Induced defence responses in *Picea abies* triggered by *Heterobasidion annosum s.l.*

Jenny Arnerup

Faculty of Natural Resources and Agricultural Sciences
Department of Forest Mycology and Pathology
Uppsala

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2011

Induced defence responses in *Picea abies* triggered by *Heterobasidion annosum s.l.*

Abstract

Norway spruce [*Picea abies* (L.)Karst.] is a key tree species for forest industry in Europe and stem and butt rot caused by *Heterobasidion parviporum* (Fr.) Niemelä & Korhonen is one of its major disease problems. The commercial gain using improved plant material could be even greater if resistance traits were included in the breeding program. The aim of this study was to increase the knowledge of the induced defences in response to *H. annosum s.l.* in Norway spruce bark.

In this thesis it has been concluded that there is a significant variation between genotypes in a full-sib family of Norway spruce. The broad sense heritability was found to be 0.11 for fungal growth. A shift in the pathway connecting primary and secondary metabolism, as detected by a transcriptional switch of *DAHP* homologues, following *H. annosum s.l.* inoculation, indicate a possible allocation of more carbon to the secondary metabolism. We also found a consistent induction of the phenylpropanoid pathway and there was an association between the phenol profile and level of resistance. For example, the level of the flavonoid (+)-catechin showed temporal variation in genotypes with higher level of resistance. Matching changes was found in the transcriptome. The R2R3-transcription factor *PaTT2-like* gene, a putative regulator of flavonoid production, was found to be induced by jasmonic acid in bark.

The responses to *H. annosum s.l.* have been shown to be non-specific but that the magnitude of the response is higher than with other types of challenges. A simultaneous up-regulation of genes related to the salicylic acid- and jasmonic acid-signalling pathway in response to fungal inoculation revealed a closer relationship between the pathways than has been observed in many angiosperms.

Finally, the clonal variation in transcriptional and chemical responses observed in this thesis demonstrates variation between genotypes that can be related to different levels of susceptibility to *H. annosum s.l.* and which can be explored for improvement in coniferous trees. It also demonstrates some of the potential of using modern molecular methods in the breeding practices.

Keywords: Picea abies, Heterobasidion, defence, transcript profiling, salicylic acid, jasmonic acid, phenylpropanoid pathway, flavonoids.

Author's address: Jenny Arnerup, SLU, Department of Forest Mycology and Pathology, Box 7026SE-750 05 Uppsala, Sweden.
E-mail: Jenny.Arnerup@slu.se

"The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability. Our business in every generation is to reclaim a little more land."

Thomas Henry Huxley

Table of Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I   Arnerup, J., Swedjemark, G., Elfstrand, M., Karlsson, B. and Stenlid, J. (2010). Variation in growth of *Heterobasidion parviporum* in a full–sib family of *Picea abies*. Scandinavian Journal of Forest Research. 25:106–110.

II Arnerup, J., Lind, M., Olson, Å., Stenlid, J. and Elfstrand, M. (2011). *Heterobasidion parviporum* infection triggers non-specific defence responses in *Picea abies*. (Submitted manuscript)

III Danielsson, M., Lundén, K., Arnerup, J., Hu J., Zhao, T., Swedjemark, G., Elfstrand, M., Borg-Karlson A-K., and Stenlid, J. (2011) Chemical and transcriptional responses of Norway spruce clones with varying susceptibility to *Heterobasidion* spp. infection. (Manuscript)

IV Arnerup, J., Lundén, K., Ihrmark K., Karlsson M., Asiegbu. F., Stenlid, J. and Elfstrand, M. (2011) Induced defence responses in Norway spruce involve an interaction between salicylic acid and jasmonic acid mediated signalling pathways. (Manuscript)

Papers I is reproduced with the permission of the publishers.

The contribution of Jenny Arnerup to the papers included in this thesis was as follows:

I Participated in the inoculation and harvest of the experiment. Performed some data analysis and wrote a large part of the manuscript.

II Participated in the experimental design, conducted most of the laboratory work and drafted the manuscript.

III Participated in the discussion about the experimental design, preformed qPCR analysis and participated in writing some of the manuscript.

IV Planed and preformed the experiment, analysed the data and written the manuscript. The assembly, annotation and clustering of transcriptome data was performed by the second author.

Abbreviations

AFLP	amplified fragment length polymorphism
BLAST	basic local alignment search tool
bZIP	basic leucine zipper domain
cDNA	complementary deoxyribonucleic acid
dpi	days post inoculation
ERF	ethylene responsive factor
ET	ethylene
H^2	broad sense heritability
HR	hypersensitive response
JA	jasmonic acid
MAMP	microbe-associated molecular pattern
MAS	marker-assisted selection
MeJA	methyl jasmonate
MeSA	methyl salicylate
mRNA	messenger ribonucleic acid
PA	proanthocyanidin
PCR	polymerase chain reaction
PP	polyphenolic
PR	pathogen related
qPCR	quantitative polymerase chain reaction
QTL	quantitative trait loci
ROS	reactive oxygen species
RZ	reaction zone
SA	salicylic acid
SAR	systemic acquired resistance

SIR	systemic induced resistance
s.l.	sensu lato – 'in the broad sense'
s.s.	sensu stricto – 'in the narrow sense'
TD	traumatic resin ducts
TDF	transcribed derived fragment
TF	transcription factor
UPS	ubiquitin / proteasome system
WRKY	refers to the WRKY amino acid DNA binding region

1 Introduction

Forests are the most widespread type of terrestrial ecological system and provide important resources for humankind. In Sweden, forest covers approximately 56 % of the total land area and in economical terms forestry is a very important industry (Fransson, 2010). Swedish forests are dominated by conifers, with Norway spruce [*Picea abies* (L.) Karst.)] and *Pinus sylvestris* making up more than 85 % of all forest (Fransson, 2010). During the long life span of coniferous trees they will interact with a wide range of microorganisms both beneficial and harmful. Conifers are able to form very close interactions with different mycorrhizal fungi, which requires intricate signalling and cross talk from both participants. Conifers have also evolved different strategies to defend themselves against attacks from pests and pathogens that include a constitutively expressed mechanical and chemical defence complemented by an inducible defence (Eyles *et al.*, 2009; Bohlmann, 2008; Franceschi *et al.*, 2005). There is a constant arms race between plants and pathogens where resistance repeatedly can be broken down or acquired. Management of plants by man has interfered with the evolutionary balance between hosts and pathogens and in some cases created opportunities for previously insignificant pathogens to become large and costly problems (Stenlid *et al.*, 2011). The increasing level of damage caused by *Heterobasidion annosum* [(Fr.) Bref.] *sensu lato (s.l.)* in Swedish forests may be an example of this. In this thesis the molecular basis underlying the response to *H. annosum s.l.* in Norway spruce will be addressed.

1.1 Background

Norway spruce belongs to the family *Pinaceae*. The genus *Picea* includes about 34 species (Ledig *et al.*, 2004; Farjon, 1998). Its natural distribution ranges across the Pyrenees, Alps and Balkans, northwards to southern

Germany and Scandinavia and eastwards through the Carpathian Mountains and Poland to the Ural Mountains (Farjón, 1990; Rushforth, 1987). Economically the most important conifer tree species in Europe is *P. abies*. One important cause of economic losses in the forest industry is rot by fungal pathogens and the major fungal pathogen on Norway spruce is *H. annosum s.l.* Fungal species in this complex cause destruction of wood and reduction in tree growth resulting in losses in the order of €790 million annually for the European forest industry (Woodward *et al.*, 1998).

The establishment of *H. annosum s.l.* infection occurs by basidiospores germinating on fresh stumps or wounds on stems and roots. The fungus then spreads to other trees through root contacts between neighbouring trees. In Norway spruce the fungus usually causes butt and stem rot and only seedlings and young trees may die as a result of the infection. Mature trees can withstand the infection and the fungus can cause a rot column as high as 8-12 m (Bendz-Hellgren *et al.*, 1998). Good forest management and stump treatment can reduce the spread of this pathogen. Stump treatment involves introducing a biological competitor, *Phlebiopsis gigantea* (Fr.) Jülich, or changing the chemical environment of the wood. A long-term investigation of the effects of stump treatment suggests that this is an effective method of preventing the establishment of *H. annosum s.l.* infection at uninfected sites. However, in already infested sites a carryover between rotations is an important factor for the health of the new generation (Oliva *et al.*, 2010a).

The *H. annosum* species complex consists of five species causing infection in a broad range of tree species, both deciduous and coniferous (Korhonen & Stenlid, 1998). Three species are native in Europe: *H. parviporum* (Fr.) Niemelä & Korhonen, with *Picea* spp. as its main host; *H. annosum* (Fr.) Bref., with *Pinus* spp. as the main host; and *H. abietinum* (Fr.) Niemelä & Korhonen, which primarily infects *Abies* species (Niemelä & Korhonen, 1998). Two North American species *H. irregulare* Otrosina & Garbelotto and *H. occidentale* Otrosina & Garbelotto (Otrosina & Garbelotto, 2010) exist with some geographic and host preferential differences. Mating compatibility studies (Chase & Ullrich, 1990; Korhonen, 1978) in parallel with molecular studies (Linzer *et al.*, 2008; Johannesson & Stenlid, 2003; Garbelotto *et al.*, 1998; Kasuga & Mitchelson, 1993) have shown that the five different species can be separated in two different clades. The Eurasian *H. parviporum* and *H. abietinum* and the North American *H. occidentale* belong to one clade and the Eurasian *H. annosum sensu stricto (s.s.)* and the North American *H. irregulare* belong to the other clade. A recent study by Dalman *et al.* (2010) regarding the evolutionary history of the *Heterobasidion*

genera argues that the *H. annosum* species complex originated in ancient Laurasia and then spread via different trajectories resulting in the emergence of different species, and that the speciation events occurred after that divergence of the host genera.
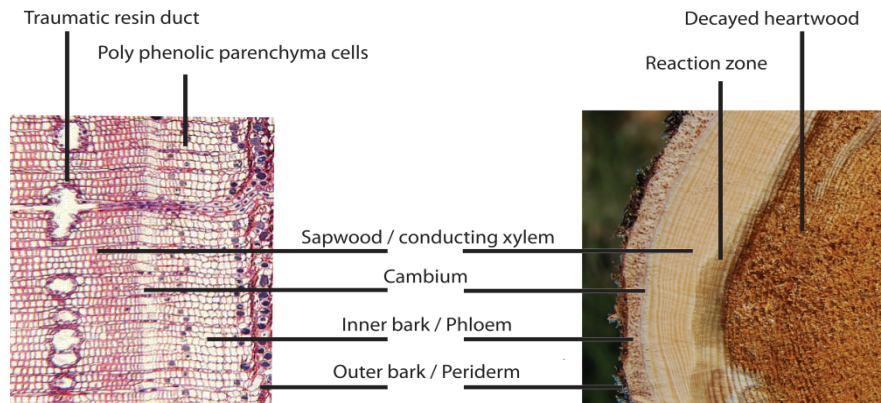

Figure 1. An overview of tissue types in the stem of Norway spruce. Pictures were kindly provided by Jonàs Oliva and The Norwegian Forest and Landscape Institute (microscopic cross-section).

1.2 Overview of plant defences with a focus on conifer defence

1.2.1 Constitutive defence

The constitutive defences of plants can be categorized as mechanical or chemical. Both types are regarded non-specific and effective against a wide range of organisms. The outer bark or the periderm constitutes the first line of defence (Fig. 1). This layer has cells that have lignified and suberised walls giving strength to the tissue and making it hydrophobic. In addition, there are cells containing phenolic compounds and there may be one or more layers of cells containing calcium oxalate crystals providing both chemical and mechanical resistance (Franceschi *et al.*, 2005).

In the secondary phloem, common defence structures for most conifers are polyphenolic parenchyma (PP) cells, cortical resin structures, stone cells and calcium oxalate crystals (Hudgins & Franceschi, 2004). PP cells are specialised parenchyma cells for synthesis and storage of phenolic compounds (Franceschi *et al.*, 1998). At the beginning of each growing season a row of PP cells differentiates from the cambial zone giving rise to one layer of PP cells each year. The PP cells will mature during following

growing seasons, becoming thick-walled round cells containing phenolic compounds such as stilbenes and flavonoids. Calcium oxalate crystals can also be present in the vacuoles of the PP cells (Hudgins *et al.*, 2003b). The PP cells are maintained as living cells and living PP cells that are over 70 years old have been identified, indicating an important role for this cell type (Krekling *et al.*, 2000).

Resin ducts are tube-like structures lined with thin-walled epithelial cells; in Norway spruce, constitutive axial resin ducts are found and radial resin ducts expand from the phloem to the secondary xylem. Axial resin ducts only occur in low numbers in the xylem (Nagy *et al.*, 2000). The composition of the resin includes three structurally diverse classes of terpenoid compounds: monoterpenes, diterpenes and sesquiterpenes. In Norway spruce, approximately 95% of the resin is composed of mono- and diterpenes in approximately equal proportions (Martin *et al.*, 2002). The resin can act as a physical defence, immobilising an invading insect (Trapp & Croteau, 2001) or may be toxic to the invading organism (Lindberg *et al.*, 1992). The xylem parenchyma is involved in the production of secondary metabolites such as phenolic compounds. The heartwood is impregnated with lignans and phenolic compounds, which provides some defence against wood rotting fungi (Franceschi *et al.*, 2005).

1.2.2 Induced defence

Constitutive defences are likely to have costs that can affect the fitness of the plant (Bolton, 2009). This leads to the need for defence mechanisms that can be induced upon an attack. The activation of inducible responses relies on recognition of the different organisms (Jones & Dangl, 2006). Plants have the ability to recognise a multitude of different microorganisms by identifying microbe associated molecular patterns (MAMPs). MAMPs are slow-evolving molecular structures unique to microbes (Jones & Dangl, 2006). One major component of the fungal cell wall is chitin, which is a polymer of N-acetyl-D-glucosamine. Chitin and chitin fragments are generally viewed as MAMPs (Hamel & Beaudoin, 2010) and can elicit defence responses in Norway spruce. (Salzer *et al.*, 1997). There is an ongoing arms race between hosts and pathogens; the pathogen can evolve different effectors that suppress MAMP-triggered defence responses and the host can in turn evolve the ability to recognise these effectors (Jones & Dangl, 2006). It has been suggested that although the recognition and subsequent reactions can vary in response to different types of pathogens (mainly necrotrophic and biotrophic), the overall signalling mechanisms that control gene expression after infection have much in common (Katagiri,

2004; van Wees *et al.*, 2003). A general induction of available defence mechanisms can be an effective tactic so that at least some may have an effect on the invading pathogen (Katagiri, 2004). However, there can be a trade-off between resistance and fitness because the defence response is energy consuming (Oliva *et al.*, 2010b; Bolton, 2009). For example, there is a negative correlation between growth and lignin content, which is an important feature of the cell wall and as a component for cell wall reinforcement during infection (Novaes *et al.*, 2010).

The induced defences in Norway spruce results in a reinforcement of the cell wall through lignifications and suberisation (Woodward & Pearce 1988) and *de novo* production of secondary metabolites such as stilbenes, flavonoids and terpenes which are induced in resin ducts, ray parenchyma and PP cells (Franceschi *et al.*, 2000; Nagy *et al.*, 2000; Lindberg *et al.*, 1992). Upon challenge the constitutively expressed stilbene glucosides are converted to free stilbene aglycones, which exhibit greater antifungal activity (Woodward & Pearce, 1988). Anatomical changes occur such as an induced additional layer of PP cells which is initiated in the phloem and traumatic resin ducts (TD) are formed in the xylem (Franceschi *et al.*, 2002). It has been shown that a pretreatment with wounding or low levels of inoculum can enhance resistance to a fungal pathogen owing to the induction of the defence mechanisms mentioned above (Krokene *et al.*, 2003).

Many of the secondary metabolites in plants are produced via the phenylpropanoid pathway (Vogt, 2010). One of the rate-limiting enzymes at the entry point of the phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL) (Dixon *et al.*, 1996) and it is common for monolignol biosynthesis and biosynthesis of flavonoids, stilbenes and lignans (Vogt, 2010). Most enzymes in the phenylpropanoid pathway belong to gene families with multiple members. These genes putatively have both specialised and overlapping functions (Shi *et al.*, 2010; Tsai *et al.*, 2006). Accordingly, many genes in the phenylpropanoid pathway are induced in conifers in response to pathogens and pests (Koutaniemi *et al.*, 2007; Ralph *et al.*, 2006; Franceschi *et al.*, 1998).

Other important features of the induced defence response include the production of reactive oxygen species (ROS) and the synthesis of pathogenesis-related (PR) proteins (Brosche *et al.*, 2010; Van Loon *et al.*, 2006). PR proteins include chitinases and glucanases that can act at the cell wall of invading fungi (Hietala *et al.*, 2004; Nagy *et al.*, 2004a; Salzer *et al.*, 1997) and peroxidases that are involved in the process of cross-linking of cell wall components and in the production of ROS (Almagro *et al.*, 2009;

Nagy *et al.*, 2004b). ROS can also mediate a hypersensitive response (HR) resulting in cell death and necrosis (Brosche *et al.*, 2010).

1.2.3 Signalling and regulation of induced defence responses.

Even though angiosperms and gymnosperms separated about 300 million years ago (Stewart & Rothwell, 1993) there are general similarities in the interactions between pathogens and plants from the two divisions. There are several connected steps in the induction of defence responses as reviewed in Zhao *et al.,* (2005). This includes ion flux increase, production of ROS, accumulation of salicylic acid (SA), induction of transcription factors and activation of downstream target genes. One of the earliest responses after the application of fungal elicitors such as chitin is the release of Cl^- and K^+ and an influx of Ca^{2+} (Zhao *et al.*, 2005; Salzer *et al.*, 1997; Salzer *et al.*, 1996) (Fig. 2). Ion fluxes subsequently induce extracellular production of ROS (Lamb & Dixon, 1997). Following the oxidative burst, cells maintain a more reducing environment owing to the accumulation of antioxidants such as SA which is an important player in the defence signalling of plants (Glazebrook *et al.*, 2003; Mou *et al.*, 2003). The increase in the level of signalling hormones, together with the change in redox potential initiates gene expression through activation of different transcription factors (TF) (Spoel *et al.*, 2010). Transcription factors belonging to the ERF, bZIP and WRKY families have been linked to a suite of mechanisms that leads to defence and stress responses (Singh *et al.*, 2002). Members of the R2R3-MYB transcription factor family have also been implicated in the regulation of plant stress responses, mainly plant secondary metabolism (Vom Endt *et al.*, 2002).

Not all pathogens elicit the same types of responses and certain responses are associated with certain types of pathogens. The two major defence signalling pathways are mediated by SA and jasmonic acid (JA)/ethylene (ET) which essentially are antagonistic (Robert-Seilaniantz *et al.*, 2007; Thomma *et al.*, 1998) and associated with biotrophic and necrotrophic pathogens, respectively (Glazebrook *et al.*, 2003) (Fig. 3). However, these pathways have several common internodes, which indicate a complex network rather than separate pathways (Katagiri & Tsuda, 2010; Tsuda *et al.*, 2009; Mur *et al.*, 2006). Furthermore, crosstalk between the SA and JA/ET pathways with other hormones, including auxin has been demonstrated, making the picture even more complex (Kazan & Manners, 2008; Llorente *et al.*, 2008; Wang *et al.*, 2007) (Fig. 3). These molecules modulate the activity of downstream transcription regulators that control a large set of
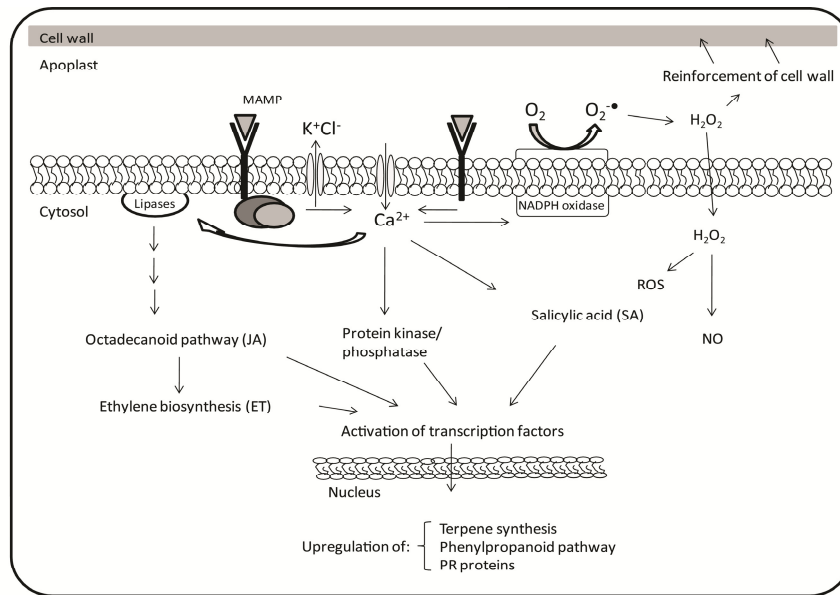
Figure 2. Scheme of the possible signalling network in the formation of induced conifer defence. Based on Zhao *et al,* 2005 and Salzer *et al.* 1996.

different defence genes. For example, SA mediates expression of pathogen related–protein 1 (PR1) and systemic–acquired resistance (SAR) through activation of the transcription factor NPR1. In an un–induced state, NPR1 is present as an oligomer in the cytosol (Mou *et al.*, 2003). Redox changes induced as a result of SA accumulation leads to conformational changes in NPR1 from an inactive oligomer to the active monomer, allowing translocation to the nucleus and activation of target gene expression (Mou *et al.*, 2003). Spoel *et al.,* (2009) discovered that the ubiquitin / proteasome system (UPS)-mediated degradation of NPR1 in the nucleus plays an essential role in regulating gene expression during plant immune responses.

Recent studies have linked the UPS to several aspects of phytohormonal signalling and regulation of biotic stress responses (Craig *et al.*, 2009; Spoel *et al.*, 2009; Delauré *et al.*, 2008; Dreher & Callis, 2007). The UPS is responsible for the degradation of un–needed and damaged proteins and for maintaining the balance between enzyme synthesis and degradation. Through a three-step cascade (E1 > E2 > E3) proteins are selected and directed for degradation by the UPS. Relatively few E1 proteins (2) and E2 enzymes (37) are present in the model plant *Arabidopsis* whereas more than 1400 genes encoding putative E3-ubiquitin ligases have been reported

(Craig *et al.*, 2009). E3–ubiquitin ligases are responsible for the final tagging of proteins, thereby conferring specificity to the degradation process. Like SA–mediated gene regulation, protein hydrolysis via the UPS pathway seems to be a prerequisite for activation of JA-responsive genes. In the presence of a JA signal the SCF^{COI1} complex interacts with the JAZ1 protein, which is targeted for degradation via the UPS system. JAZ1 is a repressor and therefore, the degradation of JAZ1 leads to transcriptional activation of JA responses (Chini *et al.*, 2007; Thines *et al.*, 2007).
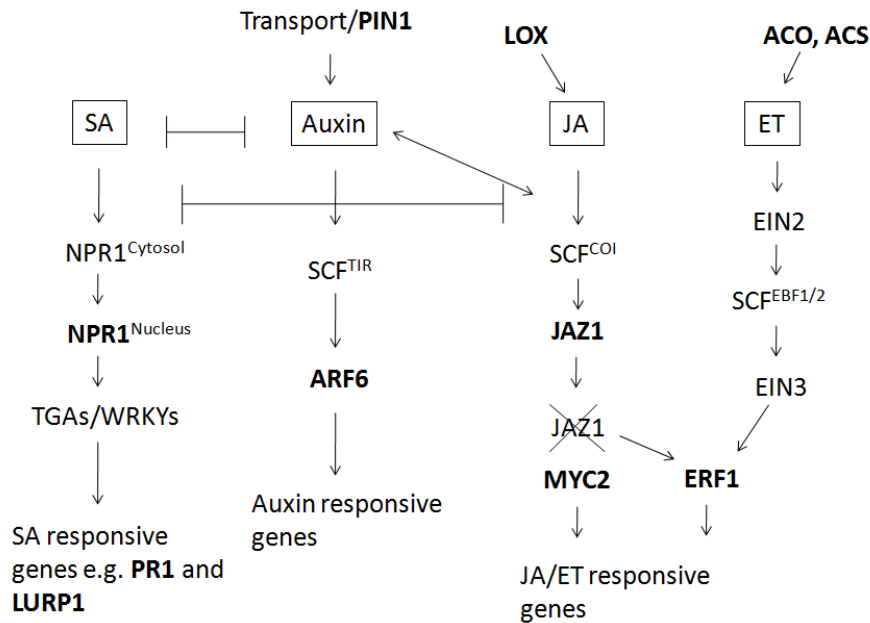
Figure 3. Schematic picture of hormonal signalling network in plant defence.
The schematic is based on Pieterse *et al.,* (2009) Nature Chemical Biology. SA–salicylic acid, JA–jasmonic acid, ET–ethylene.

The role of JA and ET as modulators of defence responses is relatively well documented in conifers (Krokene *et al.*, 2008; Miller *et al.*, 2005; Hudgins & Franceschi, 2004; Zhao *et al.*, 2004; Hudgins *et al.*, 2003a; Zhao & Sakai, 2003; Franceschi *et al.*, 2002; Martin *et al.*, 2002). For instance, exogenous application of methyljasmonate (MeJA) and ethylene have been shown to initiate similar responses as wounding e.g. induction of resin ducts, PP cells and the transcription of mono– and diterpene synthases (Miller *et al.*, 2005; Hudgins *et al.*, 2004; Hudgins & Franceschi, 2004; Fäldt *et al.*, 2003; Franceschi *et al.*, 2002; Martin *et al.*, 2002). Genes involved in the biosynthesis of ET and their induction by wounding have also been

characterised (Ralph *et al.*, 2007; Hudgins *et al.*, 2006). Hudgins and Franceschi (2004) showed that the cellular responses induced by MeJA, are in turn mediated by ET and that no induction of cellular defences could be detected after the application of methylsalicylate (MeSA). The reports of SA in conifer defence are rare and the results are not conclusive (Kozlowski *et al.*, 1999; Kozlowski & Métraux, 1998). However, Likar and Regvar (2008) observed a systemic accumulation of SA in roots and shoots of Norway spruce seedlings after inoculation with *H. annosum s.s.*, which suggests a role in defence signalling. PR proteins have also been shown to be induced after treatment with sodium salicylate (Davis *et al.*, 2002).

1.2.4 Norway spruce /*Heterobasidion* interaction

By being a facultative necrotrophic fungus *H. annosum s.l.* can live nectrotrophically, by killing host tissue but also saprotrophically, surviving on dead wood by breaking down lignin and cellulose. Modern silvicultural management facilitate the spread of these fungi by creating many new entry points such as fresh stumps (Stenlid & Redfern, 1998). The stump surface is largely unprotected and basidiospores germinating on a fresh stump escape much of the defence systems available to Norway spruce. By growing saprophytically in the heartwood, which is depleted of living cells, the fungus can extend in the wood without having to cope with host defence responses. However, as soon as the fungus comes in contact with the sapwood further growth requires a switch to necrotrophic growth and the ability to handle host defences such as reactive oxygen species and secondary metabolites. Genes involved in detoxification has been shown to be induced in this switch between different nutritional mode (Lundén, 2010). *H. annosum s.l* seems to be able to overcome most of the host´s obstacles although trade-offs might exist in the switch between saprotrophic and necrotrophic growth. A trade-off does seem to exist for the host as well because trees with a so-called reaction zone (RZ) had a lower periodic increment than infected trees without a RZ (Oliva *et al.*, 2010b; Bendz-Hellgren & Stenlid, 1997). The RZ is characterised by a high pH and a high concentration of phenols (Shain & Hillis, 1971) and functions to compartmentalise the decay.

As a potent wood decayer, *H. annosum s.l.,* destroys large quantities of wood. To access nutrients it secretes a wide range of extracellular enzymes such as cellulases, hemicellulases, pectinases, laccases and peroxidases (Asiegbu *et al.*, 2004; Maijala *et al.*, 2003; Maijala *et al.*, 1995; Karlsson & Stenlid, 1991; Maijala *et al.*, 1991). Cell wall degrading activity or MAMPs triggers the defence responses in the host and molecular studies of the

interactions between the *Heterobasidion* spp. complex and its host have revealed the induction of multiple overlapping defence strategies, as described in the sections 1.2.1 and 1.2.2. These mechanisms include (i) induction of defence related genes (Adomas *et al.*, 2007; Koutaniemi *et al.*, 2007; Asiegbu *et al.*, 2003), (ii) production of PR-proteins such as chitinases (Fossdal *et al.*, 2005; Hietala *et al.*, 2004), (iii) production of antimicrobial compounds such as phenols and terpenes (Johansson *et al.*, 2004; Nagy *et al.*, 2004b; Johansson *et al.*, 1998; Lindberg *et al.*, 1992), (iv) and shifts in primary and secondary metabolism (Adomas *et al.*, 2007). Similar anatomical responses to those induced by wounding have also been reported, with induction of TD and PP (Krokene *et al.*, 2003) and papilla formation in association with fungal hyphae (Asiegbu *et al.*, 1993).

1.3 Breeding for resistance

In the Swedish Norway spruce breeding program, the main goals are to increase the productivity and improve wood qualities (Karlsson & Rosvall, 1993). Due to the monoculture cropping of forest trees, we have created opportunities and new niches for pathogens that would otherwise be of minor importance: the growing problem of *Heterobasidion* root rot is probably an example of this. The international trade of wood products also increases the risk of introducing new pathogens to susceptible hosts (Stenlid *et al.*, 2011). It is therefore important to consider traits such as resistance or reduced susceptibility when breeding forest trees.

The generation time for Norway spruce is approximately 20-25 years and although phytohormones such as gibberellins can shorten the time from seedling to first bloom (Högberg & Eriksson, 1994) the breeding cycle is still very long compared to other crops. Conventional tree breeding is efficient for traits with high heritability, but is less efficient if the heritability is low or if the evaluation of the trait is difficult (or very time consuming). The use of marker-assisted selection (MAS) has been proposed as a tool to improve the efficiency of conventional plant breeding. However it is essential to find stable linkage between markers and DNA regions controlling the trait (called quantitative trait loci or QTLs). One single recombination event can abolish the linkage between the trait that is improved by breeding and the marker used to select genotypes. Finding stable linkage also requires a large progeny, tested in different environments and at different developmental stages (Brown *et al.*, 2003; Asíns, 2002), which makes this a costly process. As an alternative, selection can be done directly on the desired alleles of so called candidate genes known to control the trait of interest. For example,

in *Pinus taeda* (L.), candidate genes linked to growth and wood density are available (Yu *et al.*, 2006; Brown *et al.*, 2003).

Inheritance of disease resistance in forest trees has been commonly explained by polygenic models, where resistance is controlled by many genes, each with a small additive effect. However, a few exceptions have been described. Resistance to *Pissodes strob* Peck. in Sitka spruce [*Picea sitchensis* (Bong.) Carr.] has been demonstrated to have a significant genetic component with an individual tree heritability of more than 0.4 (King *et al.*, 1997). In *P. taeda* a major gene for resistance to *Cronartium quercuum* (Berk.) has been found, which has also been incorporated into the breeding program (Wilcox *et al.*, 1996). A genes for resistance to *Cronartium ribicola* (Fisch.) have also been mapped in *Pinus lambertiana* (Dougl.) (Devey *et al.*, 1995).

Experiments where Norway spruce has been inoculated with *H. annosum s.l.* have consistently shown a significant difference in fungal sapwood colonisation between different Norway spruce genotypes (Swedjemark & Karlsson, 2004; Swedjemark *et al.*, 2001; Swedjemark & Stenlid, 1997; Swedjemark *et al.*, 1997; Swedjemark & Stenlid, 1996; von Weissenberg, 1975). The heritability for fungal growth in these studies have varied between 0.09 and 0.35 which is within the same range such as other traits as growth capacity, growth rhythm and wood density (Karlsson & Swedjemark, 2006). This suggests that there may be some room for improving the resistance to *H. annosum s.l.* in Norway spruce through breeding. However, so far little is known about what constitutes the difference in susceptibility to *H. annosum s.l.* and the methods to score the relative susceptibility have some limitations. To be able to implement resistance trait in the breeding program more knowledge is needed about the underlying molecular basis for the traits and the correlation to other traits in the breeding program needs to be addressed.

2 Objectives

The overall objective of this thesis was to increase the knowledge of the molecular processes underlying the response to *H. annosum s.l.* in *P. abies*. More specifically, the objectives were to

➢ Estimate the genetic variation in susceptibility to *H. parviporum* within a full-sib family of Norway spruce (Paper I).

➢ Gain basic knowledge about the specificity of the defence responses in Norway spruce to *H. parviporum* (Paper II, IV).

➢ Investigate transcriptional changes associated with induced defence responses in Norway spruce to *H. parviporum* (Paper II, III and IV).

➢ Find associations between the induced transcriptome and chemical profiles to the level of susceptibility to *H.annosum s.l* in Norway spruce (Paper III).

➢ Investigate responses to *H. parviporum* in Norway spruce related to the salicylic acid and jasmonic acid signalling pathways (Paper IV).

3 Material and Methods

3.1 Plant and fungal material

Spruce material of different ages and genetic background were used in this study. In Papers I, II and III, cuttings from a full-sib family that originated from the Swedish breeding program were used. The cross was made in 1998 between the female parent (S21K7622162) and the male parent (S21K7621678), which both originated from the forest district Brezno in Slovakia. The original seedling (ortet) from which the cuttings were made was also included in Paper I. Unrelated spruce plants from a plant nursery were used in Papers II and IV. In Paper III, the experiment was carried out in a Norway spruce clone trial (S21S842979) established in 1984. The following fungal isolates were used: *Heterobasidion parviporum* (Rb175) in Papers I, II and IV, *H. annosum s.s.* (Sä16-4) in Paper III and *Phlebiopsis gigantea* (Rotstop) in Papers II and IV.

3.2 Inoculation methods and scoring the susceptibility

Plants were artificially inoculated: to allow the fungus to enter the plant a 5-mm circular wound was made with a cork borer through the bark. Wood plugs of Norway spruce colonised with *H. parviporum* or *P. gigantea* were prepared according to Stenlid & Swedjemark (1988) and then attached to the wound with Parafilm®.

To score the relative susceptibility, in Paper I the length of the lesion in the bark and the extension of fungal growth in the sapwood were measured. To check the fungal extension in the plants, the stems were cut into 5 mm thick discs and placed on wet filter paper in an empty Petri-plate. After 7-10 days incubation at 18ºC, the wood discs taken from the harvested trees were

checked for the presence of *H. parviporum* conidiophores (Stenlid & Swedjemark, 1988).

3.3   Molecular methods

Several different molecular techniques were used for the analyses in this thesis and are described in detail in the separate papers. The purpose of this section is to give a brief description of some of the methods used. Many of the methods used are based on the polymerase chain reaction (PCR).

3.3.1 cDNA-AFLP and quantitative PCR

Both complementary DNA-amplified fragment length polymorphism (cDNA-AFLP) and quantitative PCR (qPCR) are methods that can be used for analysing differences in steady-state messenger RNA (mRNA) levels. Quantitative PCR is a much more sensitive technique with a detection limitation of about 1 transcript per 1000 cells (Czechowski *et al.*, 2004). However, cDNA-AFLP has the advantage of not requiring prior sequence information because it uses adaptors with known sequences for the amplification (Bachem *et al.*, 1998). It also enables many different cDNA fragments to be screened at the same time.

The cDNA-AFLP technique was used in Paper II to identify the transcriptional responses in bark of *P. abies* to *H. parviporum* infection compared with the response to wounding treatment. The cDNA-AFLP method has proven successful as a screening method for differential gene expression in studies of interactions with pathogens in non-model organisms (Wang *et al.*, 2010; Durrant *et al.*, 2000). In brief, cDNA was synthesised from mRNA and then digested with the restriction enzymes EcoR1 and Mse1. Adaptors with known sequence were ligated to the fragmented cDNA and amplified with PCR using primers complementary to the adaptors. The cDNA-AFLP fragments were separated on a polyacrylamide gel and differences in the intensity of the bands were interpreted as differences in the level of expression. Fragments with different band intensities between treatments (i.e. wounded samples and *H. parviporum* inoculated samples), were cut from the gel, re-amplified and sequenced. BLAST analysis (Altschul *et al.*, 1990) was performed to find potential functions for the sequenced genes.

In Papers II, III and IV, qPCR was used to examine the differences in transcriptional level for selected genes of interest and to verify expression patterns with other techniques such as cDNA-AFLP and 454-sequencing. Total RNA was isolated as described by Chang *et al.,* (1993). To avoid

contamination of genomic DNA, total RNA was treated with DNase1 (SIGMA) prior to cDNA synthesis and transcript abundance was normalised to expression of constitutively expressed genes: phosphoglucomutase (Vestman *et al.*, 2010), eukaryotic translation initiation factor 4A (*elF4A*) (Palovaara & Hakman, 2008)*,* elongation factor 1-α *(ELF1α)* and α-tubulin (*αTUB*) which all showed low variation between samples. The relative expression was calculated using REST 2006 (Pfaffl *et al.*, 2002)

For isolation of full-length cDNA sequences of Norway spruce rapid amplification of cDNA ends (RACE), the RT reactions and 5′/3′RACE reactions were performed using the SMARTer RACE cDNA amplification kit (Clontech) according to the manufacturer's instructions.

3.3.2 454 sequencing

Total RNA was isolated in the same way as for the qPCR analysis. Purified mRNA was amplified with the MessageAmpIII kit (Ambion) according to the manufacturer's instruction. Double stranded cDNA of sufficient quality was pooled according to genotype and treatment. Two to five μg each of 24 cDNA samples representing all time points and treatments were submitted for template preparation and sequencing on a Genome Sequencer Titanium system (Roche Applied Science) at the Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo. Sequence reads and quality scores for sequences were obtained from CEES.

The sequences retrieved were assembled with the sequence assembler software Newbler v2.3 and v2.5 (Roche) (www.454.com) with default settings for cDNA assembly with the sff-files as input file. The combined sequences from all treatments were assembled into the gene-equivalent isogroups and and the plausible splice variants, isotigs. For an explanation of the terms isogroup, isotig and their connection with contigs see Ewen-Campen *et al.,* (2011). Contigs were subjected to visual inspection in ace format with the software Tablet (Milne *et al.*, 2009).The assembled reference file was annotated with the software Blast2GO (Conesa *et al.*, 2005), where the sequences got annotated to BLASTx homologies, GO terms and EC numbers as well as scanned with InterProScan. Furthermore, the data set was trimmed for fungal sequences by identification of species belonging based on the BLAST homologies with MEGAN (Mitra *et al.*, 2009).

Count data of the occurrence of the expressed genes in the individual samples were retrieved by aligning individual reads to the isogroups and isotigs. The count data were aligned in R and imported into the R-package DESeq (Anders & Huber, 2010) and normalized on number of counts and

subjected further pair-wise differential expression transcriptome analysis. The normalized count data were transformed to homoscedastic data in DESeq and clustered with JMP™ by Ward's hierarchical cluster. The contigs annotated into pathways leading to production of terpenes, stilbenes and proanthocyanidins were clustered separately.

3.4 Statistical analysis

For the analysis of variance in Paper I, the Software Proc GLM (SAS, 1996) was used. Phenotypic correlations between traits were estimated as Pearson product moment correlations and the Ryan-Joiner test was used to test for normality.

The estimates of broad-sense heritability $(\hat{H}^2)$ were obtained by $\hat{H}^2 = \hat{\sigma}^2_G / \hat{\sigma}^2_P$ where the genotypic variance components is $(\hat{\sigma}^2_G)$ and the environmental variance components is $(\hat{\sigma}^2_E)$.

4 Results and Discussion

4.1 The response to *Heterobasidion annosum s.l.* in Norway spruce is an enhanced general defence response

One of the aims of this thesis was to investigate whether *H. annosum s.l.* was able to elicit specific responses in Norway spruce or if the induced response was a generic defence response. Having specific or general induced defence responses may have implications in breeding for resistance. In order to examine the specificity of the response to *H. annosum s.l.* comparisons were made to the response after wounding (Papers II, III and IV) and *P. gigantea* infection (Papers II and IV). Unlike, for example, *Armillaria* species *Heterobasidion annosum s.l.* is not able to penetrate spruce bark in the absence of wounding (Solla *et al.*, 2002). To enable the fungus to access the sapwood, it is necessary to partially remove the bark. This inoculation method has frequently been used in other studies (Deflorio *et al.*, 2011; Koutaniemi *et al.*, 2007; Fossdal *et al.*, 2005; Hietala *et al.*, 2004; Nagy *et al.*, 2004b; Krokene *et al.*, 2003). However, when using this procedure an induced wound response in the tissue is to be expected and has been detected (Deflorio *et al.*, 2011; Krokene *et al.*, 2003; Nagy *et al.*, 2000). *H. annosum s.l.* inoculated samples showed an enhanced reaction although the overall pattern of expression was similar between treatments revealing non-specificity. Treatment with *P. gigantea* gave in principal an intermediate reaction compared with wounding or treatment with *H. parviporum*. The expression pattern for many transcribed derived fragments (TDFs) (Paper II), genes in the phenylpropanoid pathway and genes in the biosynthesis of proanthocyanidins (PAs) (Papers III and IV) was similar between treatments. Correlating changes in the phenol chemical profile between wounding or inoculation treatment further adds to this picture (Figs. 3 and 4 in Paper III). In addition, genes putatively involved in the SA and JA/(ET) signalling

pathways were also similarly expressed after wounding or inoculation and only separated by the magnitude of up-regulation (Papers II and IV, Fig. 1 in Paper IV).

The enhanced reaction after fungal inoculation is in accordance with previous observations where anatomical changes such as induction of TD and PP cells was stronger and more rapid after *H. annosum* inoculation than wounding (Krekling *et al.*, 2004). Studies have also shown that fungal inoculation enhanced the induction of chalcone synthase (Nagy *et al.*, 2004b) and *PaChi4* (Hietala *et al.*, 2004) compared with wounding. In the transcriptome data in Paper IV there were many up-regulated chitinases after wounding at 5 and 40 days post treatment (dpi). Given that the up-regulation was observed after wounding alone, it seems that the induction of chitinases is not specifically induced to target fungal hyphae but rather is a component of the general defences in Norway spruce. Together with similar observation in previous studies (Deflorio *et al.*, 2011; Fossdal *et al.*, 2005; Hietala *et al.*, 2004), this supports the hypothesis that the induced defence in response to *H. annosum s.l* is a broad non-specific defence response.

In Paper IV, we tried to separate the responses close to the point of inoculation from the responses induced by the invading fungal hyphae by analysing gene expression in samples distal to the wound and this analysis revealed some differences. In the inoculated samples, a stronger and, with time, increased response was observed for genes in the phenylpropanoid pathway as well as for the *PR1* genes and *LOX,* which are related to SA and JA signalling, respectively. The pattern of induction after wounding alone did not differ much between samples harvested at 3 or 7 dpi in the samples distal to the wound. *PR1* was the gene that showed the largest induction in the distal samples at both 3 and 7 dpi (Fig 1 in Paper IV). Induction of the R2R3-MYB transcription factor *PaMYB14,* which is known to be responsive to JA (Bedon et al., 2010), was also induced in the distal samples at 3 and 7 dpi (Table 1 in Paper IV). Attempts to follow the spatial changes in defence gene expression have previously shown that there is a substantial difference in expression levels in tissues at the wound site and in tissues spatially separated from the wound site (Deflorio *et al.*, 2011; Hietala *et al.*, 2004). Differences in gene expression have also been localised to different specialised tissue types within the bark of *P. glauca* (Abbott *et al.*, 2010). However, the signalling mediating defence responses proximal and distal to the wound site have not been investigated. Our results clearly show an induction of genes putatively involved in both JA and SA signalling in samples distal to the wound after wounding and inoculation, indicating a

transported signal from the point of inoculation. Since there was no visible lesion in the distal sample at 7 dpi but a relatively large induction of defence-related genes such as *LOX* and the *PR1* gene, we hypothesise that the increased reaction in *H. parviporum* inoculated samples was due to the advancing fungal infection. However there seems to be some signals transfusing from the point of the wound to the more distal samples because a down-regulation of *LURP1* and *ASC* was observed after wounding or *P. gigantea* inoculation.

MAMPs such as chitin can be recognised by Norway spruce, which initiates a defence response (Hebe *et al.*, 1999; Salzer *et al.*, 1996). Host-derived molecules such as cell wall fragments, created by the activity of enzymes produced by the fungus, can also elicit a defence reaction resembling the reaction initiated by MAMPs (Hématy *et al.*, 2009; Hückelhoven, 2007). Although the level of induction induced by *P. gigantea* inoculation was intermediate compared with wounding or *H. parviporum* inoculation in samples close to the wound, the reaction to *P. gigantea* inoculation was more similar to wounding in the distal samples at 7 dpi (Table 1 in Paper IV). *P. gigantea* is mainly a saprotrophic fungus living on dead wood and freshly cut stumps. Hence, in our system it is possible that the fungus has the ability to sustain itself by living on dead tissue close to the wound, consequently eliciting a stronger reaction than wounding. The stronger reaction after fungal inoculation was in accordance with previous studies where atomically changes such as induction of TD and PP cells was shown to be stronger and faster in *H. annosum* inoculation than mock inoculation (Krekling *et al.*, 2004).

4.2 The signalling pathways of salicylic acid and jasmonic acid in Norway spruce defence may be interconnected

The involvement of jasmonic acid and ethylene is relatively well documented in conifer defence (Krokene *et al.*, 2008; Miller *et al.*, 2005; Hudgins & Franceschi, 2004; Zhao *et al.*, 2004; Hudgins *et al.*, 2003a; Zhao & Sakai, 2003; Franceschi *et al.*, 2002; Martin *et al.*, 2002). However, reports of the role of SA have been rarer (Likar & Regvar, 2008; Hudgins *et al.*, 2006; Davis *et al.*, 2002; Kozlowski *et al.*, 1999). Interestingly, in our cDNA-AFLP screening (Paper II), one of the most induced TDFs was similar to the SA-mediated *PR1* gene. This TDF was recovered as many as 11 times indicating its abundance in the data set. In *Arabidopsis* and tobacco, *PR1* gene expression is particularly responsive to salicylic acid and is therefore often used as a marker for the salicylic acid-dependent SAR

response (Ryals *et al.*, 1996). In a further investigation (Paper IV), the parallel induction of SA- and JA-mediated genes was confirmed. *PR1*, in addition to the putative SA-mediated genes *LUPR1* and *NPR1* was up-regulated in both wounding treatment and inoculation with *H. parviporum* together with JA- and ET-responsive genes such as *LOX*, *JAZ*, *JAR1*, *MYC2* and *ACS* (Fig. 3) (Fig 1 and Table 1 in Paper IV). The observation of up-regulated SA-mediated genes after wounding suggests that this signalling pathway is part of a general defence response in Norway spruce and that its role in induced defence might have been overlooked. Likar and Regvar (2008) showed that free SA can accumulate in *H. annosum s.l.* inoculated Norway spruce seedlings. However, it seems that accumulation of free SA can be induced in response to MeJA treatment (Kozlowski *et al.*, 1999). In Paper IV, Norway spruce seedlings showed an induction of both *PR1* and *LURP1* after exposure to MeSA or MeJA (Fig. 2 in Paper IV). Two interpretations are possible: either MeJA itself induced transcription of *PR1* and *LURP1* or MeJA by inducing an accumulation of SA, as seen in Kozlowski *et al.,* (1999), initiated transcription of *PR1* and *LURP1*. In angiosperms the JA/ET and SA signalling pathways are generally antagonistic (Robert-Seilaniantz *et al.*, 2007; Thomma *et al.*, 1998). However, our data fit better with the theory presented by Katagari and Tsuda (2010): they proposed that the outcome of plant immunity may be determined by how a shared signalling network is used rather than being dependent on the existence of specific signalling pathways. Is seems that the antagonism observed between the different pathways observed in angiosperms (Glazebrook, 2005) does not fully hold for Norway spruce.

4.3 Involvement of the ubiquitin / proteasome system in regulating the defence responses in Norway spruce.

Analysis of the proteome of induced bark of Sitka spruce has revealed changes in the proteome as early as 2 h after treatment with *Pissodes strobi* (Lippert *et al.*, 2007). Much of the early responses are due to post-transcriptional modifications but *de novo* synthesis of proteins can be initiated rapidly by degradation of repressor proteins through the ubiquitin / proteasome system (UPS). Recent studies have linked the UPS to a number of aspects of hormone signalling and regulation of biotic stress responses (Craig *et al.*, 2009; Dreher & Callis, 2007). For example, JAZ1 is a repressor of transcriptional activation of JA responses and when JAZ1 is degraded via the UPS the repression of the transcription factor MYC2 is released and JA responses are initiated (Chini *et al.*, 2007; Thines *et al.*, 2007) (Fig. 3). The

jasmonate-resistant 1 proteins *(JAR1)* catalyses the conjugation of the amino acid isoleucine and JA and JA-Ile promotes the interaction between the SCF^COI1 E3-ubiquitin ligase complex and the JAZ protein (Thines *et al.*, 2007). In Paper II, an up-regulated TDF with similarities to *JAZ1* was found and *JAR1* and *MYC2* were up-regulated after wounding and inoculation (Paper IV), underlining the importance of the JA signalling pathway and the similarities with the defence signalling in angiosperms.

In addition, E3-ubiquitin ligases with similarities to *PUB23, ATL6* and *Xerico*, which are all induced by MAMPs (Libault *et al.*, 2007), were isolated in Paper II. In tomato the orthologue *LeATL6* is assumed to be mediated by a JA signal that, in turn, may activate an ethylene-mediated signalling pathway. Furthermore, in *Arabidopsis*, *PUB22/PUB23/PUB24* are highly induced after treatment with the MAMPs flg22, chitin and elf18 (Trujillo *et al.*, 2008; Libault *et al.*, 2007; Serrano *et al.*, 2006). Data suggest that PUB22/PUB23/PUB24 are negative regulators of the oxidative burst (Trujillo *et al.*, 2008). A necrotrophic pathogen such as *H. parviporum* can actually be assisted by the cell damage caused by an oxidative burst and the subsequent HR (Govrin & Levine, 2000). The observed up-regulation of a gene putatively involved in limiting the oxidative burst may indicate an attempt to prevent an HR response by the host (Fig. 1 in Paper II).

4.4 Carbon allocation into flavonoid biosynthesis is important for resistance in Norway spruce

The secondary metabolites synthesised through the phenylpropanoid pathway contribute substantially to the health of plants and they all derive from precursors from the shikimic acid pathway (i.e phenylalanine) (Herrmann, 1995). The modification of a limited set of core structures results in an array of different substances such as lignin, flavonoids, stilbenes and tannin that are synthesised by several different routes (Vogt, 2010). The first enzyme in the shikimic acid pathway is 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DAHP). Surprisingly, in Paper II we observed down-regulation of a TDF with similarities to *DAHP* after inoculation with *H. parviporum*. However, further analysis of two additional *DAHP* genes found in Norway spruce revealed a significant shift in transcript abundance, where *PaDAHP1* was down-regulated and *PaDAHP2* was up-regulated. This is consistent with data from the transcriptome analysis in Paper IV and in agreement with previous studies that showed that, *PaDAHP2* was up-regulated in conifers in response to both wounding (Ralph *et al.*, 2006) and inoculation with *H. annosum* (Adomas *et al.*, 2007).

33

The shift in *DAHP* expression may imply reallocation of carbon flow from protein synthesis to secondary metabolism.

Flavonoids have been shown to be relevant in the defence against pathogens and pests. For example, there seems to be a correlation between reduced susceptibility to the pathogenic fungi and accumulation of (+)-catechin in Norway spruce clones (Brignolas *et al.*, 1998; Brignolas *et al.*, 1995). In Paper III, an examination of the phenol profile in the bark of clones with different levels of resistance to *H. annosum s.* was preformed. The aim of the study was to find correlations with the level of resistance to differences in the chemical and transcriptional profile after wounding or *H. annosum s.s.* inoculation. In samples harvested at 5 dpi, a striking reduction in the level of the flavan–3-ol (+)-catechin was observed. However, at 15 dpi the level of (+)-catechin was comparable to the level of the controls (Fig. 4 in Paper III). Given that a decrease in soluble catechin is often observed in the later phase of wounded or infected Norway spruce bark while the tannin capacity of the tissue increases, it has been hypothesised that catechin, among other phenolic compounds, is converted to insoluble products such as proanthocyanidins (PA) (Schmidt. *et al.*, 2005; Brignolas *et al.*, 1995). The induction of (+)-catechin was preceded by an induction of genes in the PA pathway such as *DFR*, *LAR*, *ANS* and *ANR*, which were observed in both the transcriptome data and the qPCR analysis. The genes involved in PA biosynthesis were also analysed in Paper IV, confirming the induction seen in Paper III.

Many of the genes in the monolignol pathway have previously been shown to be up-regulated in response to *H. annosum s.s.* (Koutaniemi *et al.,* 2007). In our studies, genes in the first steps of the phenylpropanoid pathway (*PAL* and *C4H*) showed up-regulation in response to wounding and *H. annosum s.l.* inoculation (Papers II, III and IV). However, genes directly involved in monolignol formation, for example, CCR and CAD did not show a corresponding up-regulation (Paper III). These observations, together with the observed induction of genes in the flavonoid biosynthesis and PA pathways, suggest that a greater proportion of the metabolites are allocated to other downstream pathways such as the flavonoid pathway. Similar results have been observed in poplar hybrids in response to the biotrophic pathogen *Melampsora medusae* Thüm (Miranda *et al.*, 2007). This might indicate that the flavonoid pathway and PA biosynthesis is prioritised in the defence response.

In the cDNA–AFLP screening we recovered a TDF with similarities to the R2R3-MYB transcription factor *TT2* (Paper II). Over-expression of the orthologue *MYB134* in poplar resulted in an induction of genes associated

with PA biosynthesis, in addition to a plant-wide accumulation of PAs (Mellway *et al.*, 2009). In *Arabidopsis*, the regulation of PA production has also in part been assigned to TT2 (Nesi *et al.*, 2001). After recovering the full-length cDNA sequence a phylogenetic analysis was performed (Supplementary material Fig. 2 in Paper IV), that indicated that the TDF was homologous to the *TT2/MYB134* gene. The *PaTT2-like* gene was consistently up-regulation in response to both wounding and *H. parviporum* inoculation in our studies (Papers II, III and IV). This shows that the *PaTT2-like* gene is a stress-induced transcription factors and that it may be responsible for the observed induction of *DFR, LAR, ANS* and *ANR* (Fig. 6 in Paper III, Table 1 and Fig. 3 in Paper IV). The *PaTT2-like* gene was also up-regulated in response to exposure to MeJA but not to MeSA (Fig. 2 in Paper IV), suggesting that JA is involved in regulating the induction of this transcription factor and subsequently the induction of PA biosynthesis in Norway spruce.

4.5 Variation in susceptibility to *Heterobasidion annosum s.l.* and the potential for breeding for resistance

The relative susceptibility to *H. parviporum* was investigated in a full-sib family of Norway spruce by inoculating a set of 252 cloned progeny from a controlled cross (Paper I). Four ramets of each progeny were used, and to score the relative susceptibility, lesion length in the inner bark and fungal growth in the sapwood were measured. Among the progeny significant differences were found for fungal growth in the sapwood ($p<0.0005$) and the broad sense heritability (H^2) was 0.11. Previous experiments with clones of Norway spruce have shown that disease development is partly genetically controlled (Swedjemark & Karlsson, 2004; Swedjemark *et al.*, 1997) and the H^2 values found in other studies using the same isolate of *H. parviporum* range between 0.09 and 0.35 (Karlsson *et al.*, 2008; Swedjemark *et al.*, 2001; Swedjemark *et al.*, 1999; Swedjemark & Stenlid, 1997). The previous tests have been performed on cuttings of genetically unrelated Norway spruce material. The data from Paper I showed that the genetic component for susceptibility to *H. parviporum* can be detected even within a full-sib family of Norway spruce where the parents were not prior tested for resistance to *H. annosum s.l.* The approximately normal distribution of the mean values for fungal growth indicates that the response to *H. parviporum* infection is a quantitative trait under polygenic control. The aim was to use the data from this inoculation study for QTL-mapping. The construction of a genetic map for the 252 full-sib progeny has been a part of this thesis work. However,

because the map has not been completed this work will not be discussed in this thesis.

In our study we have come across some interesting variation between genotypes. In Paper III clones previously defined as more or less susceptible were used (Karlsson & Swedjemark, 2006). Clones 2405 and 7398, which were both considered as less susceptible clones showed differences in gene expression. One major difference was the observed level of induction: for example at 15 dpi the *PAL* genes were down-regulated in the wounded material in clone 7398 whereas they remained slightly up-regulated even at 28 dpi irrespective of treatment in clone 2405 (Fig. 6 in Paper III). Also the pattern of extractable (+)-catechin in bark differed between the clones. Between 15 and 28 dpi the free (+)-catechin level dropped significantly in 7398 whereas no drop in (+)-catechin level was seen in 2405 in wounded samples (Fig. 4 in Paper III).

The major goals in the Swedish breeding program for Norway spruce are to increase the productivity and to improve wood quality (Karlsson & Rosvall, 1993). The commercial gain achieved by using improved plant material could be even greater if resistance to pathogens was included in breeding programmes. In selecting more resistant genetic material for breeding it is essential that there is no negative correlation with growth traits. In an earlier assessment of the material used in Paper III there were no significant genotypic correlations between tree size and infection by rot fungi (Karlsson & Swedjemark, 2006). However, a negative correlation between stress treatments and growth has been indicated in studies on MeJA-treated Norway spruce trees where the radial sapwood growth was reduced by up to 30% (Krokene *et al.*, 2008). A negative correlation between tree lignin content and growth has also been established in several tree species (Novaes *et al.*, 2010; Kirst *et al.*, 2004). Oliva *et al,.* (2010b) showed that Norway spruce trees that formed a reaction zone (RZ) in response to decay grew less than trees with decay but without a RZ. The high level of secondary metabolites in the RZ implies that high levels of carbon resources are allocated to this zone. The shikimic acid pathway connects primary and secondary metabolism (Herrmann, 1995) and, therefore, the more carbon allocated into the shikimic acid pathway the less is left for growth. The shift in the expression of *DAHP* genes in Norway spruce under normal growth conditions and after *H. parviporum* inoculation highlights the importance of this pathway in carbon allocation. To date there has been little research directed at understanding how plants recruit energy for the defence response (Bolton, 2009). However, work with tobacco plants transformed with antisense constructs of *CAD* and *CCR* has

shown that manipulations of the carbon flow in one pathway in the secondary metabolism will have substantial effects on the partition of carbon between the primary and secondary metabolism (Dauwe *et al.*, 2007). When selecting material for breeding, the genotypic ability to balance the allocation of energy to growth and defence may be important aspects to consider. However, to address the growing problem of *H. annosum s.l.* rot, choosing more resistant material with slightly less growth potential may turn out to be the most cost beneficial.

5 Conclusion and Future prospects

The overall aim of this thesis was to provide basic knowledge about the induced defences in Norway spruce in response to *H. annosum s.l.* infection. The transcriptional changes that occur after *H. annosum s.l.* inoculation have been investigated using three different methods and in several different plant materials. Although the comprehensiveness of the methods differs the trends were similar. Several mechanisms have been highlighted as potentially important features in defence against this *H. annosum s.l.,* including the induction of the phenylpropanoid– and proanthocyanidin pathways, and the observed shift in the shikimic acid pathway. Furthermore, the transcription factor *PaTT2-like* gene can be important for regulating the production of PAs.

In Paper I we showed that there is significant variation between full–sibs within a Norway spruce family in the response to *H. parviporum* infection. The broad sense heritability (H^2) was found to be 0.11 for fungal growth, which was within the expected range. The parents of the full–sib Norway spruce family were not tested for their relative susceptibility to *H. parviporum* before the cross. It is possible that a cross between parents with different levels of resistance would give a higher H^2 and provide a good mapping population for QTL mapping of traits relevant to resistance to *H. parviporum*. In connection to the Norway spruce genome sequencing project an initiative for association mapping of several traits, including resistance traits, has been taken. A scoring of the relative susceptibility to *H. annosum s.l.* will be conducted in the mapping population with the potential to reveal genetic regions important for defence against this pathogen.

In Paper III, correlation between the constitutive phenol profile and the level of resistance to *H. annosum s.l* was found for a 30–year–old Norway spruce material. Changes in the chemical profile could also be related to observed changes in the transcriptome. The material in Paper III is an

obvious target for further investigations. More genotypes with different levels of resistance are available with several clones per genotype. They could be used to investigate the span of transcriptional variation both within and among genotypes. The sequenced genomes of Norway spruce should facilitate a survey of the promoter regions to couple transcriptional variation to defences in those regions. If correlation exists between those parameters the signature in the promotor region could work as a genetic marker for selection. Given that this clonal trial is included in the Swedish breeding programme and is beeing repeated at different locations in Sweden it should be possible to correlate differences in resistance to growth traits to evaluate this relationship more thoroughly. The plant material used in Paper III could possibly also provide potential parents for a cross between genotypes that are more and less susceptible to *H. annosum s.l.*

In this study, the difference found between the induced wound response and the response triggered by *H. annosum s.l.* was quantitative rather than qualitative. This implies that the response to *H. annosum s.l.* is a broad non-specific response (Papers II, III and IV). Adomas *et al.,* (2008) did find some differences in the induced response after challenging pine seedlings with different types of fungi (i.e a pathogenic, a saprophytic and a mutualistic fungus). However, reciprocal inoculation of roots and shoots with *Gremmeniella abietina* (Lagerberg) Morelet and *H. annosum s.s.* indicated a more organ specific defence than pathogen specific (Adomas & Asiegbu, 2006). A general induced defence controlled by several genes may have implications for breeding for resistance because polygenic resistance traits are generally more durable than major gene resistance traits (McDonald & Linde, 2002).

The induction of SA-related genes such as *PR1* and *LURP* after wounding and inoculation together with the observation that MeJA and MeSA could both induce those genes, implies a closer relationship between the SA and JA signalling pathways in Norway spruce than that observed in angiosperms (Papers II and IV). We know little of the defence signalling architecture in conifers but our data suggest a potential role for SA-dependent signalling that needs to be further dissected. Transgenic *NahG* plants, which are unable to accumulate salicylic acid, may be helpful tools in evaluating the relationship between SA and JA in Norway spruce and in determine how these signalling pathways contribute to the resistance to *H. parviporum*.

Looking at the transcriptome only shows the picture on one side of the coin. Observation of up-regulated E3-ubiquitin ligases in Paper II highlights the importance of post-translational regulatory mechanisms and, several of

the TDFs found have a documented involvement in the defence response. To get a more comprehensive picture of the regulatory changes that occur in Norway spruce in response to stress an integration of studies of transcriptional changes and changes in the proteome is needed. The whole genome sequence of Norway spruce should facilitate protein identification.

Finally, the clonal variation in transcriptional and chemical responses observed in Paper III provide some clues as to what may constitute the difference between siblings in Paper I. Palle and co-workers (2011) have elegantly shown how differences in the transcript profile can be correlated with the population structure. This study not only demonstrates a large variation in the natural population that can be explored to improve coniferous trees in plant breeding programmes but also some of the potential of using modern molecular methods in breeding practices.

References

Abbott, E., Hall, D., Hamberger, B. & Bohlmann, J. (2010). Laser microdissection of conifer stem tissues: Isolation and analysis of high quality RNA, terpene synthase enzyme activity and terpenoid metabolites from resin ducts and cambial zone tissue of white spruce (*Picea glauca*). *BMC Plant Biology*, 106.

Adomas, A. & Asiegbu, F.O. (2006). Analysis of organ-specific responses of Pinus sylvestris to shoot (*Gremmeniella abietina*) and root (*Heterobasidion annosum*) pathogens. *Physiological and Molecular Plant Pathology* 69(4-6), 140-152.

Adomas, A., Heller, G., Li, G., Olson, Å., Chu, T.M., Osborne, J., Craig, D., Van Zyl, L., Wolfinger, R., Sederoff, R., Dean, R.A., Stenlid, J., Finlay, R. & Asiegbu, F.O. (2007). Transcript profiling of a conifer pathosystem: Response of *Pinus sylvestris* root tissues to pathogen (*Heterobasidion annosum*) invasion. *Tree Physiology* 27(10), 1441-1458.

Adomas, A., Heller, G., Olson, Å., Osborne, J., Karlsson, M., Nahalkova, J., Van Zyl, L., Sederoff, R., Stenlid, J., Finlay, R. & Asiegbu, F.O. (2008). Comparative analysis of transcript abundance in *Pinus sylvestris* after challenge with a saprotrophic, pathogenic or mutualistic fungus. *Tree Physiology* 28(6), 885-897.

Almagro, L., Gómez Ros, L.V., Belchi-Navarro, S., Bru, R., Ros Barceló, A. & Pedreño, M.A. (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany* 60(2), 377-390.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* 215(3), 403-410.

Anders, S. & Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biology* 11(10), R106.

Asiegbu, F., Daniel, G. & Johansson, M. (1993). Studies on the infection of Norway spruce roots by *Heterobasidion annosum*. *Canadian Journal of Botany* 71(12), 1552-1561.

Asiegbu, F.O., Abu, S., Stenlid, J. & Johansson, M. (2004). Sequence polymorphism and molecular characterization of laccase genes of the conifer pathogen *Heterobasidion annosum*. *Mycological Research* 108(2), 136-148.

Asiegbu, F.O., Choi, W.B., Li, G.S., Nahalkova, J. & Dean, R.A. (2003). Isolation of a novel antimicrobial peptide gene (Sp-AMP) homologue from *Pinus sylvestris* (Scots pine) following infection with the root rot fungus *Heterobasidion annosum*. *FEMS Microbiology Letters* 228(1), 27-31.

Asíns, M.J. (2002). Present and future of quantitative trait locus analysis in plant breeding. *Plant Breeding* 121(4), 281-291.

Bachem, C.W.B., Oomen, R.J.F.J. & Visser, R.G.F. (1998). Transcript imaging with cDNA-AFLP: A step-by-step protocol. *Plant Molecular Biology Reporter* 16, 157-173.

Bedon, F., Bomal, C., Caron, S., Levasseur, C., Boyle, B., Mansfield, S.D., Schmidt, A., Gershenzon, J., Grima-Pettenati, J., Seguin, A. & MacKay, J. (2010). Subgroup 4 R2R3-MYBs in conifer trees: gene family expansion and contribution to the isoprenoid- and flavonoid-oriented responses. *Journal of Experimental Botany* 61(14), 3847-3864.

Bendz-Hellgren, M., Lipponen, K., Solheim, H. & Thomsen, I.M. (1998). Impact, control and managment of *Heterobasidion annosum* root and but rot in Europe and North America. In: Woodward, S.*, et al.* (Eds.) *Heterobasidion annosum biology, ecology, impact and control.* pp. 333-345. Wallingford: CAB International.

Bendz-Hellgren, M. & Stenlid, J. (1997). Decreased volume growth of *Picea abies* in response to *Heterobasidion annosum* infection. *Canadian Journal of Forest Research* 27(10), 1519-1524.

Bohlmann, J. (2008). Insect-induced terpenoid defenses in spruce. In: Schaller, A. (Ed.) *Induced Plant Resistance to Herbivory.* pp. 173 - 187. Dordrecht: Springer.

Bolton, M.D. (2009). Primary Metabolism and Plant Defense-Fuel for the Fire. *Molecular Plant-Microbe Interactions* 22(5), 487-497.

Brignolas, F., Lacroix, B., Lieutier, F., Sauvard, D., Drouet, A., Claudot, A.C., Yart, A., Berryman, A.A. & Christiansen, E. (1995). Induced responses in phenolic metabolism in two Norway spruce clones after wounding and inoculations with *Ophiostoma polonicum*, a bark beetle-associated fungus. *Plant Physiology* 109(3), 821-827.

Brignolas, F., Lieutier, F., Sauvard, D., Christiansen, E. & Berryman, A.A. (1998). Phenolic predictors for Norway spruce resistance to the bark beetle *Ips typographus* (Coleoptera: Scolytidae) and an associated fungus, *Ceratocystis polonica*. *Canadian Journal of Forest Research* 28(5), 720-728.

Brosche, M., Overmyer, K., Wrzaczek, M., Kangasjärvi, J. & Kangasjärvi, S. (2010). Stress signaling III: Reactive oxygen species (ROS). In: Pareek, A.*, et al.* (Eds.) *Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation.* pp. 91-102. Dordrecht: Springer.

Brown, G.R., Bassoni, D.L., Gill, G.P., Fontana, J.R., Wheeler, N.C., Megraw, R.A., Davis, M.F., Sewell, M.M., Tuskan, G.A. & Neale, D.B. (2003). Identification of Quantitative Trait Loci Influencing Wood Property Traits in Loblolly Pine (*Pinus taeda* L.). III. QTL Verification and Candidate Gene Mapping. *GENETICS* 164(4), 1537-1546.

Chang, S.J., Puryear, J. & Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter* 11(2), 113-116.

Chase, T.E. & Ullrich, R.C. (1990). Genetic basis of biological species in *Heterobasidion annosum*: Mendelian determinants. *Mycologia* 82, 67-72.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L. & Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 448(7154), 666-671.

Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Taón, M. & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21(18), 3674-3676.

Craig, A., Ewan, R., Mesmar, J., Gudipati, V. & Sadanandom, A. (2009). E3 ubiquitin ligases and plant innate immunity. *Journal of Experimental Botany* 60(4), 1123-1132.

Czechowski, T., Bari, R.P., Stitt, M., Scheible, W.R. & Udvardi, M.K. (2004). Real-time RT-PCR profiling of over 1400 *Arabidopsis* transcription factors: unprecedented sensitivity reveals novel root- and shoot-specific genes. *Plant Journal* 38(2), 366-379.

Dalman, K., Olson, Å. & Stenlid, J. (2010). Evolutionary history of the conifer root rot fungus *Heterobasidion annosum* sensu lato. *Molecular Ecology* 19(22), 4979-4993.

Dauwe, R., Morreel, K., Goeminne, G., Gielen, B., Rohde, A., Van Beeumen, J., Ralph, J., Boudet, A.-M., Kopka, J., Rochange, S.F., Halpin, C., Messens, E. & Boerjan, W. (2007). Molecular phenotyping of lignin-modified tobacco reveals associated changes in cell-wall metabolism, primary metabolism, stress metabolism and photorespiration. *The Plant Journal* 52(2), 263-285.

Davis, J.M., Wu, H.G., Cooke, J.E.K., Reed, J.M., Luce, K.S. & Michler, C.H. (2002). Pathogen challenge, salicylic acid, and jasmonic acid regulate expression of chitinase gene homologs in pine. *Molecular Plant-Microbe Interactions* 15(4), 380-387.

Deflorio, G., Horgan, G., Woodward, S. & Fossdal, C.G. (2011). Gene expression profiles, phenolics and lignin of Sitka spruce bark and sapwood before and after wounding and inoculation with *Heterobasidion annosum*. *Physiological and Molecular Plant Pathology* In Press, Corrected Proof.

Delauré, S.L., Van Hemelrijck, W., De Bolle, M.F.C., Cammue, B.P.A. & De Coninck, B.M.A. (2008). Building up plant defenses by breaking down proteins. *Plant Science* 174(4), 375-385.

Devey, M.E., Delfinomix, A., Kinloch, B.B. & Neale, D.B. (1995). Random amplified polymorphic DNA markers tightly linked to a gene for resistance to white-pine blister rust in sugar pine. *Proceedings of the National Academy of Sciences of the United States of America* 92(6), 2066-2070.

Dixon, R.A., Lamb, C.J., Masoud, S., Sewalt, V.J.H. & Paiva, N.L. (1996). Metabolic engineering: Prospects for crop improvement through the genetic manipulation of phenylpropanoid biosynthesis and defense responses - A review. *Gene* 179(1), 61-71.

Dreher, K. & Callis, J. (2007). Ubiquitin, hormones and biotic stress in plants. *Annals of Botany* 99(5), 787-822.

Durrant, W.E., Rowland, O., Piedras, P., Hammond-Kosack, K.E. & Jones, J.D.G. (2000). cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. *Plant Cell* 12(6), 963-977.

Ewen-Campen, B., Shaner, N., Panfilio, K., Suzuki, Y., Roth, S. & Extavour, C. (2011). The maternal and early embryonic transcriptome of the milkweed bug *Oncopeltus fasciatus*. *BMC Genomics* 12(1), 61.

Eyles, A., Bonello, P., Ganley, R. & Mohammed, C. (2009). Induced resistance to pests and pathogens in trees. *New Phytologist* 185(4), 893-908.

Fäldt, J., Martin, D., Miller, B., Rawat, S. & Bohlmann, J. (2003). Traumatic resin defense in Norway spruce (*Picea abies*): Methyl jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. *Plant Molecular Biology* 51(1), 119-133.

Farjon, A. (1998). *World Checklist and Bibliography of Conifers*. Richmond UK: Royal Botanic Gardens, Kew. ISBN 1-900347-54-7.

Farjón, A. (1990). *Pinaceae: Drawings and Descriptions of the Genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea*. Königstein: Koeltz Scientific Books.

Fossdal, C.G., Hietala, A.M., Kvaalen, H. & Solheim, H. (2005). Changes in host chitinase isoforms in relation to wounding and colonization by *Heterobasidion annosum*: early and strong defense response in 33-year-old resistant Norway spruce clone. *Tree Physiology* 26(2), 169-177.

Franceschi, V.R., Krekling, T., Berryman, A.A. & Christiansen, E. (1998). Specialized phloem parenchyma cells in Norway spruce (*Pinaceae*) bark are an important site of defense reactions. *American Journal of Botany* 85(5), 601-615.

Franceschi, V.R., Krekling, T. & Christiansen, E. (2002). Application of methyl jasmonate on *Picea abies (Pinaceae)* stems induces defense-related responses in phloem and xylem. *American Journal of Botany* 89(4), 578-586.

Franceschi, V.R., Krokene, P., Christiansen, E. & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* 167(2), 353-375.

Franceschi, V.R., Krokene, P., Krekling, T. & Christiansen, E. (2000). Phloem paremchyma cells are involved in local and distant defense responses to fungal inoculation or bark-beetle attack in Norway spruce (*Pinaceae*). *American Journal of Botany* 87(3), 314-326.

Fransson, J. (Ed.) (2010). *Forest Statistics*. Umeå, Sweden: Official Statistics of Sweden. (Swedish University of Agricultural Sciences.)

Garbelotto, M., Otrosina, W.J., Cobb, F.W. & Bruns, T.D. (1998). The European S and F intersterility groups of *Heterobasidion annosum* may represent sympatric protospecies. *Canadian Journal of Botany* 76(3), 397-409.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* 43, 205-227.

Glazebrook, J., Chen, W.J., Estes, B., Chang, H.S., Nawrath, C., Metraux, J.P., Zhu, T. & Katagiri, F. (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *Plant Journal* 34(2), 217-228.

Govrin, E.M. & Levine, A. (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Current Biology* 10(13), 751-757.

Hamel, L.-P. & Beaudoin, N. (2010). Chitooligosaccharide sensing and downstream signaling: contrasted outcomes in pathogenic and beneficial plant–microbe interactions. *Planta* 232(4), 787-806.

Hebe, G., Hager, A. & Salzer, P. (1999). Initial signalling processes induced by elicitors of ectomycorrhiza-forming fungi in spruce cells can also be triggered by G-protein-

activating mastoparan and protein phosphatase-inhibiting cantharidin. *Planta* 207(3), 418-425.

Hématy, K., Cherk, C. & Somerville, S. (2009). Host-pathogen warfare at the plant cell wall. *Current Opinion in Plant Biology* 12(4), 406-413.

Herrmann, K.M. (1995). The shikimate pathway as an entry to aromatic secondary metabolism. *Plant Physiology* 107(1), 7-12.

Hietala, A.M., Kvaalen, H., Schmidt, A., Johnk, N., Solheim, H. & Fossdal, C.G. (2004). Temporal and spatial profiles of chitinase expression by Norway spruce in response to bark colonization by *Heterobasidion annosum*. *Applied and Environmental Microbiology* 70(7), 3948-3953.

Högberg, K.-A. & Eriksson, U. (1994). Effects of root pruning and stem injections with Gibberellin A 4/7 on flowering and cone harvest in three *Picea abies* seed orchards. *Scandinavian Journal of Forest Research* 9(1), 323 - 328.

Hückelhoven, R. (2007). Cell wall-associated mechanisms of disease resistance and susceptibility. *Annual Review of Phytopathology* 45(1), 101-127.

Hudgins, J.W., Christiansen, E. & Franceschi, V.R. (2003a). Methyl jasmonate induces changes mimicking anatomical defenses in diverse members of the *Pinaceae*. *Tree Physiology* 23(6), 361-371.

Hudgins, J.W., Christiansen, E. & Franceschi, V.R. (2004). Induction of anatomically based defense responses in stems of diverse conifers by methyl jasmonate: a phylogenetic perspective. *Tree Physiology* 24(3), 251-264.

Hudgins, J.W. & Franceschi, V.R. (2004). Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiology* 135(4), 2134-2149.

Hudgins, J.W., Krekling, T. & Franceschi, V.R. (2003b). Distribution of calcium oxalate crystals in the secondary phloem of conifers: A constitutive defense mechanism? *New Phytologist* 159(3), 677-690.

Hudgins, J.W., Ralph, S.G., Franceschi, V.R. & Bohlmann, J. (2006). Ethylene in induced conifer defense: cDNA cloning, protein expression, and cellular and subcellular localization of 1-aminocyclopropane-1-carboxylate oxidase in resin duct and phenolic parenchyma cells. *Planta* 224(4), 865-877.

Johannesson, H. & Stenlid, J. (2003). Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. *Molecular Phylogenetics and Evolution* 29(1), 94-101.

Johansson, M., Lundgren, L. & Asiegbu, F.O. (1998). Differential phenol-induced laccase activity and total oxidative capacity of the S and P intersterility groups of the conifer root pathogen *Heterobasidion annosum*. *Microbiological Research* 153(1), 71-80.

Johansson, S.M., Lundgren, L.N. & Asiegbu, F.O. (2004). Initial reactions in sapwood of Norway spruce and Scots pine after wounding and infection by *Heterobasidion parviporum* and *H-annosum*. *Forest Pathology* 34(3), 197-210.

Jones, J.D.G. & Dangl, J.L. (2006). The plant immune system. *Nature* 444(7117), 323-329.

Karlsson, B. & Rosvall, O. (1993). Breeding programmes in Sweden: Norway spruce. In: Lee, S.J. (Ed.) *Progeny Testing and Breeding Strategies, Proceedings of the Nordic Group of Tree Breeding*. pp. 128-134. Edinburgh: Forestry Commission.

Karlsson, B. & Swedjemark, G. (2006). Genotypic variation in natural infection frequency of *Heterobasidion* spp. in a *Picea abies* clone trial in southern Sweden. *Scandinavian Journal of Forest Research* 21(2), 108-114.

Karlsson, B., Tsopelas, P., Zamponi, L., Capretti, P., Soulioti, N. & Swedjemark, G. (2008). Susceptibility to *Heterobasidion parviporum* in *Picea abies* clones grown in different environments. *Forest Pathology* 38(2), 83-89.

Karlsson, J.-O. & Stenlid, J. (1991). Pectic isozyme profiles of intersterility groups in *Heterobasidion annosum*. *Mycological Research* 95(5), 531-536.

Kasuga, T. & Mitchelson, K. (1993). Determination of the DNAsequence of the 5.8S ribosomal gene of *Heterobasidion annosum* and *Heterobasidion araucariae*. *Nucleic acids research* 21(5), 1320.

Katagiri, F. (2004). A global view of defense gene expression regulation – a highly interconnected signaling network. *Current Opinion in Plant Biology* 7(5), 506-511.

Katagiri, F. & Tsuda, K. (2010). Understanding the plant immune system. *Molecular Plant-Microbe Interactions* 23(12), 1531-1536.

Kazan, K. & Manners, J.M. (2008). Jasmonate signaling: Toward an integrated view. *Plant Physiology* 146(4), 1459-1468.

King, J.N., Yanchuk, A.D., Kiss, G.K. & Alfaro, R.I. (1997). Genetic and phenotypic relationships between weevil (*Pissodes strobi*) resistance and height growth in spruce populations of British Columbia. *Canadian Journal of Forest Research* 27(5), 732-739.

Kirst, M., Myburg, A.A., De Leon, J.P.G., Kirst, M.E., Scott, J. & Sederoff, R. (2004). Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of eucalyptus. *Plant Physiology* 135(4), 2368-2378.

Korhonen, K. (1978). Intersterility groups of *Heterobasidion annosum*. *Communicationes Instituti Forestalis Fenniae* 94, 25-.

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

Koutaniemi, S., Warinowski, T., Karkonen, A., Alatalo, E., Fossdal, C.G., Saranpaa, P., Laakso, T., Fagerstedt, K.V., Simola, L.K., Paulin, L., Rudd, S. & Teeri, T.H. (2007). Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology* 65, 311-328.

Kozlowski, G., Buchala, A. & Metraux, J.P. (1999). Methyl jasmonate protects Norway spruce [*Picea abies* (L.) Karst.] seedlings against *Pythium ultimum* Trow. *Physiological and Molecular Plant Pathology* 55(1), 53-58.

Kozlowski, G. & Métraux, J.P. (1998). Infection of Norway spruce (*Picea abies* (L.) Karst.) seedlings with *Pythium irregulare* Buism. and *Pythium ultimum* Trow.: Histological and biochemical responses. *European Journal of Plant Pathology* 104(3), 225-234.

Krekling, T., Franceschi, V.R., Berryman, A.A. & Christiansen, E. (2000). The structure and development of polyphenolic parenchyma cells in Norway spruce (*Picea abies*) bark. *Flora* 195(4), 354-369.

Krekling, T., Franceschi, V.R., Krokene, P. & Solheim, H. (2004). Differential anatomical response of Norway spruce stem tissues to sterile and fungus infected inoculations. *Trees-Structure and Function* 18(1), 1-9.

Krokene, P., Nagy, N.E. & Solheim, H. (2008). Methyl jasmonate and oxalic acid treatment of Norway spruce: anatomically based defense responses and increased resistance against fungal infection. *Tree Physiology* 28(1), 29-35.

Krokene, P., Solheim, H., Krekling, T. & Christiansen, E. (2003). Inducible anatomical defense responses in Norway spruce stems and their possible role in induced resistance. *Tree Physiology* 23(3), 191-197.

Lamb, C. & Dixon, R.A. (1997). The oxidative burst in plant disease resistance. *Annual Review of Plant Biology* 48, 251-275.

Ledig, E.T., Hodgskiss, P.D., Krutovskii, K.V., Neale, D.B. & Eguiluz-Piedra, T. (2004). Relationships among the spruces (*Picea, pinaceae*) of southwestern North America. *Systematic Botany* 29(2), 275-295.

Libault, M., Wan, J.R., Czechowski, T., Udvardi, M. & Stacey, G. (2007). Identification of 118 *Arabidopsis* transcription factor and 30 ubiquitin-ligase genes responding to chitin, a plant-defense elicitor. *Molecular Plant-Microbe Interactions* 20(8), 900-911.

Likar, M. & Regvar, M. (2008). Early defence reactions in Norway spruce seedlings inoculated with the mycorrhizal fungus *Pisolithus tinctorius* (Persoon) Coker & Couch and the pathogen *Heterobasidion annosum* (Fr.) Bref. *Trees-Structure and Function* 22(6), 861-868.

Lindberg, M., Lundgren, L., Gref, R. & Johansson, M. (1992). Stilbenes and resin acids in relation to the penetration of *Heterobasidion annosum* through the bark of *Picea abies*. *European Journal of Forest Pathology* 22(2-3), 95-106.

Linzer, R.E., Otrosina, W.J., Gonthier, P., Bruhn, J., Laflamme, G., Bussières, G. & Garbelotto, M. (2008). Inferences on the phylogeography of the fungal pathogen *Heterobasidion annosum*, including evidence of interspecific horizontal genetic transfer and of human-mediated, long-range dispersal. *Molecular Phylogenetics and Evolution* 46(3), 844-862.

Lippert, D., Chowrira, S., Ralph, S.G., Zhuang, J., Aeschliman, D., Ritland, C., Ritland, K. & Bohlmann, J. (2007). Conifer defense against insects: proteome analysis of Sitka spruce (*Picea sitchensis*) bark induced by mechanical wounding or feeding by white pine weevils (*Pissodes strobi*). *Proteomics* 7(2), 248-270.

Llorente, F., Muskett, P., Sanchez-Vallet, A., Lopez, G., Ramos, B., Sanchez-Rodriguez, C., Jorda, L., Parker, J. & Molina, A. (2008). Repression of the auxin response pathway increases *Arabidopsis* susceptibility to necrotrophic fungi. *Molecular Plant* 1(3), 496-509.

Lundén, K. (2010). *Heterobasidion - conifer pathosystem*. Diss. Uppsala:Swedish University of Agricultural Sciences.

Maijala, P., Fagerstedt, K.V. & Raudaskoski, M. (1991). Detection of extracellular cellulolytic and proteolytic activity in ectomycorrhizal fungi and *Heterobasidion annosum* (Fr.) Bref. *New Phytologist* 117(4), 643-648.

Maijala, P., Harrington, T.C. & Raudaskoski, M. (2003). A peroxidase gene family and gene trees in *Heterobasidion* and related genera. *Mycologia* 95(2), 209-221.

Maijala, P., Raudaskoski, M. & Viikari, L. (1995). Hemicellulolytic enzymes in P- and S-strains of *Heterobasidion annosum*. *Microbiology* 141(3), 743-750.

Martin, D., Tholl, D., Gershenzon, J. & Bohlmann, J. (2002). Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. *Plant Physiology* 129(3), 1003-1018.

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

Mellway, R.D., Tran, L.T., Prouse, M.B., Campbell, M.M. & Constabel, C.P. (2009). The wound-, pathogen-, and ultraviolet B-responsive MYB134 gene encodes an R2R3 MYB transcription factor that regulates proanthocyanidin synthesis in poplar. *Plant Physiology* 150(2), 924-941.

Miller, B., Madilao, L.L., Ralph, S. & Bohlmann, J. (2005). Insect-induced conifer defense. White pine weevil and methyl jasmonate induce traumatic resinosis, de novo formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. *Plant Physiology* 137(1), 369-382.

Milne, I., Bayer, M., Cardle, L., Shaw, P., Stephen, G., Wright, F. & Marshall, D. (2009). Tablet - next generation sequence assembly visualization. *Bioinformatics* 26(3), 401-402.

Miranda, M., Ralph, S.G., Mellway, R., White, R., Heath, M.C., Bohlmann, J. & Constabel, C.P. (2007). The transcriptional response of hybrid poplar (*Populus trichocarpa x P-deltoides*) to infection by *Melampsora medusae* leaf rust involves induction of flavonoid pathway genes leading to the accumulation of proanthocyanidins. *Molecular Plant-Microbe Interactions* 20(7), 816-831.

Mitra, S., Klar, B. & Huson, D.H. (2009). Visual and statistical comparison of metagenomes. *Bioinformatics*.

Mou, Z., Fan, W. & Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* 113(7), 935-944.

Mur, L.A.J., Kenton, P., Atzorn, R., Miersch, O. & Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology* 140(1), 249-262.

Nagy, N.E., Fossdal, C.G., Dalen, L.S., Lonneborg, A., Heldal, I. & Johnsen, O. (2004a). Effects of Rhizoctonia infection and drought on peroxidase and chitinase activity in Norway spruce (*Picea abies*). *Physiologia Plantarum* 120(3), 465-473.

Nagy, N.E., Fossdal, C.G., Krokene, P., Krekling, T., Lonneborg, A. & Solheim, H. (2004b). Induced responses to pathogen infection in Norway spruce phloem: changes in polyphenolic parenchyma cells, chalcone synthase transcript levels and peroxidase activity. *Tree Physiology* 24(5), 505-515.

Nagy, N.E., Franceschi, V.R., Solheim, H., Krekling, T. & Christiansen, E. (2000). Wound-induced traumatic resin duct development in stems of Norway spruce (*Pinaceae*): Anatomy and cytochemical traits. *American Journal of Botany* 87(3), 302-313.

Nesi, N., Jond, C., Debeaujon, I., Caboche, M. & Lepiniec, L. (2001). The Arabidopsis TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. *Plant Cell* 13(9), 2099-2114.

Niemelä, T. & Korhonen, K. (1998). Taxonomy of the genus *Heterobasidion*. In: Woodward, S., Stenlid, J., Karjalainen, R., & Hüttermann, A. (Ed.) *Heterobasidion annosum biology, ecology, impact and control.* pp. 27-33. Wallingford: CAB International.

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H. & Sederoff, R. (2010). Lignin and Biomass: A Negative Correlation for Wood Formation and Lignin Content in Trees. *Plant Physiology* 154(2), 555-561.

Oliva, J., Thor, M. & Stenlid, J. (2010a). Long-term effects of mechanized stump treatment against *Heterobasidion annosum* root rot in *Picea abies*. *Canadian Journal of Forest Research* 40(6), 1020-1033.

Oliva, J., Thor, M. & Stenlid, J. (2010b). Reaction zone and periodic increment decrease in *Picea abies* trees infected by *Heterobasidion annosum* s.l. *Forest Ecology and Management* 260(5), 692-698.

Otrosina, W.J. & Garbelotto, M. (2010). *Heterobasidion occidentale* sp. nov. and *Heterobasidion irregulare* nom. nov.: A disposition of North American *Heterobasidion* biological species. *Fungal Biology* 114(1), 16-25.

Palle, S., Seeve, C., Eckert, A., Cumbie, W., Goldfarb, B. & Loopstra, C. (2011). Natural variation in expression of genes involved in xylem development in loblolly pine (*Pinus taeda* L.). *Tree Genetics & Genomes* 7(1), 193-206.

Palovaara, J. & Hakman, I. (2008). Conifer WOX-related homeodomain transcription factors, developmental consideration and expression dynamic of WOX2 during *Picea abies* somatic embryogenesis. *Plant Molecular Biology* 66(5), 533-549.

Pfaffl, M.W., Horgan, G.W. & Dempfle, L. (2002). Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic acids research* 30(9), 10.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. & Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. *Nat Chem Biol* 5(5), 308-316.

Ralph, S.G., Hudgins, J.W., Jancsik, S., Franceschi, V.R. & Bohlmann, J. (2007). Aminocyclopropane carboxylic acid synthase is a regulated step in ethylene-dependent induced conifer defense. Full-length cDNA cloning of a multigene family, differential constitutive, and wound- and insect-induced expression, and cellular and subcellular localization in spruce and Douglas fir. *Plant Physiology* 143(1), 410-424.

Ralph, S.G., Yueh, H., Friedmann, M., Aeschliman, D., Zeznik, J.A., Nelson, C.C., Butterfield, Y.S.N., Kirkpatrick, R., Liu, J., Jones, S.J.M., Marra, M.A., Douglas, C.J., Ritland, K. & Bohlmann, J. (2006). Conifer defence against insects: microarray gene expression profiling of Sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms (*Choristoneura occidentalis*) or white pine weevils (*Pissodes strobi*) reveals large-scale changes of the host transcriptome. *Plant Cell and Environment* 29(8), 1545-1570.

Robert-Seilaniantz, A., Navarro, L., Bari, R. & Jones, J.D. (2007). Pathological hormone imbalances. *Current Opinion in Plant Biology* 10(4), 372-379.

Rushforth, K. (1987). *Conifers*. London: Helm.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y. & Hunt, M.D. (1996). Systemic Acquired Resistance. *The Plant Cell Online* 8(10), 1809-1819.

Salzer, P., Hebe, G. & Hager, A. (1997). Cleavage of chitinous elicitors from the ectomycorrhizal fungus *Hebeloma crustuliniforme* by host chitinases prevents induction of K^+ and Cl^- release, extracellular alkalinization and H_2O_2 synthesis of *Picea abies* cells. *Planta* 203(4), 470-479.

Salzer, P., Hebe, G., Reith, A., ZitterellHaid, B., Stransky, H., Gaschler, K. & Hager, A. (1996). Rapid reactions of spruce cells to elicitors released from the ectomycorrhizal fungus *Hebeloma crustuliniforme*, and inactivation of these elicitors by extracellular spruce cell enzymes. *Planta* 198(1), 118-126.

SAS (1996). *SAS/STAT Software: Changes and enhancements through release 6.11.* Cary, N.C: SAS Institute Inc.

Schmidt., A., Zeneli., G., Hietala., A.C.G., Fossdal., C.G., Krokene., P., Christiansen., E. & Gershenzon., J. (2005). Induced chemical defenses in conifers: Biochemical and molecular approaches to studying their function. In: Romeo, J. (Ed.) *Chemical ecology and phytochemistry of forest ecosystems*. pp. 1-28. Amsterdam: Elsevier. (Recent Advances in Phytochemistry; 39).

Serrano, M., Parra, S., Alcaraz, L.D. & Guzman, P. (2006). The ATL gene family from *Arabidopsis thaliana* and *Oryza sativa* comprises a large number of putative ubiquitin ligases of the RING-H2 type. *Journal of Molecular Evolution* 62(4), 434-445.

Shain, L. & Hillis, W.E. (1971). Phenole extractives in Norway spruce and their effect on *Fomes annosum*. *Phytopathology* 61, 841-845.

Shi, R., Sun, Y.-H., Li, Q., Heber, S., Sederoff, R. & Chiang, V.L. (2010). Towards a Systems Approach for Lignin Biosynthesis in *Populus trichocarpa*: Transcript Abundance and Specificity of the Monolignol Biosynthetic Genes. *Plant and Cell Physiology* 51(1), 144-163.

Singh, K.B., Foley, R.C. & Oñate-Sánchez, L. (2002). Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology* 5(5), 430-436.

Solla, A., Tomlinson, F. & Woodward, S. (2002). Penetration of *Picea sitchensis* root bark by *Armillaria mellea*, *Armillaria ostoyae* and *Heterobasidion annosum*. *Forest Pathology* 32(1), 55-70.

Spoel, S.H., Mou, Z., Tada, Y., Spivey, N.W., Genschik, P. & Dong, X. (2009). Proteasome-Mediated Turnover of the Transcription Coactivator NPR1 Plays Dual Roles in Regulating Plant Immunity. *Cell* 137(5), 860-872.

Spoel, S.H., Tada, Y. & Loake, G.J. (2010). Post-translational protein modification as a tool for transcription reprogramming. *New Phytologist* 186(2), 333-339.

Stenlid, J., Oliva, J., Boberg, J.B. & Hopkins, A.J.M. (2011). Emerging diseases in european forest ecosystems and responses in society. *Forests* 2(2), 486-504.

Stenlid, J. & Redfern, D.B. (1998). Spread within the tree and stand. In: Woodward, S.*, et al.* (Eds.) *Heterobasidion annosum: Biology, Ecology, Impact and Control.* Wallingford: CAB International.

Stenlid, J. & Swedjemark, G. (1988). Differential growth of S and P-isolates of *Heterobasidion annosum* in *Picea abies*. *Transactions of the British Mycological Society* (90), 209-213.

Stewart, W.N. & Rothwell, G.W. (1993). *Paleobotany and the Evolution of Plants* 2. ed. Cambridge: Cambridge University Press.

Swedjemark, G., Johannesson, H. & Stenlid, J. (1999). Intraspecific variation in *Heterobasidion annosum* for growth in sapwood of *Picea abies* and *Pinus sylvestirs*. *European Journal of Forest Pathology* 29(4), 249-258.

Swedjemark, G. & Karlsson, B. (2004). Genotypic variation in susceptibility following artificial *Heterobasidion annosum* inoculation of *Picea abies* clones in a 17-year-old field test. *Scandinavian Journal of Forest Research* 19(2), 103-111.

Swedjemark, G. & Stenlid, J. (1996). Variation in spread of *Heterobasidion annosum* in clones of *Picea abies* grown at different vegetation phases under greenhouse conditions. *Scandinavian Journal of Forest Research* 11, 137-144.

Swedjemark, G. & Stenlid, J. (1997). Between-tree and between-isolate variation for growth of S-group *Heterobasidion annosum* in sapwood of *Picea abies* cuttings. *Canadian Journal of Forest Research* 27(5), 711-715.

Swedjemark, G., Stenlid, J. & Karlsson, B. (1997). Genetic variation among clones of *Picea abies* in resistance to growth of *Heterobasidion annosum*. *Silvae Genetica* 46(6), 369-374.

Swedjemark, G., Stenlid, J. & Karlsson, B. (2001). Variation in growth of *Heterobasidion annosum* among clones of *Picea abies* incubated for different periods of time. *Forest Pathology* 31(3), 163-175.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A. & Browse, J. (2007). JAZ repressor proteins are targets of the SCF[COI1] complex during jasmonate signalling. *Nature* 448(7154), 661-665.

Thomma, B., Eggermont, K., Penninckx, I., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A. & Broekaert, W.F. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Sciences of the United States of America* 95(25), 15107-15111.

Trapp, S. & Croteau, R. (2001). Defensive resin biosynthesis in conifers. *Annual Review of Plant Physiology and Plant Molecular Biology* 52, 689-724.

Trujillo, M., Ichimura, K., Casais, C. & Shirasu, K. (2008). Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in *Arabidopsis*. *Current Biology* 18(18), 1396-1401.

Tsai, C.J., Harding, S.A., Tschaplinski, T.J., Lindroth, R.L. & Yuan, Y. (2006). Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. *New Phytologist* 172(1), 47-62.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J. & Katagiri, F. (2009). Network Properties of Robust Immunity in Plants. *PLoS Genet* 5(12), e1000772.

Van Loon, L.C., Rep, M. & Pieterse, C.M.J. (2006). Significance of inducible defense-related proteins in infected plants. In: *Annual Review of Phytopathology*. pp. 135-162.

van Wees, S.C.M., Chang, H.-S., Zhu, T. & Glazebrook, J. (2003). Characterization of the early response of *Arabidopsis* to *Alternaria brassicicola* infection using expression profiling. *Plant Physiology* 132(2), 606-617.

Vestman, D., Larsson, E., Uddenberg, D., Cairney, J., Clapham, D., Sundberg, E. & von Arnold, S. (2010). Important processes during differentiation and early development of somatic embryos of Norway spruce as revealed by changes in global gene expression. *Tree Genetics & Genomes* 7(2), 347-362.

Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular Plant* 3(1), 2-20.

Vom Endt, D., Kijne, J.W. & Memelink, J. (2002). Transcription factors controlling plant secondary metabolism: what regulates the regulators? *Phytochemistry* 61(2), 107-114.

von Weissenberg, K. (1975). Variation in relative resistance to spread of *Fomes annosus* in four clones of *Picea abies*. *European Journal of Forest Pathology* 5(2), 112-117.

Wang, D., Pajerowska-Mukhtar, K., Culler, A.H. & Dong, X.N. (2007). Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* 17(20), 1784-1790.

Wang, X., Liu, W., Chen, X., Tang, C., Dong, Y., Ma, J., Huang, X., Wei, G., Han, Q., Huang, L. & Kang, Z. (2010). Differential gene expression in incompatible interaction between wheat and stripe rust fungus revealed by cDNA-AFLP and comparison to compatible interaction. *BMC Plant Biology* 10.

Wilcox, P.L., Amerson, H.V., Kuhlman, E.G., Liu, B.H., Omalley, D.M. & Sederoff, R.R. (1996). Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. *Proceedings of the National Academy of Sciences of the United States of America* 93(9), 3859-3864.

Woodward, S. & Pearce, R.B. (1988). The role of stilbenes in resistance of Sitka spruce (*Picea sitchensis* (Bong) Carr.) to the entry of fungal pathogens. *Physiological and Molecular Plant Pathology* 33(1), 127-149.

Woodward, S., Stenlid, J., Karjalainen, R. & Huttermann, A. (1998). *Heterobasidion annosum: Biology, Ecology, Impact and Control*. Wallingford: CAB International.

Yu, Q., Li, B., Nelson, C., McKeand, S., Batista, V. & Mullin, T. (2006). Association of the cad-n1; allele with increased stem growth and wood density in full-sib families of loblolly pine. *Tree Genetics & Genomes* 2(2), 98-108.

Zhao, J., Davis, L.C. & Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances* 23(4), 283-333.

Zhao, J. & Sakai, K. (2003). Multiple signalling pathways mediate fungal elicitor-induced B-thujaplicin biosynthesis in *Cupressus lusitanica* cell cultures. *Journal of Experimental Botany* 54(383), 647-656.

Zhao, J., Zheng, S.H., Fujita, K. & Sakai, K. (2004). Jasmonate and ethylene signalling and their interaction are integral parts of the elicitor signalling pathway leading to B-thujaplicin biosynthesis in *Cupressus lusitanica* cell cultures. *Journal of Experimental Botany* 55(399), 1003-1012.

Acknowledgements

With this work I hope that I have uncovered enough land to keep my feet dry and although, there has been purpose and meaning in my path, it is the "pathway" that have been the labors`, worth. The words by Karin Boye have sadly become somewhat of a cliché but they are still very true for me. This book in itself is not nearly as important or dear to me as the chance it has provided to immerse myself in science for this wonderful, but also arduous journey. I would like to take the opportunity to extend my heartfelt thanks to the many people that have made this journey not only possible, but also most enjoyable.

Malin, I cannot express my gratitude strongly enough for all your support during this time. Thanks for your knowledge, your amazing ability find time to help me and your patience with my lack of math skills. I still do not get how I could get the concentration so wrong. I will remember our struggle with all the elusive AFLP markers, our fights with aggressive spruce plants and also our laughter. I consider you my friend and I hope you will find your way to Kalmar soon.

I will also like to thank my main supervisor Jan Stenlid for all the supporting knowledge, for giving me the opportunity to visit other labs and for your patience concerning my repeated absence.

I am also very grateful for the opportunity to be a part of the Research School in Forest genetics and Breeding, which has provided many memorable moments. Thanks Bengt, Jan-Erik and Gunnar for all the well organized events. Thanks Sara, Mats, Henrik, David, Jon, Ellinor, Maria, Johan and Lars for all the laughs. Thanks also to Magnus Lindén at Södra for arranging interesting projects and for giving me the opportunity to see some "Big Machines" in action. David, my 3-years-old son, is very jealous.

Even though our e-mail address only say "@slu.se", we all know that we are proud mykopats. I would like to send a warm hug to all colleagues at

Ulls väg 26 and wish you a happy move to your new address. Special thanks are required for Katta, Maria and Rena. Your kind help, hard work and friendly faces have made the work in the labs so much more agreeable. I would also like to give a special thanks to Erika and Karin for their help with administrative issues. In this day and age it is impossible for anyone to carry out their work without the help of computers and for me that means that I would not have been able to carry out my work without Les and Patrik. Thanks for not making fun of me for my total lack of computer skills. Thanks to Kalle for letting me be part of a "group".

I gratefully acknowledge Eddy, Jonàs, Elisabeth and Åke for critically reading of the manuscripts and the thesis and for providing many useful comments.

Unfortunately, I cannot say that no one has come to harm during this work. There are many spruce plants that have given the ultimate sacrifice to science. For that I am deeply grateful and I would also like to thank the people in Ekebo that have nursed them. Thanks to Bosse for help with statistics.

A warm thanks to my parents for believing in me and for teaching me to question everything and for entertaining my family.

Ingrid, food never tasted as good as when someone else cooked it. Your food tastes extra good. Thanks for helping out when things tended to pinch.

Lena, my dear sambo. Without your hospitality, your nice company and those countless cups of teas in front of the TV, this commuting life would have been miserable. It is time for me to leave the nest but you will always stay in my heart. I hope that you and your family will continue beign a part of my family.

And to my dear and deeply loved husband, Johan. Now we can finally say "Finally". Thanks for all your support, for all your love, for making me laugh when I´m down and thanks for not letting me be my grumpy self. And thanks for being the best dad ever to Clara and David. I love you all and I want to come home now!