The Efficiency of Rotstop and Sodium Borate to Control Primary Infections of *Heterobasidion* to *Picea abies* Stumps: a Serbian Study

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Abstract

North temperate coniferous forests are known to suffer from root and butt rot caused by *Heterobasidion* spp., resulting in severe economic losses. Since the mid-twentieth century, various intensities of fungus-inflicted damage have been reported in Scots pine and Norway spruce stands first in the former Yugoslavia and later on in Serbia. Despite the observed damage, the measures taken to protect tree stumps, which serve as an entry for basidiospore infections, have been rare or absent. Sodium borate (borax) has been used at certain locations to prevent *Heterobasidion* infection following damage caused by wind or snowstorms. This study aimed to compare the efficiency of sodium borate and *Phlebiopsis gigantea* to control the establishment of new *Heterobasidion* infections and to assess the cost of stump protection using these protective agents.

Two sites were chosen and both products were applied to the freshly cut stumps. Thirty stumps per treatment and 15 control stumps were analysed at both the sites after 3, 9 and 12 months; therefore, a total of 450 stumps were included in the analysis. The percentages of infection in the borax- and *P. gigantea*-treated stumps and the untreated stumps were 4, 7 and 20%, respectively. The efficiency of these treatments was very high and ranged from 78% (for borax) to 94% (for *P. gigantea*).

The stump area occupied by *P. gigantea* ranged from 20–91%, and mycelia could be observed up to 50 cm inside the stumps after 12 months of treatment.

The estimated costs of these treatments 5–5.7 Euro cents per stump, depending on the price of the product applied. Thus, the results of this research reveal that stump protection is both ecologically and economically justified.

Key words: *Picea abies*, *Heterobasidion* spp., *Phlebiopsis gigantea*, chemical control, stump treatment, economic appraisal

Introduction

Root and butt rot disease in conifers is caused by the pathogenic fungus *Heterobasidion annosum sensu lato* (s.l.), formerly known as *Fomes annosus* (Fr.) Cooke. In the literature, this species is considered to be one of the most important pathogens in the coniferous forests of the Northern Hemisphere (Tsopelas and Korhonen 1996, Woodward et al. 1998, Šķipars and Ruņģis 2011). The first recorded attack of *Heterobasidion annosum* s.l. infection was in Kopaonik Mountain (Serbia) in 1963, where the degree of infection in some stands was almost 30% (Marinković 1978). During the same period, natural spruce forests in mountains Zlatar, Durmitor, and Jahorina Mountains were also found to be infected by this species (Marinković et al. 1990).

Newly cut stumps are the weakest link in the process of disease management; this fact motivated researchers to try to find a way to protect the stump surface from basidiospores infection (Korhonen et al. 1992, Korhonen et al. 1997, LaPorta et al. 1998, Woodward et al. 1998, Dai and Korhonen 1999). In the mid-twentieth century, Rishbeth (1959) proved that 20% w/v solution of disodium octaborate tetrahydrate ($Na_2B_8O_{13}·4H_2O$) could effectively protect stumps from infection by *Heterobasidion* spp. spores. Further studies showed that application of 0.1 kg·m^{-2} sodium tetraborate (borax) to stumps (Smith 1970, Pratt and Quill 1996) could efficiently prevent the germination of basidiospores. Borax Europe Ltd. registered this active ingredient for use in forestry.

However, freshly cut stumps contain nutrients that support the growth of many microorganisms (Holdenrieder 1984). Since tissues in the tree stump continue to live after the tree is cut, they provide nutrients to support the growth of organisms such as *Heterobasidion* spp., *Phlebiopsis gigantea* (Fr.) Jülich, *Resinicium bicolor* (Alb. & Schw.: Fr.) Parm., *Fomitopsis pinicola* (Sw.: Fr.) P. Karst, and *Bjerkandera adusta* (Willd.: Fr.) P. Karst (Holdenrieder and Greig 1998). During his work, Rishbeth (1963) found that *P. gigantea* colonised stumps that originated from a thinning and it had the capability of killing and replacing *H. annosum* from the roots of these stumps (Stenlid 1987). Thereafter, several studies have investigated the efficiency of *H. annosum* control by *P. gigantea* (Greig 1976, Kallio and Hallaksela 1979, Korhonen et al. 1994, Berglund and Rönnberg 2004). Mycelium fragments and oidia of several *P. gigantea* species isolated from Finland were used to make a product that was registered under the name Rotstop® (Verdera Oy, Helsinki, Finland). Recent studies have been trying to compare the protection with fungi from the genus *Trichoderma* spp. (Berglund et al. 2005).

Thus far, there are no registered products for the control of *Heterobasidion* spp. in Serbia. However, the Ministry of Agriculture, Trade, Forestry and Water Management of Serbia approves the use of products such as borax ($Na_2B_4O_7 \cdot 10H_2O$) or Rotstop® in certain cases such as wind- and snowstorms and bark beetle outbreaks (Lazarev et al. 2005). Most tree stumps remain without protection after they are cut, or are very rare, and only in some areas, treated with borax.

The aim of this study was to (i) determine the efficiency of sodium tetraborate and *P. gigantea* in the reduction of the number of stumps infected by *Heterobasidion* spp. in a selective Norway spruce forest, (ii) monitor the colonisation and spread of *Phlebiopsis gigantea* in the treated stumps, and (iii) calculate the cost of stump treatment in a regular selection cutting.

Material and methods

Study site and experimental design

The experiment was established in August 2007 in two selective Norway spruce forests (*Piceetum excelsae oxalidetosum* Miš. et Pop. 60). The forests were located on two ridges approximately three kilometres away from each other (Table 1). The sites were situated in a forest in Kopaonik Mountain (43º19' 5" N, 20º45' 55" E) at 1,200–1,600 m above sea level. The studied sites were selected as they were deemed to be the most productive spruce stands (Table 1) according to a national forest inventory (Banković et al. 2009).

Fresh stumps regardless of the presence or absences of root and butt rot were treated. The presence of *Heterobasidion* infection was low.

Two products were tested:

(1) A suspension of *P. gigantea* mycelium and oidia administered at 2×10^6 to 2×10^7 active parts per gram (Rotstop®; Verdera Oy, Helsinki, Finland), and

(2) Borax-sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$).

The stumps were manually treated with a 5-L hand sprayer less than 1 h after the trees were felled. Approximately 1 $L \cdot m^{-2}$ *P. gigantea* was administered at a layer thickness of 1 mm (as recommended by the manufacturer). Borax was applied as a 10% w/v solution or 100 $g \cdot m^{-2}$. A total of 1,479 stumps were treated with borax or Rotstop, and 200 stumps served as the control and were sprayed with 1 $L \cdot m^{-2}$ distilled water (Table 2).

Table 1. Studied sites and stand characteristics

Site	Treatment	Number of trees/ha	Diameter ($d_{1.3}$ in cm)	Height (m)	Stand volume (m^3/ha)	Technical wood (%)
Study site 1 "Pašina bačišta"	Rotstop 1 Borax 1	493	29	20.5	409,5	40
Study site 2 "Rudno"	Rotstop 2 Borax 2	522	32	21.4	520.8	50

Sampling and laboratory analysis

Before treatment, each freshly cut stump (total 1679) was checked for root and butt rot (Table 2). The stumps were sampled 3, 9 and 12 months after the experiment was initiated. A total of 450 stumps were analysed for the presence and spread of *Heterobasidion* spp. and *P. gigantea*. Each stump was sampled only once after 3, 9 or 12 months. Thirty stumps per treatment (borax and Rotstop) and 15 control stumps were randomly selected from each of the two sites, i.e. a total of 150 stumps were analysed per sampling time. Stumps with obvious *Heterobasidion* or *Armillaria* rot were excluded from the sampling.

After three months, 30 stumps per treatment without obvious symptoms of root and butt rot were selected for sampling from each site. One 3-cm-thick disc was cut from each stump after removing a disc of 2-cm thickness from the top of the stump (Figure 1). Thereafter, the stump was sawn using a chainsaw to obtain a slice (approximately 3-5 cm thick) extending from the bark to the centre of the stump (Figure 1). These samples were immediately transferred into sterile black plastic bags and sealed. The samples were then transferred to the laboratory, and incubated for 14 days at room temperature (approximately 22ºC). Both sides of the samples were analysed under a dissecting microscope for detecting the presence of conidial stages of *P. gigantea* mycelium and *Heterobasidion* spp. The areas colonised by *P. gigantea* or *Heterobasidion* were measured using IMG Tool 2.0.

The sampling procedure was repeated after 9 and 12 months. Only the spread of *P. gigantea* was estimated in the samples collected after nine months. At 12 months, two 3-cm-thick discs and one vertical slice were sawn from each stump 2 and 20 cm below the treated surface (Figure 1). The discs were handled as described above. The areas of colonisation on the discs by fungi other than *P. gigantea* and *Heterobasidion* were recorded, and the fungi were isolated on malt extract agar (MEA-1.5%).

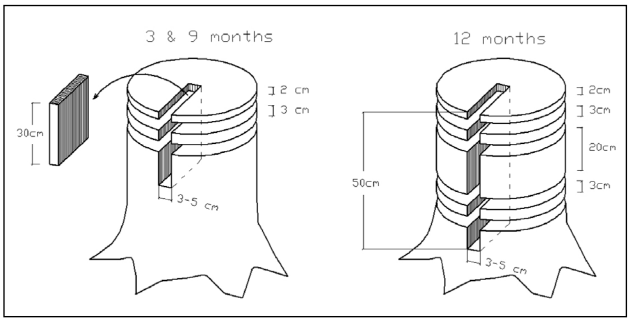

Figure 1. Stump sampling scheme at 3, 9 and 12 months after treatment

Heterobasidion spp. were isolated from both sides of each disc sampled for identification (Rönnenberg and Cleary 2011). Identification was performed by pairing the isolates with identified homokaryotic tester strains of three European *Heterobasidion* species (donated by K. Korhonen, Finnish Forest Research Institute, Vantaa, Finland) (Korhonen 1978).

treatment was expressed as a percentage of a working day (Table 5).

Statistical analysis

The incidence of infections for treatment was based on the number of stumps in which *P. gigantea* and *Heterobasidion* spp. were found. The calculated means of the areas and the number of *Heterobasidion* infections per stump were expressed as the ratio of the area of all infections to the area of the sampled discs (Berglund and Rönnberg 2004). The calculations of numbers, areas and relative areas were based on the established *Heterobasidion* infections. The efficiency of the treatment was calculated as the area of infection by *Heterobasidion* on the protected and untreated stumps.

Data from the treatments were analysed using one-way analysis of variance (ANOVA), and significant differences among the treatments were separated using Tukey's B test ($p < 0.05$).

Results

Presence of root rots at the experimental sites

The studied sites revealed the loss and damages from both *Heterobasidion* and *Armillaria* spp. Of the 1679 stumps that were assessed, 16–33% (Table 2) were found to be infected (average: 23%). At harvest, the average incidence of *Heterobasidion* spp. at both sites was close to 4% (Table 2).

Overview	Study site 1			Study site 2			Σ	Average
	Borax	Rotstop	Control	Borax	Rotstop	Control		
Total number of stumps	423	658	100	192	206	100	1,679	
Number of healthy stumps	356	499	92	129	145	66		
Number of infected stumps	67	159	16	63	61	26	392	
Percentage of infected stumps (%)	16	24	16	33	30	26		23
Percentage of stumps infected by *Heterobasidion*	3	6	2	3	5	1		4
Percentage of stumps infected by *Armillaria*	13	18	14	30	25	25		20
Basal area of treated stumps	84.79	95.92	17.57	45.85	41.49	22.32		

Table 2. Overview of cut trees and treated stumps with different treatments and from different study sites

Estimation of stump protection costs

Treatment costs included the price of the product and labour cost. The labour cost was estimated directly in the field. The process of cutting and assortment production was recorded for 5 days, and the time spent for the preparation of the suspension and application of both treatments was calculated. Based on the data collected, the average time spent for the

Of the 27 *Heterobasidion* isolates obtained from the sampled discs, 96% were identified as *H. parviporum*, and only one isolate was identified as *H. abietinum*.

Incidence of Heterobasidion

After 3 and 12 months, on average 20% and 14% of the untreated control stumps, respectively, revealed the presence of *Heterobasidion* infection (Table 3). After 3 months, the Rotstop- and borax-treated stumps had an infection incidence of 6–9% and 3–6%, respectively. After 12 months, the incidence of *Heterobasidion* infection for the Rotstop-treated stumps slightly decreased from 6% to 3% at Site 1, but the incidence did not change at Site 2. The incidence of *Heterobasidion* infection for the borax-treated stumps increased to 6% and 9% at Sites 1 and 2, respectively. None of these changes were statistically significant (p = 0.32). The control efficacy ranged from 78–84% and 82–94% after 3 and 12 months, respectively (Table 3).

Table 3. Frequency of *Heterobasidion*-infected stumps and control efficiency of two samplings

Location	Treatment	Frequency of *Heterobasidion* infected stumps		Control efficiency (reduced infected area on stumps)	
		3 months (%)	12 months (%)	3 months (%)	12 months (%)
Site 1	Rotstop 1	6 a[1]	3 a	81 a	94 a
	Borax 1	3 a	6 a	78 a	85 a
Site 2	Rostop 2	9 a	9 a	84 a	88a
	Borax 2	6 a	9 a	80 a	82 a
	Control	20 b	14 b	Not appl.	Not appl.

'Control efficiency' means a relative reduction of the total infected disc surface area in relation to the total infected disc surface area on the untreated stumps, including the uninfected stumps. Figures within the columns with different letters are significantly different. [1]p<0.05 compared with '100%'

Heterobasidion colonies

The number of established *Heterobasidion* colonies was higher in the untreated stumps than in the treated stumps at both sampling sites (Table 4). The observed differences were not statistically significant. The number of colonies in borax-treated stumps increased after 12 months.

The size of colonies ranged from 2–6 cm^2, and was significantly higher for the untreated stumps (p = 0.05) (Table 4). The highest protective efficiency was observed in the *P. gigantea*-treated stumps at Site 1 (Table 4). There was a significant difference in the size of colonies between the untreated and treated stumps (p < 0.01).

Table 4. Mean number, size and relative infected area (area of *Heterobasidion* infection/total disc surface area of the infected stumps) of *Heterobasidion* infections in infected stumps at two sampling times

		Mean number of *H.* colonies in infected stumps		Mean size of H. colonies in infected stumps		Area of H./total disc surface area of infected stumps	
	Treatment	3 months (nos.)	12 months (nos.)	3 months (cm^2)	12 months (cm^2)	3 months (%)	12 months (%)
Site 1	Rotstop 1	2 a	1 a	2.33 a	2.41 a	0.56 a	0.60 a
	Borax 1	1 a	2 a	3.92 a	2.77 a	0.83 a	0.73 a
Site 2	Rostop 2	3 a	3 a	2.50 a	3.05 a	0.78 a	0.95 a
	Borax 2	2 a	3 a	3.04 a	3.96 ab	1.01 ab	1.22 ab
	Control	6 ab	4 a	5.23 b	6.67 b	2.02 b	3.02 b

Figures within the columns with different letters are significantly different (p<0.05)

Efficiency of the treatments

Only two untreated stumps were colonised with *P. gigantea*, while 98% of the treated stumps were colonised with *P. gigantea*. A change in the wood colour observed after three months indicated good colonisation, and on average 19% of the disc area was colonised (Figure 2). First, the fungus colonises sapwood, after which it spreads into the heartwood. The colonisation was much slower in the central part of the stump. *P. gigantea* was found to have spread at least 5 cm below the stump surface (Figure 2).

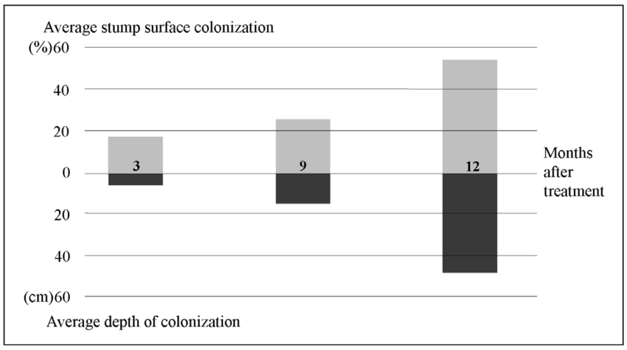

Figure 2. Average stump surface area colonized by *Phlebiopsis gigantea* and average depth of the mycelium spread inside the stump at different time points after treatment

One year after the treatment, 20–91% of the stump surface (average: 58%) showed *P. gigantea* colonisation (Figure 2). *P. gigantea* mycelia were observed up to 50 cm inside the stumps. Approximately 48% of the total area was colonised by the fungus up to 15 cm from the top of the stump. The fungus actively colonised the surface of the stumps, but it should be pointed out that it had a higher prevalence in sapwood than in heartwood.

P. gigantea formed a strong reaction zone on several stumps that had been previously occupied with *Heterobasidion* mycelia.

Estimated stump protection costs

The treatments of freshly cut stumps with Rotstop and borax cost 5.7 and 5.0 Euro cents per stump, respectively. The estimated labour cost is 0.8 euro cents per stump, i.e. 5% of the working hours per day (approximately 30 minutes per day) is spent on this task (Table 5).

Discussion

It is well-known that natural stands have a lower incidence of *Heterobasidion* infection than artificially established plantations (Woodward et al. 1998, Ol-

Table 5. The costs involved for treating freshly cut stumps with borax and Rotstop

	Treatment	
	Borax	Rotstop
Number of treated stumps	615	864
Area of treated stumps	130.64	137.41
Amount of product spent (Borax-kg, Rotstop-g)	14	140
Applied amount ($g \cdot m^{-2}$)	100	5.0
Price of product $€ \cdot kg^{-1}$ (Borax) and $€ \cdot g^{-1}$ (Rotstop)	1.83	1.50
Product price per m^2 of the stump	0.18	0.30
Labour cost ($€ \cdot day^{-1}$)	10	10
Price of product per stump (in euro cents)	4.2	4.9
Price of treatment per stump (in euro cents)	5.0	5.7

iva et al. 2008). This is primarily because there are fewer stumps in natural stands, resulting in reduced possibility for infection, since stumps increase the possibility for the establishment of infection and enable rapid spread into the root systems of neighbouring trees. Regular management of stands involving activities such as cutting and thinning results in an increased number of stumps and can consequently increase the incidence of infection and accelerate disease development (Keča and Keča 2011).

This study aimed to determine the natural infectious potential of *Heterobasidion* species in freshly cut stumps and to compare the efficiency of chemical and biological treatments to control the infection. Both borax and *P. gigantea* treatments showed an acceptable reduction in stump protection when the infection potential was low or medium. Stumps treated within 1 hour of cutting tended to be more likely to remain uninfected (Hüttermann and Haars 1987, Korhonen 2003).

The incidence of *H. parviporum* in the treated stumps ranged from 4–7%. This was 15% less than that observed in the untreated stumps. Expectedly, since most of the study area was covered with pure Norway spruce forests, most infections were caused by *H. parviporum*.

Norway spruce stands in Sweden treated with urea were reported to have *Heterobasidion* infection at an incidence of 2.7% 15 years after the treatment (Oliva et al. 2008). Chemical treatments can change species composition colonizing the stump (Vasiliauskas et al. 2004) and influence the environment if used on a large scale, but they are efficient in protecting stumps from *Heterobasidion* infection. In an Estonian study with Rotstop, Drenkhan et al. (2008) reported very good protection with average areas of 75.2 cm^2 and 4.3 cm^2 colonised by *P. gigantea* and *Heterobasidion*, respectively, similar to the findings reported in our study.

The number of basidiospores, according to the number of infections controlled (14% and 20%), was considerably lower than the results of Berglund and Rönnberg (2004), who had found that the percentage of infected stumps was as high as 80% in the control stumps after three months. These differences are probably the result of a higher inoculum potential in the surrounding area and environmental conditions (e.g. humidity and average temperature), which are more in favour of disease in Scandinavia.

At the same time, favourable conditions during September and October enabled fast colonisation of the stumps by *P. gigantea* (Berglund and Rönnberg 2004). The samples collected after 12 months showed intense colonisation, but visual inspection also revealed the presence of decay. This is very important, since large stumps are a repository of mycelia that can potentially infect the roots of surrounding trees (Rishbeth 1951, Redfern and Stenlid 1998) and since *Heterobasidion* can survive even in small stumps (approximately 30 cm) for up to 40 years (Keča 2008, Pantić et al. 2011) or 50 years in Finland (Piri 1996). Moreover, the ecological aspects of a stand, such as the efficiency in suppression of *Heterobasidion* and decomposition of stumps, are in favour of treatment with *P. gigantea*.

Economic analysis revealed that stump treatments are justified (Thor and Stenlid 2005, Thor et al. 2006), but our results revealed that the expenses are justified not only during regular cuttings but also during thinning, when the value of the timber is very low. The two-worker team required for sawing wood, in which one wields the chainsaw and the other provides support, enables maintenance of stump treatment costs at a considerably low level.

Thus, an appropriate combination of protection and good management practices can effectively maintain the level of *H. parviporum* infection at around 10%. A similar level of infection was reported in natural stands such as in strict reserves undisturbed by cutting and other man-made interventions (Garbelotto et al. 1999, Keča and Keča 2011).

Further studies should account for the establishment of new experiments that will test novel products such as Rotstop® S (Rönnberg et al. 2006), which contains a mixture of *P. gigantea* strains isolated from spruce trees in Sweden. Furthermore, it would also be useful to test the protective efficiency of *P. gigantean* isolates from Serbia.

Conclusions

In conclusion, this analysis revealed that both Rotstop® and borax could efficiently protect the tree stumps against infection by *Heterobasidion* spp. The inoculum potential during the summer cutting was low, but the number of *Heterobasidion* spp.-infected tree stumps was significantly different between unprotected and treated stumps. In addition to protecting the stumps from *Heterobasidion* colonisation, *P. gigantea* accelerated the decomposition of wood and reduced the opportunity of *Heterobasidion* to survive and produce sporophores. Further, the colonisation of sapwood by *P. gigantea* was more intense than that of heartwood. The calculated costs of stump treatment revealed that these treatments are acceptable and justifiable for preventing infection in Norway spruce stands.

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ЭФФЕКТИВНОСТЬ РОТСТОПА И БОРАТА НАТРИЯ В КОНТРОЛЕ ПЕРВИЧНЫХ ИНФЕКЦИЙ *HETEROBASIDION* НА ЕЛОВЫХ ПНЯХ: ИССЛЕДОВАНИЕ В СЕРБИИ

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Резюме

Вред, нанесеный видами комплекса *Heterobasidion* замечен на белой сосне и ели ещё в середине XX века, прежде всего в бывшей Югославии, а позже и в Сербии. Вопреки значительному вреду, защита пней, зараженных инфекциибазидиоспорами, проводилась изредка, или вовсе не проводилась. Только на некоторых участках, после ветровала или снеговала в целях предупреждения расширения инфекции видами комплекса *Heterobasidion–a* применялся борат натрия (боракс)

Целью данного исследования было сравнение эффективности бората натрия и *Phlebiopsis gigantea* в предупреждении возникновения новых инфекций видами комплекса *Heterobasidion*, а также оценка экономических рассходов защиты пней.

Выбранны два участка, где препараты применялись на недавно выкорчеванных пнях. Спустя 3 и 12 месяцев проанализированно по 30 пней, на которых применялись препараты, и столько же контрольных, всего 150 образцов пней. Соотношение возникшей нфекция видами комплекса *Heterobasidion* на пнях, где применялся боракс *Phlebiopsis gigantea* и на необработанных пнях в процентах составляло соответсевенно 4%, 7% и 20%. Эффективность применения препарата была очень высокая и составляла 78% (для боракса) и до 94% (для ротстопа).

Поверхность пней, заселенная *Phlebiopsis gigantea* составляла с 20 до 91%, а внутри пней инфекция замечена на глубине 50 см спустя 12 месяцев после появления грибницы (мицелия)ю

Стоимость такой обработки пней составляла с 5 до 5,7 EUR по пень, в зависимости от цены применявшегося препарата. Из высшеприведенного можно сделать вывод, что защита пней целесообразна как в экологическом, так и в экономическом аспектах.

Ключевые слова: *Picea abies*, *Heterobasidion* spp., *Phlebiopsis gigantean*, химический контроль, обработка пней.