Effect of the Stump Treatment with *Phlebiopsis gigantea* against *Heterobasidion* Root Rot in Estonia

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Abstract

Efficiency of the biological control agent Rotstop® against spore infection by the pathogenic basidiomycetes of the genus *Heterobasidion* was investigated in Estonia during two years. First, *in vitro* laboratory experiments were carried out for comparison of the efficiency of the basic strain of *Phlebiopsis gigantea* in ROTSTOP® with an Estonian strain of the same species against several Estonian strains of *Heterobasidion* spp. Preliminary laboratory tests on artificially infected wood pieces of *Picea abies* and *Pinus sylvestris* were conducted, as well, for the learning of visible characteristic features of rot caused by *Phlebiopsis gigantea,* and the diagnostic features of *Heterobasidion* spp. on wood surfaces. Following experiments in forests in four Norway spruce and two Scots pine before unthinned stands, in south-eastern and north-eastern Estonia, respectively, comprised in each site as a mean 83 stumps, which were treated with the suspension of Rotstop®, and 21 stumps, which were left untreated as the control.

Rotstop® had especially effectively controlled airborne infection of *Heterobasidion annosum* s. str. in *P. sylvestris* stands (average areas occupied by the fungi on treated stumps: *P. gigantea* 113.3 cm^2 and *H. annosum* s. str. 0.6 cm^2, respectively). Noticeable natural infection by the indigenous *Phlebiopsis gigantea* occurred in the control stumps as well, but it was too limited to really keep the pathogen away from fresh cuttings. In spruce the preparation Rotstop® was effective (average areas 75.2 cm^2 and 4.3 cm^2, respectively), but less than in pine, possibly because of the extremely high infection level of *Heterobasidion parviporum* in our experimental spruce stands (up to 85 % of the number of untreated stumps).

Key words: Biological control, Rotstop®, *Phlebiopsis gigantea*, *Heterobasidion* spp., Scots pine, Norway spruce

Introduction

Pathogenic *Heterobasidion* spp. cause economically important root and butt rot disease of forest trees (over 200 species, especially conifers), throughout the Northern hemisphere (Woodward *et al*. 1998). As for conifer species, even most of them have been classified as susceptible (Asiegbu *et al*. 2005). A healthy tree stand is infected by the basidiospores, disseminating in high numbers throughout the summer (Korhonen and Stenlid 1998). During the summer the pathogen inocula is considered to be a common component of the air microflora in coniferous forests. Primary infection occurs in forests mainly through freshly exposed wood surfaces: stumps or, sometimes, through wounds on roots and root collars. The spores cannot infect healthy, uninjured roots of living trees, although the fungus may be able to degrade and penetrate the bark of them to some extent (Korhonen and Stenlid 1998). In Estonia the decay is caused mainly by *Heterobasidion parviporum* Niemelä and Korhonen on Norway spruce and *H. annosum* (Fr.) Bref. (*H. annosum* s. str.) on Scots pine (Hanso and Hanso 1999a, b). The most severe damages in Estonia occur mainly on Norway spruce (*Picea abies* (L.) Karst), but quite often the disease is being found also on Scots pine (*Pinus sylvestris* L.*)* and on juniper (*Juniperus* communis L.), less on larches (*Larix* spp.), firs (*Abies* spp.) and Douglas fir (*Pseudotsuga* sp.). Even broadleaved trees may be infected. The fungus attacks host trees in every age class and the advancing decay causes weakening, decreasing of the growth and finally, death of the trees (*e.g*. Hanso and Hanso 1999b). Therefore all control methods which reduce the losses caused by the disease must be applied but first evaluated prior to practical applications.

Since the early 1950s in England stump treatment experiments had been carried out using a saprotrophic fungus, which was highly competitive against *Heterobasidion annosum* s.l., but did not infect living tree tissues: *Phlebiopsis gigantea* (Fr.) Jül. [*Phlebia gigantea* (Fr.) Donk, *Phanerochaete gigantea* (Fr.: Fr.) Rattan et al.]. However, later on the testing of several other fungal species and strains against *Heterobasid-*

ion spp. was continued in different countries, including Estonia (Hanso 1992, Hanso and Hanso 1985, 1992).

P. gigantea is a common and widely distributed saprotrophic fungus in the coniferous forests of the Northern Hemisphere, occurring especially frequently in managed forests (Vainio *et al*. 2001). Morphologically, *P. gigantea* has been regarded as a single taxonomic species throughout its wide geographical range, but clear genetic differentiation has been found between the European and North American populations of the fungus (Vainio and Hantula 2000). It forms resupinate greyish fruitbodies with characteristic conical cystidia projecting from the hymenium. In pure culture it can be identified easily on the basis of its oidial chains, encrusted upright aerial hyphae, and multiple clamp connections (Holdenrieder and Greig 1998). The ability of *P. gigantea* to form abundant oidia, together with fast colonisation of fresh wood surfaces, and its strong antagonistic reaction towards *Heterobasidion* spp. were the main reasons for choosing *P. gigantea* for the industrial-scale production of biological control preparation Rotstop®. Various *P. gigantea* preparations, based on local isolates of the fungus had been in use for decades in the UK, thereafter in Nordic countries and in Poland.

The Finnish product of *P. gigantea*, Rotstop® was registered in Estonia already in 2004 (as a preparation for biological control). Main reason for the registration might be the fact, that by that time this preparation had been already internationally acknowledged as a promising agent against *Heterobasidion* spp. The regarded negative aspect for the wide-scale use of the preparation – a single dispersive clone of the fungus will be artificially distributed over large areas of forests (Vasiliauskas *et al*. 2004) with its possible influence on the natural diversity of the local populations of *P. gigantea* – has been evaluated by other researchers as quite small (Holdenrieder and Greig 1998, Vainio *et al* 2001).

Material and methods

In this study the effect of the Estonian and Finnish strains of *Phlebiopsis gigantea* (further P.g.1 and R, respectively) against the Estonian strains of *Heterobasidion* spp. were tested in laboratory 1) as pure cultures and 2) on infected woodblocks. Thereafter the biological efficiency of Rotstop® was studied on fresh spruce and pine stumps in several experimental plots, established in the forests of north- and South-eastern Estonia.

Hanso and Hanso (1999a) had established in 80 different spruce forests located all-over in Estonia that *Heterobasidion parviporum* was prevalent on spruces (73 isolates of *H. parviporum* and only one of *H. annosum*), whereas in 69 investigated pine forests all the *Heterobasidion* spp. isolates belonged to the *Heterobasidon annosum* s.str. It means that *Heterobasidion* spp. are predominantly host-specific in Estonia.

Laboratory experiments with pure cultures

Pure cultures of different Estonian strains of *Heterobasidon annosum* s.str. (labelled as H.a.1, H.a.3, H.a.4) and *H. parviporum* (respectively H.p.1, H.p.2, H.p.3, H.p.4, H.p.5), and the strains of *P. gigantea* from Estonia (P.g.1) and from Finland (R - isolated from Finnish preparation Rotstop®) were grown in dual cultures in Petri dishes according to the standard protocols (*e.g*. Holdenrieder and Greig 1998) with the purpose to evaluate the antagonistic effect of the strains of *P. gigantea* towards the strains of *H. annosum* and *H. parviporum*. Dual cultures were grown at two different temperatures, corresponding in large scale to the mean temperatures of forest ground floor in Estonia during the warmest summer month July and the full summer (period between June to August, *i.e.* +22°C and +17°C, respectively). Dual inoculations of the strains of *Heterobasidion* spp. and *P. gigantea* were made onto the 1.5 % malt agar (OXOID, malt LP0039, agar LP0013) into the centre areas of 90 mm Petri dishes at the distance of 2 cm from each other, each variant in 5 duplications (*i.e*. in 5 different dishes). Growth rate of both the colonies and barrier zone characteristics were estimated, respectively, one and three weeks after the inoculations.

Laboratory experiments on woodblocks

The aim of these experiments was to investigate in *in vitro* conditions:

1) Ability of the colonies of the Estonian and Finnish strains of *P. gigantea* to overgrow the Estonian strains of *Heterobasidion* spp. on woodblocks (as the growth and antagonistic relations may differ depending on the kind of medium – malt agar medium or conifer wood). Altogether 40 small wooden blocks, 20 blocks of the both conifer species (from a freshly cut Norway spruce and a Scots pine tree) were placed into the Petri dishes fully grown by the colony of *P. gigantea* strain. During three weeks the fungus had colonized the woodblocks. Thereafter the blocks were placed into the new Petri dishes, where agar medium was occupied by one of the Estonian strains of *Heterobasidion* spp (*H. annosum* and *H. parviporum*, respectively). Two weeks after that the woodblocks were investigated under the stereomicroscope for the *Heterobasidion* anamorphs.

2) Characteristic features of wood colonisation by the both fungal species were verified for identification of the two fungi in *in vivo* experiments in forest. Infected/rotting wood colour variation and characteristic su-

perficial mycelium of *P. gigantea*, and appearance of the anamorphic stage (*Oedocephalum lineatum* (syn. *Spiniger meineckellus*) of *Heterobasidion* spp. on blocks, visible under the small-magnification stereomicroscope, were used as the distinguishing characteristics.

Establishment of study sites in forests

The field sites for studies were previously unthinned stands chosen by the forestry authorities. Cuttings and immediate spraying of stumps were carried out from May, the 17th until June, the 16th in 2005 and from June, the 19th until June, the 22nd in 2006, altogether in six (this way: unthinned before) stands of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*). During the trials, the stumps were identified according to numbered aluminium labels attached to the stumps.

Experiments in two spruce and two pine stands were established in the spring and the first results were analyzed already in the late autumn of the same (first) year (signed later as: 2005_1). Thereafter, two new spruce stands were left to be selected out for our experiments by the local forestry authorities in the next spring (signed as: 2006_1). Stumps on all the study sites (incl. the sites, established in 2005) were analysed in the late autumn of 2006, as well. Results of the experiments, which lasted for two years, are later signed as 2006_2. Three Norway spruce and one Scots pine study sites were situated in North-eastern Estonia, one Norway spruce and one Scots pine experimental stand situated in South-eastern Estonia (Figure 1).

Thinnings were carried out as manual man-made cuttings in South-eastern (Järvselja) experimental sites, but in North-eastern Estonia (Triigi, Rava, Ahtme) by single-grip harvester (Table 1). The stumps were left about 30-40 cm high and sprayed with Rotstop® immediately after the tree felling. Before the application over stumps, the spore viability, but not the concentration of the suspension was controlled in our experiments. The viability of oidia in suspension was determined, according to the Rotstop® instructions, a day before the treatment.

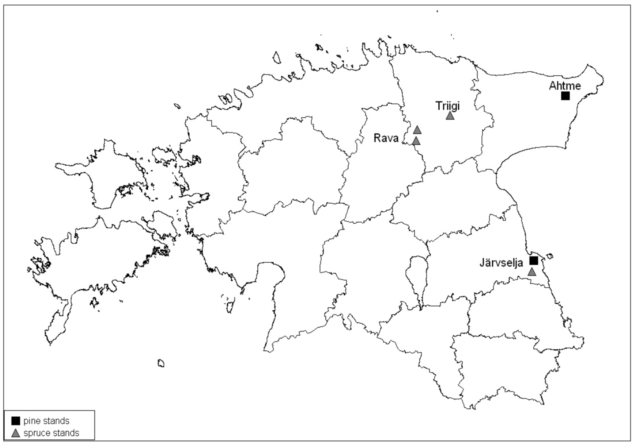

Figure 1. Location of the experimental stands

Table 1. Main characteristics of the experimental stands (study sites)

Location, forest district, compartment, subcompartment	Geographical coordinates	Tree species	Stand age (years)	Site type	Average mean height (m)	Treatment style
2005						
South-eastern Estonia, Järvselja (274, 14)	58°31'06''N 26°57'12''E	*Picea abies*	35	*Myrtillus*	29.1	Manually after cutting with chain saw
South-eastern Estonia, Järvselja (244, 13)	58°16'41''N 27°20'06''E	*Pinus sylvestris*	49	*Oxalis-Myrtillus*	27.5	Manually after cutting with chain saw
North-eastern Estonia, Ahtme (VF 221, 8)	59°17'63''N 27°44'65''E	*Pinus sylvestris*	35	Recultivated oil-shale quarry	25	Mechanically by the harvester*
North-eastern Estonia, Triigi (PX 068, 3)	59°12'18''N 26°13'30''E	*Picea abies*	41	*Hepatica*	28	Mechanically by the harvester*
2006						
North-eastern Estonia, Rava (RA222, 19)	59°06'59''N 25°49'81''E	*Picea abies*	39	*Hepatica*	30.2	Mechanically by the harvester*
North-eastern Estonia, Rava (RA222, 20)	59°06'57''N 25°49'83''E	*Picea abies*	53	*Hepatica*	23.8	Mechanically by the harvester*

* - For technical reasons the stump coverage with Rotstop® suspension ranged between 70 and 100%

Altogether 626 stumps were included into the experiments (Table 2).

Table 2. The number of stumps in the experiments on different study sites

Study site	Tree species	Number of experimental (labelled) stumps: Treated stumps	Number of experimental (labelled) stumps: Untreated (control) stumps	Number of analyzed stumps: out of treated stumps	Number of analyzed stumps: out of untreated stumps
2005					
Järvselja (274, 14)	*Picea abies*	29	25	16	17
Järvselja (244, 13)	*Pinus sylvestris*	52	15	46	10
Ahtme (VF 221, 8)	*Pinus sylvestris*	163	33	72	20
Triigi (PX 068, 3)	*Picea abies*	133	28	65	14
2006					
Rava 1 (RA222, 19)	*Picea abies*	40	15	30	15
Rava 2 (RA222, 20)	*Picea abies*	52	15	36	13
Järvselja (274, 14)	*Picea abies*			8	4
Järvselja (244, 13)	*Pinus sylvestris*			7	5
Ahtme (VF 221, 8)	*Pinus sylvestris*			40	10
Triigi (PX 068, 3)	*Picea abies*			39	12

Sampling and laboratory analysis

The discs for laboratory analyses were cut from stumps (both – treated and untreated) ca 4-5 months after treatments. In case there were signs of *Heterobasidion* decay in the butt region of the stump, this stump was excluded from further analysis as already infected. From each stump, after removal of the stump surface (3-5 cm thick), the sample disc (3-4 cm thick) was cut and immediately (the same day) transported in plastic bag to the laboratory. After incubation for 5-7 days at room temperature the discs were analysed under the stereomicroscope from both sides for the presence of the agent of the biocontrol *P. gigantea* (characteristic dark-orange wood surface discoloration + oidia) and of the pathogen *Heterobasidion* sp. (anamorphic fruitings). A small-magnification microscope was used for the analysis of the discs in laboratory. The surface area colonized by *P. gigantea* and/or *Heterobasidion* spp. on analyse discs was transferred to the white clean paper sheets using watercolour markers Edding 40 and measured (in cm^2) from the paper lists by digital planimeter.

Calculations and statistics

Statistical analysis was conducted with SAS (Statistical Analysis System). The procedure GLM (General Linear Models) was used for calculation of the efficiency of Rotstop®. To compare average values the T-test was used. The figures were prepared in Excel.

The effect of Rotstop® treatment to the amount of mycelium coverage (cm^2) on the total stump area (S) was analysed as follows:

1. The whole data set, classified in analysis as: a) both tree species, b) treatment, and c) treatment year;
2. The whole data set, classified in analysis as: a) first year after the treatment (2005_1 and 2006_1, respectively); and b) means of two years of treatments (2006_2);
3. The a) untreated and treated (with the suspension of Rotstop®) stumps after the first and second year, respectively, and b) the treatment, and c) the tree species;
4. The difference between the results on the tree species, classified in analysis: a) by treatment way, and b) by the year of treatment.

Using the statistical tools, the mean results of experiments were analysed, except Tables 3 and 4, where the data from each experimental stand are pointed out separately.

Results

Laboratory experiments with pure cultures

Both, the mycelium growth rate of both the fungi in cultures and the inhibiting effect of *P. gigantea* to the growth of *Heterobasidion* spp. in dual cultures was higher at +22°C than at +17°C. In the control culture where two inocula of the same *Heterobasidion* sp. strain were placed on one plate, the colony had occupied during the same time the total surface of the agar medium. Both, Rotstop® and the Estonian strain of *P. gigantea* inhibited the growth of the colonies of *Heterobasidion* sp. nearly by the same rate, *i.e.* without statistical differences ($p>0.05$). Already in the course of two first weeks the colonies of the both *P. gigantea* strains overgrew the colonies of all the *Heterobasidion* spp. strains in *in vitro* experiments, and their influence could be classified as strong antagonistic or competitive activity against *Heterobasidion* spp.

Laboratory experiments on woodblocks

Ability of the both strains of *P. gigantea* (isolated from the preparation Rotstop® and from Estonia, respectively) and Estonian strains of *Heterobasidion* spp. to occupy spruce- and pinewood in mono-, and dual cultures was evaluated and compared.

It was checked up whether the mycelium of *P. gigantea* was able to overgrow the *Heterobasidion* spp. colonies and how abundantly these two fungal species had colonised the wood. Visible changes on spruce- and pinewood surfaces were investigated. No formation of conidial stage of *Heterobasidion* (*Oedocephalum lineatum*) was found on the woodblocks, overgrown before with *P. gigantea*. Approximate coverage of the blocks by the colonies of both fungi was visually evaluated (Figure 2).

Both *P. gigantea* strains (R and P.g.1) colonized in *in vitro* experiments spruce- and pinewood effectively, the commercial (Finnish) strain being somewhat faster.

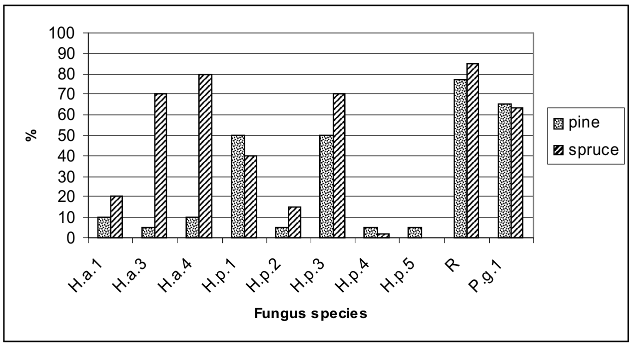

Figure 2. Growth rate of different Estonian strains of *Heterobasidion* spp. (*H. annosum* - H.a. and *H. parviporum* - H.p.) and of *Phlebiopsis gigantea* (Finnish R and Estonian P.g.1, respectively) on spruce-, and pine woodblocks in *in vitro* experiments, as evaluated one week after the inoculation (in percentages, which concern the occupied by *Heterobasidion* spp. or *P. gigantea* area from the total area, visually estimated)

Experiments on study sites in forests

Table 3 represents the results of the first year experiments (2005_1 and 2006_1 respectively), *i.e.* ca 4-5 months after the treatment.

Table 4 represents the data of second year (2006_2), *i.e.* 18 months after the treatment.

Comparison of the total stump area and occupied by *Phlebiopsis gigantea* area shown statistically significant (P<0.0001) difference. According to the results of this study the efficiency of Rotstop® could be evaluated effective: majority of the treated stumps was protected.

Table 3. Results of the experiments ca 4-5 months after the treatment

Location of field area	Tree species	Year of experiment	Treatment	Average area of stumps (cm²)	Number of stumps colonized by *Heterobasidion* sp.	Average stump area colonized by *Heterobasidon* sp. (cm²)	Stump area colonized by *Heterobasidion* sp. in the trial variant (%)	Number of stumps infected by *P. gigantea*	Average stump area infected by *P. gigantea* (cm²)	Stump area in the trial variant infected by P. *gigantea (%)*
Järvselja	*Picea abies*	2005	treated	216.7	0	0	0	16		11
			untreated	206.4	14	5.4	3	6	2.1	1
Järvselja	*Pinus sylvestris*	2005	treated	216.0	1	0.1	0	46	135.7	63
			untreated	118.1	1	1.0	1	6	40.4	34
Ahtme	*Pinus sylvestris*	2005	treated	175.7	0	0	0	72	143.7	82
			untreated	153.8	5	5.7	4	4	10.0	6
Triigi	*Picea abies*	2005	treated	219.0	23	1.1	1	57	56.6	26
			untreated	222.9	5	3.0	1	4	10.8	5
Rava 1	*Picea abies*	2006	treated	267.7	6	0.9	0.3	29	178.8	67
			untreated	184.8	13	12.1	7	3	7.1	4
Rava 2	*Picea abies*	2006	treated	241.9	10	0.5	0.2	31	153.8	64
			untreated	118.1	13	8.5	7	5	6.6	6

Table 4. Results of the experiments 18 months after the treatment

Location of field area	Tree species	Year of experiment	Treatment	Average area of stumps (cm²)	Number of stumps colonized by *Heterobasidion* sp.	Average stump area colonized by *Heterobasidon* sp. (cm²)	Stump area colonized by *Heterobasidion* sp. in the trial variant (%)	Number of stumps infected by *P. gigantea*	Average stump area infected by *P. gigantea* (cm²)	Stump area in the trial variant infected by P. *gigantea (%)*
Järvselja	*Picea abies*	2006	treated	169.9	3	0.4	0.2	6	28.6	17
			untreated	204.5	2	1.2	0.6	2	2.4	1
Järvselja	*Pinus sylvestris*	2006	treated	295.9	0	0	0	7	244.2	83
			untreated	223.0	0	0	0	2	98.4	44
Ahtme	*Pinus sylvestris*	2006	treated	188.1	0	0	0	30	125.6	67
			untreated	185.1	1	0.9	0.5	3	10.0	5
Triigi	*Picea abies*	2006	treated	213.1	1	5.6	3	37	115.3	54
			untreated	210.7	5	3.0	1.4	4	10.8	5

Efficiency of Rotstop® in preventing the *Heterobasidion* root rot infection in treated stumps was estimated and compared to the natural infection by the pathogen in untreated stumps Figure 3). For that purpose average areas of the colonies of *P. gigantea* and *Heterobasidion* spp. on stump surfaces were used, calculations were made separately for the both tree species (spruce, pine) and treatments (treated/untreated with Rotstop®).

Comparison of the total stump area with the area occupied by *Heterobasidion* spp. area shown statistically significant difference (P<0.05), as well. *Phlebiopsis gigantea* had suppressed the pathogen. *Heterobasidion* rot had occupied only a very small part of the area on pine stumps, but the coverage of the pathogen was somewhat larger on the treated spruce stumps. Difference between the treatment and untreatment variants was statistically significant (P<0.0001).

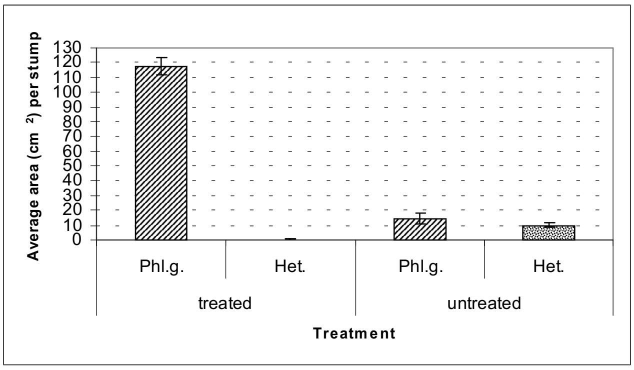

Figure 3. Efficiency of Rotstop® in preventing the *Heterobasidion* spp. infection in the treated stumps, compared to untreated (control) stumps (Phl.g. - *Phlebiopsis gigantea*; Het. - *Heterobasidion* sp.; treated – treated with suspension of Rotstop®; untreated – control, *i.e.* without treatment)

Average stump coverages by *P. gigantea* and *Heterobasidion* spp., respectively, in the first (2005_1 and 2006_1) and second (2006_2) years were compared (Figure 4). At the same time the treatment (treated, untreated) and tree species (spruce or pine) were not distinguished. The occupation of stumps by *P. gigantea* in different years had no statistically significant difference (P>0.05). *P. gigantea* had occupied treated and untreated, respectively, stumps similarly in the first year, as well as in the second year. At the same time the results were different concerning the occupation of stumps by the *Heterobasidion* spp.: the year as a characteristic differed significantly (P<0.05), and in the first years (both: in 2005_1 and 2006_1) there were more *Heterobasidion* spp. infections detected than in the second year (2006_2).

The effect of treatment to the mean area of stump surface occupied by *P. gigantea* and *Heterobasidion* spp., respectively, was analysed over tree species and experimental year (Figure 5). Concerning area occupied by *P. gigantea*, the treatment had statistically significant difference (P<0.0001) between the tree species, but the treatment year had not (P>0.05). Concerning *Heterobasidion* spp., the treatment and the experimental year were statistically significantly different (P<0.0001 and P<0.05, respectively).

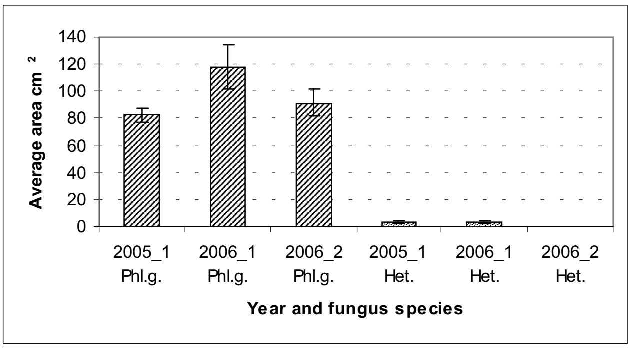

Figure 4. Stump occupation levels by *P. gigantea* and *Heterobasidon* spp., respectively, in the first (cutting) and second (after-cutting) year, expressed as the occupied areas on stump surfaces

The area occupied by *P. gigantea* in untreated stumps was statistically different between tree species (P<0.05) but it did not differ, between years (P>0.05).

In difference to that, concerning *Heterobasidion* spp., as the treatment (with Rotstop®) variant as well the year occurred to be statistically different (P<0.05).

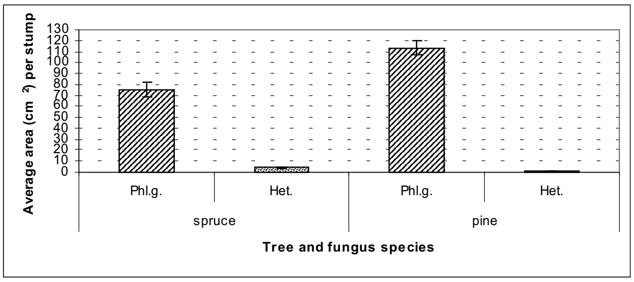

Figure 5. Efficiency of treatment of conifer stumps with biopreparation Rotstop®
(Phl.g. – *Phlebiopsis gigantea* colonies; Het. – *Heterobasidion* spp. colonies)

In case of the tree species the treatment and years were also compared. The treatment (treated, untreated), concerning the tree species (spruce and pine), and not depending of the fungus species (*Heterobasidion* spp. or *P. gigantea*), differed significantly (P<0.0001). Comparing the tree species (spruce and pine) the year in case of *P. gigantea* did not differ statistically (P>0.05). Comparing *Heterobasidion* spp. and the spruce, the infection differed statistically (P<0.05), but in case of pine no difference was found (P>0.05). As shown before, the natural infection level of pine stumps by *Heterobasidion* spp. was much lower than of spruce, and it might influence the result.

Discussion

Laboratory experiments

Laboratory experiments with pure cultures of fungi as on malt agar, as well on woodblocks, demonstrated that *P. gigantea* strain Rotstop® had a strong controlling effect against the Estonian strains of *Heterobasidion* spp., even somewhat stronger than had the tested Estonian strain of the same species (*P. gigantea*). On *Pinus sylvestris* and *Picea abies* woodblocks it assured complete protection against the pathogenic rot agent over wood surface area. Similar *in vitro* tests on stem pieces of *Pinus pinea*, carried out by Annesi *et al*. (2005) in Italy showed that the preparation Rotstop® provided a full protective effect as well against an Italian strain of *Heterobasidion annosum* after artificial inoculation of wood with the fungi. Both of the *P. gigantea* strains, a native strain from Italy and the Rotstop® strain were effective in *in vitro* experiments, preventing the following infection of woodblocks of *P. pinea* by the local strains of *Heterobasidion* spp.

Field experiments, rather effective

During two consequtive years field treatments with the biopreparation Rotstop® in Estonia were found to be effective, even in the spruce stands with the high infection level of *Heterobasidion parviporum*. Before that, good results were obtained from the investigations in Finland, Denmark and Italy.

In Finland the use of Rotstop® exhibited practically complete prevention against *Heterobasidion* spp. infection in Scots pine stumps, whereas in Norway spruce stumps the competitiveness of the saprotrophyte was somewhat lower (Vainio *et al.* 2001). The results in Finland showed that the stump treatment with Rotstop® is an effective preventive controller of *Heterobasidion* spp. in forests during the summer cuttings. In the field trials performed in southern Finland, the surface of the untreated (control) stumps had approximately ten-fold larger area occupied by *Heterobasidion* spp. than in treated stumps (Kasanen, unpublished).

In Denmark, six months after the nearly simultaneous artificial infection of fresh stumps of Norway spruce with the conidiospores of the S-type of *H. annosum* (= *Heterobasidion parviporum*?) and spraying of the stumps with Rotstop®, the level of *Heterobasidion* infection in treated with Rotstop® and untreated (control) stumps was 15% and 88%, respectively.(Thomsen 2003). The same series of experiments in Denmark resulted in the conclusion, that the efficiency of stump treatment with biopreparation Rotstop® was even somewhat higher than an alternative treatment with urea and the treatment with Rotstop® had inhibited the rate of distribution of the pathogen to the neighbouring trees.

Annesi *et al*. (2005) carried out stump treatment experiments in Italy, in a coastal *Pinus pinea* forest close to Rome with comparable research methods as were used in Estonia. *Phlebiopsis gigantea* had colonized there 100% of the surface area of subsamples, taken from the treated stumps, whereas *H. annosum* could be never recorded in the stumps protected this way. In Estonia, as in Italy, similarly some natural infection of *P. gigantea* occurred in control stumps, but the inocula was too scarce and few to naturally control the pathogen. In another Italian study the biopreparation Rotstop® demonstrated high effectiveness (70-90%, cf. La Porta *et al*. 2001), as well, on Norway spruce (*Picea abies*), whereas the local *P. gigantea* strain (as in Estonia) and the use of urea were less effective.

Field experiments, rather less effective

Thor and Stenlid (2005) compared the efficiency of different methods of stump treatment during the summer cuttings in large-scale experiments in Sweden, altogether with 1,246 Norway spruce stumps on 14 different experimental areas. They concluded, that all the control measures used during summer cuttings, similarly to the winter cuttings, decreased the infected by *Heterobasidion* stump area. Among these methods, including the use of biopreparation Rotstop®, the highest efficiency was achieved using urea. As well in Sweden, some other experimental treatments of Norway spruce stumps with Rotstop®, had resulted in only a small difference in the occurrence of the pathogen *Heterobasidion* as on treated, as well on untreated stumps (*e.g.* 76.2% and 85.7%, cf. Lygis 2005), this way showing much lower efficiency than in most other countries. Berglund and Rönnberg (2004) and Berglund *et al*. (2005), the both as well in Sweden, concluded after the similar experiments as we carried out in Estonia, that the use of the biopreaparation Rotstop® did not essentially decrease the number of spruce stumps infected by the *Heterobasidion* root rot in the course of summer cuttings.

Efficiency variation in Phlebiopsis gigantea

In different countries several local strains of *P. gigantea* have already been investigated, genetically compared between each other (*e.g.* Webber and Thorpe 2003) or evaluated in comparison with the Rotstop® strain (La Porta *et al*. 2001, Rönnberg *et al*. 2005). In southern Sweden two series of experiments were carried out (Berglund 2005 and Rönnberg *et al*. 2006) with different *P. gigantea* preparations, containing *P. gigantea* strain from Finland (Rotstop F) and several others from Sweden (*e.g.* Rotstop S). Two of the local (Swedish) *P. gigantea* strains showed the best control against airborne *Heterobasidion* infection.

Treatment efficiency depending on the surface coverage of stumps

In the evaluations of the biocontrol efficiency the stump surface area, occupied by *P. gigantea* mycelium, is one characteristic of the Rotstop® efficiency. In order to achieve the best control, the stump surface must be fully covered by the suspension (Berglund 2005). Mechanical treatment of stumps with incomplete coverage failed to control the infection by *Heteroba-*

sidion root rot (Rönnberg *et al.* 2006). In our experiments in North-eastern Estonia, although the stump coverage by the suspension was not total (varying from 70 to 100%), the efficiency of treatment was fully acceptable.

Efficiency, depending on the conifer species

In our experiments, as well in other investigations the efficiency of treatment turned out to be different in different conifer species. In Estonia it was obvious that the natural occurrence of *P. gigantea* was higher in Scots pine than in Norway spruce, which is in full accordance with the data from the literature (*e.g*. Lygis 2005). It is not known why *P. gigantea* is more actively colonising stumps of pine, but forms more abundant fruitbodies on stumps of spruce. Our investigations resulted principally in the same conclusion which was made by several other researchers (*e.g*. Vainio *et al*. 2001), that treatment of stumps of Scots pine shows higher biological efficiency than of Norway spruce stumps. However, there were some differences between the coverage of stumps. Pine stumps were more covered by *P. gigantea* and thereby more protected against *Heterobasidion* spp. infection by the suspension of Rotstop® than spruce stumps, which probably means that the vegetative growth of the *P. gigantea* mycelium was faster in the wood of pine than of spruce. As well, *Heterobasidion* spp. infection in the untreated spruce stumps was more dispersed than in pine stumps. Probably, pine stumps are already naturally more protected (*e.g.* by the resin) than spruce. The scanter growth of *P. gigantea* on the stump surface on the second year could be caused by the weather conditions, as the summer of 2006 was hot and dry (Hanso and Drenkhan 2007), therefore the stump surface analyses might betray smaller than actually extent of stump colonisation.

Duration of the efficiency of Rotstop® treatment

In our experiments in the stumps (both spruce and pine) which were analysed twice (*i.e.* during two successive autumns), less *P. gigantea* occupation was found in the first year than in the second year, but the difference was not statistically significant. Vainio *et al.* (2001) succeeded in isolating *P. gigantea* a year after the treatment with Rotstop® from 77% of the total number (altogether 53) of treated Scots pine stumps, whereby the area of stump surface colonisation was visually evaluated from 0 to 70% (with 20% as the mean). It means that the extent of the actual stump colonisation by the fungus mycelium is even larger than it can be evaluated by the investigation of the stump surface. In Estonia a year after the treatment only 10 stumps (4.6% from the 219 treated pine stumps) were characterised as not infected by *P. gigantea*, and all these 10 stumps were found in one year (2006) and from one experimental area (Ahtme). Six years after the inoculation Vainio *et al*. (2001) did not succeed in finding any fruitbodies of the fungus from the pine stumps inoculated with it. At the same time Vainio *et al*. (2001) succeeded to isolate *P. gigantea* from 64% of the Norway spruce stumps inoculated a year before. The coverage of the spruce stumps by the rot of *P. gigantea*, evaluated visually, was only 0-10%, and, as a mean, less than 5%. In contrast to pine, six years after the treatment the basidiocarp abundance on the Norway spruce stumps treated with Rotstop® was impressive: 53% of stumps were carrying basidiocarps of *P. gigantea*.

Treatment method

Thor (2005) found in Sweden, that the efficiency of manual treatment by Rotstop® was more effective than mechanical treatment. Different experimental areas were treated differently, or manually or mechanically, in our investigations, which may have affected the results.

The influence of other fungi

Also other xylophilic fungi may have effect to the results of field experiments with control agents. In this investigation the natural fungal community structure of stumps was not determined. In total 47 species of fungi were found, from pure cultures of fungi isolated from 402 wood samples, cut from 63 stumps in Sweden (Vasiliauskas *et al*. 2004). In Lithuania the fungal community in stumps of Norway spruce (*Picea abies*) was investigated (Vasiliauskas *et al*. 2002). Fruiting bodies were recorded and determined on 3,294 conifer stumps. Among others, also *P. gigantea* and *H. annosum* were often found. In stumps, treated 4 years before with Rotstop®, the fungal community was dominated by *P. gigantea*, both in terms of isolated strains and observed fruitbodies. In 6-year-old stumps, both treated and untreated, *P. gigantea* was detected seldom and no fruitbodies were observed (Vasiliauskas *et al*. 2005).

In stumps treated with Rotstop® in Italy many fungal taxa, altogether 49, were isolated (Varese *et al*. 2003), surprisingly close to the number of taxa in Sweden (47, cf. Vasiliauskas *et al*. 2004). As well abundant bacteria can colonize the upper parts of the stumps, particulary in the first 2-3 years after felling of trees (Woods *et al*. 2005).

The relations of other (incl. xylophilic) fungi to the *Heterobasiodion* spp. and *P. gigantea* are less known. They may have some influence to the results of the biocontrol of the pathogen, too. In the earlier experiments in Estonia, a total of 76 strains of different fungal species were tested as potential biocontrol agents (Hanso 1992, Hanso and Hanso 1985, 1992). Intense antagonistic or competitive activity was found in 8 of them, including *P. gigantea*.

Conclusions

Biological efficiency of the use of Rotstop® in Estonia during two years of experiments was fully acceptable. However, as in other countries before, the treatment with Rotstop® was more effective in pine than in spruce. In pine stumps the effect of Rotstop® was higher in the first year, but in spruce stumps the effect lasted longer and was even higher in the second year after the treatment. In our experimental areas the natural infection level of stumps by *Heterobasidion* spp. was much higher in spruce than in pine stumps and undoubtedly it might have some influence to the results. A clear conclusion was made: conducting the stump treatment was necessary and effective in all the six of our experimental stands, selected for the experiments by the forest authorities and not by the authors of this article. The results of this study suggest more extensive use of biological control against *Heterobasidion* root rot in Estonia.

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ЭФФЕКТ ОБРАБОТКИ КОРНЕЙ ПРОТИВ КОРНЕВОЙ ГУБКИ (*HETEROBASIDION* SPP.) ФЛЕБИЕЙ ГИГАНТСКОЙ (*PHLEBIOPSIS GIGANTEA*) В ЭСТОНИИ

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Резюме

Эффективность препарата биологической борьбы Rotstop® против споровой инфекции патогенными базидиомицетами с рода *Heterobasidion* была исследована в Эстонии в течений двух лет. В начале лабораторные эксперименты были проведены *in vitro* для сравнения эффективности штамма гриба *Phlebiopsis gigantea* в препарате Rotstop® с Эстонским штаммом того же вида против ряда Эстонских штаммов *Heterobasidion* spp. Также были проведены предварительные лабораторные испытания вышеуказаных грибов на кусочках свежей древесины как ели (*Picea abies*), так и сосны (*Pinus sylvestris*) с целью изучения характерных признаков гнили, вызываемой грибом *Phlebiopsis gigantea* и диагностических признаков *Heterobasidion* spp. на поверхности древесины. Последующие эксперименты в прежде непройденных рубками ухода лесах в четырех ельниках и двух сосняках в Южно-восточной и Северо-восточной Эстонии, соответственно, включили в среднем 83 обработанных суспенсией Rotstop® и 21 необработанных (т.е. контролных) пней на каждом участке.

Rotstop® особенно успешно контролировал споровую инфекцию *H. annosum* s. str. в насаждениях сосны (средние окупированные обеими исследованными грибами поверхности обработанных пней: соответственно *P. gigantea* 113,3 см^2 и *H. annosum* s. str. 0,6 см^2).

Заметная естественная инфекция с локальными (природными) штаммами *P. gigantea* была также регистрирована, но она была слишком ограниченной для реального исключения патогена от пней во время рубок леса. В еловых насаждениях Rotstop® был эффективным (средние окупированные обеими грибами поверхности пней 75,2 см^2 и 4,3 см^2, соответственно), хотя меньше, чем в сосновых насаждениях, возможно, по причине чрезвычайно высокого естественного инфекционного уровня *H. parviporum* в наших экспериментальных еловых насаждениях (до 85% необработанных препаратом еловых пней заражались патогеном).

Ключевые слова: биологическая борьба, Rotstop®, *Phlebiopsis* gigantea, *Heterobasidion* spp., сосна обыкновенная, ель европейская