White Rose Research Online

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/160092/

Version: Published Version

Article:
Mageroy, M.H., Wilkinson, S.W., Tengs, T. et al. (2020) Molecular underpinnings of methyl jasmonate-induced resistance in Norway spruce. Plant, Cell & Environment, 43 (8). pp. 1827-1843. ISSN: 0140-7791

https://doi.org/10.1111/pce.13774

Reuse
This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Check for updates

Received: 17 December 2019 | Accepted: 15 April 2020

DOI: 10.1111/pce.13774

ORIGINAL ARTICLE

Plant, Cell & Environment

WILEY

Molecular underpinnings of methyl jasmonate-induced resistance in Norway spruce

Melissa H. Mageroy[1] | Samuel W. Wilkinson[2] | Torstein Tengs[1,3] | Hugh Cross[1,4] | Marit Almvik[1] | Pierre Pétriacq[2,5] | Adam Vivian-Smith[1] | Tao Zhao[6] | Carl Gunnar Fossdal[1] | Paal Krokene[1]

[1]Molecular plant biology and Forest Genetics and biodiversity, Norwegian Institute of Bioeconomy Research, Ås, Norway

[2]P[3] Centre for Translational Plant and Soil Biology, Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

[3]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway

[4]Department of Anatomy, University of Otago, Dunedin, New Zealand

[5]UMR 1332 BFP, INRA, University of Bordeaux, MetaboHUB-Bordeaux, MetaboHUB, PHENOME-EMPHASIS, Villenave d'Ornon, France

[6]School of Science and Technology, Örebro University, Örebro, Sweden

Correspondence
Melissa H. Mageroy and Paal Krokene, Molecular plant biology and Forest Genetics and biodiversity, Norwegian Institute of Bioeconomy Research, Ås, Norway.
Email: melissa.mageroy@nibio.no (M. H. M.) and
Email: paal.krokene@nibio.no (P. K.)

Funding information
The Research Council of Norway, Grant/Award Numbers: 249920, 249958/F20

Abstract

In response to various stimuli, plants acquire resistance against pests and/or pathogens. Such acquired or induced resistance allows plants to rapidly adapt to their environment. Spraying the bark of mature Norway spruce (*Picea abies*) trees with the phytohormone methyl jasmonate (MeJA) enhances resistance to tree-killing bark beetles and their associated phytopathogenic fungi. Analysis of spruce chemical defenses and beetle colonization success suggests that MeJA treatment both directly induces immune responses and primes inducible defenses for a faster and stronger response to subsequent beetle attack. We used metabolite and transcriptome profiling to explore the mechanisms underlying MeJA-induced resistance in Norway spruce. We demonstrated that MeJA treatment caused substantial changes in the bark transcriptional response to a triggering stress (mechanical wounding). Profiling of mRNA expression showed a suite of spruce inducible defenses are primed following MeJA treatment. Although monoterpenes and diterpene resin acids increased more rapidly after wounding in MeJA-treated than control bark, expression of their biosynthesis genes did not. We suggest that priming of inducible defenses is part of a complex mixture of defense responses that underpins the increased resistance against bark beetle colonization observed in Norway spruce. This study provides the most detailed insights yet into the mechanisms underlying induced resistance in a long-lived gymnosperm.

KEYWORDS

defense priming, epigenetics, gymnosperm, induced resistance, jasmonic acid, *Picea abies*, terpenes, transcriptomics

1 | INTRODUCTION

Long-lived Norway spruce (*Picea abies*) trees encounter a wide range of environmental conditions and challenges throughout their lifetime. The ability of this economically and ecologically important gymnosperm species to adapt to change and to defend itself against most biotic and abiotic stresses, has helped it dominate European boreal and temperate forest ecosystems. However, due to rapid climate

This is an open access article under the terms of the Creative Commons Attribution?NonCommercial?NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non?commercial and no modifications or adaptations are made.
© 2020 The Authors. *Plant, Cell & Environment* published by John Wiley & Sons Ltd.

change, Norway spruce is expected to face more extreme weather events and higher frequencies of pest outbreaks (Hlásny et al., 2019; Marini et al., 2017; Mezei et al., 2017). Therefore, understanding the mechanisms by which Norway spruce adapts to environmental change and stresses is of great importance.

The enhancement of plant basal resistance, known as induced or acquired resistance, is an important defense strategy that allows plants to survive in hostile environments (Wilkinson et al., 2019). Defense priming is one of two mechanisms behind this phenomenon and provides increased resistance without incurring high fitness costs (Martinez-Medina et al., 2016; Wilkinson et al., 2019). Plant defenses can be primed following the exposure to specific stimuli, such as chemical elicitors, beneficial microbes, localized pathogen attack and wounding (Mauch-Mani, Baccelli, Luna, & Flors, 2017). Following the onset of priming, defenses are maintained at basal levels during stress-free conditions, but upon an attack (a 'triggering stress') defenses are upregulated faster and stronger, resulting in enhanced resistance (Martinez-Medina et al., 2016). Over the past 20 years, defense priming has been well studied in short-lived angiosperms, and this research has led to a better understanding of the molecular mechanisms underpinning the onset, maintenance and triggering of primed defense responses (Conrath, 2011; Conrath, Pieterse, & Mauch-Mani, 2002). Mechanisms that have been suggested to maintain a primed state within a plant include chromatin modifications and the accumulation of conjugated defense hormones or inactive signalling proteins (Beckers et al., 2009; Conrath, Beckers, Langenbach, & Jaskiewicz, 2015; Jaskiewicz, Conrath, & Peterhälnsel, 2011; Pastor, Balmer, Gamir, Flors, & Mauch-Mani, 2014; Schillheim et al., 2018).

In gymnosperms, defense priming has only recently been conceptualized (Krokene, 2015; Mageroy et al., 2020; Zhao, Borg-Karlson, Erbilgin, & Krokene, 2011). Methyl jasmonate (MeJA), the methylated form of the plant hormone jasmonic acid (JA), has been shown to induce long-term resistance (i.e., lasting weeks to months) in Norway spruce against both the tree-killing bark beetle *Ips typographus* and the bluestain fungi that it vectors (Erbilgin, Krokene, Christiansen, Zeneli, & Gershenzon, 2006; Mageroy et al., 2020; Zeneli, Krokene, Christiansen, Krekling, & Gershenzon, 2006). MeJA is known to induce anatomical defense responses, such as the formation of traumatic resin ducts (cavities filled with resinous terpenes), and the production of defensive chemicals, such as terpenes and polyphenolics (Fossdal, Nagy, Johnsen, & Dalen, 2007; Franceschi, Krekling, & Christiansen, 2002; Martin, Tholl, Gershenzon, & Bohlmann, 2002). However, more recent evidence has shown that although MeJA-induced resistance may persist for months, defense-related terpenes return to near basal levels within a few weeks (Mageroy et al., 2020; Zhao et al., 2011; Zulak et al., 2009). The observation of long-term acquired resistance in spruce without a persistent maintenance of upregulated defenses suggests that in addition to directly inducing defenses MeJA may also prime inducible defenses (Mageroy et al., 2020).

Almost nothing is known about the molecular mechanisms underpinning the onset, maintenance and triggering of primed defense responses in Norway spruce. While we may expect various priming mechanisms and gene regulatory networks to be conserved across the plant kingdom, some will likely differ between angiosperms and long-lived conifers due to the different life strategies and 400 million years of evolutionary separation of these plant lineages. The aim of this study was to explore the molecular aspects of defense priming in Norway spruce using transcriptomic and metabolite profiling. More specifically, our objectives were to (a) understand how the basal and wound-induced transcriptomes of Norway spruce trees change in response to MeJA treatment, (b) identify the primed defense mechanisms which may underpin the MeJA-induced resistance observed against *I. typographus* and its associated bluestain fungi, and (c) explore the mechanisms which maintain the primed state in Norway spruce.

2 | METHODS

2.1 | Plant material and field procedures

Six trees from a single clone (no. 137) were selected from a stand at the Hogsmark Experimental Farm in Ås, SE Norway (59°40′04.1″ N 10°42′46.2″ E). The trees were vegetatively propagated from cuttings and planted out in 1970 (Franceschi, Krekling, Berryman, & Christiansen, 1998). On 30 April 2013, a 3 m section of the lower stem (0.5–3.5 m height) of each tree was gently brushed with a plastic scrub brush to remove debris and loose bark scales. The same day, three trees were sprayed with a solution of 100 mM methyl jasmonate (MeJA) and 0.1% Tween, and three trees were sprayed with a solution of only 0.1% Tween (control) (Figure 1). Four weeks later, small areas of bark on all trees were mechanically wounded to elicit-induced defenses. The rest of the bark area was left intact. Wounding was done by puncturing the bark to the cambium with a push-pin about 30 times inside four 10 mm × 10 mm areas evenly distributed around the stem circumference at about 1.75 m height (Figure 1). This gave four different treatment combinations: MeJA-treated and wounded (MW), MeJA-treated and intact (MI), control and wounded (CW), and control and intact (CI) (Figure 1).

Twenty-four hours after wounding, bark samples for chemical and molecular analysis were collected from trees treated with MeJA and control trees using a 10-mm cork borer. Bark plugs of wounded bark were collected at the four wounded areas around the stem (Figure 1). Plugs of intact bark were collected at four sites situated about 18 cm below and 45° to the left of each wounded area. This sampling technique has been used in a previous study that showed induced defenses in spruce do not spread much in the tangential direction (Erbilgin et al., 2006). Each plug was split in two immediately after sampling and four half plugs of each kind were pooled into one sample and wrapped in aluminium foil (Figure 1). One sample of half plugs was used for chemical analysis and one sample was used for molecular analysis. The chemical samples were kept on dry ice and the molecular samples were kept in liquid N in the field. All the samples were moved to a −80°C freezer within 3 hr.

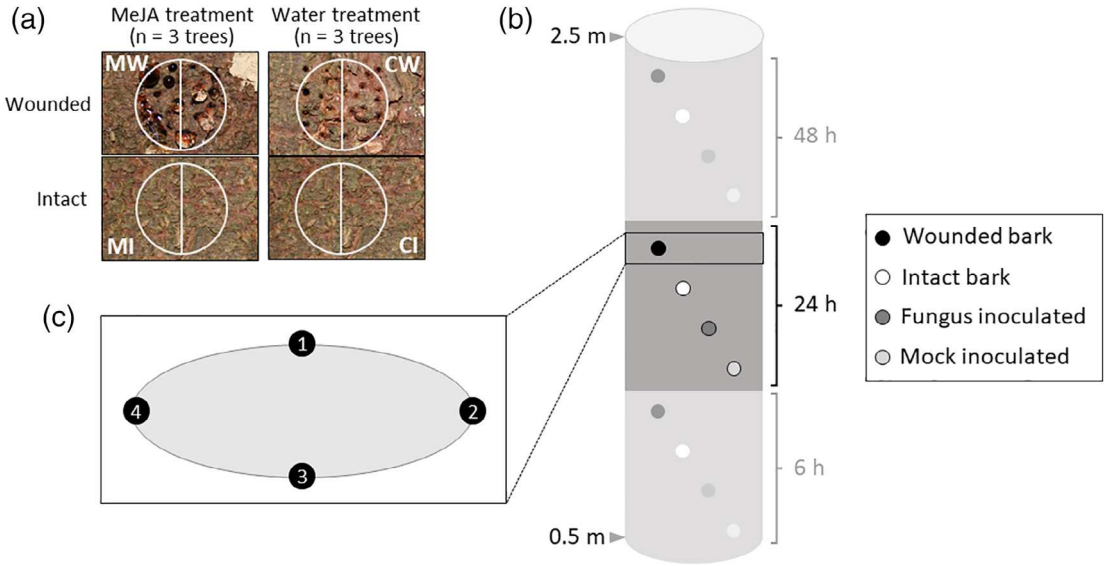


FIGURE 1 Overview of experimental design and sampling. (a) Three Norway spruce trees were sprayed with methyl jasmonate on the lower stem and three trees were sprayed with water (control). Four weeks later, small areas of bark on all trees were wounded while the rest of the bark area was left intact. This gave four treatment combinations: MeJA-treated and wounded bark, MeJA-treated and intact bark, control and wounded bark, and control and intact bark. After 24 hr, bark plugs were sampled from all treatment combinations using a cork borer. Each bark plug was split in two, and four half plugs (see panel [c]) from each treatment combination were pooled into one sample for chemical analysis and one sample for transcriptome analysis. (b) There were three similar blocks of treatments on the lower stem of all six trees. These blocks were sampled at different times after wounding, but only the 24 hr samples were included in this study. In each block, there were sampling sites for wounded bark, intact bark and bark inoculated with fungus or mock (the latter two were not used in this study). (c) Each set of samples (wounded, intact, and so forth) consisted of four sampling sites evenly distributed around the stem circumference

2.2 | Monoterpene analysis

Monoterpene extractions and quantification were performed as described by Mageroy et al. (2020). Specifically, the outer cork bark was removed from the bark plugs, and the phloem was submerged in 1.0 ml of hexane containing 0.25 mg pentadecane (Lancaster synthesis, England) as an internal standard and 0.19 mg 3-tert-butyl-4-hydroxyanisole (Fluka, Switzerland) as antioxidant. The samples were extracted in hexane at room temperature for 48 hr before the extract was transferred to new vials and kept at −25°C until analyses. The phloem plugs were dried at 80°C for 6 hr, and then weighted on a Sartorius electronic balance for absolute amount calculation.

The hexane extracts were separated, identified and quantified using a 2DGC-MS system that consisted of two Agilent 7890 A gas chromatographs (GCs), each GC combined with a 5975C inert mass-selective detection with triple axis detector. A split, splitless injector was used with a 30 s splitless injection at 225°C. The first GC was equipped with a HP-5 capillary column (Agilent, 30 m, 0.25 mm inner diameter [id], 0.25 μm film thickness) (Agilent Technologies, CA) to separate the substances in the extract. The interesting peaks were then cut from the first GC to the second GC, which was equipped with a β-cyclodextrin column (J&W Scientific, 30 m, 0.25 mm i.d., 0.25 μm film thickness, Folsom, California) for chiral separation. The sample (1 ml) was introduced to the injector and analyzed by the following temperature program: 40°C for 3 min, increasing to 260°C at a rate of 4°C/min, and remaining constant at 260°C for 10 min. The interesting peaks were individually cut to the second GC and separated by a temperature program of 50°C for 10 min, increasing to 90°C at a rate of 1°C/min, to 220°C at a rate of 10°C/min and kept at 220°C for 5 min. Helium was used as the carrier gas at a flow of 1 ml/min. Electron ionization mass spectra were acquired at 70 eV with the ion source at 150°C and a mass range of 30–400 Da. The compounds were identified by comparing retention times (RTs) and mass spectra with available authenticated standards, or by comparing retention indexes and mass spectra with reference libraries of National Institute of Standards and Technology. The amounts of terpenes were calculated relative to the internal standard and expressed as mg/g dry weight equivalent to the mass of pentadecane.

2.3 | Diterpene resin acid analysis

The diterpene resin acid extraction protocol was based on methods described by Kersten, Kopper, Raffa, and Illman (2006). Bark samples were extracted overnight in 10 μl/mg tissue methanol with shaking. Extracts were diluted 1:100 in methanol. Diterpene resin acids were quantified using LC-Q-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). An extract volume of 1 μl was injected and the resin acids separated on a UHPLC Dionex Ultimate 3000XRS (Thermo Scientific, San Jose, CA) with a Phenomenex Kinetex F5 separation column (100 mm × 2.1 mm, 1.7 μm particle diameter) at 30°C. Mobile phase

was 67% methanol in water (pH 2.6), both acidified with 0.1% formic acid, isocratic at a flow of 0.3 ml/min. The compounds were ionized to $[M+H]^+$ cations using HESI II electrospray ionization and the following tune settings: sheath gas flow rate 50; aux gas flow rate 10; spray voltage 3.50 kV; capillary temperature 300°C; S-lens RF level 50; heater temperature 350°C. The ions were collected with targeted SIM acquisition parameters at a resolution of 70,000 Full width at half maximum (FWHM), automatic gain control (AGC) target 5×10^4 and maximum ion injection time (IT) of 50 ms. The targeted ions were fragmented (ddMS2) at a resolution of 17,500 FWHM, collision energy NCE 28 (40 for dehydroabietic acid), AGC target 5×10^4, max IT 50 ms, isolation window 1.0 *m/z*, loop count 2, intensity threshold at 5.0×10^3 and apex trigger at 6–7 s with dynamic exclusion for 6.0 s. The MS was targeted on $C_{20}H_{28}O_2$ from 3 to 8 min and on $C_{20}H_{30}O_2$ from 8 to 13.5 min. Reference standards of the diterpene resin acids abietic acid, neoabietic acid, levopimaric acid, palustric acid and dehydroabietic acid were purchased from Santa Cruz Biotechnology (Dallas, Texas). The organic acids were mixed in methanol in the range 0.05–50 μg/ml. RTs were 6.1 min. For dehydroabietic acid, 9.4 min for neoabietic acid, 10.0 min for levopimaric acid, 10.5 min for palustric acid and 11.0 min for abietic acid. The mass spectrometry (MS)-data were processed with the Tracefinder 4.1 software. Identification criteria were RT match, precursor ion exact *m/z* within 2 ppm and presence of three targeted product ions produced by fragmentation of the precursor ion. An in-house library of product ion spectra (MS2) for the resin acids aided in the identification. Quantification was based on the peak height of the precursor ions. Limit of quantification was 0.05 μg/ml.

2.4 | Hormone analysis

The same bark extractions used for diterpene resin acid analysis were used for hormone analysis. Two microliters of 100 mM 3-ethoxy-4-hydroxybenzaldehyde were added to a 200 μl aliquot of stock methanol extract as an internal standard. The samples were dried under inert nitrogen for transport purposes. Samples were resuspended in 200 μl of liquid-chromatography mass spectrometry (LCMS) grade methanol. These stocks were then diluted 1:10 by placing 50 μl of stock in a new vial and adding 450 μl of methanol. Ultra Performance Liquid Chromatography (UPLC)-Quadrupole Time-of-Flight (qTOF) MS/MS analyses and relative quantification of hormones was performed according to Wilkinson, Pastor, Paplauskas, Pétriacq, and Luna (2018) and Pétriacq, Stassen, and Ton (2016). Briefly, UPLC-qTOF MS/MS analyses were conducted on an ACQUITY UPLC system coupled to a SYNAPT G2 qTOF mass spectrometer equipped with an electrospray (ESI) source (Waters, UK). An ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm, Waters) with a precolumn (VanGuard, 2.1 mm × 5 mm, 1.7 μm, Waters) and a solvent gradient from 95% water with formic acid (0.05% vol/vol) to 100% acetonitrile with formic acid (0.05% vol/vol) was used for separation. Detection with SYNAPT G2 was performed using both −ESI and +ESI. The following extracted ions were used for identification of hormones: JA (−)209, salicylic acid (SA) (−)137, indole-3-acetic acid (IAA) (+)176, JA-isoleucine (JA-ILE) (−)322.

2.5 | RNA extraction

The cork bark was removed from the bark samples and the phloem was ground in liquid nitrogen. RNA was purified from phloem powder using Epicentre MasterPure Plant RNA Purification Kit (Epicentre, Madison, WI; kit now replaced with MasterPure Complete DNA and RNA Purification Kit, Lucigene, Middleton, WI) according to the manufacturer's instructions. Contaminating DNA was removed from the total RNA samples using the above-mentioned kit, according to the supplier's protocol. The quantity of total RNA was assessed using a NanoDrop 2000 Spectrophotometer (Thermo Scientific). Total RNA preparations were then stored at −80°C until library preparation.

2.6 | Library preparations and RNA-sequencing analysis

Total RNA was sent to the Norwegian High-Throughput Sequencing Centre and sequenced with an Illumina HiSeq 2500 (Illumina Inc., San Diego, CA). A total of 12 separately barcoded libraries were made with the Illumina TruSeq RNA Library Prep Kit v2 (Illumina Inc.): three each from control intact (CI), control wounded (CW), MeJA-treated intact (MI) and MeJA-treated wounded (MW). The barcoded libraries were run over seven lanes. Sequence quality was assessed using the software FastQC (version 10-01-18; https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). A reference transcriptome (Pabies1.0-all-cds.fna) was downloaded from the PlantGenIE.org website (Sundell et al., 2015). The transcriptome file was processed by removing identical entries and sequences where all three reading frames resulted in the formation of stop codons (Supporting Information S1). The final database contained 63,522 sequences (minimal length: 150 bp). Illumina reads were mapped to the curated transcriptome using the software rsem (version 1.3.0) with the bowtie2 option (Li & Dewey, 2014). Data were normalized and analyzed for differentially expressed genes using the edgeR package in R (version 3.4.4) (McCarthy, Chen, & Smyth, 2012; Robinson, McCarthy, & Smyth, 2010). Normalization for unequal numbers of reads in sequence libraries was done using the trimmed mean of *M* values method and *p*-values were adjusted using The false discovery rate (FDR) method described by Benjamini & Hochberg (1995). The alpha parameter for threshold of statistical significance was set to 0.01. Transcripts were annotated using BLASTP (v 2.8.1) and the swissprot database (Camacho et al., 2009).

2.7 | Pfam enrichment analysis

Protein family (Pfam) assignments of amino acid sequences were performed using hmmscan, part of the HMMER software suite for protein sequence analysis, and the Pfam dataset (version 32.0) (Eddy, 2009; El-Gebali et al., 2019). Pfam enrichment analysis were performed in R (version 3.5.0) using bc3net (de Matos Simoes & Emmert-Streib, 2016).

FDR was determined using the Benjamini–Hochberg approach. An FDR adjusted *p*-value of .05 was used as the cutoff for significance.

3 | RESULTS

To gain a deeper understanding of the effects of methyl jasmonate (MeJA) treatment on Norway spruce defense responses, we sprayed the trunk of six large clonal spruce trees growing in the same forest plot with MeJA or a water control (three trees per treatment). After 4 weeks, small areas of bark on each tree were wounded to elicit inducible defenses. The rest of the bark area was left intact. This resulted in four treatment combinations: MI, MW, control (water-treated) and intact (CI), control (water-treated) and wounded (CW) (Figure 1). Twenty-four hours after wounding, bark plugs of wounded and intact bark were collected from all trees; half of each plug was used for chemical analyses and the other half for transcriptome analysis.

3.1 | Confirmation of the defense priming phenotype using metabolite analysis

Resinous terpenoids are known to be an important part of the conifer defense repertoire and thus help to provide resistance against disease and many pests, including *I. typographus*. Previous studies showed

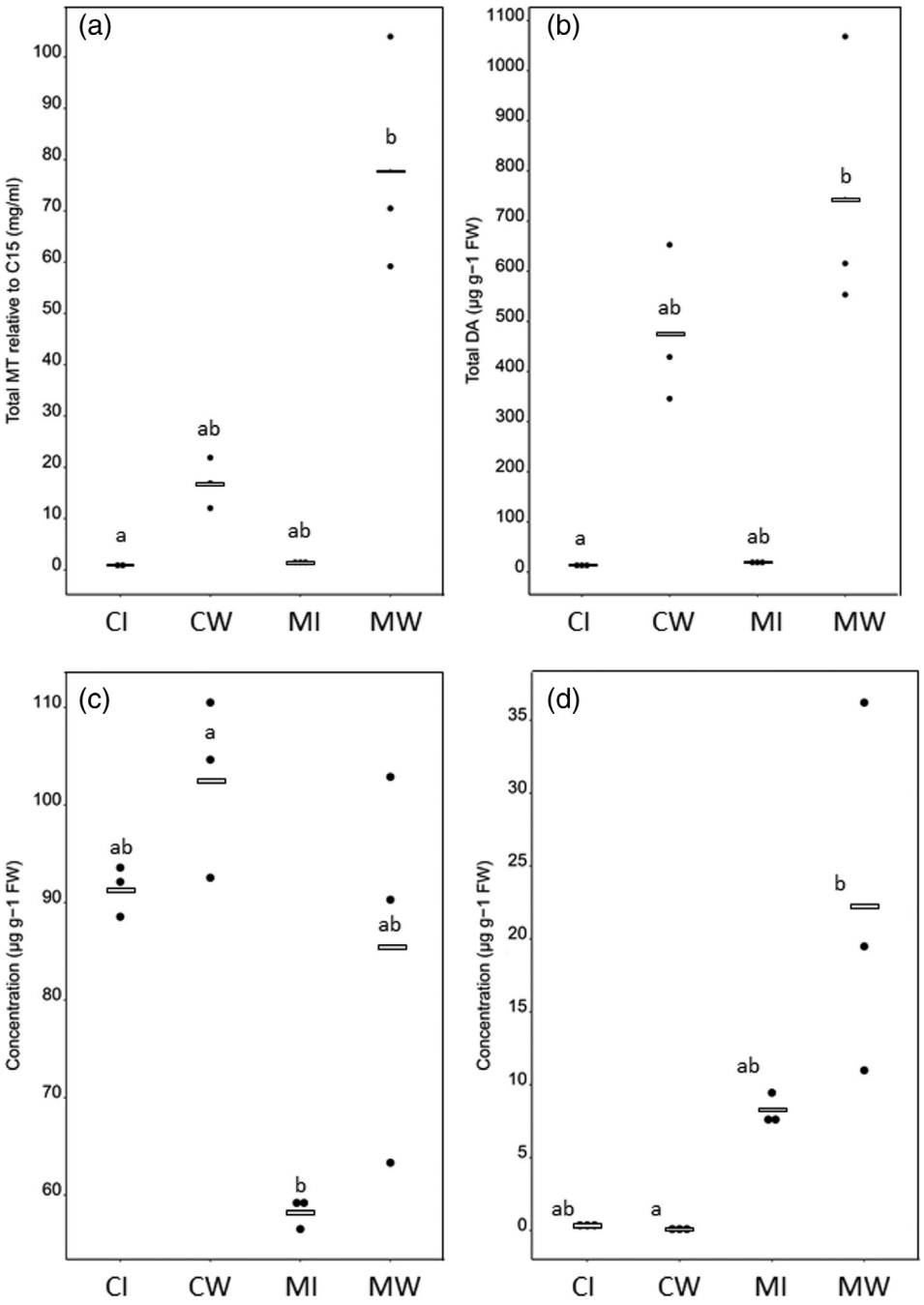


FIGURE 2 Metabolite analysis of Norway spruce bark. Bark tissue harvested 24 hr after wounding was extracted for monoterpenes (MT), diterpene resin acids (DA) or the phytohormones salicylic acid (SA) and jasmonic acid (JA). (a) The total MT concentration was determined using gas-chromatography mass spectrometry (GCMS) (n = 3 except for CI n = 2). (b) The total concentration five DA was determined using liquid-chromatography mass spectrometry (LCMS) (n = 3). The concentrations of (c) SA and (d) JA were quantified with LCMS (n = 3). Black points represent individual replicates. Grey bars represent the mean of each treatment. Significance was determined using a Kruskal–Wallis followed by a Dunn test. Letters represent significant difference between treatments (false discovery rate-adjusted p-value <.05)

that while MeJA treatment alone did not cause any sustained changes in terpene levels in Norway spruce bark, wounding of MeJA-treated bark triggered accumulation of much higher levels of terpenes than in trees that were only wounded (Mageroy et al., 2020; Zhao et al., 2011). Similarly, we observed that 4 weeks after MeJA treatment concentrations of monoterpenes and diterpene resin acids in the intact bark (MI) were similar to those in bark from untreated trees (CI) (Figure 2a,b). However, in response to wounding, MW bark accumulated significantly higher amounts of monoterpenes and diterpene resin acids than CI bark (Figure 2a,b).

SA and JA are plant hormones which are known to be important for the regulation of defense responses (Pieterse et al., 2014). We, therefore, quantified the level of these hormones across the four treatments combinations using LCMS. SA levels were significantly lower in MI bark than in CW bark, while levels in MW and CI were not significantly different from any other treatment (Figure 2c). In contrast, JA levels were significantly higher in MW bark than in CW and CI bark, while JA levels in MI were not significantly different from any other treatment (Figure 2d).

3.2 | Global impact of methyl jasmonate treatment on basal and wound-induced transcriptomes

Transcriptomes of the four treatment combinations were generated using mRNA sequencing (RNA-seq) (Figure 1). A principle component analysis (PCA) indicated that the three biological replicates of the four treatment groups were consistent and that there was a strong effect of both the MeJA treatment and wounding on the spruce transcriptome (Figure 3a). We then conducted pairwise comparisons

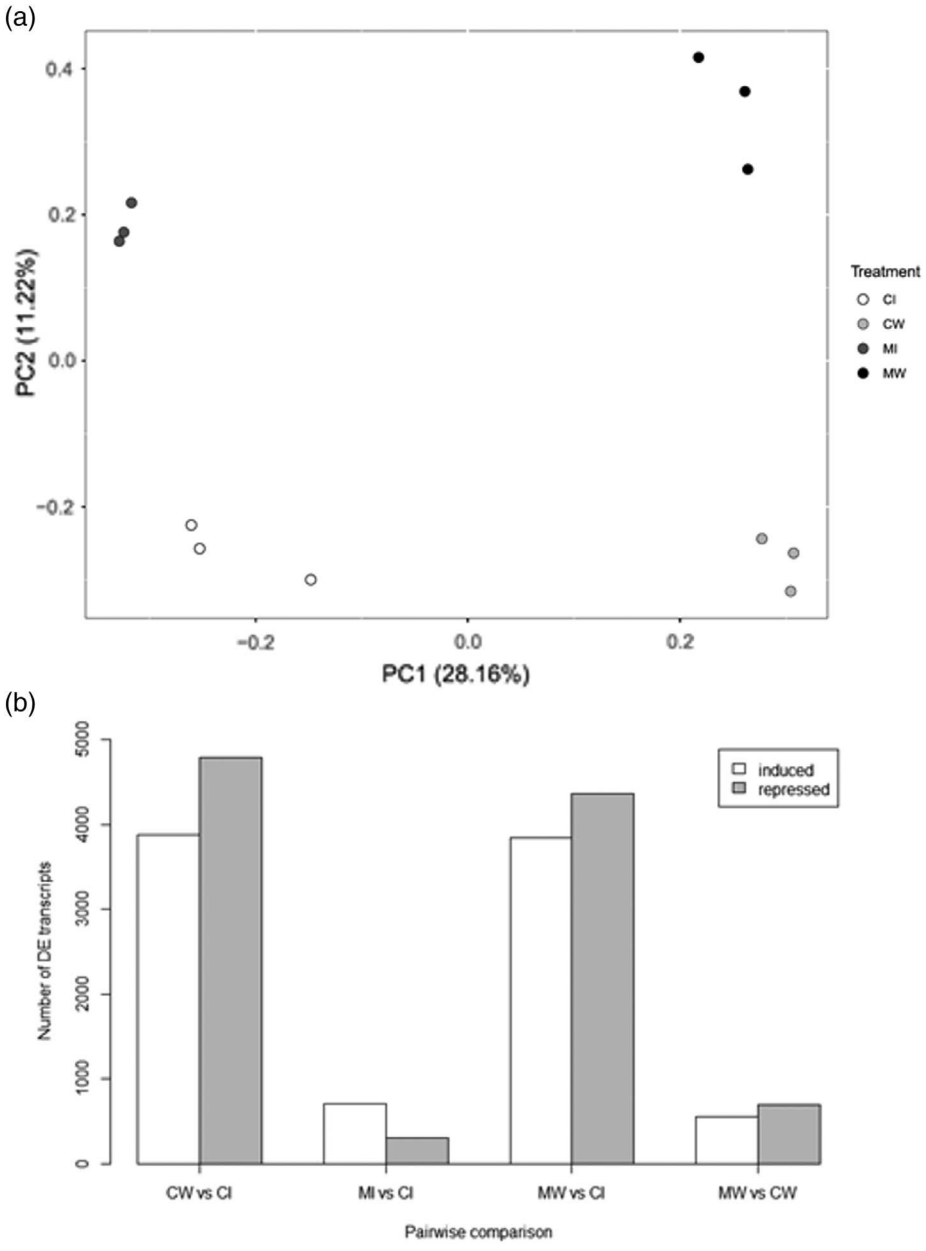
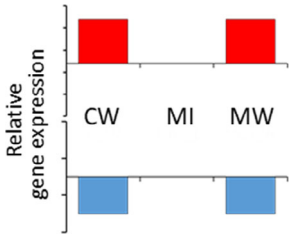
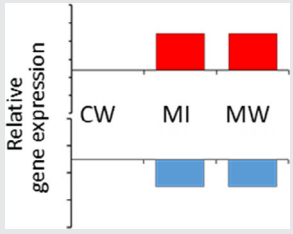
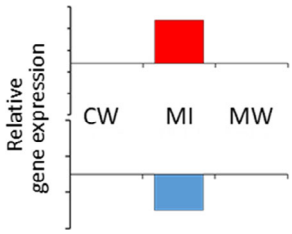
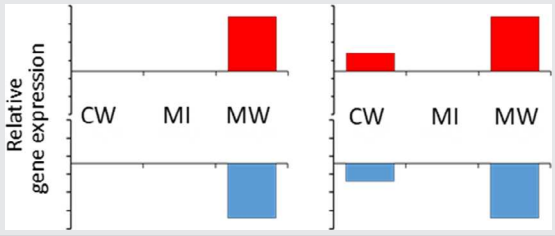


FIGURE 3 Transcriptome analysis of the bark of Norway spruce trees subjected to methyl jasmonate (MeJA) treatment and/or wounding 4 weeks later. (a) A principal component analysis (PCA) score plot showing the separation and grouping of treatments according to transfer-matrix method normalized transcript counts. Component 1 separates intact from wounded samples. Component 2 separates control from MeJA-treated samples. Abbreviations: CI, control and intact/unwounded; CW, control and wounded; MI, MeJA treated and intact; MW, MeJA-treated and wounded. (b) The number of significantly (false discovery rate-adjusted *p*-value <.01) induced and repressed transcripts for the pairwise comparisons CI-CW, CI-MI, CI-MW and MW-CW

between CI and the three other treatment combinations (CW, MI, MW) (Figure 3b). Although we had only three biological replicates per treatment, we identified thousands of differentially expressed transcripts (Figure 3b). The effect of wounding was particularly prominent, with a large majority of the differentially expressed transcript being identified in the CW versus CI and MW versus CI pairwise comparisons (Figure 3b). Although there were a similar number of differentially expressed transcripts in each of these comparisons, the PCA suggested that the MW and CW transcriptomes differed considerably (Figure 3a). Therefore, we additionally compared the effects of wounding in MeJA-treated (MW) and control (CW) bark (Figure 3b).

To decrease the complexity of analyzing the thousands of differentially expressed transcripts and to find transcripts with expression patterns which suggest they could underpin the induced defense responses, we identified four mutually exclusive transcriptional response patterns (Table 1). These patterns were based on the description of primed defense responses by Martinez-Medina et al. (2016) and the mechanisms of acquired resistance as described by Wilkinson et al. (2019). The four transcriptional response patterns were: (a) 'unprimed response to wounding' – transcripts that were differentially expressed in both CW and MW relative to CI, but were not differentially expressed between CW and MW; (b) 'prolonged response to MeJA' – transcripts that were differentially expressed in both MI and MW relative to CI (i.e., transcripts that responded to MeJA treatment and were maintained as up- or downregulated after wounding); (c) 'primed state' – transcripts that were differentially expressed in MI only (i.e., transcript that were expressed only after MeJA treatment but not after wounding); (d) 'primed response to wounding' – transcripts that were differentially expressed only in MW relative to CI or were differentially expressed in the comparisons CW versus CI and MW versus CW. These four transcriptional response patterns were based on the pairwise comparisons shown in Figure 3b and are represented graphically in Table 1.

TABLE 1 Four mutually exclusive transcriptional response patterns observed in the bark of Norway spruce trees treated with methyl jasmonate (MeJA) and then wounded (MW), treated with MeJA and left intact (MI) or not treated with MeJA and wounded (CW) [Colour table can be viewed at wileyonlinelibrary.com]

Transcriptional response pattern	Expression pattern	Transcripts
1. Unprimed response to wounding DE in CW vs. CI & DE in MW vs. CI & not DE in MW vs. CW		2,932 2,445
2. Prolonged response to MeJA DE in MI vs. CI & DE in MW vs. CI		48 71
3. Primed state DE in MI vs. CI		102 180
4. Primed response to wounding DE in MW vs. CI. Can be DE in CW vs. CI, but then also DE in MW vs. CW Not DE in MI vs. CI		413 153

Note: Transcriptional response patterns are based on the pairwise comparisons shown in Figure 3. The presence of a bar indicates that a transcript is differentially expressed (DE) between the treated and control intact (CI) bark. Differences in the height of two bars indicate that the transcript is DE between two treatments. Upregulated transcripts are shown in red, downregulated transcripts in blue.

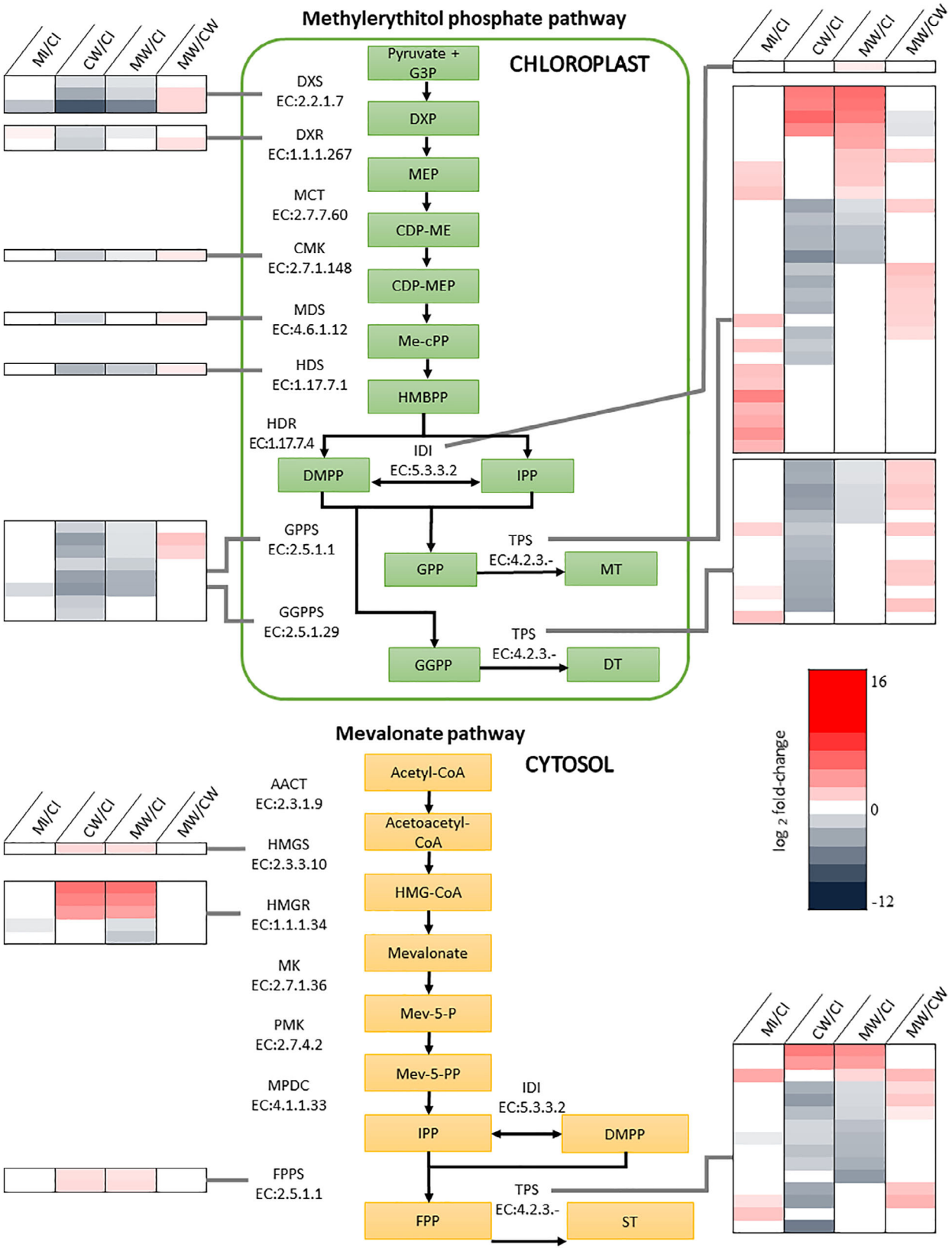


FIGURE 4 Legend on next page.

The transcriptional response pattern with the highest number of differentially expressed transcripts was 'unprimed response to wounding' (Table 1). Transcripts following this pattern were induced or repressed after wounding to a similar degree in MeJA-treated and control tissues (MW vs. CI and CW vs. CI, respectively) (Table 1). Additionally, these transcripts were not differentially expressed in intact MeJA-treated bark (MI vs. CI). A protein family (Pfam) enrichment analysis of upregulated transcripts showed that the three most significantly enriched Pfams among the 'unprimed response to wounding' transcripts were aminotransferase Class I & II (PF00155), chorismate synthase (PF01264), and WRKY DNA-binding domain (PF03106) (Supporting Information Table S1). In addition, five of the 40 significantly enriched Pfams we detected were related to glutathione detoxification of radical oxygens species and six were related to cellular transport. The top three enriched Pfams for the downregulated transcripts were cytochrome P450 (PF00067), subtilase family (PF00082), and protease associated domain (PF03106) (Supporting Information Table S2). In total, three of the 13 significantly enriched downregulated Pfams were associated with protease activity.

Transcripts following the 'prolonged response to MeJA' pattern were differentially expressed in both intact and wounded MeJA-treated bark (MI vs. CI and MW vs. CI, respectively) (Table 1). The three most significantly enriched upregulated Pfams following this pattern were cellulose synthase (PF03552), TIFY domain (PF06200), and multicopper oxidase (PF00394) (Supporting Information Table S3). Of the seven significantly enriched upregulated Pfams, three were multicopper oxidases. One Pfam, plastocyanin-like domain (PF02298), was found to be significantly enriched among downregulated transcripts.

Transcripts classified as following a 'primed state' pattern were only differentially expressed in MI bark compared to CI bark (MI vs. CI) and were restored to basal levels after wounding (Table 1). Over 280 genes followed this pattern. The one Pfam that was significantly enriched among upregulated transcripts following a 'primed state' pattern was terpene synthase (PF03936) (Supporting Information Table S5). The most significantly downregulated Pfams were sugar transport (PF00083), APETALA2 (PF00847), and major facilitator superfamily (PF07690) (Supporting Information Table S6).

Transcripts with a 'primed response to wounding' expression pattern showed a response characterized by a faster and/or stronger up- or downregulation after a triggering stress, which in our case was mechanical wounding (Table 1). Transcripts that were classified as being primed were differentially expressed in wounded MeJA-treated bark compared to both control intact and control wounded bark (MW vs. CI; MW vs. CW) and were not differentially expressed in MI bark (MI vs. CI). The top three significantly enriched Pfams for upregulated transcripts were chitinase Class I (PF00182), pectate lyase (PF00544), and glycosyl hydrolase family 17 (PF00332) (Supporting Information Table S7). Among the 20 significantly enriched upregulated Pfams, eight were pathogenesis-related proteins (PR). Six Pfams related to cell wall modification and two Pfams belonging to Leucine Rich Repeats (LRR) were also included in this list. Amongst the downregulated primed transcripts, the most significant Pfams were UDP-glycosyltransferase (PF00201), universal stress protein (PF00582), and cytochrome P450 (PF0067) (Supporting Information Table S8).

3.3 | Primed terpene accumulation is not underpinned by priming of terpene biosynthesis genes

Although the accumulation of monoterpenes and diterpene resin acids followed a primed pattern (Figure 2a,b), the expression of their biosynthesis genes did not (Figure 4; Supporting Information Table S10). Most transcripts of genes belonging to the methylerythitol phosphate pathway were downregulated in response to wounding (in both MW and CW), except for some terpene synthases (TPS) involved in monoterpene biosynthesis (Figure 4). In the mevalonate

FIGURE 4 Change in transcript expression of genes related to terpene biosynthesis in Norway spruce bark. The $\log_2$ fold-change for transcripts annotated as enzymes belonging to the mevalonate and non-mevalonate terpene biosynthesis pathway was determined by comparing the treatments MeJA-treated and intact, control and wounded (CW) and MeJA-treated and wounded (MW) to control and intact (see Table 1 for a definition of treatments). Additionally, $\log_2$ fold-change was determined between MW and CW. A false discovery rate-corrected *p*-value of .01 was used to qualify a significant change in expression. See Supporting Information Table S10 for more details. Abbreviations: AACT, acetyl-CoA C-acetyltransferase; CMK, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol kinase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; FPPS, farnesyl diphosphate synthase; G3P, D-glyceraldehyde; GGPPS, geranylgeranyl diphosphate synthase; GPPS, dimethylallyltranstransferase/geranyl diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-enyl-diphosphate reductase; HDS, (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase; HMG-R, hydroxylmethylglutaryl-CoA reductase; HMG-S, hydroxymethylglutaryl-CoA synthase; IPPI, isopentenyl-diphosphate D-isomerase; MCT, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; MDS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MK, mevalonate kinase; MPDC, diphosphomevalonate decarboxylase; PMK, phosphomevalonate kinase; HMG-CoA, hydroxymethylglutaryl-CoA; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; CDP-MEP, 2-phospho-4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; ME-cPP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, 4-hydroxy-3-methylbut-2-enyl diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, gernaylgeranyl diphosphate; MT, monoterpene; ST, sesquiterpene; DT, diterpene [Colour figure can be viewed at wileyonlinelibrary.com]

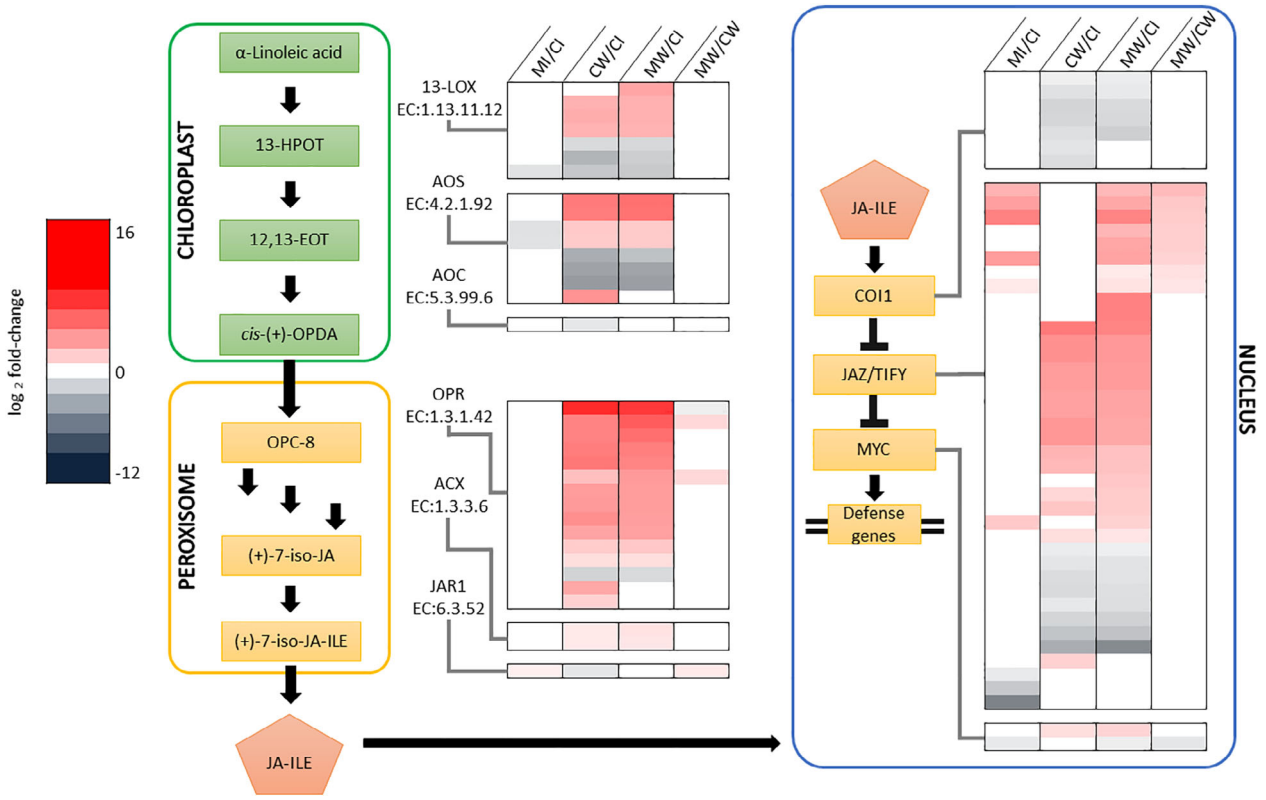


FIGURE 5 Change in transcript expression of genes related to jasmonate biosynthesis and signalling in Norway spruce bark. The $\log_2$ fold-change for transcript annotated as enzymes or proteins belonging to the jasmonate biosynthesis or signalling pathways was determined by comparing the three treatments types MeJA-treated and intact, control and wounded (CW) and MeJA-treated and wounded (MW) to control and intact (see Table 1 for a definition of treatments). Additionally, $\log_2$ fold-change was determined between MW and CW. A false discovery rate-corrected *p*-value of .01 was used to qualify a significant change in expression. See Supporting Information Table S11 for more details. Abbreviations: 13-LOX, 13-lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR, 12-oxophytodienoate reductase; ACX, acyl CoA-oxidase1; JAR1, JA-amino acid synthetase; 13-HPOT, (13S)-hydroperoxyoctadecatrienoic acid; 12,13-EOT, 12,13(S)-epoxylinolenic acid; cis-(+)-OPDA, cis-(+)-12-oxophytodienoic acid; OPC-8, 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; (+)-7-iso-JA, (+)-7-isojasmonic acid; (+)-7-iso-JA-ILE, (+)-7-isojasmonic acid isoleucine; JA-ILE, jasmonic acid isoleucine; COI1, coronatine insensitive1; JAZ/TIFY, JASMONATE-ZIM DOMAIN/TIF[F/Y]XG motif containing zinc-finger protein; MYC, bHLHzip transcription factor MYC [Colour figure can be viewed at wileyonlinelibrary.com]

pathway, most transcripts of enzymes contributing to the synthesis of the farnesyl pyrophosphate backbone were upregulated by wounding, both in MeJA-treated and untreated bark (Figure 4). Only one monoterpene synthase showed a primed upregulated response. This transcript was annotated as (+)-car-3-ene synthase 1 (Supporting Information Table S10). Overall, sesquiterpene synthases (ST-TPS) were downregulated in response to wounding (in both MW and CW) (Figure 4).

3.4 | JA could regulate primed defenses in MeJA treated plants

The faster and stronger accumulation of JA in MW bark could be driven by priming of the JA biosynthesis pathway. Many transcripts annotated as enzymes involved in the biosynthesis of JA were induced after wounding. However, very few of these enzymes showed a primed expression pattern 24 hr after wounding (Figure 5; Supporting Information Table S11).

3.5 | Priming of genes encoding pathogenesis-related proteins (PRs) may contribute to MeJA-induced resistance

Following the characterization of MeJA-induced changes in the basal and wound-induced transcriptomes, we examined defense responses that underpin MeJA-induced resistance in Norway spruce. We previously showed that Norway spruce trees treated with MeJA are more resistant to bark beetle attack 4 weeks after treatment (Mageroy et al., 2020). Two different defense strategies could underpin such long-term acquired resistance in Norway spruce and other plants: prolonged upregulation of inducible defenses and priming of inducible defenses (Wilkinson et al., 2019). In our analysis, these strategies

TABLE 2 Transcripts of pathogenesis-related proteins (PRs) in Norway spruce bark showing a primed response to wounding, as defined in Table 1

PR class	Description	Up	Down
PR 1	Sterol binding	2	0
PR 2	β-1,3-glucanase	13	0
PR 3, 4, 8, 11	Chitinase	19	0
PR 5	Thaumatin-like	4	1
PR 9	Peroxidase	10	1
PR 10	Ribonuclease-like	4	0
PR 14	Lipid transferase	1	0
Total		53	2

Note: Columns show the number of transcripts that were significantly differentially expressed in each PR class (false discovery rate-adjusted *p*-value <.01).

correlate to the 'prolonged response to MeJA' and the 'primed response to wounding' transcription patterns, respectively.

In the 'primed response to wounding' transcript category there were many transcripts related to genes encoding PRs. The PRs encompass a wide range of defensive proteins such as β-1,3-glucanases, chitinases, thaumatin family, peroxidases, and endoproteinases (Van Loon, Rep, & Pieterse, 2006). Differential expression of these genes was observed both after wounding alone (CW) and after wounding of bark that had been treated with MeJA (MW) (Supporting Information Table S12). Interestingly, PR transcripts only made up 1% of the 2,962 upregulated transcripts that followed an 'unprimed response to wounding' pattern, while they accounted for 8% of the 413 upregulated transcripts that followed a 'primed response to wounding' pattern. Additionally, several PR-related Pfams were enriched among primed upregulated transcripts. These Pfam categories included chitinase, glycosyl hydrolases family 17 (which includes β-1,3-glucanases), glycosyl hydrolase family 3 (which includes β-1,3-glucanases), peroxidase, pathogenesis-related protein Bet v I family (PR10), and tytic transglycolases (which include Barwin domain-containing proteins such as PR4) (Table 2; Supporting Information Table S7).

3.6 | Role of DNA methylation and histone modifications in the onset and maintenance of spruce defense priming

Changes in DNA methylation and post-translational histone modifications in response to priming stimuli are thought to be important for ensuring that defenses are maintained as primed (Conrath et al., 2015; Yakovlev, Carneros, Lee, Olsen, & Fossdal, 2016). In *Arabidopsis thaliana* (hereafter referred to as Arabidopsis), changes in DNA methylation have been linked to altered responsiveness of SA- and JA-dependent defenses and in turn altered resistance to the biotrophic and necrotrophic pathogens *Hyaloperonospora arabidopsidis* and *Plectosphaerella cucumerina*, respectively (López Sánchez, Stassen, Furci, Smith, & Ton, 2016). Furthermore, a priming stimuli-induced change in histone tail modifications and the decompaction of chromatin has been identified in the promotors of primed Arabidopsis defense genes (Baum et al., 2019; Jaskiewicz et al., 2011; Schillheim et al., 2018). However, there is no knowledge about the role of such epigenetic changes in the maintenance of induced resistance and defense priming in spruce.

As in other plant species, in Norway spruce changes in DNA methylation, modification of histone tails and chromatin remodelling is carried out by a suite of enzymes (Yakovlev et al., 2016). We hypothesized that the genes encoding these epigenetic regulators may be differentially expressed in response to MeJA treatment if epigenetic modifications are indeed involved in onset and maintenance of defense priming in spruce. In this experiment, we do not have gene expression data from immediately after the MeJA treatment, that is, from the onset of priming. However, wounding is also known to be a priming stimulus (Chassot et al., 2008). Furthermore, both wounding and MeJA are known to induce the formation of traumatic resin ducts, inducible terpene cavities that are thought to be key to induced resistance in spruce (Martin et al., 2002; Nagy, Franceschi, Solheim, Krekling, & Christiansen, 2000). Therefore, we analyzed the transcriptome of wounded but otherwise untreated bark (CW) to understand whether specific classes of epigenetic regulators are differentially expressed in response to a priming cue. The expression patterns of epigenetic regulators previously described by Ausin et al. (2016) and Yakovlev et al. (2016) and additional epigenetic regulators identified in our dataset by blast and Pfam domain identification were used in our analysis (Supporting Information Table S13). All DNA methylatransferases, DNA demethylases, and histone demethylases that were differentially expressed in CW bark were upregulated (Figure 6a). Additionally, most arginine methyltransferases that were differentially expressed in CW bark were upregulated. In contrast, histone methyltransferases and histone deacetylases were, in general, downregulated. Similarly, genes encoding components of the RNA-directed DNA methylation (RdDM) pathway, which performs de novo DNA methylation, and related proteins were mostly downregulated in CW bark (Figure 6a). Interestingly, there was also a general repression of genes encoding histone proteins (Figure 6a). In addition to these groups which showed a trend towards up- or downregulation, there was also groups which were neutral. Histone acetyltransferases and chromatin remodellers were two groups with equal numbers of up- and downregulated genes (Figure 6a). Similar patterns of expression for the RdDM pathway components, arginine methyltransferases, histone demethylases, histone deacetylases, and histones were observed in bark of trees that were wounded 4 weeks after MeJA treatment (MW) (Figure 6b).

4 | DISCUSSION

This study has documented how MeJA treatment primes Norway spruce defenses. Such defense priming may have important ecological implications for the world's largest terrestrial forest biomes. Priming of Norway spruce defenses following MeJA treatment was first recognized by Zhao and colleagues (Zhao et al., 2011). They found

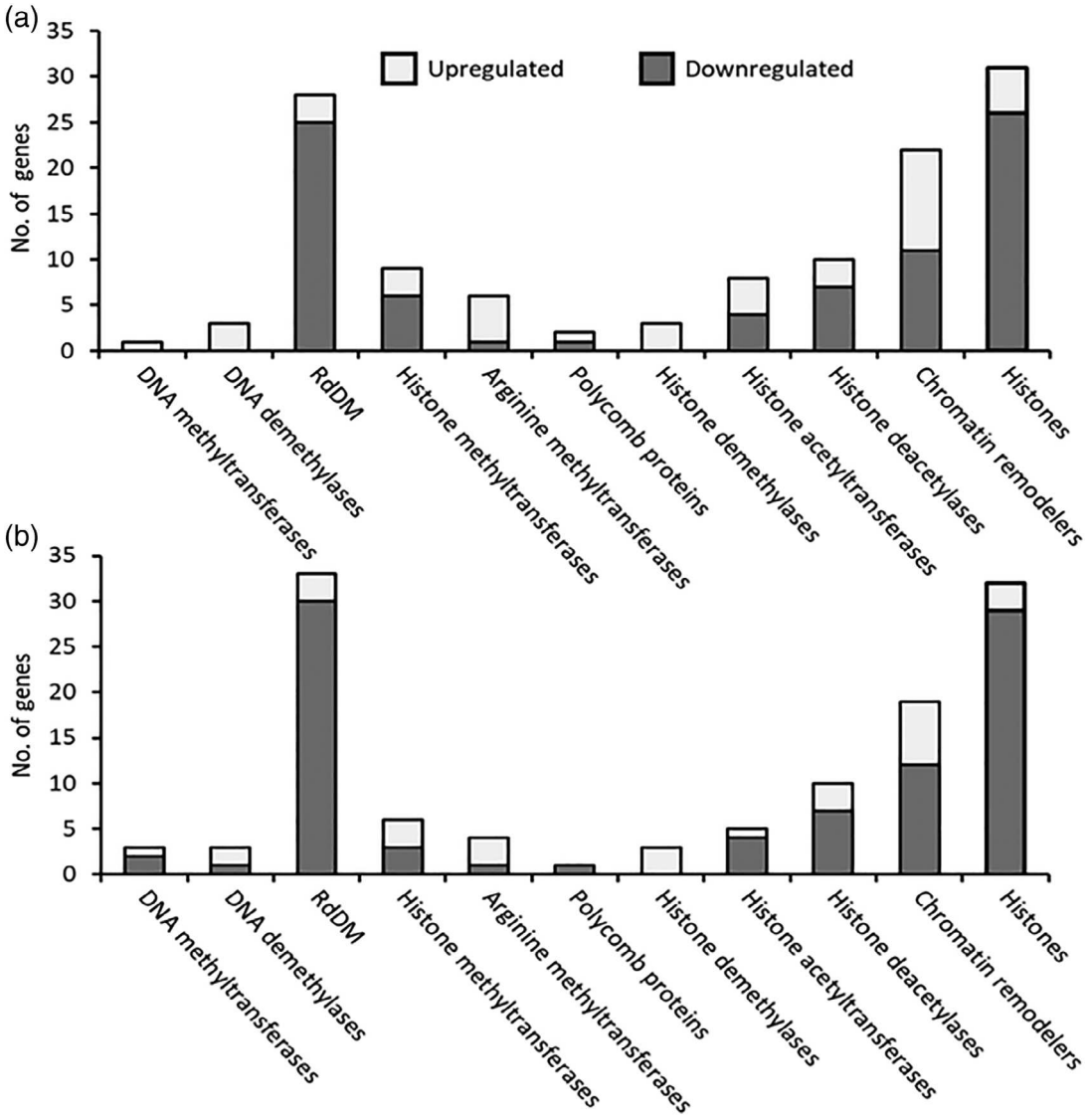


FIGURE 6 The response to wounding of different categories of epigenetic regulators, without (CW) and with (MW) prior methyl jasmonate treatment of Norway spruce bark. Bars show the numbers of epigenetic regulator encoding genes which were significantly (false discovery rate adjusted *p*-value <.01) up- (light grey) or downregulated (dark grey) in response to (a) wounding (CW) and (b) wounding after treatment with methyl jasmonate (MW)

that 4 weeks after MeJA treatment intact spruce bark only had slightly elevated total terpene levels, while wounding of MeJA-treated bark led to considerably higher terpene concentrations than in both intact and wounded control bark (Zhao et al., 2011). Later, Mageroy and colleagues showed that MeJA treatment of spruce prevents successful bark beetle attack during an epidemic outbreak (Mageroy et al., 2020). However, the molecular mechanisms underpinning this long-lasting acquired resistance in spruce have not previously been explored. Using a two-by-two experimental design (Figure 1), we have begun to dissect the effects of MeJA treatment on both the basal spruce transcriptome and the transcriptional responses to wounding. Although our study is limited in that we were only able to analyze one time point 24 hr after wounding in a single Norway spruce clone, we provide a snapshot into the complexity the spruce defense response (Figure 7). Below we describe transcriptional changes during three different inducible defense responses: unprimed response to wounding, prolonged (and unprimed) response to MeJA, and primed response to wounding (Figure 7a). Additionally, our study provides some insights into the transcriptional underpinning of the defense priming mechanism in Norway spruce by investigating differential gene expression during the three main phases of priming: onset, maintenance and triggering of primed defenses (Figure 7b).

4.1 | Unprimed response to wounding

The largest group of differentially expressed transcripts were those that followed an unprimed response to wounding pattern. The wound response is a general defense response that involves the induction of

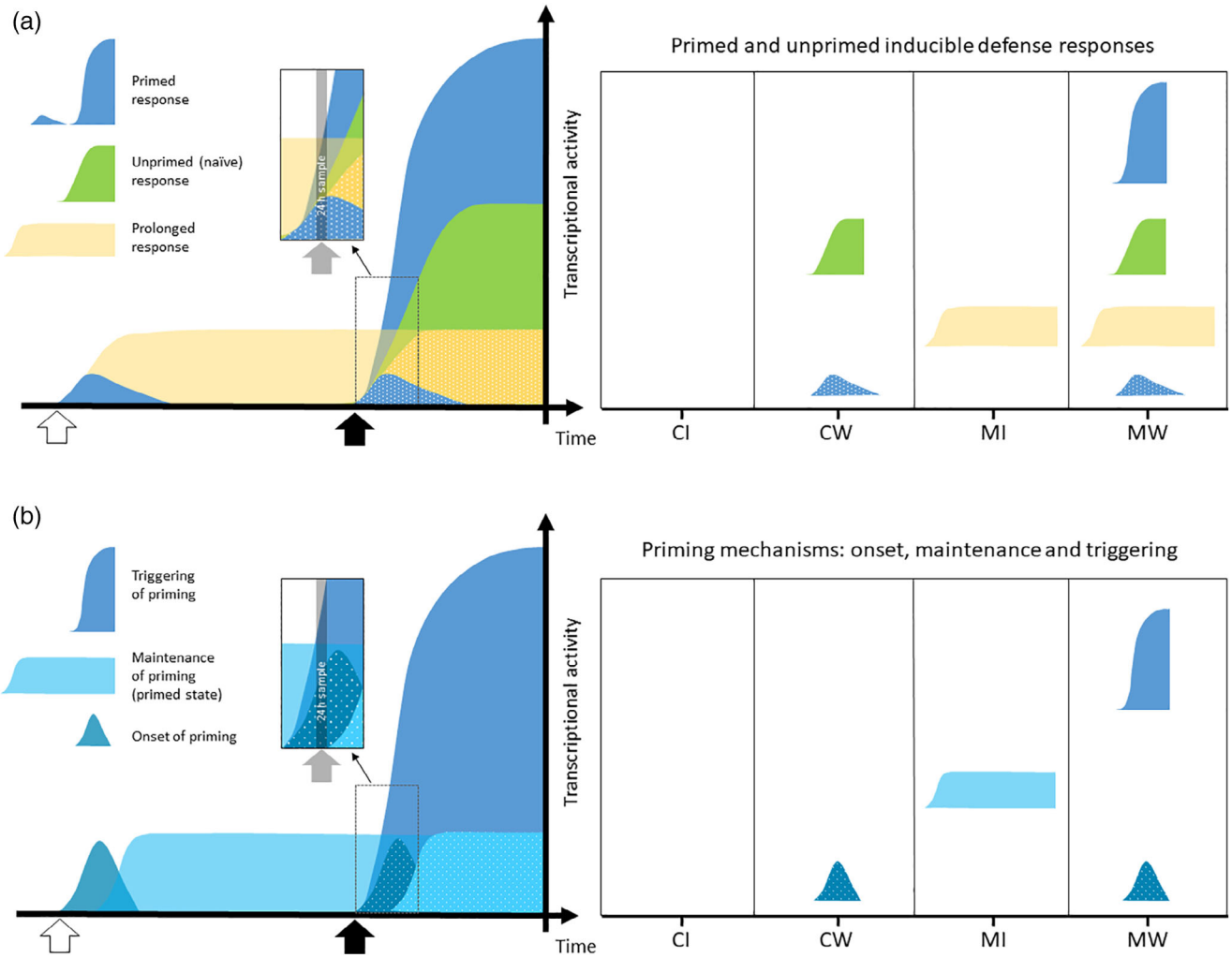


FIGURE 7 Conceptual model of Norway spruce-induced defense responses, adapted from Wilkinson et al. (2019) and Martinez-Medina et al. (2016). We used a two-by-two experimental design to dissect the transcriptional responses of spruce bark to a priming stimulus (methyl jasmonate [MeJA]) (white arrow) and a subsequent triggering stress (mechanical wounding) (black arrow). This gave four different treatment combinations that were sampled 24 hr after wounding (grey arrow): control and intact (CI), control and wounded (CW), MeJA-treated and intact (MI), and MeJA-treated and wounded (MW). (a) Using the transcript expression patterns described in Table 1, we observed three types of inducible defense responses: unprimed prolonged responses to MeJA (yellow), unprimed responses to wounding (green), and primed responses to wounding (dark blue). (b) In addition, we explored transcriptional responses during the three main phases of defense priming: onset (turquoise), maintenance (light blue), and triggering (dark blue). The right-hand panels show which treatment combinations were used to study the different types of inducible defense responses (a) and phases of the priming mechanism (b). The dotted blue and yellow shapes assume that wounding also functions as a priming stimulus [Colour figure can be viewed at wileyonlinelibrary.com]

various localized chemical and anatomical defenses, such as swelling of polyphenolic cells and the formation of traumatic resin ducts (Franceschi, Krokene, Christiansen, & Krekling, 2005). Wounding also results in the formation of callus tissue, which can be fortified with the production of polyphenolics, lignin and suberin (Franceschi et al., 2005). The most significantly enriched Pfam category among wound-induced transcripts in our study was aminotransferase Class I & II. Enzymes belonging to this category include aspartate, tyrosine, and aromatic aminotransferases. Likewise, the ethylene synthesis enzyme, 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) is known to be upregulated in response to wounding and to regulate ethylene-induced formation of traumatic resin ducts and enhance polyphenolic production (Hudgins & Franceschi, 2004; Ralph, Hudgins, Jancsik, Franceschi, & Bohlmann, 2007).

Many of the enriched Pfam categories among transcripts that were downregulated in response to wounding were plant proteases. Plant proteases are known to play important roles in plant immunity, although their specific function varies depending upon the protease type (Balakireva & Zamyatnin, 2018). Subtilases, one of the top down-regulated protease Pfam categories in wounded bark, are involved in plant cell death following *Pseudomonas syringae* infection and their expression is induced by salicylic acid (Jordá, Conejero, & Vera, 2000). It is possible that the downregulation of subtilases we observed in Norway spruce is due to negative cross-talk between SA and JA signalling

pathways (Koornneef & Pieterse, 2008). A previous study indicated that there is no antagonism between JA and SA-mediated signalling in spruce (Arnerup et al., 2013). However, our hormone profiling data suggest that there may be some antagonism between the signalling pathways, as SA levels were reduced after MeJA treatment (Figure 2c).

4.2 | Prolonged response to MeJA treatment

Given that sustained upregulation of genes is not thought to be a cost-effective defense strategy we found a surprising number of transcripts that were maintained as up- or downregulated 4 weeks after MeJA-treatment (Wilkinson et al., 2019). Two of the three most significantly enriched Pfams among prolonged upregulated transcripts were cell wall related: multicopper oxidase, which includes the laccase group of proteins, and cellulose synthase. Cell walls serve as an important first line of defense against invaders and overexpression of laccase has been shown to increase resistance to the fungal pathogen *Verticillium dahlia* in cotton (Y. Zhang et al., 2019).

The TIFY category of Pfam motifs was also one of the top three enriched categories amongst the transcripts following a prolonged upregulation pattern after MeJA treatment. The TIFY motif is found in proteins such as Jasmonate ZIM (zinc-finger inflorescence meristem)-Domain (JAZ) proteins that are transcriptional repressors of JA signaling (Bai, Meng, Huang, Qi, & Chen, 2011). Upregulation of these repressors could help to tightly regulate the JA signalling pathway.

4.3 | Onset and maintenance of defense priming after MeJA treatment

We observed differential expression of numerous epigenetic regulators that could be involved in establishing the epigenetic marks which maintain spruce defenses as primed. Both epigenetic 'writers' (i.e., methyltransferases and acetyltransferases) and 'erasers' (i.e., demethylases and deacetylases) were differentially expressed in response to wounding. The altered expression of genes encoding the epigenetic machinery could arise from the need to (a) unpack chromatin in promotors of positive regulators of JA-dependent defenses and/or the defense genes themselves, (b) condense chromatin in promotors of negative regulators and/or defense genes antagonized by JA, and/or (c) alter the chromatin structure and activity of transposable elements (TEs) which trans-regulate important defense genes from elsewhere in the genome (Wilkinson et al., 2019). Chromatin structure is influenced by covalent modifications of histone proteins. In general, the acetylation of histone lysine residues is associated with gene activation, while methylation can be either an activating or repressing mark (Pfluger & Wagner, 2007). In response to wounding, transcripts of arginine methyltransferases and histone demethylases were upregulated, while histone deacetylases and histone methylases were downregulated. Further experiments are needed to determine whether and how these changes in expression of histone modifying enzymes could influence the chromatin landscape and in turn whether this can prime the tissue for a faster and stronger response to subsequent challenge.

DNA methylation is also thought to influence chromatin structure. In a number of plant species, the RdDM pathway plays an important role in both de novo DNA methylation and the maintenance of methylation in the CHH DNA context (H = any nucleotide other than guanine) (Matzke, Kanno, & Matzke, 2014). However, in spruce, it is unclear how the RdDM pathway functions, as 24 nt sRNAs, which are known to guide the RdDM DNA methyltransferase Domains Rearranged Methyltransferase 2 (DRM2) in angiosperms, are rare in vegetative spruce tissue (Nystedt et al., 2013). In angiosperms, a non-canonical RdDM pathway has been described which involves 21 and 22 nt sRNAs (tasiRNAs) derived from Pol II generated transcripts (Matzke et al., 2014). Potentially, 21 and 22 nt sRNAs are more important than 24 nt sRNAs for directing the action of DRM2 in spruce bark. Despite our incomplete understanding of the spruce RdDM pathway, it would seem that in response to wounding many genes which are homologous to genes encoding members of the RdDM pathway in other species were downregulated. Additionally, three genes annotated as DNA demethylases (Supporting Information Table S13), including a ROS1-like gene, were upregulated. In Arabidopsis, ROS1 is a main DNA demethylase in vegetative tissue and is known to antagonize the RdDM pathway (Tang, Lang, Zhang, & Zhu, 2016; H. Zhang, Lang, & Zhu, 2018). This evidence suggests demethylation of RdDM targeted loci may occur in spruce bark in response to wounding. Potentially, these changes in DNA methylation could play a role in maintenance of induced resistance and defense priming in Norway spruce. However, methylome analysis will be required to determine if and where changes in DNA methylation occur.

4.4 | Transcriptome changes in the primed state

The transcriptome had not returned to a completely naïve state 4 weeks after MeJA treatment. Transcripts that were differentially expressed in intact bark 4 weeks after MeJA treatment (MI), but which returned to basal levels by 24 hr post wounding, contribute to the primed state. One enriched Pfam among transcripts that were upregulated in the primed state was terpene synthase. The terpene synthase Pfam included several transcripts annotated as monoterpene synthases. Interestingly, the concentration of monoterpenes was not found to be higher in MeJA-treated intact bark than in control bark. Post-transcriptional or post-translational regulation of terpenes synthases could account for this discrepancy between transcript abundance and terpene content observed in the primed state (Zulak et al., 2009).

4.5 | MeJA-primed responses to wounding

Based on our previous work and analysis of bark terpenes, we hypothesized that genes encoding terpene biosynthesis enzymes would

show a primed response to wounding (Mageroy et al., 2020; Zhao et al., 2011). Surprisingly, in our mRNA-seq dataset we did not observe this pattern for most terpene biosynthesis genes. While terpene accumulation almost certainly has a role in MeJA-induced resistance (Mageroy et al., 2020; Zhao et al., 2011), this response did not seem to be underpinned by priming of the terpene biosynthesis pathway at a transcriptional level. An explanation for the incongruence between terpene accumulation and the expression of terpene biosynthesis genes could be that any cell-specific transcriptional responses were diluted in our whole-tissue samples. Celedon and Bohlmann recently showed that transcriptome analysis of whole bark tissue can mask cell-type-specific responses to MeJA treatment (Celedon & Bohlmann, 2019). It is possible that after MeJA-induced formation of traumatic resin ducts, terpene biosynthesis is limited to the epithelial cells surrounding these ducts. Thus, upregulation of transcripts related to terpene biosynthesis could be dampened in our analysis of whole bark tissue. Future studies looking at priming of individual cell-types would be of great interest.

Unlike terpene biosynthesis enzymes, numerous PR genes were primed by MeJA at the transcription level in Norway spruce bark. For example, genes encoding enzymes annotated as chitinases were more strongly upregulated in response to wounding in bark previously treated with MeJA (MW) than in untreated bark (CW). Chitin is a major constituent of both fungal cell walls and insect exoskeletons. Plant chitinases are known to reduce fungal viability and promote fungal detection (Pusztahelyi, 2018). Thus, a greater pool of chitin-degrading enzymes would very likely enhance a plants ability to fight off attack by insect pests and fungal pathogens. In comparison to terpenes and phenolics, there has been less focus on the role of PRs in conifer defense. However, our results indicate that priming of PRs may play an important role in MeJA-induced resistance in spruce.

4.6 | JA regulation of primed spruce defenses

The accumulation of JA and not SA following wounding of MeJA-treated bark (MW) suggests that MeJA primes JA-dependent defenses in spruce. JA-dependent defenses may include PR proteins and cell wall modifying enzymes. However, it is difficult to link JA accumulation to the primed expression pattern of PRs and other defense genes without more detailed molecular analyses. One potential mechanism behind the primed accumulation of JA could be that the JA biosynthesis pathway genes are primed, but we did not observe a primed response of JA biosynthesis enzymes in our RNAseq data. This could be because transcriptome analysis 24 hr after wounding is too late to detect differences between MeJA-treated and control bark in the speed of upregulation of JA biosynthesis enzymes. From Arabidopsis, it is known that JA accumulates within a few minutes after wounding, and that JA biosynthesis enzymes are upregulated very soon after (Glauser et al., 2009, 2008). To clarify the role of JA signaling in primed defense gene expression in spruce, further investigation of earlier time points after wounding of MeJA-treated trees is required.

5 | CONCLUSION

The Norway spruce defense syndrome is a complex mixture of anatomical, chemical, and molecular defense responses that are overlapping in space and time (Franceschi et al., 2005). This study indicates that MeJA both directly induces and primes the inducible defense repertoire of Norway spruce. We have only begun to scratch the surface of the molecular underpinnings of long-term acquired resistance in this important gymnosperm species. For instance, considerable further study is required to understand the role of DNA methylation and other epigenetic modifications in spruce defense priming.

ACKNOWLEDGEMENTS

We thank Inger Heldal and technical staff at NIBIO their help with experiments. The Norwegian High-Throughput Sequencing Centre (NSC) is thanked for RNA-seq library sequencing. Bioinformatics were performed on the Abel Cluster, owned by the University of Oslo and Uninett/Sigma2, and operated by the Department for Research Computing at USIT, the University of Oslo IT-department. http://www.hpc.uio.no/ (nn9433k).

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Raw reads from mRNA sequencing have been submitted to NCBI. SRA accession: PRJNA564212. Biosamples: SAMN12687882, SAMN12687883, SAMN12687884, SAMN12687885.

AUTHOR CONTRIBUTIONS

M.H.M., H.C., C.G.F., A.V.-S. and P.K. conceived the experiment; P.K., T.Z. and C.G.F. preformed the field experiment and collections of material; T.Z., M.H.M., M.A., and P.P. performed chemical analysis; M.H.M., S.W.W., H.C., T.T., and P.K. analyzed the data; M.H.M., S.W.W., and P.K. wrote the article with contributions of all the authors.

ORCID

Melissa H. Mageroy https://orcid.org/0000-0001-7801-1007
Paal Krokene https://orcid.org/0000-0002-7205-0715

REFERENCES

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. https://doi.org/10.1007/s00425-012-1822-8

Ausin, I., Feng, S., Yu, C., Liu, W., Kuo, H. Y., Jacobsen, E. L., … Wang, H. (2016). DNA methylome of the 20-gigabase Norway spruce genome. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(50), 1–8. https://doi.org/10.1073/pnas.1618019113

Bai, Y., Meng, Y., Huang, D., Qi, Y., & Chen, M. (2011). Origin and evolutionary analysis of the plant-specific TIFY transcription factor family.

Genomics, *98*(2), 128–136. https://doi.org/10.1016/j.ygeno.2011.05.002

Balakireva, A. V., & Zamyatnin, A. A. (2018). Indispensable role of proteases in plant innate immunity. *International Journal of Molecular Sciences*, *19*(2), 1–26. https://doi.org/10.3390/ijms19020629

Baum, S., Reimer-Michalski, E., Bolger, A., Mantai, A. J., Benes, V., Usadel, B., & Conrath, U. (2019). Isolation of open chromatin identifies regulators of systemic acquired resistance. *Plant Physiology*, *181*(2), 817–833. https://doi.org/10.1104/pp.19.00673

Beckers, G. J. M., Jaskiewicz, M., Liu, Y., Underwood, W. R., He, S. Y., Zhang, S., & Conrath, U. (2009). Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. *The Plant Cell*, *21*(March), 944–953. https://doi.org/10.1105/tpc.108.062158

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*, 289. https://doi.org/10.2307/2346101

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*(1), 421. https://doi.org/10.1186/1471-2105-10-421

Celedon, J. M., & Bohlmann, J. (2019). Oleoresin defenses in conifers: Chemical diversity, terpene synthases and limitations of oleoresin defense under climate change. *New Phytologist*, *224*, 1444–1463. https://doi.org/10.1111/nph.15984

Chassot, C. C., Buchala, A., Schoonbeek, H. J., Métraux, J. P., Lamotte, O., Metraux, J.-P., & Lamotte, O. (2008). Wounding of Arabidopsis leaves causes a powerful but transient protection against *Botrytis* infection. *The Plant Journal*, *55*(4), 555–567. https://doi.org/10.1111/j.1365-313X.2008.03540.x

Conrath, U. (2011). Molecular aspects of defence priming. *Trends in Plant Science*, *16*, 524–531. https://doi.org/10.1016/j.tplants.2011.06.004

Conrath, U., Beckers, G. J. M. M., Langenbach, C. J. G. G., & Jaskiewicz, M. R. (2015). Priming for enhanced defense. *Annual Review of Phytopathology*, *53*(1), 97–119. https://doi.org/10.1146/annurev-phyto-080614-120132

Conrath, U., Pieterse, C. M. J., & Mauch-Mani, B. (2002). Priming in plant-pathogen interactions. *Trends in Plant Science*, *7*(5), 210–216. https://doi.org/10.1016/S1360-1385(02)02244-6

de Matos Simoes, R., & Emmert-Streib, F. (2016). bc3net: Gene Regulatory Network Inference with Bc3net. Retrieved from https://cran.r-project.org/package=bc3net

Eddy, S. R. (2009). A new generation of homology search tools based on probabilistic inference. *Genome Informatics. International Conference on Genome Informatics*, *23*(1), 205–211.

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., … Finn, R. D. (2019). The Pfam protein families database in 2019. *Nucleic Acids Research*, *47*(D1), D427–D432. https://doi.org/10.1093/nar/gky995

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*, 426–436. https://doi.org/10.1007/s00442-006-0394-3

Fossdal, C. G., Nagy, N. E., Johnsen, Ø., & Dalen, L. S. (2007). Local and systemic stress responses in Norway spruce: Similarities in gene expression between a compatible pathogen interaction and drought stress. *Physiological and Molecular Plant Pathology*, *70*(4–6), 161–173. https://doi.org/10.1016/j.pmpp.2007.09.002

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

Franceschi, V. R., Krekling, T., & Christiansen, E. (2002). Application of methyl jasmonate on *Picea abies* (Pinaceae) stems induces defense-related responses in phloem and xylem. *American Journal of Botany*, *89*(4), 578–586. https://doi.org/10.3732/ajb.89.4.578

Franceschi, V. R., Krokene, P., Christiansen, E., & Krekling, T. (2005). Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist*, *167*(2), 353–376. https://doi.org/10.1111/j.1469-8137.2005.01436.x

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. https://doi.org/10.1074/jbc.M109.061432

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. https://doi.org/10.1074/jbc.M801760200

Hlásny, T., Krokene, P., Liebhold, A., Montagné-huck, C., Müller, J., Qin, H., … Schelhaas, M. (2019). Living with bark beetles: Impacts, outlook and management options.

Hudgins, J. W., & Franceschi, V. R. (2004). Methyl Jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiology*, *135*(4), 2134–2149. https://doi.org/10.1104/pp.103.037929

Jaskiewicz, M., Conrath, U., & Peterhälnsel, C. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Reports*, *12*(1), 50–55. https://doi.org/10.1038/embor.2010.186

Jordá, L., Conejero, V., & Vera, P. (2000). Characterization of P69E and P69F, two differentially regulated genes encoding new members of the subtilisin-like proteinase family from tomato plants. *Plant Physiology*, *122*(1), 67–74. https://doi.org/10.1104/pp.122.1.67

Kersten, P. J., Kopper, B. J., Raffa, K. F., & Illman, B. L. (2006). Rapid analysis of Abietanes in conifers. *Journal of Chemical Ecology*, *32*, 2679–2685. https://doi.org/10.1007/s10886-006-9191-z

Koornneef, A., & Pieterse, C. M. J. (2008). Cross talk in defense signaling. *Plant Physiology*, *146*(3), 839–844. https://doi.org/10.1104/pp.107.112029

Krokene, P. (2015). Conifer defense and resistance to bark beetles. In F. E. Vega & R. W. Hofstetter (Eds.), *Bark beetles: Biology and ecology of native and invasive species* (pp. 177–207). United States: Elsevier. https://doi.org/10.1016/B978-0-12-417156-5.00005-8

Li, B., & Dewey, C. N. (2014). RSEM: Accurate transcript quantification from RNA-seq data with or without a reference genome. In *Bioinformatics: The impact of accurate quantification on proteomic and genetic analysis and research* (Vol. 12, pp. 41–74). London, UK: BioMed Central. https://doi.org/10.1201/b16589

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. *Plant Journal*, *88*(3), 361–374. https://doi.org/10.1111/tpj.13252

Mageroy, M. H., Christiansen, E., Långström, B., Borg-Karlson, A., Solheim, H., Björklund, N., … Krokene, P. (2020). Priming of inducible defenses protects Norway spruce against tree-killing bark beetles. *Plant, Cell & Environment*, *43*(2), 420–430. https://doi.org/10.1111/pce.13661

Marini, L., Økland, B., Jönsson, A. M., Bentz, B., Carroll, A., Forster, B., … Schroeder, M. (2017). Climate drivers of bark beetle outbreak dynamics in Norway spruce forests. *Ecography*, *40*(12), 1426–1435. https://doi.org/10.1111/ecog.02769

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. https://doi.org/10.1104/pp.011001

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C. M. J., Pozo, M. J., … Conrath, U. (2016). Recognizing plant defense priming. *Trends in Plant Science*, *21*(10), 818–822. https://doi.org/10.1016/j.tplants.2016.07.009

Matzke, M. A., Kanno, T., & Matzke, A. J. M. (2014). RNA-directed DNA methylation: The evolution of a complex epigenetic pathway in flowering plants. *Annual Review of Plant Biology*, *66*(1), 243–267. https://doi.org/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*(1), 485–512. https://doi.org/10.1146/annurev-arplant-042916-041132

McCarthy, D. J., Chen, Y., & Smyth, G. K. (2012). Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Research*, *40*(10), 4288–4297. https://doi.org/10.1093/nar/gks042

Mezei, P., Jakuš, R., Pennerstorfer, J., Havašová, M., Škvarenina, J., Ferenčík, J., … Netherer, S. (2017). Storms, temperature maxima and the Eurasian spruce bark beetle Ips typographus – An infernal trio in Norway spruce forests of the central European high Tatra Mountains. *Agricultural and Forest Meteorology*, *242*, 85–95.

Nagy, N. E., Franceschi, V. R., Solheim, H., Krekling, T., & Christiansen, E. (2000). Wound-induced traumatic resin duct development in stems of Norway spruce (Pinaceae): Anatomy and cytochemical traits. *American Journal of Botany*, *87*(3), 302–313. https://doi.org/10.2307/2656626

Nystedt, B., Street, N. R., Wetterbom, A., Zuccolo, A., Lin, Y.-C. C., Scofield, D. G., … Jansson, S. (2013). The Norway spruce genome sequence and conifer genome evolution. *Nature*, *497*(7451), 579–584. https://doi.org/10.1038/nature12211

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. https://doi.org/10.3389/fpls.2014.00295

Pétriacq, P., Stassen, J. H. M., & Ton, J. (2016). Spore density determines infection strategy by the plant pathogenic fungus *Plectosphaerella cucumerina*. *Plant Physiology*, *170*(4), 2325–2339. https://doi.org/10.1104/pp.15.00551

Pfluger, J., & Wagner, D. (2007). Histone modifications and dynamic regulation of genome accessibility in plants. *Current Opinion in Plant Biology*, *10*(6), 645–652. https://doi.org/10.1016/j.pbi.2007.07.013

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, *52*(1), 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pusztahelyi, T. (2018). Chitin and chitin-related compounds in plant–fungal interactions. *Mycology*, *9*(3), 189–201. https://doi.org/10.1080/21501203.2018.1473299

Ralph, S. G., Hudgins, J. W., Jancsik, S., Franceschi, V. R., & Bohlmann, J. J. (2007). Aminocyclopropane carboxylic acid synthase is a regulated step in ethylene-dependent induced conifer defense. Full-length cDNA cloning of a multigene family, differential constitutive, and wound- and insect-induced expression, and cellular and subcellular. *Plant Physiology*, *143*(1), 410–424. https://doi.org/10.1104/pp.106.089425

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: A bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, *26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Schillheim, B., Jansen, I., Baum, S., Beesley, A., Bolm, C., & Conrath, U. (2018). Sulforaphane modifies histone H3, unpacks chromatin, and primes defense. *Plant Physiology*, *176*(3), 2395–2405. https://doi.org/10.1104/pp.17.00124

Sundell, D., Mannapperuma, C., Netotea, S., Delhomme, N., Lin, Y. C., Sjödin, A., … Street, N. R. (2015). The plant genome integrative explorer resource: PlantGenIE.org. *New Phytologist*, *208*(4), 1149–1156. https://doi.org/10.1111/nph.13557

Tang, K., Lang, Z., Zhang, H., & Zhu, J. K. (2016). The DNA demethylase ROS1 targets genomic regions with distinct chromatin modifications. *Nature Plants*, *2*(11), 16169. https://doi.org/10.1038/nplants.2016.169

Van Loon, L. C., Rep, M., & Pieterse, C. M. J. (2006). Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology*, *44*, 135–162. https://doi.org/10.1146/annurev.phyto.44.070505.143425

Wilkinson, S. W., Pastor, V., Paplauskas, S., Pétriacq, P., & Luna, E. (2018). Long-lasting β-aminobutyric acid-induced resistance protects tomato fruit against *Botrytis cinerea*. *Plant Pathology*, *67*(1), 30–41. https://doi.org/10.1111/ppa.12725

Wilkinson, S. W., Magerøy, M. H., López Sánchez, A., Smith, L. M., Furci, L., Cotton, T. E. A., … Ton, J. (2019). Surviving in a hostile world: Plant strategies to resist pests and diseases. *Annual Review of Phytopathology*, *57*, 505–529. https://doi.org/10.1146/annurev-phyto-082718

Yakovlev, I., Carneros, E., Lee, Y., Olsen, J. E., & Fossdal, C. G. (2016). Transcriptional profiling of epigenetic regulators in somatic embryos during temperature induced formation of an epigenetic memory in Norway spruce. *Planta*, *243*(5), 1237–1249. https://doi.org/10.1007/s00425-016-2484-8

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. https://doi.org/10.1093/treephys/26.8.977

Zhang, H., Lang, Z., & Zhu, J. K. (2018). Dynamics and function of DNA methylation in plants. *Nature Reviews Molecular Cell Biology*, *19*, 489–506. https://doi.org/10.1038/s41580-018-0016-z

Zhang, Y., Wu, L., Wang, X., Chen, B., Zhao, J., Cui, J., … Ma, Z. (2019). The cotton laccase gene GhLAC15 enhances *Verticillium* wilt resistance via an increase in defence-induced lignification and lignin components in the cell walls of plants. *Molecular Plant Pathology*, *20*(3), 309–322. https://doi.org/10.1111/mpp.12755

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*, 691–699. https://doi.org/10.1007/s00442-011-2017-x

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*). *Plant Journal*, *60*(6), 1015–1030. https://doi.org/10.1111/j.1365-313X.2009.04020.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Mageroy MH, Wilkinson SW, Tengs T, et al. Molecular underpinnings of methyl jasmonate-induced resistance in Norway spruce. *Plant Cell Environ*. 2020; 43:1827–1843. https://doi.org/10.1111/pce.13774