Comparative Analysis on the Susceptibility of Norway spruce (*Picea abies)* in Northern and Southern Finland to *Heterobasidion parviporum* Infection

.....Thesis submitted for a M.Sc. degree in Forest Sciences and Business

University of Helsinki

Dept. of Forest Sciences

January, 2016

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HELSINGIN YLIOPISTO — HELSINGFORS UNIVERSITET — UNIVERSITY OF HELSINKI

Tiedekunta/Osasto — Fakultet/Sektion — Faculty	Laitos — Institution — Department
Faculty of Agriculture and Forestry	Department of Forest Sciences

Tekijä — Författare — Author

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Työn nimi — Arbetets titel — Title

Comparative Analysis on the Susceptibility of Norway spruce (*Picea abies*) in Northern and Southern Finland to *Heterobasidion parviporum* Infection

Oppiaine —Läroämne — Subject
Forest Ecology / Forest Pathology

Työn laji — Arbetets art — Level	Aika — Datum — Month and year	Sivumäärä — Sidoantal — Number of pages
M.Sc. Thesis	January, 2016	54 + Appendicies

Tiivistelmä — Referat — Abstract

Heterobasidion annosum s.l. is a devastating forest pathogen species complex which causes extensive damage to timber products in northern Europe. This study examined resistance of Norway spruce (*Picea abies*) in two field sites in Finland to annosum root rot (*Heterobasidion parviporum*) utilizing non-clonal stocks of *P. abies*. The northern field site in Rovaniemi does not have a historical presence of the pathogen, whereas the southern field site in Lapinjärvi has extensive historical presence of the pathogen.

The goals of the study were to assess potential difference in susceptibility between the sites, as well as to examine the differences in susceptibility between tissue types and organs in the trees examined. The study inoculated treatment trees with *H. parviporum*, as well as mock inoculations without the pathogen for control trees. Six inoculations of one treatment type were placed into each of thirty randomly selected trees at both field sites. Three inoculations were done in the stem, and three in the roots, for a total of 360 inoculations. After being left *in situ* for three months, the trees were harvested, and resulting lesions in the phloem and xylem tissues in both the roots and stem were measured to determine the extent of visible lesion extensions from the inoculation point.

Data collected from the experiment was analyzed in the context of three mixed effects models, with the assumption that larger lesions indicated lower resistance to the pathogen. The measurements considered as response variables for the models were the total length of the lesion, total width of the lesion, and total area of the lesion.

Results indicated minor overall differences in the lesion sizes between site in the lesion width and lesion area models. Significant differences were found between tissue types in the lesion width, and lesion area models. Additionally, interactions between treatment and organ, as well as treatment and tissue were significant across all models. Several other interactions were significant across some, but not all models The results indicate that further research into the potential effects of historical or geographic isolation on the resistance of *P. abies* to *H. parviporum* should include strict genetic controls with crossing of genotypes across sites, and should also consider the differences due to abiotic factors which may influence resistance in field trials.

Avainsanat — Nyckelord — Keywords
Heterobasidion annosum s.l., Heterobasidion parviporum, Picea abies, Norway spruce, resistance, forest pathology, butt rot

Säilytyspaikka — Förvaringsställe — Where deposited
Viikki Science Library, University of Helsinki

Muita tietoja — Övriga uppgifter — Further information
Supervisor: Professor Frederick Asiegbu. Assistant Supervisors : Emad Jabar and Susanna Keriö

ACKNOWLEDGMENTS

I would like to thank my supervisor, Professor Frederick Asiegbu, for his support and guidance throughout the planning and implementation of the study contained herein, and for his endless patience during countless revisions of this manuscript.

I would like to thank Dr. Emad Jaber, and Dr. Susanna Keirö, my assistant supervisors, for their patience and tutelage. Thank you for your advice based on your own experiences in conducting similar experiments regarding the implementation and analysis for the experiment contained herein.

I would like to thank Dr. Risto Kasanen for his help in coordinating with METLA regarding site selection and assistance in Lapinjärvi fieldwork for the experiment contained herein.

I would like to thank Dr. Risto Jalkanen and the Rovaniemi METLA team for assisting with the selection of the northern field site, and for their help in performing the fieldwork, as well as for their efforts in coordinating logistics for the transport of the collected samples.

I would like to thank Dr. Hui Sun, Dr. Tommaso Raffaello, Dr. Andriy Kovalchuk, Dr. Abbot Oghenekaro, Dr. Eeva Terhonen, and Dr. Hongxin Chen, for their assistance in both the field and in the lab.

I would like to thank my mother, Dr. Marianne Petersen for her assistance in reviewing this manuscript.

Finally, I would like to thank all the professors, researchers, lecturers, and lab assistants at the University of Helsinki, Department of Forest Sciences for their roles in teaching and guidance. Without you, the works herein would not have been possible.

Thank you all.

Erik Stewart

January, 2016

Contents

1 Introduction

Norway spruce (*Picea abies*) is a commercially significant conifer with a ubiquitous distribution throughout most of Finland. In Finland, Norway spruce accounts for thirty percent of total forest growing stock volume (Ylitalo, 2013). Because of its significant impact on the Finnish economy, it is important to understand and to address issues which impact the growth and production of Norway spruce.

Norway spruce established itself in northern Europe via eastern Finland approximately 6,500 years ago (Seppä et al., 2009). However, in this relatively short period of time, Norway spruce has become one of the most dominant forest trees in northern Europe, owing partially to the fact that it has high genetic plasticity and adapts well across a range of latitudes and climates (Reich et al., 1996; Chen et al., 2012). Although most populations of *P. abies* in northern Europe have been established relatively recently, some populations in ice free areas of western Scandinavia likely survived the last ice age approximately 18,500 years ago (Tollefsrud et al., 2008).

Norway spruce is a shade tolerant conifer, which grows at the final stages of ecological succession. A tolerance for shade allows *P. abies* to establish in mixed forests in the understory during early stages of growth, with increased growth opportunistically to fill in canopy gaps as they occur during the later stages of ecological succession (Jonsson and Esseen, 1990). Initial growth of *P. abies* is slow, but increases between 20-60 years of age (Kostler, 1956). Generally, *P. abies* lives for 200 years in the southern areas of its range, but can survive up to 400 years in the more northerly areas of its range (Kostler, 1956). The root systems of *P. abies* are superficial, making the tree susceptible to windfall (Kostler, 1956).

In Finland, Norway spruce is found throughout most of the country. Only small areas in the very northern parts of Finland inside the Arctic Circle are devoid of *P. abies.* Unsurprisingly, the near ubiquitous presence of *P. abies* throughout Finland and Scandinavia has led to its becoming one of the most important commercial forestry crops in northern Europe.

The most devastating pathogen affecting the growth of Norway spruce in the northern hemisphere is butt rot disease caused by the *Heterobasidion annosum s.l.* species complex. Damage to forest production in Europe from *H. annosum s.l.* is greater than 800 million euros annually. It is therefore important to study the pathogen and host to better understand the complex nature of infection biology and resistance factors. The results of such studies may hold the potential for mitigating losses and improving forest yields in areas where *H. annosum s.l.* is prevalent (Woodward et al., 1998b).

1.1 Biology and Epidemiology of *H. annosum s.l.*

The *H. annosum s.l.* species complex is comprised of several inter-sterile groups of species, each with differing host preferences. In Europe, three distinct mating types are recognized: *Heterobasidion annosum sensu stricto*, *Heterobasidion parviporum*, and *Heterobasidion abietinum,* with host preference for pine (*Pinus spp.*), spruce (*Picea spp.*), and fir (*Abies spp*.), respectively (Korhonen and Stenlid, 1998). In North America, two *H. annosum s.l.* intersterility group are recognized; *Heterobasidion occidentale,* and *Heterobasidion irregulare,* with host preference for spruce and pine respectively. Despite a preference for specific host species, several species within the *H. annosum s.l.* complex are able to infect other than their preferred hosts, albeit with less efficiency (Garbelotto and Gonthier, 2013).

H. annosum s.l. has the ability to infect a broad range of coniferous trees, as well as some angiosperms. *H. annosum s.l.* are selective necrotrophs, which cause damage by degrading lignin and cellulose in host trees. Presently, no conifer is totally resistant to the pathogen, and there is no 100% effective control against the disease. Once infection has been established within a host, death of the host tree will occur eventually due either to the pathogen or other environmental factors, such as wind throw in hosts which have compromised structural integrity. However, the degradation of host tissues to the point of lethality may take several decades. Due to the mortal nature of infections by *H. annosum s.l.,* knowledge of the life-cycle and infection biology of the pathogen is critical to understanding how best to deal with the pathogen in areas where *H. annosum s.l.* presence is a problem.

Different species of the *H. annosum s.l.* complex are generally unable to hybridize with other species within the species complex. Genetic control determining the intersterility between species within the *H. annosum s.l.* complex is regulated by at least five genes (Chase and Ullrich, 1990; Garbelotto and Gonthier, 2013). However, limited hybridization of species within the complex has been observed, mostly in laboratory settings between the North American isolates (Garbelotto et al., 1993). Garbelotto et al. (2007) speculated that the occurrence of little to no observations of hybrids in natural settings is primarily due to ecological constraints, and higher competitive abilities from pure strains within the species complex.

1.1.1 Distribution of *H. annosum s.l.* in Europe

H. annosum s.l. is widespread throughout most of the northern hemisphere. In Europe, the three intersterile groups of *H. annosum s.l.* are prevalent in many of the areas where their respective host trees are found. The European P-type intersterility group (*H. annosum s.s.*) is found throughout most of Europe, with upper limits to its geographic extent in the southern to central areas of Finland, despite the presence of suitable hosts throughout the further northern areas of Europe (Korhonen et al., 1998). The S-type intersterility group (*H. parviporum)* is found further north in Europe than that of the P-type, but its southern extent generally restricted by lack of suitable hosts in the more southern parts of Europe (Korhonen et al., 1998). The northernmost observations of *H. parviporum* have occurred just south of the northernmost distribution of *P. abies* in Finland, at approximately 68º North. Although the S-type intersterility group has been noted in these northernmost regions, it is not considered to be a problem in mechanized forestry situations, and incidences of infections are rare. In contrast with the other two intersterility groups, the F-type intersterility species of the pathogen (*H. abitenium)* is only found in southern and central Europe, with upper limits to its distribution restricted by the availability of suitable host species (Korhonen et al., 1998). Overall understanding is lacking of the ecological constraints, including temperature regimes, which limit the distribution of these pathogens from areas where suitable hosts are found, such as in far northern Europe (Korhonen et al., 1998; Witzell et al., 2011).

1.1.2 Spread and Infection of *H. annosum s.l.*

The actual infection process begins when a spore or mycelia reaches a suitable host substrate. Adhesion of spores requires suitable substrate, generally in the form of a fresh wound exposing living tissues in a host tree, or a stump through which subsequent spread to adjacent trees can occur. Germination of spores occurs when spores land on suitable host tissues, and environmental conditions are sufficient for the survival of the spores (Redfern, 1993; Redfern and Stenlid, 1998). After infection has been established, *H. annosum s.l.* utilizes lignin and cellulose as primary carbon sources for growth and proliferation within host tissues. However, *H. annosum s.l.* can also utilize other sources of carbon as well (Korhonen and Stenlid, 1998).

A recent report has shown that *H. annosum s.l.* can stay viable for at least six years in roots of at least 15mm diameter, and still maintain the ability to vegetatively infect the roots of nearby Norway spruce seedlings (Piri, 2013). In areas where conifers are intensively harvested and managed, mechanical damage due to forestry related activities provide new surfaces for inoculations, which exacerbate the problem of *H. annosum s.l.*, leading to high rates of infection and heavy losses. The primary way in which new infections are established in areas where commercial harvesting of forest trees is done is via basidiospore deposition onto freshly cut stump surfaces (Redfern and Stenlid, 1998).

Other methods by which *H. annosum s.l.* spreads is via mycelial growth within suitable host substrate. Infections also occur in the immature roots of host trees, as this is typical mode by which the pathogen spreads (Johansson and Stenlid, 1985; Asiegbu et al., 1994). Root systems of an infected tree which are near to neighboring tree's root systems, or which are grafted with the roots of another tree, can pass mycelium from the roots of one infected tree to the root systems of another tree. Contact between roots of neighboring trees is also an important natural mode for the transmission of *H. annosum s.l.* to uninfected trees within the same stand, as well as to subsequent generations of trees in instances where stumps and remaining root tissues from infected trees are left in place (Woodward et al., 1998a; Asiegbu et al., 2005; Garbelotto and Gonthier, 2013).

Reproduction of *H. annosum s.l.* requires two homokaryotic strains of differing genetic origin. With compatible mating loci, the two strains fuse to form a heterokaryotic mycelium containing the nuclei of both parental strains. With sufficient access to nutrients and favorable climate, the heterokaryotic mycelium forms a basidiocarp, generally on the lower portion of infected trees. Several methods exist for inducing fruiting in the laboratory, as reviewed by Chase and Ullrich (1985). In addition to infection from sexually produced basidiospores, conidiospores, an asexual spore produced by *H. annosum s.l.* can also start new infections, but are speculated not to be the primary source of new infections Redfern and Stenlid (1998).

1.1.3 Control of *H. annosum s.l.*

Various biotic and abiotic methods of control are used to reduce the damage done to trees by *H. annosum s.l.* In modern, highly mechanized forest timber production, one of the key routes for new infections of *H. annosum s.l.* is accidental mechanically created wounds on trees as a byproduct of harvest and maintenance. These wounds provide access to suitable host tissues for basidiospores. However most new infections occur on freshly cut stumps leftover from logging operations (Woodward et al., 1998a; Thor and Stenlid, 2005; Mäkinen et al., 2007). Primary methods for control of *H. annosum s.l.* include the careful use of equipment, along with efforts to minimize damage due to anthropogenic factors. Freshly cut stumps left in place provide an ideal suitable host substrate for *H. annosum s.l.*. Thus, one of the most efficient and widely utilized control methods is to address the issue of suitable host substrate on freshly cut stumps by means of chemical, biotic, or abiotic control in areas where infections have not been previously documented. Chemical control includes treatment of freshly cut stumps with urea, borax, or a fungicidal product like propiconizole, which can all be effective at reducing the likelihood of subsequent infection from *H. annosum s.l.* spores (Woodward et al., 1998a; Nicolotti et al., 1999; Garbelotto and Gonthier, 2013). Typical biotic treatments for the control of *H. annosum s.l.* includes the application of saprotrophic fungi *Phlebiopsis gigantea,* which colonizes available substrate (generally stumps) and out competes *Heterobasidion s.l.* (Woodward et al., 1998a; Nicolotti et al., 1999; Garbelotto and Gonthier, 2013). Silvicultural practices which are used to

reduce the damage from *H. annosum s.l.* includes removal of stumps and associated root tissues, which provide an unchallenged substrate for infections to occur on for a period of time after harvest when stump surfaces are susceptible (Vasaitis et al., 2008). Harvest of stumps and removal of sources of host material suitable for inoculation has proved to be effective in reducing the subsequent infections and rot (Oliva et al., 2010). However it is very difficult to control spread of *H. annosum s.l.* where the pathogen was established in prior generations; in these areas, stump removal after the harvest of the current generation may be insufficient for controlling spread of *H. annosum s.l.* to the subsequent generations (Piri, 1996, 2003).

1.2 Defense Systems and Resistance in Conifers

Conifers have a variety of defense systems for dealing with both biotic and abiotic threats. The major defense systems which conifers possess for dealing with pathogens are constitutive and inducible defenses. Structural, or constitutive defenses include tissues, structures, and chemicals present in parts of the tree which either prevent or reduce the possibility or severity of infection by a pathogen. Induced defenses are initiated locally and/or systemically upon the recognition of a pathogen, and include a variety of possible defense actions including secondary metabolite production, priming of system wide defenses, and changes to physical structures of the tree (Kovalchuk et al., 2013). Genetic defenses represent gene level interactions between plant and pathogen, and can confer increased resistance to a pathogen or outright immunity, depending on the tree and pathogen. The constitutive and induced systems of defense present in conifers are not mutually exclusive of one another, and interact with components of the other systems of defense within the tree on some level.

1.2.1 Constitutive Defenses in Conifers

Constitutive defenses in conifers include various types of tissues and structures which are produced during regular, unchallenged growth. Bark represents the outermost layer of defense in a conifer, and is comprised of several distinct tissues. In trees other than saplings, bark is comprised of periderm and phloem tissues. The periderm is highly

suberized and hydrophobic, which helps to inhibit adhesion and germination of fungal spores (Pearce, 1996). In most cases, it is not possible for a fungal pathogen to actively infiltrate intact outer bark tissues, a notable exception being some species of fungi in the genus *Armillaria* which can penetrate outer defenses (Pearce, 1996). However, not all structures within the bark can adequately resist fungal invasion; in a study conducted by Lindberg and Johansson (1991), *H. parviporum* was able to establish infections reaching the xylem in all test subjects with the rhytidome and phellem removed, but with inner bark tissues still intact.

Features present in the phloem tissues which allow the *P. abies* to resist damage and infection from pathogens includes polyphenolic parenchyma cells, and lignified cells. Polyphenolic parenchyma (PP) cells are present throughout the trees phloem tissues, and store phenolic compounds which are released if the cells are damaged by wounding either from mechanical forces or via pathogen growth. Phenolic compounds have various anti-microbial properties, and resistant clones of *P. abies* have been shown to have higher amounts of PP cells than susceptible clones (Franceschi et al., 1998). Xylem tissues include defense mechanisms similar to those in phloem tissues. Within the xlyem, lignified cells increase the mechanical strength of a tree, and are more resistant to fungal infections. However, much higher portions of parenchyma cells exist within the xylem in conifers, predominantly in the form of xylem rays. PP cells are also present in large numbers in the xylem tissues. Further barriers to damage from pathogens or mechanical forces include large amounts of lignified and suberized tissues.

Sapwood tissues have a variety of constituent and structural defenses, as well as the ability to induce defenses in response to a pathogen. In Norway spruce which are more resistant to fungal infection, the sapwood tissues have larger polyphenolic parenchyma cells, which can contribute to differences in resistance levels of individual trees depending on the phenotype (Nagy et al., 2004). Trees also create barriers in response to pathogens, compartmentalizing both axially and radially in response to damages or detection of a pathogen. Increases to lignification near affected tissues, plugging of vascular tissues, and programmed cell death are utilized by the tree to attempt to exclude the pathogen from vulnerable areas within the host (Shain, 1971; Shigo et al., 1977; Shigo, 1984; Pearce, 1996).

1.2.2 Inducible Defenses in Conifers

In addition to the compartmentalization of tissues surrounding a wound or pathogen within the sapwood, a reaction zone surrounding an initial wound is also created. This is characterized by increased levels of lignin in surrounding tissues, increased production of phytoalexins, free radical production from oxidative bursts, and increases in other compounds which have antimicrobial properties (Pearce, 1996; Kovalchuk et al., 2013). Increases in accumulation of antimicrobial compounds in the reaction zone help to restrict further progression of pathogens or damage which caused the response and compartmentalization of the area in the first place. The necrotic response is the activation of cellular death in localized areas in response to a pathogen; however, in the epidemiology of pathogens with necrotrophic lifestyles, such as *H. annosum s.l.*, necrotic cell death is ineffective at stopping an infection.

Non-specific chemically based defenses in conifer periderm and sapwood tissues include oleoresin based defenses, increased lignification, as well as accumulation of antimicrobial chemicals which are produced during normal plant growth. These compounds are referred to as phytoanticipins (VanEtten et al., 1994). Oleoresins are present in sapwood tissues during the normal growth of many conifer species, and are produced by a variety of specialized cells depending on the genus. For example *Pinus spp.* have well developed resin duct tissues, whereas *Abies spp.* produce resin blisters; *Picea spp.* lack centralized traumatic resin ducts, and show low levels of constituent monoterperne cyclase activity (Lewinsohn et al., 1991). Oleoresin production can greatly increase in response to wounding or pathogenic attack in genus such as *Picea* (Lewinsohn et al., 1991). Because oleoresins are produced both in normal growth as well as in response to abiotic or biotic stresses, it is appropriate to consider oleoresins as an induced defense strategy as well as a constitutive one. Phytoalexins are chemical compounds produced in response to a pathogen, and include a broad range of low molecular weight compounds; however, true phytoalexins are not known in conifers, but increased accumulation of phytoanticipins and antimicrobial compounds in response to pathogens has been observed in conifers (Bonello et al., 2006). Lignans, stilbenes and terpenoids are phytoalexin-like compounds produced in *P. abies* and other *Picea spp*. in response to fungal challenge (Pearce, 1996).

Upon infection with *H. annosum s.l.*, broad changes occur in both the host and pathogen. Specific elicitors detected by a susceptible host can initiate changes to the physiology of the host both locally and systemically. Changes in the host can include the increased production of secondary metabolites in response to the pathogen. Similarly, once the pathogen begins to encounter resistance, the pathogen can produce compounds which assist in overcoming the host defense systems. A study by Swedjemark et al. (2007) found that the priming of resistance by prior inoculation of *H. parviporum* in Norway spruce had significant effects on reducing the subsequent necrosis and fungal growth in subsequent inoculations. Other factors can prime host tree defenses, prompting physiological changes which confer enhanced resistance to pathogens. Herbivory, volatile organic compounds, and colonization of the host with certain types of rhizobacteria or mychorrizal fungi also have the potential to prime defenses in conifers (Eyles et al., 2010).

In addition to low molecular weight compounds produced for defenses, conifers also utilize protein based defenses in response to pathogens. These protein defense products are known as pathogenesis-related proteins (PR-proteins), and include proteins across seventeen well defined families (PR1-PR17), as well as several other classes of less well understood PR-proteins families (PR-18, PR-19) (Veluthakkal and Dasgupta, 2010; Kovalchuk et al., 2013).

A variety of signaling molecules are important in the induced defense in conifers. Methyl-jasmonate is an important signaling molecule which induces a wide variety of changes to conifer tissues, including traumatic resin duct formation, and increased production of terpenoid based resin defenses (Martin et al., 2002; Hudgins et al., 2003; Hudgins and Franceschi, 2004; Hudgins et al., 2004). Salicylic acid is important for systemic acquired resistance (SAR), although the exact nature by which salicylic acid enables SAR is not fully understood. Finally, ethylene is an important molecule in the signaling pathways of conifer defenses, which is influenced by the production of methyl-jasmonate (Hudgins et al., 2006). Ethylene assists in defense in the phloem of conifers, and in creating traumatic resin ducts Hudgins and Franceschi (2004).

1.3 Resistance of *P. abies* to *H. annosum s.l.*

Norway spruce has varying levels of resistance to infection by *H. annosum s.l.,* depending on a many factors including genetics, tree age, and overall health (Delatour et al., 1998; Swedjemark et al., 1998; Hietala et al., 2003). *P. abies* is not known to have outright immunity to the pathogen; once established, disease progress will eventually kill off the host tree after degrading sufficient portions of essential tissues. Because there are no known *H. annosum s.l.* immune genotypes of *P. abies*, research efforts have focused on elucidating factors which determine levels of resistance and susceptibility, as well as characterizing the interactions between host and pathogen. Resistance factors include biotic factors, such as changes to plant physiology in response to pathogens, abiotic factors, such as temperature, climate, and nutrient availability, and genetics. Abiotic factors can influence the ability of either the host or pathogen to properly defend, or infect, respectively. Factors such as soil types and availability of nutrients, pH, and moisture levels have been shown to influence the resistance of *P. abies* to infection of *H. annosum s.l.* (Lindberg and Johansson, 1992; Redfern, 1993; Asiegbu et al., 2005).

1.3.1 Host Pathogen Coevolution

Coevolution between plants and pathogens is driven by their interactions with one another over time. Differential pressures shape the way in which the organisms interact with one another, and over time can lead to resistance or susceptibility, and differing levels of virulence in the pathosystem. Theories of plant pathogen coevolution typically include the gene- for-gene model wherein one or more resistance genes (*R*) in a host are complemented with corresponding avirulence genes (*Avr*) in a pathogen (Flor, 1946). If an *Avr* gene is present in a pathogen, the corresponding gene product is recognized in an incompatible host with the corresponding resistance gene; the result is that the host recognizes the pathogen and is able to resist infection. If the necessary *R* gene is not present in the host, the outcome is a compatible reaction leading to infection. Many plant *R* gene products are nucleotide binding site leucine rich repeats (NBS-LRR), but are largely ineffective against necrotrophic pathogens, such as *H. annosum s.l.* (Glazebrook, 2005). Recent studies have examined the role of NBS-LRRs

in *P. abies* in response to a necrotrophic pathogen, but studies have found only small differences in significantly upregulated gene products between wounded and infected trees (Fossdal et al., 2012). Additional evidence exists for the importance of plant-pathogen coevolution in the positive selection of effective PR-proteins (Scherer et al., 2005). Specific mechanism which have coevolved in the interactions between host and pathogen include toxins (either general, or host specific) and effectors on the pathogen side, and elicitors and other *R* gene products on the host side. Effectors act to modulate host defense systems: this, in turn drives the host to the evolution of *R* gene products that recognize and neutralize the pathogen effectors. Over time, pathogens will evolve changes to their now unsuitable effectors to avoid the newly adapted *R* proteins. Lastly, the specific *R* or *Avr* genes do not necessarily interact directly with one another on a molecular level. In some cases, the product of an *R* gene acts as a "guard" to a target of the *Avr* product, and only initiates resistance when the target host protein ("guardee") interacts with the pathogens *Avr* product. This interaction and subsequent elicitation of further defense mechanisms within the host is called the guard hypothesis.

1.3.2 Implications for Introduced Pathogens

Overall, coevolution is a key driving factor in the development of resistant strains of a plant. However, if a potential host has been excluded from the presence of pathogen, resistance of the host may be greatly reduced or nonexistent altogether for the newly introduced pathogen. This interaction between susceptible host and non-native introduced pathogen can cause devastating damages to a species which has not had the chance to coevolve alongside the pathogen. Perhaps the best known case of an introduced pathogen having devastating effects on a new host is the introduction of *Cryphonectria parasitica,* a fungal pathogen from Asia, into the eastern areas of North America in the early 1900's. Due to the introduction of *C. parasitica*, the causal agent of chestnut blight, the American chestnut was reduced from a once dominant species across much of the eastern United States to a mere pittance (Anagnostakis, 1987). Other pathogens which have been introduced and caused great damage to native hosts include *Phytophthora ramorum,* the agent responsible for the sudden oak death, and various *Ophiostoma* species, which have caused epidemics of Dutch elm disease in both North

America and Europe.

Differences in generation times between host and pathogen can also influence the co-evolution of the species. For example, *P. abies,* as with most coniferous trees, has long generational periods, while *H. annosum s.l.* generally reproduces more rapidly, leading to faster adaptations by the pathogen. The shorter life cycles of the pathogens relative to the host, as well as both sexual, and asexual reproduction modes allow subsequent generations to select quickly for favorable traits and increased virulence against the host with its slower generation time Gilbert (2002). However, in the interactions between *H. annosum s.l.* and *P. abies*, resistance of the host and virulence of the pathogen are quantitative traits, which are under the control of many different genes. Specific genes in *P. abies* that control for resistance to *H. annosum s.l.* are not fully known. However, recent efforts have made progress towards identifying promising regions in the genome of *P. abies* for quantitative resistance traits (Lind et al., 2014). Finally, it is important to understand that most coevolutionary processes happen continuously in the context of host and pathogen interactions; either host or pathogen may eventually adapt to overcome the defenses or offenses of their complement, changing the evolutionary direction of the complement in order to adapt to the new challenge.

1.4 Lesions as an Indication of Resistance

Measuring necrotic lesions produced in response to *H. annosum s.l.* is a technique to gauge potential resistance of hosts to fungal infection, and has been utilized in many prior studies (Delatour et al., 1998). Lesions are created by the host tree in response to wounding, or from infection via a pathogen by the host tree. In cases where a pathogen, i.e., *H. annosum s.l.,* is placed into the tree, the size of the resulting lesion can be used as a gauge of the ability of the host's genotype to resist the pathogen (Swedjmark and Stenlid, 1997; Delatour et al., 1998; Woodward et al., 2007). Inoculations are generally performed utilizing a sterilized wooden dowel which has been cultured with the pathogen, or left sterile in the case of a control sample. The dowels are placed into the host in a systematic way, and left *in situ* for a period of time before measuring the resulting lesions. Most inoculation experiments with *H. annosum s.l.* have focused on stem inoculations, owing to the ease of access to above ground host biomass in field

experiments. Since the roots represent the natural infection pathway for the pathogen, additional research into the differences between lesion response in the stems and roots could be beneficial to better understanding of the pathosystem dynamics.

Genetics influence potential factors affecting resistance traits of individual trees, and resistance can be a quantitative trait, or absolute, depending on the pathosystem. For example, *Cronartium ribicola* is an invasive pathogen which causes white pine blister rust: developments in resistance via breeding programs for this pathosystem are largely based on quantitative traits. Conversely, a native fusiform rust caused by *Cronartium quercuum* f. sp. *fusiforme* which infects various pines in North America more often encounters total genetic resistance to infection versus the invasive *Cronartium ribicola* Sniezko et al. (2014). Furthermore, a study by Napierała-Filipiak and Filipiak (2012) found that resistance in Scots pine seedlings to artificial infection with *H. annosum s.s.* was higher in seeds sourced from naturally regenerated forests with high natural incidences of root rot, suggesting some amount of heritability of quantitative resistance factors.

Broad sense heritability, H^2, is defined as the variation of the genotype divided by the variation of the phenotype. Overall variance in broad sense heritability of the fungal extension in seventeen year old Norway spruce clones artificially inoculated with *H. parviporum* in a study by Swedjemark and Karlsson (2004) was estimated to be 0.18 H^2, indicating that genetics do play a potential role in the resistance of Norway spruce to *H. parviporum.* An earlier study by Swedjemark et al. (1998) similarly found fungal growth and lesion size broad sense heritability for Norway spruce clones artificially inoculated with *H. parviporum* to be 0.35 H^2 and 0.27 H^2, respectively, so variability depends on multiple factors and is not consistent. Although these studies have indicated that heritability and genetics have an influence on the resistance of *P. abies* to *H. annosum s.l.* The influence of particular genes on resistance has not been fully determined.

1.5 Mixed Effects Models in Ecology

The mixed effects model is an extension of the general linear model which incorporates both fixed and random effects. Fixed effects are treatments or experimental conditions which are known and controlled in the design of the experiment. Random effects are the effects of grouping or clustering present in the data. For example, in a study utilizing multiple randomized sites, it would be appropriate to include site as a random effect. Subject units as a group can be considered as a random effect, such as randomly selected trees within a sample plot, as well as any subsequent levels of groupings related to an individual in a group (e.g., multiple measurements by tree), or additional levels of sub grouping. The mixed effects model addresses issues with non-normal data, data from experiments which are unbalanced, data which are correlated, or other shortcomings in data which render it unsuitable for analysis utilizing standard statistical techniques such as ANOVA or simple linear models (Bolker et al., 2009). Mixed effects models have been used in limited amounts in studies of *H. annosum s.l.* and its effects upon host trees, i.e., Swedjemark and Karlsson (2004); Karlsson and Swedjemark (2006).

1.6 Introduction to Current Study

This study addresses several aspects of *H. parviporum* infections in *P. abies* in order to address the lack of knowledge about certain elements of the epidemiology of *H. annosum s.l.* root rot in *P. abies*. First, the study examines the susceptibility of *P. abies* from areas towards the upper northern extent of the range of *H. parviporum* where the pathogen has historically not been a problem. Furthermore, the study compares these results with a similar study done concurrently in Southern Finland where the pathogen is common. Secondly, this study address the natural variation of the host trees across tree organ and tissues. This study utilizes non-clonal trees for experimental units, helping to address and understand the variations in resistance that would be expected in natural populations of *P. abies.* The analysis for this study is done utilizing a mixed effect model which takes into account the inherent nesting and hierarchy of the data collected. The analysis presents the observed variation due to both fixed and random effects.

2 Objectives of the Study

2.1 Central Questions

This study seeks to answer several questions about the potential susceptibility of *P. abies* to *H. parviporum* infections in Northern Finland, and compares them with results in Southern Finland. Secondly, this study will attempt to robustly characterize the natural variation in susceptibility to *H. parviporum* across different non-clonal experimental units. Finally the study seeks to address variation in the resulting lesions produced in differing tree organs and tissues.

2.2 Hypotheses

Hypothesis 1: Norway spruce (P. abies) trees from Northern Finland are susceptible to infection by H. Parviporum.

Determination of the susceptibility of Norway spruce to *H. parviporum* in the Rovaniemi northern field site is analyzed via the inoculation experiment contained herein and compared to the inoculation experiment performed in Lapinjärvi. Although Rovaniemi does not have a strong historical presence of the pathogen, this study presumed that susceptibility will remain in *P. abies* native to the area.

Hypothesis 2: There are no genotypic difference or natural variations in the susceptibility of P. abies trees in Northern and Southern Finland to H. parviporum infections.

Because study units in both Northern and Southern Finland are the same species, it is presumed that variations in the overall response by site will be attributable to factors other than species level factors. This hypothesis is tested via comparison between the resulting data collected from this study across both study sites with univariate statistical tests, as well as in the context of a mixed effects model.

Hypothesis 3: There are no differences or variation in the lesions sizes of conifer stem or root tissues in response to H. parviporum infections.

This is tested with data collected from the study utilizing univariate statistical tests as well as in the context of a mixed model.

3 Materials and Methods

3.1 Field Sites

Two sites were used in this study, one in northern Finland (Rovaniemi) where *H. parviporum* did not have a historical presence at the site, and a site in Southern Finland in Lapinjärvi, where extensive presence of the pathogen has been historically documented. In Rovaniemi, the site was comprised of planted trees with an average age of thirty five years, from natural, non-clonal stock representative of the natural local populations of Norway spruce. In Lapinjärvi, the site was comprised of naturally regenerated trees, with an estimated average age of twenty years or less. Soils in the northern site were rockier, versus softer soils in the southern field site. Trees in Rovaniemi had visibly smaller root systems than those in Lapinjärvi. At each of the two field sites, a total of fifteen trees were randomly selected for each of either wounding control or infected treatments. Trees which were obviously damaged due to biotic or anthropogenic factors, or trees which were obviously extremely young, or otherwise unhealthy were excluded from the selection.

3.2 Inoculum Preparation

A heterokaryon isolate *H. parviporum* , #03014 courtesy of Kari Korhonen, was used for this study. The isolate was obtained from a Norway spruce in Kuhmoinen, central Finland. The culture was maintained on 2% malt extract agar and kept at 4º C. Wooden dowels of Norway spruce measuring roughly 6 mm diameter by 7 mm in length were created utilizing a drill press and a jewelers saw. Cut and formed dowels were autoclaved for thirty minutes with roughly 100 milliliters of Milli-Q water. Subsequently, autoclaved dowels for control trees were placed onto previously prepared 2% malt extract agar plates, and incubated at room temperature for two weeks. Autoclaved dowels utilized for infection were placed onto 2% malt extract agar plates which were pre-colonized with the *H. parviporum* isolate, and were similarly incubated at room temperature for two weeks.

3.3 Preparation of Study Trees and Inoculation

Inoculations in Lapinjärvi were carried out June 14th, 2013. In Rovaniemi, inoculations were carried out on the June 26th, 2013.

For this study, each tree was treated with a total of six inoculations; three stem inoculations placed at 50 cm, 100 cm, and 150 cm above the soil level, and three in the roots placed depending on the size and shape of the exposed roots. Whenever possible, the inoculations were placed on the parts of the root facing upwards towards the crown of the tree. In an attempt to ensure separate infections would not overlap during the study, a minimum of 25 cm upwards or downwards between root inoculation was maintained whenever possible in instances where a single large root was inoculated more than once. In all trees, the inoculations were performed such that the inoculation point of the stem was at the same direction to minimize any possible variance due to azimuth.

Prior to insertion of wounding (termed as control) dowels, or *H. parviporum* colonized (termed as infection) dowels, roots of the study trees were dug up carefully using garden spades, and then outer surfaces of exposed roots were cleaned of excess dirt and debris utilizing a large brush with synthetic fiber hairs. Directly before inoculating the trees, the area of the inoculation was sprayed with 70% ethanol to minimize the possibility of introducing localized contaminants into the xylem or phloem of the tree. All tools utilized in the inoculation process which had direct contact with either the host tree, or wooden inoculation dowels were sprayed with 70% ethanol and wiped clean prior to each use. Tools used for inoculations included: stainless steel forceps, for handling of the wooden dowel, and removing any excess original host tissues, a 7.0 mm interior diameter stainless steel punch for creating the bore and removing host tissue, and a large rubber headed mallet, utilized for hammering the punch.

For each stem inoculation, the steel punch was used to bore through the bark of the tree to the xylem. Host tissue from the cavity created by the bore was removed, and then inspected to ensure that the depth of the hole bored reached into the xylem. Sufficient depth was confirmed by inspecting the tissue removed from the bore; a clear delineation exists between phloem and xylem tissues in *P. abies*. Immediately after confirmation of

adequate bore depth, a wooden dowel for the specific treatment for the tree was placed into the hole and secured into place via use of forceps and the blunt end of the bore. After the dowel was secured, the area of the inoculation was wrapped with parafilm to minimize the chance of post-inoculation contamination. After trees were inoculated and inoculation points were wrapped with parafilm, the roots were covered with the loose soil previously removed to expose the roots for inoculations.

3.4 Harvest and Processing

After three months, inoculated trees were cut down for processing. Roots of the respective trees were re-dug carefully and extracted. Primary tools utilized in harvest were chainsaws. Only the lower segment of the stem containing the inoculation points were removed from the site for further processing. In root samples, as much as was feasible and reasonable was collected for each inoculation. Samples were labeled with the site (L, R, for Lapinjärvi or Rovaniemi respectively), treatment (W, for wounding control, T, for treatment), tree (1-15), and replicate number, (A, B, and C for stem representing 50, 100, and 150 cm inoculation points, and R1, R2, R3 for root inoculations, with R1 being the closest inoculation point to the root collar) before storage. Additionally, the direction of growth (i.e., towards crown) was marked on stem segments.

All samples were placed into large black trash bags (Lapinjärvi) or Kevlar sacks (Rovaniemi) once trimmed to size at the respective field site. Samples from Lapinjärvi were taken directly to the University of Helsinki and placed into cold storage at -18°C. Samples from Rovaniemi were placed in the -40°C freezer at the METLA Rovaniemi field station, and then shipped under temperature control to the University of Helsinki. Upon arrival, the samples from Rovaniemi were placed in cold storage at -18°C until further processing.

3.5 Measurements of Lesions

Before lesion measurement and analysis, the samples were processed. First, bark tissue surrounding the inoculation point on any given sample was removed utilizing a

hatchet, knife, scalpel, or other suitable tools. Care was taken not to damage phloem tissues underneath. In general, at least 10 cm of bark was cleared both above and below the inoculation point, with additional areas cleared if the lesion extended beyond the original margins of removed bark on the phloem. To the left or right of the inoculation point, 5 cm of bark was removed, again removing further tissue if lesions extended beyond the margins. After bark was removed, the initial wounding and lesion was photographed and measured in the exposed phloem tissue. A total of five measurements were taken for each lesion: 1) length of lesion extending upwards (towards the crown in stem samples, or towards the root collar in root samples) from the uppermost part of the wooden inoculation dowel, 2) length of lesion extending downward from the lowermost point of the inoculation, 3) length of lesion extension to the left and right of the inoculation point, and 4) a total length measurement taken from the uppermost point of lesion extension upwards to the lowermost point of lesion extension downwards (see Figure 1). After measurements of the phloem sample, phloem tissue was removed utilizing a mallet, scalpel, and woodworking chisels to expose the xylem tissue. Lesions present in the xylem tissue were photographed and measured in the same manner as the phloem tissue. These measurements are shown in Figure 1.

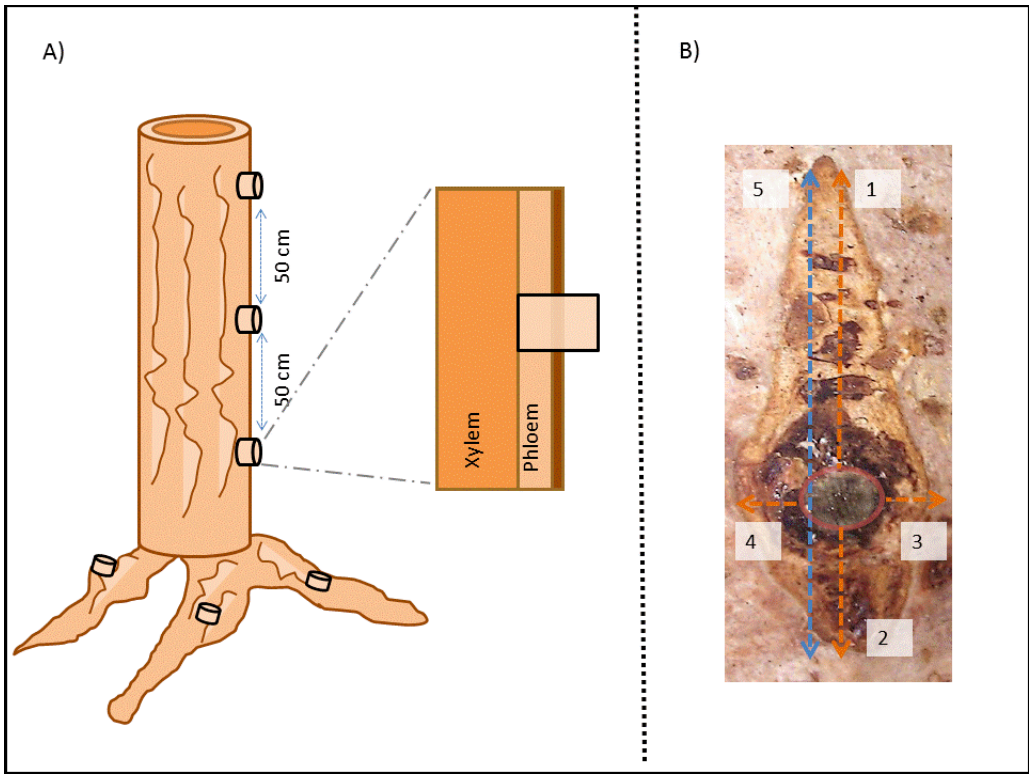

Figure 1: Schematic representation of inoculations (A) on stem and roots, and measurements taken (B) from resulting lesions after the experiment. In (B), measurements are for lesions extension upwards from inoculation dowel (1), extension downwards from inoculation dowel (2), extension to right or left from inoculation dowel (3,4), and total length of the lesion (5). The red circle in (B) outlines the inoculation dowel.

3.6 Analysis of Data

Analysis of the data was conducted using R 3.1.2 (R Core Team, 2014), and packages lme4 (Bates et al., 2013). Graphics were composed with R 3.1.2 and packages ggplot2, lattice, and gridExtra (Sarkar, 2008; Hadley, 2009; Auguie, 2012). Modification of the collected data was necessary to properly format the data, and to ensure that the data was suitable for subsequent statistical analysis. Steps taken to modify the data included taking a log_e transform of the response variables to normalize the data. Exploratory analysis with histograms and other charting techniques was utilized to explore the structure and nature of the data along all steps of modeling. A discussion of potential outliers as well as their effects is given in the appendix. Since it is not possible to address the causes for several outliers present in the data, the data was left intact for the primary analysis.

For the analysis of the susceptibility of *P. abies* to infection with *H. parviporum* at the two field sites, three separate response variables were used: log_e of total length of the lesion, log_e of total width of the lesion, and the log_e of area of the lesion. For the lesion area model it was necessary to create a single response variable with which to characterize the lesions of the individual tree; for the purposes of this study, the lesions are conceptualized as 2-dimensional rectangles based on the total length and maximal width of the lesion as measured from the center of the inoculation point. Thus, for the area model, the response variable is simply the log_e transformed product of total length and width, with a constant of 1.0 mm added to each measurement prior to multiplication to account for rare instances in which no lesion growth was visible in the given measurement direction for a sample.

Each of the three linear mixed effects model were fit using identical predictors to examine the effects that the fixed effects of site, tree, organ, and tissue had on tree response to the treatment. As repeated measurements from a single subject (e.g., tree) cannot be assumed to be independent of one another, explicit nesting among levels of the random effects was specified in building the model to ensure accurate interpretation of the data.

The mixed effects model takes the form:

$$y = X\beta + Z\gamma + \epsilon \tag{1}$$

where y is the expected response for the given model ***(total length, total width, or area***), X is the design matrix of the fixed effects variables, including all two-way interactions between main effects (***Site, Treatment, Organ, Tissue, Site*Treatment, Site*Organ, Site*Tissue, Treatment*Organ, Treatment*Tissue, Organ*Tissue)***, β is the vector of the fixed effect coefficients, Z is the design matrix of random effect variables (***Tree, Organ*** *within* ***Tree, Sample*** *within* ***Organ*** *within* ***Tree)***, γ is the vector of the random effects coefficients, and ϵ is a vector of residual errors.

Validation of the resulting models was done by visual inspection of residuals, comparison of model significance for the fitted parameters between models, and statistical tests for significance of parameter estimates. Significance of effects in the mixed models is

determined utilizing t-tests for model parameters adjusted for degrees of freedom utilizing the Satterthwaite adjustment for pooled variance estimates. Confidence intervals for parameter estimates are estimated from a bootstrap with 1,000 simulations. Finally, calculation of both the marginal and conditional R^2 for each fitted model is done to give an indication of how good the model is at describing the variance in the underlying data for the given response variable.

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4 Results

Prior to constructing the individual mixed effects models, Welch's two sample t-tests were used to determine if differences existed between the log_e of lesion growth towards the tree crown, versus growth away from the crown (*t=-1.3, df =1432.7, p-value =0.19*). Additionally, a Welch's two sample t-test was used to test differences in growth left vs. right of the inoculation dowel (*t=-0.69, df=1435.7, p-value =0.49*). The result of these tests confirmed that direction of lesion growth does not appear to have an predictive influence on overall lesion size. Because of this, only aggregated measurements of total length, width, and lesion area were used for response variables in the models.

4.1 Susceptibility of *P. abies* to *H. parviporum*

In Rovaniemi, all trees except for RT2 produced an average response larger than that produced in mock inoculated trees. Tree RT2 produced notably smaller than average lesions across all measurements than other infected trees in Rovaniemi (Fig. 3).

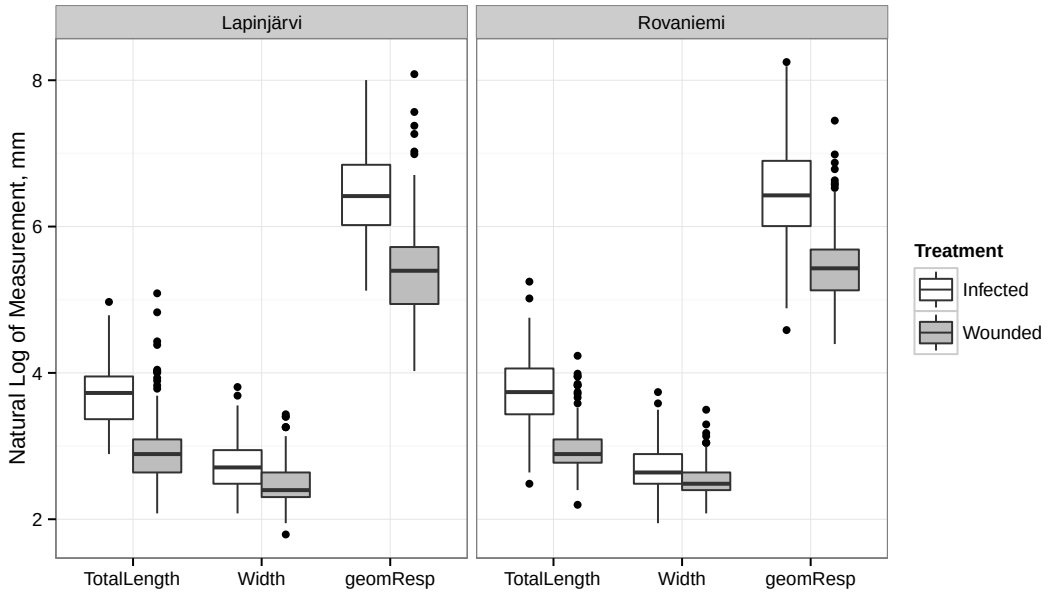
In Lapinjärvi, all trees produced visible lesions. However, tree LW8 was found to have significant amounts of heartwood decay upon harvest. The influence of this prior decay on the response of the tree to mock inoculation is clearly abnormal with regards to total length and area measurements when compared to the other mock inoculated trees in Lapinjärvi (Fig. 4).

The greatest variation in the measurements taken for the trees at both sites is in the total length measurement, which further propagates to the lesion area variable; effects of this are clearly visible when comparing lesion size measures for total length and lesion area across the trees (Figures 3, 4).

4.2 Effect of Treatment and Field Site on Response

The effect of treatment on resulting lesion size as characterized by total length, width, or area measurements was significant for all models (Fig. 2), confirming susceptibility

Figure 2: Lesion Measurements by Site and Treatment



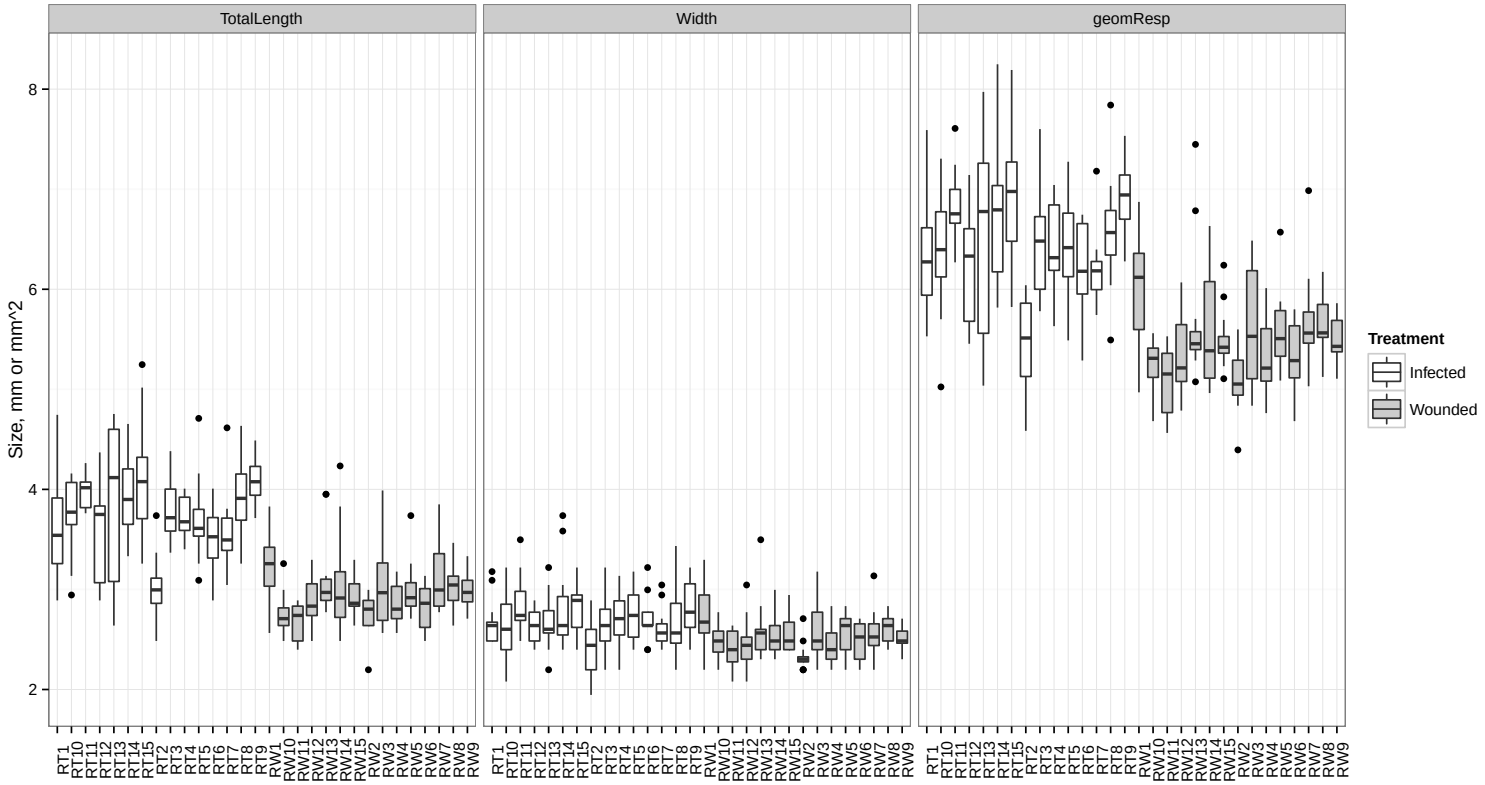
The effect of treatment is significant for all measures (tables 6,7,8). The effect of site is significant for total width (t=-2.147, df=101.1, p-value =0.03) and the lesion area (t=-2.11, d.f.=91.6, p-value=0.04), but is not significant for total length measurements. Total length and width measurements have been log_e transformed for this analysis.

of *P. abies* to *H. parviporum* in both Rovaniemi and Lapinjärvi field sites. The response variable of width and lesion area vary significantly between the two field sites (Fig. 2), but total length does not. Rovaniemi trees have larger average width and lesion area measurements than Lapinjärvi (Fig. 2, Table 1). The interaction between field site and treatment type is not significant for any of the models, indicating that the effect of treatment does not vary by site.

Table 1: Mean Lesion Extension for all Trees± Standard Deviation

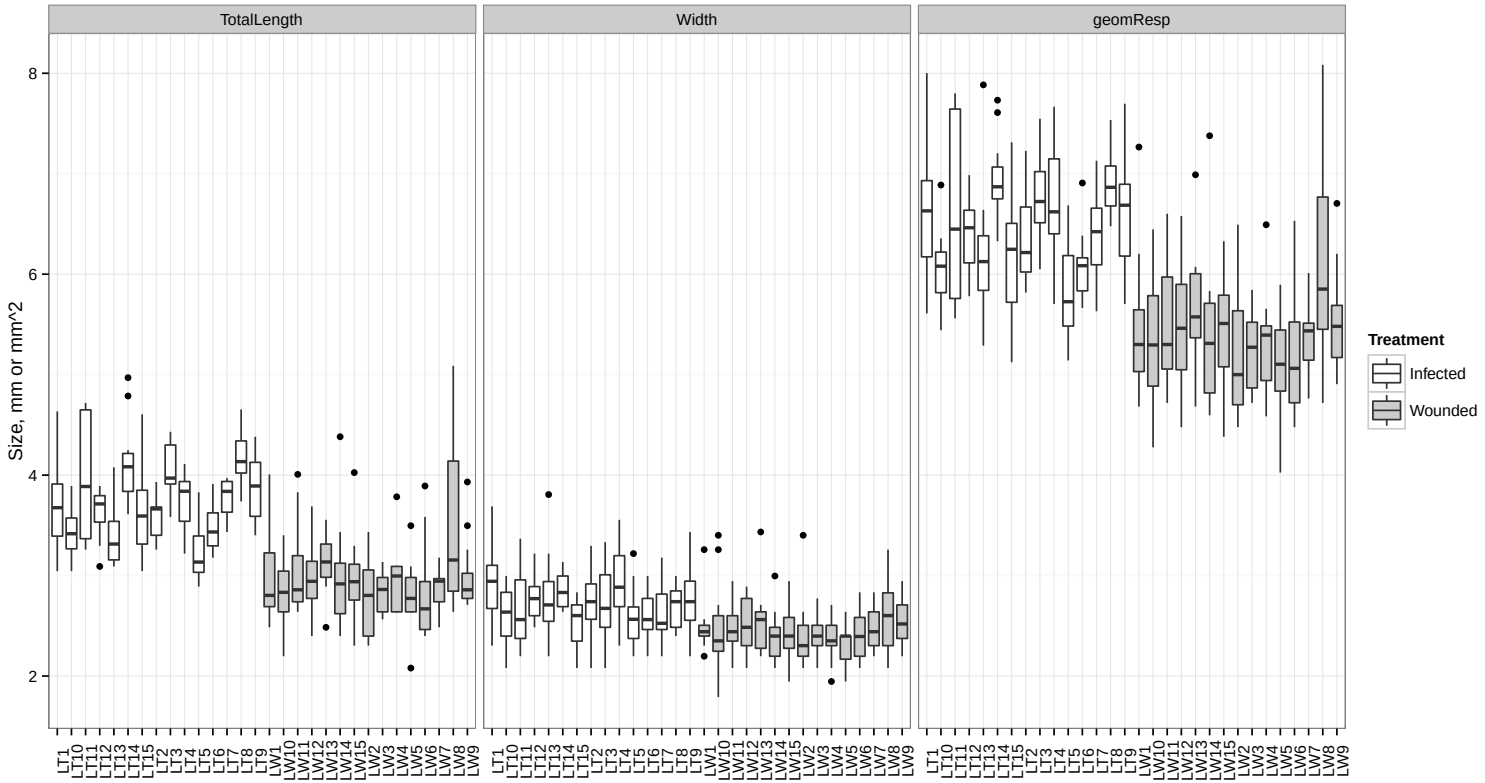
Site	**Upwards (mm)***	**Downwards (mm)***	**Width (mm)***	**Total Length (mm)***	**Area (mm^2)**
Lapinjärvi					
Infected	19.3 ± 15.1	16.8 ± 9.2	16.1 ± 5.8	45.52 ± 21.8	6.5 ± 0.6
Control	5.9 ± 6.7	7.4 ± 12.1	12.22 ± 4.2	20.0 ± 19.7	5.4 ± 0.6
Rovaniemi					
Infected	19.0 ± 14.7	19.2 ± 12.8	15.5 ± 5.1	55.6 ± 30.8	6.5 ± 0.7
Control	5.4 ± 4.1	6.2 ± 6.1	12.8 ± 3.4	20.6 ± 8.6	5.5 ± 0.5

**Measurements not log transformed for this table. Measurements include inoculation dowel size of 6.0 mm for width, area and total length measurements.*



Trees in Rovaniemi were susceptible to H. parviporum. The presented values for total length and width have been log_etransformed prior to the analysis.

Figure 3: Rovaniemi Tree Measurements



Tree LW8 exhibits large differences in measurement sizes from other mock inoculated trees. The presented values for total length and width have been log_e *transformed prior to the analysis.*

Figure 4: Lapinjärvi Tree Measurements

4.3 Effect of Organ on the Size of Lesion

The effect of organ is insignificant as a source of variation for total length (*t=0.88, df=60.6, p-value=0.38*), width (*t=0.32, df=72.1, p-value=0.74*), or area models (*t=0.79, df=62*, *p-value=0.42*). The mean total length measurements in roots and stems is $32.41 \pm 19.1\,mm$, and $35.7 \pm 26.7\,mm$, respectively. Mean width in the roots was $14.6 \pm 19.1\,mm$, and $13.8 \pm 26.7\,mm$ in the stem. The average lesion area in the root is $6.0 \pm 0.7\,mm^2$ and $5.9 \pm 0.9\,mm^2$ in the stem.

4.3.1 Interaction Between Field Site and Organ

The interaction between site and organ is significant for total length measurements (*t=2.5, df=57*, *p-value<0.05*), as well as for the lesion area (*t=2.4, df=57, p-value<0.05*). For both total length and area measurements, the difference between sites is more pronounced in the stems than it is in the roots. For root lesions, overall area and total length in Lapinjärvi are larger compared to Rovaniemi. However, in the stem, Lapinjärvi lesions are smaller compared to Rovaniemi.

4.3.2 Interaction Between Treatment and Organ

The interaction between treatment and organ is significant for all measurements, with the difference between treated and wounded lesions being more pronounced in the stem than in the roots for the total length (*t=-2.89, df=57, p-value=<0.01*), width (*t=-3.97, df=57, p-value=<0.01*), and area models *(t=-3.60, df=57, p-value=<0.01*).

Table 2: Mean Lesions Extensions in Stems for all Trees±Standard Deviation

Site	Upwards (mm)	Downwards (mm)	Width (mm)*	Total Length (mm)*	Geom. Resp. mm^2)*
Lapinjärvi					
Infected	21.2 ± 16.1	16.5 ± 8.3	16.2 ± 6.0	46.5 ± 21.8	6.5 ± 0.6
Control	4.5 ± 4.3	6.5 ± 15.6	10.8 ± 3.0	20.0 ± 19.7	5.2 ± 0.6
Rovaniemi					
Infected	21.3 ± 17.1	23.7 ± 15.4	15.8 ± 5.7	55.6 ± 30.8	6.6 ± 0.7
Control	5.6 ± 4.9	6.0 ± 4.9	12.2 ± 3.1	20.6 ± 8.6	5.4 ± 0.4

**Measurements not log transformed for this table. Measurement includes the inoculation dowel width as well as lesion extension.*

Table 3: Mean Lesions Extensions in Roots for all Trees ±Standard Deviation

Site	Upwards (mm)	Downwards (mm)	Width (mm)*	Total Length (mm)*	Geom. Resp. mm^2)*
Lapinjärvi					
Infected	17.4 ± 13.8	17.1 ± 9.9	15.9 ± 5.7	44.4 ± 21.9	6.4 ± 0.6
Control	7.3 ± 8.2	8.2 ± 6.9	13.6 ± 4.7	24.3 ± 13.8	5.6 ± 0.6
Rovaniemi					
Infected	16.7 ± 11.6	14.7 ± 7.4	15.3 ± 4.4	40.6 ± 17.1	6.3 ± 0.6
Control	5.1 ± 3.3	6.4 ± 7.3	13.5 ± 3.6	20.3 ± 8.6	5.5 ± 0.5

**Measurements not log transformed for this table. Measurement includes the inoculation dowel width as well as lesion extension.*

4.4 Effect of Tissue Type on Size of Lesion

The effect of tissue is statistically significant on the resulting size of both lesion area (*t=-10.58, df=356, p-value=<0.01*), and lesion width models (*t=-16.56, df=356, p=<0.01*). The mean lesion area is $6.15 \pm 0.76\, mm^2$ for phloem tissues, and $5.74 \pm 0.75\, mm^2$ in xylem tissues. For width measurements, the mean lesion size is $16.76 \pm 5.4\, mm$ in the phloem, and $11.59 \pm 2.67\, mm$ in the xylem tissues. Phloem and xylem tissues do not vary differently with regards to total length measurements, which have mean sizes of $34.37 \pm 22.07\, mm$, and $33.75 \pm 24.42\, mm$ respectively.

Interaction between treatment and tissue.

The interaction between treatment and tissue is significant for both the total length and width models. The effect of treatment is more pronounced in xylem for total length measurements (*t=-4.77, df=356, p-value=<0.01*). Conversely, the difference in the effect of treatment is more pronounced in the phloem for width measurements (*t=4.10, df=356, p-value=<0.01*). Due to the inverse relationship between the interactions of treatment and tissue for total length and width, the lesion area model does not indicate a significant interaction.

Interaction between site and tissue

The interaction between site and tissue is significant for the total length (*t=5.56, df=356, p-value=<0.01*), width (*t=3.08, df=356, p-value=<0.01*), and area models (*t=5.79, df=356, p-value=<0.01*), with the difference in the effect of site being greater in the

xylem than phloem. Across all models, Rovaniemi trees, on average, have larger lesions in the xylem than Lapinjärvi.

Interaction between organ and tissue

The interaction between organ and tissue is significant in both width (*t=-2.39, df=356, p-value=<0.05*) and area models (*t=-2.639, df=356, p-value=<0.01*). In both models, the difference in the size of the effect of organ on resulting lesion size in tissue is greater in the xylem tissues than it is in the phloem (Tables 4,5). For both lesion width and lesion area measurements, stems have smaller xylem lesions than in the roots xylem.

Table 4 Mean Lesion Extensions in Phloem for all Trees±Standard Deviation

Site	Upwards (mm)	Downwards (mm)	Width (mm)*	Total Length (mm)*	Geom. Resp. mm^2)*
Lapinjärvi					
Infected	19.1 ± 14.9	17.6 ± 8.9	19.7 ± 5.7	46.1 ± 21.8	6.7 ± 0.6
Control	6.8 ± 7.4	8.4 ± 12.7	14.3 ± 4.5	24.6 ± 18.4	5.7 ± 0.6
Rovaniemi					
Infected	17.6 ± 13.1	18.4 ± 10.6	18.6 ± 5.4	46.2 ± 23.6	6.6 ± 0.7
Control	5.7 ± 4.5	6.0 ± 5.7	14.6 ± 3.4	20.6 ± 8.2	5.6 ± 0.5

**Measurement includes the inoculation dowel width as well as lesion extension.*

Table 5: Mean Lesion Extensions in Xylem for all Trees±Standard Deviation

Site	Upwards (mm)	Downwards (mm)	Width (mm)*	Total Length (mm)*	Geom. Resp. (mm^2)*
Lapinjärvi					
Infected	19.6 ± 15.0	16.0 ± 9.3	12.4 ± 2.9	50.0 ± 23.0	6.2 ± 0.5
Control	5.0 ± 5.8	6.4 ± 11.3	10.1 ± 2.3	19.7 ± 15.4	5.1 ± 0.6
Rovaniemi					
Infected	20.4 ± 16.2	20.0 ± 14.8	12.7 ± 2.6	50.0 ± 28.0	6.3 ± 0.6
Control	5.0 ± 3.8	6.4 ± 6.6	11.1 ± 1.8	20.3 ± 8.9	5.3 ± 0.4

**Measurement includes the inoculation dowel width as well as lesion extension.*

4.5 Linear Mixed Effects Models for Lesion Size Measurements

For each of the models, the main (fixed) effects estimated are the effects of treatment (control or infected), site (Rovaniemi or Lapinjärvi), organ (stem or root), and tissue (phloem or xylem). Additionally, all two way interactions between these main effects are included for each model. Random effects specifications for each of the models includes three separate hierarchical grouping variables; individual tree level groupings as a base grouping effect, organ within tree level groupings a 1st level nesting of grouping effect, and sample replicates within the organ within tree level groupings as a 2nd level nested grouping effect.

4.5.1 Fixed Effects

Summary of the model parameters, 95% confidence intervals for main effects, standard error of the main effects, and *p*-values for the total length, width, and area model are given in tables 6, 7, and 8, respectively.

Table 6: Estimates of the Fixed Effects in the Total Length Model

Fixed Effect	***Estimate***	***Standard Error***	***C.I 2.5%****	***C.I. 97.5%****	***t-value***	***p-value*****
Intercept	3.72	0.07	3.58	3.87	51.10	<0.001
Treatment:Wounding	-0.55	0.10	-0.76	-0.36	-5.75	<0.001
Site:Rovaniemi	-0.17	0.07	-.034	0.01	-1.81	0.07
Organ:Stem	0.07	0.08	-0.09	0.22	0.88	0.38
Tissue:Xylem	-0.04	0.03	-0.10	0.00	-1.45	0.15
Treatment:Wounding ●Site:Rovaniemi	-0.03	0.12	-0.26	0.18	-0.30	0.76
Treatment:Wounding ● Organ:Stem	-0.27	0.09	-0.45	-0.08	-2.89	0.005
Treatment:Wounding ● Tissue:Xylem	-0.14	0.03	-0.19	-0.08	-4.77	<0.0001
Site:Rovaniemi ● Organ:Stem	0.24	0.09	0.07	0.40	2.54	0.01
Site:Rovaniemi ● Tissue:Xylem	0.16	0.03	0.10	0.22	5.66	<0.001
Organ:Stem●Tissue:Xylem	-0.05	0.03	-0.10	0.00	-1.88	0.06

**C.I. = 95% bootstrapped confidence intervals represented at the 2.5% and 97.5% intervals. Calculated utilizing the "confint" function in lme4 package (Bates et. al.) with 1000 simulations.*

*** P-value is the adjusted p-value calculated from Satterthwaite approximations to degrees of freedom*

Table 7: Estimates of the Fixed Effects in the Width Model

Fixed Effect	***Estimate***	***Standard Error***	***C.I 2.5%****	***C.I. 97.5%****	***t-value***	***p-value*****
Intercept	2.94	0.03	2.87	3.00	86.04	<0.0001
Treatment:Wounding	-0.23	0.04	-3.15	-0.15	-5.28	<0.0001
Site:Rovaniemi	-0.09	0.04	-0.18	0.00	-2.15	0.034
Organ:Stem	0.01	0.04	-0.06	0.09	0.32	0.75
Tissue:Xylem	-0.41	0.02	-0.46	-0.37	-16.57	<0.0001
Treatment:Wounding●Site:Rovaniemi	0.09	0.05	-0.01	0.19	1.73	0.09
Treatment:Wounding●Organ:Stem	-0.16	0.04	-0.23	-0.08	-3.97	<0.001
Treatment:Wounding●Tissue:Xylem	0.10	0.02	0.05	0.15	4.10	<0.0001
Site:Rovaniemi●Organ:Stem	0.06	0.04	-0.03	0.14	1.38	0.17
Site:Rovaniemi●Tissue:Xylem	0.09	0.02	0.03	0.14	3.50	<0.001
Organ:Stem●Tissue:Xylem	-0.06	0.02	-0.11	-0.01	-2.40	0.017

**C.I. = 95% bootstrapped confidence intervals represented at the 2.5% and 97.5% intervals. Calculated utilizing the "confint" function in lme4 package (Bates et. al.) with 1000 simulations.*

*** P-value is the p-value calculated from Satterthwaite approximations to degrees of freedom*

Table 8: Estimates of the Fixed Effects in the Lesion Area Model

Fixed Effect	***Estimate***	***Standard Error***	***C.I 2.5%****	***C.I. 97.5%****	***t-value***	***p-value*****
Intercept	6.7	0.10	6.58	6.8	69.1	<0.001
Treatment:Wounding	-0.80	0.13	-1.04	-0.51	-6.18	<0.001
Site:Rovaniemi	-0.27	0.13	-0.51	-0.02	-2.12	0.04
Organ:Stem	0.08	0.11	-0.13	0.29	0.80	0.42
Tissue:Xylem	-0.46	0.04	-0.53	-0.37	-10.60	<0.001
Treatment:Wounding●Site:Rovaniemi	0.06	0.16	-0.25	0.35	0.36	0.72
Treatment:Wounding●Organ:Stem	-0.43	0.12	-0.66	-0.19	-3.60	<0.001
Treatment:Wounding●Tissue:Xylem	-0.03	0.04	-0.12	0.05	-0.78	0.43
Site:Rovaniemi●Organ:Stem	0.30	0.12	0.06	0.52	2.45	0.02
Site:Rovaniemi●Tissue:Xylem	0.25	0.04	0.16	0.33	5.80	<0.001
Organ:Stem●Tissue:Xylem	-0.11	0.04	-0.197	-0.026	-2.64	0.008

**C.I. = 95% bootstrapped confidence intervals represented at the 2.5% and 97.5% intervals. Calculated utilizing the "confint" function in lme4 package (Bates et. al.) with 1000 simulations.*

*** P-value is the adjusted p-value calculated according to the Satterthwaite approximation to degrees of freedom*

4.5.2 Random Effects

The random grouping effects for total length, width, and area of the lesions are summarized in Tables 9, 10, and 11, respectively. For all models considered, the standard deviation increases for groups with increasing levels of nesting from tree, to organ within tree, to sample within organ and within tree. For all models, the residual standard deviation is higher than the standard deviation for trees level groupings as well as organ nested within tree level groupings, but is not higher than 2nd level nesting residual standard deviation of samples within organs within trees. In all three models, the highest standard deviation within the grouping random effects is attributed to the nested sample within organ and within tree groupings.

Plots of the group specific random effects are shown in figures 5, 6 and 7 for total length, width, and area models, respectively. The ordered dotplots for the models indicate that the distribution in random effects for each model is roughly normally distributed. The base level grouping of Tree has individual labels represented on the y-axis, and the most and least susceptible trees as modeled are easily determined.

Table 9: Random Effects in the Total Length Model

Random Effects	Type	Variance	Std. Deviation
Sample:(Organ:Tree)	Group Intercept	0.09	0.30
Organ:Tree	Group Intercept	0.03	0.17
Tree	Group Intercept	0.02	0.13
Residual	–	0.04	0.19

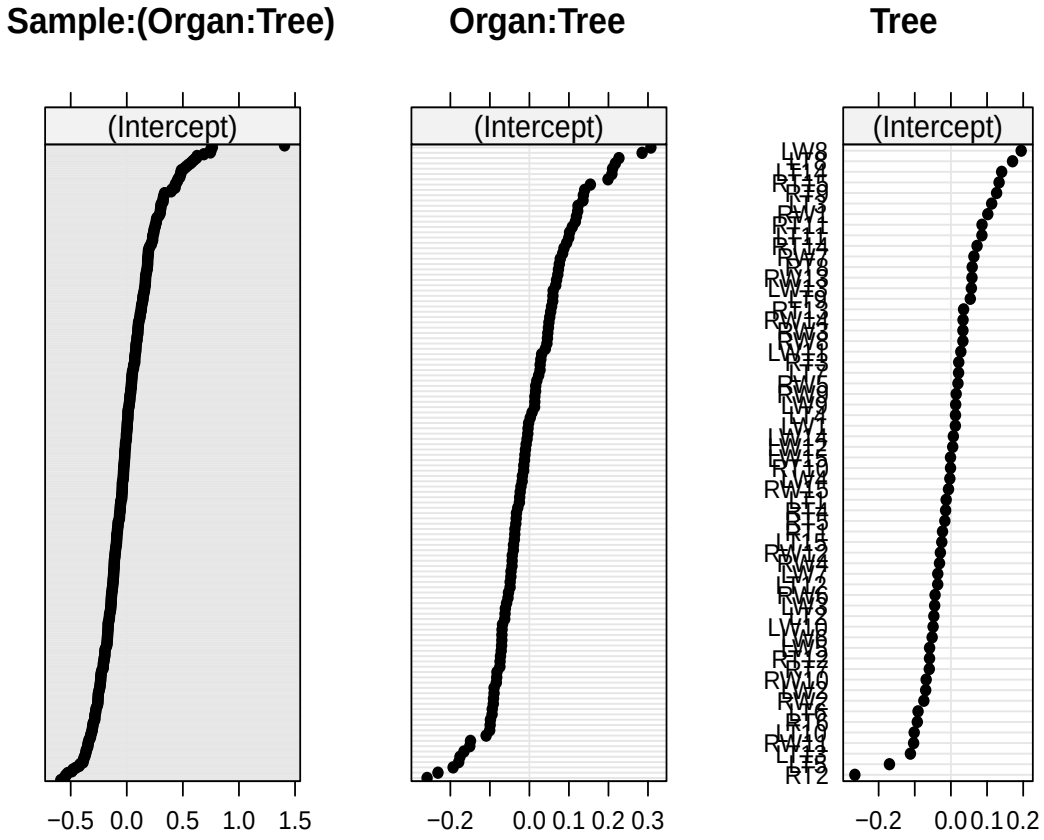


Figure 5: *Ordered Dotplots of the Total Length Model's Random Effects*

Table 10: Random Effects in the Width Model

Random Effects	Type	Variance	Std. Deviation
Sample:(Organ:Tree)	Group Effect	0.01	0.11
Organ:Tree	Group Effect	0.00	0.06
Tree	Group Effect	0.00	0.06
Residual	–	0.03	0.17

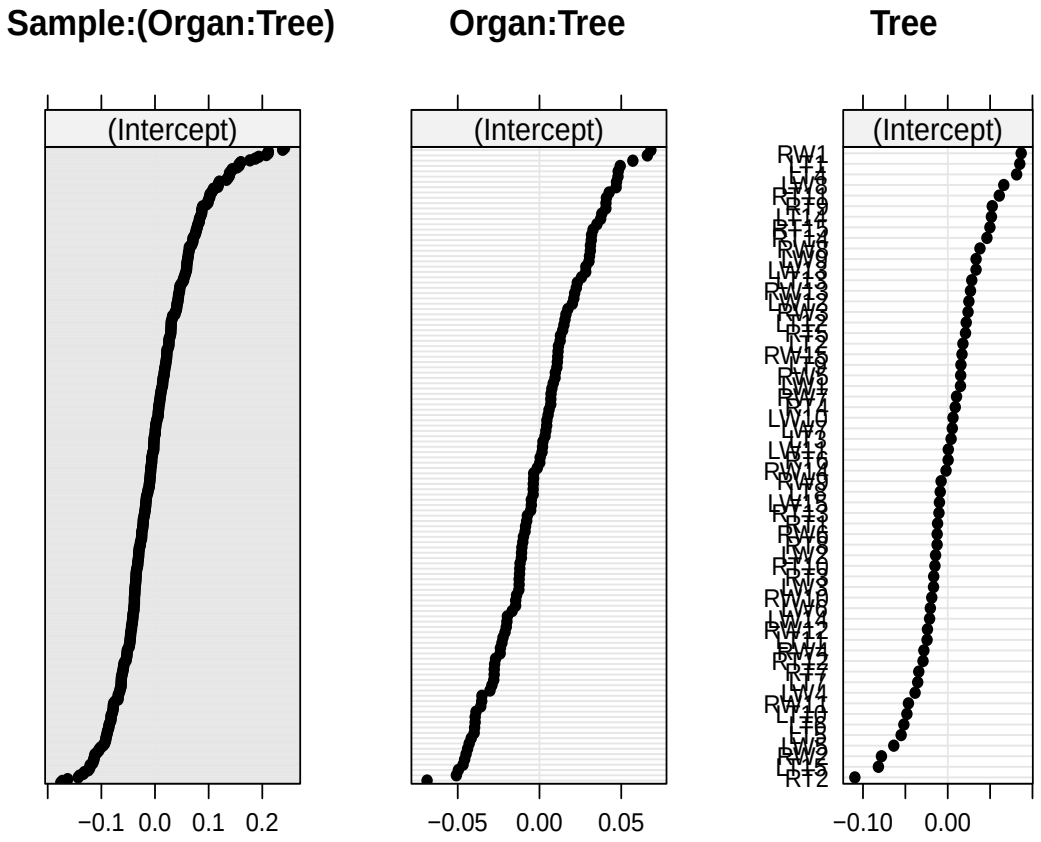


Figure 6: *Ordered Dotplots of the Width Model's Random Effects*

Table 11: Random Effects in the Lesion Area Model

Random Effects	Type	Variance	Std. Deviation
Sample:(Organ:Tree)	Group Effect	0.15	0.37
Organ:Tree	Group Effect	0.04	0.21
Tree	Group Effect	0.04	0.19
Residual	–	0.08	0.29

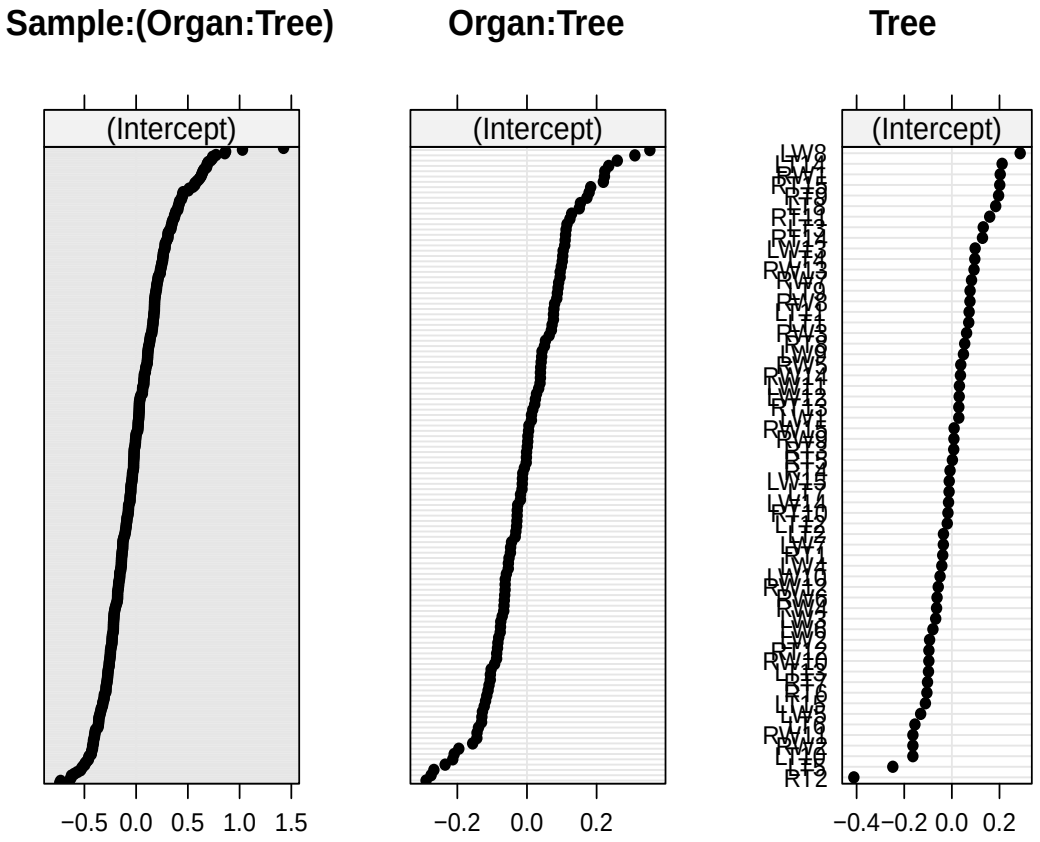
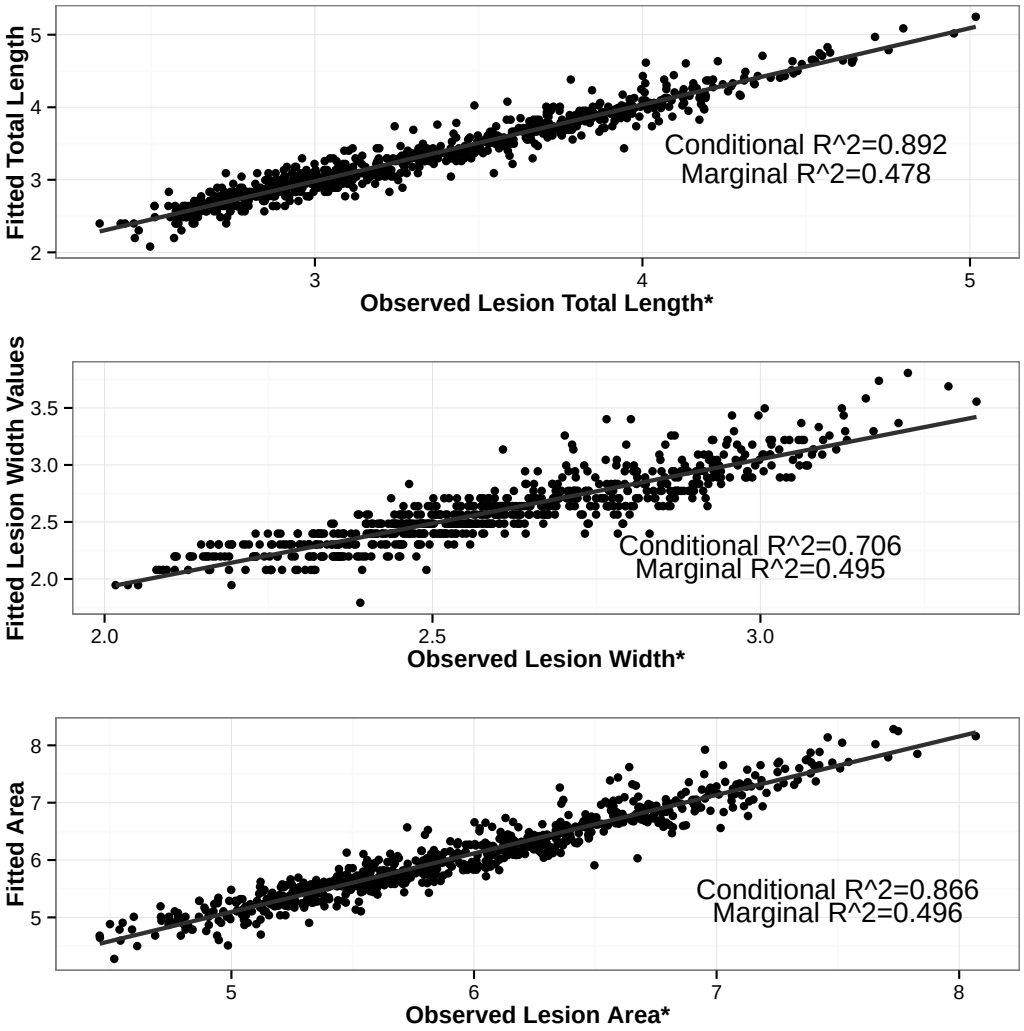


Figure 7: *Ordered Dotplots of the Area Model's Random Effects*

4.6 Model Validation and Diagnostics

Model goodness of fit was examined by plotting the response variable for each model against the fitted (predicted) values for that model. Additionally, an adaptation for the mixed effect model of the commonly utilized R^2 statistic has been calculated for each model[1]. For the total length model, the marginal R^2 is 0.478, or 47.8% of total variance explained due to fixed effects. conditional R^2 for the total length model is 0.892, or 89.2% of the total variance explained due to both fixed and random effects (Figure 11, upper panel). For the width model, the marginal R^2 is 0.495, or 49.5% of total variance explained by the fixed effects. conditional R^2 for the width model is 0.706, or 70.6% of total variance explained due to both the fixed and random effects (Figure 12, mid panel). For the lesion area model, marginal R^2 is 0.496, or 49.6% of total variance. conditional R^2 is 0.866, or 86.6% of total variance (Figure 12, lower). Subjectively, the final fits for all models range from excellent in the total length and lesion area models, to good in the total width model.

[1]The R^2 measure commonly utilized for linear regressions is not directly applicable to mixed effects models. However, Nakagawa and Schielzeth (2013) suggested a new method of calculating R^2 for mixed effects models which separates the influences of the fixed effects and random effects into two measures; marginal R^2 and conditional R^2. Marginal R^2 represents the variance attributed to the fixed effects only. Conditional R^2 includes both the influence from the fixed effects as well as the random effects in the given model.



**Observed measurements have been log transformed for this plot*

Figure 8: Plots of Model Estimate vs. Observed Lesion Sizes

5 Discussion

Based on the results from the models for total length, total width, and lesion area, the effect of field site on resulting size is inconclusive overall. The study found marginally significant evidence for a difference in the size of lesion width measurements based on site. Furthermore, the significance of the effect in width seems to propagate to lesion area model at a nearly similar level of significance. However, strong evidence for a difference in the total length measurement of the lesions was not found between sites. Given the only marginal findings of significance in a subset of the models examined, this study concludes that an overall difference in lesion size is not likely to be due to differences between the study sites when taken independent of other factors. It is worth noting that many aspects of this study are subject to analysis only in the context of the collected data and experiment performed herein. Thus, broad based inferences of the effects of historical geographical isolation from pathogen as a potential factor for differences in lesion response in *P. abies* to *H. parviporum* in Finland should not be drawn from this study alone. The results of this study partially support the work carried out by Witzell et al. (2011), who loosely concluded that difference in geographic origin might not affect overall resistance of *P. abies* to *H. annosum s.l.* in northern Fenno-Scandanavia. However, their study explored the ability of *H. annosum s.s.* versus *H. parviporum* to colonize in different geographical locations with different temperature regimes, but did not look at regions where the fungus was not known to be present prior to the study.

This study found no evidence for the effect of organ on the response in lesion size in any of the models. However, other studies, including Keriö et al. (2015), have concluded that a difference exists in the response of *P. abies* between roots and stems. Reasons for a lack of similar findings in this study could be attributed to many possible factors, such as soil type, temperature and climate, time between inoculation to harvest, and relative ability of the fungal strain to overcome host responses. Additionally, a major difference between this study and the one conducted by Keriö et al. (2015) is that this study did not utilize clonal materials with known genotypes. Other possible explanations for this observation is due to the sizes of inoculated roots. In Rovaniemi, the sizes of roots utilized for the inoculations was significantly smaller than the average

root sizes in Lapinjärvi; a study by Garbelotto and Slaughter (1997), found a positive correlation between root diameter and fungal growth. However, the interactions between site and organ in this study is significant for both the total length and lesion area models, suggesting that total length of lesion (and its contribution to the lesion area response) does vary differently between organs dependent on the site. However, other variables, such as differences in root size between the sites may be responsible for this result. Across all models, the interaction between treatment and organ was significant, with stems being less susceptible to wounding than roots.

It is worth noting that most studies where artificial inoculations have been performed on *P. abies* with *H. annosum s.l.* focused solely on stem inoculations. These types of inoculations do not mimic the natural infection pathway in *H. annosum s.l.*. Because of this, it can be difficult to judge whether or not a difference is generally assumed to exist in the responses between the two organs based on the results of the few studies which have explored root inoculations. Further complications occur due to the highly variable root structure and variability in root sizes utilized for inoculation points in this study, as well as the lack of control for genotype in this study. Differences attributable to genotype of the tree likely contribute to some of the variation among the observations.

The difference in lesion response in tissues was highly significant in this study for both the lesion width and lesion area models. In the study conducted by Keriö et al. (2015), no difference was noted between tissues in the roots, but a difference was found in the stem; however, their study found the difference in total length measurements. Different anatomical features are present in the different tissues, which may influence the ability of *H. annosum s.l.* to grow in the separate tissues (Krekling et al., 2004). Further reasons for the observed difference in the response between tissues could be due to a number of potential factors. Genetic control of the sample subjects in future experiments would help to determine if the observed differences are due to genetic, environmental, or latent factors.

The interaction between site and organ was significant in both the total length and lesion area models, with stems lesions in Rovaniemi being larger. This could be due to a number of factors which were unaccounted for in this study. Norway spruce has high genetic plasticity, which could account for differences in the response of the organs at

various sites (Reich et al., 1996; Chen et al., 2012). However, the design of the study did not control for genotype or phenotype diversity, so there is no way to account for this variance in the study. Other possible reasons why response by organ varied by site could be due to differences in the overall size of the trees, as trees of differing sizes and ages may respond differently to infections.

5.1 Technical Considerations in Experimental Design

The biggest limitation in the study is the lack of clonal material. Although the results of the study may be sufficient for drawing broad-based conclusions about aspects of the defense response of Norway spruce to infections with *H. parviporum* in natural settings. It is not possible to conclude what aspects of an individual tree in the study contributed to increased or decreased susceptibility. Clonal material would have allowed a better assessment of the influence and variability of individual tree's phenotypes. This lack of clonal material is a principal reason why trees in this study are treated as random effects as opposed to fixed effects; in a study controlled for tree genotype with sufficient numbers of ramets per clones, it would be possible to draw conclusions about the resistance of individual Norway spruce genotypes to *H. parviporum*.

The lack of control over genetics makes it difficult to know what to do with outliers present in the data; for this reason, several outliers in the traditional sense were kept in the analysis. Trees RT2 and LW8 showed extreme resistance and susceptibility to treatment, respectively. Tree LW8 had decay due to a natural unknown fungal infection prior to the experiment, but it was only noted when the tree was cut down for processing. If clonal material with known resistance phenotypes were utilized, it might be possible to determine if the tree genotype was really susceptible, or if this was an unnatural response to the treatment. Because it is not possible to positively deduce the causes for responses of trees such as RT2 and LW8, which display extreme responses with this study's experimental design, it would not be appropriate to remove the suspected outliers from the analysis.

The potential effects of historical isolation or geographical distance from the pathogen based on latitude could not be accurately determined based on this study's experimen-

tal design. Too many confounding factors could account for any potential observed differences. Additionally, only one site in each geographical region was utilized for the study; to effectively determine the effects of a site factor, more than one replicate for each area is necessary. However, prior work by Karlsson et al. (2008) supports the idea that differences in resistance exist between geographic areas. In their study, two sites in southern Europe utilizing clonal material from Greece and Italy had significant correlations between fungal growth, lesion size and other indicators of resistance, while a third field site in Sweden utilizing the same clonal material did not share significant correlations with the southern European field sites. However, the authors speculated that environmental factors could be responsible for the lack of correlations between the two regions. Further studies could attempt to address differences between trees acclimatized to certain growing environments by using a crossed design wherein trees from both genetic origins are utilized across field sites.

Additional issues with the experimental design for this study include a lack of control over external factors such as different flora and fauna at the sites; these factors could have implications in the estimation of site based effects. This is one major complication with carrying out field based experiments as opposed to those which take place in highly controlled laboratory and greenhouse settings, as temperature, weather events, and other natural influences are not controllable in field experiments. However, for drawing ecological conclusions regarding the interactions between Norway spruce and *H. annosum s.l.,* it is useful to carry out field experiments; any resulting residual variances can then be attributed to uncontrollable environmental factors, but would require that the experimental design properly controlled for both the host genotype and site factor.

5.2 Potential Issues in the Analysis

Choices of the methodology utilized to analyze and present data for this study were based on balancing several conflicting goals: how to best characterize the lesion response measurements on various levels, while avoiding over parametrization of the model, and maintaining interpretability of the final models.

In this study, the response variables of interest are the lesion total length, width, and area measurements taken as in indication of resistance or susceptibility for a given sample, with inferences then drawn for the different tissues, organs, and individual tree. In reality, the lesion presents as a three dimensional reaction to the inoculation. However, to analyze the lesions, a simplified conceptualization of the lesions was utilized, treating the lesion measurements as one-dimensional and two-dimensional geometric measurements based on the observed maximal width and length of the lesions across the point of inoculation. In reality, lesions from the inoculations performed in this experiment presented in many ways, and no single geometric shape or measurement utilized for the analysis of the data is consistent in accurately describing the overall diversity of observed lesion shapes. In hindsight, there are more accurate ways to measure the lesions. With proper photographic equipment and software, it would be possible to measure accurately the entire area of the lesion without needing to resort to constricting the conceptualization of the lesion to a single shape, utilizing a known reference measurement as a calibration. Implementing this could increase the accuracy of the parameter estimates, as well as better describe the variance of actual lesions. However, even this advanced conceptualization of the lesion lacks a third dimension (depth). The technique of utilizing digital photography and references for calculation of lesion areas has been applied in other studies with agricultural plants, but to the best of the author's knowledge, has not been implemented in studies examining lesions created from *H. annosum s.l.*.

5.3 Lesion Size as an Indicator of Resistance or Susceptibility

Differences in choices for experimental methodology include inoculation techniques. Deep tissue wounding has been used in various studies including this one, and is done by boring directly to the heartwood of a living tree to introduce the pathogen directly to susceptible tissues. (Delatour et al., 1998). In another inoculation technique, superficial wounding targets only the surface of the cambium of the tree which is inoculated with the pathogen (Delatour et al., 1998). Other variables in studies examining the resistance in conifers to *H. annosum s.l.* include number of replicates per tree, location of field sites, temperature profiles of the regions where experiments are performed, choices of

clonal material, length of incubation times, and methods for analyzing the resulting data. However, research indicates that overall sizes of respective lesions in inoculated clones may be a first step for indicating resistance or susceptibility of trees to infections from *H. annosum s.l.* (Swedjmark and Stenlid, 1997; Delatour et al., 1998; Woodward et al., 2007).

Although the author chose analysis techniques which are believed to be appropriate for the data collected in this study, the mixed effects approach does little to interpret the potential resistance or susceptibility of the trees on a biological level beyond looking at overall lesion sizes. Therefore, the results of the study and conclusions drawn from these results rely on the assumption that lesion size is a reasonable proxy for quantifying resistance and susceptibility to *H. parviporum* in *P. abies*. The lack of control for tree genotype is a troubling shortcoming in this study which prevents anything beyond generalization about tree resistance to be drawn from the data collected for this study. In future studies, use of clonal material with well characterized resistance phenotypes would be essential for comparing resistance across treatments. In future works, molecular methods should be included to find additional markers for use in quantifying measures of resistance to *H. annosum s.l.*. These might include, analysis of RNA transcripts to determine up-regulated gene products such as chitinase, terpenes, and other PR-family proteins, etc., in known resistant and susceptible genotypes. The use of molecular methods in conjunction with analysis of lesions would provide more detailed information about the resistance of trees, and would also allow for correlations to be made between specific gene products and lesion characteristics. Several prior studies have incorporated analysis of lesions and fungal growth within host tissues, along with molecular methods for assessing various aspects of tree resistance to *H. annosum s.l.* (Hietala et al., 2003; Woodward et al., 2007).

In hindsight, it is difficult to consider latitude as a reasonable effect to study for the resistance of Norway spruce to *H. parviporum*; in reality, isolation from historical presence of the pathogen is the parameter this study attempted to address, and subsequent studies should look at this along with specific environmental factors which may influence the pathology during the experiment.

6 Conclusions

H. annosum s.l. is a devastating forest pathogen which causes millions of euros in losses to annually. Many factors contribute to the capability of the pathogen to proliferate and spread under natural conditions. Despite research into forest tree breeding for resistance and use of various biotic and abiotic control methods, the pathogen still manages to cause large economic losses, and is a leading concern in the modern industrialized forestry sector. Understanding the different aspects of the epidemiology and life cycles of both the host and pathogen may hold key information to combating infections and breeding for improvements in resistance. Factors such as genetic origin, tree age and size, as well as environmental conditions all impact the resistance of Norway spruce to infection by *H. annosum s.l.*.

The study described herein attempted to examine several aspects of the resistance potential of *P. abies* to *H. parviporum* in natural conditions. Primary factors examined were the effects of site across geographic areas where the pathogen is prevalent or historically has very limited presence. Additional factors examined in this research include the potential for different responses in both host tree organ (root and stem), and tissues (phloem and xylem), as well as the statistical interactions between various crossed factors included in the design of the experiment.

The study found that overall evidence for a difference in the resistance of Norway spruce to *H. annosum s.l.* between the northern and southern field sites is minimal. In this regard, the study concludes that resistance appears similar in Norway spruce populations regardless of historical prevalence of the pathogen. Future studies should incorporate stricter controls, use of clonal materials, and if possible, crossing of geographic origin of the clonal material across environmentally distinct field sites to better control possible influences of geographic isolation from the pathogen. Further research into site based differences would also benefit from thorough monitoring and evaluation of environmental and soil based conditions.

This study did not find differences in resistance based on whether the inoculation was performed in the stem versus the root organ of a tree in any of the models examined. This is in contrast to other studies reviewed, which have found a difference between

root and stems in their susceptibility. However, this study did note a significant interaction between organ and field site for both the lesion area and lesion total length measurements. As mentioned previously, roots in Rovaniemi were smaller than those in Lapinjärvi, which may confound the observed effect of organ on the response of the lesion when taken in absence of additional information such as site. Future studies conducted in a similar fashion should attempt to incorporate root size measurements to avoid similar uncertainties in the results.

This study noted a difference in the response of the different tissues in the width and lesion area models. However, the study did not find a difference in the response of the total length of the lesion by tissue, which is in line with other reviewed works, i.e, Keriö et al. (2015). The reasons for a difference in the width and lesion area models in contrast to the null result in the lesion total length model are uncertain: possible explanations for the different results include genetics, and environmental factors, among others.

Finally, a mixed effects model proved to be a useful tool to analyze the complex nature of the data collected and to interpret the results. Several other reviewed studies have taken a similar mixed-effect approach to modeling lesion response of forest trees, e.g. Swedjemark et al. (1998); Swedjemark and Karlsson (2004); Swedjemark et al. (2007); Karlsson et al. (2008). Subsequent studies may attain valuable additional information by including random effects into their analysis and moving away from typical multiple regression or ANOVA based analysis for which collected data may violate statistical assumptions. Overall, this study is the first of its kind in Finland, and the results have led to further insights on pathogenesis of *H. parviporum*. Additionally, this study has provided a platform for future resistance field studies.

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Appendix 1

Maps

Approximate location of the field sites is indicated with a red circle on each map.

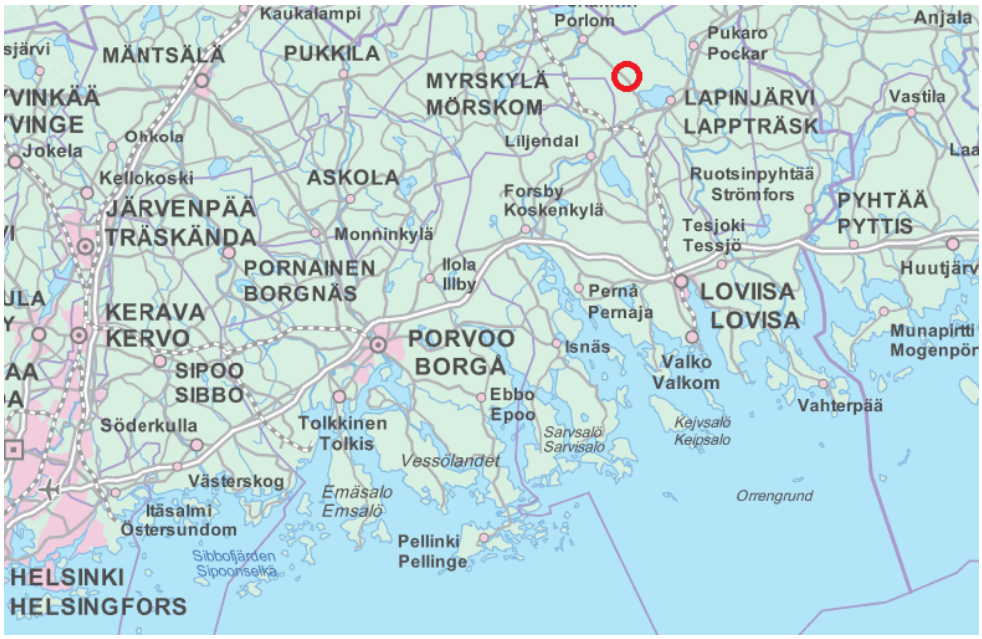

Figure A.1: Map of Lapinjärvi Field Site, Region

Figure A.2: Map of Lapinjärvi Field Site, General Area

Figure A.3: Map of Lapinjärvi Field Site, Close-Up

This map shows the southern field site at the Lapinjärvi research forest.

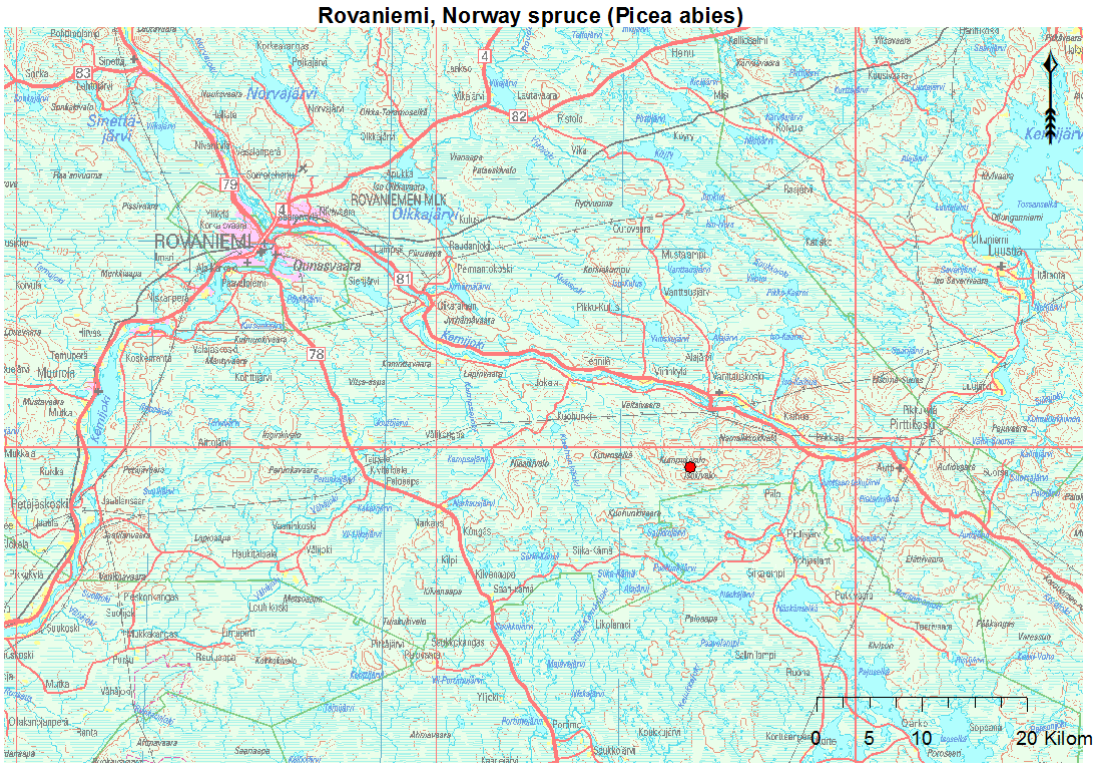

Figure A.4: Map of Rovaniemi Region, Including Field Site

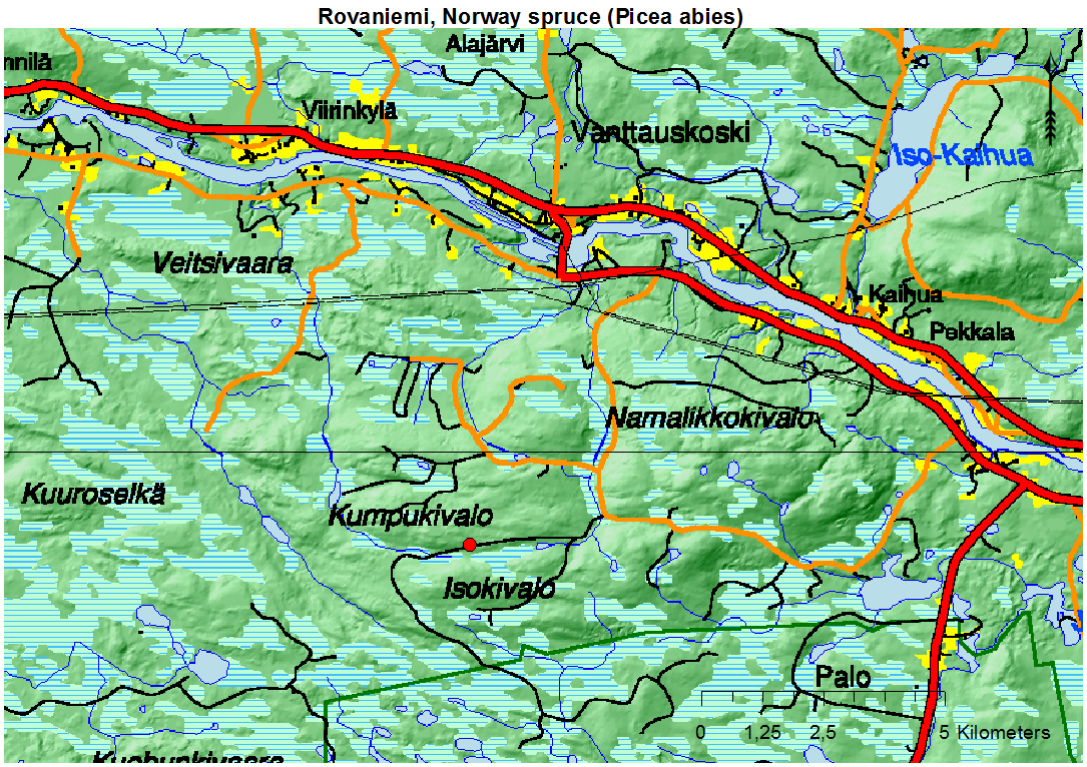

Figure A.5: Map of Rovaniemi Field Site, General Area

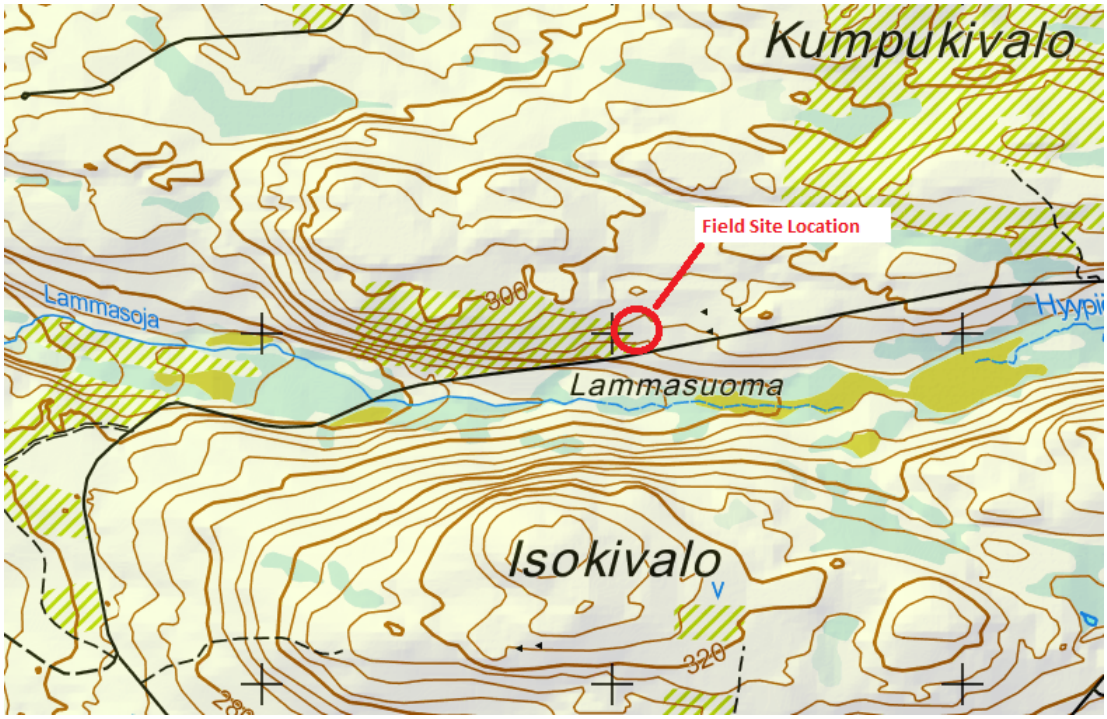

Figure A.6: Map of Rovaniemi Field Site, Close-Up

Appendix 2

Normality of Data and Outliers

The data collected from the experiment violated assumptions of normality. A log transform of the data was taken to attempt to normalize the data. Below are figures depicting the area, total length, and width measurements both before and after transformation.

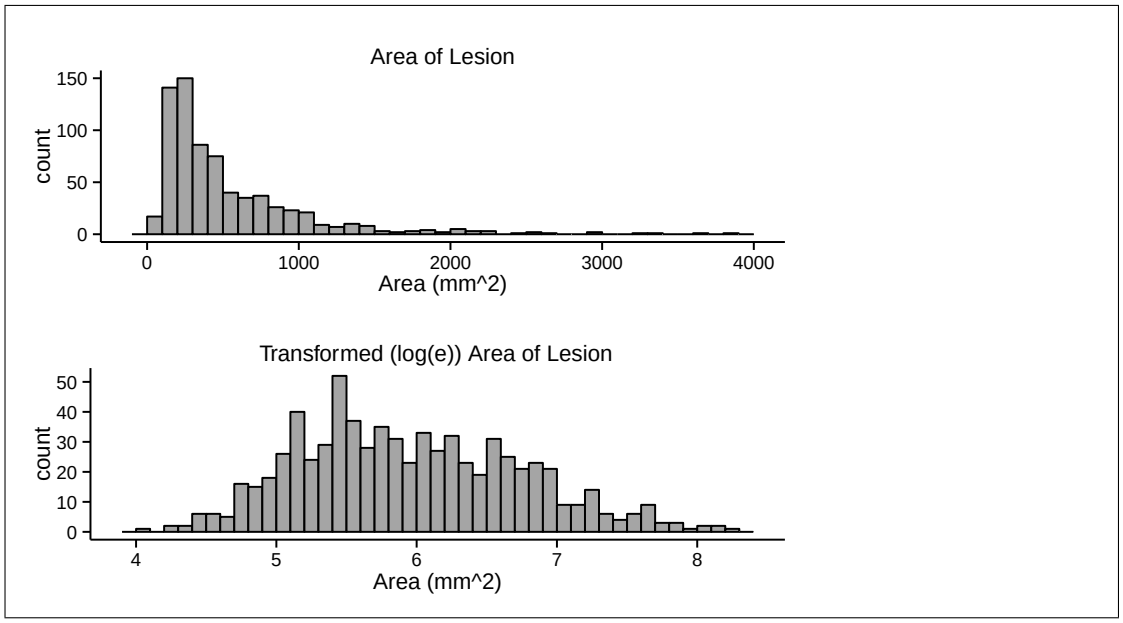

Figure A.7: *Histogram of the lesion area, and transformed area variable* $log_e(area)$*, indicating a lack of normality in the uncorrected data.*

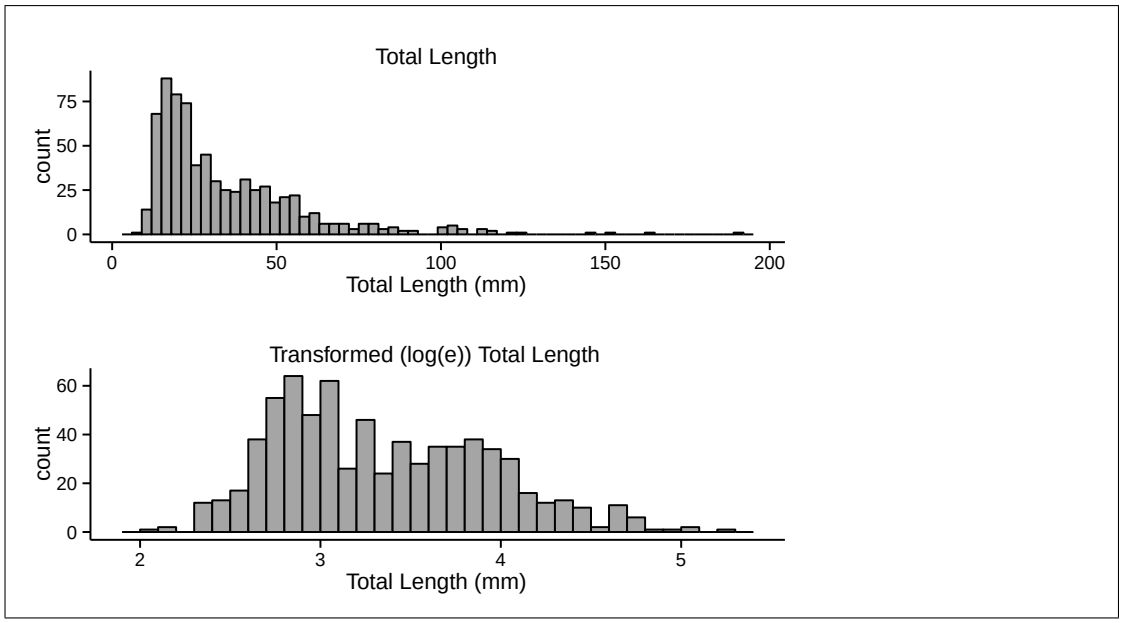

Figure A.8: *Histogram of the lesion length, and transformed length variable* $log_e(total\ length)$*, indicating a lack of normality in the uncorrected data.*

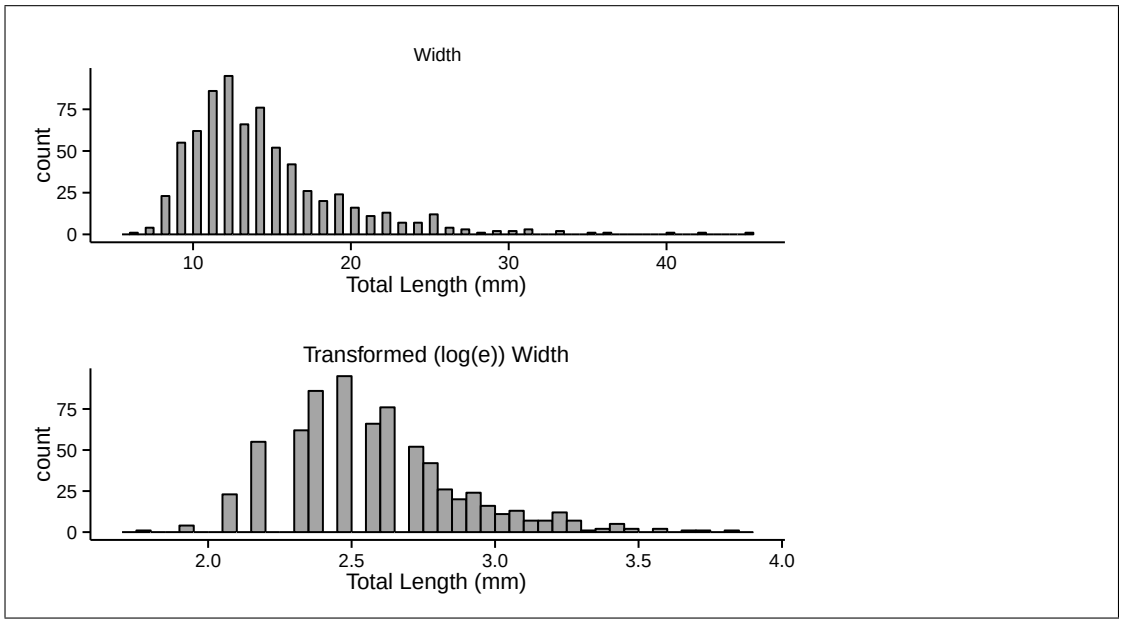

Figure A.9: *Histogram of the lesion width, and transformed width variable* $log_e(width)$*, indicating a lack of normality in the uncorrected data.*

To determine if any trees had significant influence on the parameter estimates, post-hoc investigation of the random effect of tree level groupings was performed. Cook's distance is a measure which is utilized to examine the influence of a unit on all parameters. Using the value of $4/n$, where n is the number of groups (in this case, trees) gives a cutoff value of 0.066 for determining overly influential observations. For the area and total length models, only tree RT2 is overly influential (Figs. A.6, A.7). For the width model, the only outlier is LW10 (Fig. A.8).

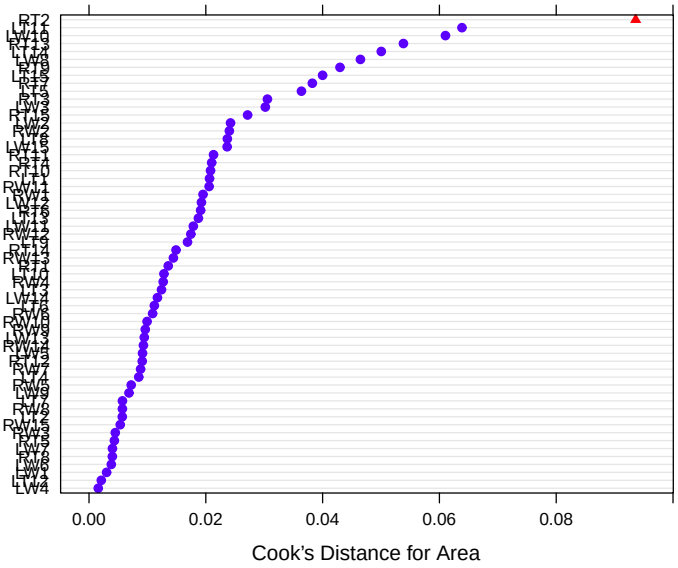

Figure A.10: Outlier Influence for Area Model

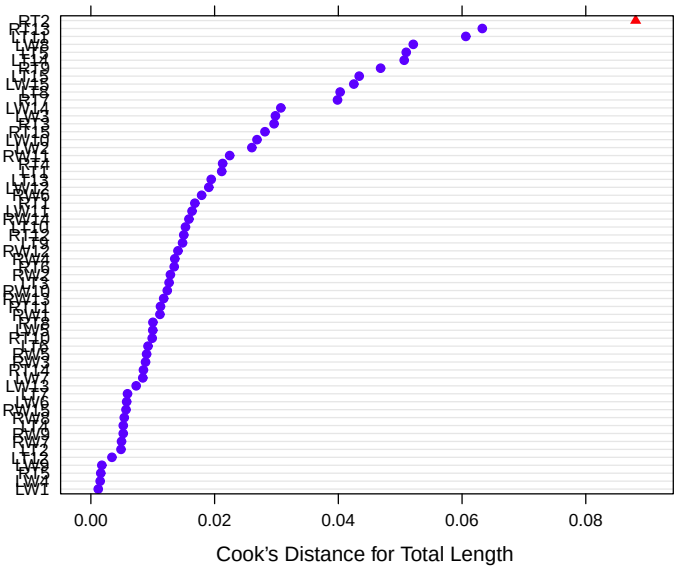

Figure A.11: Outlier Influence for Total Length Model

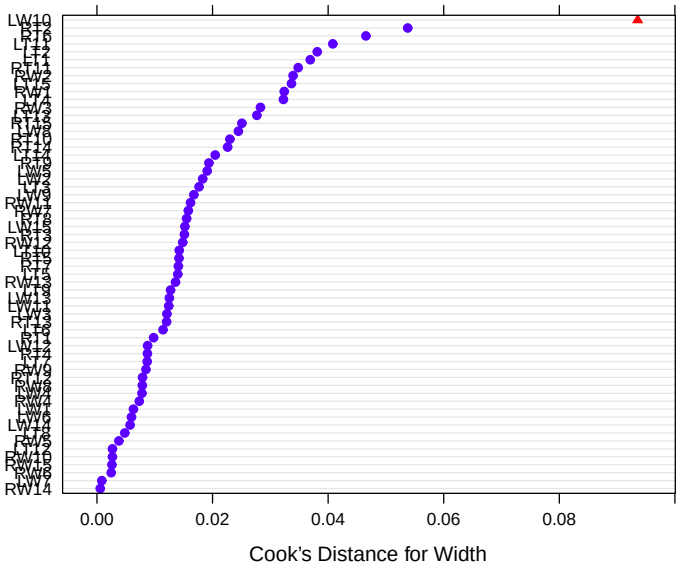

Figure A.12: Outlier Influence for Width Model

Appendix 3

Table of measurements and factors used in the mixed effects models. Length represents total length, and all measurements include the inoculation dowel. The area measurement has already been log transformed. Length and width are in millimeters, and area is millimeters squared.

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LT1	Infected	R	R1	Phloem	30	18	6.29
LT1	Infected	R	R1	Xylem	28	12	5.82
LT1	Infected	R	R2	Phloem	46	22	6.92
LT1	Infected	R	R2	Xylem	38	15	6.35
LT1	Infected	R	R3	Phloem	103	29	8
LT1	Infected	R	R3	Xylem	53	20	6.97
LT1	Infected	S	A	Phloem	29	23	6.5
LT1	Infected	S	A	Xylem	21	13	5.61
LT1	Infected	S	B	Phloem	56	40	7.71
LT1	Infected	S	B	Xylem	49	18	6.78
LT1	Infected	S	C	Phloem	41	21	6.76
LT1	Infected	S	C	Xylem	30	10	5.7
LT10	Infected	R	R1	Phloem	49	20	6.89
LT10	Infected	R	R1	Xylem	41	13	6.28
LT10	Infected	R	R2	Phloem	27	17	6.13
LT10	Infected	R	R2	Xylem	32	13	6.03
LT10	Infected	R	R3	Phloem	24	15	5.89
LT10	Infected	R	R3	Xylem	21	11	5.44
LT10	Infected	S	A	Phloem	29	17	6.2
LT10	Infected	S	A	Xylem	24	11	5.58
LT10	Infected	S	B	Phloem	32	18	6.36
LT10	Infected	S	B	Xylem	46	9	6.03
LT10	Infected	S	C	Phloem	29	16	6.14
LT10	Infected	S	C	Xylem	34	8	5.61
LT11	Infected	R	R1	Phloem	29	16	6.14
LT11	Infected	R	R1	Xylem	31	10	5.74
LT11	Infected	R	R2	Phloem	36	14	6.22
LT11	Infected	R	R2	Xylem	26	10	5.56
LT11	Infected	R	R3	Phloem	29	11	5.77
LT11	Infected	R	R3	Xylem	27	11	5.69
LT11	Infected	S	A	Phloem	106	23	7.8
LT11	Infected	S	A	Xylem	111	19	7.65
LT11	Infected	S	B	Phloem	73	29	7.66
LT11	Infected	S	B	Xylem	66	12	6.67
LT11	Infected	S	C	Phloem	104	20	7.64
LT11	Infected	S	C	Xylem	112	9	6.92
LT12	Infected	R	R1	Phloem	37	18	6.5

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LT12	Infected	R	R1	Xylem	27	12	5.78
LT12	Infected	R	R2	Phloem	22	18	5.98
LT12	Infected	R	R2	Xylem	27	15	6
LT12	Infected	R	R3	Phloem	47	23	6.99
LT12	Infected	R	R3	Xylem	49	14	6.53
LT12	Infected	S	A	Phloem	40	25	6.91
LT12	Infected	S	A	Xylem	44	14	6.42
LT12	Infected	S	B	Phloem	46	17	6.66
LT12	Infected	S	B	Xylem	42	12	6.22
LT12	Infected	S	C	Phloem	42	18	6.63
LT12	Infected	S	C	Xylem	39	12	6.15
LT13	Infected	R	R1	Phloem	22	22	6.18
LT13	Infected	R	R1	Xylem	27	12	5.78
LT13	Infected	R	R2	Phloem	24	18	6.07
LT13	Infected	R	R2	Xylem	27	13	5.86
LT13	Infected	R	R3	Phloem	59	45	7.88
LT13	Infected	R	R3	Xylem	22	15	5.8
LT13	Infected	S	A	Phloem	32	16	6.24
LT13	Infected	S	A	Xylem	29	12	5.85
LT13	Infected	S	B	Phloem	51	15	6.64
LT13	Infected	S	B	Xylem	43	13	6.33
LT13	Infected	S	C	Phloem	28	25	6.55
LT13	Infected	S	C	Xylem	22	9	5.29
LT14	Infected	R	R1	Phloem	37	20	6.61
LT14	Infected	R	R1	Xylem	40	14	6.33
LT14	Infected	R	R2	Phloem	42	23	6.87
LT14	Infected	R	R2	Xylem	65	16	6.95
LT14	Infected	R	R3	Phloem	120	19	7.73
LT14	Infected	R	R3	Xylem	144	14	7.61
LT14	Infected	S	A	Phloem	64	21	7.2
LT14	Infected	S	A	Xylem	67	14	6.84
LT14	Infected	S	B	Phloem	48	20	6.87
LT14	Infected	S	B	Xylem	70	16	7.02
LT14	Infected	S	C	Phloem	48	18	6.76
LT14	Infected	S	C	Xylem	55	15	6.72
LT15	Infected	R	R1	Phloem	46	14	6.47
LT15	Infected	R	R1	Xylem	100	15	7.31
LT15	Infected	R	R2	Phloem	45	13	6.37
LT15	Infected	R	R2	Xylem	76	13	6.9
LT15	Infected	R	R3	Phloem	50	15	6.62
LT15	Infected	R	R3	Xylem	40	8	5.77
LT15	Infected	S	A	Phloem	28	17	6.17
LT15	Infected	S	A	Xylem	21	8	5.12
LT15	Infected	S	B	Phloem	33	17	6.33
LT15	Infected	S	B	Xylem	29	9	5.56
LT15	Infected	S	C	Phloem	26	14	5.9
LT15	Infected	S	C	Xylem	24	11	5.58

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LT2	Infected	R	R1	Phloem	27	16	6.07
LT2	Infected	R	R1	Xylem	30	13	5.97
LT2	Infected	R	R2	Phloem	39	18	6.55
LT2	Infected	R	R2	Xylem	39	13	6.23
LT2	Infected	R	R3	Phloem	38	13	6.2
LT2	Infected	R	R3	Xylem	42	8	5.82
LT2	Infected	S	A	Phloem	39	20	6.66
LT2	Infected	S	A	Xylem	26	15	5.97
LT2	Infected	S	B	Phloem	51	27	7.23
LT2	Infected	S	B	Xylem	45	18	6.7
LT2	Infected	S	C	Phloem	39	25	6.88
LT2	Infected	S	C	Xylem	30	14	6.04
LT3	Infected	R	R1	Phloem	51	15	6.64
LT3	Infected	R	R1	Xylem	47	12	6.34
LT3	Infected	R	R2	Phloem	40	20	6.68
LT3	Infected	R	R2	Xylem	36	12	6.07
LT3	Infected	R	R3	Phloem	84	21	7.48
LT3	Infected	R	R3	Xylem	82	11	6.8
LT3	Infected	S	A	Phloem	79	24	7.55
LT3	Infected	S	A	Xylem	72	12	6.76
LT3	Infected	S	B	Phloem	53	19	6.91
LT3	Infected	S	B	Xylem	53	8	6.05
LT3	Infected	S	C	Phloem	55	28	7.34
LT3	Infected	S	C	Xylem	51	14	6.57
LT4	Infected	R	R1	Phloem	43	16	6.53
LT4	Infected	R	R1	Xylem	35	10	5.86
LT4	Infected	R	R2	Phloem	48	26	7.13
LT4	Infected	R	R2	Xylem	33	15	6.2
LT4	Infected	R	R3	Phloem	55	27	7.3
LT4	Infected	R	R3	Xylem	50	16	6.68
LT4	Infected	S	A	Phloem	32	22	6.56
LT4	Infected	S	A	Xylem	25	12	5.7
LT4	Infected	S	B	Phloem	47	20	6.85
LT4	Infected	S	B	Xylem	46	14	6.47
LT4	Infected	S	C	Phloem	61	35	7.67
LT4	Infected	S	C	Xylem	56	24	7.2
LT5	Infected	R	R1	Phloem	18	13	5.46
LT5	Infected	R	R1	Xylem	19	9	5.14
LT5	Infected	R	R2	Phloem	20	14	5.63
LT5	Infected	R	R2	Xylem	22	10	5.39
LT5	Infected	R	R3	Phloem	21	25	6.26
LT5	Infected	R	R3	Xylem	43	11	6.16
LT5	Infected	S	A	Phloem	24	14	5.82
LT5	Infected	S	A	Xylem	21	13	5.61
LT5	Infected	S	B	Phloem	27	17	6.13
LT5	Infected	S	B	Xylem	27	9	5.49
LT5	Infected	S	C	Phloem	40	20	6.68

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LT5	Infected	S	C	Xylem	46	12	6.31
LT6	Infected	R	R1	Phloem	26	18	6.15
LT6	Infected	R	R1	Xylem	27	12	5.78
LT6	Infected	R	R2	Phloem	31	16	6.21
LT6	Infected	R	R2	Xylem	24	12	5.66
LT6	Infected	R	R3	Phloem	32	14	6.1
LT6	Infected	R	R3	Xylem	39	9	5.86
LT6	Infected	S	A	Phloem	50	20	6.91
LT6	Infected	S	A	Xylem	43	10	6.06
LT6	Infected	S	B	Phloem	31	15	6.14
LT6	Infected	S	B	Xylem	27	11	5.69
LT6	Infected	S	C	Phloem	37	16	6.38
LT6	Infected	S	C	Xylem	29	12	5.85
LT7	Infected	R	R1	Phloem	51	19	6.88
LT7	Infected	R	R1	Xylem	52	12	6.44
LT7	Infected	R	R2	Phloem	52	24	7.13
LT7	Infected	R	R2	Xylem	53	13	6.54
LT7	Infected	R	R3	Phloem	49	15	6.6
LT7	Infected	R	R3	Xylem	51	11	6.33
LT7	Infected	S	A	Phloem	42	22	6.83
LT7	Infected	S	A	Xylem	44	10	6.09
LT7	Infected	S	B	Phloem	37	12	6.1
LT7	Infected	S	B	Xylem	31	9	5.63
LT7	Infected	S	C	Phloem	38	16	6.41
LT7	Infected	S	C	Xylem	35	12	6.04
LT8	Infected	R	R1	Phloem	60	17	6.93
LT8	Infected	R	R1	Xylem	50	13	6.48
LT8	Infected	R	R2	Phloem	42	17	6.57
LT8	Infected	R	R2	Xylem	56	12	6.51
LT8	Infected	R	R3	Phloem	58	20	7.06
LT8	Infected	R	R3	Xylem	55	15	6.72
LT8	Infected	S	A	Phloem	76	19	7.28
LT8	Infected	S	A	Xylem	75	12	6.8
LT8	Infected	S	B	Phloem	104	18	7.53
LT8	Infected	S	B	Xylem	105	12	7.14
LT8	Infected	S	C	Phloem	65	16	6.95
LT8	Infected	S	C	Xylem	79	11	6.77
LT9	Infected	R	R1	Phloem	71	31	7.7
LT9	Infected	R	R1	Xylem	62	15	6.84
LT9	Infected	R	R2	Phloem	80	25	7.6
LT9	Infected	R	R2	Xylem	62	19	7.07
LT9	Infected	R	R3	Phloem	49	15	6.6
LT9	Infected	R	R3	Xylem	37	10	5.91
LT9	Infected	S	A	Phloem	46	19	6.77
LT9	Infected	S	A	Xylem	30	10	5.7
LT9	Infected	S	B	Phloem	49	19	6.84
LT9	Infected	S	B	Xylem	50	14	6.55

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LT9	Infected	S	C	Phloem	33	16	6.27
LT9	Infected	S	C	Xylem	34	9	5.72
LW1	Wounded	R	R1	Phloem	55	26	7.27
LW1	Wounded	R	R1	Xylem	33	12	5.98
LW1	Wounded	R	R2	Phloem	17	13	5.4
LW1	Wounded	R	R2	Xylem	15	10	5.01
LW1	Wounded	R	R3	Phloem	14	11	5.04
LW1	Wounded	R	R3	Xylem	13	11	4.96
LW1	Wounded	S	A	Phloem	16	12	5.26
LW1	Wounded	S	A	Xylem	19	11	5.34
LW1	Wounded	S	B	Phloem	16	12	5.26
LW1	Wounded	S	B	Xylem	12	9	4.68
LW1	Wounded	S	C	Phloem	38	13	6.2
LW1	Wounded	S	C	Xylem	23	11	5.53
LW10	Wounded	R	R1	Phloem	21	15	5.75
LW10	Wounded	R	R1	Xylem	18	10	5.19
LW10	Wounded	R	R2	Phloem	16	26	6.03
LW10	Wounded	R	R2	Xylem	12	6	4.28
LW10	Wounded	R	R3	Phloem	21	30	6.45
LW10	Wounded	R	R3	Xylem	14	8	4.72
LW10	Wounded	S	A	Phloem	24	11	5.58
LW10	Wounded	S	A	Xylem	17	10	5.14
LW10	Wounded	S	B	Phloem	30	12	5.89
LW10	Wounded	S	B	Xylem	14	10	4.94
LW10	Wounded	S	C	Phloem	17	13	5.4
LW10	Wounded	S	C	Xylem	9	8	4.28
LW11	Wounded	R	R1	Phloem	14	12	5.12
LW11	Wounded	R	R2	Phloem	21	19	5.99
LW11	Wounded	R	R1	Xylem	16	11	5.17
LW11	Wounded	R	R2	Xylem	26	15	5.97
LW11	Wounded	R	R3	Phloem	46	16	6.6
LW11	Wounded	R	R3	Xylem	55	13	6.57
LW11	Wounded	S	A	Phloem	24	11	5.58
LW11	Wounded	S	A	Xylem	16	8	4.85
LW11	Wounded	S	B	Phloem	16	11	5.17
LW11	Wounded	S	B	Xylem	14	9	4.84
LW11	Wounded	S	C	Phloem	19	12	5.43
LW11	Wounded	S	C	Xylem	14	8	4.72
LW12	Wounded	R	R1	Phloem	28	17	6.17
LW12	Wounded	R	R1	Xylem	21	12	5.53
LW12	Wounded	R	R2	Phloem	40	18	6.58
LW12	Wounded	R	R2	Xylem	16	9	4.97
LW12	Wounded	R	R3	Phloem	22	16	5.86
LW12	Wounded	R	R3	Xylem	20	11	5.39
LW12	Wounded	S	A	Phloem	17	16	5.61
LW12	Wounded	S	A	Xylem	14	10	4.94
LW12	Wounded	S	B	Phloem	27	15	6

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LW12	Wounded	S	B	Xylem	11	8	4.48
LW12	Wounded	S	C	Phloem	18	12	5.38
LW12	Wounded	S	C	Xylem	16	10	5.08
LW13	Wounded	R	R1	Phloem	21	14	5.68
LW13	Wounded	R	R1	Xylem	12	9	4.68
LW13	Wounded	R	R2	Phloem	18	14	5.53
LW13	Wounded	R	R2	Xylem	20	9	5.19
LW13	Wounded	R	R3	Phloem	35	31	6.99
LW13	Wounded	R	R3	Xylem	31	14	6.07
LW13	Wounded	S	A	Phloem	23	12	5.62
LW13	Wounded	S	A	Xylem	23	10	5.44
LW13	Wounded	S	B	Phloem	27	15	6
LW13	Wounded	S	B	Xylem	19	11	5.34
LW13	Wounded	S	C	Phloem	29	14	6.01
LW13	Wounded	S	C	Xylem	24	9	5.38
LW14	Wounded	R	R1	Phloem	19	12	5.43
LW14	Wounded	R	R1	Xylem	14	8	4.72
LW14	Wounded	R	R2	Phloem	80	20	7.38
LW14	Wounded	R	R2	Xylem	25	12	5.7
LW14	Wounded	R	R3	Phloem	22	14	5.73
LW14	Wounded	R	R3	Xylem	21	11	5.44
LW14	Wounded	S	A	Phloem	18	10	5.19
LW14	Wounded	S	A	Xylem	13	9	4.76
LW14	Wounded	S	B	Phloem	13	12	5.05
LW14	Wounded	S	B	Xylem	11	9	4.6
LW14	Wounded	S	C	Phloem	31	11	5.83
LW14	Wounded	S	C	Xylem	14	9	4.84
LW15	Wounded	R	R1	Phloem	16	19	5.72
LW15	Wounded	R	R1	Xylem	24	13	5.74
LW15	Wounded	R	R2	Phloem	21	19	5.99
LW15	Wounded	R	R2	Xylem	22	12	5.58
LW15	Wounded	R	R3	Phloem	27	14	5.93
LW15	Wounded	R	R3	Xylem	56	10	6.33
LW15	Wounded	S	A	Phloem	15	9	4.91
LW15	Wounded	S	A	Xylem	12	7	4.43
LW15	Wounded	S	B	Phloem	16	11	5.17
LW15	Wounded	S	B	Xylem	10	8	4.38
LW15	Wounded	S	C	Phloem	21	11	5.44
LW15	Wounded	S	C	Xylem	17	10	5.14
LW2	Wounded	R	R1	Phloem	17	13	5.4
LW2	Wounded	R	R1	Xylem	11	8	4.48
LW2	Wounded	R	R2	Phloem	21	14	5.68
LW2	Wounded	R	R2	Xylem	14	10	4.94
LW2	Wounded	R	R3	Phloem	23	12	5.62
LW2	Wounded	R	R3	Xylem	16	9	4.97
LW2	Wounded	S	A	Phloem	31	11	5.83
LW2	Wounded	S	A	Xylem	17	9	5.03

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LW2	Wounded	S	B	Phloem	22	30	6.49
LW2	Wounded	S	B	Xylem	10	9	4.5
LW2	Wounded	S	C	Phloem	11	10	4.7
LW2	Wounded	S	C	Xylem	11	10	4.7
LW3	Wounded	R	R1	Phloem	23	15	5.84
LW3	Wounded	R	R1	Xylem	23	12	5.62
LW3	Wounded	R	R2	Phloem	18	13	5.46
LW3	Wounded	R	R2	Xylem	18	11	5.29
LW3	Wounded	R	R3	Phloem	19	16	5.72
LW3	Wounded	R	R3	Xylem	22	11	5.49
LW3	Wounded	S	A	Phloem	13	10	4.87
LW3	Wounded	S	A	Xylem	14	8	4.72
LW3	Wounded	S	B	Phloem	13	10	4.87
LW3	Wounded	S	B	Xylem	14	9	4.84
LW3	Wounded	S	C	Phloem	16	12	5.26
LW3	Wounded	S	C	Xylem	17	10	5.14
LW4	Wounded	R	R1	Phloem	44	15	6.49
LW4	Wounded	R	R1	Xylem	20	10	5.3
LW4	Wounded	R	R2	Phloem	18	13	5.46
LW4	Wounded	R	R2	Xylem	20	11	5.39
LW4	Wounded	R	R3	Phloem	22	13	5.66
LW4	Wounded	R	R3	Xylem	22	10	5.39
LW4	Wounded	S	A	Phloem	14	10	4.94
LW4	Wounded	S	A	Xylem	14	7	4.58
LW4	Wounded	S	B	Phloem	20	11	5.39
LW4	Wounded	S	B	Xylem	14	8	4.72
LW4	Wounded	S	C	Phloem	22	12	5.58
LW4	Wounded	S	C	Xylem	14	10	4.94
LW5	Wounded	R	R1	Phloem	16	14	5.41
LW5	Wounded	R	R1	Xylem	17	8	4.91
LW5	Wounded	R	R2	Phloem	33	11	5.89
LW5	Wounded	R	R2	Xylem	22	11	5.49
LW5	Wounded	R	R3	Phloem	14	11	5.04
LW5	Wounded	R	R3	Xylem	14	8	4.72
LW5	Wounded	S	A	Phloem	16	11	5.17
LW5	Wounded	S	A	Xylem	14	9	4.84
LW5	Wounded	S	B	Phloem	19	12	5.43
LW5	Wounded	S	B	Xylem	14	9	4.84
LW5	Wounded	S	C	Phloem	22	11	5.49
LW5	Wounded	S	C	Xylem	8	7	4.03
LW6	Wounded	R	R1	Phloem	49	14	6.53
LW6	Wounded	R	R1	Xylem	36	17	6.42
LW6	Wounded	R	R2	Phloem	12	13	5.05
LW6	Wounded	R	R2	Xylem	11	8	4.48
LW6	Wounded	R	R3	Phloem	18	13	5.46
LW6	Wounded	R	R3	Xylem	16	10	5.08
LW6	Wounded	S	A	Phloem	13	10	4.87

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
LW6	Wounded	S	A	Xylem	11	8	4.48
LW6	Wounded	S	B	Phloem	17	12	5.32
LW6	Wounded	S	B	Xylem	13	9	4.76
LW6	Wounded	S	C	Phloem	22	14	5.73
LW6	Wounded	S	C	Xylem	11	9	4.6
LW7	Wounded	R	R1	Phloem	19	16	5.72
LW7	Wounded	R	R1	Xylem	21	11	5.44
LW7	Wounded	R	R2	Phloem	17	14	5.47
LW7	Wounded	R	R2	Xylem	19	12	5.43
LW7	Wounded	R	R3	Xylem	19	10	5.25
LW7	Wounded	R	R3	Phloem	19	14	5.58
LW7	Wounded	S	A	Phloem	24	17	6.01
LW7	Wounded	S	A	Xylem	12	10	4.79
LW7	Wounded	S	B	Phloem	22	11	5.49
LW7	Wounded	S	B	Xylem	14	9	4.84
LW7	Wounded	S	C	Phloem	16	12	5.26
LW7	Wounded	S	C	Xylem	13	9	4.76
LW8	Wounded	R	R1	Phloem	18	15	5.6
LW8	Wounded	R	R1	Xylem	30	12	5.89
LW8	Wounded	R	R2	Phloem	25	26	6.48
LW8	Wounded	R	R2	Xylem	14	10	4.94
LW8	Wounded	R	R3	Phloem	57	14	6.68
LW8	Wounded	R	R3	Xylem	84	23	7.57
LW8	Wounded	S	A	Phloem	21	16	5.82
LW8	Wounded	S	A	Xylem	14	8	4.72
LW8	Wounded	S	B	Phloem	162	20	8.08
LW8	Wounded	S	B	Xylem	125	9	7.03
LW8	Wounded	S	C	Phloem	22	13	5.66
LW8	Wounded	S	C	Xylem	15	10	5.01
LW9	Wounded	R	R1	Phloem	26	19	6.2
LW9	Wounded	R	R1	Xylem	19	15	5.65
LW9	Wounded	R	R2	Phloem	16	15	5.48
LW9	Wounded	R	R2	Xylem	16	15	5.48
LW9	Wounded	R	R3	Phloem	51	16	6.7
LW9	Wounded	R	R3	Xylem	33	10	5.8
LW9	Wounded	S	A	Phloem	16	11	5.17
LW9	Wounded	S	A	Xylem	15	9	4.91
LW9	Wounded	S	B	Phloem	19	11	5.34
LW9	Wounded	S	B	Xylem	15	10	5.01
LW9	Wounded	S	C	Phloem	19	14	5.58
LW9	Wounded	S	C	Xylem	16	11	5.17
RT1	Infected	R	R1	Phloem	35	14	6.19
RT1	Infected	R	R1	Xylem	41	14	6.35
RT1	Infected	R	R2	Phloem	18	14	5.53
RT1	Infected	R	R2	Xylem	25	12	5.7
RT1	Infected	R	R3	Phloem	29	14	6.01
RT1	Infected	R	R3	Xylem	34	12	6.01

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RT1	Infected	S	A	Phloem	42	16	6.51
RT1	Infected	S	A	Xylem	85	12	6.93
RT1	Infected	S	B	Phloem	26	24	6.44
RT1	Infected	S	B	Xylem	26	12	5.74
RT1	Infected	S	C	Phloem	90	22	7.59
RT1	Infected	S	C	Xylem	115	12	7.23
RT10	Infected	R	R1	Phloem	45	22	6.9
RT10	Infected	R	R1	Xylem	40	11	6.09
RT10	Infected	R	R2	Phloem	34	15	6.23
RT10	Infected	R	R2	Xylem	42	11	6.14
RT10	Infected	R	R3	Phloem	23	13	5.7
RT10	Infected	R	R3	Xylem	19	8	5.02
RT10	Infected	S	A	Phloem	52	16	6.72
RT10	Infected	S	A	Xylem	41	12	6.2
RT10	Infected	S	B	Phloem	62	24	7.31
RT10	Infected	S	B	Xylem	64	11	6.56
RT10	Infected	S	C	Phloem	58	25	7.28
RT10	Infected	S	C	Xylem	60	14	6.73
RT11	Infected	R	R1	Phloem	56	25	7.24
RT11	Infected	R	R1	Xylem	71	15	6.97
RT11	Infected	R	R2	Phloem	61	33	7.61
RT11	Infected	R	R2	Xylem	55	16	6.78
RT11	Infected	R	R3	Phloem	44	19	6.73
RT11	Infected	R	R3	Xylem	46	15	6.54
RT11	Infected	S	A	Phloem	58	15	6.77
RT11	Infected	S	A	Xylem	65	13	6.74
RT11	Infected	S	B	Phloem	43	17	6.59
RT11	Infected	S	B	Xylem	44	12	6.27
RT11	Infected	S	C	Phloem	54	22	7.08
RT11	Infected	S	C	Xylem	57	14	6.68
RT12	Infected	R	R1	Phloem	36	17	6.42
RT12	Infected	R	R1	Xylem	43	12	6.25
RT12	Infected	R	R2	Phloem	22	14	5.73
RT12	Infected	R	R2	Xylem	18	13	5.46
RT12	Infected	R	R3	Xylem	20	12	5.48
RT12	Infected	R	R3	Phloem	18	14	5.53
RT12	Infected	S	A	Phloem	46	16	6.6
RT12	Infected	S	A	Xylem	47	14	6.49
RT12	Infected	S	B	Phloem	42	18	6.63
RT12	Infected	S	B	Xylem	45	11	6.2
RT12	Infected	S	C	Phloem	79	16	7.14
RT12	Infected	S	C	Xylem	68	11	6.62
RT13	Infected	R	R1	Phloem	27	17	6.13
RT13	Infected	R	R1	Xylem	46	13	6.39
RT13	Infected	R	R2	Phloem	16	14	5.41
RT13	Infected	R	R2	Xylem	14	11	5.04
RT13	Infected	R	R3	Phloem	21	13	5.61

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RT13	Infected	R	R3	Xylem	22	9	5.29
RT13	Infected	S	A	Phloem	87	16	7.24
RT13	Infected	S	A	Xylem	103	13	7.2
RT13	Infected	S	B	Phloem	116	25	7.97
RT13	Infected	S	B	Xylem	101	15	7.32
RT13	Infected	S	C	Phloem	82	21	7.45
RT13	Infected	S	C	Xylem	99	13	7.16
RT14	Infected	R	R1	Phloem	34	12	6.01
RT14	Infected	R	R1	Xylem	42	13	6.3
RT14	Infected	R	R2	Phloem	41	21	6.76
RT14	Infected	R	R2	Xylem	39	13	6.23
RT14	Infected	R	R3	Phloem	58	18	6.95
RT14	Infected	R	R3	Xylem	66	14	6.83
RT14	Infected	S	A	Phloem	37	11	6.01
RT14	Infected	S	A	Xylem	28	12	5.82
RT14	Infected	S	B	Phloem	91	42	8.25
RT14	Infected	S	B	Xylem	105	14	7.29
RT14	Infected	S	C	Phloem	70	36	7.83
RT14	Infected	S	C	Xylem	62	15	6.84
RT15	Infected	R	R1	Phloem	40	17	6.52
RT15	Infected	R	R1	Xylem	41	14	6.35
RT15	Infected	R	R2	Phloem	67	25	7.42
RT15	Infected	R	R3	Phloem	26	13	5.82
RT15	Infected	R	R3	Xylem	31	11	5.83
RT15	Infected	R	R2	Xylem	58	19	7
RT15	Infected	S	A	Phloem	60	18	6.98
RT15	Infected	S	A	Xylem	56	19	6.97
RT15	Infected	S	B	Phloem	76	18	7.22
RT15	Infected	S	B	Xylem	75	12	6.8
RT15	Infected	S	C	Phloem	151	22	8.11
RT15	Infected	S	C	Xylem	190	19	8.19
RT2	Infected	R	R1	Phloem	16	16	5.55
RT2	Infected	R	R1	Xylem	20	12	5.48
RT2	Infected	R	R2	Phloem	29	12	5.85
RT2	Infected	R	R2	Xylem	42	10	6.04
RT2	Infected	R	R3	Phloem	12	11	4.88
RT2	Infected	R	R3	Xylem	14	7	4.58
RT2	Infected	S	A	Phloem	22	18	5.98
RT2	Infected	S	A	Xylem	22	9	5.29
RT2	Infected	S	B	Phloem	24	15	5.89
RT2	Infected	S	B	Xylem	19	9	5.14
RT2	Infected	S	C	Phloem	20	13	5.56
RT2	Infected	S	C	Xylem	18	9	5.09
RT3	Infected	R	R1	Phloem	54	18	6.88
RT3	Infected	R	R1	Xylem	57	13	6.61
RT3	Infected	R	R2	Phloem	80	25	7.6
RT3	Infected	R	R2	Xylem	54	12	6.47

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RT3	Infected	R	R3	Phloem	47	14	6.49
RT3	Infected	R	R3	Xylem	70	15	6.96
RT3	Infected	S	A	Phloem	29	14	6.01
RT3	Infected	S	A	Xylem	36	9	5.78
RT3	Infected	S	B	Phloem	36	16	6.36
RT3	Infected	S	B	Xylem	36	9	5.78
RT3	Infected	S	C	Phloem	36	22	6.67
RT3	Infected	S	C	Xylem	33	12	5.98
RT4	Infected	R	R1	Phloem	30	17	6.23
RT4	Infected	R	R1	Xylem	40	15	6.4
RT4	Infected	R	R2	Phloem	52	21	7
RT4	Infected	R	R2	Xylem	50	14	6.55
RT4	Infected	R	R3	Phloem	55	17	6.84
RT4	Infected	R	R3	Xylem	39	12	6.15
RT4	Infected	S	A	Phloem	34	15	6.23
RT4	Infected	S	A	Xylem	31	9	5.63
RT4	Infected	S	B	Phloem	52	22	7.04
RT4	Infected	S	B	Xylem	37	11	6.01
RT4	Infected	S	C	Phloem	41	23	6.85
RT4	Infected	S	C	Xylem	38	13	6.2
RT5	Infected	R	R1	Phloem	38	15	6.35
RT5	Infected	R	R1	Xylem	35	11	5.95
RT5	Infected	R	R2	Phloem	35	22	6.65
RT5	Infected	R	R2	Xylem	32	15	6.17
RT5	Infected	R	R3	Phloem	38	18	6.53
RT5	Infected	R	R3	Xylem	58	24	7.24
RT5	Infected	S	A	Phloem	64	19	7.1
RT5	Infected	S	A	Xylem	111	13	7.27
RT5	Infected	S	B	Phloem	41	16	6.49
RT5	Infected	S	B	Xylem	36	11	5.98
RT5	Infected	S	C	Phloem	26	19	6.2
RT5	Infected	S	C	Xylem	22	11	5.49
RT6	Infected	R	R1	Phloem	18	11	5.29
RT6	Infected	R	R1	Xylem	26	14	5.9
RT6	Infected	R	R2	Phloem	20	16	5.77
RT6	Infected	R	R2	Xylem	29	14	6.01
RT6	Infected	R	R3	Phloem	34	25	6.75
RT6	Infected	R	R3	Xylem	40	20	6.68
RT6	Infected	S	A	Phloem	52	16	6.72
RT6	Infected	S	A	Xylem	35	14	6.19
RT6	Infected	S	B	Phloem	55	14	6.65
RT6	Infected	S	B	Xylem	45	11	6.2
RT6	Infected	S	C	Phloem	28	14	5.97
RT6	Infected	S	C	Xylem	34	14	6.17
RT7	Infected	R	R1	Phloem	40	15	6.4
RT7	Infected	R	R1	Xylem	45	11	6.2
RT7	Infected	R	R2	Phloem	26	21	6.3

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RT7	Infected	R	R2	Xylem	31	13	6
RT7	Infected	R	R3	Phloem	31	13	6
RT7	Infected	R	R3	Xylem	40	12	6.17
RT7	Infected	S	A	Phloem	31	12	5.92
RT7	Infected	S	A	Xylem	101	13	7.18
RT7	Infected	S	B	Phloem	21	19	5.99
RT7	Infected	S	B	Xylem	26	12	5.74
RT7	Infected	S	C	Phloem	35	14	6.19
RT7	Infected	S	C	Xylem	44	12	6.27
RT8	Infected	R	R1	Phloem	54	13	6.55
RT8	Infected	R	R1	Xylem	76	13	6.9
RT8	Infected	R	R2	Phloem	45	19	6.75
RT8	Infected	R	R2	Xylem	53	13	6.54
RT8	Infected	R	R3	Phloem	35	17	6.39
RT8	Infected	R	R3	Xylem	42	10	6.04
RT8	Infected	S	A	Phloem	26	19	6.2
RT8	Infected	S	A	Xylem	27	9	5.49
RT8	Infected	S	B	Phloem	82	31	7.84
RT8	Infected	S	B	Xylem	103	11	7.03
RT8	Infected	S	C	Phloem	47	16	6.62
RT8	Infected	S	C	Xylem	60	12	6.58
RT9	Infected	R	R1	Phloem	56	22	7.12
RT9	Infected	R	R1	Xylem	54	16	6.76
RT9	Infected	R	R2	Phloem	89	21	7.53
RT9	Infected	R	R2	Xylem	84	14	7.07
RT9	Infected	R	R3	Phloem	62	22	7.22
RT9	Infected	R	R3	Xylem	67	16	6.98
RT9	Infected	S	A	Phloem	62	16	6.9
RT9	Infected	S	A	Xylem	49	11	6.29
RT9	Infected	S	B	Phloem	50	20	6.91
RT9	Infected	S	B	Xylem	41	13	6.28
RT9	Infected	S	C	Phloem	74	25	7.52
RT9	Infected	S	C	Xylem	52	13	6.52
RW1	Wounded	R	R1	Phloem	27	27	6.59
RW1	Wounded	R	R1	Xylem	29	15	6.08
RW1	Wounded	R	R2	Phloem	23	12	5.62
RW1	Wounded	R	R2	Xylem	21	13	5.61
RW1	Wounded	R	R3	Phloem	36	19	6.53
RW1	Wounded	R	R3	Xylem	29	17	6.2
RW1	Wounded	S	A	Phloem	13	14	5.2
RW1	Wounded	S	A	Xylem	16	9	4.97
RW1	Wounded	S	B	Phloem	46	21	6.87
RW1	Wounded	S	B	Xylem	39	14	6.3
RW1	Wounded	S	C	Phloem	25	19	6.16
RW1	Wounded	S	C	Xylem	20	13	5.56
RW10	Wounded	R	R1	Phloem	14	16	5.41
RW10	Wounded	R	R1	Xylem	14	12	5.12

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RW10	Wounded	R	R2	Phloem	13	13	5.13
RW10	Wounded	R	R2	Xylem	15	13	5.27
RW10	Wounded	R	R3	Phloem	15	14	5.35
RW10	Wounded	R	R3	Xylem	16	10	5.08
RW10	Wounded	S	A	Phloem	15	11	5.11
RW10	Wounded	S	A	Xylem	12	9	4.68
RW10	Wounded	S	B	Phloem	19	12	5.43
RW10	Wounded	S	B	Xylem	26	10	5.56
RW10	Wounded	S	C	Phloem	16	14	5.41
RW10	Wounded	S	C	Xylem	20	11	5.39
RW11	Wounded	R	R1	Phloem	17	13	5.4
RW11	Wounded	R	R1	Xylem	16	11	5.17
RW11	Wounded	R	R2	Phloem	15	14	5.35
RW11	Wounded	R	R2	Xylem	12	8	4.56
RW11	Wounded	R	R3	Phloem	14	11	5.04
RW11	Wounded	R	R3	Xylem	12	9	4.68
RW11	Wounded	S	A	Phloem	18	14	5.53
RW11	Wounded	S	A	Xylem	11	11	4.8
RW11	Wounded	S	B	Phloem	16	12	5.26
RW11	Wounded	S	B	Xylem	11	9	4.6
RW11	Wounded	S	C	Phloem	17	14	5.47
RW11	Wounded	S	C	Xylem	17	10	5.14
RW12	Wounded	R	R1	Phloem	25	14	5.86
RW12	Wounded	R	R1	Xylem	16	11	5.17
RW12	Wounded	R	R2	Phloem	19	21	5.99
RW12	Wounded	R	R2	Xylem	22	12	5.58
RW12	Wounded	R	R3	Phloem	27	16	6.07
RW12	Wounded	R	R3	Xylem	17	12	5.32
RW12	Wounded	S	A	Phloem	16	12	5.26
RW12	Wounded	S	A	Xylem	17	10	5.14
RW12	Wounded	S	B	Phloem	12	10	4.79
RW12	Wounded	S	B	Xylem	14	10	4.94
RW12	Wounded	S	C	Phloem	13	10	4.87
RW12	Wounded	S	C	Xylem	21	8	5.12
RW13	Wounded	R	R1	Phloem	20	15	5.7
RW13	Wounded	R	R1	Xylem	17	13	5.4
RW13	Wounded	R	R2	Xylem	21	11	5.44
RW13	Wounded	R	R2	Phloem	19	13	5.51
RW13	Wounded	R	R3	Phloem	52	33	7.45
RW13	Wounded	R	R3	Xylem	52	17	6.78
RW13	Wounded	S	A	Phloem	18	13	5.46
RW13	Wounded	S	A	Xylem	16	10	5.08
RW13	Wounded	S	B	Phloem	18	13	5.46
RW13	Wounded	S	B	Xylem	22	10	5.39
RW13	Wounded	S	C	Phloem	18	11	5.29
RW13	Wounded	S	C	Xylem	23	11	5.53
RW14	Wounded	R	R1	Phloem	16	12	5.26

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RW14	Wounded	R	R1	Xylem	13	11	4.96
RW14	Wounded	R	R2	Phloem	22	20	6.09
RW14	Wounded	R	R2	Xylem	31	14	6.07
RW14	Wounded	R	R3	Phloem	12	14	5.12
RW14	Wounded	R	R3	Xylem	13	11	4.96
RW14	Wounded	S	A	Phloem	46	13	6.39
RW14	Wounded	S	A	Xylem	69	11	6.63
RW14	Wounded	S	B	Phloem	17	14	5.47
RW14	Wounded	S	B	Xylem	16	10	5.08
RW14	Wounded	S	C	Phloem	20	12	5.48
RW14	Wounded	S	C	Xylem	20	10	5.3
RW15	Wounded	R	R1	Phloem	17	13	5.4
RW15	Wounded	R	R1	Xylem	17	11	5.23
RW15	Wounded	R	R2	Phloem	14	16	5.41
RW15	Wounded	R	R2	Xylem	15	11	5.11
RW15	Wounded	R	R3	Phloem	27	19	6.24
RW15	Wounded	R	R3	Xylem	17	14	5.47
RW15	Wounded	S	A	Phloem	18	12	5.38
RW15	Wounded	S	A	Xylem	21	11	5.44
RW15	Wounded	S	B	Phloem	17	12	5.32
RW15	Wounded	S	B	Xylem	19	12	5.43
RW15	Wounded	S	C	Phloem	22	17	5.92
RW15	Wounded	S	C	Xylem	27	11	5.69
RW2	Wounded	R	R1	Phloem	18	15	5.6
RW2	Wounded	R	R1	Xylem	16	10	5.08
RW2	Wounded	R	R2	Phloem	18	11	5.29
RW2	Wounded	R	R3	Phloem	9	9	4.39
RW2	Wounded	R	R2	Xylem	14	10	4.94
RW2	Wounded	R	R3	Xylem	17	9	5.03
RW2	Wounded	S	A	Phloem	14	10	4.94
RW2	Wounded	S	A	Xylem	14	9	4.84
RW2	Wounded	S	B	Phloem	18	12	5.38
RW2	Wounded	S	B	Xylem	20	10	5.3
RW2	Wounded	S	C	Phloem	15	10	5.01
RW2	Wounded	S	C	Xylem	18	10	5.19
RW3	Wounded	R	R1	Phloem	41	16	6.49
RW3	Wounded	R	R1	Xylem	54	12	6.47
RW3	Wounded	R	R2	Phloem	30	16	6.17
RW3	Wounded	R	R2	Xylem	25	11	5.62
RW3	Wounded	R	R3	Phloem	13	12	5.05
RW3	Wounded	R	R3	Xylem	14	9	4.84
RW3	Wounded	S	A	Phloem	14	12	5.12
RW3	Wounded	S	A	Xylem	15	11	5.11
RW3	Wounded	S	B	Phloem	21	24	6.22
RW3	Wounded	S	B	Xylem	21	11	5.44
RW3	Wounded	S	C	Phloem	18	21	5.93
RW3	Wounded	S	C	Xylem	15	11	5.11

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RW4	Wounded	R	R1	Phloem	24	13	5.74
RW4	Wounded	R	R1	Xylem	15	10	5.01
RW4	Wounded	R	R2	Phloem	20	17	5.83
RW4	Wounded	R	R2	Xylem	15	11	5.11
RW4	Wounded	R	R3	Phloem	15	12	5.19
RW4	Wounded	R	R3	Xylem	13	9	4.76
RW4	Wounded	S	A	Phloem	16	11	5.17
RW4	Wounded	S	A	Xylem	14	9	4.84
RW4	Wounded	S	B	Phloem	20	13	5.56
RW4	Wounded	S	B	Xylem	17	11	5.23
RW4	Wounded	S	C	Phloem	24	17	6.01
RW4	Wounded	S	C	Xylem	23	10	5.44
RW5	Wounded	R	R1	Phloem	17	15	5.54
RW5	Wounded	R	R1	Xylem	15	11	5.11
RW5	Wounded	R	R2	Phloem	42	17	6.57
RW5	Wounded	R	R2	Xylem	17	14	5.47
RW5	Wounded	R	R3	Phloem	18	15	5.6
RW5	Wounded	R	R3	Xylem	20	11	5.39
RW5	Wounded	S	A	Phloem	17	14	5.47
RW5	Wounded	S	A	Xylem	19	9	5.14
RW5	Wounded	S	B	Phloem	21	17	5.88
RW5	Wounded	S	B	Xylem	18	9	5.09
RW5	Wounded	S	C	Phloem	23	14	5.77
RW5	Wounded	S	C	Xylem	26	13	5.82
RW6	Wounded	R	R1	Phloem	20	14	5.63
RW6	Wounded	R	R1	Xylem	20	14	5.63
RW6	Wounded	R	R2	Phloem	13	13	5.13
RW6	Wounded	R	R2	Xylem	12	9	4.68
RW6	Wounded	R	R3	Phloem	22	15	5.8
RW6	Wounded	R	R3	Xylem	21	15	5.75
RW6	Wounded	S	A	Phloem	16	15	5.48
RW6	Wounded	S	A	Xylem	23	10	5.44
RW6	Wounded	S	B	Phloem	13	12	5.05
RW6	Wounded	S	B	Xylem	18	9	5.09
RW6	Wounded	S	C	Phloem	14	12	5.12
RW6	Wounded	S	C	Xylem	17	10	5.14
RW7	Wounded	R	R1	Phloem	28	16	6.1
RW7	Wounded	R	R1	Xylem	31	12	5.92
RW7	Wounded	R	R2	Phloem	19	14	5.58
RW7	Wounded	R	R2	Xylem	17	12	5.32
RW7	Wounded	R	R3	Phloem	19	13	5.51
RW7	Wounded	R	R3	Xylem	16	12	5.26
RW7	Wounded	S	A	Phloem	21	14	5.68
RW7	Wounded	S	A	Xylem	25	10	5.52
RW7	Wounded	S	B	Phloem	17	15	5.54
RW7	Wounded	S	B	Xylem	17	9	5.03
RW7	Wounded	S	C	Phloem	47	23	6.99

Tree	Treatment	Organ	Sample	Tissue	Length	Width	Area
RW7	Wounded	S	C	Xylem	34	9	5.72
RW8	Wounded	R	R1	Phloem	22	17	5.92
RW8	Wounded	R	R1	Xylem	21	12	5.53
RW8	Wounded	R	R2	Phloem	18	15	5.6
RW8	Wounded	R	R2	Xylem	18	14	5.53
RW8	Wounded	R	R3	Phloem	17	14	5.47
RW8	Wounded	R	R3	Xylem	14	12	5.12
RW8	Wounded	S	A	Phloem	19	15	5.65
RW8	Wounded	S	A	Xylem	22	11	5.49
RW8	Wounded	S	B	Phloem	32	15	6.17
RW8	Wounded	S	B	Xylem	21	12	5.53
RW8	Wounded	S	C	Phloem	29	16	6.14
RW8	Wounded	S	C	Xylem	26	13	5.82
RW9	Wounded	R	R1	Phloem	16	14	5.41
RW9	Wounded	R	R1	Xylem	17	12	5.32
RW9	Wounded	R	R2	Phloem	19	12	5.43
RW9	Wounded	R	R2	Xylem	19	12	5.43
RW9	Wounded	R	R3	Phloem	21	14	5.68
RW9	Wounded	R	R3	Xylem	28	12	5.82
RW9	Wounded	S	A	Phloem	15	11	5.11
RW9	Wounded	S	A	Xylem	22	10	5.39
RW9	Wounded	S	B	Phloem	27	13	5.86
RW9	Wounded	S	B	Xylem	22	12	5.58
RW9	Wounded	S	C	Phloem	20	15	5.7
RW9	Wounded	S	C	Xylem	18	11	5.29