Efficacy of different concentrations of Rotstop® and Rotstop^S and imperfect cover of Rotstop^S against *Heterobasidion* spp. infections on Norway spruce stumps

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Summary

The effectiveness of treatment with two *Phlebiopsis gigantea* based preparations (Rotstop® and Rotstop^S) in different concentrations against the root and butt rot causing fungus *Heterobasidion annosum* s.l. on Norway spruce (*Picea abies*) thinning stumps in southern Sweden was compared. The trees were cut on three sites during the summer 2004 and 285 stumps were treated manually with 100% cover with two different amounts of spores in solution corresponding to approximately 5×10^6 spores/l and 10×10^6 spores/l. 31 stumps received mechanical part cover with the highest spore concentrations of *Phlebiopsis gigantea*, i.e. approximately 20×10^6 spores/l. Mechanical treatment was assessed for satisfactory treatment effect. Three months later, samples were collected and analyzed.

There was a significant reduction in frequency and relative areas of *Heterobasidion* spp. infections on stumps with manual application of control agents compared to untreated stumps. On average 10 to 23% of stumps subjected to manual treatments were infected compared to 52% for the untreated stumps. However, none of the concentration solutions of Rotstop® and Rotstop^S differed from the others in reduction of *Heterobasidion* spp. infections. Mechanical treatment failed to control the *Heterobasidion* spp. infections, but there was a tendency for the 85.1-95.0% cover class to provide better result than the others. Thus, despite of the incomplete control of the pathogen, stump treatment with different concentrations of *Phlebiopsis gigantea* oidia in suspensions provided equal effects at the present spore loads of *Heterobasidion* spp.

Sammanfattning

Effektiviteten av behandling med två pergamentsvampsbaserade (*Phlebiopsis gigantea)* lösningar (Rotstop® och $Rotstop^S$) i olika koncentrationer mot sporinfektioner av rotticka (*Heterobasidion* spp.) på gallringsstubbar av gran (*Picea abies*) i södra Sverige jämfördes. Träd avverkades på tre olika lokaler under sommaren 2004 och sammanlagt 285 stubbar behandlades manuellt med en hundraprocentig täckning i två olika koncentrationer motsvarande ungefär 5×10^6 sporer/l respektive 10×10^6 sporer/l lösning. 31 stubbar på en av lokalerna behandlades maskinellt med en ofullständig täckning men med den högsta koncentrationen av pergamentsvamp ($Rotstop^S$), ca 20×10^6 sporer/l lösning. Effekten av den mekaniska behandlingens partiella täckning jämfördes med den manuella fullständiga. Tre månader efter behandlingen samlades prover in för analys med avseende på förekomsten av rotticka.

Alla manuella behandlingar gav en signifikant reducering av frekvensen av och relativa arean av rottickeinfektioner jämfört med obehandlade stubbar. I genomsnitt var 10 till 23 % av de manuellt behandlade stubbarna infekterade jämfört med 52 % av de obehandlade. Ingen av de manuella applikationerna skiljde sig dock från varandra. Den mekaniska behandlingen gav inte ett tillfredsställande resultat men det fanns en tendens till att täckningsgradsklass 85,1-95,0 % fungerade bättre än de andra. Således, trots att ingen behandling gav en 100 % -ig minskning av antalet rottickeinfektioner, verkar olika koncentrationer av Rotstop® och $Rotstop^S$ ge ett likvärdigt resultat under de förhållanden i sportryck som rådde vid behandlingen.

Реферат

Сравнение эффективности применения двух биологических препаратов Rotstop® и Rotstop^{S}, основанных на оидиоспорах флебии гигантской (*Phlebiopsis gigantea*), для защиты пней ели европейской (*Picea abies*) от заражения корневой губкой (*Heterobasidion* spp.) было проведено в трех участках леса на юго-западе Швеции. В августе 2004 года одновременно с проведением рубок ухода, 285 деревьев были срублены харвестером, и поверхности пней были полностью обработаны суспензиями препаратов в различных концентрациях вручную. Дополнительно, 31 пень был обработан механически с не полным покрытием поверхности суспензией Rotstop^{S}, концентрации оидиоспор в которой, были наибольшей по сравнению с ручной обработкой. Три месяца спустя, выпиленные из пней образцы, были проанализированы. Пни, обработанные вручную, были значительно реже поражены корневой губкой, а также содержали гораздо меньшие относительные площади участков поражения по сравнению с необработанными. Результаты применения различных концентраций препаратов не отличались друг от друга статистически. Механическая обработка в целом была не эффективна, хотя пни с классом покрытия поверхности 85.1-96.0% не содержали инфекции. В среднем от 10 до 20% пней обработанных вручную были заражены, по сравнению с 52% зараженными среди необработанных. Таким образом, обработка пней различными концентрациями вышеуказанных препаратов оказывает одинаково не полный, но значительный позитивный эффект.

Content

1. Introduction

Heterobasidion spp. is the main cause of root and butt rot in conifer forests in the Northern Hemisphere (Hodges, 1969). It causes big economical losses in many countries. In Sweden the losses correspond to around 1.000.000 m^3 of Norway spruce (*Picea abies* (L.) Karst) timber per year (Bendz-Hellgren and Stenlid, 1995). A number of research studies have been carried out seeking for possible solutions to fight the disease.

Research on *Heterobasidion* spp. started nearly two centuries ago. The first genuine description of *H. annosum* s.s. was made by Elias Fries in 1821, who named it *Polyporus annosus* (Fr.). Since then the name of the fungus has changed several times due to different taxonomical ideas. The one we use now, was established by Oscar Brefeld in 1888. He created a new genus for the fungus, named *Heterobasidion.* The species name given by Fries before (*annosum*) was kept. Thus, the correct name for this organism since 1888 is *Heterobasidion annosum* (Fr. 1821) Bref. 1888 (Hütterman and Woodward, 1998).

Many years passed and despite the long investigation time of the fungus, only during the last 20 years it became clear that *H. annosum* is a complex of species. Each species differ (albeit overlapping) in distribution, host preferences and morphological characteristics. The most important for Europe is *H. annosum* s.s., which mostly inhabits *Pinus* forests, *H. parviporum* Niemelä & Korhonen that preferably attacks *Picea abies*, and *H. abietinum* Niemelä & Korhonen mostly infecting species of *Abies*. Other representatives of the *Heterobasidion* genus exist in America, Asia and Australia (Korhonen and Stenlid, 1998). Data collected on the distribution area of all species of *Heterobasidion* suggests that fungi most commonly appear in coniferous forests of the northern temperate zone (Korhonen and Stenlid, 1998). The range of host tree species is also huge. In spite of shown preferences for coniferous tree species *Heterobasidion* spp. infect broadleaf trees as well. The total number comprises nearly 150 relatively susceptible tree species (Sinclair, 1964), including 45 species of pine, 25 species of fir and 10 species of spruce (Wilson, 1927; Greig, 1976; Webb and Alexander, 1985).

Development on the control of the disease started over 100 years ago (Hütterman and Woodward, 1998). At the beginning, control strategies were erratic and did not provide satisfactory results (Hütterman and Woodward, 1998). Nowadays all measures comprise three ways of control: silvicultural, chemical and biological.

Silvicultural control aims to reduce damage caused by *Heterobasidion* spp. using forest management based on a knowledge of the biology and interactions of the fungus, host and environment (Korhonen et al., 1998). Thus, change of the tree species to more resistant ones in subsequent rotations intend to decrease susceptibility of a forest stand to the pathogen. Using of mixed stands and wider spacing restricts the spread of the fungus via root contacts. Broadleaves planted in first rotation enrich soil microflora, which competes with the pathogen. Avoiding of tree injures, slash burning and stump removal reduce inoculum potential of *Heterobasidion* spp. Early precommercial and light commercial thinnings are safer in terms of probability of infection set up. Fertilization is a preventive measure aiming to increase stand resistance (Korhonen et al., 1998).

Firstly, Petch in 1921 and Hepting and Downs in 1944 (Hütterman and Woodward, 1998) noticed the importance of stumps in epidemiology of root diseases. They hypothesized possible infections of stumps by spores (Redfern and Stenlid 1998). This appeared to be essential as stumps are often created in intensively managed forests.

In 1949 Rishbeth provided first experimental demonstration of germination of basidiospores on pine stumps (Rishbeth, 1949). In his further research (Rishbeth 1950, 1951, 1957) Rishbeth found that *H. annosum s.s.* grows from stump surfaces into the root systems and by mycelium quickly spreads within the stand via root contacts and grafts of the standing trees. Freshly cut wood surfaces become available for colonization only when the temperature is above 0°C, as basidiocarps of *H. annosum* don't release spores in cold periods (Rishbeth, 1951; Meredith, 1959). These observations provided a rationale for chemical control of the fungus by prevention of stump infection.

Consequently big efforts have been put into finding appropriate stump protectors. A number of chemical substances have been tested for stump treatment, but among the least toxic for mammals only urea and borates have shown significant effects (Brandtberg et al., 1996). They have been considered as the most appropriate and much used in practice (Pratt et al., 1998). However, urea is corrosive for mild steel and readily crystallizes, causing blockages in pipes and nozzles of expensive machinery (Pratt and Thor, 2001). Additionally, it has shown variable results (0-100%) (Johansson et al., 2002). It was also realized that urea and borates applied in big amounts could be of environmental hazard (Westlund and Nohrstedt, 2000) and with increased concern for sustainability and nature conservation the use of chemicals have been restricted. In contrast, the interest for biological treatment increased, especially in Scandinavia (Meredith, 1959).

Initially, in 1952 the potential of *Phlebiopsis gigantea* (Fr.) Jülich as a biological control agent was recognized by Rishbeth (Rishbeth, 1952). This discovery induced much research on *P. gigantea* and other possible antagonists to *H. annosum*. In 1991, a heterokaryotic strain of *P. gigantea* isolated from a *Picea abies* stump in Finland was formulated into a dry powder (Korhonen et al., 1994). Since then the preparation has been marketed in Finland and commercially available also in other countries as Rotstop®.

When the dissolved powder is applied to a stump surface, oidia of *P. gigantea* germinates and the mycelium subsequently colonizes the wood tissues. Further, the mycelia spreads downwards the stump and competes with *Heterobasidion* spp. Rotstop® has been tested in field trials in Finland, Sweden and Norway as 0,1% (1000 oidia per cm^2) and 0,5% (5000 oidia per cm^2) solutions and gave almost complete control of *Heterobasidion* spp. on stumps of both *Pinus sylvestris* and *Picea abies* (Korhonen et al., 1994).

Lately, Berglund et al. (2005) argued that new isolates of *P. gigantea* adapted to Swedish conditions could be more effective than the original Finnish strain. In his study, new Swedish strains were isolated, tested and provided better control then Rotstop®. Consequently, the isolate with the highest control efficacy was selected and formulated into a dry powder. Now it is available on the market as Rotstop^S.

In contrast to chemical protectors Rotstop® and Rotstop^S have a number of advantages: as a part of forest ecosystems *P. gigantea* is not a threat for the environment (Pratt and Thor, 2001; Westlund and Nohrstedt, 2000); dissolved spores don't corrode metal, therefore Rotstop solutions can be easily applied using harvesting machines; the amount of the product used is very small compared with the chemical equivalents (Pratt and Thor, 2001).

However, the infectivity of either *P. gigantea* or *Heterobasidion* spp. is related to their spore loads on stumps (Meredith, 1960), and the latter are unlikely to become dominant unless the balance of infecting basidiospores is greatly in their favour. Therefore, effectiveness of Rotstop® or Rotstop^S application should depend on the concentration of spores in solution.

According to M. Berglund and J. Rönnberg (personal communication), the *P. gigantea* mycelia of Rotstop^S strain produces twice the amount of oidiospores as the Finnish Rotstop® strain. Also, it is possibly more adapted to Swedish conditions and can quicker colonize stump surfaces. Due to these reasons, it is likely that even lower concentrations of Rotstop^S then Rotstop® could be effective in Swedish forests.

Therefore the aim of this study was to compare the efficacy of different concentrations of oidiospores in solutions of Rotstop® and Rotstop^S against natural infections of *Heterobasidion* spp. on spruce stumps, and if part cover from mechanized treatment with Rotstop^S with higher spore concentrations of *P. gigantea* in solution gives satisfactory treatment effect.

2. Materials and methods

The study was established in august 2004 in three unthinned first-rotation monocultures of Norway spruce on former arable land in south-western Sweden (Table 1). The study was divided into two parts: manual full cover and mechanical part cover.

Table 1. Location of the study sites and the main characteristics of the stands.

Experimental site	Location (Lat./Long.)	Total stand age* (years)	Mean diam. (cm at brh)	Mean height (m)	Site index (m)	Mean diam. of samples
Lida	56°58´N/13°34´E	28	20	18	G34	20.8
Slätteryd	56°58´N/13°37´E	35	22	20	G35	19.9
Hyltebruk	57°02´N/13°13´E	32	14.5	14	G30	15.9

* Total stand age at the height of 0.3 m

2.1 Manual full cover

In two stands 100 trees were cut, while in the third one (in Hyltebruk) 85 trees were cut by a single-grip harvester creating stumps about 60-70 cm high. The stumps were divided into 20 blocks (17 blocks in Hyltebruk), each of them containing 5 stumps close to each other in the stand (blocking on locations). The size of the stumps varied between 10 and 25 cm in diameter. Within 1 hour after harvesting the trees 10-20 cm from the top of every stump were cut to waste using a chainsaw, making stumps about 30 cm high thereafter the treatment was immediately applied. One stump within each block was left as an untreated control. The surfaces of the other four stumps were manually treated using a hand sprayer with the different solution concentrations of the treatment agents: 1g ($\approx 5\times10^6$ spores)/l (R1) and 2g ($\approx 10\times10^6$ spores)/l (R2) for Rotstop®, 0.5g ($\approx 5\times10^6$ spores)/l (RS 0.5) and 1g ($\approx 10\times10^6$ spores)/l for Rotstop^S (RS 1). Each solution included a dissolved coloring tablet for better cover control. The dissolution was made according to the prescriptions given by the manufacturer, i.e. 1 tablet/25 liters of tap water (Becker-Underwood, Inc., lowa, USA). The stumps were cross callipered. The volume of each treatment suspension was adjusted according to the diameter of each stump, the treatment agent being applied at a rate of about 1 liter per square meter, i.e. with a thickness of about one milliliter according to the prescriptions given by the manufacturer (Verdera Oy, PB 1, FI-02201 Esbo, Finland). Stumps with any visible discoloration on the cut surface, i.e. stumps with possible infection by any decay causing fungi, were excluded from the experiment. Spore concentrations in the treatment solutions were not checked in the field. The dilution method normally used is rough and it was accepted to rely on the viability tests performed at the factory by the producer.

2.2 Mechanical part cover

In another parcel at Lida 31 extra stump was produced and treated with an imperfect cover of about 85% for Rotstop^S in a 2g/l solution (≈20×10^6 spores)/l. In order to reveal the treated area, the suspensions were colored using a "coloring tablet" according to the prescriptions given by the manufacturer (Becker-Underwood, Inc., lowa, USA), i.e. 1 tablet/25 liters of suspension. Since the treatment was carried out mechanically the amount of spill was necessary to measure. The spill was estimated by putting raw paper (70x70 cm each) of known weight ("EKS electronic" scale; max. 2000g, d=1g) on the ground just before treatment. After the sprayed liquid was absorbed by paper the sawdust was easily shaken off. The weight of the paper was then measured after each treatment. The weight of the plastic jar containing the solution was measured before and after each treatment. Using this data the total amount of used solution for a stump was calculated. By subtracting the spill from the total amount of solution sprayed the actual amount of solution added to the stump was achieved.

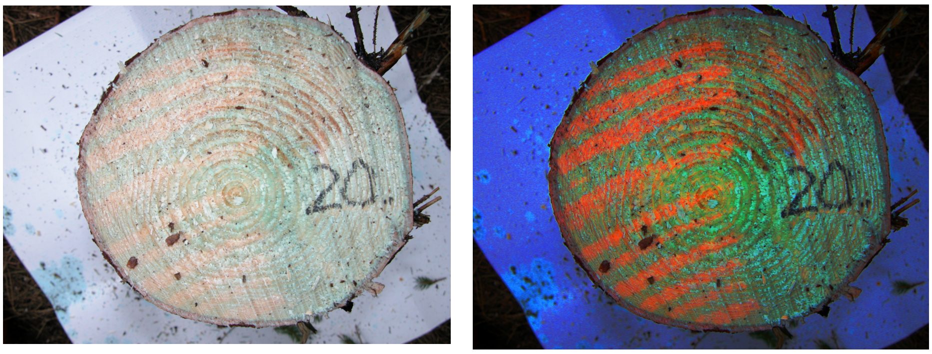
Immediately after cutting and treating the stumps the surfaces were digitally photographed (resolution of 2560x1920 pixels) and the diameter measured by cross-callipering. The pictures were transferred to a computer and reviewed to reveal the actual cover of each individual stump. The image analysis was performed using the "GNU Image Manipulation Program 2.0.4" ("GIMP"; Copyright (C) 2002-2005 Free Software Foundation, Inc. 59 Temple Place, Suite 330, Boston, MA 02111-1307 USA) and the "Able Image Analyzer" program (Copyright(c) 1999-2005 Mu Labs d.o.o, Teslova 8, Ljubljana, Slovenia).

2.3 Sampling and laboratory analysis

The sample discs were taken three months after the treatment. From each stump the top three cm was cut to waste. A five cm thick disc was then cut and immediately transferred into a plastic bag. Another disc (~ 2cm thick) was cut at the ground level to check for old infections coming from below. If there were any signs of decay, i.e. discoloration at the ground level of the stump, that disc was also immediately transferred into a plastic bag. After incubation for 7-10 days at room temperature the lower disc was analysed at one side for the presence of *Heterobasidion* spp. at it's conidial stage. If *Heterobasidion* spp. were found the stump was excluded from further analysis. The upper discs were analysed at both sides (i.e. at 3 and 8 centimetres from the top of the stump). A dissecting microscope was used for the analyses. The area (cm^2) of the disc surface colonized by *P. gigantea* and *Heterobasidion* spp. was measured. The number of colonies of *Heterobasidion* spp. as well as the location of colonies to sapwood or heartwood was registered. The amount of heartwood was based on the model presented by Wilhelmsson et al. (2000). No attempts were made to check the origin of the *P. gigantea* infection or the species of *Heterobasidion*. Identification of *P. gigantea* was based on typical dark-orange discoloration of the wood.

2.4 Image review (only for mechanical part cover)

The digital photos of each stump were analyzed to reveal the actual cover by the treatment solution. The difference in colors on the boarders between covered and uncovered part of some stumps was however not possible to distinguish by naked eye easily. Therefore, the "GIMP" program, functionally similar to "Adobe Photoshop" was used to adjust the hue, saturation and brightness parameters of the pictures. An example of this action, after which the boarders were easily seen, is given in Picture 1a and 1b.



Picture 1. a) Stump № 20 original picture
b) Stump № 20 with changed picture parameters

After the boarders were defined, the total and uncovered area of the stump was measured using the "Able Image Analyser" and expressed in pixels. Uncovered area was subtracted from the total area of the stump, and achieved size of covered part was expressed as a percent of total area. Multiplied percent of covered part by the stump size and divided by 100, revealed the covered area of each stump in square meters. The actual amount of solution added to the stump was achieved by subtracting the spill from the total amount of solution sprayed. This number was divided by the covered area and the obtained value was expressed in liters per square meter. The latter was used to judge the application rate in comparison to the 2 l/m^2 rate recommended by the producer of Rotstop^S (Verdera Oy, PB 1, FI-02201 Esbo, Finland).

2.5 Calculations and statistics

The infection frequency of *Heterobasidion* spp. and of *P. gigantea* was calculated for each treatment on each site. The positions of infections (stump level, i.e. 3 or 8 cm, and sapwood or heartwood) were not considered. Mean size and number of *Heterobasidion* spp. colonies per stump was calculated using the amalgamated data from both disc levels (3 and 8 cm). The relative area per stump occupied by *Heterobasidion* spp. or *P. gigantea* was calculated as the infected area of both disc levels in proportion of the total surface area sampled. Means for size and numbers as well as relative area of infections were calculated for infected stumps only. The treatment efficacy was calculated as mean relative area of *Heterobasidion* spp. infection in all the stumps within each site and treatment, compared with the mean relative area of *Heterobasidion* spp. infection in untreated stumps (Berglund and Rönnberg, 2004). In this analysis, stumps without infection by *Heterobasidion* spp. were also included.

A one binomial proportion (MINITAB, Minitab Inc., State College, Pennsylvania, USA) was used to compare the differences in infection frequency of *Heterobasidion* spp. or *P. gigantea* at the 5% significance level between each treatment. Treatments were compared pair-wize. The cases when the disk samples of both compared treatments were either infected or not infected were not included into the proportion. Two binomial proportions (MINITAB, Minitab Inc., State College, Pennsylvania, USA) were applied when the mechanical treatment was compared to the other treatments.

The general linear models procedure (MINITAB, Minitab Inc., State College, Pennsylvania, USA) was used to perform the analysis of variance. Three levels (site, block and treatment) were used in the model to analyze the variance of mean size, numbers and relative areas of colonies between the sites. Two levels (block and treatment) were used to analyze the variance of the same variables within each site. Tukey's test was used to separate different treatments at the 5% significance level. The normality of the data was tested through the comparison of the residual sum of squares. The results showed that the logarithmic transformation fits the best for mentioned variables. Thus, before running the model the logarithmic transformation was performed (Zar 1984).

In order to classify mechanically treated stumps with different cover, the MATLAB program (MATLAB, The MathWorks Inc., Boston, Massachusetts, USA) was used. Classification was based on infection frequency using four observations as a minimum and as much homogeneous data as possible within each class. The technique is based on the minimization of the residual sum of squares in the analysis of variance. With increasing amount of classes the residual sum of squares decreases, thus 7 classes was the optimum choice. Number of observations in each class varied from 4 to 5. The data on infection frequency was not normally distributed. Therefore the Fisher's exact test was used in the SAS program to compare infection frequency between the classes (SAS Institute Inc., Cary, NC, USA). The relative area occupied by *Heterobasidion* spp. or *P. gigantea* was compared between each class using the nonparametric Mann-Whitney test in the Minitab program (MINITAB, Minitab Inc., State College, Pennsylvania, USA). Exceptionally for the third class, there was no pair-wise comparison made with other classes for the relative *Heterobasidion* infected area, because no *Heterobasidion* infections were observed there.

During the study with mechanical part cover the spill of the applied suspension was expected to be absorbed by the paper. However, some spill drops were observed outside the paper around eighteen of thirty one stumps. The numbers of those stumps had been recorded. If unmeasured spill is valuable and not subtracted from the total amount of sprayed suspension, it would result in overestimation of the liquid added to the stump surface. In order to reveal the significance of unmeasured spill, the two sample *t*-test was performed (MINITAB, Minitab Inc., State College, Pennsylvania, USA). Total amount of liquid sprayed, suspension added to the stump and the spill, can differ in every application. While the ratio between amount of spill and the liquid added to the stump should stay the same if the diameter of the stumps is constant. This assumption was used in comparison of two means between populations of stumps with an unregistered spill and without in the *t*-test.

It could be argued that the populations cannot be compared due to the difference in stump diameter distribution. Therefore, mean diameters of the same populations has been compared using the two sample *t*-test (MINITAB, Minitab Inc., State College, Pennsylvania, USA).

3. Results

3.1 Manual 100% cover

3.1.1 Heterobasidion spp. infection frequency

Average infection frequency for untreated stumps for all sites reached 52%, and was higher ($p<0.007$) than on stumps of any other manual treatment (Table 2). However, there was a large variation between the sites (Table 3). The highest infection frequency was observed on the control stumps from Hyltebruk and Slätteryd site, while controls in Lida were the least infected. Moreover, in Lida the percentage of infected stumps subjected to any treatment, including controls, didn't differ significantly from each other ($p \geq 0.125$). Pair-wise comparison of all the treatments in Slätteryd didn't reveal significant difference between them, except R2-RS 0.5 ($p=0.031$). Being the least frequently infected, only R1 and R2 treated stumps showed significant difference from the controls ($p=0.039$ and $p=0.002$ respectively). Treatment with double concentration of Rotstop® in Slätteryd was the best overall as the stumps of that treatment were not infected at all there (Table 3). Infection frequency of stumps treated with *P. gigantea* preparations in Hyltebruk didn't differ significantly ($p \geq 0.375$), however each treatment was different from the control ($p \leq 0.004$).

Table 2. Frequency of *Heterobasidion spp.* (*H*.spp.) infections, control efficacy i.e. reduced infected area on stumps, mean number, size, relative area of *Heterobasidion* spp. infections i.e. area of *H.* spp./total disc surface area, and relative area of *P. gigantea* in infected stumps i.e. area of *P.g.*/ total disc surface area of infected stumps for all three study sites together.

Treatment	Freq. *H*.spp. inf. (%)	Control eff. (%)	Mean number *H*.spp. colonies (nos.)	Mean size *H*.spp. colonies (cm^2)	Relative area H.spp.	Relative area P.g.
Untreated	52	-	2.48	1.35	0.80	0.16
Rotstop® 1g (≈5×10^6 spores)/l	11	58	2.17	1.48	0.63	1.37
Rotstop® 2g (≈10×10^6 spores)/l	10	95	1.40	0.91	0.44	2.21
Rotstop^S 0.5g (≈5×10^6 spores)/l	21	71	1.67	1.06	0.26	0.78
Rotstop^S 1g (≈10×10^6 spores)/l	23	63	1.46	1.11	0.28	1.50
Rotstop^S 2g, Mechanic (≈20×10^6 spores)/l	48	-67	3.00	0.83	0.47	0.71

Table 3. Frequency of *Heterobasidion* ('*H.*') infected stumps and the control efficacy.

	Frequency of *H.* infected stumps (%)			Control efficacy (reduced infected area on stumps) (%)		
Treatment Site:	Lida	Slätteryd	Hyltebruk	Lida	Slätteryd	Hyltebruk
Untreated	30	50	76	-	-	-
Rotstop® 1g (≈5×10^6 spores)/l	10	16	6	-21	98	97
Rotstop® 2g (≈10×10^6 spores)/l	5	0	24	99	100	87
Rotstop^S 0.5g (≈5×10^6 spores)/l	15	30	18	54	61	96
Rotstop^S 1g (≈10×10^6 spores)/l	35	21	12	27	65	97
Rotstop^S 2g, Mechanic (≈20×10^6 spores)/l	48	not appl.	not appl.	-67	not appl.	not appl.

3.1.2 Number of Heterobasidion colonies

The results for numbers of *Heterobasidion* colonies followed the same trends as the infection frequency. Overall, control stumps showed the highest numbers of *Heterobasidion* colonies compared to other manual treatments (Table 2). For each particular site, the biggest amount of colonies was found on stumps treated with R1 in Lida (4.00 colonies per stump), while the stumps treated with R2 in Slätteryd were uninfected (Table 4). None of the stumps treated with *P. gigantea* differed from each other in numbers of *Heterobasidion* colonies in all sites ($p \geq 0.1153$). However, all the treated stumps in Hyltebruk and, R1 and R2 treated ones in Slätteryd differed from controls on the respective sites ($p \leq 0.0215$). There was no correlation between the number of *Heterobasidion* colonies in the stump and the size of the stump. The majority of *Heterobasidion* colonies appeared in the sapwood (79%).

3.1.3 Areas occupied by *Heterobasidion*

There was no difference in relative infected areas and mean colony sizes between any of the manually treated stumps at any site (Table 4). However, all treatments in Hyltebruk and, the R1 and R2 treatments in Slätteryd resulted in lower infected areas and mean colony sizes compared to control stumps ($p<0.0037$). The efficacy was highest for the stumps treated with R2, although there were no significant differences in efficacy between treatments at any site (Table 2).

Table 4. Mean number, size and relative infected area ("Area of *H.*/total disc surface area of infected stumps") of *Heterobasidion* infections in infected stumps.

	Mean number of *H.* colonies in infected stumps			Mean size of *H.* colonies in infected stumps (cm^2)			Area of *H.*/total disc surface area of infected stumps		
Treatment Site:	Lida	Slätteryd	Hyltebruk	Lida	Slätteryd	Hyltebruk	Lida	Slätteryd	Hyltebruk
Untreated	3.17	1.60	2.85	1.16	1.41	1.41	0.45	0.33	1.33
Rotstop® 1g (≈5×10^6 spores)/l	4.00	1.00	2.00	2.11	0.24	0.85	1.64	0.03	0.46
Rotstop® 2g (≈10×10^6 spores)/l	1.00	0.00	1.50	0.30	0.00	1.01	0.03	0.00	0.54
Rotstop^S 0.5g (≈5×10^6 spores)/l	2.67	1.50	1.00	1.24	1.00	0.74	0.41	0.21	0.22
Rotstop^S 1g (≈10×10^6 spores)/l	1.57	1.25	1.50	1.15	1.18	0.83	0.28	0.29	0.24
Rotstop^S 2g (≈20×10^6 spores)/l	3.00	not appl.	not appl.	0.83	not appl.	not appl.	0.47	not appl.	not appl.

3.1.4 The occurrence of *P. gigantea*

There was no significant difference found in *P. gigantea* infection frequency between the treatments at any site (Table 5). At Lida and Slätteryd all stumps with the artificial application of the fungus, except R1 treatment, were more frequently colonized ($p \leq 0.039$) than untreated stumps. However in Hyltebruk, wild *P. gigantea* appeared on control stumps with the similar frequency as strains on the treated stumps ($p \geq 0.289$). In contrast to Lida, where all the treated stumps contained bigger relative areas of *P. gigantea* than untreated stumps ($p \leq 0.0154$), in Hyltebruk they didn't differ neither from each other, nor from the controls. In Slätteryd significantly bigger relative areas of *P. gigantea* were observed for the R2 and RS1 treated stumps compared to the untreated stumps.

3.2 Mechanical part cover

Mechanically treated stumps in Lida were infected even more frequently then untreated stumps. The statistical difference ($p \leq 0.005$), however, was observed only when they were compared to treatments with R1, R2 and RS 0.5 (Table 3). Neither untreated stumps nor stumps treated with RS 1 differed ($p \geq 0.248$) from mechanically treated stumps in numbers and sizes of infections (Table 4). Relative area of *Heterobasidion* spp. as well as relative area of *P. gigantea* on mechanically treated stumps differed ($p \leq 0.004$) only from stumps treated with R2 (Table 4 and 5).

The difference in diameter distributions between populations of stumps with an unregistered spill and without was not statistically significant ($p >> 0.25$). Also, there was no significant loss of the liquid outside the paper ($p = 0.213$).

Table 5. Frequency and relative infected area of *P. gigantea* ("*P.g.*") on colonized stumps.

	Frequency of *P.g.* colonized stumps (%)			Area of *P.g.*/total disc surface area of infected stumps		
Treatment Site:	Lida	Slätteryd	Hyltebruk	Lida	Slätteryd	Hyltebruk
Untreated	35	30	65	0.37	0.06	0.08
Rotstop® 1g (≈5×10^6 spores)/l	70	63	71	2.43	0.29	1.22
Rotstop® 2g (≈10×10^6 spores)/l	100	75	53	3.28	1.33	1.43
Rotstop^S 0.5g (≈5×10^6 spores)/l	85	65	41	0.69	1.15	0.33
Rotstop^S 1g (≈10×10^6 spores)/l	85	74	47	1.54	0.54	2.64
Rotstop^S 2g (≈20×10^6 spores)/l	71	not appl.	not appl.	0.47	not appl.	not appl.

Table 6. Infections by *Heterobasidion* spp. and *P. gigantea* and number of stumps in their respective cover classes for the mechanically treated stumps.

	Cover classes						
	1	2	3	4	5	6	7
Assessment	≤65%	65.1-73.0%	73.1-76.5%	76.6-85.0%	85.1-96.0%	96.1-99.5%	99.6-100%
No of stumps	4	4	4	5	5	5	4
Aver. amount of suspension/stump (l/m^2)	10.33	3.72	3.13	3.78	2.30	2.43	3.53
H. spp. infection frequency (%)	0.5	0.75	0.75	0.4	0	0.4	0.75
Aver. number of *H.* spp. colonies (nos.)	0.5	1.5	5	1.2	0	1.4	1
Aver. size of *H.* spp. colonies (cm^2)	0.25	0.55	1.05	0.73	0	0.76	0.73
Aver. relative area colonized by *H.* (%)	0.02	0.14	1.12	0.12	0.00	0.18	0.11
Aver. relative area colonized by *P.g.* (%)	0.02	1.53	0.44	0.06	0.37	0.84	0.29
Average percent of spill (%)	15	30	41	16	22	20	19

On average the control agent covered 83% (49-100%) of the stump surface area. Stumps were assorted in 7 classes (Table 6). The 5th cover class (85.1-96.0%) contained no *Heterobasidion* infections at all in contrast to the rest of the classes. The Exact Fisher's test didn't reveal differences in the infection frequency of *Heterobasidion* spp., when that class was compared to the 4th (76.6-85.0%) and 6th (96.1-99.5%) cover classes ($p = 0.444$). Since the 5th class (85.1-96.0%) contained no *Heterobasidion* infections and number of observations for each class was too low, the relative infected area between that class and all the others was not compared. Comparison of relative area occupied by *P. gigantea* didn't show any differences between groups. However the *p*-values for comparison of 6th class with 5th and 7th were close to show the difference (p=0.0651 and p=0.115 respectively).

Amount of suspension applied per stump within each class exceeded 2 l/m^2 required by the manufacturer, and reached 4.17 l/m^2 in average. Cover class "≤65%" contained stumps with very high amounts of applied suspension. The spill was in average 23% of total suspension applied. The lowest value was observed for the cover class "≤65%".

3.3 Other results

Among the additional disks taken at ground level in order to investigate possible influence of *Heterobasidion* infections coming from the root systems, infection was found only on two samples. Thus, the frequency of infections from blow was 5% for R2 treatment in Lida and for RS1 treatment in Slätteryd. There was also one stump (Slätteryd, block 10, R1) missing in the forest.

There was no difference in stump diameters between the treatments (p=0.972), but diameters differed between the sites (p<0.001).

The number of *Heterobasidion* colonies was higher on the 3 cm level compared to the 8 cm level from the top of the stumps, however there was no significant difference neither in number of colonies, nor in their average sizes (p>0.564). The exceptions were the untreated stumps from Hyltebruk, where a higher number of *Heterobasidion* colonies were observed on the 3 cm level (p<0.001).

4. Discussion

There was no difference in reducing of *Heterobasidion* spp. infections found between treating stumps with different concentrations of Rotstop® and Rotstop^S preparations. The observations from the study support the general conclusion that *P. gigantea* may be an effective competitor to *Heterobasidion* spp. (Rishbeth 1952, Meredith 1960, Kallio 1971, Korhonen et al. 1994). However, the results do not confirm the hypothesis that Rotstop^S solutions based on Swedish isolate of *P. gigantea* would be more effective in reducing the *Heterobasidion* spp. infections then Rotstop® (Berglund et al., 2005). There may be several reasons for that.

High abundance of basidiospores in the air increases the chance for stump infection (Meredith 1959, Brandtberg et al. 1996; Solheim 1994). In present study neither spore frequency nor probable sources of infections in the immediate vicinity had been investigated. However, comparatively low infection frequencies were observed, especially at Lida site. One reason for the low infection frequencies on untreated stumps could be that before and during the work procedures at Lida and Slätteryd, those particular study sites were subjected to heavy rainfalls. High levels of precipitation decrease the amount of spores in the air (Yde-Andersen 1962). Moreover, Redfern (1993) studying the wood moisture effect on infection of Sitka spruce stumps by *Heterobasidion*, concluded that rainfalls reduce the chance of infection to be established in the sapwood. For the Norway spruce stumps the same was proved by Bendz-Hellgren and Stenlid (1998). Since the age of the planted trees varied between 28 and 35 years, heartwood comprised a minor part of the stumps wood. Therefore, low infection frequencies in the two sites might be explained by the rainfalls. In general, the high humidity level favors the sporulation of *Heterobasidion* spp. sporocarps (Sinclair 1964, Ross 1973). Thus, the third site (Hyltebruk) was rather the exception, since there was no precipitation for at least 6 hours before and after the treatment on that site and high infection frequency among the control stumps was observed.

Berglund et al. (2005) suggested that better performance of Rotstop^{S} compared to Rotstop® in their study might be explained by inability of the Rotstop® strain to effectively compete with *Heterobasidion* spp. at high spore loads. In the present study the spore loads seemed to be in contrast to those in the study by Berglund et al. (2005), where infection frequencies were 100% on most of the sites. Thus, with given low infection frequencies both preparations were equal in reduction of *Heterobasidion* infections. There was also a tendency for the significant effect of the treatment to be easier found with increasing number of infected control stumps. Therefore, it might be possible that the ability to see the statistical difference between treatments requires higher spore loads of the pathogen.

Although no viability tests were performed during the experiment, storage and application of both products were within the prescriptions given by the manufacturer. Thus, wrong handling hardly influenced the results.

The complete control of the pathogen was hard to achieve. Since the survival of even small established infections in this study is unknown, the reduction of *Heterobasidion* colonies, both in their numbers and areas by stump treatment should not be overestimated.

In this trial a bigger number of the *Heterobasidion* colonies appeared at the 3 cm level from the top of the stump, although overall not significantly different from a number of colonies at 8 cm level. The significant difference was found only once: between levels of untreated stumps at the third site. These results, however, support the assumption of correct sampling depth, which is rather important for accurate interpretation of all the other figures.

Frequent discolorations by *P. gigantea* on untreated stumps indicated the presence of wild strains of *P. gigantea* on the study sites. The discolored areas however were much smaller compared to treated stumps. Thus, the wild *P. gigantea* hardly reduced *Heterobasidion* spp. infections so, that provided a contribution to the results.

In practice, it is always hard to achieve the complete cover of the stumps with treatment solutions (Thor and Stenlid, 1998). According to Kallio (1971) and Korhonen et al. (1994) *P. gigantea* can colonize stump wood rapidly. Thus, the hypothesis of part cover effectiveness was tested in a study by Berglund and Rönnberg (2004), where mechanical treatment was imitated. In their study full cover provided better control and conclusively was more desirable. The investigation on part cover made in this study differed in methodology, and showed similarity with results found by Thor and Stenlid (1998), where better effectiveness of stump treatment with *P. gigantea* was observed for cover classes slightly below 100%. The result might be explained by intraspecific competition between *P. gigantea* spores, which occurs when the high concentrations of suspension are used. Thus, uncovered part of the stump surface provides extra substrate for the rapid *P. gigantea* colonization. However the infection by *Heterobasidion* spp. could escape the interference by *P. gigantea* if started to grow from uncovered area bigger than 15% of the stump surface. The result of the present study should be seen as indicative due to the very low number of observations within each class and the restrictions to only one site.

The technique, for the spill collection used for mechanical part cover study was not perfect, howbeit statistically justified. It could be improved by using the paper of bigger size, but that would cause difficulties in weighting procedures and take longer time. In order to reduce the spill and to get better control on the coverage of stumps the application technique should be improved. In present experiment the average spill percent is not very high (23%), however it is economically important as increases costs for the stump treatment. The measurement of applied amount of suspension was based on the assumption of equal distribution of solution on the stump surface. It could differ in reality due to different absorption abilities of the wood, and allows infections to be established in the places with lower then 2 mm thick levels of application, which might appear.

Conclusively, there was no difference in stump treatment efficacy between different concentrations of Rotstop® and Rotstop^{S}. However all concentrations of both *P. gigantea* formulations showed significant reduction of *Heterobasidion* infections. Despite that overall mechanized treatment with double concentration of oidiospores in Rotstop^{S} solution didn't provide satisfactory effect, 85.1-96.0% cover of the stump surface with the treatment solution was free from infection. Thus, an indication for the optimum cover below 100% was observed.

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