Journal of Economic Entomology, 111(1), 2018, 269–276
doi: 10.1093/jee/tox327
Advance Access Publication Date: 19 December 2017
Research Article

OXFORD

Forest Entomology

Residue Age and Attack Pressure Influence Efficacy of Insecticide Treatments Against Ambrosia Beetles (Coleoptera: Curculionidae)

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Subject Editor: Brian Sullivan

Received 29 June 2017; Editorial decision 31 October 2017

Abstract

Management of ambrosia beetles in ornamental nurseries relies, in part, on insecticide treatments to prevent beetles from boring into trees. However, data on residual efficacy of commonly used pyrethroid insecticides is needed to gauge the duration that trees are protected during spring when peak beetle pressure occurs. Residual efficacy of bifenthrin and permethrin trunk sprays was examined in field trials which used trees injected with 10% ethanol to ensure host attack pressure. Permethrin consistently reduced attacks by *Xylosandrus germanus* (Blandford; Coleoptera: Curculionidae) and other ambrosia beetles for at least 4 wk, while efficacy of bifenthrin was inconsistent and lasted only about 10 d. Since previous studies demonstrated attacks are positively correlated with host ethanol emissions, we injected trees with 2.5, 5, and 10% ethanol to determine if residual efficacy was affected by attack pressure. Preventive treatments with bifenthrin reduced ambrosia beetle attacks at all concentrations of injected ethanol compared to non-sprayed controls. There was no interaction between attack pressure and insecticide treatment with respect to total attacks or attacks by *X. germanus*. However, increasing attack pressure did increase the probability of attacks on insecticide treated trees by *X. germanus* and other Scolytinae. Results from our current study will improve the ability of growers to make decisions on frequency of protective sprays, but residual efficacy of insecticide treatments may decline as attack pressure increases. Cultural practices should therefore maximize host vigor and minimize attack pressure associated with stress-induced ethanol emissions.

Key words: *Xylosandrus*, ethanol injection, pyrethroids, ornamental trees

Exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are serious pests in ornamental tree nurseries in North America (Ranger et al. 2016a). Ambrosia beetles bore into the xylem of trees creating tunnels and galleries they inoculate with symbiotic fungi, which are the food source for adults and larvae (Wood 1982). Attacks by ambrosia beetles often lead to wilting, stem dieback, or death of nursery trees. *Xylosandrus crassiusculus* (Motschulsky; Coleoptera: Curculionidae) and *Xylosandrus germanus* (Blandford; Coleoptera: Curculionidae) are two of the most problematic exotic ambrosia beetles in ornamental nurseries (Fulcher et al. 2012, Reding et al. 2013a), but other species can also be destructive.

Ambrosia beetles overwinter as adults within their host trees and then emigrate during spring months from wooded areas into ornamental nurseries (Reding et al. 2015, Werle et al. 2015). Since the highest beetle pressure and corresponding attacks are generally associated with the overwintering adults, growers often limit their preventive applications to protect trees during peak spring flight activity (Reding et al. 2013b). The pyrethroids bifenthrin and permethrin are commonly used for managing ambrosia beetles in ornamental tree nurseries. While bifenthrin and permethrin were the most effective materials against *X. crassiusculus*, *X. germanus* and other ambrosia beetles in a series of tests, their efficacy was inconsistent (Reding et al. 2013a). Furthermore, data on residual efficacy of bifenthrin and permethrin against ambrosia beetles are largely lacking, although Frank and Sadof (2011) reported some degree of residual activity for permethrin.

Many ambrosia beetles, including *X. crassiusculus* and *X. germanus*, have broad host ranges but specifically attack physiologically stressed trees that are emitting ethanol (Weber and McPherson 1984; Kühnholz et al. 2001; Ranger et al. 2013, 2015a,b). Injecting living trees with ethanol induces attacks by ambrosia beetles, and abundance and rate of attacks are positively correlated with injected ethanol concentrations (Ranger et al. 2010, 2012). Injecting trees with ethanol has been used to maximize beetle attack pressure as part of insecticide trials (Frank and Sadof 2011, Ranger et al. 2011, Reding et al. 2013a). For example, Reding et al. (2013a) found that

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bifenthrin was generally more effective against *X. germanus* on trees injected with 10% ethanol than trees injected with 50% ethanol. Relatively high numbers of attacks also occurred on flood-stressed trees that were preventively treated with permethrin, but no attacks occurred on non-flooded trees (Ranger et al. 2016b). These data suggest that efficacy of insecticides against ambrosia beetles might decline as attack pressure increases.

The objectives of the current research were to: 1) determine the residual efficacy of bifenthrin and permethrin against ambrosia beetles, especially *Xylosandrus* species, on ornamental trees; and 2) examine the relationship between attack pressure and efficacy of insecticides at preventing ambrosia beetle attacks.

Materials and Methods

Residual Activity of Insecticides

The residual activity of bifenthrin (OnyxPro, FMC Corporation, Philadelphia, PA) and permethrin (Perm-Up 3.2 EC, United Phosphorus, Inc., Trenton, NJ) were tested in repeated experiments (2012 and 2013). Both formulations are registered for use against ambrosia beetles on trees in ornamental nurseries. Experiments were conducted on *Magnolia* × *loebneri* 'Dr. Merrill' trees 1.5 to 2 m tall, 40 to 50 mm in diameter, and potted in 27-liter containers with soilless substrate. To ensure attack pressure by ambrosia beetles, experimental trees were injected with 75 ml of 10% ethanol using the Arborjet Tree I.V. Delivery System (Woburn, MA) (Ranger et al. 2010, Reding et al 2013a). Injection sites were initiated by drilling a single 9.5 mm hole about 16 mm deep into the base of the trees. The hole was immediately plugged with an Arborjet injection port (9.5 mm diameter) and ethanol was injected through the port at a delivery pressure of 413.7 kPa (60 psi).

Experiments were conducted in spring after overwintering *X. germanus* began emerging, and were set up as randomized complete block designs (RCBD) with eight blocks. Blocks were set up in a field within 1 m of an adjacent woodland, and spaced 15 m apart. Trees within blocks were positioned 1 m apart in a single row parallel to the woodland. Insecticide treatments were applied to individual trees before trees were positioned in blocks. There were three treatments including bifenthrin, permethrin, and non-treated control (hereafter, 'control') with three residue periods per treatment. There was a replication of each treatment × residue period combination per block. Insecticides were sprayed on the trunks of trees to the point of runoff using 1.4 liter compressed air hand-triggered spray bottles (item# 65–6418, Hummert International, Earth City, MO). Insecticides were applied at the maximum labeled rate for ambrosia beetles (bifenthrin, 0.946 liters of OnyxPro per 378.5 liters of water [5 gm ai per liter of water]; permethrin, 4.730 liters of Perm-Up 3.2 EC per 378.5 liters of water [40 gm ai per liter of water]). Residual efficacy of insecticides against ambrosia beetles was compared within residue period and year.

To create different residue periods, trees were sprayed on the same date and placed in their assigned blocks. Then, a set of trees from each treatment was injected with ethanol at a specific post-spray timing (residue period), with a different set of trees used for each residue period. Experimental trees were not attractive to ambrosia beetles until they were injected (Ranger et al. 2010, 2012; Reding et al. 2013a), thus trees could be made attractive at distinct residue ages. In the 2012 experiment, trees were sprayed 1 May then sets of trees were injected 6, 20, or 34 d after spraying. In the 2013 experiment, trees were sprayed 14 May then injections were made 6, 21, or 35 d after spraying. To evaluate efficacy of treatments, trees were transported to the lab to count attacks (tunnel entrances) and excavate beetles. Ambrosia beetle flight and attack activity are influenced by weather conditions (Reding et al. 2013b), which could affect attacks on experimental trees. Therefore, injected trees remained in the field until attacks occurred on at least 5 unsprayed control trees. After injection, trees in each residue period were examined at 2 or 3-d intervals and if attacks occurred on fewer than 5 control trees that residue period was continued. Trees were transported to the lab for evaluation 2 to 7 d after injection (DAI) in 2012, and 6 to 11 DAI in 2013. Beetles were excavated from tree stems by hand pruners, and Scolytinae were identified to the species level using available keys (Wood 1982, Rabaglia et al. 2006).

Data Analysis

Data on numbers of occupied tunnels (Scolytinae present), total attacks (all tunnel entrances), and attacks by *X. germanus* (present in tunnel) were analyzed by analysis of variance for a randomized complete block design (RCBD) (Analytical Software 2003). Data were log(X+1) transformed before analysis to satisfy assumptions of homogeneity of variances and normality (Zar 1999). Efficacy of insecticides was analyzed within residue periods and years (6, 20, or 34 d in 2012; 6, 21, or 35 d in 2013). Following a significant ANOVA, means were separated using Tukey's HSD ($\alpha = 0.05$) (Analytical Software 2003).

Influence of Attack Pressure on Efficacy of Protective Sprays

The influence of attack pressure on efficacy of insecticides preventing ambrosia beetle attacks was evaluated in two separate experiments (2014 and 2016). Different levels of attack pressure (low, moderate, and high) were established by injecting trees with 75 ml of either 2.5, 5.0, or 10.0% ethanol (low, moderate, high attack pressure, respectively). Injection methods followed procedures described previously. Experiments were conducted on red maple trees (*Acer rubrum* L.) purchased bare-root and potted in 57-liter containers with soilless substrate. Different sets of trees were used in each experiment. The experiments were set up as randomized complete block designs with factorial arrangements. There was a replication of each ethanol concentration (attack pressure level) per block, and eight blocks. Attack pressure levels consisted of a pair of trees injected with 2.5, 5, or 10% ethanol; one tree of each pair was sprayed with bifenthrin (OnyxPro, FMC, Philadelphia, PA) and the other was not sprayed to use as a control (16 total trees per ethanol concentration). Bifenthrin was used because efficacy has been inconsistent against ambrosia beetles, and lack of consistency may be, in part, related to attack pressure. Furthermore, bifenthrin is a primary material used against ambrosia beetles by growers. The experiments were set up in a field adjacent to a woodland with blocks spaced 10 m apart. Trees within blocks were positioned in a single row parallel to and within 1 m of the woodland. Positions of attack pressure levels within blocks were assigned randomly and spaced 6 m apart, with trees within attack pressure levels located 0.5 m apart. This experimental design was used to provide similar spatial conditions for each level of attack pressure while minimizing potential for higher levels to influence pressure on lower levels.

Experiments were conducted in northern Ohio during spring 2014 and 2016. In 2014, trees were injected 5 May, then sprayed and set up in the field 6 May. In 2016, trees were injected 23 May, then sprayed and placed in the field 24 May. For evaluation, attacks (tunnel entrances) were counted 3, 7, 10, 14, and 21 days after spraying (DAS) in 2014, and 2, 7, 13, and 17 DAS in 2016. During evaluation,

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tunnel entrances were marked by a wax pencil to prevent recounting previous attacks. A different color pencil was used for each count date, thus excavated beetles could be associated with a specific date. Trees were transported to the laboratory for the final evaluation each year, at which time adult beetles were excavated for identification.

Data Analysis

Total attacks (all tunnel entrances) and attacks by *X. germanus* (present in tunnel) were log(X+1) transformed to meet assumptions of homogeneity of variances and normality (Zar 1999). The transformed data were analyzed within years by 2-factor repeated measures analysis of variance with insecticide treatment and ethanol concentration (attack pressure) as factors, and total attacks or *X. germanus* counts through time as the repeated measures (Proc GLM SAS) (SAS Institute 2001). When significant insecticide, ethanol concentration, insecticide × time, ethanol × time, or insecticide × ethanol concentration effects were detected for total attacks or *X. germanus* attacks, data were compared for each sample date using least square means (lsmeans) and the pdiff option in Proc GLM (SAS Institute 2001). The pdiff option in Proc GLM provides the *P*-values of differences between the lsmeans (SAS Institute 2001), which were considered significant at $P < 0.05$.

Logistic regression was used to analyze the probability of attacks by Scolytinae (total attacks) or *X. germanus* on trees treated with bifenthrin in relation to the concentration of ethanol injected (attack pressure level) (Statistix 8.1) (Analytical Software 2003). Logistic regression is used to analyze binary data (one or zero), such as presence or absence responses, against a continuous variable (Quinn and Keough 2002, Analytical Software 2003). In this case, analysis was of the presence of attacks by Scolytinae or *X. germanus* on bifenthrin treated trees. The logistic function was of the form $P = [e^{(B0+B1*X)}/(1+e^{(B0+B1*X)})]$ where *P* is the probability of bifenthrin treated trees being attacked by Scolytinae or just *X. germanus*, *X* is the concentration of ethanol injected into trees, *B0* is the constant and *B1* is the regression coefficient that measures the rate of change in probability for a given *X* (Quinn and Keough 2002). Maximum likelihood was used to estimate logistic model parameter values (Statistix 8.1) (Analytical Software 2003). For this analysis 2014 and 2016 data were combined.

Results

Residual Activity of Insecticides

In 2012, there were differences in numbers of occupied tunnels (beetles present), total attacks (all tunnel entrances), and *X. germanus* attacks among treatments at each residue period (Table 1). Because of the short time-period between injection and evaluation, all excavated beetles were considered colonizers (not offspring). Ten days after application of insecticides, there were no *X. germanus* in the permethrin treated trees ($n = 8$), while five bifenthrin trees and eight controls had *X. germanus*. At that time, there were more occupied tunnels, total attacks and *X. germanus* attacks in the control and bifenthrin treatments than permethrin, with more occupied tunnels and *X. germanus* in the controls than bifenthrin (Table1). Twenty-two days after insecticide application, there were more occupied tunnels, total attacks, and *X. germanus* attacks in the control and bifenthrin treatments than permethrin, with no other differences (Table 1). Three permethrin treated, seven bifenthrin treated, and seven control trees ($n = 8$) had *X. germanus* at that time. Forty-one DAS, there were more occupied tunnels, total attacks, and *X. germanus* attacks in the control treatment than permethrin with no other differences (Table 1). At that time, four permethrin, six bifenthrin, and seven control trees ($n = 7$) had *X. germanus*. *X. germanus* represented 93 to 94% of the beetles excavated in each residue period. *X. crassiusculus* occurred in one tree (control) in the third residue period, and represented 2% of the total beetles at that time. *Xyleborinus saxesenii* (Ratzeburg) (1 to 3% of total beetles) and *Anisandrus sayi* Hopkins (3 to 6%) were the only other Scolytinae excavated in the experiment.

In 2013, ambrosia beetle activity was low for the first and second residue periods (6 and 20 d after spraying, respectively), thus experiments were continued for 11 d and 10 d after injection, respectively (17 and 31 d after spraying) to allow more time for attacks. Seventeen days after application of insecticides there were more occupied tunnels, total attacks, and *X. germanus* attacks in trees treated with bifenthrin than permethrin, and more total attacks in the bifenthrin treatment than controls (Table 1). One permethrin, six bifenthrin, and five control trees ($n = 8$) had *X. germanus* at that time. Thirty-one DAS there were more occupied tunnels, total attacks and *X. germanus* attacks in the control trees than bifenthrin and permethrin treated trees (Table 1). At that time, no permethrin, one bifenthrin, and six control trees ($n = 7$) had *X. germanus*. Forty-one DAS, numbers of occupied tunnels, total attacks, and *X. germanus* attacks were 4 to 5 times greater in the control trees than permethrin treated, but differences were not significant (Table 1). At 41 d, *X. germanus* occurred in three permethrin, four bifenthrin, and five control trees ($n = 7$). *X. germanus* represented 87 to 96% of the beetles excavated from different residue periods in this experiment. *X. crassiusculus* represented 4 to 6% of total beetles; *Ambrosiodmus rubricollis* (Eichhoff) (0 to 4% of total beetles), *A. sayi* (0 to 9%), and *Corthylus punctatissimus* (Zimmermann) (0 to 2%) were the only other Scolytinae in the experimental trees.

Influence of Attack Pressure on Efficacy of Protective Sprays

2014

In 2014, insecticide treatments reduced attacks compared to controls by 100, 43, and 35% in trees injected with 2.5, 5, and 10% ethanol, respectively. Numbers of total attacks tended to be lower in insecticide (bifenthrin) treated trees than controls. However, effects of insecticide (bifenthrin vs unsprayed controls), insecticide × time, or insecticide × ethanol concentration (attack pressure) on total attacks were not significant (Table 2). Effects of ethanol concentration and ethanol × time on attacks were significant (Table 2), indicating different levels of attack pressure were established. Attacks tended to decline over time with greater numbers of attacks in the 10% injected trees than the 2.5% trees on all sample dates ($P < 0.05$), and than the 5% trees in all but the first sample (3 d after spraying, DAS) ($P < 0.05$). There were more attacks in the 5% ethanol trees than 2.5% 3 DAS only ($P < 0.05$). There were no attacks in the insecticide treated 2.5% ethanol trees, while attacks occurred 3 and 7 DAS in the 2.5% control trees (Fig. 1). There were significant differences among treatments on all dates, with highest numbers of attacks in control or insecticide treated trees injected with 10% ethanol ($P < 0.05$) (Fig. 1).

Effects of insecticide, ethanol concentration, insecticide × time and ethanol × time on numbers of *X. germanus* in trees were significant, but insecticide × ethanol concentration was not (Table 2). More *X. germanus* occurred in the controls than insecticide treated trees 3 and 7 d after spraying (DAS) ($P < 0.05$), with no differences thereafter (10 through 22 DAS) ($P > 0.05$). Three DAS, trees injected with 10 or 5% ethanol had more *X. germanus* attacks than 2.5%

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Table 1. Residual activity of bifenthrin and permethrin sprays for preventing attacks by ambrosia beetles on ethanol-injected trees

Year	Spray date	Injection date	Evaluation days after spraying	Treatment[a]	Statistics *n*	Mean ± SE		
						Occupied[b] tunnels	Total attacks	*X. germanus*
2012	1 May	7 May	10	Control	8	12.3 ± 1.8a	12.4 ± 1.9a	11.6 ± 1.9a
				Bifenthrin	8	1.4 ± 0.4b	8.1 ± 1.0a	1.3 ± 0.5b
				Permethrin	8	0.0c	0.1 ± 0.1b	0.0c
					F	91.4	69.4	172.0
					df	2, 14	2, 14	2, 14
					P	<0.001	<0.001	<0.001
		21 May	22	Control	8	4.5 ± 1.5a	4.8 ± 1.9a	4.3 ± 1.4a
				Bifenthrin	8	3.5 ± 1.0a	4.9 ± 0.9a	3.1 ± 1.1a
				Permethrin	8	0.5 ± 0.3b	0.8 ± 0.4b	0.5 ± 0.3b
					F	7.2	10.5	6.1
					df	2, 14	2, 14	2, 14
					P	0.007	0.002	0.013
		4 June	41	Control	7[c]	9.0 ± 2.2a	10.3 ± 2.5a	8.3 ± 1.8a
				Bifenthrin	7	5.9 ± 1.2ab	6.7 ± 1.3ab	5.4 ± 1.1ab
				Permethrin	7	1.7 ± 0.9b	2.9 ± 1.4b	1.7 ± 0.9b
					F	5.3	3.9	5.6
					df	2, 12	2, 12	2, 12
					P	0.022	0.048	0.019
2013	14 May	20 May	17	Control	8	1.4 ± 0.5ab	1.4 ± 0.5b	1.4 ± 0.5ab
				Bifenthrin	8	2.0 ± 0.4a	3.5 ± 0.6a	1.9 ± 0.4a
				Permethrin	8	0.1 ± 0.1b	0.1 ± 0.1b	0.1 ± 0.1b
					F	7.1	13.0	5.0
					df	2, 14	2, 14	2, 14
					P	0.007	0.001	0.022
2013	14 May	4 June	31	Control	7[c]	3.0 ± 1.0a	3.6 ± 1.1a	2.6 ± 0.9a
				Bifenthrin	7	0.1 ± 0.1b	0.1 ± 0.1b	0.1 ± 0.1b
				Permethrin	7	0.1 ± 0.1b	0.1 ± 0.1b	0.0b
					F	12.7	11.7	11.2
					df	2, 12	2, 12	2, 12
					P	0.001	0.002	0.002
		18 June	41	Control	7[c]	4.1 ± 1.7a	4.7 ± 1.7a	3.7 ± 1.5a
				Bifenthrin	7	2.1 ± 1.0a	2.1 ± 1.0a	1.7 ± 0.8a
				Permethrin	7	0.9 ± 0.4a	0.9 ± 0.4a	0.7 ± 0.4a
					F	2.9	2.5	2.9
					df	2, 12	2, 12	2, 12
					P	0.094	0.121	0.097

Means within injection dates and columns followed by the same letter are not significantly different (Tukey's HSD, $\alpha = 0.05$).
[a]Bifenthrin was formulated OnyxPro, and permethrin was Perm-Up 3.2 EC.
[b]Tunnels were designated occupied if Scolytinae were present.
[c]There were only 7 replications (blocks) on these dates due to loss of trees in one of the blocks.

trees ($P < 0.05$). More *X. germanus* attacked trees injected with 10% ethanol than 2.5% and 5% ethanol 7 and 10 DAS ($P < 0.05$). On most dates, the 2.5% ethanol injected control and 2.5% and 5% ethanol insecticide trees had fewer *X. germanus* than the 5% ethanol controls and both 10% ethanol treatments ($P < 0.05$) (Fig. 1).

2016

In 2016, compared to control trees insecticide treatments reduced attacks by 85, 78, and 91% in the 2.5, 5, and 10% ethanol injected trees, respectively. Effects of insecticide and ethanol concentration on total attacks by ambrosia beetles were significant, but insecticide × time, ethanol × time, and insecticide × ethanol concentration were not (Table 3). More attacks occurred in the control trees than insecticide treated trees on every count date ($P < 0.05$). Trees injected with 10% ethanol had more total attacks than trees injected with 2.5 or 5% on all but the first count date (2 DAS) ($P < 0.05$); there were no differences between the 2.5 and 5% injected trees ($P > 0.05$). The 10% ethanol control treatment had greater attacks than all insecticide treatments on all dates ($P < 0.05$), and than the 2.5 and 5% controls during all inspections except 2 DAS ($P < 0.05$) (Table 3, Fig. 2). Two DAS, control trees injected with 2.5 and 5% ethanol had more attacks than 2.5% ethanol trees sprayed with insecticide ($P < 0.05$).

In 2016, effects of insecticide, ethanol concentration, and insecticide × time on attacks by *X. germanus* were significant, but ethanol × time and insecticide × ethanol concentration were not (Table 3). Fewer attacks by *X. germanus* occurred in the insecticide treated trees than controls on all dates ($P < 0.05$). Trees injected with 10%

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Table 2. Repeated measures analysis of the effects of induced attack pressure (ethanol concentration injected) and insecticide treatment (bifenthrin) on total attacks and attacks by *Xylosandrus germanus* in 2014

Variable	Effects	Num. df	Den. df	*F*	*P*
Total attacks	Insecticide	1	42	2.27	0.14
	Ethanol concentration (attack pressure)	2	42	20.14	<0.001
	Ethanol conc.× insecticide	2	42	0.12	0.89
	Time	4	39	25.24	<0.001
	Time × insecticide	4	39	1.94	0.12
	Time × ethanol conc.	8	78	7.01	<0.001
	Time × insecticide × ethanol conc.	8	78	1.30	0.26
X. germanus	Insecticide	1	42	11.45	0.002
	Ethanol concentration (attack pressure)	2	42	11.00	<0.001
	Ethanol conc. × insecticide	2	42	0.91	0.41
	Time	4	39	18.42	<0.001
	Time × insecticide	4	39	3.15	0.025
	Time × ethanol conc.	8	78	4.36	<0.001
	Time × insecticide × ethanol conc.	8	78	0.76	0.64

Trees were injected with 2.5, 5, or 10% ethanol to produce, respectively low, moderate, or high attack pressure.

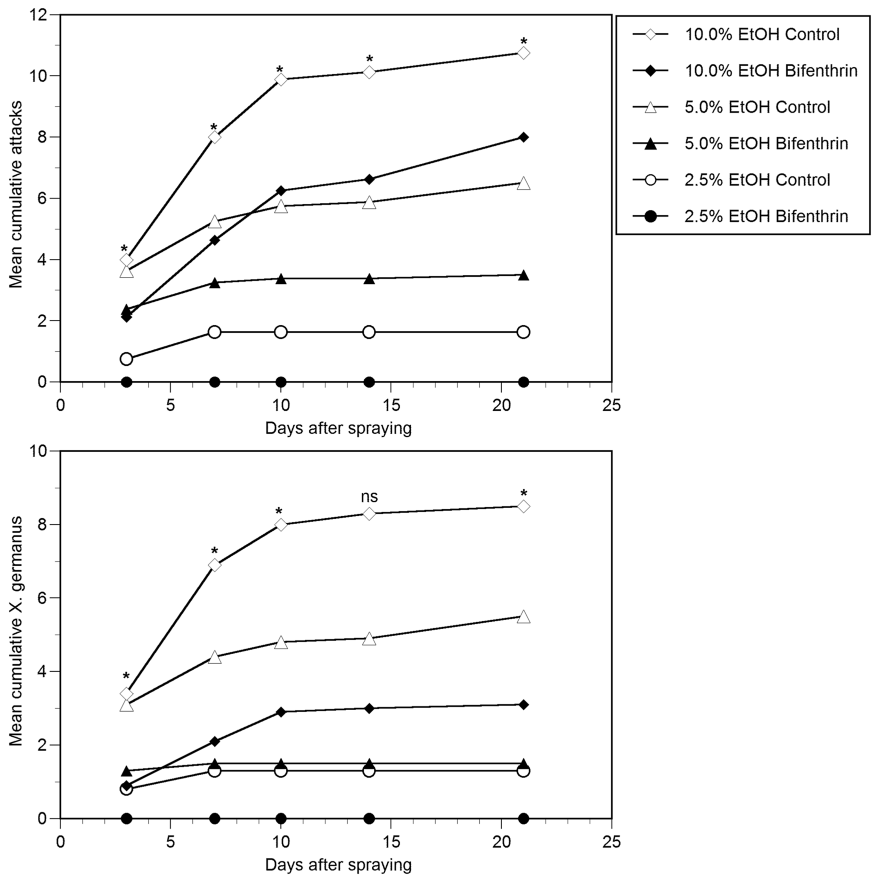

Fig. 1. Mean cumulative total Scolytinae attacks and attacks by *X. germanus* for all injected ethanol concentrations and insecticide treatments for the 2014 experiment. Asterisks indicate significant differences ($P \leq 0.05$) among means within dates (days after spraying).

ethanol had more *X. germanus* attacks than 2.5% ethanol trees 2, 7, and 13 DAS ($P < 0.05$), and than 5% ethanol trees 7 and 13 DAS ($P < 0.05$). There were no differences in numbers of *X. germanus* attacks among 2.5%, 5% or 10% ethanol injected trees treated with insecticide ($P > 0.05$). Control trees injected with 10% ethanol had more attacks by *X. germanus* than all other treatments on every date ($P < 0.05$) (Fig. 2). Attacks by *X. germanus* on insecticide treated trees injected with 2.5%, 5% or 10% ethanol were fewer than on all control concentrations 2 DAS ($P < 0.05$). There were no differences between insecticide treated trees or the 2.5% and 5% ethanol injected controls 7, 13, or 17 DAS ($P > 0.05$).

Repeated measures analysis detected no interaction effects of insecticide treatment and ethanol concentration (attack pressure) on numbers of total attacks or *X. germanus*. Therefore, logistic regression was used to examine the relationship between attack pressure and the presence of attacks on insecticide treated trees.

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Table 3. Repeated measures analysis of the effects of induced attack pressure (ethanol concentration injected) and insecticide treatment (bifenthrin) on total attacks and attacks by *Xylosandrus germanus* in 2016

Variable	Effects	Num. df	Den. df	*F*	*P*
Total attacks	Insecticide	1	42	16.07	<0.001
	Ethanol concentration (attack pressure)	2	42	3.93	0.027
	Ethanol conc.× insecticide	2	42	2.83	0.07
	Time	3	40	17.36	<0.001
	Time × insecticide	3	40	2.44	0.08
	Time × ethanol conc.	6	80	1.11	0.36
	Time × insecticide × ethanol conc.	6	80	1.15	0.34
X. germanus	Insecticide	1	42	23.29	<0.001
	Ethanol concentration (attack pressure)	2	42	3.87	0.029
	Ethanol conc. × insecticide	2	42	3.12	0.054
	Time	3	40	15.34	<0.001
	Time × insecticide	3	40	8.58	<0.001
	Time × ethanol conc.	6	80	2.17	0.054
	Time × insecticide × ethanol conc.	6	80	1.14	0.35

Trees were injected with 2.5, 5, or 10% ethanol to produce, respectively low, moderate, or high attack pressure.

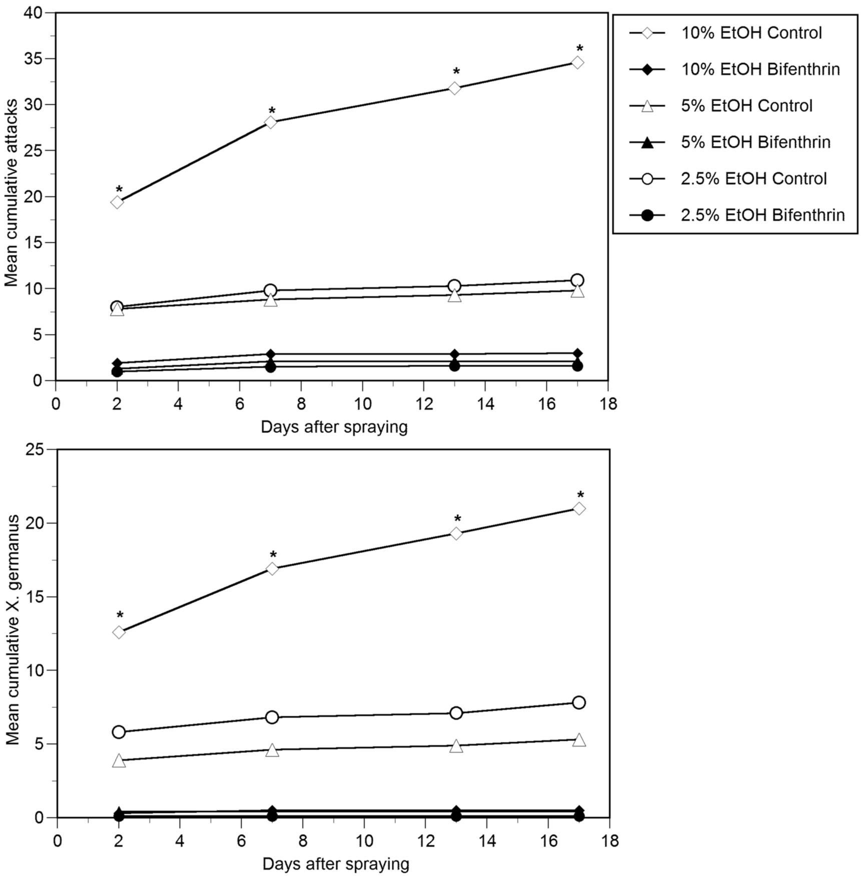

Fig. 2. Mean cumulative total Scolytinae attacks and attacks by *X. germanus* for all injected ethanol concentrations and insecticide treatments for the 2016 experiment. Asterisks indicate significant differences ($P \leq 0.05$) among means within dates (days after spraying).

Logistic regression revealed significant relationships between the presence of Scolytinae or *X. germanus* attacks on insecticide treated trees and concentration of ethanol injected (Table 4). The probability of attacks by Scolytinae and *X. germanus* increased on insecticide treated trees as the concentration of injected ethanol increased (Fig. 3).

Discussion

There were differences in residual activity of bifenthrin and permethrin for protecting nursery trees from attacks by ambrosia beetles. These materials are industry standards for managing ambrosia beetles in ornamental nurseries. Permethrin (Perm-UP) had more

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Table 4. Parameter estimates and statistics for logistic analysis of the relationship between induced attack pressure (concentration of ethanol injected) and presence of attacks by Scolytinae or *Xylosandrus germanus* on trees treated with protective sprays of bifenthrin (OnyxPro)

Variable	*n*	Parameter	df	Estimate	SE	Wald χ^2	$P > \chi^2$
Scolytinae attacks	47	*B0*	1	−1.99018	0.76041	6.86	0.009
		B1	1	0.46108	0.14643	9.92	0.002
X. germanus attacks	47	*B0*	1	−2.31587	0.75871	9.30	0.002
		B1	1	0.27791	0.10595	6.86	0.009

The basic form of the model was $P = [e^{(B0+B1*X)}/(1+e^{(B0+B1*X)})]$ where *P* is the probability of ambrosia beetle attacks or attacks by *X. germanus* on trees treated with protective sprays of bifenthrin, *X* is concentration of injected ethanol, and *B0* and *B1* are parameters to be estimated.

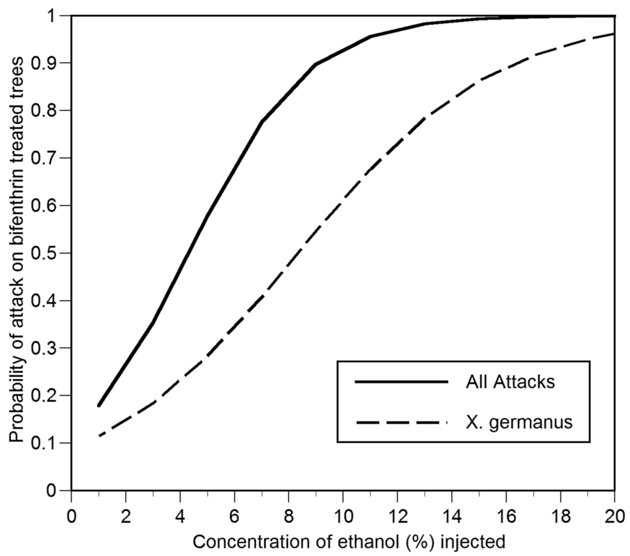

Fig. 3. Logistic regression analysis of the relationship between concentration of ethanol injected into trees and the probability of attacks on trees treated with insecticide (bifenthrin). Probabilities were derived from logistic equations based on data from trees injected with 2.5, 5.0, or 10.0% ethanol.

consistent and longer-lasting efficacy against *X. germanus* and other ambrosia beetles than bifenthrin (OnyxPro). In previous research (Reding et al. 2013a), permethrin and bifenthrin tended to reduce ambrosia beetle attacks compared to unsprayed trees, but permethrin usually performed better. In residual studies, bifenthrin often reduced numbers of *X. germanus* compared to control trees, but differences were not significant in most cases. In the present study, most bifenthrin treated trees had attacks during the shortest residue periods (10 and 17 d after spraying). However, while bifenthrin did not prevent attacks at the shortest residue periods, there were fewer occupied tunnels compared to controls. Ten DAS in 2012, only 17% of attacks on bifenthrin trees had occupied tunnels (beetles present, alive and dead) compared to 99% occupied tunnels in controls; at 17 DAS in 2013, 57% of attacks had occupied tunnels in bifenthrin trees versus 100% in controls. Abandoned tunnels were generally short and did not penetrate through the cambium. Permethrin was relatively effective through 31 d after spraying, with less than one attack per tree through that time. Frank and Sadof (2011) reported that compared to controls permethrin reduced attacks for 3 wk. Reding et al. (2013a) reported that permethrin reduced attacks compared to controls for at least 15 d in one experiment, and through 29 d in another. Some research indicates trees with less than 5 ambrosia beetle attacks can survive (Mizell and Riddle 2004). All but two permethrin treated trees had ≤ 2 attacks through 41 d after spraying in the current research.

Maintaining adequate insecticide residues on trunks of nursery trees is crucial for effective protection against ambrosia beetles. Beetles move into nurseries from surrounding habitats so sprays may not directly contact beetles (Reding et al. 2015, Werle et al. 2015). Growers should achieve acceptable protection by spraying permethrin at 21- to 28-d intervals, while intervals of bifenthrin sprays should probably not exceed 14 d. There were 9 and 11 rain events during the first 28 d of the 2012 and 2013 residual experiments, respectively. Rain events may adversely affect insecticide residues and could require shorter spray intervals. Gautam et al. (2016) demonstrated that simulated rainfall reduced residual activity of a variety of insecticides including pyrethroids. Irrigation systems that regularly deposit water on trunks of trees may also negatively affect longevity of insecticide residues.

Results from our current study indicated that efficacy of bifenthrin was influenced by attack pressure. Bifenthrin tended to reduce attacks by Scolytinae (total attacks) and *X. germanus*, but did not eliminate attacks in most cases. In 2014, bifenthrin completely prevented attacks in the low-pressure trees (2.5% ethanol) while all bifenthrin-treated moderate- (5% ethanol) and high-pressure (10% ethanol) trees had attacks. In 2016, only one low-pressure tree treated with bifenthrin was attacked, while seven moderate- and five high-pressure bifenthrin-treated trees were attacked. These results suggest that the inconsistent efficacy of bifenthrin in the residue experiments and previous research (Reding et al. 2013a) could be, in part, related to differences in attack pressure.

The presence of attacks on insecticide-treated trees was related to the amount of injected ethanol, whereby the attack pressure increased as ethanol increased. However, there was no significant interaction between insecticide treatment and concentration of injected ethanol ranging from 2.5 to 10%. A positive correlation has previously been demonstrated between concentration of ethanol emitted from lures (Klimetzek et al. 1986) and trees (Ranger et al. 2012) on attraction by generalist ambrosia beetles. Furthermore, Ranger et al. (2015) reported a positive correlation between the concentration of ethanol in bark tissue and attacks by ambrosia beetles. Since the efficacy of insecticides can decrease as populations of the target pest increase (Kain and Angello 1999, Nault and Shelton 2010), efficacy of insecticide treatments against ambrosia beetles may decline as emission of naturally-produced ethanol from stressed trees increases. A previous study found comparatively high levels of attacks occurred on flood-stressed trees that were preventively treated with permethrin (Ranger et al. 2016b). In particular, a mean of 53.0 and 33.0 attacks per tree occurred on flood-stressed *Cercis canadensis* L. that were untreated or preventively treated with permethrin, respectively, while

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118.5 and 34.2 attacks per tree occurred on *Cornus florida* L. that were untreated or preventively treated with permethrin, respectively (Ranger et al. 2016b).

In conclusion, preventive applications of permethrin and bifenthrin are used by growers in an attempt to prevent ambrosia beetles from attacking vulnerable trees. Results from our current study indicate that the residual efficacy of permethrin is longer than bifenthrin for reducing attacks. Permethrin prevented attacks from occurring for at least 4 wk, while bifenthrin was more inconsistent and lasted only about 10 d. However, our current study indicates that attack pressure can influence efficacy and that trees containing comparatively high levels of stress-induced ethanol are unlikely to be well protected. Management of ambrosia beetles will therefore benefit from cultural practices that reduce the potential for tree injury and stress-induced ethanol emissions.

Acknowledgments

We thank Betsy Anderson for technical assistance identifying ambrosia beetles. This research was supported by funding from the USDA-ARS Floriculture and Nursery Research Initiative and base funds associated with ARS research project 5082-22000-012-00D (National Program 304-Crop Protection and Quarantine). Mention of proprietary products or companies is included for the reader's convenience and does not imply any endorsement or preferential treatment by the USDA-Agricultural Research Service. USDA is an equal opportunity provider and employer.

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