Response of *Dendroctonus brevicomis* to different release rates of nonhost angiosperm volatiles and verbenone in trapping and tree protection studies

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Keywords

Pinus ponderosa, antiaggregation pheromone, nonhost volatiles, pest management, tree protection

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Received: February 29, 2008; accepted: June 25, 2008.

doi: 10.1111/j.1439-0418.2008.01317.x

Abstract

A blend of eight nonhost angiosperm volatiles (benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol) without [NAV] and with [NAVV] (–)-verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) were tested at low (L), medium (M) and high (H) release rates for their ability to reduce attraction of western pine beetle, *Dendroctonus brevicomis* LeConte, to attractant-baited (*exo*-brevicomin [racemic, 3 mg/d], frontalin [racemic, 3 mg/d] and myrcene [18 mg/d]) multiple funnel traps. NAV-L (40 mg/d) had no significant effect. Verbenone alone (50 mg/d) and NAV-M (240 mg/d) both significantly reduced attraction, but no significant difference was observed between the two treatment means. NAV-H (430 mg/d) significantly reduced catches by ~60% and 78% compared to verbenone alone and the baited control, respectively. In a second experiment, combining (–)-verbenone with NAV (NAVV) increased the effects observed in Experiment 1. NAVV-M (240 mg/d) resulted in an ~69% and 83% reduction in trap catch compared to verbenone alone and the baited control, respectively. Significantly fewer *D. brevicomis* were captured in NAVV-H (430 mg/d) than any other treatment resulting in an ~93% reduction in trap catch compared to the baited control. In a third experiment, NAVV was tested at three release rates for its ability to protect individual ponderosa pines, *Pinus ponderosa* Dougl. ex Laws., from attack by *D. brevicomis*. Cumulative release rates varied in direct proportion to tree diameter, but represented quarter, half and full NAVV rates. NAVV significantly reduced the density of *D. brevicomis* attacks, *D. brevicomis* successful attacks, and levels of tree mortality on attractant-baited trees. Only three of 15 NAVV-treated trees died from *D. brevicomis* attack while ~93% mortality (14/15) was observed in the untreated, baited control. Quarter and half rates were ineffective for reducing tree mortality.

Introduction

The western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae, Scolytinae), is a major cause of ponderosa pine, *Pinus ponderosa* Dougl. ex Laws., mortality in much of western North America (Furniss and Carolin 1977). *Dendroctonus brevicomis* prefers large diameter (>50 cm at 1.37 m) trees, but under certain conditions can aggressively attack and kill apparently healthy trees of all ages and size classes (Miller and Keen 1960). Currently, tactics for managing *D. brevicomis* infestations are limited to tree removals (thinning) that reduce stand density and presumably host susceptibility (Fettig et al. 2007) and applications of insecticides to protect individual trees (Fettig et al. 2006a).

Research on host selection by bark beetles has largely concentrated on behaviour during flight. Semiochemicals are frequently placed in attractant-baited traps to elucidate behavioural responses for the purpose of identifying and defining compounds or groups of compounds that reduce attraction, which therefore may be useful in preventing bark beetle attacks on live trees. Several nonhost angiosperm volatiles and verbenone (4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one) have been the focus of considerable study in this regard (reviewed extensively by Zhang and Schlyter 2004). Verbenone was first identified in male *D. brevicomis* by Renwick (1967) and was later demonstrated to reduce attraction of tethered, flying *D. brevicomis* females (Hughes and Pitman 1970). Bedard et al. (1980a) showed that verbenone reduced the number of *D. brevicomis* trapped at a baited source. Trap catches were further reduced by higher release rates of verbenone (Bedard et al. 1980a,b; Tilden and Bedard 1988; Bertram and Paine 1994a), and by combining verbenone with ipsdienol (Paine and Hanlon 1991), the later of which is produced by male *D. brevicomis* (Byers 1982) among other bark beetle species. It is assumed that verbenone reduces intraspecific competition by reducing crowding of developing brood within the host (Byers and Wood 1980; Byers et al. 1984).

Studies have shown that insects tend to focus host searching in patches of high host concentrations (Root 1973; Andow 1990), which increases the probability of encounters with suitable hosts. Many bark beetles use a combination of host kairomones and aggregation pheromones to locate suitable hosts (Borden 1985; Byers 1995; Zhang and Schlyter 2004). Rejection of non-hosts may occur on the basis of absence of host cues or presence of non-host cues such as green leaf volatiles or angiosperm bark volatiles, collectively termed non-host angiosperm volatiles (Borden 1997). Poland et al. (1998) were first to examine the disruptive effect of non-host angiosperm volatiles on *D. brevicomis* 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 in British Columbia, Canada (Wood 1982). Fettig et al. (2005) examined the effects of several nonhost blends on *D. brevicomis* attraction in California, USA. Combinations of six bark volatiles (benzyl alcohol, benzaldehyde, (*E*)-conophthorin, guaiacol, nonanal and salicylaldehyde), three green leaf volatiles [(*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-2-hexen-1-ol], and the nine compounds combined did not affect the response of *D. brevicomis* to traps baited with *exo*-brevicomin, frontalin and myrcene. However, a significant effect was observed when bark and green leaf volatiles were combined with verbenone reducing trap catches to levels significantly below that of verbenone alone. (*E*)-Conophthorin, a compound reported to have significant behavioural activity in a number of other bark beetles (Huber et al. 2000), was not critical to the efficacy of the overall blend. A revised blend [benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol and verbenone 82%-(–); abbreviated as NAVV] was shown to reduce trap catch by ~87% compared to the attractant-baited control (Fettig et al. 2005).

Few publications are available on development of semiochemical-based tools for protecting *P. ponderosa* from *D. brevicomis* infestations. Bertram and Paine (1994b) reported applications of verbenone and ipsdienol significantly reduced both numbers of *D. brevicomis* landing on *P. ponderosa* and densities 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. Verbenone alone appears ineffective for individual tree (Gillette et al. 2006) or small-scale stand protection (Fettig 2005). Shepherd et al. (2007) speculated that in many cases synthetic verbenone, deployed without other beetle-derived or non-host cues that more accurately reflect the complexity of the olfactory environment, may not be effective for protecting trees from bark beetle attack. Fettig et al. (2008) were first to demonstrate the successful application of a semiochemical-based technique for protecting individual *P. ponderosa* from *D. brevicomis* attack and resulting levels of *D. brevicomis*-caused tree mortality. The NAVV blend significantly reduced the density of *D. brevicomis* attacks and *D. brevicomis* successful attacks on attractant-baited trees. A significantly higher percentage of pitchouts (unsuccessful *D. brevicomis* attacks) occurred on NAVV-treated trees during two of three sample dates. The application of NAVV to individual *P. ponderosa* significantly reduced tree mortality compared to the untreated, baited control with only four of 30 NAVV-treated trees dying from bark beetle attack. While the above results are encouraging, additional studies are required to determine the minimum release rate necessary to achieve adequate levels of efficacy; to determine the effect of *D. brevicomis* populations on treatment efficacy; and to confirm effects observed in the previous study (Fettig et al. 2008).

The objectives of the present study were to determine the effect of release rate on the response of *D. brevicomis* to nonhost angiosperm volatiles and (–)-verbenone in attractant-baited traps and to attractant-baited *P. ponderosa*. The tree protection study was conducted in an area characterized by high *D. brevicomis* populations as many authors have speculated that population density affects the efficacy of semiochemical-based management techniques (Progar 2003, 2005; Bentz et al. 2005).

Materials and Methods

Trapping bioassays

Two trapping bioassays were conducted at McCloud Flats, Shasta-Trinity National Forest, Siskiyou Co., California, USA (41.30°N, 122.00°W; 1186 m elevation) during 12 July 2005 to 29 July 2005. The stand was dominated by *P. ponderosa* (mean diameter at 1.37 m (d.b.h.) ± SEM = 39.0 ± 3.7 cm) growing on soils of volcanic origin. Mean stand density was 34.8 m^2 of basal area per ha of which ~99% was *P. ponderosa* with the remainder represented by incense cedar, *Calocedrus decurrens* (Torr.) Florin. Other tree species represented in adjacent stands included white fir, *Abies concolor* (Gond. and Glend.) Hildebr, Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, California black oak, *Quercus kelloggii* Newb., and quaking aspen, *Populus tremuloides* Michx. Mean crown cover was ~40%. The topography was mainly flat (<2% slope). *Pinus ponderosa* was the only host of *D. brevicomis* present in these stands (Miller and Keen 1960).

In both experiments, 30 16-unit multiple funnel traps (Lindgren 1983) (Pherotech International Inc., Delta, BC, Canada) were deployed adjacent to a forest road. Traps were separated by >30 m to avoid interference among adjacent treatments and each was randomly assigned one of six treatments during each experiment. Traps were hung on 3-m metal poles with collection cups 80–100 cm above the ground. A 3-cm × 3-cm time-released insecticidal Prozap Pest Strip (2,2-dichlorovinyl dimethyl phosphate, Loveland Industries Inc., Greely, CO, USA) was placed in the collection cup to kill arriving insects and reduce damage or loss to predacious insects. Samples were collected and each treatment was re-randomized (5 replicates/treatment/day) daily between 06:30 and 10:00 h to avoid disturbing traps during periods of peak flight activity (Fettig et al. 2004a). Catches were immediately transported to the laboratory for storage and later analysis. Specimens were tallied and identified using voucher specimens and available keys (Wood 1982). Voucher specimens have been deposited in the USDA Forest Service Bark Beetle and Common Associates Collection housed in Placerville, California, USA.

Test compounds were selected based on performance in previous studies (Fettig et al. 2005, 2008) and were found to be antennally-active and present in nonhost trees sympatric with the distribution of *D. brevicomis* in California, USA (Shepherd et al. 2007). Semiochemicals were loaded into individual Eppendorf vials, polyvinyl chloride bubblecaps and polyethylene bottles to achieve three release rates (Pherotech International Inc.). Details concerning the source, purity, and enantiomeric purity (if chiral) of each semiochemical are provided in Fettig et al. (2005, 2008). Release devices were hung in traps in funnels seven through nine in such a manner to ensure that funnels were not obstructed. Experiment 1 was conducted to compare the effects of (–)-verbenone to three different release rates of the eight non-host angiosperm volatiles (NAV) on *D. brevicomis* response to attractant-baited traps. Treatments included: (1) unbaited control, (2) western pine beetle attractant [WPB; frontalin (racemic, 3 mg/d at 24°C), *exo*-brevicomin (racemic, 3 mg/d at 24°C), and myrcene (18 mg/d at 24°C)], (3) verbenone [V; 82%-(–); 50 mg/d at 30°C] + WPB, (4) benzyl alcohol, benzylaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol [NAV-L; 40 mg/d at 30°C] + WPB, (5) NAV-M (240 mg/d at 30°C) + WPB, and (6) NAV-H (430 mg/d at 30°C) + WPB. The experimental design was completely randomized with six treatments and 40 replicates per treatment (Fettig et al. 2006b).

Experiment 2 was conducted to compare the effects of (–)-verbenone to three different release rates of NAV + verbenone (NAVV) on *D. brevicomis* response to attractant-baited traps. Treatments included: (1) unbaited control, (2) WPB, (3) V + WPB, (4) NAV-L + V (NAVV-L) + WPB, (5) NAV-M + V (NAVV-M) + WPB, and (6) NAV-H + V (NAVV-H) + WPB. The experimental design was completely randomized with six treatments and 40 replicates per treatment.

Trap catches from unbaited controls were excluded from statistical analyses because of the heteroscedasticity among treatments that they caused (Reeve and Strom 2004). For each experiment, a test of normality was performed and appropriate transformations were used when data deviated significantly from a normal distribution (square root; Sokal and Rohlf 1995). A two-way analysis of variance (treatment and sex) was performed on the number of *D. brevicomis* caught per trap per day using $\alpha = 0.05$ (SigmaStat Version 2.0, SPSS Inc., Chicago, IL, USA). If a significant treatment effect was detected, the Tukey's multiple comparison test (Tukey's HSD) was used for separation of treatment means. Cohen's *d* was used to calculate effect size between two means (e.g. WPB and NAVV-H + WPB) among results reported in similar studies examining the effects of semiochemicals on *D. brevicomis* attraction (Cohen 1988).

Individual tree protection study

This study was conducted adjacent to Hog Flat Reservoir, Eagle Lake Ranger District, Lassen National Forest, Lassen Co., California, USA (40.43°N, 120.90°W; 1676 m elevation) from July 2006–July 2007. The forest cover type was Ponderosa-Jeffrey (Yellowpine) series (mean d.b.h. ± SEM = 35.4 ± 1.58 cm, all tree species) (Smith 1994). The stand was dominated by *P. ponderosa* (mean d.b.h. ± SEM = 32.6 ± 1.4 cm) growing on soils of volcanic origin. Mean stand density was 32.2 m^2 of basal area per ha of which ~69% was *P. ponderosa*, ~17% *A. concolor*, ~12% Jeffrey pine, *P. jeffreyi* Grev. & Balf, and ~2% lodgepole pine, *P. contorta* Dougl. ex Loud. Other tree species represented in adjacent stands included *C. decurrens* and *P. tremuloides*. Mean crown cover was ~45%. The topography was mainly flat (<4% slope). Site selection was based on reports that *D. brevicomis* was causing substantial tree mortality in this area (USDA Forest Service 2006) and subsequent surveys by field crews indicating *D. brevicomis* was actively colonizing trees in 2006.

NAVV components were formulated in four separate bubblecap bands based on similarity in chemical structure [i.e. alcohols, aldehydes, guaiacol (a phenol), and (**–**)-verbenone (a ketone)]. The full-rate NAVV treatment was applied at a rate of one bubblecap per 10 cm circumference around the bole of each tree beginning at 3 m in height with verbenone and followed by aldehydes, guaiacol and alcohols bands each of which was separated by ~20 cm (Fettig et al. 2008). The quarter and half rates [NAVV(1/4), NAVV(1/2)] were applied at one unit per 40 or 20 cm of circumference, respectively. Cumulative release rates for NAVV components therefore varied in proportion to tree diameter. For example, a 38.2 cm diameter (measured at ~3 m in height) full rate NAVV-treated tree received 12 bubblecap units per tree (22.8, 97.2, 61.2, 39.6 mg/d cumulative release rate for alcohol, aldehyde, guaiacol and verbenone bands, respectively).

The experimental design was completely randomized with four treatments and 15 replicates (trees) per treatment. Treatments included: (1) untreated *P. ponderosa* (mean d.b.h. ± SEM = 39.8 ± 0.6 cm), (2) 1/4 rate NAVV-treated *P. ponderosa* (mean d.b.h. ± SEM = 39.9 ± 0.5 cm), (3) 1/2 rate NAVV-treated *P. ponderosa* (mean d.b.h. ± SEM = 39.1 ± 0.5 cm), and (4) full rate NAVV-treated *P. ponderosa* (mean d.b.h. ± SEM = 39.1 ± 0.9 cm). There were no significant differences in tree d.b.h. among treatments ($F_{3,\ 56} = 0.8$; P = 0.53), which is thought to influence the susceptibility of *P. ponderosa* to *D. brevicomis* attack (Person 1928).

To rigorously test the efficacy of these treatments, the spacing between adjacent experimental trees was >100 m to increase the likelihood that sufficient numbers of *D. brevicomis* would be in the vicinity of each tree. All trees were baited with one *D. brevicomis* tree bait (Pherotech International Inc.) at ~3.5 m in height on the northern aspect between second (aldehydes) and third (guaiacol) bubblecap bands, and were checked biweekly for evidence of *D. brevicomis* attack. Baits were removed from all trees after 28 days when significant numbers of attacks (>100 m^2) were recorded on untreated control trees, and when natural pheromone production was likely occurring on trees under mass attack (Bedard et al. 1969; Kinzer et al. 1969; Wood et al. 1976).

For each tree (n = 60), we nondestructively sampled, using head lamps and hand lenses, the number of *D. brevicomis* successful attacks (i.e., oxidized phloem material present in pitch tubes or points of attack containing phloem boring dust and/or dry frass) and unsuccessful attacks (i.e. pitch tubes without oxidized phloem material) in 625 cm^2 (25 cm × 25 cm) sample windows at ~1.5 and 4 m in height at northern and southern aspects. These locations corresponded to bole regions directly above and below NAVV bands. Removal of bark to determine the success of egg laying and brood production was not possible without compromising the experiment. Therefore, we define 'successful attacks' as those indicative of the phloem being reached by colonizing *D. brevicomis*, which is where egg laying and early instar larval feeding occur (Miller and Keen 1960). Bark beetle colonization in the case of living hosts requires overcoming tree defences that consist of anatomical and chemical components that are both constitutive and inducible (Franceschi et al. 2005). This can only be accomplished by recruitment of a critical minimum number of beetles, which varies with changes in host vigour (Fettig et al. 2007). A significant proportion of beetles that initiate host selection are 'pitched-out' by drowning or immobilization in resin prior to reaching or feeding on the phloem (Vité and Wood 1961). Such attacks were classified as 'unsuccessful attacks'. In addition, the total number of red turpentine beetle, *D. valens* LeConte, attacks that occurred below 1.5 m in height was recorded for each tree. Data were collected at 14 (1 August 2006), 28 (15 August 2006), and 42 days (29 August 2006) after baits were applied. A test of normality was performed and appropriate transformations were used when data deviated significantly from a normal distribution (square root [attacks] and arcsine square root [% pitchouts]; Sokal and Rohlf 1995). A one-way analysis of variance (treatment) was performed on the number of *D. brevicomis* attacks, number of *D. brevicomis* successful attacks, percentage of pitchouts and number of *D. valens* attacks using $\alpha = 0.05$ for each sample period (SigmaStat Version 2.0, SPSS Inc.).

Final assessments of tree mortality were conducted 18–19 July 2007. This period of time (>11 months) was sufficient for crowns to 'fade', an irreversible symptom of tree mortality. Data on proportions of experimental trees that died from *D. brevicomis* attack were analysed by chi-square tests for comparisons between multiple proportions (Jones 1984; Huber and Borden 2001).

Results

Trapping bioassays

A total of 16 906 *D. brevicomis* were captured in multiple funnel traps during Experiment 1. Overall, the ratio of males to females was 0.55. Significantly more female than male *D. brevicomis* were collected (paired *T*-test, $P < 0.001$). There was no significant treatment × sex interaction ($F_{4,\ 390} = 0.71$; $P = 0.59$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{4,195} = 17.42$; $P < 0.0001$). NAV-L did not affect the response of *D. brevicomis* to attractant-baited traps (fig. 1). V significantly reduced *D. brevicomis* attraction by $\sim$44% compared to WPB, but no significant difference was observed between V + WPB and NAV-L + WPB (fig. 1). NAV-M + WPB caught significantly fewer *D. brevicomis* than WPB and NAV-L + WPB, but was not significantly different from V + WPB (fig. 1).

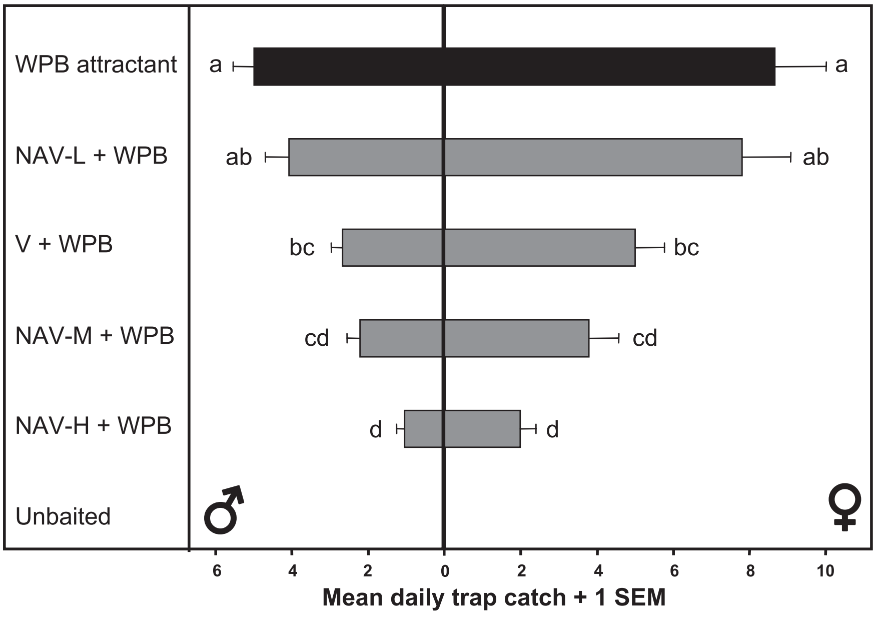

Fig. 1 Disruption of western pine beetle, *Dendroctonus brevicomis* LeConte, attraction to baited multiple funnel traps by the addition of nonhost angiosperm volatiles at three release rates and verbenone, Shasta-Trinity National Forest, California, USA (41.30°N, 122.00°W; 1186 m elevation) 12–29 July 2005. A total of 16 906 beetles were collected. WPB = attractant [*exo*-brevicomin (racemic, 3 mg/d), frontalin (racemic, 3 mg/d), and myrcene (18 mg/d)]; NAV-L = benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol (40 mg/d); NAV-M = same (240 mg/d); NAV-H = same (430 mg/d); and V = verbenone 82%-(–) (50 mg/d). Bars followed by the same letter are not significantly different (n = 40; Tukey's HSD; P > 0.05).

NAV-H + WPB significantly reduced trap catches by $\sim$60% and 78% compared to V + WPB and WPB, respectively (fig. 1). Few beetles (5) were collected in unbaited traps.

A total of 11 710 *D. brevicomis* were captured in multiple funnel traps during Experiment 2. Overall, the ratio of males to females was 0.56. Significantly more female than male *D. brevicomis* were collected (paired *T*-test, $P < 0.001$). There was no significant treatment X sex interaction ($F_{4,\ 390} = 0.80$; $P = 0.52$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{4,195} = 56.91$; $P < 0.0001$). V significantly reduced *D. brevicomis* attraction to attractant-baited traps by $\sim$44%, but no significant difference was observed between V + WPB and NAVV-L + WPB (fig. 2). NAVV-M + WPB resulted in an $\sim$69% reduction in trap catch compared to V + WPB, and an $\sim$83% reduction compared to WPB (fig. 2). Significantly fewer *D. brevicomis* were captured in NAVV-H + WPB than any other treatment. NAVV-H + WPB resulted in an $\sim$58% reduction in trap catch compared to NAVV-M + WPB, and an $\sim$93% reduction compared to WPB (fig. 2). Few beetles (8) were collected in unbaited traps.

Individual tree protection study

Dendroctonus brevicomis attacks were initially concentrated in close proximity to the tree bait and then progressed up and down the tree bole during the second and third sample periods. All experimental trees, except one NAVV-treated, were attacked by *D. brevicomis*. Attack densities, based on individual sample windows, ranged from 0 to 416 attacks/m^2. The density of *D. brevicomis* attacks was significantly lower for NAVV at 14 days ($F_{3,56} = 3.91$; $P = 0.013$), 28 days ($F_{3,56} = 3.45$; $P = 0.023$) and 42 days ($F_{3,56} = 3.87$; $P = 0.015$) than the untreated, baited control. No other significant differences were observed among treatment means for this variable (fig. 3a). The density of successful attacks was also significantly lower for NAVV at 14 days ($F_{3,56} = 4.21$; $P = 0.009$), 28 days ($F_{3,56} = 3.82$; $P = 0.015$) and 42 days ($F_{3,56} = 4.30$; $P = 0.008$) than the untreated, baited control. No other significant differences were observed among treatment means for this variable at 14 days and 28 days (fig. 3b), however at 42 days significantly higher densities of successful attacks was observed for NAVV(¼) than NAVV (fig. 3b). At 42 days, the application of NAVV resulted in an $\sim$51% reduction

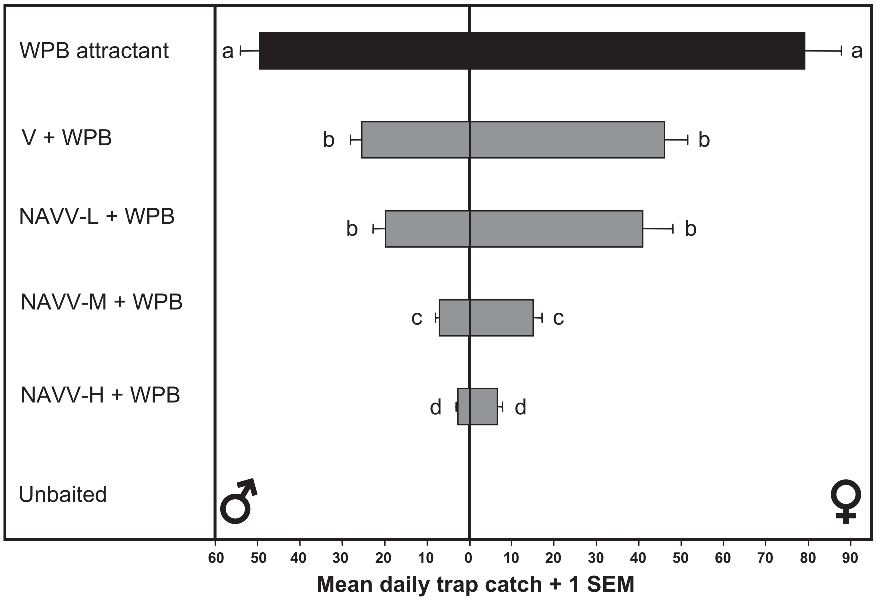

Fig. 2 Disruption of western pine beetle, *Dendroctonus brevicomis* LeConte, attraction to baited multiple funnel traps by the addition of nonhost angiosperm volatiles at three release rates with verbenone and verbenone alone, Shasta-Trinity National Forest, California, USA (41.30°N, 122.00°W; 1186 m elevation) 12–29 July 2005. A total of 11 710 beetles were collected. WPB = attractant [*exo*-brevicomin (racemic, 3 mg/d), frontalin (racemic, 3 mg/d), and myrcene (18 mg/d)]; NAVV-L = benzyl alcohol, benzaldehyde, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-2-hexen-1-ol (40 mg/d) and verbenone; NAVV-M = same (240 mg/d); NAVV-H = same (430 mg/d); and V = verbenone 82%-(–) (50 mg/d). Bars followed by the same letter are not significantly different (n = 40; Tukey's HSD; P > 0.05).

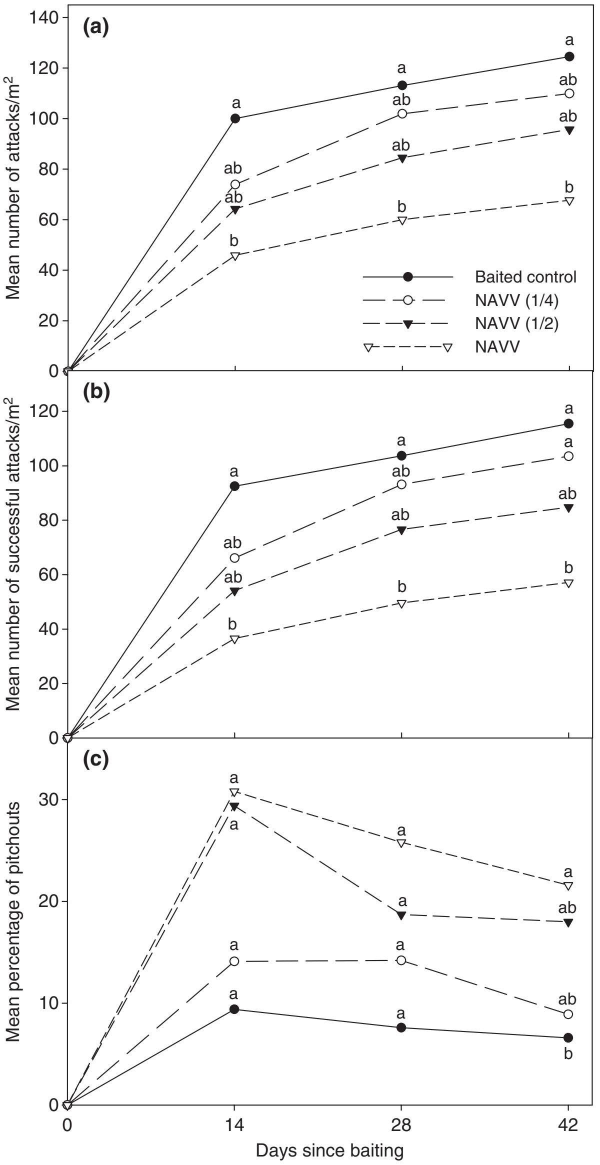

Fig. 3 Mean density of western pine beetle, *Dendroctonus brevicomis* LeConte, attacks (a), successful attacks (b), and percentage of pitch-outs (unsuccessful attacks) (c) occurring on attractant-baited ponderosa pine, *Pinus ponderosa* Dougl. ex Laws., treated with three release rates of nonhost angiosperm volatiles and (–)-verbenone [NAVV(¼), NAVV (½), NAVV] and untreated 14, 28 and 42 days after treatment, Lassen National Forest, California, USA (40.43°N, 120.90°W; 1676 m elevation) July 2006–July 2007. Means followed by the same letter within sample date and response variable are not significantly different (n = 15; Tukey's HSD; P > 0.05). Error bars are not included to enhance the visual clarity of data.

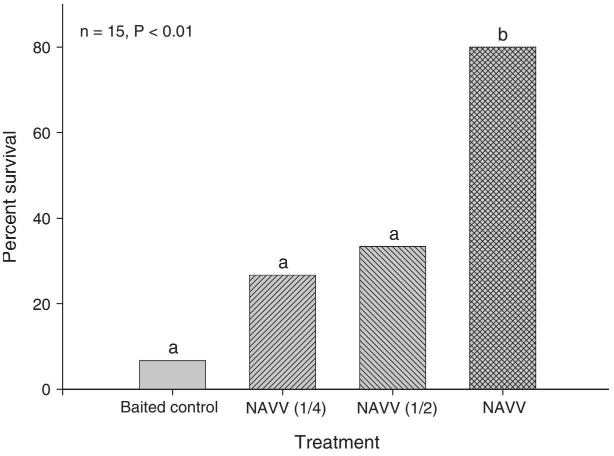

Fig. 4 Proportion of ponderosa pines, *Pinus ponderosa* Dougl. ex Laws., treated with three release rates of nonhost angiosperm volatiles and (–)-verbenone [NAVV(¼), NAVV (½), NAVV] and untreated that survived, Lassen National Forest, California, USA (40.43°N, 120.90°W; 1676 m elevation) July 2006–July 2007. Means followed by the same letter are not significantly different (n = 15; chi-square tests for comparisons between multiple proportions; P > 0.05).

in the density of successful attacks compared to the untreated, baited control. The percentage of pitch-outs (unsuccessful *D. brevicomis* attacks) was significantly greater for NAVV at 42 days compared to the untreated, baited control ($F_{3,55} = 2.8$; P = 0.048). No other significant differences were observed among treatment means on this date (fig. 3c). No significant treatment effects were observed at 14 days ($F_{3,55} = 2.4$; P = 0.075) or 28 days ($F_{3,55} = 1.4$; P = 0.258) for this variable (fig. 3c). Fewer *D. valens* attacks were observed on NAVV-treated trees than untreated, baited controls at 14 days ($F_{3,56} = 3.6$; P = 0.019; mean number of attacks per tree ± SEM = 2.5 ± 0.6 and 0.5 ± 0.03, respectively). No other significant differences were observed among treatment means on this date. No significant treatment effect was observed at 28 days ($F_{3,56} = 1.4$; P = 0.251) or 42 days ($F_{3,56} = 1.9$; P = 0.143) for this variable.

The application of NAVV to individual *P. ponderosa* significantly increased tree survival (P < 0.01; fig. 4). Only three of 15 NAVV-treated trees died from *D. brevicomis* attack while $\sim$93% mortality (14/15) was observed in the untreated, baited control. NAVV(¼) and NAVV(½) were ineffective in reducing tree mortality as their means were not significantly different from the untreated, baited control (fig. 4).

Discussion

Generally, nonhost angiosperm volatiles have been most effective for reducing bark beetle attraction when presented in combinations of two or more compounds (Zhang and Schlyter 2004). Fettig et al.

(2005) reported blends of several bark volatiles (benzyl alcohol, benzaldehyde, (*E*)-conophthorin, guaiacol, nonanal and salicylaldehyde), green leaf volatiles [(*E*)-2-hexenal, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol] and the nine nonhost angiosperm volatiles combined were ineffective for reducing *D. brevicomis* attraction in the absence of verbenone. Poland et al. (1998) reported 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 numbers of male *D. brevicomis* caught in attractant-baited traps. (*Z*)-2-Hexen-1-ol also reduced numbers of females captured (Poland et al. 1998). The greatest effect observed in their study was an ~47% reduction in trap catch compared to the attractant-baited control (Cohen's effect size, $d = 0.8$), which was greater than reported by Fettig et al. (2005) for their nonhost blends ($d = 0.5$). While it is possible that differences in *D. brevicomis* behavioral responses occur due to population level differences, the (*E*)-2-hexenal tested by Poland et al. (1998) was released at an ~3.7 × higher rate than by Fettig et al. (2005), and thus release rate is a likely cause of this particular difference. Generally, the response of bark beetles to inhibitory semiochemicals in the presence of aggregation pheromones and host kairomones depends on the ratio of inhibitory semiochemicals to attractants (Raffa and Berryman 1983; Byers et al. 1984; Tilden and Bedard 1988; Miller et al. 1995; Pureswaran et al. 2000). We therefore hypothesized that our NAV blend could be enhanced if release rates were increased. To that end, we observed a significant reduction in trap catch for NAV-M + WPB compared to WPB (fig. 1). When the release rate of NAV was further increased ~1.7 × (NAV-H), the efficacy of the blend was significantly increased compared to verbenone alone (fig. 1). These data demonstrate that our NAV blend has the ability to disrupt *D. brevicomis* attraction in the absence of verbenone, but that higher release rates are required than previously considered (Fettig et al. 2005). Both male and female portions of the population are equally responsive to nonhost angiosperm volatiles (Fettig et al. 2005), which is of practical importance as females initiate host colonization (Miller and Keen 1960).

The diverse mixture of trees encountered by most bark beetles during foraging, combined with the high costs associated with landing on nonhosts (Atkins 1966), implies that bark beetles should be able to detect and respond to olfactory cues from potential hosts and nonhosts in order to successfully and efficiently locate new hosts. Shepherd et al. (2007) identified 64 compounds present in *P. ponderosa* and nine nonhost trees sympatric with the distribution of *D. brevicomis*, 42 of which elicited antennal responses in *D. brevicomis* generally in both sexes. Only one compound (geraniol) was unique to its host. The number of nonhost volatile chemicals that *D. brevicomis* encounters and is capable of detecting, and the diversity of sources from which they emanate, highlight the complexity of the olfactory environment in which *D. brevicomis* forages. Since the mortality of *D. brevicomis* is generally great during dispersal flight, due primarily to increased predation and unfavorable abiotic conditions (Miller and Keen 1960), there are clear advantages for *D. brevicomis* to discriminate between hosts and nonhosts from a distance.

In Experiment 2, combining (–)-verbenone with NAV (NAVV) increased the effects observed in Experiment 1. To that end, an ~83% and a 93% reduction in trap catch was observed for NAVV-M and NAVV-H, respectively, compared to the untreated control. In an analogous manner to that seen in *D. brevicomis*, others have demonstrated that green leaf volatiles and verbenone significantly reduced spruce bark beetle, *Ips typographus* (L.), attraction beyond that of either compound alone (Zhang 2003; Zhang and Schlyter 2003). A diverse array of chemical cues and signals may disrupt bark beetle searching more than high doses of a single semiochemical (e.g. verbenone) or even mixtures of semiochemicals intended to mimic one type of signal (e.g. antiaggregation pheromones) because they represent heterogeneous stand conditions to foraging insects (Borden 1997; Zhang and Schlyter 2004; Shepherd et al. 2007). Because the odds of success for a searching beetle in a diverse stand are lower than in a more homogeneous stand of similar overall density (Jactel et al. 2002), a foraging beetle encountering a variety of inhibitory semiochemicals may be induced to leave the area instead of landing on and testing candidate trees by taste or close range olfaction. Jactel and Brockerhoff (2007) conducted a meta-analysis of data from 119 comparative studies of 47 different insect-tree interactions to compare herbivory between single-species and mixed-species forests. A significant reduction in herbivory occurred in more diverse forests, and diversity effects were greater when mixed forests comprised taxonomically distant tree species (e.g. angiosperms and gymnosperms). For species like *D. brevicomis* that feed on relatively few hosts there was a larger effect size. Interestingly, nonhost volatiles have also recently been shown to have strong antifeedant effects on *I. typographus* in laboratory assays (Faccoli et al. 2005; Faccoli and Schlyter 2007).

In this study, NAVV-H reduced mean trap catches compared to attractant-baited traps by levels higher ($d = 2.6$) than previously reported in most trapping bioassays (e.g. Bedard et al. 1980a; b; Paine and Hanlon 1991; Bertram and Paine 1994a; Fettig et al. 2005; Erbilgen et al. 2007). For example, Fettig et al. (2005) reported that verbenone (4 mg/d) significantly reduced *D. brevicomis* attraction by ~47% ($d = 0.7$). In a second experiment, trap catches were reduced by ~36% ($d = 0.7$) and ~48% ($d = 1.0$) by 4 and 50 mg/d of verbenone, respectively. In another recent study, verbenone (~4.9 mg/d) reduced trap catch by <50% ($d = 0.7$; Erbilgen et al. 2007). Acetophenone, recently shown to reduce attraction in southern pine beetle, *D. frontalis* Zimmermann (Sullivan 2005), significantly reduced *D. brevicomis* trap catch by ~60% ($d = 0.9$; Erbilgen et al. 2007). Combining verbenone with other semiochemicals, such as ipsdienol (Paine and Hanlon 1991; Bertram and Paine 1994a; Strom et al. 2001) or nonhost volatiles (Fettig et al. 2005), generally results in higher reductions in trap catch, but typically by <90%. One exception is a study by Tilden and Bedard (1988) in which very high release rates of verbenone (~614 mg/d) resulted in a reduction in median trap catch of 99% compared to attractant-baited traps. It must be acknowledged that such comparisons are somewhat confounded by differences in the strength of the attractant used in bark beetle trapping bioassays (e.g. Hayes and Strom 1994 for *D. brevicomis*).

Some authors have reported that the effectiveness of verbenone for tree protection varies with changes in the population density of bark beetles. For example, Progar (2003, 2005) examined the ability of verbenone to deter mass attack of mountain pine beetle, *D. ponderosae* Hopkins, on *P. contorta*. Initially, verbenone was very effective in reducing attacks, but efficacy declined in later evaluations. The author hypothesized that the reduction in response to verbenone over time was at least partially related to increasing *D. ponderosae* populations. In our study, the density of *D. brevicomis* attacks and *D. brevicomis* successful attacks on untreated, baited controls were higher during each sample period than reported by Fettig et al. (2008) (fig. 3). For example, at 14 days the density of total attacks and successful attacks were ~1.3 × and 3.1 × higher, respectively. Similarly, levels of pitch-outs (i.e. a measure of the interaction between host tree vigour and attack density) were lower during each sample period in the current study than previously reported by Fettig et al. (2008). Despite this, the application of NAVV significantly reduced levels of tree mortality compared to the other treatments. It is plausible that NAVV(¼) or NAVV(½) could provide adequate levels of tree protection if *D. brevicomis* populations were lower.

The effects on *D. valens* observed here may simply be an artefact of the reduction in *D. brevicomis* attack densities on NAVV-treated trees. *Dendroctonus valens* attacks often occur on trees colonized by *D. brevicomis*. It is thought that *D. valens* responds to monoterpenes released from host trees (Hobson et al. 1993) upon attack by *D. brevicomis* and presumably other congeners (Fettig et al. 2004b). While we observed significantly fewer *D. valens* attacks on NAVV-treated trees at 14 days, no significant treatment effects were observed at 28 and 42 days. Since no other bark beetle species were found colonizing experimental trees, tree mortality in our experiment was directly attributed to *D. brevicomis*. Furthermore, the *D. brevicomis* attack densities observed in this study were quite high (fig. 3a; Miller and Keen 1960).

Bark beetle outbreaks and associated tree mortality not only affect watershed, timber and wildlife resources, but also cultural and recreational values. Furthermore, tree losses due to bark beetle infestation in residential, recreational, or administrative sites generally result in undesirable impacts such as reduced shade, screening, and aesthetics (Helm and Johnson 1995). Dead trees pose potential hazards to public safety while costs associated with their removal can be substantial (Johnson 1981). Property values may be severely reduced by mortality of adjacent shade and ornamental trees (McGregor and Cole 1985). Trees growing in progeny tests, seed orchards, or those genetically resistant to forest diseases are also of increased value. In recent years, the amount of tree mortality attributed to *D. brevicomis* reached unprecedented levels in southern California, USA (USDA Forest Service 2002) and California is most highly ranked (~1.45 million ha) among all USA states for risk (defined as >25% of stand density will die due to bark beetle infestation in the next 15 years) of bark beetle-caused tree mortality (Krist et al. 2007). These and other situations emphasize the need for developing effective treatments that protect individual *P. ponderosa* from *D. brevicomis* attack. In this study, we demonstrated the release rate of both NAV and NAVV significantly affected the response of *D. brevicomis* to attractant-baited traps. In general, higher release rates resulted in greater reductions in trap catch. More importantly, we demonstrated the ability of NAVV to significantly reduce the density of *D. brevicomis* attacks, *D. brevicomis* successful attacks, and levels of tree mortality on attractant-baited trees, and identified a release rate suitable for individual tree protection during high *D. brevicomis* populations. The potential use of NAVV for tree protection will likely be most significant in settings in which insecticides are used (Fettig et al. 2006a) or in small, ecologically-unique or sensitive areas where applications of insecticides are not permissible (Fettig et al. 2008). Further investigations will allow us to improve and refine our NAVV blend and explore its effectiveness for other uses, such as small-scale (5 ha) stand protection, and bark beetle-host complexes.

Acknowledgements

We thank D.R. Cluck (Forest Health Protection, USDA Forest Service) for technical assistance, and S.L. Smith (Forest Health Protection, USDA Forest Service) and A. Vasquez (Lassen National Forest, USDA Forest Service) for locating the individual tree protection study site. We also thank two anonymous reviewers for their critiques, which improved an earlier version of this manuscript. This research was supported, in part, by a USDA Western Bark Beetle Initiative grant (2005-3) to C.J.F., D.P.W.H., B.T. Sullivan (Southern Research Station, USDA Forest Service) and S.L.S., a U.S. President's Early Career Award for Scientists and Engineers to C.J.F., the Pacific Southwest Research Station, and The University of Northern British Columbia.

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