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Ethanol Injection of Ornamental Trees Facilitates Testing Insecticide Efficacy Against Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae)

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ABSTRACT Exotic ambrosia beetles are damaging pests in ornamental tree nurseries in North America. The species *Xylosandrus crassiusculus* (Motshulsky) and *Xylosandrus germanus* (Blandford) are especially problematic. Management of these pests relies on preventive treatments of insecticides. However, field tests of recommended materials on nursery trees have been limited because of unreliable attacks by ambrosia beetles on experimental trees. Ethanol-injection of trees was used to induce colonization by ambrosia beetles to evaluate insecticides and botanical formulations for preventing attacks by ambrosia beetles. Experiments were conducted in Ohio, Tennessee, and Virginia. Experimental trees injected with ethanol had more attacks by ambrosia beetles than uninjected control trees in all but one experiment. *Xylosandrus crassiusculus* and *X. germanus* colonized trees injected with ethanol. In most experiments, attack rates declined 8 d after ethanol-injection. Ethanol-injection induced sufficient pressure from ambrosia beetles to evaluate the efficacy of insecticides for preventing attacks. Trunk sprays of permethrin suppressed cumulative total attacks by ambrosia beetles in most tests. Trunk sprays of the botanical formulations Armorex and Veggie Pharm suppressed cumulative total attacks in Ohio. Armorex, Armorex + Permethrin, and Veggie Pharm + Permethrin suppressed attacks in Tennessee. The bifenthrin product Onyx suppressed establishment of *X. germanus* in one Ohio experiment, and cumulative total ambrosia beetle attacks in Virginia. Substrate drenches and trunk sprays of neonicotinoids, or trunk sprays of anthranilic diamides or tolfenpyrad were not effective. Ethanol-injection is effective for inducing attacks and ensuring pressure by ambrosia beetles for testing insecticide efficacy on ornamental trees.

KEY WORDS *Xylosandrus*, ornamental nursery, Pyrethroid, plant-based essential oil

Exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are serious wood-boring pests in ornamental tree nurseries in North America (Oliver and Mannion 2001, Hale 2007, Frank and Sadoff 2011). *Xylosandrus* species have become especially problematic in states east of the Mississippi river (Hudson and Mizell 1999, Oliver and Mannion 2001, Reding et al. 2010). *Xylosandrus crassiusculus* (Motshulsky) appears more prevalent in Southeastern and Atlantic states while *Xylosandrus germanus* (Blandford) appears more common in Midwestern and Northeastern states (Hudson and Mizell 1999; Oliver and Mannion 2001; Hale 2007, Reding et al. 2010, 2011; Ranger et al. 2011). Both species are native to Asia and have wide host ranges that include primarily deciduous trees (Wood 1982, Solomon 1995). Only the females fly and primarily colonize physiologically stressed trees (Hoffman 1941; Wood 1982; Weber and McPherson 1984; Ranger et al. 2010, 2012b). Trees under physiological stress emit ethanol (Moeck 1970, Kimmerer and Kozlowski 1982, Kelsey and Joseph 2001), which acts as a primary attractant for ambrosia beetles including *X. crassiusculus* and *X. germanus* (Graham 1968; Cade et al. 1970; Moeck 1970; Montgomery and Wargo 1983; Oliver and Mannion 2001; Ranger et al. 2010, 2012a). Weber and McPherson (1984) found that *X. germanus* were more likely to colonize black walnut (*Juglans nigra* L.) trees with slower growth rates, and concluded that beetles could differentiate between slight differences in host vigor. Furthermore, Ranger et al. (2010) demonstrated that *X. germanus* and other ambrosia beetles would attack trees injected

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with ethanol, while uninjected trees or those injected with water were not attacked.

X. crassiusculus and *X. germanus* overwinter as adults in the galleries of infested trees (Weber and McPherson 1983, Oliver and Mannion 2001, Reding et al. 2010). In spring, female beetles emerge from overwintering sites to search for new hosts to colonize. After locating a suitable host, the foundress bores into the tree's xylem and establishes a gallery, which she inoculates with a symbiotic fungus that is the source of food for the adults and larvae (Hoffman 1941, Weber and McPherson 1983, Solomon 1995). *Xylosandrus* galleries generally consist of tunnels and one or more brood chambers (Hoffman 1941, Wood 1982, Weber and McPherson 1983, Solomon 1995). Newly colonized nursery trees often appear healthy when they break dormancy, then, the leaves wilt and the trees die. The causes of mortality are uncertain, but may include mechanical injury from tunneling beetles, pathogenicity of the symbiotic fungi, incidental pathogens that enter through the tunnel entrances, blockage of the trees' vascular tissues by fungal growth, or combinations of these factors (Weber and McPherson 1983, Oliver and Mannion 2001, Dute et al. 2002, Kuhnholz et al. 2003).

In ornamental tree nurseries, growers rely primarily on trunk sprays of conventional insecticides to prevent attacks by ambrosia beetles and other wood-boring beetles. However, the efficacy of insecticides recommended for preventing attacks by ambrosia beetles have not been thoroughly field tested against *X. crassiusculus* and *X. germanus* on nursery trees. A constraint to testing insecticide efficacy against ambrosia beetles has been the lack of reliable colonization of experimental trees. Ranger et al. (2010) developed a technique in which injecting trees with ethanol induced colonization of live nursery trees by ambrosia beetles. Containerized *Magnolia virginiana* L. injected with ethanol were subsequently colonized (attacked) by *X. germanus* and other ambrosia beetles (Ranger et al. 2010). Ranger et al. (2011) used the ethanol-injection technique to test commercially available plant-based essential oil mixtures for preventing attacks by ambrosia beetles on *M. virginiana,* and found variability in efficacy of the selected botanical insecticides. Frank and Sadoff (2011) also used ethanol-injection to test efficacy of permethrin against ambrosia beetles on red maple trees in North Carolina, however, colonization rates by ambrosia beetles were relatively low. Further evaluation of the ethanol-injection technique on nursery trees is warranted to assess its reliability in inducing attacks by *Xylosandrus* spp. and other ambrosia beetles in different geographic locations, and to field-test existing and new insecticides against ambrosia beetles.

The objective of the current research was to determine whether ethanol-injection of live trees would reliably induce attacks by ambrosia beetles, especially *X. crassiusculus* and *X. germanus,* in numbers sufficient to facilitate testing efficacy of preventive treatments in three geographic regions. The second objective was to field-test a variety of materials for efficacy in controlling *X. crassiusculus, X. germanus,* and other ambrosia beetles on ornamental trees.

Materials and Methods

General Methods. Experiments were conducted in 2009 and 2010 in Ohio, Tennessee, and Virginia (2009 only). These states have experienced significant damage to ornamental nursery trees from ambrosia beetles. The experiments were set up as randomized complete block designs. The experiments were set up along the wooded borders of nurseries or research farms with replicated blocks positioned at least 10 m apart and single-tree replicates within blocks at least 1.0 m apart. Unless otherwise specified, the test trees were containerized *Magnolia virginiana* L. planted in soil-free substrate in 11.4 liter containers. To induce colonization by ambrosia beetles, the trees were injected with ethanol using the Arborjet Tree I.V. Delivery System (Arborjet, Woburn, MA) (Ranger et al. 2010, 2011). Injection sites were initiated by drilling a single 9.5 mm hole ≈16 mm deep into the base of the trees. The hole was immediately plugged with an Arborjet injection port (9.5 mm diameter), then ethanol was injected through the port at a delivery pressure of 414 kPA (60 psi). Unless otherwise specified, experimental trees were injected with 75 ml of 50% ethanol (Ranger et al. 2010). The test trees were at least 40 mm in diameter at the drill or injection port site. In smaller diameter trees, the drill bit tended to go completely through the stems or the stems broke at the drill site. All experiments included a noninsecticide treated ethanol-injected treatment (hereafter referred to as injected controls). Unless otherwise stated, the experiments also included an uninjected, noninsecticide treated treatment. Data from the uninjected treatments were compared only with the injected controls and not included in the analyses of insecticide efficacy. Trunk sprays of insecticides were applied 1 d before ethanol injections, and container substrate drenches were applied 7 d (Ohio and Virginia) or 34 d (Tennessee) before ethanol injection. There were at least three postinsecticide treatment evaluations for each experiment. On each evaluation date, circles were drawn around new tunnel entrances to prevent recounting. To evaluate the success of colonization and determine the species of beetles attacking the trees, the trees were cut into bolts on the last evaluation date, then placed in bags labeled by treatment and replication and transported to the lab. The bolts were then either incubated in the lab (at least 5 wk) to allow emergence of beetles (Ohio 2009 experiment) or dissected using hand pruners to extract the colonizing beetles (Ohio and Tennessee 2010 experiments). After emergence or extraction, the beetles were stored in 70% ethanol. Scolytinae were identified to the species level using available keys (Wood 1982, Rabaglia et al. 2006).

Ohio Experiments. In the Ohio experiments, there were eight replications per treatment and the trunk sprays were applied to the point of runoff using 1.4 liter compressed air hand-triggered spray bottles

Table 1. Insecticides and botanical formulations used in ambrosia beetle experiments and their application rates and manufacturers

State	Formulated material	Active ingredient (a.i.)	Rate per 379 liters of water (a.i.)	Supplier
OH, TN, VA[a]	Acelepryn 1.67SC	Chlorantraniliprole	0.95 liters (190.5 g)	Dupont, Wilmington, DE
OH, TN	Armorex T&O	Sesame, rosemary, garlic, eugenol, white pepper oils	10%	Soil Technologies, Fairfield, IA
TN	Cinnacure	Cinnamaldehyde	10%	ProGuard, Suisun, CA
VA	Cyazypyr 10SC	Cyantraniliprole	0.95 liters (113.4 g)	Dupont, Wilmington, DE
TN	EcoTrol EC	Rosemary, peppermint, wintergreen oils	10%	EcoSmart Technologies, Franklin, TN
OH, VA	Flagship 25WG	Thiamethoxam	230 g (58 g)	Syngenta, Greensboro, NC
OH, VA	Hachi-Hachi	Tolfenpyrad	0.62 liters (93 g)	SePRO, Carmel, IN
OH, TN, VA	OnyxPro	Bifenthrin	0.95 liters (227 g)	FMC, Philadelphia, PA
TN	Pentra-Bark	Alkylphenol ethoxylate, polysiloxane polyether, copolymer, propylene glycol	0.95 liters	Quest Products, Westminster, CO
TN	Perm-UP 3.2EC[b]	Permethrin	4.73 liters (1816 g)	United Phosphorus, Trenton, NJ
OH, TN, VA	Safari 20SG	Dinotefuran	680 g (136 g)	Valent, Walnut Creek, CA
OH, TN	Scimitar	Lambda-cyhalothrin	0.15 liters (15.6 g)	Syngenta, Greensboro, NC
OH	Tengard SFR[b]	Permethrin	4.73 liters (1816 g)	United Phosphorus, Trenton, NJ
OH, TN	Veggie Pharm	Cottonseed, garlic, peppermint, rosemary oils	10%	Pharm Solutions, Port Townsend, WA

All experiments included an ethanol-injected non-insecticide treated control (injected-control).
[a] OH, Ohio; TN, Tennessee; VA, Virginia.
[b] Perm-UP 3.2EC and Tengard SFR are the same formulation with 383.5 g of permethrin per liter, and the same cis: trans ratio 42: 58.

(item no. 65-6418, Hummert International, Earth City, MO). In 2009, the experiment was conducted five through 27 May. There were seven insecticide treatments including trunk sprays of Acelepryn, Hachi-Hachi, Onyx, Safari, and Scimitar, and container substrate drenches of Flagship and Safari (see Table 1 for information on insecticide active ingredients, application rates, and manufacturers for all experiments). The Flagship and Safari substrate drenches were applied by pouring 1,000 ml and 355 ml of solution, respectively, onto the substrate of each container. In 2010, the experiment compared plant-based essential oil mixtures (Armorex and Veggie Pharm) with conventional recommended insecticides, and was conducted 4 May through 2 June. There were four insecticide treatments including trunk sprays of Armorex, Onyx, Tengard, and Veggie Pharm (Table 1), the test trees were injected with 75 ml of 10% ethanol, and there was no uninjected treatment. Ranger et al. (2011) showed that ethanol injections at concentrations as low as 5% (75 ml) induced ambrosia beetle attacks in sufficient numbers for testing efficacy of essential oils against ambrosia beetles in Ohio. To evaluate the experiments, the trees were thoroughly examined for tunnel entrances (attacks) at 1, 6, 13, and 22 d after injection (DAI) with ethanol in 2009, and 2, 6, 10, 16, 22, and 29 DAI in 2010.

Tennessee Experiments. In 2009, there were two experiments, one conducted in spring (experiment-1, 21 April to 22 May) that focused on conventional insecticides and one in summer (experiment-2, 22 June to 16 July) that included plant-based essential oil mixtures compared with a standard conventional insecticide treatment. Trunk sprays were applied to the point of runoff by a 1.4 liter compressed air hand-triggered spray bottle (item no. 65-6418). Experiment 1 had six replications per treatment and experiment 2 had five. In experiment 1, there were seven insecticide treatments including trunk sprays of Onyx, Perm-Up, Safari, Safari + Pentra Bark, and Scimitar, and container substrate drenches of Acelepryn and Safari (Table 1). Pentra Bark is a surfactant designed to facilitate penetration of the bark by insecticides (Table 1). The Acelepryn and Safari substrate drenches were applied by pouring 355 ml of solution onto the substrate of each container. Experiment 1 did not have an uninjected treatment. In experiment 2, the test trees were containerized *Oxydendrum arboreum* (L.) DC. (sourwood) potted in 11.4 liter containers. There were five insecticide treatments, including trunk sprays of Cinnacure, EcoTrol, Perm-Up, Cinnacure + Pentra Bark, and Perm-Up + Pentra Bark (Table 1). The trees in experiment 2 were injected with 75 ml of 10% ethanol. For evaluation, trees were examined for new attacks 3, 6, 9, 14, 20, 24, and 31 DAI in experiment 1, and 3, 4, 5, 8, 9, 10, 11, 18, and 25 DAI in experiment 2. The species of ambrosia beetles attacking the trees were not determined for either experiment.

In 2010, plant-based essential oil mixtures were the primary focus of the experiment with seven replications per treatment. The experiment was conducted from 20 May to 7 June, with five insecticide treatments including trunk sprays of Armorex, Perm-Up, Veggie Pharm, Armorex + Perm-Up, and Veggie Pharm + Perm-Up (Table 1). Trunk sprays were applied as previously stated for the 2009 experiments. Trees were examined for new attacks at 3, 6, 8, 10, 15, 17, and 20 DAI. Only the extracted *X. crassiusculus*, *X. germanus*, and *Cnestus mutilatus* (Blandford) (camphor shot borer) (formerly *Xylosandrus mutilatus*) species were identified in this experiment.

Virginia Experiments. The Virginia experiment was conducted 14–28 April 2009. There were six insecticide treatments including trunk sprays of Acelepryn, Cyazypyr, Hachi-Hachi, and Onyx, and container substrate drenches of Flagship and Safari (Table 1). Spray

Table 2. Mean ± SEM ambrosia beetle tunnel entrances (new and cumulative) and *Xylosandrus germanus* emerged from trees in the 2009 Ohio insecticide efficacy exp

Treatment	*n*	New ambrosia beetle tunnel entrances				Cumulative entries	*X. germanus* emerged[b]
		1 DAI	6 DAI	13 DAI	22 DAI		
Injected control	8	0.0	2.6 ± 0.9ab	6.0 ± 1.5	7.9 ± 1.9	16.5 ± 2.7	43.6 ± 16.2
Acelepryn	8	0.3 ± 0.2	2.4 ± 0.7ab	4.5 ± 1.3	9.4 ± 2.5	16.5 ± 3.6	33.6 ± 6.4
Flagship drench	8	0.0	0.9 ± 0.4b	6.8 ± 2.8	11.1 ± 3.5	18.8 ± 6.1	20.1 ± 10.0
Hachi-Hachi	8	0.0	0.8 ± 0.3ab	4.9 ± 1.9	6.1 ± 1.8	11.8 ± 2.9	15.6 ± 7.7
Onyx	8	0.0	0.4 ± 0.3b	7.9 ± 1.6	6.0 ± 1.6	14.3 ± 2.8	26.4 ± 10.4
Safari drench	8	0.1 ± 0.1	1.6 ± 0.8ab	3.5 ± 1.7	6.5 ± 2.3	11.7 ± 4.5	19.3 ± 13.3
Safari spray	8	0.1 ± 0.1	3.1 ± 0.6a	3.4 ± 0.7	9.1 ± 1.4	15.7 ± 1.6	17.4 ± 6.5
Scimitar	8	0.0	2.4 ± 0.6ab	8.3 ± 1.4	9.0 ± 1.5	19.7 ± 3.0	27.6 ± 6.5
Uninjected[a]	8	0.0	0.0	0.0	0.0	0.0***	—
F		1.27	4.13	1.65	0.70	0.91	1.92
df		7, 49	7, 49	7, 49	7, 49	7, 49	7, 49
P		0.280	0.001	0.145	0.670	0.506	0.087

Data were log(X+1) transformed before analysis. Data within each sampling date and the cumulative total were analyzed by analysis of variance (ANOVA) for a randomized complete block design. Following a significant ANOVA, the treatment means were separated by Tukey's HSD ($\alpha = 0.05$). Means followed by the same letter or no letters (ANOVA $P > 0.05$) are not significantly different. DAI refers to days after injection with ethanol. New tunnel entrances were accumulated during time periods 0–1, 1–6, 6–13, and 13–22 DAI (1, 6, 13, and 22 DAI, respectively).

[a] The Uninjected treatment was not included in the analysis of variance comparing insecticide efficacy, but rather, data are shown for comparison with the Injected Control trees. ***Represents a significant difference in the total entries between the uninjected and ethanol-injected control treatments ($F = 47.39$; df = 1, 7; $P < 0.001$).

[b] Trees were cut into bolts, placed in resealable plastic bags and kept at room temp to allow emergence of ambrosia beetles. Only *Xylosandrus germanus* emerged from the bolts.

treatments were applied to runoff using a single boom 2-liter CO_2 sprayer at 276 kPa (40 psi) (R&D Sprayers, Opelousas, LA). The Flagship and Safari substrate drenches were applied by pouring 2,000 and 355 ml of solution, respectively, onto the substrate of each container. There were eight replications per treatment. Trees were deployed for 2 wk and examined for attacks 10, 12, and 14 DAI.

Data Analysis. Data were analyzed separately by state, year, and experiment. Analysis of variance (ANOVA) for a randomized complete block design (RCBD) was used to analyze data on new attacks for each sampling date and cumulative total attacks (Zar 1999, Analytical Software 2003). Data on total *Xylosandrus* and individual *Xylosandrus* species (includes *C. mutilatus*) in the Ohio experiments were analyzed by ANOVA for RCBD. Data on attacks and *Xylosandrus* species were log(X+1) transformed before performing ANOVA to meet assumptions of homogeneity of variances and normality (Zar 1999). In the 2010 Tennessee experiment, the transformation did not correct the normality of the combined *Xylosandrus* and individual species data. Therefore, those data were analyzed by Friedman's test, which is a nonparametric rank-based test with a χ^2 test statistic and a −1 df that can be used for RCBD analysis (Zar 1999). Following a significant ANOVA model, means were separated by Tukey's honestly significant difference (HSD) ($\alpha = 0.05$). A separate ANOVA was performed to compare cumulative total attacks between the injected controls and uninjected treatments (Analytical Software 2003). For treatments receiving ethanol injections, regression analysis was used to examine the relationship between cumulative ambrosia beetle attacks and DAI (Analytical Software 2003). In experiments where the relationship was curvilinear, the natural log (ln) of DAI was used in the regression analysis.

Results

Induction of Ambrosia Beetle Attacks and Insecticide Efficacy. *Ohio Experiments.* In Ohio in 2009, there were more cumulative total attacks on the injected control trees than the uninjected trees (132 and 0 attacks, respectively) (Table 2). In the insecticide efficacy analysis, there were no differences in the numbers of new attacks among treatments 1, 13, and 22 DAI or in cumulative total attacks (Table 2). There were more new attacks in the Safari spray than the Flagship drench and Onyx spray treatments at 6 DAI, but there were no other differences among treatments (Table 2). In 2009, only *X. germanus* emerged from the bolts cut from experimental trees with no differences among treatments (Table 2).

In 2010, there were more new attacks 2, 6, and 10 DAI and more cumulative total attacks on the injected control trees than Armorex, Tengard, and Veggie Pharm treated trees (Table 3). There were more new attacks on the injected controls than the Onyx treated trees 6 and 10 DAI (Table 3). Onyx treated trees had more new attacks 2 DAI and more cumulative total attacks than the Tengard treated trees. In 2010, only *X. germanus* were detected in the experimental trees with more extracted from the injected controls than the Onyx or Tengard treatments. No other differences were detected among treatments (Table 3).

Tennessee Experiments. In 2009 in experiment 1, there were differences in new ambrosia beetle attacks 3, 6, and 9 DAI, and in cumulative total attacks (Table 4). There were more attacks in the injected control treatment than the Onyx and Perm-Up treatments at

Table 3. Mean ± SEM ambrosia beetle tunnel entrances (new and cumulative) and *Xylosandrus germanus* extracted from trees in the 2010 Ohio insecticide exp

Treatment	*n*	New ambrosia beetle tunnel entrances						Cumulative entries	*X. germanus*[a]
		2 DAI	6 DAI	10 DAI	16 DAI	22 DAI	29 DAI		
Injected control	8	5.5 ± 1.4a	5.1 ± 1.1a	2.3 ± 0.6a	1.1 ± 0.8	0.5 ± 0.3	0.3 ± 0.2	14.8 ± 3.6a	5.9 ± 2.2a
Armorex T&O	8	1.3 ± 0.5bc	1.1 ± 0.6b	0.3 ± 0.2b	0.1 ± 0.1	0.0	0.3 ± 0.2	3.0 ± 1.2bc	1.6 ± 0.9ab
Onyx	8	4.0 ± 1.4ab	1.9 ± 1.1b	0.4 ± 0.2b	0.3 ± 0.2	0.0	0.1 ± 0.1	6.6 ± 2.8ab	0.6 ± 0.5b
Tengard	8	0.0c	0.1 ± 0.1b	0.0b	0.0	0.0	0.0	0.1 ± 0.1c	0.1 ± 0.1b
Veggie Pharm	8	0.9 ± 0.4bc	1.1 ± 0.5b	0.0b	0.0	0.3 ± 0.2	0.0	2.3 ± 0.7bc	1.0 ± 0.6ab
F		10.37	7.43	8.30	1.74	2.55	1.13	10.87	4.00
df		4, 28	4, 28	4, 28	4, 28	4, 28	4, 28	4, 28	4, 24
P		<0.0001	0.0003	0.0002	0.169	0.062	0.363	<0.0001	0.011

Data were log(X+1) transformed before analysis. Data within each sampling date and the cumulative total were analyzed by analysis of variance (ANOVA) for a randomized complete block design. Following a significant ANOVA, the treatment means were separated by Tukey's HSD ($\alpha = 0.05$). Means followed by the same letter or no letters (ANOVA $P > 0.05$) are not significantly different. DAI refers to days after injection with ethanol. New tunnel entrances were accumulated during time periods 0–2, 2–6, 6–10, 10–16, 16–22, and 22–29 DAI (2, 6, 10, 16, 22, and 29 DAI, respectively).

[a] Only *Xylosandrus germanus* were found in the experimental trees.

3 DAI, and more in the Acelepryn and Safari drench treatments than the Perm-Up treatment at 6 DAI (Table 4). At 9 DAI, the ANOVA indicated a significant difference, but Tukey's HSD test was unable to identify differences among treatment means. Perm-Up had fewer cumulative total attacks than the injected control, Acelepryn, Scimitar, and all Safari treatments (Table 4). In experiment 2, attack activity was low and insecticide efficacy was analyzed only for cumulative total attacks, but no differences were detected among treatments ($F = 0.87$; df = 5, 20; $P = 0.52$). Mean cumulative total attacks ranged from 1.8 per tree in the Perm-Up treatment to 6.8 in the injected control treatment. There were no differences in cumulative total attacks between the injected control trees and the uninjected trees ($F = 0.57$; df = 1, 4; $P = 0.49$). Unlike Ohio, the uninjected trees had some ambrosia beetle attacks, but there were 2.6× more cumulative total attacks on the injected control trees.

In 2010, there were again ambrosia beetle attacks on the uninjected trees, but more cumulative total attacks occurred on the injected control trees (Table 5). There were differences in new ambrosia beetle attacks among insecticide treatments 3, 10, and 15 DAI, and in cumulative total attacks (Table 5). There were more new attacks in the injected control treatment than all other treatments at 3 and 10 DAI, and more in the injected control than Armorex, Perm-Up, and Veggie Pharm + Perm-Up at 15 DAI. There were no other differences in new attacks among treatments (Table 5). The injected control treatment had more cumulative total attacks than Armorex, Armorex + Perm-Up, and Veggie Pharm + Perm-Up, but there were no differences among other treatments (Table 5). In total, 18 *X. crassiusculus*, 6 *X. germanus*, and 12 *C. mutilatus* were extracted from the trees in this experiment. There were no differences among treatments in the total numbers of *Xylosandrus* (includes *C. mutilatus*) ($\chi^2 = 10.28$; df = 5; $P = 0.068$), *X. crassiusculus* ($\chi^2 = 7.65$; df = 5; $P = 0.176$), or *C. mutilatus* ($\chi^2 = 4.39$; df = 5; $P = 0.494$) (data not shown). There were differences in the numbers of *X. germanus* among treatments ($\chi^2 = 13.85$; df = 5; $P = 0.017$) with *X. germanus* extracted from only the injected control and Armorex + Perm-Up treatments.

Table 4. Mean ± SEM new and cumulative ambrosia beetle tunnel entrances in the 2009 Tennessee conventional insecticide efficacy exp (exp 1)

Treatment	*n*	New ambrosia beetle tunnel entrances							Cumulative totals
		3 DAI	6 DAI	9 DAI	14 DAI	20 DAI	24 DAI	31 DAI	
Injected control	6	4.3 ± 0.9a	4.7 ± 2.6ab	0.8 ± 0.4	1.2 ± 1.2	0.5 ± 0.3	0.0	0.2 ± 0.2	11.7 ± 3.5a
Acelepryn drench	6	3.0 ± 0.6ab	6.3 ± 2.8a	2.0 ± 0.5	0.7 ± 0.3	0.5 ± 0.2	0.0	0.0	12.5 ± 2.7a
Onyx	6	0.2 ± 0.2b	5.5 ± 3.8ab	0.8 ± 0.5	0.3 ± 0.2	0.2 ± 0.2	0.0	0.0	7.0 ± 4.2ab
Perm-Up	6	0.0b	0.2 ± 0.2b	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.0	0.0	0.8 ± 0.2b
Safari drench	6	2.2 ± 1.3ab	7.5 ± 3.2a	4.0 ± 1.3	1.2 ± 1.0	0.7 ± 0.5	0.0	0.2 ± 0.2	15.7 ± 5.2a
Safari + Pentra bark	6	3.0 ± 1.2ab	2.3 ± 0.6ab	1.2 ± 0.8	1.0 ± 0.3	0.5 ± 0.2	0.0	0.2 ± 0.2	8.2 ± 1.8a
Safari spray	6	1.8 ± 1.0ab	2.3 ± 1.1ab	4.3 ± 3.0	0.5 ± 0.3	0.8 ± 0.5	0.0	0.0	9.8 ± 3.1a
Scimitar	6	3.5 ± 1.6ab	3.2 ± 0.8ab	1.2 ± 0.5	1.8 ± 0.7	0.2 ± 0.2	0.0	0.0	9.8 ± 2.3a
F		2.95	2.68	2.29	0.80	0.73	na	1.00	5.46
df		7, 35	7, 35	7, 35	7, 35	7, 35	7, 35	7, 35	7, 35
P		0.015	0.025	0.050	0.59	0.65	na	0.45	0.0003

Data were log(X+1) transformed before analysis. Data within each sampling date and the cumulative total were analyzed by analysis of variance (ANOVA) for a randomized complete block design. Following a significant ANOVA, the treatment means were separated by Tukey's HSD ($\alpha = 0.05$). Means followed by the same letter or no letters (ANOVA $P > 0.05$) are not significantly different. DAI refers to days after injection with ethanol. New tunnel entrances were accumulated during time periods 0–3, 3–6, 6–9, 9–14, 14–20, 20–24, and 24–31 DAI (3, 6, 9, 14, 20, 24, and 31 DAI, respectively).

Table 5. Mean ± SEM new and cumulative ambrosia beetle tunnel entrances in the 2010 Tennessee insecticide efficacy exp

Treatment	n	New ambrosia beetle tunnel entrances							Cumulative entries
		3 DAI	6 DAI	8 DAI	10 DAI	15 DAI	17 DAI	20 DAI	
Injected control	7	6.1 ± 1.6a	1.4 ± 1.4	2.4 ± 2.3	3.7 ± 1.1a	3.4 ± 1.6a	0.4 ± 0.5	0.4 ± 0.4	18.0 ± 5.0a
Armorex T&O	7	0.9 ± 0.9b	2.7 ± 2.0	0.3 ± 0.3	0.6 ± 0.3b	0.4 ± 0.4b	0.0	0.0	4.9 ± 2.9b
Arm + Perm[a]	7	0.1 ± 0.1b	1.0 ± 0.7	0.0	0.1 ± 0.1b	1.1 ± 0.4ab	0.0	0.0	2.4 ± 0.8b
Perm-Up	7	0.0b	3.4 ± 1.5	0.9 ± 0.3	0.3 ± 0.2b	0.1 ± 0.1b	0.1 ± 0.1	1.6 ± 1.3	6.4 ± 2.6ab
Veggie Pharm	7	0.9 ± 0.5b	2.3 ± 1.3	1.0 ± 0.5	0.7 ± 0.4b	0.9 ± 0.5ab	0.4 ± 0.2	0.4 ± 0.2	6.6 ± 1.7ab
Vegg + Perm[a]	7	0.1 ± 0.1b	3.1 ± 1.9	0.1 ± 0.1	0.3 ± 0.2b	0.1 ± 0.1b	0.1 ± 0.1	0.0	4.0 ± 1.8b
Uninjected[b]	7	0.1 ± 0.1	2.9 ± 2.0	0.0	0.3 ± 0.3	0.0	0.0	0.1 ± 0.1	3.4 ± 1.9**
F		12.86	0.46	1.27	6.15	3.49	0.90	1.25	3.53
df		5, 30	5, 30	5, 30	5, 30	5, 30	5, 30	5, 30	5, 30
P		<0.0001	0.805	0.305	0.0005	0.013	0.495	0.309	0.013

Data were log(X+1) transformed before analysis. Data within each sampling date and the cumulative total were analyzed by analysis of variance (ANOVA) for a randomized complete block design. Following a significant ANOVA, the treatment means were separated by Tukey's HSD ($\alpha = 0.05$). Means followed by the same letter or no letters (ANOVA $P > 0.05$) are not significantly different. DAI refers to days after injection with ethanol. New tunnel entrances were accumulated during time periods 0–3, 3–6, 6–8, 8–10, 10–15, 15–17, and 17–20 DAI (3, 6, 8, 10, 15, 17, and 20 DAI, respectively).

[a] The treatments Arm + Perm and Vegg + Perm represent Armorex + Perm-Up and Veggie Pharm + Perm-Up, respectively.

[b] The Uninjected treatment was not included in the analysis of variance comparing insecticide efficacy, but rather, data are shown for comparison with the Injected Control trees. **Represents a significant difference in the total entries between the Uninjected and Injected Control treatments ($F = 14.96$; df = 1, 6; $P < 0.01$).

Virginia Experiment. In 2009, the injected control trees had more cumulative total attacks (37) than the uninjected trees (1) (Table 6). The Acelepryn treatment had more new attacks than Onyx at 12 DAI, Flagship, Onyx, and Safari at 14 DAI, and cumulative total attacks than Onyx (Table 6). No other differences were detected among treatments.

The Relationship Between Days After Ethanol-Injection and Ambrosia Beetle Attacks. In 2009 and 2010, there were significant positive relationships between cumulative ambrosia beetle attacks and DAI with ethanol in Ohio and Tennessee (statistics are presented in Table 7). In the 2009 Ohio experiment, regression analysis indicated the relationships between DAI and cumulative ambrosia beetle attacks represented a simple linear response for all ethanol-injected treatments (Fig. 1). There were no differences in the slopes associated with the different treatments ($F = 2.43$; df = 7, 16; $P = 0.07$). In the 2010 Ohio and 2009 and 2010 Tennessee experiments, the relationships were represented by logarithmic (curvilinear) responses (Figs. 1 and 2). In the 2010 Ohio experiment, significant differences were detected between the slopes associated with the injected control compared with Armorex ($F = 56.7$; df = 1, 8; $P = 0.0001$), Onyx ($F = 37.7$; df = 1, 8; $P = 0.0003$), Tengard ($F = 86.7$; df = 1, 8; $P < 0.0001$), and Veggie Pharm ($F = 61.2$; df = 1, 8; $P = 0.0001$). In the 2009 Tennessee experiment 1, a significant difference was detected between the slopes associated with the injected control compared with Perm-Up ($F = 23.2$; df = 1, 10; $P = 0.0007$). In the 2010 Tennessee experiment, significant differences were detected between the slopes associated with the injected control compared with Armorex ($F = 29.6$; df = 1, 10; $P = 0.0003$), Armorex + Perm-Up ($F = 42.9$; df = 1, 10; $P = 0.0001$), Perm-Up ($F = 19.1$; df = 1, 10; $P = 0.001$), Veggie Pharm ($F = 22.1$; df = 1, 10; $P = 0.0008$), and Veggie Pharm + Perm-Up ($F = 29.3$; df =

Table 6. Mean ± SEM new and cumulative ambrosia beetle tunnel entrances in the 2009 Virginia insecticide efficacy exp

Treatment	n	New ambrosia beetle tunnel entrances			Cumulative total entrances
		10 DAI	12 DAI	14 DAI	
Injected control	8	0.0 ± 0.0	1.1 ± 0.6ab	3.5 ± 1.4ab	4.6 ± 1.8ab
Acelepryn	8	1.0 ± 0.4	4.5 ± 0.9a	5.6 ± 0.8a	11.1 ± 1.6a
Cyazypyr 10SC (DPX-HGW86)	8	0.0 ± 0.0	1.4 ± 0.4ab	2.8 ± 0.6ab	4.1 ± 1.0ab
Flagship drench	8	1.8 ± 1.5	3.4 ± 1.4ab	1.5 ± 0.7b	6.6 ± 3.5ab
Hachi–Hachi	8	0.8 ± 0.5	2.0 ± 0.3ab	4.3 ± 0.7ab	7.0 ± 0.7ab
Onyx	8	0.0 ± 0.0	1.0 ± 0.4b	1.5 ± 0.5b	2.5 ± 0.8b
Safari drench	8	0.8 ± 0.8	2.4 ± 0.8ab	2.1 ± 0.4b	5.3 ± 1.6ab
Uninjected[a]	8	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1**
F		1.76	2.68	4.01	2.38
df		6, 42	6, 42	6, 42	6, 42
P		0.131	0.027	0.003	0.046

Data within each sampling date and the cumulative total were analyzed by analysis of variance (ANOVA) for a randomized complete block design. Following a significant ANOVA, the treatment means were separated by Tukey's HSD ($\alpha = 0.05$). Means followed by the same letter or no letters (ANOVA $P > 0.05$) are not significantly different. DAI refers to days after injection with ethanol. New tunnel entrances were accumulated during time periods 0–10, 10–12, and 12–14 DAI (10, 12, and 14 DAI, respectively).

[a] The Uninjected treatment was not included in the analysis of variance comparing insecticide efficacy, but rather, data are shown for comparison with the Injected Control trees. **Represents a significant difference in the total entries between the Uninjected and Injected Control treatments ($F = 13.18$; df = 1, 7; $P < 0.01$).

Table 7. Statistics for regression analysis of the relationship between cumulative ambrosia beetle attacks and days after trees were injected with ethanol in the Ohio and Tennessee experiments

State	Year	Treatment	*n*	r^2	*F*	df	*P*
Ohio	2009	Injected control	4	0.99	274.4	1, 3	0.004
		Acelepryn	4	0.96	71.3	1, 3	0.014
		Flagship	4	0.93	39.0	1, 3	0.025
		Onyx	4	0.93	43.2	1, 3	0.022
		Safari drench	4	0.97	84.5	1, 3	0.012
		Safari spray	4	0.96	67.9	1, 3	0.014
		Scimitar	4	0.98	145.8	1, 3	0.007
		Tolfenpyrad	4	0.95	62.2	1, 3	0.016
	2010	Injected control	6	0.95	88.5	1, 5	<0.001
		Armorex	6	0.90	44.6	1, 5	0.003
		Onyx	6	0.86	31.7	1, 5	0.005
		Tengard	6	0.59	8.2	1, 5	0.046
		Veggie Pharm	6	0.81	21.8	1, 5	0.010
Tennessee	2009 exp 1[a]	Injected control	7	0.83	31.21	1, 6	0.003
		Acelepryn	7	0.76	20.15	1, 6	0.007
		Onyx	7	0.64	11.49	1, 6	0.020
		Perm-Up	7	0.92	74.11	1, 6	<0.001
		Safari drench	7	0.82	27.64	1, 6	0.003
		Safari + Pentra bark	7	0.93	82.69	1, 6	<0.001
		Safari spray	7	0.85	35.96	1, 6	0.002
		Scimitar	7	0.88	45.44	1, 6	0.001
	2010	Injected control	7	0.91	65.31	1, 6	<0.001
		Armorex	7	0.86	36.63	1, 6	0.002
		Armorex + Perm-Up	7	0.93	75.91	1, 6	<0.001
		Perm-Up	7	0.85	36.01	1, 6	0.002
		Veggie Pharm	7	0.99	770.9	1, 6	<0.001
		Veggie Pharm + Perm-Up	7	0.72	16.5	1, 6	0.010

[a] Regression analysis was not conducted on the data from the Tennessee botanical exp (exp 2) or the Virginia data.

1, 10; $P = 0.0003$). More than 50% of the attacks in most treatments occurred within 6 DAI with ethanol in the 2010 Ohio and 2009 Tennessee experiments and within 8 d in the 2010 Tennessee experiment. In the 2009 Ohio experiment, < 50% of the attacks occurred within 13 DAI in five out of eight treatments, while 52–58% occurred in the others by that time. The Virginia data were not subjected to regression analysis

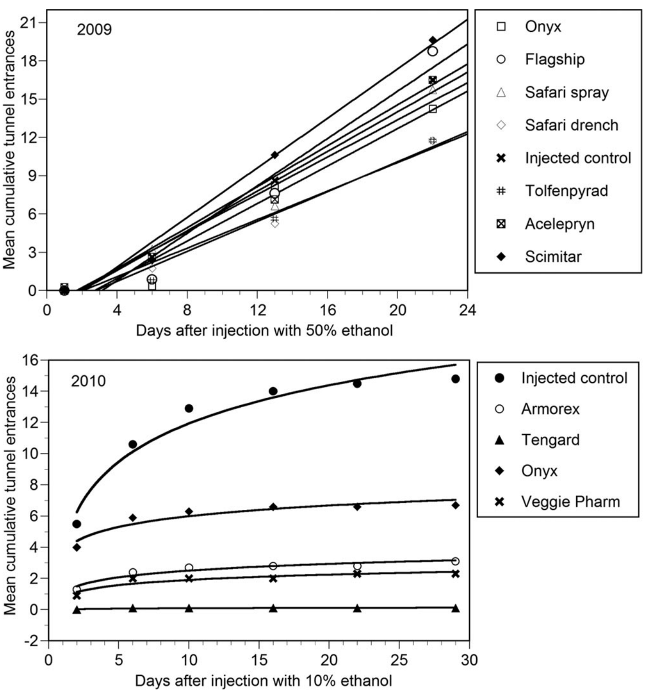

Fig. 1. Cumulative tunnel entrances in relation to days after injection (DAI) with ethanol in the Ohio experiment. The trees were injected with 75 ml of 50 or 10% ethanol in 2009 and 2010 experiments, respectively. In 2009, the relationship between cumulative tunnel entrances (Y) and DAI (X) was Y = aX + b, and in 2010 Y = alnX + b.

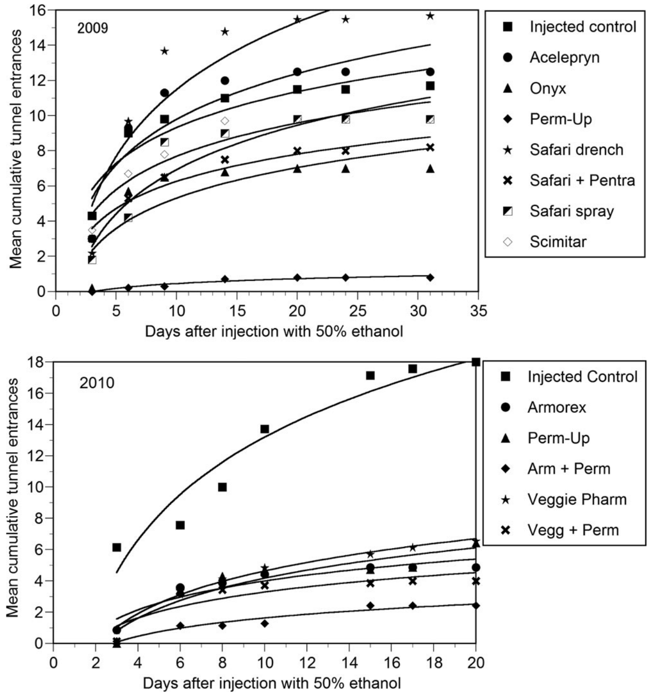

Fig. 2. Cumulative tunnel entrances in relation to days after injection (DAI) with ethanol in the Tennessee experiments. Regression analysis was performed on the conventional insecticide experiment (experiment 1) in 2009 only. The relationship between cumulative tunnel entrances (Y) and DAI (X) was Y = alnX + b. The treatments Arm + Perm and Vegg + Perm refer to Armorex + Perm-Up and Veggie Pharm + Perm-Up, respectively.

because there were only three evaluation dates over a period of 4 d.

Discussion

In the current study, ethanol-injection induced sufficient attacks on ornamental trees by ambrosia beetles, including *X. crassiusculus* and *X. germanus*, in all three states to evaluate efficacy of insecticides. In all but one experiment that included uninjected trees (Tennessee 2009 experiment 2), significantly more ambrosia beetle attacks occurred on the noninsecticide treated ethanol-injected trees than the uninjected trees. These results demonstrate that ethanol-injection ensures colonization of nursery trees by ambrosia beetles at rates that facilitate testing preventive insecticide treatments. Further research to determine the optimum concentration and volume of injected ethanol would refine this technique for testing insecticides against ambrosia beetles on nursery trees.

Differences in new attacks among treatments usually occurred within the first 2 wk after ethanol was injected. Ambrosia beetle attacks tended to peak during that time, which may have made treatment differences more detectable. The emission rates of ethanol from injected trees tend to peak within the first few days after injection and then decline (Ranger et al. 2012a). Ranger et al. (2012a) demonstrated that increasing concentrations of injected ethanol increased attacks by ambrosia beetles. The results of the regression analysis support the conclusion that beetle attacks decline as time after ethanol-injection increases. A lack of differences among insecticide treatments at later postinjection times might have been related to a decline in insecticide residual activity over time.

Efficacy of insecticides was variable and inconsistent with no product completely preventing attacks by ambrosia beetles. Pyrethroids such as bifenthrin (Onyx) and permethrin (Perm-Up and Tengard were used in the current study, and are the same formulation) are standard recommended materials for management of Scolytinae including ambrosia beetles (Svihra et al. 2004, Fettig et al. 2006, Frank and Sadof 2011). In the current study, the permethrin products were the most effective in Ohio and Tennessee, while bifenthrin (Onyx) was the most effective product tested in Virginia. The permethrin products reduced the cumulative total attacks by 64–98% compared with the injected controls; however, the differences were not always statistically significant. DeGomez et al. (2006) and Fettig et al. (2006) effectively prevented attacks by Scolytinae species on conifers using the highest labeled rate of Onyx. The highest labeled rate of Onyx for nursery trees was used in the experiments of the current study; however, Onyx did not effectively prevent attacks by ambrosia beetles in Ohio and Tennessee. In the 2010 Ohio experiment, the numbers of new tunnel entrances were relatively high in the

Onyx treated trees, but colonization by ambrosia beetles was relatively low. As observed during extraction of ambrosia beetles from the trees, none of the tunnels in the Onyx treated trees penetrated much beyond the cambium, and callus tissue had formed in the entrances. In contrast, complete galleries occurred in the injected control, Armorex, and Veggie Pharm treatments in the same experiment.

Some of the variability in efficacy among experiments in different geographic regions could be related to the species of ambrosia beetles involved. There may be differential susceptibility among the *Xylosandrus* species to certain insecticides. Hastings et al. (2001) reported differences in the efficacy of preventive treatments among bark beetle species and geographic regions. *X. crassiusculus* is more prevalent and problematic in Tennessee and Virginia than Ohio (Reding et al. 2010, 2011). Furthermore, *C. mutilatus* occurred in the Tennessee experimental plot, but was not detected in the Ohio experiments.

The inconsistent efficacy of the recommended ambrosia beetle treatments in our experiments presents problems for developing ambrosia beetle management programs in ornamental nurseries. Nursery growers have no tolerance for ambrosia beetle attacks. Growers cull trees when attacks are noticed, which leads to the perception that treatments should be 100% effective. However, Mizell and Riddle (2004) reported that nursery trees with <5 attacks by *X. crassiusculus* survived. Furthermore, the presence of callus tissue in the entrances of aborted tunnels (2010 Ohio experiment) suggests wound healing can occur when attacks fail. If the effective control level was four or less attacks per tree, the permethrin products would have been effective in three out of four experiments. Therefore, insecticide treatments that do not completely prevent attacks, but maintain relatively low attack pressure, may still be effective tools for managing *Xylosandrus* species. Further research on the susceptibility of different *Xylosandrus* species to insecticides and the residual activity of insecticides is needed. In addition, greater knowledge pertaining to ethanol emission rates from trees and beetle response to those rates following ethanol injections might determine the duration insecticide trials should be performed using this injection technique. Research is also needed on the relationship between attacks by ambrosia beetles and the mortality of nursery trees.

The neonicotinoids (Flagship and Safari), the anthranilic diamides (Acelepryn and Cyazypyr), and tolfenpyrad (Hachi-hachi) were not effective at preventing attacks by ambrosia beetles in the current study. In laboratory bioassays, Acelepryn and Cyazypyr reduced survival of the bark beetle *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae) (Fettig et al. 2010), but neither material was tested against Scolytinae in the field. Systemic insecticides have not been effective in previous trials against Scolytinae when trunk applied or soil drenched (DeGomez et al. 2006, Grosman et al. 2009), and our results with substrate drenches of Flagship and Safari or trunk sprays of Safari and Acelepryn were similar.

The plant-based essential oil mixtures Armorex and Veggie Pharm suppressed attacks by ambrosia beetles in the current study. Armorex reduced cumulative total attacks in the 2010 Ohio and Tennessee experiments, while Veggie Pharm reduced new attacks on three dates in the 2010 Ohio experiment. Ranger et al. (2011) also found Armorex and Veggie Pharm effective at reducing attacks by ambrosia beetles in Ohio. However, neither material significantly reduced the number of *X. germanus* compared with noninsecticide treated trees in the current study.

The low tolerance nursery growers have for ambrosia beetle attacks increases the challenge of using an integrated approach to their management, and also increases the likelihood that conventional insecticides will continue to play a primary role in their control within nurseries. The pyrethroid insecticides appear to be one of the most effective groups for suppressing attacks by ambrosia beetles, including *Xylosandrus* species. Previous (Ranger et al. 2010, 2012a) and current research demonstrated that *Xylosandrus* species were attracted almost exclusively to trees emitting ethanol (from injection), which is an indicator of physiological stress (Moeck 1970, Kimmerer and Kozlowski 1982, Kelsey and Joseph 2001). Observations suggest insecticides are less effective at preventing attacks on extremely stressed trees (unpublished data). Therefore, maintaining healthy trees should reduce ambrosia beetle attack pressure, and be an important component for their management in nurseries. Furthermore, insecticide treatments should be more effective at protecting trees from ambrosia beetles when attack pressure is low.

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References Cited

Analytical Software. 2003. Statistix 8 User's Manual. Analytical Software, Tallahassee, FL.

Cade, S. C., B. F. Hrutfiord, and R. I. Gara. 1970. Identification of a primary attractant for *Gnathotrichus sulcatus* isolated from Western hemlock logs. J. Econ. Entomol. 63: 1014–1015.

DeGomez, T. E., C. J. Hayes, J. A. Anhold, J. D. McMillin, K. M. Clancy, and P. P. Bosu. 2006. Evaluation of insecticides for protecting southwestern ponderosa pines from attack by engraver beetles (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. 99: 393–400.

Dute, R. R., M. E. Miller, M. A. Davis, F. M. Woods, and K. S. McLean. 2002. Effects of ambrosia beetle attack on *Cercis canadensis.* IAWA J. 23: 143–160.

Fettig, C. J., K. K. Allen, R. B. Borys, J. Christopherson, C. B. Dabney, T. J. Eager, K. E. Gibson, E. G. Hebertson, D. F. Long, A. S. Munson, et al. 2006. Effectiveness of bifenthrin (Onyx) and carbaryl (Sevin SL) for protecting individual, high-value conifers from bark beetle attack (Coleoptera: Curculionidae: Scolytinae) in the western United States. J. Econ. Entomol. 99: 1691–1698.

Fettig, C. J., C. J. Hayes, S. R. McKelvey, and S. R. Mori. 2010. Laboratory assays of select candidate insecticides for control of *Dendroctonus ponderosae.* Pest Manag. Sci. 67: 548–555.

Frank, S. D., and C. S. Sadof. 2011. Reducing insecticide volume and nontarget effects of ambrosia beetle management in nurseries. J. Econ. Entomol. 104: 1960–1968.

Graham, K. 1968. Anaerobic induction of primary chemical attractancy for ambrosia beetles. Can. J. Zoo. 46: 905–908.

Grosman, D. M., S. R. Clarke, and W. W. Upton. 2009. Efficacy of two systemic insecticides injected into loblolly pine for protection against southern pine bark beetles (Coleoptera: Curculionidae). J. Econ. Entomol. 102: 1062–1069.

Hale, F. A. 2007. Entomological research priorities for nursery and landscape ornamentals, pp. 17–21. *In* B. L. James (ed.), Proceedings 52nd Annual Southern Nursery Association Research Conference. Atlanta, GA. Southern Nursery Association, Inc., Acworth, GA.

Hastings, F. L., E. H. Holsten, P. J. Shea, and R. A. Werner. 2001. Carbaryl: a review of its use against bark beetles in coniferous forests of North America. Environ. Entomol. 30: 803–810.

Hoffman, C. H. 1941. Biological observations on *Xylosandrus germanus* (Bldfd.). J. Econ. Entomol. 34: 38–42.

Hudson, W., and R. Mizell. 1999. Management of Asian ambrosia beetle, *Xylosandrus crassiusculus,* in nurseries, pp. 198–201. *In* B. L. James (ed.), Proceeding 44th Annual Southern Nursery Association Research Conference. Atlanta, GA. Southern Nursery Association, Inc. Acworth, GA.

Kelsey, R. G., and G. Joseph. 2001. Attraction of *Scolytus unispinosus* bark beetles to ethanol in water-stressed Douglas-fir branches. For. Ecol. Manage. 144: 229–238.

Kimmerer, T. W., and T. T. Kozlowski. 1982. Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. Plant Physiol. 69: 840–847.

Kuhnholz, S., J. H. Borden, and A. Uzunovic. 2003. Secondary ambrosia beetles in apparently healthy trees: adaptations, potential causes and suggested research. Integrated Pest Manage Rev. 6: 209–219.

Mizell, R. F., and T. C. Riddle. 2004. Evaluation of insecticides to control Asian ambrosia beetle, *Xylosandrus crassiusculus,* pp. 152–155. *In* B. L. James (ed.), Proceeding 49th Annual Southern Nursery Association Research Conference. Atlanta, GA. Southern Nursery Association, Inc., Acworth, GA.

Moeck, H. A. 1970. Ethanol as the primary attractant for the ambrosia beetle *Trypodendron lineatum* (Coleoptera: Scolytidae). Can. Entomol. 102: 985–995.

Montgomery, M. E., and P. M. Wargo. 1983. Ethanol and other host-derived volatiles as attractants to beetles that bore into hardwoods. J. Chem. Ecol. 9: 181–190.

Oliver, J. B., and C. M. Mannion. 2001. Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. Environ. Entomol. 30: 909–918.

Rabaglia, R. J., S. A Dole, and A. I Cognato. 2006. Review of American Xyleborina (Coleoptera: Curculionidae: Scolytinae) occurring north of Mexico, with an illustrated key. Ann. Entomol. Soc. Am. 99: 1034–1056.

Ranger, C. M., M. E. Reding, A. B. Persad, and D. A. Herms. 2010. Ability of stress-related volatiles to attract and induce attacks by *Xylosandrus germanus* (Coleoptera: Curculionidae, Scolytinae) and other ambrosia beetles. Agric. Forest Entomol. 12: 177–185.

Ranger, C. M., M. E. Reding, J. B. Oliver, P. B. Schultz, J. J. Moyseenko, and N. Youssef. 2011. Comparative efficacy of plant-derived essential oils for managing ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) and their corresponding mass spectral characterization. J. Econ. Entomol. 104: 1665–1674.

Ranger, C. M., M. E. Reding, P. B. Schultz, and J. B. Oliver. 2012a. Ambrosia beetle (Coleoptera: Curculionidae) responses to volatile emissions associated with ethanol-injected *Magnolia virginiana* L. Environ. Entomol. 41: 636–647.

Ranger, C. M., M. E. Reding, P. B. Schultz, and J. B. Oliver. 2012b. Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate. Agric. Forest Entomol. (in press).

Reding, M., J. Oliver, P. Schultz, and C. Ranger. 2010. Monitoring flight activity of ambrosia beetles in ornamental nurseries with ethanol-baited traps: influence of trap height on captures. J. Environ. Hort. 28: 85–90.

Reding, M. E., P. B. Schultz, C. M. Ranger, and J. B. Oliver. 2011. Optimizing ethanol-baited traps for monitoring damaging ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) in ornamental nurseries. J. Econ. Entomol. 104: 2017–2024.

Solomon, J. D. 1995. Guide to insect borers of North American broadleaf trees and shrubs. Agric. Handbook No. 706. USDA–Forest Service, Washington, DC.

Svihra, P., D. F. Crosby, and B. Duckles. 2004. Emergence suppression of bark and ambrosia beetles in infested oaks. J. Arboric. 30: 62–66.

Weber, B. C., and J. E. McPherson. 1983. Life history of the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 76: 455–462.

Weber, B. C., and J. E. McPherson. 1984. Attack on black walnut by the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Forest Sci. 4: 864–870.

Wood, S. L. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. Great Basin Nat. Mem. 6: 1–1359.

Zar, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

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