Norwegian University
of Life Sciences

Master's Thesis 2021 60 ECTS
Faculty of Environmental Sciences and Natural Resource Management

Priming of inducible defenses in Norway spruce: effects of putative defense priming chemicals on tree resistance against the bluestain fungus *Grosmannia penicillata* and insights into the underlying mechanisms

Ngan Bao Huynh
Master of Science in Ecology

Preface

This thesis concludes my journey as a Master's student at the Norwegian University of Life Sciences (NMBU). It was a short journey but a very eventful and essential part of my life.

I could not have completed the thesis without guidance from my supervisors. I want to thank Melissa Magerøy for being both a supportive supervisor and a caring friend. Thank you for spending time guiding me through the laboratory works, answering all my questions and helping me whenever I was stuck, regardless of your maternal leave. I also appreciate your check-up messages and our conversations on things other than my project. I also want to thank Paal Krokene for giving me detailed feedback on my thesis drafts. Your feedback was essential in correcting and polishing my thesis. Many thanks to my co-supervisor, Line Nybakken, for helping me with the phenolic analysis. Furthermore, I also want to thank Annie Aasen, Hans Ragnar Norli and Inger Heldal for assisting me with the laboratory works.

In addition, I thank my family and friends for all their support during the stressful period. I thank my sister, Ngoc Huynh Bao, for taking care of me. I thank Claire Devos, who gave me a lot of helpful advice from her experience. I am also grateful to have Cassandra Ugland and Krister Hovde as a fantastic group of friends for sharing the good and bad experiences with the thesis. I also thank Ida Marie Munthe Sakseide for being a great colleague and a great friend. And I could not have made it through all the emotional breakdowns without my boyfriend, Marius Agard Stenberg. Thank you for always being there for me.

Finally, I want to thank the Department of Molecular Plant Biology at the Norwegian Institute of Bioeconomy Research (NIBIO) for having me working in the lab and treating me warmly. I am grateful to Carl Gunnar Fossdal for providing me the lab entry permission during the COVID-19 lockdown.

Ås, June 2021

Ngan Bao Huynh

Abstract

Priming of inducible defenses in Norway spruce could be a cost-effective strategy to protect trees from herbivores and pathogens. In this study, my aim was to identify chemical priming agents that can increase the resistance of Norway spruce plants to pathogenic fungi and investigate the mechanisms underlying defense priming. To test different priming agents, I treated 1-year-old spruce plants with methyl jasmonate (MeJA), β-amino butyric acid (BABA), hexanoic acid or thiamine, and assessed plant resistance by inoculating plants with the bark beetle-associated bluestain fungus *Grosmannia penicillata* 4 weeks later. To study defense priming mechanism, I quantified the expression level of selected defense-related genes (*ACS*, *PAL1*, *LOX*, *TPS-Car*, *Chi4* and *NRPE1*) at specific time points post-treatment and post-challenge (fungal inoculation). I also investigated phenolic and terpene responses at the same time points by quantifying traumatic resin duct (TRD) formation in the sapwood and concentrations of phenolics and terpenes in the phloem. Because the plants developed negligible symptoms to inoculation with *G. penicillata* I could not compare the resistance of plants treated with different priming chemicals and thus could not identify novel spruce priming agents. Considering the mechanical wounding inflicted by fungal inoculation as a defense triggering stimulus, I observed several types of defense responses, categorized by the response patterns, with respect to gene expression, TRD formation and chemical analyses. Most assessed genes were differentially expressed in MeJA-treated plants only during the post-treatment period or showed a prolonged induced defense response to MeJA treatment. Although TRD formation showed a primed response to MeJA treatment and wounding, terpenes did not. For the first time, flavan-3-ols were shown to have a primed response to MeJA treatment. Furthermore, I found evidence that MeJA treatment represses DNA methylation from the downregulation of *NRPE1* expression. Overall, my results confirm that MeJA is a potential priming stimulus for Norway spruce. Contrastingly, I could not draw any solid conclusions about BABA in this study. Further studies with successful fungal inoculation are needed to identify new chemical priming agents in Norway spruce. To better understand the mechanisms of priming of inducible defenses in Norway spruce, future studies should also investigate how ontogeny influences Norway spruce defenses.

Table of contents

I – Introduction

Norway spruce (*Picea abies*) trees have high economic and ecological value (Hannrup et al., 2004). Spruce has a long history of cultivation as a forest tree, providing wood for timber constructions and pulp for paper (Caudullo et al., 2016). Spruce-dominated forests also provide many other ecosystem services, such as protection against erosion, essential habitat for biodiversity, and recreation opportunities (Caudullo et al., 2016). Like most other plants, Norway spruce is attacked by herbivores and phytopathogens. A common and destructive herbivore pest is the tree-killing bark beetle *Ips typographus* (Krokene, 2015). In addition to feeding on the inner bark of trees *I. typographus* has mutualistic relationships with many symbionts, including fungi and bacteria. These symbionts help the beetles penetrate the trees' defense barriers and increase the damage caused by each beetle attack (Krokene, 2015). The most studied bark beetle symbionts are necrotrophic fungi called 'bluestain fungi' (Krokene, 2015). One important bluestain fungus associated with *I. typographus* is *Grosmannia penicillata*, which detoxifies the trees' chemical defenses by breaking down stilbenes and flavonoids (Zhao et al., 2019). To protect the valuable spruce forests from bark beetles and pathogens it is important to understand the trees' defense system and develop new methods to strengthen it.

Norway spruce trees have an elaborate defense system to protect themselves from attackers, including mechanical and chemical defenses (Christiansen et al., 1987; Krokene, 2015; Lieutier & Battisti, 2004). Mechanical defenses are structural components that stop an invader by making the tissues tough and thick (Freeman & Beattie, 2008). Certain molecules, such as lignin or suberin polymers, reinforce tissues and make it more difficult for herbivores and pathogens to penetrate, degrade or ingest tissues (Krokene, 2015). Chemical defenses consist primarily of secondary metabolites with toxic or inhibitory characteristics (Freeman & Beattie, 2008; Krokene, 2015). This differs from primary metabolites that are involved in growth or reproduction. One important group of defense chemicals in conifers is terpenes. Even though they are costly to synthesize terpenes are produced in large quantities and are toxic to both bark beetles and their symbionts (Keeling & Bohlmann, 2006; Krokene, 2015). Terpenes make up most of the trees' viscous and sticky resin, which can trap attackers and seal wounds (Krokene, 2015). Phenolics also play essential roles in conifer defense but are less well studied than terpenes (Faccoli & Schlyter, 2007; Lieutier & Battisti, 2004). Phenolics are considered to have antifungal properties and to act as feeding inhibitors for insects (Chong et al., 2009; Faccoli &

Schlyter, 2007). Phenolic compounds are synthesized, modified, and stored in specialized cells called polyphenolic parenchyma (PP) cells, as well as in other cell types (Franceschi et al., 1998; Franceschi et al., 2000).

Plant defense responses can also be classified as constitutive or inducible. Constitutive (or preformed) defenses form the first line of defense and are present even in the absence of an attack. In Norway spruce, constitutive defenses can be both mechanical and chemical, such as preformed resin and PP cells. Inducible defenses, on the other hand, are activated upon attack. The response time from induction to activated defense can vary from minutes to weeks, depending on the type of defense. Inducible defenses include many reactions, such as induced resin production in traumatic resin ducts or activated axial resin ducts, activation of PP cells, and wound repair mechanisms (Krokene, 2015; Magerøy et al., 2020a).

According to the optimal defense theory, all defenses represent a fitness cost for trees. Therefore, trees cannot afford to invest heavily in all types of defense at the same time, but should distribute their resources optimally (Rosenthal & Janzen, 1979). By activating inducible defense directly upon a specific attack, trees can avoid unnecessary investment in constitutive defense when enemies are absent. However, there is still a risk that the tree will suffer damage during the time-delay before induced defenses have become fully functional (Frost et al., 2008). To reduce this vulnerability, trees have developed a system of long-term inducible defenses called acquired resistance.

Acquired resistance (AR) may enhance resistance to future attacks and shorten the time-delay of direct inducible defense (Wilkinson et al., 2019). AR in Norway spruce was first studied by Christiansen et al. (1999), who showed that trees which had been wounded and subjected to sub-lethal fungal inoculations became much more resistant to a subsequent massive fungal inoculation compared to unwounded control trees. Since then, many studies have shown that AR in Norway spruce can be induced by mechanical wounding, sub-lethal fungal inoculations, or chemical treatment with e.g. methyl jasmonate (MeJA) (e.g. Erbilgin et al., 2006; Krokene et al., 2000; Krokene et al., 2008a; Martin et al., 2002; Zeneli et al., 2006). AR can be based on two non-exclusive mechanisms: prolonged upregulation of inducible defenses or priming of inducible defenses (Wilkinson et al., 2019). Prolonged upregulation of inducible defenses might be a costly strategy, as the inducible defenses that are triggered by a stimulus are continuously upregulated to provide AR against future attack (Mauch-Mani et al., 2017; Wilkinson et al., 2019). Priming of inducible defenses, on the other hand, may be a more cost-

effective strategy than prolonged upregulation of inducible defenses (Conrath, 2009; Martinez-Medina et al., 2016). Compared to unprimed, naïve plants primed plants are characterized by a mild defense response to the priming stimulus and a vigorous response to the subsequent challenge (Martinez-Medina et al., 2016; Mauch-Mani et al., 2017).

Plant defenses can be primed by different stimuli, including chemical compounds and stimuli derived from pathogens, beneficial microbes, and arthropods (Mauch-Mani et al., 2017; Wilkinson et al., 2019). MeJA has been used to prime defenses in Norway spruce (Krokene, 2015; Magerøy et al., 2020a; Wilkinson et al., 2019). Although MeJA is an effective and commonly used chemical priming agent it may also have some unwanted side effects. Several studies have demonstrated that MeJA application can reduce height growth in spruce and other conifers (Gould et al., 2009; Heijari et al., 2005; Moreira et al., 2012; Sampedro et al., 2011; Vivas et al., 2012; Zas et al., 2014). Fedderwitz et al. (2020) attempted to utilize this characteristic of MeJA treatment to regulate height growth in nursery-produced Norway spruce trees but found further negative effects of MeJA treatment such as reduced root growth and delayed development of freezing tolerance. Other negative side effects of MeJA include reduced tracheid cell lumen area and reduced net photosynthetic rate (Heijari et al., 2005), reduced sapwood growth (Krokene et al., 2008b), and stomatal closure (Heijari et al., 2005; Scalschi et al., 2013). Because MeJA can have these negative effects, it is interesting to look for alternative, less harmful chemical priming agents for Norway spruce.

Many other chemicals have been found to act as resistance- and priming-inducing stimuli in various crop and model plants. These chemicals include azelaic acid, pipecolic acid, β-amino-butyric acid (BABA), hexanoic acid and thiamine. BABA, hexanoic acid and thiamine seem to be the most potent priming chemicals that have been tested on the model plant *Arabidopsis thaliana* and various crop plants (Conrath et al., 2015; Pastor et al., 2013). BABA is a non-protein amino acid which can act as a resistance inducing stimulus (Jakab et al., 2001). Many defense-related genes in different plants are found to be primed when treated with BABA and this is thought to be the mechanism behind the AR effect of BABA (Conrath, 2009; Pastor et al., 2014). Hexanoic acid can also act as a priming chemical against many types of fungal infection on tomato (*Solanum lycopersicum*), fortune mandarin (*Citrus clementina* x *Citrus reticulata*) and *Arabidopsis thaliana* (Finiti et al., 2014; Kravchuk et al., 2011; Llorens et al., 2015; Scalschi et al., 2013). Thiamine is also reported to be a potent chemical protector for many crop plants, such as rice (*Oryza sativa*) or cucumber (*Cucumis sativus*). Thiamine can

induce resistance against many types of pathogens through priming of defenses when applied exogenously (Ahn et al., 2005; Ahn et al., 2007; Bahuguna et al., 2012; Boubakri et al., 2016; Huang et al., 2016).

The molecular mechanisms underpinning priming have been studied in *Arabidopsis thaliana* and various crop plants (Conrath et al., 2015; Kim & Felton, 2013; Mauch-Mani et al., 2017; Pastor et al., 2013; Wilkinson et al., 2019). Plant responses during the priming establishment phase include a burst of reactive oxygen species (ROS) that activates the mitogen-activated protein kinase signal transduction pathway (Conrath, 2009; Mauch-Mani et al., 2017; Pastor et al., 2013) and accumulation of inactive hormone conjugates in the vacuole (Pastor et al., 2013; Wilkinson et al., 2019). Epigenetic changes have also been shown to be involved in defense priming (Mauch-Mani et al., 2017; Pastor et al., 2013). Epigenetic changes are inheritable non-mutational changes that affect the phenotype or gene expression at cellular or organismal levels (Mauch-Mani et al., 2017; Waddington, 2012; Wilkinson et al., 2019; Wilkinson, 2020). Epigenetic changes that modify the chromatin state can result in changes in gene expression (Wilkinson et al., 2019). When chromatin is tightly packed (heterochromatin) transcription is repressed and when chromatin is loosened (euchromatin) transcription is promoted. Chromatin density is controlled by histone methylation/acetylation and DNA methylation (Conrath et al., 2015; Mauch-Mani et al., 2017; Wilkinson et al., 2019). For example, DNA methylation is associated with heterochromatin and unmethylated DNA is associated with euchromatin (Wilkinson et al., 2019).

Very few studies have investigated defense priming and the underlying mechanisms in conifers (Krokene, 2015; Magerøy et al., 2020b). Earlier studies in Norway spruce focused on the direct responses of some defense-related genes following treatment with resistance-inducing stimuli, such as pathogen infection or MeJA treatment (Arnerup et al., 2011; Hietala et al., 2004; Oliva et al., 2015; Schmidt et al., 2011; Yaqoob et al., 2012). A few studies have suggested that MeJA treatment enhances gene expression and boosts tree defenses to a subsequent challenge (Erbilgin et al., 2006; Magerøy et al., 2020a; Zeneli et al., 2006). However, little is known about changes that take place in gene expression and metabolite production at time points both before and after challenge treatments in Norway spruce. In this study, I investigated some representative genes involved in defense and signaling pathways and which have been shown to be upregulated in response to MeJA treatment in previous studies (Arnerup et al., 2013; Devos, 2020; Magerøy et al., 2020a; Magerøy et al., 2020b; Schmidt et al., 2011; Wilkinson, 2020; Yaqoob et al., 2012; Zulak et al., 2009). In addition, I quantified phenotypic defense

responses such as traumatic resin duct formation in the sapwood and terpene and phenolic concentrations in the bark.

The main objectives of this thesis are (i) to identify chemical priming agents that can increase the resistance of young Norway spruce plants to the bluestain fungus *G. penicillata* and (ii) to investigate the mechanisms underlying defense priming. To test different priming agents, I treated plants with MeJA, BABA, hexanoic acid or thiamine, and assessed plant resistance by inoculating them with *G. penicillata* 4 weeks later. I also quantified how the different priming agents affected plant growth rate. To study the priming mechanism, I quantified the expression level of six defense-related genes at four time points post-chemical treatment and two time points post-challenge. At the same time points, I also investigated phenolic and terpene responses.

II – Materials and methods

1. Overview of plant materials and experimental setup

I planted 145 overwintered 1-year-old Norway spruce plants obtained from Skogplanter Østnorge AS on 28 April 2020. The plants were planted in a commercial soil mixture (Plantejord – TJERBO, Torv & Jordprodukter) in individual 0.8-liter pots (7.5 cm × 7.5 cm × 12 cm; Nelson Garden, Product No. 5726) and kept in a growth room throughout the experiment. The temperature in the growth room was between 20 and 22 °C and the photoperiod was 20 hours of light and 4 hours of darkness, with a light source providing 1400 lumen (Osram L36W/77 FLUORA, Germany).

On 2 June 2020, plants were treated with methyl jasmonate (MeJA, n = 35 trees), β-amino butyric acid (BABA, n = 35 trees), hexanoic acid (n = 20 trees), thiamine (n = 20 trees), or a control solution (n = 35 trees). All solutions contained 100 ml distilled water and 100 µl Tween, with 50 mM of the chemical of choice, except for the control solution which only contained distilled water and Tween. The solutions were painted onto the plant stem using a soft brush (Décopatch No. 5, 5 cm × 9 cm × 1 cm) from the bottom of the apical shoot to the soil line (Figure 1).

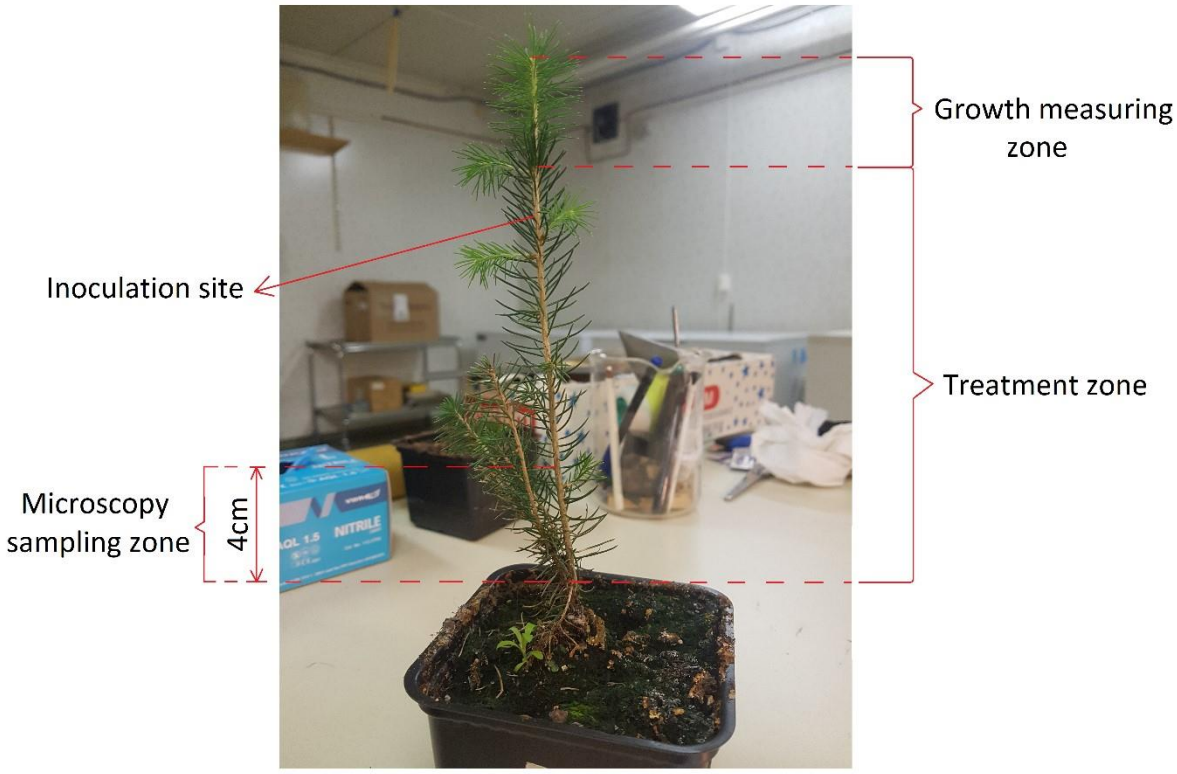


Figure 1. A 1-year-old Norway spruce plant showing the apical shoot (growth measurement zone), the fungal inoculation site, the treatment zone for application of defense priming chemicals, and the microscopy sampling zone.

The experiment consisted of two sub-experiments (Figure 2). In sub-experiment A, I tested plant resistance to a sub-lethal fungal infection. On 1 July 2020, four weeks after chemical treatment, I inoculated 10 trees from each treatment with the fungus *Grosmannia penicillata* and mock-inoculated 10 other trees, as described below. Nine weeks after inoculation (2 September 2020), I measured the length of necrotic lesions in the bark to determine plant resistance to fungal infection.

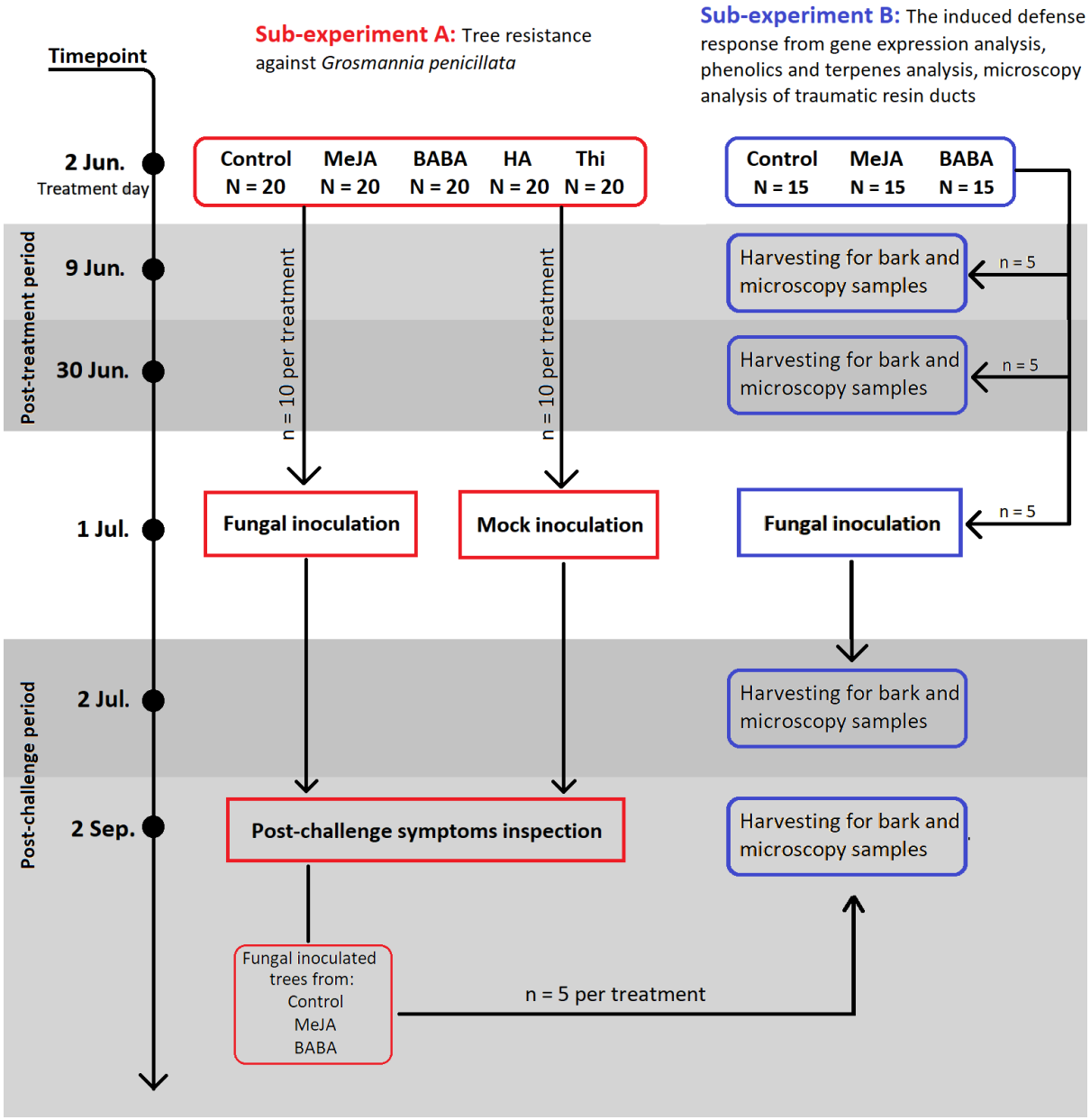


Figure 2. Experimental outline with timeline for the two sub-experiments. The different chemicals used on the treatment day are: water and Tween (Control), methyl jasmonate (MeJA), β-amino butyric acid (BABA), hexanoic acid (HA) and thiamine (Thi).

In sub-experiment B, I quantified induced defense responses in treated plants using gene expression analysis, chemical analysis of phenolics and terpenes in the bark and microscopy

analysis of traumatic resin ducts (TRDs) in the sapwood. These analyses were done at four different time-points: 1 and 4 weeks after chemical treatment (9 June and 30 June 2020, respectively) and 24 hours and 9 weeks after fungal inoculation (2 July 2020 and 2 September 2020, respectively). Due to time and cost restrains only five plants from each of the Control, MeJA and BABA treatments were sampled at each time point in sub-experiment B, giving a total of 60 plants (5 plants × 3 treatments × 4 time points). Fifteen of these 60 plants came from the 9-week time point in sub-experiment A (Figure 2). At 4 weeks post-treatment, some plants were dead (one BABA-treated plant and four MeJA-treated plants) and thus unsuitable for RNA extraction. To increase my sample size, I treated 13 previously unused plants with MeJA (five plants), BABA (four plants) and control solution (four plants) and harvested these plants after 4 weeks. Again, some of the trees died (one control plant and two MeJA-treated plants), giving me a combined total of eight control plants, four MeJA-treated plants and eight BABA-treated plants for the 4-week time point. At 9 weeks after inoculation, four out of 10 fungus-inoculated MeJA-treated plants were dead. Therefore, I sampled all six remaining trees instead of five.

In addition to the two sub-experiments, plant height growth was measured as shown in Figure 1 at three time points: before chemical treatment, 4 weeks after chemical treatment, and 9 weeks after fungal inoculation. Height growth was measured for all trees used in both sub-experiments.

2. *Grosmannia penicillata* inoculation assay

Plants were inoculated with an isolate of *G. penicillata* from the culture collection at the Norwegian Institute of Bioeconomy Research (isolate number NFRI number 1980-91/54, collected 24 July 1980 from a Norway spruce tree in Slørstad, Ås and stored at -152 °C). The fungus was taken out from the freezer on 29 May 2020 and cultured on malt agar in 100 ml Petri dishes. For the mock inoculations (control), only sterile malt agar was used. The malt agar was sliced, put in a 60 ml syringe, and homogenized by squeezing it from one 60 ml syringe to another several times. The homogenized inoculum was then transferred to a 5 ml syringe (without a needle) for easier application to the small inoculation wound on the trees. A bark flap was cut on the upper stem (Figure 1) using a scalpel, a dollop of inoculum was placed directly into the wound (Figure 3A), and the bark flap was closed gently by wrapping parafilm around the stem.

Figure 3. (A) Illustration of the inoculation procedure: a dollop of inoculum was placed under a bark flap cut on the first internode of 1-year-old Norway spruce trees. (B) Inoculation site 9 weeks after inoculation with the bluestain fungus *Grosmannia penicillata*.

Nine weeks after fungal inoculation, I measured the size of necrotic lesions in the bark of all plants. The parafilm was removed and the bark flap was carefully removed to reveal the inoculation site (Figure 3B). The full length of the necrotic lesion (if present) was measured using a digital vernier caliper (Cocraft, Clah Olson, England).

3. Tissue sampling and processing for microscopy, gene expression and metabolite analysis

As described above, five plants from each of the MeJA, BABA and Control treatments were sampled at different time points for gene expression analysis, metabolite analysis and microscopy. Before sampling, I removed all the branches from the plants. For microscopy analysis, I collected the lower 4 cm of the main stem whole without further processing (Figure 1). For gene expression and metabolites analysis, I split the remaining part of the stem into bark and wood. Only bark tissues were used in this study. All samples were wrapped in labeled aluminum foil and flash frozen in liquid nitrogen before stored in a -80 °C freezer. Frozen bark samples were ground by hand under liquid nitrogen using a mortar and pestle and transferred to a 2 ml Eppendorf tube for RNA extraction and metabolite analyses. Frozen wood samples were not used in this study.

4. Molecular biological analysis

a. RNA extraction

Total RNA was extracted from bark samples using the MasterPure^{TM} Complete DNA & RNA Purification Kit (Cat. No. 21855, Lucigen, USA). For each sample the Master Mix consisted of 600 µl Tissue and Cell Lysis Solution, 6 mg Polyvinylpyrrolidone (Sigma-Aldrich, P-5288),

3 µl β-mercaptoethanol (Sigma-Aldrich, M3148), and 1 µl Proteinase K. I placed 10 to 15 mg bark powder in a 2 mL Eppendorf tube and added 610 µl Master Mix. Samples were incubated in a Thermo-Shaker PHMT-PSC24N (Grant-bio, UK) at 56 °C and 1400 rpm for 15 minutes. The tubes were then centrifuged in an Eppendorf 5424R Centrifuge (Hamburg, Germany) at 4 °C at max speed (15000 rpm) for 10 minutes to separate the supernatant from the sample residues. The supernatant was transferred to a new tube and chilled on ice for 3 to 5 minutes. Then 250 µl of MPC Protein Precipitation Reagent was added to each tube and the content was vortexed vigorously for 10 seconds. The tubes were centrifuged again (4 °C, 15000 rpm) for 10 minutes to separate the supernatant. The supernatant was transferred to a new tube and 400 µl of LiCl 7.5M precipitation solution was added to each tube and thoroughly mixed by inverting the tubes 30 to 40 times. The tubes were incubated in a freezer at -20 °C for 40 to 50 minutes. For the 4- and 9-week post-inoculation time points, the incubation time was increased from 15 to 75 minutes due to low RNA yield using the 15 minutes incubation time. After incubating in the freezer, the tubes were centrifuged for 30 minutes (4 °C, 15000 rpm). A pellet with nucleic acid formed at the bottom of each tube. The pellet was rinsed twice by adding 800 µl of 75% ethanol and centrifuging at 4 °C and 15000 rpm for 10 minutes for each rinse. The pellets were air-dried for around 5 minutes. Alternatively, the tubes were spun in the bench centrifuge for a few seconds to remove the remaining droplets of ethanol using a pipette and speed up the air drying. The pellets were then re-suspended in 30 µl nuclease-free water at 56 °C. The tubes were chilled on ice for 10 minutes before being stored in a -80 °C freezer.

b. cDNA synthesis

Following RNA extraction, I synthesized cDNA for Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) using the Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNAase (Thermo Fisher Scientific, USA) according to the product protocol. Before cDNA synthesis, the concentration of RNA in each sample was determined using the NanoDrop 2000 spectrophotometer (NanoDrop Technologies, USA). Template RNA was diluted to a concentration of 25 ng μl^{-1} for a total of 200 ng per reaction. The finished cDNA samples were then stored at -80 °C until they were ready for qPCR analysis.

c. RT-qPCR

I quantified expression levels for three housekeeping genes (*actin*, *α-tubulin*, *ubiquitin*) and six defense-related target genes (*ACS*, *TPS-Car*, *LOX*, *PAL1*, *Chi4*, *NRPE1*). Transcripts were quantified by RT-qPCR using the ViiA7 Real-Time PCR System (Applied Biosystems, ThermoFisher Scientific, USA) with the associated QuantStudio Real-Time PCR Software

(v1.3, Applied Biosystems). A 10 µl reaction volume was used, with 5 µl Fast SYBR Green Master Mix (Applied Biosystems, Lithuania), 3 µl nuclease-free water, 1 µl of the target gene's primer (4 µM) and 1 µl cDNA. A non-template control was run for each primer in every RT-qPCR run. The primer sequences are listed in Table 1. The thermocycle conditions were: 1 cycle of 95 °C for 20 seconds; 40 cycles of 95 °C for one second, followed by 20 seconds at 60 °C; 1 cycle of 95 °C for 15 seconds, followed by 60 seconds at 60 °C, and finally 15 seconds at 95 °C.

Table 1. Information on primers used in Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR). *Ubiquitin*, *α-tubulin* and *actin* were used as housekeeping genes.

Gene[a]	Primers (F: Forward; R: Reverse)	References
Ubiquitin	F: 5'-GTTGATTTTTGCTGGCAAGC-3' R: 5'-CACCTCTCAGACGAAGTAC-3'.	Schmidt and Gershenzon (2007)
α-Tubulin	F: 5' -GGCATACCGGCAGCTCTTC- 3' R: 5' -AAGTTGTTGGCGGCGTCTT- 3'	Hietala et al. (2004)
Actin	F: 5'-GGCATACCGGCAGCTCTTC-3' R: 5'-AAGTTGTTGGCGGCGTCTT-3'	Hietala et al. (2003)
TPS-Car	F: 5'-GGTGGTACCAGGCAGACAGG-3' R: 5'-CAGTGTAGCCATCTCGATAATTGT-3'	Zulak et al. (2009)
PAL1	F: 5'-GGCAGATCATTTGGGTGATC-3' R: 5'-TAAAGTTCCATTTTCAACTATAGGACTAAT-3'	Koutaniemi et al. (2007)
Chi4	F: 5'-GCGAGGGCAAGGGATTCTAC-3' R: 5'-GGTGGTGCCAAATCCAGAAA-3'	Hietala et al. (2003)
NRPE1	F: 5'-GGTCTGGCAAAGCTAAATTCATGT-3' R: 5'-CAGGTATCTTTCTCCCAGCCCTTA-3'	Wilkinson (2020)
ACS	F: 5'-CAAGCAGAATCCCTATGATGCCGAAA-3' F: 5'-TCTGGATGAGACTTGAGCCAACCTTC-3'	Yaqoob et al. (2012)
LOX	F: 5'-ACCCTTGGTATAGCCCTCATA-3' R: 5'-ATCGTCACTCCATTCTCTCGT-3'	Arnerup et al. (2011)

[a] Target genes: *ACS: aminocyclopropane-1-carboxylic acid synthase; LOX: 13-lipoxygenase, PAL1: phenylalanine ammonia-lyase 1, TPS-Car: 3-carene synthase, Chi4: chitinase 4, NRPE1: Nuclear RNA Polymerase E1*

Relative gene expression was calculated based on the cycle threshold (Ct) values. ΔΔCt values were calculated as follows:

Step 1: Since three housekeeping genes were used, I calculated the geometric mean of all three:

$$\text{Geometric\_mean\_housekeeping} = \sqrt[3]{Ct(Actin) * Ct(\alpha Tubulin) * Ct(Ubiquitin)}$$

Step 2: Calculating ΔCt:

$$\Delta Ct = Ct(treatment) - Geometric\_mean\_housekeeping$$

Step 3: Calculating ΔΔCt:

$$\Delta\Delta Ct = -(\Delta Ct(treatment) - average\ \Delta Ct(control))$$

Positive ΔΔCt values represent an up-regulation of a target gene, and a negative ΔΔCt represents a down-regulation of a target gene.

5. Phenolic analysis

a. Phenolic extraction

Ground bark samples were freeze-dried for two days using a LyoQuest -55 freeze-drying machine (Azbil Telstar Technologies S.L.U., Spain) and stored in a -20 °C freezer. Before extraction, the samples were thawed for 24 hours at room temperature and then weighed on a Mettler Toledo XP6 weight. Each approximately 10 mg bark sample was transferred to a Precellys vial. If the samples were not as finely ground as desired, two stainless steel balls were added to the vial for better homogenization later in the extraction process. Some samples weighed less than 10 mg due to tissue loss during the grinding process or because the plants were too small.

For each sample, 400 µl methanol (MeOH) was added and the samples were homogenized for 30 seconds using a Precellys 24 Lysis & Homogenization machine (BERTIN Technologies, France). The vials were then placed in an ice bath for 15 minutes before being centrifuged for 3 minutes at full speed (16,400 rpm) in an Eppendorf centrifuge 5417C (Hamburg, Germany) to separate the solid parts from the supernatant. The supernatant was then transferred to a 5 ml tube (75 mm × 12 mm) using a 150 mm disposable glass pipette. Then 400 µl MeOH was added to the residues in each Precellys vial and the content was homogenized for 30 seconds and then centrifuged for 3 minutes at full speed (16,400 rpm). The supernatant was again transferred to the 5 ml tube. This process was repeated three times, or until the residues were colorless. All the MeOH in the tubes was evaporated using an Eppendorf Concentrator Plus vacuum centrifuge (Hamburg, Germany). Afterward, all tubes were stored at -20 °C until they were ready for High Performance Liquid Chromatography (HPLC) analysis.

b. HPLC analysis

The sample tubes were thawed for 20 minutes and 200 µl MeOH and 200 µl pure water from a Purelab CHORUS 1 (ELGA Labwater, Veolia Water Technologies, USA) was added. Samples were dissolved by putting them in an Ultrasonic Cleaners water bath (USC200TH, VWR, Malaysia). The samples were then transferred to Eppendorf tubes and centrifuged for 3 minutes at maximum speed (16,400 rpm) to remove residues before transferring them to HPLC vials. Low-molecular-weight phenolic compounds were analyzed using the Agilent Technologies HPLC system (Agilent Technologies 1100 series, Waldbronn, Germany) with the associated Agilent ChemStation software for LC 3D systems. The Agilent system consists of a G1312A binary pump with a G1322A degasser, a G1330A thermostat module, a G1329A autosampler, a G1315 diode array detector, and a G1316A column compartment. A 50 mm × 4.6 mm HPLC column (ODS HYPERSIL 3 µm, Reversed-phased C18, Octadesyl-silica, Thermo Fisher Scientific, USA) was used. For the mobile phase, samples were eluted in solution A [30 ml tetrahydrofuran, 5 ml orthophosphoric acid (85%) and pure water for a total volume of 2000 ml] and solution B [HPLC gradient grade MeOH (VWR Chemical, France)], with a flow rate of 2 ml min^{-1}, using the gradients given in Table 2. The injection volume was 20 µl and the temperature was 30 °C during the whole analysis. The absorption spectra at 270 and 320 nm, along with respective retention times, were used to identify low-molecular weight phenolic compounds and to calculate concentrations by using an in-house library created by running different commercial standards on the HPLC system.

Table 2. The High Performance Liquid Chromatography (HPLC) gradient used to quantify low-molecular weight phenolics.

Time (min)	Solution A (%)	Solution B (%)
Initial	100	0
5	100	0
10	85	15
20	70	30
40	50	50
45	50	50
Rinsing	0	100
Equilibration	100	0

c. Tannin analysis

MeOH-soluble tannins were analyzed from the remaining samples after HPLC analysis. MeOH-insoluble tannins were analyzed from the solid residues at the bottom of the Precellys

vials after the extraction of soluble phenolics. Each sample was divided and transferred to two glass tubes. For MeOH-insoluble tannins, each tube contained a minimum of 1 mg and a maximum of 3 mg of solid residues. For MeOH-soluble tannins, each tube contained 100 µl sample (or 50 µl sample if there was not enough remaining liquid in the HPLC vial). I then added 400 µl MeOH (or 450 µl MeOH for the tubes containing only 50 µl sample of MeOH-soluble tannins), 100 µl ferric reagent and 3 ml butanolic acid to each tube. The tubes were closed with a cap, boiled in a water bath (VWB2 26) at 99 °C for 50 minutes, and then cooled to room temperature. The liquid in each tube was transferred to a 4.5 ml VWR Cuvettes PS Macro. Absorbance at 550 nm was determined using a UV spectrophotometer (UV-1800, Shimadzu Corporation, Japan) equipped with UVProbe 2.62 software. The concentration of the MeOH-insoluble tannin in the samples by the formula:

$$\text{Concentration} = \frac{(absorbance) - 0.02112}{0.008 * (sample\ weight)}\ \text{mg g}^{-1}\ \text{of dry weight}$$

6. Terpene analysis

a. Terpene extraction

Approximately 10 mg of ground bark from each plant was placed in a 2 ml vial (12 mm x 32 mm, Ø 9 mm) and submerged in 1 ml hexane containing 10 µg ml^{-1} pentadecane as an internal standard. The vials were closed tightly and shaken on an IKA VIBRAX VXR orbital shaker with a VX7 platform for 24 hours. The supernatant from each vial was then transferred to a new vial and placed in a -20 °C freezer until the day of the analysis. The remaining bark in the vials was dried in a fume hood for 3 days and weighed to determine tissue dry weight.

b. Terpene analysis by GC-MS

Terpene extracts were analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS) system with a Varian 3400 gas chromatograph (Hewlett Packard (HP) equipped with a DB-wax capillary column (30 m × 0.25 mm × 0.25 mm, J&W Scientific, CA, USA), and connected to a Finnigan SSQ 7000 mass spectrometer.

Due to the large number of terpenes present in Norway spruce I only quantified selected terpenes with known biological functions. The quantified terpenes were well represented in terms of both quantity and the proportion of plants in which they were found. Terpenes were identified and quantified by searching for components in the National Institute of Standards and Technology (NIST) database using the AMDIS software.

To calculate the concentration of each terpene (TP) per sample, the area of each peak in the chromatogram at a specific retention time was divided by the area of the peak for the internal standard pentadecane (PD) and divided by the sample dry weight (DW):

$$\text{Concentration} = \frac{TP/PD}{DW} \text{ (µg g}^{-1} \text{ of dry weight)}$$

7. Quantification of traumatic resin ducts by microscopy

Formation of traumatic resin ducts (TRDs) in the xylem was evaluated using microscopy. Thin and level cross-sections were made using a platinum-coated razor blade (Feather, Japan). The cross-sections were then examined in a microscope (Leica Microsystems, Wetzlar GmbH, Germany), equipped with Leica Application Suite software (version 4.13.0). Cross-sections were examined under three magnifications: 2.5× was used to measure total xylem area per section and to note the presence or absence of TRDs; 5× and 10× were used for closer inspection of TRDs and measurement of TRD lumen area (Figure 4). The coverage of TRDs in each cross-section was calculated by:

$$\text{TRD coverage} = \frac{Sum\ of\ TRD\ area\ at\ 5\times\ or\ 10\times\ magnification}{Xylem\ area\ at\ 5\times\ or\ 10\times\ magnification} \text{ (µm}^2 \text{ per µm}^2 \text{ xylem area)}$$

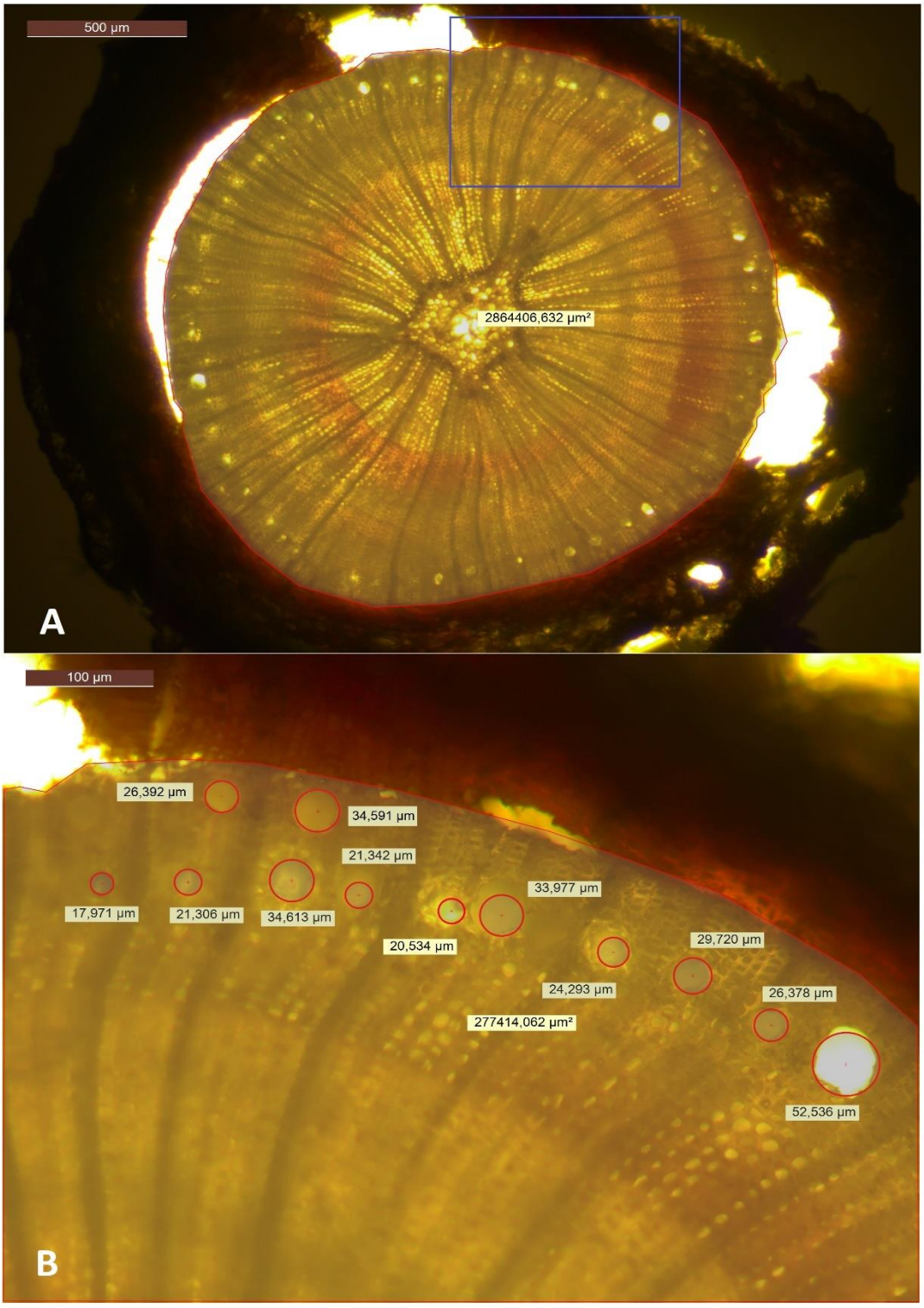


Figure 4. (A) Cross-section of the stem of a methyl jasmonate-treated Norway spruce plant 9 weeks after inoculation with the fungus *Grosmannia penicillata* (2.5× magnification). The large red circle delineates the perimeter of the xylem and was made to measure total xylem area. (B) Close-up of the cross-section shown in panel A (blue rectangle) (10× magnification). The duct lumen of individual traumatic resin ducts is outlined by red circles. Numbers show the diameter measurement made by the microscopy software.

8. Statistical analysis:

Statistical analysis was done in R (version 4.0.3) using RStudio version 1.3.1093. For all statistical models used I first checked that the assumptions of normality, constant variance, and independency were not violated by making diagnostic plots using the command "plot([model name])". Descriptive barplots were made using the packages "ggplot2" and "gridExtra". Analysis of variance (ANOVA) was performed using the packages "car" and "mixlm". Tests of interaction effects between treatments and time points for relative gene expression (ΔΔCt), phenolic and terpene concentrations were done using 2-way ANOVA. To compare the main effect of the treatment on gene expression and the concentration of each component compound, the dataset was divided into four smaller subsets, one for each time point. A 1-way ANOVA model was fitted for each time point, followed by a Tukey's pairwise post-hoc test.

Plant growth and TRD quantification were tested with 1-way ANOVAs, followed by a Tukey's pairwise post-hoc test to compare the main effect of the treatment on the response variable.

III – Results

1. Growth analysis

I first assessed if the priming agents affected the height growth of the trees by measuring the length of the apical leader at three time points. Since tree height varied at the start of the experiment and since trees were measured at a different time points post-treatment, I calculated the growth rate of the trees, i.e. the length increase of the apical leader per week:

$$\text{Growth rate} = \frac{Last\ measurement - First\ measurement}{No.of\ weeks\ between\ two\ measurements}\ (\text{cm week}^{-1})$$

I found no significant effect of the five different treatments on the growth rate of the trees (Figure 5)

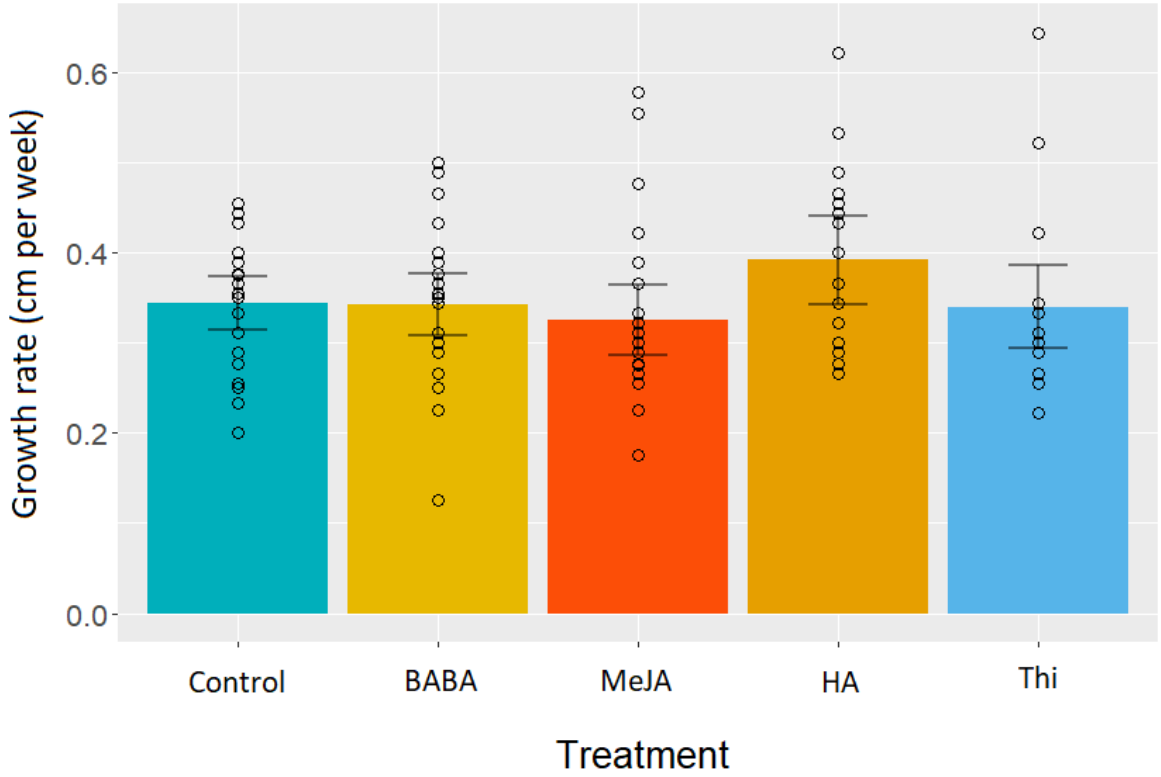


Figure 5. Growth rate of the apical leader of Norway spruce trees after treatment with water and Tween (control), methyl jasmonate (MeJA), β-amino butyric acid (BABA), hexanoic acid (HA), or thiamine (Thi). There were no significant differences between treatments (1-way ANOVA, $p > 0.05$).

2. *Grosmannia penicillata* pathogenicity assay

In sub-experiment A, I observed no visible necrotic lesion extending beyond the inoculation wound in any trees for any of the five treatments 9 weeks after inoculation. This indicated that any variation in wound size depended on the size of the bark flap that was cut during inoculation. The wounding of the plants may have served as a triggering stimulus for primed defense responses. Hence, in the following I refer to the post-inoculation time points in sub-experiment B as 'post-wounding'.

3. Gene expression analysis

In sub-experiment B, I examined transcript levels of several defense-related genes across treatments. These genes included the ethylene biosynthesis gene *1-aminocyclopropane-1-carboxylic acid synthase* (*ACS*), the jasmonic acid biosynthesis gene *lipoxygenase* (*LOX*), the phenolic biosynthesis gene *phenylalanine ammonia-lyase 1* (*PAL1*), the monoterpene synthase gene *terpene synthase 3-carene* (*TPS-Car*), the RNA-directed DNA methylation related gene *DNA-directed RNA polymerase V subunit 1* (*NRPE1*), and the pathogenesis-related gene *chitinase 4* (*Chi4*). There was a significant interaction effect between time points and treatments on the relative expression level of *ACS*, *Chi4*, *NRPE1* and *TPS-Car* (2-way ANOVA, Table 3). Thus, changes in expression levels of these transcripts were affected simultaneously by treatment and time. Expression of *PAL1* was significantly affected by time point only (Table 3). The mean expression level in control trees remained at a basal level for all transcripts (Figure 5), whereas gene expression levels in trees treated with priming chemicals varied between time points. Most genes returned to near basal expression levels by 9 weeks post-wounding.

Table 3. P-values from 2-way ANOVAs of relative gene expression levels in Norway spruce bark at different time points following treatment with different defense priming chemicals. See Table 1 for full names of the target genes. Values in bold indicate significant p-values (< 0.05)

Target genes	Treatment	Time point	Treatment × Time point
ACS	**0.036**	**0.045**	**0.002**
LOX	0.389	0.438	0.416
NRPE1	**< 0.001**	**0.033**	**0.002**
PaChi4	**< 0.001**	0.219	**< 0.001**
PAL1	0.323	**0.011**	0.181
TPS-Car	**< 0.001**	**< 0.001**	**0.037**

The effect of chemical treatments on the relative expression levels of each gene was investigated for each time point separately (Figure 5). There was no significant treatment effect on expression levels of *PAL1* and *LOX* at any time point. *ACS* and *Chi4* were significantly upregulated 1 week and 4 weeks after MeJA treatment, but returned to basal expression levels post-wounding. *TPS-Car* was also significantly upregulated in MeJA-treated bark, and remained upregulated post-wounding. *NRPE1*, on the other hand, was significantly down-regulated in MeJA-treated trees 1 and 4 weeks after treatment and 24 hours post-wounding. For BABA-treated trees, *Chi4* and *TPS-Car* were significantly downregulated 1 week after treatment and 24 hours post-wounding, respectively.

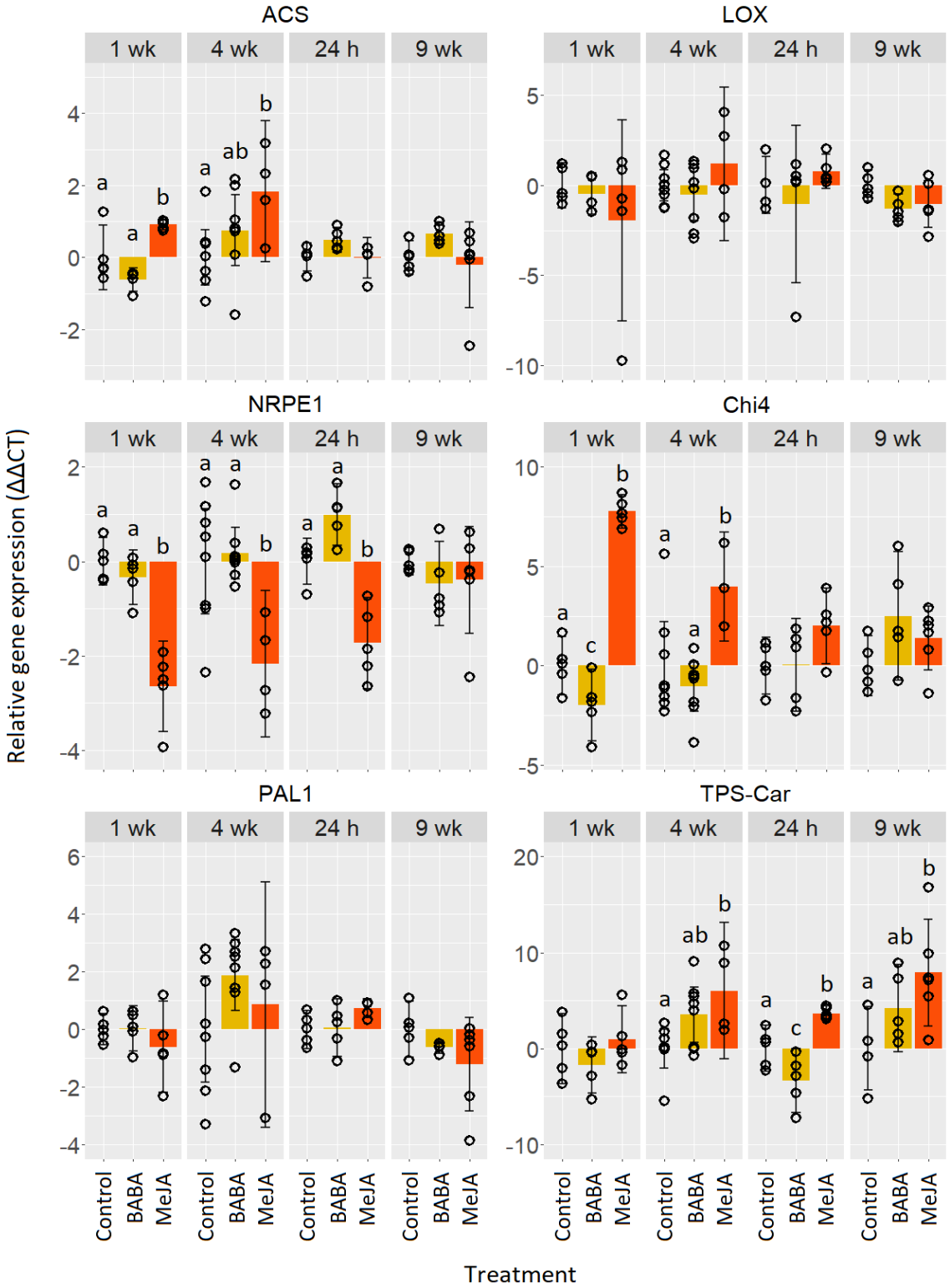


Figure 6. Mean relative gene expression of *ACS*, *LOX*, *NRPE1*, *Chi4*, *PAL1* and *TPS-Car* (see Table 1 for full gene names) in Norway spruce bark following different chemical treatments: water and Tween (control), methyl jasmonate (MeJA), and β-amino butyric acid (BABA). Gene expression was assessed 1 and 4 weeks after chemical treatment (1 wk, 4 wk) and 24 hours and 9 weeks after wounding and inoculation with a fungal pathogen (24 h, 9 wk). Error bars represent 95% confidence interval and circles represent individual data points. For each transcript and time point, treatments with different letters are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise treatment comparisons, $p < 0.05$).

4. Traumatic resin duct formation

Microscopy analysis of traumatic resin duct formation in the xylem was done to quantify anatomical defense responses. The analysis was only done on samples collected 9 weeks post-wounding, since exploratory microscopy of earlier time points did not reveal any traumatic resin ducts in any trees. MeJA-treated trees had significantly more traumatic resin ducts than control trees and trees treated with BABA (Figure 7).

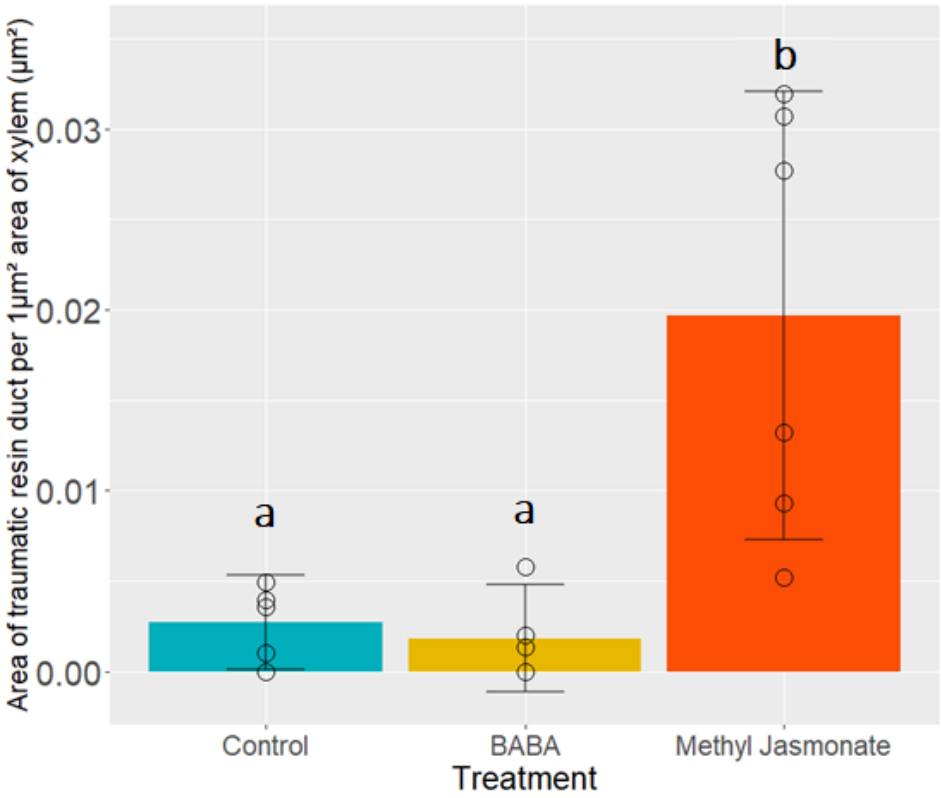


Figure 7. Mean area of traumatic resin ducts per xylem cross-sectional area in Norway spruce 13 weeks after treatment with water and Tween (control), methyl jasmonate, or β-amino butyric acid (BABA). Error bars represent 95% confidence interval and circles represent individual data points. Treatments with different letters are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments, $p < 0.05$).

5. Terpene analysis

The trees' induced terpene response was investigated by quantifying the levels of different terpenes in the bark. A total of 12 monoterpenes, three sesquiterpenes and two diterpenes were quantified by GC-MS (Table 4). Only seven terpenes– (all monoterpenes) showed a significant interaction between treatments and time points. The compounds showing no significant interaction effect were either significantly affected by the main effect of time (four

monoterpenes, one sesquiterpene and two diterpenes) or were not affected by either treatments or time points. The total concentration of all mono-, sesqui-, and diterpenes also differed significantly only between time points.

Table 4. F-values from 2-way ANOVAs of concentrations of terpene compounds in Norway spruce bark at different time points following treatment with different defense priming chemicals. Asterisks (*) denotes the level of significance (*p < 0.05, **p < 0.01, ***p < 0.001).

Type	Compound	Treatment ($F_{2,60}$)	Time point ($F_{3,60}$)	Treatment x Time point ($F_{6,60}$)
Mono-	3-Carene	0.56	2.75	2.51 *
	α-Pinene	0.38	5.14 **	0.70
	β-Pinene	0.09	7.89 ***	1.22
	β-Myrcene	2.04	19.54 ***	1.86
	β-Phellandrene	2.41	20.27 ***	1.43
	Camphene	3.66 *	0.59	2.74 *
	Eucalyptol	3.09	0.8	0.73
	γ-Terpinene	2.30	5.6 **	3.11 *
	Limonene	0.19	4.82 **	2.55 *
	p-Cymene	6.91 **	2.69	2.98 *
	Sabinen	0.79	5.37 **	3.05 *
	Terpinolen	1.91	5.37 **	3.72 **
	Monoterpenes	0.17	10.87 ***	2.02
Sesqui-	α-Gurjunene	0.97	3.01 *	0.74
	α-Longipinene	0.55	1.48	0.52
	Germacrene D	0.55	7.22 ***	0.18
	Sesquiterpenes	0.52	8.2 ***	0.23
Di-	Thunbergene	0.31	5.79 **	0.93
	Verticiol	1.19	5.68 **	0.93
	Diterpenes	0.62	5.95 **	0.95
	Total	0.35	12.41 ***	1.91

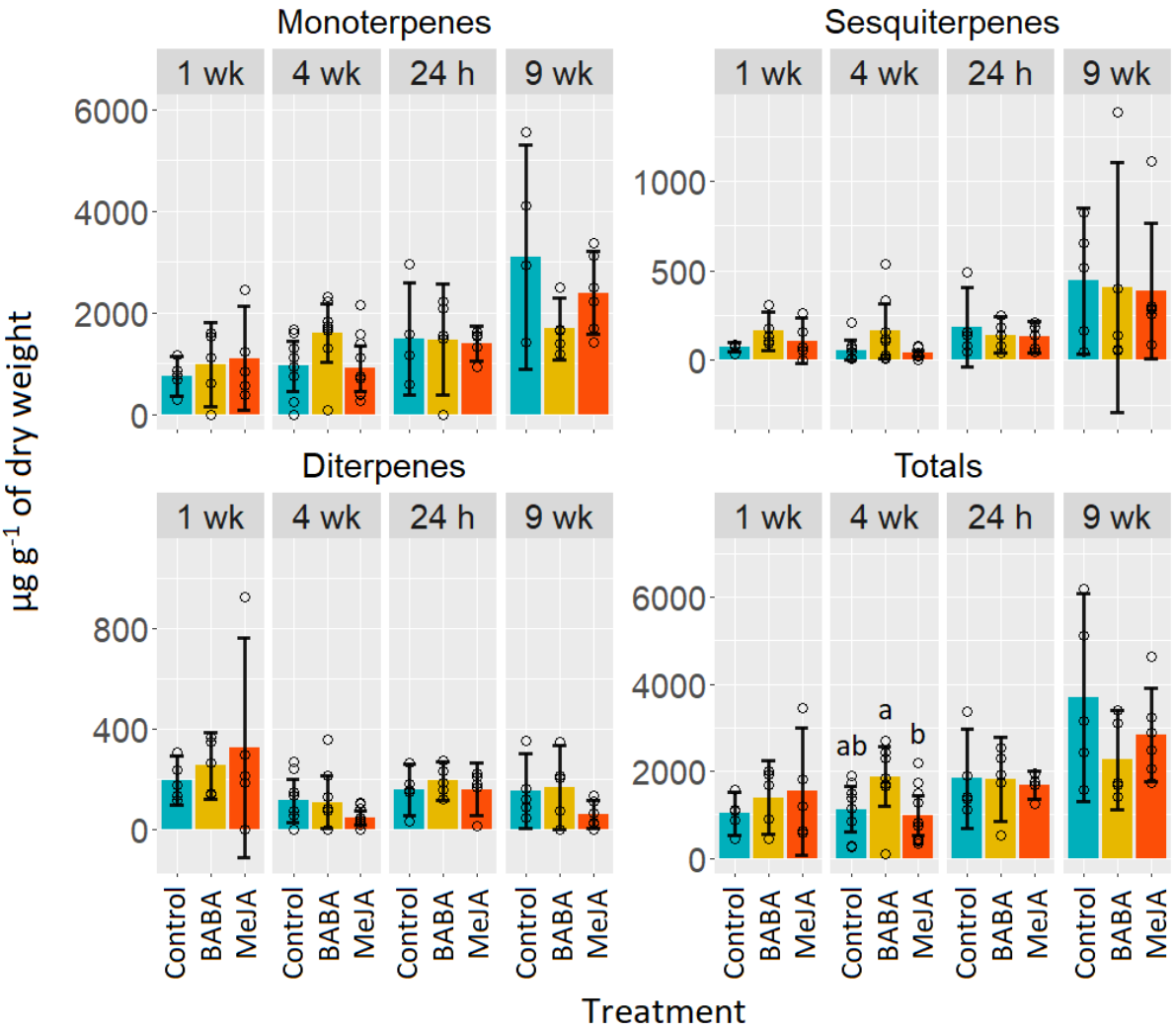


Figure 8. Mean concentrations (µg g^{-1} dry weight) of monoterpenes, sesquiterpenes, diterpenes and total terpenes in response to different treatments: water and Tween (Control), methyl jasmonate (MeJA), and β-amino butyric acid (BABA). Terpene concentrations were measured 1 and 4 weeks after chemical treatment (1 wk, 4 wk) and 24 hours and 9 weeks after wounding and inoculating with a fungal pathogen (24 h, 9 wk). Error bars show 95% confidence interval and circles represent individual data points. For each compound and time point, treatments with different letters are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise treatment comparisons, $p < 0.05$).

After looking at how treatment and time impacted terpene concentrations together, I investigated the main effect of treatment for each time point individually using 1-way ANOVA followed by a Tukey post hoc test for pairwise comparisons (Figure 8; Appendix 2a and 2b). Total terpene concentrations in MeJA-treated trees did not differ from control trees at any time point. While MeJA-treated trees had significantly more traumatic resin ducts than control trees, the concentration of terpenes in the bark did not differ between treatments. The total concentration of terpenes in MeJA-treated trees was significantly lower than in BABA-treated trees 4 weeks after treatment. However, no traumatic resin ducts were found in any treatment

groups before wounding and traumatic resin duct area in BABA-treated trees was not higher than in MeJA-treated tree post-wounding. The individual monoterpenes camphene, β-myrcene and β-phellandrene also had significantly lower concentrations in MeJA-treated trees than in BABA-treated trees after treatment – camphene at 1 week after treatment and β-Myrcene and β-Phellandrene at 4 weeks after treatment. *p*-cymene, on the other hand, was the only compound showing significantly higher concentration in MeJA-treated plants than in both control and BABA-treated plants 4 weeks after treatment.

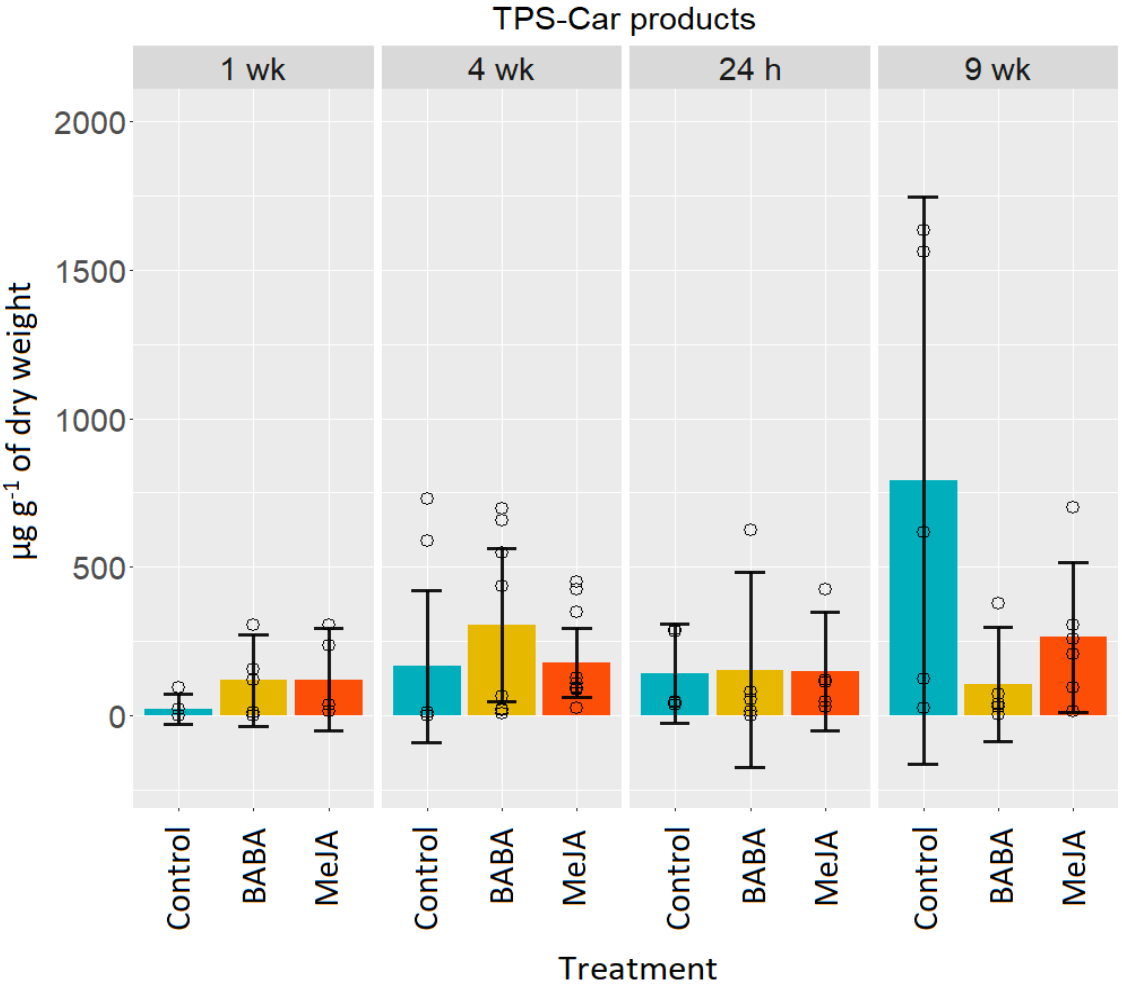


Figure 9. Mean concentrations (µg g^{-1} dry weight) of major products of the monoterpene synthases TPS-Car in response to different treatments: water and Tween (Control), methyl Jasmonate (MeJA), β-amino butyric acid (BABA). The concentration was assessed 1 and 4 weeks after chemical treatment (1 wk, 4 wk) and 24 hours and 9 weeks after wounding and inoculating with a fungal pathogen (24 h, 9 wk). Error bars represent 95% confidence interval and circles represent individual data points. 1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments showed no significant result ($p > 0.05$).

There was no correspondence between the expression level of *TPS-Car* and its products' concentration – 3-Carene and terpinolene (Fäldt et al., 2003; Roach et al., 2014). While there was significant upregulation of the TPS-Car's expression level in the MeJA-treated trees 4 weeks after treatment and post-wounding, there was no significant difference in its products concentration between treatments at any time points (Figure 9).

6. Phenolic analysis

To assess further the induced chemical defensive responses, I investigated the differences in the presence and quantity of phenolic compounds between treatments. Phenolic compounds, including flavonoids, stilbenes, and condensed tannins (Table 5, Appendix 1a and Appendix 1b) were quantified in Norway spruce bark by HPLC.

Table 5. F-values from 2-way ANOVAs of concentrations of phenolic compounds in Norway spruce bark at different time points following treatment with different defense priming chemicals. Asterisks (*) denotes the level of significance (*p < 0.05, **p < 0.01, ***p < 0.001).

Compound	Treatment ($F_{2,60}$)	Time point ($F_{3,60}$)	Treatment x Time point ($F_{6,60}$)
Picein	4.2406 *	1.3181	1.5061
Gallocatechin	10.391 ***	27.354 ***	2.886 *
(+) Catechin	21.996 ***	21.388 ***	5.066 ***
Dihydromyricetin 1	2.619	6.583 ***	2.389 *
Dihydromyricetin 2	1.262	0.242	0.532
Dihydromyricetin 3	3.328 *	1.392	0.644
Dihydromyricetin 4	0.393	1.991	0.832
Quercetin glycoside	0.135	1.006	1.404
Monocoumaryl astragallin 1	0.253	6.032 **	1.105
Monocoumaryl astragallin 2	3.020	1.525	0.644
Dicoumaryl astragallin	1.296	11.622 ***	1.189
Sum of flavonoids	6.111 ***	5.624 **	1.873
Piceatannol glycoside	5.274 **	23.023 ***	3.251 **
Resveratrol glycoside	2.077	13.462 ***	2.094
Iso-rhapontin glycoside	2.268	13.469 ***	1.868
E-astringin	0.665	3.442 *	2.828 *
Piceatannol aglycon	0.793	4.250 **	1.857
Piceatannol	1.248	27.606 ***	1.211
Unknown stilbene 1	1.170	3.890 *	1.03
Unknown stilbene 2	2.461	9.712 ***	0.794
Sum of stilbenes	3.284 *	17.779 ***	2.396 *
Total of low-molecular-weight phenolic compounds	1.703	12.098 ***	2.600 *
MeOH-soluble tannins	2.011	46.917 ***	3.755 *
MeOH-insoluble tannins	6.804 **	2.615	4.922 ***

The total amount of flavonoids was impacted by only the main effects of treatment and time points but not their interaction effect. On the other hand, the total amount of stilbenes and total amount of low-molecular-weight phenolics were impacted by treatment and time point interaction. However, when looking at the compounds individually, only seven phenolic compounds' concentrations – 3 flavonoids, 2 stilbenes and 2 tannins – were significantly affected by the interaction between the treatments and time point (Table 5). All remaining component stilbenes without interaction effects were significantly impacted by the main effect of different time points. This result indicated that most of the variations in individual stilbenes were due to the different stages of the experiment, not by the treatments. This result also corresponded to the gene expression level of *PAL1* being affected only by time point. For flavonoids without interaction effects, only dihydromyricetin 3's concentration was different due to the main effects of treatment, while monocoumaryl astragallin 1 and dicoumaryl astragalin concentrations varied over time. The acetophenone picein was affected by only the main effect of different treatments.

The main effect of the treatments on the concentration of each compound was investigated at different time points individually by 1-way ANOVA followed by a Tukey post hoc test for pairwise comparison (Appendix 1a and 1b; Figure 10). Total flavonoid concentration was only significantly higher in MeJA-treated than control trees post-wounding. This was mainly due to the contribution of catechin. Catechin was significantly higher in MeJA-treated plants than control plants 1 week after treatment, but returned to basal level 4 weeks after treatment. 24 hours and 9 weeks post-wounding, catechin level in MeJA-treated plants was higher than both BABA-treated and control plants. Aside from catechin, gallocatechin concentrations were significantly different between treatments with higher concentrations in BABA-treated than MeJA-treated trees. After wounding, its concentration no longer differed between treatments, and almost disappeared at 9 weeks after wounding.

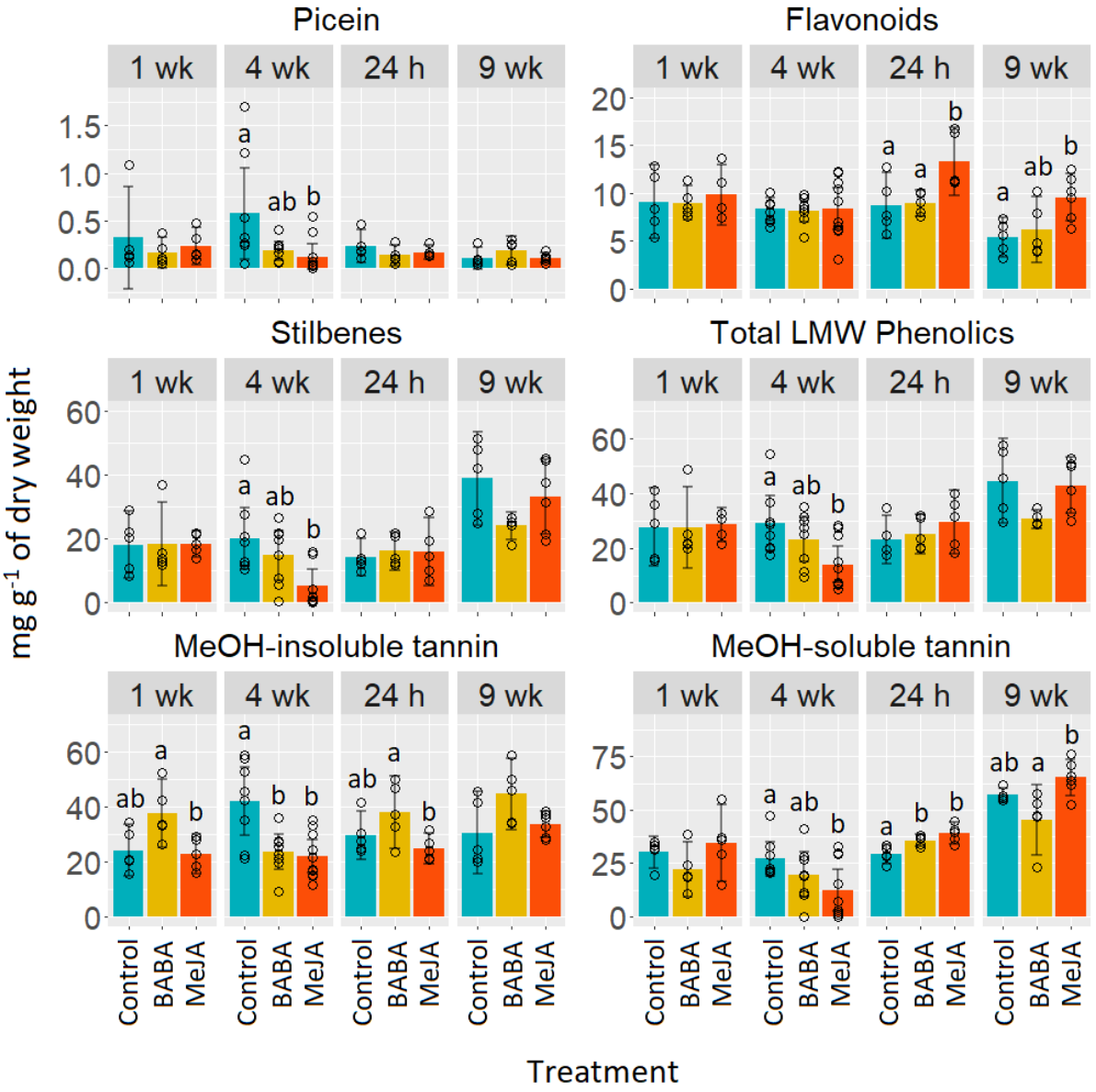


Figure 10. Mean concentrations (mg g^{-1} of dry weight) of picein, flavonoids, stilbenes, total amount of low-molecular-weight (LMW) phenolics, MeOH-insoluble tannin and MeOH-soluble tannin in response to different treatments: water and Tween (control), methyl jasmonate (MeJA), β-amino butyric acid (BABA). The concentration was assessed 1 and 4 weeks after chemical treatment (1 wk, 4 wk) and 24 hours and 9 weeks after wounding and inoculating with a fungal pathogen (24 h, 9 wk). Error bars represent 95% confidence interval and circles represent individual data points. For each compound and time point, treatments with different letters are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments, $p < 0.05$).

Total stilbenes concentration only showed significant difference between treatment once before challenge at 4 weeks after treatment (Figure 10, Appendix Table 1a). At this time point, its concentration in the MeJA-treated trees was significantly lower than control and BABA-treated plants. Piceatannol glycoside and iso-rhapontin glycoside, which are the most abundant stilbenes, and resveratrol glycoside also showed this pattern. Contrastingly, piceatannol

glycoside concentration at 9 weeks post-wounding was significantly lower in the BABA-treated plants than in the control and MeJA-treated plants. The same trend was also observed in iso-rhapontin glycoside and consequently in the total stilbenes (Tukey HSD's test, $0.1 > p\text{-value} > 0.05$). The concentration of other component compounds was not high in abundance at any time points (less than 1 mg g^{-1} of dry weight), so even though a few other compounds show significant differences between treatment groups at other time points, they did not affect the total stilbenes concentration. The total amount of low-molecular-weight phenolic compounds showed the same pattern as total stilbenes – a significantly lower concentration in the MeJA-treated trees than control and BABA-treated plants 4 weeks after treatment (Figure 6).

The concentration of the MeOH-insoluble tannins in the MeJA-treated trees did not vary much over time. There was a decrease of MeOH-insoluble tannins in the BABA-treated and MeJA-treated trees and an increase in the control plants at 4 weeks after treatment. This resulted a significant difference in MeOH-insoluble tannins between treated and control plants at this time point.

Before challenge at 4 weeks after treatment, the concentration of MeOH-soluble tannins in the MeJA-treated trees was significantly lower than the control trees, while BABA-treated plants had a concentration between MeJA-treated and control plants. 24 hours post-wounding both MeJA- and BABA-treated plants had significantly higher levels of MeOH-soluble tannins than control plants, with MeJA-treat plants having a 3-fold higher concentration and BABA-treated plants having a 1.8-fold higher concentration than the day before challenge. The highest concentration of MeOH-soluble tannins for all groups was observed 9 weeks after wounding with BABA-treated plants having a significantly lower concentration than MeJA-treated plants and control plants being intermediate to the treatments.

IV – Discussion

This study consisted of two sub-experiments. In sub-experiment A, I treated plants with MeJA, BABA, hexanoic acid or thiamine, and assessed plant resistance by inoculating them with *G. penicillata*. In sub-experiment B, I used RT-qPCR, HPLC, GC-MS and microscopy to quantify some defense response parameters with the aim of studying the priming mechanism. Below, I discuss the results in both sub-experiments by explaining the meaning of the findings and relating them to similar studies. Additionally, I point out some limitations in my study and make suggestions for further research.

1. Sub-experiment A: effects on tree resistance against *Grosmannia penicillata*

There were no visible symptoms from fungal inoculation in any plants at the end of the experiment. A possible alternative way to evaluate tree resistance potential is to look at the traumatic resin ducts (TRD) and terpene concentration data from sub-experiment B. Schmidt et al. (2011) showed that there were more TRD induced by MeJA in the Norway spruce resistant family comparing to the susceptible one. Schiebe et al. (2012) also observed that trees with higher terpenes concentration and successfully induced TRD 4 weeks after treatment survived the subsequent bark beetles mass attack. Thus, there is a correlation between TRD and tree resistance. However, such correlation was concluded based on the concrete evidence of tree resistance in both studies, which I did not obtain in my study. In addition, a correlation is not strong enough evidence comparing to direct symptoms. Therefore, I could not determine if the different chemical treatments primed or induced tree resistance against fungal inoculation.

The unsuccessful fungal inoculation was unexpected. *Grosmannia penicillata* is known for its ability to colonize the phloem and create necrotic lesions (Kirisits, 2004). The same fungal isolate I used has been used in previous studies where it caused extensive symptoms, both in seedlings (1- and 2-year-old plants) and mature trees (Hansen, 2020; Wilkinson, 2020; Zhao et al., 2015; Zhao et al., 2019). Other studies have also observed extensive necrotic lesions by *G. penicillata* inoculation on 2-year-old (Jankowiak & Kolařík, 2010) and 4-year-old (Repe et al., 2015) Norway spruce seedlings. Studies with non-significant symptoms from experimental inoculation of virulent bluestain species tend not to be published. Munch (1907), as cited in Horntvedt et al. (1983), tried to inoculate spruce trees with another type of blue stain fungus, *Ceratocystis* spp., but also failed to see any symptoms. The low oxygen levels in the sapwood

were thought to be important for the development of fungi in the trees (Horntvedt et al., 1983). Bad execution of wrapping parafilm around the inoculation site may have resulted in too high air content and poor fungal growth. Furthermore, repeated subculturing in the laboratory can reduce the virulence and pathogenicity in some fungi and oomycetes (Krokene & Solheim, 2001; Shah et al., 2007; Songe et al., 2014). The fungal isolate used in this study was originally isolated in 1980 and has been used repeatedly in several studies. Thus, the fungus may have reduced vitality.

The absence of visible symptoms could also have been due to the ontogeny of the trees. Some studies have shown that younger conifer trees are more resistant against pathogen attack than older trees (Christiansen et al., 1987; Plattner et al., 2008). Seedlings and juvenile plants in woody species are suggested to be more chemically defended and have more secondary compounds than mature trees (Barton & Koricheva, 2010; Bryant & Julkunen-Tiitto, 1995). However, the ontogenetic variation in defense traits can vary from species to species and there is a lack of studies on ontogeny in Norway spruce.

In addition to the possible effects of fungal and tree traits discussed above, I also think the inoculation method for seedlings or young trees could be improved. There are several studies on fungal inoculation of Norway spruce seedlings ranging from 1- to 4-year-old (Arnerup et al., 2011; Hansen, 2020; Jankowiak & Kolařík, 2010; Krokene & Solheim, 1998; Krokene & Solheim, 2001; Repe et al., 2015; Wilkinson, 2020; Yaqoob et al., 2012). Generally, the inoculation procedure in all these studies was to use a sterilized scalpel to cut a small bark flap with an “approximate” size. This approximation was sometimes defined (e.g. 5-10 mm in length) (Arnerup et al., 2011; Krokene & Solheim, 1998; Krokene & Solheim, 2001; Repe et al., 2015; Wilkinson, 2020; Yaqoob et al., 2012) and sometimes not (Hansen, 2020; Jankowiak & Kolařík, 2010). I used the bark flap inoculation procedure but did not define the wound size, and this may have contributed to the inconsistent results and resulted in misleading variation of lesion length. The inoculation methodology used in mature trees uses a precise wound size made with a cork borer to create the hole for inserting the inoculum (Franceschi et al., 1998; Krokene et al., 2000; Magerøy et al., 2020a; Nagy et al., 2014). Compared to that, the inoculation method in seedlings can be very imprecise in wound size, depending on the person doing it and this might affect the accuracy of the lesion measurement. It is not possible to use a cork borer on very small trees. However, it is desirable to mimic its precision. In future studies, to inoculate the small trees, I would suggest defining and marking (with marker or tape) the dimension of the wound on all trees prior to cutting the flap for better accuracy.

Plant mortality throughout the experiment was also puzzling. Plants started dying even before treatment or fungal inoculation. Furthermore, dead plants occurred in every chemical treatment, including the control plants. Thus, plant mortality might not have been caused by either chemical treatment or fungal inoculation. Since this phenomenon was first observed shortly after potted, I suspect the problem came from the plants. Dead trees probably failed to overwinter and suffered re-potted stress. In addition, I also observed parasitic plants occurring in the roots of some seedlings before planting. Unfortunately, I could not provide a concrete explanation as there are several factors that could inflict plant mortality.

2. Sub-experiment B: the underlying mechanisms of defense priming

In sub-experiment B, I investigated the underlying mechanism of priming of inducible defenses. There are few studies on defense priming in Norway spruce. Defense priming was first clearly observed in Norway spruce by Zhao et al. (2011). They found a more vigorous induction in total terpenes in MeJA-treated trees than in untreated trees after wounding, whereas there were only minor differences between treatments before wounding. A recent study explored the differences between defense priming in Norway spruce, induced by MeJA application, and prolonged upregulation of inducible defenses, induced by sub-lethal fungal inoculation (Magerøy et al., 2020a). Magerøy and co-workers showed that both MeJA treatment and sub-lethal fungal inoculation increased tree resistance to bark beetle colonization. However, the defense - activation prior to the beetle attack was much lower in MeJA-treated trees than in inoculated trees.

To further understand the molecular mechanisms of priming in Norway spruce bark, Magerøy and co-workers designed a study to look at transcriptional responses to different treatment combinations: control application of water, control and subsequent wounding, MeJA-application, and MeJA-application and subsequent wounding (Magerøy et al., 2020b). This design allowed the authors to explore defense response types and phases of defense priming. My experimental design with sampling at different time points served the same aim as the different treatment combinations in Magerøy et al. (2020b). I investigated gene expression, traumatic resin duct formation, terpene concentrations and phenolic concentrations at different time points after chemical treatment and wounding: 1 week after treatment (1wk), 4 weeks after treatment (4wk), 24 hours after wounding (24h) and 9 weeks after wounding (9wk). Based on the criteria formulated by Magerøy et al. (2020b), I categorized the response patterns found

at the different time points in this study into four response types: unprimed response to wounding, prolonged response to chemical treatment, primed state, and primed response to wounding (Figure 11). The response patterns categorized as “unprimed response to wounding” have a different degree of response between after and before wounding (was not tested for significance in this study), but no differences between treated and control at both periods (Figure 11a). The response patterns categorized as “prolonged response to chemical treatment” have a different degree of response between treated and control at both before and after wounding (Figure 11b). The response patterns categorized as “primed state” have a different degree of response between treated and control only before wounding (Figure 11c). The response patterns categorized as “primed response to wounding” have a different degree of response between treated and control trees only after wounding (Figure 11d, e and g). Additionally, the response pattern can show a slight difference in the degree of response between treated and control trees immediately after treatment and a more pronounced degree of response after wounding (Figure 11f).

Below, I go through each analysis on different defense parameters. I identify the response types of the response patterns found in each analysis and discuss further on how and why such pattern was found. In addition, I discuss factors that could have affected the accuracy of the results I obtained.

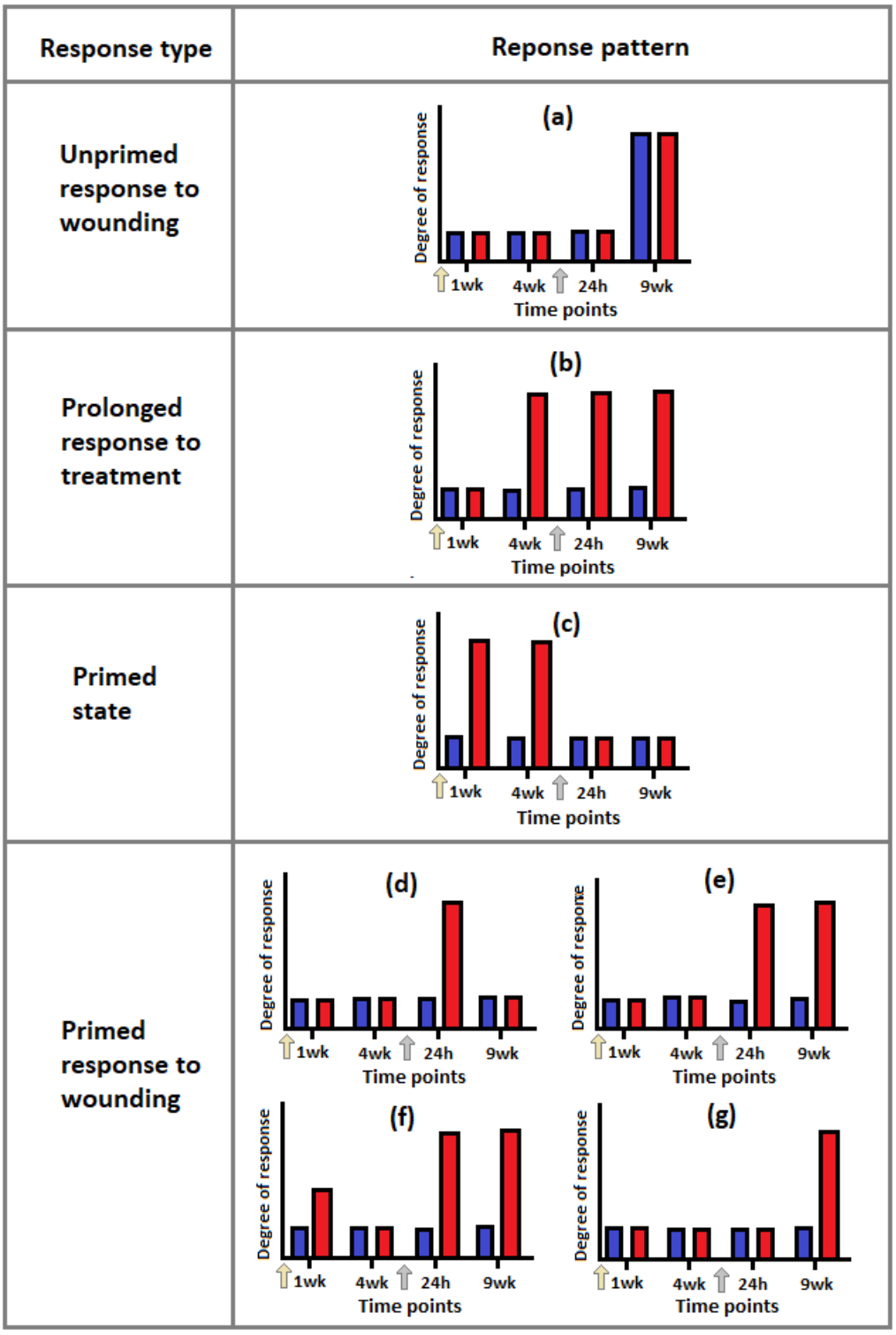


Figure 11. Different defense response patterns in gene expression, traumatic resin ducts, terpenes and phenolics accumulation found in this study in Norway spruce plants treated with putative priming chemicals (yellow arrow) and subsequent mechanical wounding (grey arrow). The response patterns group into four response types based on the differences in response between control-treated (blue) and chemically treated (red) plants before and after wounding. Trees were sampled 1 and 4 weeks after chemical treatment (1wk, 4wk), 24 hours after wounding (24h) and 9 weeks after wounding (9wk).

Gene expression analysis

To explore further the molecular mechanisms of priming, I selected representative genes from defense hormone signaling pathways, defense chemical biosynthesis pathways, and an epigenetic regulator. LOX is an enzyme catalyzing α-linolenic acid in the first step of JA synthesis (Wasternack & Song, 2017). Although the application of MeJA is known to upregulate the expression of jasmonic acid (JA) pathway biosynthesis genes in other plant species (Wasternack & Song, 2016), *LOX* expressions were found to be unresponsive to MeJA at all time points in my study. Expression of *PAL1*, which plays an important role in the salicylic acid (SA) defense signaling pathway (Arnerup et al., 2011), followed the same pattern as *LOX*. On the other hand, expression of *ACS*, which is involved in the regulation of ethylene (ET) synthesis (Xu & Zhang, 2014), was upregulated by MeJA 1 and 4 weeks after chemical treatment, but then returned to basal levels at 24h and 9wk after wounding. This response pattern seems to indicate that *ACS* expression was induced in a MeJA-primed state (Figure 11c). Hudgins and Franceschi (2004) suggested that JA and ET act together in inducible defense regulation in conifers. In previous studies, *PAL* and *LOX* were upregulated by MeJA treatment (Magerøy et al., 2020a; Schmidt et al., 2011; Wilkinson, 2020; Yaqoob et al., 2012). Interestingly, some of these studies also investigated the expression of *ACS* after MeJA treatment, but found no significant upregulation (Wilkinson, 2020; Yaqoob et al., 2012). The differences in *ACS* and *PAL1* expression found by Yaqoob et al. (2012) and in my study might be explained by antagonism between the JA/ET and SA pathways. The SA pathway which regulates defense against biotrophic pathogen can antagonize JA/ET pathways which regulate defense against herbivores and necrotrophic pathogens in several species, including the model plant *Arabidopsis thaliana* (Arnerup et al., 2013; Wilkinson, 2020). However, there is conflicting evidence whether antagonistic-crosstalk between these two pathways takes place in Norway spruce (Arnerup et al., 2013). Wilkinson (2020) found that genes from both pathways were upregulated, including *PAL* and *LOX*, regardless of the antagonism between the two pathways. However, since these two genes are just two representatives among several genes involve in both pathways, it is not possible to draw a solid conclusion about whether crosstalk exists. Furthermore, the lack of a *LOX* response in my study could be samples were collected too late relative to wounding. In *Arabidopsis*, enzymes involved in JA biosynthesis can be upregulated within minutes after wounding (Glauser et al., 2008; Glauser et al., 2009), while I collected the samples 24 hours after wounding. Hence, further study on JA pathway in priming of Norway spruce defense should include much earlier sampling time points after wounding.

Chitinase (Chi4) is known for its ability to degrade chitin in fungi and insects' exoskeleton (Davis et al., 2002). Thus, the enhanced expression of chitinase can be beneficial during attacks by insects and fungi. *Chi4* expression was upregulated 1 and 4 weeks after MeJA treatment but then returned to basal levels at 24h and 9wk after wounding. This defense response pattern indicated that *Chi4* was upregulated in the MeJA-induced primed state (Figure 11c). Similar upregulation of *Chi4* after MeJA treatment was observed in previous studies on 2- and 48-year-old Norway trees (Magerøy et al., 2020a; Yaqoob et al., 2012). In another study, *Chi4* was found to have a primed response to wounding in mature trees (Magerøy et al., 2020b). Devos (2020), however, did not find *Chi4* to be responsive to MeJA in 10-week-old seedlings after wounding. The mixed results from these different studies suggest further research on MeJA effects on Chi4.

MeJA treatment also upregulated *TPS-Car* before wounding, but not until 4wk after MeJA treatment. The upregulation was maintained after wounding at both the 24h and 9wk time points. This pattern of response suggests that it had a prolonged response to MeJA treatment (Figure 11b). *TPS-Car* has been previously found to be directly upregulated by MeJA in Norway spruce, but the effect was detected sooner after treatment than in this study. It was upregulated two days after MeJA treatment in the bark of 2-year-old plants (Zulak et al., 2009) and the stems of 3-year-old apical leader (Hall et al., 2011). The effect decreased the following days gradually. In the bark of 48-year-old Norway spruce trees, *TPS-Car* was upregulated by MeJA along with another *TPS* gene 14 days after treatment (Magerøy et al., 2020a). However, none of these experiments investigated gene expression levels after MeJA treatment and subsequent wounding and therefore cannot say anything about the primed defense responses. Results from mRNA sequencing found only one part of the sequenced *TPS-Car* to have a primed response to wounding following MeJA-treatment, while the other parts showed un-primed responses to wounding or prolonged responses to MeJA (Magerøy et al., 2020b). TPS-Car's main product is 3-carene (Fäldt et al., 2003; Roach et al., 2014). Higher level of 3-carene was found to increase the resistance of Norway spruce to bark beetles (Storer and Speight, 1996) and lodgepole pine (*Pinus* spp.) to Douglas fir pitch moth (*Synanthedon novaroensis)* (Rocchini et al., 2000). However, TPS-Car is just one member of the large terpene synthase family. Thus, the results on TPS-Car are not sufficient to draw solid conclusions on the effect of MeJA treatment on terpene synthase in Norway spruce. Other TPS genes should be included in future research.

The epigenetic regulator *NRPE1* was downregulated by MeJA at 1wk, 4wk and 24h after wounding, with the level of repression weakened over time. NRPE1 is the largest sub-unit in RNA-polymerase V, which is important for the RNA-directed DNA methylation in plants (López Sánchez et al., 2016; Matzke et al., 2015). Our results on NRPE1 agreed with the finding of Wilkinson (2020), who also found repression of *NRPE1* by MeJA treatment, with a gradual loss of effect over time. Our results, however, showed a more prolonged effect until 24 hours after wounding, while in Wilkinson (2020), the effect wore off before the second challenge. Unmethylated DNA indicates the state of euchromatin, which makes genes more accessible for transcription. Methylated DNA on the other hand indicates heterochromatin state, which makes genes less accessible for transcription. Repression of *NRPE1* by MeJA indicated that MeJA can reduce DNA methylation and increase gene transcriptional susceptibility. Studies on *Arabidopsis thaliana NRPE1*-mutants observed that several defense-related genes had constitutive primed effects (Wilkinson et al., 2019). Thus, it is possible that the repression of *NRPE1* induced by MeJA also primes the defense-related genes in Norway spruce. Further research on which genes are targeted and how they are regulated by NRPE1-dependent DNA methylation should be conducted in the future.

Contrary to MeJA treatment, which altered the expression of several transcripts, BABA treatment only affected *Chi4* and *TPS-Car* expression. Both transcripts were down-regulated by BABA, *Chi4* at 1 week after treatment and TPS-Car at 24h after wounding. These response patterns indicated that *Chi4* had a short-term repressed response and *TPS-Car* had a primed response to wounding (Figure 11d) after BABA treatment. These results might suggest that BABA reduces the resistance of Norway spruce. However, this was the first study to find a significant effect of BABA treatment in Norway spruce. Devos (2020) did not see any effects from BABA on 10-week-old seedlings after wounding. In addition, the significant results from BABA were not abundant in analyses of other defense parameters and did not follow a certain pattern. Therefore, it is not possible to draw a certain conclusion, and further research on BABA treatment on Norway spruce is necessary.

Traumatic resin ducts and terpene analysis

Traumatic resin ducts (TRDs) were not detected in any treatment until 9 weeks after wounding. The amount of traumatic resin ducts was significantly higher in MeJA-treated trees than in both control and BABA-treated trees. Previous studies have shown that MeJA application can

induce TRD formation in Norway spruce (Erbilgin et al., 2006; Schmidt et al., 2011; Zeneli et al., 2006). However, MeJA-induced TRDs in these studies were detected at the latest 4 weeks after treatment, much earlier than in this study (13 weeks after treatment). Furthermore, Zeneli et al. (2006) found no TRDs in untreated control trees, unlike the few TRDs observed in untreated trees in my study. This indicated that wounding in my study also acted as a triggering stimulus for TRD. Thus, TRD formation in MeJA-treated trees showed a primed response to wounding (Figure 11g). This result agrees with the primed response to wounding in MeJA-treated trees observed in (Zhao et al., 2011). TRD formation is an important part of the inducible defense system of Norway spruce. When bark beetles attack a tree, induced TRD formation can increase tree resistance to subsequent attacks by opportunistic pathogens and help kill the eggs and larvae of the bark beetles (Krokene, 2015). However, TRD formation takes several weeks and rarely makes it in time to protect the trees from bark beetle attacks (Krokene, 2015). Thus, a primed TRD response to wounding might benefit the trees, as the response to subsequent attack will be faster and more extensive.

In contrast to what I observed for TRD formation, metabolite analyses of terpenes in the bark showed no response to either MeJA or BABA treatment. There were no differences between treated and untreated trees either before or after wounding for concentrations of monoterpenes, sesquiterpenes, diterpenes or total terpenes. Magerøy et al. (2020a) also found no significant increase in terpene levels following MeJA treatment in the bark of 48-year-old Norway spruce trees. However, they found a massive terpene increase after wounding. These results contrasted with Erbilgin et al. (2006), who found significantly higher terpene levels in 60-year-old trees 3 weeks after MeJA treatment. After wounding, the total terpene concentration in my study seemed to undergo an unprimed response to wounding (Figure 11a). Total terpene levels in all treatment groups seemed to peak at 9 weeks after wounding compared to before wounding (no test was conducted for significance). However, there were no significant differences between treated and untreated trees (Figure 8). This result contrasted with the primed response to wounding observed in MeJA-treated trees by Zhao et al. (2011) and Magerøy et al. (2020a). More surprisingly, although MeJA-treated trees showed a significantly higher TRD formation than control trees 9 weeks after wounding, such a pattern was not found in the terpene concentration in the bark. The lack of response to MeJA in terpene concentration is therefore puzzling.

Some previous studies also found no significant increase of terpene concentration in the bark of MeJA-treated trees. Martin et al. (2002) showed the induced accumulation of resin

terpenoids by MeJA was only found in the wood but was barely detectable in the bark in 2-year-old Norway spruce. Schmidt et al. (2011) also inspected the terpene accumulation in 6-year-old trees with more traumatic resin ducts after treated with MeJA. The study found that even though high producing TRD trees had much higher terpene levels in the sapwood, such differences were not found in the bark (Schmidt et al., 2011). Nybakken et al. (2021) also found that the total mono-, sesqui-, and diterpenes did not respond significantly to MeJA or MeJA combining with wounding in the bark of both 1-year-old nursery trees and 3-year-old naturally regenerated trees. All these studies showed no significant induction of terpene caused by MeJA in the bark, and they all worked with young seedlings (2 to 6-year-old trees) like my study (1-year-old trees). On the other hand, studies that showed a significantly higher level of terpene induced by MeJA or MeJA and wounding worked with mature Norway spruce stands (Erbilgin et al., 2006; Magerøy et al., 2020a; Zhao et al., 2011). The differing results from mature and young trees might be caused by the plant ontogeny in chemical defense strategies. Terpenes are very metabolically expensive to produce due to their high level of chemical reduction (Gershenzon, 2017). They also have high cost for storage as they require special storage structure to avoid autotoxicity (Gershenzon, 1994). Induced monoterpene production by MeJA was more vigorous in the mature jack pine (*Pinus banksiana*) than in young trees (Erbilgin & Colgan, 2012). The study suggested that mature trees had more resources in production and storage for higher levels of inducible defense (Erbilgin & Colgan, 2012). Thus, young spruce trees might not have enough resources yet for inducing more terpenes in the bark and they would rather save the resources for growth. It would be interesting to carry out more studies on the ontogeny of Norway spruce defense traits in the future for a clearer explanation.

Phenolic analysis

In addition to terpenes, phenolic compounds are also important defense chemicals in Norway spruce (Krokene, 2015). In our study, the total stilbenes, which account for the major proportion in the total of low-molecular-weight phenolic concentration, was not significantly induced by MeJA or BABA both before and after wounding. Many previous studies also saw no significant induction of phenolic, mostly stilbenes, in Norway spruce when triggered by wounding or MeJA (Brignolas et al., 1995; Deflorio et al., 2011; Erbilgin et al., 2006; Jyske et al., 2020; Nybakken et al., 2021; Viiri et al., 2001; Zeneli et al., 2006). However, I might have underestimated the phenolic concentration. I only extracted and quantified the soluble phenolic compounds, but not the cell-wall bound phenolics, which require a different extraction

procedure. Previous studies on phenolic compounds in Norway quantified both the soluble and cell-wall bound form (Brignolas et al., 1995; Cvikrová et al., 2006; Fossdal et al., 2012). I also quantified mostly the glycosides form and not the aglycones of phenolic. Metsämuuronen and Sirén (2019) suggested that phenolic glycosides are less active than their corresponding aglycones. Thus, a repeat study with a more thorough investigation on phenolic would be interesting.

Although there was no apparent response in stilbenes to MeJA or wounding, the flavonoid concentration was found to be significantly higher in MeJA-treated trees than control post-wounding at 24h and 9wk. When looking more closely at the individual compounds, I suspected that the response pattern in flavonoids was dependent on the flavan-3-ol catechin. Catechin was significantly higher in MeJA-treated than control trees at 1wk, then returned to basal level at 4wk and increased again but more vigorously at 24h and 9wk. MeOH-soluble-tannins, which include polymers of catechin, were also significantly higher in MeJA-treated than in control trees at 24h and 9wk. Thus, all response patterns observed from catechin, total flavonoid concentration and MeOH-soluble-tannins show a MeJA-induced primed response to wounding (Figure 11e and 11f). Unlike stilbenes, catechin and its polymers have been reported in many studies to be induced upon wounding and fungal inoculation (Brignolas et al., 1995; Danielsson et al., 2011; Evensen et al., 2000; Jyske et al., 2020). However, this is the first study to show that catechin and condensed tannins had a primed response to wounding by MeJA treatment in Norway spruce. Flavan-3-ols are effective against fungal infection and herbivorous attack (Bueno et al., 2012). Catechin at 0.1% concentration reduces tunneling of *Ips typographus* males by 50% (Faccoli & Schlyter, 2007). The primed response to wounding of catechin and condensed tannins might have connection with the primed gene *ACS*. Even though PAL1 - an important biosynthesis gene in the phenylpropanoid pathway - was unresponsive to MeJA, *ACS* was in primed state by MeJA. Hudgins and Franceschi (2004) showed that in the JA-pathway, ethylene could elicit phenolic response in PP cell, a type of axial phloem parenchyma in which catechin was found to be localized (Jyske et al., 2020). Thus, the primed state of *ACS* might have aided the biosynthesis of catechin in PP cells and elicit the primed response to wounding of this compound. Further research on catechin and other flavan-3-ol compounds in Norway spruce priming defense are necessary. It would be interesting to include the regulation of genes involving in the flavan-3-ols synthesis, such as *leucoanthocyanidin reductase* (*LAR*) and *anthocyanidin reductase* (*ANR*) (Tanner et al., 2003;

Xie et al., 2004). In addition, the mechanism of polymerization of flavan-3-ol monomers into condensed tannins should also be inspected.

Suggestions for future research

The differences in my experimental design and methods from previous studies might contribute to inconsistency with previous findings. In this study, I only used the bark tissue for all the analyses in sub-experiment B. Many studies have shown significant differences between the bark and the sapwood in both gene expression and metabolite analyses (Deflorio et al., 2011; Martin et al., 2002; Oliva et al., 2015; Schmidt et al., 2011; Yaqoob et al., 2012; Zeneli et al., 2006). The overall defense response of the tree may have been underestimated by only analyzing the bark tissue. Furthermore, when sampling for the time points after wounding, I used the whole bark rather than taking the samples near the wound site. Most studies on wound-induced defense in Norway spruce sampled locally near the wound site (Brignolas et al., 1995; Deflorio et al., 2011; Jyske et al., 2020; Nagy et al., 2014; Viiri et al., 2001; Yaqoob et al., 2012). Sampling the whole bark might dilute the effect of some responses by including non-specialized or non-systemic response cells (Magerøy et al., 2020b). A more local sampling system could have been used for more accurate results.

Plant mortality throughout the experiment was also puzzling. Plants started dying even before treatment or fungal inoculation. Furthermore, dead plants occurred in every chemical treatment, including the control plants. Thus, plant mortality might not have been caused by either chemical treatment or fungal inoculation. Since this phenomenon was first observed shortly after potted, I suspect the problem came from the plants. Dead trees probably failed to overwinter and suffered re-potted stress. In addition, I also observed parasitic plants occurring in the roots of some seedlings before planting. Unfortunately, I could not provide a concrete explanation as several factors could inflict plant mortality.

Additionally, the contrasts between my study and previous studies could be due to the different concentrations of MeJA and application methods used in different studies. MeJA doses have varied from 10mM to 100mM concentration and have been applied by spraying or painting (Magerøy et al., 2020a; Martin et al., 2002; Schmidt et al., 2011; Yaqoob et al., 2012; Zhao et al., 2011). Martin et al. (2002) and Erbilgin et al. (2006) demonstrated that the different doses of MeJA could vary the effects. In my study, I applied 50mM of MeJA by painting. The moderate dose could also have caused the insignificant reduction of growth in MeJA-treated

trees (Figure 5). A thorough assessment for suitable dose usage for the experimental design should be done in future research on MeJA as a priming chemical in Norway spruce.

I suspected that wounding as the second challenge was not a sufficient stimulus to trigger primed response. Using wounding as the second challenge was a reluctant change in the experimental design when the fungal inoculation did not work. Many studies showed that the induced defense responses toward fungal inoculation were usually more vigorous than just mechanical wounding (Arnerup et al., 2013; Brignolas et al., 1995; Danielsson et al., 2011; Deflorio et al., 2011; Evensen et al., 2000; Hammerbacher et al., 2014; Jyske et al., 2020). Magerøy et al. (2020b) pointed out that wounding itself can either act as the triggering stimulus that induced primed response to wounding, or it could just be another priming stimulus that turned the trees into primed state only. Most of the defense response patterns in my study were non-responsive to wounding. The induced response mostly happened before wounding or nothing happened at all time points. This might indicate that wounding in my study was not strong enough to be perceived as a triggering stimulus to elicit the primed response to wounding. It might only act as a priming signal to turn the trees into primed state. In that case, the response pattern of TPS-Car might not be a prolonged response to MeJA. TPS-Car was probably "primed" the second time when received wounding. This led to the upregulation of the gene but lacking the corresponding induction of its products – 3-carene and terpinolene – after wounding (Figure 6 and 9). This response pattern was also found in Magerøy et al. (2020a) and they conclude that the trees were primed based on that. Therefore, a repeat experiment with successful fungal inoculation – a more prominent triggering stimulus – is necessary to observe more accurate defensive responses in Norway Spruce.

V – Conclusions:

Priming of inducible defense in Norway spruce is a cost-effective strategy to protect trees from herbivores and pathogens. I wanted to discover new effective Norway spruce priming stimuli and study the underlying mechanism of priming in Norway spruce. Due to the failure of *Grosmannia penicillata* fungal inoculation, I could not observe tree resistance and therefore could not identify novel chemicals as priming stimuli. Using wounding as the subsequent stimulus, I observed many different response types in gene expression and metabolites analysis the MeJA-treated trees. However, wounding was suspected to be a less potent triggering stimulus than fungal inoculation. Thus, a repetition of this study with successful fungal inoculation is desirable to elicit a stronger response. Furthermore, it would also be interesting to study the ontogeny of Norway spruce defense traits in future research on priming of inducible defense in Norway spruce.

Most genes were in primed state or had prolonged response to MeJA treatment. TRD had priming response to wounding, but terpenes were not accumulated in MeJA-treated trees. In addition, the first evidence of flavan-3-ols having primed response to wounding by MeJA treatment was found. Moreover, I found evidence of MeJA ability to repress DNA methylation from the downregulation of *NRPE1* expression. Overall, my results confirm that MeJA is a potential priming stimulus for Norway spruce. Future research should be conducted to better understand the effects of MeJA treatment on flavan-3-ol accumulation and epigenetic modifications. In contrast, I did not observe any clear results from BABA-treated trees. Further research on the efficacy of this chemical as a priming stimulus in Norway spruce is needed.

VI – References:

Ahn, I.-P., Kim, S. & Lee, Y.-H. (2005). Vitamin B1 Functions as an Activator of Plant Disease Resistance. *Plant Physiology*, 138 (3): 1505. doi: 10.1104/pp.104.058693.

Ahn, I.-P., Lee, Y.-H. & Suh, S.-C. (2007). Vitamin B1-Induced Priming Is Dependent on Hydrogen Peroxide and the NPR1 Gene in *Arabidopsis*. *Plant Physiology*, 143: 838-48. doi: 10.1104/pp.106.092627.

Arnerup, J., Lind, M., Olson, A., Stenlid, J. & Elfstrand, M. (2011). The pathogenic white-rot fungus *Heterobasidion parviporum* triggers non-specific defence responses in the bark of Norway spruce. *Tree Physiology*, 31: 1262-72. doi: 10.1093/treephys/tpr113.

Arnerup, J., Nemesio-Gorriz, M., Lundén, K., Asiegbu, F. O., Stenlid, J. & Elfstrand, M. (2013). The primary module in Norway spruce defence signalling against *H. annosum* s.l. seems to be jasmonate-mediated signalling without antagonism of salicylate-mediated signalling. *Planta*, 237 (4): 1037-1045. doi: 10.1007/s00425-012-1822-8.

Bahuguna, R., Joshi, R., Shukla, A. & Kumar, J. (2012). Thiamine primed defense provides reliable alternative to systemic fungicide carbendazim against sheath blight disease in rice (*Oryza sativa L.*). *Plant physiology and biochemistry : PPB / Société française de physiologie végétale*, 57: 159-67. doi: 10.1016/j.plaphy.2012.05.003.

Barton, Kasey E. & Koricheva, J. (2010). The Ontogeny of Plant Defense and Herbivory: Characterizing General Patterns Using Meta-Analysis. *The American Naturalist*, 175 (4): 481-493. doi: 10.1086/650722.

Boubakri, H., Gargouri, M., Mliki, A., Brini, F., Chong, J. & Moez, J. (2016). Vitamins for enhancing plant resistance. *Planta*, 244. doi: 10.1007/s00425-016-2552-0.

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. doi: 10.1104/pp.109.3.821.

Bryant, J. P. & Julkunen-Tiitto, R. (1995). Ontogenic development of chemical defense by seedling resin birch: Energy cost of defense production. *Journal of Chemical Ecology*, 21 (7): 883-896. doi: 10.1007/BF02033796.

Bueno, J. M., Ramos-Escudero, F., Sáez-Plaza, P., Muñoz, A. M., José Navas, M. & Asuero, A. G. (2012). Analysis and Antioxidant Capacity of Anthocyanin Pigments. Part I: General Considerations Concerning Polyphenols and Flavonoids. *Critical Reviews in Analytical Chemistry*, 42 (2): 102-125. doi: 10.1080/10408347.2011.632312.

Caudullo, G., Tinner, W. & de Rigo, D. (2016). *Picea abies* in Europe: distribution, habitat, usage and threats.

Chong, J., Poutaraud, A. & Hugueney, P. (2009). Metabolism and roles of stilbenes in plants. *Plant Science*, 177 (3): 143-155.

Christiansen, E., Waring, R. H. & Berryman, A. A. (1987). Resistance of conifers to bark beetle attack: Searching for general relationships. *Forest Ecology and Management*, 22 (1): 89-106. doi: https://doi.org/10.1016/0378-1127(87)90098-3.

Christiansen, E., Krokene, P., Berryman, A. A., Franceschi, V. R., Krekling, T., Lieutier, F., Lönneborg, A. & Solheim, H. (1999). Mechanical injury and fungal infection induce acquired resistance in Norway spruce. *Tree Physiology*, 19 (6): 399-403. doi: 10.1093/treephys/19.6.399.

Conrath, U. (2009). Priming of induced plant defense responses. *Advances in Botanical Research*, 51: 361-395.

Conrath, U., Beckers, G. J., Langenbach, C. J. & Jaskiewicz, M. R. (2015). Priming for enhanced defense. *Annual Review of Phytopathology*, 53: 97-119.

Cvikrová, M., Malá, J., Hrubcová, M. & Eder, J. (2006). Soluble and cell wall-bound phenolics and lignin in Ascocalyx abietina infected Norway spruces. *Plant Science*, 170 (3): 563-570. doi: https://doi.org/10.1016/j.plantsci.2005.10.011.

Danielsson, M., Lundén, K., Elfstrand, M., Hu, J., Zhao, T., Arnerup, J., Ihrmark, K., Swedjemark, G., Borg-Karlson, A.-K. & Stenlid, J. (2011). Chemical and transcriptional responses of Norway spruce genotypes with different susceptibility to Heterobasidion spp. infection. *BMC Plant Biology*, 11 (1): 154. doi: 10.1186/1471-2229-11-154.

Davis, J., Wu, H., Cooke, J., Reed, J., Luce, K. & Michler, C. (2002). Pathogen challenge, salicylic acid, and jasmonic acid regulate expression of chitinase gene homologs in pine. *Molecular Plant-Microbe Interactions : MPMI*, 15 4: 380-7.

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*, 75 (4): 180-187. doi: https://doi.org/10.1016/j.pmpp.2011.02.002.

Devos, C. C. (2020). *Effect of seed treatment with putative defence priming chemicals on defence-related gene expression and pathogen resistance in Norway spruce (Picea abies) seedlings*: M.Sc. thesis. Norwegian University of Life Sciences, Ås.

Erbilgin, N., Krokene, P., Christiansen, E., Zeneli, G. & Gershenzon, J. (2006). Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and

reduces host colonization by the bark beetle *Ips typographus*. *Oecologia*, 148 (3): 426-436.

Erbilgin, N. & Colgan, L. J. (2012). Differential effects of plant ontogeny and damage type on phloem and foliage monoterpenes in jack pine (Pinus banksiana). *Tree Physiology*, 32 (8): 946-957. doi: 10.1093/treephys/tps047.

Evensen, P. C., Solheim, H., Høiland, K. & Stenersen, J. (2000). Induced resistance of Norway spruce, variation of phenolic compounds and their effects on fungal pathogens. *Forest Pathology*, 30 (2): 97-108. doi: https://doi.org/10.1046/j.1439-0329.2000.00189.x.

Faccoli, M. & Schlyter, F. (2007). Conifer phenolic resistance markers are bark beetle antifeedant semiochemicals. *Agricultural and Forest Entomology*, 9 (3): 237-245. doi: https://doi.org/10.1111/j.1461-9563.2007.00339.x.

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. doi: 10.1023/A:1020714403780.

Fedderwitz, F., Björklund, N., Anngren, R., Lindström, A. & Nordlander, G. (2020). Can methyl jasmonate treatment of conifer seedlings be used as a tool to stop height growth in nursery forest trees? *New Forests*, 51. doi: 10.1007/s11056-019-09737-6.

Finiti, I., de la O. Leyva, M., Vicedo, B., Gómez-Pastor, R., López-Cruz, J., García-Agustín, P., Real, M. D. & González-Bosch, C. (2014). Hexanoic acid protects tomato plants against *Botrytis cinerea* by priming defence responses and reducing oxidative stress. *Molecular Plant Pathology*, 15 (6): 550-562.

Fossdal, C. G., Nagy, N. E., Hietala, A. M., Kvaalen, H., Slimestad, R., Woodward, S. & Solheim, H. (2012). Indications of heightened constitutive or primed host response affecting the lignin pathway transcripts and phenolics in mature Norway spruce clones. *Tree Physiology*, 32 (9): 1137-1147. doi: 10.1093/treephys/tps073.

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. doi: https://doi.org/10.2307/2446529.

Franceschi, V. R., Krokene, P., Krekling, T. & Christiansen, E. (2000). Phloem parenchyma 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. doi: https://doi.org/10.2307/2656627.

Freeman, B. C. & Beattie, G. A. (2008). An overview of plant defenses against pathogens and herbivores. *The Plant Health Instructor*.

Frost, C. J., Mescher, M. C., Carlson, J. E. & De Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, 146 (3): 818-824.

Gershenzon, J. (1994). Metabolic costs of terpenoid accumulation in higher plants. *Journal of chemical ecology*, 20 (6): 1281-1328.

Gershenzon, J. (2017). The cost of plant chemical defense against herbivory: a biochemical perspective. In *Insect-plant interactions*, pp. 105-176: CRC Press.

Glauser, G., Grata, E., Dubugnon, L., Rudaz, S., Farmer, E. E. & Wolfender, J.-L. (2008). Spatial and Temporal Dynamics of Jasmonate Synthesis and Accumulation in *Arabidopsis* in Response to Wounding*. *Journal of Biological Chemistry*, 283 (24): 16400-16407. doi: https://doi.org/10.1074/jbc.M801760200.

Glauser, G., Dubugnon, L., Mousavi, S. A. R., Rudaz, S., Wolfender, J.-L. & Farmer, E. E. (2009). Velocity Estimates for Signal Propagation Leading to Systemic Jasmonic Acid Accumulation in Wounded *Arabidopsis**. *Journal of Biological Chemistry*, 284 (50): 34506-34513. doi: https://doi.org/10.1074/jbc.M109.061432.

Gould, N., Reglinski, T., Northcott, G. L., Spiers, M. & Taylor, J. T. (2009). Physiological and biochemical responses in *Pinus radiata* seedlings associated with methyl jasmonate-induced resistance to *Diplodia pinea*. *Physiological and Molecular Plant Pathology*, 74 (2): 121-128.

Hall, D. E., Robert, J. A., Keeling, C. I., Domanski, D., Quesada, A. L., Jancsik, S., Kuzyk, M. A., Hamberger, B., Borchers, C. H. & Bohlmann, J. (2011). An integrated genomic, proteomic and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce (*Picea sitchensis*) genotypes that are resistant or susceptible to white pine weevil. *The Plant Journal*, 65 (6): 936-948. doi: https://doi.org/10.1111/j.1365-313X.2010.04478.x.

Hammerbacher, A., Paetz, C., Wright, L. P., Fischer, T. C., Bohlmann, J., Davis, A. J., Fenning, T. M., Gershenzon, J. & Schmidt, A. (2014). Flavan-3-ols in Norway Spruce: Biosynthesis, Accumulation, and Function in Response to Attack by the Bark Beetle-Associated Fungus *Ceratocystis polonica*. *Plant Physiology*, 164 (4): 2107. doi: 10.1104/pp.113.232389.

Hannrup, B., Cahalan, C., Chantre, G., Grabner, M., Karlsson, B., Bayon, I. L., Jones, G. L., Müller, U., Pereira, H. & Rodrigues, J. C. (2004). Genetic parameters of growth and wood quality traits in *Picea abies*. *Scandinavian Journal of Forest Research*, 19 (1): 14-29.

Hansen, H. P. (2020). *Impacts of abiotic stress on priming of defense responses and pathogen resistance in Norway spruce*: M.Sc. thesis. Ås, Norwegian University of Life Sciences.

Heijari, J., Nerg, A.-M., Kainulainen, P., Viiri, H., Vuorinen, M. & Holopainen, J. (2005). Application of methyl jasmonate reduces growth but increases chemical defence and resistance against *Hylobius abietis* in Scots pine seedlings. *Entomologia Experimentalis et Applicata*, 115: 117-124. doi: 10.1111/j.1570-7458.2005.00263.x.

Hietala, A., Kvaalen, H., Schmidt, A., Jøhnk, 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: 3948-53. doi: 10.1128/AEM.70.7.3948-3953.2004.

Hietala, A. M., Eikenes, M., Kvaalen, H., Solheim, H. & Fossdal, C. G. (2003). Multiplex real-time PCR for monitoring *Heterobasidion annosum* colonization in Norway spruce clones that differ in disease resistance. *Applied and environmental microbiology*, 69 (8): 4413-4420. doi: 10.1128/AEM.69.8.4413-4420.2003.

Horntvedt, R., Christiansen, E., Solheim, H. & Wang, S. (1983). Artificial inoculation with *Ips typographus*-associated blue stain fungi can kill healthy Norway spruce trees. *Meddelelser fra Skogforsk*.

Huang, W.-K., Ji, H.-L., Gheysen, G. & Kyndt, T. (2016). Thiamine-induced priming against root-knot nematode infection in rice involves lignification and hydrogen peroxide generation. *Molecular Plant Pathology*, 17 (4): 614-624. doi: https://doi.org/10.1111/mpp.12316.

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. doi: 10.1104/pp.103.037929.

Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Métraux, J.-P. & Mauch-Mani, B. (2001). β-Aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology*, 107 (1): 29-37.

Jankowiak, R. & Kolařík, M. (2010). Diversity and pathogenicity of ophiostomatoid fungi associated with Tetropium species colonizing *Picea abies* in Poland. *Folia microbiologica*, 55 (2): 145-154.

Jyske, T., Kuroda, K., Keriö, S., Pranovich, A., Linnakoski, R., Hayashi, N., Aoki, D. & Fukushima, K. (2020). Localization of (+)-Catechin in *Picea abies* Phloem: Responses to Wounding and Fungal Inoculation. *Molecules*, 25 (12). doi: 10.3390/molecules25122952.

Keeling, C. I. & Bohlmann, J. (2006). Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytologist*, 170 (4): 657-675.

Kim, J. & Felton, G. W. (2013). Priming of antiherbivore defensive responses in plants. *Insect Science*, 20 (3): 273-285.

Kirisits, T. (2004). Fungal Associates of European Bark Beetles With Special Emphasis on the Ophiostomatoid Fungi. In Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C. & Evans, H. F. (eds) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*, pp. 181-236. Dordrecht: Springer Netherlands.

Koutaniemi, S., Warinowski, T., Kärkönen, A., Alatalo, E., Fossdal, C. G., Saranpää, P., Laakso, T., Fagerstedt, K. V., Simola, L. K., Paulin, L., et al. (2007). Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology*, 65 (3): 311-328. doi: 10.1007/s11103-007-9220-5.

Kravchuk, Z., Vicedo, B., Flors, V., Camañes, G., González-Bosch, C. & García-Agustín, P. (2011). Priming for JA-dependent defenses using hexanoic acid is an effective mechanism to protect *Arabidopsis* against *B. cinerea*. *Journal of plant Physiology*, 168 (4): 359-366.

Krokene, P. & Solheim, H. (1998). Assessing the virulence of four bark beetle-associated bluestain fungi using Norway spruce seedlings. *Plant Pathology*, 47 (4): 537-540. doi: https://doi.org/10.1046/j.1365-3059.1998.00268.x.

Krokene, P., Solheim, H. & Långström, B. (2000). Fungal Infection and Mechanical Wounding Induce Disease Resistance in Scots Pine. *European Journal of Plant Pathology*, 106 (6): 537-541. doi: 10.1023/A:1008776002248.

Krokene, P. & Solheim, H. (2001). Loss of pathogenicity in the blue-stain fungus *Ceratocystis polonica*. *Plant Pathology*, 50 (4): 497-502. doi: https://doi.org/10.1046/j.1365-3059.2001.00588.x.

Krokene, P., Nagy, N. E. & Krekling, T. (2008a). Traumatic Resin Ducts and Polyphenolic Parenchyma Cells in Conifers. In Schaller, A. (ed.) *Induced Plant Resistance to Herbivory*, pp. 147-169. Dordrecht: Springer Netherlands.

Krokene, P., Nagy, N. E. & Solheim, H. (2008b). 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. doi: 10.1093/treephys/28.1.29.

Krokene, P. (2015). Conifer defense and resistance to bark beetles. In *Bark Beetles*, pp. 177-207: Elsevier.

Lieutier, F. & Battisti, A. (2004). *Bark and wood boring insects in living trees in Europe: a synthesis*: Springer, Dordrecht.

Llorens, E., Scalschi, L., Fernández-Crespo, E., Lapena, L. & García-Agustín, P. (2015). Hexanoic acid provides long-lasting protection in 'Fortune'mandarin against *Alternaria alternata*. *Physiological and Molecular Plant Pathology*, 91: 38-45.

López Sánchez, A., Stassen, J. H. M., Furci, L., Smith, L. M. & Ton, J. (2016). The role of DNA (de)methylation in immune responsiveness of *Arabidopsis*. *The Plant Journal*, 88 (3): 361-374. doi: https://doi.org/10.1111/tpj.13252.

Magerøy, M. H., Christiansen, E., Långström, B., Borg-Karlson, A. K., Solheim, H., Björklund, N., Zhao, T., Schmidt, A., Fossdal, C. G. & Krokene, P. (2020a). Priming of inducible defenses protects Norway spruce against tree-killing bark beetles. *Plant, Cell & Environment*, 43 (2): 420-430.

Magerøy, M. H., Wilkinson, S. W., Tengs, T., Cross, H., Almvik, M., Pétriacq, P., Vivian-Smith, A., Zhao, T., Fossdal, C. G. & Krokene, P. (2020b). Molecular underpinnings of methyl jasmonate-induced resistance in Norway spruce. *Plant, Cell & Environment*, 43 (8): 1827-1843. doi: https://doi.org/10.1111/pce.13774.

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.

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C. M., Pozo, M. J., Ton, J., van Dam, N. M. & Conrath, U. (2016). Recognizing plant defense priming. *Trends in Plant Science*, 21 (10): 818-822.

Matzke, M. A., Kanno, T. & Matzke, A. J. M. (2015). RNA-Directed DNA Methylation: The Evolution of a Complex Epigenetic Pathway in Flowering Plants. *Annual Review of Plant Biology*, 66 (1): 243-267. doi: 10.1146/annurev-arplant-043014-114633.

Mauch-Mani, B., Baccelli, I., Luna, E. & Flors, V. (2017). Defense priming: an adaptive part of induced resistance. *Annual review of Plant Biology*, 68: 485-512.

Metsämuuronen, S. & Sirén, H. (2019). Bioactive phenolic compounds, metabolism and properties: a review on valuable chemical compounds in Scots pine and Norway spruce. *Phytochemistry Reviews*, 18 (3): 623-664. doi: 10.1007/s11101-019-09630-2.

Moreira, X., Zas, R. & Sampedro, L. (2012). Genetic variation and phenotypic plasticity of nutrient re-allocation and increased fine root production as putative tolerance mechanisms inducible by methyl jasmonate in pine trees. *Journal of Ecology*, 100 (3): 810-820.

Nagy, N. E., Sikora, K., Krokene, P., Hietala, A. M., Solheim, H. & Fossdal, C. G. (2014). Using laser micro-dissection and qRT-PCR to analyze cell type-specific gene expression in Norway spruce phloem. *PeerJ*, 2: e362.

Nybakken, L., Fløistad, I. S., Magerøy, M., Lomsdal, M., Strålberg, S., Krokene, P. & Asplund, J. (2021). Constitutive and inducible chemical defences in nursery-grown and naturally regenerated Norway spruce (*Picea abies*) plants. *Forest Ecology and Management*, 491: 119180. doi: https://doi.org/10.1016/j.foreco.2021.119180.

Oliva, J., Rommel, S., Fossdal, C. G., Hietala, A. M., Nemesio-Gorriz, M., Solheim, H. & Elfstrand, M. (2015). Transcriptional responses of Norway spruce (*Picea abies*) inner sapwood against *Heterobasidion parviporum*. *Tree Physiology*, 35 (9): 1007-1015. doi: 10.1093/treephys/tpv063.

Pastor, V., Luna, E., Mauch-Mani, B., Ton, J. & Flors, V. (2013). Primed plants do not forget. *Environmental and Experimental Botany*, 94: 46-56.

Pastor, V., Balmer, A., Gamir, J., Flors, V. & Mauch-Mani, B. (2014). Preparing to fight back: generation and storage of priming compounds. *Frontiers in Plant Science*, 5: 295.

Plattner, A., Kim, J.-J., DiGuistini, S. & Breuil, C. (2008). Variation in pathogenicity of a mountain pine beetle–associated blue-stain fungus, Grosmannia clavigera, on young lodgepole pine in British Columbia. *Canadian Journal of Plant Pathology*, 30 (3): 457-466. doi: 10.1080/07060660809507543.

Repe, A., Bojović, S. & Jurc, M. (2015). Pathogenicity of ophiostomatoid fungi on *Picea abies* in Slovenia. *Forest Pathology*, 45 (4): 290-297. doi: https://doi.org/10.1111/efp.12170.

Roach, C., Hall, D., Zerbe, P. & Bohlmann, J. (2014). Plasticity and Evolution of (-)-3-Carene Synthase and (-)-Sabinene Synthase Functions of a Sitka Spruce Monoterpene Synthase Gene Family Associated with Weevil Resistance. *The Journal of Biological Chemistry*, 289. doi: 10.1074/jbc.M114.571703.

Rocchini, L. A., Lindgren, B. S. & Bennett, R. G. (2000). Effects of resin flow and monoterpene composition on susceptibility of lodgepole pine to attack by the Douglas-fir pitch moth, Synanthedon novaroensis (Lep., Sesiidae). *Journal of Applied Entomology*, 124 (2): 87-92. doi: https://doi.org/10.1046/j.1439-0418.2000.00449.x.

Rosenthal, G. A. & Janzen, D. H. (1979). *Herbivores their interaction with secondary plant metabolites*: Academic Press, Inc.

Sampedro, L., Moreira, X. & Zas, R. (2011). Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology*, 99 (3): 818-827. doi: https://doi.org/10.1111/j.1365-2745.2011.01814.x.

Scalschi, L., Vicedo, B., Camañes, G., Fernandez-Crespo, E., Lapeña, L., González-Bosch, C. & García-Agustín, P. (2013). Hexanoic acid is a resistance inducer that protects tomato plants against *P. seudomonas syringae* by priming the jasmonic acid and salicylic acid pathways. *Molecular Plant Pathology*, 14 (4): 342-355.

Schiebe, C., Hammerbacher, A., Birgersson, G., Witzell, J., Brodelius, P. E., Gershenzon, J., Hansson, B. S., Krokene, P. & Schlyter, F. (2012). Inducibility of chemical defenses in Norway spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle. *Oecologia*, 170 (1): 183-198. doi: 10.1007/s00442-012-2298-8.

Schmidt, A. & Gershenzon, J. (2007). Cloning and characterization of isoprenyl diphosphate synthases with farnesyl diphosphate and geranylgeranyl diphosphate synthase activity from Norway spruce (*Picea abies*) and their relation to induced oleoresin formation. *Phytochemistry*, 68 (21): 2649-2659. doi: https://doi.org/10.1016/j.phytochem.2007.05.037.

Schmidt, A., Nagel, R., Krekling, T., Christiansen, E., Gershenzon, J. & Krokene, P. (2011). Induction of isoprenyl diphosphate synthases, plant hormones and defense signalling genes correlates with traumatic resin duct formation in Norway spruce (*Picea abies*). *Plant Molecular Biology*, 77 (6): 577-590. doi: 10.1007/s11103-011-9832-7.

Shah, F., Allen, N., Wright, C. & Butt, T. (2007). Repeated in vitro subculturing alters spore surface properties and virulence of Metarhizium anisopliae. *FEMS microbiology letters*, 276: 60-6. doi: 10.1111/j.1574-6968.2007.00927.x.

Songe, M. M., Thoen, E., Evensen, Ø. & Skaar, I. (2014). In vitro passages impact on virulence of Saprolegnia parasitica to Atlantic salmon, Salmo salar L. parr. *Journal of fish diseases*, 37 (9): 825-834. doi: 10.1111/jfd.12175.

Tanner, G. J., Francki, K. T., Abrahams, S., Watson, J. M., Larkin, P. J. & Ashton, A. R. (2003). Proanthocyanidin Biosynthesis in Plants: PURIFICATION OF LEGUME LEUCOANTHOCYANIDIN REDUCTASE AND MOLECULAR CLONING OF ITS cDNA*. *Journal of Biological Chemistry*, 278 (34): 31647-31656. doi: https://doi.org/10.1074/jbc.M302783200.

Viiri, H., Annila, E., Kitunen, V. & Niemelä, P. (2001). Induced responses in stilbenes and terpenes in fertilized Norway spruce after inoculation with blue-stain fungus, *Ceratocystis polonica*. *Trees*, 15 (2): 112-122. doi: 10.1007/s004680000082.

Vivas, M., Martín, J., Gil, L. & Solla, A. (2012). Evaluating methyl jasmonate for induction of resistance to *Fusarium oxysporum*, *F. circinatum* and *Ophiostoma novo-ulmi*. *Forest Systems*, 21 (2): 289-299.

Waddington, C. H. (2012). The Epigenotype. *International Journal of Epidemiology*, 41 (1): 10-13. doi: 10.1093/ije/dyr184.

Wasternack, C. & Song, S. (2016). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, 68 (6): 1303-1321. doi: 10.1093/jxb/erw443.

Wasternack, C. & Song, S. (2017). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, 68 (6): 1303-1321.

Wilkinson, S. W., Magerøy, M. H., López Sánchez, A., Smith, L. M., Furci, L., Cotton, T. A., Krokene, P. & Ton, J. (2019). Surviving in a hostile world: plant strategies to resist pests and diseases. *Annual Review of Phytopathology*, 57: 505-529.

Wilkinson, S. W. (2020). *Investigating the Mechanisms of Long-Lasting Within Genertion Jasmonate Induced Resistance*: Ph.D Thesis. England, The University of Sheffield.

Xie, D.-Y., Sharma, S. B. & Dixon, R. A. (2004). Anthocyanidin reductases from Medicago truncatula and *Arabidopsis thaliana*. *Archives of Biochemistry and Biophysics*, 422 (1): 91-102. doi: https://doi.org/10.1016/j.abb.2003.12.011.

Xu, J. & Zhang, S. (2014). Regulation of Ethylene Biosynthesis and Signaling by Protein Kinases and Phosphatases. *Molecular Plant*, 7. doi: 10.1093/mp/ssu059.

Yaqoob, N., Yakovlev, I., Krokene, P., Kvaalen, H., Solheim, H. & Fossdal, C. G. (2012). Defence-related gene expression in bark and sapwood of Norway spruce in response to *Heterobasidion parviporum* and methyl jasmonate. *Physiological and Molecular Plant Pathology*, 77. doi: 10.1016/j.pmpp.2011.11.001.

Zas, R., Björklund, N., Nordlander, G., Cendán, C., Hellqvist, C. & Sampedro, L. (2014). Exploiting jasmonate-induced responses for field protection of conifer seedlings against a major forest pest, *Hylobius abietis*. *Forest Ecology and Management*, 313: 212-223. doi: https://doi.org/10.1016/j.foreco.2013.11.014.

Zeneli, G., Krokene, P., Christiansen, E., Krekling, T. & Gershenzon, J. (2006). Methyl jasmonate treatment of mature Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. *Tree Physiology*, 26 (8): 977-988.

Zhao, T., Borg-Karlson, A.-K., Erbilgin, N. & Krokene, P. (2011). Host resistance elicited by methyl jasmonate reduces emission of aggregation pheromones by the spruce bark beetle, *Ips typographus*. *Oecologia*, 167 (3): 691-699. doi: 10.1007/s00442-011-2017-x.

Zhao, T., Axelsson, K., Krokene, P. & Borg-Karlson, A.-K. (2015). Fungal Symbionts of the Spruce Bark Beetle Synthesize the Beetle Aggregation Pheromone 2-Methyl-3-buten-2-ol. *Journal of Chemical Ecology*, 41 (9): 848-852. doi: 10.1007/s10886-015-0617-3.

Zhao, T., Kandasamy, D., Krokene, P., Chen, J., Gershenzon, J. & Hammerbacher, A. (2019). Fungal associates of the tree-killing bark beetle, *Ips typographus*, vary in virulence, ability to degrade conifer phenolics and influence bark beetle tunneling behavior. *Fungal ecology*, 38: 71-79.

Zulak, K. G., Lippert, D. N., Kuzyk, M. A., Domanski, D., Chou, T., Borchers, C. H. & Bohlmann, J. (2009). Targeted proteomics using selected reaction monitoring reveals the induction of specific terpene synthases in a multi-level study of methyl jasmonate-treated Norway spruce (*Picea abies*). *The Plant Journal*, 60 (6): 1015-1030. doi: https://doi.org/10.1111/j.1365-313X.2009.04020.x.

Appendix

Appendix Table 1a. Mean concentrations (mg g^{-1} of dry weight ± SE) of phenolics quantified by HPLC analysis of bark tissues of Norway spruce (*Picea abies*) trees sampled 1 and 4 weeks after treatment with water and Tween (Control), methyl jasmonate (MeJA) or β-amino butyric acid (BABA). For each timepoint and phenolic compound, treatments with different letters in bold are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments, $p < 0.05$).

		1 week after treatment			4 weeks after treatment		
Type		BABA	Control	MeJA	BABA	Control	MeJA
Acetophenon	Picein	0.16 ± 0.06	0.32 ± 0.19	0.24 ± 0.07	0.18 ± 0.04 ab	**0.58 ± 0.20 a**	**0.12 ± 0.06 b**
Flavonoids	Gallocatechin	**4.06 ± 0.22 a**	3.05 ± 0.54 ab	**1.33 ± 0.58 b**	**1.59 ± 0.24 a**	1.13 ± 0.50 ab	**0.11 ± 0.08 b**
	Catechin	1.84 ± 0.34 ab	**1.56 ± 0.30 a**	**3.80 ± 0.91 b**	1.28 ± 0.22	1.41 ± 0.20	1.44 ± 0.57
	Dihydromyricetin 1	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
	Dihydromyricetin 2	0.12 ± 0.02	0.18 ± 0.06	0.20 ± 0.02	0.15 ± 0.02	0.19 ± 0.03	0.18 ± 0.06
	Dihydromyricetin 3	0.02 ± 0.01	0.03 ± 0.02	0.04 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02
	Dihydromyricetin 4	0.04 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
	Quercetin glycoside	0.65 ± 0.15	0.40 ± 0.12	0.72 ± 0.30	0.55 ± 0.12	0.76 ± 0.09	0.65 ± 0.15
	Monocoumaryl astragalin 1	0.17 ± 0.01	0.29 ± 0.14	0.18 ± 0.06	0.25 ± 0.03	0.34 ± 0.06	0.34 ± 0.05
	Monocoumaryl astragalin 2	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.02
	Dicoumaryl astragallin	2.07 ± 0.29	3.47 ± 1.22	3.43 ± 0.48	4.22 ± 0.62	4.39 ± 0.42	5.55 ± 0.71
Sum of flavonoids		8.99 ± 0.65	9.11 ± 1.39	9.84 ± 1.13	8.14 ± 0.48	8.33 ± 0.49	8.41 ± 0.97
Stilbenes	Piceatannol glycoside	4.12 ± 0.90	4.66 ± 0.79	3.16 ± 0.25	2.91 ± 0.59 ab	**4.69 ± 0.69 a**	**1.18 ± 0.45 b**
	Piceatannol aglycon	**0.12 ± 0.04 a**	0.08 ± 0.03 ab	**0.00 ± 0.00 b**	0.06 ± 0.03	0.08 ± 0.04	0.04 ± 0.02
	Resveratrol glycoside	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0008 ± 0.00 ab	**0.0014 ± 0.00 a**	**0.0003 ± 0.00 b**
	Iso-rhapontin glycoside	13.71 ± 3.82	13.01 ± 3.45	14.79 ± 1.36	11.61 ± 2.73 ab	**14.85 ± 3.77 a**	**4.07 ± 1.77 b**
	E-astringin	0.23 ± 0.07	0.30 ± 0.10	0.44 ± 0.09	0.19 ± 0.06	0.32 ± 0.12	0.09 ± 0.02
	Piceatannol	0.03 ± 0.02	0.02 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
	Unknown stilbene 1	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
	Unknown stilbene 2	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Sum of stilbenes		18.23 ± 4.70	18.13 ± 3.80	18.44 ± 1.52	14.85 ± 3.27 ab	**20.04 ± 4.11 a**	**5.47 ± 2.22 b**
Total of low-molecular-weight phenolic compounds		27.39 ± 5.37	27.56 ± 5.10	28.51 ± 2.25	23.17 ± 3.43 ab	**28.95 ± 4.25 a**	**14.00 ± 2.94 b**
MeOH-insoluble condensed tannins		**37.54 ± 4.49 a**	24.14 ± 3.45 ab	**22.89 ± 2.57 b**	**23.64 ± 2.76 b**	**42.00 ± 5.21 a**	**21.93 ± 2.62 b**
MeOH-soluble condensed tannins		22.14 ± 4.59	30.15 ± 2.71	34.37 ± 6.52	19.60 ± 4.49 ab	**27.00 ± 3.34 a**	**12.15 ± 4.29 b**

Appendix Table 1b. Mean concentrations (mg g^{-1} of dry weight ± SE) of phenolics quantified by HPLC analysis of bark tissues of Norway spruce (*Picea abies*) trees sampled 24 hours and 9 weeks after wounding and inoculation with *Grosmannia penicillata*. For each timepoint and phenolic compound, treatments with different letters in bold are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments, $p < 0.05$).

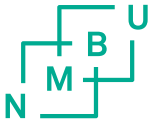
		24 hours after inoculation			9 weeks after inoculation		
Type		BABA	Control	MeJA	BABA	Control	MeJA
Acetophenon	Picein	0.14 ± 0.04	0.23 ± 0.06	0.17 ± 0.03	0.18 ± 0.05	0.11 ± 0.04	0.11 ± 0.02
Flavonoids	Gallocatechin	3.02 ± 0.82	1.80 ± 0.27	2.09 ± 0.48	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.30
	Catechin	**2.36 ± 0.18 a**	**1.96 ± 0.31 a**	**5.84 ± 0.80 b**	**2.42 ± 0.27 a**	**3.55 ± 0.49 a**	**7.13 ± 0.94 b**
	Dihydromyricetin 1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02	0.07 ± 0.03
	Dihydromyricetin 2	**0.13 ± 0.03 a**	0.18 ± 0.02 ab	**0.22 ± 0.03 b**	0.17 ± 0.01	0.14 ± 0.02	0.15 ± 0.01
	Dihydromyricetin 3	0.05 ± 0.01	0.03 ± 0.02	0.07 ± 0.02	**0.01 ± 0.01 a**	0.05 ± 0.01 ab	**0.06 ± 0.01 b**
	Dihydromyricetin 4	0.08 ± 0.03	0.06 ± 0.03	0.07 ± 0.03	0.10 ± 0.02	0.10 ± 0.03	0.08 ± 0.03
	Quercetin glycoside	0.33 ± 0.09	0.68 ± 0.11	0.62 ± 0.15	0.65 ± 0.15	0.36 ± 0.08	0.39 ± 0.08
	Monocoumaryl astragalin 1	0.24 ± 0.07	0.21 ± 0.06	0.29 ± 0.03	0.19 ± 0.08	0.08 ± 0.02	0.08 ± 0.03
	Monocoumaryl astragalin 2	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01
	Dicoumaryl astragallin	2.73 ± 0.56	3.73 ± 1.14	4.10 ± 0.61	2.63 ± 0.97	1.09 ± 0.28	1.29 ± 0.23
Sum of flavonoids		**8.95 ± 0.5 a**	**8.67 ± 1.24 a**	**13.33 ± 1.28 b**	6.19 ± 1.24 ab	**5.41 ± 0.74 a**	**9.57 ± 0.96 b**
Stilbenes	Piceatannol glycoside	3.83 ± 0.75	2.97 ± 0.26	4.09 ± 1.27	**5.32 ± 0.62 b**	**10.45 ± 1.7511 a**	8.55 ± 1.36 ab
	Piceatannol aglycon	0.00 ± 0.00	0.03 ± 0.03	0.07 ± 0.04	0.08 ± 0.03	0.15 ± 0.02	0.14 ± 0.03
	Resveratrol glycoside	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Iso-rhapontin glycoside	11.67 ± 1.34	10.83 ± 1.86	11.43 ± 2.64	18.44 ± 1.51	27.85 ± 3.74	24.09 ± 3.62
	E-astringin	**0.57 ± 0.09 b**	**0.32 ± 0.04 a**	**0.25 ± 0.05 a**	0.20 ± 0.05	0.29 ± 0.08	0.31 ± 0.10
	Piceatannol	0.03 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.02	0.12 ± 0.02	0.08 ± 0.01
	Unknown stilbene 1	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
	Unknown stilbene 2	**0.00 ± 0.00 a**	0.01 ± 0.01 ab	**0.02 ± 0.00 b**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sum of stilbenes		16.14 ± 2.11	14.22 ± 2.06	15.92 ± 3.80	24.15 ± 1.57	38.89 ± 5.29	33.20 ± 4.53
Total of low-molecular-weight phenolic compounds		25.22 ± 2.60	23.12 ± 3.16	29.43 ± 4.14	30.53 ± 1.20	44.40 ± 5.52	42.87 ± 4.01
MeOH-insoluble tannins		**38.02 ± 4.77 a**	29.61 ± 3.18 ab	**24.80 ± 1.99 b**	44.62 ± 4.76	30.46 ± 5.41	33.55 ± 1.83
MeOH-soluble tannins		**35.58 ± 1.06 b**	**29.25 ± 1.68 a**	**38.98 ± 1.89 b**	**45.49 ± 5.93 a**	56.95 ± 1.24 ab	**65.23 ± 3.35 b**

Appendix Table 2a. Mean concentrations (µg g^{-1} of dry weight ± SE) of terpenes quantified by GC-MS analysis of bark tissues of Norway spruce (*Picea abies*) trees sampled 1 and 4 weeks after treatment with water and Tween (Control), methyl jasmonate (MeJA) or β-amino butyric acid (BABA). For each timepoint and phenolic compound, treatments with different letters in bold are significantly different (1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments, $p < 0.05$).

Type	Compound	1 week after treatment			4 weeks after treatment		
		BABA	Control	MeJA	BABA	Control	MeJA
Monoterpenes	3-Carene	103.22 ± 50.11	20.77 ± 16.15	109.69 ± 55.42	262.22 ± 94.60	145.52 ± 95.28	169.45 ± 48.08
	α-Pinene	335.85 ± 109.54	261.45 ± 52.23	295.38 ± 98.59	398.89 ± 91.39	238.26 ± 47.91	259.72 ± 72.96
	β- Pinene	272.45 ± 91.65	307.43 ± 65.74	508.97 ± 203.65	537.91 ± 114.27	377.26 ± 88.29	315.64 ± 74.66
	β-Myrcene	27.49 ± 10.08	18.30 ± 4.82	20.01 ± 9.59	**50.45 ± 10.05 a**	25.21 ± 6.22 ab	**13.81 ± 5.13 b**
	β-Phellandrene	125.33 ± 51.14	95.24 ± 14.99	128.09 ± 46.50	**175.01 ± 36.89 a**	88.86 ± 18.52 ab	**51.14 ± 16.17 b**
	Camphene	**41.63 ± 14.74 a**	9.88 ± 4.87 ab	**3.50 ± 2.27 b**	28.60 ± 5.31	14.24 ± 3.93	28.30 ± 4.93
	Eucalyptol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.75 ± 1.02
	γ-Terpinene	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.60 ± 1.34	1.47 ± 1.05	0.52 ± 0.52
	Limonene	46.54 ± 20.03	36.40 ± 8.52	16.14 ± 7.43	84.47 ± 33.56	29.19 ± 9.15	35.21 ± 12.93
	p-Cymene	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	**0.00 ± 0.00 a**	**0.00 ± 0.00 a**	**2.76 ± 0.91 b**
	Sabinen	6.00 ± 3.10	0.97 ± 0.97	3.22 ± 3.22	17.62 ± 6.20	7.93 ± 5.21	10.70 ± 3.54
	Terpinolen	15.64 ± 6.02	1.99 ± 1.99	11.98 ± 6.48	44.50 ± 14.72	20.96 ± 13.03	10.10 ± 6.46
Total monoterpenes		974.15 ± 300.43	752.43 ± 143.18	1096.98 ± 368.87	1602.27 ± 246.93	948.9 ± 211.79	899.11 ± 202.38
Sesqui-	Germacrene D	137.34 ± 44.67	45.28 ± 6.73	80.20 ± 46.15	131.60 ± 65.81	36.94 ± 22.67	13.05 ± 6.10
	alfa-Gurjunene	19.85 ± 14.87	7.27 ± 3.83	9.18 ± 4.40	14.81 ± 6.47	7.32 ± 3.21	3.83 ± 1.43
	alfa-Longipinene	7.68 ± 7.68	22.52 ± 7.78	19.48 ± 8.48	18.78 ± 7.40	14.09 ± 7.95	22.82 ± 6.75
Total sesquiterpenes		164.86 ± 38.86	75.07 ± 10.08	108.85 ± 45.77	165.19 ± 65.11	58.35 ± 23.91	39.70 ± 7.27
Di-	Thunbergene	153.59 ± 29.45	115.77 ± 21.54	208.10 ± 89.14	68.24 ± 27.94	70.20 ± 22.19	36.21 ± 8.78
	Verticiol	99.86 ± 19.22	77.86 ± 13.89	117.65 ± 71.87	38.64 ± 16.48	44.96 ± 15.70	8.04 ± 5.40
Total diterpenes		253.45 ± 48.58	193.64 ± 35.33	325.75 ± 158.23	106.89 ± 44.19	115.16 ± 36.24	44.25 ± 12.34
Total terpene		1392.46 ± 307.91	1021.13 ± 181.08	1531.58 ± 525.43	**1874.35 ± 287.54 a**	1122.42 ± 219.06 ab	**983.06 ± 206.15 b**

Appendix Table 2b. Mean concentrations (µg g^{-1} of dry weight ± SE) of terpenes quantified by GC-MS analysis of bark tissues of Norway spruce (*Picea abies*) trees sampled 24 hours and 9 weeks after wounding and inoculation with *Grosmannia penicillata*. 1-way ANOVA followed by a Tukey HSD post hoc test for pairwise comparisons between treatments showed no significant result ($p > 0.05$).

Type	Compound	24 hours after inoculation			9 weeks after inoculation		
		BABA	Control	MeJA	BABA	Control	MeJA
Mono-	3-Carene	131.42 ± 105.19	121.18 ± 51.39	126.60 ± 61.13	84.96 ± 62.23	654.14 ± 286.80	222.45 ± 84.86
	α-Pinene	352.30 ± 94.65	356.04 ± 70.41	400.79 ± 26.20	445.84 ± 91.08	540.33 ± 84.99	588.91 ± 91.98
	β- Pinene	516.63 ± 149.79	651.34 ± 224.42	477.46 ± 122.74	651.55 ± 47.36	887.60 ± 180.06	989.07 ± 193.53
	β-Myrcene	66.11 ± 19.06	54.70 ± 15.44	46.18 ± 5.98	76.30 ± 15.96	131.23 ± 35.71	87.94 ± 12.09
	β-Phellandrene	223.28 ± 78.44	189.21 ± 33.26	141.88 ± 37.10	310.08 ± 50.21	437.06 ± 94.57	303.82 ± 37.95
	Camphene	22.17 ± 8.07	25.09 ± 7.22	27.89 ± 2.51	22.93 ± 6.98	18.04 ± 3.38	29.29 ± 6.36
	Eucalyptol	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.30 ± 1.30
	γ-Terpinene	1.59 ± 1.59	0.99 ± 0.71	0.75 ± 0.75	0.94 ± 0.94	12.56 ± 5.84	3.55 ± 1.46
	Limonene	120.02 ± 73.99	58.03 ± 13.11	139.23 ± 44.87	59.18 ± 27.4	210.80 ± 69.50	95.17 ± 25.66
	p-Cymene	0.00 ± 0.00	0.00 ± 0.00	0.29 ± 0.29	0.00 ± 0.00	1.18 ± 0.85	0.82 ± 0.41
	Sabinen	11.85 ± 7.14	7.94 ± 4.99	11.14 ± 4.03	9.22 ± 3.78	62.31 ± 27.83	22.79 ± 7.81
	Terpinolen	23.22 ± 13.00	20.22 ± 8.58	21.17 ± 10.96	20.58 ± 7.39	138.11 ± 57.00	41.22 ± 12.73
Total monoterpene		1468.59 ± 394.91	1484.76 ± 400.08	1393.39 ± 127.49	1681.58 ± 221.12	3093.35 ± 797.77	2386.33 ± 316.99
Sesqui-	Germacrene D	94.43 ± 47.47	143.98 ± 78.03	80.30 ± 26.79	371.42 ± 252.44	372.67 ± 134.67	341.77 ± 146.9
	α-Gurjunene	21.04 ± 5.35	19.54 ± 6.81	22.76 ± 5.44	15.85 ± 6.35	26.78 ± 7.77	15.62 ± 6.62
	α-Longipinene	25.36 ± 9.19	21.00 ± 5.96	27.92 ± 6.57	21.48 ± 9.43	44.18 ± 23.64	31.61 ± 9.85
Total sesquiterpene		140.83 ± 36.55	184.52 ± 79.22	130.98 ± 32.01	408.76 ± 251.97	443.63 ± 145.71	389.01 ± 147.80
Di-	Thunbergene	129.19 ± 17.68	115.54 ± 26.43	108.02 ± 24.46	111.90 ± 40.71	101.37 ± 34.15	60.12 ± 22.12
	Verticiol	64.58 ± 10.31	41.74 ± 19.17	51.07 ± 13.98	55.62 ± 20.45	51.31 ± 19.43	0.00 ± 0.00
Total diterpene		193.78 ± 27.64	157.28 ± 36.96	159.09 ± 37.91	167.52 ± 60.70	152.67 ± 53.34	60.12 ± 22.12
Total terpene		1803.19 ± 348.49	1826.56 ± 406.86	1683.46 ± 117.06	2257.85 ± 408.10	3689.65 ± 856.94	2835.45 ± 420.80



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway