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Influence of the Ethanol Lure and Concentration on Captures of Ambrosia Beetles in Tree Fruits and Ornamentals

Ramkumar Govindaraju[1] | Jensen Hayter[2] | Juang Horng Chong[3] | Alejandro I. Del Pozo-Valdivia[4] | Ted E. Cottrell[5] | James F. Walgenbach[2] | Thomas W. Scheyer[6] | Brett R. Blaauw[6] | Michael E. Reding[7] | Christopher M. Ranger[7] (ID) | Shimat V. Joseph[1] (ID)

[1]Department of Entomology, University of Georgia, Griffin, Georgia, USA | [2]Entomology & Plant Pathology, North Carolina State University, Mountain Horticultural Crops Research & Extension Center, Mills River, North Carolina, USA | [3]SePRO Corporation, Carmel, Indiana, USA | [4]Department of Entomology, Virginia Polytechnic Institute and State University, Virginia Beach, Virginia, USA | [5]Southeastern Fruit and Tree Nut Research Laboratory, USDA-ARS, 21 Dunbar Road, Byron, Georgia, USA | [6]Department of Entomology, University of Georgia, Athens, Georgia, USA | [7]USDA-Agricultural Research Service, Horticultural Insects Research Laboratory, Wooster, Ohio, USA

Correspondence: Shimat V. Joseph (svjoseph@uga.edu)

ABSTRACT

Xylosandrus crassiusculus (Motschulsky) and *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) are major ambrosia beetle pests in tree nut and fruit orchards and ornamental nurseries in the eastern United States (USA). Ethanol-baited bottle traps and ethanol-infused tree stem sections (i.e., bolts) have been used to monitor ambrosia beetles, but limited studies exist on the influence of ethanol-lure release rate on ambrosia beetle trap captures and bolt attacks. We designed this study to compare low-release (LR) and high-release (HR) ethanol lures in bottle traps for capturing invasive ambrosia beetles. We also compared beetle attacks among bolts pre-soaked in ethanol solutions of low (10%) and high (90%) concentrations and bolts cored and filled with the same low and high ethanol concentrations. In 2022, experiments were conducted in ornamental nurseries and apple, peach, or pecan orchards in five USA states. Higher numbers of *X. crassiusculus* and *X. germanus* were captured in bottle traps baited with the HR ethanol lure compared to the LR lure at most of the study sites. More attacks per bolt by *X. crassiusculus* and *X. germanus* were observed at most sites on pre-soaked and filled bolts with 90% compared to 10% solutions of ethanol. Bolts soaked in low (10%) ethanol solutions sustained more attacks from both *X. crassiusculus* and *X. germanus* than cored bolts filled with low ethanol. These results will assist with monitoring the flight activity of invasive ambrosia beetles within nut, fruit, and ornamental tree crops.

1 | Introduction

Many species of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in the tribe Xyleborini are secondary pests that infest dying and dead trees. However, some species attack living trees undergoing physiological stress and cause serious economic losses in ornamental nurseries and orchards (Ranger et al. 2021; Tobin and Ginzel 2023). In the eastern

USA, the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) and the black stem borer, *Xylosandrus germanus* (Blandford), are destructive pests of apple and nut orchards and ornamental nurseries (Agnello et al. 2015; Addesso et al. 2019; Monterrosa et al. 2021; Ranger et al. 2016; Tobin and Ginzel 2023). Females tunnel into the heartwood of trees to excavate galleries to grow their nutritional fungal symbionts and rear offspring (Hulcr and Stelinski 2017). For *Xylosandrus* spp., densely packed sawdust resulting from the boring activity, known vernacularly as 'noodles', protrudes from the bark, which indicates the presence of beetle attack (Gugliuzzo et al. 2021; Ranger et al. 2016). Sap oozes from the entry holes, appearing stained and necrotic on the trunks and affect the marketability of trees.

Overwintered beetles emerge from their natal galleries and start flying during the spring when temperatures reach about 20°C for a few consecutive days (Reding et al. 2013b). As xyleborine males are flightless, mated adult females represent the dispersing stage. Xyleborine ambrosia beetles find stressed trees use ethanol as a host-derived cue to locate suitable hosts to colonise (Ranger et al. 2010, 2021). Ethanol production by plants is induced in response to anaerobic respiration and the compound is then emitted from the bark and leaf tissue along with other stress-induced volatiles (MacDonald and Kimmerer 1991). Flood and low-temperature stress have been demonstrated to predispose ornamental and fruit trees to infestation by xyleborine ambrosia beetles (Ranger et al. 2021).

In nurseries and orchards, growers manage ambrosia beetle attacks with repeated applications of pyrethroid insecticides, such as bifenthrin and permethrin (Brown et al. 2020; Williamson, Blaauw, and Joseph 2023). To prevent attacks on trees, it is critical to spray insecticides before or at the start of flight events (Gugliuzzo et al. 2021; Reding et al. 2013a; Brown et al. 2020). For this reason, several studies attempted to develop monitoring tools that can reliably detect dispersing females, thus aiding in the timing of insecticide applications (Gugliuzzo et al. 2021; Reding and Ranger 2018). Ethanol-baited traps, as well as ethanol-soaked/filled stem sections (i.e., bolts) or ethanol-injected trees, have been tested in several studies to attract these dispersing females (Ranger et al. 2016). Nonetheless, no study simultaneously compared the efficacy of these different techniques in monitoring xyleborine ambrosia beetles. Ethanol-based trapping techniques have been practiced to monitor ambrosia beetle activity in production systems, such as open-field nurseries (Galko et al. 2019; Gugliuzzo et al. 2021). However, ethanol lures with various release rates may influence adult trap captures. Ethanol-baited bottle traps and bolts soaked in solutions of ethanol or cored and filled with ethanol have been used to monitor ambrosia beetles (Monterrosa et al. 2022; Reding and Ranger 2018, 2020), but there is limited information on the relative effectiveness of these techniques to ambrosia beetles.

The following objectives were addressed as part of this multi-location and multi-crop study: (1) compare trap captures of ambrosia beetles using low (LR) and high release (HR) ethanol lures, (2) assess ambrosia beetle attacks in soaked versus cored bolts infused with diluted and concentrated solutions of ethanol; and (3) correlate *X. crassiusculus* and *X. germanus* adult captures in bottle traps baited with LR and HR ethanol lures to attacks on bolts infused with diluted and concentrated solutions of ethanol in apple, peach, pecan, and ornamental production systems.

2 | Materials and Methods

2.1 | Study Area

In 2022, experiments were conducted in several apple, peach and pecan orchards and ornamental nurseries in the states of Ohio (OH), Virginia (VA), North Carolina (NC), South Carolina (SC), and Georgia (GA) in the USA (Table 1). In an apple orchard in NC, 3- to 8-year-old trees were planted 1 m apart within a row and 4.6 m between rows, with a tree density of approximately 2400 trees per ha. In a peach orchard in GA, 8- to 14-year-old trees were planted 4.5 m apart with row spacing of 6 m. In a pecan orchard in GA, 12 to 50 years old trees were planted at ~12 m spacing. The apple, peach, and pecan trees were not irrigated.

In ornamental nurseries, trees were planted at ~1.5 m spacing across all sites. The species and age of trees planted varied within each nursery and among sites. Each tree nursery site had three or more tree species planted during the experiment (Table 1). The OH site consisted of three nurseries and one farm field (two replications per site) adjacent to a woodlot. One nursery and the farm field were in Wayne County, OH, and two nurseries were in Lake County, OH. In most nurseries, trees were pruned in the spring before bud break. All ornamental trees were on drip irrigation. The woodlot surrounding each nursery consisted of diverse tree and shrub species (Table 1). Insecticides were not applied to nursery trees during the experiment.

2.2 | Trap Types

2.2.1 | Bottle Traps

Either non-tornado square or tornado round (1.5–2 L) plastic bottle traps were used at all the sites (Table 2). Two ~5 cm × ~19 cm rectangular vents at two opposite sides of a bottle were cut to provide access to adults into the trap baited with a lure; and traps were hung from two holes that were drilled into the bottle bottom (Figure 1A). Two types of commercially available ethanol lures were tested. A low-release (LR) lure consisted of a heat-sealed, permeable membrane pouch containing 15 mL of 95% ethanol releasing 16 mg/d at 20°C according to the manufacturer with > 120 days estimated field life (Manufacturer: ChemTica Internacional, S.A., San Jose, Costa Rica; Distributor: AgBio Inc., Westminster, Colorado, USA) (Figure 1A,B). The high-release (HR) lure consisted of a collapsible soft plastic squeeze tube containing a gel matrix infused with 120 mL of 95% denatured ethanol and a 1 cm (diam.) opening for releasing ethanol at 1000 mg/d at 20°C with a > 60 days estimated field life per manufacturer (ChemTica Internacional). Using a wooden stake or shepherd's hook and zip ties, the bottles were suspended upside down. At some sites (as specified in Table 2 and Figure 1B), tornado connectors were used to attach 500-mL plastic bottles, which served as a collecting device, to the bottle traps. At sites where tornado connectors were not used, beetles were trapped in the bottom of bottles below the rectangular vents (Figure 1A). To effectively trap, kill, and preserve beetles, ~200 mL of a

TABLE 1 | Details of the field sites used in 2022 to test ambrosia beetle trapping tactics.

Site no.	Cropping system	County, State	Location	Production area (ha)	Common trees in the production area	Common trees in the woodlot	Time of experiment
1	Peach	Oconee County, GA	33.8863, −83.4167	1.8	Peach	*Acer* spp., *Liquidambar styraciflua*, *Quercus* spp., *Pinus* spp.	27 April—01 June
2	Apple	Henderson Count, NC	35.3987, −82.350	16	Apple	*Acer* spp., *Quercus* spp., *Pinus* spp., *Liriodendron tulipifera*	13 April—1 June
3[a]	Pecan	Peach County, GA	1: 32.6588, −83.7275 2: 32.6583, −83.7247 3: 32.6558, −83.7347 4: 32.6566, −83.7327	10 10 4 4	Pecan	*Quercus* spp., *Pinus* spp.	03 March—08 April
4	Ornamental	Pike County, GA	33.2242, −84.6983	8.6	Cherry, crepe myrtle	*L. styraciflua*, *Acer* spp., *Quercus* spp., *Pinus* spp., *Ligustrum* spp.	03 March—07 April
5[b]	Ornamental	Wayne County, Lake County, OH	1:40.4545, −81.5434 2: 40.5202, −82.0238 3: 41.4915, −81.0210 4: 41.4808, −81.0729	131 4.2 14.2 42.3	Many species and varieties	*Acer* spp., *Quercus* spp., *Ulmus* spp., *Carya* spp., *Fagus* spp.	04 May—15 June (Wayne Co.) 05 May—16 June (Lake Co)
6	Ornamental	Florence Co., SC	34.3012, −73.7500	8	Many species	*Acer* spp., *Carya* spp., *Ligustrum* spp., *L. styracuflua*, *Quercus* spp.	04 March—08 April
7	Ornamental	Virginia Beach[c], VA	36.8875–76.17476	1.7	Many species and varieties	*Pinus* spp., *Quercus* spp.	1 June—7 July

[a]Multiple locations were used for the pecan site.
[b]Set up at Wayne County and Lake County using three nurseries and one farm field (two replications per site) adjacent to a woodlot.
[c]Independent city.

TABLE 2 | Details of bolt (soaked and cored) and bottle traps used to test ambrosia beetle monitoring tactics in 2022.

		Bolt[a]				Bottle trap[b]				
				Total ethanol volume absorbed in six bolts					Tornado connector (no: Figure 1A) or yes; Figure 1B)	
Site no.	Host	Length (cm)	Diameter (cm)	10% ethanol	90% ethanol	Shape	Size (L)	Manufacturer		Preservative used
1	Cherry	20	5	140 mL	200 mL	Round	2	Southeastern Container, Asheville, NC	Yes	Soap water
2	Crape myrtle	25	3.5–7.6	A: 1000 mL B: 1050 mL C: 550 mL	A: 1000 mL B: 1250 mL C: 1300 mL	Square	1.9	Berlin Packaging LLC, Chicago, IL	Yes	Soap water
3	Pecan	16	4	A: 34 mL B: 100 mL C: 66 mL	A: 52 mL B: 35 mL C: 81 mL	Round	1.7	VTM LLC, Lexington, KY.	Yes	Soap water
4	Red maple	10	3–4	A: 250 mL B: 535 mL C: 100 mL	A: 400 mL B: 450 mL C: 500 mL	Square	1.7	VTM LLC, Lexington, KY.	No	Soap water
5	Red maple	16	4	—	—	Round	2	NASCO, Fort Atkinson, WI	Yes	Propylene-glycol
6	Redbud	25	3.6–5.3	A: 150 mL B: 150 mL C: 140 mL	A: 200 mL B: 200 mL C: 110 mL	Round	2	Pepsi Bottling, Longs, SC	Yes	Propylene-glycol
7	Red maple	30	5.5–7	—	—	Round	2	Pitsco Education, Pittsburg, KS	Yes	Soap water

Note: Red maple (*Acer rubrum* L.), cherry (*Prunus avium* L.), crape myrtle, (*Lagerstroemia indica* L.) and pecan, *Carya illinoinensis* (Wangenh). A, B, and C represent bolts changed at 0, 3 and 5 weeks after deployment, respectively.

[a]10 mL of 10% and 90% ethanol was added to cores for cored bolt treatments; bolts soaked 24 h for the soaked bolt treatments.

[b]AgBio LR (low release) or HR (high release) ethanol lures were used for the bottle trap treatments.

FIGURE 1 | (A) Non-tornado square bottle trap with low release ethanol lure. (B) Tornado round bottle trap with high release ethanol lure, and (C) cored bolt. Photo credits (A) Shimat Joseph (University of Georgia), (B) Aaron Yilmaz (USDA-ARS), and (C) Zia Williamson (University of Georgia). [Colour figure can be viewed at wileyonlinelibrary.com]

soapy or propylene glycol solution was added to each bottle trap through the rectangular vent or to the plastic tube (Table 2). The soapy solution was prepared by adding ~0.5 mL of detergent soap (Dawn, P&G, Cincinnati, OH, USA) in 200 mL of water. Propylene glycol (~25%) was not diluted (Table 2). Bottle traps were suspended ~1 m (bottom end) above the ground level in all sites. Bottle traps were collected, and fresh kill-preservation solution was added at 7-day intervals for 6 weeks.

2.2.2 | Cored Bolts

For these studies and across sites, a bolt consisted of a section cut from tree trunks/branches of various tree species (Table 2). The tree species used for the bolt preparation was consistent at each site but not among sites. Freshly prepared bolts were used at all sites except in NC apple, GA peach, and SC ornamental sites, where previously cut bolts were kept in a freezer (−18°C) between 2 to 4 weeks before use. Once the bolts had been harvested from trees, bolts were prepared and used within 3 weeks. At all sites, bolts were ~15–30 cm long and 3–7.6 cm in diameter. A 10 cm deep ×12 mm diameter core was vertically drilled in the centre of the heartwood and filled with 10 mL of either 10% or 90% ethanol. Ethanol was refilled on a 7-day interval. The cores were then sealed using cork stopppers in most sites except in the VA ornamental site, where rubber stoppers were used (Figure 1C), and in GA pecan and SC ornamental sites, where silicone stoppers were used. The bolts were suspended ~1 m (bottom end) above the ground level using shepahrd's hooks or wooden stakes and string at all sites (Figure 1C).

2.2.3 | Soaked Bolts

Bolts were prepared as described in the previous section, except they were not cored in the centre but rather soaked. The same tree species used for cored bolts was used for these soaked bolts

at each site. Before deployment, bolts were soaked by fully immersing the bolts in either 10% or 90% ethanol in plastic bags for 24 h. The bolts were individually weighed before and after immersion in ethanol. The net weight gain was documented for 10% and 90% ethanol-soaked bolts (Table 2). The bolts were deployed immediately at most sites except in OH, where they were temporarily stored for 24 h at 4°C in sealed plastic bags before deployment. Bolts were suspended ~1 m above the ground surface using shepard's hooks or wood stakes as previously described.

2.3 | Experimental Design

Treatments included in the study were as follows: (1) bolt soaked in 10% ethanol, (2) bolt soaked in 90% ethanol, (3) cored bolt filled with 10% ethanol, (4) cored bolt filled with 90% ethanol, (5) bottle trap baited with LR ethanol lure, (6) bottle trap baited with HR ethanol lure, (7) bolt non-cored not soaked and not filled with ethanol [control bolts] and (8) non-baited bottle trap [control traps]. These treatments were arranged in a randomised complete block design with six replications at all sites except NC and OH, where treatments were replicated eight times. A replicate is an individual trap or bolt along the transect. Both soaked and cored bolts were replaced twice with new bolts at 14-day intervals beginning 14 days after initial deployment. The duration of the experiment was 6 weeks at all sites. The treatments (bottle trap or bolt) were deployed 10 m apart along the woodline and 1.5–5 m inside the production area from the woodline. The spacing between blocks was 20 m at all sites. The experiments were conducted during peak ambrosia beetle activity (Table 2).

2.4 | Evaluation

For bolt treatments, the number of entry holes on bolts was marked (circled) with coloured wax pencils each week and counted. After 14 days of exposure, bolts from each field site

were removed, transported, and temporarily stored at 4°C. Later, ambrosia beetle adults were removed from the bolts from all sites except the NC apple site. Adults were retrieved after vertically splitting the bolts across the diameter four times, producing eight vertical splits in most sites except the OH and SC sites. In the OH site, bolts were dissected using hand pruners to retrieve the adult beetles. In the SC site, bolts were split vertically four ways, then cut horizontally to follow each tunnel to excavate individual beetles. The recovered beetles were stored in 70% ethanol for species identification.

For bottle trap treatments, the insects collected in each trap bottle were emptied by unscrewing the bottle's lid into a coffee filter paper placed over a mesh strainer in some sites (Table 2). The coffee filters with beetles were transported in plastic bags and temporarily stored at −18°C. In other sites where a tornado connector and a collection bottle was used, the collection bottles were replaced with new ones with fresh trap solutions. The trap bottles (where the ethanol lures were placed) were not replaced. The collection bottles with insects were transported to the laboratory, where they were sorted and stored in 70% ethanol until identification. Traps were serviced at 7-day intervals.

Ambrosia beetles retrieved from bolts and collected in bottle traps were identified to species using taxonomic keys (Baker et al. 2009; Smith et al. 2019; Bateman and Hulcr 2017). Key invasive species were identified to species, namely, *X. crassiusculus*, *X. germanus*, and *Anisandrus maiche* (Stark) (detected in OH only).

2.5 | Statistical Analyses

For bottle traps, the numbers of *X. crassiusculus*, *X. germanus*, and *A. maiche*, and the total numbers of all ambrosia beetle species over 6 weeks were organised by treatment and replication to generate a single data set for each site. For the bolt traps, the numbers of entry holes were combined over 6 weeks by treatment and replication to generate a single data set. The entry-hole and adult beetle data were analysed separately by site, and thus, the entry-hole data among sites were not directly compared, although the eight treatments were deployed together within the same replicates, design, and experiment. The adult beetle and entry hole count data were distinct, that is, not all the beetles collected in the bottle traps would create successful entry holes on bolts; therefore, the two data sets were analysed in different statistical models. In addition, various types of agents, such as propylene glycol or soap water, were added to the collection device of bottle traps to trap and preserve the captured beetles (Table 2). These agents were not used in monitoring bolts at each site.

Adult beetle and entry hole counts were subjected to one-way analysis of variance (ANOVA) for each site using generalised linear mixed models (PROC GLIMMIX; SAS Institute 2024) with log-link function and Poisson distribution because discrete beetle counts or entry holes were not normally distributed. The estimation method used was Laplace. Adult beetle and entry hole count data from each site were analysed and

interpreted separately. Because GA peach and pecan sites were zero-inflated, a value of one was added to the *X. crassiusculus* data from bottle traps. A value of one was added to data from NC apple and OH ornamental sites, as no entry holes were detected on some bolts. The treatments and blocks were fixed and random effects, respectively. Means and standard errors were calculated after back transformation using the PROC PLM procedure in SAS, and means were separated by the Tukey–Kramer test ($\alpha = 0.05$). Pearson's correlation analysis was conducted to detect the correlations between the numbers of *X. crassiusculus* and *X. germanus* and LR and HR ethanol lures in bottle traps and between the numbers of entry holes and low and high ethanol concentrations in the soaked- or filled-bolts (PROC CORR; SAS Institute 2024).

## 3 | Results

3.1 | Bottle Traps

For both the GA peach and VA ornamental sites, *X. crassiusculus* captures were significantly greater in traps baited with LR lures compared to HR lures and non-baited treatments (Figure 2A,G and Table 3). In contrast, the numbers of *X. crassiusculus* were significantly greater in the HR than LR treatment in the NC apple, GA pecan, and GA and SC ornamental sites (Figure 2B–D,F and Table 3). There were no differences in *X crassiusculus* among treatments at the OH site (Figure 2E and Table 3).

In GA pecan, and GA and OH ornamental sites, the numbers of *X. germanus* were significantly greater in the LR and HR lure treatments than in the non-baited treatment (Figure 3A–D and Table 3). In NC apple, and GA and OH ornamental sites, the counts of *X. germanus* adults were significantly greater in the HR lure than LR lure treatment, followed by the non-baited treatment (Figure 3B–D and Table 3). *Anisandrus maiche* adults were collected only in OH, with no differences among treatments (Figure 3E and Table 3).

In NC apple, GA pecan, GA, OH, and SC ornamental sites, the numbers of total ambrosia beetles collected in bottle traps were significantly greater for the HR compared with the LR lure treatment followed by the non-baited treatment (Figure 4B–F and Table 3). In GA peach and VA sites, the total ambrosia beetle captures were significantly greater for the HR and LR lure treatments than for the non-baited treatment (Figure 4A,G and Table 3).

3.2 | Bolt Traps

The numbers of entry holes were not significantly different among ethanol-soaked, ethanol-filled, or untreated bolts at the GA peach site (Figure 5A and Table 4). In NC apple site, entry holes on bolts were significantly more numerous in the order: 90% ethanol-soaked > 10% ethanol-soaked > 90% ethanol-cored > 10% ethanol-cored = 0% ethanol (control) treatments (Figure 5B and Table 4). For GA pecan site, the counts were significantly greater in the order of 90% ethanol-cored > 10% ethanol-cored > 90% ethanol-soaked > 0% ethanol treatments
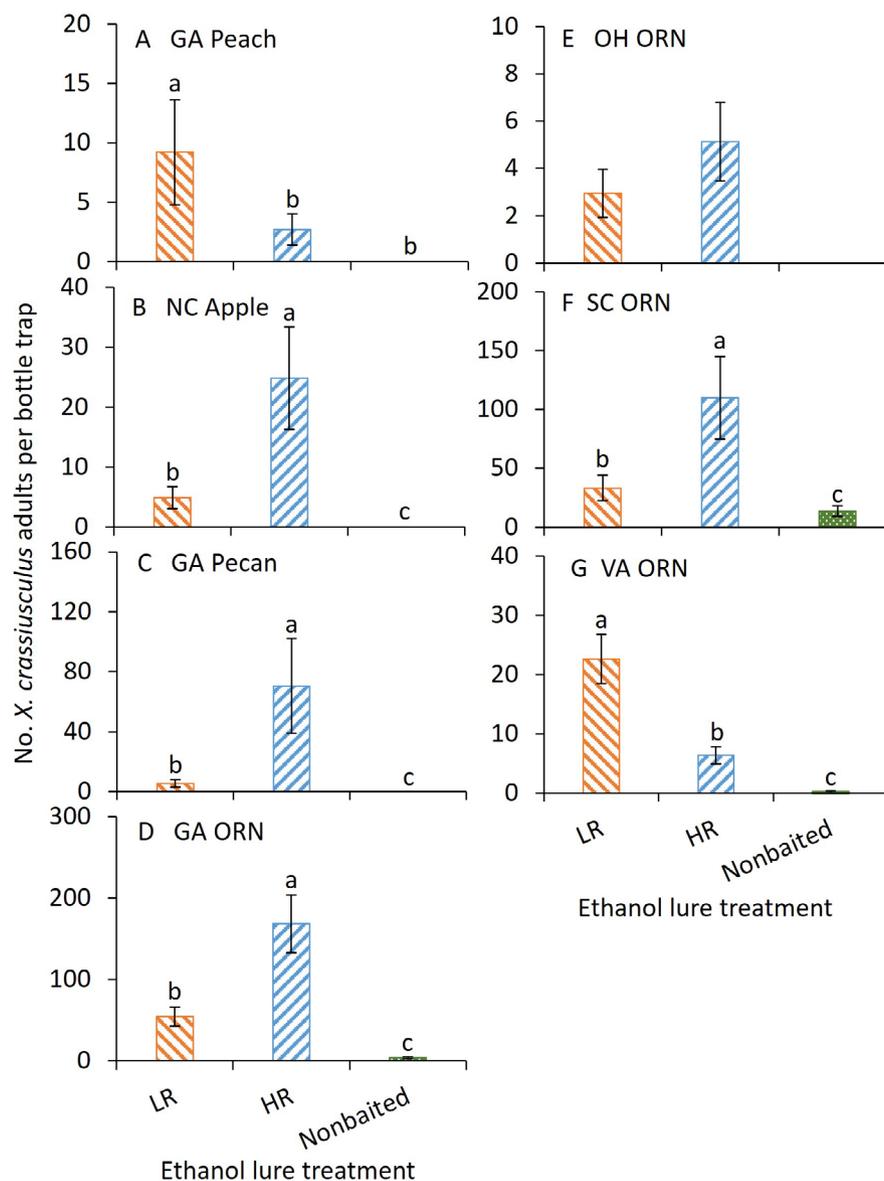
FIGURE 2 | Mean (±SE) *X. crassiusculus* adults collected from various bottle trap treatments (LR, HR and non-baited) through 6 weeks from (A) GA peach, (B) NC, (C) GA pecan, (D) GA ornamental, (E) OH, (F) SC and (G) VA sites in 2022. Bars with the same letters within a figure are not significantly different at (α = 0.05) using the Tukey–Kramer test. Where no differences were observed among treatments, no letters are given. The abbreviation, ORN, ornamentals. [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 5C and Table 4). At this site, the counts of holes on 10% ethanol-soaked treatment were not significantly different from either of the ethanol-cored treatments. For GA ornamental site, the number of entry holes was significantly greater in the order of 90% ethanol-soaked > 90% ethanol-cored > 10% ethanol-soaked > 10% ethanol-cored > 0% ethanol treatments (Figure 5D and Table 4). For the OH site, the number of holes was significantly greater in the following order: 90% ethanol-cored > 90% ethanol-soaked > 10% ethanol-cored and soaked > 0% ethanol treatments (Figure 5E and Table 4). For the SC site, significantly greater numbers of holes recorded in 90% ethanol-soaked, followed by 10% ethanol-soaked, 90% ethanol-cored, and 0% ethanol treatments > 10% ethanol-cored treatments (Figure 5F and Table 4). Finally, at the VA site, the number of holes was significantly greater in 90% ethanol-cored, 90% ethanol-soaked, and 10% ethanol-soaked

treatments, compared to 10% ethanol-cored and 0% ethanol treatments (Figure 5G and Table 4).

When bolts were dissected, *X. crassiusculus* was the dominant species recovered from all sites except the OH site, where *X. germanus* was most abundant (Figure 6).

The numbers of *X. crassiusculus* captured from the LR and HR ethanol lures in bottle traps were positively correlated with the numbers of entry holes on bolts soaked with low- and high-concentration ethanol (Table 5). The number of *X. crassiusculus* captured with the HR ethanol lure was associated with the number of entry holes in 10% ethanol for both (soaked and cored), whereas the number of *X. crassiusculus* captured in the LR ethanol lure was associated with the number of entry holes in 90% ethanol in cored bolts. The *X. germanus* counts recovered from

TABLE 3 | Analysis of variance statistical output associated with ambrosia beetles collected in ethanol-baited bottle traps in 2022.

Sites	*X. crassiusculus*			*X. germanus*			*A. maiche*			**All ambrosia beetle species combined**[h]		
	F	df	*p*	*F*	df	*p*	*F*	df	*p*	*F*	df	*p*
GA Peach[a]	18.4	2,6	0.003	—	—	—	—	—	—	37.4	2,6	<0.001
NC Apple[b]	81.3	2,10	<0.001	63.9	2,10	<0.001	—	—	—	274.7	2,10	<0.001
GA Pecan[c]	233.7	2,10	<0.001	4.6	2,10	0.038	—	—	—	284.0	2,10	<0.001
GA ORN[d]	331.2	2,10	<0.001	33.9	2,10	<0.001	—	—	—	442.8	2,10	<0.001
OH ORN[e]	3.4	2,14	0.061	340.4	2,14	<0.001	3.9	2,14	0.044	503.4	2,14	<0.001
SC ORN[f]	314.9	2,10	<0.001	—	—	—	—	—	—	320.3	2,10	<0.001
VA ORN[g]	37.2	2,10	<0.001	—	—	—	—	—	—	78.8	2,10	<0.001

Note: Other ambrosia beetle species collected in the samples were randomly identified but were not quantified.

[a]*Xylosandrus compactus, Xyleborus ferrugineus, Xyleborinus saxesenii, Cyclorhipidion* spp., *Hypothenemus* spp.

[b]*X. saxesenii* and *Monarthrum mali.*

[c]*X. compactus, Xyleborus* spp., *X. saxesenii, Ambrosiodmus* spp., *Cnestus mutilatus* and *Hypothenemus* spp.

[d]*X. compactus* and *X. saxesenii.*

[e]Other species of ambrosia beetles were not identified.

[f]*X. compactus, Xyloborus affinis, X. ferrugineus, Xyloborus glabratus, Xyleborus intrusus, Xyleborus pubescens, Xyleborinus attenuates, X. saxesenii, Anisandrus dispar, Ambrosiodmus lecontei, C. mutilatus, Cyclorhipidion bodoanum, Euwallacea validus* (Eichhoff), *Hylastes porculus, Hypothenemus crudiae* (Panzer), *Hypothenemus dissimilis* and *Hypothenemus eruditus.*

[g]*X. compactus, X. xylographus, X. rubicollis, X. saxesenii, C. mutilatus* and *D. onoharanse.*

[h]*X. crassiusculus* and *X. germanus* included.

the LR ethanol lure were significantly correlated with entry holes on 10% cored bolts (Table 5).

4 | Discussion

Xylosandrus crassiusculus and *X. germanus* were collected from tree fruit and nut orchards and ornamental nurseries. Although all ethanol lures captured *X. crassiusculus* adults, specific attraction to LR and HR ethanol lures was inconsistent across all sites. At sites where there was high activity of *X. crassiusculus*, such as at GA and SC ornamental and GA pecan sites, more adults of this species were captured with the HR compared to the LR lure. Conversely, at sites with low *X. crassiusculus* activity, such as VA ornamental and GA peach sites, more adults were captured with the LR lure than with the HR lure. In North America, *X. crassiusculus* populations are genetically diverse (Storer et al. 2017). The innate genetic variations in the *X. crassiusculus* populations in various sites could also contribute to unique behaviour and sensitivity to ethanol or its concentrations across the sites.

The numbers of *X. germanus* captures were greater in the HR ethanol lure than in the LR ethanol lure in all three sites (OH ORN, NC Apple, and GA ORN). This result is consistent with a previous study where more *X. germanus* were captured in traps baited with the HR lure than those baited with the LR lure (Ranger et al. 2011). Thus, results in current and previous studies indicate that response to ethanol release rates may be species-specific.

A. maiche was first reported in Pennsylvania, OH, and West Virginia in 2005 (Rabaglia, Vandenberg, and Acciavatti 2009) and now spread to nine states in the USA (Rabaglia et al. 2019).

A. maiche attacks ethanol-soaked bolts prepared with red maple (*Acer rubrum* L.), sassafras (*Sassafras albidum* (Nutt.) Nees), and shingle oak (*Quercus imbricaria* Michx.) (Reding and Ranger 2020). In the current study, *A. maiche* was captured from the OH site but was not affected by the ethanol lure types. This study documented variable responses of three ambrosia beetle species to ethanol concentrations. Our data suggest that more research is warranted to understand the relationship between ethanol lures with varying release rates exposed to varying temperatures and *X. crassiusculus*, *X. germanus*, and *A. maiche* adult captures.

In the current study, more ambrosia beetle attacks were observed in the 90% ethanol concentration than in the 10% ethanol concentration when ethanol was added to cored bolts in all sites. Cavaletto et al. (2021) showed that more *X. crassiusculus* attacks occurred on 5% ethanol-filled bolts than on 90%, whereas *Xyleborinus saxesenii* (Ratzeburg) did not show any preferential attractiveness between 5% and 90% ethanol. In the current study, the ethanol-soaked bolts sustained more ambrosia beetle attacks in the 90% ethanol than in the 10% ethanol concentration in most sites (GA, SC, and OH ornamental, and NC apple); however, there were either no differences or 10% ethanol soak resulted in more attacks than 90% in GA pecan and VA. Previous studies also reported contrasting results, where an increase in ethanol concentration from 4% to 12% reduced *X. germanus* adult attacks on bolts (Rassati et al. 2020). These current and past studies indicate that more research should be conducted on how these varying ethanol concentrations interact with the ambrosia beetle species and the environment in which they thrive.

Bolts soaked with ethanol received more or similar numbers of ambrosia beetle attacks than the ethanol-infused cored bolts
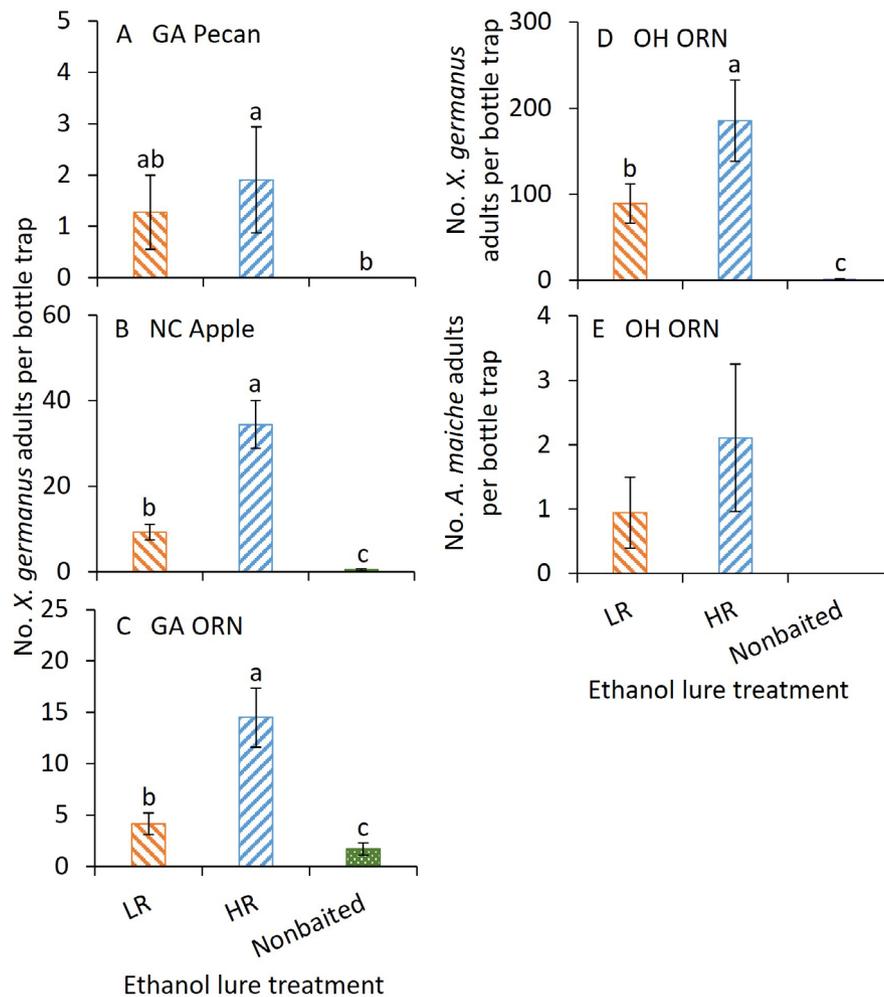
FIGURE 3 | Mean (±SE) *X. germanus* adults collected in (A) GA pecan, (B) NC, (C) GA ornamental, (D) OH and (E) *A. maiche* adults in OH sites from various bottle trap treatments (LR, HR and non-baited) through 6 weeks in 2022. Bars with the same letters within a figure are not significantly different at (α = 0.05) using the Tukey–Kramer test. The abbreviation, ORN, ornamentals. Where no differences were observed among treatments, no letters are given. [Colour figure can be viewed at wileyonlinelibrary.com]

regardless of ethanol concentration (10% or 90%). This was perhaps related to how readily ethanol was released from the soaked bolts. Although bolts were soaked in ethanol for 24 h in the current study, they may not have penetrated deep into the bolt beyond the bark tissue, and ethanol may have been released more quickly than ethanol added to cored holes. It is likely that the movement of ethanol from the cored area of bolts to the bark delayed and reduced the total amount released, and probably resulting in a lower release rate. At the OH site, where *X. germanus* was most abundant, more attacks occurred in high-concentration cored versus soaked bolts. The reason for these contrasting results is not clear; perhaps *X. germanus* responds more favourably to ethanol released slowly from cored bolts as opposed to quickly released from soaked bolts. When a high-ethanol concentration was injected into trees, considerably higher attacks were observed than those injected with a low ethanol concentration (Addesso et al. 2019). Cavaletto et al. (2021) suggested that *X. crassiusculus* responded more readily to ethanol-soaked bolts and ethanol in cored bolts than *X. saxesenii*. Also, ethanol may be the primary olfactory signal for *X. crassiusculus* that initiates boring activity, whilst *X. saxesenii*

may require a more complex mixture containing ethanol and other host-related volatiles (Chen et al. 2021; Yang, Kim, and Kim 2018). The optimal ethanol concentration is more critical than the host species itself for *X. crassiusculus* as a primary cue that drives attacks (Reding et al. 2017). Moreover, the treatment comparisons were limited to a specific site, and analyses of entry holes were not conducted among sites across commodities and states.

Trapping strategies evaluated in this study bring unique value to the nursery, tree nut, and tree fruit industries for monitoring ambrosia beetles. The LR and HR ethanol lures used in our current study are commercially available and can be purchased and paired with bottle traps. Plastic bottles can be easily modified and prepared to accommodate the lure pouches for monitoring. The drawback of ethanol pouches is that the lure may need replacement depending on the type of pouches used. Data show that the LR lure is effective up to 8 weeks after deployment (Monterrosa et al. 2021). Also, the bottle requires a killing a preserving solution, such as glycol or soap water, which also require weekly replacements to minimise
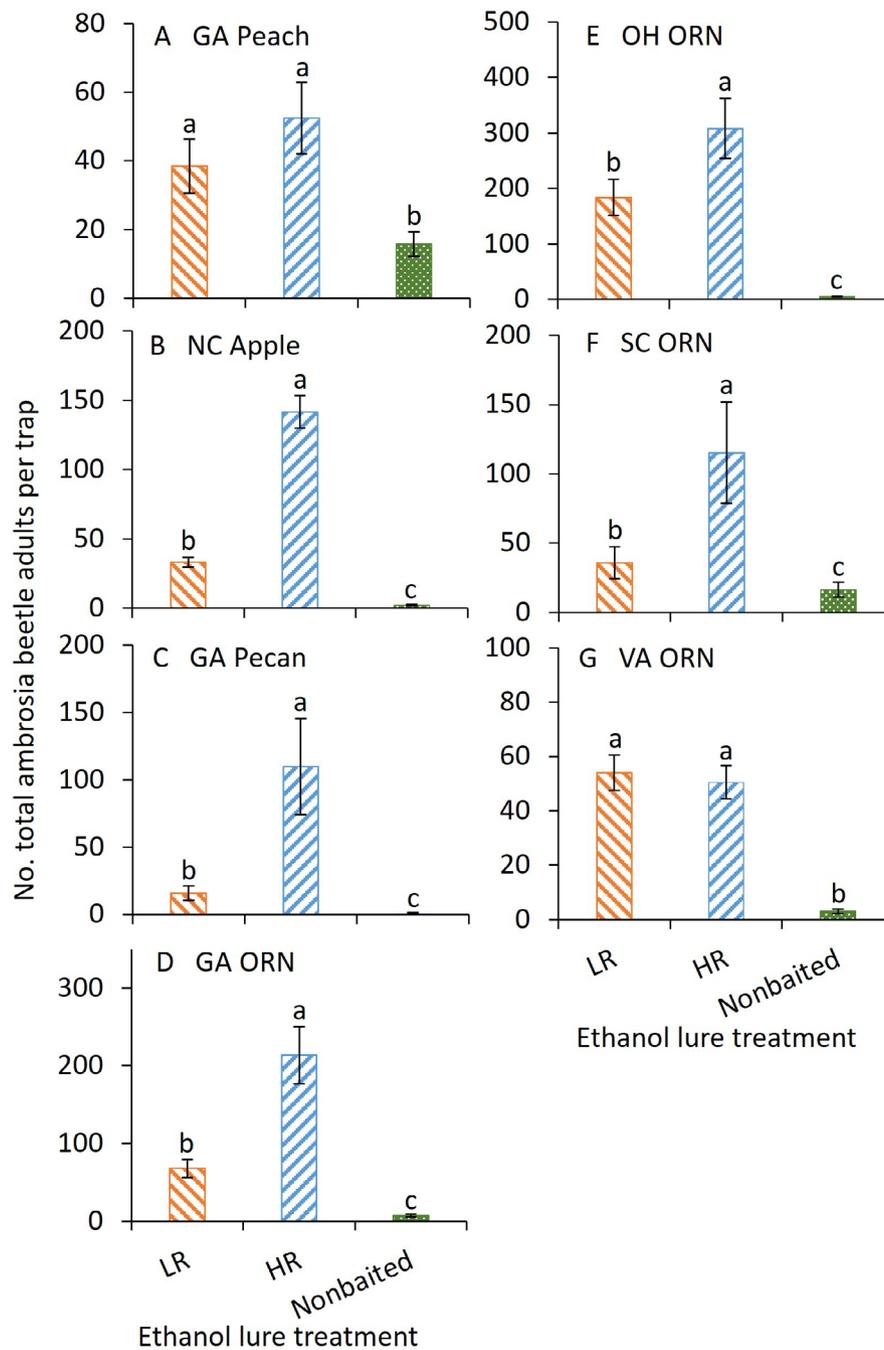
FIGURE 4 | Mean (±SE) adults of all ambrosia beetle species (including *X. crassiusculus*, *X. germanus*, and *A. maiche*) collected through 6 weeks from various bottle trap treatments from (A) GA peach, (B) NC, (C) GA pecan, (D) GA ornamental, (E) OH, (F) SC and (G) VA sites in 2022. Bars with the same letters within a figure are not significantly different at ($\alpha = 0.05$) using the Tukey–Kramer test. The abbreviation, ORN, ornamentals. [Colour figure can be viewed at wileyonlinelibrary.com]

deterioration of the collected beetles. The effort needed to create and deploy bottle traps in the field is relatively lower than for the bolt traps. Growers will need training to visually differentiate ambrosia beetles from other beetles or insects in bottle traps, whilst the bolt method does not require knowledge of beetle identification (Rassati et al. 2020); instead, the bolt method only requires knowledge of the protrusion of compacted sawdust 'noodles'. However, these 'noodles' can break off with rain or wind. These bolts may need replacement as they can become hardened after exposure to ambient

factors for a prolonged period, as the exact period of exposure could vary with biotic factors, such as host tree, age, etc., and abiotic factors, such as temperature, wind, etc. The effects of these factors on the bolt have not been systematically studied. Regardless, both traps are practical for monitoring ambrosia beetles for management decisions.

In summary, these results showed that bolts soaked with 90% ethanol solution for up to 24 h attracted more *X. crassiusculus* and *X. germanus* adults than those with 10% ethanol solution
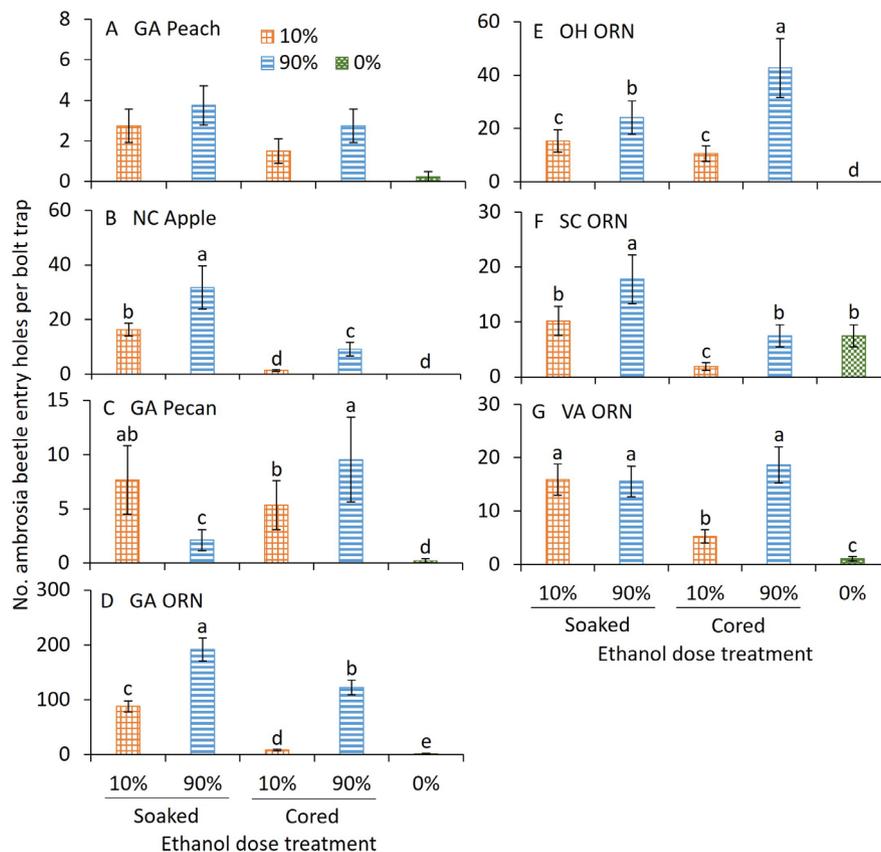
FIGURE 5 | Mean (±SE) entry holes from adult ambrosia beetle documented through 6 weeks from various bolt trap treatments from (A) GA peach, (B) NC, (C) GA pecan, (D) GA ornamental, (E) OH, (F) SC and (G) VA sites in 2022. Bars with the same letters within a figure are not significantly different at ($\alpha = 0.05$) using the Tukey–Kramer test. Where no differences were observed among treatments, no letters are given. The abbreviation, ORN, ornamentals. [Colour figure can be viewed at wileyonlinelibrary.com]

when deployed during peak flight periods. Although cored bolts infused with a high concentration of ethanol (90%) attracted *X. crassiusculus* and *X. germanus*, they had fewer attacks compared with bolts soaked in ethanol in the current study. Sites used different host species as bolt traps, which could influence beetle activity due to other volatile components of the bolts. Thus, more research is warranted to understand the length of time soaked or cored bolts with ethanol remain attractive to ambrosia beetles, keeping host species of bolts constant. Data also suggests that the HR ethanol lures used in this study consistently captured more *X. germanus* compared to the LR ethanol lure. The captures of *X. crassiusculus* with the different lures were not consistent across all sites. Also, lure release rates might be influenced by some unknown factors, such as temperature, wind speed, and direction, relative humidity, or may be mediated by inherent differences among different geographic populations of ambrosia beetles. The results from this study might have been affected by the variation in population levels, surrounding and competing wooded areas, and the host tree species at various sites. Tobin et al. (2024) showed that captures of *X. crassiusculus*, *X. germanus*, and *A. maiche* adults were high when a LR ethanol lure was combined with sticky cards. Sticky traps were rarely evaluated in ornamental nursery systems. Because *A. mache* was not captured in the high numbers in the current study, we could not assess how the trapping method or the ethanol lure type influenced this species.

TABLE 4 | Analysis of variance statistical output associated with entry holes from ambrosia beetle attacks observed on various bolt traps in 2022.

	No. entry holes		
Sites	**F**	**df**	**p**
GA Peach	2.3	4,12	0.114
NC Apple	48.9	4,20	<0.001
GA Pecan	15.8	4,20	<0.001
GA ORN	218.7	4,20	<0.001
OH ORN	81.7	4,28	<0.001
SC ORN	21.4	4,20	<0.001
VA ORN	22.6	4,20	<0.001

Abbreviation: ORN = Ornamentals.

Overall, these findings emphasise that ethanol concentration and release rate can significantly influence ambrosia beetle captures, though responses vary by species and site-specific factors. Further research may be needed to optimise trap design and ethanol lure formulations to enhance early detection and management strategies across different environments, beetle populations, and cropping systems.
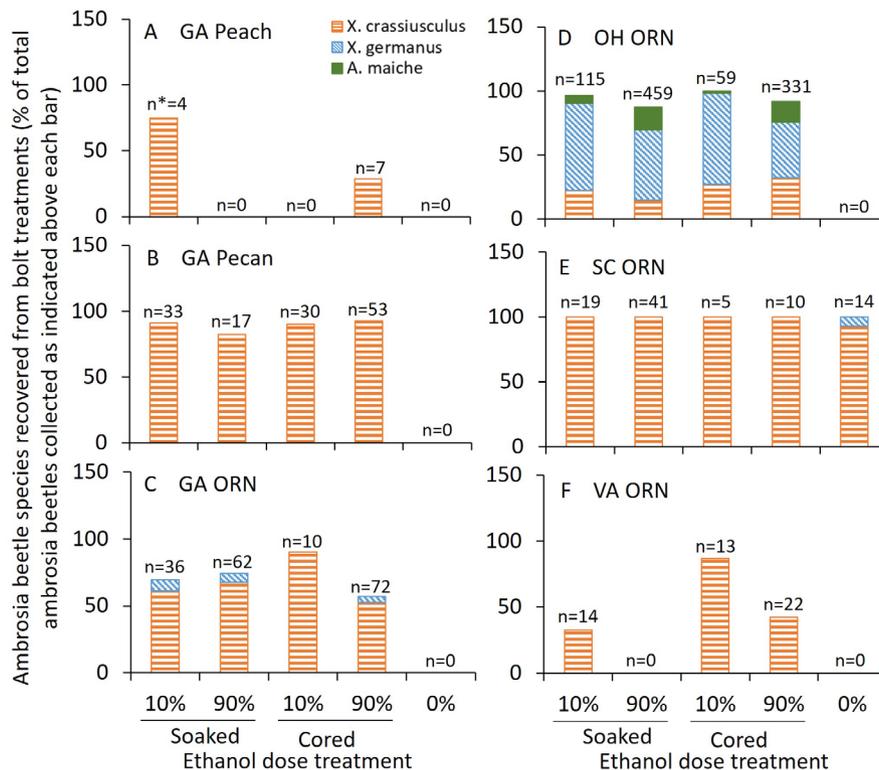
FIGURE 6 | The percentages of *X. crassiusculus*, *X. germanus* and *A. maiche* adults recovered from various bolt trap treatments from (A) GA peach, (B) GA pecan, (C) GA ORN, (D) OH ORN, (E) SC ORN, and (F) VA ORN sites in 2022. *Indicate the total number of ambrosia beetles recovered from the bolt treatment. Ambrosia beetle adults representing only *X. crassiusculus*, *X. germanus* and *A. maiche* were presented in the figure. The abbreviation, ORN, ornamentals. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 5 | Pearson's correlation coefficient (r) between the number of entry holes on bolt treatments and numbers of *X. crassiusculus* and *X. germanus* collected in the bottle lure treatments.

Number of entry holes	*X. crassiusculus* (n = 42)		*X. germanus* (n = 26)	
	LR lure	HR lure	LR lure	HR lure
Soaked 10% ethanol	0.49**	0.54***	NS	NS
Soaked 90% ethanol	0.46**	0.53***	NS	NS
Cored 10% ethanol	NS	0.34*	0.49**	NS
Cored 90% ethanol	0.35*	NS	NS	NS

Abbreviations: HR = high release ethanol lures, LR = low release, NS = not significant.
*$p < 0.5$.
**$p < 0.01$.
***$p < 0.001$.

Author Contributions

Christopher M. Ranger conceived the research idea and designed the experiment; Ramkumar Govindaraju, Jensen Hayter, Christopher M. Ranger, Juang Horng Chong, Alejandro I. Del Pozo-Valdivia, Ted E. Cottrell, James F. Walgenbach, Thomas Scheyer, Brett R. Blaauw, Michael E. Reding, and Shimat V. Joseph conducted the experiments, collected data; Shimat V. Joseph analysed the data; Ramkumar Govindaraju and Shimat V. Joseph prepared the initial draft; Jensen Hayter, Christopher M. Ranger, Juang Horng Chong, Alejandro I. Del Pozo-Valdivia, Ted E. Cottrell, James F. Walgenbach, Thomas Scheyer, Brett R. Blaauw, and Michael E. Reding provided editorial comments on the manuscript draft. All authors are accountable for all aspects of the work.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are deposited in public repository https://knb.ecoinformatics.org/view/urn%3Auuid%3A198825d9-dda1-40f5-a25f-9d6118b740e3

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