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Permethrin Residual Activity Against Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae) Attacks Following Field Aging and Simulated Rainfall Weathering

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Abstract

Adult ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) bore into ornamental nursery trees resulting in trunk vascular tissue damage, which can potentially kill trees. Ambrosia beetle exposure to surface-applied insecticides is minimal after internal trunk galleries are formed, so effective management requires insecticide treatments to be applied near the time of infestation or to have residual activity on the bark. Tree trunk sections (bolts) were used to determine the effect of field aging or irrigation (i.e., simulated rainfall weathering) on permethrin residual activity against ambrosia beetles. In all experiments, 30-cm-long bolts from *Liriodendron tulipifera* L. (Magnoliales: Magnoliaceae) were hollowed and filled with 70% ethanol at field deployment to induce ambrosia beetle attacks over a 2-wk period. To evaluate insecticide residual activity, permethrin was sprayed onto tree bolts at 0, 8, 17, or 24 d before ethanol addition, and then bolts were deployed along a wooded border in fall 2017 and spring 2018. Tree bolts with permethrin residues ≤17 d old had significantly fewer ambrosia beetle attacks than bolts with 24-d-old residues or the non-permethrin-treated control bolts. To evaluate simulated rainfall weathering, permethrin was applied to tree bolts 8 or 22 d before ethanol (spring 2018) or 10 or 24 d before ethanol (fall 2018) with half of the bolts receiving regular irrigation events. Irrigation had no significant effect on permethrin residual activity against ambrosia beetles during either test. This study determined ambrosia beetle control was affected by permethrin residue age more than simulated rainfall weathering, and a reapplication interval of ≤17 d maximized beetle control.

Key words: insecticide, *Xylosandrus*, nursery, residue weathering

Ambrosia beetles are important pests of ornamental nurseries that bore into trees creating small entrance holes (Oliver and Mannion 2001) and subsequently rear progeny by feeding on symbiotic fungi inoculated within tunnels in the xylem or pith (Beaver 1989). In particular, the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), was ranked the third most important arthropod pest in a survey of southeastern nursery growers (Fulcher et al. 2012). Other species, such as *Xylosandrus germanus* (Blandford), *Xylosandrus compactus* (Eichhoff), and *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), may be more or less problematic in different regions of the country. In addition to entrance holes that ruin tree aesthetics and marketability, ambrosia beetle tunneling also exposes trees to secondary pathogen infection (Kessler 1974, Agnello et al. 2017), and the symbiotic fungi damage the vascular system (Dute et al. 2002). Additionally, some species, such as the granulate ambrosia beetle, can kill trees with as few as five attacks (Mizell and Riddle 2004). Ambrosia beetle control is challenging due to beetle ecology, which includes a wide host range (Ranger et al. 2015), fungi as the primary food source (i.e., systemic insecticides in woody tissues are ineffective) (Beaver 1989, Reding et al. 2013), and cryptic tunneling behavior that obscures attacks and protects the beetles from surface-applied insecticides. Adult female ambrosia beetles are well adapted to colonizing new hosts because they carry their future food resource (i.e., symbiotic fungi in specialized structures called mycangia) and have no need to search for mates as most species exhibit female-skewed sex ratios with male development from unfertilized eggs and sibling or maternal inbreeding.

Nursery-attacking ambrosia beetle species typically colonize stressed trees that produce ethanol (Ranger et al. 2013, 2015), which is a known attractant of some ambrosia beetles (Ranger et al. 2010). Ethanol is produced by plants in response to a variety of stress factors, including flooding, drought, disease, wounding, hypoxia, pollutants, and low- and high-temperature extremes (Kimmerer and Kozlowski 1982, Anderson 1994, Kelsey and Joseph 1998, Manter and Kelsey 2008, Rottenberger et al. 2008, Bourtsoukidis et al. 2014, Kelsey et al. 2016). Maintaining healthy plants is important for preventing ambrosia beetle attacks, although trees may not display symptoms of stress before ambrosia beetle attacks begin.

The damage thresholds for ambrosia beetles can be as low as zero attacks in nursery production because beetle entrance holes can result in unmarketable trees or rejection by state horticulture inspectors (Ranger et al. 2016). Pyrethroid insecticides, especially permethrin, are the most effective insecticide treatments currently available against ambrosia beetles, but insecticides do not reliably prevent ambrosia beetle attacks (Mizell et al. 1998, Mizell and Riddle 2004, Reding et al. 2013, Ranger et al. 2016, Frank et al. 2017). Combining knowledge about ambrosia beetle biology, monitoring, and control strategies as part of an integrated pest management (IPM) plan provide the best opportunity to achieve effective ambrosia beetle management. Insecticide efficacy is maximized by timing applications appropriately during peak beetle activity with additional considerations to the effects of optimal or sub-optimal environmental conditions on spray residues.

Ambrosia beetles spend most of their life cycle inside trees, so surface-applied insecticides are most effective against beetles dispersing among hosts. Adult female beetles emerge in the spring to search for new hosts and primarily attack trees around the time of dormancy break (Oliver and Mannion 2001), but attacks can continue throughout the summer and fall if trees are stressed. Trap monitoring to determine beetle emergence and peak activity is important for timing insecticide applications. Also, knowledge of insecticide residual activity is required to determine reapplication intervals.

Currently, 3 wk is the recommended permethrin application interval for trunk sprays against ambrosia beetles (Fulcher and White 2012), which is supported by residual activity studies (Frank and Sadof 2011). Understanding the appropriate timing of insecticide applications and the effects of weathering on residues could reduce insecticide use and thereby minimize the negative impacts on the environment and nontarget organisms (Smith and Stratton 1986, Weston et al. 2005), reduce grower application costs, and minimize secondary pest outbreaks (Frank and Sadof 2011). The objective of this study was to determine permethrin residual activity against ambrosia beetles after field aging and simulated rainfall weathering.

Materials and Methods

Experimental Design

Field experiments were conducted to determine the residual activity of permethrin against ambrosia beetles. Tulip poplar (*Liriodendron tulipifera* L.) (Magnoliales: Magnoliaceae) trunks with ~5 cm diameter were cut into 30-cm sections (tree bolts), and an 11.5-cm deep hole was drilled into one end of the bolt with a 1.6-cm drill bit. To attract ambrosia beetles, the drilled chambers in the tree bolts were filled with 15 ml of 70% ethanol (Product no# 793213, MilliporeSigma, St. Louis, MO), and the open end was plugged with a number 21d (bottom: 17 mm, top: 22 mm, height: 25 mm) Versilic silicone stopper (Saint-Gobain Performance Plastics, Malvern, PA). Tree bolts were suspended ~1 m above the ground along a wooded border on metal trap rods. Ethanol-filled tree bolts are an effective method for evaluating insecticides against ambrosia beetles for up to 14 d (Reding and Ranger 2020). Tree bolts were monitored for ~ 2 wk after deployment at 2–7 d intervals for new ambrosia beetle attacks, and new attacks were circled with a wax pencil to prevent re-counting of attacks on subsequent rating dates.

Permethrin Residual Activity After Field Aging

During fall 2017 and spring 2018, Perm-Up 3.2EC (36.8% permethrin) (United Phosphorous, Inc., King of Prussia, PA) was sprayed onto tree bolts at 0, 8, 17, or 24 d before the addition of ethanol to determine residual activity against ambrosia beetles. The initiation of spray treatments was staggered so that the ambrosia beetle ethanol attractant could be added to all treatments on the same date. Perm-Up treated tree bolts were sprayed to runoff using the Coleopteran borer and bark beetle maximum labeled rate of 12.5 ml Perm-Up 3.2EC/ liter of water (38.3 kg AI/ 100 liters of water) with a model 640 trigger sprayer that delivered 3.5 ml/ pump (manufacturer no# 110804; Tolco Corp., Toledo, OH). Both tests had six replications arranged in a randomized complete block design (RCBD). Before the addition of ethanol, permethrin-treated bolts were suspended under the edge of a carport at the Tennessee State University Otis L. Floyd Nursery Research Center, McMinnville, TN (35.707617N; −85.740862W) (TSU-NRC) to reduce exposure to rainfall and sunlight. To suspend bolts, a wire was passed through an eyebolt screw inserted into the end of the bolts, and bolts were spaced ~45 cm apart on the wire. After ethanol addition to 2017 tree bolts, treatments were moved from the carport to the edge of a wooded border at the TSU-NRC from 15 to 27 September with 5 m between treatments and 10 m between replications. After ethanol addition to 2018 tree bolts, treatments were moved from the carport at the TSU-NRC and were deployed along a wooded border at a commercial nursery (35.792747N; −85.77819W) from 23 May to 7 June. At the commercial nursery, tree bolt treatments and replications were spaced as previously described.

Permethrin Residual Activity After Simulated Rainfall Weathering

During spring 2018 and fall 2018, permethrin-treated tree bolts were irrigated to simulate rainfall weathering and determine the effect on insecticide residual activity. To prepare insecticide treatments at the TSU-NRC, tree bolts were sprayed to the point of runoff with Perm-Up 3.2 EC as previously described at 22 or 8 d before ethanol in spring 2018 and 24 or 10 d before ethanol in fall 2018. As before, the initiation of spray timings was staggered so that the ambrosia beetle ethanol attractant could be added to the tree bolt treatments on the same date for test deployment. For Perm-Up application treatment timings, half of the bolts were randomly selected and exposed to biweekly simulated rainfall weathering, while the other half remained dry under the carport. Tree bolts receiving irrigation treatments were immediately returned to the carport with the nonirrigated bolts after watering to reduce the likelihood of natural rain exposure before test deployment. In addition to these irrigated and nonirrigated permethrin treatments, a control treatment consisted of bolts that received no permethrin or irrigation. To simulate rainfall weathering, tree bolts were suspended ~1 m above the ground on a metal trap rod near a lawn sprinkler for 1 h (rotating tree bolts 180° after 30 min to ensure both sides received similar irrigation exposure).

For the spring 2018 test, a rectangular sled lawn sprinkler (model: 8826GF; Gilmour, Middleton, WI) (22.7 liter/ min flow rate; 250 m^2 coverage area) was used to deliver a downward oriented water droplet similar to rainfall. Bolts sprayed 22 d before ethanol were irrigated 1, 3, 8, 10, 15, and 17 d after spraying, while bolts sprayed 8 d before ethanol were irrigated 1 and 3 d after spraying (Table 1). To estimate tree bolt exposure to irrigation, four 400-ml beakers (top diameter 7.2 cm) were placed on the ground near suspended tree bolts during each irrigation exposure event. Total irrigation exposure for tree bolts sprayed 22 and 8 d before ethanol was 249 ml (rainfall equivalent of 6.12 cm/ha) and 84.8 ml (rainfall equivalent of 2.08 cm/ha), respectively (Table 1). After ethanol addition, all tree bolt treatments were moved from the TSU-NRC carport and deployed along a wooded border from 23 May to 7 June 2018 at the previously described commercial nursery using four replications spaced as described before in an RCBD.

During the fall 2018 test, a light duty (model 084020-1304) adjustable rectangular sprinkler (Gilmour, Middleton, WI) was used to deliver simulated rainfall, and beakers near tree bolts were used to estimate irrigation exposure as previously described. Bolts sprayed 24 d before ethanol were irrigated 1, 3, 8, 10, 15, and 17 d after spraying, while bolts sprayed 10 d before ethanol were irrigated 1 and 3 d after spraying (Table 1). Total irrigation exposure for tree bolts sprayed 24 and 10 d pre-ethanol was 271 ml (rainfall equivalent of 6.66 cm/ha) and 83 ml (rainfall equivalent of 2.04 cm/ha), respectively (Table 1). After ethanol addition, all tree bolt treatments were moved from the TSU-NRC carport and deployed along a wooded border from 28 September to 12 October 2018 as previously described for the spring 2018 test. During the fall 2018 test, purple colored plastic plates were attached between eyebolt screws to create a roof over the tree bolts and further limit rain exposure and minimize permethrin weathering to just the experimental irrigation treatments. Due to low ambrosia beetle attack rates, tree bolt treatments received a second and third addition of 15 ml of 70% ethanol at 5 and 10 d, respectively.

Data Analysis

Total cumulative ambrosia beetle attacks were compared among treatments using a Generalized Linear Interactive Model (GLIM) with a log link and assuming a negative binomial distribution, and pairwise comparisons were made using Tukey's test ($\alpha = 0.05$) (Proc GENMOD; SAS Institute, Cary, NC) (Agresti 2002). The area under the ambrosia beetle attack progress curve (AUAPC) was calculated by summing the area of the trapezoids between each adjacent pair of observation dates using the same procedure developed for estimating plant disease severity progression with time (Madden et al. 2007). Cumulative AUAPC values were calculated at each observation date by summing the cumulative area under the curve up to that date. Cumulative AUAPC was compared among treatments at each post-ethanol sampling date using a Generalized Linear Interactive Model (GLIM) with a log link and assuming a gamma distribution, and pairwise comparisons were made using least-squared means ($\alpha = 0.05$) (Proc GENMOD; SAS Institute, Cary, NC) (Agresti 2002). Treatments having all replicates with zero values had 0.5 randomly added into one of the replicates to meet the requirements of the GLIM analysis.

Results

Permethrin Residual Activity After Field Aging

In the fall 2017 test, 0, 8, 17, or 24 d permethrin spray treatments had significantly reduced total ambrosia beetle attacks compared to control treatment bolts, and the 0, 8, or 17 d treatments had less attacks than the 24 d treatment (Table 2). The AUAPC had a gradual increase in attacks that began at 3, 7, and 12 d after ethanol for the 24, 17, and 8 d permethrin treatments, respectively (Fig. 1). The 0 d permethrin treatment had no attacks during the study (Fig. 1). On all post-ethanol sampling dates, no significant differences were detected in AUAPC values among 0, 8, or 17 d permethrin spray treatments, but all of these treatments had less attacks than the 24 d permethrin treatment (Fig. 1). Average (±SE) air temperature during the fall test was 23.0 ± 0.2°C, and rainfall totaled 1.0 cm (occurring on 19 September) (NCEI 2020).

During spring 2018 test, the pattern of total ambrosia beetle attacks was similar to the fall 2017 test with 0, 8, or 17 d permethrin spray treatments having significantly lower total ambrosia beetle attacks compared to the control or 24 d permethrin treatments (Table 2). Unlike fall 2017, no differences were detected between the 24 d permethrin and control treatments (Table 2). As in 2017, the AUAPC had a gradual increase in attacks for all treatments with a trend for more attacks in treatments with older permethrin residues (Fig. 2). The first beetle attacks beginning at 8 d after ethanol for the 0 d permethrin treatment and 6 d after ethanol for the other treatments (Fig. 2). Unlike fall 2017, the 0 d permethrin treatment had lower AUAPC values than the other treatments up to 8 d after ethanol. By 15 d after ethanol, the 0 d permethrin treatment still had significantly less attacks than all treatments, except the 8 d permethrin treatment (Fig. 2). On most post-ethanol sampling dates, permethrin treatments applied ≤17 d before ethanol had fewer attacks than the 24 d permethrin treatment (Fig. 2). Average (±SE) air temperature during the spring test was 23.8 ± 0.4°C, and rainfall totaled 6.8 cm (NCEI 2020). Rainfall occurred on 28 May (0.6 cm), 29 May (1.4 cm), 30 May (0.2 cm), 31 May (0.9 cm), and 1 June (3.7 cm) (NCEI 2020).

Table 1. Average water volumes sampled during irrigation events of permethrin-treated tree bolts

Test	Permethrin spray timing (days before ethanol)	Average (±SE) irrigation vol. (ml) at different days after permethrin spray[a]						
		1	3	8	10	15	17	Total[b]
Spring 2018	22	47.00 ± 3.14	38.25 ± 1.55	35.25 ± 2.21	43.75 ± 2.50	43.50 ± 1.94	41.25 ± 1.70	249.0
	8	43.50 ± 1.94	41.25 ± 1.70					84.8
Fall 2018	24	75.25 ± 3.20	30.75 ± 2.50	46.75 ± 2.17	35.25 ± 1.11	36.50 ± 1.71	46.50 ± 4.57	271.0
	10	36.50 ± 1.71	46.50 ± 4.57					83.0

[a]Tree bolts were suspended ~1 m above the ground from a metal trap rod and irrigated with a rectangular sled lawn sprinkler. Irrigation amount was averaged among four beakers placed on the ground near suspended tree bolts.

[b]Total is the sum of the average irrigation collections.

Table 2. Average (±SE) and total ambrosia beetle attacks on tree bolts treated with permethrin at different times (0, 8, 17, or 24 d) before introducing ethanol to attract ambrosia beetles

Permethrin spray timing (days before ethanol)	Fall 2017 Average (±SE) total attacks/tree bolt	Fall 2017 Total attacks	Spring 2018 Average (±SE) total attacks/tree bolt	Spring 2018 Total attacks
Control	9.17 ± 2.07a	55	4.67 ± 1.20a	28
24	4.17 ± 1.62b	25	4.33 ± 1.74a	26
17	0.33 ± 0.21c	2	1.67 ± 0.42b	10
8	0.17 ± 0.17c	1	1.67 ± 0.42b	10
0	0.00 ± 0.00c	0	1.17 ± 0.31b	7

Different letters in the last column represent significant differences among permethrin spray timing treatments in total cumulative attacks ($\alpha = 0.05$). Average total cumulative attacks were used for the GLIM analysis (Fall 2017: $\chi^2 = 37.4$, df = 4, $P < 0.0001$; Spring 2018: $\chi^2 = 13.92$, df = 4, $P = 0.0076$).

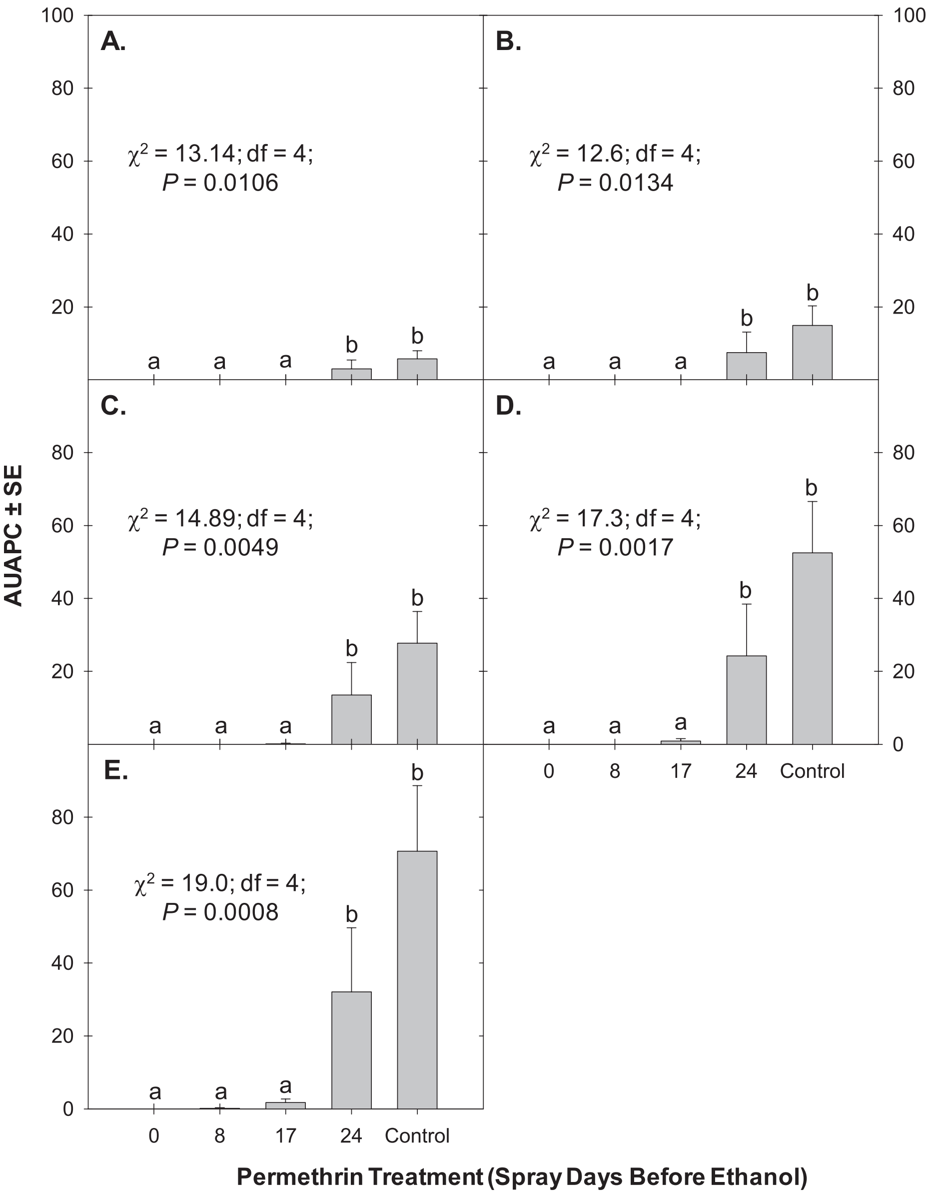

Fig. 1. Average area under the ambrosia beetle attack progress curve (±SE) (AUAPC) for different permethrin spray treatments during fall 2017 test at (A) 3, (B) 5, (C) 7, (D) 10, or (E) 12 sampling days after ethanol addition. The AUAPC is a modification of methods to calculate disease progression over time (Madden et al. 2007) and represents the area under the curve of cumulative ambrosia beetle attacks calculated by summing the areas of the trapezoids between each adjacent pair of observation dates. The AUAPC values listed for each sampling date sub-graph represent the cumulative area under the curve up to that time. Bars with different letters were significantly different within each sampling date sub-graph (General Linear Interactive Model using a Gamma Distribution [Proc Genmod, SAS Institute, Cary, NC]).

Permethrin Residual Activity After Simulated Rainfall Weathering

In the spring 2018 test, the irrigated and nonirrigated 8 and 22 d permethrin spray treatments had significantly less total ambrosia beetle attacks than the non-treated control (Table 3). For the 8 d permethrin treatments, no differences were detected in AUAPC values between irrigated and nonirrigated treatments on any of the post-ethanol sampling dates (Fig. 3). However, for the 22 d permethrin treatments, the AUAPC values were higher for the nonirrigated treatment at 1 and 6 d after ethanol (Fig. 3). All treatments increased in ambrosia beetle attacks with time with the control having the highest attack rates (Fig. 3). Average (±SE) air temperatures during the spring test was 23.7 ± 0.5°C, and rainfall totaled 6.8 cm (NCEI 2020). Rainfall occurred on the same dates as listed for the spring test in the previous section.

During the fall 2018 test, all permethrin treatments had less total ambrosia beetle attacks than the non-treated control (Table 3). No differences were detected in total ambrosia beetle attacks between irrigated and nonirrigated treatments at 10 or 24 d, respectively (Table 3). As in the spring 2018 test, the AUAPC values increased for all treatments with time (Fig. 4). The AUAPC values for 10 d permethrin sprays were actually higher for nonirrigated

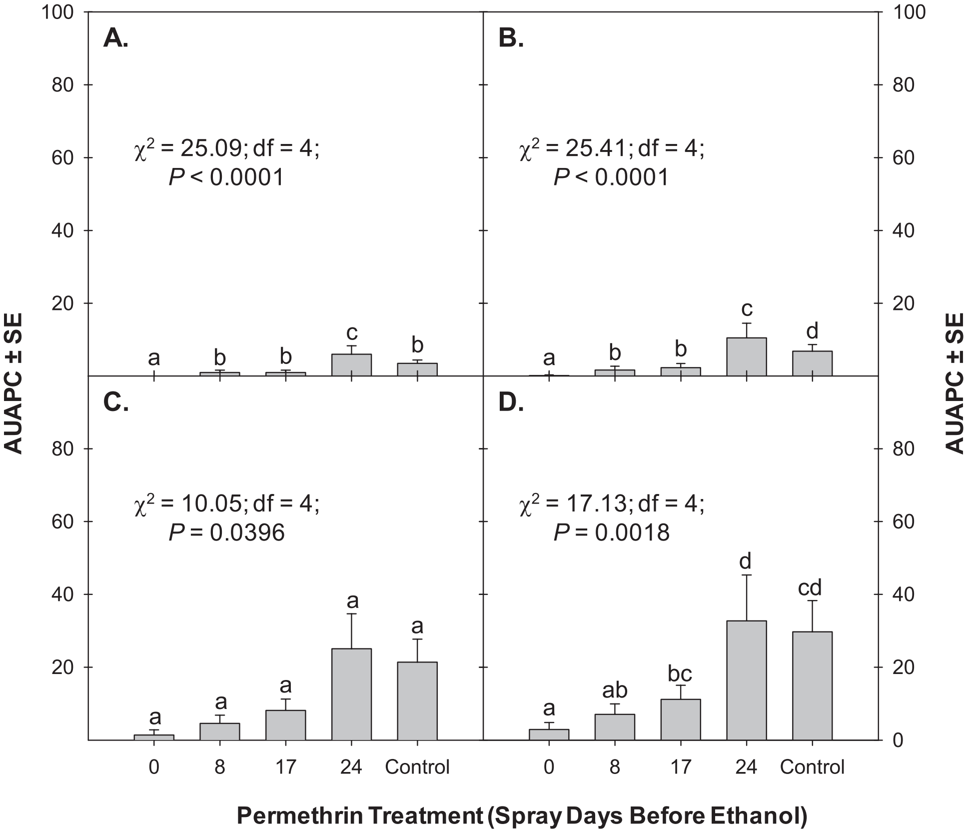

Fig. 2. Average area under the ambrosia beetle attack progress curve (±SE) (AUAPC) for different permethrin spray treatments during spring 2018 test at (A) 6, (B) 8, (C) 13, or (D) 15 sampling days after ethanol addition. The AUAPC is a modification of methods to calculate disease progression over time (Madden et al. 2007) and represents the area under the curve of cumulative ambrosia beetle attacks calculated by summing the areas of the trapezoids between each adjacent pair of observation dates. The AUAPC values listed for each sampling date sub-graph represent the cumulative area under the curve up to that time. Bars with different letters were significantly different within each sampling date sub-graph (General Linear Interactive Model using a Gamma Distribution [Proc Genmod, SAS Institute, Cary, NC]).

Table 3. Average (±SE) cumulative ambrosia beetle attacks on irrigated and nonirrigated tree bolts treated with permethrin at different times (8–10 or 22–24 d) before introducing ethanol to attract ambrosia beetles

		Spring 2018		Fall 2018	
Permethrin spray timing (days before ethanol)	Irrigation	Average (±SE) total attacks/ tree bolt	Total attacks	Average (±SE) total attacks/ tree bolt	Total attacks
Control	No	6.25 ± 0.63a	25	15.75 ± 3.28a	63
22–24	Yes	2.75 ± 0.63b	11	4.50 ± 0.65b	18
22–24	No	3.25 ± 1.25b	13	2.25 ± 0.85bc	9
8–10	Yes	3.00 ± 1.35b	12	0.25 ± 0.25c	1
8–10	No	1.75 ± 0.85b	7	1.25 ± 0.95bc	5

Different letters in the last column represent significant differences among permethrin spray timing treatments in total cumulative attacks ($\alpha = 0.05$). Average total cumulative attacks were used for the GLIM analysis (Spring 2018: $\chi^2 = 12.3$, df = 4, $P = 0.0153$; Fall 2018: $\chi^2 = 114.39$, df = 4, $P < 0.0001$).

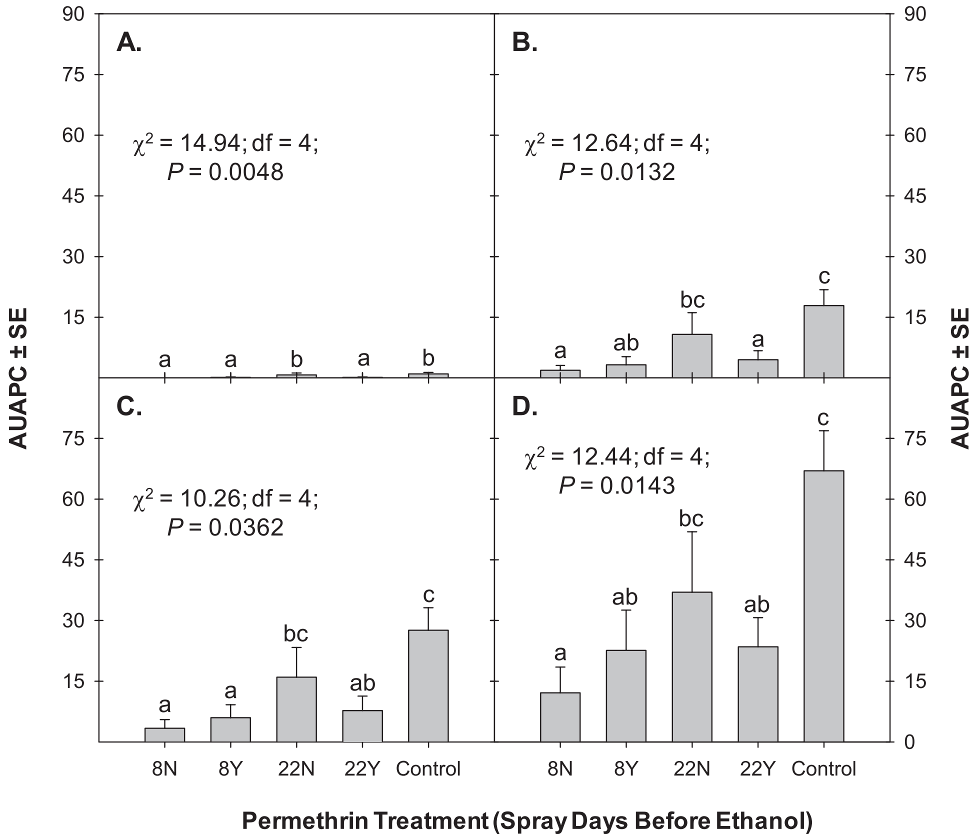

Fig. 3. Average area under the ambrosia beetle attack progress curve (±SE) (AUAPC) for different permethrin spray treatments during spring 2018 test at (A) 1, (B) 6, (C) 8, or (D) 15 sampling days after ethanol addition. Permethrin spray treatments include 8 or 22 d before ethanol with irrigation (Y) or no irrigation (N). The AUAPC is a modification of methods to calculate disease progression over time (Madden et al. 2007) and represents the area under the curve of cumulative ambrosia beetle attacks calculated by summing the areas of the trapezoids between each adjacent pair of observation dates. The AUAPC values listed for each sampling date sub-graph represent the cumulative area under the curve up to that time. Bars with different letters were significantly different within each sampling date sub-graph (General Linear Interactive Model using a Gamma Distribution [Proc Genmod, SAS Institute, Cary, NC]).

treatments than irrigated treatments at 7, 10, 12, and 14 d after ethanol (Fig. 4). The AUAPC values for the 24 d permethrin sprays also were initially higher in the nonirrigated treatment than the irrigated treatment at 3 d after ethanol, but no differences were detected on subsequent sampling dates (Fig. 4). The 24 d permethrin treatments had higher AUAPC values than the 10 d permethrin irrigated treatments on most post-ethanol sampling dates (Fig. 4). Average (±SE) air temperature during the fall test was 22.8 ± 1.1, and rainfall totaled 2.4 cm, respectively (NCEI 2020). Rainfall occurred on 1 October (1.3 cm), 3 October (0.4 cm), and 11 October (0.7 cm).

Discussion

Permethrin effectively reduced ambrosia beetle attacks on ethanol-filled tree bolts compared with untreated control bolts, but no permethrin treatment completely prevented attacks (even bolts sprayed with permethrin the same day ethanol was added). In other studies, pyrethroid insecticides (permethrin, bifenthrin) provided optimal protection against ambrosia beetles, but likewise did not completely prevent attacks (Mizell and Riddle 2004, Reding et al. 2013). In this study, ethanol introduction into hollowed bolts functioned as a stress signal to induce ambrosia beetle attacks (Ranger et al. 2010, 2015; Reding and Ranger 2020). Tree bolts treated with permethrin ≤17 d before ethanol provided equivalent levels of protection against ambrosia beetles for the duration of the 12 d post-ethanol observation period in one test. However, in another test, bolts treated 0 d before ethanol provided superior protection to bolts treated 8 or 17 d before ethanol. Tree bolts treated with permethrin 24 d before ethanol provided significantly less protection than all other permethrin treatment timings. In other studies, permethrin reduced attacks by ambrosia beetles (*X. crassiusculus* and *X. germanus*) up to 15 to 28 d (Frank and Sadof 2011, Reding et al. 2013, Reding and Ranger 2018), which is consistent with the present study for the entire pre- and post-ethanol residue period. The longest reported permethrin residual activity (28 d) was primarily against *X. germanus* (Reding and Ranger 2018), but *X. crassiusculus* is a more important pest in southern states (Mizell et al. 1998, Oliver and Mannion 2001, Schultz et al. 2002, Mizell and Riddle 2004, Fulcher et al. 2012). Even permethrin applied as often as weekly using rates twice the labeled Perm-up 3.2EC rate did not prevent all ambrosia beetle attacks on nursery trees (Frank and Sadof 2011), but it is possible the termiticide permethrin product used was not adequately formulated for tree trunk surfaces. Thus, permethrin cannot prevent all ambrosia beetle attacks even at high rates and application frequencies, but maximizing residue levels will increase the likelihood of treatment success.

The addition of simulated rainfall weathering was not a significant factor in permethrin efficacy against ambrosia beetles. Tree bolts were not exposed to irrigation until at least 24 h after permethrin application, so dried residues on the tree trunk surface appear to be impervious to water removal. Permethrin residues

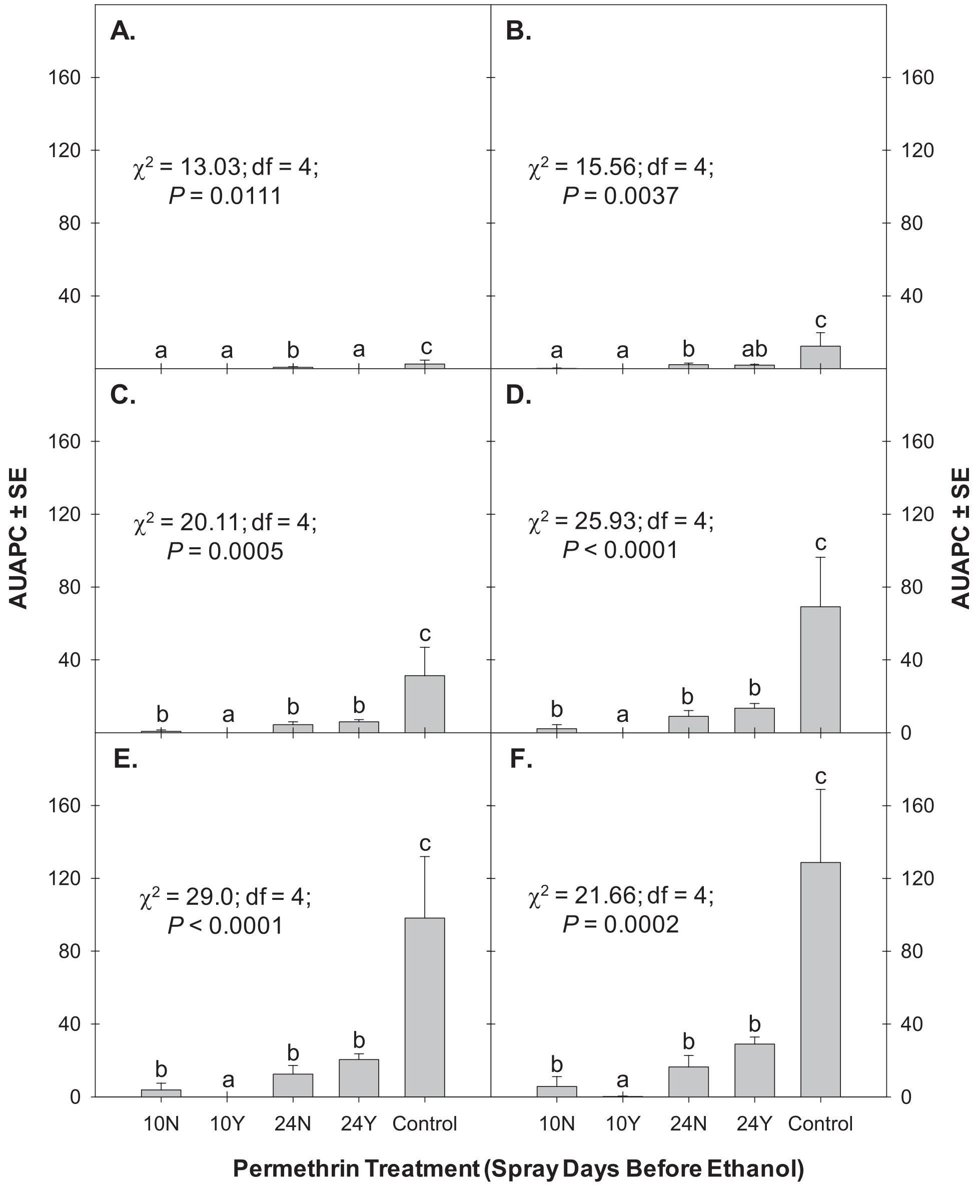

Fig. 4. Average area under the ambrosia beetle attack progress curve (±SE) (AUAPC) for different permethrin spray treatments during fall 2018 test at (A) 3, (B) 5, (C) 7, (D) 10, (E) 12, or (F) 14 sampling days after ethanol addition. Permethrin spray treatments include 10 or 24 d before ethanol with irrigation (Y) or no irrigation (N). The AUAPC is a modification of methods to calculate disease progression over time (Madden et al. 2007) and represents the area under the curve of cumulative ambrosia beetle attacks calculated by summing the areas of the trapezoids between each adjacent pair of observation dates. The AUAPC values listed for each sampling date sub-graph represent the cumulative area under the curve up to that time. Bars with different letters were significantly different within each sampling date sub-graph (General Linear Interactive Model using a Gamma Distribution [Proc Genmod, SAS Institute, Cary, NC]).

are more resistant to rain-related removal with increasing time after application (Willis et al. 1986, 1992), and most permethrin weathering occurs in the initial 3 mm of rainfall (Willis et al. 1994). Permethrin likely adheres to the tree surface better with longer drying times before rain events. Although the irrigated bolts with older residues (22 or 24 d) were exposed to six simulated rainfall weathering events compared to two events for those with more recently applied residues (8 or 10 d) (Table 1), permethrin residues were likely similar based on the observed treatment efficacy against ambrosia beetles (Figs. 3 and 4; Table 3). Pyrethroids are extremely lipophilic (Elliott and Janes 1978), which may contribute to water removal resistance. Other pyrethroid weathering studies have observed similar results with bifenthrin efficacy against Japanese beetles (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae), where insecticidal activity was negatively affected by field age, but not rainfall weathering (Hulbert et al. 2011).

Hydrolysis (i.e., chemical breakdown due to reaction with water) and oxidation (i.e., chemical breakdown due to reaction with oxygen) are the main pathways affecting degradation of pyrethroid insecticides (Lord et al. 1982). Other environmental factors that could affect permethrin residual activity after field aging include photodegradation (Katagi 2004), plant or microbial metabolism (Van Eerd et al. 2003), or volatilization (although permethrin has relatively low volatility) (NPIC 2009). Permethrin is more stable to light exposure compared with other pyrethroids (WHO 1990). When a thin film of permethrin was exposed to daylight indoors through a window, >50% of the applied permethrin remained after 3 wk, although other environmental factors also may have played a role in decomposition (Elliott et al. 1973). In this study, tree bolts were stored under a carport, which partially protected them from photodegradation by ultraviolet (UV) light. Thus, pesticide weathering on tree trunks in nurseries may be greater than observed in this study due to higher exposure to UV radiation. The physical properties of the trunk surface for different tree species also may affect insecticide residue levels via ease of removal or bark crevice protection from environmental factors like sunlight. The tulip poplar bolts used in this study had relatively smooth bark with small and exposed crevices, so it is unlikely bark crevices prevented most weathering effects. Tree species also can affect ambrosia beetle attraction and pressure on treatments (Reding and Ranger 2020), but the tulip poplar bolts used in this study received adequate attacks to assess treatment effects.

Ambrosia beetle-attacked trees can be unmarketable due to the gallery entrance holes and pathogen introduction, and therefore growers have a low threshold for ambrosia beetle attacks. Mizell and Riddle (2004) reported that 5–10 attacks by *X. crassiusculus* killed most trees. Thus, ≥5 attacks per tree would be an unacceptable economic threshold for growers. In this study, all permethrin-treated bolts averaged fewer than five ambrosia beetle attacks per tree bolt, but bolts treated 24 d before ethanol averaged more than four attacks in both fall and spring tests (Table 2), which is approaching an assumed grower threshold. Consequently, a reapplication interval of 24 d may put vulnerable nursery trees at risk of being killed by ambrosia beetles. Since ambrosia beetle entrance holes may ruin the marketability of trees and jeopardize plant approval by state horticultural inspectors, grower damage thresholds may be even lower than five attacks. In addition, this study was only performed for 15 d, but field nursery trees with inadequate protective treatments may continue to add more attacks with time, eventually exceeding acceptable economic thresholds.

The inability to completely prevent attacks with permethrin emphasizes the importance of a multi-strategy IPM approach for ambrosia beetle control. Future research should focus on combining insecticide treatments with other management tactics like cultural practices to reduce tree stress and beetle attraction in order to maximize insecticide efficacy (Frank et al. 2017). This study only examined total beetle attacks on tree bolts but did not evaluate whether female beetles were present in initiated galleries. Because the experiments were only monitored for 2 wk, other indicators of successful colonization like ambrosia fungal growth and brood presence could not be assessed. Permethrin treatments may be more effective if measured in terms of beetle colonization success, since disruption of fungal pathogen establishment and egg laying (Hudson and Mizell 1999, Ranger et al. 2016), and gallery abandonment have been associated with permethrin treatments (Schultz et al. 2002, Reding et al. 2013, Reding and Ranger 2018). In contrast, Castrillo et al. (2016) concluded that unsuccessful colonization could be undesirable if it led to foundress beetles exiting galleries and attacking more adjacent trees or if the female beetle made more attacks on the same tree.

Ethanol-filled tree bolts were used to attract ambrosia beetles to evaluate permethrin residual efficacy. Tree bolts that were untreated or had older permethrin residues (i.e., sprayed 24 d before ethanol) had new ambrosia beetle attacks on every sampling date, demonstrating that ethanol-filled bolts were reliably attractive to ambrosia beetles. Ethanol-filled bolts have an attractive period no longer than 14 d (Reding and Ranger 2020), so the ~2 wk post-ethanol observation period in this study was appropriate for evaluating permethrin residues. However, ethanol-filled tree bolts may not accurately simulate a stressed nursery tree, since ethanol volatiles were likely much higher than naturally produced by stressed trees (Ranger et al. 2010, 2013). Reding and Ranger (2018) did not observe differences in attack rates on bifenthrin-treated trees injected with 2.5, 5.0, or 10.0% ethanol, but these concentrations may still exceed natural ethanol production levels in stressed trees. If the ethanol-filled tree bolts were more attractive than a naturally stressed nursery tree (e.g., root flooding stress), then permethrin treatments could be more effective in a typical nursery setting where treatments are protecting trees with lower beetle attraction rates. The branches or foliage of intact nursery trees also may provide some protection to trunk residues from direct rainfall and sunlight. Indeed, Reding and Ranger (2018) reported longer permethrin residual activity against *X. germanus* (28 d) when using ethanol-injected trees rather than ethanol-filled tree bolts. Additional research contrasting the residual activity of insecticides on trees versus tree bolts in the same study would be important to validate these assumptions. Ethanol-filled bolts are reported to be less attractive than ethanol-soaked bolts (Reding and Ranger 2020), so the former may be a better choice for insecticide residue testing when not using live stressed trees emitting more natural ethanol levels. Ethanol-filled bolts also allow greater precision and flexibility in ethanol amount, which could be manipulated to better replicate the attractiveness of naturally stressed trees.

In conclusion, tree bolts treated with permethrin at 0, 8, or 17 d before ethanol had similar 2-wk post-ethanol efficacy against ambrosia beetles, but attacks were more likely to occur with time on bolt treatments with the oldest residues. All permethrin treatments in this study had some protective benefit, even though attack rates in bolts with 24-d-old residues would likely exceed acceptable grower damage thresholds. Results indicate a 1 or 2 wk permethrin application interval may have no increased benefit over a 3 wk interval, even though trees will likely experience some attacks with all application intervals. Simulated rainfall weathering had no negative effect on permethrin efficacy in this study. Therefore, growers using these study results may be able to achieve effective ambrosia beetle control using a 3 wk permethrin spray interval even if the weather is rainy, but adequate permethrin drying time before rain events is likely important to maximize insecticide residues. Because of the challenges associated with ambrosia beetle management, insecticides remain an important component of an integrated strategy to control these pests. Understanding the appropriate use of insecticides, such as application interval, should optimize the effectiveness of insecticide treatments, while saving growers money, reducing environmental contamination and nontarget organism impacts, and avoiding secondary pest outbreaks.

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Mentioning of product names is for informational purposes only and does not imply an endorsement by the authors or their institutions.

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