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Effect of Thinning and *Phlebiopsis gigantea* Stump Treatment on the Growth of *Heterobasidion parviporum* Inoculated in *Picea abies*

MATTIAS PETTERSSON, JONAS RÖNNBERG, GUDMUND VOLLBRECHT and PER GEMMEL

Swedish University of Agricultural Sciences, Southern Swedish Forest Research Centre, P.O. Box 49, SE-230 53 Alnarp, Sweden

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The spread of *Heterobasidion parviporum* Niemelä & Korhonen in roots of Norway spruce was studied in three unthinned first rotation stands of Norway spruce [*Picea abies* (L.) Karst.] on former agricultural land in south-western Sweden. *Heterobasidion parviporum* was inoculated at stump height into the trunk of 135 standing trees in a randomized block design. One year after inoculation, two-thirds of the trees were thinned out and one-third was left standing. Half of the stumps left by thinning were treated with spores of *Phlebiopsis gigantea* (Fr.) Jül and half were left untreated. The spread of *H. parviporum* was examined both 3 and 5 yrs after inoculation. The rate of spread of *H. parviporum* and the proportion of infected roots were found to be significantly higher in the root systems of the stumps than in those of the standing trees. Treatment with *P. gigantea* had no significant effect on the development of *H. parviporum* in the stumps. There was a tendency 5 yrs after inoculation, however, for a lower proportion of *H. parviporum*-infected roots in the stumps treated with *P. gigantea* than in the untreated stumps. In conclusion, thinning of infected Norway spruce was found to increase the rate of spread of *H. parviporum* in the root systems of the infected trees, which could increase the risk of a rapid build-up of infection in the remaining stand. *Key words: Butt rot, Heterobasidion annosum, incidence, Norway spruce, root rot, spread rate.*

Correspondence to: M. Pettersson, e-mail: mattias.pettersson@ess.slu.se

INTRODUCTION

Heterobasidion parviporum Niemelä & Korhonen (European S intersterility group of *Heterobasidion annosum* s.l.) and *Heterobasidion annosum* (Fr.) Bref. s.s. (European P intersterility group of *H. annosum* s.l.) are the primary causes of root and butt rot in Norway spruce [*Picea abies* (L.) Karst.] in Sweden (Stenlid & Wästerlund 1986), resulting in major financial losses to the forestry sector (Bendz-Hellgren & Stenlid 1997). *Heterobasidion* is dispersed primarily by basidiospores that colonize fresh wounds and freshly cut stumps (Rishbeth 1951*a*, Isomäki & Kallio 1974). The secondary vegetative spread of *Heterobasidion* from infected stumps and trees to adjacent trees takes place through root contacts and grafts (Rishbeth 1951*a*, *b*). Growth rates for *Heterobasidion* of between 7 and 12 cm per year in the roots of living Norway spruce have been reported (Schönhar 1978, Stenlid & Johansson 1987, Bendz-Hellgren et al. 1999). The growth rates of *Heterobasidion* in the stump roots of Norway spruce have been reported to be two to three times as high as in the roots of living trees (Schönhar 1978, Bendz-Hellgren et al. 1999).

Stump treatment with *Phlebiopsis gigantea* (Fr.) Jül can be an effective silvicultural method for reducing spore infections on freshly cut and healthy stumps during the spore dispersal season (Rishbeth 1963, Korhonen et al. 1994, Thor & Stenlid 1997). When already present in a stump, however, *Heterobasidion* can survive for 15–60 yrs (Greig & Pratt 1976, Piri 1996). Since an infected stump can thus serve as a source of infection for an extended period, the effect of stump treatment in infected stands can be questioned. Korhonen et al. (1994) found *P. gigantea* to have the potential for becoming a competitor to *Heterobasidion* in Norway spruce stumps by restricting the spread of the *Heterobasidion* in the root systems. Since these results were based on only a small sample, no practical conclusions could be drawn before further investigation.

The aim of this study was to evaluate the effect of thinning and subsequent stump treatment with *P.*

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gigantea on the growth of *H. parviporum* inoculated into the trunks of standing Norway spruce. The major hypotheses were (1) that the growth rate of *H. parviporum* increases after an infected tree has been cut, and (2) that stump treatment with *P. gigantea* reduces the growth rate of *H. parviporum* in already infected stumps compared with untreated stumps.

MATERIALS AND METHODS

The experiment to be reported was started in 1995 in three unthinned first rotation Norway spruce stands on former agricultural land in the Tönnersjöheden Experimental Forest in south-western Sweden (56°40′ N, 13°10′ W). The experiment was conducted as a randomized block design. Each stand consisted of 15 blocks with three trees in each block, each of them separated by at least one other tree, trees of the latter type not being included in the experiment. The trees selected were uninjured and showed no discoloration in bore cores taken at stump height. The difference in diameter at breast height between the thickest and the thinnest tree in a block was less than 4 cm. For the three stands, the mean tree diameters were 13.6, 14.2 and 13.3 cm, the mean heights were 9.8, 11.3 and 9.1 m, and the age of the stands was 27, 36 and 27 yrs, respectively. The three trees in each block were inoculated with cutter shavings infected by a single heterokaryotic isolate of *H. parviporum*. The isolate, Rb 175, was deposited at 4°C for future somatic incompatibility tests. Inoculation was performed by removing a bore core directed at the pith of the tree and filling the hole with infected cutter shavings. The increment borer used had an outer diameter of 10 mm. Inoculation was performed 10 cm above ground and above the buttressing root closest to the south.

In 1996, two trees in each block were selected randomly and felled; the remaining tree was left standing. The surface of one of the two stumps in each block was treated manually with a suspension of *P. gigantea* oidiospores (Rotstop®, Kemira Agro Oy) in accordance with the manufacturer's instructions; the other stump was left untreated. The height of the discoloration in the stems of the felled trees was measured by cutting transverse sections until no further discoloration could be detected. A disc was cut from each tree at stump height. Each disc was placed immediately in a plastic bag and was incubated at 20°C for 10 days. The identification of *H. parviporum* was based on the presence of its conidial stage.

In autumn 1998, seven of the 15 blocks in each stand were selected randomly. The standing tree that remained in each of these blocks was then felled, the height of the discoloration in the stem being measured just as it was in 1996. A disc was cut from the stump of each of the felled trees. Buttressing roots with visible discoloration were sampled in each of the stumps. Roots that appeared healthy, i.e. which had no visible discoloration, were regarded as uninfected. The length of the discoloured sections of the roots was measured. Discs were taken at a distance of 20, 40 and 60 cm from the inoculation point and 10 cm beyond the end of the discoloured section. In the stumps treated with *P. gigantea*, discs were also cut from roots with no visible discoloration. The area of discoloured stump surface was measured for each of the stumps. Discs were handled and were analysed for *H. parviporum* just as in 1996. Identification of *P. gigantea* was based on discoloration and on the presence of oidia in mycelia taken from the sampled discs at stump height and in the roots.

In autumn 2000, seven of the eight blocks that remained in each stand were selected randomly. The remaining tree standing in each block was felled. The height of the discoloration was measured, a disc was cut from the stump surface, and the roots of the new stumps were sampled in the same way as in 1998. Both the treated and the untreated stumps were excavated. The general degradation of the stumps made it difficult to follow the discoloration in the roots. Accordingly, in all of the buttressing roots discs were cut 20, 40 and 60 cm from the inoculation point. Discs were removed and examined in the same way as in 1998. The area of discoloration on the surface of the stump was only measured for the new stumps, since in the old stumps it was difficult to distinguish *H. parviporum* discoloration from discoloration caused by other fungi. In both 1998 and 2000 the total number of buttressing roots was counted for each of the stumps. To confirm that the *H. parviporum* reisolated from the root systems was identical to the strain that was inoculated, somatic incompatibility tests were performed on 58 randomly selected samples in 1998 and on 11 randomly selected samples in 2000.

Calculations and statistics

The length of the spread of *H. parviporum* was calculated in two ways: (1) on the basis of the presence of conidia in the root samples; and (2) on the basis of measurements on the visible discoloration found in the

roots. A mean value per stump and a mean for each treatment and year were calculated. Since in 1998 there was only a single root in the standing trees in which conidia were found, this treatment could not be included in the statistical comparisons for that year. In addition, since it is possible that the maximum length of spread of *H. parviporum* in the roots of the treated and the untreated stumps had already been reached in 2000, no measures of the length of spread were calculated. Instead, the proportion of buttressing roots in which *H. parviporum* conidia were found to be present 20, 40 and 60 cm from the inoculation point was calculated for each stump. For the sites as a whole, the mean of these proportions was calculated for each treatment and year. The frequencies were transformed according to Bartlett (1937), followed by arcsine square-root transformation of each of the frequencies (Zar 1984). Calculations of the height of the discoloration in stems of the felled trees were based on the trees in which *H. parviporum* conidia were present at stump height. The same criterion was used for calculating the area of the discoloured stump surface. The general linear models procedure in SAS (Anon. 1999) was used in performing the statistical tests. Differences between individual treatments were evaluated using Tukey's significant difference mean separation test. Pearson correlation coefficients were computed in SAS to investigate the relationship between stump diameter and the height of discoloration in the stems of the felled trees.

RESULTS

In 1998 *H. parviporum* could only be detected in one of the root systems of the standing trees. In that root system the length of the spread as shown by the presence of conidia was 20 cm and the length of discoloration 30 cm. There was no difference in that year between the treated and untreated stumps in the length of the spread as shown by the presence of conidia in the roots (Table 1). For the treatments as a whole in 1998 and for the standing trees in 2000, conidia were found to be present in the last sample, i.e. the sample taken furthest from the inoculation point, in about 40% of the roots. The mean length of the dicoloration in the roots was significantly greater for the stumps than for the standing trees, whereas there was no difference in this respect between the untreated and the treated stumps (Table 1).

In 1998 and in 2000, the proportion of roots infected by *H. parviporum* at a distance of 20, 40 and 60 cm from the inoculation point was significantly lower in the standing trees than in the other treatments, except at 60 cm in 1998, where no such difference was found (Fig. 1). There were no significant differences in the proportion of infected roots between treated and untreated stumps.

In 1998 the proportion of the surface area of the stump that was discoloured was the same for the untreated and the treated stumps (Table 1). For the standing trees in that year there was only one stump in which active *H. parviporum* was present at stump height, 26.1% of the surface of the stump being discoloured.

The average height of the discoloration in the stems was 41.10 cm in 1996 ($n = 90$) and 71.3 cm in 2000 ($n = 13$). The maximum spread was 90 and 125 cm, respectively, for these years. For the one standing tree in 1998 in which *H. parviporum* was active at stump height, discoloration was found up to a height of 100 cm. No significant correlation between stump diameter and the height of discoloration was found for any of the years ($p > 0.05$).

Table 1. *Mean length of spread of Heterobasidion parviporum from the inoculation point into roots of Picea abies as shown by the presence by either conidia or discoloration and the proportion of stump surface area that was discoloured*

	Spread length, conidia (cm)				Spread length, discoloration (cm)				Proportion of discoloured stump surface area (%)			
Treatment	1998	(*n*)	2000	(*n*)	1998	(*n*)	2000	(*n*)	1998	(*n*)	2000	(*n*)
Standing trees	–	–	50.7	(14)	31.3a	(23)	40.1	(21)	–	–	20.4	(13)
Treated stumps	43.1a	(26)	–	–	55.0b	(59)	–	–	54.0a	(13)	–	–
Untreated stumps	36.8a	(30)	–	–	59.1b	(65)	–	–	50.2a	(14)	–	–

All means in a given column for which the letter designations differ have been shown to differ significantly from one another ($p < 0.05$), the number of roots or stumps on which the mean value in question is based being indicated by *n*.
Treated stumps: stumps treated with *Phlebiopsis gigantea*.

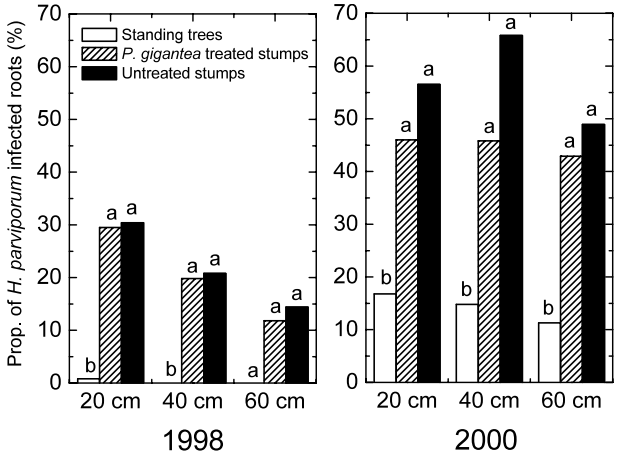

Fig. 1. Proportions of buttressing roots in which *Heterobasidion parviporum* was identified by the presence of conidia at different distances from the inoculation point. For a given year and distance, those proportions (bar lengths) for which the letter designations differ were found to differ significantly from one another ($p < 0.05$).

In both 1998 and in 2000, 91% of the isolates from the roots were of the same genotype as the isolate that was inoculated. Extensive contamination of cultures by unidentified microorganisms in 2000 led to the number of somatic incompatibility tests carried out being lower in 2000 than in 1998. *Phlebiopsis gigantea* was found in 19% of the treated stumps in 1998 and in 83% of them in 2000. In stumps in which *H. parviporum* was absent, the presence of *P. gigantea* was of the same magnitude as in the stumps in which *H. parviporum* was present.

DISCUSSION

In accordance with the first hypothesis presented, the average rate of spread of *H. parviporum* increased significantly when a tree already partly colonized by the pathogen was felled. The same was found according to the proportion of infected buttressing roots being higher for the stumps than for the standing trees. This suggests that the thinning of Norway spruce stands infected by *H. parviporum* increases the rate of spread of the fungus in the root system, thus creating the possibility of a more rapid build-up of infection in the remaining stand. In line with these results, Schönhar (1978) and Bendz-Hellgren et al. (1999) showed that *Heterobasidion* grew more rapidly when inoculated into stump roots than when inoculated into tree roots.

Treatment of the stumps with *P. gigantea* had no effect during any of the years on the length of the spread of *H. parviporum*. In 1998, the proportion of roots infected by *H. parviporum* was found to be the same in the treated as in the untreated stumps. In 2000, however, the proportion of infected roots showed a tendency towards becoming higher in the untreated than in the treated stumps. It is possible that the difference between the treated and untreated stumps in the colonization by *H. parviporum* increases over time. *Phlebiopsis gigantea* may have no chance of catching up with *H. parviorum* if it is already present in a stump. *Phlebiopsis gigantea* may, however, colonize the roots that are not yet infected by *H. parviporum* and by competition block their colonization by *H. parviporum* (Korhonen et al. 1994). This would possibly reduce the future spread of *H. parviporum* at the stand level. The period for the study may have been too short to reveal such an effect of *P. gigantea* treatment on the spread of *H. parviporum* within the stumps. The study was also not designed to evaluate the effects of stump treatment at the stand level.

Korhonen et al. (1994) reported growth rates for *P. gigantea* of more than 20 cm during a 3 month period. This suggests that *P. gigantea* may have the potential quickly to compete with and possibly overgrow *H. parviporum* in the stumps. In the present study, however, no such rapid colonization could be found in 1998 and only a few buttressing roots in the treated stumps appeared to be colonized by *P. gigantea*.

In that same year, discoloration could be noted and measured but almost no conidia were detected in the roots or at stump height in the standing trees. In 2000, in contrast, conidia could be detected in the roots or at stump height in more than half of the trees that were sampled. Since the samples were taken at some distance from the inoculation point, the fungus may not yet have reached the point where the first sample was located, and thus would have been missed in 1998. In that year, however, infection by *H. parviporum* could not be detected at stump height either. It is possible that in 1998 *H. parviporum* was present in the wood without being identified by the presence of its conidial stage.

The proportion of roots infected by *H. parviporum* increased between 1998 and 2000 for all treatments. Since in the roots samples were taken at the same distance from the inoculation point, similar proportions of infected roots could have been expected for both sampling years. However, since inoculation was carried out on one side of the stump, the *H.*

parviporum infection had to grow across the fibre direction to reach the roots of the opposite side. Transverse growth across the fibre direction may thus have slowed down the development of the infection (Rennerfelt 1946).

The average vertical growth of *H. parviporum*, measured as visible discoloration in the stems, appears to have ceased between 1996 and 2000. An explanation for this could be that aeration of the wood decreased as the inoculation holes closed, slowing down the development of *H. parviporum*. This conclusion is supported by two factors: (1) the development of decay behind a wound usually ceases when the wound has been sealed completely through the growth of callus (Shigo 1986); and (2) oxygen deficiency slows down the development of heartrot fungi (Highley et al. 1983).

Zycha & Kató (1967) found a positive correlation between the mean annual ring width and the development of *Heterobasidion* in stems of Norway spruce. In the present study, no correlation was found between stump diameter and the growth of *H. parviporum* in the stem. It is possible, however, that the sample ($n = 90$ in 1996 and $n = 13$ in 2000) was too small to indicate such a correlation.

Almost all cultures of *H. parviporum* that were reisolated in 1998 and in 2000 were of the same individual as the one inoculated. Consequently, other genotypes of *H. parviporum* could have had no more than a minor effect in the experiment.

In about 40% of the measurements of spread length based on the presence of conidia, the exact spread length could not be measured owing to conidia being found in the outermost samples taken in the roots. Accordingly, the estimates obtained of the absolute length of spread of *H. parviporum* in the roots can be questioned. Nevertheless, since the results for the proportion of the buttressing roots infected by *H. parviporum* show the same relationship to treatment as the length of spread, this has no effect on the conclusions to be drawn from the study.

As a whole, this study shows that the felling of Norway spruce trees infected by *H. parviporum* increases the rate of spread of the fungus in the root systems of the felled trees, thus increasing the risk of a rapid build-up of infection in the remaining stand. Treatment with *P. gigantea* of stumps infected by *H. parviporum* was not found to reduce significantly the development of *H. parviporum* infections within a period of 4 yrs after treatment, although it is possible that the period involved was too short to reveal such an effect.

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REFERENCES

Anon. 1999. SAS OnlineDoc®. Version 8. SAS Institute, Cary, NC.

Bartlett, M. S. 1937. Some examples of statistical methods of research in agriculture and applied biology. J. R. Statist. Soc. Suppl. 4: 137–170.

Bendz-Hellgren, M. & Stenlid, J. 1997. Decreased volume growth of *Picea abies* in response to *Heterobasidion annosum* infection. Can. J. For. Res. 27: 1519–1524.

Bendz-Hellgren, M., Brandtberg, P.-O., Johansson, M., Swedjemark, G. & Stenlid, J. 1999. Growth rate of *H. annosum* in *Picea abies* established on forest land and arable land. Scand. J. For. Res. 14: 402–407.

Greig, B. J. & Pratt, J. E. 1976. Some observations on the longevity of *Fomes annosus* in conifer stumps. Eur. J. For. Path. 6: 250–253.

Highley, T. L., Bar-Lev, T. K. & Larsen, M. J. 1983. Influence of O_2 and CO_2 on wood decay by heartrot and saprot fungi. Phytopathology 73: 630–633.

Isomäki, A. & Kallio, T. 1974. Consequences of injury caused by timber harvesting machines on the growth and decay of spruce (*Picea abies* (L.) Karst.). Acta For. Fenn. 136: 25.

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* Johansson, M. & Stenlid, J. (eds). Proceedings of the 8th International Conference on Root and Butt Rots, pp. 675–685. 9–16 Aug. 1993. IUFRO Working Party S2.06.01. Univ. Agric. Sci., Uppsala, Sweden. ISBN 91-576-4803-4.

Piri, T. 1996. The spreading of the S type of *Heterobasidion annosum* from Norway spruce stumps to the subsequent tree stand. Eur. J. For. Path. 26: 193–204.

Rennerfelt, E. 1946. Om rotrötan (*Polyporus annosus* Fr.) i Sverige. Dess utbredning och sätt att uppträda. Medd. Statens Skogsforskningsinstitut 35(8): 88p. (In Swedish with German summary.)

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: 1–21.

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.

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

Schönhar, S. 1978. Artificial infection of vigorous and moribund roots of *Picea abies* with *Fomes annosus* (*Heterobasidion annosum*), Allg. Forst. J. Ztg. 149: 73–74. (In German with English summary.)

Shigo, A. L. 1986. A New Tree Biology. Facts, Photos, and Philosophies on Trees and Their Problems and Proper Care. Shigo and Trees, Associates, Durham, NH. ISBN 0-943563-05-4.

Stenlid, J. & Johansson, M. 1987. Infections of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*. II. Early changes in phenolic content and toxicity. Eur. J. For. Path. 17: 217–226.

Stenlid, J. & Wästerlund, I. 1986. Estimating the frequency of stem rot in *Picea abies* using an increment borer. Scand. J. For. Res. 1: 303–308.

Thor, M. & Stenlid, J. 1997. *In* Thor, M. (ed.) Stump treatment against *Heterobasidion annosum* – techniques and biological effect in practical forestry. Licentiate thesis, SLU. ISBN 91-576-5372-0.

Zar, J. H. 1984. Biostatistical Analysis. 2nd edn. Prentice-Hall, Englewood Cliffs, NJ. ISBN 0-13-077595-9.

Zycha, H. & Kató, F. 1967. Untersuchungen über die Rotfäule der Fichte. Schriftenreihe der Forstlichen Fakultät der Universtität Göttingen 39: 1–120. (In German.)