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Efficacy of *Phlebiopsis gigantea* against *Heterobasidion* conidiospore and basidiospore infection in spruce wood

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Treatment of freshly cut stumps with biological control agents containing *Phlebiopsis gigantea* spores effectively restricts the spread of new *Heterobasidion* infections in conifer forests. To test the control efficacy of different *P. gigantea* strains, conifer stumps or billets cut from tree stems can be artificially infected with asexual *Heterobasidion* conidiospores or sexual basidiospores or left for natural basidiospore infection. Currently, no information is available about whether the control efficiency of *P. gigantea* in Norway spruce wood is affected by *Heterobasidion* spore type. In the present study, the impact of four *P. gigantea* strains (including the commercial product Rotstop®) on initiation and development of *Heterobasidion* basidiospore and conidiospore infections as well as the relationship between the area occupied by *P. gigantea* and control efficacy were analysed in spruce billets. The mean size of the area occupied by *P. gigantea* was larger, and the efficacy of *P. gigantea* against *Heterobasidion* was significantly higher in billets left for natural basidiospore infection compared to treatment with *Heterobasidion* conidiospore suspension. The control efficacy against *Heterobasidion* infection was high, although only a small area of the billet surface was occupied by *P. gigantea* and even when there was no visible discoloration caused by *P. gigantea* infection on wood surfaces.

Keywords: ***Picea abies*, Billets, Conidiospores, Basidiospores**

Introduction

Infection of freshly cut stump surfaces by airborne spores is the most common way of *Heterobasidion* species establishment into previously uninfected conifer stands. From infected stump roots, the fungus spreads to adjacent healthy trees causing root and butt rot in the residual stand (Redfern & Stenlid 1998, Witzell et al. 2011). An effective way to restrict *Heterobasidion* spore infections of conifer stumps is to treat the stump surfaces with biological or chemical control agents (Holdenrieder & Greig 1998, Pratt et al. 1998). Biological preparations containing asexual spores of *Phlebiopsis gigantea* – an antagonist of *Heterobasidion* species – are very effective in pine stumps but can be less effective in spruce stumps (Sun et al. 2009 and literature therein). However, good control efficiency can also be achieved in spruce wood when the spore concentration of treatment suspension is high, *i.e.*, 5 million spores L^{-1} (Korhonen 2003). A commercial preparation of *P. gigantea*, Rotstop®, was developed almost 30 years ago in Finland and is the most commonly used biological control agent against *Heterobasidion* root rot in Europe (Korhonen et al. 1994, Thor et al. 2006). However, the use of a genetically homogenous preparation may negatively affect fungal communities, therefore different studies were carried out to evaluate the impact of the biological control agent on below ground and stump colonizing fungal communities (Holdenrieder & Greig 1998, Vainio et al. 2001, Roy et al. 2003, Vasiliauskas et al. 2004, Vasiliauskas et al. 2005, Menkis et al. 2012, Terhonen et al. 2013). Previous studies indicate that local *P. gigantea* strains can be as effective, or even better, than the Finnish strain used in Rotstop® (Berglund et al. 2005). Therefore, studies have been carried out in several countries to identify local *P. gigantea* strains that could be used for controlling *Heterobasidion* root rot (Kenigsvalde et al. 2016 and literature therein).

Field testing of the efficiency of *P. gigantea* strains is usually done in stumps at sites with natural *Heterobasidion* spore infection (Korhonen et al. 1994, La Porta et al. 2003, Berglund & Rönnberg 2004, Annesi et al. 2005, Berglund et al. 2005, Nicolotti & Gonthier 2005, Rönnberg et al. 2006, Covert et al. 2013, Kenigsvalde et al. 2016). However, under *in vivo* conditions, the results can be influenced by several factors such as erratic densities of airborne *Heterobasidion* spores, as well as variable background levels of natural *P. gigantea* spore load (Berglund & Rönnberg 2004, Gaitnieks et al. 2018). Laborious and long-lasting field experiments may even be inconclusive due to a lack of *Heterobasidion* infections in control stumps (Korhonen et al. 1994). The growth rate and efficiency of *P. gigantea* against *Heterobasidion* infection is also strongly dependent on the characteristics of individual trees (Sun et al. 2009). In several infection experiments, billets cut from tree stems have been used instead of stumps, or billets are used in conjunction with stumps (Holdenrieder 1984, Korhonen et al. 1994, Korhonen 2003, La Porta et al. 2003, Roy et al. 2003, Annesi et al. 2005, Sun et al. 2009). Cutting several billets from each tree reduces the variation due to differences between individual trees and enables the use of more controlled experimental conditions to efficiently acquire information about growth rate of *P. gigantea* strains and competitive ability against *Heterobasidion*.

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Although both artificial inoculation with conidial suspensions and natural or artificial basidiospore infection have been widely used in several studies testing the efficiency of *P. gigantea* preparations, only a few studies (and only using stumps of *Pinus* spp.) have been carried out to compare the efficiency of *P. gigantea* against both *Heterobasidion* basidio- and conidiospore infections under the same experimental conditions. Both in *Pinus echinata* and *P. nigra* ssp. *laricio* stumps, *P. gigantea* proved to be more efficient against infection caused by *Heterobasidion* conidiospores than by basidiospores (Kuhlman & Hendrix 1964, Tubby et al. 2008). As the host tree species influences the control effect of *P. gigantea* as well as susceptibility to *Heterobasidion* species (Thomsen & Jacobsen 2003, Garbelotto & Gonthier 2013, Gonthier 2019, Zaluma et al. 2019), the results obtained in one tree species may not be applicable to others. Besides Scots pine stumps, Norway spruce stumps are the most important targets of stump treatment in northern and most of central Europe. Increased knowledge of interactions between *P. gigantea* and infection of Norway spruce by *Heterobasidion* with different spore types would assist in testing the efficiency of novel local *P. gigantea* strains.

The aim of the present study was: (i) to evaluate the efficacy of different Latvian *P. gigantea* strains to control *Heterobasidion* conidio- and basidiospore infections in spruce wood in maximally standardised Norway spruce wood (billets); and (ii) to analyse the relationship between occupied area and control efficacy of *P. gigantea* against infection caused by both types of *Heterobasidion* spores.

Materials and methods

Preparation of billets

This experiment was initiated in May 2010. Three trees of Norway spruce (*Picea abies* [L.] H. Karst; dbh: 13-21 cm) without visual signs of decay at stump level and with long branchless stems were felled in the experimental forests in Kalsnava, eastern Latvia (56° 40′ 21″ N, 25° 57′ 40″ E). For transport from the forest to the experimental location, the stems were cut into one meter long logs. Immediately before treatment, the logs were cut into 20-30 cm long segments (billets). Billets were numbered starting from the root collar. To avoid the effect of individual wood properties of each tree on the results, billets for each treatment variant were chosen randomly from all trees and also from different heights.

Preparation of treatment suspensions

Three Latvian *P. gigantea* strains and the biological control agent Rotstop® were used in the experiment. The Latvian strains of *P. gigantea* were selected based on the characteristics obtained in laboratory (Tab. 1) and field experiments (unpublished data except the data concerning the Latvian strain G1 – Kenigsvalde et al. 2016, Zaluma et al. 2019). For preparation of treatment suspension, each *P. gigantea* strain was cultured in six Petri dishes on malt extract agar medium for 3 weeks at 20 °C in dark conditions. Spore suspensions were prepared by washing the spores several times from one Petri dish with unsterilized tap water, agitating the colony gently three times with a glass triangle. Tap water was added to the spore suspension obtained to a final volume of one liter. To count the number of spores in the suspensions, 0.5 mL was transferred to a Petri dish containing malt extract agar medium and spread evenly.

P. gigantea spores were counted under a microscope (magnification 100×) within 30 sight fields distributed systematically over the dish. The total number of spores in suspension was calculated taking into account the number of spores in the sight field, the area of the sight field and the area of the Petri dish. Treatment suspensions were prepared 2-4 hours before the experiment and the spore concentration in suspension was adjusted to ca. 5000 spores mL^{-1}. Suspension of *Heterobasidion* conidiospores was prepared as a mixture from two heterokaryotic *H. annosum* (ISm15, VMa15) and two *H. parviporum* (No.66 and S37-9.8) strains of Latvian origin. Suspensions were prepared by washing spores several times from one Petri dish of each *Heterobasidion* strain with tap water, agitating the colony gently three times with a glass triangle and creating a mixture of all four strains. Spore concentration in suspension was adjusted to ca. 500 spores mL^{-1}.

Treatments

The upper surface of each billet was divided in two sectors leaving a two cm wide buffer zone between sectors to avoid cross contamination. Half of the billet upper surface was covered with a paper sheet while the other half was treated with a suspension of *P. gigantea* until the surface was wet, *i.e.*, an approx. 1 mm thick layer. One hour after application of *P. gigantea*, the entire surface of 28 billets was inoculated with *Heterobasidion* conidiospore suspension. After treatment, these billets (treated with *P. gigantea* and *Heterobasidion*) were placed in the field (56° 40′ 51″ N, 25° 57′ 53″ E). To avoid contact with soil, folding garden fabric was used and billets were watered regularly to provide appropriate moisture content for fungal growth.

The remaining 32 billets, treated with only *P. gigantea* strains, were transported to an experimental site (56° 41′ 39″ N, 25° 54′ 14″ E) – a Norway spruce stand growing on drained peat soil; forest type: *Oxalidosa turf. mel.*; stand age: 65 years – and exposed to natural infection by *Heterobasidion* basidiospores. Billets were placed in a radius of 4 metres surrounding spruce logs and stumps with abundant *Heterobasidion* fruit body development. Development of fruit bodies was favoured by shaded location rich in vegetation. Exposure time in the experimental site was five days. The billets were then transported to the field and placed next to the other billets. In total, billets were incubated in the field for 4 weeks. Mean daily air temperature during incubation was 16 °C. In total, 60 billets (7 repetitions for each *P. gigantea* strain treated with *Heterobasidion* conidiospores and 8 repetitions for basidiospore infection of *Heterobasidion*) were analysed.

Sampling and laboratory analysis

After incubation in the field, one sample disc (3 cm thick) was cut from the top of the billets and a second disc 2-3 cm lower so that the underside of the second disc was 8 cm from the top of the billet. The discs were transported to the laboratory, debarked and washed with a stiff brush under running tap water. After that the discs were placed in loosely closed transparent plastic bags and incubated for 7 days at room temperature in the daylight. The lower side of the discs at depth of 3 cm and 8 cm from the top of the billet were examined. A plastic grid consisting of 0.49 cm^2 squares was fixed on each disc with pins. A dissection microscope was used to examine each square for the presence of *Heterobasidion* conidiophores (area colonised by the fungus was marked on the disc with red dots). The area occupied by *P. gigantea* was identified by the typical orange brown colouration in wood. The surface area occupied by the fungus was redrawn on transparent paper and measured using a planimeter (PLANIX 10S "Marble", Tamaya, Japan).

Calculations and statistics

Efficacy of *P. gigantea* treatment was cal-

Tab. 1 - Properties of the *P. gigantea* strains grown on malt extract agar medium at 20 °C. (*): Three repetitions per strain; (**): Two repetitions per strain.

Strain	Host tree species	Growth rate (mm day^{-1})*	Growth rate over *Heterobasidion* colony (mm day^{-1})**		Spore production (million/ Petri dish)*
			H. parviporum	*H. annosum*	
J4	*Pinus sylvestris*	8.0	1.4	0.9	12.8
Kn107E	*Picea abies*	6.5	0.8	0.7	18.5
G1	*P. abies*/*P. sylvestris*	7.1	1.4	0.9	47.3
Rotstop®	*P. abies*	7.8	0.9	0.8	42.9

culated taking into account the area occupied by *Heterobasidion* on sectors of the disc treated with *P. gigantea* (sapwood and heartwood included) and the area of *Heterobasidion* on the untreated (control) sector. The efficacy was calculated at depths of 3 and 8 cm from the billet surface, and results were obtained from 7 or 8 billet replicates. The following formula was used to calculate the efficacy of different *P. gigantea* strain treatments (eqn. 1):

$$E(\%) = 100 - \left(100 \cdot \frac{n_t}{n_u}\right) \quad (1)$$

where n_t and n_u represent the percentage of area occupied by *Heterobasidion* in treated and untreated sectors, respectively.

The correlation between the area occupied by *P. gigantea* and its control efficacy was calculated. Area occupied and efficacy between different *P. gigantea* strains were compared using the Mann-Whitney test in R (R Core Team 2017). Percentages were arcsin transformed before calculations.

Results

Area occupied by P. gigantea

After *Heterobasidion* conidiospore infection, the mean area occupied by *P. gigantea* strains varied from 5.8% to 21.6% and from 0.6% to 5.1% at 3 and 8 cm depths, respectively (Tab. 2). All strains occupied a greater proportion of the disc area at a depth of 3 cm compared to 8 cm depth; moreover, the values differed significantly for strains J4, G1 and Rotstop® ($p<0.05$). Mean area occupied by strain G1 was significantly smaller in both analyzed depths compared to Rotstop® ($p<0.05$). Differences between strains were not significant at the 8 cm depth.

After *Heterobasidion* basidiospore infection, the mean area occupied by *P. gigantea* strains compared to total disc area varied from 23.6% to 31.4% and from 14.3% to 22.4% at 3 and 8 cm depths, respectively. No significant differences were found in the area occupied between *P. gigantea* strains at either analysed depth.

Mean area occupied by *P. gigantea* strains was significantly larger in billets infected by *Heterobasidion* basidiospores compared to conidiospores at a depth of 3 cm for the strain G1. Correspondingly, at a depth of 8 cm the *P. gigantea* strains J4, G1 and Kn107E occupied a significantly larger area after *Heterobasidion* basidiospore infection compared to conidiospore infection.

Area occupied by Heterobasidion

The area occupied by *Heterobasidion* mycelium after conidiospore inoculation was significantly smaller in sectors treated with *P. gigantea* compared to sectors without *P. gigantea* treatment. This applied to all analysed variants at both depths ($p<0.05$), except for treatment with the *P. gigantea* strain J4 at a depth of 8 cm (Tab. 3). Mean area occupied by *Heterobasidion* in control sectors after conidiospore inoculation was significantly larger at a depth of 3 cm compared to 8 cm depth: 52.6% and 28.7%, respectively.

The area occupied by *Heterobasidion* mycelium after natural basidiospore infection was significantly smaller in sectors treated with *P. gigantea* compared to sectors that were untreated in all analysed variants at both depths ($p<0.05$). Variation of the areas occupied by *Heterobasidion* in control sectors was quite large. After conidiospore treatment, the area occupied by *Heterobasidion* ranged from 14.9% to 80.7% (average 52.6%) and from 3.9% to 62.3% (average 28.7%) at a depth of 3 and 8 cm, respectively. After basidiospore infection, the area occupied by *Heterobasidion* varied from 2.9% to 70.3% (average 36.3%) and from 9.7% to 70.9% (average 34.6%) at a depth of 3 and 8 cm, respectively.

Differences in the area occupied by *Heterobasidion* (in sectors treated with *P. gigantea*) at both analysed depths were significant between basidiospore and conidiospore infections. In addition, in the control sector (without *P. gigantea* treatment) at a depth of 3 cm, the difference in area occupied by *Heterobasidion* was significant: 52.6% after conidiospore and 36.3% after basidiospore infection. In the control sector at a depth of 8 cm, there were no significant differences between basidiospore and conidiospore infection in relation to the size of area colonized by *Heterobasidion*.

Efficacy of P. gigantea against Heterobasidion

Mean efficacy of native *P. gigantea* strains and Rotstop® against conidiospore infection of *Heterobasidion* varied from 47% to 89% (Tab. 4). Efficacy of Latvian strains of *P. gigantea* was significantly lower compared to Rotstop® at a depth of 3 cm ($p<0.05$). At a depth of 8 cm, however, there were no statistical differences be-

Tab. 2 - Mean area (%, ± standard error) occupied by *P. gigantea* after *Heterobasidion* treatment with conidiospores/basidiospores. (Strain): *P. gigantea* strain used for treatment.

Strain	Conidiospore infection		Basidiospore infection	
	Depth: 3 cm	Depth: 8 cm	Depth: 3 cm	Depth: 8 cm
J4	10.5 ± 4.1	1.2 ± 0.7	23.6 ± 6.6	22.4 ± 7.0
G1	5.8 ± 3.0	0.6 ± 0.4	28.3 ± 7.7	20.9 ± 7.7
Kn107E	10.1 ± 5.5	2.3 ± 1.5	25.6 ± 6.9	18.3 ± 5.7
Rotstop®	21.6 ± 7.6	5.1 ± 1.6	31.4 ± 6.3	14.3 ± 4.5
Mean	12.0 ± 3.4	2.3 ± 1.0	27.2 ± 1.7	19.0 ± 1.8

Tab. 3 - Mean area (%, ± standard error) occupied by *Heterobasidion* in sectors treated with *P. gigantea* and control sectors, analysed at depths of 3 and 8 cm.

Infection	Strain	Depth: 3 cm		Depth: 8 cm	
		Treated	Control	Treated	Control
Conidiospore	J4	22.6 ± 6.4	46.6 ± 8.5	16.5 ± 7.3	19.9 ± 6.9
	G1	27.4 ± 5.3	59.7 ± 5.1	9.5 ± 4.3	25.3 ± 4.6
	Kn107E	26.9 ± 5.1	54.7 ± 4.0	11.8 ± 5.0	31.9 ± 3.0
	Rotstop®	5.6 ± 2.1	49.4 ± 5.4	12.0 ± 3.1	37.7 ± 6.6
	Mean	20.6 ± 5.1	52.6 ± 2.9	12.5 ± 1.5	28.7 ± 3.9
Basidiospore	J4	3.0 ± 1.0	36.9 ± 5.9	1.8 ± 0.7	35.6 ± 5.1
	G1	2.9 ± 0.8	41.6 ± 4.9	2.7 ± 1.1	38.3 ± 5.2
	Kn107E	4.2 ± 2.1	33.3 ± 7.0	2.9 ± 1.8	33.8 ± 6.5
	Rotstop®	1.2 ± 0.6	33.4 ± 6.8	1.0 ± 0.4	30.6 ± 7.2
	Mean	2.8 ± 0.6	36.3 ± 2.0	2.1 ± 0.4	34.6 ± 1.6

Tab. 4 - Mean efficacy of *P. gigantea* strains at a depth of 3 and 8 cm in Norway spruce billets.

Infection	Depth	Strain Efficacy (%)			
		J4	G1	Kn107E	Rotstop®
Conidiospore	3 cm	51.6	54.1	50.8	88.7
	8 cm	47.1	62.5	63.1	68.3
Basidiospore	3 cm	91.8	93.0	87.2	96.4
	8 cm	94.8	93.0	91.3	96.9

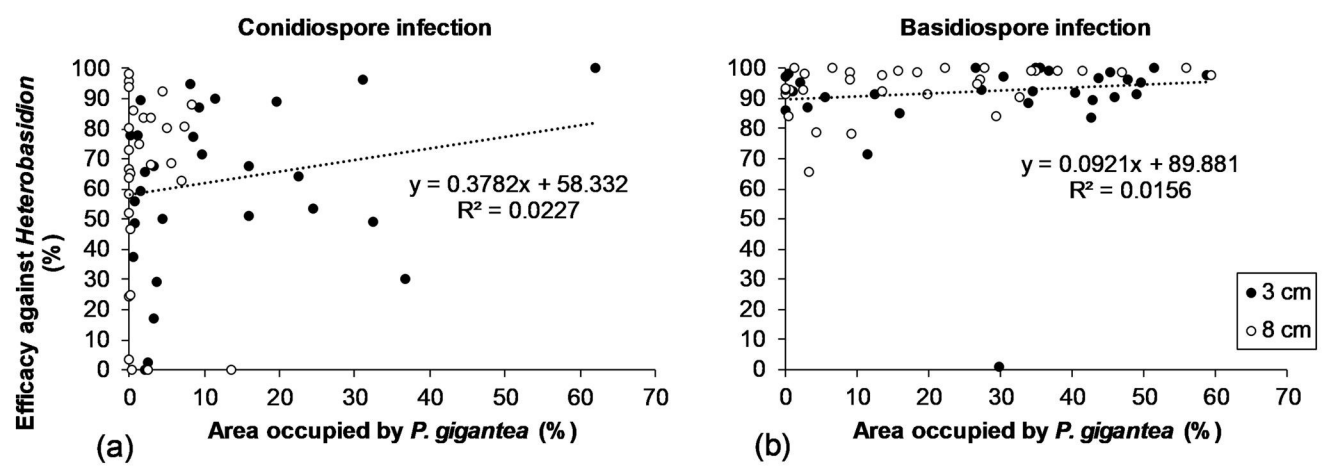

Fig. 1 - Relationship between area occupied by *P. gigantea* and efficacy against conidiospore (a) and basidiospore (b) infection of *Heterobasidion* in billets at a depth of 3 and 8 cm.

tween *P. gigantea* strains (p>0.05). The efficacy at a depth of 8 cm was lower for *P. gigantea* strains J4 and Rotstop® but higher for G1 and Kn107E compared to a depth of 3 cm.

Mean efficacy of *P. gigantea* strains against basidiospore infection of *Heterobasidion* varied from 87% to 96% and from 91% to 97% in a depth of 3 and 8 cm, respectively. Rotstop® showed the highest efficacy at both analysed depths, but differences between the strains were not significant at either of the depths analysed. Mean efficacy of the three Latvian *P. gigantea* strains against conidiospore infection of *Heterobasidion* at a depth of 3 and 8 cm combined was 54.9% and for the Rotstop® strain 78.5%. Mean efficacy of the Latvian *P. gigantea* strains against basidiospore infection of *Heterobasidion* (depths of 3 and 8 cm combined) was 91.9% compared to 96.7% for the Rotstop® strain.

Mean efficacy (combined data from both analysed depths) of *P. gigantea* was significantly higher in variants after *Heterobasidion* natural basidiospore infection instead to artificial conidiospore infection (p<0.05).

Relationship between the area occupied by *P. gigantea* and efficacy against *Heterobasidion* (combined data of both analysed depths: 3 and 8 cm) was not significant (p>0.05) in either conidiospore or in basidiospore infection (Fig. 1a, Fig. 1b).

Discussion

In this study, the efficacy of three Latvian *P. gigantea* strains and Rotstop® (commercial preparation of *P. gigantea*) against *Heterobasidion* infections caused naturally by basidiospores and artificially by conidiospores was evaluated in Norway spruce billets. In several studies analyzing the efficacy of chemical and biological control agents against *Heterobasidion* infection, conifer stumps and logs were treated with conidio- or basidiospores of the pathogen or left for airborne *Heterobasidion* inoculation (Nicolotti et al. 1999, Korhonen 2003, Annesi et al. 2005, Nicolotti & Gonthier 2005, Berglund et al. 2005, Rönnberg et al. 2006, Tubby et al. 2008, Lehtijärvi et al. 2011, Kenigsvalde et al. 2016, Oliva et al. 2017, Gonthier 2019). To our knowledge, our experiment is the first study where treatment efficacy of a *P. gigantea* isolate against *Heterobasidion* conidiospores (artificial infection) and basidiospore (natural) infection was characterized in a maximally standardized substrate such as spruce billets from the same trees. Four weeks after treatment, the area occupied by *P. gigantea* strains was larger in billets exposed to natural infection of *Heterobasidion* basidiospores than in billets artificially inoculated with *Heterobasidion* conidiospores. Because our experiment only lasted four weeks, it is possible that in the longer term the relationship regarding the areas occupied by *P. gigantea* and *Heterobasidion* may change.

As shown in earlier studies, number of both *P. gigantea* and *Heterobasidion* spores may be critical to the infection success and the following wood occupation (Korhonen et al. 1994, Berglund et al. 2005). In our study, *Heterobasidion* occupied a larger area on average after conidiospore infection in sectors treated with *P. gigantea* than after natural basidiospore infection. The advantages of artificial inoculation with *Heterobasidion* have been indicated in several studies (Thomsen 2003, Berglund & Rönnberg 2004, Tubby et al. 2008). The advantage of artificial infection compared to natural spore infection was also demonstrated for *P. gigantea* in experiments with *Pinus resinosa* logs (Roy et al. 2003). In the previously mentioned study, two months after artificial infection with *P. gigantea*, the mycelium was found at a depth of 5 cm but at the depth of only one centimeter after natural infection. In our experiment, the billet surface was completely covered by *P. gigantea* suspension. Also the amount of spores in the treatment suspension was sufficiently high (ca. 5000 spores mL^{-1}) to effectively prevent *Heterobasidion* infections in spruce wood (Korhonen 2003). For natural *Heterobasidion* basidiospore infection, the billets were placed close to fruit bodies to ensure sufficient spore load. The vast majority of the released basidiospores are deposited within a distance of a few meters from the fruit body (Kallio 1970, Stenlid 1994). The method used in our experiment ensured a high *Heterobasidion* infection rate (area occupied by *Heterobasidion* was on average 36.3% at a depth of 3 cm) in analysed control sector of billets, whereas in similar studies in Sweden average *Heterobasidion* occupied area in control *Picea abies* stumps ranged from 0.70% to 2.12% (Berglund & Rönnberg 2004, Rönnberg et al. 2006). Consequently, it is unlikely that a low density of basidiospores or conidiospores (*i.e.*, 500 spores mL^{-1}) had limited *Heterobasidion* infection in the present study. Thus, taking in account the area occupied by *Heterobasidion* in control sector, better efficiency of *P. gigantea* treatment against natural basidiospore than conidiospore infections may not be due to inadequate level of basidiospore inoculum. On the other hand, very high natural infection rate of *Heterobasidion* spores (Berglund & Rönnberg 2004, Berglund et al. 2005, Kenigsvalde et al. 2016) as well as season of application (Gonthier 2019) can negatively affect the efficacy of biological control agents.

Norway spruce can be infected by both *H. parviporum* and *H. annosum*. However, *H. parviporum* is better adapted for spruce wood (Vasiliauskas & Stenlid 1998, Oliva et al. 2013). As shown in Fig. 1, a greater variance of *P. gigantea* efficacy was unexpectedly observed after artificial treatment with *Heterobasidion* conidiospores, even though *Heterobasidion* basidiospore development may be more affected by other wood colonizing fungi in comparison to conidiospore development (Tubby et al. 2008, Oliva et al. 2013). Moreover, physical conditions, especially moisture, may have a

greater impact on natural *Heterobasidion* basidiospore infection in the upper layers of stumps (Redfern 1982, 1993). In our experiment, conidiospore suspension contained both *H. annosum* and *H. parviporum* strains. The main reason for the high variance might probably be interspecies competition between different *Heterobasidion* genotypes (Redfern et al. 1997).

For the natural basidiospore infection the billets were exposed near *H. parviporum* fruit bodies (unpublished data). Therefore, the majority of basidiospores causing natural infection were probably derived from *H. parviporum.* Inoculation experiments carried out by Gunulf et al. (2012) showed the competitive advantage of *H. parviporum* over *H. annosum*: *H. parviporum* totally replaced *H. annosum* in Norway spruce billets inoculated with a mixture of homokaryotic conidiospores of both species. Moreover, as also demonstrated in a study by Gunulf et al. (2012), *H. parviporum* grows successfully deeper in spruce wood compared to *H. annosum*. Dominance of *H. parviporum* may partly explain the result of our study indicating that infections caused by basidiospores were larger in area in the lower part of the control sector in billets.

In our experiment, we used billets cut from three individual trees instead of stumps in order to decrease variability due to differences between wood characteristics and of the individual trees. Despite maximizing the homogeneity of wood material, the variation in fungal colonization was high. The area occupied by *Heterobasidion* varied greatly both after conidiospore (4%-81%) and natural basidiospore (3%-71%) infections. A similar range in area occupied by *Heterobasidion* in Sitka spruce stumps after infection with *Heterobasidion* basidiospores (0.02%-56.6%), was found in a study by Morrison & Redfern (1994). In our study with spruce billets, the area occupied by Rotstop® was on average 22.8%. Whereas, a study in Sweden showed that area occupied by *P. gigantea* after treatment with Rotstop® in spruce stumps at depths of 2-12 cm was 5.9% (Berglund & Rönnberg 2004). Stumps can remain alive at least for 10 years after cutting if they have root contact with neighbouring trees (Redfern 1993). Unlike *Heterobasidion*, *P. gigantea* colonizes only deadwood, thus growth of *P. gigantea* is more likely to be inhibited in stumps than in billets (Vainio et al. 2001 and literature therein, Tubby et al. 2008). Thus, our results indicate that *P. gigantea* grows faster and may be more efficient against *Heterobasidion* infections in Norway spruce billets than in Norway spruce stumps.

In several studies *P. gigantea* efficacy is related to its occupied area (Korhonen 2003, Berglund & Rönnberg 2004, Tubby et al. 2008). An earlier Latvian study indicated that when the area occupied by *P. gigantea* exceeds 10% of stump surface area, occurrence of *Heterobasidion* is significantly decreased (Kenigsvalde et al. 2016). Interestingly, in the present study, the efficacy of *P. gigantea* against conidiospore infection has been demonstrated even when *P. gigantea* occupied a relatively small area or when there was no discoloration in wood. Similar data was obtained in research carried out in Finland (Sun et al. 2011). It is possible that *P. gigantea* mycelium can be present in the wood and affect growth of *Heterobasidion*, but wood discoloration by *P. gigantea* appears only after longer period of incubation (K. Korhonen personal communication). This is supported by the results obtained by Oliva et al. (2017). By quantifying the biomass of *H. annosum* and *P. gigantea* in Norway spruce stumps, they reported that visual assessment after incubation may be a poor measure of presence or absence of both fungi. In Rotstop® treated stumps, no differences in biomass of *P. gigantea* could be found between areas with visual presence or absence of *P. gigantea* after incubation. For *Heterobasidion*, a significant difference in *Heterobasidion* biomass between areas with or without growth of *Heterobasidion* after incubation was found in stumps artificially inoculated with conidia suspension but not in naturally infected stumps (Oliva et al. 2017).

In several studies, the efficacy of the biological control agent Rotstop® varies from 50 to 100% (Korhonen 2003, Berglund & Rönnberg 2004, Berglund et al. 2005, Nicolotti & Gonthier 2005, Rönnberg et al. 2006, Cech et al. 2008, Kenigsvalde et al. 2016). In our study, the average efficacy of Rotstop® was 55% to 96 %. The average area occupied by the three Latvian *P. gigantea* strains after treatment with *Heterobasidion* conidiospores was smaller than after treatment with Rotstop®. However, indigenous *P. gigantea* strains showed slightly higher occupation areas at a depth of 8 cm after *Heterobasidion* basidiospore infection. Efficacy of the local *P. gigantea* strain G1 used in our experiment has also been demonstrated in previous studies (Kenigsvalde et al. 2016). To limit the spread of *Heterobasidion* in the long term (*via* secondary infection) at a stand level, it is critical to restrict the growth of *Heterobasidion* mycelium deeper in the wood (Korhonen et al. 1994, Pettersson et al. 2003, Berglund & Rönnberg 2004). Therefore, the obtained results demonstrate the potential of local *P. gigantea* strains to limit *Heterobasidion* infection in long term. Although in our experiment with *Heterobasidion* conidiospores local *P. gigantea* strains showed lower efficacy than Rotstop®, with high ambient *Heterobasidion* natural basidiospore level, efficacy of the same *P. gigantea* strains was high.

Conclusions

Efficacy of *P. gigantea* treatment in a representative site was lower in Norway spruce billets after inoculation with conidiospores from four *Heterobasidion* strains belonging to two species than after natural infection through *Heterobasidion* basidiospores. Therefore, spruce billets artificially inoculated with conidiospore suspension provide a reliable method for screening effective *P. gigantea* strains for controlling *Heterobasidion* spore infections on Norway spruce stumps. The commercial biological control agent Rotstop® was more effective against *Heterobasidion* conidiospore and natural basidiospore infection in spruce wood compared to the local *P. gigantea* strains used in the study. However, local *P. gigantea* strains have the potential to effectively limit advance of *Heterobasidion* infection deeper in the wood, thereby decreasing vegetative spread of *Heterobasidion*.

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