Nonhost Angiosperm Volatiles and Verbenone Protect Individual Ponderosa Pines from Attack by Western Pine Beetle and Red Turpentine Beetle (Coleoptera: Curculionidae, Scolytinae)

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ABSTRACT

Nonhost angiosperm volatiles (NAV) and verbenone were tested for their ability to protect individual ponderosa pines, Pinus ponderosa Dougl. ex. Laws., from attack by western pine beetle (WPB), Dendroctonus brevicomis LeConte, and red turpentine beetle (RTB), Dendroctonus valens LeConte (Coleoptera: Curculionidae, Scolytinae). A combination of (−)-verbenone and eight NAVs [benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (E)-2-hexenal, (E)-2-hexen-1-ol, and (Z)-2-hexen-1-ol] (NAV) significantly reduced the density of WPB attacks and WPB successful attacks on attractant-baited trees. A significantly higher percentage of pitchouts (unsuccessful WPB attacks) occurred on NAV-treated trees during two of three sample dates. In addition, significantly fewer RTB attacks were observed on NAV-treated trees during all sampling dates. The application of NAV to individual ponderosa pines significantly reduced tree mortality, with only 4 of 30 attractant-baited trees dying from bark beetle attack while 50% mortality (15/30) was observed in the untreated, baited control. To our knowledge, this is the first report establishing the effectiveness of NAVs and verbenone for protecting individual ponderosa pines from WPB attack.

Keywords: Pinus ponderosa, Dendroctonus brevicomis, Dendroctonus valens, pest management, tree protection

The western pine beetle (WPB), Dendroctonus brevicomis LeConte (Coleoptera: Curculionidae, Scolytinae), is a major cause of ponderosa pine, Pinus ponderosa Dougl. ex. Laws., mortality in much of the western United States and, particularly, in California (Furniss and Carolin 1977). Under certain conditions, this species can attack and kill apparently healthy trees of all ages and size classes (Miller and Keen 1960). Currently, techniques for managing WPB infestations are limited to tree removals (thinning) that reduce stand density and presumably host susceptibility (Fettig et al. 2007a) and the application of insecticides to protect individual trees (Fettig et al. 2006a). In many cases, these options are not viable because of regulatory and social constraints (Wood et al. 1985). In addition, sufficient markets or labor sources may be lacking in some areas.

The red turpentine beetle (RTB), Dendroctonus valens LeConte, is a common bark beetle species found throughout much of North America. Usually, attacks are confined to basal portions of previously stressed, weakened, or dead and dying trees (Furniss and Carolin 1977) or those under attack by other bark beetles such as WPB (Hall 1983, Fettig et al. 2004). Typically, attacks are not considered a significant threat to tree health. However, tree mortality was attributed to RTB in a 17-year-old ponderosa pine plantation in northern California (Rappaport et al. 2001), for several trees in stands where logging residues were chipped and retained on site in northern California (Fettig et al. 2006b), and of Pinus tabuliformis Carriere in China (Li et al. 2001). In recent years, there has been an interest in developing semiochemical-based management tools for RTB (Rappaport et al. 2001; Sun et al. 2003; Fettig et al. 2005b, 2007b; Gillette et al. 2006). The species has become one of the most economically important forest pests in China and effective control measures are urgently needed (Li et al. 2001, Sun et al. 2003, Zhang et al. 2006).

Studies have shown that insects concentrate host searching in areas of high host concentrations (e.g., Andow [1990]). To reproduce, bark beetles must successfully locate and colonize suitable hosts while avoiding nonhosts. In mixed forests, rejection of nonhosts may occur, in flight, on the basis of absence of host cues or presence of nonhost cues such as green leaf volatiles or angiosperm bark volatiles, collectively termed nonhost angiosperm volatiles (NAV; Borden [1997]). NAVs have been the focus of several recent studies that established these compounds as capable of reducing aggregation in Dendroctonus spp., including WPB, southern pine beetle, Dendroctonus frontalis Zimmerman, mountain pine beetle (MPB), Dendroctonus ponderosae Hopkins, and Douglas-fir beetle, Dendroctonus pseudotsugae Hopkins (Zhang and Schlyter 2004). For many bark beetle species, mass attack on suitable hosts is mediated by aggregation pheromones that are biosynthesized from de novo pathways or host tree precursors (Seybold et al. 2000) in combination with host kairomones (Borden 1985). For example, after initiation of attack, female WPB produce exo-brevicomin, which in...
combination with the host monoterpene myrcene is highly attractive to conspecifics (Bedard et al. 1969). Frontalin, produced by male WPB (Kinzer et al. 1969), enhances attraction.

Poland et al. (1998) were the first to examine the disruptive effect of NAVs on WPB attraction, but their study was limited in scope to green leaf volatiles only and was conducted at the periphery of the beetle's native range (Wood 1982). The aldehyde, $\text{(E)}$-2-hexenal, and two alcohols, $\text{(E)}$-2-hexen-1-ol and $(Z)$-2-hexen-1-ol, significantly reduced numbers of male WPB caught in attractant-baited traps. $(Z)$-2-hexen-1-ol also reduced numbers of female WPB captured (Poland et al. 1998).

Verbenone (4,6,6-trimethylcyclo[3.1.1]hept-3-en-2-one) is an antiaggregation pheromone of several *Deuteroconus* spp., including WPB (Borden 1997, Skillen et al. 1997). In 1970, verbenone was shown to elicit a negative response on tethered, flying female WPBs (Hughes and Pitman 1970). Bedard et al. (1980a) showed that verbenone reduced the number of WPB trapped at a baited source. Trap catches were reduced further by higher release rates of verbenone (Bedard et al. 1980a, 1980b; Tilden and Bedard 1988). In a more recent study, verbenone significantly reduced WPB attraction to attractant-baited traps, but no significant difference was observed between 4 and 50 mg/24-hour release rates (Fettig et al. 2005a). It is assumed that verbenone reduces intraspecific competition by reducing crowding of developing brood within the host (Byers et al. 1984).

In western North America, the use of verbenone to mitigate bark beetle-caused tree mortality has concentrated on small-scale (i.e., less than 5 ha) stand protection, primarily from MPB infestations in lodgepole pine, *Pinus contorta* Dougl. ex. Loud, forests (Amman et al. 1989, 1991; Lindgren et al. 1989; Gibson et al. 1991; Shea et al. 1992; Shore et al. 1992; Lindgren and Borden 1993; Progar 2003; Bentz et al. 2005). Results have been favorable but inconsistent at times (Bentz et al. 1989, 2005; Gibson et al. 1991; Progar 2005), which may be caused by, in part, photoisomerization of verbenone to behaviorally inactive chrysanthenone in the presence of UV light (Kostyk et al. 1993) or other factors (Zhang and Schlyter 2004). Verbenone alone does not appear to be effective for reducing WPB caused tree mortality. For example, the 5-g verbenone pouch (50 mg/24-hour release rate) applied at 125 U/ha was ineffective for protecting ponderosa pine stands from WPB attack in northern California (Fettig 2005). A sprayable formulation of verbenone failed to protect individual ponderosa pines from WPB attack (Gillette et al. 2006). Bertram and Paine (1994) reported that application of ipsdienol and verbenone significantly reduced both numbers of WPB landing on ponderosa pines and the density of attacking beetles. In their study, paired treated and untreated trees were baited with aggregation pheromones to stimulate mass attack, but tree mortality rates were not determined. Lister et al. (1990) reported that verbenone was ineffective for protecting individual ponderosa pines from MPB attack.

Fettig et al. (2005a) examined the effect of several bark volatiles (benzyl alcohol, benzaldehyde, *trans*-conophorin, guaiacol, nonanal, and salicylaldehyde), green leaf volatiles [(E)-2-hexenal, (E)-2-hexen-1-ol, and $(Z)$-2-hexen-1-ol], and a blend of these NAVs on WPB attraction in trapping bioassays. The NAV blend did not significantly affect the response of WPB to attractant-baited traps; however, NAV augmented the disruptive effect of 4 and 50 mg/24-hour (←)verbenone to levels significantly below that of verbenone alone. Additional work revealed that *trans*-conophorin was not essential to the efficacy of this blend. The revised NAVV blend [benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (E)-2-hexenal, (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and verbenone (82%←; 50 mg/24-hours)] reduced trap catch by 87% compared with the attractant-baited control. Both males and females were equally affected (Fettig et al. 2005a). The authors suggested that applications of NAVV to ponderosa pine may reduce WPB colonization rates and presumably subsequent levels of tree mortality (Fettig et al. 2005a).

Others have shown that blends of NAVs, with or without antiaggregation pheromones, may disrupt aggregation of bark beetles on individual host trees and in forested stands (Wilson et al. 1996, Borden et al. 1998, Huber and Borden 2001, Jakus et al. 2003, Zhang and Schlyter 2004). The effectiveness of NAVs to protect individual trees from WPB attack has not been established but is promising given recent identification of an inhibitory blend in trapping bioassays (Fettig et al. 2005a) and positive results with other coniferophagous bark beetles. The objective of this study was to document the efficacy of NAVV to protect individual ponderosa pines from attack by WPB and RTB.

**Methods**

**Study Site**

This study was conducted adjacent to Butte Lake Road, Eagle Lake Ranger District, Lassen National Forest, Lassen County, California (41.60°N, 121.28°W; 1,760-m elevation) from July 2005 to July 2006. Site selection was based on surveys indicating that WPB was causing significant amounts of tree mortality in this area (Bohne and Rios 2005). The cover type was Ponderosa-Jeffrey (Yellowpine) series (mean diameter at 1.37 m [dbh] ± SEM = 27.9 ± 1.5 cm, all species; Smith [1994]). The stand was dominated by ponderosa pine (dbh ± SEM = 29.0 ± 1.8 cm) growing on soils of volcanic origin. Mean stand density was 20.5 m² of basal area per hectare of which 54.6% was ponderosa pine (11.2 ± 2.4 m²/ha); 33.7% Jeffrey pine, *Pinus jeffreyi* Grev. & Balf. (6.9 ± 5.1 m²/ha); 11.2% white fir, *Abies concolor* (Gond. and Glend.) Hildebr. (2.3 ± 1.1 m²/ha), and 0.5% quaking aspen, *Populus tremuloides* Michx. (0.1 ± 0.1 m²/ha). Mean crown cover was 25%. The topography was mainly flat. Ponderosa pine was the only host of WPB present in these stands (Miller and Keen 1960).

**Experimental Design and Treatments**

The experimental design was completely randomized with two treatments and 30 replicates (trees) per treatment. Treatments included baited and otherwise untreated ponderosa pine (mean dbh = 41.5 ± 0.9 cm) and baited NAVV-treated ponderosa pine (mean dbh = 38.2 ± 0.6 cm). NAVV components were formulated in four separate bubblecap bands (Table 1) based on similarity in chemical structure (i.e., alcohols, aldehydes, guaiacol (a phenol), and (←)-verbenone (a ketone)) and applied at a rate of 1 U/10 cm circumference around the bole of each tree beginning at 3 m in height in a manner similar to Huber and Borden (2001). Cumulative release rates for NAVV components varied in direct proportion to tree diameter. For example, a 38.2-cm diameter (measured at ~3 m in height) NAVV-treated tree received 12 bubblecap units per tree (22.8, 97.2, 61.2, 39.6 mg/24-hour cumulative release rate for alcohol, aldehyde, guaiacol, and verbenone bands, respectively; Table 1). Adjacent bands were separated by 20 cm. To rigorously test the efficacy of these treatments, the spacing between adjacent experimental trees was greater than 100 m to increase the likelihood that...
Table 1. Description of semiochemicals used in a tree protection study (Lassen National Forest, Lassen County, California, 2005–2006).

<table>
<thead>
<tr>
<th>Semichemical band (a)</th>
<th>Components</th>
<th>Source (b)</th>
<th>Purity (%)</th>
<th>Release device</th>
<th>Release rate (mg/24 hr) (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>(E)-2-hexen-1-ol</td>
<td>Bedoukian</td>
<td>98</td>
<td>Phero Tech bubblecap</td>
<td>0.6 (20° C)</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>(Z)-2-hexen-1-ol</td>
<td>Bedoukian</td>
<td>95</td>
<td>Phero Tech bubblecap</td>
<td>0.8 (20° C)</td>
</tr>
<tr>
<td></td>
<td>Benzyl alcohol</td>
<td>Fisher Scientific</td>
<td>98</td>
<td>Phero Tech bubblecap</td>
<td>0.5 (20° C)</td>
</tr>
<tr>
<td></td>
<td>(E)-2-hexenal</td>
<td>Bedoukian</td>
<td>97</td>
<td>Phero Tech bubblecap</td>
<td>2.8 (20° C)</td>
</tr>
<tr>
<td></td>
<td>Benzyaldehyde</td>
<td>Fisher Scientific</td>
<td>99</td>
<td>Phero Tech bubblecap</td>
<td>2.9 (20° C)</td>
</tr>
<tr>
<td></td>
<td>Nonanal</td>
<td>Sigma-Aldrich</td>
<td>95</td>
<td>Phero Tech bubblecap</td>
<td>1.5 (20° C)</td>
</tr>
<tr>
<td></td>
<td>Salicylaldehyde</td>
<td>Sigma-Aldrich</td>
<td>99</td>
<td>Phero Tech bubblecap</td>
<td>1.9 (20° C)</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>Guaiacol</td>
<td>Sigma-Aldrich</td>
<td>98</td>
<td>Phero Tech bubblecap</td>
<td>5.1 (20° C)</td>
</tr>
<tr>
<td>Verbenone</td>
<td>Verbenone [82%–(-)]</td>
<td>Phero Tech</td>
<td>97</td>
<td>Phero Tech bubblecap</td>
<td>3.3 (20° C)</td>
</tr>
<tr>
<td>WBP tree bait</td>
<td>Frontalin (racemic)</td>
<td>Phero Tech</td>
<td>98</td>
<td>250 µl Eppendorf vial</td>
<td>3.0 (24° C)</td>
</tr>
<tr>
<td></td>
<td>exo-Brevicomin (racemic)</td>
<td>Phero Tech</td>
<td>97</td>
<td>250 µl Eppendorf vial</td>
<td>3.0 (24° C)</td>
</tr>
<tr>
<td></td>
<td>Myrcene</td>
<td>Sigma-Aldrich</td>
<td>90</td>
<td>1.8 µl x 2 Eppendorf vials</td>
<td>18.0 (24° C)</td>
</tr>
</tbody>
</table>

(a) Band applied at rate of 1 bubblecap U per 10-cm circumference (e.g., 12 U for 38-cm diameter at 3-m height).
(c) Measured in the laboratory at specified temperature after 10 days. Overall release rate of each bubblecap is the addition of individual components.

Results and Discussion

The density of WBP attacks and WBP successful attacks was significantly lower for NAVV at 14 days (df = 1, 58; t = −3.409, P < 0.001) and 28 days (df = 1, 58; t = −5.738, P < 0.001), and 42 days (df = 1, 58; t = −7.050, P < 0.001) after treatment (Figure 1, A and B). At 42 days, we observed a 68% reduction in total attacks and a 76% reduction in successful attacks in the NAVV treatment. The percentage of pit-chouts (unsuccessful WBP attacks) was significantly greater for the NAVV treatment at 28 days (df = 1, 58; t = −5.956, P < 0.001) and 42 days (df = 1, 58; t = −6.864, P < 0.001) after treatment (Figure 1A and B). During initial phases of host colonization (e.g., 14-day sample), the percentage of pit-chouts may not be an appropriate measure of treatment efficacy. Few beetles enter the bark and, typically, trees are capable of covering these wounds and encapsulating beetles (Bedard et al. 1985; Figure 1A–C). However, during mass attack (e.g., 28- and 42-day samples) resin flow and pressure per attack site also is a function of attack density and therefore likely a more effective measure of treatment efficacy. Successful colonization of living host trees requires overcoming tree defenses, which only can be accomplished by recruitment of a critical minimum number of beetles (Wood 1972). This varies with changes in host vigor (Fettig et al. 2007a). If a tree does not sustain a threshold number of attacks, it likely will retain the ability to defend itself by pitching out arriving beetles (Raffa and Berryman 1983).

Borden et al. (1998) reported that a combination of nonhost bark volatiles and verbenone significantly reduced MPB attack densities on attractant-baited trees. In another study, Borden et al. (2003) reported no significant difference in the attack density of MPB (attacks per square meter at eye level) among pheromone-baited P. contorta treated with and without NAVs and verbenone. However, the proportion of mass attacked trees (greater than 17.5-cm dbh) was significantly lower in treatments containing high release rates of verbenone and NAVs.

Fewer RTB attacks were observed on NAVV-treated trees at 14 days (df = 1, 58; t = −6.721, P < 0.001), 28 days (df = 1, 58; t = −7.372, P < 0.001), and 42 days (df = 1, 58; t = −8.248, P < 0.001).
We have established the effectiveness of a blend of eight NAVs and verbenone for reducing WPB colonization densities and associated levels of tree mortality. Others have shown similar effects in other systems (Huber and Borden 2001, Jakus et al. 2003, Zhang and Schlyter 2004). There continues to be considerable interest in the potential use of NAVY for single or multiple tree protection likely will be most significant in residential, recreational (i.e., campgrounds), or administrative sites, similar to settings in which insecticides are used (Fettig et al. 2006a).

Tree losses in these environments generally result in undesirable impacts such as

Figure 2. Mean number (±SEM) of RTB (D. valens LeConte) attacks per tree (below 1.5-m height) on ponderosa pine (P. ponderosa Dougl. ex. Laws.) treated with (dashed line, n = 30) and without (solid line, n = 30) NAV and (−)-verbenone (NAVy) at 14, 28, and 42 days after treatment (Lassen National Forest, Lassen County, California, 2005-2006). Asterisks denote a significant difference between treatments on that date (*test; **P < 0.001).
reduced shade, screening, and aesthetics (Helms and Johnson 1995). Dead trees also pose potential hazards to public safety (Johnson 1981). In addition, NAVV may be of use in small, ecologically unique or sensitive areas where applications of insecticides are not permissible. Additional studies are required to determine the minimum release rate necessary to achieve adequate levels of efficacy, to potentially reduce the total number of compounds in our blend, to determine optimal spacing and formulation on individual trees, to determine the influence of WPB population density on treatment efficacy, and to determine the potential of NAVV for stand protection.

Literature Cited


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