with *P. gigantea* across different sites, where dots represent individual stumps. The boxplots show the large variation of stump coverage across sites. The n value above each bar represents the number of stumps examined, the thick lines in each boxplot indicate the median stump treatment coverage and colors indicate the degree of coverage for individual stumps.

being positive for *P. gigantea*. Only in site eight did all ten stumps test positive for *P. gigantea*. There was no correlation between PCR results and the presence or relative infected area of *Heterobasidion* on the stumps ($p > .90$).

4. Discussion

We investigated the application of *P. gigantea* in the field at the end-user level and found that the provided protection was considerably

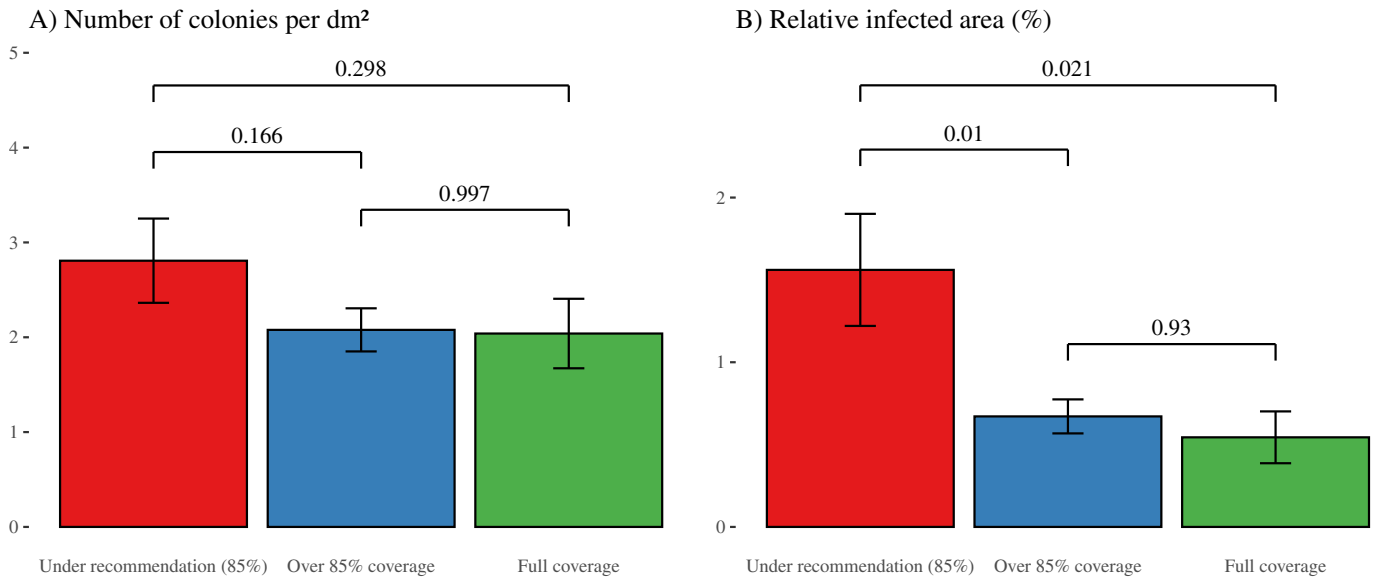

Fig. 3. On both graphs the x-axis shows three groups of different coverage levels during the application of *P. gigantea*. Above the bars are the pairwise p-values from a linear mixed-effects model. The figure on the left: A) the number of colonies per dm^2. The figure on the right: B) the relative infected area in percentage.

poorer than previous controlled studies have reported (Blomquist et al., 2020; Oliva et al., 2010; Pellicciaro et al., 2021; Rönnberg et al., 2006). The reason for this is likely that this study followed the normal practice during commercial felling operations whereas previous studies were conducted under strictly controlled conditions and are therefore not representing real-world efficacy. We assessed the application of *P. gigantea* on its presence on stumps using PCR and the stump area covered with treatment. Both are critical aspects of the successful colonization of *P. gigantea. Heterobasidion* spp. colonies were noted on discs six to seven weeks after treatment.

From the results we draw the following conclusions: 1. Almost every other stump became infected by *Heterobasidion* spp. despite being mechanically treated with Rotstop®S and even stumps considered to have 100 % coverage had an infection frequency of 45 %. 2. Forest operators rarely achieved full coverage and failed in all sites except one to cover all stumps with treatment at the minimum of 85 % stipulated by the distributor. 3. In roughly-one quarter of all analyzed stumps, *P. gigantea* DNA was not detected. The percentage of stumps where we could detect *P. gigantea* varied considerably across stands ranging from 100 % positive in one site to as low as 30 % in another. One possible explanation may be that only 10 stumps per site were analyzed in this study, which could be too few. 4. Results of coverage versus recovery of *P. gigantea* were varied; in some of the fully covered stumps we could not detect *P. gigantea*, while in some of the less covered stumps we could find the biocontrol agent. Two of the sites stood out; Stand eight had the best score based on PCR results, but the coverage was poor. Site nine was the only site that had more than 50 % of all stumps entirely covered by treatment and reached the distributor's recommended minimum coverage (85 %) on all stumps, but this was the second worst site in terms of detection of *P. gigantea* spores, present in only on six out of ten stumps.

As sampling was conducted during normal felling operations it was not possible to include controls (untreated stumps). The lack of controls made the extrapolation of disease pressure on different sites and efficacy calculations of *P. gigantea* meaningless. Nevertheless, the stumps were treated with a spore-solution of unknown viability coupled with low coverage of stump surfaces. Our results suggest that the true potential of *P. gigantea* is not realized in practice and hence stump treatment does not guarantee the successful colonization of *P. gigantea* and the expected effect against *Heterobasidion* spp..

The adoption of biological control of pathogens in forestry has been limited in practice (Prospero et al., 2021) and the usage of *P. gigantea* against H*eterobasidion* spp. is one of the few examples where biological control is used in the field. If proper treatment is performed at an early stage of the rotation, *Heterobasidion* has less time to colonize and spread in the stand before final felling (Cleary et al., 2013; Wang et al., 2015). The effect of biological control of *Heterobasidion* with *P. gigantea* also seems to be effective in the long term. Stands initially investigated by Thor and Stenlid (2005), where *Heterobasidion* was effectively controlled by *P. gigantea*, was reevaluated 13 years later by Oliva et al. (2010), who found that the stump treatment had a persistent effect on *H. annosum*, with less infections in stands established in former agricultural land and less genetic variation of *Heterobadision* spp. in stands established in former forest land. Interestingly, when different application techniques were tested and the control agents were applied through the bar, showed a very uneven coverage, two out of three sites had the lowest coverage in the study and performed equally or worse compared to non-treated controls. Application through the bar is the same method that forest operators are using in practical stump treatment today, and in our study. Thor and Stenlid (2005) also found that manual application resulted in less *Heterobasidion* infections.

Earlier studies have shown that as coverage increases so does also protection, with the best protection achieved at 100 % coverage (Berglund and Rönnberg, 2004; Oliva et al., 2017; Rönnberg et al., 2006). An explanation could be that *P. gigantea* can outcompete *Heterobasidion* for resources, although there are likely several important features which gives advantage to *P. gigantea* if applied as a plant protection agent (Prospero et al., 2021). However, the protective effect of *P. gigantea* seems to be local, and if the coverage is less than complete, *Heterobasidion* spp. will be able to colonize untreated parts of the stump. This might slow down the infection of the stump initially, but over time *Heterobasidion* grows quicker down the stump compared to *P. gigantea* (Berglund and Rönnberg, 2004), which further points at the need to treat the whole stump surface.

Several studies have shown that smaller infections have a lower viability long term, while larger colonies are more likely to survive (Berglund and Rönnberg, 2004; Dimitri et al., 1971; Morrison and Johnson, 1978; Rönnberg and Cleary, 2012). In this study, few stumps (19 %) were fully covered with treatment and increasing treatment coverage levels significantly decreased the size of *Heterobasidion* spp.. Interestingly, no such effect was found for the number of infections per dm^2.

The poor efficacy in this study is probably due to problems at the end user level and the discrepancy between previous results from meticulous application by scientists and real-world application by harvesters likely explains the continued skepticism by forest managers around stump treatment. One issue could be that *P. gigantea* needs to be kept in cold storage before use and failures in the cold chain can have a detrimental effect. If there are no viable spores in the stump treatment solution the coverage and treatment itself become irrelevant. Thor et al. (1997) found that the temperature maximum for *P. gigantea* iodiospores is around 35 °C, and that temperatures and pressure in the harvester during normal mechanical usage does not severely affect the spores. However, *P. gigantea* needs to be handled with caution also before the application as the shelf life decreases with increasing temperatures and time (Rotröta 2019). A further complication is that the amount of detected *P. gigantea* on treated stumps were highly variable between sites, and that the efficacy of *P. gigantea* also can be influenced by site-specific factors such as humidity and temperature (Berglund and Rönnberg, 2004). Our samples were gathered during the summer of 2018, which was drier and hotter than usual, as well as during more normal autumn conditions in 2017 (Fig. S1). It seems unlikely that both these contrasting weather conditions would have a negative effect on *P. gigantea* on discs collected six to seven weeks post treatment.

Our results point at the importance of investigating points of failure on the chain from production to application of *P. gigantea.* Future studies should focus on developing training methods for operators on how to handle *P. gigantea* to assure that the biocontrol agent is viable enough to provide the desired protection both before and after the spore solution is applied on the stump. It is also essential to improve coverage during commercial thinning operations, as well as assisting forest operators in making relevant decisions on when breakthrough infections are inevitable and other tools could be selected, e.g. (Blomquist et al., 2020) found that *P. gigantea* failed to provide a similar level of protection as urea on small stumps.

5. Conclusions

The practical implications of our findings are that the chain from producer to end user needs to undergo a quality control overhaul to identify the likely points of failures. It seems evident that operators need to be trained for quality control, machinery improved to secure persistent and complete stump coverage and there is a need to have field tools that make it possible to evaluate the vitality of the biological control agent when treating stumps. If there is a lack of positive experience from forest operators and forest managers to the efficacy of biological control agents it could spill-over to future tools. It is essential that scientific outreach assists in developing protocols to address the failing points and to unlock the potential of *P. gigantea* as a biological control agent against *Heterobasidion* spp..

CRediT authorship contribution statement

Mimmi Blomquist: Investigation, Writing – original draft, Writing – review & editing, Visualization. **Michelle Cleary:** Writing – original draft, Writing – review & editing. **Patrick Sherwood:** Validation, Investigation, Writing – original draft, Writing – review & editing. **Wiebke Schultz:** Investigation, Writing – original draft. **Sebastian Larsson Herrera:** Formal analysis, Data curation, Visualization. **Diana Marčiulynienė:** Investigation, Supervision, Writing – review & editing. **Mohammed Elsafy:** Investigation. **Itzhak Bakal:** Investigation. **Anna Nilsson:** Writing – original draft. **Jonas Rönnberg:** Conceptualization, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bendz-Hellgren, M., Lipponen, K., Solheim, H., Thomsen, I.M., 1998. The nordic countries. Heterobasidion annosum. Biology, ecology, impact and control/Ed. Woodward, S. , Stenlid, J. , Karjalainen, R. & Hüttermann, A.

Berglund, M., 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. https://doi.org/10.1111/j.1439-0329.2004.00363.x.

Blomquist, M., Larsson Herrera, S., Hofmann, J., Beram, R.C., Cleary, M., Rönnberg, J., 2020. Size matters but is big always better? Effectiveness of urea and Phlebiopsis gigantea as treatment against Heterobasidion on Picea abies stumps of variable size. For. Ecol. Manage. 462, 117998 https://doi.org/10.1016/j.foreco.2020.117998.

Brandtberg, P., Johansson, M., Seeger, P., 1996. Effects of season and urea treatment on infection of stumps of Picea abies by Heterobasidion annosum in stands on former arable land. Scand. J. For. Res. 11, 261–268. https://doi.org/10.1080/02827589609382935.

Cleary, M.R., Arhipova, N., Morrison, D.J., Thomsen, I.M., Sturrock, R.N., Vasaitis, R., Gaitnieks, T., Stenlid, J., 2013. Stump removal to control root disease in Canada and Scandinavia: A synthesis of results from long-term trials. For. Ecol. Manage. 290, 5–14. https://doi.org/10.1016/j.foreco.2012.05.040.

Dimitri, L., Zycha, H., Kliefoth, R., 1971. Untersuchungen über die Bedeutung der Stubbeninfektion durch Fomes annosus für die Ausbreitung der Rotfäule der Fichte. Forstwiss. Cent.bl. 90, 104–117. https://doi.org/10.1007/BF02753240.

Hori, C., Ishida, T., Igarashi, K., Samejima, M., Suzuki, H., Master, E., Ferreira, P., Ruiz-Dueñas, F.J., Held, B., Canessa, P., Larrondo, L.F., Schmoll, M., Druzhinina, I.S., Kubicek, C.P., Gaskell, J.A., Kersten, P., St John, F., Glasner, J., Sabat, G., Splinter BonDurant, S., Syed, K., Yadav, J., Mgbeahuruike, A.C., Kovalchuk, A., Asiegbu, F. O., Lackner, G., Hoffmeister, D., Rencoret, J., Gutiérrez, A., Sun, H., Lindquist, E., Barry, K., Riley, R., Grigoriev, I.V., Henrissat, B., Kües, U., Berka, R.M., Martínez, A. T., Covert, S.F., Blanchette, R.A., Cullen, D., 2014. Analysis of the Phlebiopsis gigantea genome, transcriptome and secretome provides insight into its pioneer colonization strategies of wood. PLoS Genet. 10, e1004759.

Inglis, P.W., Pappas, M., de, C.R., Resende, L.V., Grattapaglia, D., 2018. Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. PLoS One 13, e0206085.

Kallio, T., 1971. Protection of spruce stumps against Fomes annosus (Fr.) Cooke by some wood-inhabiting fungi. Acta For. Fenn. 0. 10.14214/aff.7551.

Korhonen, K., Lipponen, K., Bendz, M., Johansson, M., Ryen, I., Venn, K., Seiskari, P., Niemi, M., 1994. Control of Heterobasidion annosum by stump treatment with "Rotstop", a new commercial formulation of Phlebiopsis gigantea, in: Proceedings of the Eight International Conference on Root and Butt Rots. Wik, Sweden and Haikko, Finland, August 9-16, 1993/Ed. Johansson, M. & Stenlid, J.(eds.). Uppsala.

Kvakkestad, V., Sundbye, A., Gwynn, R., Klingen, I., 2020. Authorization of microbial plant protection products in the Scandinavian countries: A comparative analysis. Environ. Sci. Policy 106, 115–124. https://doi.org/10.1016/j.envsci.2020.01.017.

Lenth, R., Singmann, H., Love, J., Buerkner, P., Herve, M., 2018. Emmeans: Estimated marginal means, aka least-squares means. R package version 1, 3.

Morrison, D.J., Johnson, A.L.S., 1978. Stump colonization and spread of Fomes annosus 5 years after thinning. Can. J. For. Res. 8, 177–180. https://doi.org/10.1139/x78-028.

Oliva, J., Thor, M., Stenlid, J., 2010. Long-term effects of mechanized stump treatment against Heterobasidion annosum root rot in Picea abies. Can. J. For. Res. 40, 1020–1033. https://doi.org/10.1139/x10-051.

Oliva, J., Messal, M., Wendt, L., Elfstrand, M., 2017. Quantitative interactions between the biocontrol fungus Phlebiopsis gigantea, the forest pathogen Heterobasidion annosum and the fungal community inhabiting Norway spruce stumps. For. Ecol. Manage. 402, 253–264. https://doi.org/10.1016/j.foreco.2017.07.046.

Pellicciaro, M., Lione, G., Ongaro, S., Gonthier, P., 2021. Comparative Efficacy of State-of-the-Art and New Biological Stump Treatments in Forests Infested by the Native and the Alien Invasive Heterobasidion Species Present in Europe. Pathogens 10. https://doi.org/10.3390/pathogens10101272.

Prospero, S., Botella, L., Santini, A., Robin, C., 2021. Biological control of emerging forest diseases: How can we move from dreams to reality? For. Ecol. Manage. 496, 119377 https://doi.org/10.1016/j.foreco.2021.119377.

R Core Team, 2021. R: A Language and Environment for Statistical Computing.

Rishbeth, J., 1957. Some Further observations on Fomes Annosus Fr. Forestry: An Int. J. For. Res. https://doi.org/10.1093/oxfordjournals.forestry.a063099.

Rishbeth, J., 1951a. Observations on the Biology of Fomes annosus, with Particular Reference to East Anglian Pine Plantations: II. Spore Production, Stump Infection, and Saprophytic Activity in Stumps. Ann. Bot. 15, 1–22. https://doi.org/10.1093/oxfordjournals.aob.a083264.

Rishbeth, J., 1951b. Observations on the Biology of Fomes annosus, with Particular Reference to East Anglian Pine Plantations: III. Natural and Experimental Infection of Pines, and Some Factors affecting Severity of the Disease. Ann. Bot. 15, 221–246. https://doi.org/10.1093/oxfordjournals.aob.a083278.

Rishbeth, J., 1952. Control of Fomes Annosus Fr. Forestry 25, 41–50. https://doi.org/10.1093/oxfordjournals.forestry.a063039.

Rishbeth, J., 1963. Stump protection against Fomes annosus. Ann. Appl. Biol. 52, 63–77. https://doi.org/10.1111/j.1744-7348.1963.tb03728.x.

Rönnberg, J., Cleary, M.R., 2012. Presence of Heterobasidion infections in Norway spruce stumps 6 years after treatment with Phlebiopsis gigantea. For. Pathol. 42, 144–149. https://doi.org/10.1111/j.1439-0329.2011.00731.x.

Rönnberg, J., Sidorov, E., Petrylaité, E., 2006. Efficacy of different concentrations of Rotstop® and Rotstop® S and imperfect coverage of Rotstop®S against Heterobasidion spp. spore infections on Norway spruce stumps. Forest Pathol. 10.1111/j.1439-0329.2006.00476.x.

Rotröta [WWW Document], 2019. Interagroskog. URL https://www.interagroskog.se/rotrota-skogens-varsta-skadegorare/ (accessed 9.27.22).

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089.

Thor, M., Bendz-Hellgren, M., Stenlid, J., 1997. Sensitivity of Root Rot Antagonist Phlebiopsis gigantea spores to high temperature or pressure. Scand. J. For. Res. 12, 356–361. https://doi.org/10.1080/02827589709355423.

Thor, M., Stenlid, J., 2005. Heterobasidion annosum infection of Picea abies following manual or mechanized stump treatment. Scand. J. For. Res. 20, 154–164. https://doi.org/10.1080/02827580510008338.

Thor, 2003. Operational stump treatment against Heterobasidion annosum in European forestry–current situation, in: Root and Butt Rots of Forest Trees: Proceedings of the IUFRO Working Party. pp. 170–175.

Wang, L., Gunulf, A., Pukkala, T., Rönnberg, J., 2015. Simulated Heterobasidion disease development in Picea abies stands following precommercial thinning and the economic justification for control measures. Scand. J. For. Res. 30, 174–185. https://doi.org/10.1080/02827581.2014.978887.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open Source Softw. 4, 1686. 10.21105/joss.01686.