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Interaction of pre-attack and induced monoterpene concentrations in host conifer defense against bark beetle-fungal complexes

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Abstract Two pine species (*Pinus resinosa, P. banksiana*) responded to inoculation with fungi carried by bark beetles by rapidly increasing monoterpene concentrations at the entry site. Changes in total monoterpenes were more pronounced than changes in proportionate compositions. The extent and rate of host response was affected by fungal species, the viability of the inoculum, and host tree species. In general, host responses were highest to fungi that are phytopathogenic and consistently associated with the major bark beetles in the study region. Simple mechanical wounding cannot account for the observed allelochemical changes, as aseptic inoculations elicited only minor reactions. Similarly, inoculation with autoclaved inviable fungi generally elicited intermediate responses, suggesting that both structural and metabolic fungal properties are important. Responses by jack pine, *P. banksiana*, were generally more rapid and variable than those of red pine, *P. resinosa*. Dose-toxicity experiments with synthetic compounds demonstrated that monoterpene concentrations present in vivo only a few days after simulated attack are lethal to most beetles. Constitutive (pre-attack) monoterpene levels can also exert some toxicity. Because bark beetles engage in pheromone-mediated mass attacks that can deplete host defenses, constitutive monoterpene levels, while a necessary early phase of successful plant defense, appear insufficient by themselves. Such interactions between constitutive and induced defense chemistry may be important considerations when evaluating general theories of plant defense.

Key words Induced resistance · Plant-insect interactions · Insect-fungal interactions · Terpenes · Bark beetles

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Introduction

Bark beetles (Coleoptera:Scolytidae) exploit the subcortical tissues of woody plants. The available habitat for most species is limited to dead or dying plants or plant parts (Raffa et al. 1993). However, a few species that colonize primary stems can kill relatively healthy trees. Successful development disrupts translocation, removes or degrades the living phloem and is almost always fatal to the host (Raffa and Berryman 1983a). Several species in the genera *Dendroctonus, Ips,* and *Scolytus* can cause extremely high rates of tree mortality, and are important agents of forest succession (Schowalter et al. 1981; Raffa and Berryman 1987). The ability to exploit the subcortical stem tissue of living trees is facilitated by aggregation pheromones that coordinate mass attacks, mutualistic associations with phytopathogenic fungi that help kill the tree, and chemosensory abilities to detect tree stress physiology (Hemmingway et al. 1977; Borden 1982; Whitney 1982; Wood 1982; Raffa and Berryman 1987).

Conifers possess several traits that can reduce the likelihood of bark beetle success (Lorio 1986, 1993; Nebeker et al. 1992, 1993). For example, resins contain monoterpenes and phenolics that are insecticidal and fungistatic at high doses (Cobb et al. 1968; Coyne and Lott 1976; Raffa and Berryman 1982a; Raffa et al. 1985; Langenheim 1994). The concentrations of these compounds in phloem tissue can increase rapidly following invasion by bark beetle-microbial complexes (Christiansen and Horntvedt 1983; Cook and Hain 1986; Miller et al. 1986; Paine et al. 1988, 1993; Lewinsohn et al. 1991; Popp et al. 1991; Raffa 1991; Lieutier 1993; Klepzig et al. in press). Successful defense is accompanied by increased allelochemical concentrations and the formation of necrotic lesions in advance of the beetle-microbial complex (Berryman 1972). It has been proposed that these constitutive (pre-attack) and inducible properties have evolved partly in response to selective pressures exerted by scolytids (Mitton and Sturgeon 1982; Raffa and Berryman 1987).

As with many plant-herbivore relationships (e.g., Chapin et al. 1985; Rhoades 1985; Myers and Williams 1987; Karban and Myers 1989), the precise role of inducible reactions in conifer resistance to bark beetle-fungal complexes remains unclear. Although elevated allelochemical concentrations in response to natural and simulated attacks have often been demonstrated (reviewed in Raffa 1991), we have little understanding of whether or when *in vivo* concentrations are sufficient to affect beetles. The element of time is critical to the dynamics of bark beetle-host interactions, because successful aggregation can overwhelm host defenses. That is, a tree's defensive response may be depleted if the first beetles to enter survive long enough to attract a threshold number of recruits (Raffa and Berryman 1983a). Conversely, rapid host responses to initial entry can repel, incapacitate, or kill the "pioneer" beetles, thus terminating the colonization sequence.

The purpose of this study was to evaluate the role of constitutive and localized rapidly induced host allelochemistry in conifer-bark beetle interactions. We quantified the time sequence of monoterpene concentrations following simulated attack, and the effects of these concentrations on adult beetle survival. Experiments were conducted with two pine species, red pine, *Pinus resinosa* Ait., and jack pine, *P. banksiana* Lamb. The pine engraver, *Ips pini* (Say), and its associated blue-staining fungi *Ophiostoma ips* (Rumb.) Nannf., and *O. nigrocarpa* (R.W. Davidson) De Hoog were assayed. The pine engraver is the major tree-killing bark beetle associated with pines in the upper Great Lakes region (Klepzig et al. 1991). Previous studies demonstrated that all *I. pini* in this region carry *O. ips*, and *O. nigrocarpa* is often but not always present on these same beetles (Klepzig et al. 1991). Host responses to the related fungus *Leptographium terebrantis* (Barras and Perry) were also evaluated, because of its role in *I. pini* ecology: Lower-stem- and root-infesting beetles, such as *Dendroctonus valens* LeConte, are vectors of *L. terebrantis* into below-ground tissue (Klepzig et al. 1994b). Infection is not lethal, but may sufficiently stress trees to reduce their resistance to stem colonization by *I. pini-Ophiostoma* complexes. Previous work with this and related systems demonstrated that inoculations with fungal associates of bark beetles elicit responses qualitatively and quantitatively similar to natural attacks (Raffa and Berryman 1982a,b; Christiansen and Ericsson 1986; Miller et al. 1986; Paine et al. 1988; Raffa 1991).

Materials and methods

General

We administered controlled inoculations of the three test fungi to mature trees to simulate beetle entry, sampled resulting lesions at varying intervals, and determined the monoterpene contents of the reaction tissue. We incorporated a corresponding range of synthetic monoterpenes and controls into ground phloem, placed *I. pini* adults into this medium, and monitored beetle survival.

Field inoculation

Fungi were cultured as described by Raffa and Smalley (1988a,b). Pure stock cultures of *O. ips* (W-20–84), *O. nigrocarpa* (W41-84), and *L. terebrantis* (W-14–85) provided the source for hyphal tip transfers that were grown for 5–7 days in petri plates on Potato Deptrose Agar at 22°C.

The inoculations were administered to mature *P. resinosa* and *P. banksiana* at a site in the Sauk Co. Forest, Wisconsin (Longitude 90′ 11″ W: Latitude 43′ 9″ N). The site is characterized by sandy soil and level terrain. Twenty-five trees of each species were inoculated with one of the three fungi for a total of 150 trees. The red pines were in a single-species plantation, and originated from local seed sources collected and propagated by the Wisconsin Department of Natural Resources. Trees inoculated with *O. ips* or *O. nigrocarpa* were 21 years old, and those inoculated with *L. terebrantis* were 25 years old. The average diameters were 14.3 ± 0.2 cm (x ± SE), 14.3 ± 0.2 cm and 14.4 ± 0.2 cm for the trees inoculated with *O. ips*, *O. nigrocarpa*, and *L. terebrantis*, respectively. The jack pines were in a naturally seeded single species portion of the same forest, located adjacent to the red pine plantation. The trees were 31.4 ± 0.8 years, 29.8 ± 0.4 years, and 23.2 ± 0.4 years old, with diameters of 16.1 ± 0.4 cm, 15.2 ± 0.4 cm and 16.2 ± 0.7 cm, for assays with *O. ips, O. nigrocarpa,* and *L. terebrantis*, respectively. No trees growing along edges were selected.

O. ips and *O. nigrocarpa* were inoculated into the main stem at 1.5 m height to simulate introduction by *I. pini*. *L. terebrantis* was inoculated at soil level to simulate its more typical introduction by *D. valens* (Klepzig et al. 1991). Inoculations were administered by boring a hole to the sapwood with a 4-mm-diameter cork borer, removing a plug from the leading edge of the mycelium in the Petriplate, applying the fungus to the sapwood surface, and replacing the outer bark. Inoculations were administered on 14–15 July 1986.

Each tree received six inoculations with living mycelium, one inoculation with autoclaved mycelium, and one aseptic mechanical wound. Inoculations were spaced evenly about the circumference of the tree. One inoculation site per tree was sampled at 3, 7, 14, 21, and 28 days. For half of the trees, one inoculation was sampled at 10 days and for the other half, one site was sampled at 35 days, to more finely characterize the time course. The autoclaved mycelium – and aseptic mechanical injury – treatments, and a section of unwounded control phloem were sampled at 21 days. For each tree, the first site of fungal inoculation sampling was determined randomly and thereafter proceeded clockwise. Previous work revealed no directional or systemic effects on host response (Raffa and Smalley 1988a,b).

We sampled tissue by removing the outer bark and phloem surrounding the wound response with a hammer and chisel, and then excising the clearly visible phloem lesion with a scalpel. Because lesion width shows little variation among treatments (Raffa and Smalley 1988), only a very narrow section (about,3 cm wide × 12 cm long) was removed from each sample. This allowed for sufficient distances between samples to avoid any interfering effects (Raffa 1991). Any flow of liquid resin from the wound was included in the sample. The tissue was placed into a vial and immediately stored over dry ice in the field. The samples were returned to the laboratory, and stored at –10°C until analysis.

Chemical analysis

Monoterpene contents were determined during January to September 1987, using previously described methods (Raffa and Berryman 1982a,b, 1983b; Raffa and Steffeck 1988). Briefly, phloem samples were finely chopped with a razor blade, and extracted in 10 ml pentane for 24 h. The pentane solution contained 0.1% paracymene as an internal standard. This monoterpene is not present in either *P. banksiana* or *P. resinosa* phloem, is easily separated from the naturally present monoterpenes, and provides consistent response ratios (Raffa and Steffeck 1988). The extract was separated from the phloem by vacuum filtration, and dried over calcium chloride for 1 h.