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Priming of inducible defenses protects Norway spruce against tree-killing bark beetles

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Abstract

Plants can form an immunological memory known as defense priming, whereby exposure to a priming stimulus enables quicker or stronger response to subsequent attack by pests and pathogens. Such priming of inducible defenses provides increased protection and reduces allocation costs of defense. Defense priming has been widely studied for short-lived model plants such as *Arabidopsis*, but little is known about this phenomenon in long-lived plants like spruce. We compared the effects of pretreatment with sublethal fungal inoculations or application of the phytohormone methyl jasmonate (MeJA) on the resistance of 48-year-old Norway spruce (*Picea abies*) trees to mass attack by a tree-killing bark beetle beginning 35 days later. Bark beetles heavily infested and killed untreated trees but largely avoided fungus-inoculated trees and MeJA-treated trees. Quantification of defensive terpenes at the time of bark beetle attack showed fungal inoculation induced 91-fold higher terpene concentrations compared with untreated trees, whereas application of MeJA did not significantly increase terpenes. These results indicate that resistance in fungus-inoculated trees is a result of direct induction of defenses, whereas resistance in MeJA-treated trees is due to defense priming. This work extends our knowledge of defense priming from model plants to an ecologically important tree species.

KEYWORDS

defense priming, *Ips typographus*, methyl jasmonate, *Picea abies*, resistance

1 | INTRODUCTION

Conifers are large gymnosperm trees that dominate boreal and temperate forests. They include some of the world's most long-lived, abundant, and economically important plant species and provide vital ecosystem services and important raw materials. From a research perspective, conifers have some biological attributes that make them hard to study, including large size, long generation times, and extremely large and intractable genomes (Nystedt et al., 2013). Still, thanks to their economic importance, the defense mechanisms of conifers are relatively well studied. These defenses include constitutive defenses, such as thick impregnable cork bark and an elaborate

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network of ducts containing terpenoid resin, as well as numerous inducible defenses, such as formation of traumatic resin ducts and activation of polyphenol-containing cells (Celedon & Bohlmann, 2019; Franceschi, Krokene, Christiansen, & Krekling, 2005; Hammerbacher et al., 2013; Krokene, 2015).

A major cause of mortality in mature conifers is attack by tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae; Krokene, 2015). In Europe, the Eurasian spruce bark beetle *Ips typographus* may overwhelm the resistance of healthy Norway spruce (*Picea abies*) trees in pheromone-coordinated mass attack (Hlásny et al., 2019). The beetles also increase the virulence of their attacks by introducing necrotrophic blue-stain fungi, such as *Endoconidiophora polonica*, into the tree (Krokene & Solheim, 1998). The beetles oviposit in characteristic egg galleries in the inner bark of successfully colonized trees, and the developing larvae make feeding tunnels away from the egg galleries (Hlásny et al., 2019). The terpenoid resin in the bark and sapwood is toxic to the beetles and is a key component in the constitutive and inducible defenses in conifers (Celedon & Bohlmann, 2019). Trees that rapidly increase their resin concentrations in the bark are more resistant to beetle attack than trees with a weaker or slower response (Schiebe et al., 2012; Zhao, Krokene, et al., 2011).

Research over the last 20 years has shown that conifers can become more resistant to attacks if they have experienced prior stress. This has been described variably as acquired resistance, systemic acquired resistance, induced systemic resistance, or "vaccination" (Bonello, Gordon, & Storer, 2001; Christiansen et al., 1999; Krokene, 2015; Martin, Tholl, Gershenzon, & Bohlmann, 2002). Acquired resistance to fungal infection was first demonstrated in the 1990s in Norway spruce, where wounding and fungal inoculation made trees more resistant to a subsequent massive fungal infection (Christiansen et al., 1999). Increased tree resistance following conditioning with fungal infection, mechanical wounding, or stem application of the phytophormone methyl jasmonate (MeJA) has since been described in several other conifer species, including Scots pine (*Pinus sylvestris*), loblolly pine (*Pinus taeda*), ponderosa pine (*Pinus ponderosa*), and Monterey pine (*Pinus radiata*; Franceschi, Krekling, & Christiansen, 2002; Heijari, Nerg, Kainulainen, Vuorinen, & Holopainen, 2008; Martin et al., 2002; Reynolds, Gordon, & McRoberts, 2016; Swett & Gordon, 2017). Application of MeJA has also been shown to increase resistance to bark beetle attack 1 year after application in Norway spruce (Erbilgin, Krokene, Christiansen, & Gershenzon, 2006). However, because total terpene levels are only slightly elevated and phenolics are not significantly induced in MeJA-treated spruce trees (Erbilgin et al., 2006), it remains unclear how MeJA treatment increases spruce resistance.

Two mechanisms of inducible defenses may explain acquired resistance in spruce and other conifers following MeJA treatment: (a) prolonged up-regulation of inducible defenses and (2) priming of defense responses (Wilkinson et al., 2019). Prolonged up-regulation of inducible defenses occurs when defenses induced by, for example, pathogen infection, remain up-regulated for weeks or months and provide resistance against subsequent attacks. However, this resistance is costly as resources are allocated away from growth and reproduction to defense over a long period (Wilkinson et al., 2019). A more cost-effective form of inducible resistance is defense priming. In a primed plant, inducible defenses are sensitized following exposure to a priming stimulus (Conrath, Beckers, Langenbach, & Jaskiewicz, 2015). This sensitization of defense responses allows for defenses to be maintained at basal or weakly induced levels and then be rapidly activated upon subsequent attack (Pastor, Luna, Mauch-Mani, Ton, & Flors, 2013). Because defense priming does not induce a strong instant activation of inducible defenses overall, resource allocation to defenses is reduced and delayed (Martinez-Medina et al., 2016). Priming stimuli may be abiotic or biotic stress, beneficial organisms, or chemical compounds (Mauch-Mani, Baccelli, Luna, & Flors, 2017). The primed state can be maintained for days, weeks, or possibly years (Erbilgin et al., 2006; Eyles, Bonello, Ganley, & Mohammed, 2010; Frost, Mescher, Carlson, & De Moraes, 2008; Mauch-Mani et al., 2017).

Most of our knowledge about defense priming in plants comes from laboratory studies of model plants such as *Arabidopsis* (Balmer, Pastor, Gamir, Flors, & Mauch-Mani, 2015; Conrath et al., 2015; Mauch-Mani et al., 2017). Controlled studies with genetically defined model plants have been used to elucidate the molecular mechanism underpinning defense priming. These mechanisms include changes in DNA methylation and histone modifications that alter gene expression, accumulation of dormant signalling kinases, primed deposition of callose, and the accumulation of glycosylated hormones (Annacondia, Magerøy, & Martinez, 2018; Conrath, 2011; Hilker & Schmülling, 2019; Wilkinson et al., 2019). Although studies in model plants are foundational to our understanding of plant defense priming, they do not give much information about costs and benefits of priming in an ecological context. For example, model plant studies often demonstrate a faster and stronger up-regulation of inducible defenses in primed plants but rarely evaluate plant performance and fitness in natural environments (Martinez-Medina et al., 2016).

In this paper, we describe a field experiment demonstrating that pretreatment with fungal inoculation or MeJA application can protect Norway spruce trees from colonization by the tree-killing bark beetle *Ips typographus*. Our experimental design allowed us to parse the effects of prolonged induction of defense by the fungus and priming of defense by MeJA treatment. Thus, we extend the demonstration of defense priming from model plants to an ecologically and economically important tree species.

2 | MATERIALS AND METHOD

2.1 | Treatment of trees with MeJA and fungal inoculations

In spring 2008, we selected 60 healthy-looking trees along a 200-m stand edge in a planted 48-year-old Norway spruce stand of a local provenance in Tönnersjöheden Experimental Forest, Halland, Sweden (56°41′N, 13°4′E). On April 22, every third tree along the stand edge was inoculated with the blue-stain fungus *E. polonica*, sprayed with MeJA, or left untreated as a control (n = 20 trees per treatment;

Figure S1). The stem of fungus-treated trees was inoculated between 0.8 and 3.8 m above the ground at a density of 20 inoculations m^{-2} bark surface using a 5-mm cork borer. Approximately 30 μl of inoculum was used for each inoculation. Inoculum consisted of mycelium of strain NFLI 1993-208/115 growing on malt agar (Zhao, Borg-Karlson, Erbilgin, & Krokene, 2011). On MeJA-treated trees, the corresponding 3-m stem section was sprayed with 100-mM MeJA in water with 0.1% Tween 20, as described in Erbilgin et al. (2006). Application was always done on dry bark, and the bark was kept wet for a minimum of 5 min by repeated application of MeJA if necessary. The trees averaged 20.9 ± 2.7 cm (±SD) in diameter at breast height, with no significant differences between trees allotted to the different treatments (one-factor analysis of variance: $F(2, 57) = 0.14$, $P = .87$).

2.2 | Bark sampling

Immediately prior to the treatment, a single bark sample was taken from each tree at 1.3-m height using a hollow punch (6-mm diameter) and immediately frozen on dry ice for subsequent transcript quantification. Additional samples were taken 14 and 35 days after treatment at 1.3-m height using a 5-mm cork borer to quantify induced terpene levels in the treated stem section of MeJA-treated and inoculated trees. In fungus-inoculated trees, the bark sample was taken immediately above an inoculation hole within the necrotic lesion. To reduce the influence of previous sampling, new bark samples were taken from the opposite side of the trunk and at least 30 cm away from previous samples. A full timeline of tissue sampling and experimental treatments is given in Figure S1.

2.3 | Chemical procedures

The cork bark was discarded from the bark plugs, and terpenes in the inner bark (phloem) were extracted in hexane and analysed as described in Zhao, Krokene, et al. (2011). Briefly, the phloem was extracted for 48 hr at room temperature in 1.0-ml of hexane containing 0.20-mg pentadecane (Lancaster synthesis, England) as an internal standard and 0.12-mg 3-tert-butyl-4-hydroxyanisole (Fluka, Switzerland) as antioxidant. After extraction, phloem plugs were dried and weighed. The hexane extracts were analysed using a Varian 3400 gas chromatograph 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. Terpenes were identified by comparing retention times and mass spectra with available authenticated standards. Alternatively, compounds were identified using Massfinder 3.0 (Hochmuth Scientific Consulting, Germany) and by comparing to the reference libraries of NIST (National Institute of Standards and Technology). The concentration of terpenes was calculated relative to the internal standard and expressed as mg g^{-1} dry weight of phloem tissue.

2.4 | Quantitative PCR procedures

Real-time quantitative PCR (RT-qPCR) of terpene-related genes (Table S1) from bark tissue was performed at Max Planck Institute for Chemical Ecology, Jena, Germany, using the procedure described by Schmidt et al. (2011). Briefly, RNA isolation and complementary DNA synthesis were carried out by using a Plant RNA Isolation Kit (Stratec, Berlin, Germany) including a DNA digestion step (RNase Free DNase Set, Qiagen, Hilden, Germany) and Superscript III (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-qPCR was done with Brilliant SYBR Green qPCR Master Mix (Stratagene) using a standard protocol with an annealing temperature of 53°C on a Stratagene MX3000P thermocycler according to the instruction manual. Transcript abundance was normalized by using ubiquitin as a reference gene. Primer sequences were adopted from previous studies and are given in Table S1 (Schmidt et al., 2010; Schmidt & Gershenzon, 2007, 2008; Zulak et al., 2009).

RT-qPCR of phenylpropanoid and defense-related gene transcripts (Table S1) from bark tissue was performed at the Norwegian Institute of Bioeconomy Research, Ås, Norway. RNA was isolated using an RNAqueous total RNA isolation kit and Plant RNA Isolation Aid (Ambion, Austin, TX, USA) following the manufacturer's instructions. Contaminating DNA was degraded using DNA-Free (Ambion). Complementary DNA synthesis was carried out using 100 ng of total RNA that was reverse transcribed with TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA, USA). RT-qPCR was done with SYBR Green PCR Mastermix (P/N 4309155; Applied Biosystems) using a standard protocol with an annealing temperature of 60°C on an ABI Prism 7500 thermocycler (Applied Biosystems). Transcript abundance was normalized by using actin as a reference gene. Primer sequences were adopted from previous studies and are given in Table S1 (Fossdal et al., 2012; Hietala, Eikenes, Kvaalen, Solheim, & Fossdal, 2003; Koutaniemi et al., 2007).

2.5 | Bark beetle attack success

On Day 35 after treatment, after the last chemical samples had been collected, a 40-cm-long pheromone dispenser tape (Hercon® type releasing methylbutenol, *cis*-verbenol, and ipsdienol at a ratio of about 160:7:1, Hercon Environmental, Emigsville, PA, USA, Bakke, Sæther, & Kvamme, 1983) was attached at 0.75-m height on a pole placed ~40-cm from the SW side of each tree to attract bark beetles (Mulock & Christiansen, 1986). At the same time, one sticky trap (10 × 15-cm, Pherobank®, Wijk bij Duurstede, the Netherlands) was attached to the bark at 1.5-m height on the SW side of each tree to monitor beetle landing rates. The number of spruce bark beetles on the sticky traps was recorded 50 days after treatment (15 days after pheromone dispensers were put out) and expressed as beetles m^{-2}.

All trees were inspected for beetle colonization 65 days after treatment (30 days after placement of pheromone dispensers). We determined beetle attack density as attacks m^{-2} bark surface by counting all beetle entrance holes on the bark surface within a 0.2-m band

around the stem (1.7–1.9 m above the ground). Extent of beetle tunneling was determined by carefully removing the outer bark and exposing three randomly selected attacks within each band and recording the length of all egg galleries. Beetle tunneling was expressed as tunnel length per bark surface area (m m^{-2}). We also recorded the developmental stage of any offspring present (i.e., whether there were pupae, larvae, or eggs present). In several MeJA-treated or fungal-inoculated trees, beetle attacks were so sparse that we could find only one to two gallery systems in the inspected band. The following spring (March 2009), we inspected the trees again and recorded if they were alive or dead, based on needle discoloration. See Figure S1 for a full timeline of beetle sampling.

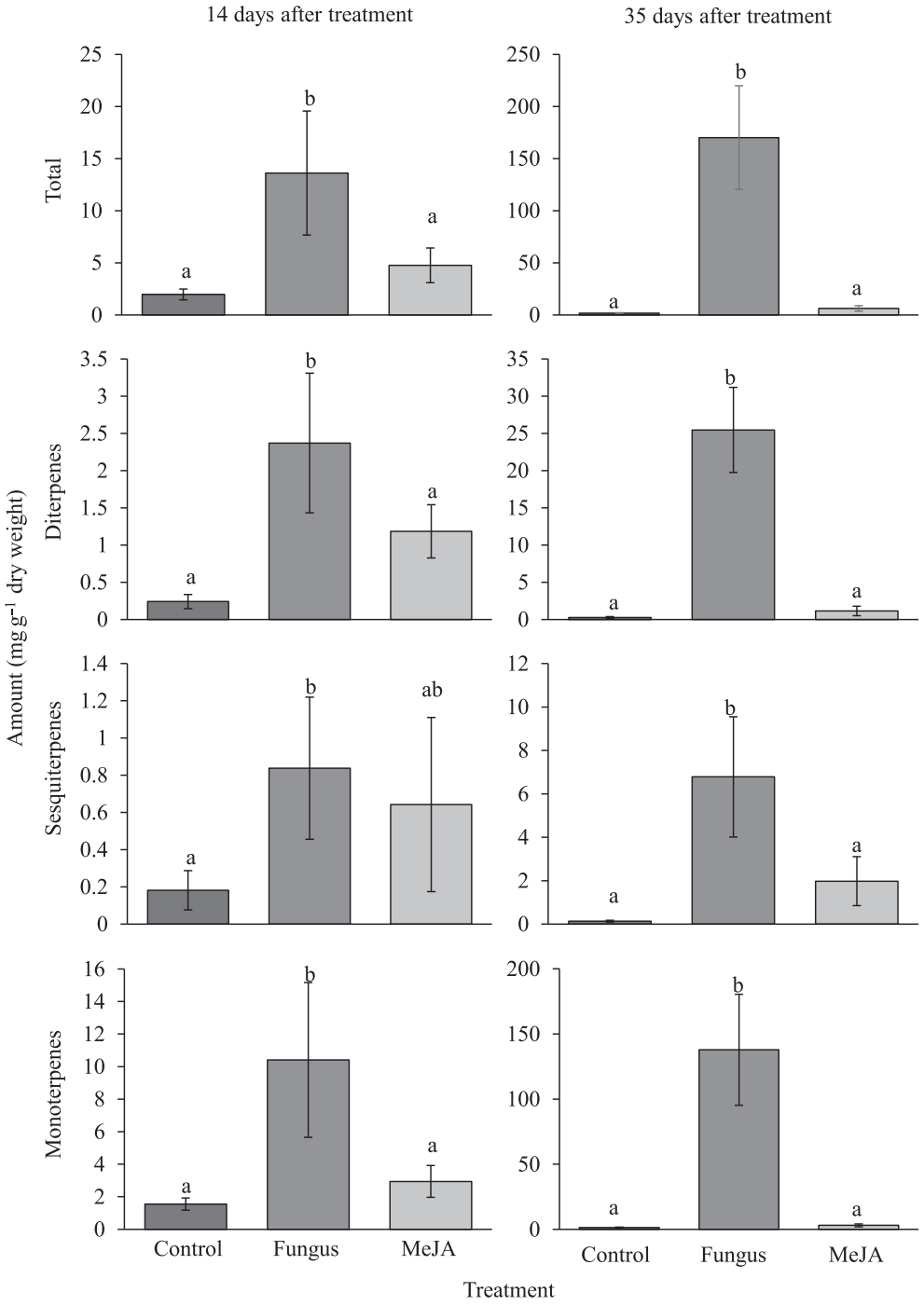

FIGURE 1 Terpene concentrations in the bark of untreated Norway spruce (*Picea abies*) trees (control) and trees conditioned by sublethal fungal inoculations (fungus) or application of methyl jasmonate (MeJA) on the outer bark. Monoterpenes, sesquiterpenes, and diterpenes were extracted from bark plugs collected 14 and 35 days after treatment and quantified using GC–MS. Letters indicate significant difference between treatments ($P < .05$) tested with Tukey's honest significant difference following one-factor analysis of variance. Error bars represent 95% confidence intervals. $n = 20$ trees per treatment, except MeJA ($n = 19$)

2.6 | Statistical methods

Statistical analysis was performed using R (Version 3.5.1) in R studio (Version 1.1.456) and with packages: lm {stats}, anova {stats}, and glht {multcomp} (Hothorn, Bretz, & Westfall, 2008; R Core Team, 2018).

3 | RESULTS

3.1 | Induction of terpene defenses

A total of 18 monoterpenes, 11 sequiterpenes, and five diterpenes were quantified (Table S2). Bark samples taken 14 days after treatment showed that trees inoculated with fungus differed significantly from control and MeJA-treated trees, with sevenfold more total terpenes than control trees. Total terpene concentrations in MeJA-treated trees did not differ from control trees (Figure 1). At 35 days after treatment, trees inoculated with fungus had 27- and 91-fold higher levels of total terpenes than MeJA-treated and control trees, respectively. MeJA-treated and control trees did not differ significantly from each other. Total concentrations of monoterpenes, sesquiterpenes, and diterpenes differed between treatments in a similar way as total terpene concentrations (Figure 1). The same was true for individual terpenes (Table S2).

3.2 | Induction of defense-related gene transcription

Using RT-qPCR, we found a significant impact of MeJA treatment and fungal inoculation on defense-related gene expression compared with the control trees at 14 days after treatment. We examined the transcript level of three groups of genes: (a) two pathogenesis-related genes (the chitinase *Chi4* and the peroxidase *PX3*); (b) four phenylpropanoid biosynthesis genes known to be up-regulated by mechanical wounding or insect feeding (phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, cinnamoyl-CoA reductase, and hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase); and (c) five genes involved in terpene biosynthesis (three isoprenyl diphosphate synthases [*IDS1*, *IDS2*, and *IDS5*], (+)-3-carene synthase [*TPS-Car*], and (−)-limonene synthase [*TPS-Lim*]; Fossdal et al., 2012; Schmidt et al., 2011).

Transcripts of the pathogenesis-related genes, *Chi4* and *PX3*, were significantly up-regulated in both fungus-inoculated and MeJA-treated trees (Figure 2a). Additionally, all quantified phenylpropanoid-related genes were significantly up-regulated in these two treatments relative to the control (Figure 2a). Of terpene-related genes, the monoterpene synthases TPS-Car and TPS-Lim were significantly up-regulated in MeJA-treated trees as compared with control, but not in fungus-inoculated trees (Figure 2b). Surprisingly, there was no correspondence between up-regulation of these two specific monoterpene synthases in MeJA-treated trees and levels of their metabolic products (considered to be 3-carene and terpinolene for TPS-Car, Roach, Hall, Zerbe, & Bohlmann, 2014, and (−)-limonene for TPS-Lim, Martin, Fäldt, & Bohlmann, 2004). As shown in Figure 3, none of the major

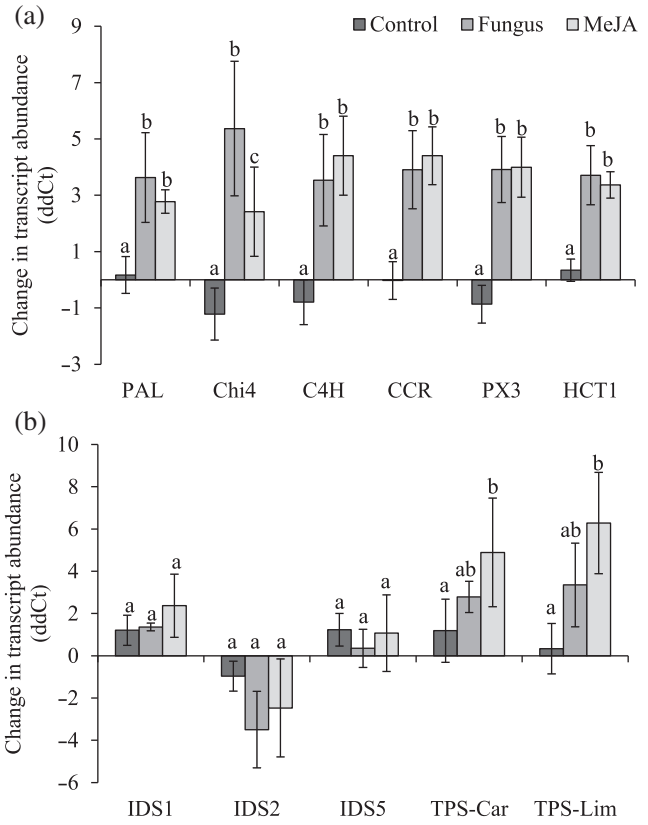

FIGURE 2 RT-qPCR-based gene expression analysis in the bark of untreated Norway spruce (*Picea abies*) trees (control) and trees conditioned by sublethal fungal inoculations (fungus) or application of methyl jasmonate (MeJA) on the outer bark. Bars show differences in gene expression from before treatment to 14 days after treatment. (a) Pathogenesis-related (*Chi4* and *PX3*) and phenylpropanoid-related genes (*PAL*, *C4H*, *CCR*, and *HCT1*) were normalized to actin. (b) Terpenoid biosynthesis-related genes (*IDS1*, *IDS2*, *IDS5*, *TPS-Car*, and *TPS-Lim*) were normalized to ubiquitin. Error bars represent 95% confidence intervals, and letters show significant difference between treatments ($P < .05$) tested with Tukey's honest significant difference following one-factor analysis of variance. Control: $n = 12$; fungus: $n = 6$; and MeJA: $n = 9$

products of these monoterpene synthases increased significantly in MeJA-treated trees relative to the control, neither at 14 nor 35 days after treatment.

3.3 | Bark beetle attack success

Significantly, more beetles landed on sticky traps placed on MeJA-treated trees than on control trees, with trees inoculated with fungus being intermediate, $F(2, 57) = 6.75$, $P = .002$; Figure 4. Despite the lower number of landing beetles, control trees had significantly more beetle entrance holes and tunneling in the bark than both MeJA-treated and fungus-inoculated trees (entrance holes: $F(2, 57) = 22.31$, $P < .0001$; tunneling: $F(2, 57) = 46.39$, $P < .0001$; Figure 4). The number of entrance holes per landing beetle was 38-fold higher on control trees than on MeJA-treated trees. The higher

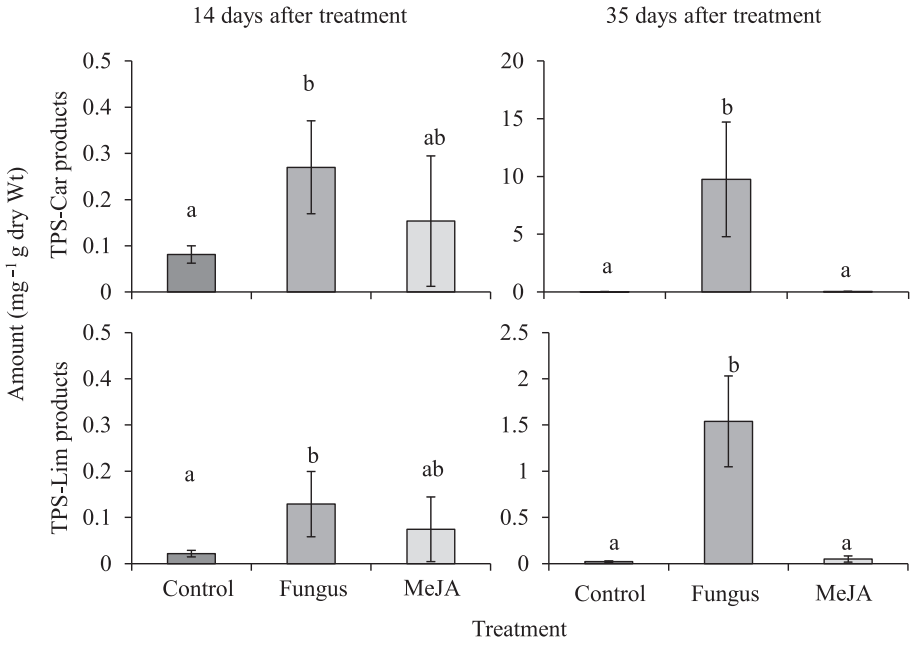

FIGURE 3 Concentration of major products of monoterpene synthases TPS-Car and TPS-Lim 14 and 35 days after treatment in the bark of untreated Norway spruce (*Picea abies*) trees (control) and trees conditioned by sublethal fungal inoculations (fungus) or application of methyl jasmonate (MeJA) on the outer bark. Monoterpene products of TPS-Car and TPS-Lim were extracted and quantified from bark plugs collected 14 and 35 days after treatment as described in Figure 1. Error bars represent 95% confidence interval, and letters show significant difference between treatments ($P < .05$) tested with Tukey's honest significant difference following one-factor analysis of variance. Control: $n = 20$; fungus: $n = 20$; and MeJA: $n = 19$ for monoterpene products; control: $n = 12$; fungus: $n = 6$; and MeJA: $n = 9$ for monoterpenes synthase transcripts

landing rate but lower colonization rate on MeJA-treated trees translated into a dramatically reduced likelihood that beetles landing on trees treated with MeJA would enter and tunnel in the bark.

Of the 20 MeJA-treated trees, only three trees were found to have incipient breeding gallery systems (>5-mm tunnel length), with a male-produced mating chamber and one or more short egg galleries extending from this chamber. The average number of incipient egg galleries per gallery system in these three trees was 1.11, and average egg gallery length was only 7.28-mm. Beetle attacks were much more successful on control trees, where incipient or well-developed gallery systems were found in all 20 trees, with an average of 1.84 egg galleries per gallery system and 49.11-mm egg gallery length (Table S3). Tunneling length per landing beetle was more than 320-fold higher in control trees as compared with MeJA-treated trees. Beetle brood development also differed dramatically between treatments; we found no eggs, larvae, or pupae in MeJA-treated trees, whereas all developmental stages were present in most of the sampled gallery systems in control trees (Figure 5). Beetle reproduction was likewise decreased in fungus-inoculated trees, and tunneling was intermediate between MeJA-treated and control trees (Figure 5 and Table S3).

The following spring (March 2009), 11 of the 20 control trees (55%) were dead, whereas none of the MeJA-treated or fungus-inoculated trees had died (pairwise comparisons of MeJA and fungus vs. control: $P < .0001$ using Fisher's exact test). Developing broods (larvae or pupae) had been observed in all the dead trees the previous year. We had observed eggs in a few surviving trees the previous summer (three control trees and two fungus-inoculated trees), but the remaining 44 surviving trees had no signs of beetle reproduction.

4 | DISCUSSION

MeJA has for almost two decades been known to induce defenses and increase resistance in spruce and other conifers but has not been clearly demonstrated to act as a defense-priming stimulus. Previous studies have suggested that MeJA triggers the direct induction of defenses, such as traumatic resin duct formation, swelling of polyphenolic cells, and transient up-regulation of terpenes and their biosynthetic enzymes (Franceschi et al., 2002; Martin et al., 2002; Zulak et al., 2009). Therefore, MeJA has been presumed to increase tree resistance through direct and prolonged up-regulation of inducible defenses. By comparing the resistance to a bark beetle attack of trees pretreated with MeJA or inoculation with fungus, we now demonstrate that MeJA not only directly induces tree defenses but also primes inducible defenses. Additionally, the setting of this experiment in a forest environment enabled us to evaluate costs and benefits of different defensive spruce phenotypes (i.e., trees with different levels of inducible defenses) under ecologically relevant conditions. Below, we first discuss the evidence that MeJA is a priming stimulus that establishes an immunological-like memory in Norway spruce. We then summarize the allocation costs and fitness benefits associated with three different defensive phenotypes by which spruce trees can face

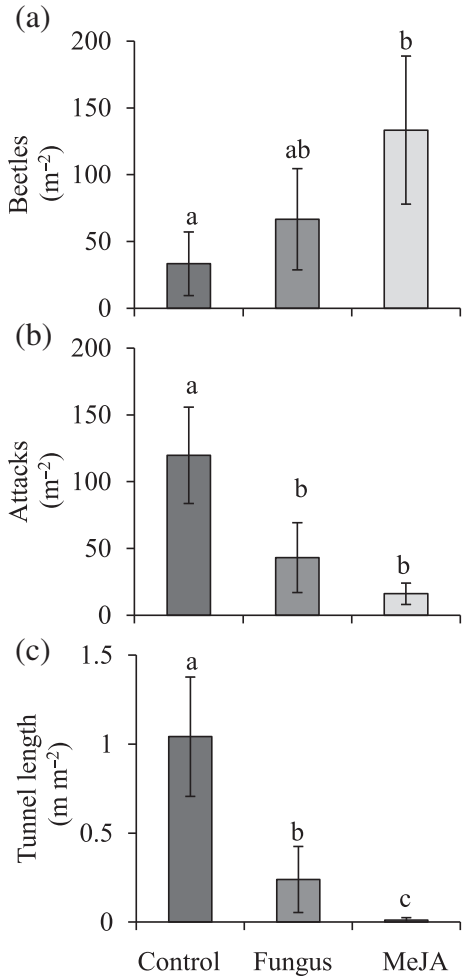

FIGURE 4 Bark beetle colonization of untreated Norway spruce (*Picea abies*) trees (control) and trees conditioned by sublethal fungal inoculations (fungus) or application of methyl jasmonate (MeJA) on the outer bark. (a) Density of beetles trapped in sticky traps on the tree trunks between May 27 and June 11. (b) Density of beetle entrance holes in the bark. (c) Density of length of egg galleries based on data from the three gallery systems per tree. Entrance holes and egg gallery lengths were recorded June 26. Error bars represent 95% confidence interval, and letters show significant difference between treatments ($P < .05$) tested with Tukey's honest significant difference following one-factor analysis of variance. $n = 20$

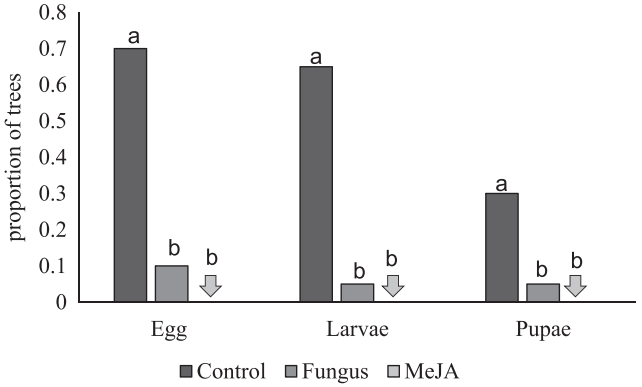

FIGURE 5 Bark beetle brood development in untreated Norway spruce (*Picea abies*) trees (control) and in trees conditioned by sublethal fungal inoculations (fungus) or application of methyl jasmonate (MeJA) on the outer bark prior to beetle attack. For each treatment, the proportion of trees containing bark beetle egg, larvae, and pupae was determined by exposing three breeding gallery systems on each tree ($n = 20$), or fewer if we could not find more gallery systems following repeated sampling. Arrows indicate zero values. Letters show significant differences in pairwise comparisons between treatments ($P < .05$) using Fischer's exact test

bark beetle attack: (a) direct induction of defenses in immunologically naïve trees, (b) prolonged up-regulation of inducible defenses, and (c) priming of defenses (Figure 6). These three defensive phenotypes correspond to our three experimental treatments: untreated control trees, fungus-inoculated trees, and MeJA-treated trees, respectively.

4.1 | MeJA as a priming stimulus

Two key features of defense priming are that it involves a "memory" mechanism and that it "superinduces" the defenses of primed plants (Martinez-Medina et al., 2016). Formation of an immunological-like memory allows the plant to store information about the priming stimulus and maintain a primed state. We directly demonstrated the presence of such a memory in Norway spruce by exposing our experimental trees to two sequential events (Figure 6). First, trees were given a priming stimulus (MeJA application) that established the primed state or memory. Then, 35 days later, trees received a triggering stimulus that let them recall the memory and superinduce their defenses. The nature of the immunological memory in Norway spruce is completely unknown, but based on evidence from *Arabidopsis*, it is likely to involve epigenetic modifications and possibly accumulation of inactive precursor molecules (Conrath et al., 2015; Pastor, Balmer, Gamir, Flors, & Mauch-Mani, 2014; Wilkinson et al., 2019).

The greatly enhanced resistance to bark beetle colonization observed in trees conditioned by MeJA application cannot fully be explained by direct induction of defenses, as there was no significant increase in terpene levels in the bark of these trees relative to naïve control trees 14 or 35 days after MeJA application. Previous studies have reported an induction of terpenes after MeJA treatment, with concentrations peaking around 16 days after treatment and returning to near-normal levels by 32 days after treatment (Erbilgin et al., 2006; Martin et al., 2002; Zulak et al., 2009). However, the reported increases were moderate, ranging from 1.2- to 2.5-fold increase in total terpene concentrations (Erbilgin et al., 2006; Martin et al., 2002). We also observed a moderate 2.2- and 3.4-fold increase in terpenes in our MeJA-treated trees 14 and 35 days after treatment, respectively, but these changes were not statistically significant and were marginal compared with the massive 91-fold increase observed in fungus-inoculated trees 35 days after treatment. At the transcript level, we saw a strong and significant increase in expression of the monoterpene synthases TPS-Car and TPS-Lim in MeJA-treated trees. These trees, however, did not produce significantly higher levels of the TPS-Car and TPS-Lim products 3-carene, terpinolene, or (−)-

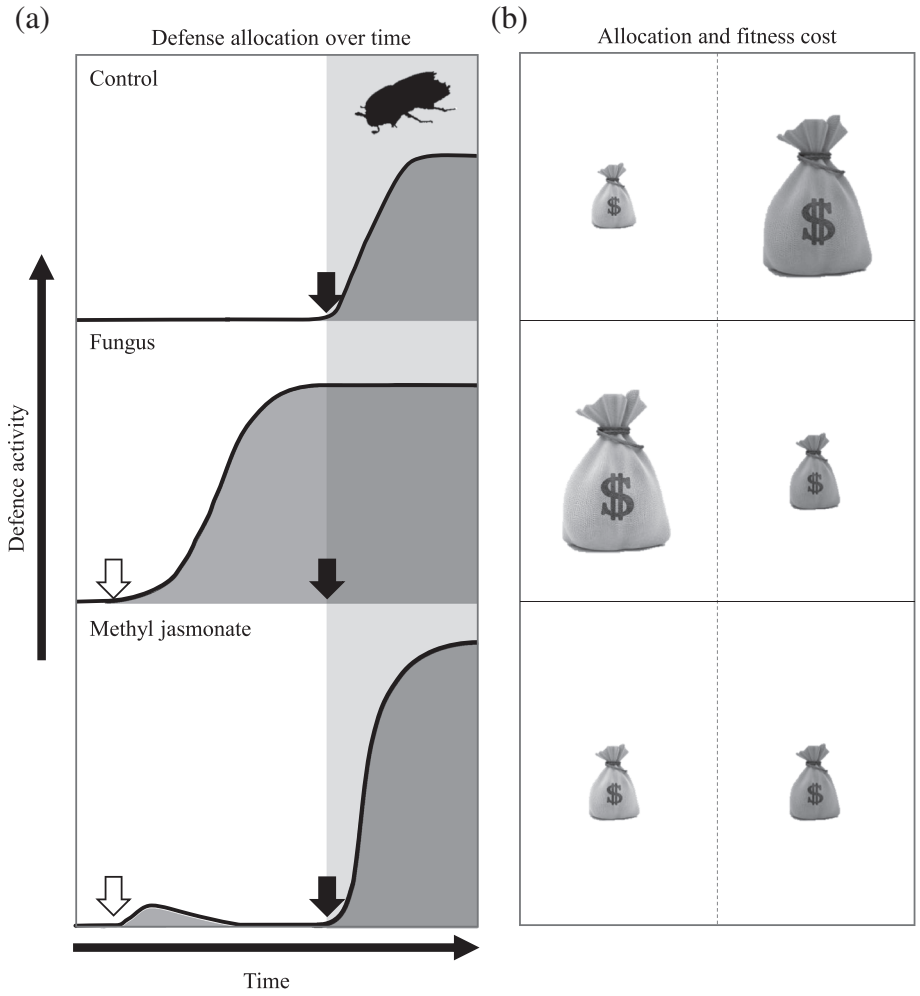

FIGURE 6 Conceptual model of (a) induced defense allocation over time and (b) magnitude of allocation costs (left) and fitness costs (right) in Norway spruce (*Picea abies*). Defenses in immunologically naïve control trees (top) are directly induced upon bark beetle attack (black arrow) but too slowly and weakly to effectively resist attack. Defenses in trees preinfected by fungus (middle) are directly induced by fungal infection (middle, white arrow) and maintained as up-regulated by the continued presence of the fungus. This provides resistance to the subsequent beetle attack (black arrow), but at a high-allocation cost. Treatment with methyl jasmonate (bottom, white arrow) not only causes a slight and transient direct induction of defenses but also activates defense priming. When the beetles attack the primed trees (black arrow), they trigger the superinduction of defenses. This makes the trees highly resistant to attack at a minimal allocation cost

limonene. It is possible that these and other defense-related transcripts, or their protein products, undergo some kind of posttranscriptional or posttranslational modification that allows them to be inactivated until needed (Conrath et al., 2015; Pastor et al., 2014). Unfortunately, we cannot test this hypothesis with our current data. Overall, MeJA treatment alone seemed to induce a very weak defense response compared with the massive and prolonged up-regulation of defenses observed in fungal-inoculated trees.

Because we allowed our experimental trees to be exposed to actual bark beetle colonization rather than simulating beetle attacks with mechanical wounding, we did not collect samples after we had launched the triggering stress. Hence, we do not have direct chemical evidence for a superinduction of defense responses in primed trees from this study. However, in a previous study, we have shown that mechanical wounding of Norway spruce trees 1 month after MeJA treatment results in a 26-fold increase in diterpene concentrations within 24 hr, as compared with an unprimed control tree (Zhao, Krokene, et al., 2011). We have also previously shown that the spruce bark beetle emits very little aggregation pheromone and attracts fewer flying beetles when they tunnel into MeJA-treated trees, presumably because the beetles are physiologically stressed by the trees' primed defenses (Erbilgin et al., 2006; Zhao, Krokene, et al., 2011). This host environment is likely due to the "release of priming" induced by wounding as the beetles chew into the bark.

In contrast to MeJA treatment, pretreatment with sublethal fungal inoculations probably led to direct and prolonged defense induction, because fungus-inoculated trees showed massive up-regulation of terpene levels 35 days after treatment. We cannot rule out that fungal inoculation also activated defense priming in the trees, thus adding to the trees' resistance to the subsequent bark beetle attack. Either way, beetles would be met by a slew of already induced defenses immediately upon entering the trees, and this was probably the main reason for the resistance to beetle attack observed in fungus-inoculated trees.

4.2 | Balancing costs and benefits: Three different defensive phenotypes

Determining the cost–benefit trade-offs of inducible defense can be difficult, as it is challenging to accurately quantify costs and benefits (Neilson, Goodger, Woodrow, & Møller, 2013). This is especially true for large spruce trees with a generation time of >50 years, which makes it almost impossible to directly assess the impact of defense allocation on overall fitness. In our study, we used tree survival after bark beetle colonization as a proxy for fitness, as heavily colonized trees died and could no longer reproduce. As a proxy for allocation costs of direct-induced defenses, we used terpene induction at 35 days after treatment.

We argue that our three experimental treatments illustrate three contrasting defensive phenotypes that spruce trees can face bark beetle attack with (Figure 6): (a) the control treatment represents immunologically naïve defense response that relies on basal defenses and does not yet have acquired immunity, (b) the fungal inoculation treatment shows a prolonged up-regulation of defenses response with massive resource allocation to the synthesis of terpenes, and (c) the MeJA treatment shows a primed defense response that is only fully activated upon bark beetle attack and thus conserves valuable resources.

The control trees have constitutive defenses but are otherwise unprepared for a bark beetle attack. When the attack occurs, these trees must induce their defenses de novo. Some of these inducible defenses, such as the formation of traumatic resin ducts, may take more than 2 weeks to form (Nagy, Franceschi, Solheim, Krekling, & Christiansen, 2000). By this time, the critical phase in the interaction between beetle attack and tree defenses may already be over (Toffin, Gabriel, Louis, Deneubourg, & Grégoire, 2018) and the beetles can successfully colonize and reproduce in many of the trees, with high-tree mortality as a result. For these trees, the allocation of resources to defense comes too late and the trees suffer the ultimate fitness cost: death (Figure 6).

Fungus-inoculated trees undergo a prolonged up-regulation of defenses response induced by the actively growing fungus. The trees remain on high alert with their defenses fully induced for an extended period, as illustrated by their higher terpene content than control trees 35 days after treatment. Thus, when a beetle attack finally comes, it is met with already elevated defenses that reduce levels of bark beetle tunneling and reproduction. This resistance probably comes at a high-allocation cost as defenses are maintained over a long period. Nevertheless, relative to control trees, it seems that the benefits of prolonged up-regulation of defenses outweigh the costs (Figure 6), as all fungus-inoculated trees survived the beetle attack.

MeJA-treated trees have a primed defense response. Their defenses are on standby, and the trees are not investing heavily in terpenoid defenses. These trees did not have a prolonged increase in terpene concentrations but were nevertheless nearly immune to bark beetle attack, as they suffered negligible beetle tunneling in the bark and no mortality. The MeJA-induced immunological memory seemed to maximize fitness as it allowed trees to resist bark beetle attack at a lower cost by postponing investments in costly defenses until they were needed (Figure 6). In this regard, defense priming appears to represent a win-win situation. However, defense priming may come with some other costs that we have not quantified.

5 | CONCLUSIONS

In this paper, we have shown that treatment with MeJA is effective in reducing bark beetle attack success in Norway spruce trees under field conditions. Although there was no significant induction of defensive terpenes in intact MeJA-treated bark, the number of bark beetle entry holes and length of beetle egg galleries were significantly reduced. Most likely, increased resistance in MeJA-treated spruce trees is due to priming of tree defenses and not direct induction of defense. Understanding the mechanisms by which the priming memory is established requires further investigation. Likely, many of the mechanism known to underpin defense priming in *Arabidopsis* are conserved in gymnosperms, such as changes in DNA methylation and histone modifications (Annacondia et al., 2018; Hilker & Schmülling, 2019; Schillheim et al., 2018). However, due to their vastly different life histories, we may find some important differences between this short-lived angiosperm and a long-lived conifer, particularly in the longevity of the primed state (Wilkinson et al., 2019). Additionally, we were not able to evaluate all potential costs associated with the three defensive phenotypes represented in our study (Figure 6). Priming with MeJA may incur other costs such as reduced ability to form symbiotic relationships and/or increased susceptibility to other attackers (de Román et al., 2011; Martinez-Medina et al., 2016; Vos, Pieterse, & van Wees, 2013). Further work is required to determine the extent and impact of such other costs. Overall, this study illustrates the need for more experiments on nonlaboratory and nonmodel species in order to understand and apply plant defense strategies in a real-world context.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

E.C., B.L., H.S., and P.K. conceived the experiment. E.C., B.L., H.S., T.Z., N.B., and P.K. preformed the field experiment and collections of material. T.Z. and A.K.B.-K. performed the chemical analysis. A.S. and C.G.F. performed the qPCR analysis. A.S., C.G.F., T.Z, M.H.M., and P.K. analysed the data. M.H.M and P.K. wrote the article with contributions of all the authors.

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REFERENCES

Annacondia, M. L., Magerøy, M. H., & Martinez, G. (2018). Stress response regulation by epigenetic mechanisms: Changing of the guards. *Physiologia Plantarum*, *162*(2), 239–250. https://doi.org/10.1111/ppl.12662

Bakke, A., Sæther, T., & Kvamme, T. (1983). *Mass trapping of the spruce bark beetle Ips typographus. Pheromone and trap technology. (Report no. 38.3)*. Ås, Norway: Norwegian Forest Research Institute.

Balmer, A., Pastor, V., Gamir, J., Flors, V., & Mauch-Mani, B. (2015). The "prime-ome": Towards a holistic approach to priming. *Trends in Plant Science*, *20*(7), 443–452. https://doi.org/10.1016/j.tplants.2015.04.002

Bonello, P., Gordon, T. R., & Storer, A. J. (2001). Systemic induced resistance in Monterey pine. *Forest Pathology*, *31*(2), 99–106. https://doi.org/10.1046/j.1439-0329.2001.00230.x

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

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

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., Langenbach, C. J. 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

de Román, M., Fernández, I., Wyatt, T., Sahrawy, M., Heil, M., & Pozo, M. J. (2011). Elicitation of foliar resistance mechanisms transiently impairs root association with arbuscular mycorrhizal fungi. *Journal of Ecology*, *99*(1), 36–45. https://doi.org/10.1111/j.1365-2745.2010.01752.x

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

Eyles, A., Bonello, P., Ganley, R., & Mohammed, C. (2010). Induced resistance to pests and pathogens in trees. *New Phytologist*, *185*(4), 893–908. https://doi.org/10.1111/j.1469-8137.2009.03127.x

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. https://doi.org/10.1093/treephys/tps073

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

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*, 818–824. https://doi.org/10.1104/pp.107.113027

Hammerbacher, A., Schmidt, A., Wadke, N., Wright, L. P., Schneider, B., Bohlmann, J., … Paetz, C. (2013). A common fungal associate of the spruce bark beetle metabolizes the stilbene defenses of Norway spruce. *Plant Physiology*, *162*(3), 1324–1336. https://doi.org/10.1104/pp.113.218610

Heijari, J., Nerg, A.-M. M., Kainulainen, P., Vuorinen, M., & Holopainen, J. K. (2008). Long-term effects of exogenous methyl jasmonate application on Scots pine (*Pinus sylvestris*) needle chemical defence and diprionid sawfly performance. *Entomologia Experimentalis et Applicata*, *128*(1), 162–171. https://doi.org/10.1111/j.1570-7458.2008.00708.x

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. https://doi.org/10.1128/AEM.69.8.4413-4420.2003

Hilker, M., & Schmülling, T. (2019). Stress priming, memory, and signalling in plants. *Plant, Cell & Environment*, *42*(3), 753–761. https://doi.org/10.1111/pce.13526

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. From Science to Policy 8. European Forest Institute.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Koutaniemi, S., Warinowski, T., Kärkönen, A., Alatalo, E., Fossdal, C. G., Saranpää, P., … Teeri, T. H. (2007). Expression profiling of the lignin biosynthetic pathway in Norway spruce using EST sequencing and real-time RT-PCR. *Plant Molecular Biology*, *65*(3), 311–328. https://doi.org/10.1007/s11103-007-9220-5

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.

Krokene, P., & Solheim, H. (1998). Pathogenicity of four blue-stain fungi associated with aggressive and nonaggressive bark beetles. *Phytopathology*, *88*(1), 39–44. https://doi.org/10.1094/phyto.1998.88.1.39

Martin, D. M., Fäldt, J., & Bohlmann, J. (2004). Functional characterization of nine Norway spruce TPS genes and evolution of gymnosperm terpene synthases of the TPS-d subfamily. *Plant Physiology*, *135*(4), 1908–1927. https://doi.org/10.1104/pp.104.042028

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

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

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

Mulock, P., & Christiansen, E. (1986). The threshold of successful attack by *Ips typographus* on *Picea abies*: A field experiment. *Forest Ecology and Management*, *14*(2), 125–132. https://doi.org/10.1016/0378-1127(86)90097-6

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

Neilson, E. H., Goodger, J. Q. D., Woodrow, I. E., & Møller, B. L. (2013). Plant chemical defense: At what cost? *Trends in Plant Science*, *18*, 250–258. https://doi.org/10.1016/j.tplants.2013.01.001

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

Pastor, V., Luna, E., Mauch-Mani, B., Ton, J., & Flors, V. (2013). Primed plants do not forget. *Environmental and Experimental Botany*, *94*, 46–56. https://doi.org/10.1016/j.envexpbot.2012.02.013

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria. URL: R Foundation for statistical computing. https://www.R-project.org/

Reynolds, G. J., Gordon, T. R., & McRoberts, N. (2016). Quantifying the impacts of systemic acquired resistance to pitch canker on Monterey pine growth rate and hyperspectral reflectance. *Forests*, *7*(1), 1–10. https://doi.org/10.3390/f7010020

Roach, C. R., Hall, D. E., 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. *Journal of Biological Chemistry*, *289*(34), 23859–23869. https://doi.org/10.1074/jbc.M114.571703

Schiebe, C., Hammerbacher, A., Birgersson, G., Witzell, J., Brodelius, P. E., Gershenzon, J., … 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. https://doi.org/10.1007/s00442-012-2298-8

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

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. https://doi.org/10.1016/j.phytochem.2007.05.037

Schmidt, A., & Gershenzon, J. (2008). Cloning and characterization of two different types of geranyl diphosphate synthases from Norway spruce (*Picea abies*). *Phytochemistry*, *69*(1), 49–57. https://doi.org/10.1016/j.phytochem.2007.06.022

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. https://doi.org/10.1007/s11103-011-9832-7

Schmidt, A., Wachtler, B., Temp, U., Krekling, T., Seguin, A., & Gershenzon, J. (2010). A Bifunctional geranyl and geranylgeranyl diphosphate synthase is involved in terpene oleoresin formation in *Picea abies*. *Plant Physiology*, *152*(2), 639–655. https://doi.org/10.1104/pp.109.144691

Swett, C. L., & Gordon, T. R. (2017). Exposure to a pine pathogen enhances growth and disease resistance in *Pinus radiata* seedlings. *Forest Pathology*, *47*(1), 1–10. https://doi.org/10.1111/efp.12298

Toffin, E., Gabriel, E., Louis, M., Deneubourg, J. L., & Grégoire, J. C. (2018). Colonization of weakened trees by mass-attacking bark beetles: No penalty for pioneers, scattered initial distributions and final regular patterns. *Royal Society Open Science*, *5*(1). https://doi.org/10.1098/rsos.170454

Vos, I. A., Pieterse, C. M. J., & van Wees, S. C. M. (2013). Costs and benefits of hormone-regulated plant defences. *Plant Pathology*, *62*(S1), 43–55. https://doi.org/10.1111/ppa.12105

Wilkinson, S. W., Magerøy, M. H., Sánchez, A. L., 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-082718-095959

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

Zhao, T., Krokene, P., Hu, J., Christiansen, E., Björklund, N., Långström, B., … Borg-Karlson, A.-K. (2011). Induced terpene accumulation in Norway spruce inhibits bark beetle colonization in a dose-dependent manner. *PLoS ONE*, *6*(10), e26649. https://doi.org/10.1371/journal.pone.0026649

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 the article.

Figure S1. Timeline of experimental treatments, tissue sampling, and evaluation of bark beetle attack. We pre-treated Norway spruce (*Picea abies*) trees with sublethal inoculations of the fungus *Endoconidiophora polonica* or by spraying the bark with 100 mM methyl jasmonate. Five weeks later, we placed pheromone dispensers next to the trees to elicit bark beetle attack. One month after this, we evaluated the level of bark beetle attack and colonization of the bark. In March of the following year, we counted the number of surviving and dead trees.

Table S1. Primer sequences for real time quantitative PCR of genes in the inner bark of Norway spruce (*Picea abies*) trees.

Table S2. Terpenes quantified by GC–MS analysis of Norway spruce (*Picea abies*) trees treated with sublethal inoculations of the fungus *Endoconidiophora polonica*, trees sprayed with 100 mM methyl jasmonate (MeJA) on the outer stem bark, and trees that remained untreated as controls.

Table S3. Number of spruce bark beetle (*Ips typographus)* egg galleries per gallery system and average egg gallery length in Norway spruce (*Picea abies*). Three gallery systems were dissected in each of 20 trees. Letters show significant difference between treatments ($p < 0.05$) tested using Tukey's honest significant difference following one-factor ANOVA.

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