

Green leaf volatiles disrupt responses by the spruce beetle, *Dendroctonus rufipennis*, and the western pine beetle, *Dendroctonus brevicomis* (Coleoptera: Scolytidae) to attractant-baited traps

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ABSTRACT

We tested the hypothesis that green leaf volatiles (GLVs) disrupt the response of spruce beetles, *Dendroctonus rufipennis* Kirby, and western pine beetles, *Dendroctonus brevicomis* LeConte, to attractant-baited traps. Two green leaf aldehydes, hexanal and (*E*)-2-hexenal, reduced the number of spruce beetles captured to intermediate levels and one green leaf alcohol, hexanol, significantly reduced spruce beetle trap catches. Together, the green leaf alcohols and aldehydes reduced trap catches by 78.7 and 89.3% for males and females, respectively. The green leaf aldehyde, (*E*)-2-hexenal, and two green leaf alcohols, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol, significantly reduced the numbers of male western pine beetles captured and the latter compound also reduced the numbers of female western pine beetles captured. The greatest disruptive effect for the western pine beetle was 46.7% for (*Z*)-2-hexen-1-ol on males. These results support the hypothesis that GLVs common to non-host angiosperms are disruptive to pheromone and kairomone attraction of conifer-attacking bark beetles. While general GLVs are disruptive to several scolytid species, the most disruptive individual GLV components and blends differ by scolytid species and may reflect differences in the volatile characteristics of their particular ecosystems.

Keywords: Spruce beetle, *Dendroctonus rufipennis*, western pine beetle, *Dendroctonus brevicomis*, Coleoptera, Scolytidae, antiaggregant, green leaf volatiles, 1-hexanol, hexanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol.

INTRODUCTION

Volatile stimuli associated with host and non-host plants are important in mediating behavioral responses by phytophagous insects (Visser 1986). Most bark beetles are monophagous or oligophagous, attacking only one or a few species within a genus (Sturgeon and Mitton 1982). Suitable hosts are characteristically well scattered throughout mixed species forests, and are distributed unevenly in space and time (Atkins 1966). Therefore, bark beetles commonly utilize specialized and complex semiochemical messages, including host kairomones and aggregation pheromones to locate suitable breeding material (Borden 1985).

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In seeking suitable hosts, bark beetles encounter and reject many unsuitable hosts and non-host trees. Rejection can be based on a lack of host characteristics or the presence of repellent or deterrent stimuli. Some of the latter stimuli may be green leaf volatiles (GLVs), six carbon alcohols, aldehydes, and derivative esters that are general odor components commonly found in green plants (Visser *et al.* 1979; Whitman and Eller 1990). While GLVs occur across a wide variety of plant families, they are especially abundant in herbaceous plants and deciduous shrubs and trees (Visser *et al.* 1979).

GLVs have been the focus of several recent studies demonstrating that compounds commonly found in non-host angiosperms reduce attraction of conifer-attacking bark beetles to attractant-baited traps. Hexanal and 1-hexanol disrupted attraction of the southern pine beetle, *Dendroctonus frontalis* Zimmermann, to traps baited with attractant semiochemicals and hexanal had a similar effect on the eastern five-spined ips, *Ips grandicollis* (Eichhoff), and the small southern pine engraver, *Ips avulsus* (Eichhoff) (Dickens *et al.* 1992). A blend of four green leaf alcohols disrupted attraction by the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, to pheromone-baited traps, whereas, a blend of two green leaf aldehydes was inactive (Wilson *et al.* 1996). The two most disruptive alcohols, (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol, reduced the number of mountain pine beetles captured in attractant-baited traps to levels found in unbaited control traps and also reduced attacks on attractant-baited trees. Green leaf alcohols have also been shown in trapping experiments to disrupt the response to aggregation pheromones by conifer-infesting ambrosia beetles, including the striped ambrosia beetle, *Trypodendron lineatum* (Olivier) (Borden *et al.* 1997), *Gnathotrichus sulcatus* (LeConte), and *G. retusus* (LeConte) (Deglow and Borden 1998a, b). For *T. lineatum* and *G. sulcatus* the two aldehydes, hexanal and (*E*)-2-hexenal, enhanced the response to pheromone (Borden *et al.* 1997; Deglow and Borden 1998a). An exception occurs for the bark beetle, *Pityogenes knechteli* Swaine, which uses 1-hexanol as a male-produced multifunctional pheromone (Savoie *et al.* 1998). Finally, 1-hexanol was one of four disruptant volatiles for the mountain pine beetle that were collected from the bark of trembling aspen, *Populus tremuloides* Michx., the most common non-host angiosperm associated with the beetle's principal host, lodgepole pine, *Pinus contorta* var. *latifolia* Engelmann (Borden *et al.* 1998). It was the only component that was disruptive on its own.

The disruptive effects of common GLVs on attraction of the southern pine beetle (Dickens *et al.* 1992) and the mountain pine beetle (Wilson *et al.* 1996) suggest that they might also be effective disruptants for other important tree-killing *Dendroctonus* spp. Two of these in Western North America are the spruce beetle, *Dendroctonus rufipennis* Kirby, and the western pine beetle, *D. brevicomis* LeConte. The spruce beetle is the most destructive insect pest of mature spruce forests throughout its range (Safranyik 1988), and the western pine beetle is the most damaging insect affecting growth and yield of ponderosa pine, *Pinus ponderosa* Laws (Smith 1990). Our objectives were to test common GLVs, alone and combined, as potential disruptants for the spruce beetle and the western pine beetle.

MATERIALS AND METHODS

In 1995 and 1996, six field trapping experiments (Exp.) were conducted with 12-unit multiple funnel traps laid out in randomized complete blocks with at least 15 m between traps. A small section of vapona no-pest strip was placed in each trap to kill captured insects.

Exp. 1-3 tested the effect of common GLVs (Table 1) on the spruce beetle. They were conducted in mature stands of Engelmann spruce, *Picea Engelmannii* Parry, and subalpine fir, *Abies lasiocarpa* (Moench.) Voss, near Princeton British Columbia. Spruce beetle lures (Phero Tech Inc., Delta, BC) consisted of the aggregation pheromone frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane) and the host kairomone α -pinene (2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene), with release rates as in Table 1. Exp. 1, conducted from 20 April to 5 June 1995, compared the disruptive effect of green leaf aldehydes, alcohols, or both added to spruce beetle lures. It comprised 10 replicates of five treatments: 1) unbaited control, 2) spruce beetle lure, and spruce beetle lures with 3) green leaf alcohols, 4) green leaf aldehydes, or 5) the full GLV blend. Exp. 2, conducted at the same time as Exp. 1, tested the two green leaf aldehydes alone and combined against the spruce beetle lure, with 10 replicates of five treatments: 1) unbaited control, 2) spruce beetle lure, and spruce beetle lures with 3) hexanal, 4) (*E*)-2-hexen-1-al, or 5) both green leaf aldehydes. Exp. 3, conducted from 18 June to 2 July 1996, tested the four green leaf alcohols alone and combined against spruce beetle lure, with seven replicates of seven treatments: 1) unbaited control, 2) spruce beetle lure, and spruce beetle lures with 3) hexanol, 4) (*E*)-2-hexen-1-ol, 5) (*Z*)-2-hexen-1-ol, 6) (*Z*)-3-hexen-1-ol, or 7) all green leaf alcohols.

Table 1

Description of semiochemicals employed in trapping experiments for the spruce beetle and western pine beetle.

Semiochemical	Source ^a	Release Device ^b	Release Rate mg per 24 hr
frontalin	Phero-Tech	400 μ l Eppendorf	2
α -pinene	Phero-Tech	1.5 ml Eppendorf	50-80
<i>exo</i> -brevicomin	Phero-Tech	400 μ l Eppendorf	1.7
myrcene	Phero-Tech	15 ml polyethylene bottle	15-25
hexanal	Aldrich	bubble cap	13
(<i>E</i>)-2-hexenal	Aldrich	bubble cap	13
1-hexanol	Aldrich	bubble cap	4
(<i>E</i>)-2-hexen-1-ol	Aldrich	bubble cap	4
(<i>Z</i>)-2-hexen-1-ol	Bedoukian	bubble cap	4
(<i>Z</i>)-3-hexen-1-ol	Aldrich	bubble cap	4

^a Aldrich = Aldrich Chemical Company, Milwaukee, WI; Bedoukian = Bedoukian Research Inc., Danbury, CT.

^b Release devices prepared by Phero-Tech, Inc., Delta, BC with semiochemicals stabilized with 1.2% (wet weight) Ethanox® 330 antioxidant, Ethyl Chemicals Group, Baton Rouge, LA. Release rates determined in the laboratory at 20 °C.

Exp. 4-6 were similar to Exp. 1-3, except that they tested whether attraction of the western pine beetle was disrupted by GLVs and were set up in ponderosa pine stands near Nelson, BC. The attractive lure consisted of the pheromones *exo*-brevicomin (*exo*-7-ethyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane) and frontalin (1,5-dimethyl-6,8-dioxabicyclo[3.2.1] octane) plus the host kairomone myrcene (2-methyl-6-methylene-2,7-octadiene) with release rates as in Table 1. Treatments were identical to those for Exps. 1-3, respectively, except that western pine beetle lures replaced spruce beetle lures. Numbers of replicates and dates were as follows: Exp. 4, 10 replicates from 3-10 June and

10 replicates from 15-19 July 1996; Exp. 5, 10 replicates from 10-14 June and 10 replicates from 19-24 July 1996; Exp. 6, 24 replicates with six replicates at a time conducted in 3 to 8 day periods during 14-29 June 1996. For experiments in which replicates were conducted in multiple time periods, all lures were collected after the initial set of replicates, and the experiments were repeated with additional replicates laid out in new randomized complete blocks.

Insects captured in all experiments were collected at regular intervals and held at -18°C until counted and sexed. The numbers of insects captured were transformed by $\log_{10}(x+1)$ and analyzed by ANOVA (GLM procedure, SAS Institute Inc. 1990) for a randomized complete block design, treating replicates as blocks. For Exp. 4-6, in which replicates were conducted over 2 or more time periods, time was included as a blocking factor. The Ryan-Einot-Gabriel-Welsch multiple Q-test (REGW test) (SAS Institute Inc. 1990, Day and Quinn 1989) was used to determine differences between treatment means in all experiments.

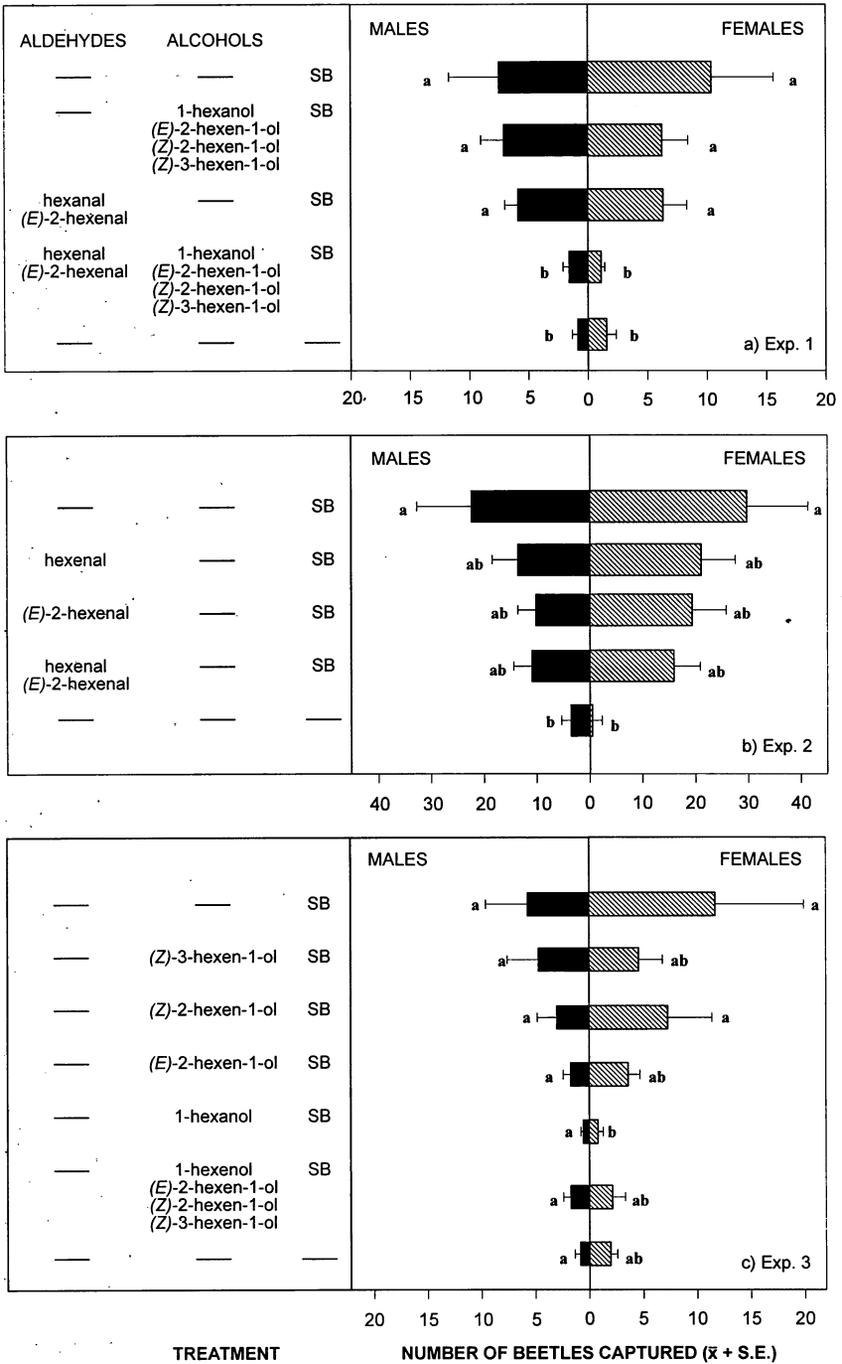
RESULTS

The complete blend of all six GLVs in Exp. 1 caused 78.7 and 89.3% reduction of spruce beetle males and females, respectively, in attraction to spruce beetle lures and reduced the number of beetles captured to levels that did not differ significantly from those in unbaited control traps (Fig 1a). Neither the aldehyde nor the alcohol blends significantly reduced trap catches. Trap catches in Exp. 2 were reduced by the two green leaf aldehydes alone or together to levels that were statistically intermediate between those in unbaited control traps and in traps baited with spruce beetle lures alone (Fig 1b). In Exp. 3, there were no significant differences between treatments for males, but attraction of females was significantly reduced by 1-hexanol (Fig. 1c). The highest numbers of females captured were in traps baited with spruce beetle lures alone. The numbers of females captured in all other treatments, including unbaited control traps, were at statistically intermediate levels, except captures in traps with spruce beetle lures plus (*Z*)-3-hexen-1-ol, which did not differ from spruce beetle lures alone. The total numbers of spruce beetles captured were 436, 1189, and 335 in Exp. 1-3, respectively.

For the western pine beetle, the responses to GLVs were weak and inconsistent. The aldehyde (*E*)-2-hexenal significantly reduced the number of males captured in Exp. 5 (Fig. 2b). In Exp. 6, (*Z*)-2-hexen-1-ol significantly reduced the number of beetles of both sexes captured and (*E*)-2-hexen-1-ol reduced the numbers of males. The complete alcohol blend reduced the numbers of males captured in Exp. 6, but not in Exp. 4 (Fig. 2a,c). The maximum reduction achieved was 46.7% for (*Z*)-2-hexen-1-ol on males. The total numbers of western pine beetles captured were 4237, 4727, and 32,081 in Exp. 4-6, respectively.

DISCUSSION

The results for the western pine beetle differ from those for the spruce beetle. Whereas, attraction of both male and female spruce beetles was strongly disrupted by the full GLV blend (Fig 1a), no such reduction was seen for the western pine beetle (Fig. 2a). For the spruce beetle, no reduction in attraction was seen for the aldehyde blend or the alcohol blend, suggesting that some combination of both alcohols and aldehydes is required to achieve significant disruption. No aldehyde and only one alcohol was active



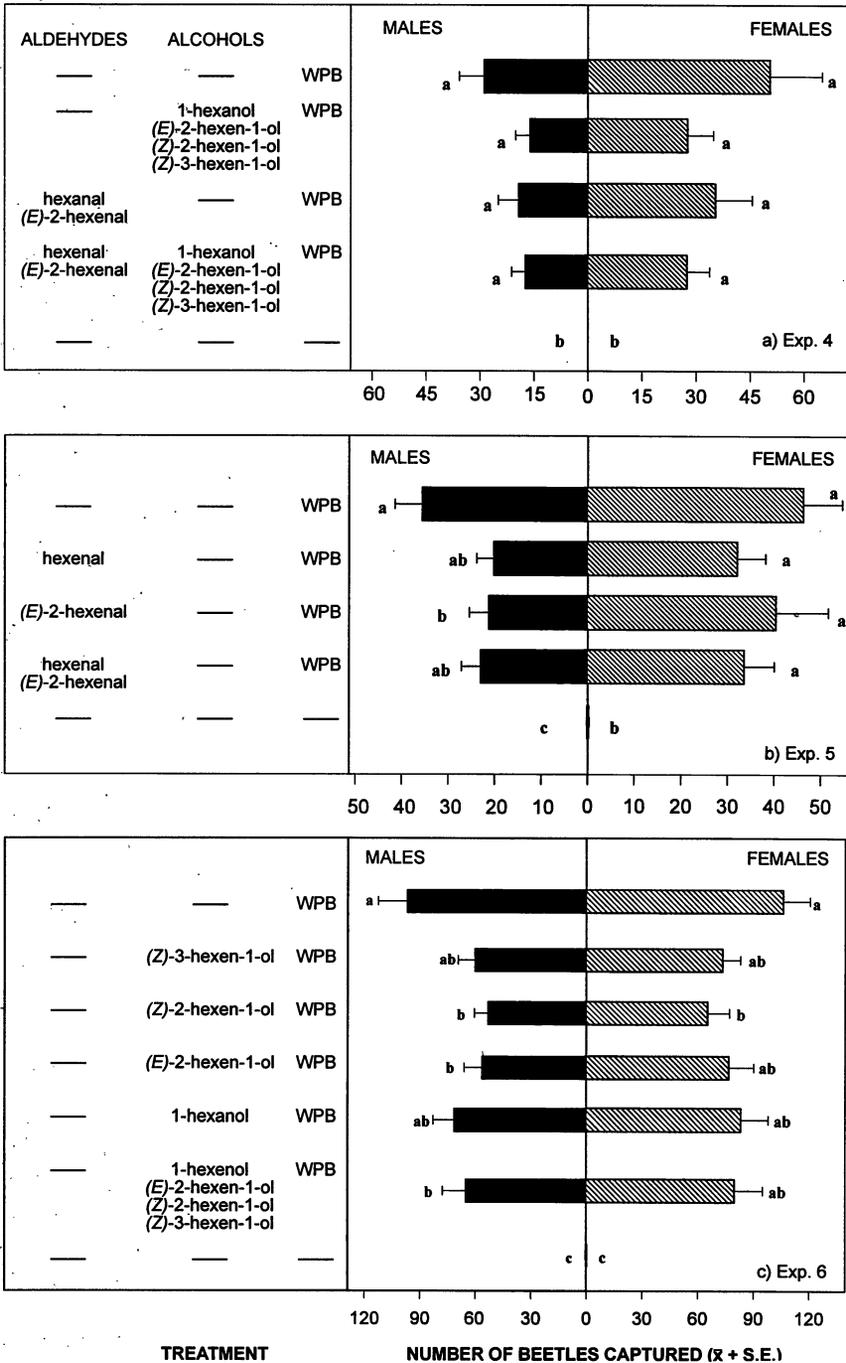


Figure 2. Effect of GLVs released, as in Table 1, on the capture of male and female western pine beetles in attractant-baited multiple funnel traps in Exp. 4-6, near Nelson, BC. Western pine beetle lures (WPB) consisted of *exo*-brevicomin, frontalin and myrcene released as in Table 1. $N=20$, 20 and 24 for Exp. 4-6, respectively. Bars for each sex with the same letter are not significantly different, REGW test, $P<0.05$.

alone against the spruce beetle, but one aldehyde and two alcohols were active alone against the western pine beetle. The weak and inconsistent responses by the western pine beetle to GLVs are typical of those to stimuli of low bioactivity offered at threshold doses.

The results for the spruce beetle and western pine beetle further contrast with the congeneric mountain pine beetle and southern pine beetle. Neither of the aldehydes was disruptive for the mountain pine beetle, and all of the alcohols were disruptive, with the two most effective being (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol (Wilson *et al.* 1996). For the southern pine beetle, both 1-hexanol and hexanal were disruptive (Dickens *et al.* 1992), but neither was effective against the western pine beetle, and only 1-hexanol disrupted the response of the spruce beetle (Figs. 1, 2).

The increased deterancy of spruce beetle attraction by the full GLV blend compared to the alcohols, aldehydes or individual components suggests an additive or dose dependent effect of the combined stimuli. The individual GLV components may have similar dose-dependent disruptive effects, thus differences in response to the full blend may be due to the higher overall release rate of the combined GLVs.

Our results for the spruce beetle and western pine beetle are consistent with the hypothesis that GLVs common to non-host angiosperms are disruptive to conifer-attacking bark and ambrosia beetles. As summarized by Deglow *et al.* (1998a) the pheromone-positive responses of 10 species of conifer-infesting scolytid beetles are now known to be disrupted by green leaf volatiles, presumed to be produced by non-host angiosperms, and for *P. knechteli* 1-hexanol is a repellent pheromone at doses ≥ 15 mg per 24 h (Savoie *et al.* 1998). It would be adaptive for these beetles to recognize and avoid general volatile compounds that are commonly found in a wide variety of non-host deciduous and herbaceous species rather than recognizing precise species-specific volatiles for each non-host species (Borden *et al.* 1998). In this way, several species of non-host trees with partially overlapping blends of common volatile compounds could be perceived and avoided during host location. On the other hand, certain specific compounds found in host trees and the most prevalent non-host species could be important for close range host selection. Precise blends of specific host and pheromone components would further enhance specificity in host selection and maintain breeding isolation between sympatric species of bark beetles.

Only for the mountain pine beetle do GLVs appear to be sufficiently potent to have the potential when used alone to be operationally effective in deterring attack (Wilson *et al.* 1996). For other species, combinations of disruptants, including any or all of GLVs, other non-host compounds, antiaggregation pheromones, repellent synomones, and resistant host kairomones may be required for optimal protection of hosts from attack (Borden 1997).

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