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Trap Tree and Interception Trap Techniques for Management of Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) in Nursery Production

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Abstract

The majority of wood-boring ambrosia beetles are strongly attracted to ethanol, a behavior which could be exploited for management within ornamental nurseries. A series of experiments was conducted to determine if ethanol-based interception techniques could reduce ambrosia beetle pest pressure. In two experiments, trap trees injected with a high dose of ethanol were positioned either adjacent or 10–15 m from trees injected with a low dose of ethanol (simulating a mildly stressed tree) to determine if the high-dose trap trees could draw beetle attacks away from immediately adjacent stressed nursery trees. The high-ethanol-dose trees sustained considerably higher attacks than the low-dose trees; however, distance between the low- and high-dose trees did not significantly alter attack rates on the low-dose trees. In a third experiment, 60-m length trap lines with varying densities of ethanol-baited traps were deployed along a forest edge to determine if immigrating beetles could be intercepted before reaching sentinel traps or artificially stressed sentinel trees located 10 m further in-field. Intercept trap densities of 2 or 4 traps per trap line were associated with fewer attacks on sentinel trees compared to no traps, but 7 or 13 traps had no impact. None of the tested intercept trap densities resulted in significantly fewer beetles reaching the sentinel traps. The evaluated ethanol-based interception techniques showed limited promise for reducing ambrosia beetle pressure on nursery trees. An interception effect might be enhanced by applying a repellent compound to nursery trees in a push–pull strategy.

Key words: mass trapping, *Xylosandrus crassiusculus*, *Xylosandrus germanus*, *Cnestus mutilatus*, trap crop

Several species of nonnative ambrosia beetles in the tribe Xyleborini (Coleoptera: Curculionidae: Scolytinae) have become serious pests of woody ornamental nursery crops across the United States. Among these pests, the most widely destructive species include granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) (Oliver and Mannion 2001, LeBude et al. 2011, Reding et al. 2011, Ranger et al. 2016), with other emerging pests such as camphor shot borer, *Cnestus mutilatus* (Blandford) (Oliver et al. 2012, Olatinwo et al. 2014), *Anisandrus maiche* Stark (Ranger et al. 2015b), and black twig borer, *Xylosandrus compactus* (Eichhoff) (Chong et al. 2009, Greco and Wright 2015) becoming more prevalent in recent years. Recently introduced ambrosia beetles like members of the *Euwallacea* nr. *fornicatus* complex are becoming pests of increasing concern in California and

Florida forests and tree fruit production (Eskalen et al. 2012, Lynch et al. 2016, Kendra et al. 2017, Owens et al. 2018).

Ambrosia beetles have a unique lifestyle, which makes them difficult to control with traditional pesticide applications. The adult female beetles bore into the trunks and branches of host trees, creating galleries for rearing larvae (Hoffman 1941, Weber and McPherson 1983). The beetles and larvae do not feed on the tree tissue but rather on symbiotic fungi inoculated onto the gallery walls (Hoffman 1941, Weber and McPherson 1983, Hulcr and Cognato 2010). Both contact and systemic insecticides are of limited value since the beetles are protected from direct sprays under the bark and do not feed directly on vascular tissues. The efficacy of pesticide trunk sprays for these insects relies on proper timing to coincide with spring emergence and the beginning of tree attacks. Predicting

spring emergence is challenging since the beetles overwinter as mated adults without a temperature-dependent larval development period that can be patterned with growing degree days. Previous studies of *X. germanus* reported that no attacks or trap captures occurred before 1–2 d of at least 20°C maximum daily temperatures (Reding et al. 2013b), which may occur in February (Werle et al. 2015) in southernmost states, March in Tennessee (Oliver and Mannion 2001), and April in Ohio (Reding et al. 2013b). Repeated applications of insecticides throughout the spring flight period are required to obtain a reasonable level of control, but often treatments are still inconsistent (Oliver and Mannion 2001, Mizell and Riddle 2004, Frank and Sadof 2011, Reding et al. 2013a).

The ambrosia beetle species targeting nursery stock attack only stressed trees emitting ethanol (Ranger et al. 2010, 2015a), but there are no practical methods currently available to growers for measuring tree stress in the field. Variation in response by individual trees, tree cultivars, and species to stress caused by flood, frost, or other factors results in spatially and temporally unpredictable attack patterns by the beetles (La Spina et al. 2013, Ranger et al. 2013a, Reed et al. 2015). While prophylactic sprays of insecticides are not encouraged by pest management practitioners, the difficulty of predicting when and where attacks will occur is often addressed by blanket sprays of pyrethroids or other labeled products on fields of preferred host tree species.

Recent studies of ambrosia beetles have confirmed that source populations in nursery production originate from overwintering sites in forests adjacent to production fields (Ranger et al. 2013a, Reding et al. 2015, Werle et al. 2015). Therefore, one non-chemical avenue of management for ambrosia beetles in nursery production is to intercept beetles dispersing into ornamental nurseries from adjacent forests. Trees injected with increasing doses of ethanol attracted greater numbers of ambrosia beetles (Ranger et al. 2012). The dose-dependent response to ethanol suggests that artificially stressed trap trees may be able to draw ambrosia beetles away from other nursery stock.

Ambrosia beetles are routinely monitored with ethanol-baited traps (Klimetzek et al. 1986, Reding et al. 2011, Ranger et al. 2012). Other potential host plant attractants such as conophthorin (VanDerLaan and Ginzel 2013, Ranger et al. 2014, Miller et al. 2015) or α -pinene (Miller and Rabaglia 2009, Ranger et al. 2011) have been evaluated in combination with ethanol, but they did not consistently improve trap captures for nursery species, evidence which supports ethanol as the primary attractant. Deploying ethanol-baited traps along the borders of nursery fields to intercept beetles from adjacent forested areas may reduce the number of ambrosia beetles entering nursery fields, provided that the traps are more attractive than nearby stressed nursery trees.

Based on the aforementioned studies, we hypothesized that ethanol-based interception techniques could be useful for reducing ambrosia beetle pest pressure on crop trees grown in ornamental nurseries. Thus, the objective of this study was to investigate two trapping methods: (1) ethanol-injected trap trees and (2) ethanol-baited soda bottle traps—as a means of protecting nursery trees from ambrosia beetle attacks. The results of this study will inform future directions of management research for ambrosia beetle pests of nursery stock.

Materials and Methods

Trees and Ethanol Injection Procedures

Two species of host tree, zelkova (*Zelkova serrata* Makino [Thunb.]) (Rosales: Ulmaceae) and sugar maple (*Acer saccharum* Marshall)

(Sapindales: Sapindaceae), were purchased as 1.5 cm bare root liners and transplanted into 11.3-liter (number 3) black plastic nursery containers (Hummert International, St. Louis, MO) with Pro-Gro Mix (Barky Beaver, Moss, TN; 78% pine bark, 12% peat moss, 10% sand, and 4.8 kg lime/m³ with a manufacturer's reported bulk density range of 240.3–256.3 kg/m³) amended with fertilizer (18N-6P-12K Osmocote fertilizer with micronutrients, ICL Fertilizers Company, Dublin, OH) and maintained with overhead irrigation until use in field tests. Histological grade ethanol (70%) (Sigma Aldrich, St. Louis, MO) was diluted with tap water before use. Ethanol was injected into trees using a Tree I.V. (Arbojet, Woburn, MA) designed for injecting trees with insecticides (Ranger et al. 2010).

Trap Trees Arranged Parallel With Forest Edge

The purpose of the following study was to evaluate the capability of ethanol-injected trap trees to protect nearby stressed nursery trees. As part of tests conducted in 2013 and 2015, sugar maple tree pairs were oriented parallel to the forest edge, simulating trees in the first row of a nursery block (Fig. 1A).

Two ethanol injection treatments included a high (75 ml of 50% ethanol [trap tree]) or low (75 ml of 1% ethanol [simulated stressed nursery tree]) dosage of ethanol. For the two ethanol injection treatments, two spacing treatments were tested including low- and highdose treatments adjacent (i.e., containers touching) or low- and high-dose treatments widely spaced (i.e., 15 m spacing) (Fig. 1A). All treatments were placed 10 m from the edge of a deciduous forest with container orientation parallel to the forest edge. Treatments within a block were spaced 15 m apart with 20 m between replicates. Each treatment pair was replicated six times in 2013 (31 May to 21 June) and 2015 (22 April to 13 May) in a randomized complete block design. Attacks on trees were counted three times per week for the duration of each test to determine if the high-dose 'trap' tree would pull beetles away from the low-dose 'simulated stressed nursery' tree. Tree pairs (adjacent or distant) were re-randomized weekly throughout each test. The tests were conducted at the Tennessee State University Otis L. Floyd Nursery Research Center in McMinnville, TN (TSU-NRC; 35.70747°N, -85.74467°W) along the edge of a forested area of oak-hickory adjacent to the Collins River.

Trap Trees Arranged Perpendicular With Forest Edge

The purpose of this study was to further evaluate the capability of ethanol-injected trap trees to protect nearby stressed nursery trees. As part of tests conducted in 2014 and 2015, zelkova tree pairs were oriented perpendicular to the forest edge, simulating trees in the first and second or first and fourth rows of a nursery block (Fig. 1B).

As before, trees were injected with high (75 ml of 50% ethanol) or low (75 ml of 1% ethanol) dosages of ethanol. Low- and highdose tree pairs were arranged adjacent (i.e., containers touching) or widely spaced (15 m spacing) in an orientation perpendicular to the edge of a deciduous forest (Fig. 1B). For each tree pair, the tree closest to the forest was 10 m from the edge and low- or high-dose treatments were assigned randomly. Procedures for the experimental design were identical to the previous experiment except each treatment pair was replicated in a randomized complete block design four times in 2014 (1–21 May) and six times in 2015 (21 April to 11 May [three replicates] and 15 May to 8 June [three replicates]). The tests were conducted at the TSU-NRC.

Trap Line Interception Test

The purpose of the trap line study was to evaluate the effect of different ethanol-baited trap densities positioned along a forest edge for reducing attacks on sentinel trees or captures in sentinel traps positioned farther within a nursery field (Fig. 2). The experiment was conducted along the edge of a predominantly deciduous forest of oak-hickory with some mixed pine on a power line right-of-way at Arnold Engineering Development Corporation Air Force Base (AEDC), Tullahoma, TN (35.3925°N, -86.08583°W). Plots consisted of 60-m trap lines with traps within lines spaced at 5, 10, 20, or 60 m apart for a total of 13, 7, 4, or 2 traps per treatment, respectively (Fig. 2). An additional treatment had no traps (control treatment). Trap lines were positioned 5 m from the forest edge. Each trap line density treatment was replicated four times in a randomized complete block design with 60 m between each trap line within a replicated block and 100 m between blocks.

Ethanol-baited intercept traps similar to previously published designs (Oliver et al. 2004, Klingeman et al. 2017) were constructed using 2-liter soda bottles hung upside down 1 m above the ground from metal rods. Each soda bottle had three window slots 8 cm wide by 15 cm tall cut in the bottle side to allow beetle entry. The mouthend of a 50-ml plastic collection tube was attached to the mouth-end of a 2-liter soda bottle with a threaded tube ('Tornado Tube', item #WTUB-500, Steve Spangler Science, Englewood, CO). The inside of the tornado tube was enlarged with a drill to allow beetles to fall more easily into the collection container. The collection containers were filled with ethanol-free Splash RV & Marine antifreeze (Splash Products Inc., St. Paul, MN; composed of propylene glycol, proprietary plant-derived additive corrosion inhibitor, and dye) as an ambrosia beetle drowning and preserving solution. All traps on

the trap line were baited with an ethanol lure hung from the inside top of the soda bottle trap (AgBio, Inc., Westminster, CO; 65 mg/d release rate). Soda bottle traps were deployed on 28 April 2016, and beetles were collected weekly for the duration of the study.

Sentinel traps used to assess the efficacy of the trap lines were made from a sheet of clear plastic that was rolled and stapled to create a cylinder 34.1 cm long by 12.8 cm diameter and coated in Pestick insect glue (Phytotronics, Earth City, MO). Each sentinel trap was baited with the same lure used in the intercept traps, and this was positioned at the top, center of the cylinder. Sentinel traps were divided into quadrants facing in four directions and were labeled as follows: northeast = trap quadrant facing north to east $(0-90^{\circ})$, southeast = trap quadrant facing east to south $(90-180^{\circ})$, southwest = trap quadrant facing south to west (180-270°), and northwest = trap quadrant facing west to north (270-360°). The forest border was oriented north-east from all sentinel traps, thus, the half of the cylinder oriented toward the forest included both the northeast and southeast quadrants. Sentinel traps were placed 10 m from the intercept trap line into the open field (Fig. 2). Ambrosia beetles on the sentinel traps were collected weekly from 28 April to 25 May 2016.

On 25 May 2016, sentinel traps in the field were replaced with potted sugar maple trees placed 10 m from the intercept trap line (Fig. 2). Due to low beetle response, sugar maple sentinel trees were injected with 75 ml of 10% ethanol on 15 June 2016, and on 22 June 2016, they received a second injection with 75 ml of 50% ethanol to

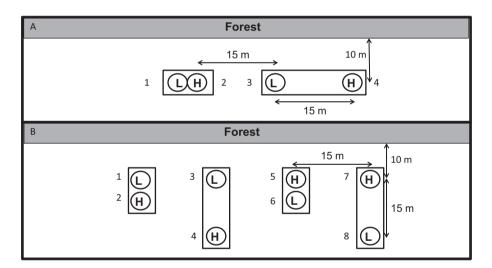


Fig. 1. (A) Field layout used to test the capability of ethanol-injected 'high-dose' trap trees to reduce attacks on 'low-dose' trees when positioned in parallel with the forest edge, simulating trees in the first row of a nursery block. Numbers 1–4 represent individual trees. (B) Field layout used to test the capability of ethanol-injected 'high-dose' trap trees to reduce attacks on 'low-dose' trees when positioned perpendicular to the forest edge, simulating trees in the first and second or first and fourth rows of a nursery block. L = tree received low dose (75 ml injection of 1% ethanol) (simulates stressed nursery tree), H = tree received high dose (75 ml injection of 50% ethanol) (trap tree).

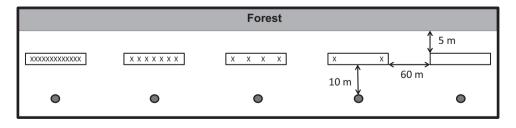


Fig. 2. Field layout used to test the effect of different ethanol-baited trap densities positioned along a forest edge for reducing attacks on sentinel trees or captures in sentinel traps positioned farther within a nursery field. An 'X' represents an ethanol-baited soda bottle trap; a rectangle indicates a 60-m trap line with traps spaced at 5, 10, 20, and 60 m; a filled circle represents a sentinel trap or trap tree.

further increase beetle response. The sentinel trees were checked for attacks three times per week once attacks began to occur. Trap trees were removed on 15 July 2016 and trap line traps were removed on 19 July 2016 (4 wk of collection). Trees were watered as needed. Beetles captured in traps and reared from trees were identified to species (Wood 1982, Gomez et al. 2018).

Statistics

Count data for ambrosia beetle trapping and attacks were fit to a negative binomial distribution and analyzed with a generalized linear interactive model in SAS 9.3 (PROC GENMOD; SAS Institute 2018). Experiments were analyzed separately by year. Higher order models were evaluated first and non-significant factors and interactions were removed from the final model. The model for the parallel-to-forest experiment was: attacks = tree spacing + ethanol dosage; for the perpendicular-to-forest experiment, beetle catches = placement + tree spacing + ethanol dosage; and the trap line intercept experiment, (1) intercept trap catches = trap spacing and (2) sentinel trap catches = trap spacing. Percent change in sentinel trap captures was similarly fit to a normal distribution and analyzed with a generalized linear model (percent change = trap spacing; PROC GENMOD). Treatment means were separated by a pairwise Tukey–Kramer test (α = 0.05).

Results

Trap Trees Arranged Parallel With Forest Edge

Acer saccharum trap trees injected with ethanol and positioned in parallel along a forest edge were subsequently attacked by ambrosia beetles (Fig. 1A, Table 1). Trap trees injected with a high (50%) dose of ethanol had significantly more ambrosia beetle attacks than trees injected with a low (1%) dose of ethanol in both 2013 and 2015 (Table 1). Following both test periods, a combined total of 4,110 Scolytinae beetle attacks occurred on the high-dose trap trees compared to 50 attacks on the low-dose trees.

In 2013, more attacks occurred on the low-dose trees when they were spaced 15 m from their corresponding high-dose trap trees compared to low-dose trees that were in close proximity to the

high-dose trap trees (Table 1). However, a spacing effect was not observed in 2015, and no difference in attacks occurred on the low-dose trees, whether they were distant or in close proximity to the high-dose trap trees.

Trap Trees Arranged Perpendicular With Forest Edge

Zelkova serrata trap trees injected with ethanol and positioned perpendicular to a forest edge were likewise discriminated by ambrosia beetles (Fig. 1B, Table 2). Trap trees injected with a high (50%) dose of ethanol had more attacks by ambrosia beetles than trees injected with a low (1%) dose of ethanol in both 2014 and 2015 (Table 2). Following both test periods, a combined total of 4,293 Scolytinae beetle attacks occurred on the high-dose trap trees compared to 38 attacks on the low-dose nursery trees.

In 2014 and 2015, no difference occurred in attacks on the low-dose trees that were in close versus distant proximity to the high-dose trap trees (Table 2). Proximity of the low- and high-dose trees at 5 versus 15 m from the forest edge also did not affect the number of attacks on *Z. serrata* trees.

Trap Line Interception Test

The density of ethanol-baited intercept traps (2-13 traps per 60-m trap line) did not significantly affect the number of beetles collected in these traps (Figs. 2 and 3). Sentinel A. saccharum trees positioned in front of trap lines containing two and four intercept traps per 60 m were associated with fewer total attacks compared to trap lines without any intercept traps or trap lines with seven intercept traps (Fig. 4). However, sentinel sticky traps positioned in front of trap lines containing any number of intercept traps were not associated with fewer total ambrosia beetle catches or pest ambrosia beetle catches than lines with no intercept traps. Sentinel traps associated with lines with four intercept traps captured significantly more total pest species than any other treatment. However, Ambrosiodmus rubricollis Eichhoff captures in sentinel traps with two intercept traps were significantly lower compared to trap lines containing no intercept traps per trap line (Fig. 4). Additionally, the percentage changes in sentinel trap captures in the presence of any number of intercept traps (relative to lines without traps) were not statistically significant (Table 3).

Table 1. Ambrosia beetle attacks on ethanol-injected Acer saccharum trees placed parallel to a forest edge

Test year	Treatment number ^a	Tree spacing b	Ethanol dosage ^c	Mean ± SE ambrosia beetle attacks/tree ^d	Total attacks
2013	1	Adjacent	Low	1.2 ± 0.6c	7
	2		High	$119.3 \pm 37.8a$	716
	3	Distant	Low	6.0 ± 2.5 b	36
	4		High	$192.8 \pm 41.5a$	1,157
	χ^2	4.22	29.19		
	df	1	1		
	P-value	0.04	< 0.0001		
2015	1	Adjacent	Low	$1.0 \pm 0.2b$	6
	2		High	$192.7 \pm 14.9a$	1,156
	3	Distant	Low	$0.2 \pm 0.1b$	1
	4		High	$180.2 \pm 9.1a$	1,081
	χ^2	0.79	38.75		
	df	1	1		
	P-value	0.37	< 0.0001		

^aTreatment number corresponds to numbers on tree plot arrangement in Fig. 1A.

^bAdjacent, trees with containers touching. Distant, trees with containers spaced 15 m apart.

^cAll trees received a 75 ml volume injection of ethanol at a concentration of either 1% (low dosage) or 50% (high dosage).

 $[^]d$ Treatments were fit to a negative binomial distribution and analyzed with a generalized linear interactive model (PROC GENMOD; SAS Institute 2018), and treatment means were separated by pair-wise Tukey–Kramer test (P < 0.05). Means followed by different letters were significantly different.

Table 2. Ambrosia beetle attacks on ethanol-injected Zelkova serrata trees placed perpendicular to a forest edge

Test year	Treatment nnumber ^a	Placement ^b	Tree spacing ^c	Ethanol dosage ^d	Mean ± SE ambrosia beetle attacks/tree ^e	Total attacks
2014	1	Near	Adjacent	Low	0.0 ± 0.0b	0
	2	Far		High	$196.0 \pm 24.7a$	784
	3	Near	Distant	Low	$0.3 \pm 0.3b$	1
	4	Far		High	$84.0 \pm 52.6a$	336
	5	Near	Adjacent	High	$157.0 \pm 62.4b$	628
	6	Far		Low	$0.0 \pm 0.0b$	0
	7	Near	Distant	High	$147.0 \pm 21.8b$	588
	8	Far		Low	$0.3 \pm 0.3 \text{ b}$	1
	χ^{2e}	0.08	0.35	55.08		
	df	1	1	1		
	P-value	0.78	0.55	< 0.0001		
2015	1	Near	Adjacent	Low	$4.7 \pm 0.8b$	28
	2	Far		High	$100.8 \pm 7.4a$	605
	3	Near	Distant	Low	$0 \pm 0b$	0
	4	Far		High	$48.5 \pm 3.7a$	291
	5	Near	Adjacent	High	$67.8 \pm 4.9a$	407
	6	Far		Low	$1.0 \pm 0.2b$	6
	7	Near	Distant	High	$109.0 \pm 8.4a$	654
	8	Far		Low	$0.3 \pm 0.1b$	2
	χ^2	0.54	3.6	43.12		
	df	1	1	1		
	P-value	0.46	0.06	< 0.0001		

^aTreatment number corresponds to numbers on tree plot arrangement in Fig. 1B.

 $^{^{\}circ}$ Treatments were fit to a negative binomial distribution and analyzed with a generalized linear interactive model (PROC GENMOD; SAS Institute 2018), and treatment means were separated by pair-wise Tukey–Kramer test (P < 0.05). Means followed by different letters were significantly different.

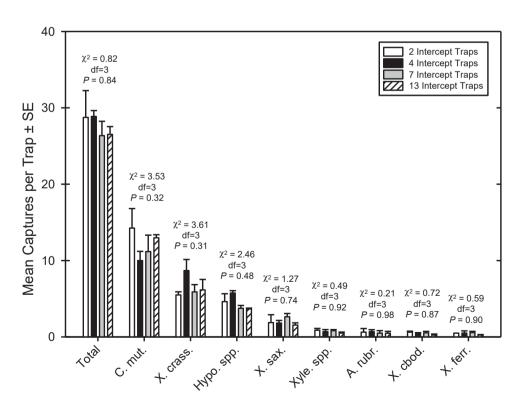


Fig. 3. Average number of borers captured per intercept trap (±SE) for different trap densities. Total = all Scolytinae species combined; C. mut. = Cnestus mutiliatus; X. crass. = Xylosandrus crassiusculus; Hypo. spp. = Hypothenemus spp.; X. sax. = Xyleborinus saxesenii; Xyle. spp. = Xyleborus spp.; A. rubr. = Ambrosiodmus rubricollis; X. cbod. = Cyclorhipidion bodoanum; X. ferr. = Xyleborus ferrugineus.

^bNear, trees placed next to forest edge. Far, trees placed interior to first tree.

^cAdjacent, trees with containers touching. Distant, trees with containers spaced 15 m apart.

^dAll trees received a 75 ml volume injection of ethanol at a concentration of either 1% (low dosage) or 50% (high dosage).

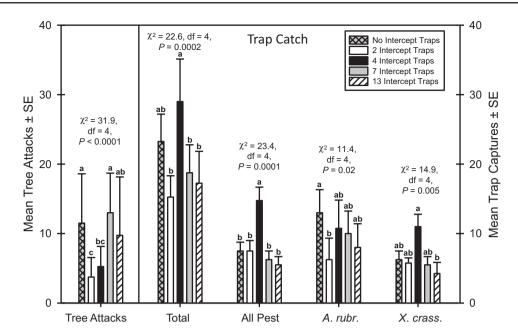


Fig. 4. Average number of ambrosia beetle attacks on field sentinel trees (±SE) or total beetles captured on field sentinel traps (±SE) placed in front of plots with different intercept trap densities. Attacks = galleries found on sugar maple trees; All = all ambrosia beetle species trapped; All Pest = trap captures of all species known to attack nursery trees combined including *Xylosandrus crassiusculus*, *X. germanus*, *Cnestus mutilatus*, and *Xyleborinus saxesenii*. A. rub. = *Ambrosiodmus rubricollis* trapped; X. crass. = *Xylosandrus crassiusculus* trapped. Other species had too few trap captures for statistical analysis. Means with different letters within groups were different by pair-wise Tukey–Kramer test (*P* < 0.05).

Table 3. Total number of most common scolytine beetles in each intercept trap density treatment

Intercept trap distance (m)	No. of intercept traps ^a	Intercept trap total ^b	Sentinel ^c total	% Change in capture ^d
0	0	_	93	0ab
60	2	187d	61	-34.4b
20	4	354c	116	+24.7a
10	7	597b	75	-19.4b
5	13	1,127a	69	-25.8b
χ^{2e}		56.72	6.21	14.01
df		3	4	4
P-value		< 0.0001	0.18	0.01

"Traps were arranged in a 60-m trap line with the specified intercept trap densities equally spaced within that 60-m line.

*Count treatments were fit to a negative binomial distribution and analyzed with a generalized linear model (PROC GENMOD; SAS Institute 2018), and treatment means were separated by pair-wise Tukey–Kramer test (P < 0.05). % Change in capture was fit to a normal distribution and analyzed in the same manner. Means followed by different letters were significantly different.

Higher number of traps in each 60-m trap line also increased the total number of beetles trapped within the line. For instance, 187 total specimens were collected in intercept traps in plots containing two traps compared to 1,127 specimens in plots containing 13 intercept traps (Table 3).

Overall, all intercept trap lines captured 2,809 Scolytinae beetles during the study, with the four most abundant species being C. mutilatus, X. crassiusculus, Xyleborinus saxesenii (Ratzeburg), and A. rubricollis (Table 4). The top four most abundant species caught in the sentinel field traps were A. rubricollis, X. crassiusculus, C. mutilatus, and Xyleborus ferrugineus (Fabricius). Orientation of all Scolytinae landings on the sentinel sticky traps was not concentrated in any single quadrant (Table 5). However, for all Scolytinae and A. rubricollis, the northwest quadrant (right angles to the direction of the forest and intercept traps) trapped

significantly fewer beetles than either the northeast (facing the forest) or southwest quadrants.

The four species excavated from attacked sentinel *A. saccharum* trees were *C. mutilatus*, *X. crassiusculus*, *A. rubricollis*, and *X. saxesenii*. The non-native species *C. mutilatus* and *X. crassiusculus* accounted for 94.6% of total attacks on the sentinel trees (Table 4).

Discussion

The goal of this study was to explore the use of trap trees or other trap interception methods as a tool for managing ambrosia beetles in nursery fields, which often border forested areas. Based on previous research regarding the dose-dependent nature of ambrosia beetle attraction to ethanol (Klimetzek et al. 1986, Reding et al.

^bNursery pest beetles in this analysis included A. rubricollis, Cyclorhipidion bodoanum (Reitter), X. crassiusculus, X. germanus, X. ferrugineus, X. saxesenii, and C. mutilatus.

^{&#}x27;Sentinel total includes both field sentinel sticky trap catches and field sugar maple tree attack totals.

^dPercentage change was compared to an absence of intercept traps (93 beetles total).

Table 4. Beetle species collected during interception tests

Species	Trap captures				Beetles dissected from	
	Interception traps		Sentinel field traps		sentinel trees	
	Total	%	Total	%	Total	%
Cnestus mutilatus	1,262	44.9ª	19	4.6	152	81.7
Xylosandrus crassiusculus	668	23.8	131	31.9	24	12.9
Hypothenemus spp.	425	15.1	17	4.1	0	0.0
Ambrosiodmus rubricollis	55	2.0	192	46.7	5	2.7
Xyleborinus saxesenii	199	7.1	11	2.7	5	2.7
Xyleborus ferrugineus	41	1.5	14	3.4	0	0.0
Cyclorhipidion bodoanum (Reitter)	40	1.4	5	1.2	0	0.0
Xyleborus affinis Eichoff	22	0.8	5	1.2	0	0.0
Xylosandrus germanus	16	0.6	5	1.2	0	0.0
Thysanoes spp.	14	0.5	2	0.5	0	0.0
Bostrichidae spp.	13	0.5	0	0.0	0	0.0
Pityophthorus spp.	13	0.5	0	0.0	0	0.0
Cnesinus strigicollis LeConte	11	0.4	0	0.0	0	0.0
Monarthrum fasciatum (Say)	9	0.3	2	0.5	0	0.0
Monarthrum mali Wood & Bright	6	0.2	1	0.2	0	0.0
Unknown Scolytinae	1	0.0	5	1.2	0	0.0
Xyleborus impressus Eichoff	3	0.1	0	0.0	0	0.0
Dryocoetes spp.	2	0.1	1	0.2	0	0.0
Pseudopityophthorus spp.	2	0.1	0	0.0	0	0.0
Dryoxylon onoharaensum (Murayama)	1	0.0	0	0.0	0	0.0
Euwallacea validus (Eichoff)	1	0.0	0	0.0	0	0.0
Hylesinini spp.	1	0.0	0	0.0	0	0.0
Orthotomicus caelatus (Eichoff)	1	0.0	0	0.0	0	0.0
Pityoborus comatus (Zimmerman)	1	0.0	0	0.0	0	0.0
Pseudopityophthorus pruinosus (Eichoff)	1	0.0	0	0.0	0	0.0
Xyleborus celsus Eichoff	1	0.0	0	0.0	0	0.0
Xyleborus xylographus Say	0	0.0	1	0.2	0	0.0
Total	2,809		411		186	

Table 5. Mean ± SE trap captures in field sentinel sticky traps by cardinal direction for two common ambrosia beetle species and pooled Scolytinae

Direction of trap quadrant	Xylosandrus crassiusculus	Ambrosiodmus rubricollis	All Scolytinae	
Northeast	$2.3 \pm 0.5a^a$	3.3 ± 0.6a	6.9 ± 0.9a	
Southeast	$1.3 \pm 0.3a$	$3.7 \pm 0.7a$	$6.3 \pm 0.9a$	
Southwest	$1.4 \pm 0.4a$	$1.8 \pm 0.6ab$	$4.3 \pm 0.7ab$	
Northwest	$1.7 \pm 0.4a$	$0.9 \pm 0.2b$	3.2 ± 0.5 b	
χ^2	5.22	19.72	16.48	
df	3	3	3	
P-value	0.16	0.0002	0.0009	

 $^{^{}a}$ Means followed by different letters were significantly different at P < 0.05 by pair-wise Tukey–Kramer test.

2011, Ranger et al. 2012), we hypothesized that trees injected with artificially high doses of ethanol could be used to pull beetles away from trees with relatively low levels of stress. In addition, we predicted that mildly stressed nursery trees would suffer more attacks when spaced farther (15 m) from 'protective' highethanol-dose trap trees. In both test years, 32- to 1,000-fold more beetles attacked the trap trees than low-stress trees. The pattern was observed in both the adjacent and distant treatments and is consistent with observations in nursery fields where some trees can have hundreds of attacks, whereas neighboring trees have a few or no attacks. While the difference between attacks on high-and low-ethanol-dose trees was consistent, the difference in attack levels observed between adjacent and distantly spaced trap and low-stress trees was not.

Despite the strength of the trap trees to attract beetles, the low-dose trees still suffered some attacks. In one of the two tests (Table 1, trap lines parallel to forest, 2013), a significant number of beetle hits were observed on low-dose trees when spaced 15 m from their paired trap tree (6.0 ± 2.5 attacks per tree). This level of damage would not be acceptable to most growers. On small trees, six attacks could be sufficient to girdle a tree trunk. However, attack levels were much lower on the low-dose trees in 2014. It is possible that the differences observed between years were due to a higher level of constitutive stress in the low-dose trees before injection in 2013. While all trees were handled in the same manner during tests, prior damage from frost (La Spina et al. 2013), flooding (Ranger et al. 2013a) or other stressful environmental conditions could have played a role in the greater number of attacks on those trees. Any one of these factors

may have contributed to the observed variation but it is impossible to know whether the plants experienced any stressors prior to purchase. Differences in beetle activity or populations could be another factor in attack variability among years. However, the high-ethanol trees caught almost identical numbers of beetles in both years. This would indicate that either the population was the same in both years or that beetles rejected the host plants once the trees reached a specific level of infestation. In one of the treatments in tests with trees arranged perpendicular to the forest, low-dose ethanol trees placed adjacent to trap trees experienced unacceptably high numbers of attacks (28 hits total) during 2 y of testing; otherwise low-dose ethanol trees, regardless of spacing, had few or no attacks. Again, we suspect that the higher numbers of attacks on some individual trees was due to higher initial stress levels, since nearly 80% of those attacks were on two tree replicates.

Our design for the trap tree experiments was based on the typical nursery field production scheme of planting 5-10 rows of trees with a road break between blocks for tractor access. Depending on the planting density, 15 m spacing would approximate a distance between the first and fourth tree row in a nursery block. While this is a reasonable distance to consider in nursery production, it may be too close to achieve independence between the high- and lowdose trees. A lack of consistent distance effect in the parallel and perpendicular experiments makes it difficult to conclude definitively that the trap trees did or did not provide significant shielding to lowstress trees from attack. Spacing trees in check treatments more than 15 m apart might have resulted in a stronger relationship between proximity and number of attacks. It is also possible that the trap trees created a spillover effect, where beetles were drawn to the area of the trap tree but upon landing on the low-stress tree, found it suitable for attack. A trapping program developed for Gnathotrichus sulcatus (LeConte) in sawmills observed similar spillover from pheromone baited traps onto adjacent piles of lumber (McLean and Borden 1977). Based on this observation, the authors recommended that slabbing be placed adjacent to the traps in order to capture the few stray beetles that land nearby. This material could then be mulched to kill the developing larvae. If spillover of nursery pests from trap trees onto nearby hosts is unavoidable, it would be worthwhile to investigate how far that spillover effect extends. If a trap tree increases attacks on one or two neighboring trees, but protects the remainder of a field from attack, trap trees may nonetheless be a viable management option. In addition, trap trees may provide better protection if located in the interior of the nursery away from the forest edge. Conversely, trap trees placed internally to the forest edge may prevent beetles from dispersing into neighboring nurseries. If future investigations can demonstrate the usefulness of trap trees in one of the aforementioned scenarios, culled trees can be used for trap tree management programs without growers incurring additional crop losses. Trap trees could then be burned or mulched to prevent larval development.

While the forest border intercept trap method did capture thousands of Scolytinae beetles, hundreds more were able to reach the sentinel traps and trees. Among the key nursery pest species captured in this study, at least $\sim 20\%$ (n=539) were able to penetrate the intercept trap line. These results are in agreement with a separate mass-trapping experiment where mean ambrosia beetle captures at the nursery interior were not significantly different for plots that were either protected or unprotected by a row of edge intercept traps (Werle et al. 2017). In the Werle et al. study, despite nearly 90% of total beetle captures occurring in the intercept traps, the presence of these ethanol-baited traps at the nursery edge appeared to draw more beetles toward the plots from outside, and thus facilitated dispersal

into the nursery interior. Similarly, in the present study, an increase in the number of intercept traps increased the number of beetles that were trapped at the site (Table 3). It is therefore unlikely that a field border of intercept traps between the forest and the desired area of protection (i.e., where were located the sentinel sticky traps or sugar maples simulating a stressed nursery crop) would remove sufficient beetle numbers from the surrounding environment to protect a nursery field on its own. Other ethanol-sensitive ambrosia beetles, such as coffee berry borer (Hypothenemus hampei [Ferrari]), have been evaluated for efficacy of similar mass trapping programs (Dufour and Frérot 2008). Mass trapping for coffee berry borer is effective perhaps due to the limited availability of host plants adjacent to the coffee plantations. In contrast, ambrosia beetle pests of nursery trees have an abundant supply of wild host material in adjacent forested areas, making management plans based on removing beetles from the landscape perhaps unrealistic. In addition, the maintenance of ethanol-baited traps at a density higher than 60 m spacing evaluated here would not be feasible under most production scenarios based on the authors' conversations with growers. If the trap line method is investigated further, tests should focus on traps deployed at distances greater than 60 m and deeper into the forest border to deter beetles from dispersing into adjacent fields.

The northwest quadrant of the sentinel traps captured fewer scolytines than the northeast quadrant which faced the forest edge. The directionality of trap capture was not as strong as expected. If ambrosia beetles circle an odor source prior to landing, such behavior could explain the lack of resolution.

Here, we presented two methods proposed as 'pull' components for a potential 'push-pull' management program. Neither trapping method was sufficient to protect nursery trees alone. Several studies have been undertaken to evaluate the efficacy of repellent 'push' semiochemicals like verbenone for management of ambrosia beetle pests of the nursery industry (Burbano et al. 2012, Ranger et al. 2013b, Hughes et al. 2017). The results of these 'push' studies have been variable across ambrosia beetle species, both spatially and temporally, and none of the investigated repellents resulted in complete protection of host trees. Like the pull components evaluated here, it is unlikely these push methods will be successful alone.

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