

ENEMIES AT THE GATE: TERPENOIDS AND DEFENSE AGAINST SPRUCE BARK BEETLE (*IPS TYPOGRAPHUS*)

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INTRODUCTION

The spruce bark beetle (*Ips typographus*) (Coleoptera, Scolytidae) is considered the single most destructive of the bark beetles that inhabit the coniferous forests of the Palaearctic region (Berryman, 1972; Wood, 1982). A keystone species, it causes both small-scale and large-scale disturbances, thus driving forest succession in Eurasia (Christiansen and Bakke, 1988). The last outbreak (1971–1981) killed the equivalent of 5 million m³ of spruce timber within a 140,000 km² area of southeastern Norway (Christiansen and Bakke, 1988).

As the adult bark beetles enter the tree they introduce a variety of microorganisms, including phytopathogenic fungi. Bark beetles and their associated fungi represent one of the most serious threats to coniferous forests worldwide (Paine et al. 1997). Thus it is not surprising that conifers appear to have evolved sophisticated constitutive and inducible defence mechanisms to protect themselves against bark beetle colonization (Franceschi et al., 2005).

The best studied chemical defense of *P. abies* and other conifers is the oleoresin found in foliage, stems and other organs, a defense system that has existed for at least 50 million years (Labandeira et al., 2001). Oleoresin is composed largely of terpenes, the largest class of plant secondary compounds (Gershenzon and Kreis, 1999). Terpenes are formed by the fusion of C₅ isopentenoid units and classified by the number of such units present in their basic skeletons. Oleoresin has long been believed to play a crucial role in conifer defense because of its physical properties (i.e. viscosity) and repellency to many herbivores and pathogens. In *P. abies*, oleoresin is found constitutively but may also be induced by herbivore or pathogen attack (Nagy et al., 2000). Since wounding itself can cause the loss of resin, especially the volatile components, we explored the utility of a non-invasive procedure for resin induction involving the application of methyl jasmonate (MJ), an elicitor of plant defense responses in many species. In the present investigation, we have studied the host colonization

process of the spruce bark beetle *Ips typographus* (L.) in mature Norway spruce *Picea abies* (L.) Karst. trees. To test if induced responses of *P. abies* to MJ also have defensive potential against bark beetles, we applied MJ to mature trees in a wild stand and challenged the treated trees with *I. typographus* attracted by pheromone dispensers.

MATERIAL AND METHODS

Field experiment

In the spring, twelve trees were randomly selected in an open, pure stand of mature Norway spruce (60 years old, tree height ca. 28 m. On 26 May, a stem section between 1.5 and 4.5 m above ground was divided into east - and west-facing halves by two vertical lines, using a water-based latex paint. One half of each tree was treated with 100 mM MJ and the other half was left untreated to serve as a control (MJC). MJ was sprayed onto the stem using a small spray gun, while carefully avoiding contamination of the control side.

Three weeks after MJ application (16 June) four samples containing the bark and outermost sapwood (1.6 cm wide × 5 cm high × 1 cm deep) were removed for anatomical investigation from each tree at 1.5 and 3.5 m above ground, two on the treated side and two on the control side. At each site a smaller sample (1.6 × 1.6 × 1 cm) for analysis of terpenes and phenolics was removed, quickly frozen on liquid N₂ and transferred to a –80 °C freezer.

On 17 June, an Ipslure pheromone dispenser (Borregaard, Sarpsborg, Norway) was placed on each tree 2 m above ground to induce attack by *I. typographus*. The dispensers were placed on the north side of the trees on the border between the MJ-treated and untreated sides.

Because the beetle population in the area was relatively low and the main flight of the beetles already had taken place, an additional Ipslure dispenser was added three days later to enhance attraction. Beetle aggregation remained moderate. The pheromone dispensers remained on the stems until 25 July, when the trees were sampled to assess the beetle's attack success.

For sampling, the outer cork bark was carefully shaved away on both sides of trees at the dispenser

height. A transparent plastic sheet (210 × 297 mm) was placed on the stem within the shaved area, with the long side oriented vertically, and well away from the dividing lines between the two treatments. All entrance holes covered by the sheet and penetrating into the live phloem were marked, and more developed beetle galleries were traced. On 26 August, the trees were sampled again immediately above the first sampling site, using the same method.

In the laboratory we recorded the number of entrance holes and incipient galleries (i.e. tunnels longer than 10 mm), and total length of all maternal galleries on the plastic sheets. When multiple galleries extended from a single entrance hole, we recorded the sum of their lengths. On 24 July, 2003, 12 other Norway spruce trees in the same stand (diameter at breast height 28.59 ± 3.19 cm) were treated with MJ as described above to see if MJ treatment in one year would have any effects on beetle colonization the following year. Samples for anatomical and chemical analyses were removed from the trees the following spring (12 May 2004) as described above, and two days later the trees were baited with pheromone dispensers to induce attack by *I. typographus*. At this stage, there was extensive resin flow in some trees on bark that had been treated with MJ, and resin flow was assessed qualitatively on a scale from 0 (no resin) to 4 (extensive resin flow). On 16-17 June 2004, the outer bark was removed and the outcome of the beetle attacks was assessed as described above.

Chemical analyses

Terpene extractions were based on the procedures of Zeneli et al. (2006). A Hewlett-Packard 6890 GC-MSD system, using a DB-5 MS column (30 m × 0.25 mm × 0.25 µm, J&W Scientific, Folsom, CA) was used for the GC-MS analysis of monoterpenes and sesquiterpenes. Split injections (1 µL ethereal extract) were made at a ratio of 1:5 for wood and 1:10 for bark samples with an injector temperature of 220°C. The instrument was run under the same program described by Zeneli et al. (2006). Analysis of diterpene constituents was performed on the same GC-MS instrument fitted with the same DB-5 MS column. Injections were made with 1 µL of the concentrated, derivatized ethereal extracts. GC-MS split ratios were 1:10 (for both wood and bark extracts) with an injector temperature of 220°C.

The temperature programs for the instrument are described by Zeneli et al. (2006). GC-MS generated peaks were quantified using Hewlett-Packard Chemstation software. For quantitative analysis of monoterpenes, sesquiterpenes and diterpenes, the MS detector was operated in the

SIM mode. The selected ions for the internal standards, monoterpenes, sesquiterpenes and diterpene methyl esters are described by Zeneli et al. (2006). The total monoterpene, sesquiterpene, or diterpene resin acid content was calculated as the sum of the individually quantified compounds.

Statistical analyses

Data were analyzed using analysis of variance. Each variable was tested to satisfy assumptions of normality and homogeneity of variances by graphical analysis of residuals. If the variance was non-homogeneous, variables were transformed to square root, which provided distributions that satisfied these assumptions in all cases. Beetle colonization data were analyzed on a single-tree basis, by using the calculated differences between MJ treated and untreated bark within trees as the response variable. The data were subjected to one-sample t-tests using SYSTAT (SPSS Inc., Illinois, USA). A Protected LSD test was used for multiple comparisons of means.

RESULTS AND DISCUSSIONS

Methyl jasmonate increased the number of traumatic resin ducts and the accumulation of terpene resin constituents

Anatomical analyses showed that in both years there were significantly more traumatic resin ducts (TDs) in the xylem of MJ-treated sections of trees than in the xylem of untreated control sections (2003: 27.7% vs. 1.8% of sapwood circumference, $P = 0.001$; 2004: 14.2 vs. 5.9%, $P = 0.04$; one-sample t-test). There was no significant difference in TD abundance in MJ-treated sections between years ($P = 0.11$, t-test).

The concentrations of monoterpenes, diterpenes and total terpenes were significantly higher in MJ treated bark and sapwood than in control tissues (Fig. 1).

This was true both for the year of MJ-application (2003) and the following year, but the response was much weaker in 2004, particularly in the bark. Total terpene concentration in bark was 2.5 fold higher after MJ-treatment in 2003 than in untreated portions of trees, but only 1.3 fold higher in 2004. In wood, the corresponding fold-differences were 3.0 and 2.1. There were no qualitative differences in terpene composition in MJC vs. MJ-treated bark or wood ($R^2 > 0.99$ for linear regression of percent composition of individual terpenes in MJC and MJ-treated tissues).

A total of 27 different terpenes were detected with the monoterpenes, α - and β -pinene and limonene, the sesquiterpene germacrene D, and the diterpenes, dehydroabietic acid, isopimaric acid and neoabietic acid, making up nearly three-quarters of the total terpenes.

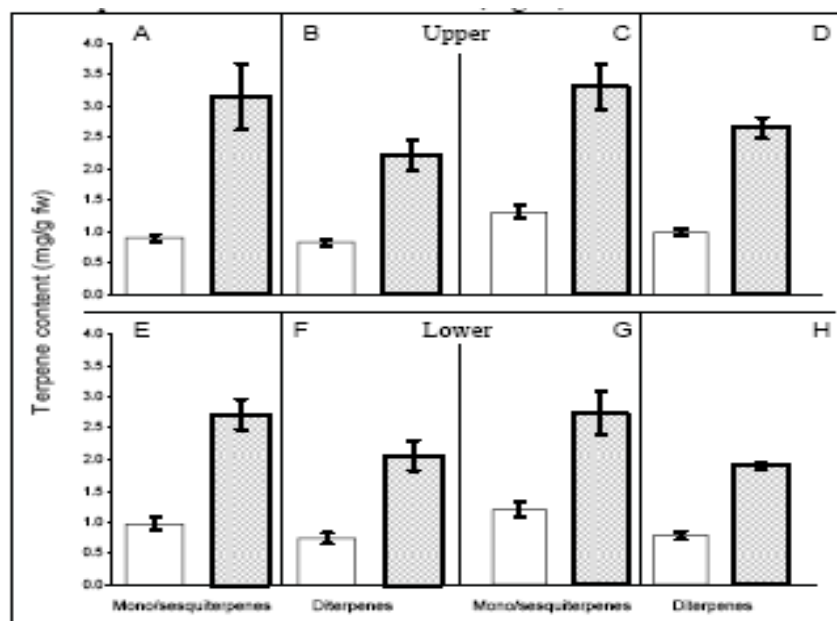


Fig. 1. Effect of methyl jasmonate (MJ) on terpenes of *P. abies* during the year of treatment (2003) at two different heights above the ground. Untreated controls = white bars; MJ treatment = gray bars. (A), (B), (E) and (F) = wood samples; (C), (D), (G) and (H) = bark samples.

The total amount of terpenes was roughly the same in the bark and wood.

Methyl jasmonate (MJ) reduced Ips typographus colonization of Picea abies bark After MJ treatment of portions of the bark surface of twelve 60-year-old *P. abies*, the attack of *I. typographus* was induced three weeks later by pheromone dispensers attached to the trees. Although one tree was mass-attacked and killed in 2003, attacks on the other 11 trees were all aborted. No tunnels extended more than 50 mm from the entrance hole in the eleven surviving trees and no oviposition took place; this allowed comparison of the number and length of attacks between MJ-treated and untreated bark without considering their success.

The application of the pheromone was very effective and beetle attacks did not extend far from the pheromone dispenser. Nearly all were confined to the sampling area.

MJ-treated bark suffered much less bark beetle colonization than did untreated bark on the same tree (referred to as "MJC"), both at the lower and upper sampling positions ($P = 0.0001 - 0.05$). Treated bark had an average of 31 % fewer entrance holes and 69 % fewer galleries, and gallery length was 82 % shorter than in untreated bark. All of these differences were statistically significant. The difference between MJC and MJ-treated bark was significantly greater with regard to total gallery length (MJC:MJ ratio of 6.53) than to number of incipient galleries and entrance holes (MJC:MJ ratios of 2.40 and 1.66, respectively ($F = 4.48$, $P = 0.015$)). This suggests that the negative impact of MJ increased as the beetles proceeded along the

colonization sequence from first entry into the bark to sustained tunneling activity. Beetle colonization also varied significantly with sampling position, with more colonization on the lower position, which was closer to the pheromone source ($F = 4.24 - 13.75$, $P = 0.0006 - 0.04$ for the different colonization variables).

The anatomical and chemical changes induced by MJ application to *P. abies* in this study may be responsible for the increase in bark beetle resistance. MJ treatment has been suggested to increase resistance to herbivores and pathogens in previous studies in a similar manner (Franceschi et al., 2002; Martin et al., 2002; Hudgins and Franceschi, 2004; Zeneli et al., 2006). However, further investigation is required to confirm this conclusion since MJ could have also acted by increasing other chemical or physical defenses that were not measured. In the current study, MJ induced the formation of numerous traumatic resin ducts in the sapwood, and so can be assumed to have increased the volume of resin available for repelling biological invaders and sealing off wounds. An increase in traumatic ducts has been implicated in defense against *C. polonica*, a pathogenic fungal associate of *I. typographus* (Krokene et al., 2003; Zeneli et al., 2006).

ABSTRACT

In the present investigation, the effect of chemical induced defense in host colonization process of the spruce bark beetle *Ips typographus* (L.) in mature Norway spruce were studied. We used methyl jasmonate (MJ), a wellknown inducer of plant defense responses, to manipulate the biochemistry and anatomy of mature *Picea abies* trees and test their resistance to attack by *Ips typographus*. Stem sections of *P. abies*

treated with MJ had a significantly higher concentration of mono-, sesqui- and diterpenes than untreated sections. Bark sections of *P. abies* treated with MJ had significantly less *I. typographus* colonization than bark sections in the controls and exhibited shorter parental galleries and fewer eggs deposited. The increased amount of terpenoid resin present in MJ-treated bark could be directly responsible for the observed decrease in *I. typographus* colonization and reproduction.

CONCLUSIONS

This study demonstrates that MJ application to mature Norway spruce growing in a wild stand induces resistance against bark beetle attack in Norway spruce. Bark sections of *P. abies* treated with MJ had significantly less *I. typographus* colonization than control bark, with shorter parental galleries excavated and fewer eggs deposited. The numbers of beetles that emerged and mean dry weight per beetle were also significantly lower in MJ-treated

bark. The anatomical and chemical changes induced by MJ application to *Picea abies* in this study may be responsible for the increase in bark beetle resistance.

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