ENTOMOLOGICAL
SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY

OXFORD

Ecosystem Ecology

Ornamental nurseries adjacent to hardwood-dominated woodlots pose more risk from exotic ambrosia beetles (*Xylosandrus* spp.) (Coleoptera: Curculionidae: Scolytinae) than pine-dominated woodlots

Shimat V. Joseph[*,] and **Ramkumar Govindaraju**

Department of Entomology, University of Georgia, Griffin, GA, USA

*Corresponding author. Department of Entomology, University of Georgia, 1109 Experiment Street, Griffin, GA 30223, USA (Email: svjoseph@uga.edu).

Subject Editor: Lukasz Stelinski

Exotic granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), black stem borer, *Xylosandrus germanus* (Blandford), and black twig borer, *Xylosandrus compactus* (Eichhoff) are serious pests of woody ornamental trees in nurseries. Woodlots often surround ornamental nurseries, where the ambrosia beetles fly into nurseries and attack young trees. The woodlots are either dominated by hardwood trees, such as Oaks (*Oak* spp.), maples (*Acer* spp.), or pine trees, mainly loblolly pine (*Pinus taeda* L.). It is unclear if the woodlot type would influence the abundance of ambrosia beetle pests flying outside the woodlot. Thus, this study aimed to determine whether the hardwood or pine-dominated stand affects the relative abundance of ambrosia beetle pests outside the woodlot. In 2023 and 2024, experiments were conducted by deploying 3 ethanol-lured plastic bottle traps in 4 hardwood and 4 pine-dominated woodlots in mid-Georgia (USA). The overall captures of *X. crassiusculus* and *X. germanus* were significantly greater in the hardwood than in the pine-dominated woodlots in both years. The numbers of *X. crassiusculus* and *X. germanus* collected in traps were not consistently significantly different between the hardwood and pine-dominated woodlots for most sampling dates. The captures of *X. compactus* were not influenced by woodlot type. This suggests that although ambrosia beetle pests were collected from both hardwood and pine-dominated woodlots in both years, the risk of infestation in nurseries is greater from the adjacent hardwood than pine-dominated woodlots.

Keywords: Xylosandrus crassiusculus, Xylosandrus germanus, monitoring, nursery, pecan

Introduction

The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky), black stem borer, *Xylosandrus germanus* (Blandford), and black twig borer, *Xylosandrus compactus* (Eichhoff) are serious exotic ambrosia beetle pest species in the United States (Reding et al. 2010, Ranger et al. 2016, Addesso et al. 2019), including Georgia (USA) (Monterrosa et al. 2022, Joseph et al. 2023). Adults of *X. crassiusculus*, *X. germanus*, and *X. compactus* attack young trees in ornamental nurseries and fruit and nut orchards (Werle et al. 2015, Ranger et al. 2016, Agnello et al. 2017, Tobin et al. 2024). In 2023, the annual market value of the green industry was estimated at ~$846 million USD, where field nurseries contributed 13.6% in Georgia (GG 2024). Females of *X. crassiusculus*, *X. germanus*, and

X. compactus attack trees in > 200 plant families, although they do not feed on wood (Ranger et al. 2016). Once females bore into the tree trunk, they construct galleries and colonies in the heartwood. As *X. crassiusculus*, *X. germanus,* and *X. compactus* belong to the tribe Xyleborini, adults and larvae of ambrosia beetles are mycetophagous as they exclusively feed on specialized symbiotic ambrosia fungi (Hulcr and Stelinski 2017). These ambrosia beetle species farm these symbiotic ambrosia fungi inside galleries after they attack the host (Ranger et al. 2016). Some infested trees develop branch dieback (Hara and Beardsley 1979, Ranger et al. 2016, Brockerhoff and Liebhold 2017, Gugliuzzo et al. 2020, 2021), whereas others succumb to infestations. The mechanism causing tree mortality is not completely understood. *Xylosandrus compactus* adults attack

pencil-diameter twigs, which can cause extensive damage to trees (Ngoan et al. 1976). Predicting attacks by ambrosia beetles is challenging, and damaged trees are rarely marketed (Ranger et al. 2016). Thus, growers resort to insecticide use in the spring, increasing production costs through additional expenditure on insecticide products, equipment, and labor.

Mated females overwinter in woodlots (Addesso et al. 2019), and they emerge from dead or decaying trees and fly in high numbers out of woodlots, especially when the temperatures increase in the late winter and spring (Ranger et al. 2016, Addesso et al. 2019). Males are flightless and rarely leave the colony (Ranger et al. 2016). Trees exposed to repeated floods (Ranger et al. 2015) and frost events (La Spina et al. 2013) become stressed and produce elevated levels of ethanol (Ranger et al. 2010, 2015), especially before or during bud-break in the spring (Oliver and Mannion 2001). Females of *X. crassiusculus* and *X. germanus* use ethanol as a signal to attack trees, including trees in ornamental nurseries (Ranger et al. 2016). *Xylosandrus compactus* adults are not specifically attracted to ethanol signals and can also attack healthy trees (Ranger et al. 2016).

Management of ambrosia beetles primarily involves timely preventative trunk applications of pyrethroids, such as permethrin and bifenthrin (Ranger et al. 2016, Reding and Ranger 2018, Williamson et al. 2023). Ethanol-lured bolts and bottle traps are recommended to determine the early flight activity of ambrosia beetles during the late winter and early spring for timely pyrethroid truck applications (Joseph et al. 2019, Monterrosa et al. 2021). Because management options to manage ambrosia beetle attacks in ornamental nurseries, fruit, and nut orchards are limited, it is critical to explore and evaluate possible alternate strategies that could reduce the risk of ambrosia beetle infestation. Ornamental nurseries in the southeastern US are often surrounded by mixed stands either dominated by hardwood or pine trees. The coniferous stands in mid-Georgia are mostly comprised of loblolly pine (*Pinus taeda* L.) plantings. Typically, 988 to 1729 pine seedlings are planted per hectare (Cunningham et al. 2008), and the density of young trees decreases as they are harvested from 12 to 17 yr after planting for up to 35 to 40 yr (Nebeker et al. 1985). The pine stands range in size but are at least 8 ha stand trees with other trees and shrubs, such as privet [*Ligustrum sinense* Lour. (ITIS)] or growing on the edges of some pine stands. In contrast, hardwood-dominated woodlots in mid-Georgia primarily comprise trees that naturally grow in heterogeneous spacing and density. A diverse species of hardwood trees, such as oaks (*Oak* spp.), sweetgum (*Liquidambar styraciflua* L.), maples (*Acer* spp.), and so on, are found in the hardwood stands of mid-Georgia. Pine trees typically constitute < 10% of the hardwood stands (SVJ Per. Observation). Adults of *X. crassiusculus*, and *X. germanus* were captured inside pine stands in the southern states, including Georgia (Miller and Rabaglia 2009). Adult *X. germanus* densities increased with increased management of pine forests (Gossner et al. 2019). In the Midwest, captures of adult *X. crassiusculus* and *X. germanus* were not different between the hardwood and pine stands (Baniszewski et al. 2024). Baniszewski et al. (2024) evaluated the seasonal abundance of exotic ambrosia beetles inside rather than outside the woodlot. The biotic factors, such as tree species composition, tree density, canopy height, and so on, and abiotic factors, such as temperature, relative humidity, wind speed, and so on, can be distinctly different inside and outside woodlots. Unlike woodlots, trees in ornamental nurseries are predominantly young, planted in specific spacing, and managed for pests, diseases, and tree growth. In addition, some ornamental nurseries have open areas, as trees are harvested and delayed new tree plantings. It is unclear whether ornamental trees in nurseries or orchards adjacent to hardwood or pine woodlots pose a specific risk for ambrosia beetle attacks. Thus, the objective of this study was to determine the relative abundance of trap captures of ambrosia beetles when traps were deployed adjacent to hardwood or pine woodlots.

Materials and Methods

Study Sites

In 2023 and 2024, experiments were conducted along the borderline of woodlots in mid-Georgia. Woodlots were either dominated by hardwood or pine trees (Table 1). The tree species observed in hardwood woodlots included sweetgum, eastern redbud (*Cercis canadensis* L.), various species of oaks (*Quercus* spp.), maple (*Acer* spp.), privets (*Ligustrum* spp.), poplar (*Populus* spp.), and pine (*Pinus* spp.). The planted pine woodlots were dominated by loblolly pine, while in other sites, natural pine stands had various pine species, such as short-leaf pine (*Pinus echinata* Mill.), long-leaf pine (*Pinus palustris* P. Mill.); however, privets, maples, and sweetgum were present mostly on the edges of these pine stands. The tree species on each site were surveyed by assessing the number of tree species present in a 10 × 10 m area within 1 m from the edge of the woodlot. The location details of sites and the major tree species found in the woodlot are listed in Table 1. The satellite images of hardwood (Fig. 1A–E) and pine-dominated woodlot sites (Fig. 1F–J), in-ground images of a hardwood (Fig. 1H) and a pine-dominated woodlot site are presented (Fig. 1I). Trees in hardwood woodlots were of various ages and at various canopy heights. Trees in pine-dominated woodlots were 17 to 20 yr old and were approximately 6 to 12 m tall. Natural pine trees on various lots were older than 20 yr and taller than 12 m.

Bottle Trap

An ethanol-lured plastic bottle trap was used to determine the flight activity of ambrosia beetles. A clear, rectangular-shaped 1774 mL plastic bottle (VTM LLC, Lexington, KY) was used to construct a bottle trap. On the opposite sides of a plastic bottle, two ~5 cm × ~9 cm rectangular windows were created. The plastic bottle was suspended upside down on a shepherd's hook so that the bottom end of the plastic bottle was ~1 m above the ground level. This allowed flying ambrosia beetles to access the plastic bottle. Two holes (0.4 cm diameter) were drilled at the bottom of the bottle to hang a plastic bottle. A zip tie was inserted through these two holes to hang an ethanol pouch inside the plastic bottle, and another zip tie was inserted through the first zip tie to hang the plastic bottle. Soap solution (2 ml Dawn dish soap per 10 ml water, P&G, Kansas City, KS) was added to the plastic bottle to trap and drown the fallen ambrosia beetles. The plastic bottle lid was unscrewed to empty the collected insects, including the ambrosia beetles. When the bottle was serviced, the contents were emptied onto a coffee filter and placed on a mesh strainer.

We used a low-release ethanol pouch lure inside the plastic bottle. About 15 ml of 95% ethanol was added to a permeable membrane pouch and heat-sealed (Manufacturer: ChemTica Internacional, S.A., San Jose, Costa Rica; Distributor: AgBio Inc., Westminster, Colorado, USA). According to the manufacturer, the pouch releases 16 mg per d ethanol at 20°C, with a > 120 d estimated field life, although the ethanol lures were replaced at 4-wk intervals according to the manufacturer's recommendation.

Experiment Design and Evaluation

The treatments were traps placed along (i) hardwood and (ii) pine-dominated woodlots. The treatments were replicated 4 times (sites)

Table 1. Details of the field sites, and major tree species in hardwood and pine-dominated woodlots in 2023 and 2024.

	Hardwood-dominated stand			Pine-dominated stand	
Site	GPS coordinates	Trees species[a]	Site	GPS coordinates	Trees species[a]
2023					
1	33°24′44″N 84°36′50″W	90%: Oak, sweetgum, maple, poplar; < 10%: Pine	1	33°02′17″N 84°20′17″W	98%: Pine[b]; 2%: Privet
2	33°01′58″N 84°19′45″W	90%: Oak, eastern redbud; <10%: Pine	2	33°15′43″N 84°18′21″W	98%: Pine[b]; 2%: Privet
3	33°03′28″N 84°14′48″W	95%: Oak, sweetgum; 5%: Pine	3	32°59′26″N 84°04′59″W	70%: Pine[c]; 30%: Oak
4	33°15′15″N 84°18′14″W	95%: Oak, sweetgum, privet; 5%: Pine	4	32°58′2.97″N 84°07′5.601″W	95%: Pine[c]; 5%:
2024					
1	32°59′51″N 84°04′59″W	95%: Oak, sweetgum, maple, poplar; 5%: Pine	1	33°02′17″N 84°20′17″W	98%: Pine[c]; 2%: Privet
2	33°01′58″N 84°19′45″W	90%: Oak, eastern redbud; <10%: Pine	2	33°15′43″N 84°18′21″W	98%: Pine[b]; 2%: Privet
3	33°03′28″N 84°14′48″W	95%: Oak, sweetgum; 5%: Pine	3	32°59′26″N 84°04′59″W	70%: Pine[b]; 30%: Oak
4	33°15′15″N 84°18′14″W	95%: Oak, sweetgum, privet; 5%: Pine	4	32°56′41″N 84°14′17″W	98%: Pine[b]; 2%: Privet

[a]Based on the survey of trees within 10 × 10 m² within each woodlot;

[b]Loblolly pine (*Pinus taeda* L.) plantation.

[c]Natural pine stand with short leaf pine (*Pinus echinata* Mill.), long leaf pine (*Pinus palustris* P. Mill.), and loblolly pine. Note: Oak (*Oak* spp.), sweet gum (*Liquidambar styraciflua* L.), maple (*Acer* spp.), eastern redbud (*Cercis canadensis* L.), privet (*Ligustrum* spp.), poplar (*Populus* spp.), pine (*Pinus* spp.).
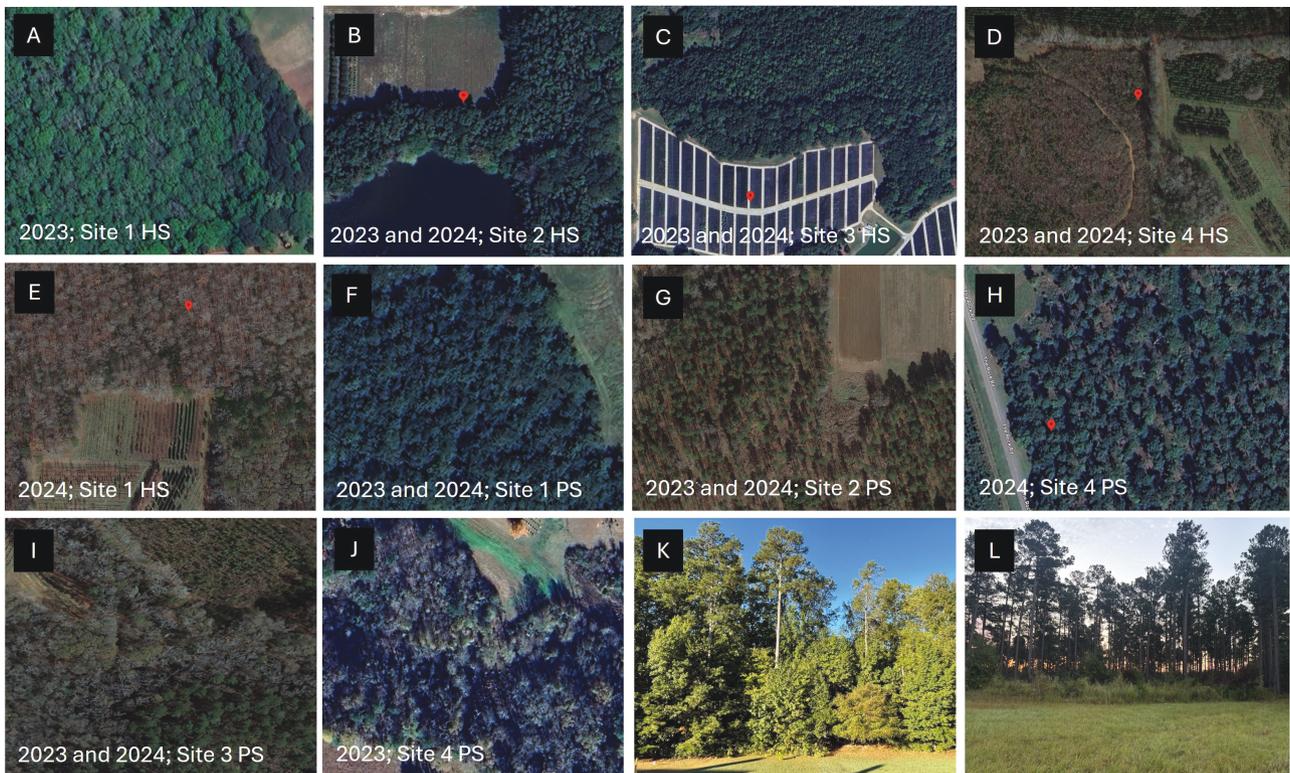

Fig. 1. Satellite images (Google Maps; detailed in Table 1) of sites for (A-E) hardwood and (F-J) pine-dominated stands; (K) hardwood and (L) pine-dominated stands from the ground. Three ethanol-lured bottle traps were deployed along the edge of each woodlot 10 m apart in 2023 and 2024 in mid-Georgia (USA). Abbreviation: HS, hardwood-dominated stand; PS, pine-dominated stand.

(Table 1). At each site, 3 plastic bottle traps were set up ~1 m outside the borderline of the woodlot and 10 m apart from each other. In 2023 and 2024, experiments were initiated during the first spring flight in February. In 2023, traps were deployed on 23 February, and in 2024, traps were deployed on 22 February in all sites. The experiment was concluded on 20 July and 20 June in 2023 and 2024,

Table 2. Analysis of variance of ambrosia beetle pests (*Xylosandrus* spp.) collected in hardwood and pine-dominated stands in mid-Georgia (USA) in 2023 and 2024.

Year	2023[a]			2024[b]		
	F	df	P	F	df	P
X. crassiusculus						
Woodlot	56.1	1,129	< 0.001	51.5	1,99	< 0.001
Sampling date	71.8	21,129	< 0.001	339.9	16,99	< 0.001
Woodlot × Sampling date	4.3	21,129	< 0.001	8.6	16,99	< 0.001
X. germanus						
Woodlot	5.3	1,129	0.023	7.9	1,99	0.006
Sampling date	11.5	21,129	< 0.001	38.3	16,99	< 0.001
Woodlot × Sampling date	2.9	21,129	< 0.001	9.9	16,99	< 0.001
X. compactus						
Woodlot	0.9	1,129	0.335	2.2	1,99	0.141
Sampling date	1.0	21,129	0.455	1.9	16,99	0.021
Woodlot × Sampling date	0.9	21,129	0.608	0.5	16,99	0.941

[a]Samples collected from 2 March to 28 July;
[b]Samples collected from 29 February to 20 June.

respectively. Traps were serviced at 7 d intervals, and the coffee filter paper with ambrosia beetles placed in plastic Ziploc bags was transported to the entomology laboratory at the University of Georgia, Griffin Campus. The samples were temporarily placed in a freezer (–18°C) until sorted in 70% ethanol and identified to species using a lucid key for Southeast Asian Xyleborini Species (Smith et al. 2019) and Bateman and Hulcr's ambrosia beetle guide (Bateman and Hulcr 2017). The identification was restricted to the pest species, such as adults of *X. crassiusculus*, *X. germanus*, and *X. compactus*, as they were the dominating species in ornamental and orchards in Georgia (Monterrosa et al. 2022).

Statistical Analyses

Ambrosia beetle trap capture data were statistically analyzed using SAS software (SAS Institute 2024). For the 2023 and 2024 ambrosia beetle species (*X. crassiusculus*, *X. germanus*, and *X. compactus*) data, one value was added to the ambrosia beetle counts as there were many zeros (Govindaraju et al. 2024). For each ambrosia beetle species, data were subjected to the two-way analysis of variance (ANOVA) with interaction using a generalized linear mixed model analysis (PROC GLIMMIX) in SAS with a log link function and a Poisson distribution. The treatments were woodlot type (hardwood and pine-dominated stands), sampling date, and their interaction, where the sampling date was the repeated measure. The hardwood and pine-dominated stand sites were replications in the analyses. Means were separated post-ANOVA, using the Tukey Kramer method (α = 0.05) for the woodlot type.

The interaction between woodlot type and sampling date was evaluated for all ambrosia beetle species. If the interaction was significantly different for each ambrosia beetle species, trap capture data for each ambrosia beetle species were subjected to one-way ANOVA using a generalized linear mixed model analysis (PROC GLIMMIX) in SAS with a log link function and a Poisson distribution by each sampling date in 2023 and 2024. Means were separated post-ANOVA, using the Tukey Kramer method (α = 0.05).

Data for each analysis were back-transformed using the PROC PLM procedure in SAS to obtain estimates of the least square means and standard errors to differentiate differences between woodlot types. The mean and standard error for the non-transformed data (without adding the value of one) were also calculated using the PROC MEAN procedure in SAS.

Results

In 2023, of 3,517 ambrosia beetles captured, 84.5%, 13.9%, and 1.6% were females of *X. crassiusculus*, *X. germanus*, and *X. compactus*, respectively, whereas, in 2024, of 6,666 ambrosia beetles captured, 86.9%, 11.9%, and 1.1% were adults of *X. crassiusculus*, *X. germanus*, and *X. compactus*, respectively. In both years, the captures of *X. crassiusculus* and *X. germanus* adults significantly differed for woodlot type, sampling date, and their interaction (Table 2). However, for adults of *X. compactus*, there were no significant differences in woodlot type and interaction between woodlot type and sampling date (Table 2). The non-transformed numbers of adult *X. crassiusculus* and *X. germanus* captured in 2023 and 2024 are in Fig. 2A and C, respectively. Greater estimated numbers of adult *X. crassiusculus* and *X. germanus* were captured for the hardwood than in the pine-dominated woodlots in 2023 (Fig. 2B) and 2024 (Fig. 2D). The number of adult *X. compactus* differed significantly by sampling dates only in 2024 (Table 2).

Because the interactions between woodlot type and sampling date for adult *X. crassiusculus* and *X. germanus* were significantly different, one-way ANOVAs were performed to understand the effects of woodlot by sampling date on captures by adult *X. crassiusculus* and *X. germanus*. In 2023, the captures of adult *X. crassiusculus* peaked twice, and they were in March, and late April-early May (Fig. 3A). The numbers of adult *X. crassiusculus* were significantly greater for the hardwood than for the pine-dominated woodlots on 2, 9 March, 12, 27 April, 4, 25 May, 8 June, and 20 July (Table 3; Fig. 3B). There were no significant differences between the hardwood and pine-dominated woodlots on the remaining sampling dates (Table 3; Fig. 3B). The captures of adult *X. germanus* peaked during early March and then peaked and remained steady from late March to early May (Fig. 3C). The numbers of adult *X. germanus* were significantly greater for the hardwood than for the pine-dominated woodlots on 2 March and 20 April whereas, captures were significantly greater for the pine than for the hardwood-dominated woodlots on 30 March (Table 3; Fig. 3D). On the remaining sampling dates, there were no significant differences in adult *X. germanus* captures between the hardwood and pine-dominated woodlots (Table 3; Fig. 3D).

In 2024, the captures of adult *X. crassiusculus* peaked first during mid-March, peaked by early April, and remained moderately high up to early May (Fig. 4A). The numbers of adult *X. crassiusculus* were
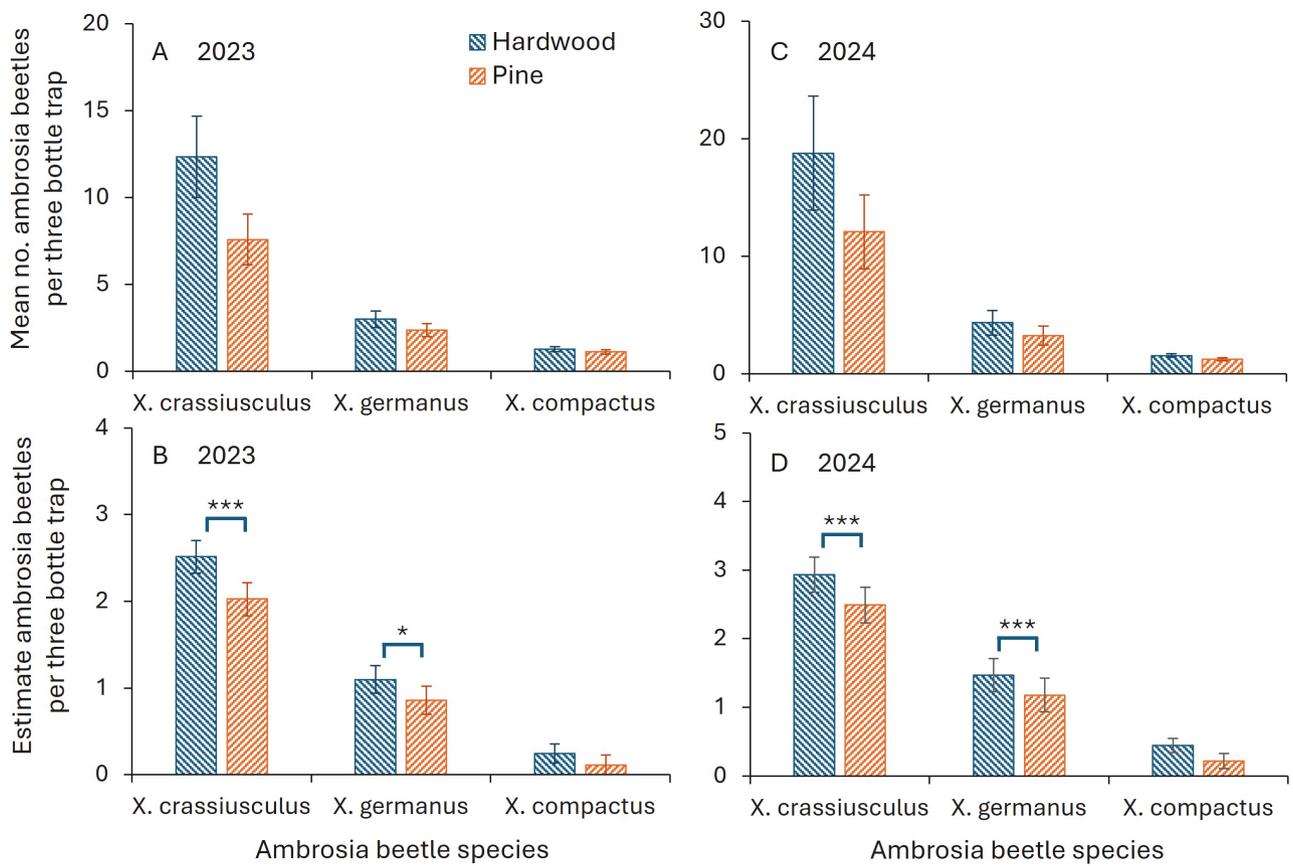
Fig. 2. Overall mean (± SE) of non-transformed numbers of *Xylosandrus* spp. captured in (A) 2023, (C) 2024 using 3 ethanol-lured bottle traps in each site, and overall least square (LS) mean (± SE) estimates of back-transformed numbers of *Xylosandrus* spp. captured in (B) 2023 and (D) 2024. For B and D, asterisks above the bars indicate significant differences between hardwood and pine dominated stands for each *Xylosandrus* spp. at (α = 0.05) using the Tukey-Kramer test. Where no differences were observed among treatments, no asterisks are given. The number of asterisks indicates *P*-value: ***, < 0.001; *, < 0.05.
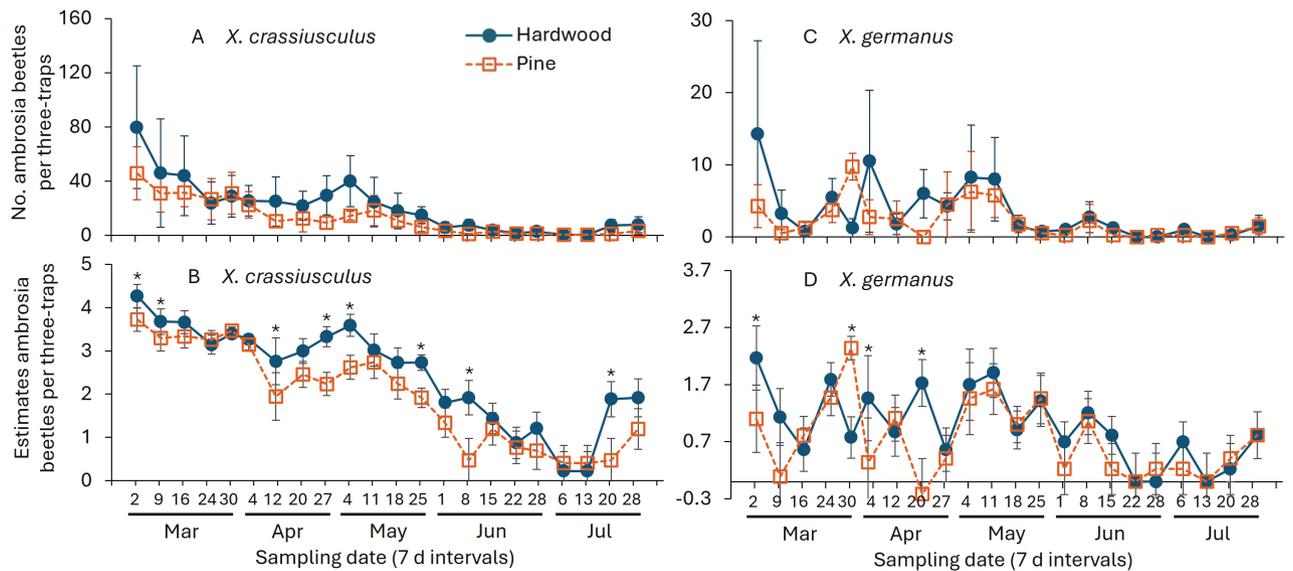

Fig. 3. Mean (± SE) of non-transformed numbers of (A) *X. crassiusculus*, (C) *X. germanus* captured in 3 ethanol-lured bottle traps in each site, and LS mean (± SE) estimates of back-transformed numbers of (B) *X. crassiusculus*, and (D) *X. germanus* in 2023. For B and D, asterisks above the SE lines indicate significant differences between hardwood and pine-dominated stands for each sampling date at (α = 0.05) using the Tukey-Kramer test. Where no differences were observed among treatments, no asterisks are given.

significantly greater for the hardwood than for the pine-dominated woodlots on 20 March, 3, 17, 26 April, 3, 23, 30 May, and 6 June (Table 3; Fig. 4B). There were no significant differences between the hardwood and pine-dominated woodlots on remaining sampling dates (Table 3; Fig. 4B). The captures of adult *X. germanus* peaked 3 times, mid-March, and then in early and late April (Fig.

Table 3. Analysis of variance of ambrosia beetle pests (*Xylosandrus* spp.) from wood line adjacent to hardwood and pine-dominated stands in mid-Georgia (USA) in 2023 and 2024.

Sampling date	X. crassiusculus			X. germanus			X. compactus		
	F	df	P	F	df	P	F	df	P
2023									
2-Mar	35.4	1,3	0.009	17.8	1,3	0.024	1.1	1,3	0.369
9-Mar	11.3	1,3	0.044	4.8	1,3	0.116	1.8	1,3	0.270
16-Mar	7.9	1,3	0.066	0.3	1,3	0.652	0.1	1,3	0.761
24-Mar	0.7	1,3	0.467	1.1	1,3	0.375	0.4	1,3	0.575
30-Mar	0.3	1,3	0.607	18.2	1,3	0.024	7.9	1,3	0.067
4-Apr	0.7	1,3	0.459	14.2	1,3	0.033	0.0	1,3	1.000
12-Apr	21.2	1,3	0.019	0.4	1,3	0.592	0.1	1,3	0.761
20-Apr	9.8	1,3	0.052	13.3	1,3	0.036	0.0	1,3	1.000
27-Apr	36.5	1,3	0.009	0.0	1,3	0.888	0.1	1,3	0.781
4-May	42.6	1,3	0.007	0.9	1,3	0.398	0.4	1,3	0.575
11-May	3.5	1,3	0.159	1.3	1,3	0.341	4.3	1,3	0.131
18-May	6.7	1,3	0.081	0.1	1,3	0.841	0.0	1,3	1.000
25-May	12.8	1,3	0.038	0.1	1,3	0.799	0.0	1,3	1.000
1-Jun	2.2	1,3	0.232	0.7	1,3	0.470	0.0	1,3	1.000
8-Jun	13.6	1,3	0.035	0.1	1,3	0.731	1.3	1,3	0.340
15-Jun	0.5	1,3	0.543	1.1	1,3	0.369	0.0	1,3	1.000
22-Jun	0.1	1,3	0.833	0.0	1,3	1.000	0.0	1,3	1.000
28-Jun	1.5	1,3	0.312	0.1	1,3	0.761	0.0	1,3	1.000
6-Jul	0.1	1,3	0.783	1.9	1,3	0.470	0.0	1,3	1.000
13-Jul	0.1	1,3	0.783	0.0	1,3	1.000	0.0	1,3	1.000
20-Jul	12.9	1,3	0.036	0.1	1,3	0.783	1.8	1,3	0.270
28-Jul	5.9	1,3	0.092	0.0	1,3	1.000	0.0	1,3	1.000
2024									
29-Feb	1.9	1,3	0.262	4.5	1,3	0.123	0.1	1,3	0.761
6-Mar	0.1	1,3	0.833	0.8	1,3	0.438	0.0	1,3	1.000
13-Mar	6.6	1,3	0.083	2.5	1,3	0.209	3.6	1,3	0.153
20-Mar	14.6	1,3	0.032	34.9	1,3	0.009	1.7	1,3	0.275
27-Mar	0.4	1,3	0.594	6.3	1,3	0.086	0.1	1,3	0.761
3-Apr	28.7	1,3	0.013	53.3	1,3	0.005	0.2	1,3	0.670
10-Apr	9.5	1,3	0.560	17.3	1,3	0.025	0.1	1,3	0.799
17-Apr	59.8	1,3	0.005	13.5	1,3	0.035	0.1	1,3	0.799
26-Apr	11.4	1,3	0.043	0.9	1,3	0.406	0.1	1,3	0.833
3-May	81.4	1,3	0.003	0.2	1,3	0.703	3.6	1,3	0.153
10-May	0.4	1,3	0.597	3.0	1,3	0.182	0.1	1,3	0.437
17-May	1.6	1,3	0.289	5.1	1,3	0.109	0.4	1,3	0.575
23-May	11.2	1,3	0.044	3.6	1,3	0.153	0.0	1,3	1.000
30-May	24.4	1,3	0.016	3.6	1,3	0.153	0.0	1,3	1.000
6-Jun	18.3	1,3	0.024	4.3	1,3	0.131	0.0	1,3	1.000
13-Jun	8.9	1,3	0.058	3.6	1,3	0.152	0.0	1,3	1.000
20-Jun	1.4	1,3	0.325	1.1	1,3	0.377	0.0	1,3	1.000

4C). The numbers of adult *X. germanus* were significantly greater for the hardwood than for the pine-dominated woodlots on 3 April whereas, captures were significantly greater for the pine than for the hardwood-dominated woodlots on 30 March, 10 and 17 April (Table 3; Fig. 4D). There were no significant differences in adult *X. germanus* captures between the hardwood and pine-dominated woodlots for the remaining sampling dates (Table 3; Fig. 4D).

The adults of *X. compactus* were captured in early and late March, early May and June, and mid-July 2023 (Fig. 5A). In 2024, adults of *X. compactus* were captured from mid-March to mid-May (Fig. 5B).

Discussion

We sought to understand the flight activity of adult *X. crassiusculus*, *X. germanus,* and *X. compactus* in the spring and early summer along the borderline of hardwood and pine-dominated woodlots in mid-Georgia. Overall, captures of adult *X. crassiusculus* and *X. germanus* were greater in traps deployed adjacent to hardwood than pine-dominated woodlots. Although the exact reasons are unclear, this could be due to several variables. First, the hardwood tree species in hardwood-dominated woodlots are more diverse, and even with diverse niches and stratifications for exotic ambrosia beetles, such as *X. crassiusculus* and *X. germanus*, than pine-dominated woodlots. Second, the hardwood-dominated woodlots are likely older than pine-dominated woodlots. It is known that older hardwood-woodlots have more dead or dying trees (Harmon et al. 1986), whereas pine-dominated woodlots are mostly monoculture of single tree species of similar age. Ambrosia beetle populations thrive in older hardwood-dominated woodlots with dead or dying trees (Campbell et al. 2008, Reed and Muzika 2010). Finally, advanced hardwood-dominated woodlots are rich in hardwood trees with various trunk and branch sizes. Because exotic ambrosia beetles are mostly generalists (Ranger et al. 2016), trees at various trunk
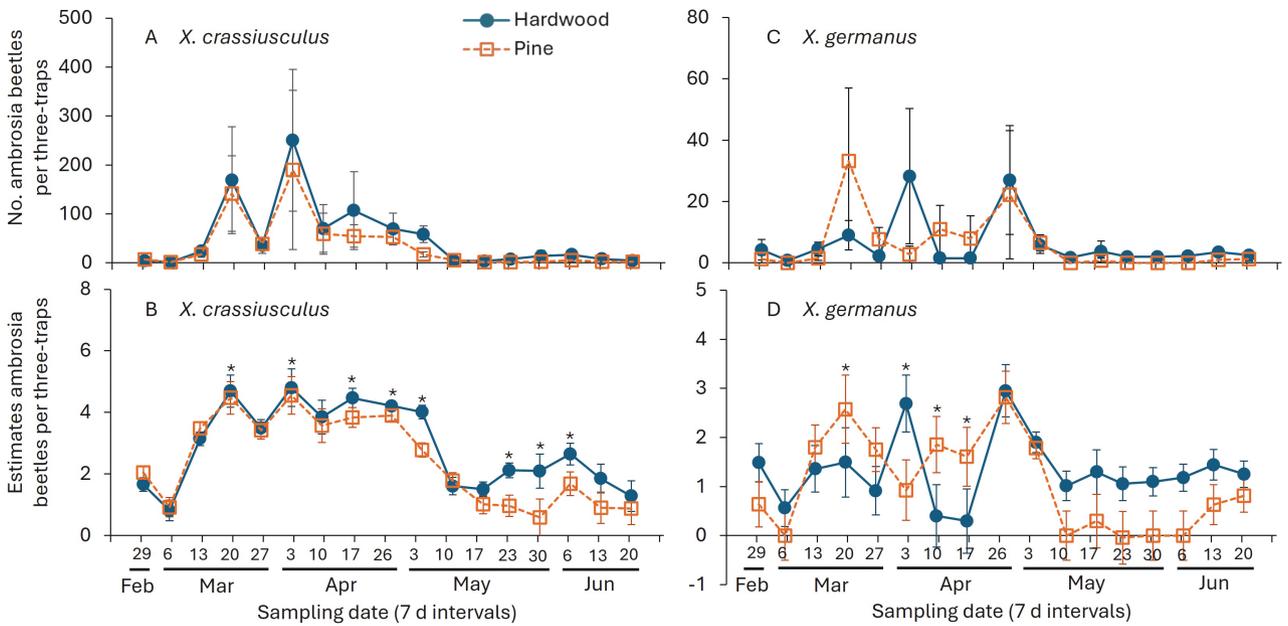
Fig. 4. Mean (± SE) of non-transformed numbers of (A) *X. crassiusculus*, (C) *X. germanus* captured in 3 ethanol-lured bottle traps in each site, and LS mean (± SE) estimates of back-transformed numbers of (B) *X. crassiusculus*, and (D) *X. germanus* in 2024. For B and D, asterisks above the SE lines indicate significant differences between hardwood and pine-dominated stands for each sampling date at (α = 0.05) using the Tukey-Kramer test. Where no differences were observed among treatments, no asterisks are given.
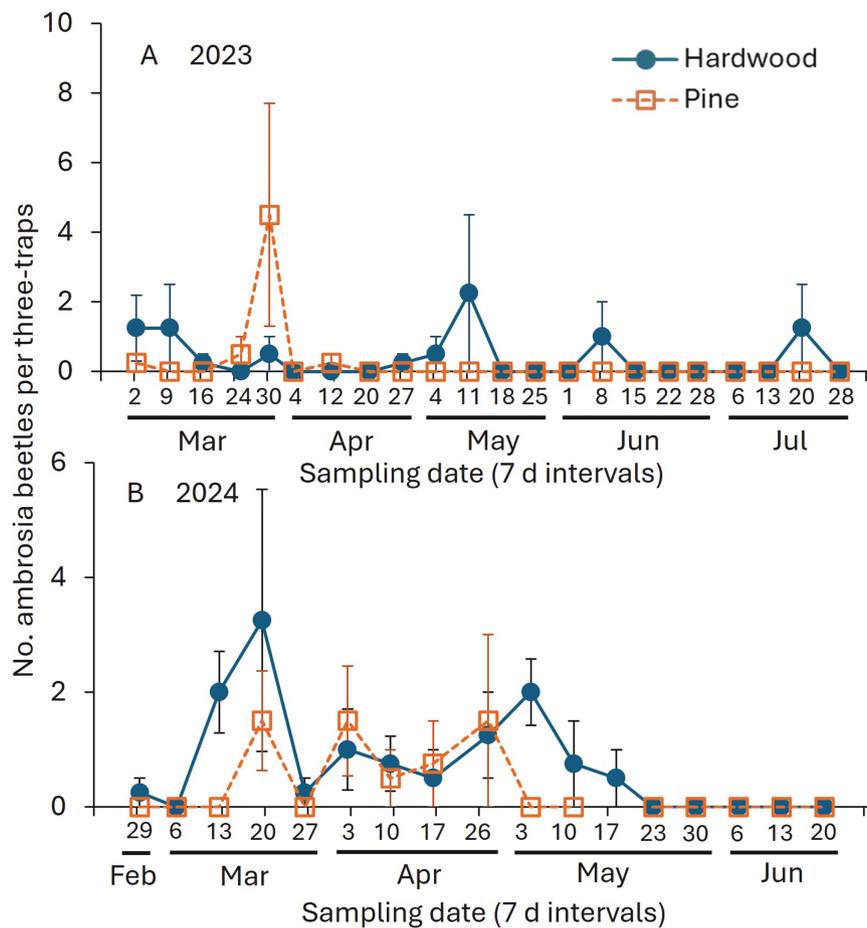
Fig. 5. Mean (± SE) of non-transformed numbers of *X. compactus* in (A) 2023 and (B) 2024. Where no differences were observed among treatments, no asterisks are given.

diameters favor the population growth of exotic ambrosia beetles. In contrast, the densities of adult *X. germanus* and *X. crassiusculus* were similar in the deciduous and coniferous woodlots in midwestern forests (Baniszewski et al. 2024). In Baniszewski et al. (2024), ethanol lure traps were placed inside the woodlot rather than outside or along the woodline, as in the current study. The behavior of ambrosia beetle species, especially flight activity, can differ within and outside the woodlot (Werle et al. 2015).

In the current study, captures of adult *X. germanus* were greater in pine than in hardwood-dominated woodlots in certain weeks. Adult *X. germanus* were found on conifers, such as Norway spruce (*Picea abies* (L.) Karst.) (Henin and Versteirt 2004). Adult *X. germanus* uses aggregation pheromone, and they tend to aggregate in trees infested with mutualistic fungi (Gugliuzzo et al. 2023), suggesting a high population of *X. germanus* is likely in certain niches in the pine woodlots leading to mass flight events certain weeks. Overall, captures of adult *X. germanus* along the borderline of woodlots were only 11.9% to 13.9% compared to 84.5% to 86.9% of adult *X. crassiusculus*. The frequency of inbreeding in *X. germanus* populations is very high (Keller et al. 2011). This suggests that the dispersal of *X. germanus* adults out of the woodlot could be limited. Similarly, *X. compactus* captures were low and were not affected by the woodlot type in both years. Ethanol-lured traps are used to detect the seasonal flight activity of *X. compactus* (Burbano et al. 2012), although they are not particularly attracted to ethanol in trees (Ranger et al. 2016).

In > 50% of dates during peak flight, the numbers of adult *X. crassiusculus* and *X. germanus* captures in traps were greater in the hardwood than in pine-dominated woodlots. However, there were no differences in captures between hardwood and pine-dominated woodlots for many sampling dates during peak activity. First, it is unclear if ethanol-lured traps attracted ambrosia beetles beyond adjacent woodlots where traps were placed as the flight distance and capability of *X. crassiusculus* and *X. germanus* females seeking new hosts through seasons are poorly understood. Second, abiotic conditions, especially temperature and relative humidity within hardwood-dominated woodlot, could be varied due to tree canopy thickness and height and the topography of the woodlot. These conditions might have created a distinct microclimate within the woodlot.

Xylosandrus crassiusculus, *X. germanus,* and *X. compactus* are the major ambrosia beetles in production systems, such as ornamental nurseries, pecan, and apple orchards in Georgia (Monterrosa et al. 2021, 2022, Williamson et al. 2023, Govindaraju et al. 2024). Native ambrosia beetles collected from production sites were much lower than exotic species, such as *X. crassiusculus*, *X. germanus,* and *X. compactus* (Monterrosa et al. 2021, 2022). Similarly, exotic ambrosia beetle species, *X. germanus*, *X. crassiusculus,* and *Anisandrus maiche* (Stark), outnumbered native ambrosia beetle species in the mid-western states (Baniszewski et al. 2024). The flight activity of exotic species occurred 85°C days earlier than native species, suggesting that they are likely to fly, attack, and colonize trees earlier than native species (Baniszewski et al. 2024). Although the peak flight activity of adult *X. crassiusculus* and *X. germanus* lasted until early June 2023 and the end of April 2024, they flew throughout the sample periods in both years at lower densities. This result is consistent with Baniszewski et al. (2024), in which the duration of flight activity of exotic species was substantially longer than that of native ambrosia species.

In summary, results showed that the number of adult *X. crassiusculus* and *X. germanus* adults were greater in traps deployed adjacent to hardwood than pine-dominated woodlots. Moreover,

the number of pestiferous ambrosia beetle adults was lower in pine-dominated woodlots; adults were still flying, and they could potentially cause economic damage to young trees in ornamental nurseries or orchards. The age of the pine plantation could be an important factor. In the case of a newly planted pine plantation adjacent to the production site, the risk of ambrosia beetle infestation from the young woodlot to young trees in ornamental nurseries and orchards could be negligible, at least for initial years. As the pine trees age, they are vulnerable to getting infested over time and develop a high ambrosia beetle population on stressed or fallen trees. This study clearly indicates that both hardwood and pine-dominated woodlots can be a repository for ambrosia beetles over time and can threaten young trees in ornamental nurseries, as well as orchards. More research is warranted to understand the effects of overall health parameters, such as levels of disease, management (eg thinning, harvest, prescribed fire), natural disasters (eg wildfire, storms), and age of tree species on the ambrosia beetle dispersal patterns. Although flight activities of adult *X. crassiusculus* and *X. germanus* were observed adjacent to both hardwood and pine-dominated woodlots during the late winter and early spring, the risk of infestation is greater for the hardwood than pine-dominated woodlots. In pine-dominated woodlots, the age of the stand should be considered carefully for pest management decisions, as *X. crassiusculus* and *X. germanus* thrive in old or mature trees that are either stressed, dying, or dead.

Author contributions

Shimat Villanassery Joseph (Conceptualization [lead], Data curation [lead], Formal analysis [lead], Funding acquisition [lead], Investigation [equal], Methodology [lead], Project administration [equal], Resources [lead], Software [lead], Supervision [lead], Validation [lead], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), and Ramkumar Govindaraju (Investigation [equal], Project administration [equal], Writing—review & editing [equal])

References

Addesso KM, Oliver JB, Youssef N, et al. 2019. Trap tree and interception trap techniques for management of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in nursery production. J. Econ. Entomol. 112:753–762. https://doi.org/10.1093/jee/toy413

Agnello AM, Breth DI, Tee EM, et al. 2017. *Xylosandrus germanus* (Coleoptera: Curculionidae: Scolytinae) occurrence, fungal associations, and management trials in New York apple orchards. J. Econ. Entomol. 110:2149–2164. https://doi.org/10.1093/jee/tox189

Baniszewski JA, Barnett J, Reding ME, et al. 2024. Seasonal dominance of exotic ambrosia beetles compared to native species within deciduous

and coniferous woodlots. Biol. Invasions 26:1651–1668. https://doi.org/10.1007/s10530-024-03255-z

Bateman C, Hulcr J. 2017. A guide to Florida's common bark and ambrosia beetles. IFAS Extension and University of Florida. FOR321:1-32. https://edis.ifas.ufl.edu/pdffiles/fr/fr38900.pdf (Accessed on 13 November 2024).

Brockerhoff EG, Liebhold AM. 2017. Ecology of forest insect invasions. Biol. Invasions 19:3141–3159. https://doi.org/10.1007/s10530-017-1514-1

Burbano EG, Wright MG, Gillette NE, et al. 2012. Efficacy of traps, lures and repellents for *Xylosandrus compactus* (Coleoptera: Curculionidae) and other ambrosia beetles on *Coffea arabica* plantations and *Acacia koa* nurseries in Hawaii. Environ. Entomol. 41:133–140. https://doi.org/10.1603/EN11112

Campbell JW, Hanula JL, Outcalt KW. 2008. Effects of prescribed fire and other plant community restoration treatments on tree mortality, bark beetles, and other saproxylic Coleoptera of longleaf pine, *Pinus palustris* Mil., on the Coastal Plain of Alabama. For. Ecol. Manag 254:134–144. https://doi.org/10.1016/j.foreco.2007.07.032

Cunningham K, Barry J, Walkingstick T. 2008. Managing loblolly pine stands… from A to Z. Fayetteville (AR): University of Arkansas Cooperative Extension Service FSA5023 [Accessed on 8 October 2024]. https://www.uaex.uada.edu/environment-nature/forestry/managing-woodlands/FSA-5023.pdf

[GG] Georgia Grown. 2024. Nurseries. https://georgiagrown.com/find-georgia-grown/horticulture/nurseries/#:~:text=Georgia's%20ornamental%20horticulture%20industry%20has,of%20over%20%24846%20million%20annually. (Accessed on 6 October 2024).

Gossner MM, Falck K, Weisser WW. 2019. Effects of management on ambrosia beetles and their antagonists in European beech forests. Forest Ecol. Manag. 437:126–133. https://doi.org/10.1016/j.foreco.2019.01.034

Govindaraju R, Hayter J, Chong JH, et al. 2024. Influence of the ethanol lure and concentration on captures of ambrosia beetles in tree fruits and ornamentals. J. Appl. Entomol. 149:74–87. https://doi.org/10.1111/jen.13361

Gugliuzzo A, Criscione G, Biondi A, et al. 2020. Seasonal changes in population structure of the ambrosia beetle *Xylosandrus compactus* and its associated fungi in a southern Mediterranean environment. PLoS One 15:e0239011. https://doi.org/10.1371/journal.pone.0239011

Gugliuzzo A, Biedermann PHW, Carrillo D, et al. 2021. Recent advance toward the sustainable management of invasive *Xylosandrus* ambrosia beetles. J. Pest Sci. 94:615–637.

Gugliuzzo A, Kreuzwieser J, Ranger CM, et al. 2023. Volatiles of fungal cultivars act as cues for host-selection in the fungus-farming ambrosia beetle *Xylosandrus germanus*. Fron. Microbiol 14:1151078. https://doi.org/10.3389/fmicb.2023.1151078

Hara A, Beardsley J. 1979. The biology of the black twig borer, *Xylosandrus compactus* (Eichhoff) in Hawaii. Proc. HI Entomol. Soc 13:55–70.

Harmon ME, Franklin JF, Swanson FJ, et al. 1986. Ecology of coarse woody debris in temperate ecosystems. Adv. Ecol. Res. 15:133–276.

Henin JM, Versteirt V. 2004. Abundance and distribution of *Xylosandrus germanus* (Blandford 1894) (Coleoptera, Scolytidae) in Belgium: New observations and an attempt to outline its range. J. Pest Sci. 77:57–63. https://doi.org/10.1007/s10340-003-0030-5

Hulcr J, Stelinski LL. 2017. The ambrosia symbiosis: from evolutionary ecology to practical management. Annu. Rev. Entomol. 62:285–303. https://doi.org/10.1146/annurev-ento-031616-035105

Joseph SV, Pugliese PJ, Hudson W. 2019. Granulate ambrosia beetle: Biology and management. University of Georgia Circular#1160.

Joseph SV, Acebes AL, Blaauw B. 2023. A survey on ambrosia beetle problems in the ornamental and pecan industry in Georgia. J. Entomol. Sci. 58:471–483. https://doi.org/10.18474/JES23-06

Keller L, Peer K, Bernasconi C, et al. 2011. Inbreeding and selection on sex ratio in the bark beetle *Xylosandrus germanus*. BMC Evol. Biol. 11:359. https://doi.org/10.1186/1471-2148-11-359

La Spina S, De Cannière C, Dekri A, et al. 2013. Frost increases beech susceptibility to scolytine ambrosia beetles. Agic. Forest Entomol 15:157–167. https://doi.org/10.1111/j.1461-9563.2012.00596.x

Miller DR, Rabaglia RJ. 2009. Ethanol and (-)-alpha-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. J. Chem. Ecol. 35:435–448. https://doi.org/10.1007/s10886-009-9613-9

Monterrosa A, Acebes AL, Blaauw B, et al. 2021. Effects of trap, and ethanol lure type and age on attraction of ambrosia beetles (Coleoptera: Curculionidae). J. Econ. Entomol. 114:1647–1654. https://doi.org/10.1093/jee/toab089

Monterrosa A, Joseph SV, Blaauw B, et al. 2022. Ambrosia beetle occurrence and phenology of *Xylosandrus* spp. (Coleoptera: Curculionidae: Scolytinae) in ornamental nurseries, tree fruit and pecan orchards in Georgia. Environ. Entomol. 51:998–1009. https://doi.org/10.1093/ee/nvac064

Nebeker TE, Hodges JD, Karr BK, et al. 1985. Thinning practices in southern pines – with pest management recommendations. United States Department of Agriculture, Forest Service Technical Bulletin 1703. p. 36.

Ngoan ND, Wilkinson RC, Short DE, et al. 1976. Biology of an introduced ambrosia beetle, *Xylosandrus compactus*, in Florida. Ann. Entomol. Soc. Am. 69:872–876. https://doi.org/10.1093/aesa/69.5.872

Oliver JB, Mannion CM. 2001. Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. Environ. Entomol. 30:909–918. https://doi.org/10.1603/0046-225x-30.5.909

Ranger CM, Reding ME, Persad AB, et al. 2010. Ability of stress-related volatiles to attract and induce attacks by *Xylosandrus germanus* and other ambrosia beetles. Agric. Forest Entomol. 12:177–185. https://doi.org/10.1111/j.1461-9563.2009.00469.x

Ranger CM, Schultz PB, Frank SD, et al. 2015. Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. PLoS One 10:e0131496. https://doi.org/10.1371/journal.pone.0131496

Ranger CM, Reding ME, Schultz PB, et al. 2016. Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. J Integr Pest Manag 7:1–23. https://doi.org/10.1093/jipm/pmw005

Reding ME, Ranger CM. 2018. Residue age and attack pressure influence efficacy of insecticide treatments against ambrosia beetles (Coleoptera: Curculionidae). J. Econ. Entomol. 111:269–276. https://doi.org/10.1093/jee/tox327

Reding M, Oliver J, Schultz P, et al. 2010. Monitoring flight activity of ambrosia beetles in ornamental nurseries with ethanol-baited traps: influence of trap height on captures. J. Environ. Hortic. 28:85–90. https://doi.org/10.24266/0738-2898-28.2.85

Reed SE, Muzika RM. 2010. The influence of forest stand and site characteristics on the composition of exotic dominated ambrosia beetle communities (Coleoptera: Curculionidae: Scolytinae). Environ. Entomol. 39:1482–1491. https://doi.org/10.1603/EN09374

SAS Institute 2024. SAS Institute *Inc.*, Version 9.4. Cary, NC.

Smith SM, Beaver RA, Cognato AL, et al. 2019. Southeast Asian Ambrosia beetle ID. Fort Collins, CO: USDA APHIS Identification Technology Program (ITP) and Michigan State University. https://idtools.org/id/wbb/sea-ambrosia/

Tobin KN, Lizarraga S, Acharya R, et al. 2024. Comparison of ethanol-baited trap designs for ambrosia beetles in orchards in the eastern United States. J. Econ. Entomol. 117:1476–1484. https://doi.org/10.1093/jee/toae145

Werle CT, Chong JH, Sampson BJ, et al. 2015. Seasonal and spatial dispersal patterns of select ambrosia beetles (Coleoptera: Curculionidae) from forest habitats into production nurseries. Fla. Entomol. 98:884–891. https://doi.org/10.1653/024.098.0311

Williamson VZ, Blaauw B, Joseph SV. 2023. Effects of permethrin residues on ambrosia beetles (Coleoptera: Curculionidae) in ornamental nurseries. J. Insect Sci. 23:1–8. https://doi.org/10.1093/jisesa/iead052