TESTING THE ‘ROTSTOP’ BIOLOGICAL PREPARATION FOR CONTROLLING
HETEROBASIDION ROOT ROT IN LATVIA

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Abstract

In Latvia, Picea abies and Pinus sylvestris as the commercially most valuable species make more than half of all woodlands. It has been found that about 23% of spruce stands are infected by root rot, caused predominantly by fungal pathogen Heterobasidion annosum s.l. To restrict the spread of root rot in coniferous forests of Latvia, the Joint Stock Company ‘Latvijas valsts meži’ collaborated with Latvian State Forest Research Institute ‘Silava’ in 2006 and launched a project for testing the Rotstop biological preparation, containing a suspension of Phlebiopsis gigantea spores. This project had a general task to accomplish Rotstop using technology approbation in Latvia and develop control system. Starting with the year 2008, preparation was used for stump treatment during thinning operations. When analysing the field data, a conclusion made in other studies was confirmed - P. gigantea colonizes pinewood more intensively even in case of improper stump treatment. It implies that the quality of stump treatment is decisive when using Rotstop for rot control in spruce stands.

Key words: Phlebiopsis gigantea, Heterobasidion annosum, thinning, quality control, Rotstop.

Introduction

With forests covering 50.9% of the country’s territory and the conifers accounting for 54% of the total woodland area (2014 State Forest Service data) about 23% of spruce stands are infected by root rot, caused predominantly by such fungal pathogen as Heterobasidion annosum s.l. (Arhipova et al., 2010). H. annosum spreads by fungal mycelium when infected tree roots come into contact with the roots of adjacent healthy trees, and by basidiospores, which infect tree stumps right after felling (Hodges, 1969). In standing trees the H. annosum infection causes white rot of tree roots and stem, which may spread as high as 12 m (Stenlid and Wästerlund, 1986). In the EU, the annual losses to forestry caused by this pathogen are estimated at EUR 790 million (Woodward et al., 1998). As a result, the timber quality is lower and the forests become more susceptible to different biotic and abiotic damages. The spread of H. annosum is closely related to the existing management practices. Mechanized timber harvesting favours the infection of tree stumps and damages stems and roots by fungal spores both in the remaining stands and restocked cutovers.

It is possible to control the spread of H. annosum at any stage of its life cycle (Asiegbu et al., 2005). Silvicultural methods of control include restocking the respective site by the tree species other than the infected one, for instance, by pine P. sylvestris or broadleaves instead of spruce P. abies (Piri, 1996; Piri and Korhonen, 2001). There is a possibility also to establish mixed stands which are less susceptible to H. annosum (Arhipova et al., 2010), or reduce the number of young trees per unit area, thus reducing the need for thinning out the dominant species (Epstein, 1978), or carry out thinning operations in winter (Rönnberg, 2000). One of the methods that could be used is no thinning in heavily infected sites, thus reducing the rotation cycle of infection-sensitive stand (Bendz-Hellgren et al., 1999). Stubbing of infected stumps appears to be one of the most efficient methods for mitigating the risk of H. annosum infection (Rishbeth, 1951; Hodges, 1969; Vasaitis et al., 2008; Cleary et al., 2013), including removal of all infected wood from the forest site (Schütt and Schuck, 1979; Müller et al., 2007; Stívriňa et al., 2010). The other method that could be used is the treatment during logging operations of fresh stump surfaces by borate and carbamide solvents, or using preparations which contain a suspension of Phlebiopsis gigantea spores (Woodward et al., 1998).

In forestry, thinning, carried out properly and in due time, is one of the major preconditions for achieving a tree stand of high productivity in the future. Still, in thinning operations we leave a lot of stumps behind, which is one of the risk factors that the future crop trees might be rot-infected. Unlike the Scandinavian countries, known as an avanguard of modern forestry, in Latvia the stump treatment in thinnings by H. annosum controlling biological preparations was not practiced. In 2006, the Joint Stock Company ‘Latvijas valsts meži’ (LVM), after studying the experience of Finland and Sweden in this respect, decided to test the above mentioned method and implement it in practical forestry. The LVM, involving the leading research scientists, Finnish experts, and local service providers in forest management, launched a project for field-testing the above mentioned method, including personnel training. The method of applying the Rotstop preparation in fungal pathogen...
control, the related regulatory documentation and the quality control system are among the outputs of this project. In the meantime, the efficacy of the Rotstop preparation was tested so as to achieve its official registration in Latvia. Starting with 2008, in order to reduce the risk of root rot infection, the LVM has been using the biological fungicide Rotstop for treating the stumps in thinning conifer stands. To evaluate the efficiency of this method, starting with 2008 samples of stumpwood for laboratory analyses are annually collected in randomly chosen twenty recently cut stands. The objective of this study was to evaluate the quality of Rotstop treatment and the presence of Rotstop and natural P. gigantea strains in treated conifer stumps. Project experts regularly take LVM personal trainings to improve knowledge about root rot and Rotstop using.

Materials and Methods

This Rotstop approbation experiment was organised in Zemgale and Ziemeļkurzeme LVM regional branches in the year 2006 in thinning (stamps treated to harvest 5448 m³ conifer wood) and finally cuttings (stamps treated to harvest 7200 m³ conifer wood).

Potential for restricting root rot

Field-tests in thinning of the preparations for controlling the Heterobasidion root rot suggest that for the conditions of Latvia those containing the spore suspension of P. gigantea appear to be the most efficient ones. Among the known preparations of this kind the Polish preparation PG-IBL, in which P. gigantea spores are in a mix with conifer sawdust, the British PG Suspension, and the Rotstop of similar composition developed in Finland, which was the most efficient one for Latvia also with regard to the manufacturing methods and supplies are worth mentioning. The latter has been used for controlling H. annosum since 1993 in Scandinavian countries.

Preparing the Rotstop spray solution and the method of its application

The preparation should be kept in a refrigerator or cold storage. To keep the P. gigantea spores alive, in preparing the spray solution its temperature should not exceed 40 °C.

The Rotstop spray solution is prepared as provided by the manufacturer’s instructions (www.verdera.fi), dissolving the preparation in water at a ratio one gram per one litre of water. The final solution looks somewhat muddy. One or two tablets of the TurfMark dye, depending on the operator’s experience, should be added to the solution to control the quality of stump treatment. The spray solution should be used up within 24 hours. Taking into account the ecological and economic aspects of pathogen control, in the process of thinning, it is recommended to treat the stumps by this preparation in tree stands on mineral soils, where the proportion of conifers exceeds 50% and the area of the respective site is not less than one hectare.

Methods of Rotstop application

The Rotstop solution may be applied to stumps simultaneously with the harvester’s felling head severing the tree stem from the stump or by a manual sprayer after specified intervals. The former method is the most efficient one, as the spray solution is pressure-applied through holes in the guide bar of the cutter at the moment the tree stem is cut down. This method ensures a high quality of stem treatment, which is essential for efficient Rotstop application. The use of manual sprayer is recommended in case of motor-manual felling. The treatment ought to be carried out within an hour after felling, i.e. after each successive instance of refilling the fuel tank; the average time span between refillings is about 45 minutes (2014 Verdera).

Quality control of stump treatment

The quality control of stump treatment by root-suppressing agents is necessary because for the forest manager this operation is normally an outsourced service. For this purpose a special method of quality control has been worked out, which envisages three levels of control. In the first level of control the harvester’s operator is obliged to follow up the course of stump treatment. To make this job easier, a dye marker is added to the spray solution, and there is a manual measuring device, which shows to what extent the stump surface is covered with the solution. As provided by quality requirements, about 85% of the stump surface should be covered with the solution. As the dye marker biologically disintegrates in three days, the job supervisor is supposed to check the quality of stump treatment during this time span (2014 Verdera). This is the second level of control. A special electronic form has been worked out in this project to facilitate decision-making regarding stump treatment quality control. The readings of the measuring device are entered into this form and a mathematical algorithm (based on excel) incorporated therein calculates an average for the proportion of stump surface treated.

The third level of control, following a method worked out in this project at the Latvian State Forest Research Institute ‘Silava’, goes with collecting the wood samples from treated stumps and laboratory analyses to determine the occurrence of P. gigantea in these samples. Normally, the mentioned control is done once a year in cases we doubt the job quality of one or another service provider. Sampling is done not earlier than 30 days after the Rotstop application.
5 to 20 stumps are randomly chosen for sampling, depending on the stand composition and the stump mean diameter. A disk about 5 cm thick is sawn off the stump surface, leaving the disk on-site. For the needs of analyses another disk is sawn off. The wooden disks thus prepared are accordingly labelled and placed into plastic bags. The laboratory analyses are done in one or two days after preparing the disks. In the laboratory the disks are debarked and washed under tap water using a brush. After drying the disks are placed in plastic bags and incubated for 5 to 7 days. Incubation results in the signs of *P. gigantea* presence that appear as orange brown colour on the disk surface. A wood sample, 2 – 3 cm wide and 7 – 10 mm deep, is taken using a flame-sterilized chisel from the disk surface at the spot where the *P. gigantea* staining is the biggest. Then rectangular-shaped small pieces of wood are cut out by using a sterile scalpel and placed on sterile Petri dish containing malt extract agar medium. The dish is placed in a thermostat and kept there for about two weeks at the ambient temperature +20 °C till the fungus monocolon is ready for further use. In order to determine whether the *P. gigantea* found on the wooden disk belongs to the Rotstop genotype, tiny pieces (size about 2 × 4 mm) of its mycelium are placed on the Petri dish with malt extract agar medium using a sterile dropper. Then a piece of agar with the tester’s culture is placed on the said dish some 1.5 – 2 cm away from the culture to be tested. The tester’s culture is derived from the Rotstop preparation, putting a small amount of it on the malt extract agar medium. Similarity between the culture to be tested and the Rotstop genotype is evaluated within 3 – 4 weeks by observing the changes in the tester’s culture and an emergence of demarcation or confrontation lines between the two cultures. Emergence of demarcation line is a proof that the respective cultures belong to different genotypes (Stenlid, 1985). In this experiment more than 200 wooden disks were analysed.

**Results and Discussion**

*Use of the Rotstop in forestry practices*

Stumps should be treated by Rotstop preparations in warm weather when the air temperature is above 5 °C and *H. annosum* fruiting bodies intensively sporulate. In Latvian conditions the sporulation is found to be most active from April till November. For example, you can see dynamics of sporulation of *H. annosum* fruiting bodies in the year 2012, in Figure 1. (Brūna, unpublished data).

In 2006, the LVM as a manager of state-owned forests started testing the methods of Rotstop application and worked out regulatory documents for its application and efficiency control. By 2007 the procedure of registering the Rotstop preparations in Latvia had been completed. In 2008, before the Rotstop implementation in forestry practices, a training session, involving the experts (partners from approbation experiment) of the Latvian State Forest Research Institute ‘Silava’, was organized for the LVM staff and its cooperation partners, training 400 persons in total. The implementation of Rotstop methods was a step-by-step process, first applying them in thinning to some 1,200 ha (2008). The aim was to increase the application of this method so as to fully cover the required volume of thinnings in conifer stands in warm weather. Because of the 2009 economic crisis, the Rotstop use was discontinued and resumed again in 2010 with the forest areas thus treated increasing steadily since then (Fig. 2).

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**Figure 1.** Sporulation intensity of *H. annosum* fruiting bodies in the year 2012.
Quality control of the jobs done

The LVM employees and service providers use the Rotstop preparations for reduction of the spread of root rot and carry out the controls following the methods described in chapter ‘Quality control of stump treatment’. The related analyses are done on an annual basis at the Latvian State Forest Research Institute ‘Silava’. For the past three years no *P. gigantea* genotype derived from the Rotstop preparation has been detected on the average in 25% of the stumpwood samples analysed (Fig. 3). This indicator does not reflect the overall situation with the use of Rotstop at the LVM, but only the situation in particular cutting areas as the control refers only to those sites where we reasonably doubted the quality of treatment.

When evaluating the results of 2011 analyses in greater detail, *P. gigantea* is found more often in pinewood, taking up a higher proportion of the stump surface (Table 1). In pinewood *P. gigantea* occupies from 1.04 to 98.00% of the stump surface, while for the wood of spruce this indicator is 0.08 – 75.00%, respectively. It confirms the conclusion made in other studies that *P. gigantea* colonizes pinewood more intensively even in case of improper stump treatment (Thor, 2005), which may be explained by the biology of *P. gigantea*, normally colonizing pinewood (Lipponen, 1991; Korhonen, 2001). It implies that the quality of stump treatment is decisive when dealing with spruce (Thor, 2005).

In analyses of the 2011 samples for the occurrence of natural infection by *P. gigantea* and that of the Rotstop isolate, no differences were detected between the samples of pine and spruce. It implies that natural infection by *P. gigantea* competes with artificially provoked one by stump treatment with Rotstop. This is a positive sign, indicating that the nature itself provides significant natural competition to *H. annosum*, which is likely to appear after thinning. A number of factors like site location, forest type, air temperature, ground cover vegetation, moisture, season of the year, competition of other fungi, and *H. annosum* in particular, affect the development of *P. gigantea*. If *H. annosum* infection background in the stand is high, the development of *P. gigantea* is limited (Berglund and Rönnberg, 2004; Berglund et al., 2005).
The proportion between the natural *P. gigantea* and Rotstop strain in the isolates analysed was 38% and 62% for pine, and 36% and 64% for spruce. According to the studies done in Sweden, in rot control the efficacy of local *P. gigantea* strains may be higher than that of Rotstop ones (Berglund et al., 2005). That is why in a number of countries research is under way to find efficient local *P. gigantea* strain suitable for stump treatment against root rots. Similar research has also been done in Latvia, and the efficacy of *P. gigantea* isolate of Latvian origin was not lower than that of Finnish preparations (Kenigsvalde et al., 2011). Regardless of a popular belief that natural *P. gigantea* cannot protect the stumps from the infection by *H. annosum* basidiospores (Drenkhan et al., 2008; Rönnberg, 2006), our results confirm the natural *P. gigantea* to be efficient for suppressing the development of *H. annosum* especially in stumps of small-dimension pines (Gaitnieks, unpublished data). A task for the future is to identify the factors favouring the development of natural *P. gigantea*.

**Conclusions**

1. Following the Scandinavian experience and the test results in Latvia the Rotstop preparation, containing *P. gigantea* spores, may be used for controlling *H. annosum*.

2. When analysing stumpwood samples from the cutting areas where there was reasonable doubt of improper Rotstop application, no *P. gigantea* genotype, existing in the Rotstop preparation, was detected on the average in 25% of the stumpwood samples.

3. Analyses of stumpwood samples confirm a conclusion made in other studies that *P. gigantea* colonize pinewood more intensively even in case of improper stump treatment by the Rotstop preparation.

**Acknowledgements**

We owe a debt of gratitude to Mikko Söderling of Verdera Oy, Bo Axelsson of Bohult Maskin AB, Kari Korhonen of the Finnish Forest Research Institute METLA, and Arto Kaurala of Ponsse PLC for support and advice in testing the Rotstop preparations in Latvian conditions.

This study was performed by financial support of the JSC ‘Latvijas valsts meži’, European Regional Development Fund project (No. L-KC-11-0004) ‘Methods and technologies for increasing forest capital value’ and the Grant project No.426/2012 of the Latvian Council of Science ‘Evaluation of factors affecting the efficacy of *Phlebiopsis gigantea* against *Heterobasidion* root rot’.

**References**


