

# Effectiveness of treatment of Norway spruce stumps with *Phlebiopsis gigantea* at different rates of coverage for the control of *Heterobasidion*

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## Summary

The natural establishment of the root and butt rot causing fungus *Heterobasidion annosum* s.l. on Norway spruce (*Picea abies*) thinning stumps treated with *Phlebiopsis gigantea* was investigated on seven sites in southern Sweden. The trees were cut during summertime and the stumps were treated with different patterns simulating the effect of mechanical stump treatment with a single-grip harvester. Sampling was conducted 3 and 12 months after treatment. At both samplings, the best control was obtained when 100% of the stump surface was covered by *P. gigantea*: in contrast, untreated control stumps showed the highest incidences of *H. annosum* s.l. infection at both sampling times. However, 30 and 26% of the fully covered stumps at the first and second samplings, respectively, were diseased, and question the efficacy of treating Norway spruce stumps with this biological control agent in Sweden.

## 1 Introduction

Root and butt rot in stands of Norway spruce (*Picea abies* (L.) Karst.) results in severe economic losses to forestry in Scandinavia (HOLMSGAARD et al. 1968; BENDZ-HELLGREN et al. 1998). The decay is mainly caused by *Heterobasidion annosum* s.s. (Fr.) Bref. (commonly referred to as the European P intersterility group of *Heterobasidion annosum* s.l.) and *Heterobasidion parviporum* Niemelä and Korhonen (commonly referred to as the European S intersterility group of *Heterobasidion annosum* s.l.) (STENLID and WÄSTERLUND 1986). In this paper, both are referred to as *Heterobasidion*. The primary route of infection for *Heterobasidion* is by airborne basidiospores which infect freshly cut stumps and wounds (RISHBETH 1951a; ISOMÄKI and KALLIO 1974). Subsequent spread by mycelia to adjacent healthy trees takes place through root contacts and grafts (RISHBETH 1951b; MORRISON and REDFERN 1994).

Stump treatment, using *Phlebiopsis gigantea* (Fr.) Jül. can be an effective biocontrol method to reduce spore infections on freshly cut healthy stumps, especially during the season when spores of the pathogen are dispersed (RISHBETH 1963; KORHONEN et al. 1994; THOR and STENLID 1998). In Sweden, about 35 000 ha of Norway spruce thinnings are treated yearly (THOR 2003). Most stump treatment in Sweden is mechanical (THOR 2003). However, it can be difficult to fully cover the stump surfaces without using an excess of stump-treatment agent (PRATT and THOR 2001). Because *P. gigantea* can colonize stump wood rapidly (KALLIO 1971; KORHONEN et al. 1994), full coverage of *P. gigantea* may not be needed to prevent colonization of stumps by *Heterobasidion* following successful basidiospore infections. Intriguingly, THOR and STENLID (1998) observed that *Heterobasidion* may actually be stimulated to germinate by the presence of a suspension of

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*P. gigantea* oidia covering the complete stump surface: this effect was not evident in stumps where treatment covered <100%.

The aim of this study was to investigate the effect of varying rates of stump coverage of *P. gigantea* on the establishment of natural *Heterobasidion* basidiospore infections in Norway spruce.

## 2 Material and methods

### 2.1 Study sites and experimental design

The experiment was established during August 2001 in seven unthinned first-rotation Norway spruce stands planted on former arable land in southern Sweden (Table 1). All sites were situated in forested areas where *Heterobasidion* infections are endemic, and sporocarps of *Heterobasidion* were common on old stumps and on roots of windthrown trees in surrounding older stands.

On each of the seven sites, 200 trees were randomly selected and were felled by a single grip harvester leaving stumps approximately 60–70 cm high. Stumps selected for the trials were uninjured, had no signs of decay on the surface, and were between 10 and 30 cm in diameter. Before treatment, 10–20 cm of the top of each stump was cut to waste, to produce stumps 50 cm tall. The surface of each stump was then treated without delay with a suspension of *P. gigantea* oidiospores (Rotstop<sup>®</sup>, Verdera Oy, Helsinki, Finland) using a hand sprayer. The stumps were treated <1 h after the trees were felled. The dose of *P. gigantea* suspension was adjusted according to the diameter of each stump, the treatment agent being applied at a rate of about 1 l/m<sup>2</sup>, i.e. with a thickness of about 1 ml. This accords to the manufacturer's instructions for the use of Rotstop<sup>®</sup>, and corresponds to approximately 200–1000 oidiospores/cm<sup>2</sup> on the stump surface. On each site, 40 stumps were treated at each of five different rates of coverage: (i) the whole stump surface covered (100%), (ii) three quarters of the stump surface covered (75%), (iii) one half of the stump surface covered (50%H), (iv) half of the stump surface covered in a striped pattern (50%S), (v) untreated control (0%). The different rates of coverage used were chosen to resemble patterns that can be seen on the stumps after mechanized stump treatment with a single-grip harvester in Sweden. The 75% and the 50%H treatments simulate an imperfect application through a nozzle located on the underside of the guide bar. The striped pattern (50%S) simulates an imperfect treatment

Table 1. Location of the study sites

Experimental site	Location (Lat./Long.)	Tree age (years)	Mean diam. (cm)	Site index (m)
Emmaboda Klenemåla (EK)	56°17'N/15°45'E	37	17.7	30
Emmaboda Ödevata (EÖ)	56°17'N/15°40'E	34	17.7	30
Hult (H)	56°53'N/12°52'E	29	15.8	34
Kalvsvik (K)	56°44'N/14°46'E	22	15.2	30
Mästocka (M)	56°38'N/13°17'E	30	19.8	32
Unnaryd Rotatorpet (UR)	56°58'N/13°33'E	26	16.0	30
Unnaryd Vare (UV)	57°00'N/13°28'E	22	15.5	34

'Tree age', (age at stump height at approximately 50 cm above ground), 'Mean diam.' (mean diameter of cut trees approximately 50 cm above ground), and 'Site index' (the productivity of the sites measured on the dominant tree species (Norway spruce) as the height of the two biggest trees at breast height at a total age of 100 years).

using a drilled guide bar. To obtain the striped pattern, a Plexiglas board out of which longitudinal parallel subsections had been cut, was placed on top of the stump prior to treatment. When lifting the board from the stump after application of the *P. gigantea* suspension, the surface tension of the liquid was broken and consequently the suspension spread slightly to the sides. Therefore, to get the correct thickness at treatment the distance between the subsections (24 mm) had to be greater than the width of the subsections (16 mm).

Before each stump was treated, the diameter of the heartwood was measured. The spatial extent of the heartwood was determined using a wood moisture meter (Gann Hydromette HT65, Gann Mess- und Regeltechnik GmbH, Stuttgart, Germany), to define the border between heart- and sapwood by virtue of their different moisture content.

## 2.2 Sampling and laboratory analysis

Three months after the establishment of the experiment, 20 stumps of each treatment on each site were randomly selected for sampling. From each of these stumps, two discs each 2-cm thick were cut, one from the top of the stump and one 10 cm below the top. All discs were immediately transferred into polythene bags that were sealed, and were kept refrigerated before being incubated in darkness for 10 days at room temperature (approximately 20°C). Assessment of the presence of *Heterobasidion* in its conidial stage was made with a dissecting microscope on the underside of each disc. This provided samples at depths of 2 and 12 cm below the treated stump surface. Discrete colonies, i.e. aggregations of conidiophores without visible mycelial connections, were assumed to represent individual infections, and the number of such infections on each disc was counted. The smallest area assigned to a single colony was 10 mm<sup>2</sup>. The aggregated infected area for each disc was measured. Colonies of *P. gigantea* were identified based on their characteristic brownish-orange discoloration of the disc and the presence of oidia in mycelial samples taken from discoloured wood. The area of the disc surface colonized by *P. gigantea* was measured.

Preliminary results from the first sampling indicated that *Heterobasidion* in some stumps might originate from infections established in the root systems before the trees were felled. Therefore, 6 months after the establishment of the experiment, stumps with little or no *Heterobasidion* present on the top disc but with extensive *Heterobasidion* infections at the 12-cm level (five to seven stumps per site) were selected on each site (except at Mästocka which overall had very low infection incidence). From each of the selected stumps, one disc was cut at ground level. The discs were incubated and analysed for the presence of *Heterobasidion* in its conidial stage.

One year after the establishment of the experiment, 10 of the 20 remaining stumps of each treatment on each site were randomly selected. Four discs, each 2 cm thick, were cut from each stump to provide samples for assessment at 2, 12, 27 and 42 cm below the treated surface. Discs were handled and analysed as described above. Additionally, *Resinicium bicolor* (Alb. & Schw. ex Fr.) Parm. was recorded based on the presence of its characteristic mycelia and the presence of cystidia on hyphae of mycelial samples checked in a microscope. The area occupied by *R. bicolor* was not measured.

Isolations were made from conidiophores of *Heterobasidion* on 61 randomly chosen discs using the method of SWEDJEMARK and STENLID (1993). The isolations were made at both sampling times and from all the study sites, providing up to five samples from each sampling time and site. Identification of the species of *Heterobasidion* was made according to KORHONEN (1978) and was based on the ability of the isolated strain to heterokaryotize homokaryotic strains of the *H. parviporum* and *H. annosum* s.s. species.

### 2.3 Calculations and statistics

The incidence of infection for a site was based on the number of stumps in which *Heterobasidion* or *P. gigantea* were found, regardless of their position (stump level and sapwood or heartwood) in the stump. The calculated means for the areas and numbers of *Heterobasidion* infections per stump were based on amalgamated data from all disc levels, and included infections in both sapwood and heartwood. Relative area of infection per stump was calculated as the aggregated area infected by *Heterobasidion* or *P. gigantea* in all disc levels in proportion of the total surface area sampled. The calculations of numbers, areas and relative areas of infections were based on infected stumps only. The efficacy of stump treatment was calculated as the mean relative area of *Heterobasidion* infection in all the stumps within each site and treatment compared with the mean relative area of *Heterobasidion* infection in untreated stumps. In this analysis, stumps without infection by *Heterobasidion* were also included. The general linear model for block design (MINITAB, Minitab Inc., State College, Pennsylvania, USA), with sites as blocks, was used to perform analysis of variance. Values for incidence were arc-sine transformed before statistical calculations were performed (ZAR 1984), and Tukey's test was used to separate the different treatments at the 5% significance level. The homogeneity of variances was tested for all data using Bartlett's test (ZAR 1984). The variances for area of *Heterobasidion* infection 12 months after the treatment, the relative area of *Heterobasidion* infection 12 months after the treatment and the relative area of *P. gigantea* colonization 3 months after the treatment were not equal between treatments. The logarithms for these data were thus used in the statistical analyses (ZAR 1984). A general linear model with three levels (site, treatment, and sampling time), was used to analyse changes in incidence and extent of infection between the two sampling times. Again, because of heterogeneity of the variances for number of *Heterobasidion* colonies, relative area of *Heterobasidion* infected area and relative area of *P. gigantea* colonization the logarithms for these data were used in the statistical analyses (ZAR 1984).

As the presence of *Heterobasidion* and *P. gigantea* was recorded at four different levels in the stump 1 year after treatment, an attempt was made to compare the vertical growth rates down the stumps of the two fungi. The comparison was based only on the presence of *Heterobasidion* and *P. gigantea* on the different disc levels and did not take into account the areal distribution of the fungi. Only stumps where both fungi were present were used for this comparison. Because the observations could not be considered as normally distributed, a randomization test was performed to reveal the difference in growth between the fungi.

## 3 Results

### 3.1 Incidence of *Heterobasidion*

In untreated control stumps, the average incidence of *Heterobasidion* infection was 80 and 69% for the first and second sampling times, respectively (Table 2). There was a large variation between sites in these results (Table 3): 20% at Mästocka compared with 100% at Emmaboda (Klenemåla) and Unnaryd (Rotatorpet) 3 months after the treatment. During the second sampling, the lowest infection level (10%) occurred again at Mästocka and the highest (90%) at Emmaboda (Klenemåla), Unnaryd (Rotatorpet) and Unnaryd (Vare). The frequency of *Heterobasidion*-infected stumps was lowest for the 100% treatment at both times of sampling, being 30 and 26% at the first and second sampling time, respectively (Table 2). There was a significant decrease in the frequency of infected stumps between the first and second sampling time ( $p < 0.001$ ), but there was no

Table 2. Frequency of *Heterobasidion* ('H.') infected stumps and the control efficacy at the two samplings

Treatment	Frequency of <i>H.</i> infected stumps		Control efficacy (reduced infected area on stumps)	
	3 months (%)	12 months (%)	3 months (%)	12 months (%)
100%	30 a	26 a	71 a	78 a
75%	51 b	44 ab <sup>1</sup>	24 a	70 a
50%H	65 b	40 ab	31 a	66 a
50%S	65 b	54 bc	25 a	10 a
Untreated	80 c	69 c	Not appl.	Not appl.

'Control efficacy' means the reduction of relative infected total disc surface area in relation to the relative infected total disc surface area on untreated stumps, including uninfected stumps. Figures within columns with different letters are significantly different.  
<sup>1</sup>p = 0.0511 compared with '100%'.

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

Experimental site	Frequency of <i>H.</i> infected stumps at first and second sampling (%)					Control efficacy (reduced infected area on stumps) (%)			
	100%	75%	50%H	50%S	Untreated	100%	75%	50%H	50%S
Emmaboda Klenemåla (EK)	70/20	80/60	95/60	90/60	100/90	86/98	58/85	23/40	-5/69
Emmaboda Ödevata (EÖ)	30/40	50/60	75/40	60/50	95/80	-27/-22	16/-24	-65/97	-76/-247
Hult (H)	11/0	33/0	50/18	42/20	63/45	99/100	16/100	55/100	73/91
Kalvsvik (K)	50/60	75/50	70/70	80/80	85/80	54/71	71/58	46/39	71/-38
Mästocka (M)	5/0	16/9	10/0	10/20	20/10	89/100	-156/100	31/100	-4/88
Unnaryd Rotatorpet (UR)	25/40	60/60	85/40	90/70	100/90	97/97	69/95	67/94	52/93
Unnaryd Vare (UV)	21/20	45/70	70/50	81/80	95/90	99/100	91/78	57/-9	62/16

'Control efficacy' means the reduction of relative infected total disc surface area in relation to the relative infected total disc surface area on untreated stumps, including uninfected stumps. Figures for the different sampling times are given within columns separated with '/'.  
<sup>1</sup>p = 0.0511 compared with '100%'.

interaction between treatment and sampling time ( $p = 0.245$ ), i.e. no treatment changed more than the other.

### 3.2 Number of *Heterobasidion* colonies

Untreated stumps showed the highest and the 100% treatment the lowest numbers of *Heterobasidion* colonies at both sampling times (Table 4). Overall, the number of colonies was lower at the second sampling ( $p < 0.001$ ), but there was no interaction between treatment and sampling time ( $p = 0.291$ ). There was no correlation between the number of *Heterobasidion* colonies in a stump and the size of the stump. The majority of *Heterobasidion* colonies were found in the sapwood at both sampling times, namely 85.2 and 73.8%, respectively.

Table 4. Mean number, size and relative infected area ('area of *H.*/total disc surface area of infected stumps') of *Heterobasidion* ('*H.*') infections in infected stumps at the two sampling times

Treatment	Mean number of <i>H.</i> colonies in infected stumps		Mean size of <i>H.</i> colonies in infected stumps		Area of <i>H.</i> /total disc surface area of infected stumps	
	3 months (nos.)	12 months (nos.)	3 months (cm <sup>2</sup> )	12 months (cm <sup>2</sup> )	3 months (%)	12 months (%)
100%	2.01 a	1.02 a	2.1 a	1.7 a	0.86 a	0.76 a
75%	2.94 a	1.36 ab	2.4 a	3.5 ab	0.95 ab	1.24 a
50%H	3.99 ab	1.76 ab	3.4 a	4.6 ab	1.42 ab	2.41 a
50%S	3.73 ab	2.52 ab	3.2 a	7.0 ab	1.40 ab	2.04 a
Untreated	6.35 b	3.15 b	5.3 a	8.8 b	2.12 b	3.75 a

Figures are averages for all levels in the infected stump, i.e. including discs with infections in the stump but excluding stumps without any infection. Figures within columns with different letters are significantly different.

### 3.3 Areas occupied by *Heterobasidion*

The relative infected area (the surface area of *Heterobasidion* colonies divided by the total disc surface area on discs from all levels), was significantly smaller for the 100% treatment than for the untreated stumps at the first sampling (Table 4). At the second sampling, no significant differences were found. There was no significant increase in the relative infected area between sampling times ( $p = 0.876$ ). The efficacy was highest for fully treated stumps at both sampling times. However, there were no significant differences in efficacy between treatments at any sampling (Table 2).

### 3.4 The occurrence of *P. gigantea*

The frequency of untreated stumps colonized by *P. gigantea* was lower ( $p < 0.05$ ) than for stumps where *P. gigantea* was applied artificially at first sampling (Table 5). There was no significant difference in the frequency of stumps colonized by *P. gigantea* for the treated stumps at any of the sampling times. The frequency of colonized stumps was higher at the second sampling time ( $p < 0.001$ ), but there was no interaction between treatment and sampling time ( $p = 0.126$ ).

Table 5. Frequency of *P. gigantea* ('*P.g.*') colonized stumps

Treatment	Frequency of <i>P.g.</i> colonized stumps		Area of <i>P.g.</i> /total disc surface area for colonized stumps	
	3 months (%)	12 months (%)	3 months (%)	12 months (%)
100%	71 a	91 ab	5.98 a	29.8 a
75%	60 a	93 a	4.17 ab	24.8 a
50%H	62 a	90 ab	4.51 ab	26.2 a
50%S	60 a	93 ab	1.67 bc	21.8 ab
Untreated	19 b	74 b	0.74 c	8.39 b

The relative colonized area is an average for colonized stumps including all levels in the stump, i.e. including discs with no colonization in the stump but excluding stumps without any colonization. Figures within columns with different letters are significantly different.

Three months after the treatment the relative area of stump surface colonized by *P. gigantea* was significantly greater for the fully treated stumps than for untreated stumps or for stumps with a 50% coverage in a striped pattern (Table 5). At the second sampling the relative area covered by *P. gigantea* was lower for untreated stumps than for the other treatments except the 50%S. Overall, the surface area occupied by *P. gigantea* was greater at the second than at the first sampling time ( $p < 0.001$ ). There was a tendency for an interaction between treatment and sampling time ( $p = 0.072$ ), where the 50%S and untreated stumps showed the biggest change.

### 3.5 Other results

A total of 146 stumps were found to contain both *Heterobasidion* and *P. gigantea*. In 81 of these stumps, the two fungi had spread equally deep. *Heterobasidion* was found to have spread deeper than *P. gigantea* in 44 stumps and in 21 stumps *P. gigantea* had spread deeper than *Heterobasidion*. The randomization test used to compare the growth rates of the two fungi down the stumps showed that *Heterobasidion* had grown significantly quicker than *P. gigantea* ( $p = 0.0016$ ).

Ninety-three per cent of the strains of *Heterobasidion* isolated from stumps were *H. parviporum*. On four sites *H. annosum* s.s. was also identified.

On the additional discs taken at ground level, to investigate the possible influence of *Heterobasidion* infections originating from the root systems infection was found only on one sample.

At the second sampling time, *R. bicolor* was present on the top disc in 57% of the stumps, and on about 16% of the stumps it was also present at the 12 cm level. However, on the two lowest disc levels *R. bicolor* was almost absent. There was no difference between treatments in the incidence of *R. bicolor* ( $p > 0.05$ ).

There was no difference in the sizes of stumps between treatments ( $p = 0.904$ ), but sizes differed between sites ( $p < 0.001$ ). On average, sapwood occupied 83% of the total stump surface, the actual amount varied with site ( $p < 0.001$ ) but not on the treatment within the site ( $p = 0.131$ ). Some stumps had not yet developed heartwood and in others the sapwood occupied only 32% of the total area.

## 4 Discussion

Stump treatment experiments may be evaluated by analysing the frequency of infected stumps, the size and number of infections, the relative area in the stump occupied by *Heterobasidion* or a biocontrol agent, and the control efficacy. To use only one measure might be misleading (REDFERN 1982). The different ways of presenting the results of this study all indicate that complete coverage of the stump surface is clearly desirable, although the overall effectiveness of the stump treatment agent used in these trials is debatable. The results presented here for the different ways of evaluating the effect of the stump treatment show the same trends in spite of large variation between the sites in the amount of infection of untreated, control stumps against which the effectiveness of treatment has to be compared. The problem here is that the number of observations will always be low for treatments with few infected stumps, making statistical analysis difficult unless a larger sample is provided than was the case in this study. This was particularly evident for the second sampling time when rarely more than three of the 10 stumps per site from the complete-coverage treatment were infected. Given these low frequencies, it was perhaps surprising that there were significant differences between treatments in the frequency of infected stumps, the number and size of colonies as well as the relative area of stump surfaces occupied by *Heterobasidion* and *P. gigantea*.

In this trial, stumps which had been fully covered with *P. gigantea* were more extensively colonized by *Heterobasidion* than in other studies using Rotstop® (K. Korhonen pers. comm.; L. Holmer pers. comm.; RYEN 1993; KORHONEN et al. 1994; SOUTRENON et al. 1998; THOMSEN 2003), and might lead to the conclusion that there could be limited economic benefit from doing stump treatment with *P. gigantea*. However, making comparisons with earlier trials is always bedevilled by the unknown effects of using different methodologies.

Only one strain of *P. gigantea*, formulated into Rotstop®, was applied as a treatment. Infection of control stumps by *P. gigantea* (Table 5) suggests there was wild *P. gigantea* present in the area, but its contribution to the overall levels of control was unlikely to be high. The evidence from this trial is that the *P. gigantea* was poor at competing with *Heterobasidion* in many stumps on all sites, albeit there was a significant treatment effect on the frequency and extent of *P. gigantea* colonization. The reasons for this partial failure are not known. The product containing *P. gigantea* was from a standard commercial batch, and it was handled and stored according to the prescriptions given by the manufacturer. However, no viability tests were performed during the trial.

Some studies (K. Korhonen, pers. comm.; THOMSEN 2003) have relied on artificial inoculation of *Heterobasidion*, using conidia with a certain number of different genets or a single genet, or basidiospores. One disadvantage of the use of artificial inoculation with large numbers of spores might be increased intraspecific competition as indicated by REDFERN et al. (1997), decreasing the growth of the *Heterobasidion* and giving an apparent (but artificial) advantage to the *P. gigantea* treatment. An advantage of artificial inoculation is that dose–response curves can be constructed for treatment materials, and these can provide reassurance that the observed effects are real. When basidiospores are abundant, the risk for infection is higher (MEREDITH 1959): spore frequency was not measured in this study, but at some sites all the untreated stumps were infected, and this might explain the rather poor result obtained for those stumps which were fully covered by the *P. gigantea* treatment.

As only few harvesters are able to provide constant, high-quality applications (PRATT and THOR 2001) the results from the partially covered stumps are important. Three months after the treatment, covering a portion of the stump surface also seemed better compared with the untreated stumps. However, after the second sampling this pattern was not as clear anymore. It could be argued that none of the 50% treatments are sufficient to justify the treatment at all. A ranking of the two 50% treatments is statistically questionable. However, the mean values 12 months after treatment indicate that the 50%H should be preferred to the 50%S treatment. These two treatments simulate partial treatments using different application techniques, namely application through a nozzle located on the underside of the guide bar (50%H) and application through a drilled guide bar (50%S). Consequently using a nozzle would be the preferred technique. As the results are unclear, further studies would be needed before making practical recommendations.

It is generally accepted that *P. gigantea* out-competes *Heterobasidion*, especially in pine stumps (MEREDITH 1960), and in stumps of Norway spruce (KALLIO 1971; KORHONEN et al. 1994), and is evident in this trial by the reduced incidence of *Heterobasidion* infections on treated compared with untreated stumps. However, at the second sampling, *Heterobasidion* appeared to have grown down the stumps more rapidly than *P. gigantea* in those stumps where both were present. It is evident that *Heterobasidion* may survive for many years in Norway spruce stumps (PIRI 1996), while *P. gigantea* disappears relatively soon postinfection (VAINIO et al. 2001), and *Heterobasidion* may become established in some of the roots. It would be blocked from infecting the complete root system if *P. gigantea* gets first into other roots. However, there is little evidence for this, and it is prudent to conclude that infections of *Heterobasidion* in stumps treated with *P. gigantea* may still be as important in the spread of the disease as in untreated stumps.



The reduction in the incidence of stumps with *Heterobasidion* between the first and second sampling (Table 2) requires some explanation. It is possible that infections present at 3 months might disappear with time as suggested by DIMITRI et al. (1971) and MORRISON and JOHNSON (1978), and is supported by the coincident reduced number of *Heterobasidion* colonies (Table 4). Colony size may also influence the survival of *Heterobasidion* in stumps (MORRISON et al. 1986; BENDZ-HELLGREN and STENLID 1998), with the larger colonies having a greater potential for survival. In this study, the colonized areas of stumps with different treatments and at different sampling times were more or less the same statistically, although the number of stumps at the second sampling was reduced to 10 for each treatment and site, and only one fourth of the fully treated stumps actually were infected by *Heterobasidion*. The potential of *Heterobasidion* colonies of different sizes for further spread of the disease has not been measured (MORRISON et al. 1986; REDFERN 1993; BENDZ-HELLGREN and STENLID 1998) and the importance of small or few infections should not be neglected.

In Sitka spruce (*Picea sitchensis* (Bong.) Carr.), the location of spore infection in heartwood or sapwood may be crucial for the survival and further development of *Heterobasidion* in stumps of that species (REDFERN 1993; MORRISON and REDFERN 1994). Given the same relationship for Norway spruce, it is possible that the heartwood of these stumps may be less prone to infections compared with the sapwood. Infection established in heartwood may not spread to neighbouring trees via root contacts as easily as an infection established in sapwood. In these trials, the amount of heartwood in stumps was not correlated with treatments, and the comparison between treatments in relation to future risks remains valid. In practice, only a small proportion of the pathogen's colonies were located in heartwood and their role for the future spread of the disease is unknown.

In some of the stumps from the first sampling, the surface areas colonized by *Heterobasidion* were much larger at the level of the lower disc than at the top. It seems unlikely that the origins for this infection could be from pre-existing disease within the root system as it was not possible to find evidence of *Heterobasidion* at discs taken from the ground level on those particular stumps. Colonization of stumps by both organisms is a dynamic process, and when evaluating different stump treatment agents' efficiency, the importance of the correct sampling depth is obvious. For example, in this study, the frequency of *Heterobasidion* infection at the upper disc level appeared to decline from the first to the second sampling: had the assessments been confined to the upper part of stumps at 12 months, the results might be misleading. In practice, the safest trials are those which are sufficiently long-term to allow full disease expression in residual or following crops (e.g. THOMSEN 2003).

Conclusively, this study was designed to show the effect of different coverage of the stump treatment agent, and the results are more or less clear. A different type of trial would be needed to show the long-term effect of stump treatment on the incidence of *Heterobasidion* in the remaining stand after thinning, because the survival and infectiousness of colonies of *Heterobasidion* in these stumps is unknown. In stumps which were fully covered with *P. gigantea*, colonies of *Heterobasidion* may be overwhelmed by *P. gigantea* after a relatively short time, but the role they play in the development of disease in the residual stand should not be underestimated. However, more long-term studies are needed to quantify this important factor. Still, this study raises concerns about the effectiveness of stump treatment with this stump treatment agent in Norway spruce in Sweden.

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### Résumé

*Efficacité de traitements de souches d'Epicéa commun avec Phlebiopsis gigantea en utilisant différents taux de couverture des souches pour lutter contre Heterobasidion annosum*

L'étude porte sur l'établissement naturel d'*Heterobasidion annosum* s.l. sur des souches d'éclaircies d'Epicéa commun traitées avec *Phlebiopsis gigantea*, dans sept sites du sud de la Suède. Les arbres ont été abattus pendant l'été et traités selon différentes modalités simulant le traitement mécanique des souches par un combiné d'exploitation à une seule pince. L'échantillonnage a été réalisé trois et douze mois après traitements. Quelle que soit la date d'échantillonnage, la meilleure efficacité a été obtenue quand la surface totale de la souche avait été traitée avec *P. gigantea* et la plus forte incidence d'infection par *Heterobasidion* chez les témoins non traités. Toutefois, même chez les souches totalement traitées, des taux de 30% et 26% d'infection ont été observés respectivement aux deux dates d'échantillonnage, ce qui pose la question de l'efficacité de ce type de traitement des souches d'Epicéa commun en Suède.

### Zusammenfassung

*Wirksamkeit der Behandlung von Fichtenstümpfen mit Phlebiopsis gigantea bei unterschiedlich vollständiger Oberflächenbehandlung gegen Heterobasidion*

An sieben Standorten in Südschweden wurde die natürliche Besiedelung von Fichtenstümpfen (*Picea abies*) mit *Heterobasidion annosum* s.l. in Durchforstungen nach einer Behandlung mit *Phlebiopsis gigantea* untersucht. Die Bäume wurden während des Sommers gefällt und die Stumpfoberflächen nach unterschiedlichen Mustern behandelt, welche die mechanische Stumpfbehandlung mit einem einarmigen Vollernter simulieren sollten. Die Stümpfe wurden drei und zwölf Monate nach der Behandlung beprobt. An beiden Probeterminen wurde die beste Wirkung bei hundertprozentiger Bedeckung der Stumpfoberfläche mit *P. gigantea* erzielt. Unbehandelte Stümpfe hatten jeweils die höchsten Infektionsraten mit *Heterobasidion*. Jedoch waren 30% bzw. 26% der Stümpfe mit vollkommener Oberflächenbehandlung am ersten bzw. zweiten Probetermin ebenfalls mit *Heterobasidion* infiziert, was Fragen nach der Wirksamkeit dieser Behandlung von Fichtenstümpfen in Schweden aufwirft.

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