Interruption of the Semiochemical-Based Attraction of Ambrosia Beetles to Ethanol-Baited Traps and Ethanol-Injected Trap Trees by Verbenone

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ABSTRACT We examined the extent to which verbenone, a bark beetle antiaggregation pheromone, interrupted the semiochemical-based attraction of ambrosia beetles. Field trapping studies conducted in Ohio showed that a verbenone dispenser with a release rate of 50 mg/d at 25°C reduced the attraction of *Anisandrus sayi* Hopkins, *Euwallacea validus* (Eichhoff), *Hypothenemus dissimilis* (Zimmermann), *Xylosandrus germanus* (Blandford), and *Xyleborinus saxesenii* (Ratzeburg) to ethanol-baited traps. A verbenone dispenser attached to ethanol-injected *Magnolia virginiana* L. trap trees deployed in Ohio also reduced ambrosia beetle attacks compared to trap trees without a verbenone dispenser. Subsequent field trials demonstrated a direct relationship between distance from a verbenone dispenser and ambrosia beetle attacks on trap trees in Ohio in 2011 and 2012 and Tennessee in 2012, but not in Tennessee and Virginia in 2011. Assessment of the influence of verbenone on the probability of attacks above a density threshold found that although attacks occurred on trap trees regardless of their proximity to a verbenone dispenser, the higher density of attacks per tree occurred on trap trees farthest away from the verbenone source in Ohio and Tennessee. Verbenone alone could be somewhat useful for discouraging ambrosia beetle attacks on individual trees or on a small spatial scale, but deployment of verbenone might be most effective when integrated as part of a "push-pull" strategy.

KEY WORDS verbenone, ambrosia beetle, Scolytinae, *Xylosandrus germanus*, *Xylosandrus crassiusculus*

Ambrosia beetles can pose significant challenges to the production of ornamental nursery crops. Two species can be especially problematic in ornamental nurseries, namely, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae) and *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae) (Oliver and Mannion 2001, Reding et al. 2010, Ranger et al. 2013). *Xylosandrus germanus* first was reported on greenhouse-grown grape vines from Long Island, NY in 1932 and has since been reported from many states within the continental United States and Hawaii (Hoffman 1941, Solomon 1995, LaBonte et al. 2005, Rabaglia et al. 2006, Cognato and Rubinoff 2008). *Xylosandrus crassiusculus* first was reported on peach trees from Summerville, SC in 1974 and has also been reported from many states in the United States, including Hawaii (Anderson 1974, Solomon 1995, LaBonte et al. 2005, Rabaglia et al. 2006, Cognato and Rubinoff 2008). *Xylosandrus germanus* tends to be the most common species of ambrosia beetle attacking nursery stock in Ohio and other midwestern states (Ranger et al. 2010, Reding et al. 2010), although *X crassiusculus* is more abundant and problematic in mid-Atlantic, southeastern, and southern states (Oliver and Mannion 2001, Frank and Sadof 2011).

Both *X germanus* and *X crassiusculus* are capable of attacking a wide range of hosts including >200 and 120 species, respectively, but deciduous hosts are preferred over coniferous tree species (Schedl 1962, Weber and McPherson 1983). Some of the more common hosts in ornamental nurseries include dogwood (*Cornus* spp.), magnolia (*Magnolia* spp.), redbud (*Cercis* spp.), and Japanese snowbell (*Styrax* spp.). Despite a large host range, host quality plays an important role in the host-selection behavior of *X germanus* and *X crassiusculus* (Ranger et al. 2013).

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Ethanol is emitted from stressed and dying hosts and is also the most attractive semiochemical known for *X germanus* and *X crassiusculus* (Montgomery and Wargo 1983; Kelsey 2001; Ranger et al. 2010, 2011a, 2012, 2013). An efficient olfactory mechanism aids *X germanus* and *X crassiusculus* in distinguishing among host qualities and locating trees emitting ethanol and other semiochemicals, but neighboring trees not emitting ethanol generally are not attacked (Weber and McPherson 1984; Ranger et al. 2010, 2012, 2013; P.B.S., unpublished data). Stem injection of ethanol into living trees induces attacks on specific trees by *X germanus*, *X crassiusculus*, and other ambrosia beetles, but neighboring noninjected or water-injected trees usually are not colonized (Ranger et al. 2010, 2012, 2013; Frank and Sadof 2011; Reding et al. 2013). Ethanol-injected trap trees have been critical for measuring the efficacy of conventional and botanically-based insecticides (Ranger et al. 2011a, Frank and Sadof 2011, Reding et al. 2013), and could be useful for attracting ambrosia beetles away from valuable nursery stock as part of a "push-pull" management strategy (Pyke et al. 1987, Miller and Cowles 1990, Cook et al. 2007).

Given the important role of olfaction in the host-location behavior of *X germanus* and *X crassiusculus* (Ranger et al. 2010, 2012, 2013), repellent compounds can be useful for discouraging ambrosia beetle attacks on nursery stock. Previous studies have found verbenone, 4,6,6-trimethylbicyclo[3.1.1] hept-3-en-2-one, acts as a bark beetle antiaggregation pheromone by interrupting the attraction of several *Dendroctonus* spp. (Coleoptera: Curculionidae) and a few *Ips* spp. (Coleoptera: Curculionidae) to conspecific pheromones, host-derived volatiles, trap trees, or all of these (Borden et al. 2003, Bentz et al. 2005, Gillette et al. 2006, Graves et al. 2008). Verbenone was first identified from the hindgut of the southern pine beetle, *Dendroctonus frontalis* Zimmerman, and the western pine beetle, *Dendroctonus brevicomis* LeConte (Renwick 1967). The primary route of production results from bark beetles ingesting α-pinene, which is metabolized into *cis*- and *trans*-verbenonol and then into verbenone by yeasts within the beetles' digestive tract and their galleries (Leufvén et al. 1984, Hunt and Borden 1990, Borden et al. 2003). Verbenone has also been isolated from coniferous (Cool and Zavarin 1992, Dallara et al. 2000) and deciduous plant tissues (Buttery et al. 2000).

Compared with bark beetles, considerably fewer studies have assessed the influence of verbenone on ambrosia beetle orientation, but Dudley et al. (2006) found verbenone reduced the attractiveness of ethanol-baited traps to *X crassiusculus*, *Xylosandrus compactus* (Eichhoff) (Coleoptera: Curculionidae), and *Xyleborinus saxesenii* (Ratzeburg) (Coleoptera: Curculionidae). Similarly, Dodds and Miller (2010) determined verbenone reduced the attraction of *X germanus* and *Gnathotrichus materiarius* (Fitch) to red pine, *Pinus resinosa* Aiton, trap trees, which were created by stem injections of the herbicide Dicamba (3,6-dichloro-*o*-anisic acid).

Conventional insecticides currently are used in ornamental nurseries for protecting nursery stock against *X germanus*, *X crassiusculus*, and other ambrosia beetles. However, manipulating ambrosia beetle behavior using repellents could provide a more economical, user- and environmentally-friendly, and sustainable alternative to synthetic insecticides. We sought to assess the extent to which verbenone interrupts attraction by *X germanus*, *X crassiusculus*, and other ambrosia beetles. We hypothesized verbenone would reduce the attraction of *X germanus*, *X crassiusculus*, and other ambrosia beetles to ethanol-baited traps. We also hypothesized that verbenone would reduce ambrosia beetle attacks on ethanol-injected trees, and that attacks per tree would decrease with decreasing proximity to a verbenone dispenser.

Materials and Methods

Field Trapping. We tested the efficacy of verbenone for reducing ambrosia beetle attraction to ethanol-baited traps under field conditions in Ohio and North Carolina. Bottle traps were constructed according to Ranger et al. (2010) and baited with ethanol alone, verbenone alone, or ethanol + verbenone by attaching the dispensers within the upper portion of the trap. Empty traps served as a blank control. Ethanol dispensers consisted of a heat-sealed, permeable membrane pouch containing 95% ethanol (65 mg/d at 30°C; AgBio, Westminster, CO). Verbenone dispensers consisted of a heat-sealed, permeable membrane pouch containing 92% verbenone (BeetleBlock-Verbenone; 50 mg/d at 25°C; AgBio). Propylene glycol was used in the bottle traps as a killing and preserving agent.

Traps were arranged in a randomized complete block design (RCBD) along the edge of a service road passing through a primarily deciduous woodlot at the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH at ≈0.6 m above ground level and with 6 m between traps and 15 m between blocks. Traps were held under field conditions in Ohio from 30 April 2010 to 25 May 2010. Traps also were arranged in a RCBD alongside a woodlot next to a commercial nursery in Fuquay-Varina, NC at ≈1.5 m above ground level and held under field conditions from 28 February 2011 to 11 May 2011. In total, eight complete blocks were used in Ohio and North Carolina; thus, eight replicates for each treatment.

Ambrosia beetles were identified to species and total trap captures were transformed using $\log_{10}(y + 1)$ before statistical analyses to equalize variance and normalize distribution. One-way analysis of variance (ANOVA) was used to compare ambrosia beetle transformed-counts among traps baited with ethanol alone, verbenone alone, ethanol + verbenone, and a blank control. When the overall model was significant ($P < 0.05$), Tukey's (HSD) test at $\alpha = 0.05$ was used to test for differences among treatment means (PROC GENERAL LINEAR MODEL, SAS Institute 2001).

Trap Tree Protection. *Experiment 1.* The efficacy of verbenone for reducing ambrosia beetle attacks on

individual sweetbay magnolia, *Magnolia virginiana* L., was assessed under field conditions. To ensure ambrosia beetle pressure under field conditions, an Arborjet Tree I.V. system (Woburn, MA) was used to inject *M virginiana* with ethanol according to Ranger et al. (2010, 2012). Trees were ≈4 yr old, 1 m tall, and growing in 13-liter containers with a mixture of aged pine bark, peat, and coarse sand (60:30:10 vol:vol). Injection sites were initiated by drilling a single 9.5-mm hole ≈16 mm deep into the base of the trees, which had a base diameter of 40–50 mm. The hole immediately was plugged with an Arborjet injection port (9.5 mm in diameter) and 75 ml of 5% aqueous ethanol solutions were injected at a delivery pressure of 4.14 bar (60 psi). Control trees were injected with 75 ml of water.

Trees were injected with either ethanol or water on 31 May 2010 and then arranged on the same day in randomized complete blocks along the edge of a service road passing through a primarily deciduous woodlot at the OARDC. Treatments in each block included: 1) ethanol-injected tree with a verbenone dispenser attached to the stem at ≈1 m above the potting media, 2) ethanol-injected tree without a verbenone dispenser, and 3) a water-injected tree without a verbenone dispenser. Previous studies have demonstrated that water-injected trees were not attacked by ambrosia beetles (Ranger et al. 2010, 2011b, 2012); thus, water-injected trees with a verbenone dispenser were not included as part of our current study. Trees within each block were 5 m apart and replicated blocks were separated by 10 m. In total, seven complete blocks were used; thus, there were seven replicates for each treatment. Trees were maintained under field conditions until 21 June 2010, during which time they were examined thoroughly for new attacks at 1, 6, 11, 16, and 21 d after treatment. Attacks on individual trees were circled with a waterproof marker (Sharpie, Oak Brook, IL), which allowed for attacks to be quantified over several points in time (Ranger et al. 2010, 2011a, 2012). Previous field studies in Ohio have found *X germanus* to be the predominant species of ambrosia beetle attacking ethanol-injected trees; thus, beetles were not excavated from the galleries of attacked trees (Ranger et al. 2010, Reding et al. 2013).

Total attacks per tree were quantified as described previously and were transformed using $\log_{10}(y + 1)$ before statistical analyses to equalize variance and normalize distribution. Repeated measures ANOVA was used to initially test for between-subject effects in ambrosia beetle attacks among the trap tree treatments (PROC GLM, SAS Institute 2001). Repeated measures ANOVA was used because repeated measurements were made on the same experimental units, and the number of attacks was cumulative and dependent in part on the previous number of attacks (Littell et al. 1998). When a significant between-subject effect was detected ($P < 0.05$), the number of attacks at each time point were compared among the three treatments by using one-way ANOVA and Tukey's HSD ($\alpha = 0.05$; SAS Institute 2001). The overall equality of slopes associated with cumulative attacks on the three trap tree treatments and days after deployment were also compared (PROC GLM, SAS Institute 2001). Predetermined contrast statements were then used to make specific comparisons among and between slopes (PROC GLM, SAS Institute 2001).

Experiment 2. The relationship between distance from a verbenone dispenser and attacks on ethanol-injected *M virginiana* trap trees was assessed under field conditions to determine if attacks per tree decreased with decreasing proximity to a verbenone dispenser. The relationship between distance from a woodlot, which serves as a refuge for overwintering adults, and attacks on trap trees also was examined. Four 20-m^2 plots separated by ≥50 m were arranged adjacent to primarily deciduous woodlots in Ohio, Tennessee, and Virginia in 2011, and in Ohio and Tennessee in 2012. The minimum distance between each plot and the nearest woodlot was ≈2–16 m, and the distance between each tree and the nearest woodlot ranged from 3 to 34 m across all plots and years. One potted *M virginiana* trap tree injected with 75 ml of 5% ethanol was placed in the center of each plot with a verbenone dispenser attached to the stem ≈1 m above the potting media. Additional ethanol-injected *M virginiana* trap trees were then randomly positioned within each plot at varying distances ranging from 2 to 10 m from the verbenone dispenser. We used 15 trees per plot in Tennessee (2011 and 2012), 22–23 trees per plot in Virginia (2011), and 19–24 trees per plot in Ohio (2011 and 2012). Trees were deployed from 10 May 2011 to 25 May 2011 and 30 April 2012 to 13 May 2012 in Ohio, from 14 June 2011 to 15 July 2011 and 11 June 2012–6 July 2012 in Tennessee, and 12 April 2011 to 26 May 2011 in Virginia. Ambrosia beetles were not excavated from galleries as part of the current study, but previous studies have found *X germanus* to be the predominant species of ambrosia beetle attacking ethanol-injected trees in Ohio (Ranger et al. 2010, 2012; Reding et al. 2013) and *X crassiusuculus* in Tennessee and Virginia (Reding et al. 2013).

The total number of ambrosia beetle attacks were recorded on each tree and counts were transformed using $\log_{10}(y + 1)$ for statistical analyses to equalize variance and normalize distribution. For each year (2011 and 2012) and state (Ohio, Tennessee, and Virginia), we assessed the main effects of distance from the verbenone source, distance from the nearest woodlot, and their interaction, on the log-transformed number of attacks (PROC GLM, SAS Institute 2001).

We also examined the relationship between the probability of attack above a density threshold and distance from the verbenone source. In this analysis, we restricted the data sets to Ohio (2011 and 2012) and Tennessee (2012) because we were specifically interested in the location of the higher density of attacks relative to the verbenone source ($N = 236$ trees). Also, at these sites and years, there was a reasonable range of attacks per tree, from 0 to 100 (median = 10), 0–148 (median = 16), and 0–63 (median = 7.5) in Ohio (2011), Ohio (2012), and Tennessee (2012), respectively. In contrast, the range of attacks per tree in

Table 1. Influence of verbenone on the attractiveness of ethanol-baited traps to ambrosia beetles

Species	Trapping location	Mean (± SE) total trap counts				*F*	*P*
		Ethanol	Verbenone	Ethanol + verbenone	Blank		
Ambrosiodmus rubricollis	NC	0.0 ± 0.0a	0.0 ± 0.0a	0.03 ± 0.03a	0.0 ± 0.0a	1.00	0.41
Ambrosiophilus atratus	NC	0.03 ± 0.03a	0.03 ± 0.03a	0.05 ± 0.03a	0.0 ± 0.0a	0.68	0.57
Anisandrus sayi	OH	7.1 ± 2.8a	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	20.51	<0.0001
Euwallacea validus	OH	1.1 ± 0.3a	0.1 ± 0.1b	0.3 ± 0.2b	0.0 ± 0.0b	7.56	0.0007
Hypothenemus dissimilis	OH	0.4 ± 0.2a	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0b	4.20	0.014
Hypothenemus eruditus	OH	0.0 ± 0.0a	0.1 ± 0.1a	0.0 ± 0.0a	0.0 ± 0.0a	1.00	0.41
Monarthrum mali	OH	0.1 ± 0.1a	0.0 ± 0.0a	0.4 ± 0.3a	0.1 ± 0.1a	0.86	0.47
Phloeotribus limnaris	NC	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.03a	0.0 ± 0.0a	2.33	0.096
Xyleborinus alni	OH	0.3 ± 0.3a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	1.00	0.41
Xyleborinus saxesenii	OH	0.9 ± 0.3a	0.1 ± 0.1b	0.0 ± 0.0b	0.0 ± 0.0b	7.50	0.0008
	NC	0.2 ± 0.1a	0.03 ± 0.03a	0.1 ± 0.1a	0.03 ± 0.03a	2.32	0.097
Xyleborus horridus	NC	0.03 ± 0.03a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	1.00	0.41
Xylosandrus crassiusculus	NC	0.4 ± 0.2a	0.03 ± 0.03a	0.4 ± 0.2a	0.0 ± 0.0a	2.55	0.076
Xylosandrus germanus	OH	139.3 ± 19.3a	0.8 ± 0.4b	4.3 ± 0.8b	0.9 ± 0.4b	152.99	<0.0001
	NC	0.03 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	1.00	0.41
Xyloterinus politus	OH	5.1 ± 2.7a	1.5 ± 0.8ab	2.6 ± 1.0ab	0.1 ± 0.1b	5.30	0.005
	NC	0.0 ± 0.0a	0.0 ± 0.0a	0.03 ± 0.03a	0.03 ± 0.03a	0.67	0.57

Different letters within a row indicate significant differences (ANOVA; Tukey's HSD; $\alpha = 0.05$; df = 3, 28 for Ohio [OH] and df = 3, 28 for North Carolina [NC]).

Virginia (2011) was 0–11 (median = 0), and 0–32 (median = 1.5) in Tennessee (2011).

We considered the combined range of attacks per tree from Ohio (2011 and 2012) and Tennessee (2012) and scored each tree a value of one if the attacks on that tree exceeded a density threshold and 0 otherwise. We used a density threshold of 0 as a baseline (i.e., attacked or not), and then used density thresholds corresponding to the 25th, 50th, 75th, and 90th percentile of the combined range of attacks per tree, which were 5, 12, 26, and 48, respectively. We then analyzed this set of binary variables as a function of the distance from the verbenone source using the likelihood ratio chi-squared (G^2) in logistic regression (PROC LOGISTIC, SAS Institute 2001).

Results

Field-Trapping. As part of the trapping experiment conducted in Ohio, more *X germanus* ($F = 152.99$; df = 3, 28; $P < 0.01$), *Anisandrus sayi* Hopkins ($F = 20.51$; df = 3, 28; $P < 0.01$), *Euwallacea validus* (Eichhoff) ($F = 7.56$; df = 3, 28; $P < 0.01$), *Xyleborinus saxesenii* (Ratz.) ($F = 7.50$; df = 3, 28; $P < 0.01$), and *Hypothenemus dissimilis* (Zimmermann) ($F = 4.20$; df = 3, 28; $P = 0.01$) were collected in traps baited with ethanol alone compared with traps baited with verbenone alone, verbenone + ethanol, and nonbaited (Table 1). More *Xyloterinus politus* (Say) were collected in traps baited with ethanol alone compared with the nonbaited traps, but differences were not detected between ethanol alone, verbenone alone, and ethanol + verbenone ($F = 5.30$; df = 3, 28; $P < 0.01$) (Table 1). No significant difference among trap treatments were detected for *Hypothenemus eruditis* Westwood, *Monarthrum mali* (Fitch), and *Xyleborinus alni* (Niisima) in Ohio ($P > 0.05$; Table 1).

Nine species of ambrosia beetle were collected in Ohio during this study. In total, 1,324 ambrosia beetle specimens were collected in ethanol-baited traps deployed in Ohio. *Xylosandrus germanus* represented 91.2 ± 1.9% (mean ± SE) of total trap captures for ethanol-baited traps deployed in Ohio, which was higher compared with *A sayi* (4.4 ± 1.5%), *X politus* (2.7 ± 0.9%), *E validus* (0.7 ± 0.2%), *X saxesenii* (0.6 ± 0.2%), and *X alni* (0.1 ± 0.1%) trap captures in Ohio ($F = 1194.04$; df = 6, 49; $P < 0.01$).

Eight species of ambrosia beetle were collected in NC, but no significant differences were detected for any species among traps baited with ethanol, verbenone, ethanol + verbenone, and nonbaited treatments ($P > 0.05$; Table 1).

Trap Tree Protection. *Experiment 1.* We detected a significant between-subjects effect among the treatments in a repeated measures ANOVA ($F = 15.76$; df = 2, 18; $P < 0.01$). Differences in mean (±SE) attacks were detected among all three treatments at 1 d after deployment for ethanol-injected *M virginiana* trees without verbenone (9.0 ± 1.5), ethanol-injected trees with verbenone (1.1 ± 0.5), and water-injected trees without verbenone (0.0 ± 0.0) ($F = 45.76$; df = 2, 18; $P < 0.01$; Fig. 1). Differences in mean (±SE) attacks were also detected among all three treatments at 21 d after deployment for ethanol-injected *M virginiana* trees without verbenone (23.1 ± 5.6), compared with ethanol-injected trees with verbenone (3.4 ± 1.2), and water-injected trees without verbenone (0.0 ± 0.0) ($F = 39.70$; df = 2, 18; $P < 0.01$; Fig. 1).

Differences were detected in the overall equality of slopes associated with cumulative attacks over time on ethanol-injected *M virginiana* trees with and without verbenone and water-injected trees without verbenone ($F = 5.63$; df = 2; $P < 0.01$). Subsequent specific contrasts determined the slope associated with ethanol-injected trees without a verbenone dispenser (0.71) was larger than ethanol-injected trees with a verbenone dispenser (0.1) and the water injected control (0.0) ($F = 11.08$; df = 1; $P < 0.01$);

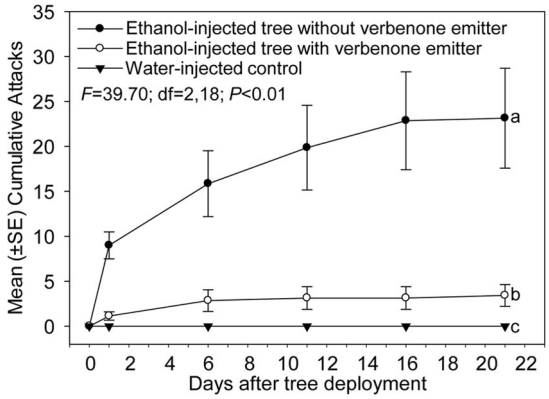

Fig. 1. Mean (±SE) cumulative ambrosia beetle attacks on ethanol-injected *M virginiana* with and without a verbenone dispenser and a water-injected control deployed in Ohio. Different letters indicate significant differences between treatments 21 d after tree deployment (one-way ANOVA; Tukey's HSD).

however, differences were not detected between the latter two treatments ($F = 0.18$; df = 1; $P = 0.67$).

Experiment 2. An effect of distance from the verbenone dispenser on trap tree attacks was detected in Ohio in 2011 ($F = 14.2$; df = 1, 78; $P < 0.01$; Fig. 2A) and 2012 ($F = 44.2$; df = 1, 92; $P < 0.01$; Fig. 2A) (intercept ± SE = 0.85 ± 0.07; $t = 12.9$; $P < 0.001$; slope ± SE = 0.05 ± 0.01; $t = 4.7$; $P < 0.001$), and in Tennessee in 2012 ($F = 4.3$; df = 1, 57; $P = 0.04$; Fig. 2B) (intercept ± SE = 0.54 ± 0.18; $t = 3.1$; $P = 0.004$; slope ± SE = 0.05 ± 0.03; $t = 2.1$; $P = 0.048$), such that attacks on trap trees increased with increasing distance from the verbenone dispenser. We also detected an influence of distance from the nearest woodlot in Ohio in 2011 ($F = 7.2$; df = 1, 78; $P < 0.01$; Fig. 3A) and 2012 ($F = 10.6$; df = 1, 92; $P < 0.01$; Fig. 3A) (intercept = 1.51 ± 0.10; $t = 14.8$; $P < 0.001$; slope = −0.03 ± 0.01; $t = -4.1$; $P < 0.001$), such that attacks on individual trap trees increased with decreasing proximity to a woodlot. No significant main effect or interaction effect was detected in Tennessee in 2011 and 2012 and Virginia in 2011 ($P > 0.05$; Fig. 3B).

When considering the Ohio data set, we did not observe an interaction effect between year and distance from the verbenone source ($F = 0.02$; df = 1, 172; $P = 0.88$), or between year and distance from the nearest woodlot ($F = 0.6$; df = 1, 172; $P = 0.59$), suggesting that we could pool the data for Ohio across years. We also did not observe an effect of distance from the verbenone source when considering any level of attack on a host tree (i.e., attack threshold = 0; $G^2 = 2.1$; $P = 0.14$). However, we did detect a significant effect when considering all other density thresholds: five ($G^2 = 8.7$; $P < 0.01$), 12 ($G^2 = 9.6$; $P < 0.01$), 26 ($G^2 = 20.5$; $P < 0.01$), and 48 ($G^2 = 18.4$; $P < 0.01$) (Fig. 4). In all these cases, the host trees with the higher density of attacks were located farther from the verbenone source. Thus, although attacks were present on trap trees regardless of their proximity to the verbenone source, the higher density of attacks per tree occurred on trees located farther away from the dispenser.

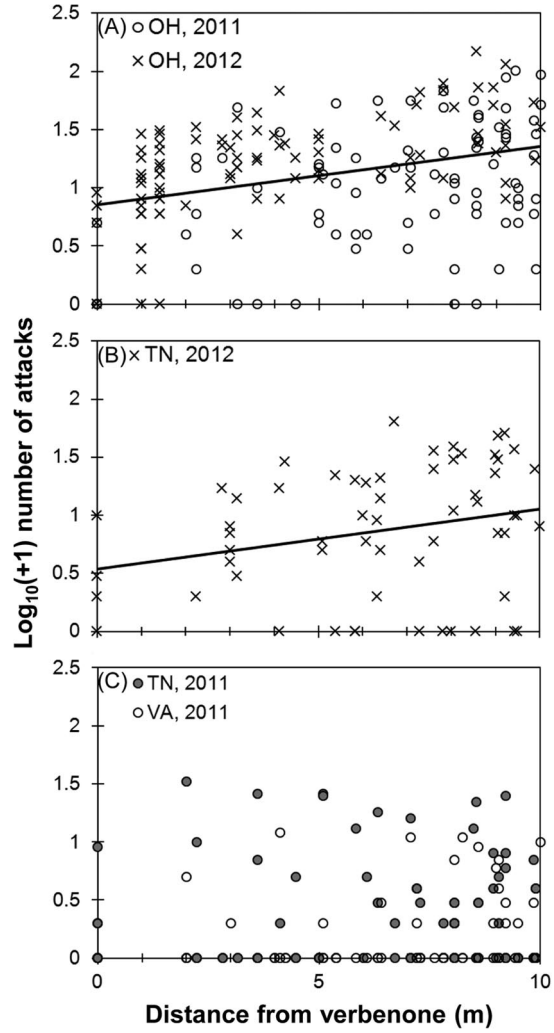

Fig. 2. A–C. Relationship between distance from verbenone dispenser and cumulative ambrosia beetle attacks on ethanol-injected M. virginiana trap trees deployed in (A) Ohio, (B) Tennessee, and (C) Tennessee and Virginia.

Discussion

These results demonstrate the efficacy of verbenone at manipulating the behavioral response of selected ambrosia beetles to ethanol-baited traps and ethanol-injected trap trees. As part of the trapping experiment conducted in Ohio in 2010, verbenone interrupted the response of *A sayi*, *E validus*, *H dissimilis*, *X germanus*, and *X saxesenii* to ethanol-baited traps. In particular, verbenone resulted in a 96.9% reduction in *X germanus* captures in traps baited with ethanol + verbenone compared with ethanol alone. This finding is consistent with Dudley et al. (2006), who also reported that verbenone interrupted the attraction of *X crassiusculus*, *X compactus*, and *X sax-*

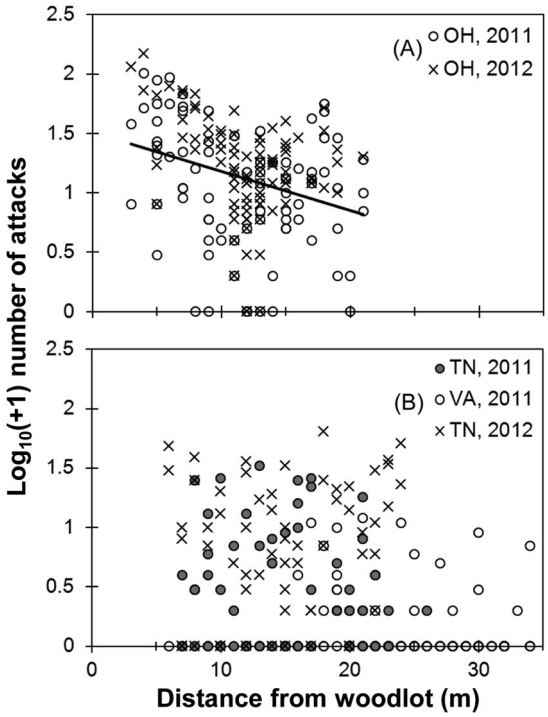

Fig. 3. A–B. Relationship between distance from woodlot and cumulative ambrosia beetle attacks on ethanol-injected M. virginiana trap trees deployed in (A) Ohio and (B) Tennessee and Virginia.

esenii to ethanol-baited traps. Verbenone did not reduce the response of *X crassiusculus* and other ambrosia beetles to ethanol-baited traps as part of the experiment conducted in North Carolina in 2011. However, in this case, it is possible that low numbers of ambrosia beetle trap captures throughout the duration of the experiment in North Carolina, regardless of treatment, hindered our ability to detect statistical differences among treatments.

Traps baited with verbenone alone were not more attractive than blank traps to any species of ambrosia beetle collected in Ohio or North Carolina, and no evidence that verbenone acts as an attractant to ambrosia beetles was obtained as part of our current study. Lindgren and Miller (2002) found *Trypodendrum lineatum* (Oliver) (Coleoptera: Curculionidae) exhibited a positive dose-dependent attraction to verbenone as part of a late season experiment after peak flight had passed, but the phenomenon was not detected by the authors in additional experiments.

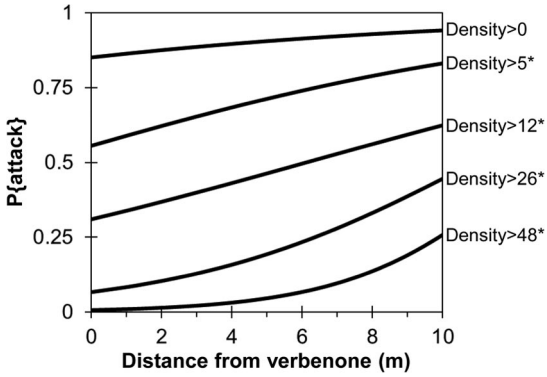

Fig. 4. Relationship between distance from a verbenone dispenser and probability of attacks occurring above various density thresholds.

Results from our trap tree experiment conducted in Ohio in 2011 demonstrated that a verbenone dispenser attached to an individual ethanol-injected *M virginiana* tree reduced ambrosia beetle attacks by 85%, but did not completely prevent them from occurring. In some cases, attacks occurred on tissue directly underneath the verbenone emitter. *Dendroctonus ponderosae* also reportedly attacked verbenone-treated trap trees directly underneath the dispenser (Lindgren et al. 1989, Lindgren and Borden 1993). Ambrosia beetles were not excavated from attacked trees as part of our study, but previous studies have found *X germanus* to be the predominant species of ambrosia beetle attacking ethanol-injected trees in Ohio (Ranger et al. 2010, 2012; Reding et al. 2013). Verbenone reduced the attraction of *X germanus* and *G materiarius* to herbicide-injected *P resinosa* trap trees, but was also not completely effective at preventing ambrosia beetle attacks (Dodds and Miller 2010). No ambrosia beetle attacks occurred on neighboring water-injected trees as part of our current study, which supports previous observations regarding a lack of attacks on neighboring trees not emitting ethanol (Ranger et al. 2010, 2012, 2013; Frank and Sadof 2011; Reding et al. 2013).

Our subsequent multistate trap tree experiments demonstrated that a direct relationship was detected between distance from the verbenone dispenser and ambrosia beetle attacks on trap trees in Ohio (2011 and 2012) and Tennessee (2012). Although attacks occurred on trap trees regardless of their proximity to a verbenone dispenser, the higher density of attacks per tree occurred on trap trees farthest away from the verbenone source in Ohio and Tennessee. The absence of a relationship detected for Tennessee and Virginia in 2011 may be attributed to a large number of trees within the 20 m^2 blocks that were not attacked. Collectively, these results indicate that additional studies are warranted to assess the efficacy of verbenone dispensers when positioned in a grid formation. Results from Ohio in 2011 and 2012 demonstrated a relationship between proximity to overwintering sites and ambrosia beetle attacks, such that attacks on individual trap trees increased with decreasing proximity to a wood lot. Thus, deployment of highly attractive traps or trap trees, especially as part of a "push-pull" strategy, could be most effective for intercepting beetles when deployed as a protective barrier in between nursery stock and ambrosia beetle overwintering sites in neighboring wood lots.

A number of studies have assessed the efficacy of verbenone to protect forested stands against bark beetles, but efficacy has been inconsistent (Kostyk et al. 1993, Bentz et al. 2005, Progar 2005). Verbenone dis-

pensing strategies, densities of beetle populations, and stand susceptibilities may account for differences in efficacy (Gillette et al. 2009). The deployment of many small, point-source releasers, such as verbenone-releasing flakes, could provide improved efficacy over plastic pouches or bubblecaps (Gillette et al. 2009). Individual membrane-type dispensers were used as part of our current study. Thus, additional studies that compare different verbenone release strategies and formulations, along with application techniques for deploying verbenone-impregnated flakes within ornamental nurseries, could potentially enhance the effectiveness of this management strategy.

Lindgren and Miller (2002) assessed the effect of verbenone on five species of bark beetles with differences in host-age preference. Verbenone interrupted the response of species requiring relatively fresh host tissue, but species that colonize decaying tissue were not affected. In particular, the degree to which verbenone interrupted a species' response appeared to be related to a preference for fresh host tissue (Lindgren and Miller 2002). The apparent preference of *X germanus* for relatively fresh host tissue can provide a basis for verbenone to interrupt their semiochemical-based response. Yet, despite a large host range and preference for relatively fresh tissue, recent studies have indicated that trees within ornamental nurseries are not randomly attacked by *X germanus* (C.M.R., unpublished data). Instead, tissues emitting ethanol and potentially other stress-related volatiles are selectively targeted for colonization, with *X germanus* efficiently locating the source of host-derived ethanol, whereas neighboring trees not emitting ethanol are rarely landed on (Ranger et al. 2013).

Based on our current results, verbenone alone would not appear to be an effective management tactic, particularly for protecting trees emitting ethanol in response to abiotic, biotic stress, or both. A preliminary experiment involving the deployment of verbenone dispensers among a block of dogwood trees (*Cornus* sp.) subjected to flood-stress in a commercial nursery in Ohio failed to prevent ambrosia beetle attacks (C.M.R., personal observation). Therefore, verbenone did not prevent ambrosia beetle attacks even when ethanol emission levels were probably closer to natural tree production under stress (i.e., flooding) compared with potentially higher levels resulting from stem injections of ethanol. However, trunk applications of synthetic or botanical insecticides also do not always protect ornamental trees from being attacked when they are emitting ethanol (Ranger et al. 2011b, Reding et al. 2013, P.B.S., personal observation; J.B.O., personal observation). The low esthetic threshold for wood-borer damage to ornamental nursery stock would also minimize the efficacy of verbenone as the sole management tool. Verbenone might be useful for protecting small blocks when integrated with other tactics, particularly when used in conjunction with highly attractive traps or trap trees as part of "push-pull" strategy. The propensity of *X germanus*, *X crassiusculus*, and other ambrosia beetles to efficiently locate and attack trees emitting ethanol, even despite the presence of conventional insecticides, attests to the importance of maintaining host vigor as the primary foundation for an ambrosia beetle management strategy (Ranger et al. 2013). However, challenges associated with maintaining host vigor in ornamental nurseries growing diverse stock that likely vary in their tolerance to abiotic and biotic stressors necessitates the need for a multifaceted management plan.

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