A study of root distribution and the effect of *Heterobasidion* spp. root infection on the growth of live Scots pines (*Pinus sylvestris*) in Southern Sweden

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Master Thesis no. 198

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Abstract

Stump treatment against *Heterobasidion* spp. root infection has not been commonly conducted on Scots pine (*Pinus sylvestris* L.) plantations in Sweden. To assess whether it should be, growth and volume losses caused by *H.* spp. was studied in a 36-year old Scots pine stand in southern Sweden. A total of 24 Scots pine trees were extracted and checked for *H.* spp. root infection, followed by a stem analysis reconstructing the volume growth. Root morphology and location of infection were studied and different above-ground indicators were tested to assess the incidence of infection. *H.* spp. were detected on 87.5% of the studied trees and colonized 9.7% of root volume in average (0.0-32.3%), which resulted in a growth reduction of 1.93 m^3 per hectare in the latest five years. Roots and infections were located unevenly in cardinal directions. Large primary roots were most susceptible to infections. Needle retention, crown length, DBH and presence of *Heterobasidion* fruiting bodies were not accurate enough as an infection indicator. In conclusion, the root morphology and data of infection may be used to calibrate some root disease models and it is recommended that stump treatment on Scots pine is conducted to maintain the site productivity in southern Sweden.

Key words: *Heterobasidion* spp., *Pinus sylvestris,* stump treatment, forest management, root morphology

1 Introduction

Scots pine (*Pinus sylvestris* L.) is the second most important conifer species for industry in Sweden (Skogsstyrelsen 2007). During 2009, total volume of round wood used by the forest industry was 67.6 million m³ sub (solid wood under bark), of which Scots pine accounted for 38%, Norway spruce (*Picea abies L.*) 52%, birch (*Betula pendula L., B. pubescens L.*) 8% and 1% for other broad-leaved species (Skogsstyrelsen 2010). Most Scots pine logs are sold to saw mills for timber production. In Sweden, high quality Scots pine timber is more valuable than spruce timber (Mellanskog 2012)

Establishment and maintenance of vigorous Scots pine stands can be hampered by abiotic damages, insect pests and fungal diseases, e.g. winter drought, pine weevils (*Hylobius abietis*) and root rot (*Heterobasidion* spp.), respectively (Jacek et al. 1998; Örlander and Nillson 1999; Rönnberg et al. 2006). Of those, root rot caused by species of *Heterobasidion* frequently leads to growth reduction and mortality in Scots pine stands (Gibbs and Greig. 2002). A study in Poland showed that middle-aged (45 years) Scots pine stands infected by *Heterobasidion* spp. decreased the annual increment by 50-60% i.e. standing volume of 27.8-69.5 m^3ha^{-1}, as compared to a healthy stand (Sierota, 2003a; Rykowski and Sierota 1984). In UK, great mortality has been observed in Scots pine stands infected by *Heterobasidion* spp. (Burdekin, 1972) and resulted in some limitations in later thinnings due to the reduced amount of trees that remained.

Two species of *Heterobasidion* can decay or kill roots of Scots pine and lead to growth losses in Sweden, namely *Heterobasidion annosum* (Fr.) Bref. s.s. and *Heterobasidion parviporum* Niemelä & Korhonen (Niemelä 1998). In the active growing season when the temperatures exceed 5°C (Brandtberg 1996), airborne spores of *Heterobasidion* spp. germinate on freshly cut stumps created at thinnings or final felling. Fungal mycelia colonize stumps and subsequently spread to neighboring live trees via root contacts and root grafts (Rishbeth, 1951b). Infected Scots pine might remain alive throughout a rotation even with heavily diseased or dead roots, but without trees showing obvious

symptoms of infection (Greig, 1998). Sometimes infections can be diagnosed from crown symptoms, i.e. shorter needles, low needle retention and thin crowns (Kurkela, 2002a). However, because *Heterobasidion* spp. seldom causes visible decay at the cut surface of pine stumps (Bendz-Hellgren et al., 1998), there is a tendency among Swedish foresters to associate crown symptoms and dead or dying pines with insect attacks followed by root infections being a secondary but less important factor (Rönnberg et al. 2006). Hence, mortality or growth loss of Scots pine caused by *Heterobasidion* spp. might have been underestimated in the past. This is in sharp contrast to Norway spruce, in which root decay may extend several meters up in the stem.

Spread of *Heterobasidion* infection and the impacts on growth of Scots pine could be simulated and predicted by root disease models based on root morphology and infection data e.g. incidence, rate of spread etc.(Pukkala et al. 2005). Such models may be useful to forest managers in production forecast and forest management, yet none of which was derived from morphological study of Scots pine root system in Sweden.

Primary infection by *Heterobasidion* spp. can be prevented by applying chemical (Pratt, 1998) or biological agents on freshly cut stumps (Greig, 1976). Chemical treatment is commonly represented by urea and Timbor, while biological control using *Phlebiopsis gigantea* (Fr.) Jül (Korhonen et al. 1998) is commercially available as Rotstop ™. In Sweden, stump treatment with *P. gigantea* has been practiced for about 20 years, and is generally preferred over the use of chemicals in the forest (Thor 2003). Most of the stump treatment is conducted on Norway spruce (Rönnberg et al. 2006) and Scots pine may only occasionally be treated, since the impact of *Heterobasidion* spp. on yield of Scots pine is largely unknown to economically justify costs for stump treatment.

So far, there has been no study assessing the non-lethal effect of *Heterobasidion* spp. on growth loss of Scots pine or any cost analysis to evaluate the potential economic benefit

doing stump treatment (Rönnberg et al. 2006). Consequently, the aim of this study was to quantify growth loss of Scots pine infected by *Heterobasidion* spp. and to justify costs of stump treatment applied during thinning.

2 Material and Method

2.1 Site

The experimental site, with a area of 25.1 ha, was located at Vittskövle (55° 51' 0" N, 14° 8' 0" E) in southern Sweden (Fig. 1) on former pasture land with a site index of 26m for Scots pine (dominant height at the age of 100 years). The site vegetation is 36-year old pine plantation with sandy, alkaline soils. Two thinnings were carried out in the summer 2002 and spring 2009. Stumps were not treated against root rot at either thinning. Damages from rodents and a storm in year 2005 were reported (personal communication with the site manager, 2011).

Fig.1 Location of the experiment site

2.2 Sample plots, measurement and extraction of trees

Two pine stumps, with and without *Heterobasidion* spp. fruiting bodies, were randomly selected as plot centers. The stump without fruiting bodies was regarded as diseased-free or less infected compared to the other stump. Two 8 m radius plots (0.02 ha) centered at each stump were established. The plot without fruiting bodies of *Heterobasidion* spp. on the center stump was defined as 'Healthy Plot' and the one with fruiting bodies as 'Infected Plot'. Incidence of *Heterobasidion* spp. in standing trees was consequently expected to be lower for the healthy plot.

All live trees in plots were numbered and marked with ribbons. For every selected tree, the distance to the plot center and the relative angle to the north were recorded so that it could then be projected onto a plot map (Fig. 2-3). In addition, diameter at breast height (DBH) and visible crown lengths in cardinal directions of each tree were measured (a,

b), with which crown area was calculated using the ellipse formula ($S = \pi ab$).

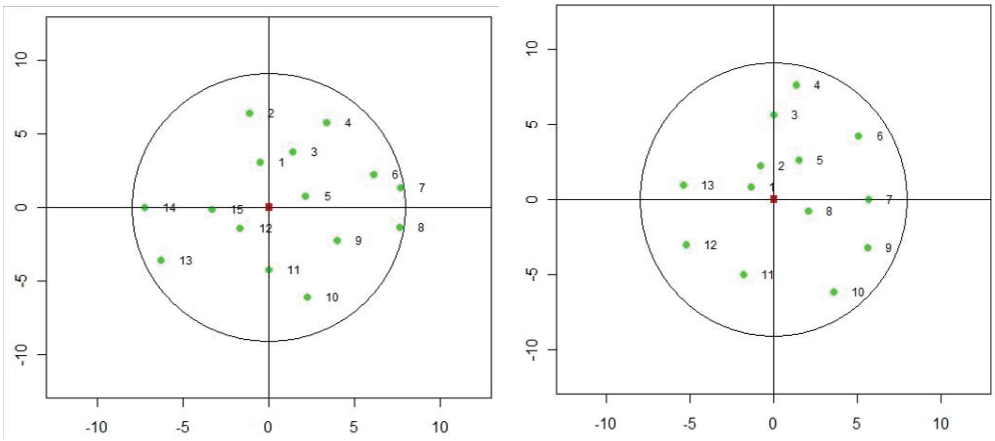


Fig.2-3 Map of the healthy (left) and disease plot (right). Green dots stand for live trees in plot. Red square stands for the centering stump.

The number of each tree was painted on the north side of the stem. A single grip harvester (Machine: Ecolog 580B; Head: logmax 7000) then pulled out all trees. Each tree was carefully laid down on the ground near the original location. Any remaining roots missed by the excavator were dug up manually, marked and tied up to the stump.

Five branches were randomly sampled from the upper, middle and lower part of each crown and needle retention was measured by counting the number of annual cohorts that retained the needles.

2.3 Stump measurement

After cleaned in field, stumps were transported to a warehouse where each of them was elevated by a floor crane. Diameters of roots in cardinal directions (N/S, E/W) of each stump were then measured to calculate the area of the root plate with the ellipse formula.

Roots thicker than 1cm at the root collar were classified into three levels: Primary (incl. tap roots), secondary and tertiary roots (Fig. 4). Smaller roots (roots with a diameter at the root collar < 1cm) and branches of tertiary roots were cut off from the stumps. All

root classes were labeled (Fig. 5) and angles to the north direction were registered for primary roots.

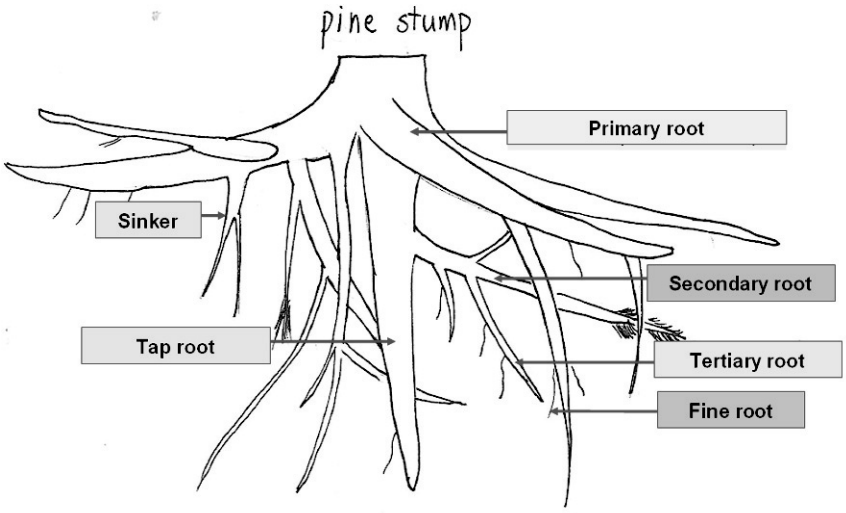


Fig. 4 Classification of roots as primary, secondary, tertiary and fine

Root length and the distance to stump surface were measured. Diameter was measured at the root collar and at every 50cm distal until roots were less than 1cm. Where root breakage and grafts occurred, its location was registered including where and which root it grafted with, and whether it was a tap root or sinker root.

2.4 Determination of infection incidence and severity

After measurement, roots were sterilized with 70% ethanol and cut off at the collar using an electronic saw. All cuts on the root were sterilized and wrapped with plastic bags to avoid contamination. Wrapped roots were cut to sections at every 25cm from the root collar. A 5-cm disc at the proximal end of each root section was sampled (Fig. 5). All discs were labeled, sealed in separate plastic bags and then checked for *Heterobasidion* spp. conidiophores under a dissecting microscope (20X magnification) after incubation at 20-25℃ for 7-10 days. If conidiophores were found on either side of a root disc, the entire 25-cm root length section was considered infected. A tree was

classified healthy only when no infections existed on any of the roots. Incidence of infection (inf. %) per hectare was calculated as percentage of the infected trees out of the total amount of trees.

Root volumes (V_R) were calculated for each section using the frustum of a cone formula (Fig. 5). For each tree severity of infection at the time of sampling was described as a percentage of infected volume in the total root volume.

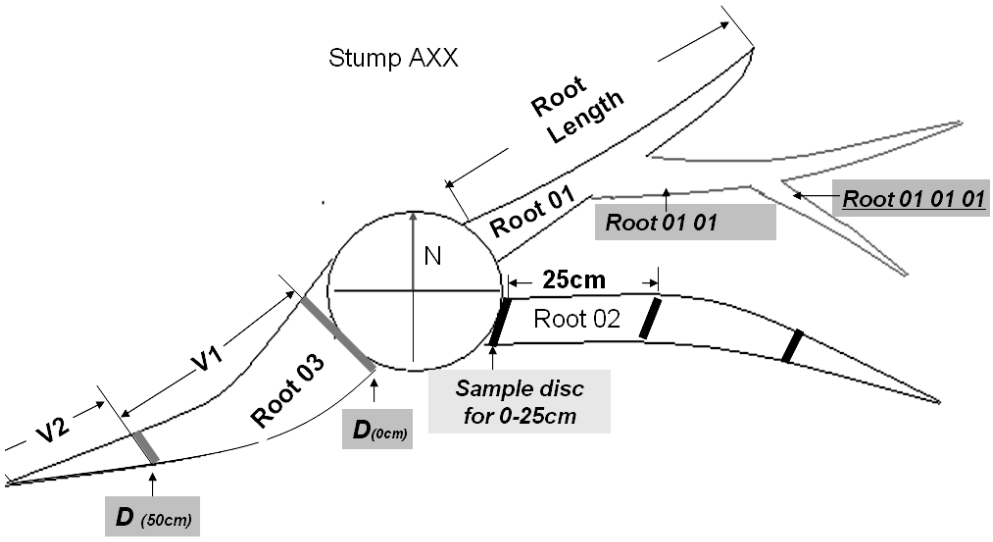


Fig. 5 Root labeling and sampling: D (0cm) stands for the diameter at the root collar. D (50 cm) stands for the diameter at 50cm. V_1 and V_1 stand for root volume from 0-50cm and 50cm to the end.

2.5 Stem analysis

Tree height was measured and cross-sectional discs (1.5 cm thick) were cut using a chainsaw at 0m, 0.5 m, 1 m, 1, 3 m, 2 m and every 2 m until the stem was less than 5cm diameter.

The north direction was indicated by an arrow on the surface of stumps and stem discs. Discs were marked with tree number and height, and then placed separately in a ventilated basement for drying. After 2 weeks, one side of the dried discs was sanded on a belt sander until tree rings were clearly visible and scanned (Fig. 6).

Fig. 6 Scanned stem disc

The software Windendro (2005a) was used for calculating the ring-width, disc-radius and basal areas. Software Winstem (2005a) reconstructed volume dynamics by summing up cones delimited by two consecutive discs.

2.6 Growth calculation

Tree volumes (V) and volume increment (VI) at every year were determined from stem analysis. To eliminate the influence of tree size, the volume increment rate (IV %) was calculated for each year e.g. $IV_{2011}\% = VI_{2011} / V_{2010}$. A liner regression was constructed for the IV % and the percent of infected root volume (Inf. %).

Trees were equally sorted to two groups by the Inf. %, regarded as 'healthy / lightly-infected' and 'medium-infected' group, respectively. The average VI% of each group were calculated and compared. The volume of medium-infected group was calculated as they were healthy / lightly-infected, and the difference between the result and their actual volume was regarded as the growth loss.

2.7 Cost analysis of stump treatment

The costs for stump treatment at each felling in a rotation were estimated and discounted to the year of study (2011) at an interest rate of 3%, and compared with the value of the growth losses.

Table 1 Accumulated Costs of stump treatment for Scots pine discounted to the 2011 at 3%

Year	Operation	Harvested volume, ha		Total harvested vol.m³	Prices			
		Pulp, m³	Timber,m³		Pulp, SEK/m³	Timber[a] SEK/m³,sub	Treatment cost[b] ,SEK/ m³ sub	Treatment cost[c] , SEK/ha
26	1st TH[d]	23	1	24	275	385	9.8	326
35	2nd TH	26	14	40	275	435	7.35	637
45	3rd TH	26	31	57	275	510	11	1132
60	4th TH	22	51	73	275	570	6.6	1377
75	F.F[e]	16	313	329	275	585	3.31	1731
	Sum, m³:	113	410	Average log price at present: 331 SEK/ m³ sub				

[a]Mellanskog, 2012 [b]Magnus Thor, 1996 [c] discounted to 2011 at 3% [d] Thinning [e]Final felling

2.8 Survey of *Heterobasidion* fruiting bodies on live Scots pines

The incidence of *Heterobasidion* infected Scots pine based on the presence of fruiting bodies was investigated for 240 trees in four monoculture Scots pine plantations at Degeberga (55° 50' 0" North, 14° 5' 0" East) and Gualöv (56° 3' 0" North, 14° 25' 0" East) in Southern Sweden. Lines with 15m intervals were walked through the stands. Ground vegetation and soil around the base of trees on the route was removed and the base was checked for the presence of *Heterobasidion* fruiting bodies. Once fruiting bodies were present, the rate of defoliation and needle discoloration of the host tree were observed and noted. Trees that surrounded a stump or tree with fruiting bodies were investigated even if they were outside the route.

2.9 Statistic methods

T-test in Minitab (ver 16, Minitab Inc., State College, PA, USA) was used to examine the difference in distribution and size of roots and growth characteristics of trees between two plots. P-values were calculated at the mean time.

3 Results

3.1 Incidence and severity of *Heterobasidion* infection in plots

Twenty-four trees extracted from the two sample plots were used for further analysis. Table 2 shows the incidence of *Heterobasidion* spp. and the severity of infection as percent of infected root volume. In total, three trees were free from infection and all of them were from the 'Plot A'. Among all infected trees, the percent of infected root volume varied from 3.0% to 32.3%. However, both the incidence and severity of infection did not significantly differ between the two plots.

Table 2 Incidence of trees infected with *Heterobasidion* spp. and severity of infection in the healthy and infected plots

Plot	No. of trees included	Incidence of trees infected with *H.* spp. (%)	Average percent (%) of infected root volume, (min-max)
A	12	75	9.2 (0.0-21.1)
B	12	100	11.8 (3.7-32.3)
Mean	12	**87.5**	**9.7**

3.2 Root distribution of a stump

A total of 1365 roots from the 24 trees were analyzed. Of those, 14% (n=191) were primary, 53% (n=716) were secondary and 33% (n=158) were tertiary. All roots were located within three meters from the stem. For every stump, the average amount of roots observed 0-25cm, 25-50cm, 50-75cm and 75-100cm from the stem were 24, 32, 33, and 29, respectively. The amount of secondary roots was higher than primary ($p=0.009$) and tertiary roots ($p=0.03$) within 1.5 meters from the stem (Fig.7).

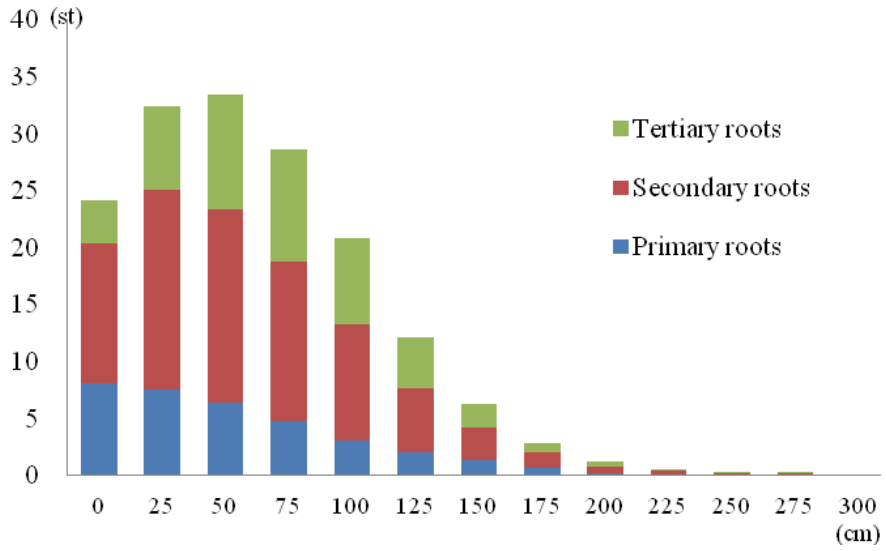
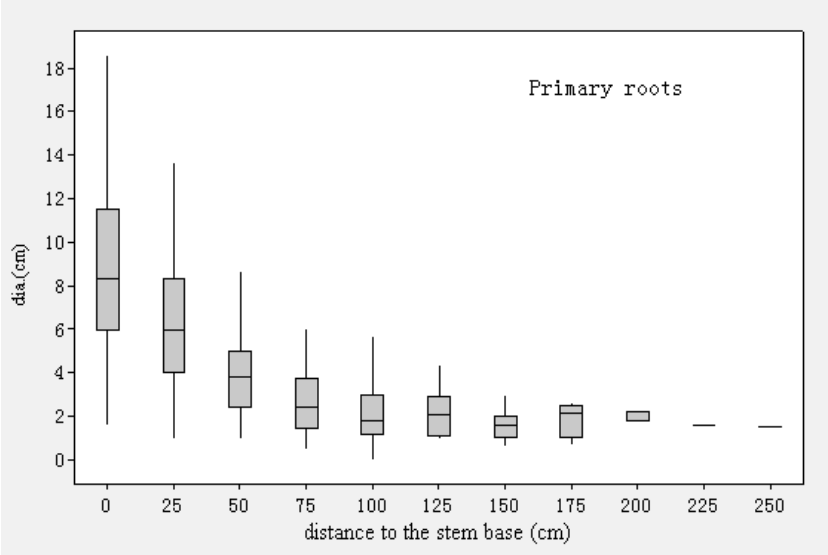
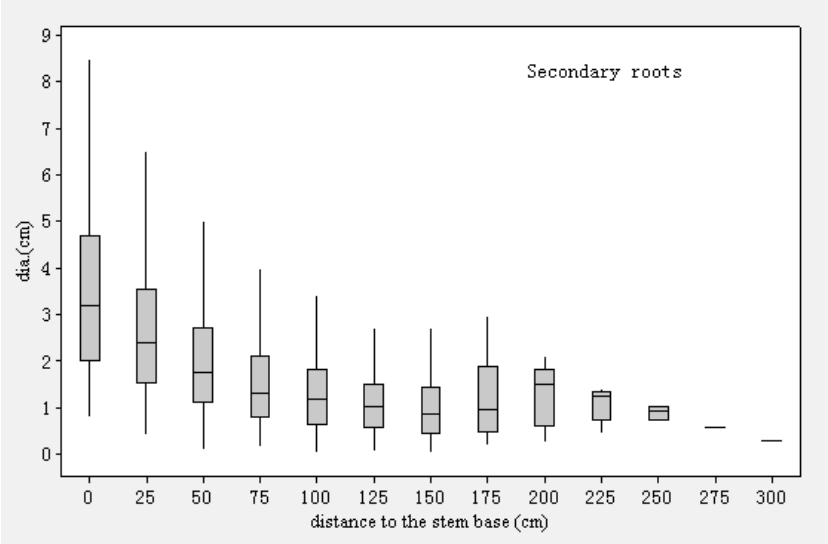


Fig. 7 Root distribution of primary, secondary and tertiary roots. X axis represents the distance to the stem (cm) and Y axis is the amount of roots.

Within one meter from the stem, a decrease appeared on the size of primary, secondary and tertiary roots at every 25 cm (fig.8-10). The average diameter of the infected root collars for primary, secondary and tertiary roots (9.46 cm, 4.80cm, 3.18cm, respectively) tended to be larger than the healthy roots (8.96cm, 3.2cm, 2.09cm, respectively), and the difference was significant for secondary ($p<0.001$) and tertiary roots ($p=0.023$).





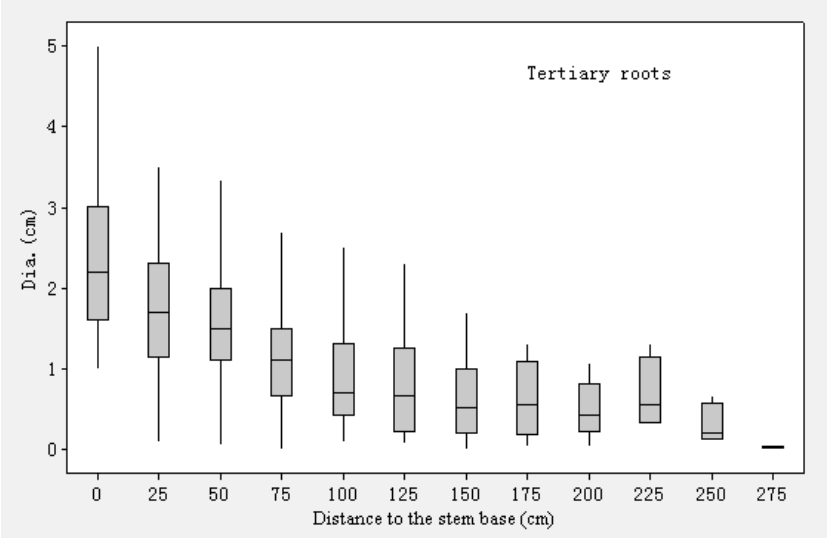


Fig. 8-10 Distribution of root dimensions in the stump. X axis denotes the distance to the stem base (cm) and Y axis is the diameter at every 25cm (cm). Fig 8, 9, 10 represent primary, secondary and tertiary roots, respectively.

Roots were located unevenly in cardinal directions (N/S, E/W) as shown in figure 11. The proportion of roots to the south (S/SW/SE) was 14% less than the north (N/NE/NW). Fewer roots were found in the east and west of the stump (4%) than the north and south (15%). The average amount of secondary roots is larger than the primary ($p=0.013$) and tertiary roots ($p=0.043$) for each stump.

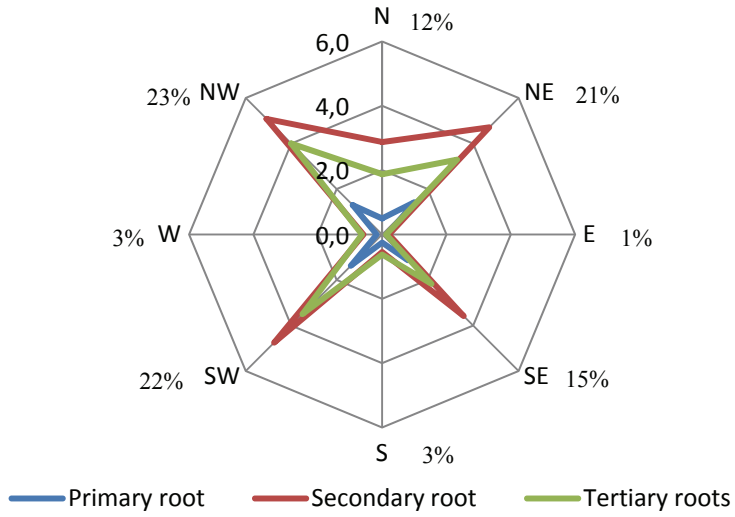


Fig. 11 Proportion of roots and amount of primary, secondary and tertiary roots in cardinal directions (N/S, E/W).

3.3 Infection characteristics

More than 8% (n=116) of the total number of roots, equivalent to 9% of the total root volume, were infected by *Heterobasidion* spp. Distribution of infection among primary, secondary and tertiary roots was shown in Table 3. Primary roots were the most diseased in terms of the incidence and severity of infection. Around 69.3% of all infections were located 0-50cm from the stem, 20.3% between 50 -75cm, and 10.3% between 75-150cm. Primary roots on the east of the stump were most diseased (50%), followed by primary roots in the north (42%, Fig. 12).

Table 3 Distribution of infection in primary, secondary and tertiary roots. Figures within columns with different letters are significantly different ($p<0.000$).

Root level	No. of infected roots	No. of healthy roots	Incidence of infection, (%)	Infected root volume, dm^3.	Total root Volume dm^3.	Percent of infected root volume (%)
Primary	49	142	26	57 [a]	459[a]	12
Secondary	55	661	8	19 [b]	293[a]	6
Tertiary	12	446	3	2 [b]	69 [b]	3
Sum	116	1249	8	78	821	9

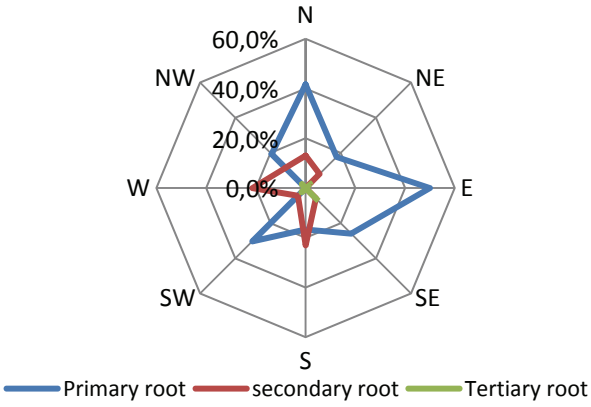


Fig. 12 Incidence of infections in all root classes in cardinal directions

More than 9% of the total infected root volume was contributed by tap roots, and 5% and 2% were from secondary and tertiary roots, respectively (Tab. 4).

Table 4 Infection distribution among sinkers of primary, secondary and tertiary roots

Root class	No. of sinkers	No. of infected sinkers	Incidence of infection (%)	Proportion of sinkers (%)	Volume proportion of infected sinkers (%)
Primary	23	5	22	12	9
Secondary	199	9	5	28	5
Tertiary	117	7	6	26	2
Sum	339	21	6	25	16

3.4 Frequency of visable *Heterobasidion* infections

In the frequency survey, out of 240 living Scots pines, *Heterobasidion* fruiting bodies were present on eight trees or 3,3%Of those, five were of no difference from the surrounding trees in terms of DBH and visual crown characteristics (defoliation, area). The other three were of small DBH or crowns; however, there were signs of disturbing factors e.g. insect attack and suppression and one was much younger than the other trees.

3.5Tree characteristics and growth vs. root infection

3.5.1 Characteristics of trees and roots vs. root infection

The characteristics of all trees in the two plots were shown in table 5. Average DBH of trees in Plot A and B was 17.1cm and 18.3cm, respectively. Percent of infected root volume was not correlated with DBH, crown area or needle retention.

Table 5 Characteristics of all trees in the plots. 'V.I.' means volume increment.

Tree	DBH dm	needle retention	crown area m^2	root area m^2	Tree Vol dm^3	2002-2006 V.I. dm^3	2007-2011 V.I. dm^3	Root volume dm^3	Infection%
A02	140	3.5	10.6	1.6	117	28	47	9.9	0.0%
A03	146	3.1	11.5	2.1	161	46	56	19.0	21.1%
A04	70	3.5	6.7	3.2	22	5	5	3.4	0.0%
A05	179	3.1	31.4	7.0	232	64	68	33.4	11.9%
A06	182	3.0	16.5	3.8	264	82	90	31.0	8.0%
A07	169	3.6	15.1	2.0	215	59	71	50.5	0.0%
A08	197	3.5	9.3	2.5	269	89	91	37.6	3.0%
A09	210	3.2	-	-	291	89	80	39.2	17.0%
A10	183	3.5	18.3	3.1	226	56	87	32.9	13.9%
A11	170	3.5	10.9	3.5	192	48	60	26.1	11.9%
A12	206	3.8	11.9	2.7	284	77	94	36.8	11.3%
A14	208	2.8	17.5	3.2	320	95	111	33.0	12.2%
B01	165	3.5	9.4	2.3	192	34	32	19.3	8.3%
B02	122	3.1	4.4	1.5	99	25	29	11.3	7.1%
B03	205	3.2	14.5	1.4	274	87	69	53.8	4.1%
B04	197	2.9	-	-	264	65	92	44.2	3.7%
B05	157	3.0	7.2	2.5	156	53	41	16.8	32.3%
B06	170	3.0	16.8	3.1	158	46	31	23.5	23.5%
B07	135	2.7	8.4	3.8	111	26	31	15.3	9.1%
B08	223	3.7	14.6	5.1	324	101	102	51.1	14.2%
B10	212	2.9	18.0	2.7	298	78	96	48.8	5.9%
B11	235	3.5	20.5	11.5	383	102	124	70.6	5.5%
B12	141	3.2	11.5	3.1	121	46	19	16.5	20.2%
B13	244	3.3	28.2	16.6	396	82	93	96.5	7.9%

3.5.2 Location of trees, crowns and root plates.

Crown areas were positively correlated with root areas ($p<0.000$) (Fig. 13-14).

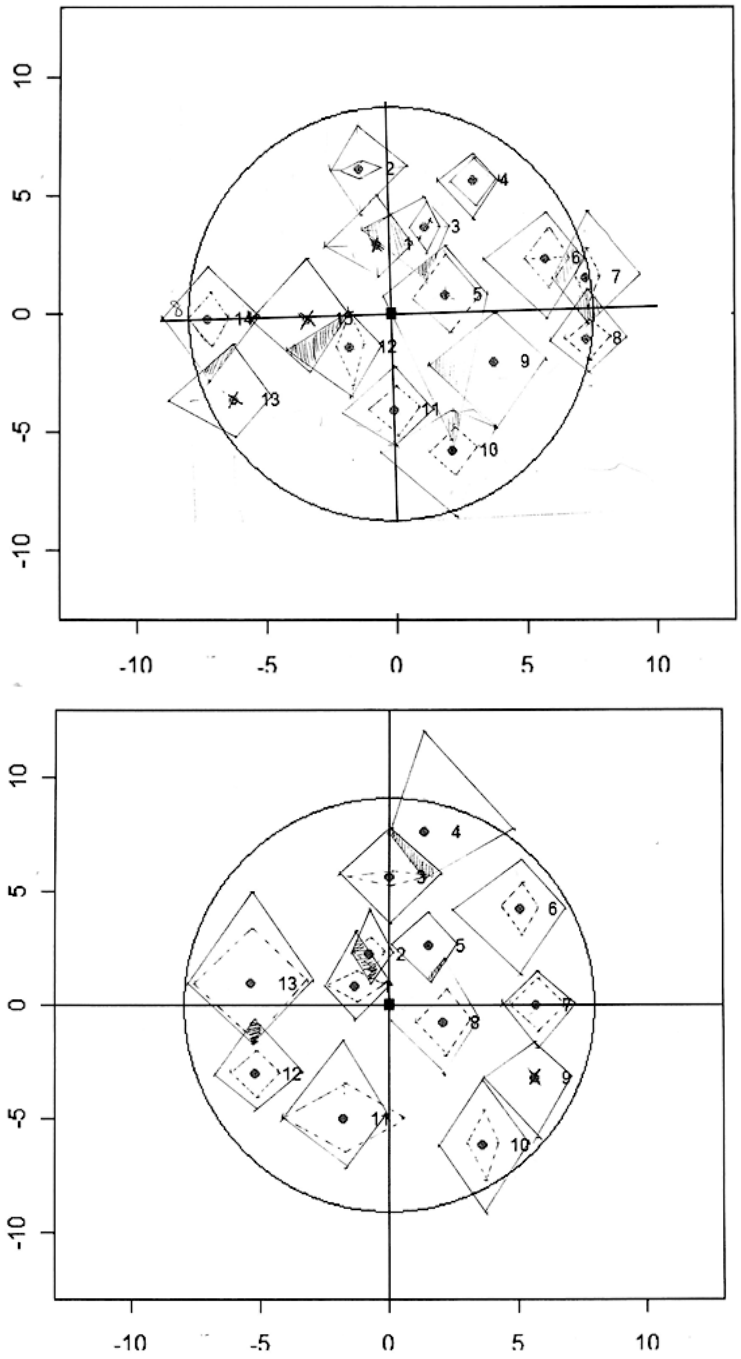


Fig. 13-14 Crown and root cover of each tree in the Healthy plot (Fig. 13) and Infected plot (Fig.14). Solid lines indicate crown cover and dashes indicate root area. Overlaps were marked in shade.

3.5.3 Comparing volume increment rate between healthy and infected trees

Volume increment rate (IV%) of infected trees was lower than the healthy trees in 1998

and 1999 as well as the latest three years than in other years between 1992 and 2011 (P<0.000) (Fig. 15).

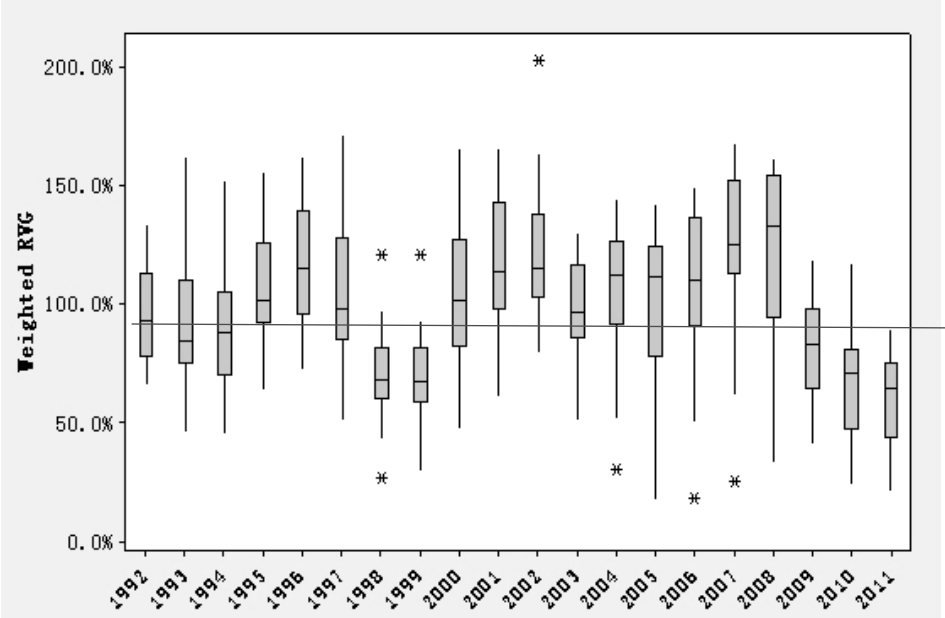


Fig. 15 Comparison between IV% $_{healthy}$ and IV% $_{infected}$ during 1992 and 2011. X axis represents the year and Y axis is the weighted IV%. '*' are outliers and were discarded from the comparisons.

3.5.4 Volume increment rate and volume losses

The average volume increment rate (IV %) decreased from 2002 to 2011 (Fig 16) and a negative correlation appeared between IV% and percent infected root volume in 2010 and 2011 (p=0.006 and p=0.005, respectively).The average IV%$_{2007\text{-}2011}$ was smaller than 2002-2006, and their difference was correlated with percent of infected root volume (p=0.008). (Fig. 17)

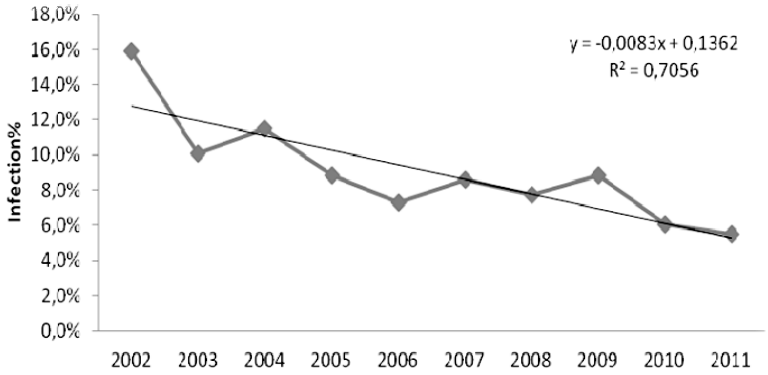


Fig. 16 Average IV% over 2002-2011 X-axis represents the year and Y-axis represents the severity of infection.

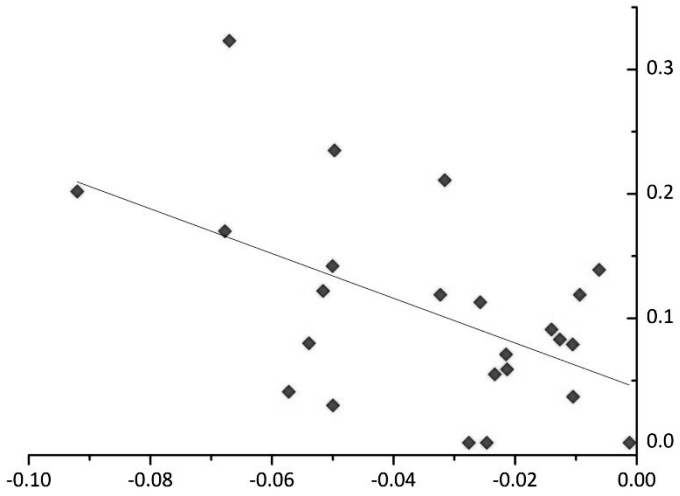


Fig. 17 Differences between mean IV% $_{2002-2006}$ and IV% $_{2009-2011}$correlated with severity of infection: X axis represented the difference of the average IV% $_{2002-2006}$ and IV% $_{2009-2011}$; the Y represented the percent of infected root volume.

The 24 trees were equally sorted to two groups by the severity of infection (Inf. %). One group, with Inf. % less than 8.3%, was defined as healthy or lightly-infected (Group H) and the other group with Inf. % from 8.4% to 32.3% was medium-infected (Group Inf.). A regression equation was constructed for the average IV% $_{2007-2011}$ and the percent of infected root volumes (Fig.18):

$IV\%_{2007\text{-}2011} = -0.09244 \times \text{Inf. } \% + 9.544$

Based on the equation, the IV% of a healthy tree (Inf. %=0) is 9.55%. When Inf. % reaches 8.3%, the IV% is 8.79%. Therefore the average IV % for Group H is 9.17%. For group Inf., IV% is 8.78% and 6.57% when Inf. % is 8.4% and 32.3%, respectively, so the average IV% is 7.67%. The gap between IV percent of the two groups is 1.50%.

Assuming the trees in Group Inf. were healthy or lightly-infected, the IV% would be 1.50% higher, result in a increase in the total volume by 1.93m^3 per hectare from 2007-2011 (Tab. 6).

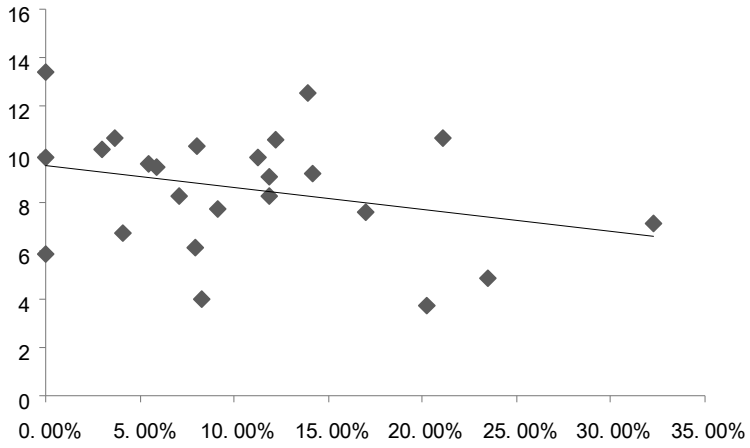


Fig. 18 Regression between mean $IV\%_{2007\text{-}2011}$ and percent of infected root volume (inf. %): X axis represented the inf. %; the Y represented the $IV\%_{2007\text{-}2011}$. R^2=0.088.

Table 6 Volume calculations per hectare and the five-year volume losses

	$Vol._{2011}$ m^3/ha	$VI_{2002\text{-}2006}$ m^3/ha, year	$VI_{2007\text{-}2011}$ m3/ha, year	$IV\%_{2001\text{-}2006}$ year	$IV\%_{2007\text{-}2011}$ year	If healthy / lightly infected	$VI_{2007\text{-}2011}$ $_m{}^3$/ha, year	Vol. loss, m^3/ha. year	Vol. loss 5 years m^3/ha
Plot A	130	7.38	8.6	14.8	9.9	Plot A	9.12	0.52	2.61
Plot B	139	7.45	7.59	11.7	7.5	Plot B	7.84	0.25	1.25
Average	134	$Vol._{2002,}$ m^3/ha		$Vol._{2007,}$ m^3/ha		Average	8.48	0.39	**1.93**
	Plot A	49.75		86.65		Stems/plot		Stems/ha	
	Plot B	63.6		100.85		12		600	

3.5.5 Economic analysis

The present price of wood is calculated to be 331 SEK/m^3 (Tab. 1), and therefore the economic value of the reduced volume increment (1.93 m^3/ha) between 2007 and 2011 is 639 SEK per hectare. Discounted to the year of study (2011), the cost of stump treatment at the first and second thinning is 326 SEK and 312 SEK, respectively, and 637 SEK in total (Tab. 1). The cost and assumed value saved from the treatment is almost equal.

4. Discussion

4.1 Growth reduction caused by *Heterobasidion* spp. in Scots pine

Scots pine trees may reduce the rate of volume growth after a certain period of infection by *Heterobasidion* spp., and consequently results in considerable losses in volume growth through the rotation. In general, this conclusion agrees with former studies on growth losses caused by *Heterobasidion* spp. in Scots pine (Sierota, 2003; Rykowski. and Sierota 1984a) and other conifer species e.g. Slash pine (*Pinus elliottii var. elliottii Engelm.*), loblolly pine (*Pinus taeda L.*) and Norway spruce (Froelich et al. 1977; Bradford et al. 1978; Bendz-Hellgren 1995; Bendz-Hellgren and Stenlid 1997). However, this was the first study to correlate above-ground growth and percent of infected root volume on Scots pine. The result suggested that there was a trend showing infected Scots pines started to grow less than the healthy, and the more infection detected in the stump the less growth in the stem.

Growth losses due to *Heterobasidion* infections measured in this study may be due to a direct loss of root tissue restricting the water uptake (Kozlowski 1969) and induced defenses that divert the resources from growth (Franceschi 2005). During the present experiment, diseased pine trees were frequently observed to have resin soaked and discolored roots. The resin exudation observation might be attributed to the general defense response of a tree against injury or infection. The discoloration might also be caused by the accumulation of phenolic compounds in the barrier zone between healthy and infected wood (Shain 1971). In this study, such roots had died before the extraction and none of their discs were found with conidiophores of *Heterobasidion* spp. The defense starts in advance of fungal penetration and continue till the tree dies (Shain 1979; Shigo 1984) consuming the energy otherwise allocated to growth. This could be supported by a recent study that the decrease of volume growth in Norway spruce were only evident on *H. annosum*-infected trees with signs of host defense to *H.* spp. (Oliva et al. 2010).

4.2 Time of infection vs. stump treatment

Heterobasidion infections observed in the present study could be introduced via three possible channels at different times: 1) diseased trees on the former pasture before the plantation got established (1976), 2) stumps created at the first thinning (2002), and 3) exposed stump surface in the experiment (2011). Stump treatment, to prevent the primary infection, is only suggested when the first thinning served as the trigger of the infection. The high incidence (87.5%) of *Heterobasidion* spp. in our study was unlikely to be caused by only several scattered oaks or pines on the former pasture land, and besides, neither old trees nor retained stumps that may transmit *Heterobasidion* spp. to the new plantations were observed during the site inventory. The likelihood of any infections being initiated during this experiment should be small too, considering the spread rate (25-128 cm per year) of *Heterobasidion* in coniferous roots (Bendz-Hellgren 1999; Chavez Jr. et.al. 1980). The lack of correlation between the amount of infections per stump and the time of storage seems to support this conclusion as well. Therefore, it seems prudent to consider the first thinning in 2002 as the starting point of the *Heterobasidion* infections, and stump treatment could hence have been applied to prevent the infection via fresh stumps.

4.3 Incidence of *Heterobasidion* spp.

Incidence of trees infected by *Heterobasidion* spp. was higher in the present study (87.5%) compared to the previous assessment (73%) in southern Sweden (Rönnberg, 2006). Firstly the present study was conducted on only one site that might not necessarily have been the most representative for all sites in Sweden. The difference may though also be partly explained by the tap roots, which were excluded from Rönnberg's assessment and more or less ignored by similar studies (Froelich et al. 1977). In the present study, more than 12% of incidence was found in tap roots alone. One tap root of an infected pine was found resin soaked and dead since long. Tap roots of red pine (*Pinus resinosa*) killed by *Heterobasidion* infection was also reported in a

previous study (Shigo 1983). In general, *Heterobasidion* spp. are considered less active in deep soils where the tap roots grow, however, excluding tap roots may lead to some underestimation of the disease situation.

Considering the fairly short period for infection spread (10 years), compared to a whole rotation, *Heterobasidion* spp. did spread rapidly in this study. This may be attributed to the sandy, alkaline soils in southern Sweden that favors the spread of *Heterobasidion* spp. (Froelich et al., 1966) and a lower amount of competitive forest fungi due to the former land use as a pasture (Rönnberg et al. 2006). Sites with the similar conditions in southern Sweden may have a high incidence of *Heterobasidion* root infection as well, and may have benefit from stump treatment at thinnings.

4.4 Location of infections in stump

In this study*, Heterobasidion* spp. occurred more frequently in primary roots that are large in diameter at the root collar and adjacent to the stem. The result is supported by a study on white fir (Abies concolor (Gord. & Glend.) Lindl), suggesting that the colonization of *Heterobasidion annosum* was greater in large-sized roots and close to the root collar or stump surface. This might be caused by water flows towards the root collars and a greater air movement near the stem (Garbelotto 1997), which help direct the spread of pathogen. The infection was less frequent in smaller roots in the present study, which though seems to contradict Rishbeth (1951) who suggested that smaller roots are generally infected first and spread rapidly due to less defence by the infected trees. However, Rishbeth's conclusion was made on Douglas fir (Latin) with less resin exudation than pine species in general (Rishbeth, 1951a). The limited space of smaller pine roots could be easily soaked with resin and thus stop the invasion of fungus (Garbelotto 1997). As observed in this study, smaller roots of Scots pine were more often resin soaked and free from infection. Therefore it seems reasonable to assume that compared with the large primary roots, smaller secondary and tertiary roots may be of less importance in terms of assessing incidence of infection.

Infections might have been initiated through the stumps from the thinnings in the plots, however it was not possible to determine the points of entry where the infections were transferred into the stump in this study, due to lack of obvious roots contact with stumps (Fig. 13-14). This could be caused by the mechanical extraction that broke the grafted roots, and could to some extent have been investigated by extracting also the stumps to cross check infections by *Heterobasidion* spp..

4.5 Above-ground indicators of infection

Needle retention and visible crown lengths were not accurate indicators due to a lack of correlation with the incidence or severity of disease, therefore cannot be used to estimate the situation of infection on the site, as addressed by former studies (Kurkela 2002, Rönnberg 2006). Visible crown length may help estimate the area of root plate, and assess the possibility of root contacts or grafts underground, but not to determine the actual incidence of *Heterobasidion* spp. and the effect on volume growth. This seems to be in agreement with Froelich's finding (1977) of an even higher incidence of *Heterobasidion* infection in trees with vigorous crowns. The lack of correlation between severity of infection and DBH or tree volumes suggest that the latter two are not accurate indicators of infection at this stage either. Though the two plots were differentiated by presence of fruiting bodies on the central stump, there was no statistical difference in incidence and severity of infection in the study. Therefore it might also be inaccurate to estimate the situation of infection based on the amount of fruiting bodies occurring on stumps or trees on a site.

Other indicators used in former studies to determine the infection severity were either based on visual observations or derived from relatively limited amount of root samples, e.g. dead or resin soaked roots, decay at the stem bottom or incidence of infected lateral roots etc. (Bradford et al. 1978; Froelich 1977; Gonthier et al. 2012; Rönnberg, 2006). Compared to checking the presence of *Heterobasidion* conidiophores over the entire stump under the microscope, these indicators were of some uncertainty. Thus the

incidence and severity presented in this study may be considered more accurate.

4.6 Root morphology and disease models

The morphological study of roots and incidence of *Heterobasidion* spp. may be used to build or calibrate models of the spread and impacts of *Heterobasidion* spp. on Scots pine stands e.g. Rotstand (Pukkala et al. 2005). The incidence of infection modeled by Rotstand is much lower compared to the result in the present study. Regarding growth and mortality, this study shows that the IV% was influenced during the spread of infections in roots, while in Rotstand the growth was set to decrease only after the infection reached the stem base. The distance of fungal mycelia spread within a stump was set as the radius of the root plate in Rotstand, however in reality the disease traveled along the roots to the root collar. Therefore the root length measured in the study may be more accurate in terms of modeling the spread of infection in a stump.

The growth calculation was based on 24 Scots pines, and the amount of healthy trees (3) was smaller than the infected trees (21). Besides, there was some variance within the healthy trees which may disturb the comparison. By enlarging the group of healthy trees with lightly infected trees, this relatively small amount of healthy trees will not influent the regression between the growth and the infection since it was based on a various infection severity (0% - 32.3%). However considering the unknown precision of the comparison caused by the sample size, more studies need to be done to verify the relation between infection and volume increment and the calculation of growth losses in the study should be treated with care.

4.7 Other disturbing issues

Volume and growth differences due to ground competition were not fully considered in the analysis, as crowns and roots were only occasionally overlapping, indicating weak competition. Other factors to cause growth loss are windstorm of 2005 and damages

from rodents or insects. However, no signs of such damages were apparent at tree level.

4.8 Cost justification of treatment

The result shows till the year of study (2011) i.e. the mid-rotation, the costs of treatment could already be paid back by the value of the reduced volume increment measured in the present study, and it has not taken into account any losses due to increased mortality caused by root infections (Yde-Andersen 1989). Moreover, with the infections growing in roots and spreading to neighboring trees, more increment loss is expected, and the benefit from stump treatment can be substantially higher over a full rotation. However, considering the uncertainty in forests and economic situation, it might be speculative to calculate the volume and treatment costs throughout a rotation, so the economic analysis after the year of 2011 was not performed here.

The prices of treatment used in the study were from 1996; however the inflation may be compensated by the added-value from the improved technology and efficiency of treatment in these years.

As the cost justification plays a vital role in the decision of stump treatment and may influence the planning process, further investigations on difference age classes of Scots pine are needed.

5. Conclusions and practical implications

Incidence of *Heterobasidion* spp. and severity of infection are seldom detectable in mid-rotation (35-50 years) Scots pine stands. Consequently, growth loss caused by the infection is probably neglected or related to damaging agents other than *Heterobasidion* spp. Results of the current study suggest that volume losses of Scots pine monocultures due to root infections by *Heterobasidion* spp. started after the first thinning and account for at least 1.93 m^3 in the latest five years. The value of the growth losses was more or less equal to the costs of stump treatment at the time of study; however with the spread of disease and any losses due to increased mortality caused by the infection, the benefit from stump treatment may be realized more throughout the rotation. Therefore we recommended that stump treatment on Scots pine is conducted to maintain the site productivity on high hazard sites i.e. sandy, alkaline soil with first generation of forests in Southern Sweden.

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Annex:

Crown measurement: Marta (left), Rickard (right)

Extraction of trees

Measuring of annual height increment

Cutting a stem into discs with a chainsaw

Discs – marked with the northerly direction

Drying of stem discs

Cleaning of stumps

Transportation out of the forest and back to the campus in Alnarp

Stump lifted by a floor crane and measured

Root discs **Resin soaked root disc**