A host monoterpenone influences *Ips typographus* (Coleoptera: Curculionidae, Scolytinae) responses to its aggregation pheromone

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**Abstract**

1. Host tree terpenones can influence attraction of conifer-infesting bark beetles to their aggregation pheromones, and both synergistic and inhibitory effects have been reported.
2. We tested a gradient of ratios of (–)-α-pinene, the predominant monoterpenone in Norway spruce, to the pheromone of *Ips typographus*, a major pest of Norway spruce.
3. Attraction of *I. typographus* increased as the release rate of (–)-α-pinene increased. The two highest (–)-α-pinene : pheromone ratios (526 : 1 and 2595 : 1) attracted twice as many *I. typographus* as pheromone alone, whereas low to intermediate ratios (56 : 1, 274 : 1) did not differ from pheromone alone.
4. Our results are in agreement with a proposed model, which suggests that bark beetles display unique response profiles to host terpenones depending on the physiological condition of the host trees that they typically colonize. *Ips typographus*, which is an aggressive species capable of colonizing and killing healthy trees, showed an increased attraction to monoterpenone : pheromone ratios, and this may be high enough to inhibit attraction of less aggressive beetle species typically colonizing dead, dying or stressed trees.
5. Attraction of associates of *I. typographus* was also modified by (–)-α-pinene. *Ips duplicatus*, a competitor of *I. typographus*, showed increased attraction to the pheromone of *I. typographus* across all concentrations of (–)-α-pinene.

**Keywords** Attraction, bark beetles, host colonization, host terpenones, inhibition, *Ips*, Norway spruce, α-pinene, pheromone.

**Introduction**

Olfactory cues from plants play a crucial role in host location and selection by herbivorous insects and in their intra- and inter-specific interactions (Bernays, 2000). Interactions between conifer-infesting bark beetles (Coleoptera: Curculionidae, Scolytinae) and their coniferous hosts are of particular interest because several bark beetle species are capable of killing healthy trees, thus causing significant economic losses (Wood S.L., 1982). Olfactory cues provided by host trees can mediate host selection and colonization by bark beetles by providing long- and short-range cues for attraction and, in some cases, synergizing beetle aggregation pheromones during host colonization (Wood D.L., 1982; Byers, 1995).

Monoterpenones are volatile compounds that occur in conifers and other plants. Many beetles use oxygenated monoterpenones as aggregation pheromones. These may be metabolized from host monoterpenones, produced *de novo* after stimulation by host compounds, converted from host compounds by beetle-associated microorganisms, or generated by various combinations of the above (Hendry et al., 1980; Byers, 1989; Ivarsson et al., 1993; Seybold et al., 1995a). Different release rates of host monoterpenones may either enhance or inhibit the attraction of bark beetles to their aggregation pheromones (Rudinsky et al., 1971; Erbilgin & Raffa, 2000; Miller & Borden, 2000a). Furthermore, many monoterpenones are toxic and the pitch in which they occur is viscous and sticky, causing entrapment and suffocation of beetles and associated...
fungi (Raffa & Berryman, 1982; Byers, 1989; Delorme & Lietz, 1990; Raffa & Smalley, 1995; Klepzig et al., 1996).

Erbilgin et al. (2003) showed that attraction of Ips pini (Say), a secondary bark beetle species in the Great Lakes region of the U.S.A., to its aggregation pheromone was modified by the dominant monoterene in bark tissues of the host phloem, α-pinene. Although I. pini showed stronger attraction to pheromone released together with moderate amounts of α-pinene than to pheromone alone, higher α-pinene release reduced its attraction to the pheromone. Erbilgin et al. (2003) hypothesized that different species of bark beetles display unique response profiles to monoterpene : pheromone ratios and that this response is linked to the physiological condition of the host trees they typically colonize (i.e. healthy vs. dying or stressed trees). In the present study, we tested this hypothesis to predict the response of the spruce bark beetle Ips typographus (L.) in a Norway spruce, Picea abies (L.) Karst., forest in Norway.

Materials and methods

Study system

Ips typographus is the most aggressive tree killing bark beetle in Europe and has killed more than 50 million m³ of Norway spruce in large outbreaks from the late 1940s onward (Worrell, 1983; Christiansen & Bakke, 1988). The monoterene (–)-α-pinene was chosen to represent the host terpenes in the present study because it is the dominant monoterene in bark tissues of Norway spruce in Scandinavia (Zeneli et al., 2006) and it increases attraction of I. typographus to its aggregation pheromone (Reddemann & Schopt, 1996; Jakuš & Blaženec, 2003).

Field sites and experimental design

Field experiments were conducted in clear cut areas within a naturally regenerated Norway spruce stand in Ås, southeast Norway from 3–18 June 2005. The treatments consisted of four ratios of (–)-α-pinene to the aggregation pheromone of I. typographus [methyl-3-buten-2-ol (MB) and (S)-cis-verbenol (cV)], the aggregation pheromone alone, and a blank control (six treatments in total). The lures were suspended from multiple funnel traps (NoveFella, KMK TrapTech Inc., Norway) and particular attention was given to suspend the (–)-α-pinene dispensers away from the pheromone source on each trap. Traps were deployed in a randomized complete block design on clear cuttings made within the preceding year. The distance between treatments within blocks was approximately 15 m, and the distance between blocks (a total of five blocks) was approximately 60 m. Treatments were re-randomized every 3–4 days to avoid spurious effects due to trap positions. Trapped insects were identified to species and tabulated. All I. typographus individuals were sexed if trap catches were below 50 and, for trap catches above 50, a random sample of 50 individuals were sexed. Due to unfavourable weather conditions for bark beetle flight, particularly low temperatures and frequent rains with winds, one sample was removed, leaving a total of 30 replicates per treatment for statistical analyses.

Release rates of monoterpenes and pheromones

Pheromone of I. typographus, obtained from Lars Skattebøl (University of Oslo, Norway) and Yngve Stenstrøm (Norwegian University of Life Sciences, Norway), was released from dispensers placed inside triple polyethylene bags. The amount of pheromone released was about 170 mg over 180 days in the laboratory at 21 °C, or average of 0.95 mg/day. The initial ratio between MB and cV in the dispensers was approximately 6 : 1 before deploying them in the field. (–)-α-Pinene was obtained from two sources: Sigma-Aldrich Chemical Co. (St Louis, Missouri) (chemical purity approximately 99%, enantiomeric purity approximately 97%) and Phero Tech, Inc. (Canada) (chemical purity approximately 99.5%, enantiomeric purity approximately 95%). Two different release devices for (–)-α-pinene were obtained from Phero Tech, Inc. The first release devices were 15-mL polyethylene bottles filled with 14 mL of (–)-α-pinene. Each bottle also contained 100 mg BHT (butylhydroxytoluene) (Sigma-Aldrich Chemical Co.) as an antioxidant. The 15-mL bottles released about 103.4 ± 11.3 mg/day of (–)-α-pinene at 20 ± 4.3 °C with 6.2 ± 3.3 km/h wind velocity for 30 days in the field. The second release device (Ultra High Release bags, Phero Tech Inc., Canada) released approximately 2000 ± 85.3 mg/day under the same conditions. The desired (–)-α-pinene : pheromone ratios were achieved by deploying different numbers and combinations of both release devises (Table 1). To obtain a reduced release rate, some of the 15-mL dispensers were modified by covering part of the surface area with non-permeable, oil-based paint, giving a release rate of 53.4 ± 3.9 mg/day at the meteorological conditions specified above.

Statistical analysis

Trap catches were analysed with Poisson regression models for over-dispersed Poisson distributed responses (counts) to address the over-dispersion possibly arising from repeated measurements (McCulloch & Searle, 2001). Count data often do not conform to simple variance assumptions (a variance equal to its mean, and the expectation that the events being counted are independent) implied in using the binomial or multinomial distribution. The over-dispersion error was assumed to be normally distributed. The maximum likelihood ratio test with the Bonferroni adjustment was applied for multiple comparisons for an experiment-wise error rate of 0.05. SAS PROC MIXED procedures were used to estimate parameters (SAS Institute, Cary, North Carolina). Sex ratio data for I. typographus were transformed by arcsin √y.

Results

The most abundant insect species caught in the traps were three species of bark beetles, I. typographus (7316 males, 11 681 females), Ips duplicatus Sahlg˚berg (n = 1262) and Dryocoetes autographus (Rätzegb) (n = 408). There were
Ips duplicatus

Ips typographus in a Norway spruce forest in Norway

| Treatment | 5  | 21.44 | 0.0007 |
| Block     | 4  | 13.83 | 0.0079 |
| Treatment × Block | 20 | 26.23 | 0.1583 |
| Time      | 5  | 20.63 | 0.001  |

Ips duplicatus

Trt: 15 mL 3| 1
| 4  | 9.78  | 0.0443 |
| Treatment × Block | 20 | 14.11 | 0.8249 |
| Time      | 5  | 21.22 | 0.0007 |

Dryocoetes autographus

Trt: 5 21.44 | 0.097 |
| Block     | 4  | 9.78  | 0.0443 |
| Treatment × Block | 20 | 14.11 | 0.8249 |
| Time      | 5  | 21.22 | 0.0007 |

Discussion

Varying release rates of (–)-α-pinene had an effect on attraction of Ips typographus to its pheromone. Ips typographus showed an increased attraction to (–)-α-pinene : pheromone ratios of 526 or higher, whereas attraction to the ratios below 526 was similar to that of pheromone alone. This response differed from a less aggressive cogeneric bark beetle, I. pini, in the Great Lakes region in the U.S.A. (Erbilgin et al., 2003). Ips pini was more strongly attracted to an intermediate monoterpene : pheromone ratio (500 : 1) than to pheromone alone, whereas a higher ratio (5000 : 1) reduced its attraction (Erbilgin et al., 2003).

Our results support a model proposed by Erbilgin et al. (2003). The model predicts that aggressive bark beetles, which are capable of colonizing and killing healthy trees, show maximum attraction at higher monoterpene : pheromone ratios than less aggressive species, which could attack only dead, dying or weakened trees. Thus, the different responses between I. pini and Ips typographus may reflect the differences in host colonization behaviour of these two species. Ips typographus, which is by far the more aggressive of the two, displays two distinctive host colonization behaviours. When its population level is low, Ips typographus typically colonizes dying or recently wind-felled trees, similar to I. pini in the Great Lakes region. However, during outbreaks, Ips typographus is capable of colonizing and killing healthy trees, a behaviour that has never been observed for I. pini in the Great Lakes region. As predicted by the model, attraction increases for both species over relatively low monoterpene : pheromone ratios, but as the concentration of monoterpene increases, attraction of I. pini is reduced, whereas attraction of Ips typographus increases.

Unlike Erbilgin et al. (2003), Miller & Borden (2000a) reported an increased attraction of I. pini in British Columbia, Canada to monoterpene : pheromone ratios that were inhibitory to I. pini in the Great Lakes region. Some of the discrepancy

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between these two studies may be related to regional differences in host colonization behaviours of *I. pini* because this species has a very large host and geographical range in North America (Wood S.L., 1982) with distinctive semiochemical and behavioural variation (Seybold *et al*., 1995b). For example, in British Columbia, *I. pini* is attracted to β-phellandrene alone (Miller & Borden, 1990), the most abundant monoterpene in lodgepole pine phloem (Raffa & Berryman, 1982), whereas host monoterpenes alone are not attractive to *I. pini* in the Great Lakes region (Erbilgin & Raffa, 2000).

Some of the variation between Miller & Borden (2000a) and Erbilgin *et al.* (2003) may also be due to different associations of *I. pini* with other bark beetle species in the two systems. In lodgepole pine stands in British Columbia, *I. pini* is frequently associated with the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, which kills several pine species throughout western North America during periodic outbreaks. *Ips pini* and *D. ponderosae* display niche separation when they attack the same host trees: *I. pini* attacks the mid-and upper-bole, whereas *D. ponderosae* generally infests the lower bole (Furniss & Carolin, 1980). It appears that this niche separation is mediated by semiochemicals because both species can discriminate the pheromone of the other (Miller & Borden, 2000b). Furthermore, unlike *I. pini* in the Great Lakes region, *I. pini* in western North America apparently has the ability to increase its population level and kill healthy trees after *D. ponderosae* outbreaks or long-lasting drought or forest fires (Amman & Ryan, 1991). Thus, *I. pini* in the western North America, including British Columbia, is likely to have evolved olfactory mechanism and behaviour for detecting high concentration of host monoterpenes released from healthy trees upon attacks by *D. ponderosae*. By contrast, *I. pini* in the Great Lakes region is associated with secondary beetle species that usually breed in dead, dying, or recently felled trees (Erbilgin & Raffa, 2002), and may thus have a different strategy for detecting low concentrations of monoterpenes emitted from stressed or weak trees.

In nature, the amount of monoterpenes emitted from trees has been correlated to host resistance against bark beetles and/or their associated microorganisms (Raffa & Berryman, 1982; Leufvén & Birgersson, 1987; Klepzig *et al*., 1995; Phillips & Croteau, 1999; Franceschi *et al*., 2005). During early stages of host colonization, high rates of host terpenes released may actually reduce attraction of most bark beetle species to their pheromones (Cook & Hain, 1986; Lieutier & Berryman, 1988; Byers, 1995). For example, Birgersson & Bergström (1989) measured volatiles released from entrance holes of *I. typographus* on Norway spruce during the first week of an attack and found much higher amounts of monoterpenes released by the host tree compared with pheromones shortly after the males had bored into the tree. This may arise from physical obstruction of resin (e.g. sticky properties and toxicity) and a reduced ability to produce and emit pheromones by males surrounded by resin (Berryman, 1972; Cates & Alexander, 1982; Cook & Hain, 1988; Raffa, 2001).

*Ips duplicatus* showed an increased attraction to the pheromone of *I. typographus* with increased amounts of (–)-α-pinene in the present study. Behavioural responses of *I. duplicatus* to *I. typographus* pheromone components and host monoterpenes are not surprising. The two species are competitors and partition the phloem resources within Norway spruce trees, and they share pheromone components (Schlyter *et al*., 1992; Schlyter & Anderbrant, 1993). Colonization by *I. duplicatus* is usually limited to upper stems of trees (diameter < 20 cm) whereas *I. typographus* commonly colonize the lower parts (Schlyter & Anderbrant, 1993). Our future studies in Norway will focus on the response of *I. duplicatus* to its own pheromone combined with various release rates of (–)-α-pinene to further test the model proposed by Erbilgin *et al.* (2003).

### Acknowledgements

This study was supported by the Norwegian Forest and Landscape Institute and University of California–Berkeley (College of Natural Resources). Lars Skattebøl and Yingve Stenstrom kindly provided pheromones and modified dispensers for *Ips typographus*. The manuscript benefited from comments by Kenneth F. Raffa (University of Wisconsin, Madison), David L. Wood (University of California, Berkeley), and Brice A. McPherson (University of California, Berkeley). The authors are also thankful three anonymous reviewers for their help on the previous draft of this manuscript. We gratefully acknowledge the permission to carry out the field trials in the municipal forest in Ås, Norway.
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Accepted 1 January 2007