Resistance of Norway spruce (*Picea abies*) to root and butt rot *(Heterobasidion parviporum)* in peatland and mineral soil

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Tiivistelmä — Referat — Abstract
Root and butt rot is the most harmful fungal disease affecting Norway spruce in southern Finland. In approximately 90 % of cases the causal agent is *Heterobasidion parviporum*. Root and butt rot infections have not been reported in Finnish peatlands. However, the increase in logging operations in peatlands means there is a risk that the fungus will eventually spread to these areas.

The aim of this study was to find out the impact of growing site on the resistance of Norway spruce to *Heterobasidion parviporum* infections. This was investigated by artificially inoculating *H. parviporum* to spruce trees in pristine mire, drained peatland and mineral soil and comparing the defence reactions. Additionally, the effect of genotype on resistance was studied by comparing the responses of spruce clones representing different geographic origins. The roots and stems of the trees to be sampled were wounded and inoculated with wood dowels pre-colonised by *H. parviporum* hyphae. The resulting necrosis around the point of inoculation was observed. It was presumed that increased length of necrosis indicates high susceptibility of the tree to the disease. The relationship between growth rate and host resistance was also studied.

The results indicated that growing site does not have a statistically significant effect on host resistance. The average length of necrosis around the point of inoculation was 35 mm in pristine mire, 37 mm in drained peatland and 40 mm in mineral soil. It was observed that growth rate does not affect resistance, but that the genotype of the tree does have an effect. The most resistant spruce clone was the one with Russian origin. The results suggest that the spruce stands in peatlands are not more resistant to root and butt rot infections than those in mineral soil. These findings should be taken into consideration when logging peatland forests.

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Norway spruce, *Picea abies*, root and butt rot, *Heterobasidion parviporum*, resistance, peatland, mineral soil, drainage

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Tiivistelmä — Referat — Abstract

Kuusentyvilaho on pahin kuusen sienitauti Etelä-Suomessa. Noin 90 % tapauksista on kuusenjuurikäävän (*Heterobasidion parviporum*) aiheuttamia. Juurikääpää ei toistaiseksi ole havaittu soilla, mutta hakkuumäärien lisääntyminen suometsissä lisää riskiä, että sieni leviää myös näille alueille.

Tämän tutkimuksen tarkoituksena oli selvittää, vaikuttaako kasvupaikka kuusen taudinkestävyyteen. Sitä tutkittiin tartuttamalla luonnontilaisella suolla, ojitetulla turvekankaalla ja kivennäismaalla kasvaviin kuusiin juurikääpää ja tutkimalla puiden puolustusreaktioita. Lisäksi tutkittiin puun perimän vaikutusta taudinkestävyyteen vertailemalla eri maantieteellistä alkuperää edustavien kloonikuusten reaktioita. Tutkittavien puiden runkoja ja juuria haavoitettiin, ja haavoihin asetettiin kuusenjuurikäävän rihmaston peittämä puutappi. Tartutuskohdan ympärille muodostui kuollut soluvyöhyke, joka on sitä suurempi mitä alttiimpi puu on taudille. Myös puiden kasvunopeuden ja taudinkestävyyden yhteyttä selvitettiin.

Tutkimuksen tulokset osoittivat, ettei kasvupaikka vaikuta tilastollisesti merkitsevästi taudinkestävyyteen. Kuolleen soluvyöhykkeen pituus tartutuskohtien ympärillä oli luonnontilaisella suolla keskimäärin 35 mm, ojitetulla turvekankaalla 37 mm ja kivennäismaalla 40 mm. Myöskään puiden kasvunopeuden ei todettu vaikuttavan taudinkestävyyteen. Puun perimällä sen sijaan on tilastollisestikin merkitsevä vaikutus. Paras taudinkestävyys havaittiin venäläistä alkuperää olevalla kuusikloonilla. Tutkimuksen perusteella turvemaiden kuusikot eivät ole kestävämpiä tyvilaholle kuin kivennäismaiden kuusikot. Tämä tulisi ottaa huomioon suopuuston hakkuissa.

Avainsanat — Nyckelord — Keywords
kuusi, *Picea abies*, kuusenjuurikääpä, *Heterobasidion parviporum*, taudinkestävyys, suo, kivennäismaa, ojitus

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1 INTRODUCTION

1.1 Background

The resistance of forest trees to fungal diseases is an important factor when it comes to growing timber for industrial use. However, knowledge of forest tree resistance as a disease control method is still undervalued (Delatour *et al.* 1998). In southern Finland, 10 % of the forest land area is affected by fungal damage (Tomppo *et al.* 2011). If resistance factors are taken into account in silvicultural operations and included in tree breeding programmes, the damage caused by pathogenic fungi can be better managed.

Root and butt rot fungus *Heterobasidion annosum* (Fr.) Bref. *sensu lato* is one of the most destructive forest pathogens in the northern hemisphere (Asiegbu *et al.* 2005). It causes decay in the most valuable part of conifers – the heartwood. Norway spruce stands in southern Finland are particularly susceptible to the disease. Peatlands, on the other hand, account for one third of the forestry land in Finland and over half of which has been drained, mainly between the 1960's and 1980's. The majority of forest stands in drained peatlands will be harvested during the next decades, which means they are of particular economic importance. Peatlands have traditionally been considered free of root and butt rot risk, as little or no *H. annosum s.l.* infections has been recorded at these sites (Päivänen 2007).

The absence of this fungus in peatlands is probably due to the characteristics of peat or peatland trees. *H. annosum s.l.* is an effective coloniser of freshly cut stumps and spreads via root connections to healthy trees. Therefore the fact that the peatland forest stands are still relatively young and have only been harvested a few times may be one of the reasons for the absence of the fungus. This study aims to investigate the influence of growing site, growth rate and genotype on the resistance response of mature Norway spruce trees to *H. parviporum* infection. The field experiments were carried out on naturally-born, mature Norway spruce trees in pristine mire, drained peatland and mineral soil, which constitute pioneering work in the field of Heterobasidion research. A clonal spruce stand was also included in the study.

1.2 Resistance of coniferous trees to fungal pathogens

1.2.1 Disease virulence and resistance

There are three components needed for the development of a disease: host, pathogen and favourable environment (Agrios 2005). This so called disease triangle illustrates that if any of these components is absent, there is no disease. Koch's postulates are commonly used as criteria for defining the pathogen as the causal agent of a disease:

> *1. The suspected causal agent must be present in every diseased host organism examined;*
>
> *2. The suspected causal agent must be isolated from the diseased host organism and grown in pure culture;*
>
> *3. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host, the host must reproduce the specific disease;*
>
> *4. The same causal agent must be recovered again from the experimentally inoculated and infected host, i.e. the recovered agent must have the same characteristics as the organism in step 2* (Agrios 2005).

To enable the disease development, the environmental conditions have to be either favourable for pathogen growth or harmful for host resistance (Agrios 2005).

Virulence is the degree of a pathogen's ability to cause a disease, whereas avirulence is its inability to infect a certain plant (Agrios 2005). The success of the infection depends on the response of the plant host. The outcome of this interaction is expressed either as resistance or susceptibility of a host. According to Merrill (1992), resistance is an infinite term that together with reciprocal term susceptibility forms a continuum that describes plant response to pest or pathogen. As a relative term, it must be measured by comparing the response of two or more genetic strains of the suscept to the same strain of the pathogen.

Delatour *et al.* (1998) defined resistance as "the restriction of disease development by host factors". Agrios (2005) places types of resistance into three categories. The first being non-host resistance, meaning that a plant is resistant because it does not

belong to the host range of the pathogen. The second being plants that possess genes for resistance (i.e. R genes) against the avirulence genes of the pathogen. The third, miscellaneous category contains plants that are for various reasons tolerant or able to escape from the pathogen.

1.2.2 Abiotic factors affecting resistance

Abiotic factors such as light, temperature, soil and weather affect both host trees and pathogens. The site conditions of a certain biotope may affect a pathogen's life cycle, preventing it from causing a disease, even though it may prove possible to develop that disease in experimental conditions. This is also called ecological resistance (Delatour *et al.* 1998).

It is commonly known that temperature affects both pathogen development and the expression of host resistance (Pariaud *et al.* 2009). The optimal temperature for experimental development of a fungal disease in conifers is about 20°C (Delatour *et al*. 1998). Fertile sites with high nitrogen level, such as former agricultural lands, and clay and sandy soils offer a more favourable environment for fungal infection (Redfern 1984, Hüttermann & Woodward 1998, Mattila and Nuutinen 2007). Several studies have shown that peat soil improves host resistance against fungal pathogens (e.g. Redfern 1984, Lindberg and Johansson 1991).

Some studies have reported that abiotic stress decreases host resistance. According to Lindberg and Johansson (1992) and Swedjemark (1995), the risk of infection is higher in seedlings growing in dry soil with a poorly developed root system than in seedlings growing in moist soil with well-developed fine roots. In addition, the amount of phenolic compounds, that inhibit the growth of pathogenic fungi in inner bark, is in some cases found to decrease in spruce trees stressed by drought or nutrient deficiency (Woodward 1992).

1.2.3 Biotic factors characteristic of Norway spruce

Picea abies (P.) Karsten is a shade-tolerant species which spreads to all growing sites in its distribution area – with the exception of those with very poor soil – during the final stage of ecological succession (Blomqvist 1891, Sarvas 1964, Järvinen 1996). It grows best on fertile, well-drained moraine hillsides (Valkonen 2005). Norway spruce is a relatively long-lived species, as its biological age can reach 250–400 years (Sarvas 1964, Valkonen 2005). Norway spruce trees develop quite slowly in the beginning, but at the age of 25–50 it starts to grow at high rate (Valkonen 2005). Older trees will continue to grow, though more slowly – unlike Scots pine, which at a certain stage stop growing completely (Kärkkäinen 2007).

It has generally been assumed that vigorously growing trees are more resistant to fungal and insect attacks. In contrast, it has also been suggested that increased growth could result in decreased resistance, as growth and resistance are competing for the same carbon resources within the tree (Kytö et al. 1996, Baier *et al.* 2002). Resin duct area in Norway spruce is negatively correlated to relative tree growth, which is considered as physical trade-off between allocation to defence and growth (Baier *et al.* 2002). Observations from naturally infected sites indicate that Heterobasidion root rot infections are more frequent in trees with large stem diameter (Piri *et al.* 1990).

As the Norway spruce seedlings may grow in the understory for a long time, the species has developed bad-tasting chemicals to prevent itself from being eaten by mammals (Väre and Kiuru 2006). Norway spruce is in many ways less tolerant to damage than Scots pine: it has thin bark and therefore is not as resistant to fire as (Sarvas 1964). The spruce also produces less resin than the pine, which makes it more vulnerable to insect attack, especially from Coleoptera (Väre and Kiuru 2006).

Norway spruce has superficial roots (Sarvas 1964, Valkonen 2005) which makes it very vulnerable to windthrow (Kärkkäinen 2007). Kalliokoski (2011) found that radial spread of Norway spruce roots increases as site fertility decreases. In his studies the maximum depth of Norway spruce roots is 1.62 meters, whereas the roots

of Scots pine and silver birch can reach over 2.6 meters in depth (Kalliokoski 2011). The superficial roots of the spruce may be one of the reasons for development of root connections between different tree individuals; therefore, growing Norway spruce mixed stand is recommended in some cases to avoid the spread of fungal infections (Piri *et al.* 1990, Kärkkäinen 2007). Superficial roots are also vulnerable to logging wounds when harvesters drive in the stand.

1.2.4 Coevolution of trees and pathogenic fungi

The close interaction between trees and fungi in nature indicates that they have been evolving together. Coevolution is a stepwise process, where the host and the pathogen continuously create resistance and virulence, respectively, maintaining a dynamic equilibrium over time (Agrios 2005). Forest management has a major impact on trees and their associated organisms. A good example is the root and butt rot fungus *H. annosum s.l.*, which has benefitted from forestry as it is relatively rare in natural forests, but very common in managed boreal forests (Swedjemark 1995). Understanding of pathogen population genetics and evolution is important when developing resistance-breeding strategies that aim to attain durable disease resistance in plants (McDonald & Linde 2002). This approach is equally valid for forest tree management.

Pathogens cause mortality and reduce the growth and fertility of individual plants. Therefore, they are responsible for numeric changes in host populations and create selection pressure on those host characteristics involved in tree's defence against pathogens (Gilbert 2002). As well as being destructive agents, pathogens help maintaining the plant diversity, facilitating successional processes and enhancing the structure of host populations (Gilbert 2002). The diseases that affect wild plants can be classified into eight categories: 1) seed decay, 2) seedling diseases, 3) foliage diseases, 4) systemic infection, 5) parasitic plants, 6) cankers, wilts and diebacks, 7) root and butt rots and 8) floral diseases (Gilbert 2002).

The gene-for-gene coevolution concept is the most used model in research on the evolution of plant resistance to parasites (Thompson & Burdon 1992). The basis of

the gene-for-gene concept lies in Flor's (1946) work with a leaf pathogen *Melampsora lini*. He discovered that each resistance gene in a host has a corresponding avirulence factor in its pathogen, and each virulence gene in a pathogen has a corresponding susceptibility gene in the host. Resistance is a dominant gene, whereas virulence is a recessive gene, which means that in cases where the host lacks the resistance gene and/or the pathogen lacks the avirulence gene, the disease will occur (Thompson & Burdon 1992).

The authors indicate that the gene-for-gene concept is not always valid: most examples of the concept are interactions with biotrophic pathogens, and it does not apply to most necrotrophic pathogens. Additionally, in natural conditions where the host is regularly attacked by several parasite species, evolution of resistance is likely to be polygenic. In 1963, Van der Plank was first to introduce the idea that there are two types of resistance: 1) vertical resistance, which is strong, specific and controlled by a few major resistance genes, and 2) horizontal resistance, which is weak, general and determined by minor resistance genes (Van der Plank 1984, Agrios 2005).

According to McDonald and Linde (2002), pathogens that are most likely to break down the major resistance genes of the host have mixed reproduction system, high potential for gene flow, large effective population sizes and high mutation rates. Similarly, pathogens that are able to reproduce only asexually and have low mutation rates are less risky for the host. In forest ecosystems, the generation time of trees is much longer than that of most pathogens, which could result in a situation where the pathogens may pass through various generations under high selection pressure for increased virulence before resistant host trees reach maturity for reproduction (Gilbert 2002). In the coevolution arms race of host and pathogen, trees may also suffer from low genetic variability and rates of out-crossing rates (Gilbert 2002). In addition, genetic drift and gene flow play an important role in coevolution (Thompson & Burdon 1992).

1.3 Defence at cellular level

1.3.1 Mechanisms of defence

Plants, including trees, have two kinds of defence mechanisms: constitutive and induced. Constitutive defence mechanisms are present in all cells and include pre-existing morphological and/or chemical barriers that prevent the penetration and colonisation of fungi. Induced defence, or active defence, is only possible in living cells, and involves morphological and chemical barriers that are activated by the presence of a pathogen (Merrill 1992).

In forest trees, the bark is the most important defensive barrier protecting the wood material of a tree. Bark consists of periderm, cortex, phloem and cambial tissues (Pearce 1996). Outer bark layers contain suberin, which makes it highly durable and hydrophobic. Antimicrobial compounds, such as terpenes and polyphenols, also contribute to its defensive properties (Pearce 1996). According to Swedjemark (1995), the bark phenolics in Norway spruce have an inhibitory effect on spore germination and hyphal growth. In gymnosperms (a group to which most coniferous tree species belong, including Norway spruce) wounding or infection stimulates resin production (Pearce 1996).

Healthy sapwood includes both living and non-living cells. In sapwood, living parenchyma cells are in charge of active response to infection (Pearce 1996). The cells produce zones that are rich in fungistatic and fungitoxic compounds (Shain 1971). In addition, high water potential, which causes a lack of oxygen, restricts the growth of fungi in sapwood (Swedjemark 1995). There are no active defence mechanisms in heartwood, as there are no living cells. Therefore, necrotrophic pathogens may colonise a considerable portion of the inner tissues (heartwood) of the tree, even if the tree is still alive (Swedjemark 1995). Compared to Scots pine, the heartwood of Norway spruce contains lower quantities of oleoresin and other inhibitory substance, which makes it more susceptible to heart rot (Shain 1971).

Constitutively produced resin is the first defensive barrier against parasitic invasion, followed by secondary resin and monoterpene production induced by pathogen attack (Schmidt *et al.* 2005, Woodward *et al.* 2007). Hypersensitive reaction (HR) is another form of plant defence response to fungal attack, which often leads to the death of plant cells (Agrios 2005). HR is an effective way to restrict the growth of biotrophic pathogens; however, it cannot restrict the invasive growth of necrotrophic fungi, as they are capable of utilising dead host cells and even kill living plant cells (Rayner and Boddy 1988).

It is likely that active response to pathogen attack is partially controlled by genes (Delatour *et al.* 1998). Pathogens produce so-called *elicitor molecules*, which are recognised by specific receptors in the plant. This receptor–elicitor recognition activates the plant's defence response, which often leads to death of the infected plant cell and inhibition of the pathogen (McDonald & Linde 2002). Mutation from avirulence to virulence leads to a change in the elicitor, which complicates the receptor's recognition. Frequent mutations in the virulence gene of the pathogen will ultimately result in reduced efficacy of the host resistance gene, leading to a breakdown of resistance (McDonald & Linde 2002).

1.3.2 Interaction between host and pathogen

During the initial stages of infection, the host and the pathogen compete with each other: the host produces defensive chemicals and cell wall barriers, and the fungus produces enzymes that oxidise and degrade lignin and cellulose (Asiegbu *et al.* 1993, 1998, Swedjemark 1995). After successful infection, the host tries to restrict the growth of the pathogen. Pearce (1996) presents four principal models for the process of wood decay restriction: the heart rot concept, compartmentalisation, the reaction zone and environmental restriction of fungal colonisation. The idea of heartrot concept is that decay is a saprotrophic process, occurring only in non-living heartwood. Deep wounds and dead organs allow fungi to enter the heartwood. The fungus normally produces a fruiting body to interact with external environment (Pearce 1996).

Compartmentalisation of decay (CODIT) is a defence mechanism whereby a tree isolates injured tissues in order to restrict the spread of a pathogen (Shigo 1984). According to the CODIT model, decay is bounded by mechanical barriers, or "walls": wall "1" restricts the axial growth of infection across the xylem, wall "2" restricts the radial growth inwards from the wound, wall "3" restricts lateral spread across the surface of the annual ring and wall "4" eventually closes the wound by forming wound periderm (Shigo 1984). Wall "4" is the most durable and most important of the walls; it isolates the youngest wood and cambial tissues, thus guaranteeing the future survival and growth of the tree (Pearce 1996).

Reaction zone is an active host response to injury or infection. It contains dead parenchyma and is produced in sapwood before the spread of pathogen (Shain 1971). The reaction zone moves ahead of a continuously advancing decay front, first turning sapwood into transition zone, then reaction zone, which eventually becomes part of the spreading lesion (Pearce 1996). Oxidative enzymes produced by the host mediate the colour change observed in reaction zones (Shain 1971). Reaction zone contains more extractives, particularly phenols, than neighbouring tissues (Shain 1971), as wells as phytoalexin-like anti-fungal compounds, which retard the advance of the colonising fungus (Pearce 1996). Reaction zone and incipiently decayed wood are more resistant to decay than sound heartwood and sapwood (Shain 1971). The results of Shain's (1971) studies on Heterobasidion also suggest that the susceptibility of spruce to extensive central-stem rot is due to the lack of inhibitory substances in its heartwood, whereas accumulation of inhibitory substances in a reaction zone helps limit invasive growth in sapwood.

Boddy & Rayner (1983) criticised the concepts of active host defence models. They argue that microenvironmental restrictions, particular the high moisture content and low oxygen levels found in sapwood, may be the principal factors limiting the growth of decay organisms. Pearce (1996) reported that none of the above mentioned models is alone sufficient to fully describe the interaction between living sapwood and wood-inhabiting microorganisms, but the models together give an idea of the spectrum of interaction.

1.3.3 Necrosis as a resistance response

Resistance of individual trees can be tested using direct inoculation (e.g. Swedjemark 1995, Swedjemark *et al.* 1997, Delatour *et al.* 1998), i.e. artificially transferring the disease agent into a host (Agrios 2005). One effective way to inoculate a tree is using a wood dowel that has been pre-colonised with the pathogen mycelium *in vitro* (Delatour *et al.* 1998). Results can be assessed by measuring necrosis in the sapwood and inner bark (Swedjemark *et al.* 1997, Delatour *et al*. 1998). Most inoculation experiments have been conducted on stem wounds, as methods for root inoculation are not well developed at this time (Delatour *et al*. 1998). Information on root inoculation is essential, as the initial stage of recognition and resistance phenomenon takes place at the point where host root and fungus make contact (Asiegbu *et al.* 1998).

Necrosis is often associated with the death of host tissue (Tainter and Baker 1996) and could be induced by pathogen attack (Pariaud *et al.* 2009). If living phellogen or phelloderm cells are wounded, necrotic tissue will start to form within hours. Necrotization as a process involves brown-staining, thickening of cell walls and accumulation of phenolic and resinous compounds in cell vacuoles (Lindberg & Johansson 1991, Johansson *et al.* 2004). After spreading through the phloem, the rate of necrotization gradually decreases.

Lindberg and Johansson (1991) suggest that the eventual size of the necrotic area depends on the eliciting power of the wounding injury and the capacity of living parenchyma cells to synthesise wound periderm. Necrotic area is obviously an obstacle for the growth of Heterobasidion hyphae, which normally grow intracellularly. Thickened phenol-containing cell walls of periderm and phloem, tissues with decreased starch and protein content, activated hydrolysing enzymes, and accumulated resinous and phenolic compounds form an effective, though not impenetrable, resistance barrier (Lindberg and Johansson 1991).

According to Swedjemark *et al.* (1997), there is a positive correlation between fungal growth and the length of necrosis. Lindberg & Johansson (1991) suggest that length of necrosis in bark is an indicator of a pathogen's ability to penetrate living bark

tissue. This leads to the conclusion that tree individuals with limited necrotic reactions possess a high level of resistance in early stages of fungal infection (Swedjemark 1995, Swedjemark and Stenlid 1997). Thus, individuals with extensive necrotic lesions are more susceptible than those with reduced necrotic reaction (Woodward *et al.* 2007).

1.4 Characteristics of *Heterobasidion annosum s.l.*

1.4.1 General information

Heterobasidion annosum sensu lato is a white-rot fungus that causes root and butt rot in coniferous trees in the northern hemisphere. The term *white rot* refers to the bleached colour of the delignified wood. Basidiomycetes consume first lignin, then cellulose and hemicellulose, and, over time, are capable of completely breaking down the structure of wood (Rayner and Boddy 1988). Butt rot originates in the roots or root collar of a tree, then spreads upward into the heartwood of the trunk (Rayner and Boddy 1988). *H. annosum s.l.* is one of the most destructive forest pathogens in Europe (Asiegbu *et al.* 2005) and the primary cause of decay in Norway spruce in southern Finland (Kallio and Norokorpi 1972, Hallaksela 1984, Piri *et al.* 1990, Kaarna-Vuorinen 2000).

Root and butt rot causes economic loss in several ways: it reduces the quality of timber; impedes tree-growth; increases the risk of windthrow, death and infection in future generations; and shortens rotations (Bendz-Hellgren *et al.* 1998). In southern Finland, approximately 5 % of stumpage earnings are lost due to root and butt rot during a single rotation of Norway spruce (Korhonen *et al.* 2000). In total, direct and indirect loss caused by *Heterobasidion annosum s.l.* is estimated at 50 million euros annually (Kansallinen metsäohjelma... 2011). According to the estimations of Korhonen *et al.* (2010), butt rots in general cause 30–35 million euro's worth of damage to Norway spruce every year, of which 75 % is due to *Heterobasidion parviporum*.

H. annosum s.l. has a wide range of host tree species, but it causes the most damage in trees of the genera *Pinus, Picea* and *Abies* (Niemelä and Korhonen 1998). *H. annosum s.l.* has been classified in three European intersterility groups. This means that the species is differentiating into three new species which no longer breed with each other, yet they are still morphologically similar enough that it is impossible to distinguish them from each other without special techniques (Mitchelson and Korhonen 1998). The intersterility groups are as follows (Niemelä & Korhonen 1998):

- P group mainly attacks *Pinus* trees, but also *Picea* and *Juniperus. Heterobasidion annosum* (Fr.) Bref. and *Heterobasidion annosum sensu stricto* are synonymous, indicating that only the P group of *H. annosum* is in question;
- S group mostly inhabits *Picea abies.* S group is also known as *Heterobasidion parviporum* Niemelä & Korhonen;
- F group mainly infects *Abies* species and is now called *Heterobasidion abietinum* Niemelä & Korhonen.

This study is primarily focused on *H. parviporum* for two reasons: 1) in southern and western Finland, the fungus is nine times more common than *H. annosum* (Piri *et al*. 1990) and 2) *Picea abies* as a host is more susceptible than other economically important tree species in Finland (Piri 1996).

H. parviporum has a perennial, resupinate, semi-resupinate or pileate basidiocarp, which has a creamy-white pore surface and a cigar-brown upper side (Niemelä 2005). The main anatomical differences compared to *H. annosum s.s.* are the smaller pores (4–6 pores in a millimetre) and a soft, felt-like layer in the upper side (Niemelä 2005). The distribution of *H. parviporum* follows the natural distribution of *Picea abies*: the northernmost record being 68°N in Finland; the southernmost, being 41°N in northern Greece. In the west, *H. parviporum* has even been found outside the natural distribution of *P. abies,* on spruce plantations. In the east, the fungus has even been discovered in the Ural Mountains (Korhonen *et al*. 1998a).

1.4.2 Infection biology

In most cases *H. annosum s.l.* is not able to infect intact, healthy trees due to active and passive host resistance; however, the fungus is a successful coloniser of fresh wood surface (Redfern and Stenlid 1998). Freshly cut stumps at logged forest sites are the main entrance point through which airborne basidiospores of *H. annosum s.l.* infect existing wood material in new areas (Redfern and Stenlid 1998). The fungus has the capacity to infect living cells, which gives it an edge over other competing fungi (Rayner and Boddy 1988). There is a limited susceptibility period in which infection is possible: stumps of Norway spruce remain susceptible for less than a month (Redfern and Stenlid 1998), after which the competition from other decaying fungi, such as *Phlebiopsis gigantea*, is too strong for *H. annosum s.l.* to survive. Spore infection is also possible through stem or root wounds caused by logging operations; for example, by harvesting machines or falling trees (Stenlid & Redfern 1998).

After colonising stumps, *H. annosum s.l.* spreads into the root system. The mycelium of the fungus can spread from tree to tree via root contacts. However, each clone of the fungus infects only 1.8 trees on average (Piri *et al.* 1990). The fungus can attack the host in two ways, either growing epiphytically under the outer bark scales prior to actual penetration, or by invading the root bark directly (Stenlid & Redfern 1998). *H. annosum s.l.* cannot grow on soil, but it is capable of surviving in roots for decades, and can infect neighbouring trees (Stenlid & Redfern 1998). Piri (1996) found a living mycelium of *Heterobasidion parviporum* in a Norway spruce stump cut 46 years ago, which suggests that the fungus may be able to live within a stump for over 50 years. The stumps of large spruce trees that are extensively colonised by *H. annosum s.l.* before felling are particularly important infection sources in the stand (Piri 1996).

H. annosum s.l. is also able to infect trees of the next generation (Stenlid and Redfern 1998). According to Piri and Korhonen (2001), 53 % of seedlings were infected via the vegetative spread of the fungus through root contact with overstory trees. This means that all other infections in the next regeneration must have started from spore infection of injured roots (Piri and Korhonen 2001). Norway spruce seedlings that

are planted on infested sites become damaged by Heterobasidion root and butt rot at between 10 and 20 years of age, and infection rates tend to increase steadily until the first thinning (Piri 2003). However, disease frequency in future generations will gradually decrease due to degeneration of old Heterobasidion genets (Piri and Korhonen 2007).

Basidiocarps form on stumps, logs and dead trees, as well as living, infected trees; fruiting may also occur in litter accumulations between buttress roots (Redfern & Stenlid 1998). Temperature and humidity are the main factors influencing spore production in basidiospores (Redfern & Stenlid 1998). In boreal conditions, *H. annosum s.l.* mainly produces basidiospores during the summertime, as spores are inactive at temperatures below zero (Kallio 1970). In moist conditions, the fungus also produces conidiospores (Korhonen and Stenlid 1998). There are two major differences between basidiospores and conidiospores: 1) asexual conidiospores are less diverse in genotype than sexual basidiospores that are formed through meiosis and 2) the production of basidiospores in basidiocarp is more effective than that of conidiospores in conidiophores on top of mycelia (Korhonen and Stenlid 1998). Compared to other polypores, *H. annosum s.l.* is unusual in that it forms conidiophores (Korhonen and Stenlid 1998); this makes the hyphae of *H. annosum s.l.* easy to recognise.

1.4.3 Pathogenicity factors

Merrill (1992) defines pathogenicity as the ability of an organism to cause disease in at least some members of the host population. Pathogenicity is often intimately associated with parasitism; however, there a significant differences. A disease tends to cause much greater damage to the host: apart from removal of nutrients, the pathogen is also able to secrete substances that interfere with the essential functions of the host (Agrios 2005). *H. annosum s.l.* possesses a wide range of extracellular enzymes that degrade and detoxify the host's defence mechanisms. It also secretes several groups of enzymes that degrade polysaccharides, lignin and phenols (Asiegbu *et al*. 1998).

The ability to change from necrotrophy to saprotrophy is probably *H. annosum s.l.*'s secret of success. Necrotrophic fungal species produce toxins in order to kill plant cells and convert them into resources for growth (Pariaud *et al.* 2009); they are also capable of inducing a hypersensitivity reaction in a host, as well as tolerating the response (Asiegbu *et al.* 1998). Lindberg and Johansson (1991) observed the necrotrophic behaviour of *H. annosum* when the hyphae reached the front of the wound-induced necrotic tissue in their study. The necrotic border expanded continuously in front of the infection, making the eliciting effect of the pathogen evident. A necrotroph's induction of HR seems to be a prerequisite for subsequent saprotrophic colonisation of *H. annosum s.l.* (Asiegbu *et al.* 1998). It has been proven that *H. annosum s.s.* is more aggressive than *H. parviporum*, because it secretes more pectinase (Johansson 1988) and degrades wood more efficiently (Daniel *et al.* 1998).

The pathogenicity of a pathogen population may vary considerably over time as a result of the strong influence of environmental conditions (McDonald and Linde 2002). McDonald and Linde (2002) have also postulated that, there may be so-called *pathogenicity allele*, which encode enzymes that inactivate preformed chemical inhibitors, allowing a pathogen to counteract a plant's resistance genes. Pariaud *et al.* (2009) suggest that the fungus maintains a trade-off between toxin production and fitness, just as its host maintains a trade-off between growth and resistance.

1.4.4 Control methods

There are three approaches to preventing the germination and growth of *H. annosum s.l.*: silvicultural control, chemical control and biological control. Intensive forest management has created a significant amount of fresh wood surface in the forests of northern hemisphere, enabling *H. annosum s.l.* to spread at a remarkable rate. Silvicultural control attempts to exploit biological knowledge of the fungus in order to make forest-management decisions that will minimise the impact of *H. annosum s.l.* (Korhonen *et al.* 1998b); for example: choosing resistant tree species or mixed stand when possible (Piri *et al.* 1990); keeping the number of thinnings during rotation of a stand to a minimum; avoiding logging scars; and changing the tree

species of infected sites (Korhonen *et al*. 1998b). Probably the most important control method is the avoidance of logging during the summertime, when spores are disseminating. If logging is carried out when the temperature is above 0°C, stumps should be treated with protectant (Korhonen et al. 1998b). Over the past years, stump removal in Finland has increased, which has helped reduce the risk of *H. annosum s.l.* infection on clear-felled sites (Vasaitis *et al.* 2008).

Biological control and prevention of *H. annosum s.l.* infection is mainly carried out via *Phlebiopsis gigantea* stump treatment. *P. gigantea* is a common white-rot fungus found in boreal and temperate forests, and a primary coloniser of fresh wood (Holdenrieder & Greig 1998). Stumps treated with *P. gigantea* are rapidly colonised by the fungus, so *H. annosum s.l.* is no longer able to grow. The principle advantage of biological control is that it is biodegradable, and it brings with it none of the ecological hazards related to the use of artificial fungicides (Holdenrieder & Greig 1998). Biological stump treatment is especially effective at sites where *H. annosum s.l.* is not yet present.

Stump treatment may also be carried out chemically, and today urea and borates are commonly used as control chemicals (Pratt *et al.* 1998). According to Risbeth´s studies in 1959, borates provided an immediate and toxic barrier to colonisation by basidiomycetes (Pratt *et al.* 1998). Urea, on the other hand, has proved not to be toxic to all fungi. The effect of urea is based on the hydrolysing ability of the stump; it causes ammonia to form, raising pH to a level at which *H. annosum s.l.* is unable to survive (Johansson *et al.* 2002). The use of chemical treatment is restricted to sites where the disease is still absent. Both biological and chemical stump treatments are most usually applied together with mechanical harvesting, making it relatively cost-efficient. In 2008, stump treatment against root and butt rot was performed on 62 500 hectares of logging area across Finland and the overall cost of *H. annosum s.l.* control was 5.4 million euros (Juntunen and Herrala-Ylinen 2009).

1.5 Characteristics of Finnish peatlands

1.5.1 Definition and classification

Peatland can be defined in terms of botanical, ecological, geological or silvicultural concepts (Päivänen 2007). In this study, peatland is defined in silvicultural terms, as this definition is also used by the National Forest Inventories (NFI). According to the NFI's instructions, a growing site is defined as a peatland if the organic layer covering the mineral soil is formed by peat, or more than 75 % of the vegetation is peat-producing. Peat itself is an accumulation of dead organic matter, mainly the remains of *Sphagnum* and *Carex* species (Laine *et al*. 2000). In a broad sense, the term peatland includes both pristine and drained peatlands. Undrained, pristine peatlands are called mires (Päivänen 2007). Drained peatlands are considered peatlands as long as the peat layer still exists. Once the peat layer has disappeared due to the drying effect of drainage, the growing site is classified as mineral soil (Hökkä *et al.* 2002). According to the tenth NFI, the entire land area of Finland is 30.41 million hectares, of which the total forestry land area is 26.08 million hectares. Of that forestry land, 34 % (8.9 mill. ha) is peatland, over half of which (4.76 mill. ha) has been drained (Peltola and Ihalainen 2009).

The classification of mire site types has been an issue since the beginning of the twentieth century (Päivänen 2007). The basis for the classification, as well as that of mineral soil forest types, has been ground vegetation: the idea is that similar mire plant communities can be sorted into a series that reflects the variation of different site-type factors, such as fertility and moisture (Päivänen 2007). The main gradient is seen within the fertility of the site. If the site gets its nutrients from rain water only, the site is *ombrotrophic;* whereas, if, in addition to rain water, it gets its nutrients from the surrounding environment via surface and ground water, it is *minerotrophic* (Päivänen 2007). However, it has been argued that vegetation is not an indicator of the growth potential of a site. According to Laine and Vasander (2008), mire site types should be classified into two main groups: 1) genuine forested mires and 2) treeless and composite mires. This division, together with nutrition and moisture gradients, forms a coordinate with 31 different mire types existing in Finland.

There are three English terms that cover the Finnish mire types (Laine *et al.* 2004):

A *bog* is an ombrotrophic, normally pine-dominated mire that can be either genuine forested, composite or treeless;

A *fen* is a minerotrophic treeless or composite mire;

A *swamp* is normally a minerotrophic, spruce-dominated genuine forested mire.

The aim of drainage is to change the output of a site from peat-producing mire ecosystem into timber-producing forest ecosystem (Päivänen 2007). Draining the excess water out of the surface peat increases the air-filled porosity and leads to changes in vegetation composition and structure (Laiho 2008). Over the course of several decades, the mire will then gradually become drained peatland forest, as mire species are outcompeted by forest species (Vasander and Laine 2008). Drained peatland forests can be classified into seven categories that are comparable to those of forests growing in mineral soil (Laine and Vasander 2008). However, in the latest classification, the *Vaccinium myrtillus* and *V. vitis-idaea* site types have both been subdivided into two: site type I, which contains genuine forested mire types; and site type II, which contains treeless and composite mires (Vasander and Laine 2008). The majority of drainage operations have been carried out on genuine forested mire types or minerotrophic composite mire types that already have forest cover, even before drainage; this has made them a promising prospect for silviculture (Päivänen 2007).

1.5.2 Fungal diseases in peatland forests

Trees growing on pristine mires often suffer from a scarcity of nutrients and an excess of water which limits their growth and development (Päivänen 2007). Several growing disorders have been reported that have turned out to be caused by sites which are lacking in nutrients. In addition, trees that are suffering from abiotic stress are often susceptible to biotic pathogens (Tainter and Baker 1996). According to the eighth NFI, fungi are the most common causal agents of peatland damage (Hökkä *et al.* 2002). However, the fungal species causing damage has not always been identified by the people compiling these inventories.

As drained peatlands are often colder and more humid sites than the surrounding environment, pine stands are susceptible to Scleroderris canker caused by the fungus *Gremmeniella abietina* (Päivänen 2007). Chrysomyxa rust in spruce, caused by *Chrysomyxa ledi*, is a fungal disease that commonly occurs in peatland spruces, as its intermediary host, labrador tea (*Ledum palustre*), is a very common peatland plant. Damage caused by root and butt rot fungus (*Heterobasidion annosum s.l.*) has not been recorded in peatlands (Päivänen 2007).

1.5.3 *H. parviporum* in peatlands

In Finland, the areas where risk of *H. parviporum* infection is highest are found on the south-west coast, where the maritime climate and long growing periods favour fungal growth (Mattila & Nuutinen 2007). The role that soil plays in disease severity is unclear. The most severe damage to trees has been recorded on sites with high pH, low organic matter content and high sand content. For this reason, it has been postulated that poorly drained peatland offers the lowest risk of disease (Stenlid & Redfern 1998).

According to the probability test of root and butt rot in spruce stands, the damage risk for stands in peatland that has a thin peat layer is about half that of those growing in mineral soil; at sites where the peat layer is more than 30 cm thick, the risk is less than one-third that of mineral soil sites (Mattila & Nuutinen 2007). If quick conclusions were drawn, this would indicate that the thicker the peat layer, the smaller the risk of Heterobasidion infection.

What is, then, the reason that *H. annosum s.l.* has not managed to infect peatland forests? One reason could be differences in nutrient concentration and nutrient availability between peat and mineral soil. Compared to mineral soil, peat contains more nitrogen and less potassium; on average, it is also more acidic (Päivänen 2007). According to Redfern (1984), sandy soils with high pH and low organic matter content have the greatest risk of disease development, which also supports the hypothesis that *H. annosum s.l.* avoids peatlands. Kasanen (2009) suggests that peat,

which is a suppressive growing plate with high microbe activity, inhibits the growth of *H. annosum s.l.* Bacteria and archaea take charge of microbial activities in anoxic conditions, whereas fungi are the main decomposers in oxic conditions (Laiho 2008).

An excess of water may also contribute to the absence of *H. annosum s.l.*, as lack of oxygen limits fungal respiration (Redfern 1984, Lindberg and Johansson 1992). Interestingly, Mattila and Nuutinen's (2007) results indicate that drainage of sites reduces the risk of root and butt rot damage. According to Laiho (2008), drainage alters the abundance and structure of soil fauna and microbe communities. The change is dependent on the former wetness of the site; the surface peat of initially wet mires may increase in fungal biomass after drainage, whereas at drier sites the fungal biomass may even decrease (Laiho 2008). Although the effect of soil is evident, it is also the case that planting distance and root frequency play an important role in the spread of the disease via root contact (Redfern 1984).

1.5.4 Economic significance

Finland is the most densely mired country in the world (Virtanen 2008), a fact that has direct bearing on the Finnish economy. The total volume of timber in Finnish peatland forests is 479 million cubic meters, which is 23 % of the country's total forest-timber volume (Tomppo 2005). The annual growth of Finnish peatland forests is estimated to be 3.9 m^3 ha^{-1}. In the ninth NFI, the estimation of total annual forest growth is 86.7 million m^3, out of which 24.4 % is peatland forest, 18.08 million m^3 is drained peatland forest and 3.11 million m^3 is pristine peatland forest (Tomppo 2005).

The rate of drainage grew significantly from 1960s to 1980s. In the third NFI (years 1951–1953), the total area of pristine peatland is 8.83 million hectares, whereas in the eighth NFI (1986–1994), it is only 4.23 million hectares. Due to the beneficial effects of drainage on growing condition, the increment of peatland growth from the third to ninth NFI is 138 %. In comparison, during the same time period, the increment of growth for mineral soil was only 45 % (Tomppo 2005). New drainages of pristine peatlands are no longer carried out, because the Finnish society no longer funds these actions and the certification prohibits it (Päivänen 2007).

Even-aged forest stands in mineral soils are easily classified into development stages according to the timber volume of the stand. The classification of peatland stands is a bit more complex, because they are often of uneven age: trees usually die naturally and stands are not burnt or clear-cut (Päivänen 2007). Despite this, peatlands are still classified by development stages in National Forest Inventories. The majority of drained peatland forest stands are young thinning stands (southern Finland 46 %, northern Finland 67 %). Only 7 % of peatland stands in southern Finland and 3 % of peatland stands in northern Finland are ready for harvesting; in contrast, a much greater percentage of mineral soil sites are harvest-ready (Finnish Forest Research Institute, in Päivänen 2007).

Because of the development class distribution of peatland, it is estimated that peatland logging will increase to make up more than 20 % of total logging in Finland by the year 2025 (Nuutinen *et al.* 2000). This will markedly affect the Finnish forestry industry. Because the logging of peatland forest has been going on for a relatively short period of time, there is little empirical information concerning the reaction of pathogenic fungi to large amounts of fresh wood (stumps). If fungi are able to spread to peatland conifers, there is a huge risk of new root and butt rot infections within the next decades, which means that control methods should also be applied at peatland forest sites (Kasanen 2009).

Interest in growing uneven-aged forest with no clear-cutting has increased over the past few years (Valkonen and Hallikainen 2006). Uneven-aged forestry is based on selective cutting and the utilisation of seedlings growing in the understory. The most promising tree species is Norway spruce, as it tolerates shade better than other commercially important tree species (Valkonen 2010). One disadvantage of uneven-aged forestry is that selective cutting increases the risk of root and butt rot damage in the stand (Mattila & Nuutinen 2007). Advance growth of Norway spruce should not be used for regeneration at heavily infested sites, as it may be infected, even if there are no visual symptoms (Piri and Korhonen 2001). As peatland has traditionally been considered free of root and butt rot infection, partial and selective cutting has been suggested as a sound option for peatland forestry. In the case that Heterobasidion is able spread to peatland, methods for controlling fungal diseases in uneven-aged forestry need to be carefully planned.

2 OBJECTIVES OF THE STUDY

2.1 Central questions

The main objective of this study is to evaluate the resistance of Norway spruce to root and butt rot infections at different growing sites, with particular focus on peatland sites. The resistance responses of Norway spruce were compared on pristine peatland, drained peatland and mineral soil. Furthermore, the connection between growth rate and resistance in a single tree was evaluated. Finally, the effects of genotype and geographic origin on resistance were also studied.

This study aims to answer the following questions.

1. Does growing site affect the resistance of Norway spruce to *Heterobasidion parviporum* infection?

2. How does resistance response differ in different parts of the tree?

3. Are trees with high growth rate more susceptible to *H. parviporum* infection than trees with low growth rate?

4. How does the genotype and geographic origin of the host tree affect resistance?

2.2 Hypotheses

Hypothesis 1: The growing site affects the resistance of Norway spruce to Heterobasidion parviporum infections.

It is presumed that the resistance of mature Norway spruce trees to *H. parviporum* infection is significantly different at different growing sites. This was tested by artificially infecting mature Norway spruce stems and roots with *H. parviporum*. It is expected that trees growing in mineral soil will result in large necrotic lesions.

Hypothesis 2: The difference in necrotic response between trees at different growing sites is more pronounced in the roots than in the stems.

It is expected that the variation in necrotic response of trees at different sites is higher in root inoculations than in stem inoculations, because it is likely that the growing site affects below-ground conditions more than it does above-ground conditions.

Hypothesis 3: High growth rate in Norway spruce trees correlates negatively with resistance to H. parviporum.

A tree's allocation to growth is expected to result in weak resistance. The height of the tree, crown-height relation and the mean annual growth in diameter were used as measures of growth. These measures are expected to correlate positively with length of necrosis.

Hypothesis 4: The genotype and geographic origin of a tree affect its resistance.

It is expected that the effect of genotype on resistance is significant, as is growing site. This was tested with planted Norway spruce ramet clones growing on former agricultural land with a similar experimental setting as described in Hypothesis 1 and Hypothesis 2. Instead of different growing sites, there were Norway spruce trees of 15 different clones. It is presumed that the variation in the necrotic reactions of spruce clones growing at the homogeneous growing site is significant.

3 MATERIAL AND METHODS

3.1 Fungal isolate, growth medium and inoculum

The fungal isolate, *H. parviporum* (01053/2), was obtained courtesy of Dr. Kari Korhonen (Finnish Forest Research Institute). The fungus was maintained on malt extract agar (2% malt extract, 2% agar, Bacto) at room temperature. The fungus had been growing on agar plates for four weeks before its use in infection of the wood dowels.

The wood dowels were prepared from frozen Norway spruce (*P. abies*) disk. Cylinders 10 mm in diameter and 9 mm in length were cut using an upright drill and a manual saw. The dowels were then autoclaved (20 ml of milliQ water for every set of 200 dowels) at 120 °C for 20 minutes and stored in sterile containers until used.

The sterile wood dowels were placed on malt extract agar plates (5-10 dowels per plate) colonised by *H. parviporum* (figure 1). Control dowels were placed on sterile malt extract agar plates (approximately 20 dowels per plate). The plates with dowels were incubated for three weeks at room temperature. The success of colonisation was estimated visually by inspecting the surface of the dowels for the presence of hyphae.

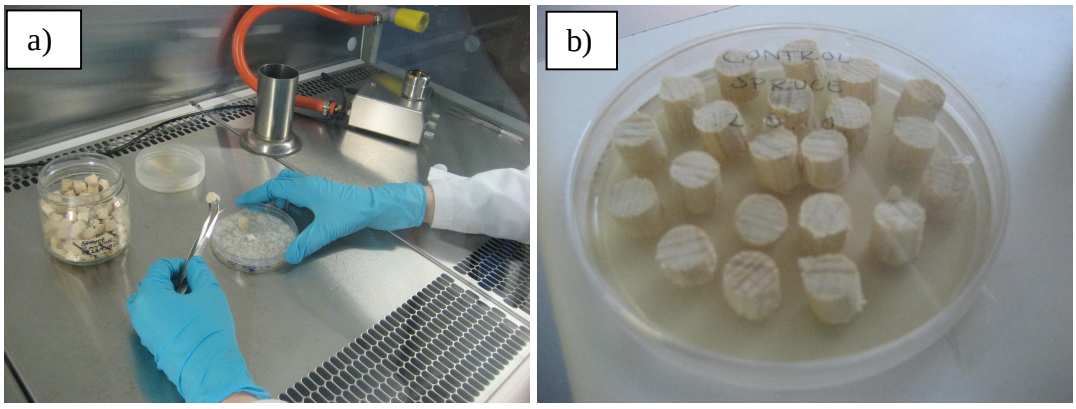


Figure 1a-b. **a)** Autoclaved spruce dowels were placed on agar plate colonised by *H. parviporum* hyphae and incubated for three weeks. **b)** Control dowels were incubated on pure agar plate for three weeks.

3.2 Location and characteristics of sites

3.2.1 Sites in Lakkasuo

Three sites were selected in Lakkasuo mire located in Orivesi, Central Finland (61°40'N24°21'E). The experimental plots were established in pristine mire, drained peatland and mineral soil (appendix 1). The following growing sites were selected:

- Vaccinium myrtillus spruce swamp
- Drained V. myrtillus site type I
- Myrtillus type forest

Site 2 (Vaccinium myrtillus spruce swamp) is a pristine mire with relatively small trees (table 1, figure 2a). The experimental trees were selected among the biggest trees. The peat layer is approximately 1.5 m thick. The proportion of dead trees within the experimental plot is 37.5 %, of which the majority has diameters less than 5.0 cm. Ground vegetation consists mainly of blueberry (*Vaccinium myrtillus*), though lingonberry (*Vaccinium vitis-idaea*) and *Carex globularis* are also fairly common. The moss layer is comprised of *Sphagnum girgensohnii, S. russowii, Hylocomium splendens, Pleurozium schreberi, Polytrichum commune* and *Dicranum majus* (Laine *et al.* 2004).

Site 5 (Drained V. myrtillus site type I) is a former V. myrtillus spruce swamp (figure 2b), and the experimental plot was delimited by ditches from three sides. The site was drained in 1965, and ditch maintenance was carried out in 1988 (Laine *et al.* 2004). The peat layer is 1.8 m thick (Laine *et al*. 2004). The vegetation has changed over the drainage succession: the *Sphagnum* mosses have mostly been replaced by the moss species typical to mineral soil such as *Pleurozium schreberi* and *Dicranum sp.*, and *Vaccinium myrtillus* is the dominant dwarf shrub in the field layer (Laine *et al.* 2004). Growth has been extremely good since the site was drained. In 1998 the measured annual growth was 8.3 m^3 ha^{-1}, which is even higher than the average for Myrtillus type forest (Laine *et al.* 2004).

Site 6 (Myrtillus type forest) has sandy soil with a thick humus layer (figure 2c). The site is gradually changing into Vaccinium type forest further up in the slope (see the map in appendix 1). There are many broken tree tops around the site, caused by snow damage during previous winter. The site is protected from forest management. Ground vegetation consists of *Pleurozium schreberi, Hylocomium splendens* and *Dicranum majus* mosses (Laine *et al.* 2004).

Table 1. Diameter of breast height (D1.3) of all living trees in the experimental plot, and D1.3 and age of experimental trees of Lakkasuo sites.

Site	D1.3 of all living trees, cm	D1.3 of experimental trees, cm	Age of experimental trees, yrs
2	10.8	14.6	132
5	24.1	25.1	132
6	13.9	15.1	106

At each site, 20 experimental trees were selected: 10 of them were wounded and inoculated with dowels colonised by *H. parviporum* (H for Heterobasidion) and the other 10 only wounded and inoculated with sterile wood dowels (WC for wounded control). Trees were selected based on diametric distribution such that each diameter class was represented, corresponding to the distribution of all trees at the site. Spatial distribution was also taken into account such that the experimental trees were evenly located across the plot area and were not too close together; in this way, root contact and shading effect was avoided (appendix 2). Only living, healthy-looking, non-damaged trees were chosen as experimental trees (H and WC).

3.2.2 Site in Röykkä

The site in Röykkä, Nurmijärvi, southern Finland (60°30'N24°42'E) is an experimental Norway spruce stand, which has been established on former agricultural land with fertile loamy clay soil (figure 2d). Originally, the stand has been established to test the growth performance of various Norway spruce progenies

(Foundation for Forest Tree Breeding, Finnish Forest Research Institute, experiment 657/01). Altogether 62 different Norway spruce progenies representing different genetic and geographic origins were planted to the site, of which 52 were ramet clones.

The area of the site is 0.12 ha, and it has been divided into five slots. On each slot, four Norway spruce plants representing the same genetic origin were planted into a 2 x 2 m square at 1 x 1 m intervals. The location of the trees within the slots was randomised. The Norway spruce plants were planted in 1977; however the biological age of the trees was 40-43 years at the time of this experiment. The stand was thinned in 1988 and in 1997 (Finnish Forest… 1992, Napola 1999). The average diameter of the experimental trees is 13.2 cm (appendix 4).

For the current study, 15 Norway spruce clones representing different geographic origins were selected (appendix 3). The ramets were propagated during 1969-1972 from seedlings that originated either from seed material produced by controlled crossings where both parent trees are known, or from open-pollinated seed material where only the mother tree is known (appendix 3) (Finnish Forest Research Institute 1992, Napola 1999). From each selected clone, four to five trees were selected as experimental trees with a total of 68 trees used for the study. Separate trees within each clone were either wounded and infected with *H. parviporum*, or wounded and inoculated with sterile wood dowels.

Figure 2a-d. **a)** Site 2: Vaccinium myrtillus spruce swamp (pristine mire) **b)** Site 5: Drained V. myrtillus site type I (drained peatland). **c)** Site 6: Myrtillus type forest (mineral soil). **d)** Clonal spruce stand in Röykkä.

3.3 Inoculation

The inoculations at Lakkasuo sites were carried out between the 5th and 11th of June 2010, when the temperatures were around +15 °C. Method of inoculation was similar as described for example by Solla *et al.* (2002). First, the main roots of each H and WC tree were dug out by hand with a small hoe and brushed with a scrubbing brush. Three root inoculations were performed for each tree. The inoculation distance from the root collar among all root inoculations varied between 15 and 65 cm. Stem inoculations were carried out at three heights: 50, 100 and 150 cm above ground level, measured from the buttress, at the original point of germination (figure 3).

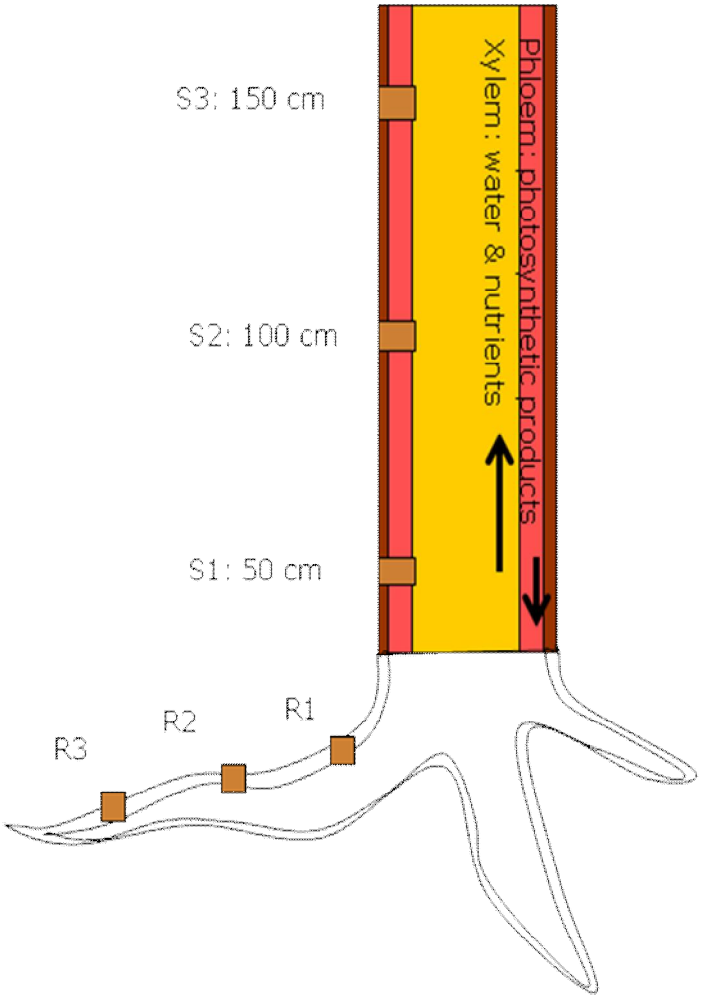


Figure 3. Schematic illustration of inoculations of trees at Lakkasuo sites. Wood dowels were placed to reach xylem surface. The tissues (phloem and xylem) are equal in stem and roots.

Both root and stem inoculations were carried out using a comparable procedure. All the tools were sterilised with 70 % ethanol, and protective laboratory gloves were used. The bark around the point of inoculation was first sprayed with 70% ethanol, then a circular wound was created using a 10 hole puncher and a hammer. The wounds were bored deep enough to reach the xylem surface, in order to avoid the resistance factors and defence reactions exhibited by bark and cambium (Delatour *et al*. 1998). In order to keep the inoculum sterile, the agar plates containing the wood dowels were opened only when needed and a dowel was quickly placed in the wound using tweezers. The inoculum was inserted firmly to the wound using the non-cutting end of the hole puncher and a hammer (figure 4). The inoculations were wrapped

firmly using Parafilm® in spruce swamp and saran wrap in drained peatland and mineral soil (figure 5).

Figure 4a-d. Inoculation. **a)** Making the wound with a hole puncher and a rubber hammer. **b)** Bark and cambium was removed. **c)** Wound reached the xylem surface. **d)** Inserting a wood dowel to the wound.

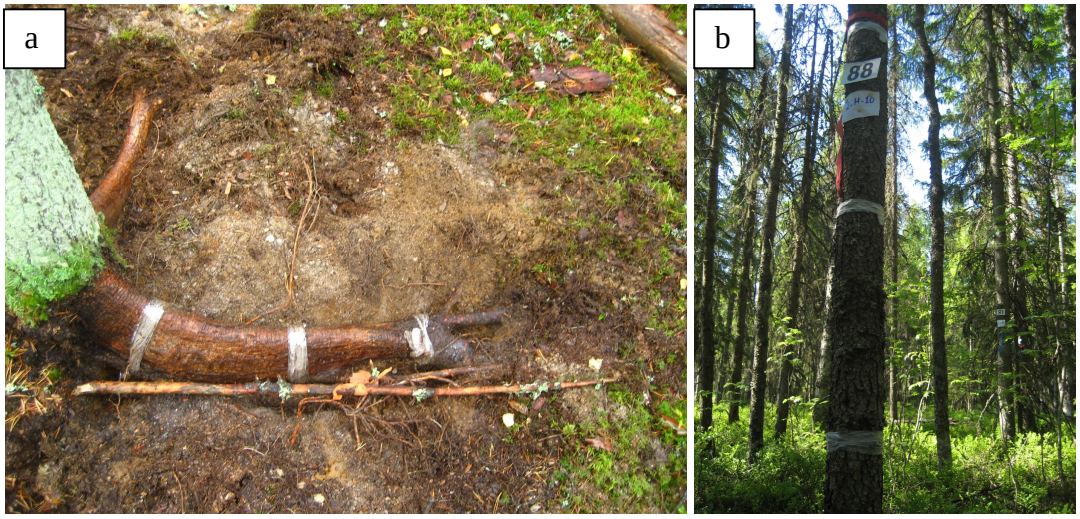


Figure 5a-b. **a)** Root inoculations of a tree in mineral soil**. b)** Stem inoculations of a tree in pristine mire.

The trees at clonal spruce stand in Nurmijärvi were inoculated between 26th and 28th of June 2010, when the temperature was around +20 °C. The procedure used was the same as that used at the Lakkasuo sites, with the exception that only four inoculations were performed on each tree: stems were inoculated at two heights (50 and 100 cm) and roots were inoculated at two distances from the buttress. Parafilm® was used to wrap the inoculations.

3.4 Sample collection

The Lakkasuo samples were harvested between 20th and 28th of September 2010, during which period the temperature was 5–15°C. For logistical reasons, the sites were harvested in the following order: 1) Myrtillus type forest, 2) Drained V. myrtillus site type I, 3) Vaccinium myrtillus spruce swamp. Each tree to be sampled (WC and H trees) was first cut down with a chainsaw. The overall height and the height of the living crown were measured from the stump, using a tape measure.

The stem was sawn into three logs, 50 cm in length, ensuring that the inoculations were in the middle of each log (25 cm away from both ends). Larger diameter logs (over 22 cm) were chopped into smaller pieces with an axe. Each sample was marked with a sample code, and with a code indicating its location in the tree (S1, S2, S3,

R1, R2 or R3: stem/root, distance from the butt) (figure 3). The samples were stored in a fridge at +5°C for a maximum of three days, waiting for transportation. The samples were then transported to Helsinki and stored in a freezer at –18°C.

The samples at Röykkä were collected between 2nd and 9th of November 2010, when the temperature was 0–10°C. The procedure employed was similar to that used at Lakkasuo.

3.5 Measurements and calculations

Measurement of the Lakkasuo samples was carried out in Helsinki between October and November 2010, Röykkä samples between January and February 2011. First, the bark of the sample was peeled off using a small axe and a knife. Then, the length of the necrotic lesion in the phloem was measured. The length and width of the necrosis relative to the nearest edge of the inoculation were measured using a ruler, to the nearest millimetre (figure 6). The total lengths and widths of each sample were also measured, taking the inoculation point into account. The phloem was then removed with a chisel and a hammer, and necrotic lesion measurements were taken from the xylem in a similar fashion. The measurements of necrotic lesions were equally done in the xylem. After each series of measurements, the sample was photographed.

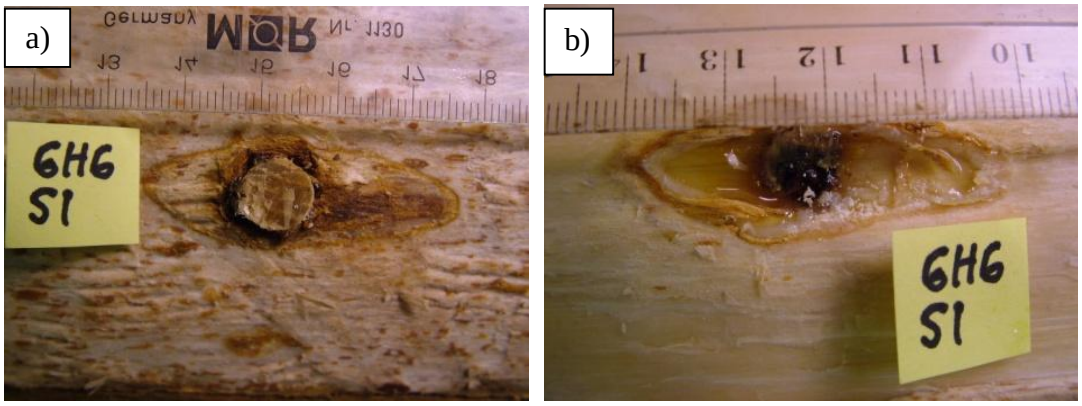


Figure 6a-b. Necrotic reaction in Heterobasidion infected stem sample in mineral soil. **a)** Phloem tissue. **b)** Xylem tissue.

A disc was sawn from the stump of every experimental tree to discover its age and annual growth rate. The discs were kept at –18°C until measured. Tree rings were measured with stereomicroscope and Measure J2X Tree Ring Measuring Program.

The annual growth in diameter was calculated as the mean of the width of the annual rings in the disc. Crown-height relation was calculated by dividing the living crown height of a tree by the overall height of a tree. The axial growth was not measured in the field; however, a rough estimation of annual axial growth was carried out by simply dividing the tree height by the age of the tree.

3.6 Statistical analysis

As there was no clear variation in the widths of the necrotic lesions, total length of necrosis was selected as a measure of resistance. For the Lakkasuo data, this was calculated as the mean of the necrosis measurements at the three different distances of inoculation (S1, S2 and S3 in the stem and R1, R2 and R3 in the root). For the Röykkä data, this was calculated as the mean of the two measurements (S1 and S2 in stem and R1 and R2 in root). Finally, four values, namely phloem and xylem responses in roots and stems, remained for each tree.

Generalised linear models with robust covariance structure were used to analyse the differences between the necrotic responses at Lakkasuo. The least significant difference (LSD) test was used for pair-wise comparisons between sites (pristine mire, drained peatland and mineral soil), treatment (H and WC), tree organ (stem and root) and tissue (phloem and xylem). Total length of necrosis was used as dependent value and was converted into a logarithmic scale in order to reach normal distribution.

Tests similar to those carried out on the Lakkasuo data were used to analyse the necrotic responses in the clonal spruce stand at Röykkä; the main difference being that the factor “site” was replaced with the factor “clone”. Some of the trees were suffering from prior decay, which only became apparent at the time of sample collection. All trees belonging to the clones numbered 14, 35, 39, 45, 54 and 60 were excluded from the analysis; the results, therefore, are based on the rest 9 clones (appendix 3).

Correlations and Regression analyses were used to identify potential correlations between resistance and growth measurements from the trees at Lakkasuo. Necrosis length in stem xylem was selected as a measure of resistance. Only Heterobasidion infected trees were included in these analyses, because the treatment was found effective in first tests.

Statistical analyses were completed with PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA). All graphs were drawn with Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA).

3.7 Re-isolation of *H. parviporum*

To make sure that the infected trees were really infected by *H. parviporum* and not by any other fungi and that control samples were not contaminated, some re-isolation from sampled tissues were carried out. Altogether 35 samples were tested, and they were from 5 Lakkasuo trees and from 5 Röykkä trees (appendix 7). Samples were chosen evenly from root and stem samples and from phloem and xylem tissues. Circular pieces of tissue samples (5 mm in diameter) were taken next to the point of inoculation, on the border of the necrotic zone.

The samples were surface sterilised using a three-step sterilising procedure: the pieces of tissue were dipped for 5–10 seconds firstly in 70 % ethanol, then in 10 % sodium hypochlorite, then in sterile milliQ water. Pieces were placed in 2 % malt agar plates and incubated in room temperature. The presence of *H. parviporum* hyphae and conidiophores were checked after 5, 10 and 20 days after isolation.

The compatibility of *H. parviporum* strains was tested by mating test with pure culture of *H. parviporum* isolated from the tissue and of the original isolate that was used in the inoculation. The two strains were placed to grow on a same malt agar plate. Interference was expected to occur in case the two strains would not have been originated from the same strain. Isolate from the sampled xylem tissue of tree number 35H1 stem.

4 RESULTS

4.1 Extent of necrosis at Lakkasuo sites

4.1.1 Effect of site and treatment

There was no clear difference (P=0.39) between the responses in different growing sites. In addition, when sites were compared pair-wise (treatments separately), no pair was found to differ significantly (P>0.05). However, the trend followed the hypothesis that the necrotic reactions of mineral soil are longer than those of peatland. As shown in table 2, the Heterobasidion infected trees growing in pristine mire exhibited the lowest average length of necrosis (35 mm), whereas those in mineral soil exhibited the highest (40 mm). Interestingly, control trees in peatland sites seemed to react with higher necrosis to wounding than control trees in mineral soil.

The treatment, however, seemed to have been effective (P<0.001), and the fungus had an effect on the resistance response of the *H. parviporum* infected trees (based on comparison with the control trees). Infected trees responded with necrotic reactions that were nearly twice as long as the reactions of the trees that were only wounded. The average length of necrosis in infected trees is 37 mm and in control trees 20 mm (table 2).

Table 2. Mean length of necrosis (mm) of all sample trees in pristine mire, drained peatland and mineral soil. The results are means of measurements in both tissues and roots and stems combined.

	Heterobasidion infected		Wounded control	
Site	Mean	S.D.	Mean	S.D.
Pristine mire	35	11.9	20	5.4
Drained peatland	37	13.8	21	5.8
Mineral soil	40	17.7	18	1.8
Average	37	14.5	20	4.3

4.1.2 Effect of organ and tissue

Tree organs, in this case stem and root, seemed to react differently to inoculations (table 3). The difference in extent of stem necrosis compared to root necrosis was statistically significant ($P<0.001$) at all sites in Lakkasuo. This indicates that these organs differed in their response to artificial wounding. Necrotic reactions in stems were generally longer (33 mm) than those in roots (25 mm).

In general, this study did not find any remarkable differences in the resistance of different tree tissues ($P=0.12$). As shown in table 3, the average length of necrosis in phloem is 29 mm, and 28 mm in xylem. Mean extent of necrosis at all sites, organs and tissues within the two treatments separately are shown in table 4. The extent of necrosis in all trees individually is shown in appendix 5.

Table 3. Mean length of necrosis (mm) of all sample trees in pristine mire, drained peatland and mineral soil. The results are means of measurements in all three sites and two treatments combined.

	Phloem		Xylem	
Organ	Mean	S.D.	Mean	S.D.
Stem	33	9.7	31	9.2
Root	25	9.8	25	8.9
Average	29	9.7	28	9.0

In the stem inoculations it was notable that the trees reacted with higher responses to root and but rot in peatland sites than in mineral soil (Figure 7). The highest resistance was exhibited by the trees growing in pristine mire. These results were more pronounced in xylem than in phloem. For the necrosis length in *H. parviporum* infected stem xylem, the difference between sites was statistically significant when compared pristine mire to mineral soil ($P=0.04$) (table 4a).

The root inoculations did not follow the trend that was hypothesised. As shown in table 4b, the trees with most susceptible roots seemed to be those in drained peatland (necrosis length 32 mm in phloem and 33 mm in xylem) and the most resistant seem to be those in mineral soil (necrosis length 32 mm in phloem and 28 mm in xylem). However, this difference was not statistically significant (P>0.05).

Table 4a-b. Least significant difference (LSD) test for comparing the extent of necrosis (mm) in **a)** stem samples and **b)** root samples in mineral soil with peatland sites (pristine and drained). n=10.

a)

	Heterobasidion infected						Wounded control					
	Phloem			Xylem			Phloem			Xylem		
Site	Mean	S.D.	Sign.	Mean	S.D.	Sign.	Mean	S.D.	Sign.	Mean	S.D.	Sign.
pristine	43	12.8	0.36	35	7.7	0.04	25	8.5	0.45	20	4.4	0.33
drained	42	16.9	0.30	41	12.9	0.30	21	2.6	0.17	21	3.6	0.06
mineral	47	14.3		51	25.1		23	3.0		18	1.5	

b)

	Heterobasidion infected						Wounded control					
	Phloem			Xylem			Phloem			Xylem		
Site	Mean	S.D.	Sign.	Mean	S.D.	Sign.	Mean	S.D.	Sign.	Mean	S.D.	Sign.
pristine	32	13.7	0.83	32	13.3	0.36	19	4.5	0.00	17	4.0	0.14
drained	32	13.0	0.72	33	12.6	0.28	20	7.1	0.00	21	9.8	0.04
mineral	32	18.8		28	12.8		15	1.7		15	0.9	

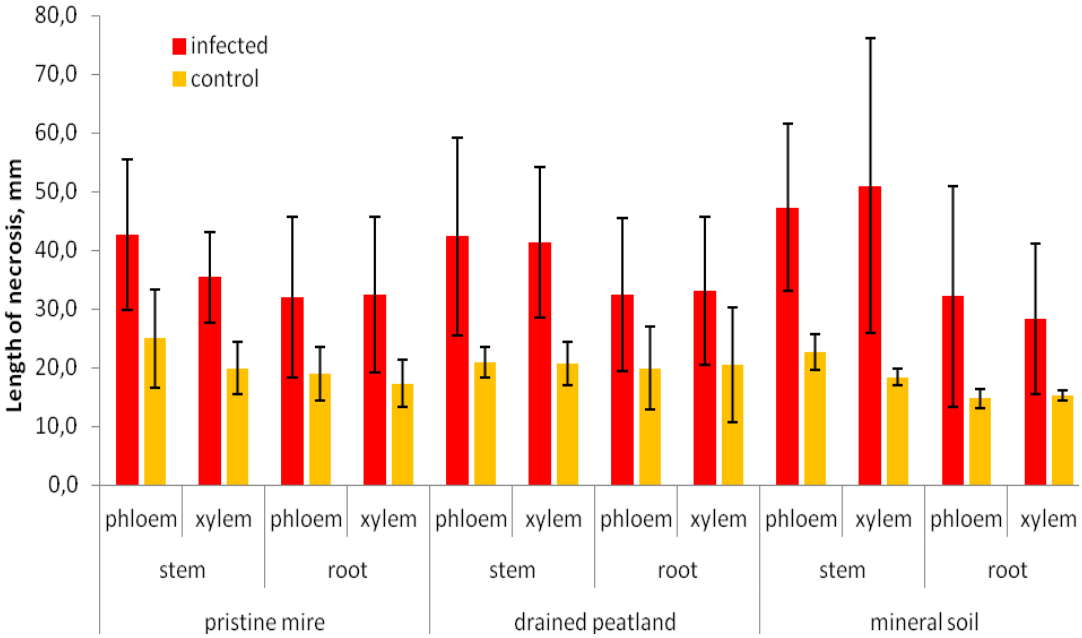


Figure 7. Necrosis length in different spruce tree tissues and organs at all Lakkasuo sites. Error bars indicate the standard deviation. n=10.

4.2 Correlation between growth and resistance at Lakkasuo sites

4.2.1 Growth in diameter

Trees that grew rapidly in diameter were not more susceptible to Heterobasidion infections than slow-growing trees. Length of necrosis and mean annual growth in diameter were not well correlated (figure 8).

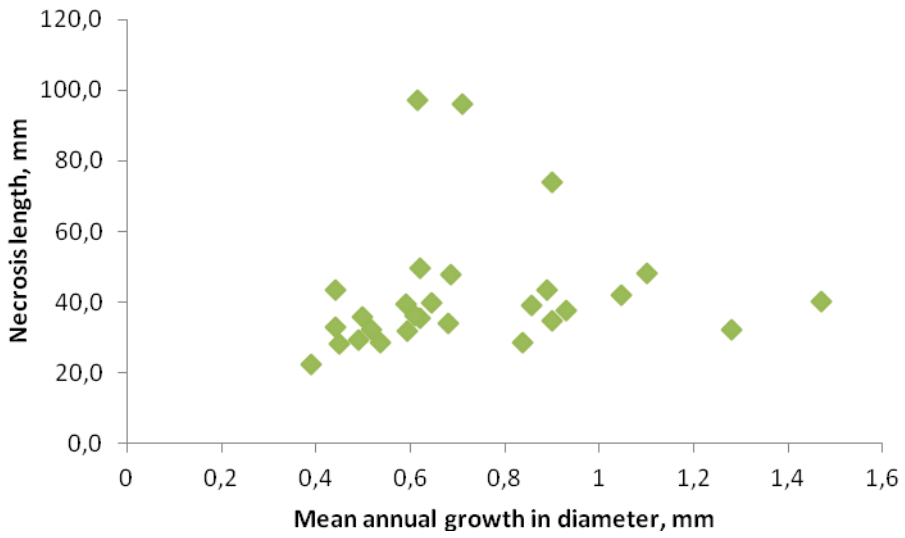


Figure 8. Correlation of necrosis length in *H. parviporum* infected stem xylem and mean annual growth in diameter for the trees at Lakkasuo sites. Pearson Correlation = 0.058. R^2 = 0.012. P = 0.575.

4.2.2 Living crown

Trees that had large living crowns relative to their height did not seem to be any more or less resistant to root and butt rot. There was a very low negative correlation (–0.113) between necrosis length and living-crown size (figure 9).

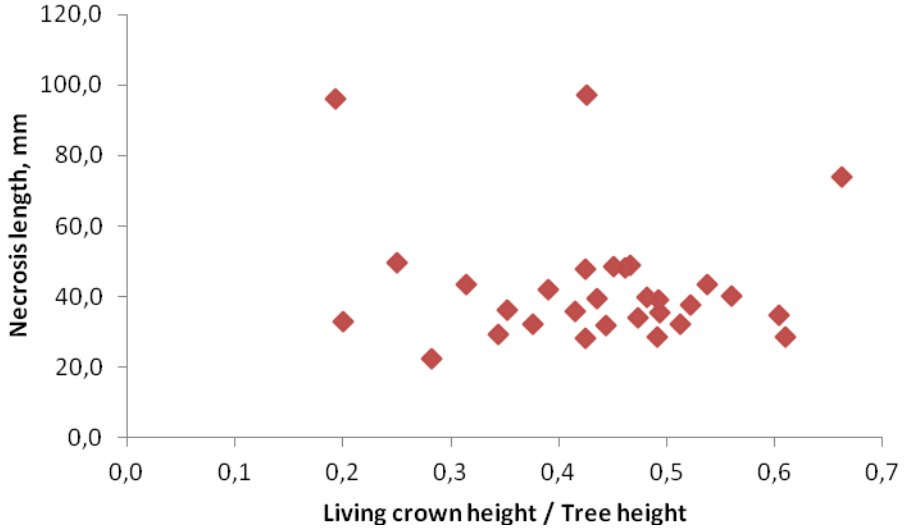


Figure 9. Correlation of necrosis length in *H. parviporum* infected stem xylem and crown-height relation for the trees at Lakkasuo sites. Pearson Correlation = –0.113. R^2 = 0.013. P = 0.553.

4.2.3 Axial growth

Trees with rapid axial growth did not seem to be less resistant to Heterobasidion infections than those with slow growth. There was a very low positive correlation (0.115) between necrosis length and mean annual axial growth (figure 10).

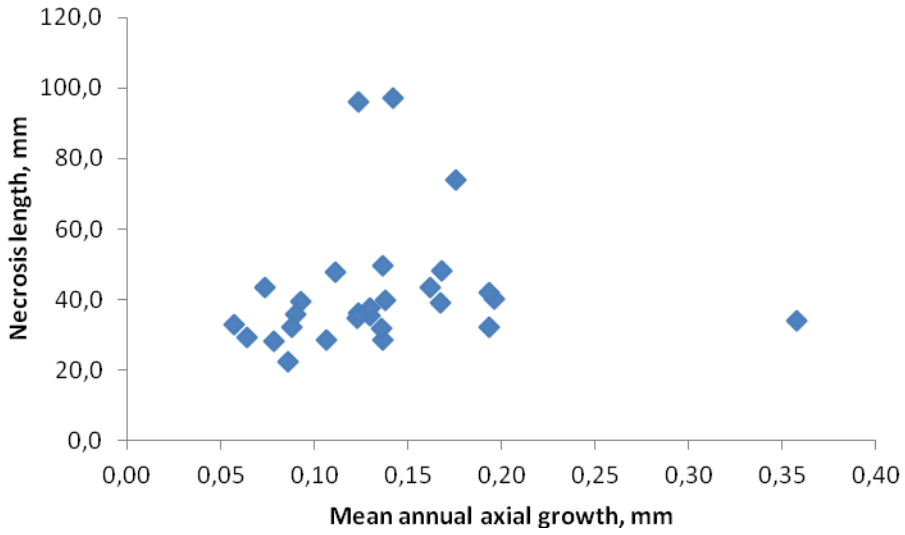


Figure 10. Correlation of necrosis length in *H. parviporum* infected stem xylem and mean annual axial growth for the trees at Lakkasuo sites. Pearson Correlation = 0.115. R^2 = 0.013. P = 0.56.

4.3 Variation between the different clones in Röykkä

4.3.1 Stem

Genotype seemed to have affected tree resistance. There were significant differences between the necrotic reactions of different clones (P<0.001). However, a comparison of stem and root inoculations showed that those responses varied significantly (P=0.04). Of the stem inoculations, the most susceptible clone was number 28, which originated from central Finland (Pieksämäki) (figure 11). The trees belonging to this clone exhibited very strong necrotic reactions in both tissues, with and average necrotic reaction of 112 mm in phloem and 130 mm in xylem. The most resistant

clone was number 20, which was of Russian origin (Novgorod), with an average necrotic reaction of 20 mm in phloem and 18 mm in xylem (table 5).

With some trees the results differed slightly in phloem and xylem, although the difference between tissues was not statistically significant (P=0.65). The average length of necrotic reactions in Heterobasidion infected trees was 39 mm in phloem and 37 mm in xylem (table 5). The difference between Heterobasidion infected and wounded control trees in the clonal spruce stand at Röykkä was statistically significant (P<0.001), just as it was at the Lakkasuo sites.

Table 5. Necrosis length (mm) in the stems of different Norway spruce clones. n=2 or 3 (appendix 3).

	Heterobasidion infected				Wounded control			
	Phloem		Xylem		Phloem		Xylem	
Clone	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
12	33	14.0	27	5.9	16	1.8	17	0.4
15	22	8.1	22	1.4	20	9.9	22	10.3
16	32	0.4	33	0.7	31	20.5	30	20.9
20	20	2.8	18	4.6	19	6.0	21	3.5
27	43	10.3	27	2.3	38	27.2	25	6.0
28	112	42.8	130	132.2	18	1.4	15	2.8
31	35	26.1	28	10.0	24	13.1	30	20.5
36	26	1.1	26	0.7	75	78.8	55	49.5
62	34	1.1	29	1.1	24	7.4	17	2.5
Average	39	12.1	37	16.5	28	16.8	25	11.7

Although clone number 28 clearly showed the strongest necrotic response, the standard deviation was high, meaning that this may be due to the results of a single tree (28H1) within the clone (appendix 6). This also affects the overall results, as it increases the total deviation in the analysis. However, even after the exclusion of the values of the tree named 28H1, the clone 28 would still have been ranked as the most susceptible one.

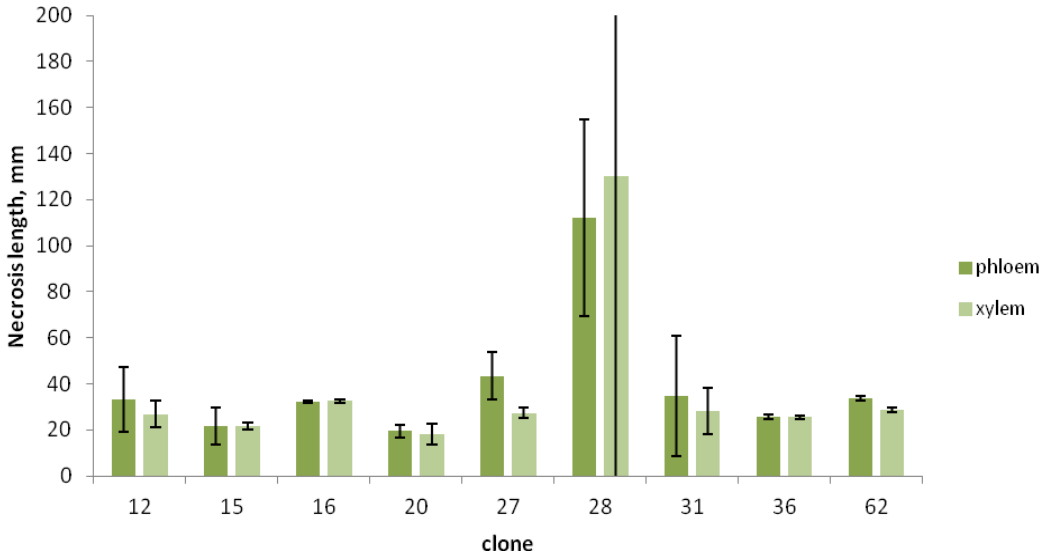


Figure 11. Necrosis length in the Heterobasidion infected stems of different Norway spruce clones. n=2 or 3.

4.3.2 Roots

Roots were generally more susceptible to Heterobasidion infection than stems. The average length of necrotic reaction was 61 mm in infected root phloem and 62 mm in infected root xylem (table 6). The most susceptible clone was number 31, a Finnish-German hybrid (Loppi x Schmiedewald) (fiure 12). Clone number 27, from central Finland (Pieksämäki), was also found to be very highly affected by necrosis. The Russian clone (number 20) was the most resistant, in root as well as stem. The clones 27 and 28 were both highly susceptible to infection in stems and roots. These clones also had a common parent (appendix 3).

Table 6. Necrosis length (mm) in the roots of different Norway spruce clones.

	Heterobasidion infected				Wounded control			
	Phloem		Xylem		Phloem		Xylem	
Clone	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
12	46	14.8	37	8.7	15	1.8	15	0.7
15	33	21.2	35	14.1	14	1.1	18	0.7
16	37	0.0	70	43.5	15	0.0	15	0.7
20	22	5.7	33	12.4	15	0.7	15	0.0
27	90	47.3	81	68.8	23	6.4	19	3.5
28	75	5.7	67	3.9	15	2.1	16	2.8
31	125	44.8	115	20.8	20	4.9	24	11.3
36	34	3.2	33	0.4	16	1.4	17	0.7
62	90	67.5	90	60.8	30	22.3	30	15.9
Average	61	23.3	62	25.9	18	4.5	19	4.0

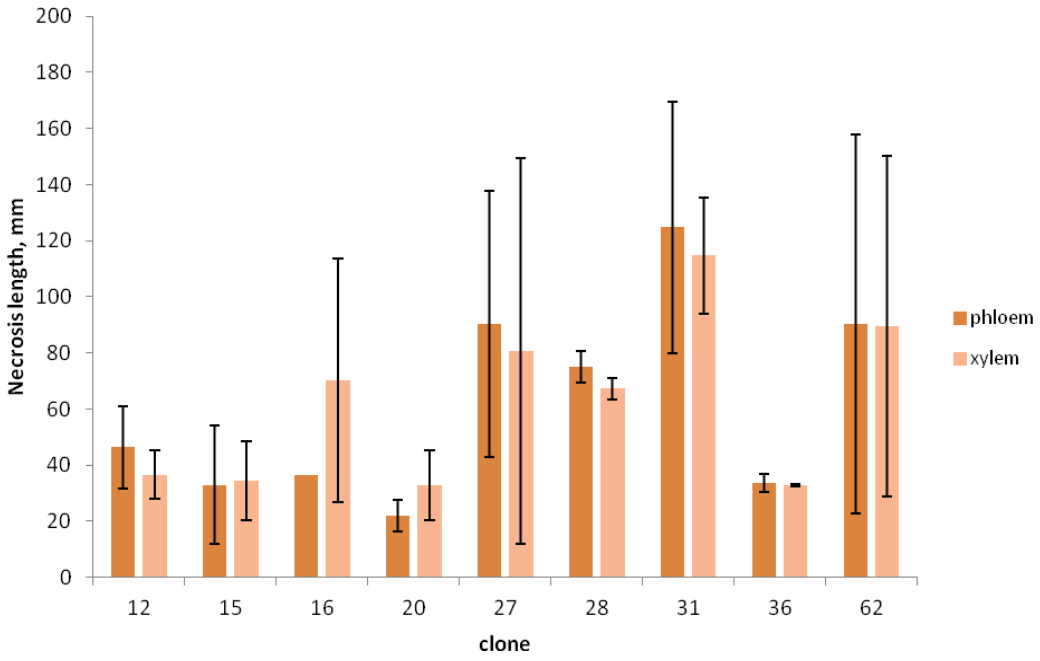


Figure 12. Necrosis length in the Heterobasidion infected roots of different Norway spruce clones. n=2 or 3.

4.4 Re-isolation of *H. parviporum*

H. parviporum was successfully isolated from two experimental trees of Röykkä site: from root sample of 28H1 and from stem sample of 35H1 (appendix 7). Conidiophores were observed only in xylem sample of 35H1, where they were present already after 5 days of incubation. Hyphae were observed in 12 out of 35 samples after 10 days of incubation. Most of these were hyphae of unidentified fungi, not *H. parviporum*, as no conidiophores were observed.

The mating test indicated that the *H. parviporum* strain isolated from the sample was the same that was used for inoculation. The two strains were growing on each other.

5 DISCUSSION

5.1 Factors affecting resistance response

5.1.1 Site properties

The results of this study revealed that site has no effect on the resistance of Norway spruce to Heterobasidion infection. These results support the findings of Piri *et al.* (1990), although that study focused on infection rate, and not host resistance. Piri *et al.* (1990) did not find significant differences in Heterobasidion root rot frequency between forest site types, when the occurrence of *H. annosum s.l.* in southern Finnish spruce stands was investigated.

In inoculation experiments on Sitka spruce plantations aged between 25 and 30 years, Redfern (1984) found that infection rates were much higher at two mineral soil sites than they were at peatland sites. Lindberg and Johansson (1991) carried out experiments on the bark-wound inoculation of spruce trees between 22 and 23 years; they found that, of the roots growing in clay soil, 75 % became infected; in moraine soil, 65 %; and in peat soil, only 21 %. The results of this study with respect to stem xylem follow previous findings and support the hypothesis that trees growing on pristine peatland are the most resistant. Even though no statistically significant difference between sites was recorded in the rest of the results, the trend was similar in stem phloem.

The fact that the results for stem xylem are clearer than those for phloem may be explained by the direction of liquid flow in these tissues. In xylem, water and nutrients are transported from the ground upwards; in phloem, photosynthetic sugars are transported from the leaves – or needles – downwards to the roots (figure 3). It is, therefore, a possibility that the effect of soil on resistance is reflected in the response of xylem tissue, considering that intracellular signals are transported along the gradient of liquid flow.

If the root results were similar to the stem results, it would indicate that peatland trees are slightly more resistant to root and butt rot than mineral soil trees. In this case, no clear statements about the effect of site can be made, as the differences are small. The fact that the roots of the trees in mineral soil had the highest resistance makes analysis of the results even more complex.

5.1.2 Growth measures

According to Kytö *et al.* (1998) there is a trade-off between growth and carbon-based defence mechanisms. In Swedjemark and Stenlid's (1997) studies with cuttings the extent of necrosis was negatively correlated with height, diameter and root condition. In contrast Swedjemark and Karlsson (2004) found a significant, positive correlation between necrosis length and diameter and height when 17-year-old Picea abies clones were inoculated with Heterobasidion.

In this study, growth was measured based on the axial growth rate, the diametric growth rate and the size of the crown (the crown-height relation). None of these measures correlated with length of necrosis in stem. The possible allocation between growth and resistance cannot be seen in the results of this study. Judging by the results of this and previous studies, it is clear that finding a connection between growth and resistance will be difficult. The most likely explanation for this is that the high production potential of the site – fertility and proper drainage – favours the growth of the fungus rather than well growing trees.

5.1.3 Tree genotype

Inevitably, the genotype of an individual tree will affect its resistance. Swedjemark's (1995) results – which are supported by the findings of several other authors – indicate that the growth of *H. annosum* in sapwood varies significantly across different Norway spruce clones. 35 % of the variation in her study was explained by the genetic constitution of the clones.

This study finds that the genotype of a tree seems to play a significant role in resistance. Notable differences between clones suggest that resistance is mostly determined by genes. Geographic origin may also have affected the results. The Finnish-German hybrids and the two related clones originating from Central Finland were the most susceptible to Heterobasidion infection, which may indicate that trees with foreign origin are more susceptible to fungal diseases than those with purely local origin. However, the clone that was considered to be the most resistant, originated from North Eastern Russia. This and the similar level of susceptibility of the two related clones from Central Finland indicate that the inherent genetic factors play a key role in the resistance of Norway spruce against *H. parviporum* infections.

The results from the clonal spruce stand showed that even on a very homogenous growing site, the genotypic differences are reflected by the resistance responses of the trees. In this light, it is probable that the results gained from the genetically heterogenous and naturally regenerated mature tree material in the Lakkasuo sites reflect the different genotypes of the trees, and not so much the effect of the site on the tree resistance. Therefore, the genetically heterogenous tree material may have masked the effect of the growing site on the resistance responses. Thus, the question, whether the growing site affects the resistance of the trees, remains inconclusive.

It is interesting to see that the roots of clonal spruce stand are markedly more suscpetible to infection than the stems, since the opposite is true at the Lakkasuo sites. It could be that *H. parviporum* grows more readily on fertile field than it does at the sites in Lakkasuo. No comparisons can be made between Lakkasuo and Röykkä, as the microclimate and topography of these sites, as well as the age and origin of the trees, differ greatly. How stem and root can react in such different ways and so inconsistently remains unclear.

5.2 Evaluation of the study

5.2.1 Objectives

The main objective was to find out whether spruce trees growing on peatland are more resistant to root and butt rot than those growing in mineral soil. *Resistance* is a complicated term, which cannot be explained in terms of necrotic response alone. Measuring necrosis is just one method for assessing the resistance response of an individual tree: the resistance in seedlings is normally assessed using mortality rate, and resistance in older trees can be gauged via infection rate; fungal development can also be employed as an indicator and can be calculated based on the extension of mycelium in host tissues (Delatour *et al.* 1998). This study, however, utilised the necrosis measurement method, as is relatively simple.

Dissecting the research question into smaller pieces helped to manage the complexity of the subject. With clear hypotheses, it was relatively easy to figure out the most important aspects to focus on. The connection between growth and resistance could have been more carefully studied, as growth measurements were carried out as an extension to the scope of the work and were not thoroughly planned.

The tests with clonal material were not an original part of this study, but were added in order to explore the genetic aspects of resistance. However, as explained previously, no comparisons could be made between the clonal stand and the Lakkasuo sites – if the clonal spruce site was situated in Lakkasuo and offered conditions similar to the other sites, it would have served this research better.

5.2.2 Material used

To be completely sure which traits affect resistance, tree material should be homogeneous (Delatour *et al.* 1998). Unfortunately, this is not a possibility with Finnish peatland forests, which rely on naturally born seedlings; this means that the trees are, at best, half-siblings. However, natural growing sites and tree maturity were, in this study, more desirable factors than complete uniformity. Furthermore, the latter cannot be achieved in Finnish forests, only in nurseries.

Lakkasuo was selected as the place to set out the peatland experiment, as the area itself is well studied and documented, and its close proximity to Hyytiälä Forestry Field Station provided easy access to the resources required for field work. It was not possible to find equal-aged and equal-sized spruce stands that fulfilled the other site requirements of the study. The Myrtillus type forest site (mineral soil) was possibly a gradient undergoing change into Vaccinium type forest at the edges, as it was not as fertile as Myrtillus type forests normally, and relatively few plant species typical to this forest type were found across the site.

Heterobasidion normally infects the roots of a tree rather than the stem, so why did this study employ stem inoculation? Stem inoculation was carried out in order to standardise the amount of woody tissue and the distance from the root collar (Swedjemark & Stenlid 1997). It also clearly demonstrates between-tree variations in host tissue (Delatour *et al.* 1998), which means it provides more reliable comparisons between trees. Stem inoculations are further justified in this case by the fact that, in spruce, the disease develops naturally as decay in the stem heartwood (Delatour *et al.* 1998).

At Lakkasuo, the stem samples were in every way more uniform than the root samples, which may indicate that the stem results from these sites were in fact more reliable. The roots were all unique individuals, as they differed in shape, diameter and depth from the surface. In addition, the thickness of the bark on the roots differed remarkably, whereas on the stem it was somewhat uniform. Root inoculations, in general, were more laborious and difficult to perform: each root must be dug up and

cleaned of mud, and then the inoculation must be carried out from an uncomfortable working position. This may have had an effect on the quality of the root results – if equal-sized and equal-shaped roots were easily available, the material might have been more consistent. The amount of inoculum to each experimental tree was not necessarily completely equal, since the wood dowels were not tested on agar plates whether they were equally colonised by *H. parviporum*.

5.2.3 Research methods

Inoculation of Heterobasidion is a rather commonly used method for testing the resistance of trees to infection. It seems to work pretty well, as the differences between Heterobasidion infected and wounded control samples were statistically significant at all sites. In addition, the extent of the necrotic reaction in the control samples is in line with previous studies. Woodward *et al*. (2007) studied Sitka spruce response to colonisation by *Heterobasidion annosum* using control samples of two types: wounded, and wounded and infected. In their study, the necrosis of wounded control samples extended less than 3 mm from the point of wounding, which is comparable to results of this study.

Swedjemark and Stenlid (1997) used an inoculation period of 34 days for seedlings, as the fungus needs a couple of weeks to make its way to the sapwood. In small seedlings, the inoculation period must not be too long, otherwise the infection will become so extensive that comparative assessment is difficult. When working with spruce trees between 22 and 23 years of age, Lindberg and Johansson (1991) harvested samples 70–72 days after inoculation. Many experiments on the Heterobasidion inoculation of mature Norway spruce trees (between 60 and 100 years old) have been carried out with incubation periods of 12–30 months (Delatour *et al.* 1998). In this study, an inoculation period of three months was chosen, in order to provide sufficient time for the resistance response to appear in mature trees – it is difficult to say whether this period was too long, or not long enough.

One major obstacle for this study was the logistics of sample collection. As Asiegbu *et al.* (2005) noted, “mature trees do not readily lend themselves to laboratory-based studies on account of their large size and long life span”. According to Lindberg and Johansson (1991), cutting down a tree dramatically affects the turgor of the sieve tubes in the phloem, which in turn creates favourable conditions for fungal activity and host oxidation. This effect could have been mitigated using water saturation, or by storing samples in liquid nitrogen; however, the resources required for this were not available in the field.

There could have been more replicates at each site. However, since logistics matters relating to mature trees are difficult, 10 replicates per site, as well as treatment at Lakkasuo, was a fair achievement, and provided sufficient results for statistical analysis. At Röykkä, it is possible that more replicates per clone and fewer clones in total would have given more reliable results. On the other hand, looking for a resistant genotype is like looking for a needle in a hay stack, which is why many genotypes must be screened before any differences between genetic origins can be observed.

Some clones exhibited a high standard deviation with regards the extent of their necrotic lesions, which means that the individual trees within a clone reacted differently to infection. This means that it would be useful to have not only more replicates per tree, but additionally to study traits of resistance other than necrotic response. The results of Baier *et al.* (2002) show that hypersensitivity after wounding and inoculation causes not only necrotic response but also remote defence reactions. According to the authors, this could at least partially explain the extraordinarily long necrotic reactions they observed. This study also found that some trees presented remarkably long necrotic reactions, which may indicate that these individuals were hypersensitive to inoculation.

The measurements taken by this study did not include the depth of necrosis. Width and superficial area of necrosis were measured, as well as the extent of the necrosis up, down, left and right of the point of inoculation. However, these measurements were not used in the analysis, only the total length of necrosis. The spatial distribution of the trees within the stand may have had an effect on resistance. The

shading effect, competition with other trees and topographic conditions may all have influenced the growth of the fungus or the resistance of the tree.

Several authors have created vigour indices which predict stand growth based on various tree measurements, such as height, diameter, basal area and living-crown size (Baier *et al*. 2002, Kytö *et al.* 1998, Swedjemark *et al.* 2001, Waring *et al.* 1980). Attempts have been made to find a correlation between these measurements and resistance factors; however, these attempts have been unsuccessful, and results have been inconsistent. The measures of growth employed by this study could have been further developed into a growth index, but it was decided that they should be used separately. Axial growth of trees should have been measured in the field in order to have more accurate results on annual axial growth than only an estimation.

For the re-isolation of *H. parviporum* from the tissues, the surface sterilisation method was perhaps too strong. The re-isolations should also be more comprehensive to represent all samples. However, since the difference between the extent of necrosis in *H. parviporum* infected and wounded control trees was statistically significant at all sites, it can be assumed that the higher necrosis is caused by *H. parviporum* and not by any other fungus.

6 CONCLUSIONS

Growing site does not have a significant effect on the resistance of Norway spruce to root and butt rot. This study could find no clear difference between the resistance responses of spruce trees growing on pristine mire, drained peatland or mineral soil. Even though the sample size was rather small, the results of this study suggest that Norway spruce trees growing on peatland are not markedly more resistant to *Heterobasidion parviporum* than those growing in mineral soil.

However, it is possible that the genetically heterogeneous tree material made it difficult to observe the effect of the site on the resistance responses. In the future, research should be carried out on other forest and peatland types – and in other parts of the country – in order to validate these findings. The measurement of necrotic response should be complemented with other methods for assessing tree resistance, such as decay testing and gene expression analysis of induced defence in tree tissues. Additionally, it would be interesting to test how tree material representing the same genetic origin would respond to *H. parviporum* infection when grown on the various sites.

There is no negative or positive correlation between the growth rate of a single tree and its resistance; therefore, no conclusion can be drawn as to whether fast- or slow-growing trees are more resistance than others. In subsequent research, perhaps more focus should be placed on allocation of a single tree: Are there any trade-offs between growth and resistance? How should these trade-offs be measured? The only factor affecting tree resistance seems to be the genotype of the tree. In Finnish forests, the trees all have different genotypes, as no industrial clonal forestry is practiced. This means that variation in resistance across trees, even those in the same area, may be high. Resistance breeding could, however, be a future solution for sites with a previous history of root and butt rot. Tree breeders want to find genotypes that simultaneously offer excellent growth and resistance – is it possible to find these traits in a single tree?

Or is infection just a matter of bad luck? What part does chance play in an individual tree becoming infected? For a disease to form, three things need to come together in time and space: a pathogen that is capable of overcoming the host's constitutive and induced defence mechanisms; a host that offers sufficient quantities of tissue to feed the pathogen; and an environment that favours the growth of the pathogen and/or complicates the survival of the host. Normally, the onset of disease requires the synchronisation of so many factors that the probability of infection is relatively low (Tainter and Baker 1996).

In Norway spruce, damage caused by *H. parviporum* cannot be seen before trees are harvested, at which point the most valuable part of the timber is already lost. For this reason, advance measures should be taken to prevent fungal infection, and the disease should not be permitted to spread to new areas. If peatland forests are proven not to be risk-free of infection from root and butt rot, there is a very real need to apply control methods to peatland forestry. This may be a serious problem, as stump treatments cost money, and, in Finland, these kinds of forest management operation are already beset by a lack of public funding (Niemi 2011). A great deal of peatland forest stands will be harvested soon, which makes the issue of root and butt rot control a topical issue. The risk that root and butt rot infection poses to uneven-aged forests growing on peatland should also be taken very seriously.

In the future, greater emphasis should perhaps be placed on soil properties and tree resistance, and the ability of *H. parviporum* to grow in peat should be further examined. What are the microbiological factors present in peat that inhibit the growth of the fungus, if any? Is peat able to inhibit the growth of the fungus even if there is a large amount of fresh wood surface available for the spores to germinate on? The more that is known about tree characteristics affecting resistance – as well as other factors related to disease development – the better forest management operations can be planned. If we know where, when and what to harvest and replant in order to avoid root and butt rot infection, we may be able to guarantee that growing timber for industrial use remains possible, and profitable, for forest owners.

REFERENCES

Agrios, G.N. 2005. Plant pathology. 5th edition. Elsevier Academic Press. Burlington-San Diego-London. 922 p.

Asiegbu, F.O., Adomas, A., & Stenlid, J. 2005. Conifer root and butt rot caused by Heterobasidion annosum (Fr.) Bref. s.l. Molecular Plant Pathology 6: 395-409.

Asiegbu, F.O., Daniel, G., Johansson, M. 1993. Studies on the infection of Norway spruce roots by Heterobasidion annosum. Canadian Journal of Botany 71: 1552-1561.

Asiegbu, F.O., Johansson, M., Woodward, S. & Hüttermann, A. 1998. Biochemistry of the host-parasite interaction. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 167-195.

Baier, P., Führer, E., Kirisits, T. & Rosner, S. 2002. Defence reactions of Norway spruce against bark beetles and the associated fungus Ceratocystis polonica in secondary pure and mixed species stands. Forest Ecology and Management 159: 73-86.

Bendz-Hellgren, M., Lipponen, K., Solheim, H. & Thomsen, I.M. 1998. The Nordic countries. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 333-345.

Blomqvist, A.G. 1891. Suomen puulajit metsänhoidolliselta kannalta. Keisarillisen senaatin kirjapaino. Helsinki. 165 s.

Boddy, L. & Rayner, A.D.M. 1983. Origins of decay in living deciduous trees: the role of moisture content and re-appraisal of the expanded concept of tree decay. New Phytologist 94: 623-641.

Daniel, G., Asiegbu, F.O. & Johansson, M. 1998. The saprotrophic wood-degrading abilities of Heterobasidion annosum intersterility groups P and S. Mycological Research 102: 991-997.

Delatour, C., von Weissenberg, K., & Dimitri, L. 1998. Host resistance. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 143-166.

Finnish Forest Research Institute. 1992. Metsänjalostuksen koeviljelys 657/01, form number 41 a/1992.

Flor, H.H. 1946. Genetics of pathogenicity in Melampsora lini. Journal of Agricultural Research 73: 335-357.

Gilbert, G.S. 2002. Evolutionary ecology of plant diseases in natural ecosystems. Annual Review of Phytopathology 40:13-43.

Hallaksela, A.-M. 1984. Causal agents of butt-rot in Norway spruce in southern Finland. Silva Fennica 18: 237-243.

Holdenrieder, O. & Greig, B.J.W. 1998. Biological Methods of Control. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 235-258.

Hüttermann, A. & Woodward, S. 1998. Historical aspects. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 1-41.

Hökkä, H., Kaunisto, S., Korhonen, K.T., Päivänen, J., Reinikainen, A. & Tomppo, E. 2002. Suomen suometsät 1951-1994. Metsätieteen aikakauskirja 2B/2002: 201-357.

Johansson, M. 1988. Pectic enzyme activity of spruce (S) and pine (P) strains of Heterobasidion annosum (Fr.) Bref. Physiological and Molecular Plant Pathology 33: 333-349.

Johansson, S.M., Lundgren, L.N., & Asiegbu, F.O. 2004. Initial reactions in sapwood of Norway spruce and Scots pine after wounding and infection by Heterobasidion parviporum and H. annosum. Forest Pathology 34: 197-210.

Johansson, S.M., Pratt. J.E. & Asiegbu, F.O. 2002. Treatment of Norway spruce and Scots pine stumps with urea against the root and butt rot fungus Heterobasidion annosum – possible modes of action. Forest Ecology and Management 157: 87-100.

Juntunen, M.-L. & Herrala-Ylinen, H. 2009. Silviculture. In: Finnish Statistical Yearbook of Forestry 2009. Finnish Forest Research Institute. Vammalan kirjapaino Oy. Sastamala. p. 107-152.

Järvinen, L. 1996. Puulajit. Suomen Metsäyhdistys ry. Painotalo Miktor. Helsinki. 43 p.

Kaarna-Vuorinen, L. 2000 Kuusen (Picea abies (L.) Karst.) lahoisuus, sen taloudelliset vaikutukset ja syyt Kaakkois-Suomen päätehakkuuleimikoissa. Publications from the Department of Forest economics. University of Helsinki. 82 p. In Finnish (Summary: Rot frequency and the ensuing economic losses, and the causes of butt-rot in final fellings on Norway spruce (Picea abies (L.) Karst.) stands in south-eastern Finland.

Kallio, T. 1970. Aerial distribution of the root-rot fungus Fomes annosus (Fr.) Cooke in Finland. Acta Forestalia Fennica 107: 1-55.

Kallio, T. & Norokorpi, Y. 1972. Kuusen tyvilahoisuus. Silva Fennica 6: 39-51. (In Finnish. Summary: Butt rot in a spruce stand)

Kalliokoski, T. 2011. Root system traits of Norway spruce, Scots pine and silver birch in mixed boreal forests: an analysis of root architecture, morphology and anatomy. Department of Forest Sciences. 67 p.

Kansallinen metsäohjelma 2015 – metsäalasta biotalouden vastuullinen edelläkävijä. 2011. Ministry of Agriculture and Forestry. Juvenes Print. 52 p.

Kasanen, R. 2009. Metsäpuiden sienitaudit. Metsäkustannus Oy. 221 p.

Korhonen, K., Capretti, P., Karjalainen, R. & Stenlid, J. 1998. Distribution of Hetrobasidion annosum Intersterility Groups in Europe. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 93-104.

Korhonen, K., Delatour, C., Greig, B.J.W. & Schönhar, S. 1998. Silvicultural control. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. p. 283-313.

Korhonen, K., Lipponen, K. & Mäkelä, M. 2000. Laho ja sen torjunta. Finnish Forest Research Institute & Metsäteho Oy. Tuokinprint Oy. Helsinki.

Korhonen, K., Lipponen, K. & Piri, T. 2010. Kuusen tyvilaho. Finnish Forest Research Institute. Available at: http://www.metla.fi/metinfo/metsienterveys/lahontorjunta/kuusi-tyvilaho.htm. [Cited 16 March 2011].

Korhonen, K. & Stenlid, J. 1998. Biology of Heterobasidion annosum. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 43-70.

Kytö, M., Niemelä, P. & Annila, E. 1996. Vitality and bark beetle resistance of fertilized Norway spruce. Forest ecology and management 84: 149-157.

Kytö, M., Niemelä, P. & Annila, E. 1998. Effects of vitality fertilization on the resin flow and vigour of Scots pine in Finland. Forest Ecology and Management 102: 121-130.

Kärkkäinen, M. 2007. Puun rakenne ja ominaisuudet. Metsäkustannus Oy. Karisto Oy. 468 s.

Laiho, R. 2008. From a mire ecosystem to a drained peatland forest ecosystem. In: Korhonen, R., Korpela, L. & Sarkkola, S. (eds.) Finland – Fenland. Maahenki Ltd. Finnish Peatland Society. p. 152-157.

Laine, J., Komulainen, V.-M., Laiho, R., Minkkinen, K., Rasinmäki, A., Sallantaus, T., Sarkkola, S., Silvan, N., Tolonen, K., Tuittila, E.-S., Vasander, H. & Päivänen, J. 2004. Lakkasuo – a guide to mire ecosystem. Publications from the Department of Forest Ecology, University of Helsinki 31. Helsinki. 123 p.

Laine. J., Minkkinen, K., Laiho, R., Tuittila, E-S. & Vasander, H. 2000. Suokasvit – turpeen tekijät. University of Helsinki. Publications from the Department of Forest Ecology 24: 1-55.

Laine, J. & Vasander, H. 2008. Suotyypit ja niiden tunnistaminen. 2nd edition. Metsäkustannus Oy. Karisto Oy. Hämeenlinna. 110 p.

Lindberg, M. & Johansson, M. 1991. Growth of Heterobasidion annosum through bark of Picea abies. European Journal of Forest Pathology 21: 377-388.

Lindberg, M., & Johansson, M. 1992. Resistance of Picea abies seedlings to infection by Heterobasidion annosum in relation to drought stress. European Journal of Forest Pathology 22: 115-124.

Mattila, U., & Nuutinen, T. 2007. Assessing the incidence of butt rot in Norway spruce in Southern Finland. Silva Fennica 41: 29-43.

McDonald, B.A. & Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology 40: 349-379.

Merrill,W. 1992. Mechanisms of resistance to fungi in woody plants. a historical perspective. In: Blanchette, R.A. & Biggs, A.R. Defense mechanisms of woody plants against fungi. Springer-Verlag, Berlin, Heidelberg. p. 1-11.

Mitchelson, K. & Korhonen, K. 1998. Diagnosis and Differentiation of Intersterility Groups. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 71-92.

Napola, M-L. 1999. Kuusen V-kloonien testaustuloksia – vuosina 1969-74 valitut aineistot. Metsänjalostussäätiön työraportteja 62. Foundation for Forest Tree Breeding.

Niemelä, T. 2005. Käävät – puiden sienet. Botanical Museum, Finnish Museum of Natural History, Helsinki. Helsinki University Press. 320 p.

Niemelä, T. & Korhonen, K. 1998. Taxonomy of the Genus Heterobasidion. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 27-33.

Niemi, S. 2011. Juurikäävän torjunnan määrärahat lopussa. Maaseudun Tulevaisuus, 20 June. p. 18.

Nuutinen, T., Hirvelä, H., Hynynen, J., Härkönen, K., Hökkä, H., Korhonen, K.T. & Salminen, O. 2000. The role of peatlands in Finnish wood production – an analysis based on large-scale forest scenario modelling. Silva Fennica 34: 131-153.

Pariaud, B., Ravigne, V., Halkett, F., Goyeau, H., Carlier, J., Lannou, C. 2009. Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology 58: 409-424.

Pearce, R.B. 1996. Antimicrobial defences in the wood of living trees. New Phytologist 132: 203-233.

Peltola, A. & Ihalainen, A. 2009. Forest resources. In: Finnish Statistical Yearbook of Forestry 2009. Finnish Forest Research Institute. Vammalan kirjapaino Oy. Sastamala. p. 29-78.

Piri, T. 1996. The spreading of the S type of Heterobasidion annosum from Norway spruce stumps to the subsequent tree stand. European Journal of Forest Pathology 26: 193-204.

Piri, T. 2003. Early development of root rot in young Norway spruce planted on sites infected by Heterobasidion in southern Finland. Canadian Journal of Forest Research 33: 604-611.

Piri, T. & Korhonen, K. 2001. Infection of advance regeneration of Norway spruce by Heterobasidion parviporum. Canadian Journal of Forest Research 31: 937-942.

Piri, T. & Korhonen, K. 2007. Spatial distribution and persistence of Heterobasidion parviporum genets on a Norway spruce site. Forest Pathology 37: 1-8.

Piri, T., Korhonen, K. & Sairanen, A. 1990. Occurrence of Heterobasidion annosum in pure and mixed spruce stands in southern Finland. Scandinavian Journal of Forest Research 5: 113-125.

Pratt, J.E., Johansson, M. & Hüttermann, A. 1998. Chemical control of Heterobasidion annosum. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 259-282.

Päivänen, J. 2007. Suot ja suometsät – järkevän käytön perusteet. Metsäkustannus Oy. Karisto Oy, Hämeenlinna. 368 p.

Rayner, A.D.M. & Boddy, L. 1988. Fungal Decomposition of Wood. A Wiley-Interscience publication. John Wiley & Sons Ltd. Chichester-New York-Brisbane-Toronto-Singapore. 587 p.

Redfern, D.B. 1984. Factors affecting spread of Heterobasidion annosum in plantations. In: Kile, G.A. Proceedings of the sixth international conference on root and butt rots of forest trees. CSIRO, Melbourne, Australia. p. 104-114.

Redfern, D.B. & Stenlid, J. 1998. Spore Dispersal and Infection. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 105-124.

Sarvas, R. 1964. Havupuut. WSOY. Porvoo. 518 p.

Schmidt, A., Zeneli, G., Hietala, A.M., Fossdal, G.-G., Krokene, E., Christiansen, E. & Gershenzon, J. 2005. Induced chemical defences in conifers: biochemical and molecular approaches to studying their function. In: Romeo, J.T. Chemical Ecology and Phytochemistry in Forest ecosystems. Elsevier, Amsterdam. p. 1-28.

Shain, L. 1971. The response of sapwood of Norway spruce to infection by Fomes annosus. Phytopathology 61: 301-307.

Shigo, A.L. 1984. Compartmentalization. a conceptual framework for understanding how trees grow and defend themselves. Annual Review of Phytopathology 22:189-214.

Solla, A., Tomlinson, F., & Woodward, S. 2002. Penetration of Picea sitchensis root bark by Armillaria mellea, Armillaria ostoyae and Heterobasidion annosum. Forest Pathology 32: 55-70.

Stenlid, J. & Redfern, D.B. 1998. Spread within the tree and stand. In: Woodward, S., Stenlid, J., Karjalainen, R. & Hüttermann, A. (eds.) Heterobasidion annosum Biology, Ecology, Impact and Control. Wallingford, UK. CAB International. p. 125-142.

Swedjemark, G. 1995. Heterobasidion annosum root rot in Picea abies: variability in aggressiveness and resistance. Swedish University of Agricultural Sciences. Uppsala. 45 p.

Swedjemark, G., & Karlsson, B. 2004. Genotypic variation in susceptibility following artificial Heterobasidion annosum inoculation of Picea abies clones in a 17-year-old field test. Scandinavian Journal of Forest Research 19: 103-111.

Swedjemark, G., & Stenlid, J. 1997. Between-tree and between-isolate variation for growth of S-group Heterobasidion annosum in sapwood of Picea abies cuttings. Canadian Journal of Forest Research 27: 711-715.

Swedjemark, G., Stenlid, J. & Karlsson, B. 1997. Genetic variation among clones of Picea abies in resistance to growth of Heterobasidion annosum. Silvae Genetica 46: 369-376.

Swedjemark, G., Stenlid, J. & Karlsson, B. 2001. Variation in growth of Heterobasidion annosum among clones of Picea abies incubated for different periods of time. Forest Pathology 31: 163-175.

Tainter, F.H. & Baker, F.A. 1996. Principles of forest pathology. John Wiley & Sons, Inc. New York. 805 p.

Thompson, J.N. & Burdon, J.J. 1992. Gene-for-gene coevolution between plants and parasites. Nature 360: 121-125.

Tomppo, E. 2005. Suomen suometsät 1951-2003. Metsäntutkimuslaitoksen tiedonantoja 947:26-38.

Tomppo, E., Heikkinen, J., Henttonen, H.M., Ihalainen, A., Katila, M., Mäkelä, H., Tuomainen, T. & Vainikainen, N. 2011. Designing and conducting a forest inventory – case: 9th National Forest Inventory of Finland. Managing Forest Ecosystems 22: 1-269.

Valkonen, S. 2005. Puulajit. In: Rantala, S. (ed.) Metsäkoulu. 5th edition. Metsäkustannus Oy. Karisto Oy. Hämeenlinna. p. 35-54.

Valkonen, S. & Hallikainen, V. 2006. In: Jalonen, R., Hanski, I., Kuuluvainen, T., Nikinmaa, E., Pelkonen, P., Puttonen, P., Raitio, K. & Tahvonen, O. (eds.) Uusi Metsäkirja. Gaudeamus. Helsinki. p. 254-261.

Valkonen, S. 2010. Missä edellytyksiä, mihin sopii? In: Valkonen, S., Sirén, M., Piri, T. (eds.) Poiminta- ja pienaukkohakkuut – vaihtoehtoja avohakkuulle. Metsäkustannus Oy, Tampere. 125 s.

Van der Plank, J. E. 1984. Disease resistance in plants. 2nd edition. Academic Press, Inc. Orlando, Florida. 194 p.

Vasander, H. & Laine, J. 2008. Site type classification on drained peatlands. In: Korhonen, R., Korpela, L. & Sarkkola, S. (eds.) Finland – Fenland. Maahenki Ltd. Finnish Peatland Society. p. 146-151.

Vasaitis, R., Stenlid, J., Thomsen, I.M., Barklund, P. & Dahlberg, A. 2008. Stump removal to control root rot in forest stands. A literature study. Silva Fennica 42: 457-458.

Virtanen, K. 2008. The origin and development of mires since the last ice age. In: Korhonen, R., Korpela, L. & Sarkkola, S. (eds.) Finland – Fenland. Maahenki Ltd. Finnish Peatland Society. p. 12-20.

Väre, H. & Kiuru, H. 2006. Suomen puut ja pensaat. Metsäkustannus Oy. Karisto Oy. Hämeenlinna. 249 p.

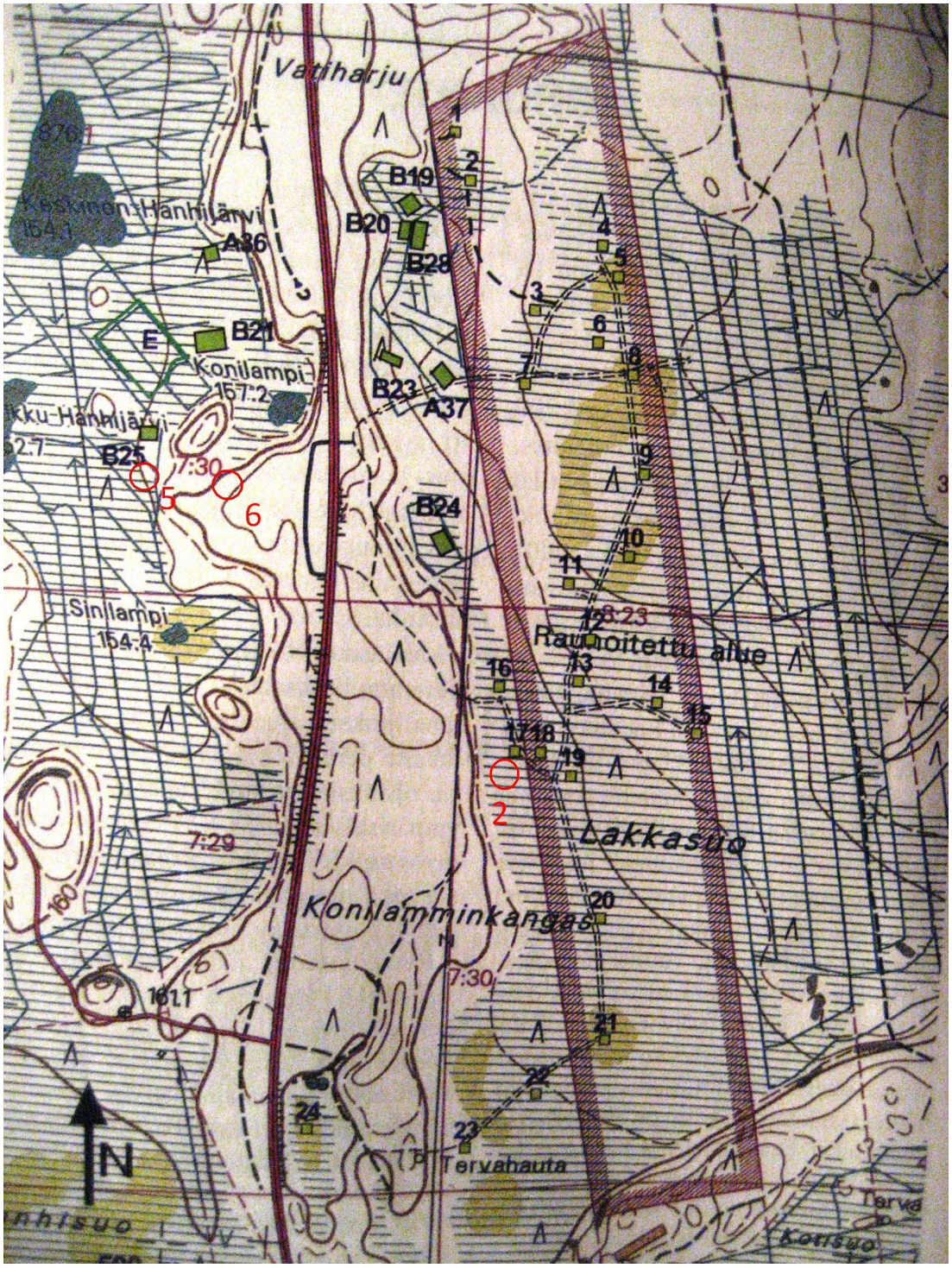
Woodward, S. 1992. Responses of gymnosperm bark tissues to fungal infections. In: Blanchette, R.A. & Biggs, A.R. Defense mechanisms of woody plants against fungi. Springer-Verlag, Berlin, Heidelberg. p. 62-75.

Woodward, S., Bianchi, S., Bodles, W.J.A., Beckett, L. & Michelozzi, M. 2007. Physical and chemical responses of Sitka spruce (Picea sitchensis) clones to colonization by Heterobasidion annosum as potential markers for relative host susceptibility. Tree Physiology 27: 1701-1710.

Waring, R.H., Thies, W.G., Muscato, D. 1980. Stem growth per unit of leaf area: a measure of tree vigor. Forest Science: 26:112-117.

Appendix 1

Map of Lakkasuo, Orivesi. The sites are marked with red circles. Number 2 refers to V. myrtillus spruce swamp (pristine mire), number 5 to drained V. myrtillus site type 1 (drained peatland) and number 6 to Myrtillus type forest (mineral soil).



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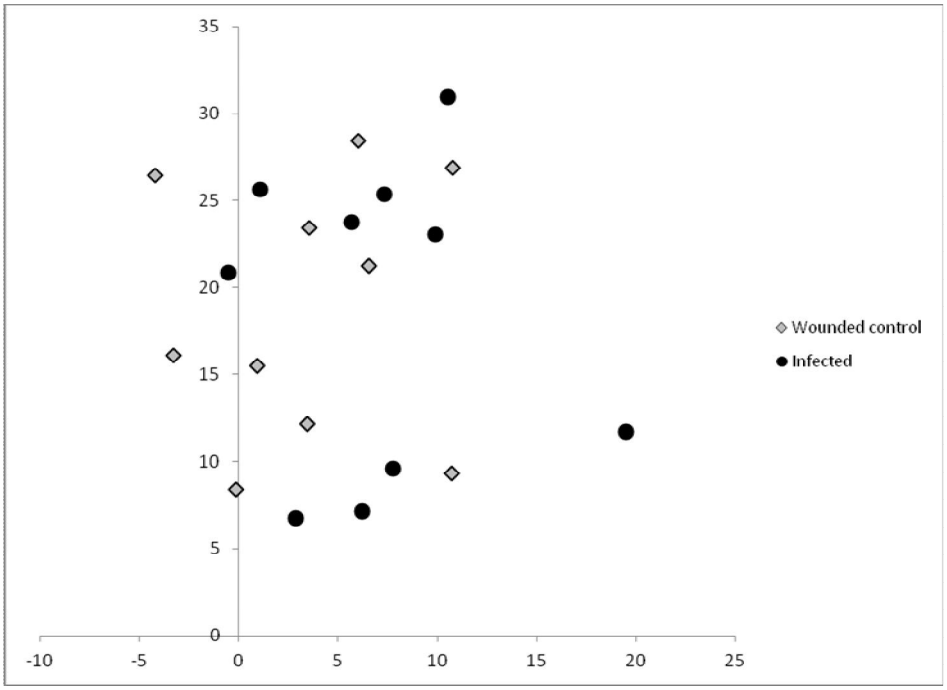
Appendix 2a-c

Measurements and spatial distribution of experimental trees in the sites of Lakkasuo.

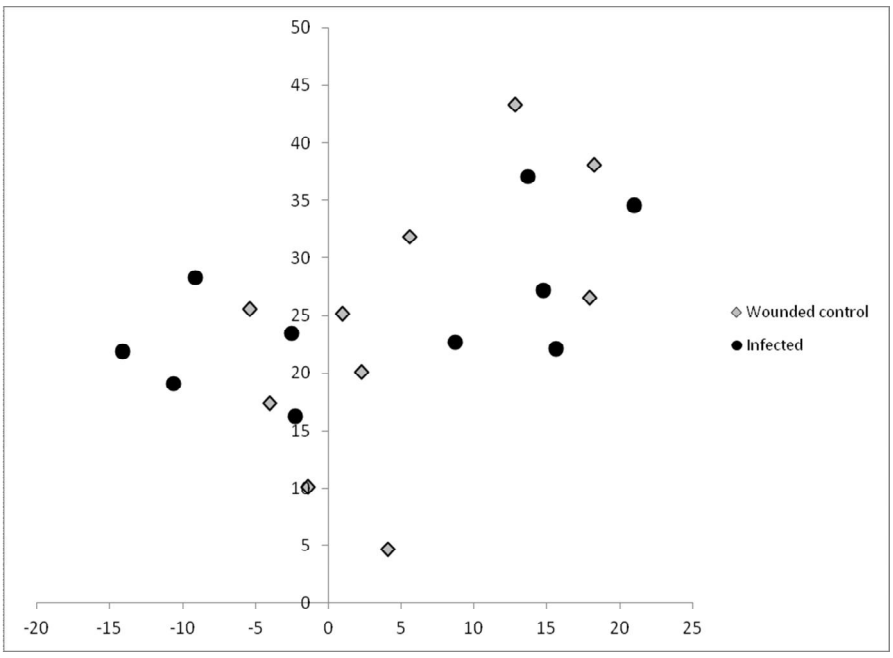
a) Site 2, Vaccinium myrtillus spruce swamp.

Tree no	Treatment	Label	D1.3, cm	Tree height, m	Living crown height, m	Age, yrs
1	H	2-H-1	17.8	14.8	7.3	114
15	H	2-H-2	23.5	18.9	8.8	
22	H	2-H-3	14.1	16.2	5.7	131
9	H	2-H-4	22.2	18.2	9.5	140
19	H	2-H-5	8.8	6.4	2.2	100
38	H	2-H-6	11.5	9.5	1.9	167
4	H	2-H-7	13.7	13.1	3.7	152
74	H	2-H-8	12	10.2	3.2	139
79	H	2-H-9	16.4	13.8	6	149
88	H	2-H-10	10.4	10.6	4.5	135
6	WC	2-WC-1	16.2	15.8	6.2	138
14	WC	2-WC-2	15.4	14	4.7	143
2	WC	2-WC-3	10.7	10.1	3.4	107
20	WC	2-WC-4	14.3	14.7	7.9	136
48	WC	2-WC-5	15	14.9	7.1	131
86	WC	2-WC-6	10.9	8.9	1.7	113
35	WC	2-WC-7	22.4	17.1	7.3	133
51	WC	2-WC-8	16.7	15.5	6	136
95	WC	2-WC-9	9.3	7.7	3.4	124
65	WC	2-WC-10	11.4	9.9	3.7	126
Average			14.6	13.0	5.2	132



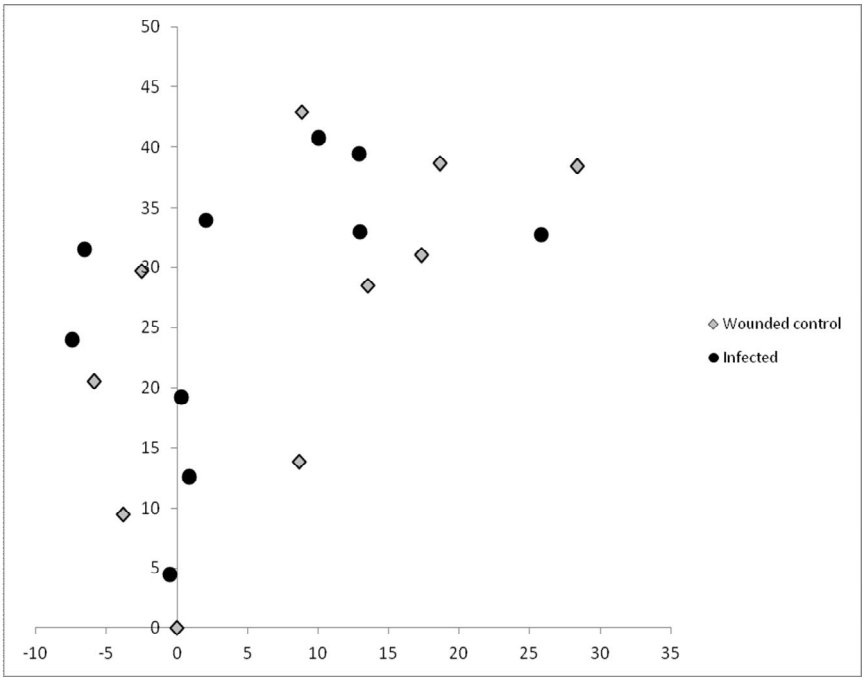
b) Site 5, Drained V. myrtillus site type 1.

Tree no	Treatment	Label	D1.3. cm	Tree height, m	Living crown height, m	Age, yrs
6	H	5-H-1	29	23.4	10.8	139
19	H	5-H-2	29.1	24.2	12.4	125
21	H	5-H-3	28	24.9	16.5	142
32	H	5-H-4	32.6	25.9	14.5	132
36	H	5-H-5	25.7	23.6	9.2	122
38	H	5-H-6	19.7	16.4	10.0	120
39	H	5-H-7	26.6	20.7	12.5	168
47	H	5-H-8	21	18.6	8.8	52
55	H	5-H-9	15.1	11.8	4.9	131
58	H	5-H-10	25.4	22.5	12.1	139
4	WC	5-WC-1	24.2	19.0	10.1	124
12	WC	5-WC-2	31.3	22.6	7.2	110
17	WC	5-WC-3	25	22.0	10.6	141
23	WC	5-WC-4	28.5	25.8	9.8	122
30	WC	5-WC-5	23.6	22.7	13.2	124
34	WC	5-WC-6	28.5	19.9	10.9	146
44	WC	5-WC-7	25.7	21.0	14.0	120
57	WC	5-WC-8	15.9	12.9	8.0	118
63	WC	5-WC-9	28.2	23.3	16.2	139
67	WC	5-WC-10	19.1	19.5	10.5	233
Average			25.1	21.0	11.1	132



c) Site 6, Myrtillus type mineral soil.

Tree no	Treatment	Label	D1.3, cm	Tree height, m	Living crown height, m	Age, yrs
14	H	6-H-1	14	15.5	6.6	109
26	H	6-H-2	21.1	19.1	9.4	114
40	H	6-H-3	15	12	5.1	108
53	H	6-H-4	14.7	16	7.7	116
73	H	6-H-5	12.5	11	5.4	103
82	H	6-H-6	28.5	23.1	10.4	
96	H	6-H-7	10.3	9.3	3.5	106
108	H	6-H-8	12.5	10.9	2.1	88
113	H	6-H-9	12.2	13.3	5.9	98
114	H	6-H-10	10.8	11.6	2.9	85
1	WC	6-WC-1	15.6	16.2	6.4	115
15	WC	6-WC-2	10.3	10.2	5.3	108
19	WC	6-WC-3	20.2	22	12	111
29	WC	6-WC-4	10.5	10.3	2.1	106
50	WC	6-WC-5	27.9	24.2	17.2	112
62	WC	6-WC-6	15.2	13.1	7.1	115
68	WC	6-WC-7	10.8	10.3	2.5	111
69	WC	6-WC-8	15	14.6	7.6	102
86	WC	6-WC-9	13.7	14	4	95
100	WC	6-WC-10	11.8	12.8	4.6	111
Average			15.1	14.5	6.4	106



Appendix 3

Information on the Norway spruce clones in Röykkä.

Clone	Origin code	Geographic origin	Mother x father	Biological age, yrs	Number of wounded trees	Number of infected trees
12	V48	Miehikkälä (FIN)	68-92-44*	42	2	3
14**	V304	Loppi (FIN)	68-H 3753 x E 2653	42	2	2
15	V315	Loppi (FIN)	68-E 7079*	42	2	2
16	V323	Hronov (CZE)	Pc-Cs-547*	43	2	2
20	V330	Novgorod (RUS)	66-517*	43	2	2
27	V374	Pieksämäki (FIN)	K 1399 x K 1398	43	2	3
28	V375	Pieksämäki (FIN)	K 1398 x K 1395	43	2	2
31	V477	Loppi (FIN) x Schmiedewald (GER)	H 3499 x E 4277	40	2	3
35**	V481	Loppi (FIN) x Schielbach (GER)	H 3367 x E 4297	40	2	3
36	V483	Loppi (FIN) x Carlsfeld (GER)	H 3505 x E 4284	40	2	2
39**	V488	Loppi (FIN) x Schielbach (GER)	E 5708 x E 4324	40	2	2
45**	V494	Loppi (FIN)	68-H 5181*	40	3	2
54**	V3012	Loppi (FIN)	68-H 3550*	40	3	2
60**	V3027	Loppi (FIN)	68-H 3882*	41	2	2
62	V3031	Loppi (FIN)	68-H 3024*	41	2	2

*Ramets taken from open-pollinated progenies (father tree unknown). **Excluded from the statistical analyses.

Appendix 4

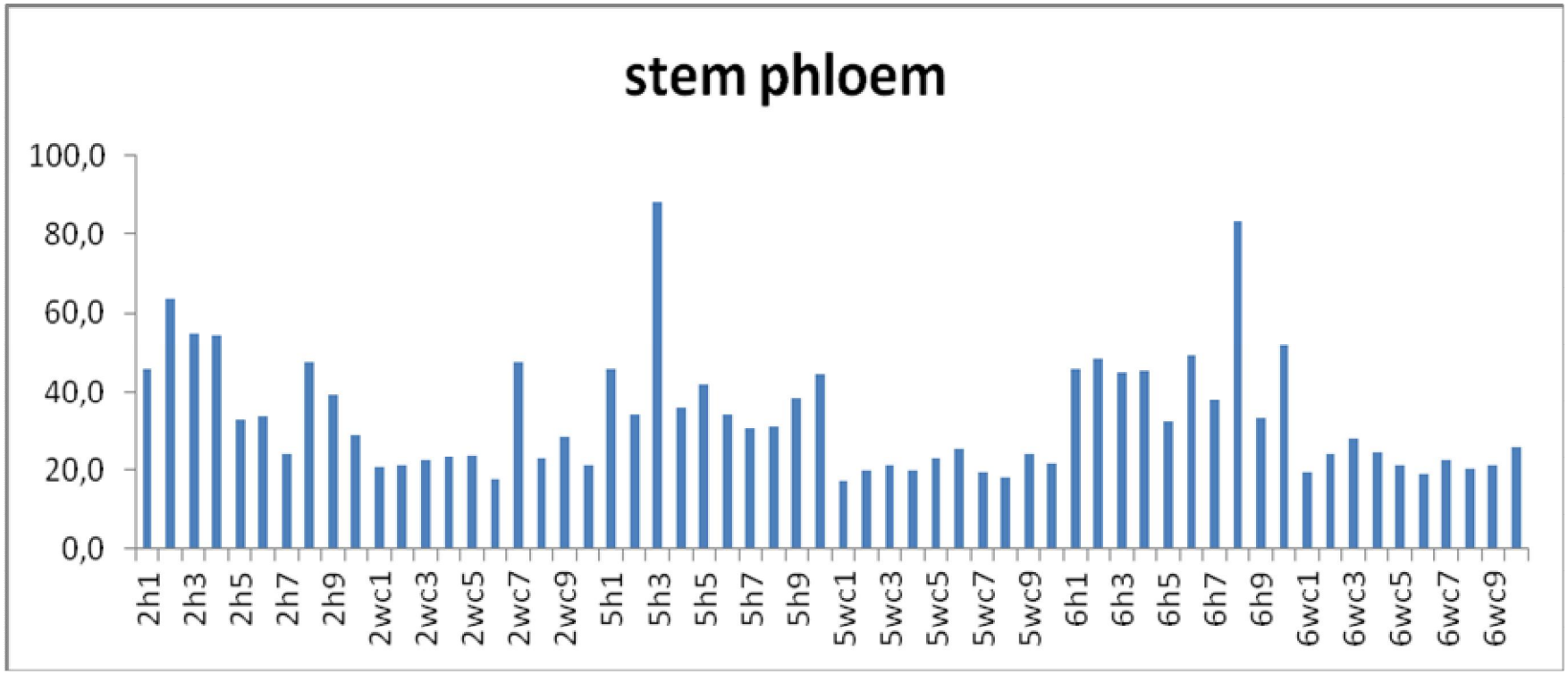
Measurements of experimental trees in Röykkä.

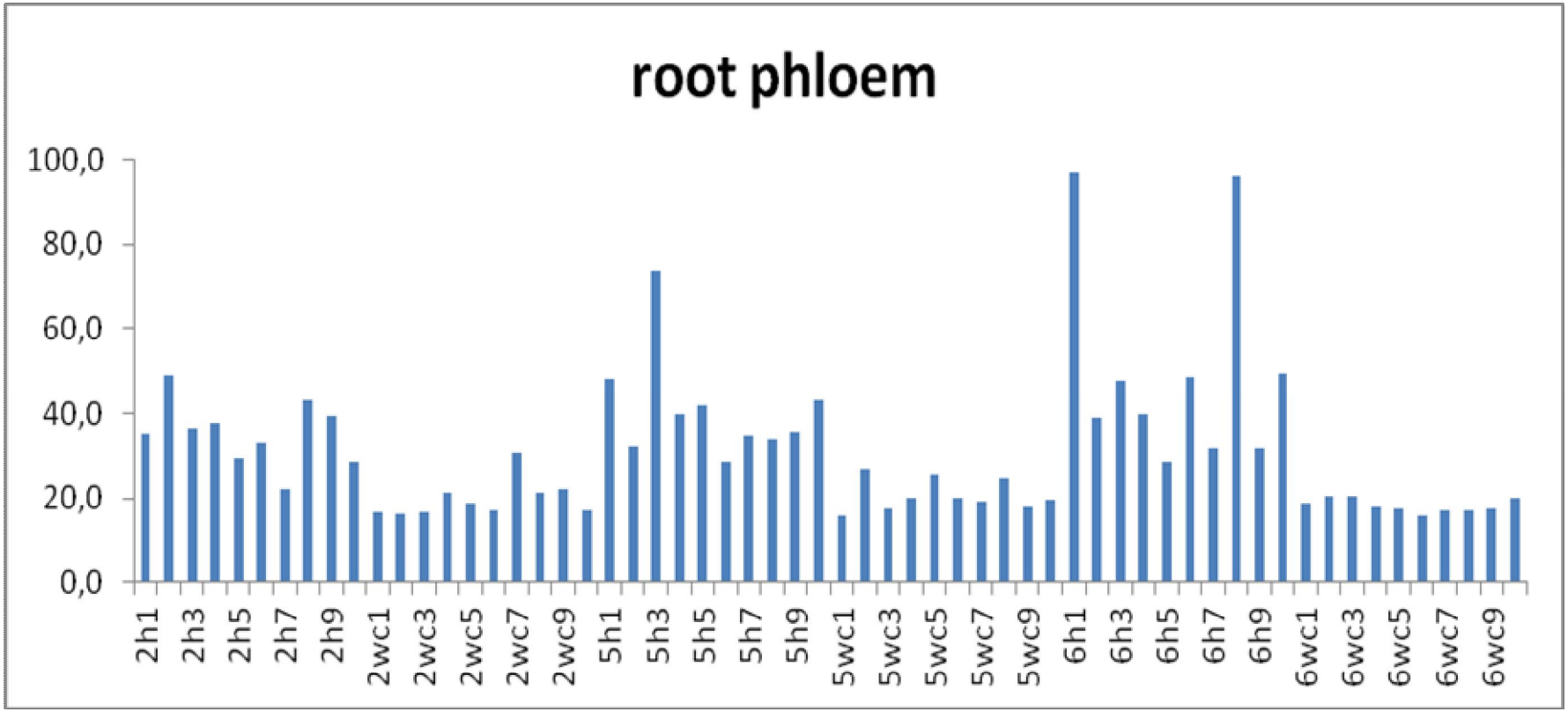
Clone number	Treatment	Label	D1.3, cm	Tree height, m	Living crown height, m
12	H	12-H-1	12.9	18.0	9.0
12	H	12-H-2	16.4	19.0	10.0
12	H	12-H-3	18.7	19.0	12.5
14	H	14-H-1	14.3	17.0	9.0
14	H	14-H-2	15.4	16.5	10.5
15	H	15-H-1	8.9	12.5	7.0
15	H	15-H-2	14.5	18.0	8.5
16	H	16-H-1	13.5	16.0	7.0
16	H	16-H-2	8.9	11.0	3.0
20	H	20-H-1	14.9	13.0	4.0
20	H	20-H-2	13.0	15.0	9.5
27	H	27-H-1	9.8	15.0	6.0
27	H	27-H-2	12.8	16.0	10.5
27	H	27-H-3	14.1	15.5	8.0
28	H	28-H-1	9.1	16.0	7.5
28	H	28-H-2	13.2	17.5	10.0
31	H	31-H-1	13.2	16.5	7.5
31	H	31-H-2	11.4	15.0	7.0
31	H	31-H-3	12.3	16.5	8.5
35	H	35-H-1	6.2	10.0	5.5
35	H	35-H-2	11.8	15.5	9.5
35	H	35-H-3	11.0	15.0	9.0
36	H	36-H-1	19.2	20.5	11.0
36	H	36-H-2	13.9	20.0	11.5
39	H	39-H-1	20.4	18.5	9.0
39	H	39-H-2	8.4	13.0	5.0
45	H	45-H-1	17.3	19.5	10.5
45	H	45-H-2	12.8	16.5	7.5
54	H	54-H-1	16.7	19.0	8.0
54	H	54-H-2	12.9	18.5	10.5
60	H	60-H-1	9.4	12.0	4.5
60	H	60-H-2	14.8	16.5	7.5
62	H	62-H-1	9.2	12.5	5.5
62	H	62-H-2	14.9	17.0	10.5

12	WC	12-WC-1	13.5	17.0	7.0
12	WC	12-WC-2	18.4	19.5	10.0
14	WC	14-WC-1	17.5	19.0	9.0
14	WC	14-WC-2	13.9	19.5	11.5
15	WC	15-WC-1	13.5	18.0	10.0
15	WC	15-WC-2	9.6	13.5	6.5
16	WC	16-WC-1	11.1	13.5	6.5
16	WC	16-WC-2	8.7	10.5	3.0
20	WC	20-WC-1	12.7	16.0	6.5
20	WC	20-WC-2	16.0	17.0	10.5
27	WC	27-WC-1	16.4	18.0	10.0
27	WC	27-WC-2	12.3	16.0	8.0
28	WC	28-WC-1	14.5	18.0	9.0
28	WC	28-WC-2	9.0	15.0	7.5
31	WC	31-WC-1	9.4	12.5	8.0
31	WC	31-WC-2	14.0	16.5	8.5
35	WC	35-WC-1	9.7	15.0	9.0
35	WC	35-WC-2	12.5	18.0	10.5
36	WC	36-WC-1	15.6	20.5	11.5
36	WC	36-WC-2	20.1	20.0	11.5
39	WC	39-WC-1	16.2	18.0	7.0
39	WC	39-WC-2	11.2	16.0	7.0
45	WC	45-WC-1	15.5	16.5	5.5
45	WC	45-WC-2	13.6	16.5	7.5
45	WC	45-WC-3	12.4	17.0	6.0
54	WC	54-WC-1	12.7	19.0	9.0
54	WC	54-WC-2	13.6	18.5	12.0
54	WC	54-WC-3	14.3	18.5	10.0
60	WC	60-WC-1	9.8	13.0	5.5
60	WC	60-WC-2	14.3	16.0	9.0
62	WC	62-WC-1	15.6	18.5	10.5
62	WC	62-WC-2	9.8	14.5	5.5
Average			13.2	16.4	8.3

Appendix 5

Mean length of necrosis (mm) in experimental trees in Lakkasuo.

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2h5
2h7
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2wc3
2wc5
2wc7
2wc9
5h1
5h3
5h5
5h7
5h9
5wc1
5wc3
5wc5
5wc7
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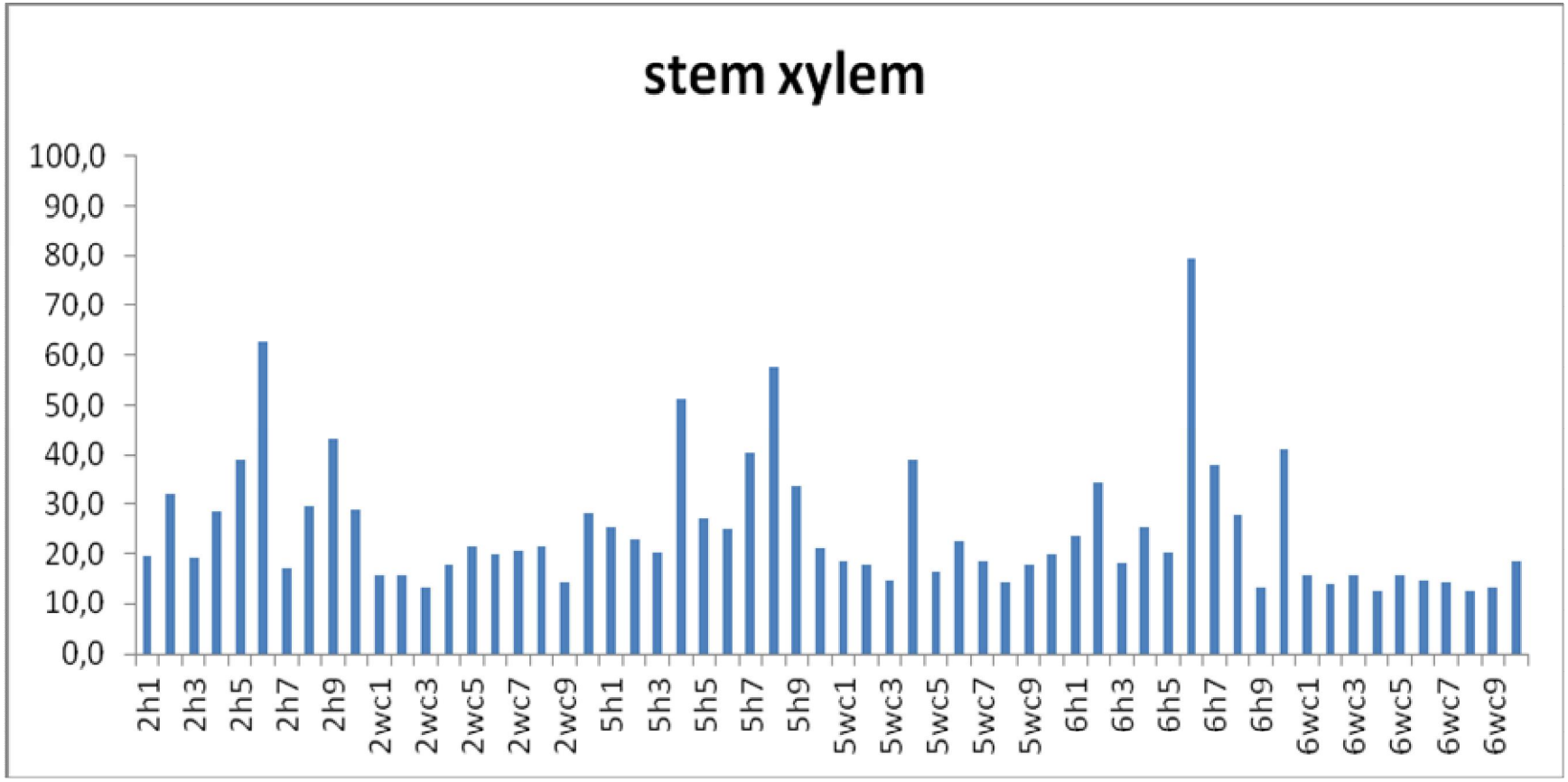
6h3
6h5
6h7
6h9
6wc1
6wc3
6wc5
6wc7
6wc9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20,0
10,0
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2h1
2h3
2h5
2h7
2h9
2wc1
2wc3
2wc5
2wc7
2wc9
5h1
5h3
5h5
5h7
5h9
5wc1
5wc3
5wc5
5wc7
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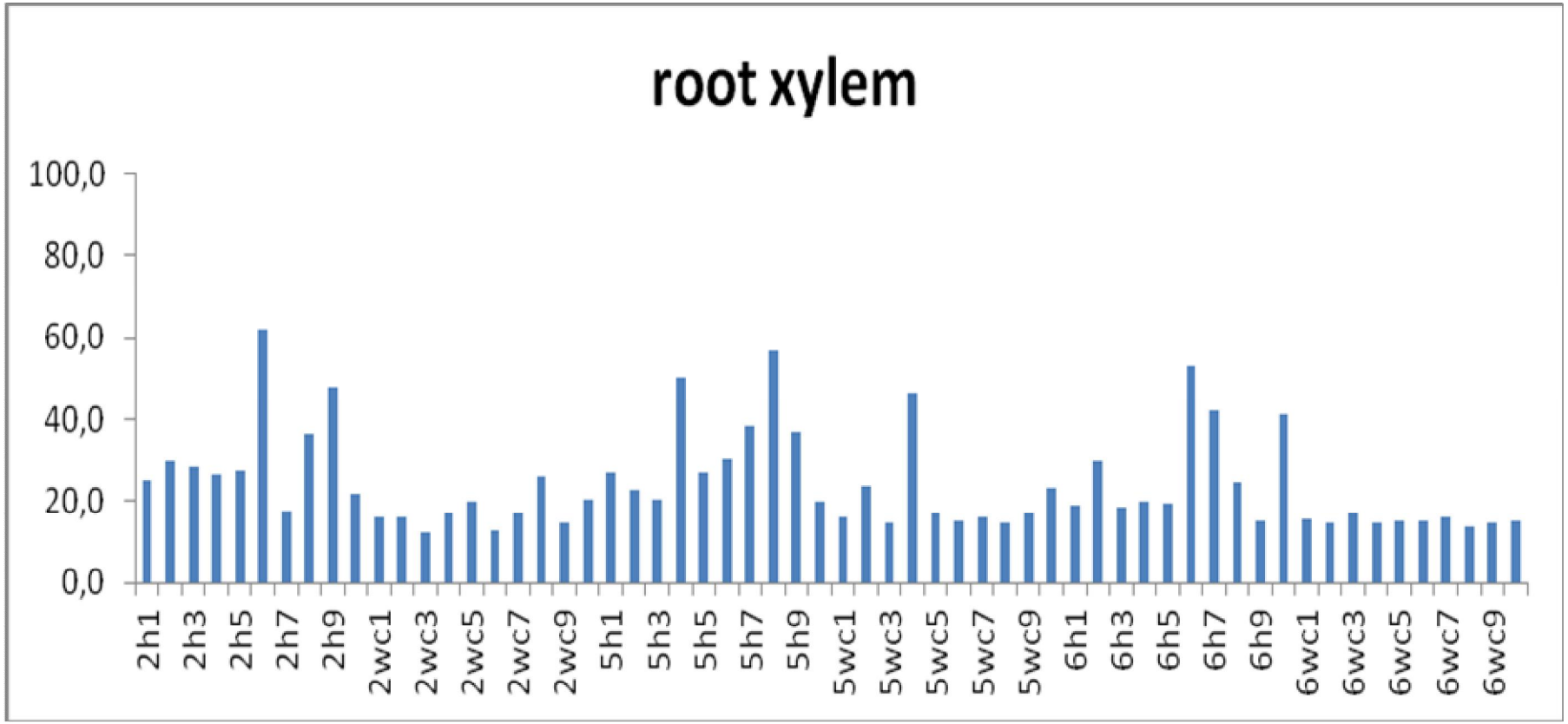
6h9
6wc1
6wc3
6wc5
6wc7
6wc9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2h3
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2h7
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2wc3
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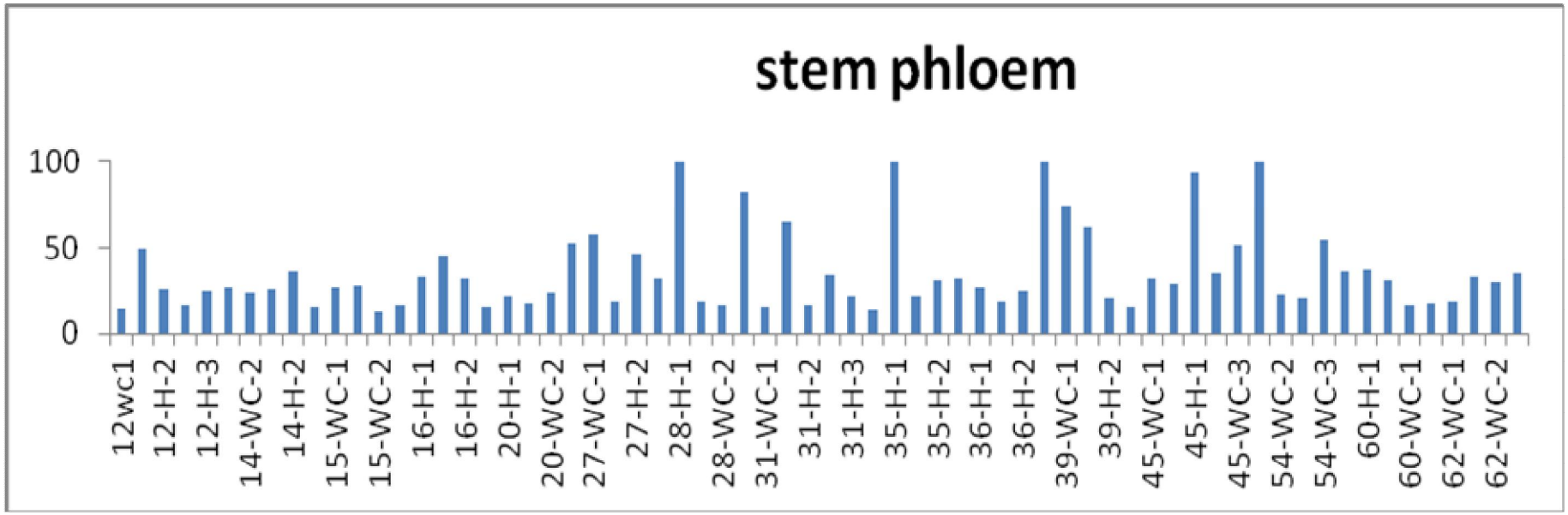
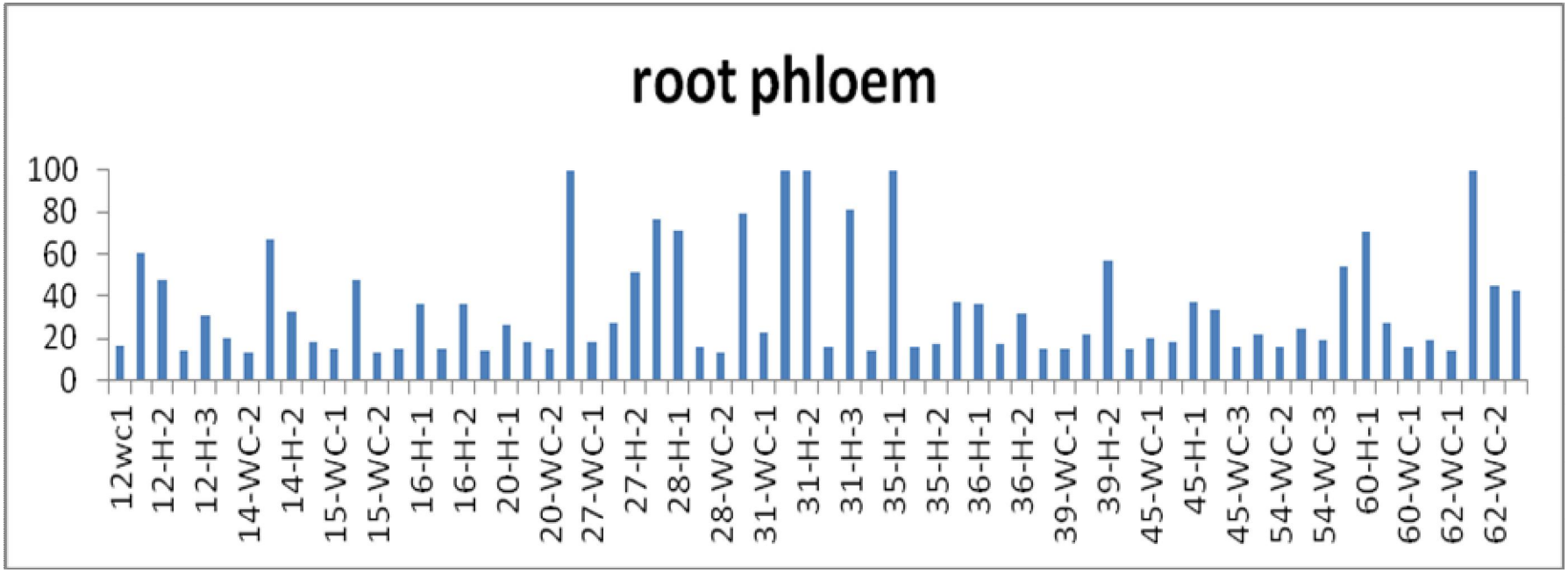
2wc7
2wc9
5h1
5h3
5h5
5h7
5h9
5wc1
5wc3
5wc5
5wc7
5wc9
6h1
6h3
6h5
6h7
6h9
6wc1
6wc3
6wc5
6wc7
6wc9

Appendix 6

Mean length of necrosis (mm) in experimental trees in Röykkä.

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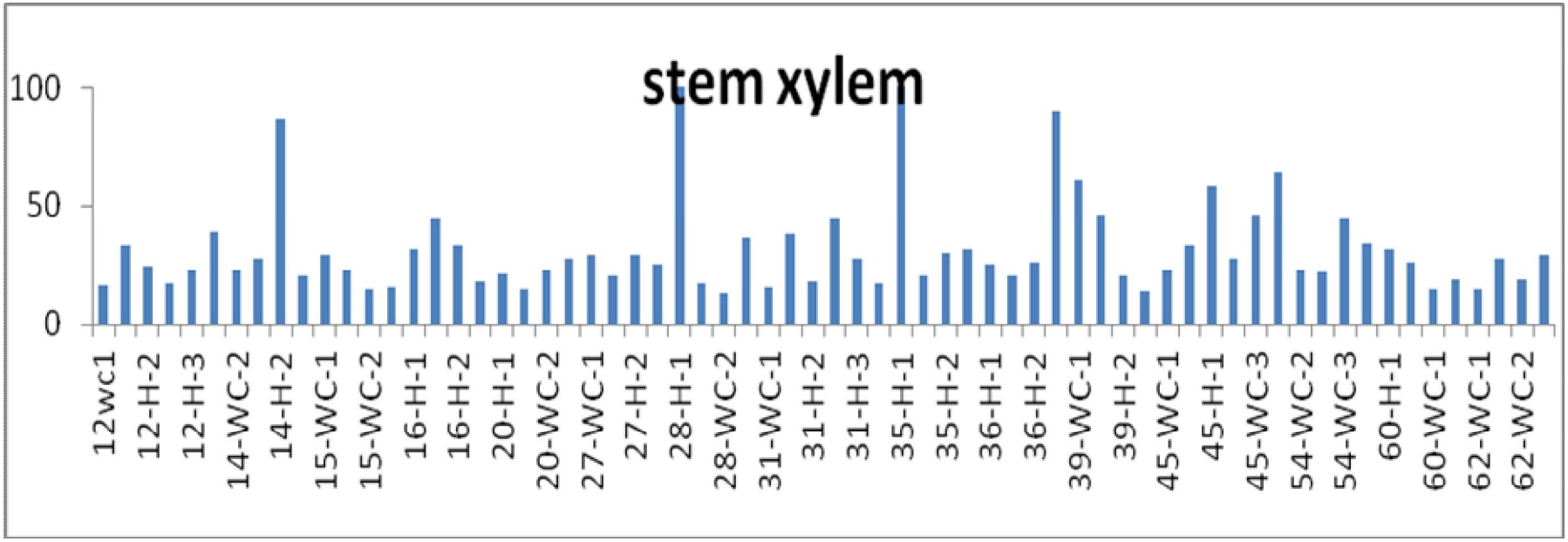
62-WC-2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15-WC-2
16-H-1
16-H-2
20-H-1
20-WC-2
27-WC-1
27-H-2
28-H-1
28-WC-2
31-WC-1
31-H-2
31-H-3
35-H-1
35-H-2
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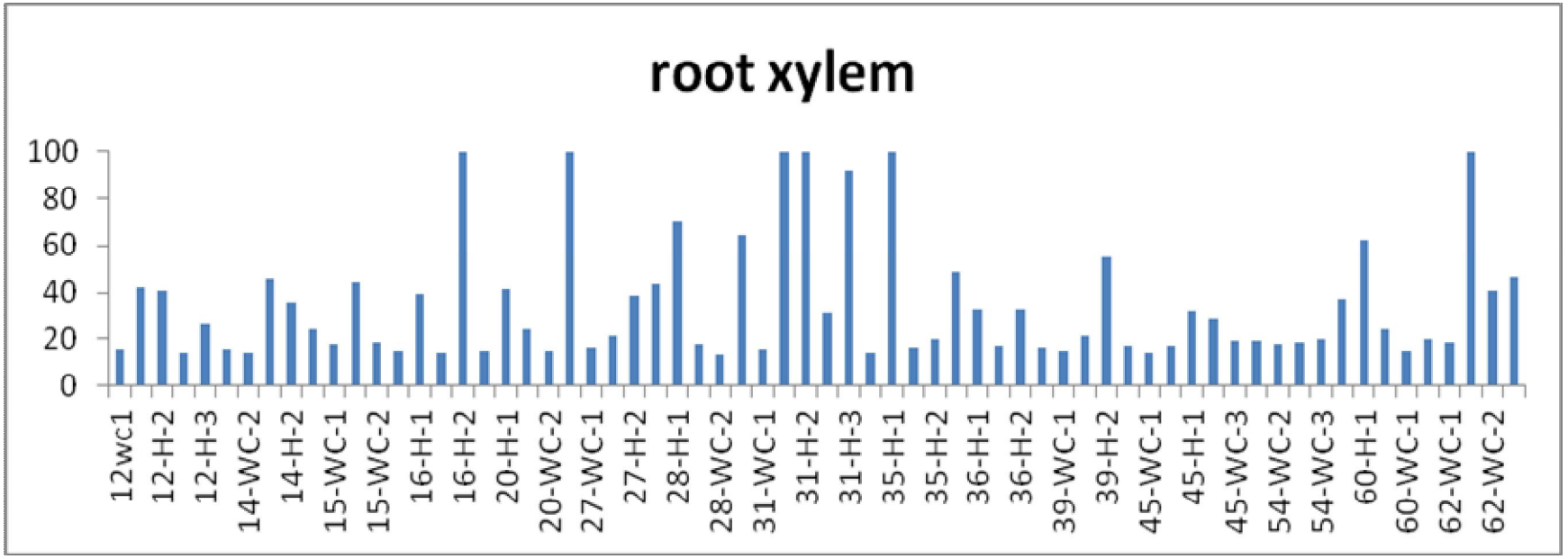
62-WC-2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15-WC-2
16-H-1
16-H-2
20-H-1
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27-WC-1
27-H-2
28-H-1
28-WC-2
31-WC-1
31-H-2
31-H-3
35-H-1
35-H-2
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39-WC-1
39-H-2
45-WC-1
45-H-1
45-WC-3
54-WC-2
54-WC-3
60-H-1
60-WC-1
62-WC-1
62-WC-2

Appendix 7

Occurrence of hyphae and conidiospores of *H.parviporum* and signs of other fungi in isolations of tissue samples.

			date:	4.3.2011		date:	9.3.2011		date:	21.3.2011	
Sample		piece no	Hyphae	Conidiop.	Else	Hyphae	Conidiop.	Else	Hyphae	Conidiop.	Else
2h1	s1p0	1	no	no	no	no	no	no	no	no	no
2h1	s1x0	1	no	no	yes	no	no	no	no	no	mayteen-
2h1	s1x0	2	no	no	yes	no	no	yes rinkula	no	no	yeast
2wc7	s2x0	1	no	no	no	no	no	no	no	no	no
2wc7	s2p0	1	yes	no	?	yes	no	no	yes	no	contam-
5h8	r1x0	1	no	no	yes	no	no	yes, rinkula	no	no	rinkula
5h8	r1p0	1	yes	no	?	yes	no	no	no	no	contam-
5wc4	r1p0	1	no	no	yes	no	no	yes, orange stuff	no	no	orange
5wc4	r1p0	2	no	no	yes	no	no	"	yes	no	hyphae
5wc4	r1x0	1	no	no	yes	no	no	yes rinkula	no	no	rinkula
5wc4	r1x0	2	no	no	yes	no	no	yes	no	no	rinkula
6h6	s1x0	1	no	no	no	no	no	no	no	no	no
6h6	s1p0	1	no	no	no	no	no	yes orange stuff	no	no	orange
28h1	r1x0	1	no	no	?	no	no	no	no	no	no
28h1	r1x0	2	no	no	?	no	no	no	no	no	no
28h1	r1p0	1	yes	no	?	yes	no	no	yes	no	hyphae
35h1	s2x0	1	yes	yes	?	yes	yes	no	yes	yes	no
35h1	s2p0	1	no	no	?	yes	no	no	yes	no	black
36wc2	s1p0	1	no	no	no	yes	no	no	yes	no	yellow's spots
36wc2	s1x0	1	no	no	yes	no	no	yes rinkula	no	no	rinkula
36wc2	s1x0	2	no	no	yes	no	no	yes "	no	no	"
36wc2	s1x0	3	no	no	yes	no	no	yes "	no	no	"
54h2	s2x0	1	yes	no	?	yes	no	?			
54h2	s2x0	2	no	no	?	no	no	yes rinkula			
54h2	s2p0	1	yes	no	?	yes	no	no	yes	no	black
54h2	s2p0	2	no	no	?	no	no	no	no	no	no
54h2	s2p0	3	no	no	?	no	no	no	no	no	no
54wc1	s2x0	1	yes	?	yes	yes	no	yes yellow stuff	yes	no ?	yellow
54wc1	s2x0	2	no	no	yes	yes	no	yes "	yes	?	"
54wc1	s2x0	3	yes	?	yes	yes	no	yes "	yes	?	"
54wc1	s2p0	1	no	no	no	no	no	yes orange stuff	no	no	orange
28H1	S2X0	1				no	no	no	no	no	no
"	"	2				no	no	no	no	no	no
28H1	S2P0	1/2				yes	no	mystic [illegible]	yes	no	black