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Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines

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Summary. Green leaf volatiles were shown to interrupt responses to aggregation pheromones of three species of bark beetles (Coleoptera: Scolytidae) which infest pines in the southern United States [the southern pine beetle, *Dendroctonus frontalis* Zimm.; the four-spined engraver, *Ips avulsus* (Eichhoff); and the five-spined engraver, *Ips grandicollis* (Eichhoff)]. The order of effectiveness of the compounds tested for each species was hexanal > hexanal + hexan-1-ol > hexan-1-ol. Neither hexanal, hexan-1-ol nor hexanal + hexan-1-ol was as effective in interrupting pheromone responses of *D. frontalis* as verbenone, a known inhibitor of this species. Other than interspecific chemical signals, this is the first report of an interruptant for *Ips* species, and the only report of a pheromone interruptant active for both *Ips* and *Dendroctonus* species.
Key words. Inhibitor; antiaggregation pheromone; bark beetle; green leaf volatile; *Dendroctonus frontalis*; *Ips avulsus*; *Ips grandicollis.*

We have discovered that green leaf volatiles interrupt pheromone responses of three species of cohabiting bark beetles (Coleoptera: Scolytidae) [the southern pine beetle, *Dendroctonus frontalis* Zimm.; the four-spined engraver, *Ips avulsus* (Eichhoff); and the five-spined engraver, *Ips grandicollis* (Eichhoff)] which infest pines in the southern United States. This is the first report of an interruptant for *Ips* species other than response interruption by chemicals emitted by cohabiting *Ips* species[1,2], and the only report of a pheromone interruptant active for both *Ips* and *Dendroctonus* species. We hypothesize that the green leaf volatiles have their effect by providing an inappropriate host stimulus, i.e. a broad-leafed plant, and that, therefore, these compounds may serve to interrupt response of other forest insects to their attractants. The orientation of bark beetles to conspecifics and host trees is largely governed by insect-produced aggregation pheromones and host tree-produced volatiles which may enhance responses to the pheromones[3,4]. Among sympatric bark beetle species, interspecific interactions may occur in which pheromones produced by one species may either enhance responses of other species to their aggregation pheromones[5], or inhibit responses of other species to their aggregation pheromones[1,2]. Antiaggregation pheromones, such as verbenone for *Dendroctonus frontalis* (Zimm.)[6], also may be produced and function to prevent overcrowding by switching of attack from previously attractive trees.

Green leaf volatiles are six carbon alcohols, aldehydes, and their derivatives, e.g. acetates, which are produced by plants as a product of oxidation of surface lipids[7]. These compounds have been assigned several roles in insect behavior, including: 1) host plant finding by phytophagous insects[8-10]; 2) enhancement of insect pheromones[11-13]; and 3) host finding by parasitoids of lepidopterous larvae[14,15].

A series of field tests were conducted involving one or more of the sympatric bark beetle species to determine the effects of the green leaf volatiles, hexan-1-ol and hexanal, on their responses to aggregation pheromone blends. Our first field test investigated the effects of the green leaf volatiles on responses of *D. frontalis* to its aggregation pheromone, frontalin, and a host synergist, southern pine turpentine[16,17]. Verbenone, a known antiaggregation pheromone of *D. frontalis*[6], also was included. Both hexan-1-ol, hexanal, and the combination of the two odors, interrupted the pheromone response of *D. frontalis* (table, A.; $p < 0.05$). The interruptant effect

Mean number of southern pine bark beetles (*Dendroctonus frontalis*, *Ips grandicollis* and *I. avulsus*) captured in traps baited with aggregation pheromones and the green leaf volatiles, hexan-1-ol and hexanal. Verbenone, a known inhibitor for *Dendroctonus frontalis*, was included in tests with this species.

Treatment	Mean number of insects/ replicate ± SE (Mean % of total)[1]
A *Dendroctonus frontalis*[2]	
Frontalin (F) + turpentine (T)	55.8 ± 11.7 (33.5)a
F + T + hexan-1-ol	38.2 ± 7.9 (23.2)b
F + T + hexan-1-ol + hexanal	35.0 ± 13.7 (19.8)b
F + T + hexanal	30.0 ± 10.8 (16.2)b
F + T + verbenone	7.4 ± 1.8 (5.4)c
B *Ips grandicollis*[3]	
Ips pheromone mix (IPM)	48.6 ± 9.4 (32.4)a
IPM + hexan-1-ol	48.2 ± 10.1 (32.3)ab
IPM + hexan-1-ol + hexanal	27.6 ± 5.2 (18.4)abc
IPM + hexanal	22.4 ± 5.7 (14.9)cd
Blank	2.8 ± 1.0 (1.9)d
C *Ips avulsus*[3]	
Ips pheromone mix (IPM)	31.0 ± 4.5 (41.8)a
IPM + hexan-1-ol	18.8 ± 6.2 (25.3)ab
IPM + hexan-1-ol + hexanal	14.2 ± 3.2 (19.1)bc
IPM + hexanal	9.6 ± 3.2 (12.9)bcd
Blank	0.6 ± 0.6 (1.0)d

[1] Mean number of insects are from five replicates. Means followed by different letters are significantly different ($p < 0.05$; Duncan's new multiple range test). Traps were rotated for all tests so that each trap occurred in each position for one collection period.

[2] Racemic frontalin (99%, Pherotech Inc., Vancouver, BC, Canada) was released from two Eppendorf capsules while steam distilled turpentine from loblolly pine (*Pinus taeda* L.) was released from a 250-ml amber bottle via a cotton wick at a rate of 3.6 g/trap/day[20]. Hexan-1-ol (99%, Aldrich Chemical Co., Milwaukee, WI, USA), hexanal (99%, Aldrich Chemical Co.), and verbenone (83% (−):17% (+)-verbenone, Bedoukian Research Inc., Danbury, CN, USA) were released at a rate of 0.3 ml/dispenser/day through a 5 cm × 10 cm polyethylene bag containing a sponge saturated with 4–5 ml of test chemical. Two dispensers of each compound were placed in each trap, except for tests involving two chemicals where one dispenser of each was placed in a trap.

[3] The *Ips* pheromone mix (IPM) consisted of 1% racemic ipsdienol (81%, Borregaard Industries, Sarpsborg, Norway) + 1% ipsenol (89%, Borregaard Industries) + 2% (S)-*cis*-verbenol (85%, Borregaard Industries) in an inert petroleum carrier by volume. IPM was released at a rate of 0.1 g/day by placing 10 g of paste in a 5 cm × 10 cm polyethylene bag. This mixture was previously shown to be attractive to the three southern *Ips* spp. including *I. grandicollis* and *I. avulsus*[18–20]. Hexan-1-ol and hexanal were released at a rate of 0.3 ml/dispenser/day through a 5 cm × 10 cm polyethylene bag containing a sponge saturated with 6 ml of test chemical. Two dispensers of each compound were placed in each trap, except for tests involving two chemicals where one dispenser of each was placed in a trap.

of the green leaf volatiles was significantly less than the effect observed for verbenone.

The attractant pheromone for the two *Ips* species consisted of a mixture of *Ips* pheromone components (racemic ipsdienol, ipsenol, and (S)-cis-verbenol in the ratio 1:1:2) previously shown to be attractive to the cohabiting southern *Ips* including *I. avulsus* and *I. grandicollis*[18–20]. Responses of both *I. grandicollis* and *I. avulsus* to the pheromonal attractant were significantly interrupted by hexanal (table, B and C) ($p < 0.05$). While the combination of hexan-1-ol + hexanal was an effective interruptant of the pheromone response of *I. avulsus*, the combination of odorants did not significantly interrupt responses of *I. grandicollis* to the pheromonal attractant. Hexan-1-ol did not significantly interrupt pheromone responses of either *Ips* species.

Our results show that green leaf volatiles interrupt pheromonal responses in the bark beetle guild which inhabits pines in the southern United States. The ability of these compounds to interrupt pheromonal communication in bark beetles is surprising since green leaf volatiles previously had been shown to serve as host plant attractants[8–10], and enhancers of pheromones[11–13] or other host plant odors[10] among insects associated with green plants. However, bark beetles release their pheromone from the bole or branches of pine trees in a monoterpene rich environment[21], frequently above bushes and deciduous trees. The ability of bark beetles to detect and respond to green leaf volatiles could facilitate orientation to pheromone-producing conspecifics on pine trees, and provide a mechanism to avoid nonhosts and predators associated with green plants. The observation that fir and pine monocultures suffer heavier attacks by bark beetles than mixed forests is commonly explained quantitatively in that there simply are more host trees. The potential effect of green leaf volatiles in mixed stands may be an additional explanation for lower infestation levels which supports efforts for a more diversified silviculture.

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