Effects of Insect Growth Regulators on Ambrosia Beetles (Coleoptera: Curculionidae)[1]

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Abstract Ambrosia beetles, especially the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae), are serious pests in ornamental nurseries in Georgia during the spring months. Growers spray pyrethroid insecticides to prevent ambrosia beetle attacks on the tree trunk around bud break. Repeated pyrethroid insecticide applications can harm beneficial arthropods and cause a resurgence of minor, secondary pests. Insect growth regulators (IGRs), such as novaluron and azadirachtin, have demonstrated transovarial activity on many insect pests, for which the viability of the eggs was reduced after adult exposure. IGRs, particularly azadirachtin, are also repellent to many insect pests. Thus, the objective of this study was to determine the transovarial and repellent activity of IGRs on ambrosia beetles. Two experiments were conducted in ornamental nurseries in 2019 and 2021. In the first experiment, novaluron, azadirachtin, and permethrin were sprayed as stand-alone and combination treatments with permethrin on maple (*Acer*) tree bolts. The number of ambrosia beetle attacks was significantly lower in treatments with permethrin in both years. Novaluron and azadirachtin treatments neither reduced ambrosia beetle attacks on the ethanol-infused bolts nor suppressed the recovery of *X. crassiusculus* from the bolts, suggesting the lack of repellent and transovarial activity, respectively. For the second experiment, novaluron alone and in combination with 1×, 4×, and 8× bark penetrant at the label rate were sprayed on ethanol-infused bolts. None of the novaluron treatments with or without bark penetrant elicited transovarial activity in *X. crassiusculus,* as the beetle recovery was similar among treatments.

Key Words *Xylosandrus crassiusculus*, Scolytinae, novaluron, azadirachtin, permethrin

Ambrosia beetles, particularly the granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae), and *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae), are serious pests in woody ornamental nurseries (Reding et al. 2010, Ranger et al. 2016, Addesso et al. 2019). In 2018, the estimated value of ornamental horticultural crops in Georgia was $888 million USD, with field nurseries accounting for 14.1% of those incurred costs (Wolfe and Stubbs 2019). *Xylosandrus crassiusculus* can colonize 120 tree species (Schedl 1963), and *X. germanus* can attack >200 tree species (Weber and McPherson 1983). Some of the major tree species attacked in the field nurseries in the southeastern United States include redbud (*Cercis* spp.), ornamental cherry (*Prunus* spp.), maple (*Acer* spp.), dogwood (*Cornus* spp.), magnolia (*Magnolia* spp.), and oak (*Quercus*

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spp.) (Weber and McPherson 1983, Ranger et al. 2016). *Xylosandrus* spp. preferentially attack the trunks of these trees (Ranger et al. 2016).

In the spring, the mated females of ambrosia beetles typically emerge from overwintering trees in the woodlot and then seek new hosts to infest and colonize, which are often in adjacent nurseries and orchards (Ranger et al. 2016, Addesso et al. 2019). These mass emergences and flight events coincide with temperatures above 20°C for at least two consecutive days (Reding et al. 2013b) and with bud break of the ornamental trees. During bud break, ethanol levels spike in young, stressed trees (Ranger et al. 2010). Ambrosia beetles bore into the heartwood of colonized trees, where they inoculate symbiotic fungi into the galleries before oviposition. Females and developing larvae consume farmed fungal colonies in the galleries (Ranger et al. 2016). Newly colonized trees may appear wilted with branch dieback (Hara and Beardsley 1979, Ranger et al. 2016), reducing the marketability of ornamental trees (Ranger et al. 2016). Although some affected trees die or remain unsalable, most of them recover from the infestation depending on the tree size and infestation level.

Because ambrosia beetles colonize the heartwood region of trees, it is challenging to reach the adults and developing larvae in the galleries using systemic insecticides or trunk sprays of contact insecticide (Reding et al. 2010, VanDerLaan and Ginzel 2013). Thus, ambrosia beetles are currently managed using preventative trunk sprays during peak adult flight periods (Oliver and Mannion 2001, VanDerLaan and Ginzel 2013, Ranger et al. 2016). Pyrethroids, especially cypermethrin, permethrin, and bifenthrin, reduced beetle attack densities on ethanol-baited trees or experimental bolts (Mizell and Riddle 2004, Frank and Sadof 2011, Reding et al. 2013a, Reding and Ranger 2020). For prolonged control, repeated insecticide applications are required to maintain active ingredient concentrations necessary to repel or deter beetle trunk boring (Frank and Sadof 2011, Ranger et al. 2016). Other insecticides, such as acephate, cyfluthrin, thiamethoxam, chlorantraniliprole, cyantraniliprole, dinotefuran, endosulfan, fenpropathrin, imidacloprid, and tolfenpyrad, were deemed ineffective in preventing *Xylosandrus* spp. infestation (Reding et al. 2013a, Ranger et al. 2016).

Insecticide use, especially repeated applications of pyrethroid insecticides, can harm nontarget organisms and beneficial arthropods in the nursery (Frank and Sadof 2011) and cause the outbreak of secondary pests, such as scale insects and spider mites. Thus, there is a critical need for alternate strategies to manage ambrosia beetles in nurseries. Insect growth regulators (IGRs) are known to transovarially affect the viability of insect eggs when adult females are exposed. Previous studies showed that coleopterans (Zepp et al. 1979, Cowles 2004, Alyokhin et al. 2009) elicit transovarial activity upon exposure; however, little is known about their effects against ambrosia beetles, especially *Xylosandrus* species, such as *X. crassiusculus*. Ambrosia beetles do not feed on wood but may be exposed to the IGRs through contact or by chewing the tunnel, regardless of ingestion. The objectives of this study were to determine the transovarial and repellent effects of (a) novaluron and azadirachtin and (b) novaluron combined with various doses of a bark penetrant when sprayed on bolt traps. If effective, IGRs would reduce egg viability and stop the development of the ambrosia beetle colony inside the heartwood.

Materials and Methods

Study sites. The experiments were conducted in woody ornamental nurseries in central Georgia in 2019 and 2021. At the nursery sites (sites 1 and 2), 0- to 4-yr-old *Magnolia* spp., *Prunus* spp., *Cercis* spp., *Illex* spp., *Ginkgo* spp., *Lagerstroemia* spp., *Stewartia* spp., *Camellia* spp., *Acer* spp., and *Cupressus* spp. trees were planted. These ornamental trees are susceptible to *X. crassiusculus* and *X. germanus* attacks (Ranger et al. 2010, 2016). The ornamental trees in the nurseries were drip irrigated and pruned the entire year. In the spring of 2019 and 2021, the trees at both sites received multiple sprays of bifenthrin to prevent ambrosia beetle attacks and dinotefuran for scale control. Both nursery sites were surrounded by wood lots with various trees and shrubs, such as *Liquidambar styraciflua* L., *Acer* spp., *Quercus* spp., *Pinus* spp., and *Ligustrum* spp.

Insecticide. Two IGRs, namely, novaluron (Pedestal®, 10% active ingredient [a.i.]; OHP Inc., Bluffton, SC), and azadirachtin (Azatin-O®, 4.5% a.i.; Botanical Insecticide, OHP Inc.), and a pyrethroid, permethrin (Perm-UP® 3.2 EC, 36.8% a.i.; FMC Corp., Philadelphia, PA), were used. The rates of active ingredients used in all assays were 58.1, 54.6, and 58.1 g a.i./ha for novaluron, azadirachtin, and permethrin, respectively. For Experiment 1, novaluron, azadirachtin, and permethrin were used without a surfactant. For Experiment 2, novaluron, permethrin, and a nonionic surfactant product containing alkylphenol ethoxylate, polysiloxane polyether copolymer, and propylene glycol (DiAqua PS, 99.8% a.i.; Regal Chemical Co., Alpharetta, GA) were used. The 1× application rate of DiAqua PS was 58.1 g/ha. Because the spray water volume generally varies between 280.6 and 560.7 L/ha in nursery production, an intermediate water volume of 373.9 L/ha was selected to determine the insecticide concentration in the experiments. The insecticides were applied using a CO_2-powered single-boom (one nozzle) handheld sprayer at 206.8 kPa.

Bolt trap and deployment. Woody bolt traps, ~0.5 m long and 6 cm in diameter, were prepared using fresh branches of red maple (*Acer rubrum* L.). The bolts were deployed in the field within 3 weeks after cutting the tree branches. An approximately 25.4-cm-deep hole was drilled at the center of the bolt, and 10 ml of ethanol (70%) was poured into the hole. The hole was closed using a woody cork. The ethanol was replaced in each bolt weekly. In the field, the bolts were deployed 20 m apart, at least 1 m from the wood line, and 9.1 m from the border row of the trees in the nursery. A string was attached to the bolt using screws, and the bolt was hung from a 1.21-m-tall Shepherd hook (Fig. 1A). The ambrosia beetle attacks left entry holes or strands of woody material (toothpicks) characteristic of *Xylosandrus* beetles (Fig. 1B), and new entry holes were quantified weekly. The counted entry holes were circled using a permanent marker pen (Sharpie®; Newell Brands, Atlanta, GA) during quantification. The color of the permanent marker pen was changed every week.

IGR-type experiment. This experiment was conducted in 2019 and 2021. Red maple bolts were used in this experiment and were deployed along the edge of an ornamental nursery at Meansville, GA, in 2019 and Barnesville, GA, in 2021. The details of the trap preparation and deployment are previously described. The treatments were (a) novaluron, (b) azadirachtin, (c) novaluron + permethrin, (d)

Fig. 1. Bolt trap deployed in the nursery (A) and wood material "toothpicks" characteristic of *Xylosandrus* beetles on the bolt (white arrow) when beetles bore through the bark into the heartwood (B).

azadirachtin + permethrin, (e) permethrin, and (f) nontreated control. The treatments were replicated six times in a randomized complete block design (RCBD). The experiments were initiated before the peak flight periods of ambrosia beetles in the nursery. The bolts were deployed on 12 March 2019 and 11 March 2021. The details of the insecticide spray on the bolt traps are explained in the previous section. Treatments were spray applied on bolts at 7-d intervals during 2019 on 12 and 19 March and during 2021 on 11 and 18 March using insecticide formulations and rates previously described.

Novaluron-surfactant experiment. This experiment was conducted in 2021 to determine whether adding the surfactant DiAqua PS would help elicit transovarial activity in ambrosia beetles. Red maple bolts were used in the experiment and were deployed along the edge of an ornamental nursery at Meansville, GA, in 2021. The details of the trap preparation and deployment are previously described. The treatments were (a) novaluron, (b) novaluron + (1×) DiAqua PS, (c) novaluron + (4×) DiAqua PS, (d) novaluron + (8×) DiAqua PS, (e) permethrin, and (f) nontreated control. The treatments were replicated five times in a RCBD. The 1×, 4×, and 8× application rates of DiAqua PS were 58.1, 232.4, and 464.8 g a.i./ha, respectively. The DiAqua PS surfactant was selected for the study because this product is specifically developed for trunk spray application (RCC 2021). Additionally, this surfactant is widely sold and tank mixed with many insecticides for trunk sprays by ornamental nursery growers in Georgia, specifically to prevent the incidence of ambrosia beetle attacks. The application rate of permethrin was 58.1 g a.i./ha. The insecticide treatments were sprayed on the bolts only once on 31 March 2021. The insecticide formulations and application rates were previously described.

Evaluation. The bolt traps were assessed weekly for both experiments, beginning 1 week after trap deployment for up to 3 weeks. For the IGR-type experiment, weekly observations were conducted on 12, 19, and 26 March 2019 and on 11, 18, and 24 March 2021. For the novaluron-surfactant experiment, weekly observations were conducted on 7, 14, and 21 April 2021. On the bolts, the number of entry holes and toothpicks were marked and quantified. The bolts were individually bagged and transported to the University of Georgia Entomology Lab in the third week. The bolts were not opened to document fungal development in the heartwood, as it could damage or reduce the survival of developing larvae in the galleries. The bolts were stored in a room at 28°C (day) and 21°C (night) for 3 mo. The curculionid beetles that emerged from the bolts were trapped inside the plastic bags. The beetles recovered from the bags were sorted and stored in 70% ethanol and identified to the genus or species level using the keys of Smith et al. (2019) and Bateman and Hulcr (2017).

Statistical analyses. The statistical analyses for all of the experiments were conducted using SAS software (SAS Institute 2012). For the IGR-type experiment in 2019 and 2021 and the novaluron-surfactant experiment in 2021, the numbers of holes caused by ambrosia beetle attacks during three sample dates were combined. The numbers of total beetles and various species or genera of adult beetles recovered from the bolt traps were recorded. To determine how many beetles emerged from the entry holes on the bolt for each treatment, the numbers of beetles (all the species or genera combined) per hole were calculated. All the data were subjected to one-way analysis of variance using the generalized linear model procedure (PROC GLIMMIX) with log link function and distribution as the negative binomial. The method used was laplace. The insecticide treatments and replications were fixed and random effects, respectively. The analysis was conducted on all the ambrosia beetle species or genera of ambrosia beetles as long as the beetle counts recovered were sufficient for analysis. The least squares means were separated by a pairwise *t* test ($P < 0.05$).

To determine the association between attack holes on the bolts and ambrosia beetles recovered from the bolts, Pearson's correlation analysis was performed (PROC CORR) in SAS for both experiments. Similarly, Pearson's correlation analysis also was performed between attack holes on the bolts and ambrosia beetles recovered per hole from the bolts by treatment and all treatments combined.

Results

IGR-type experiment. In 2019, significantly lower numbers of holes were found in the bolts treated with permethrin, novaluron + permethrin, and azadirachtin + permethrin than in those treated with novaluron, azadirachtin, and nontreated bolts ($F = 10.8$; df = 5, 25; $P < 0.001$; Fig. 2A). A significantly lower number of emerged ambrosia beetles per attack hole was observed in the azadirachtin + permethrin and permethrin treatments than in the novaluron and the novaluron + permethrin treatments followed by azadirachtin treatment ($F = 5.0$; df = 5, 25; $P = 0.003$; Fig.

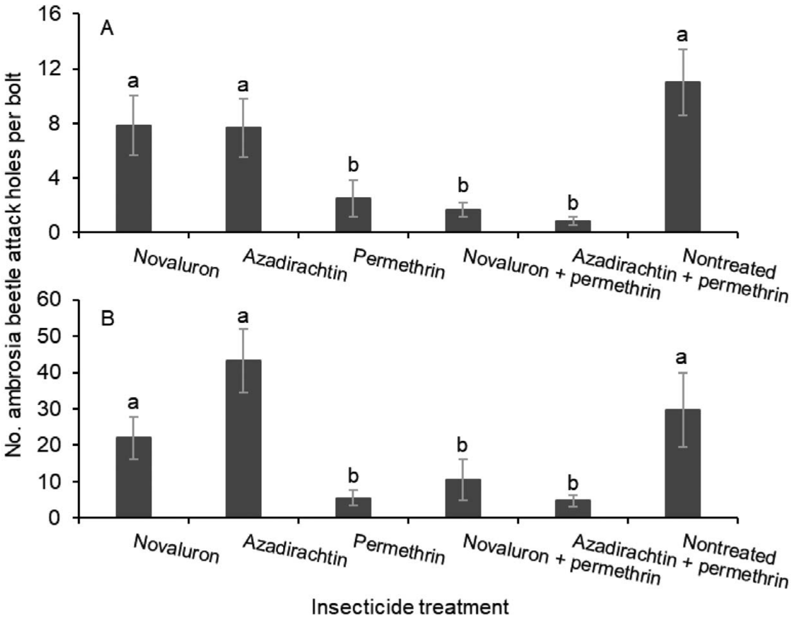

Fig. 2. Mean ± SE number of ambrosia beetle attack holes on the bolt in 2019 (A) and 2021 (B) from the insect growth regulator-type experiment. The bars with the same letters are not significantly different (pairwise *t* test, $P = 0.05$).

3A). There were no significant differences in ambrosia beetles per attack hole among the permethrin, azadirachtin + permethrin, and nontreated treatments. The numbers of *X. crassiusculus* recovered from the bolts were significantly lower in the azadirachtin + permethrin and permethrin treatments than those in the novaluron treatment followed by azadirachtin and the nontreated treatments (Table 1). However, the densities of *X. crassiusculus* recovered from the bolts were not significantly different among the novaluron, novaluron + permethrin, and permethrin treatments. Because low numbers of *X. germanus* and *Ambrosiodmus rubricollis* (Eichhoff) were recovered from the bolts, the effect of treatments on the combined numbers of ambrosia beetles recovered was similar to the numbers of *X. crassiusculus* recovered from the bolts.

In 2021, the numbers of attack holes were significantly lower for permethrin, azadirachtin + permethrin, and novaluron + permethrin treatments than for novaluron, azadirachtin, and nontreated treatments ($F = 9.3$; df = 5, 25; $P < 0.001$; Fig. 2B). No differences were detected in the numbers of ambrosia beetles per hole among treatments ($F = 1.8$; df = 5, 22; $P = 0.157$; Fig. 3B). The numbers of *X. crassiusculus*, other species, and the total beetles recovered from the bolts were not significantly different among treatments (Table 1).

The Pearson's correlation analysis showed that the combined number of attack holes and recovered ambrosia beetle densities were positively correlated in 2019

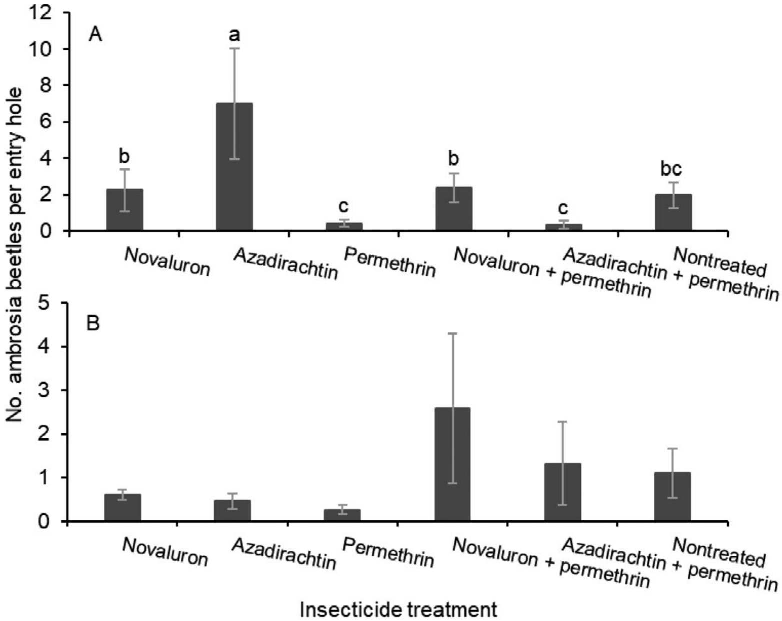

Fig. 3. Mean ± SE number of ambrosia beetles per attack hole on the bolt in 2019 (A) and 2021 (B) from the insect growth regulator-type experiment. All ambrosia beetle taxa recovered from the bolts were combined. The bars with the same letters are not significantly different, and bars without letters are not significantly different (pairwise *t* test, $P = 0.05$).

(Table 2). For the azadirachtin and the novaluron + permethrin treatments, there were positive correlations between attack holes and recovered beetles but not for other treatments. There was no association between the numbers of attack holes on the bolts and recovered beetles per attack hole from the bolt traps for each treatment or all treatments combined (Table 2). In 2021, the combined number of attack holes and recovered ambrosia beetle densities were positively correlated ($P > 0.05$; Table 2). For the novaluron + permethrin treatment, a significant, positive correlation between attack holes and recovered beetles was observed but not for other treatments. When the number of attack holes on the bolts and recovered beetle densities per attack hole from the bolt traps was assessed, a negative association was observed for novaluron treatment, although the attack holes and recovered beetles per attack hole on the bolts for the remaining individual treatments and combined treatments were not significantly correlated ($P > 0.05$; Table 2).

Novaluron-surfactant experiment. Significantly fewer attack holes were found in the bolts treated with permethrin than in the bolts treated with novaluron, novaluron + DiAqua PS (1×), novaluron + DiAqua PS (4×), novaluron + DiAqua PS (8×), and nontreated treatments ($F = 4.3$; df $= 5, 20$; $P = 0.008$; Fig. 4A).

Table 1. Mean ± SE number of ambrosia beetle species recovered from ethanol-baited wooden bolt traps treated with insect growth regulator treatments in 2019 and 2021*.

Year	Treatment	*X. crassiusculus*	*X. germanus*	*Xyleborinus* spp.	*Xyleborus* spp.	*A. rubricollis*	Others	Total
2019	Novaluron	8.0 ± 3.0bc	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	8.2 ± 3.1bc
	Azadirachtin	35.0 ± 9.6a	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	35.0 ± 9.6a
	Novaluron + permethrin	5.0 ± 2.3cd	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 2.3cd
	Azadirachtin + permethrin	0.5 ± 0.3e	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.3e
	Permethrin	1.5 ± 0.6de	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.6de
	Nontreated	21.2 ± 10.7a	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	21.5 ± 10.6a
	F; df1, df2	10.1; 5, 25	—	—	—	0.0; 5, 25	—	10.5; 5, 25
	P value	<0.001	—	—	—	0.996	—	<0.001
2021	Novaluron	9.8 ± 1.4	0.5 ± 0.3	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.7 ± 1.5
	Azadirachtin	15.8 ± 3.7	0.3 ± 0.3	0.8 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	17.2 ± 4.2
	Novaluron + permethrin	8.0 ± 3.5	0.7 ± 0.4	0.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.0 ± 3.7
	Azadirachtin + permethrin	5.0 ± 3.6	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5.2 ± 3.8
	Permethrin	3.7 ± 1.4	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	3.8 ± 1.3
	Nontreated	23.5 ± 10.1	0.3 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	24.0 ± 10.2
	F; df1, df2	2.1; 5, 25	0.4; 5, 25	1.4; 5, 25	—	—	—	2.3; 5, 25
	P value	0.098	0.866	0.269	—	—	—	0.077

* Weekly observations were conducted on 12, 19, and 26 March in 2019 and on 11, 18, and 24 March 2021, and weekly data were combined to calculate means and errors. Within the column, ambrosia beetles recovered without letters or the same letters indicate no significant differences among treatments (pairwise *t* test, $P = 0.05$).

Table 2. Pearson's correlation coefficients between the number of total attack holes and recovered beetles per attack hole from the bolts in 2019 and 2021.

Attack Holes	Treatments†	Beetles	Beetles per Attack Hole
2019	Combined	0.665***	
	Novaluron		
	Azadirachtin	0.871*	
	Novaluron + permethrin	0.897*	
	Azadirachtin + permethrin		
	Permethrin		
	Nontreated		
2021	Combined	0.458**	
	Novaluron		−0.868*
	Azadirachtin		
	Novaluron + permethrin	0.836*	
	Azadirachtin + permethrin		
	Permethrin		
	Nontreated		

† $n = 36$ for the combined; and $n = 6$ for all other individual treatments.
The asterisks indicate the level of significance (*, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$) between variables. Nonsignificant coefficients are not presented ($P > 0.05$).

There were no significant differences between the novaluron + DiAqua PS treatments and permethrin treatment. The numbers of ambrosia beetles per attack hole observed on the bolts were not significantly different among treatments ($F = 1.9$; df = 5, 18; $P = 0.128$; Fig. 4B). The numbers of recovered *X. crassiusculus* and all of the ambrosia beetles combined from the bolts were not significantly different for novaluron alone, novaluron + various dose treatments of DiAqua PS, and permethrin treatments compared with those of the nontreated group (Table 3). No differences were detected in the numbers of recovered ambrosia beetles combined, *X. crassiusculus*, or other species among treatments (Table 3).

Pearson's correlation analysis showed that the numbers of attack holes and recovered ambrosia beetle densities were positively correlated in 2019 ($r = 0.374$; $P = 0.042$; $n = 36$) but not for individual treatments. However, the associations between the numbers of beetles per attack hole recovered from the bolt traps and for each treatment or all treatments combined were not significantly different ($P > 0.05$).

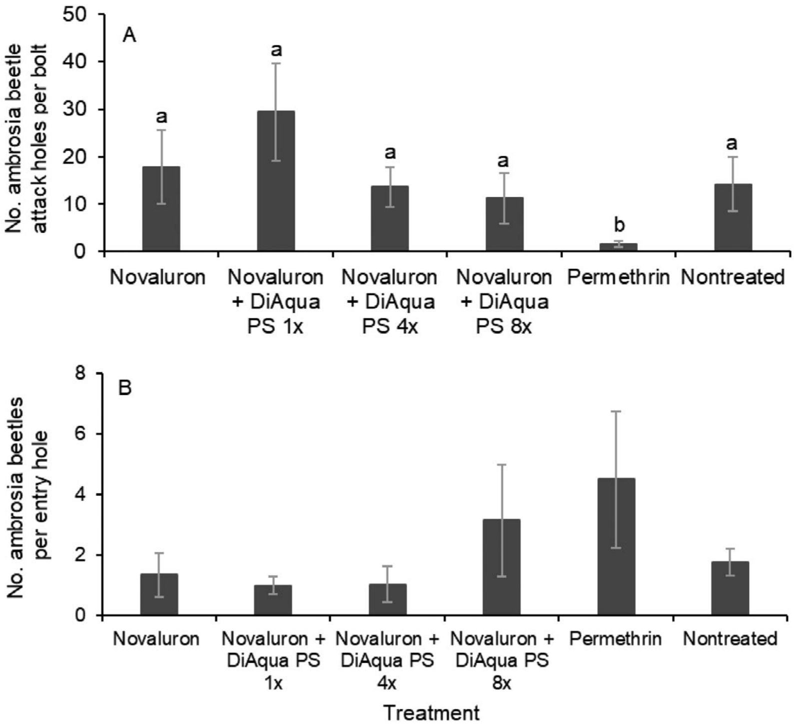

Fig. 4. Mean ± SE number of ambrosia beetle attack holes on the bolt (A) and ambrosia beetles per attack hole (B) from the insect growth regulator surfactant experiment in 2021. All ambrosia beetle taxa recovered from the bolts were combined. The bars with the same letters are not significantly different, and bars without letters are not significantly different (pairwise *t* test, $P = 0.05$).

Discussion

The results did not provide conclusive evidence for transovarial activity with reduced egg viability in ambrosia beetles, as more adults emerged from the bolts than after adult exposure to dried residues of IGRs, such as novaluron and azadirachtin. In previous research in which transovarial activity was documented in coleopterans, such as the Colorado potato beetle (*Leptinotarsa decemlineata* [Say]) (Alyokhin et al. 2009), vine weevil (*Otiorhynchus sulcatus* [F.]) (Cowles 2004), and red flour beetle (*Tribolium castaneum* [Herbst]) (Trostanetsky and Kostyukovsky 2008), insects were fed an IGR-treated diet. The invading ambrosia beetles, primarily *X. crassiusculus* in the current study, did not feed on the IGR-treated bark before boring through the bolts. Other studies showed that hemipterans, for example, (*Bagrada hilaris* [Burmeister]) (Joseph 2017), *Stephanitis pyrioides* [Scott]) (Joseph 2019), and sweetpotato whitefly (*Bemisia tabaci* Gennadius) (Lewis and Joseph 2021), exposed to dry residues of IGRs developed transovarial

Table 3. Mean ± SE number of ambrosia beetle species recovered from ethanol-baited wooden bolt traps treated with novaluron and various rates of bark penetrant in 2021.

Treatment	*X. crassiusculus*	*X. germanus*	*Xyleborinus* spp.	*Xyleborus* spp.	*A. rubricollis*	*Cnestus mutilatus*	*X. compactus*	Total
Novaluron	30.6 ± 16.6	0.0 ± 0.0	5.8 ± 3.5	0.2 ± 0.2	0.0 ± 0.0	1.8 ± 0.4	0.0 ± 0.0	39.2 ± 18.2
Novaluron + ADJ* 1×	21.4 ± 8.1	0.2 ± 0.2	1.8 ± 1.1	0.2 ± 0.2	0.0 ± 0.0	1.0 ± 0.8	0.0 ± 0.0	26.4 ± 10.1
Novaluron + ADJ 4×	13.2 ± 10.5	0.0 ± 0.0	1.2 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	14.8 ± 11.1
Novaluron + ADJ 8×	20.4 ± 15.8	0.0 ± 0.0	6.2 ± 5.2	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	27.2 ± 16.4
Permethrin	2.6 ± 0.9	0.0 ± 0.0	1.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	4.8 ± 1.7
Nontreated	14.0 ± 7.5	0.0 ± 0.0	4.2 ± 2.6	0.4 ± 0.4	0.2 ± 0.2	0.6 ± 0.6	0.2 ± 0.2	20.8 ± 7.7
F; df1, df2	2.2; 5, 20	—	1.2; 5, 20	0.2; 5, 20	—	0.9; 5, 20	—	2.6; 5, 20
P value	0.096	—	0.353	0.839	—	0.455	—	0.060

* Adjuvant, DiAqua PS (99.8% a.i.) and alkylphenol ethoxylate, polysiloxane polyether copolymer, propylene glycol. Weekly observations were conducted on 7, 14, and 21 April 2021, and weekly data were combined to calculate means and errors. Within the column, no significant differences were observed among treatments and hence, no letters are given (pairwise *t* test, $P > 0.05$).

effects. It is unclear whether the approaching ambrosia beetles sufficiently contacted the treated residues of IGR on the bark before boring through the bark into the heartwood.

The invading ambrosia beetles were not repelled by fresh residues of IGRs, especially azadirachtin deposited on the bark. Azadirachtin or neem oil has demonstrated repellency against many insect pests (Brahmachari 2004, Weathersbee and McKenzie 2005, Seljåsen and Meadow 2006, Mikami and Ventura 2008, Kamminga et al. 2009, Hasan and Ansari 2011, Ikeura et al. 2013, Rehman et al. 2014). Similarly, novaluron did not elicit any repellent effects. Novaluron elicited signs of repellency with *S. pyrioides* in a laboratory study (Joseph 2020). Consistent with previous research (Frank and Sadof 2011, Reding and Ranger 2020), fewer entry holes were observed on the bolt traps when permethrin was applied. The reduction in entry holes suggests that permethrin can kill or repel invading ambrosia beetles, in addition to it having toxic effects.

Xylosandrus crassiusculus was the major species of ambrosia beetles detected from the bolt traps. These results were consistent with previous research, where 82–94% and 40–46% of all ambrosia beetles collected were *X. crassiusculus* in 2019 and 2020, respectively, using ethanol-baited bottle traps deployed in ornamental nurseries in central Georgia (Monterrosa 2021). Other pest species, for example, *X. germanus* and *Xylosandrus compactus* (Eichhoff), also were collected from the bolts in the current study; however, their densities were too low to assess the impact of IGRs on their behavior or reproductive fitness. More research is warranted to determine transovarial activity on other pest species of ambrosia beetles.

In summary, the transovarial activity of IGRs on *X. crassiusculus* was not evident in the current study. The presence of IGR products on the surface of bolts did not slow the establishment of *X. crassiusculus* colonies inside the tree trunk after infestation. Additionally, the number of emerging beetles per gallery in IGR-treated bolts was similar that of untreated bolts. When the repellency of IGRs and permethrin was assessed against ambrosia beetles, permethrin consistently reduced entry holes on the tree trunk. The study also showed that *X. crassiusculus* continues to be the major pest species of ambrosia beetles in central Georgia ornamental nurseries.

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