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Author(s): Christopher J. Fettig, Stephen R. McKelvey, Christopher P. Dabney, and Dezene P. W. Huber
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Responses of *Dendroctonus brevicomis* (Coleoptera: Curculionidae) in Behavioral Assays: Implications to Development of a Semiochemical-Based Tool for Tree Protection

CHRISTOPHER J. FETTIG,[1,2] STEPHEN R. MCKELVEY,[1] CHRISTOPHER P. DABNEY,[1] AND DEZENE P. W. HUBER[3]

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ABSTRACT Currently, techniques for managing western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae, Scolytinae), infestations are limited to tree removals (thinning) that reduce stand density and presumably host susceptibility, and/or the use of insecticides to protect individual trees. There continues to be significant interest in developing an effective semiochemical-based tool for protecting trees from *D. brevicomis* attack, largely as an alternative to conventional insecticides. The responses of *D. brevicomis* to tree volatiles and verbenone were documented in eight experiments (trapping assays) conducted over a 4-yr period in which 88,942 individuals were collected. Geraniol, a tree volatile unique to *Pinus ponderosa* that elicits female-specific antennal responses in *D. brevicomis*, did not affect *D. brevicomis* behavior. Blends of two green leaf alcohols [hexanol + (Z)-3-hexen-1-ol] tested at two release rates (5.0 and 100.0 mg/d) had no effect on the response of *D. brevicomis* to attractant-baited traps. A nine-component blend [benzaldehyde, benzyl alcohol, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone; NAVV] and subsequent revisions of this blend disrupted the response of *D. brevicomis* to attractant-baited traps in all experiments. The inhibitory effect of a revised five-component blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone; NAVV5] on the response of mountain pine beetle, *D. ponderosae* Hopkins, to attractant-baited traps was also documented. Acetophenone significantly reduced *D. brevicomis* attraction, but was not as effective as verbenone alone. Acetophenone increased the effectiveness of NAVV5 in one of two experiments. Furthermore, by adding acetophenone to NAVV5 we were able to remove the aldehydes from NAVV5 without compromising effectiveness, resulting in a novel four-component blend [acetophenone, (*E*)-2-hexen-1-ol + (Z)-2-hexen-1-ol, and (–)-verbenone; Verbenone Plus]. We discuss the implications of these and other results to development of Verbenone Plus as a semiochemical-based tool for management of *D. brevicomis* and *D. ponderosae* infestations.

KEY WORDS acetophenone, *Dendroctonus ponderosae*, nonhost angiosperm volatile, pest management, *Pinus ponderosa*

The western pine beetle, *Dendroctonus brevicomis* LeConte (Coleoptera: Curculionidae, Scolytinae), is a major cause of ponderosa pine, *Pinus ponderosa* Douglas ex Lawson, mortality in much of western North America (Furniss and Carolin 1977). *D. brevicomis* prefers large diameter (>50 cm at 1.37 m) trees, but under certain conditions may aggressively attack and kill apparently healthy trees of all ages and size classes (Miller and Keen 1960). During colonization females release *exo*-brevicomin, which in combination with the host monoterpene myrcene is attractive to conspecifics (Bedard et al. 1969). Frontalin, produced by males (Kinzer et al. 1969), enhances attraction and mass attack ensues (Wood 1972, Bedard et al. 1985). Currently, tactics for managing *D. brevicomis* infestations are generally 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).

Development of semiochemical-based tools to protect trees from bark beetle infestations in western coniferous forests has centered on the use of aggregation pheromones to attract the subject species for purposes of retention and later destruction (e.g., Ross and Daterman 1997), or antiaggregation pheromones to reduce host finding and colonization success (e.g., Gibson et al. 1991; Shea et al. 1992; Shore et al. 1992;

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[1] Ecosystem Function and Health Program, Pacific Southwest Research Station, USDA Forest Service, Davis, CA 95618.

[2] Corresponding author, e-mail: cfettig@fs.fed.us.

[3] Ecosystem Science and Management Program, University of Northern British Columbia, Prince George, BC, V2N 4Z9 Canada.

Lindgren and Borden 1993; Huber and Borden 2001; Borden et al. 2003; Holsten et al. 2003; Progar 2003, 2005; Bentz et al. 2005; Negrón et al. 2006; Gillette et al. 2006, 2009a,b). Many efforts have met with less than satisfactory results (e.g., Fettig et al. 2009a for *D. brevicomis*). Recently, efforts to more fully explore host and nonhost volatiles have led to the use of systems-level concepts in the development of new semiochemical-based tools and tactics for tree protection (Shepherd et al. 2007). Many bark beetles are believed to use a combination of host kairomones and aggregation pheromones to locate suitable hosts (Zhang and Schlyter 2004). Rejection of nonhosts may occur on the basis of absence of host cues; the presence of nonhost cues such as green leaf volatiles or angiosperm bark volatiles, collectively termed nonhost angiosperm volatiles; or both (Borden 1997).

Research on host selection by bark beetles has largely concentrated on behavior during flight. Semiochemicals are frequently placed in attractant-baited traps to elucidate behavioral responses for the purposes 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 (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 1994), and by combining verbenone with ipsdienol (Paine and Hanlon 1991), the latter of which is produced by male *D. brevicomis* (Byers 1982), and with nonhost angiosperm volatiles (Fettig et al. 2005). Acetophenone, produced by some bark beetles (Pureswaran and Borden 2004), has also been shown to reduce *D. brevicomis* attraction (Erbiligen et al. 2007, 2008).

Poland et al. (1998) were first to examine the effects of nonhost angiosperm volatiles on *D. brevicomis* attraction. Fettig et al. (2005) reported that combinations of six bark volatiles [benzaldehyde, benzyl alcohol, (*E*)-conophthorin, guaiacol, nonanal, and salicylaldehyde], three green leaf volatiles [(*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-2-hexen-1-ol], or the nine compounds combined did not affect the response of *D. brevicomis* to attractant-baited traps. However, when bark and green leaf volatiles were combined with verbenone, they reduced trap catches to levels significantly below that of verbenone alone. A revised blend [benzaldehyde, benzyl alcohol, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; NAVV] reduced trap catch by 87% compared with the attractant-baited control (Fettig et al. 2005). Based on these data, Fettig et al. (2008) were first to demonstrate the successful application of a semiochemical-based tool for protecting *P. ponderosa* from mortality attributed to *D. brevicomis.*

Fettig et al. (2009b) showed that *D. brevicomis* trap catches were further reduced by increasing the release rate of NAVV in attractant-baited traps. Fewer *D. brevicomis* were captured in traps containing NAVV released at 430 mg/d (all components, exclusive of verbenone) than in any other treatment [including verbenone (50 mg/d), NAVV (40 mg/d), and NAVV (240 mg/d)] resulting in an ≈93% reduction in trap catch compared with the attractant-baited control. In another experiment, the NAVV blend was examined at several release rates for protecting individual *P. ponderosa* from mortality attributed to *D. brevicomis* attack. Cumulative release rates varied in direct proportion to tree diameter, but the highest rates significantly reduced the density of *D. brevicomis* attacks, *D. brevicomis* successful attacks, and levels of tree mortality attributed to *D. brevicomis* attack on attractant-baited trees. Only 3 of 15 NAVV-treated trees died from *D. brevicomis* attack while ≈93% mortality was observed in the untreated, baited control (Fettig et al. 2009b).

Shepherd et al. (2007) assayed stem volatile extracts from trees sympatric with *D. brevicomis* by gas chromatographic-electroantennographic detection (GC-EAD) analysis to isolate antennal responses. Extracts were analyzed from *P. ponderosa*, two nonhost angiosperms, and seven nonhost conifers. Sixty-four compounds were identified, 42 of which elicited antennal responses in *D. brevicomis*, usually in both sexes. In addition, several synthetic compounds, including a number of the antennally active compounds from the extracted trees and some bark beetle pheromone components, elicited antennal responses in a manner similar to that seen with the extracts. This information highlights the complexity of the olfactory environment in which *D. brevicomis* forages, and was used as a foundation to guide behavioral assays. The primary objective of this study was to determine the responses of *D. brevicomis* to nonhost angiosperm volatiles and verbenone in attractant-baited traps in hopes of improving the efficacy of the NAVV blend for tree protection, and reducing the number of components involved.

Materials and Methods

Study Sites. Eight experiments were conducted on the Shasta-Trinity National Forest, CA (2006–2009; 41.30° N, 122.00° W; 1,186-m elevation) for *D. brevicomis;* and one experiment was conducted on the Uinta-Wasatch-Cache National Forest, UT (2006, 40.90° N, 110.83° W; 2,576-m elevation) for mountain pine beetle, *D. ponderosae* Hopkins. In California, stands were dominated by *P. ponderosa* (mean diameter at 1.37 m; diameter at breast height [dbh]) ± SEM = 35.8 ± 3.6 cm). Mean stand density was 31.5 m^2 of basal area per hectare of which ≈96% was *P. ponderosa* with the remainder represented by incense cedar, *Calocedrus decurrens* (Torr.) Florin, white fir,

Table 1. Description of semiochemicals and release devices used in behavioral assays, 2006–2009

Semiochemical	Code	Source[a]	Purity (%)	Release device	Release rate (mg/d)[b]
(*E*)-2-Hexenal	—	Bedoukian	97	Phero Tech bubblecap	3.5 (20°C)
(*E*)-2-Hexen-1-ol	—	Bedoukian	98	Phero Tech bubblecap	3.8 (20°C)
(*Z*)-2-Hexen-1-ol	—	Bedoukian	95	Phero Tech bubblecap	3.8 (20°C)
Acetophenone	A	Sigma-Aldrich	99	Phero Tech 15 ml polyethylene bottle	18.0 (20°C) 29.4 (25°C)[c]
Benzaldehyde	B	Fisher Scientific	>99	Phero Tech flexlure	3.5 (20°C)
Benzyl alcohol	BA	Fisher Scientific	98	Phero Tech bubblecap	1.3 (20°C)
Geraniol	GE	Sigma-Aldrich	98	Phero Tech bubblecap	3.5 (20°C)
Guaiacol	G	Sigma-Aldrich	>98	Phero Tech bubblecap	5.0 (20°C)
Nonanal	—	Sigma-Aldrich	95	Phero Tech flexlure	3.5 (20°C)
Salicylaldehyde	S	Sigma-Aldrich	99	Phero Tech bubblecap	5.0 (20°C)
Verbenone [82%-(−)]	V	Phero Tech	97	5-g Phero Tech pouch	50.0 (30°C)
Verbenone [77%-(−)]	V7	Phero Tech[d]	97	7-g Phero Tech pouch	50.0 (20°C) 68.1 (25°C)[c]
Verbenone [80%-(−)]	V7.5	Synergy	96	7.5-g Synergy pouch	100 (30°C)
Hexanol (*Z*)-3-Hexen-1-ol	GLV	Synergy	98	Synergy bubblecap (1:1 blend)	5.0 (30°C)
Hexanol (*Z*)-3-Hexen-1-ol	EGLV	Synergy	98	Synergy pouch (1:1 blend)	100 (30°C)
(*E*)-2-Hexen-1-ol (*Z*)-2-Hexen-1-ol	—	Bedoukian	97	Phero Tech pouch (1:1 blend)	50.0 (20°C)
75% (*E*)-2-Hexen-1-ol 25% (*Z*)-2-Hexen-1-ol	—	Bedoukian	97	Phero Tech pouch (75:25 blend)	50.0 (20°C) 71.6 (25°C)[c]
D. ponderosae attractant:					
trans-verbenol	MPB	Phero Tech	97	Phero Tech bubblecap	1.0 (20°C)
exo-brevicomin (racemic)			99	Phero Tech flexlure	0.3 (25°C)
myrcene			90	Phero Tech bottle	270 (20°C)
D. brevicomis attractant:					
frontalin (racemic)	WPB	Phero Tech	99	400 μl Eppendorf vial	2.8 (20°C)
exo-brevicomin (racemic)			97	205 μl Eppendorf vial	0.5 (20°C)
myrcene			90	1.8 ml × 2 Eppendorf vials	5.5 (20°C)

[a] Bedoukian = Bedoukian Research Inc., Danbury, CT; Sigma-Aldrich = Sigma-Aldrich Canada Ltd., Oakville, ON, Canada; Fisher Scientific = Fisher Scientific International Inc., Hampton, NH; Phero Tech = Phero Tech Inc., Delta, BC, Canada (now Contech Enterprises Inc.); Synergy = Synergy Semiochemicals Corp., Burnaby, BC, Canada.

[b] Reported by manufacturer of release device and measured in the laboratory at specified temp.

[c] Measured in our laboratory at specified temp.

[d] Phero Tech phased in the 7-g pouch in 2006 and fully replaced the 5-g pouch with the 7-g pouch in 2008. The 7-g pouch was used in all treatments since experiment 6 (Aug. 2007).

Abies concolor (Gordon and Glendinning) Lindley ex Hildebrand, Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, California black oak, *Quercus kelloggii* Newberry, and quaking aspen, *Populus tremuloides* Michaux. Mean crown cover was 27%. The topography was mainly flat. In Utah, stands were dominated by lodgepole pine, *P. contorta* Douglas ex Loudon (mean dbh ± SEM = 20.1 ± 0.5 cm). Mean stand density was 21.3 m^2 of basal area per ha of which ≈94% was *P. contorta* with the remainder represented by *Po. tremuloides.* Mean crown cover was 65%, and slopes averaged 4%.

Experimental Design. All experiments were conducted using similar protocols and a completely randomized design (Fettig et al. 2006b). Sixteen-unit multiple funnel traps (Lindgren 1983) were deployed along forest roads. Trap locations were separated by 25–30 m to avoid interference among adjacent treatments. Traps were hung on 3-m metal poles with collection cups 80–100 cm above the ground. Each trap location was randomly assigned among treatments in each experiment. A 3 × 3-cm Prozap Pest Strip (2,2-dichlorovinyl dimethyl phosphate, Loveland Industries Inc., Greeley, CO) was placed in the collection cup to kill arriving insects and reduce damage or loss to predacious insects. Samples were collected and treatments were rerandomized daily. Catches were transported to the laboratory for storage and analysis. Specimens were tallied and identified using available keys (Wood 1982) and voucher specimens. Details concerning the source, purity, enantiomeric purity (if chiral), release device, and release rate for each semiochemical are provided in Table 1.

Trap catches from unbaited controls were excluded from statistical analyses because of heteroscedasticity among treatments that they caused (Reeve and Strom 2004). 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 (ANOVA) (treatment and sex) was performed on the number of *D. brevicomis* (or *D. ponderosae*, experiment 3) caught per trap per day using $\alpha = 0.05$. If a significant treatment effect was detected by ANOVA, the Tukey's multiple comparison test (Tukey's honestly significant difference [HSD]) was used for separation of treatment means.

Experiments. Experiment 1 was conducted to determine if benzaldehyde (B), benzyl alcohol (BA), and salicylaldehyde (S) were critical to the nine-component NAVV blend [benzaldehyde, benzyl alcohol, guaiacol, nonanal, salicylaldehyde, (*E*)-2-hexenal,

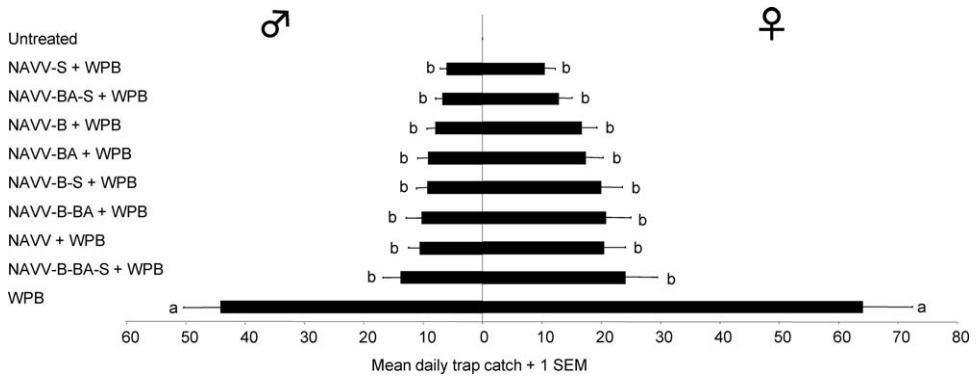

Fig. 1. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and a nine-component NAVV blend [benzaldehyde (B), benzyl alcohol (BA), guaiacol, nonanal, salicylaldehyde (S), (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; NAVV] (Table 1), Shasta-Trinity National Forest, CA, 1–10 July 2006. Bars followed by the same letter are not significantly different ($n = 30$; Tukey's HSD; $P > 0.05$).

(*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; Fettig et al. 2005, 2009b] for inhibiting the response of *D. brevicomis* to attractant-baited traps, 1–10 July 2006. Treatments included: 1) unbaited control, 2) *D. brevicomis* attractant (WPB), 3) NAVV + WPB, 4) NAVV-B + WPB, 5) NAVV-BA + WPB, 6) NAVV-S + WPB, 7) NAVV-B-BA + WPB, 8) NAVV-B-S + WPB, 9) NAVV-BA-S + WPB, and 10) NAVV-B-BA-S + WPB ($n = 30$; Table 1).

Experiment 2 was conducted to determine if guaiacol (G) was critical to the six-component NAVV blend [guaiacol, nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; NAVV6) for inhibiting the response of *D. brevicomis* to attractant-baited traps, 4–13 August 2006. Treatments included: 1) unbaited control, 2) WPB, 3) NAVV6 + WPB, and 4) NAVV6-G + WPB ($n = 40$; Table 1).

Experiment 3 was conducted to determine the effect of NAVV6 on the response of *D. ponderosae* to attractant-baited traps; and to determine if G was critical to the effectiveness of this blend, 14–23 July 2006. Treatments included: 1) unbaited control, 2) *D. ponderosae* attractant (MPB), 3) NAVV6 + MPB, and 4) NAVV6-G + MPB ($n = 70$; Table 1).

Experiment 4 was conducted to compare the effect of acetophenone (A) with other *D. brevicomis* inhibitors, and combined with the five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; NAVV5] in attractant-baited traps, 13–22 June 2007. Treatments included: 1) unbaited control, 2) WPB, 3) A + WPB, 4) (–)-verbenone (5-g, V) + WPB, 5) A + V + WPB, 6) NAVV5 + WPB, and 7) NAVV5 + A + WPB ($n = 40$; Table 1).

Experiment 5 was conducted to compare the effect of green leaf volatiles [hexanol + (*Z*)-3-hexen-1-ol; GLV] with other *D. brevicomis* inhibitors in attractant-baited traps, 13–22 June 2007. Treatments included: 1) unbaited control, 2) WPB, 3) GLV + WPB, 4) V + WPB, 5) GLV + V + WPB, and 6) NAVV5 + WPB ($n = 40$; Table 1).

Experiment 6 was conducted to compare the effect of a new (–)-verbenone release device (7-g, V7) with other *D. brevicomis* inhibitors in attractant-baited traps, 15–24 August 2007. Treatments included: 1) unbaited control, 2) WPB, 3) A + WPB, 4) V7 + WPB, 5) A + V7 + WPB, 6) NAVV5 (substituting V7) + WPB, and 7) NAVV5 (substituting V7) +A + WPB ($n = 30$; Table 1).

Experiment 7 was conducted to determine the effect of geraniol (GE) on the response of *D. brevicomis* to unbaited and attractant-baited traps, 15–24 August 2007. Treatments included: 1) unbaited control, 2) GE, 3) WPB, and 4) GE + WPB ($n = 40$; Table 1).

Experiment 8 was conducted to determine the effect of an enhanced GLV blend [hexanol + (*Z*)-3-hexen-1-ol; EGLV], another (–)-verbenone release device (7.5-g, V7.5), and other *D. brevicomis* inhibitors in unbaited (V only) and attractant-baited (all others) traps, 15–24 August 2007. Treatments included: 1) unbaited control, 2) V, 3) WPB, 4) EGLV + WPB, 5) V + WPB, 6) V7.5 + WPB, and 7) NAVV5 + WPB ($n = 30$; Table 1).

Experiment 9 was conducted to compare the effect of NAVV5, and a new blend [A, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol, and V; Verbenone Plus (VP)] in attractant-baited traps, 24 June through 1 July 2009. Blends of (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol were examined in both 1:1 (VP) and 75:25 (*E*/*Z*) ratios (VP75:25). Treatments included: 1) unbaited control, 2) WPB, 3) NAVV5 + WPB, 4) VP + WPB, and 5) VP75:25 + WPB ($n = 56$; Table 1).

Results

Experiment 1. In total, 9,767 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.57. There was no significant treatment × sex interaction ($F_{8, 522} = 0.19$; $P = 0.99$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{8, 261} = 15.8$; $P < 0.0001$). All inhibitors significantly reduced trap catches com-

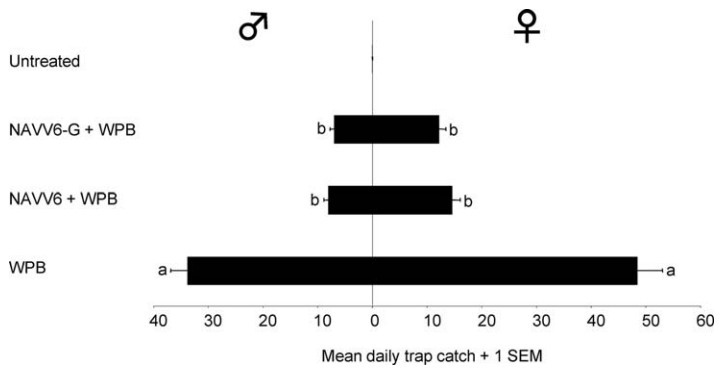

Fig. 2. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and a six-component NAVV blend [guaiacol (G), nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone; NAVV6] (Table 1), Shasta-Trinity National Forest, CA, 4–13 August 2006. Bars followed by the same letter are not significantly different ($n = 40$; Tukey's HSD; $P > 0.05$).

pared with WPB, but no other significant differences were observed (Fig. 1). The six-component NAVV blend (NAVV-B-BA-S + WPB) resulted in a 64.9% reduction in trap catch compared with WPB. Very few beetles (a total of three) were collected in unbaited traps.

Experiment 2. In total, 4,971 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.65. There was no significant treatment × sex interaction ($F_{2, 234} = 0.08$; $P = 0.92$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{2, 117} = 63.7$; $P < 0.0001$). NAVV6 + WPB and NAVV6-G + WPB significantly reduced trap catches compared with WPB (Fig. 2). No significant difference was observed between NAVV6 + WPB and NAVV6-G + WPB (Fig. 2). The five-component NAVV blend (NAVV6-G + WPB) resulted in a 76.7% reduction in trap catch compared with WPB. Very few beetles (a total of three) were collected in unbaited traps.

Experiment 3. In total, 3,575 *D. ponderosae* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.97. There was no significant treatment × sex interaction ($F_{2, 414} = 0.04$; $P = 0.96$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{2, 207} = 220.3$; $P < 0.0001$). NAVV6 + MPB and NAVV6-G + MPB significantly reduced trap catches compared with MPB (Fig. 3). No significant difference was observed between NAVV6 + MPB and NAVV6-G + MPB (Fig. 3). The five-component NAVV blend (NAVV6-G + MPB) resulted in a 97.7% reduction in trap catch compared with MPB.

Experiment 4. In total, 17,578 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.76. There was no significant treatment × sex interaction ($F_{5, 468} = 0.18$; $P = 0.97$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{5, 234} = 69.4$; $P < 0.0001$). All inhibitors significantly reduced trap catches compared with WPB (Fig. 4). NAVV5 + A + WPB and A + V + WPB had the lowest trap catches resulting in 87.0 and 77.9% reductions in trap catch, respectively, compared with WPB. No significant differences were observed among A + V + WPB, NAVV5 + WPB, and V + WPB. Acetophenone (A + WPB) was least effective, reducing trap catch by 35.8% compared with WPB, but when added to NAVV5 (NAVV5 + A +

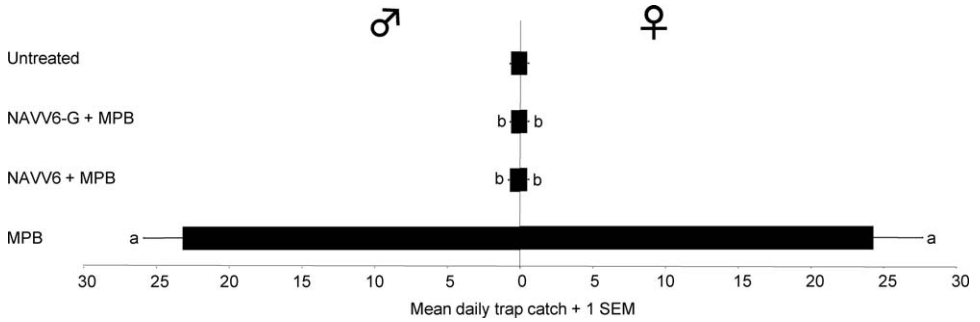

Fig. 3. Captures of *D. ponderosae* in multiple funnel traps baited with and without the *D. ponderosae* attractant (MPB), or with the attractant and a six-component NAVV blend [guaiacol (G), nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone; NAVV6] (Table 1), Uinta-Wasatch-Cache National Forest, UT, 14–23 July 2006. Bars followed by the same letter are not significantly different ($n = 70$; Tukey's HSD; $P > 0.05$).

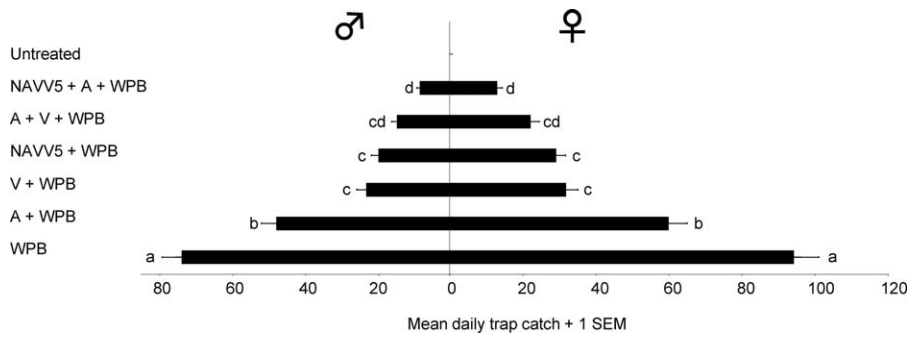

Fig. 4. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and acetophenone (A), verbenone (V), A + V, a five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (−)-verbenone; NAVV5], and NAVV5 + A (Table 1), Shasta-Trinity National Forest, CA, 13–22 June 2007. Bars followed by the same letter are not significantly different ($n = 40$; Tukey's HSD; $P > 0.05$).

WPB) significantly increased effectiveness of that treatment (Fig. 4). Very few beetles (a total of three) were collected in unbaited traps.

Experiment 5. In total, 7,638 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.98. There was no significant treatment × sex interaction ($F_{4, 390} = 0.47$; $P = 0.76$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{4, 195} = 35.6$; $P < 0.0001$). GLV had no effect on the response of *D. brevicomis* to WPB (Fig. 5). All other inhibitors significantly reduced trap catches compared with WPB, but no other significant differences were observed overall (Fig. 5). NAVV5 + WPB resulted in a 73.1% reduction in trap catch compared with WPB. While no gender effect was observed overall, NAVV5 + WPB significantly reduced attraction compared with V + WPB for males, but not females (Fig. 5). Very few beetles (a total of two) were collected in unbaited traps.

Experiment 6. In total, 11,024 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.67. There was no significant treatment × sex interaction ($F_{5, 348} = 0.19$; $P = 0.97$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{5, 174} = 32.0$; $P < 0.0001$). All inhibitors significantly reduced trap catches compared with WPB (Fig. 6). Acetophenone (A + WPB) was least effective, reducing trap catch by 40.4% compared with WPB. NAVV5 + A + WPB resulted in an 88.5% reduction in trap catch compared with WPB, but was not significantly different from NAVV5 + WPB and A + V7 + WPB (Fig. 6). However, NAVV5 + A + WPB was the only treatment that caught significantly fewer *D. brevicomis* than V7 + WPB (Fig. 6). Few beetles (a total of 22) were collected in unbaited traps.

Experiment 7. In total, 10,959 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.73. There was no significant treatment × sex interaction ($F_{3, 312} = 2.3$; $P = 0.075$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{3, 156} = 166.4$; $P < 0.0001$). Geraniol had no effect on the response of *D. brevicomis* to unbaited traps [0.1 ± 0.1 (GE) and 0.4 ± 0.2 (unbaited control); mean ± SEM] or WPB [117.8 ± 19.7 (GE + WPB) and 155.7 ± 19.7 (WPB)]. GE + WPB and WPB caught significantly more *D. brevicomis* than GE and the unbaited control. No other significant differences were observed.

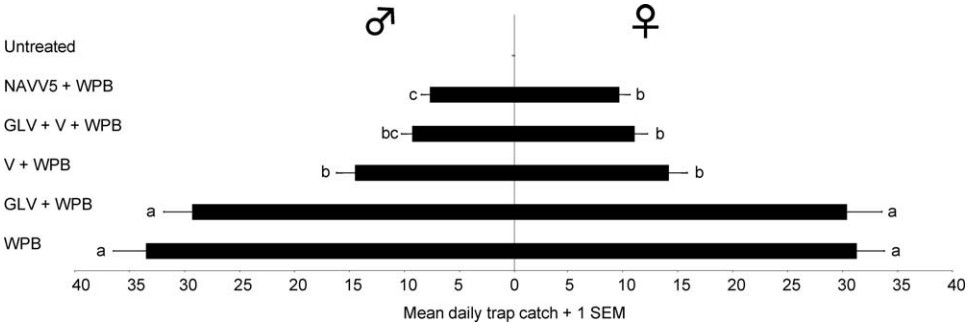

Fig. 5. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and hexanol + (*Z*)-3-hexen-1-ol (GLV), verbenone (V), GLV + V, and a five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (−)-verbenone; NAVV5] (Table 1), Shasta-Trinity National Forest, CA, 13–22 June 2007. Bars followed by the same letter are not significantly different ($n = 40$; Tukey's HSD; $P > 0.05$).

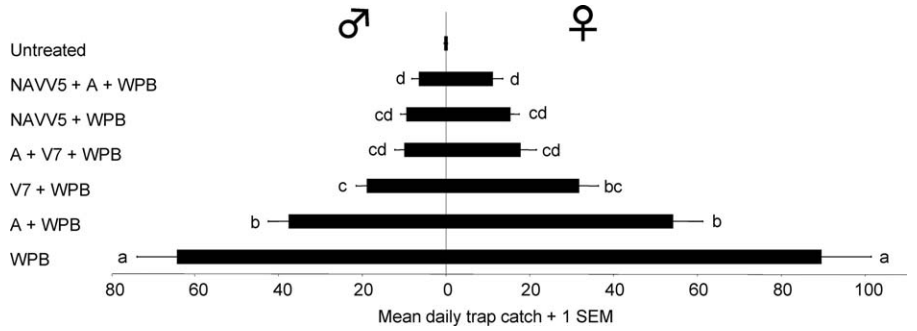

Fig. 6. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and acetophenone (A), verbenone (V7, 7-g pouch), A + V7, a five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone 7-g; NAVV5], and NAVV5 + A (Table 1), Shasta-Trinity National Forest, CA, 15–24 August 2007. Bars followed by the same letter are not significantly different ($n = 30$; Tukey's HSD; $P > 0.05$).

Experiment 8. In total, 16,744 *D. brevicomis* were captured in multiple funnel traps over the 10-d period. Overall, the ratio of males to females was 0.57. There was no significant treatment × sex interaction ($F_{4, 290} = 0.20$; $P = 0.94$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{4, 145} = 13.1$; $P < 0.0001$). EGLV had no effect on the response of *D. brevicomis* to WPB (Fig. 7). Overall, all other inhibitors significantly reduced trap catches compared with WPB (Fig. 7). Significantly fewer beetles were captured in NAVV5 + WPB than V7.5 + WPB. No significant difference was observed between V7.5 + WPB and V + WPB. NAVV5 + WPB resulted in a 77.9% reduction in trap catch compared with WPB, but was not significantly different from V + WPB (Fig. 7). Few beetles (a total of 33) were collected in unbaited traps (V and untreated control).

Experiment 9. In total, 10,261 *D. brevicomis* were captured in multiple funnel traps over the 8-d period. Overall, the ratio of males to females was 0.73. There was no significant treatment × sex interaction ($F_{3, 447} = 1.2$; $P = 0.95$), and therefore results pertain equally to both male and female responses. A significant treatment effect was observed ($F_{3, 223} = 27.7$; $P < 0.0001$). All inhibitors significantly reduced trap catches compared with WPB (Fig. 8), but no other significant differences were observed (Fig. 8). VP75:25 + WPB resulted in an 89.2% reduction in trap catch compared with WPB.

Discussion

Bark beetles use a variety of contextual cues to interpret chemical messages and tailor foraging decisions. These include visual cues such as bole reflectance (Strom et al. 2001), tactile or gustatory cues once in direct contact with host trees (Elkinton and Wood 1980), pheromone or allomone cues from con- or heterospecifics (Byers et al. 1984), and other olfactory cues from intermixing plumes of host and nonhost volatiles. In the case of *D. brevicomis*, chemical cues have been shown to be more important than visual cues (Strom et al. 2001). The diverse mixture of tree species encountered by most bark beetles during foraging, combined with the high biological costs associated with landing on unsuitable hosts and nonhosts, suggests that bark beetles should be able to discriminate among olfactory cues to locate suitable hosts and associated habitats (Shepherd et al. 2007).

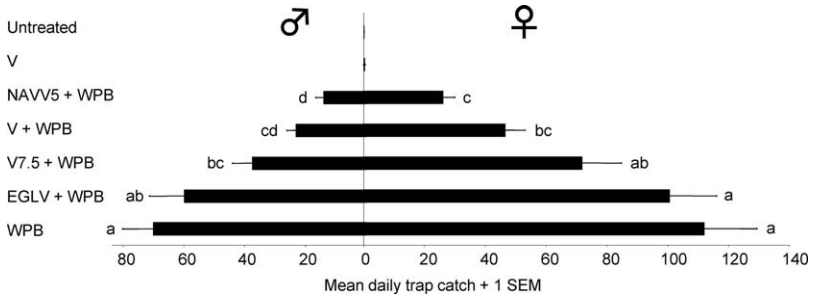

Fig. 7. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), verbenone (V) without the attractant, or with the attractant and hexanol + (*Z*)-3-hexen-1-ol (EGLV), V, verbenone (V7.5, 7.5-g pouch), and a five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (–)-verbenone; NAVV5] (Table 1), Shasta-Trinity National Forest, CA, 15–24 August 2007. Bars followed by the same letter are not significantly different ($n = 30$; Tukey's HSD; $P > 0.05$).

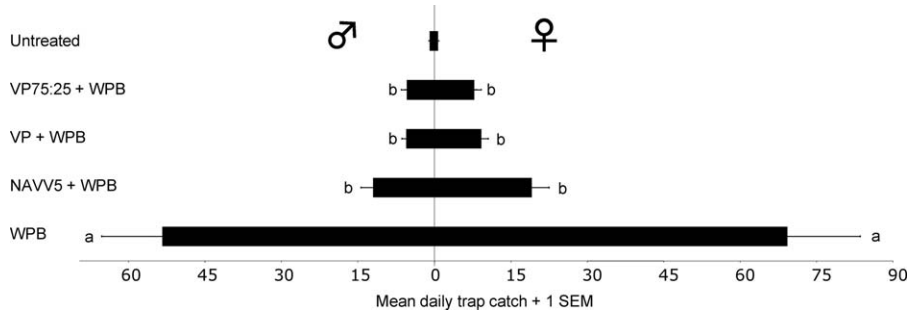

Fig. 8. Captures of *D. brevicomis* in multiple funnel traps baited with and without the *D. brevicomis* attractant (WPB), or with the attractant and a five-component NAVV blend [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol and (–)-verbenone; NAVV5] and four-component blend [acetophenone, (*E*)-2-hexen-1-ol + (*Z*)-2-hexen-1-ol, and (–)-verbenone] with 1:1 (VP) or 75:25 (VP75:25) blend of (*E*)-2-hexen-1-ol:(*Z*)-2-hexen-1-ol (Table 1), Shasta-Trinity National Forest, CA, 24 June through 1 July 2009. Bars followed by the same letter are not significantly different ($n = 56$; Tukey's HSD; $P > 0.05$).

Based on the semiochemical-diversity hypothesis (Zhang and Schlyter 2004), this seems critical for species like *D. brevicomis* that have narrow host ranges and often forage in mixed forests (Miller and Keen 1960). In the context of pest management, a diverse array of chemical cues and signals (e.g., Verbenone Plus) 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 (Zhang and Schlyter 2004, Shepherd et al. 2007). Because the odds of success for a searching beetle in a diverse stand are likely lower than in a more homogeneous stand of similar overall tree density (Jactel et al. 2002), a foraging beetle encountering a variety of inhibitory semiochemicals (e.g., Verbenone Plus) may be induced to leave the area instead of landing on and testing candidate trees by taste or close range olfaction.

Verbenone is an antiaggregation pheromone of several *Dendroctonus* spp., including *D. brevicomis* (Borden 1997), and is naturally derived from three sources: 1) the beetles themselves, 2) auto-oxidation of α-pinene and subsequently *cis*- and *trans*-verbenol to verbenone (Hunt et al. 1989), and 3) conversion of *cis*- and *trans*-verbenol to verbenone by microorganisms, typically yeasts, associated with bark beetles (Hunt and Borden 1990). Verbenone has been demonstrated to disrupt the response of *D. brevicomis* to attractant-baited traps in many (Bedard et al. 1980a,b; Tilden and Bedard 1988; Paine and Hanlon 1991; Bertram and Paine 1994; Shea and Wentz 1994; Fettig et al. 2005, 2009b; Erbilgin et al. 2007, 2008), but not all (Hayes and Strom 1994) studies. It is assumed that verbenone reduces intraspecific competition by altering adult behavior to minimize overcrowding of developing brood within the host tree (Byers and Wood 1980, Byers et al. 1984). Lindgren et al. (1996) further proposed that verbenone is an indicator of host tissue quality and that its quantity is a function of microbial degradation, thus providing cues as to host suitability. Despite this, verbenone alone has been ineffective for protecting *P. ponderosa* from mortality attributed to *D. brevicomis* (Gillette et al. 2006, Fettig et al. 2009a). Poor results have been linked to several factors (reviewed in detail in Fettig et al. 2009a) including, but not limited to, photoisomerization of verbenone to behaviorally inactive chrysanthenone (Kostyk et al. 1993); inconsistent or inadequate release (Bentz et al. 1989); rapid dispersal of verbenone (Gibson et al. 1991, Negrón et al. 2006); and/or limitations in the range of inhibition (Fettig et al. 2009a), particularly when populations are high. Research suggests that while verbenone is a relatively weak inhibitor of *D. brevicomis*, it is critical to the successful development of a semiochemical-based tool for *D. brevicomis* (Fettig et al. 2005, 2008, 2009a,b).

The nine-component NAVV blend (Fettig et al. 2005, 2009b) and subsequent revisions (Figs. 1–2, 4, 6, and 8) were effective for inhibiting the response of *D. brevicomis* to attractant-baited traps in all experiments. Both male and female portions of the population were equally responsive (Fettig et al. 2005, 2009b; Figs. 1, 2, and 4–8). Although there are exceptions (Huber et al. 1999, 2000), nonhost angiosperm volatiles are usually only effective for reducing bark beetle attraction when presented in combinations of two or more compounds (reviewed in detail in Zhang and Schlyter 2004). Poland et al. (1998) reported (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and (*Z*)-2-hexen-1-ol significantly reduced the number of male *D. brevicomis* caught in attractant-baited traps. (*Z*)-2-Hexen-1-ol also reduced the number of female *D. brevicomis* captured. Fettig et al. (2005) reported combinations of six bark volatiles (benzyl alcohol, benzaldehyde, *trans*-conophthorin, guaiacol, nonanal, 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 *D. brevicomis* response to attractant-baited traps. However, inhibition was observed when the bark and green leaf volatiles were combined with verbenone (Fettig et al. 2005). Later, Fettig et al. (2009b) demonstrated that complex blends of nonhost angiosperm volatiles could disrupt *D. brevicomis* attraction in the absence of verbenone,

but that higher release rates were required than previously considered (>200 mg/d).

In earlier research, geraniol was the only compound found to be unique to the primary host *P. ponderosa* that also elicited a sex-specific response from female *D. brevicomis* antennae (Shepherd et al. 2007). However, only 57.8% of total trap catch (and 50% of the GE trap catch) was represented by females in our study. Because females initiate host colonization (Miller and Keen 1960), we thought that this compound may impart a behavioral response, most likely as an attractant, which could be isolated in trapping assays. Based on experiment 7, geraniol appears to have no behavioral effect on *D. brevicomis* adults at the release rate examined (Table 1). Recent evidence indicates that geraniol is a component of the aggregation pheromone of the ambrosia beetle *Platypus koryoensis* Wood & Bright (Coleoptera: Curculionidae), and synthetic blends that included geraniol were attractive to both males and females (Kim et al. 2009). Additional tests of geraniol at higher release rates in both unbaited and attractant-baited traps are merited for *D. brevicomis.*

Many of our behavioral assays were designed based on data from GC-EAD analyses. For example, in experiment 1 we documented that salicylaldehyde was not critical to NAVV (Fig. 1). Salicylaldehyde elicited no detectable antennal responses in synthetic form, but elicited responses from both sexes of *D. brevicomis* in the tree extracts (Shepherd et al. 2007). Similarly, only female antennae responded significantly to synthetic benzyl alcohol and synthetic benzaldehyde (Shepherd et al. 2007), and we subsequently found both unnecessary to the efficacy of NAVV (Fig. 1). In these cases, behavioral assays partially confirmed observations based on GC-EAD recordings, and allowed reduction of the NAVV blend from nine to six components [guaiacol, nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone]. However, in experiment 2 we were able to remove guaiacol from NAVV6 (Fig. 2) despite guaiacol being found to be antennally active in both sexes of *D. brevicomis* in synthetic and tree forms (Shepherd et al. 2007). This suggests that while *D. brevicomis* antennae detect guaiacol, guaiacol elicits no behavioral response when included in NAVV6, but may still impart a behavioral response (e.g., when presented alone) that could not be detected in our assay. Alternatively, positive antennal responses do not necessitate a behavioral effect (Byers 1989). Regardless, guaiacol could be problematic to registration if retained in the blend because of its corrosive nature and status as an eye irritant. While behavioral assays permitted reduction in the number of components in the NAVV blend from nine to five [nonanal, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (Z)-2-hexen-1-ol, and (–)-verbenone] without reduction in inhibition (Figs. 1 and 2), we no longer observed significant reductions in trap catch between verbenone and NAVV blends in two experiments (Figs. 4 and 6).

Acetophenone has recently been shown to reduce attraction in Douglas-fir beetle, *D. pseudotsugae* Hopkins (Pureswaran and Borden 2004), southern pine beetle, *D. frontalis* Zimmermann (Sullivan 2005), and *D. brevicomis* (Erbilgin et al. 2007, 2008). Erbilgin et al. (2007) reported that both acetophenone and verbenone reduced the capture of *D. brevicomis* in attractant-baited traps, but no significant difference was observed between the two treatments. In another study, acetophenone was reported to be superior to verbenone for disrupting *D. brevicomis* attraction (Erbilgin et al. 2008). In experiment 4, we found that verbenone was more effective than acetophenone for reducing *D. brevicomis* attraction to attractant-baited traps, reducing trap catches by 67.1% (V + WPB) compared with only 35.8% for acetophenone (A + WPB). A similar effect was observed in experiment 6 (Fig. 6). The differences in the relative effectiveness of acetophenone and verbenone in these studies may be an artifact of release rates (Table 1; Erbilgin et al. 2008). Acetophenone increased the effectiveness of NAVV5 for disrupting the response of *D. brevicomis* to attractant-baited traps in one of two experiments (Figs. 4 and 6). Furthermore, by adding acetophenone to our NAVV5 blend we were able to remove the aldehydes, nonanal, and (*E*)-2-hexenal, without compromising the effectiveness for disrupting *D. brevicomis* attraction (Fig. 8). This is of practical importance in developing a semiochemical-based tool as aldehydes are problematic for long-term storage in conventional release devices (J.H.B., personal communication). As indicated, we have called the resulting four-component blend Verbenone Plus [A, (*E*)-2-hexen-1-ol + (Z)-2-hexen-1-ol, and V]. Our data indicates that the 75:25 blend of (*E*)-2-hexen-1-ol + (Z)-2-hexen-1-ol is suitable for disrupting *D. brevicomis* attraction (Fig. 8), which decreases the cost by ≈25% compared with the 1:1 mixture (C.J.F., unpublished data). Ongoing research (not presented here) indicates that Verbenone Plus may be effective for protecting *P. ponderosa* from mortality attributed to *D. brevicomis* attack when applied in three separate release devices (Table 1) to individual trees at release rates similar to those used in this study (C.J.F., unpublished data).

Unlike *D. brevicomis*, substantial research has documented the response of *D. ponderosae* to nonhost angiosperm volatiles and verbenone (Zhang and Schlyter 2004). Experiment 3 was the first demonstration of the inhibitory effect of the NAVV6 blend on the response of *D. ponderosae* to attractant-baited traps. A similar study was repeated in 2008 with Verbenone Plus, but failed to produce meaningful results because of adverse weather conditions that hampered *D. ponderosae* flight. In that study, 4.9 ± 1.5 and 0.2 ± 0.1 *D. ponderosae* (mean ± SEM, $n = 42$) were collected in the baited control (MPB) and VP + MPB, respectively. Of forests in the United States, ≈8% are classified at risk (i.e., defined as >25% of stand density will die within the next 15 yr) to insect and disease outbreaks, and *D. ponderosae* is ranked as the most damaging of all agents considered (Krist et al. 2007). *Dendroctonus ponderosae* ranges throughout most of western North America and is able to colonize many native pine species (Furniss and Carolin 1977), al-

though most tree mortality attributed to *D. ponderosae* occurs in *P. contorta* and *P. ponderosa.* Ongoing research (not presented here) indicates that Verbenone Plus is effective for protecting *P. contorta* from mortality attributed to *D. ponderosae* (C.J.F., unpublished data), and its efficacy compared with verbenone alone is being evaluated in that system. Should Verbenone Plus prove more effective than verbenone alone for protecting *P. contorta* from *D. ponderosae,* such data would be useful in facilitating commercialization of Verbenone Plus (i.e., as the only effective semiochemical-based tool for *D. brevicomis*) given the recent impacts of *D. ponderosae* to forest resources (Bentz 2009). Based on our research, we suggest future work for *D. brevicomis* should concentrate on Verbenone Plus applied at the individual tree and small-scale stand levels as well as in other systems. Finally, use of semiochemical-based tools should be considered among all management techniques in an integrated approach.

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