

Research Article

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Ethanol concentration can influence the outcomes of insecticide evaluation of ambrosia beetle attacks using wood bolts

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Abstract: The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), and the black stem borer, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), are important pests of ornamental, fruit and nut trees in the eastern USA. A commonly employed method of evaluating insecticide efficacy against ambrosia beetles relies on treating tree bolts infused with $\geq 50\%$ ethanol, which are highly attractive to ambrosia beetles. We conducted experiments at three locations to test the hypothesis that the efficacy of marginally effective insecticides may be better demonstrated using bolts infused with 10% ethanol than $\geq 50\%$ ethanol, where the latter may be so attractive that the ambrosia beetles overcome the effects of the insecticides. The insecticides tested in this study were half and fully labeled application rates of bifenthrin, which are highly effective in reducing ambrosia beetle attacks, and the full rate of indoxacarb, which was reported to have marginal efficacy. On bolts infused with 50% ethanol, attacks (i.e. numbers of entry holes) were lower on those treated with the full and half-rate of bifenthrin than the untreated ones. Attacks on bolts infused with 10% ethanol were too low to allow for the detection of statistical differences among the insecticide treatments. We confirmed that indoxacarb was ineffective in reducing attacks regardless of the

ethanol concentration. We conclude that the current evaluation system should continue to employ $\geq 50\%$ ethanol.

Keywords: bifenthrin; *Xylosandrus crassiusculus*; *Xylosandrus germanus*; ethyl alcohol; wood bolt

Resumen: El escarabajo ambrosia granulada, *Xylosandrus crassiusculus* (Motschulsky), y el barrenador negro del tallo, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) son importantes plagas de árboles ornamentales, frutales y de nuez en el lado este de los Estados Unidos. Un método común empleado para la evaluación de la eficacia de insecticidas para controlar los escarabajos ambrosia se basa en tratar troncos de madera con soluciones de etanol iguales o mayores al 50% de concentración. Estas concentraciones de etanol han probado ser altamente atractivas para los escarabajos ambrosia. Experimentos fueron conducidos en tres lugares para probar la hipótesis de que insecticidas marginalmente eficaces podrían mejorar sus evaluaciones usando troncos de madera tratados con concentraciones de etanol al 10%, comparado con concentraciones iguales o mayores al 50%. Altas concentraciones de etanol podrían ser demasiado atractivas para los escarabajos ambrosia, haciendo que estos superen el efecto de los insecticidas. En estos experimentos, los insecticidas probados fueron: bifentrina aplicada a la dosis completa y al 50% de la dosis, y indoxacarb a la dosis completa. Bifentrina es altamente efectiva para reducir los ataques de estos escarabajos, y indoxacarb ha sido marginalmente efectivo contra los escarabajos. Troncos de madera tratados con etanol al 50% y aplicados con bifentrina a la dosis completa y la mitad de la dosis recibieron menos ataques (hoyos de entrada) que los troncos de madera controles que no tuvieron insecticidas. El número de ataques en los troncos tratados con etanol al 10% fue muy bajo, lo que impidió el análisis estadísticos del efecto de los insecticidas. Se confirmó que indoxacarb fue inefectivo para reducir los ataques de estos escarabajos independientemente de la concentración de etanol. Se concluyó que el sistema actual de evaluación debe continuar usando etanol con concentraciones iguales o mayores al 50%.

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Palabras claves: bifentrina; *Xylosandrus crassiusculus*; *Xylosandrus germanus*; alcohol étílico; tronco de madera

1 Introduction

Tree stress is a major driver for ambrosia beetle attacks on young trees in ornamental tree nurseries and fruit and nut orchards in the eastern USA (Ranger et al. 2010, 2015). Major pestiferous species in the eastern USA are the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), and the black stem borer, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) (Gugliuzzo et al. 2021; Monterrosa et al. 2022; Ranger et al. 2016). These beetles emerge from overwintering sites and bore into the heartwood of young trees. They create galleries and brood chambers, where they cultivate symbiotic fungi with spores stored in their mycangia (Ranger et al. 2016). Females oviposit, and adults and larvae feed on the fungal mycelia (Hulcr and Stelinski 2017). Infested young trees develop branch dieback or general wilting, and some die. Growers incur losses through culling or the inability to sell the infested trees (Ranger et al. 2016).

Environmental stresses, such as flooding, predispose trees to attacks by ambrosia beetles (Ranger et al. 2015). A flooded root system is deprived of oxygen (Cambronero-Heinrichs et al. 2024a). This anaerobic condition alters the normal metabolism of carbohydrates in cells, leading to the accumulation of ethanol in wood tissue (Bailey-Serres and Voesenek 2008; Gibbs and Greenway 2003; Greenway and Gibbs 2003). Ambrosia beetles use ethanol emitted by stressed trees (MacDonald and Kimmerer 1991) as a host-finding cue (Ranger et al. 2015). Exploiting this behavior, ethanol-baited monitoring traps have been used to detect ambrosia beetle flight activity in tree nurseries and orchards (Monterrosa et al. 2021; Oliver et al. 2004; Reding et al. 2010). Ethanol-soaked or ethanol-infused bolts have been used to monitor ambrosia beetles in various environmental settings as well as to investigate either ecological mechanisms related to host selection and colonization or to determine the efficacy of insecticides sprayed on the bolt surface (Cavaletto et al. 2021; Govindaraju et al. 2025; Ranger et al. 2016). Ethanol-infused bolts also have been used extensively in research to determine and refine management tactics, such as documenting the efficacy of insecticides sprayed on the surface of the bolts (Govindaraju and Joseph 2025a; Joseph 2022a,b; Williamson et al. 2023).

Ethanol concentration is one of the key variables affecting the outcome of studies involving ethanol-infused bolts. In fact, a number of studies highlighted that ambrosia beetle response to traps, bolts, or potted trees can be affected by the amount of ethanol released by a given source or present within wood tissues (Cambronero-Heinrichs et al. 2024b; Cavaletto et al. 2021; Govindaraju and Joseph 2025b). For evaluating the efficacy of

insecticides against ambrosia beetles, ethanol concentrations of 50 % or higher have been used in previous studies infusing experimental bolts (Joseph 2022a, b; Williamson et al. 2023). However, a recent study showed that the ethanol concentration emitting from flood-stressed, intact young trees was approximately 10 % (Ranger, unpublished data), suggesting that the ethanol concentration typically used in bolt traps could have been higher than the concentration typically released from stressed trees. A separate study conducted in seven ornamental, nut, and fruit tree sites demonstrated attraction of ambrosia beetles to bolts soaked and filled with 10 % ethanol, albeit significantly less attraction than to bolts soaked and filled with 90 % ethanol (Govindaraju et al. 2025). The higher (and arguably less natural) ethanol concentration used in infused bolts in previous research has significant implications for the design and interpretation of insecticide efficacy experimentation using these bolts. The high concentration of ethanol may enhance the attractiveness of these bolts to such a point that ambrosia beetles may not be influenced by the repellency or lethality of insecticides and attack the bolts, which creates a situation where insecticides or application rates that may be effective or marginally effective in reducing ambrosia beetle attacks are reported to be ineffective. Some of the reported ineffective insecticides or application rates may have less non-target impacts than pyrethroids (Frank and Sadof 2011), which are reportedly the most effective and commonly used insecticides in ambrosia beetle management programs (Joseph 2022a, b).

It is unclear whether bolts infused with 10 % ethanol can attract sufficient numbers of ambrosia beetles to accurately evaluate insecticide efficacy. We conducted experiments in three states (Georgia, South Carolina, and Virginia) to test the hypotheses that bolts infused with 10 % ethanol are (1) similarly attractive to bolts infused with 50 % ethanol, and (2) better at detecting the efficacy of marginally effective insecticides (indoxacarb and reduced application rate of bifenthrin) in reducing ambrosia beetle attacks than those infused with 50 % ethanol. If our hypotheses are supported, growers may be able to use a lower ethanol concentration in their bolt traps (which lowers cost) and insecticide alternatives to pyrethroids (which mitigates environmental and non-target risks), and researchers may be able to develop a testing system that better reflects the field-realistic conditions or ethanol release rate in young stressed trees, and ultimately better identify insecticide alternatives.

2 Materials and methods

2.1 Study site

Four experiments were conducted in 2024, with two experiments in nurseries in Georgia (GA), one in an agricultural

field in South Carolina (SC), and one near a woodlot adjacent to a golf course in Virginia (VA). In all sites, bolts were hung from wooden stakes or metal shepherd's hooks (about 1 m from the ground) along a woodlot. The bolts were hung in transects about 1 m to the woodlot and 10 m to the adjacent bolts. Transects were placed at least 20 m apart. The woodlots were composed of diverse tree and shrub species, such as sweetgum (*Liquidambar styraciflua* L.; Altingiaceae), maple (*Acer* spp.; Sapindaceae), oak (*Quercus* spp.; Fagaceae), hickory (*Carya* spp.; Juglandaceae), pine (*Pinus* spp.; Pinaceae), and privet (*Ligustrum* spp.; Oleaceae).

In nurseries in GA, 1–3 years old sweetbay magnolia (*Magnolia virginiana* L.; Magnoliaceae), oak, maple, etc., were grown under drip irrigation. These trees received routine cultural and pest management practices, such as pruning, fertilization, and pesticide applications. Insecticides were used in nurseries to manage ambrosia beetles and scale insects (Hemiptera: Coccoidea) in the spring but were not used where experiments were conducted. The agricultural field in SC was cultivated with soybeans during the previous year but was not cultivated during the experimental period. In VA, the selected woodlot was next to a water body which was prone to flooding. In the SC and VA sites, insecticides were not used during the experiment to manage pests.

2.2 Chemicals

In the GA and SC sites, bifenthrin (Onyx Pro[®], 23.4 % active ingredient, FMC, Philadelphia, Pennsylvania) was applied at 2,337.5 mL per ha (full rate) or 1,168.8 mL per ha (half rate). In the VA site, bifenthrin (Talstar[®] P, 7.9 % active ingredient, FMC, Philadelphia, Pennsylvania) was applied at 641.8 mL per ha (full rate) or 320.9 mL per ha (half rate). Thus, three times more bifenthrin was applied in GA and SC than in VA. Indoxacarb (Provaunt[®] WDG, 20 % active ingredient, Syngenta, Greensboro, North Carolina) at 525.4 g per ha was used in all experiments except in Experiment 1 in GA. The application volume used in all experiments was 935.4 L per ha. In GA and SC, the insecticide solution was sprayed on bolts using a single nozzle, CO₂-powered sprayer at 206.8 kPa. In VA, a non-ionic surfactant (Audible[™] 90, 90 % active ingredient glycerin, diethylene glycol, and alkyl polyglucoside, Exacto Inc., Sharon, Wisconsin) was mixed at 88.7 mL per ha in insecticide solutions. These solutions were sprayed (for approximately 10 s per bolt) using a compressed air spray bottle (Tough Guy, Grainger, Inc., Lake Forest, Illinois). Bolts were deployed after they had been air-dried in all sites.

2.3 Bolt trap and experimental design

In GA and VA, red maple (*Acer rubrum* L.; Sapindaceae) branches were used to prepare bolts, whereas, in SC, sweetgum (*L. styraciflua*) was used. In all sites, the bolts were approximately 30 cm long × 6 cm diameter. A vertical hole (10 cm deep × 1.2 cm diameter) was drilled in the center of the heartwood of each bolt. After adding 10 mL of 10 % or 50 % ethanol to each hole, corks were used to seal the hole. Ethanol was refilled weekly during the experimental period.

The treatments were 1) 10 % ethanol [10%EtOH]; 2) 10% EtOH + full rate of bifenthrin; 3) 10%EtOH + half rate of bifenthrin; 4) 10%EtOH + full rate of indoxacarb; 5) 50 % ethanol [50%EtOH]; 6) 50%EtOH + full rate of bifenthrin; 7) 50%EtOH + half rate of bifenthrin; and 8) 50%EtOH + full rate of indoxacarb. Indoxacarb treatments were included in the second GA experiment and in the SC and VA experiments but not in the first GA experiment. In all sites, treatments were arranged in randomized complete block design with six replications. Bifenthrin and indoxacarb were sprayed only once on bolts when experiments were initiated. A bolt trap was the experimental unit. The experiments were initiated on 27 February (Experiment 1) and 5 April 2024 (Experiment 2) in GA, 31 March 2024 in SC, and 10 April 2024 in VA, corresponding to the respective major flight periods at the experimental sites.

2.4 Evaluation

The bolts were examined and the numbers of fresh entry holes were recorded at 7 day intervals for 3 or 4 weeks post-deployment. Bolts were evaluated on 5, 12, 19, and 26 March 2024 for Experiment 1 and on 12, 19, and 26 April 2024 for Experiment 2 in GA, 7, 14, 21, and 28 April 2024 in SC, and 17, 24 April, 1, and 8 May 2024 in VA. Fresh entry holes were marked using various colored wax pencils (one color per assessment day) to avoid duplication of counts. The bolts were recovered from the field after 3 or 4 weeks and transported in separate plastic bags to the laboratory. In all the sites, three bolts representing three replications per treatment were randomly selected and were vertically split in eight ways. The ambrosia beetles dislodged from the tunnels inside bolts were collected and preserved in 70 % ethanol for future identification to genus and species using keys in Baker et al. (2009), Bateman and Hulcr (2017), and Smith et al. (2019). Voucher specimens are retained at the Department of Entomology, University of Georgia, Griffin Campus, Griffin, Georgia, Department of Entomology, Virginia Polytechnic Institute and State University, Virginia

Beach, Virginia, and Clemson University, Pee Dee Research and Education Center, Florence, South Carolina.

2.5 Statistical analysis

The data (i.e., total numbers of entry holes across the entire experimental period) from each site were analyzed separately because of the differences in settings among the sites, i.e., ornamental tree nurseries in GA, uncultivated agricultural field in SC, and woodlot near a golf course in VA. The data were subjected to a two-way analysis of variance using a generalized linear model (PROC GLIMMIX) (SAS Institute 2024), where ethanol concentration, insecticide rate and their interaction were included as the fixed factors and block as the random factor. The model was set with a log-link function and using a Poisson distribution. The treatment and replication were fixed and random effects in the model, respectively. The estimates of Least Significant Means were separated by the Tukey–Kramer test ($\alpha < 0.05$). Means and standard errors were calculated from non-transformed data using the PROC MEAN procedure in SAS for plotting the figures. Species diversity of ambrosia beetle adults recovered from bolts were not subjected to statistical analysis.

3 Results and discussion

We conducted a series of experiments across four sites in three states to test the hypotheses that bolts infused with 10 % ethanol, which are more similar in ethanol concentration released from stressed young trees (Ranger, unpublished data), are similarly attractive to bolts infused with 50 % ethanol in terms of number of adult entry holes, and would therefore be better at detecting the efficacy of marginally effective insecticides than bolts infused with 50 % ethanol. Results from our experiments refuted both hypotheses. Bolts infused with 50 % ethanol had significantly more ambrosia beetle attacks than those infused with 10 % ethanol across all sites (Table 1). Insecticide treatments were significantly different in their efficacy in reducing ambrosia beetles at all sites, but not as expected based on our hypotheses. Significant interactions between ethanol concentrations and insecticide treatments were detected in GA and VA but not in SC.

The numbers of ambrosia beetle attacks on bolts infused with 50 % ethanol were significantly different among insecticide treatments in experiments at all sites (Figures 1 and 2). Bolts treated with half and full rates of bifenthrin consistently received significantly fewer attacks than the untreated bolts or bolts that were treated with indoxacarb.

Table 1: Analysis of variance of ethanol concentration, insecticide, and their interaction on entry holes caused by ambrosia beetles on ethanol-infused wooden bolts.

Treatment	<i>F</i>	<i>df</i>	<i>p</i>
Georgia Experiment 1 ^a			
Ethanol conc.	21.4	1.25	<0.001
Insecticide	11.1	2.25	<0.001
Ethanol conc. × insecticide	50.9	2.25	<0.001
Georgia Experiment 2			
Ethanol conc.	118.7	1.28	<0.001
Insecticide	27.7	3.28	<0.001
Ethanol conc. × insecticide	5.1	3.28	0.006
South Carolina			
Ethanol conc.	65.3	1.35	<0.001
Insecticide	21.4	3.35	<0.001
Ethanol conc. × insecticide	1.2	3.35	0.324
Virginia			
Ethanol conc.	655.6	1.35	<0.001
Insecticide	164.9	3.35	<0.001
Ethanol conc. × insecticide	49.3	3.35	<0.001

^aIndoxacarb treatment was not included.

There was no difference in the number of attacks between untreated bolts and indoxacarb-treated bolts in GA Experiment 2 and SC (Figure 2). In VA, the number of entry holes was significantly lower for the indoxacarb-treated bolts than for the untreated bolts. Bolts treated with half rate of bifenthrin received more attacks than those treated with the full rate of the same insecticide active ingredient in GA Experiment 1 (Figure 1) and VA; however, the difference between full and half rate was not significant in GA Experiment 2 and SC (Figure 2). The bifenthrin product used in GA and SC sites had approximately three times more bifenthrin concentration than in the product used in VA site.

Although the numbers of attacks on bolts infused with 10 % ethanol were significantly different among insecticide

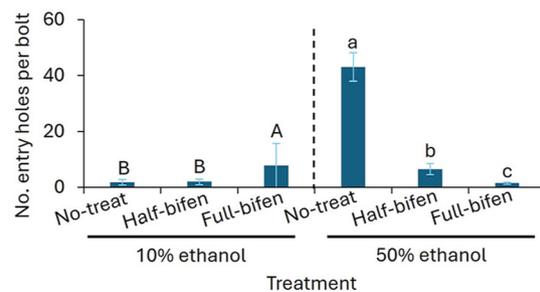


Figure 1: Ambrosia beetle entry holes (accumulated over 3 weeks; mean \pm SE) on bolts infused with 10 % or 50 % ethanol and treated with half or full rates of bifenthrin (bifen) in a Georgia ornamental nursery (Experiment 1) in 2024. The same case letters above bars (within 10 % or 50 % ethanol panel) indicate no significant difference among insecticide treatments within an ethanol concentration using the Tukey–Kramer test ($\alpha = 0.05$).

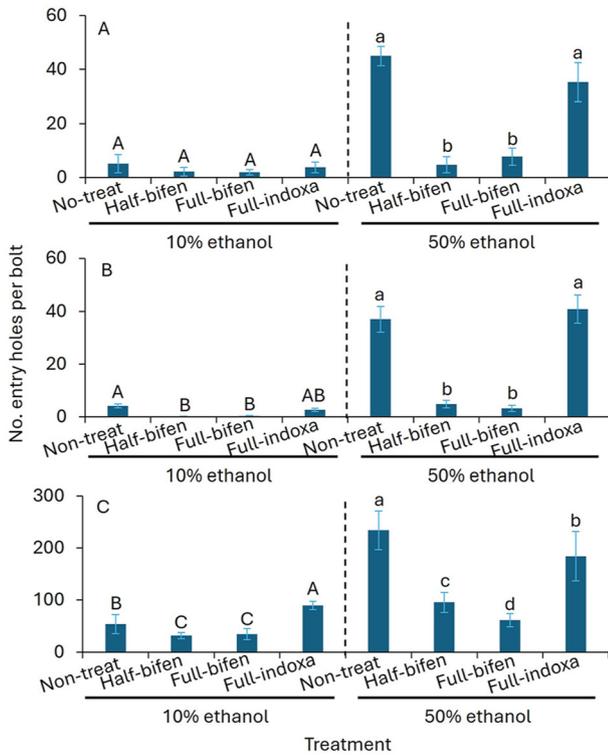


Figure 2: Numbers of ambrosia beetle entry holes (accumulated over 3 or 4 weeks; mean \pm SE) on bolts infused with 10 % and 50 % ethanol and treated with half or full rates of bifenthrin (bifen) or indoxacarb (indoxa) in (A) a nursery in Georgia (GA) (Experiment 2), (B) a farrow agricultural field in South Carolina (SC), and (C) a woodlot near to a golf course in Virginia (VA) in 2024. The same case letters above bars (within 10 % or 50 % ethanol panel) indicate no significant difference among insecticide treatments within an ethanol concentration using the Tukey–Kramer test ($\alpha = 0.05$).

treatments in GA Experiment 1 (Figure 1), SC and VA (Figure 2), ad hoc mean separation tests did not detect the same pattern of means separation as observed on bolts infused with 50 % ethanol. Results generated from bolts infused with 10 % were also inconsistent across test sites. In GA Experiment 1, bifenthrin treatment at the full application rate resulted in greater numbers of attacks than the untreated bolts or those treated with the half application rate (Figure 1). In GA Experiment 2, no significant insecticide treatment effect was observed (Figure 2). In SC, untreated bolts suffered greater numbers of attacks than those treated with bifenthrin (both rates), which were the same numbers on bolts treated with indoxacarb. In VA, bolts treated with indoxacarb suffered more attacks than untreated bolts and bolts treated with bifenthrin (both rates; Figure 2).

The major ambrosia beetle species recovered from the bolts was adult *X. crassiusculus* in all three sites and were mostly from bolts treated with 50 % ethanol (Figure 3). Low numbers of adult *X. germanus* and *Xylosandrus compactus* (Eichhoff) also were collected from bolts in all sites. Adult

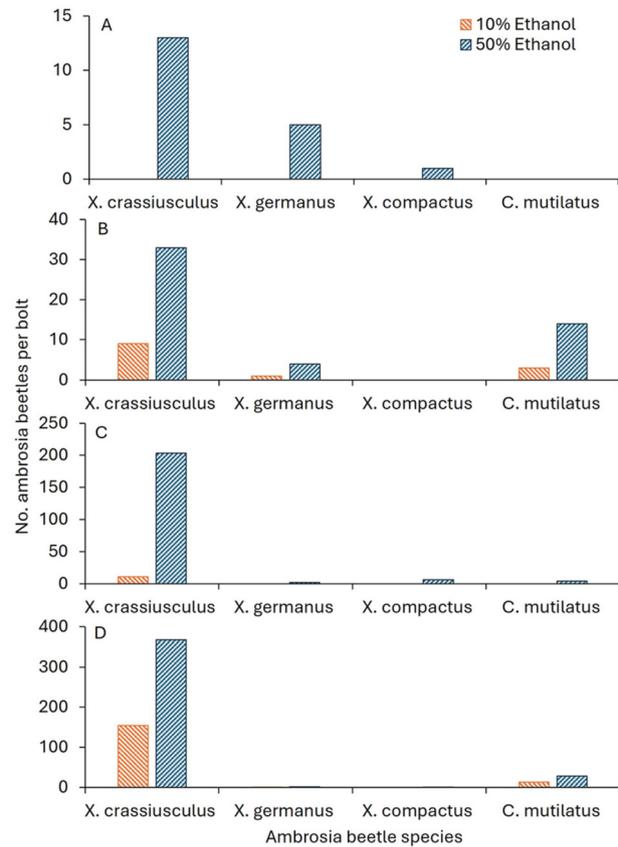


Figure 3: Ambrosia beetles including *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), *Xylosandrus compactus* (Eichhoff) and *Cnestus mutilatus* (Blandford) collected from 10 % and 50% ethanol infused bolts in (A) Georgia (GA) experiment 1, (B) Georgia (GA) experiment 2, (C) South Carolina (SC), and (D) Virginia (VA) in 2024.

Cnestus mutilatus (Blandford) were only collected from GA and VA sites.

Although bolts infused with 10 % ethanol also were attacked, the numbers of attacks they suffered were significantly less than bolts infused with 50 % ethanol. Similar results had been observed in Govindaraju et al. (2025), where the numbers of *X. crassiusculus* and *X. germanus* attacks per bolt were lower on pre-soaked and bolts filled with 10 % rather than 90 % ethanol solution.

Based on results from all sites, differences among insecticide treatments followed similar trends on bolts infused with 50 % ethanol, where the full rate of indoxacarb was not effective, but bifenthrin was highly effective in preventing ambrosia beetle attacks. The full rate of bifenthrin was significantly more effective than half the rate of the same insecticides in two of four sites. On bolts infused with 10 % ethanol, however, differences among insecticide treatments were not consistent across all test sites. Indoxacarb was more effective in preventing attacks than the untreated control in one of three sites where this insecticide

was evaluated, and the full rate of bifenthrin was more effective than the half rate at only one in four sites (VA site). Our data suggests that 10 % ethanol concentration did not consistently produce sufficient attacks to determine the efficacy of the insecticide evaluated. Similar to Govindaraju and Joseph (2025a), the numbers of ambrosia beetle attacks were low on 10 % ethanol treated bolts in the GA and SC sites in this study. Ambrosia beetle attacks in the VA site were approximately five times greater than in the GA and SC sites. We suggest that evaluating insecticide efficacy using bolts infused with 10 % ethanol may help researchers only when the ambrosia beetle population would be expected to be high, even though 10 % ethanol is a close approximation of the concentration produced and released in young flood-stressed trees (Ranger, unpublished data).

Our study results confirm previous reports that indoxacarb was ineffective in reducing ambrosia beetle attacks (Joseph et al., unpublished data). This and previous studies have failed to identify reliable alternatives to pyrethroids in managing ambrosia beetles in ornamental, fruit, and nut tree systems. Research should continue evaluating new chemistries as they become available from agrochemical companies. Based on the results from the current study, future studies should continue to use 50 % ethanol as the attractant in experimental wood bolts in order to generate sufficient attacks to perform any insecticide efficacy experimentation.

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