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

Lauma Brūna (1), Dārta Kļaviņa (1), Astra Žaļuma (1), Kristine Kenigsvalde (1), Natālija Burneviča (1), Vizma Nikolajeva (2), Tālis Gaitnieks (1), Tuula Piri (3)

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 & Sterlind 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, Visslaukas et al. 2004, Visslaukas 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 (Korhunvalde et al. 2016 and literature therein).

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

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 stumps have been used instead of stumps, or billets are used in conjunction with stumps (Holdenrieder 1984, Korhonen et al. 1994, Korhonen et al. 2003, La Porta et al. 2003, Roy et al. 2005, 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.
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 stamps 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 et al. 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 stumps 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* (ISim15, 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. 5000 spores mL⁻¹.

Treatments

The upper surface of each billet was disinfected 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.

| Strain | Host tree species | Growth rate (mm day⁻¹) | Growth rate over *Heterobasidion* colony (mm day⁻¹) | Spore production (million/Petri dish) |
|--------|-------------------|------------------------|---------------------------------------------------|-------------------------------------|
| J4     | *P. sylvestris*    | 8.0                    | 1.4                                              | 0.9                                 |
| Kn107E | *P. abies*         | 6.5                    | 0.8                                              | 0.7                                 |
| G1     | *P. abies/P. sylvestris* | 7.1                   | 1.4                                              | 0.9                                 |
| Rotstop® | *P. abies*     | 7.8                    | 0.9                                              | 0.8                                 |

Calculations and statistics

Efficacy of *P. gigantea* treatment was cal-
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* strains compared to conidiospore infection. Kn107E occupied a significantly larger area by strains at either analysed depth. The area occupied between 8 cm depth. Differences between strains were not significant 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 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.

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 area colonized by *H. annosum* (rotted by *Heterobasidion*) at both analysed depths were significant: 52.6% after conidiospore and 36.3% after basidiospore treatment, 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-

**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.

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*.

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**Phlebiopsis gigantea efficacy against Heterobasidion infection in spruce wood**

**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            | 12.0 ± 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 | Treat | 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 (%) |
|---------------|-------|--------|--------------|
| Conidiospore  | 3 cm  | J4     | 51.6         |
|               | 8 cm  | G1     | 54.1         |
|               |       | Kn107E | 50.8         |
|               |       | Rotstop® | 88.7       |
| Basidiospore  | 3 cm  | J4     | 91.8         |
|               | 8 cm  | G1     | 93.0         |
|               |       | Kn107E | 87.2         |
|               |       | Rotstop® | 96.4       |
|               | 8 cm  | Rotstop® | 91.3         |
|               |       | Rotstop® | 96.9         |
between 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 Heterobasidion strains against conidiospore infection of Heterobasidion at a depth of 3 and 8 cm combined was 54.6% 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, 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. 2002). 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 billies were exposed near H. parviporum fruit bodies (unpublished data). Therefore, the majority of basidiospores causing natural infection were primarily derived from H. parviporum. Inoculation experiments carried out by Gunuf et al. (2012) showed the competitive advantage of H. parviporum over H. annosum: H. parviporum totally replaced H. annosum in Norway spruce billies inoculated with a mixture of homokaryotic conidiospores of both species. Moreover, as also demonstrated in a study by Gunuf 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 billies.

In our experiment, we used billies 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 conidio- spore (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 billies, 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 billies (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 billies 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). Interest-ingly, 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 study of Morrison 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, Niccolotti & 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 Norwegian spruce billies after inoculation with conidiospores from four Heterobasidion strains belonging to two species than after natural infection through Heterobasidion basidiospores. Therefore, spruce billies artificially inoculated with conidiospore suspension provide a reliable method for screening effic- eent 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 effec- tively limit advance of Heterobasidion infec- tion deeper in the wood, thereby decreasing vegetative spread of Heterobasidion.

Acknowledgements

The authors thank Kari Korhonen for valu- able comments, and Dains Edgars Rungis for language revision. The authors grate- fully thank three anonymous referees for their suggestions to improve manu- script quality.

Study was financially supported by Joint stock company “Latvia’s State Forests” project No. 5 5 5 0 0 0 4 1 0 1 6 4 “Investi- gation of the factors limiting the spread of root rot”, Latvian Council of Science fundamental and applied research project No. Izp-2018/1-0431 “Investigations on the role of Phlebiopsis gigantea in restraining vegetative spread of Heterobasidion spp. in stumps of Norway spruce and Scots pine” and in accordance with the contract No. 1.2.1.1/18/A/004 between Forest Sector Competence Centre of Latvia Ltd. and the Central Finance and Contracting Agency, the study “Development of biological pre- paration for reducing root rot caused losses in conifer stands” is conducted by LSFRI Silava with support from the ERDF within the framework of the project “Forest Sec- tor Competence Centre of Latvia”.

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