

Attraction of Invasive Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) to Ethanol-Treated Tree Bolts

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Abstract

Ethanol-treated bolts (tree stem sections) have potential as monitoring and pesticide screening tools for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Bolts were infused with ethanol by immersing them for at least 24 h. Attacks on ethanol-treated bolts by *Xylosandrus* species were compared with captures in ethanol-baited traps. Bolts infused in ethanol were usually as attractive or more attractive to *Xylosandrus germanus* (Blandford) than ethanol-baited bottle traps. *Xylosandrus crassiusculus* (Motschulsky) were more attracted to bolts than trap in some experiments, but numbers were low and differences were usually not significant. Two techniques for treating bolts with ethanol were compared. Attraction of ambrosia beetles to ethanol-infused bolts were compared with bolts with a drilled cavity filled with ethanol. Drilled bolts filled with ethanol were attractive to *X. germanus* and were reliably attacked, but numbers of beetles were often lower than in traps and infused bolts. Aged and fresh ethanol-infused bolts were compared with evaluate residual attractiveness. Bolts aged 7 d usually had fewer *X. germanus* than fresh bolts and traps, and bolts aged 14 d had no beetles. Ethanol-infused bolts from different species of trees were compared. *Xylosandrus germanus* attacked all species tested with more attacks usually in red maple (*Acer rubrum* L.). *Anisandrus maiche* Stark was attracted to ethanol-infused bolts indicating it may attack trees emitting ethanol. Bolts attracted fewer nontarget species than traps, but residual attraction was much less. The selectivity of ethanol-treated bolts for *Xylosandrus* species should make them useful for monitoring and screening pesticides against those species.

Key words: *Xylosandrus germanus*, *Anisandrus maiche*, red maple, elm, residual attraction

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are serious pests of ornamental tree nurseries and other tree crops in North America. Damaging species colonize tree stems by boring into the xylem and creating galleries that they inoculate with symbiotic fungi (Wood 1982, Weber and McPherson 1983, Ranger et al. 2015). Symbiotic fungi are the food source for adults and larvae and some species lay eggs only after their symbiotic fungi start growing (French and Roeper 1972, Weber and McPherson 1983, Peer and Taborsky 2005). Attacks by ambrosia beetles often lead to wilting, stem die-back, or death of nursery trees. However, the small size and cryptic nature of ambrosia beetles make them difficult to detect before damage occurs.

Management of ambrosia beetles in tree crops relies on maintaining health of trees to reduce attractiveness (Ranger et al. 2013) and application of trunk sprays of insecticides to prevent entry of beetles into trees (Frank and Sadof 2011). There are currently no effective rescue treatments for eliminating ambrosia beetles within trees once beetles are established. The invasive species *Xylosandrus*

crassiusculus (Motschulsky) and *Xylosandrus germanus* (Blandford) are two of the most damaging ambrosia beetles in ornamental tree nurseries in North America (Oliver and Mannion 2001, Fulcher et al. 2012, Reding et al. 2013b). Both species overwinter as adults in host trees, then emerge in spring to colonize new hosts. *Xylosandrus crassiusculus* and *X. germanus* colonize primarily deciduous trees and have large host ranges (>120 and >200 species, respectively) (Wood 1982). Both species preferentially colonize physiologically stressed trees (Ranger et al. 2012, Reding et al. 2013b), and tend to emerge from overwintering sites during postdormancy development of common ornamental tree species (Oliver and Mannion 2001, Reding et al. 2013a). *Xylosandrus crassiusculus*, *X. germanus*, and many other species are attracted to ethanol emitted from trees under certain types of physiological stress (Ranger et al. 2010, 2013). Furthermore, ethanol is beneficial to the symbiotic fungi of *X. crassiusculus* and *X. germanus*, and may be crucial for successful colonization (Ranger et al. 2018).

Ethanol-baited traps have been used to monitor flight activity of *X. crassiusculus*, *X. germanus*, and other ambrosia beetles in tree crops (Oliver and Mannion 2001, Reding et al. 2011). Monitoring can be used by growers to synchronize protective treatments with ambrosia beetle activity. However, ethanol baited traps capture many species of Scolytinae, wherein growers may have difficulty determining whether damaging species are present. Development of more selective techniques for monitoring damaging ambrosia beetles should make monitoring easier for growers. Ethanol-injected trees are attractive to *Xylosandrus* species and usually attract fewer other species of Scolytinae than ethanol-baited traps (Reding et al. 2011, 2013b, 2015, 2017). Ethanol-infused sections of tree stems (bolts) are attractive to *X. crassiusculus* and *X. germanus* (Mayfield and Hanula 2012, Ranger et al. 2012, Reding et al. 2013a), and might be similar to ethanol-injected trees in regard to selectivity. Ethanol-treated bolts may also be useful for screening insecticides against ambrosia beetles. Injecting living trees with ethanol has been effective for ensuring attack pressure by ambrosia beetles for testing protective treatments against them (Frank and Sadof 2011, Ranger et al. 2011a, Reding et al. 2013b). However, using living trees to screen a wide variety of materials is expensive and labor intensive. Using ethanol-treated bolts for preliminary screening of materials would be more economical and less laborious. Then, materials that demonstrate the most potential could be more thoroughly tested on living trees.

The primary objectives of the current research were 1) to test attraction of ethanol-treated bolts to damaging ambrosia beetles, especially *X. crassiusculus* and *X. germanus*; 2) evaluate two methods for treating bolts with ethanol; 3) examine the time-period that bolts were attractive; 4) evaluate attractiveness of ethanol-infused bolts from different species of trees.

Materials and Methods

General Methods

Experiments were conducted in commercial nurseries in Lake, Lorain, and Wayne counties, Ohio during 2012, and 2014 through

2017. All nurseries were adjacent to a woodland. Experiments were set up as randomized complete block designs with replications as blocks, at least two blocks per nursery, and blocks at least 50 m apart. Experiments were positioned in a single row parallel to and within 1 m of adjacent woodlands, with positions of treatments within blocks assigned randomly and spaced 20 m apart. Ethanol-baited bottle traps were used in all experiments as a standard technique for monitoring ambrosia beetles (Reding et al. 2011, 2013a), and to compare with bolts for attractiveness and selectivity for *Xylosandrus* spp. Numbers and species of ambrosia beetles excavated from bolts were compared with captures in traps. Traps were baited with commercially available pouch-style dispensers (lures) loaded with 10 ml of 95% ethanol with a release rate of 65 mg/d at a constant 30°C (Standard Release ethanol lures, AgBio, Westminster, CO) (Reding et al. 2011). Each experiment had an ethanol-infused bolt treatment in which bolts were immersed in 10% ethanol for at least 24 h. After removal from ethanol, infused bolts were placed in re-sealable plastic bags and stored at 5°C until use the following day. Bolts were cut from the main stems of young deciduous trees, and were at least 20 cm long, and 2.5–5.0 cm in diameter. A 3.1 cm long screw-eye hook was screwed into the end of each bolt to use for hanging. Bolts and traps were hung 0.5 m above the ground from steel rods. Untreated bolts (no ethanol) were not included in experiments because Ranger et al. (2012) demonstrated that bolts without ethanol were not attractive to *X. germanus*. The ambrosia beetles of main interest were *X. germanus* and *X. crassiusculus*. At the end of each experiment, bolts and specimens in traps were collected to identify beetles. In the laboratory, hand pruners were used to excavate ambrosia beetles from bolts. All Scolytinae from bolts and traps were identified to the species level using available keys (Wood 1982, Rabaglia et al. 2006). Details for all experiments below are given in Table 1.

Attractiveness of Ethanol-Infused Bolts

The goal of these experiments was to compare attractiveness of ethanol-infused bolts and ethanol-baited bottle traps (standard monitoring tools) to *X. germanus* and *X. crassiusculus*. There were four experiments, three conducted in 2012 and one in 2014. Bolts

Table 1. Treatment and timing details for bolt experiments

Experiments	Exp. num.	Dates	Nurseries	Bolt Treatments ^a	n ^b
Attractiveness of Ethanol-Infused Bolts	1	21–28 Mar. 2012	5	red maple	20
	2	12–19 Apr. 2012	5	red maple and American elm	10, 10, 20
	3	19–25 Apr. 2012	5	red maple	20
	4	8–14 May 2014	6	American elm	24
Attractiveness of Drilled + Filled versus Infused Bolts	1	22–29 May 2014	5	red maple	14
	2	5–12 June 2014	5	American hornbeam	12
	3	28 May–10 June 2015	4	American elm	10
	4	18–25 May 2016	5	red maple	10
Attractiveness of Bolts over Time	1	8–16 July 2015	4	red maple fresh, 7-d aged	10
	2	7–12 July 2016	5	red maple fresh, 7-d, 14-d aged	10
Attractiveness of Bolts from Various Tree Species	1	26 Apr.–3 May 2017	5	red maple, black cherry, dogwood	10
	2	17–24 May 2017	5	red maple, black cherry, sumac	10
	3	8–14 June 2017	5	red maple, sassafras, sycamore	10
	4	6–19 July 2017	5	red maple, sassafras, shingle oak	10

There were at least two complete blocks per nursery.

^aAll experiments included ethanol-baited bottle traps as a standard treatment. Bolts were 30 cm long in experiments during 2012 through 2015, 25 cm long in 2016, and 20 cm long in 2017. Red maple (*Acer rubrum* L.), American elm (*Ulmus americana* L.), American hornbeam (*Carpinus caroliniana* Walter), black cherry (*Prunus serotina* Ehrh.), dogwood (*Cornus florida* L.), sumac (*Rhus typhina* L.), sassafras (*Sassafras albidum* (Nutt.) Nees), sycamore (*Platanus occidentalis* L.), shingle oak (*Quercus imbricaria* Michx.).

^bThe replications of bolts and traps for each experiment. In Attractiveness of Bolts and Traps experiment 2, there were 10 replications of each bolt species and 20 replications of traps.

were immersed in 10% ethanol for 24 h, and experiments were set up the day after bolts were removed from ethanol.

Attractiveness of Drilled + Filled Versus Infused Bolts

The goal of these experiments was to compare two methods of treating bolts with ethanol. Drilled + filled bolts could be more compatible with insecticide screening than infused, because immersing bolts in ethanol might influence efficacy of tested materials. There were four experiments, with two conducted in 2014 and one each in 2015 and 2016. The treatments in this set of experiments were drilled + filled bolts, infused bolts, and ethanol-baited traps. Drilled + filled treatments consisted of bolts with a 10 cm long 12.7 mm diameter cavity bored into one end, that was filled with 95% ethanol just prior to deployment. A drill press with a 12.7 mm (0.5 inch) auger bit (Irwin Industrial Tools, Huntersville, NC) was used to bore cavities in the drilled + filled bolts. Then, the drilled bolts were placed in re-sealable plastic bags and stored at 5°C until use the following day. In the field, each cavity was filled with 10 ml of 95% ethanol and the opening was plugged with a cork. Infused bolts were immersed in ethanol for 24 h and handled following procedures previously described.

Attractiveness of Bolts over Time

There were two experiments (one in 2015 and 2016) designed to determine attractiveness of infused bolts over time. Bolts were infused following previously described procedures. Bolt treatments were fresh (used within 24 h after infusion), or aged wherein bolts were immersed in 10% ethanol for 24 h, then placed on a bench in the lab for 7 or 14 d before being used in experiments. After the specified time for aging, bolts were placed in re-sealable plastic bags and stored at 5°C until use the following day.

Attractiveness of Ethanol-Treated Bolts from Various Species of Trees

Experiments were designed to compare attractiveness of ethanol-infused bolts from a variety of tree species commonly planted in nurseries and common in local woodlands. There were four experiments conducted in five nurseries during 2017. All bolts were 20 cm long and infused in 10% ethanol for 72 h. Red maple (*Acer rubrum* L.) was included in all experiments as a standard because it is common in local nurseries and woodlands, and it provided a link to previous experiments.

Data Analysis

All data were log(X+1) transformed for analysis to satisfy assumptions of homogeneity of variances and normality, which were successful. Analysis of variance for a randomized complete block design was used to analyze data on numbers of *X. germanus*, *X. crassiusculus*, and other Scolytinae (non-*Xylosandrus*) with blocks as replicates (Analytical Software 2003). In experiments with more than two treatments, Tukey's HSD ($\alpha = 0.05$) was used to separate means following a significant ANOVA ($P \leq 0.05$). Drilled + Filled experiment 3 was deployed for 2 wk with attacks counted each week; therefore, repeated-measures analysis was used to analyze effects of treatment and treatment \times time on numbers of *X. germanus*.

Results

Ethanol-baited traps tended to attract more nontarget species (non-*Xylosandrus*) than bolts. Twenty-one species of Scolytinae were collected in the various experiments with 19 species collected from traps and 11 from bolts (Tables 2 and 3). There were 10 species that occurred only in traps (Tables 2 and 3) and two found only in bolts

Table 2. Species of Scolytinae collected in the bolt and trap experiments of 2012 through 2016

Species	Total Beetles									Grand total
	Bolts versus Traps ^a		Drilled versus Infused vs Traps ^a			Fresh versus Aged vs Traps ^a				
	Bolts	Traps	Drilled	Infused	Traps	Fresh	7-d	14-d	Traps	
<i>Ambrosiophilus atratus</i> Eichhoff	0	16	0	0	2	0	0	0	0	18
<i>Anisandrus maiche</i> Stark	0	0	0	0	0	59	23	0	16	98
<i>Anisandrus sayi</i> Hopkins	17	36	24	6	30	7	1	0	2	123
<i>Cyclorhipidium bodoanum</i> (Reitter)	0	3	0	0	1	0	0	0	0	4
<i>Euwallacea validus</i> (Eichhoff)	0	9	0	0	2	0	0	0	0	11
<i>Hylocurus rudis</i> (LeConte)	0	2	0	0	0	0	0	0	0	2
<i>Hypothenemus dissimilis</i> Zimmerman	1	324	2	1	255	0	0	0	1	583
<i>Hypothenemus eruditus</i> Westwood	0	0	0	0	11	0	0	0	1	12
<i>Micrasis swainei</i> Blackman	0	3	0	0	0	0	0	0	0	3
<i>Monarthrum fasciatum</i> (Say)	1	0	0	0	0	0	0	0	0	1
<i>Monarthrum mali</i> (Fitch)	0	0	0	0	1	0	0	0	0	1
<i>Phloetribus limnaris</i> (Harris)	0	0	0	0	1	0	0	0	0	1
<i>Pityophthorus lautus</i> Eichhoff	0	0	1	0	1	0	0	0	0	2
<i>Scolytus rugulosus</i> (Müller)	16	1	0	0	0	0	0	0	0	17
<i>Xyleborinus attenuatus</i> (alni) Nijijima	0	2	0	0	0	0	0	0	0	2
<i>Xyleborinus saxesenii</i> (Ratzeburg)	69	29	5	4	17	1	0	0	1	126
<i>Xylosandrus crassiusculus</i> (Motschulsky)	46	4	3	2	2	4	7	0	0	68
<i>Xylosandrus germanus</i> (Blandford)	2052	1817	362	567	638	210	96	0	228	5,970
<i>Xyloterinus politus</i> Say	0	5	0	0	4	0	0	0	0	9

Species in bold occurred only in traps throughout all experiments (see also Table 3).

^aBolt versus Trap experiments were conducted late Mar., mid- to-late April, and mid-May; Drilled versus Infused bolt experiments were in late May and early June; Aged bolt experiments occurred in early to mid-July.

Table 3. Species of Scolytinae collected from the bolt species experiments in 2017

Experiment	Species	Total beetles			
		Trap	Maple	Cherry	Dogwood
Exp-1 26 Apr. to 3 May	<i>Ambrosiophilus atratus</i>	6	0	0	0
	<i>Anisandrus sayi</i>	28	16	5	4
	<i>Cyclorhpidium bodoanum</i>	1	0	0	0
	<i>Euwallacea validus</i>	2	0	0	0
	<i>Hypothenemus dissimilis</i>	90	0	0	0
	<i>Hypothenemus eruditus</i>	4	0	0	0
	<i>Phloetribus limnaris</i>	0	0	8	0
	<i>Xyleborinus saxesenii</i>	5	8	22	7
	<i>Xylosandrus crassiusculus</i>	1	5	3	1
	<i>Xylosandrus germanus</i>	155	387	105	255
	<i>Xyloterinus politus</i>	2	0	0	0
Exp-2 17 to 24 May	<i>Ambrosiophilus atratus</i>	2	0	0	0
	<i>Anisandrus maiche</i>	0	1	0	0
	<i>Anisandrus sayi</i>	35	27	11	23
	<i>Cyclorhpidium bodoanum</i>	1	0	0	0
	<i>Dryoxylon onoharaensum</i>	0	1	0	0
	<i>Hypothenemus dissimilis</i>	88	1	0	0
	<i>Hypothenemus eruditus</i>	3	0	0	0
	<i>Monarthrum mali</i>	1	0	0	0
	<i>Phloetribus limnaris</i>	0	0	1	0
	<i>Xyleborinus saxesenii</i>	5	6	7	9
	<i>Xyleborus affinis</i> Eichhoff	1	0	0	0
	<i>Xylosandrus crassiusculus</i>	4	9	3	19
	<i>Xylosandrus germanus</i>	265	330	94	159
Exp-3 8 to 14 June	<i>Anisandrus maiche</i>	19	44	28	0
	<i>Anisandrus sayi</i>	15	16	31	8
	<i>Hypothenemus dissimilis</i>	26	0	0	0
	<i>Hypothenemus eruditus</i>	12	0	0	0
	<i>Monarthrum fasciatum</i>	0	0	9	0
	<i>Monarthrum mali</i>	0	0	29	0
	<i>Pityophthorus lautus</i>	2	0	0	0
	<i>Xyleborinus saxesenii</i>	0	3	0	0
	<i>Xylosandrus crassiusculus</i>	0	5	3	0
	<i>Xylosandrus germanus</i>	85	184	44	56
Exp-4 6 to 19 Jul	<i>Anisandrus maiche</i>	63	122	27	63
	<i>Anisandrus sayi</i>	19	5	2	0
	<i>Hypothenemus dissimilis</i>	5	0	0	0
	<i>Hypothenemus eruditus</i>	5	0	0	0
	<i>Xyleborinus saxesenii</i>	1	0	0	1
	<i>Xylosandrus crassiusculus</i>	1	14	0	0
	<i>Xylosandrus germanus</i>	50	95	24	6

Species in bold occurred only in traps throughout all experiments (see also Table 2).

(*Dryoxylon onoharaensum* Murayama and *Monarthrum fasciatum* Say). *Xylosandrus germanus* was the most common species in all experiments, except experiment 4 in 2017 (19 July) when attacks by *Anisandrus maiche* Stark were greater than other species.

Attractiveness of Ethanol-Infused Bolts

Attraction of *Xylosandrus* species to bolts was comparable or better than attraction to traps (Fig. 1). Seven species of Scolytinae occurred in bolts while 13 were captured in traps, with 95 and 81% of the beetles being *Xylosandrus* spp. in bolts and traps, respectively (Table 2). In experiment 1, there were more *X. germanus* in bolts than traps ($F = 7.30$; $df = 1, 19$; $P = 0.014$), no differences in *X. crassiusculus* between treatments ($F = 1.08$; $df = 1, 19$; $P = 0.312$), and more

other Scolytinae in traps than bolts ($F = 7.06$; $df = 1, 19$; $P = 0.016$) (Fig. 1). In experiment 2, there were no differences in *X. germanus* or *X. crassiusculus* among elm bolts, red maple bolts, and traps ($F = 1.10$; $df = 2, 18$; $P = 0.35$; $F = 2.06$; $df = 2, 18$; $P = 0.156$, respectively), but more other Scolytinae occurred in traps than either bolt species ($F = 6.54$; $df = 2, 18$; $P = 0.007$) (Fig. 1). In experiment 3, more *X. germanus* and *X. crassiusculus* occurred in bolts than traps ($F = 12.46$; $df = 1, 19$; $P = 0.002$; $F = 8.12$; $df = 1, 19$; $P = 0.010$, respectively), with no differences in other Scolytinae ($F = 3.16$; $df = 1, 19$; $P = 0.09$) (Fig. 1). In experiment 4, there were no differences in *X. germanus* between bolts and traps ($F = 0.56$; $df = 1, 23$; $P = 0.46$), while more other Scolytinae occurred in traps ($F = 35.8$; $df = 1, 23$; $P < 0.0001$) (Fig. 1); one *X. crassiusculus* occurred in each treatment.

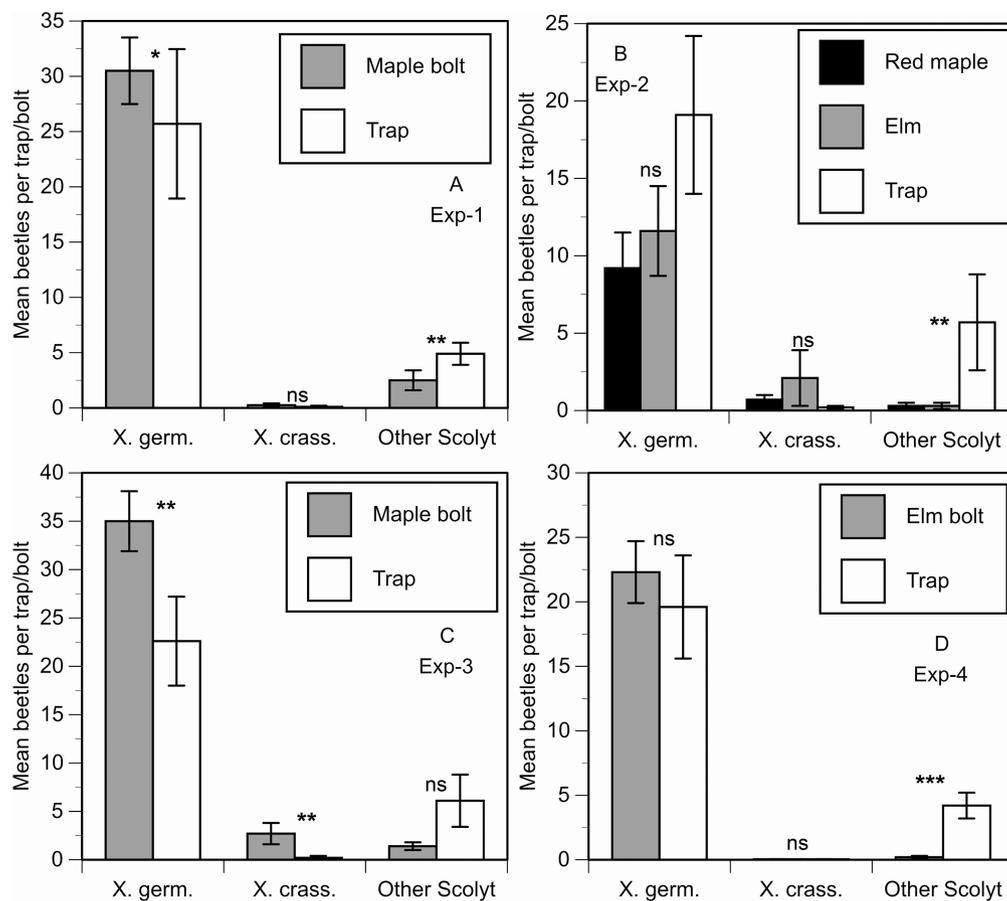


Fig. 1. Mean \pm SE (error bars) total *Xylosandrus germanus* (*X. germ.*), *Xylosandrus crassiusculus* (*X. crass.*), and other Scolytinae (other Scolyt) found in ethanol-infused bolts versus ethanol-baited traps. Experiment 1 was conducted 21–28 March 2012 by using red maple bolts (A), experiment 2 was conducted 12–19 April 2012 with red maple and elm bolts (B), experiment 3 was conducted 19–25 April 2012 with red maple bolts (C), and experiment 4 was conducted 8–14 May 2014 with elm bolts (D). Asterisks indicate significance at P -value *0.05, **0.01, and ***0.001, and ns = not significant.

Attractiveness of Drilled + Filled Versus Infused (immersed) Bolts

Drilled + filled bolts were attractive to *Xylosandrus* species, but usually had fewer attacks by *X. germanus* than infused bolts (Fig. 2). Six species of ambrosia beetles occurred in bolts while 13 were captured in traps, with *Xylosandrus* spp. 95 and 66% of the beetles in bolts and traps, respectively (Table 2). In experiment 1, more *X. germanus* occurred in infused bolts and traps than drilled bolts ($F = 13.4$; $df = 2, 26$; $P = 0.0001$), and more other Scolytinae occurred in traps than either bolt treatment ($F = 85.9$; $df = 2, 26$; $P < 0.0001$) (Fig. 2); only four *X. crassiusculus* were collected with two each from drilled bolts and traps. In experiment 2, ambrosia beetle activity was low; no *X. crassiusculus* were detected, and there were no differences in *X. germanus* or other Scolytinae among treatments ($F = 0.92$; $df = 2, 22$; $P = 0.41$; $F = 3.12$; $df = 2, 22$; $P = 0.06$, respectively) (Fig. 2). In experiment 3, repeated-measures analysis of *X. germanus* data detected no treatment or treatment \times time effects ($F = 0.71$; $df = 2, 27$; $P = 0.50$; $F = 0.38$; $df = 2, 27$; $P = 0.69$, respectively) (Fig. 2). Due to low numbers of other Scolytinae, only cumulative numbers were analyzed with more in traps than drilled + filled bolts ($F = 3.86$; $df = 2, 18$; $P = 0.040$). Only one *X. crassiusculus* occurred (drilled bolt) in this experiment. In experiment 4, more *X. germanus* occurred in infused bolts and traps than drilled bolts ($F = 9.77$; $df = 2, 18$; $P = 0.001$) (Fig. 2). More other Scolytinae occurred in traps than drilled and infused bolts ($F = 11.51$; $df = 2, 18$; $P = 0.0006$

(Fig. 2). Only two *X. crassiusculus* occurred in this experiment, both in infused bolts.

Attractiveness of Bolts Over Time

Fresh bolts were generally more attractive to target species than aged bolts (Fig. 3). Five species of ambrosia beetles occurred in bolts while six were captured in traps, with 98% of beetles in bolts and traps being either *A. maiche* or *Xylosandrus* spp. (Table 2). In experiment 1, there were no significant differences in *X. germanus* among treatments ($F = 1.03$; $df = 2, 18$; $P = 0.38$) (Fig. 3). Only two *X. crassiusculus* occurred in this experiment (both in fresh bolts). *Anisandrus maiche* an exotic and potentially damaging ambrosia beetle, occurred in numbers sufficient for analysis with no significant differences among treatments ($F = 2.08$; $df = 2, 18$; $P = 0.15$) (Fig. 3). Only two other Scolytine beetles (*A. maiche* not included) occurred in this experiment. In experiment 2, more *X. germanus* were collected from fresh bolts than all other treatments, with more from traps than 14 d bolts ($F = 19.18$; $df = 3, 27$; $P < 0.0001$) (Fig. 3). *Xylosandrus crassiusculus* were collected from fresh and 7 d bolts only, with no differences among treatments ($F = 2.87$; $df = 3, 27$; $P = 0.055$). Numbers of *A. maiche* were again sufficient for analysis with more in fresh bolts than all other treatments ($F = 15.23$; $df = 3, 27$; $P < 0.0001$) (Fig. 3). There were no differences in other Scolytinae (*A. maiche* not included) among treatments ($F = 2.12$; $df = 3, 27$; $P = 0.12$) (Fig. 3).

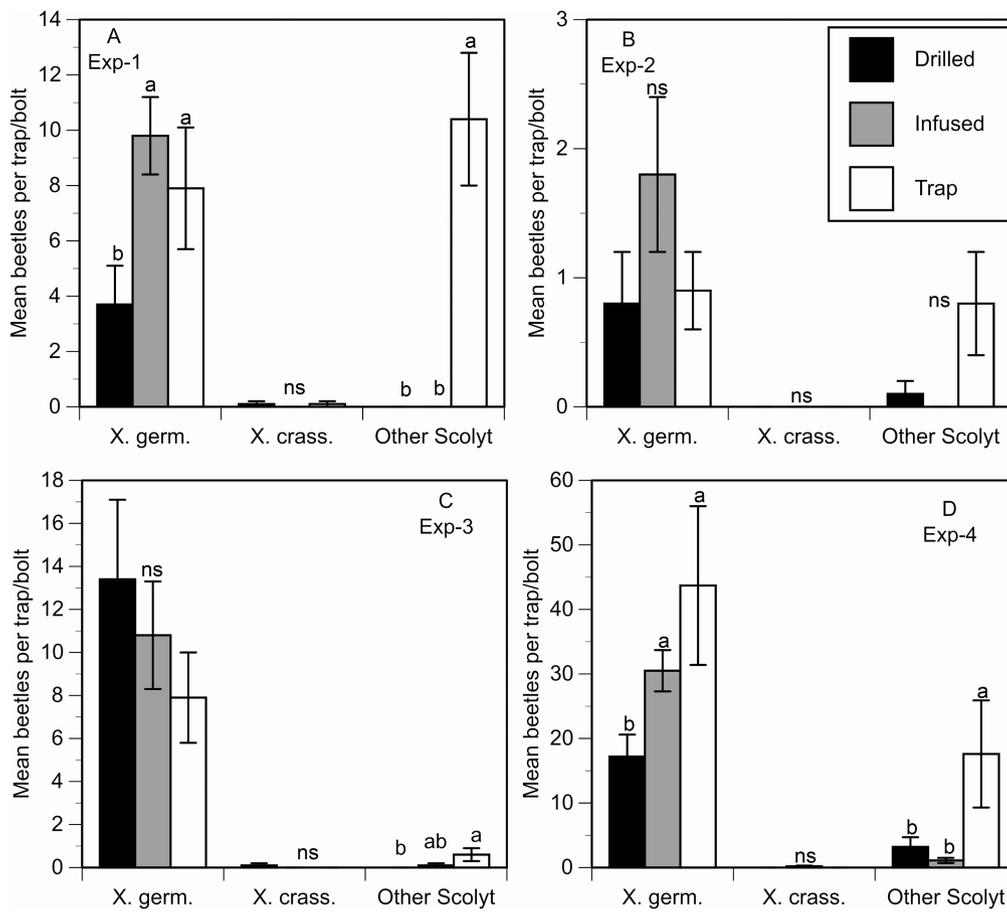


Fig. 2. Mean \pm SE (bars) total *X. germanus*, *X. crassiusculus*, and other Scolytinae attracted to ethanol-treated bolts and ethanol-baited traps. Experiments were conducted 22–29 May 2014 with red maple bolts (A), 5–12 June 2014 hornbeam bolts (B), 28 May–10 June 2015 with elm bolts (C), and 18–25 May 2016 with red maple bolts (D). Within species and experiment, columns with the same letter were not significantly different (Tukey's HSD), ns = not significant.

Attractiveness of Ethanol-Treated Bolts From Various Species of Trees

Red maple tended to attract more *Xylosandrus* sp. than other bolt species, but differences were not always significant (Fig. 4). Ten species of ambrosia beetles occurred in bolts while 14 were captured in traps, with 76 and 64% of beetles in target species (*A. maiche* and *Xylosandrus*) in bolts and traps, respectively (Table 3). In experiment 1, more *X. germanus* occurred in red maple and dogwood bolts than cherry or traps ($F = 13.9$; $df = 3, 27$; $P < 0.0001$) (Fig. 4). The analysis of variance was significant for *X. crassiusculus*, ($F = 2.96$; $df = 3, 27$; $P = 0.050$), but Tukey's test could not identify differences among means. Numbers of other Scolytinae were greater in traps than all bolt species ($F = 9.96$; $df = 3, 27$; $P = 0.0001$) (Fig. 4). In experiment 2, there were more *X. germanus* in red maple bolts than cherry or traps ($F = 6.60$; $df = 3, 27$; $P = 0.002$), with no other differences among treatments (Fig. 4). More *X. crassiusculus* occurred in sumac than cherry bolts or traps ($F = 7.14$; $df = 3, 27$; $P = 0.001$), with no other differences among treatments (Fig. 4). Traps had higher numbers of other Scolytinae than cherry or sumac bolts ($F = 6.56$; $df = 3, 27$; $P = 0.002$), with no other differences among treatments (Fig. 4). In experiment 3, numbers of *X. germanus* were greater in red maple than sassafras and sycamore ($F = 6.22$; $df = 3, 27$; $P = 0.002$) (Fig. 4). There were no differences in numbers of *X. crassiusculus* among treatments ($F = 2.71$; $df = 3, 27$; $P = 0.065$). *Anisandrus maiche* occurred in sufficient

numbers for analysis, with fewer in sycamore than all other treatments ($F = 10.5$; $df = 3, 27$; $P = 0.0001$) (Fig. 4). Numbers of other Scolytinae were fewer in sycamore than all other treatments ($F = 10.5$; $df = 3, 27$; $P = 0.0001$), with no other differences (Fig. 4). In experiment 4, more *X. germanus* occurred in red maple than oak ($F = 3.97$; $df = 3, 27$; $P = 0.018$), with no other differences (Fig. 4). More *X. crassiusculus* occurred in red maple than all other treatments ($F = 4.98$; $df = 3, 27$; $P = 0.007$), with no other differences among treatments (Fig. 4). Numbers of *A. maiche* were greater in red maple than sassafras and oak ($F = 8.76$; $df = 3, 27$; $P = 0.0003$), with no other differences among treatments (Fig. 4). More other Scolytinae occurred in traps than all bolt species ($F = 7.54$; $df = 3, 27$; $P = 0.001$) (Fig. 4).

Discussion

Numbers of attacks by *X. germanus* on ethanol-infused bolts were statistically similar to their captures in ethanol-baited traps in most experiments. Infused and drilled + filled bolts were reliably attacked; however, numbers of total attacks and *X. germanus* attacks were usually greater in infused bolts. Drilled + filled bolts may have emitted lower amounts of ethanol than infused bolts. Ranger et al. (2015) demonstrated that ambrosia beetle attacks increased as the concentration of ethanol in bark tissue increased. Other studies have shown that captures of ambrosia beetles increase as ethanol emitted

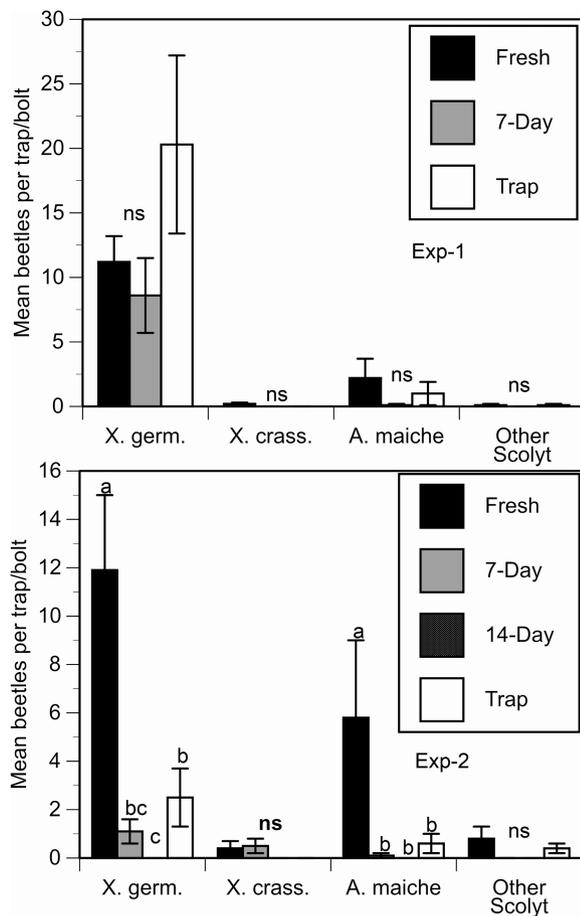


Fig. 3. Mean \pm SE (bars) *X. germanus*, *X. crassiusculus*, *A. maiche*, and other Scolytinae in experiments testing residual attractiveness of ethanol-infused bolts. Within species and experiment, columns with the same letter were not significantly different (Tukey's HSD, $\alpha = 0.05$) (ns = not significant). (A) 2015, (B) 2016.

from traps increased (Klimentzek et al. 1986, Ranger et al. 2011b, Reding et al. 2011). Emission rates of ethanol from bolts were not measured and are unknown.

Various tree species were used for bolts in the experiments comparing ethanol immersion to a drilled + filled technique, which might have influenced comparisons of these techniques. However, relative to captures in traps, attacks on bolts were comparable across those experiments. While fewer attacks often occurred on drilled + filled bolts than infused, attacks were still plentiful indicating drilled + filled bolts should be useful for screening insecticides. Previous research demonstrated that insecticides are less effective preventing attacks on highly attractive trees (Ranger et al. 2016, Reding and Ranger 2018). Therefore, additional research is needed on bolts to determine a level of attraction that induces attacks by ambrosia beetles while not overwhelming the ability of insecticides to prevent attacks. This could be done by testing different volumes and concentrations of ethanol in the cavity of drilled + filled bolts.

Attractiveness of ethanol treated bolts was inconsistent after 1 wk. In one experiment, infused bolts aged 7 d attracted similar numbers of *X. germanus* as traps or fresh bolts. In the other experiment, bolts aged 7 d attracted very few *X. germanus* compared with fresh bolts while 14-d bolts attracted no beetles. In a third experiment where attacks on infused and drilled bolts were monitored

for 2 wk, attacks were similar each week. Attacks on trees injected with 10% ethanol generally decline after 10–14 d (Reding et al. 2013b, Reding and Ranger 2018), presumably when emission of ethanol decreases below a level attractive to beetles (Ranger et al. 2012). Diameters of bolts used were similar in all experiments being at least 2.5 cm and not exceeding 5.0 cm. Larger diameter bolts might absorb more ethanol, and since larger bolts have greater volume to surface area they should emit ethanol for longer periods of time. Also, larger cavities that hold more ethanol could be bored into larger bolts, which might increase the time period they are attractive. Relatively short-term attractiveness may make bolts less practical for monitoring than traps baited with commercial ethanol lures, which are attractive for 8 wk or more in Ohio. Bolts attracted fewer nontarget species than traps, however, which may be beneficial for growers trying to determine activity of damaging species. Furthermore, drilled bolts could be refilled in the field to extend their attractiveness, but there would be a limit as bolts tend to desiccate and dry conditions are unfavorable for *Xylosandrus* species. Growers of ornamental trees should have regular supplies of bolt material, because they cull trees annually.

Bolts from all tree species were attacked by ambrosia beetles, with attacks easier to detect on bolts with smooth bark than rough. Bolt materials (saplings) were collected from local woodlands; however, all species are grown in ornamental nurseries in northern Ohio. American elm infused with ethanol was as attractive as red maple, but the rough bark made attacks difficult to see. Black cherry was tested in two experiments and had the fewest attacks by *X. germanus* in each test. Sassafras, sycamore, and shingle oak were generally less attractive than red maple in the current research. Sassafras was tested because previous research and field observations suggested *A. maiche* might be especially attracted to sassafras. Results of the current research do not support that hypothesis because sassafras did not attract more *A. maiche* than other species in tests. Shingle oak and sycamore are very common in Ohio woodlands and would be readily available as ambrosia beetle hosts or bolt material. Sumac is common along roadways and early successional woodlands, and would be readily available as a host species for ambrosia beetles and easily collected for bolt material. However, sumac produces large amounts of sticky sap, wherein after soaking in 10% ethanol for 72 h sumac bolts remained relatively sticky from sap. All but one sumac bolt had abandoned tunnels discharging sap. The sticky and copious sap may deter colonization of living sumac by ambrosia beetles.

Xylosandrus crassiusculus occurred in 13 of 14 experiments, but their numbers were relatively low. *Xylosandrus crassiusculus* appeared to prefer bolts to traps occurring in bolts in 13 experiments and traps in only eight. However, differences in *X. crassiusculus* between bolts and traps occurred in only two experiments, which could be related to their low numbers. Attack pressure by *X. crassiusculus* was influenced by species of bolt in 2017, wherein *X. crassiusculus* did not occur in sycamore or oak. Furthermore, in one of the aged bolt experiments *X. crassiusculus* occurred in bolts aged 7 d while none occurred in traps. Ethanol-treated bolts may emit volatiles in addition to ethanol which could make them more attractive to *X. crassiusculus* than traps emitting ethanol only. Mayfield and Hanula (2012) and Mayfield et al. (2013) reported *X. crassiusculus* were attracted to bolts from certain species of trees, that were not treated with ethanol.

Anisandrus maiche is a relatively new ambrosia beetle in Ohio. We initially detected it in 2011 in nurseries monitored annually since 2006 (M.E.R., unpublished data). In Ohio, *A. maiche* starts emerging in June (M.E.R., unpublished data), which is later than *X.*

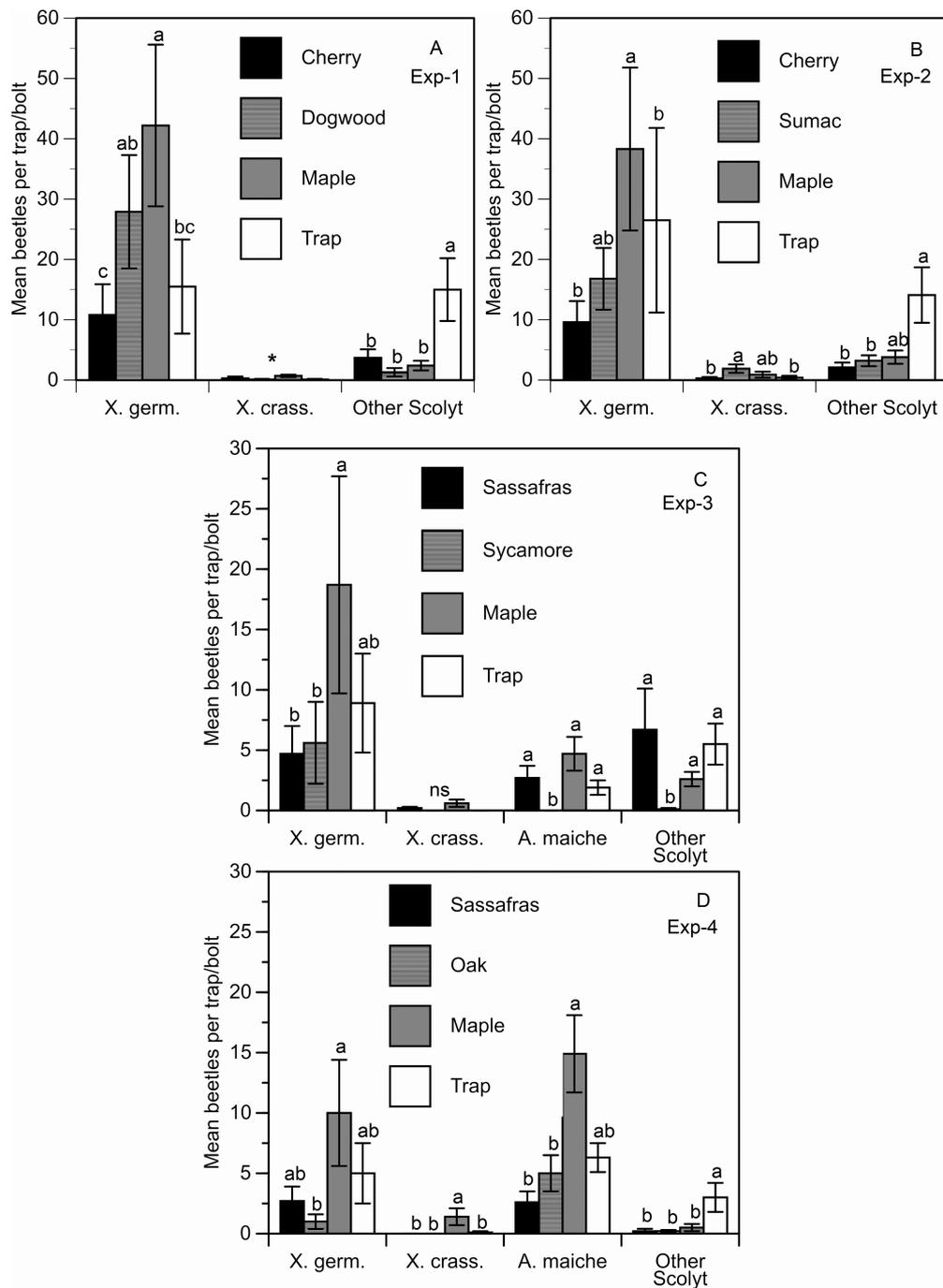


Fig. 4. Mean \pm SE (bars) *X. germanus*, *X. crassiusculus*, *A. maiche*, and other Scolytinae attacking bolts and captured in ethanol-baited traps. Within beetle species and experiment, columns with the same letter were not significantly different (Tukey's HSD, $\alpha = 0.05$). Species of bolts were cherry (*Prunus serotina*), dogwood (*Cornus florida*), maple (*Acer rubrum*), Oak (*Quercus imbricaria*), sassafras (*Sassafras albidum*), Sumac (*Rhus typhina*), and sycamore (*Platanus occidentalis*).

germanus and *X. crassiusculus*. The later emergence is probably the reason *A. maiche* occurred only in experiments run in June and July. The fact *A. maiche* occurred in ethanol treated bolts suggests it might attack physiologically stressed trees emitting ethanol and become a pest in nurseries during summer. *Xylosandrus* species are considered problems primarily during spring and growers time protective treatments to coincide with their spring activity. The activity period of *A. maiche* could be problematic because protections against ambrosia beetles are not usually applied during summer.

The current research demonstrated ethanol-infused bolts from a variety of tree species are attractive to damaging species of ambrosia beetles, and attacks are easier to detect on smooth bark than rough. Bolts tend to be more selective for damaging species than traps; however, their period of residual attractiveness is much less. Attractiveness of ethanol-treated bolts 2.5–5.0 cm in diameter should not be expected to last more than 7 d. Attack pressure on bolts filled with ethanol was reliable, which should make them useful for screening insecticides against ambrosia beetles.

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