The impact of stumps' height on secondary spread of *Heterobasidion parviporum* in precommercial thinning stands of Norway spruce (*Picea abies*)

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ABSTRACT

The economic losses caused *by Heterobasidion* spp. infection is an important issue in the Swedish forestry sector where Scots pine and Norway spruce make up to 80% of the standing volume. Both of the fungi species naturally existing in Sweden: *H. annosum* and *H. parviporum* cause decay of the wood and decrease quality of the timber. In spruce dominated stands the risk of primary infection occurred even in small sized (2-14 cm at stump height) percommercial thinning stumps. Therefore, there is a need to develop useful methods of preventing the primary infection and secondary spread the in young spruce stands. The paper investigates the impact of stump's height created during precommercial thinning operations on transferring *H. parviporum* infection into adjacent trees due to root contact. Ninety Norway spruce trees were cut down in three precommercial thinning spruce dominated stands in southern Sweden to create two types of donor stump high- (100 cm above the ground) and low stumps (15 cm above the ground). Four years after artificial inoculation of *H. parviporum* sample discs were collected from donor stumps and the four the biggest trees within 2 m radius in order to examine the secondary spread of the infection. The infection frequency has reached 16,2%. In most cases it was naturally occurred contamination, the artificially inoculated strain of *H. parviporum* was found only on four out of 51 infected discs (0,7 % infection frequency). Trees surrounding low stumps were infected more frequent than those adjacent to high stumps, 31 and 18 infected trees respectively. It means that 63% of the infection was found in the trees adjacent to low stump and 37% of infected trees occurred next to high stumps. However, the differences of infection frequency occurred in the blocks with high and low stumps is too slightly to be significantly important (p-value equal 0,0691) when all investigated strains of Heterobasidion are taken into account.

Key Words: stumps' height, *Norway spruce*, *Heterobasidion parviporum,* precommercial thinning

STRESZCZENIE

‘Wpływ wysokości pni utworzonych podczas czyszczeń późnych na wegetatywne rozprzestrzenianie się Korzeniowca drobnoporego (*Heterobasidion parviporum*) w drzewostanach świerkowych’

Straty gospodarcze wywołane przez grzyby rodzaju *Heterobasidion* (korzeniowiec) są istotnym zagadnieniem szwedzkiego leśnictwa gdzie gatunki iglaste, podatne na infekcje, stanowią 80% zapasu drewna na pniu. Oba gatunki huby korzeniowej naturalnie występujących we Szwecji: *H. annosum* i *H. parviporum* są przyczyną zgnilizny twardzieli, co powoduje obniżenie jakości drewna. Zarodniki mogą zainfekować pniaki nie dużych rozmiarów (2-14 cm na wysokości pnia), które powstają podczas czyszczeń późnych w drzewostanach świerkowych. W związku z tym istnieje potrzeba opracowania skutecznych metod ograniczenia infekcji pierwotnej i następującego po niej wegetatywnego rozprzestrzenianie się zakażenia w młodych drzewostanach świerkowych. W niniejszej pracy zbadano wpływ wysokości pniaków powstałych podczas czyszczeń późnych na rozprzestrzenianie się zakażenia *H. parviporum* do sąsiednich osobników poprzez kontakt pomiędzy systemami korzeniowymi. Długo okresowy eksperyment został ustanowiony w trzech młodnikach świerkowych w południowej Szwecji. Na każdej powierzchni utworzono dwa rodzaje pni: wysokie (100 cm) i niskie (15 cm), oba rodzaje pniaków zostały sztucznie zakażone szczepem *H. Parviporum* Rb175. Cztery lata po wszczepieniu sztucznej infekcji zebrano próbki z pni i czterech drzew o największej pierśnicy w promieniu 2 m od zakażonych odziomów. Wskaźnik infekcji osiągnął 16,2%. Sztucznie wprowadzony szczep *H. parviporum* stwierdzono tylko w czterech z 51 zainfekowanych dysków (0,7% częstotliwości infekcji). W większości przypadków drzewa zostały zarażone przez zarodniki naturalnie występujące w badanym siedlisku. Drzewa otaczające niskie pnie zostały zainfekowane częściej niż w sąsiedztwie wysokich pni, odpowiednio 31 i 18 zainfekowanych drzew. Oznacza to, że 63% infekcji stwierdzono u drzew przylegających do niskich pni, a 37% zakażonych drzew wystąpiło przy wysokich pniach. Jednakże, różnice częstotliwości zakażenia pomiędzy blokami z wysokimi i niskimi pniami są zbyt małe aby być statystycznie istotnie ($p = 0{,}0691$).

Słowa kluczowe: wysokość pnia, świerk pospolity, *Heterobasidion parviporum*, czyszczenia późne

Content

1. INTRODUCTION

Heterobasidion spp. infection is widely spread in the Northern Hemisphere and has been the major cause of root and butt rot in Scandinavian conifer forests for over one century (Woodward *et al.*, 1998). The economic losses caused by *Heterobasidion* spp. infection are an important issue in the Swedish forestry sector where Scots pine and Norway spruce make up to 80% of the standing volume (Skogsstyrelsen, 2014).

As reported by Olsson (2010), approximately 20% of stems are affected by root rot in southern Sweden. Moreover, this figure has increased twice since the 1970s (Olsson, 2010). It is estimated that loses caused by rot (rötangrepp) reach 0,5-1 billion SEK per year (Witzell, 2009). Therefore, biological and chemical means of control of *Heterobasidion* spp. are one of the main issues in the Swedish forestry sector.

1.1. Taxonomy of the species

Two *Heterobasidion* species occur in Sweden: *H. annosum* (Fr.) Bref. s.s. that attacks mainly Pine (*Pinus),* but may infect Spruce *(Picea)* stands as well and *H. parviporum* that usually inhabits Norway spruce (*Picea abies)* (Niemelä & Korhonen, 1998). However, *H. annosum* infection affects *Picea abies* and *Pinus sylvestris* in different ways. In Norway spruce stands the infection is not the direct reason of trees death. It causes root decay and butt rot column in the stumps which influence the vitality of the trees and make them more susceptible for other disease. Damages for Scots pine attacked by *H. annosum* are more severe than in case of Norway spruce. The infection kills cambium at the stem base, when it affects the whole root collar, the tree dies (Bendz-Hellgren et al., 1998)

1.2. Infection strategy

Infection of *Heterobasidion* spp. has two stages: primary and secondary infection. The most common way of primary infection are airborne sexual spores - basidiospores realised from fruiting body as long as it is not frozen or suffer from severe drought. The spores infect fresh wood - surface of recent created stumps or wounds (Rishbeth, 1950). When spores have reached appropriate substrate, mycelia start to grow into the wood towards roots (Rishbeth, 1951). The secondary infection is caused by vegetative spread of mycelia through root contact of infected and non-infected individual trees (Rishbeth, 1951).

1.3. Spread rate

The spreading ratio of *Heterobasidion* spp. infection in the wood of a living tree depends on many factors like host species and its vitality, moisture of the wood, competition with other fungi inhabiting the substrate and etc. In general, in Nordic countries the average rate of spreading is 20-30 cm/ year^{-1} and the figure tends to increase in the southern part of the region (Korhonen & Stenlid, 1998).

The infection of *Heterobasidion* spp. may also spread vegetative via the root system. It is proved that the spread rate is from two to three times faster in dead roots then in those of a living tree where growth rate is about 7-12 cm, year^{-1} (Pettersson *et al.*, 2003; Bendz-Hellgren *et al.*, 1999).

1.4. Means of control

There are several ways to protect stand from *Heterobasidion* spp. infection. There are silviculture methods to reduce the risk of the transferring infection. Furthermore, chemical or biological treatment can be applied to the stumps to protect the surface from the colonisation by *Heterobasidion* spp. spores.

1.5. Silvicultural vs. chemical control

The most important silviculture measures to decrease the risks of *Heterobasidion* spp. infection include:

- Establishment of resistant tree species, e.g. broadleaved or mixed stands;
- Thinning and harvesting during winter time when the probability of spore infection is low;
- Use of stumps treatment after cuttings during warm season to prevent spore infection;
- Avoidance of logging scars which might be a point of establishing primary infection;
- Reduction of the rotation age in greatly infected stands to reduce spread of the infection and provide harvest before timber gets rotten ;
- Removal of the old stumps before the establishment of the 2nd conifer generation on heavily infected sites to avoid secondary infection of the new seedlings by the contact with residues of the infected old roots (Korhonen et al., 1998).

Since the middle of the 20th century many experiments has been carried out using chemical and biological compounds to find an optimal way to control the fungus. The most commonly considered that can be used for stump treatment: urea, DOT (boron) and *Phlebiopsis gigantea* (Thor, 2005).

Both urea and easy to store without losing efficiency and easily accessible (Thor, 2005). However, high concentration of boron is toxic for plants growing near treated stumps (Westlund & Nohrstedt, 2000). Therefore, DOT is not registered as pesticide in Sweden and are not allowed to use in practical forestry for stumps treatment (Thor, 2005). Urea due to ability of rising pH of the stump makes the surface resistant to primary infection of *Heterobasidion* spp.. Urea is registered as a pesticide in Sweden since 2008 (Kemikalieinspektionen, 2012) but it is not widely used in practical Swedish forestry today (Thor, 2005).

Phlebiopsis gigantea is a saprotrophic fungus that naturally inhabits fresh *Pinus* and *Picea* wood (Holdenrieder & Greig, 1998). Artificial application of *P. gigantea* on stumps prevents *Heterobasidion* spp. infection to a great extent (Thor, 2005; Holdenrieder & Greig, 1998) There are at least four products based on *P. gigantea* across the Europe: PG Suspension in the UK, PG IBL in Poland, Rotstop® in Finland and RotstopS in Sweden (Sidorov, 2005; Pratt et al.,, 2000). They were created from various strains of *P. gigantea,* using different concentrations of the fungus oidia and ways of application. However, the main mechanism is the same i.e. they use antagonist ability of *P. gigantea* towards *Heterobasidion* spp..

So far the products which contain *P. gigantea* are commonly used to prevent spreading of *Heterobasidion* spp. infection in conifer stands due to their low impact on environment and acceptable efficiency (Thor, 2005).

1.6. Silviculture program for Norway spruce stands in Sweden

Norway spruce is the second after Scot pine most the numerous species in Sweden and makes up 42 % of standing volume (Skogsstyrelsen, 2014). A frequent silviculture programme for spruce dominated sites in Swedish conditions starts with establishing of the stand, is followed by one or two precommercial thinning operation, than two commercial thinning and ending with final felling when it reach cutting age (Näslund et al., 2010). In the last period 2011-2013 the area where precommercial thinning operations were carried out was in average 265 300 hectares/year (Skogsstyrelsen, 2014).

1.7. Pre-commercial thinning

When spruce dominated stand reach two to six meters in height the precommercial thinning is usually performed to stimulate growth of preferred trees by removing trees with undesirable features or positions (Pettersson *et al.*, 2007). Precommercial thinning operation are labour-intensive and costly but it improves stand stability against snow and wind pressure and in the long-term brings positive impact for stock's economy (Pettersson *et al.*, 2007). During the precommercial thinning small dimensions stumps are created.

It was assumed that precommercial thinning stumps are unable to spread *Heterobasidion* spp. decease because of unsuccessful primary infection on small sized stumps surface (Vollbrecht *et al.*, 1995). However, the recent research conducted by Gunulf et al (2013) shows that small sized Norway spruce stumps (diameter 2-14 cm at stump height) created during precommercial thinning in Norway spruce stands manage to transfer *H. parviporum* infection into surrounding trees to great extent. Moreover, the result suggests that there is no limitation, within the investigated size span, under with the spread of *H. parviporum* is not taking place. Therefore, performing precommercial thinning operations in winter time or stump treatment is advisable to avoid spread of *Heterobasidion* spp. infection (Gunulf *et al.*, 2013).

There could be also another solution which might inhibited the spread of infection - creating high instead of low stumps during precommercial thinning. Recently, a few tools which are able to create high stumps during precommercial thinning operation has been developed (Anon, 2010; Ligné et al., 2005) which make this technique simpler to carry out. However, the method of creating high stumps is not yet commonly use at practice.

Advantages of high stumps

As it is reported by Karlsson et al. (2002) creating about one meter height stumps instead of standard 15-20 cm height is more efficient and reduces the costs of precommercial thinning operations. High stumps has also positive impact to the quality of remaining trees by stimulating natural clearance of branches from the trees from below (Karlsson et al., 2002).

It was also assumed that high stumps are more resistant for infection of *Heterobasidion spp.* than low stumps. High stumps are expose to more windy and sunny microclimate then low stumps, therefore, they have dryer wood surface which is not preferable by *Heterobasidion* spp. spores. However, the hypothesis wasn't confirmed by the study conducted in spruce dominated precommercial thinning stands in Southern Sweden. The results shows that height of stumps has no significant influence on infection frequency (McCarthy, 2011).

There is also another knowledge gap which is going to be investigate in this paper: if the height of the precommercial thinning stump influence on spreading secondary infection of *H. parviporum* into surrounding living trees. The hypothesis is that high stumps inhibit spread of infection because of two reasons:

- the infection need to overcome a longer distance through wood tissues before it reaches root system and transfer the infection into surrounding trees by root contact;
- expansion of mycelia develops faster in dead wood then in a living tissues, high stumps need more time to dry out which slows down spread of the infection compering to low stumps which dry out faster (Bendz-Hellgren *et al.*, 1999).

1.8. The aim of the study

The aim of the study is to investigate if the height of the stump created during precommercial thinning operation in Norway spruce stands has an impact on the secondary spread of *Heterobasidion* spp.

2. MATERIALS AND METHODS

2.1. Establishment of a long-term study

The experiment was established in June 2010 on five precommercial thinning sites of Norway spruce in the southern part of Sweden (table 1) as a part of the research for Rebecka McCarthy's master thesis (McCarthy, 2011).

Sixty trees were cut on each site creating 30 high and 30 low stumps alternately. The spruce trees had the diameter from 2 to 14 cm at height of stumps surface (15 cm above ground for low stumps and 1 m above ground for high stumps) and were surrounded by other trees, most preferably by at least four spruces within 2 m radius (McCarthy, 2011).

Table 1 Long-term study site, diameters was measured the height of 1 m and are the averages from all samples within the site (data from 2010) (McCarthy, 2011)

Site	Location	County	Coordinates (RT90)	Stand age	Site Index	Stems/ha	Average Diam. on a high of 1 m
S1	Tönnersjöheden	Halland	6288804, 1335074	15	G32	2500	6.3
S2	Åryd 497	Småland	6298980, 1450741	6	G26	2700	4.2
S3	Åryd 200	Småland	6303559, 1449943	6	G26	4100	3.5
S4	Åryd 268	Småland	6301429, 1450354	11	G28	3120	4.3
S5	Kullaskogen 65	Scania	6241540, 1403207	11	G28	4750	5.9

A chainsaw was used to create high stumps (1 m above the ground) and low stumps (15 cm above the ground). There was at least 10 meters distance between the stumps and the border of the stand. The layout of the study reflected the shape of the stand and turned around whenever it reached the edge of the stand. Metal labels with identification numbers from 1 to 300 were attached to the stumps. Moreover, four largest surrounding trees were marked with fluorescent spray and plastic ribbons to facilitate the allocation of the stumps in the future (McCarthy, 2011).

On each site the low and high stumps were artificially infected by one individual of *H. parviporum* (RB 175) isolated from a Norway spruce tree in southern Sweden (Stenlid, 1987) in order to investigate if the spreading of the *Heterobasidion* spp. infection depends on stump height.

The spores of RB 175 were sprayed to each stump with the concentration of 50 spores/ cm^2 directly after cuttings. The solution contained 1000 spores/ ml. Before the field work started, the strain of RB 175 was cultivated in the laboratory on Petri dishes containing Hagem agar to provide a sufficient amount of spores. A new solution was prepared every day before it was applied on the stumps (McCarthy, 2011).

2.2. Pilot study

Apart from 60 donator stumps, 3 high and 3 low test stumps were established in each site. The test stumps were inoculated in the same way as the donor stumps. Five centimetres thick discs from the test stumps were taken in spring 2011, approximately 6-7 months after the establishment of the long term study, in order to ensure that *H. parviporum* infected the stumps successfully. The sample discs were store in temperature 4˚C before further investigation in the laboratory. Then the discs were incubated in room temperature for 7-8 days before they were analysed under a dissecting microscope. Both sides of the discs were investigated. The results are presented in the table below (Table 2) The infection was establish successfully on most of the high stumps, no conidia of Rb 175 were found on low stumps.

Table 2 **Number of infected test stump on each site**

Type	N of stump infected by Rb 175
S1	
H	**3**
L	**0**
S2	
H	**1**
L	**0**
S3	
H	**3**
L	**0**
S4	
H	**1**
L	**0**
S5	
H	**3**
L	**0**

Due to inoculation success my master thesis study was conducted in the sites with the numbers S1, S3 and S5 (see table 1) where the infection of Rb 175 was found most frequently on sample discs cut from the pilot study stumps.

2.3. Process of detecting Rb 175 infection

To be able to investigate if the infection of Rb 175 has spread into trees adjacent the donator stumps the following steps were taken:

- Discs collection in the field;
- Laboratory examination;
- Data analysis.

All the steps are described below.

2.3.1. Field work

The field work took place in Tönnersjöheden, Åryd 200 and Kullaskogen 65, in July 2014 (Table 1), four years after the long term study was established. The research area contains pre-commercial thinning stands dominated by spruce in the age of 10-19 years old .

Thirty out of 60 existing stumps on each of the three sites were used in this study, i.e. every second block (high stumps plus low stump) was selected. The sample discs were collected from the donator stumps and 4 largest spruces within 2 m radius from the donator stumps (Gunulf *et al.*, 2013).

Sample discs collection:

One sample disc was collected from each low stump at 12 cm above the ground and two sample discs were collected from the high stumps: first at 65 cm and the second 15 cm above the ground. The bark was removed in the place of cutting and the stumps were sprayed with 70% solution of ethanol. The sample discs were 2-3cm thick and cut using a hand bow saw.

Four spruces with the biggest diameter growing within 2 m radius from the donor stump were cut at 50 cm above the ground level by a chainsaw. The bark was sprayed with 70% solution of ethanol and then 2-3 cm thick sample discs were cut at 15cm above the ground using a hand bow saw. The distance between the donor stump and the neighbouring trees was measured.

Figure 1 One of the low stump at the site S3 Ĺryd 200

The blade of the hand bow saw was sterilized with 70% ethanol before every cutting. The top part of every sample disc was marked and put into labelled plastic bag immediately after collecting. Afterwards they were kept in a cold place at the temperature about 4^{o}C until the further investigation was carried out.

During field work 437 discs were collected: 45 from low stump, 90 from high stump (top, and bottom discs) and 302 from surrounding trees.

Figure 2 A low stump after collecting sample disc

Figure 3 One of adjacent tree after collecting of sample disc

2.3.2. Laboratory analysis

Discs detection

Figure 4 Examined disc under a dissecting microscope

The discs were incubated at room temperature from 7 to 10 days before they were analysed under a dissecting microscope. Marking was put on the discs to simplify the work with the surface. The surface investigation was made between the stripes under 15 magnification. The bottom side of every disc was examined. The identification of *Heterobasidion* spp. conidiophores was used to detect infection. The infected area found on the disc surface was marked. In cases when there were more than one colony of *Heterobasidion* spp. found on one disc the two biggest colonies were chosen for further investigation. *Heterobasidion* spp. clean cultures were made by touching the top of the conidiophores with a sterile needle under 40 magnification when the heads of conidia were clearly visible. Then it was placed into a Petri dish containing Hagem agar extract. In about 4 days mycelium grew to the size which could be clearly visible. It was then moved from the initial agar and put on a new Petri dish where it grew for approximately 7 days. The extraction from the initial agar was made in order to avoid any undesirable contaminations. Then, the agar plate with clean culture of *Heterobasidion* spp. was covered with parafilm to ensure better protection from contamination and was stored in the fridge at the temperature of 2 ^{o}C for 2-4 weeks until all discs were investigated with regards to the presence of the infection.

Moreover, the diameter of every disc was measured perpendicularly with a ruler (rounded off to 0,5 cm) and the annual rings were counted.

Somatic incompatibility test

Finally, the somatic compatibility test was done to initially determined if the trees were infected with the same individual of the *H. parviporum,* i.e. Rb 175, as was artificially applied on the donor stumps. A piece of the Hagem agar extract, containing clean culture of *Heterobasidion* spp., found in the sample discs and the mycelium of the Rb 175 were put in the same Petri dish containing Hagem agar extract. Afterwards the test Petri dishes were put into plastic bags and stored at the room temperature for 6 weeks. Then the observation took place to see if the mycelium from the two samples grows together or stays apart. If it grows together without visible border, it will represent the same strain of *H. parviporum* (Rb 175) making it therefore possible to recognize the infection found on the sample discs i.e. Rb 175. In case the mycelium from the two samples stays apart it means that another strain of *H. parviporum* had infected the spruce trees. When the mycelia create dense border between the two samples it means that the examined disc was infected by *H. annosum.*

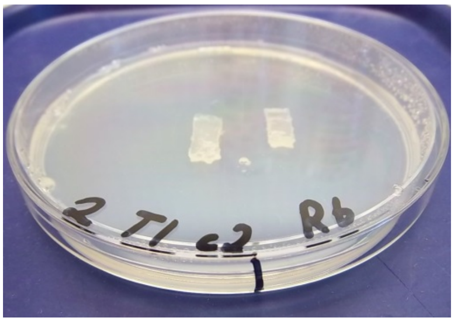

Figure 5 The Petri dish: on the left sample from the infected disc, on the right mycelium of Rb 175

2.3.3. Statistical analyses

The collected data were analysed by the means of two statistical software: Minitab 17 and SAS 9.3. The model needed to take into account that infection frequency is dependent on examined factors: height of the stump, diameter of the stump surface were the primary infection took place, distance between donor stump and infected trees, age of the examined trees and the site. To investigate if distance, age, diameter and site have a significant influence on infection frequency a T-test was performed.

Moreover, to analyse height of the stump it should be consider that there might be dependence between the trees adjacent to the same donor stump. Therefore, the standard model of binomial distribution cannot be used without considering over-dispersion. However, there is a way to analyse the data.

Ninety blocks were created, they consider type of the stump (45 blocks with low stumps and 45 blocks with high stumps), there were divided into the sites and each of them contains the stump and investigated adjacent trees. The results from T-test showed that distance, age and diameter do not influence infection frequency significantly, therefore, this factors were excluded from further analyses. Next, the frequency of infected trees in block was calculated. Then calculation in GENMOD using over-dispersion in the model provided by SAS 9.3 software was carried out for the block to investigate the dependency between height of the stump and infection frequency. The calculation was performed in two alternatives: take into account the species of *Heterobasidion* infecting the samples (*H. annosum* or *H. parviporum*) and sum up them together focusing on the fact of infection.

3. RESULTS

The infection of *Heterobasidion* spp. was found on 51 discs (49 trees and 2 donor stump) which is equal to 16,2 % infection rate (table 4).

3.1. High and low stump differences

The table below shows number of trees infected by *Heterobasidion* spp. depending on height of donor stump (table 3). Trees surrounding low stumps were infected more frequent than those adjacent to high stumps, 31 and 18 infected trees respectively. It means that 63% of the infection was found in the trees adjacent to low stump and 37% of infected trees occurred next to high stumps. However, the differences of infection frequency occurred in the bocks with high and low stumps is too slightly to be significantly important (p-value equal 0,0691) when all investigated strains of *Heterobasidion* are taken into account..

Nevertheless, when the significance is calculated for every species separately height of the stump has significant influence on infection frequency by *H. annosum* - the most common pathogen (Table 4) (p-value 0, 0481). It occurred on 10 discs from the blocks with high stump and 22 discs from blocks with low stump. The height of the stumps has not influence infection frequency by *H. parviporum* (p-value – 0, 1808)

Table 3: Number of trees infected by *Heterobasidion spp.* depending on height of donor stump

Type of donor stump	No. of infected trees	No. of trees infected by Rb 175
Site 1		
High	4	0
Low	10	0
Site 3		
High	14	2
Low	13	0
Site 5		
High	0	0
Low	8	0
Total	H = 18, L = 31	H = 2, L = 0

3.2. Species of Heterobasidion found on the discs

The somatic incompatibility test allowed to tentatively assess the species of *Heterobasidion* occurred on investigated discs. The results of the test are showed in the table below (Table 4).

Table 4: Number of discs infected by *Heterobasidion spp.* on each site , results from somatic incompatibility test: Rb 175- the known individual of. *H. parviporum*, Hp- another individual of *H. parviporum* excluding Rb 175, Ha- *H. annosum*, undef sp.- undefined species of Heterobasidion - results of the test was unclear.

site	number of samples		infected stumps stumps		infected tree									
	trees	stumps			Rb 175		*H. parviporum*		*H. annosum*		undef. sp.		total	
	n	*n*	*n*	*[%]*	*n*	*[%]*	*n*	*[%]*	*n*	*[%]*	*n*	*[%]*	*n*	*[%]*
1	103	45	1	2,2	0	0,0	0	0,0	14	13,6	0	0,0	14	13,6
3	97	45	0	0,0	2	2,1	10	10,3	11	11,3	4	4,1	27	27,8
5	102	45	1	2,2	0	0,0	1	1,0	7	6,9	0	0,0	8	7,8
sum	302	135	2	2,2	2	0,7	11	3,6	32	10,6	4	1,3	49	16,2

The most common pathogen was *H. annosum,* it was found on 63% of infected discs(32 out of 51infected samples).

The known individual, Rb 175, occurred only on four discs, two from donor stumps and two from trees. The infection was found on both type of stumps: one low and one high. Moreover, both of infected trees were associated with high stumps. However, the infected by Rb 175 donor stump and trees weren't situated in the same blocks. Two trees and the high stump infected by Rb 175 came from site no. 3, the low donor stump with Rb 175 conidiophores came from site no 1. The distances between the artificially inoculated stumps and infected tree were 91 and 156 cm.

Heterobasidion parviporum excluding the known individual Rb 175 were recognized on 11 out of 51 infected discs. Almost all of the trees infected by *H. parviporum* were found in the site no. S3, only one tree came from the site no. S5.

There were difficulties to identify species of Heterobasidion in 4 of the examined discs. The results from mating test were unclear, therefore, the exact species of *Heterobasidion* weren't recognised. However, the appearance of mycelia in the undefined test Petri dishes allowed excluding infection by Rb 175.

3.3. Site

There is a significant differences in the infection frequency between the sites, p-value equals 0, 0075. The infection rate has reached 13,6% for S1, 27,8% for S2 and 7,8% for S3 (Figure 6).

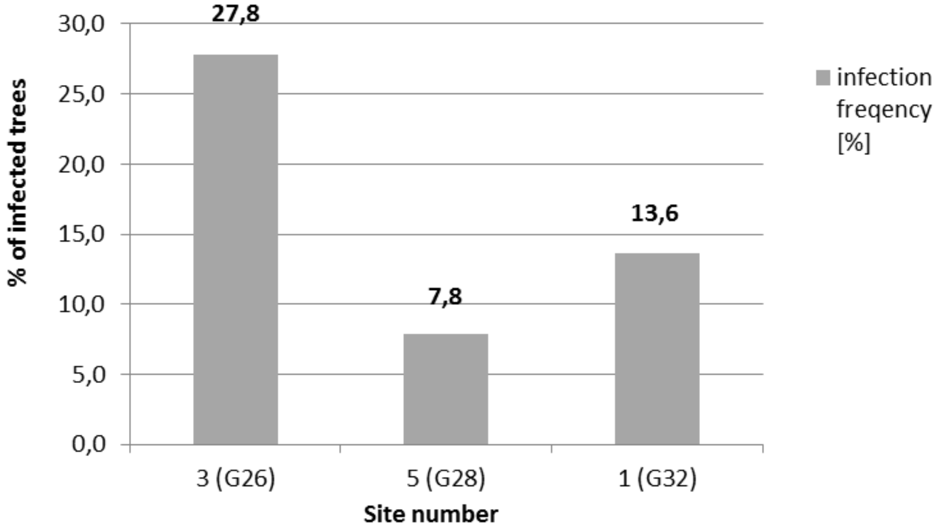

Figure 6 Infection frequency on each site

3.4. Distance, diameter, age

According to T- test distance, diameter and age have no significant influence on the infection rate (p- value 0,538; 0,460; 0,661 respectively).

Distance

Distance between potential donor stumps and adjacent trees was usually more than 1 m, nearly 80% of the trees in the blocks were growing more than 1,25 cm from the stumps (Figure 7). No infection was found in the trees close to donor stump (1- 50 cm distance). The largest infection frequency occurred in class 76-100 cm (28,6%). Distance did not influence the number of infected individuals (p-value 0,538).

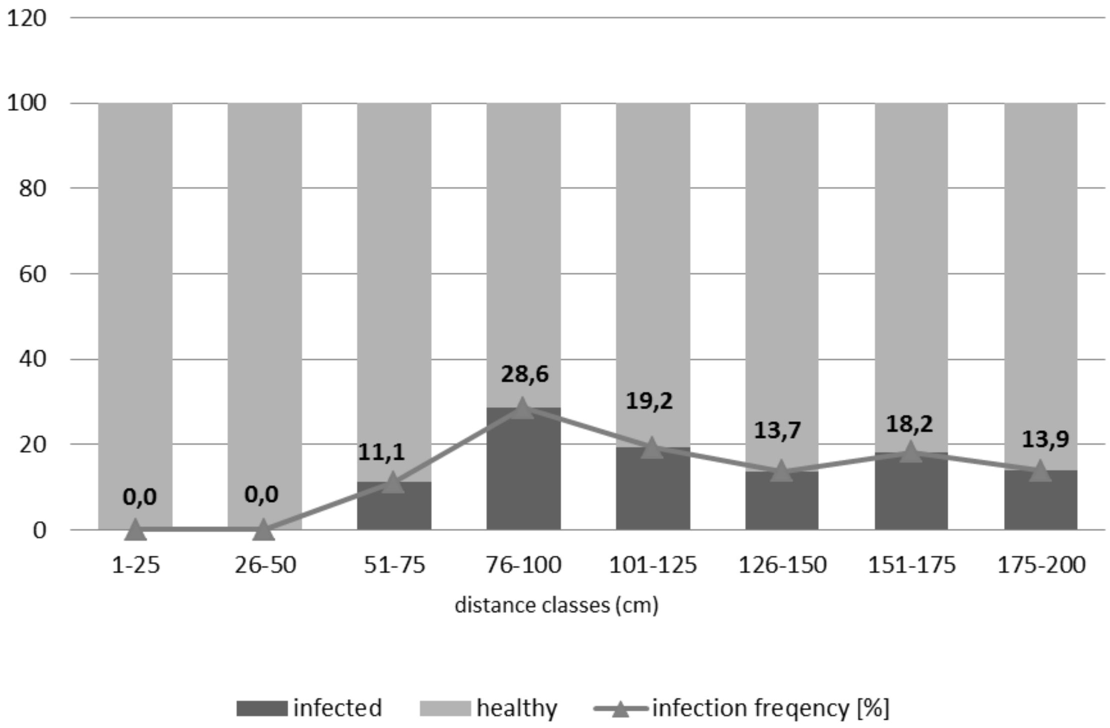

Figure 7 **Infected and healthy trees divided into distance classes**

Diameter of infected discs

The diameter of investigated trees (at the height of 15 cm) varied from 2,0 to 22,0 cm. The smallest diameter class 0,0 -2,0 cm and two the largest diameter classes (18,1-20,00 cm; 20,1-22 cm) were represented by not more than three individuals with no detected infection.

The infected discs have diameter from 2,5 to 16,75 cm. The highest infection rate occurred in discs with dimension from 6,1cm to 12,0 cm (Figure 8).

The size of discs taken from donor stumps (at the height 12 cm for low stumps and 65 cm for high stumps) vary from 2,5 cm to 13,00 cm. The infection of *Heterobasidion spp.* were found on two stumps: one high and one low with dimension 5 cm and 7,25cm respectively.

The results of statistics analyses shows that there is no significant influence between infection frequency and size of collected discs, p-value is 0,460.

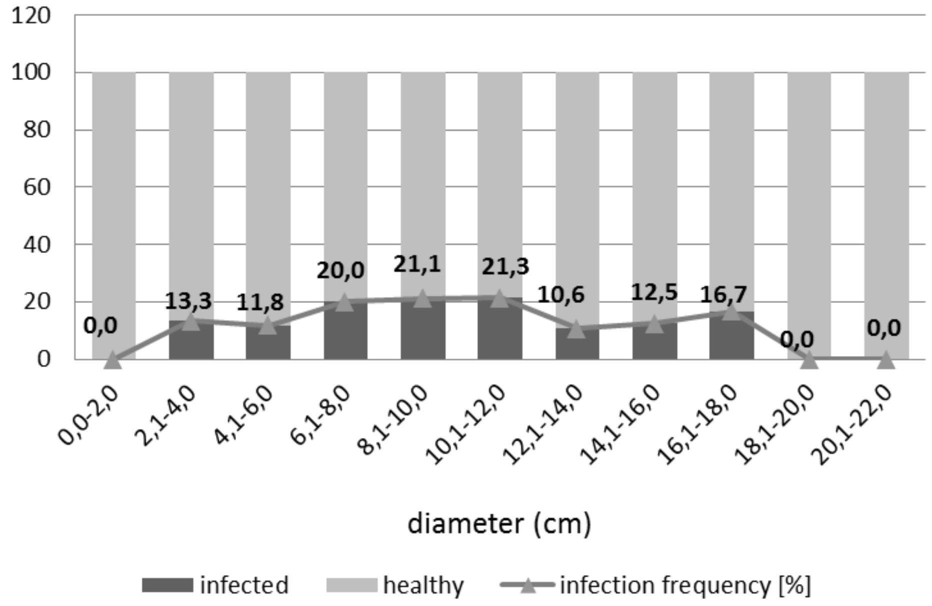

Figure 8 **Infected and healthy trees divided into diameter classes**

Age

There is one year differences in the age of the stands calculated from the time when they were established, it is 14 years for the site S3 and 15 years for the site S1 and S5. However, depending on the number of annual rings the age variation is much wider: from 9 to 22 years old.

The age doesn't influence infection frequency (p-value = 0,661). There are quite big differences in the number or trees in every age class and infection rate in each of them (Figure 9). There are two age classes represents only by one tree. It is the youngest class - 9 year old and 17 years old class which have 100% and 0% infection rate respectively. There is another class where no infection occurred - 10 years old class represents by two trees. Apart of that tow extreme (0% and 100% infection rate) the infection frequency in the other age classes vary from 6,3 to 25,0 with the mean of 18,7% infection rate. The differences in the value of infection frequency between two the most numerous classes (14 and 15 years old) is 9%.

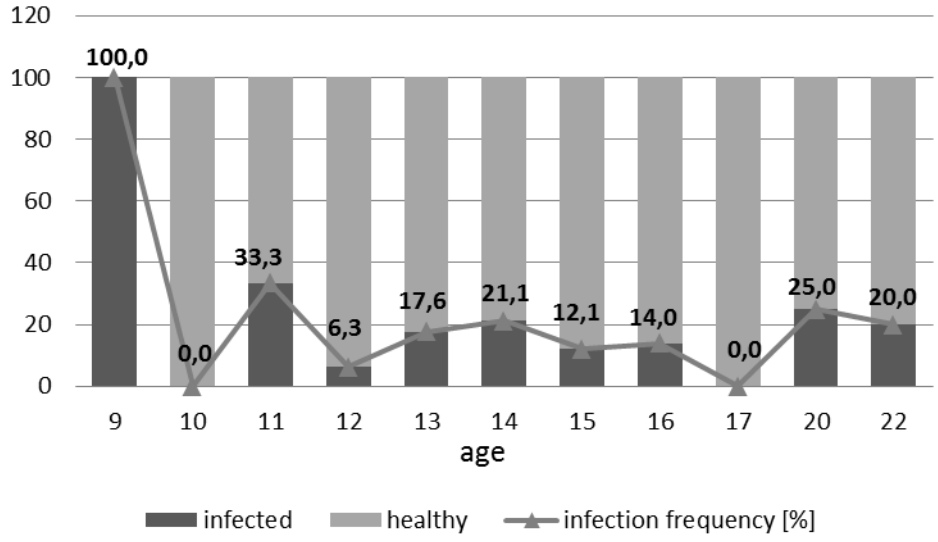

Figure 9 **Infected and healthy trees depending on the age**

3.5. Blocks

The block contains one donor stump and the four largest neighbouring trees within 2 m radius but there was some block with less than 4 trees because of the gaps in the stand. In this study 90 blocks were investigated, 56,6% of them contained 4 trees, 24,4% contained 3 trees and 16,6% of the blocks included 2 trees. There were 2 block which contained only one tree (Figure 10).

The infection of *Heterobasidion* spp. was found in 27 blocks. Usually only one tree in a block was infected. In two blocks infection were found in all of 4 individuals (Figure 10).

In the cases when infection were found in more than one tree in the block usually the same species of *Heterobasidion* were found in all of the contaminate trees.

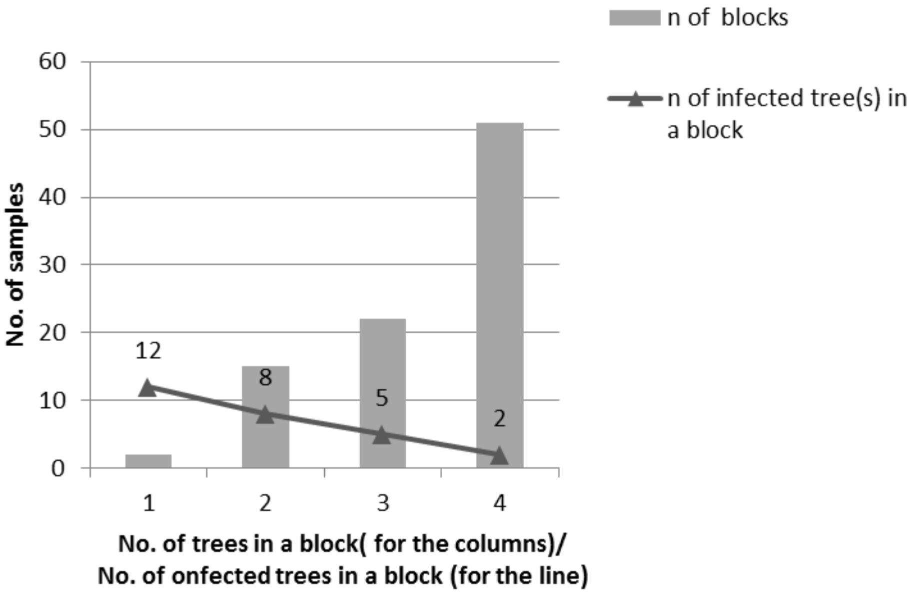

Figure 10 The number of trees in a blocks (columns) and number of infected tee(s) in a blocks (line)

4. DISCUSSION

4.1. The major findings

In general, the results show that height of the donor stump has no significant influence on the secondary spreading frequency of *Heterobasidion* spp. infection , p-value equals 0,0691. In my study conidia of Heterobasidion spp. were found on 31 trees adjacent to low stumps and 19 trees adjacent to high stumps.

The infection of Rb 175 was found only on two trees and two donor stumps (2,2% spread ratio). The trees infected by Rb 175 were associated with high stumps. Most of the trees were infected naturally by other strains of *H. parviporum* or *H. annosum* (11 and 32 trees respectively).

The site has an impact to infections rate (p-value 0,0075), the largest infection frequency – 27,8 % were found on the site with the lower site index - G26. In the case of this study distance, diameter and age have no significant influence on the infection rate.

4.2. Spread of Rb 175

In the study by Gunulf et al. (2013) 65% of the donor stumps had spread Rb 175 infection into at least one of the neighbouring trees. In my study it was 2,2%, only two out of 90 donor stumps had transferred the artificially inoculated Rb 175. There are a few possible explanations of that fact.

Firstly, the artificial establishment of Rb 175 infection might have failed at least on the low stumps. The result from pilot study conducted in the examined sites showed that 6-7 months after inoculation the infection of Rb 175 was found on 73% of test discs from the high stumps but none of the tested low stumps were contaminated. The fact that only high stump were infected in the pilot study could explain why both of Rb 175 infected trees found in the present study were associated with high stumps. However, there are too few observations to draw further conclusions.

Moreover, the donor stumps might be too small to spread the infection to a greater extent. The proportion of heartwood to sapwood increases with the size of tree. The study by Oliva et al. (2011) showed that infection of *H. annosum* and *H. parviporum* in Norway spruce prefer to develop in heartwood rather than in sapwood. It is also proved that infection frequency increases with the size of stumps (Gunulf *et al.*, 2013; Berglund *et al.*, 2007). The present study was performed on pre-commercial thinning sites where average diameter of donor stump varied from 4,75 to 8,5 cm depending on the sites. In many cases the ratio heartwood to sapwood could decrease probability of primary infection and its further spread through the root system into adjacent trees.

Another reason for low transferring rate of Rb 175 infection in my study could be too short time period, the infection could have not manage to reach all the trees within 2 m radius from the donor stumps yet. As reported from many studies there are big variations in the growth rate of *Heterobasidion* spp. in stem and root systems of trees. The growth rate of *H. annosum* in stump of *Picea abies* is reported to vary from 29 to 99 cm/year (Swedjemark & Karlsson, 2004). Additionally, the spread ratio in roots differ depending on its vitality; in living roots *H. annosum* could spread 7-12 cm year^{-1} and in dead roots about 20-30 cm year^{-1}(Pettersson *et al.*, 2003; Woodward *et al.*, 1998; Bendz - Hellgren, 1997). Taking into consideration the lowest reported growth rate the infection of Rb 175 could spread only 30 cm from the donor stump in the case of high stump. However, the distance between the high donor stumps with manage to transfer Rb 175 infection and affected trees in my study was much bigger (91 and 156 cm) which showed that the infection was able to grow further then the lowest reported spread ratio.

4.3. Impact of stump height on the infection frequency

The trees adjacent to low stumps showed a slight tendency to have a higher rate of infection comparing to those that are close to the high one (63% and 37% of the infected discs respectively). However, from the statistical point of view, when all of the *Heterobasidion* spp. are taken into account, the height of the stump does not significantly influence the infection frequency: P-value, that is equal to 0.0691, is slightly above significance level set up to 0,05. Moreover, the pattern seen from the most common species initially determined on investigated discs- *H. annosum,* allows to say that for this particular strain height of the stump has an significant impact on infection frequency (p=0,0481).

Nevertheless, there is another way to interpret the data which can show that p-value is below significance level of 0,05. The one-side hypothesis is to take as a fact from the beginning that the infection of *Heterobasidion* species spreads faster in low stumps then in high one. Therefore, the calculated p-value should be divided by two. In case of this study the p-value for one-side hypothesis is equal to 0,03455 (0,0691/2). However, it is not recommended to use one-side hypothesis in the study since it has not been proved yet that the height of the lower stumps influences the infection rate of *Heterobasidion* spp. One-side hypothesis is a sort of ethical issue in the statistical analyses which allows selecting the data to match it to the pre-convinced assumption which should not be used in scientific research.

Moreover, the impact of the stump height cannot be clearly seen from this study since the source of the infection, in most of the cases, is undefined. The majority of the trees were infected naturally by other strains of *Heterobasidion* spp. occurring wild on the sites (not by the known individual of Rb 175 that was used for artificial stump inoculation). The previous land use type of all investigated sites was forest. For this reason the infection found on the discs might have come from roots of old stumps.

On the other hand, the infection could be spread by the airborne spores that colonise the surface of fresh stumps. The fact that the trial plot was established during summer, when the spores of *Heterobasidion* spp. are very active, also proves this theory.

4.4.*Heterobasidion annosum* vs. *Heterobasidion parviporum*

Both *H. annosum* and *H. parviporum* inhabits Norway spruce in southern Sweden. It is proved that *H. parviporum* is more common in Norway spruce stands then *H. annosum* (Gunulf et al., 2012; Oliva et al., 2011; Hanso et al., 1993, Swedjemark & Stenlid, 1993). The result from my study shows that *H. annosum* infection occurring more frequent then *H. parviporum* on spruce trees which stay in contradiction with the sources mentioned above. However, there is report from other trial which suggest that *H. parviporum* is not over competing *H. annosum* in spruce dominated stands, in Gonthier *et al.* (2003) study both of the *Heterobasidion* species occur fairly equal in spruce dominated stand.

4.5. Potential sources of errors

Field work:

Two disc from low stump and 3 discs from the sample trees were lost in the field that is why they weren't examined in the laboratory. However, the infection wasn't found on the discs from the adjacent trees so most probably there was no infection on the lost discs as well.

There was an error in the establishment of the experiment in the site no S5 i.e. the tree no 261 was supposed to be a high stump according to the sample path but in the field it turned out to be a low stump so it was decided to skip the block with the trees no. 261-262 and collect the sample discs from the trees 263-264 instead.

Lab work:

The somatic incompatibility test used in the study is easy and reliable method to initially determine the species of *Heterobasidion* and investigate population structure of the fungi. However, to analyse the degree of relatedness of the isolates and determine the exact species further investigation such us sexual incompatibility is needed (Stenlid, 1985). Therefore, the result of the study based on somatic incompatibility test tentatively assess *Heterobasidion* spp., not determine the species more precisely which could be done using sexual incompatibility test.

Another inaccuracy may influence on the age class distribution presented in the study. There were difficulties to distinguish the annual rings at some of the discs. It usually applies to discs with small diameter up to 6 cm and the samples from donor stumps among with many were partly decayed. In that cases the age of the trees were estimated based on the number of visible annual rings, the diameter of the disk and the time when the stand was established. As a consequence of that the age class distribution presented in the paper may differ a little from the reality. However, as the calculation shows the age has no significant effect on infection frequency.

5. CONCLUSIONS

Four years after donor stumps were inoculated with the know individual of *H. parviporum* - Rb 175, the infection was found only on two out from 90 investigated stumps (one low and one high). Just two high donor stumps manage to spread the infection to neighbour trees. The infection of Rb 175 occurred on two samples out of 302 investigated spruce trees.

Take into consideration all of *Heterobasidion* spp. found on the samples discs, height of the stumps has no significant impact on infection frequency in precommercial thinning stands of Norway spruce (p-value equals 0,0691).

The most common pathogen was *H. annosum* which was tentatively assess on 32 out of 51 infected discs. The height of the stumps has significant influence on transferring *H. annosum* infection (p-value 0, 0481). It occurred on 10 discs from the blocks with high stump and 22 discs from blocks with low stump.

5.1. Practical implications

The study shows that both types of donor stumps – high and low are able to spread wild infection of *Heterobasidion* spp. in four year period in young spruce stand. The pattern seen from the most common species initially determined on investigated discs- H. annosum, allows to say that for this particular strain height of the stump has an significant impact on infection frequency (p=0,0481).The result could be a premise to create high instead of low stump during the precommercial thinning to inhibit spread of *Heterobasidion* spp. infection.

Moreover, there is a need for further research to investigate the impact of heights of the stump on transferring *Heterobasidion s*pp. infection. In the case of my study, most of the infected sampled discs were colonised by species of *Heterobasidion* naturally inhibiting the stand site. It is unsure if the source of infection was the donor stump created for the study or rather the old roots maintaining in the sites after previous generation of trees. There was unexpectedly low infection frequency of the artificially inoculated indicator strain of *H. parviporum* - Rb 175. It was found only on 2 donor stumps and 2 investigated trees; therefore it is too less sample to make any further conclusions.

Perhaps another way of inoculation Rb 175 should be consider in further study to be more successful. Witzell et al. (2010) compared inoculation by asexual spores sprayed on the stump surface with infection by colonized cutter shavings applied directly into the hole drilled in trees. Using the first method the establishment of the infection were mainly unsuccessful in contrast to the second technique where *Heterobasidion spp*. were detected in over 50% of inoculated trees (Witzell *et al.*, 2010).

When spruce trees were infected by Rb 175 directly to pith of the tree by colonized wood chips almost all mycelium of *H. parviporum* found 3 and 5 years letter were the same strain as used for infection (Pettersson *et al.*, 2003), However, the further root samples were taken 60 cm from the inoculation point so the distance was much lower than two meters radius use in my study (Pettersson *et al.*, 2003).

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