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Ethanol release patterns and captures of *Xylosandrus* spp. (Coleoptera: Curculionidae) in ornamental nursery

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Xylosandrus crassiusculus Motschulsky and *Xylosandrus germanus* Blandford are serious ambrosia beetle pests in ornamental nurseries. Three ethanol baits, AgBio low release (LR), AgBio high release (HR), and Trécé are commercially available for use in bottle traps to determine flight activity of adult *Xylosandrus* spp. However, release patterns of ethanol from these baits under varying temperatures and captures of *Xylosandrus* spp. are poorly understood. Thus, the objectives of this study were (i) to determine ethanol release rates from these baits under constant and variable temperatures and (ii) to compare relative adult *Xylosandrus* spp. captures using these baits in ornamental nurseries. When 3-d difference (3-d difference) bait weights were recorded under constant 15.6, 21.1, 26.7, and 32.2 °C, an increase in release rates was recorded with an increase in temperature from 15.6 to 32.2 °C for LR bait. At 32.2 °C, no increase or reduction in the 3-d-difference weights was found for the HR and Trécé baits, respectively, compared to LR bait. The 3-d-difference weights were steady with all 3 baits when temperatures gradually increased and decreased in variable sequence for 30 d. In 2022, 2023, and 2024, although all 3 baits captured adult *X. crassiusculus* and *X. germanus*, LR bait captured significantly more numbers of *X. crassiusculus* than the other 2 baits in 2022 and 2023, and in 2024, *X. crassiusculus* captures were greater in traps with the Trécé bait than the other 2 baits. Thus, ethanol baits can effectively detect the early flight activity of *Xylosandrus* spp. adults in ornamental nurseries.

Keywords: AgBio low release, AgBio high release and Trécé bait, granulate ambrosia beetle, black stem borer

Introduction

The granulate ambrosia beetle, *Xylosandrus crassiusculus* Motschulsky, and the black stem borer, *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae), are serious wood-boring pests of young trees in ornamental nurseries in the eastern United States (Ranger et al. 2016, Reding and Ranger 2018, Addesso et al. 2019, Williamson et al. 2023). Woody ornamental trees are valued at $888 million USD in Georgia, USA (Wolfe and Stubbs 2019). Adult females attack trunks of many tree hosts, such as dogwood (*Cornus* spp.), redbud (*Cercis* spp.), red maple (*Acer rubrum* L.), ornamental flowering cherry (*Prunus serrulate* Lindl.), Japanese maple (*Acer palmatum* Thunb.), crapemyrtle (*Lagerstroemia indica* L.), Chinese elm (*Ulmus parvifolia* Jacq.), magnolia (*Magnolia grandiflora* L.), fig (*Ficus carica* L.), and rhododendron (*Rhododendron arboreum* Sm.) (Ranger et al. 2016). The adult beetles bore through the vascular layer and the boring activity pushes out compacted sawdust and excrement as "noodles" emerging from entry holes on trunks and branches (Gugliuzzo et al. 2021). Branch dieback and wilting of trees are sometimes noticed after boring activity (Ranger et al. 2016, Gugliuzzo et al. 2021). Economic loss is reported when affected trees are culled or not marketed (Ranger et al. 2016).

In the eastern United States, mated females of *X. crassiusculus* and *X. germanus* overwinter in fallen and dying trees in woodlots (Ranger et al. 2016). In Georgia (USA), as the weather warms up in the late winter or early spring, females emerge from infested trees and fly, seeking new hosts to colonize. Weather events, such as drought, flood, or frost, can induce physiological stress on young trees in ornamental nurseries, and these stresses induce trees to produce and release ethanol in response (Ranger et al. 2015). The flying females are attracted to traces of ethanol released from stressed trees in fields adjacent to wooded overwintering sites and those trees are attacked (Ranger et al. 2010, 2015). The females horizontally bore through the trunk (Weber and McPherson 1983) and make extensive galleries in the heartwood of the branches or trunk (Ranger et al. 2016). Areas of these galleries form brood chambers where females inoculate symbiotic fungi that they carry in their mycangia. *Ambrosiella roeperi* T.C. Harr. & McNew and *Ambrosiella grosmanniae* C. Mayers, McNew & T.C. Harr., are the symbionts of *X. crassiusculus* and *X. germanus*, respectively (Harrington et al. 2014, Mayers et al. 2015). Females oviposit in the newly developed fungal gardens to initiate colonies (Ranger et al. 2016). The larvae develop feeding only on the fungi (Hoffman 1941) without consuming wood tissue (Ranger et al. 2016). Because all stages of *X. crassiusculus and X. germanus* do not feed on wood tissue and colonize inside the heartwood of the tree, it is challenging to manage them using systemic insecticides, such as neonicotinoids, which are typically transported through active xylem vessels (Reding et al. 2013a, Joseph 2022).

In ornamental nurseries, adult *X. crassiusculus* and *X. germanus* are mainly managed using pyrethroids, such as bifenthrin and permethrin (Reding and Ranger 2018, Williamson et al. 2023). These insecticides are applied preventatively before flight activity, effectively reducing attacks on tree trunks (Ranger et al. 2016). Post-attack or curative application of insecticides has limited or no effects on boring adults or developing larvae and in reducing damage symptoms, such as branch dieback. Therefore, monitoring the flight activity of females, especially during the late winter or spring, is critical for timely management decisions (Reding and Ranger 2018). Because adult *X. crassiusculus* and *X. germanus* are attracted to ethanol signals (Ranger et al. 2015, 2021), ethanol is used as a monitoring bait (Monterrosa et al. 2021). Ethanol baits are commercially available and are used in bottle traps. Three common ethanol baits are AgBio low release (LR), AgBio high release (HR), and Trécé. Once seasonal ambient temperatures reach 20 to 21 °C for 2 consecutive days, adult *X. germanus* flight and attack activities are observed (Reding et al. 2013b). However, the daily temperatures in the spring typically fluctuate between 4 and 22 °C with occasional frost and > 22 °C during early morning and midday, respectively in central Georgia. In some cases, temperatures persist in a cooler range for a few days. These fluctuations in temperature could affect the plasticity of ethanol baits, causing inconsistent rates of release and influencing the early captures of adult *X. crassiusculus* and *X. germanus* using commercial ethanol baits. There are limited studies that systematically evaluated ethanol release patterns from the commercially available baits and further compared adult *X. crassiusculus* and *X. germanus* captures. Thus, the objectives of this study were (i) to determine ethanol release rates from these baits under constant and sequential temperatures and (ii) to compare relative adult *Xylosandrus* spp. captures using these baited traps in ornamental nurseries.

Materials and Methods

Ethanol Release at Constant Temperatures

Experiments were conducted in controlled environmental chambers with temperatures set at constant temperatures in the University of Georgia, Griffin Campus, Griffin, GA. Three commercially available ethanol baits, LR and HR (distributed by AgBio Inc., Westminster, CO; Manufacturer: ChemTica Internacional, S.A., San Jose, Costa Rica) and Trécé (Trécé Inc., Adair, OK), were used in the experiment as treatments (Fig. 1). The LR bait contains 7 to 8 mL of 95% ethanol, and the release rate is 65 mg per day at 30°C (Monterrosa et al. 2021). The HR bait has 120 ml of 95% denatured ethanol with a release rate of ~1,000 mg per day (item# AP551-UHR, Chemtica, USA LLC). The Trécé bait (# TRE 1785), ~1,100 mg of 83% ethanol, has a release rate of ~30 mg per day at 26.7°C, per the manufacturer. These 3 baits were exposed to constant temperatures: (i) 15.6, (ii) 21.1, (iii) 26.7, and (iv) 32.2°C (treatments) in separate controlled environmental chambers (Percival Scientific, Intellus control system, Perry, IA). The relative humidity (RH) inside the chamber was recorded using 3 relative humidity and temperature sensors (Acu-RITE, model#00619hdsba3, Lake Geneva, WI) and they were approximately 65, 40.3, 41.3, and 27.6% for 15.6, 21.1, 26.7, and 32.2°C treatments, respectively. Four baits (replicates) of each type were used in each chamber set to a specific temperature. Baits were individually weighed before deploying inside chambers and then weighed at 3-d intervals using a weighing balance (Ohaus Cooperation USA, model#AX423/E, Parsippany, NJ). The weighing balance was installed on a potable cart and moved near the chambers, and the sides and top of the balance were completely covered during weighing baits. For analysis, the change in weight

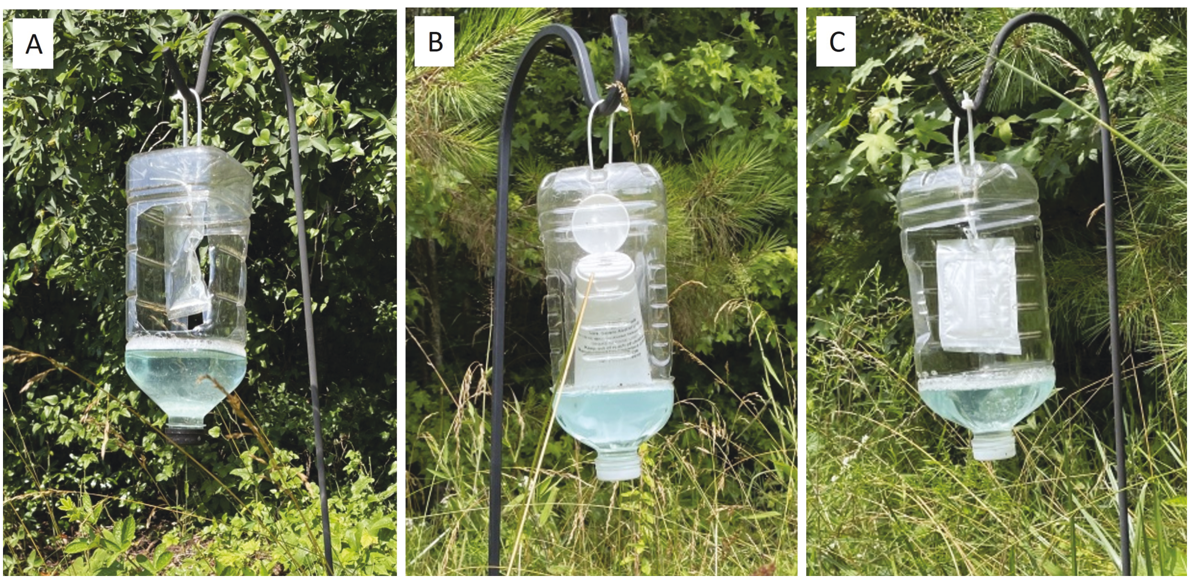

Fig. 1. (A) LR, (B) HR, and (C) Trécé baits installed in bottle traps deployed on shepherd's hooks along wood lines in ornamental nurseries in 2022, 2023, and 2024.

measurements at 3-d intervals was determined and is referred to as a 3-d difference or 3-d difference. The experiment was set up at lighted conditions using 25 W (Phillips, Fluorescent light, F25T8-TL841, Somerset, NJ). The chamber temperature was recorded hourly using Onset HOBO loggers (HOBO, Pendant temp/light, Bourne, MA).

Ethanol Release at Sequential Temperatures

A variable sequence of temperatures was programmed in the chamber to simulate field conditions where temperature gradually increases and then decreases after reaching a maximum during a cycle. The chamber was programmed with temperatures with specific duration and sequence (a–d) was (a) 15.6 °C for 8 h, (b) 21.1 °C for 11 h, (c) 26.7 °C for 3 h; and (d) 32.2 °C for 2 h. The RH inside the chamber was set at 70% for the duration of the experiment. The variable sequence was repeated 30 times, simulating 30-d intervals. Three bait treatments were (i) LR, (ii) HR, and (iii) Trécé, and were replicated 4 times within a single chamber. Similar to the experiment determining ethanol release at constant temperatures, baits were individually weighed before deploying inside chambers and then weighed at 3 d intervals using a weighing balance. The change in weight measurements at 3 d intervals was determined for analysis. The experiment was set up on fluorescence at 650 µmol, 4 incandescence lights with 29 W and 300 lumen (Ecosmart, F1 daylight, Göd, Hungary) light conditions. In addition, temperatures were recorded using 3 Onset HOBO loggers, which record hourly temperatures.

Field Exposure and Trap Captures

In 2022, 2023, and 2024, one field experiment was conducted in woody ornamental nurseries in central GA. In 2022, the experiment was conducted in a woody containerized nursery in Meriwether County, whereas in 2023 and 2024, experiments were conducted in an in-ground woody ornamental nursery in Pike County. In nurseries, a wide range of tree hosts, magnolia (*M. virginiana*. L.), maples (*Acer* spp.), eastern redbud (*C. canadensis* L.), hydrangea (*Hydrangea* spp.), cherry (*Prunus* spp.), camelia (*Camellia* spp.), juniper (*Juniperus communis* L.), etc., were planted. The trees were planted in rows, or tree containers were placed at varied spacings in blocks with a network of roads running between blocks to access those trees. The experiments were conducted along the wood line, and various trees and shrubs, such as *Acer* spp., *Pinus* spp., *Quercus* spp., and *Ligustrum* spp., were present in the woodlots. The trees in the nurseries were drip-irrigated throughout the year. In the spring, the nursery managers preventatively sprayed pyrethroids to protect trees from ambrosia beetles and other bark beetle attacks in all 3 yr.

A transparent 1.7 L plastic bottle (VTM LLC, Lexington, KY) was used to build a bottle trap for all 3 yr. The bottle was modified with two 5 × 19 cm rectangular vents cut on opposite sides to enable adults access to the bottle where the bait was suspended. Two holes were drilled into the bottom of the bottle to hang and secure a bait (LR, HR, or Trécé) pouch using a zip tie (Fig. 1). A bottle was hung upside down on a shepherd's hook. To trap beetles, a soap solution was prepared with 2 ml dish soap (Dawn P&G, Kansas City, KS) in 10 ml water, and 100 ml of soap solution was added to the bottle trap to drown and trap the visiting ambrosia beetles. The lid of the bottle was unscrewed to empty the collected beetles in the bottle using a coffee filter placed on a mesh strainer. The collected ambrosia beetles were sorted and stored in 70% ethanol for future identification.

The treatments were (i) LR, (ii) HR, (iii) Trécé, and (iv) nonbaited control. Treatments were replicated 6 times, following a randomized complete block design. Bottle traps were deployed along the wood line with 10 m spacing between treatments and 20 m between blocks. Experiments were conducted in the spring each year when temperatures spiked around bud break, and ambrosia beetle flight activity was high. Traps for this experiment were deployed on 20 April 2022, 22 March 2023, and 23 March 2024 for 4, 11, and 4 wk, respectively. In 2024, in addition to captures of ambrosia beetles, baits were individually weighted before deployment and at 7 d intervals for up to 4 wk. The weighing balance was positioned flat when baits were weighed in the field.

The ambrosia beetle adults were identified as species using a lucid key for Southeast Asian Xyleborini species (Smith et al. 2019) and the ambrosia beetle guide by Bateman and Hulcr (Bateman and Hulcr 2017). To interpret the beetle captures, temperature data were obtained. In 2022, temperature data were retrieved from a climate weather model from the Prism Climate Group, Oregon State University (Prism Climate Group 2023). The 2023 and 2024 temperature data were retrieved from Onset HOBO loggers (Supplementary Fig. 1). Because relative humidity measures moisture saturation of air only at a certain temperature, vapor pressure deficit (hPa) readings were used. Vapor pressure deficit measures the difference between saturation and the current amount of moisture in the air, in terms of pressure, and is independent of temperature. The minimum and maximum vapor pressure deficit data were retrieved from Prism (Prism Climate Group 2024; https://prism.oregonstate.edu/explorer/) for Meriwether County, Georgia in 2022 and Pike County, Georgia in 2023 and 2024 (Supplementary Fig. 2).

Statistical Analyses

To determine the effect of temperature, observation date, and interaction for each bait type, the 3-d-difference ethanol pouch weight data were subjected to a 2-way analysis of variance (ANOVA) using a generalized linear model (PROC GLIMMIX) in SAS (SAS Institute 2024). The model was set with a log-link function and using a Poisson distribution. To determine the effects of observation date on each temperature treatment and bait type, the 3-d-difference ethanol bait weight data were subjected to 1-way ANOVA in a general linear model (PROC GLM) in SAS after log transformation (ln[x + 1]). The means were separated by the Tukey Honestly Significant Difference (HSD) test ($\alpha < 0.05$). To determine the effects of temperature treatment on each sampling date and bait type, the 3-d-difference ethanol bait weight data were subjected to 1-way ANOVA in a general linear model (PROC GLM) in SAS after log transformation (ln[x + 1]). The means were separated by the Tukey HSD test ($\alpha < 0.05$).

For each bait type, the effect of observation time on sequential temperature treatment and 3-d-difference ethanol bait weight data were subjected to 1-way ANOVA in the general linear model (PROC GLM) in SAS after log transformation (ln[x + 1]). The means were separated by the Tukey HSD test ($\alpha < 0.05$). To compare the 3-d-difference weights from the sequential program with 3-d-difference weights when exposed to constant temperatures, the mean 3-d-difference weights at 26.7, 21.1, and 15.6 °C treatments were utilized. Both data sets were compared using *t*-test analysis (PROC TTEST) in SAS. The pooled method was used to check the statistical significance of the 3-d-difference weight data for each bait type ($\alpha < 0.05$). The 32.2 °C treatment was excluded when the mean was calculated because 32.2°C was not attained in the controlled environmental chamber within 2 h in the sequential program experiment (Fig. 3A), although 32.2 °C was set for 2 h.

To determine the effects of bait types across all years, the total number of ambrosia beetles, adult *X. crassiusculus* and *X. germanus* captured in bottle traps were subjected to 2-way ANOVA (PROC

GLIMMIX) in SAS. Because the interactions for most of the total ambrosia beetles, adult *X. crassiusculus* and *X. germanus*, collected were statistically significant, 1-way ANOVA (PROC GLIMMIX) in SAS by sampling date using the generalized linear mixed model was employed. The model included a log-link function and Poisson distribution, with treatments and replicates as fixed and random effects, respectively. In 2024, the data on 7-d-difference weights were analyzed with a negative binomial distribution. The 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.

Results

Ethanol Release at Constant Temperatures

For LR, HR, and Trécé baits, when 3-d-difference weights were analyzed, the temperature, observation time, and their interaction treatments were significantly different (Table 1). Because interactions were significantly different, 1-way ANOVA was conducted by observation time (3 d post-deployment interval) for temperature treatment. For LR bait, 3-d-difference weights were significantly greater for the 26.7 and 32.2 °C treatments than for the 21.1 and 15.6 °C treatments during all 3 d post-deployment intervals (Supplementary Table 1; Fig. 2A). On 3, 9, 21, and 24 d post-deployment, 3-d-difference weights of LR bait were significantly greater for the 32.2 °C treatment than for the 26.7 °C treatment (Supplementary Table 1). There were no significant differences in the 3-d-difference weights of LR bait between 26.7 and 32.2 °C treatments for the remaining 3 d post-deployment intervals. For all 3 d post-deployment intervals except at 15 d, significantly greater 3-d-difference weights were documented for the 21.1°C treatment than for the 15.6 °C treatment (Supplementary Table 1; Fig. 2A). The 3-d-difference weights of LR bait were significantly different across times of observation for all the temperature treatments (Supplementary Table 2; Fig. 2A).

For HR bait, 3-d-difference weights were significantly greater for the 32.2 °C treatment than for the remaining treatments during all 3 d post-deployment intervals (Supplementary Table 1; Fig. 2B). Between 26.7, 21.1, and 15.6 °C treatments during all 3-d post-deployment intervals, there were no consistent significant differences (Supplementary Table 1; Fig. 2B). The 3-d-difference weights of HR bait were significantly different across times of observation for all temperature treatments (Supplementary Table 2; Fig. 2B).

For Trécé bait, 3-d-difference weights were significantly lower for the 32.2°C treatment than for the remaining temperature treatments at 2, 12, and 18 d post-deployment, and there were no significant differences between each other for other 3 d post-deployment intervals (Supplementary Table 1; Fig. 2C). Between 26.7, 21.1, and 15.6 °C treatments, there were no significant differences in 3-d difference weights at 3, 12, and 18 d post-deployment. The 3-d-difference weights of Trécé bait were significantly different across observation times for the 26.7, 21.1, and 15.6 °C treatments except for the 32.2 °C treatment (Supplementary Table 2; Fig. 2C).

Table 1. Two-way analysis of variance of 3-d difference in ethanol lure weight at 3-d intervals to determine the effects of temperature, time of observation (up 27 d post-exposure), and their interactions when exposed to specific temperatures in the controlled environmental chambers

Variable	*F*	df	*P*
LR			
Treatment (temperature)	1,090.5	3,107	<0.001
Observation time	37.9	8,107	<0.001
Treatment × observation time	7.3	24,107	<0.001
HR			
Treatment	16,005.3	3,107	<0.001
Observation time	2,796.9	8,107	<0.001
Treatment × observation time	351.6	24,107	<0.001
Trécé			
Treatment	6,959.8	3,104	<0.001
Observation time	1,874.5	8,104	<0.001
Treatment × observation time	280.4	23,104	<0.001

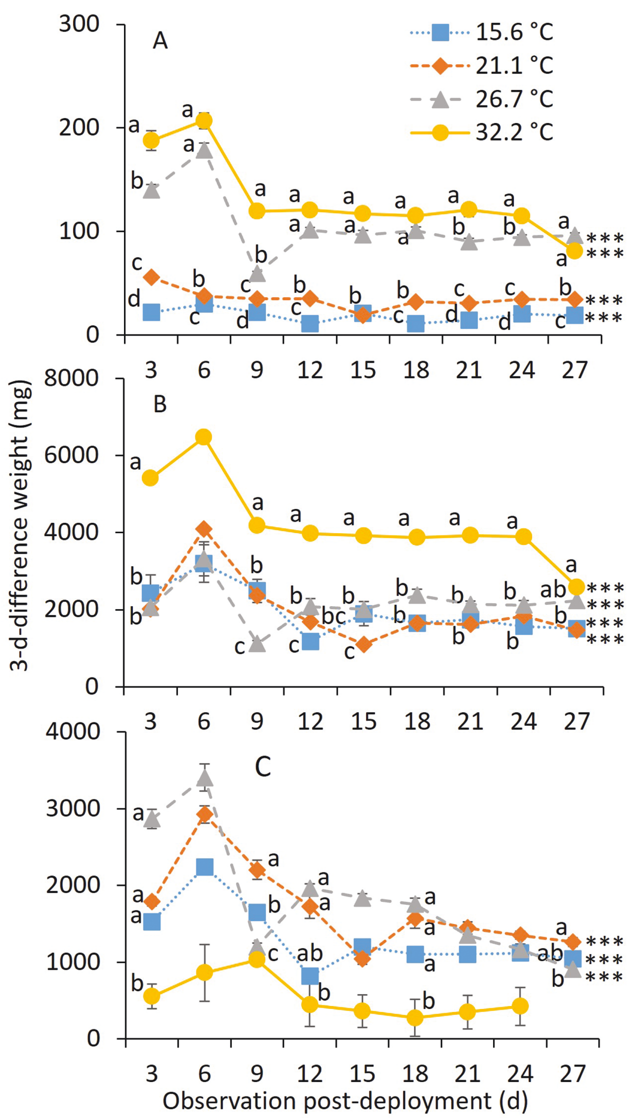

Fig. 2. Mean ± (SE) 3-d-difference weights for (A) LR, (B) HR, and (C) Trécé baits after exposing to 15.6, 21.1, 26.7, and 32.2 °C for up to 27 d post-deployment in controlled environmental chambers. The same letters between temperature treatments at each 3 d interval within each figure (ethanol bait) are not significantly different using Tukey's HSD test ($\alpha = 0.05$). Asterisks (*** $P < 0.001$) are provided corresponding to each temperature treatment within each figure (ethanol bait) when there were significant differences in 3-d-difference weights between 3 d intervals for each temperature using Tukey's HSD test ($\alpha = 0.05$). Where no differences were observed, no letters or asterisks are given.

Ethanol Release at Sequential Temperatures

The HOBO logger readings showed that temperatures set in the controlled environmental chambers were consistent with the program (Fig. 3A). For LR bait, 3-d-difference weights with sequential program at all the 3 d post-deployment intervals were not significantly different from mean 3-d-difference weights for 26.7, 21.1, and 15.6 °C treatments (Fig. 3B). For HR and Trécé baits, 3-d-difference weights with sequential program were not significantly different from mean 3-d-difference weights for 26.7, 21.1, and 15.6 °C treatments at most 3 d intervals except at 6, 21, and 24 d post-deployment (Fig. 3C and D). The 3-d-difference weights with the sequential program significantly differed by time (3 d post-deployment intervals).

For the sequential program, 3-d-difference weights varied at 3 d post-deployment intervals for all 3 baits (LR: $F = 10.4$; df = 9, 27; $P < 0.001$ [Fig. 3B]; HR: $F = 15.8$; df = 9, 27; $P < 0.001$[Fig. 3C]; and Trécé: $F = 5.4$; df = 9, 27; $P < 0.001$ [Fig. 3D]).

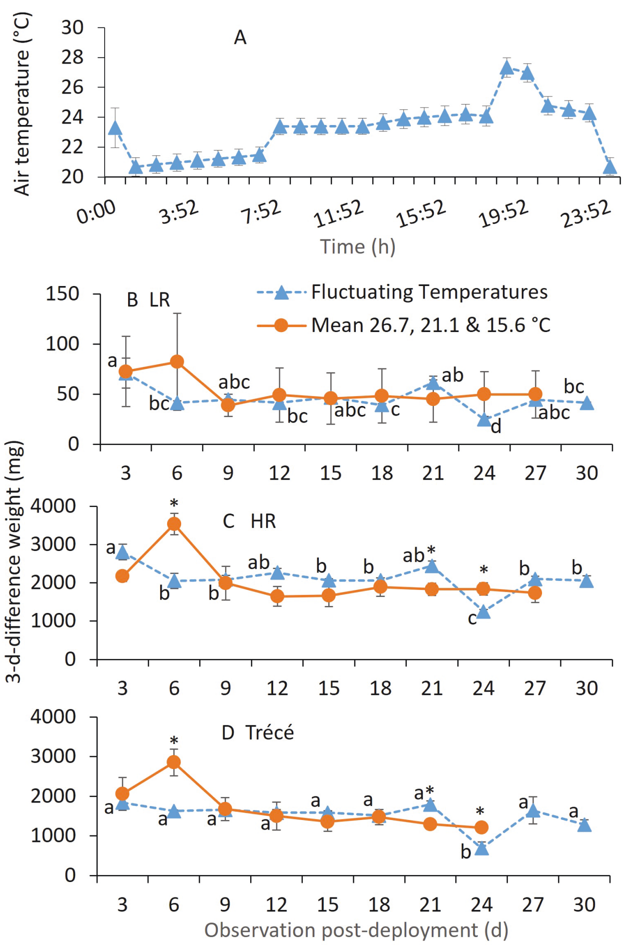

Fig. 3. (A) Temperatures recorded by HOBO data loggers placed inside the controlled environmental chamber for 30 d post-deployment and mean ± (SE) 3-d difference weights when exposed to sequential program (cyclical sequence of temperatures) and mean 15.6, 21.1, and 26.7°C treatments for (B) LR, (C) HR, and (D) Trécé baits for 30 d post-deployment in the controlled environmental chamber. The HOBO temperature data (A) were not subjected to ANOVA or post-hoc tests. The same letters between 3 d intervals after exposed to the cyclical temperature program within each bait type (B-D) are not significantly different using Tukey's HSD test ($\alpha = 0.05$). Asterisks indicate a significant difference between 3-d-difference weights when exposed to a sequential program (cyclical sequence of temperatures) and mean 15.6, 21.1, and 26.7 °C treatments at certain 3 d intervals (PROC TTEST in SAS). Where no differences were observed, no asterisks are given.

Field Study

In 2022, 2023, and 2024, the bait type treatment, sampling date, and interaction treatments significantly differed for total ambrosia beetles and adult *X. crassiusculus*. For adult *X. germanus*, bait type treatment, sampling date, and interaction significantly differed in 2022 (Table 2). In 2023, bait type treatment, sampling date, and interaction were not significantly different for adult *X. germanus*. In 2024, only the sampling date and interaction between bait type treatment and sampling date significantly differed for adult *X. germanus* (Table 2).

2022

At 7, 14, and 21 d post-deployment, the numbers of total ambrosia beetles and adult *X. crassiusculus* were significantly greater for the LR treatment, followed by HR and Trécé treatments, than for the non-baited treatment (Supplementary Table 3; Fig. 4A and B). There was no significant difference between HR and Trécé treatments for the numbers of total ambrosia beetles and adult *X. crassiusculus* at 7, 14, and 21 d post-deployment. At 28 d post-deployment, the numbers of total ambrosia beetles and adult *X. crassiusculus* were significantly greater for the HR treatment than for the remaining treatments (Supplementary Table 3; Fig. 4A and B). Adults of *X. germanus* were collected in significantly more numbers in the LR treatment than in the non-baited treatment at 7, 14, and 21 d post-deployment (Supplementary Table 3; Fig. 4C). At 28 d post-deployment, the number of adult *X. germanus* was significantly greater for the HR treatment than for the remaining treatments (Supplementary Table 3; Fig. 4C).

2023

At 7, 14, 21, 28, 49, 56, 70, and 76 d post-deployment, the numbers of total ambrosia beetles and *X. crassiusculus* adults were significantly greater for the LR treatment than for the remaining treatments (Supplementary Table 3; Fig. 5A and B). The number of adult *X. germanus* was significantly greater for the LR treatment than for the HR and non-baited treatments at 7 d post-deployment (Supplementary Table 3; Fig. 5C). No other differences were observed on other dates.

2024

The 7-d-difference weights of bait treatment were significantly different ($F = 10.8$; df = 2, 55; $P < 0.001$), whereas the sampling date ($F = 0.4$; df = 3, 55; $P = 0.744$) and interaction between bait treatment and sampling date ($F = 0.7$; df = 6, 55; $P = 0.666$) were not significantly different (Fig. 6A). The 7-d-difference weights were greater for the HR and Trécé treatments than for the LR treatment at 28 d post-deployment ($F = 5.1$; df = 2, 10; $P = 0.030$) but not significantly different at 7 ($F = 3.2$; df = 2, 10; $P = 0.082$), 14 ($F = 2.9$; df = 2, 10; $P = 0.105$), and 21 d ($F = 3.2$; df = 2, 10; $P = 0.279$; Fig. 6A).

The numbers of total ambrosia beetles and adult *X. crassiusculus* were significantly greater for the Trécé treatment than for the LR or HR treatments at 21 d post-deployment (Supplementary Table 3; Fig. 6B and C). At 28 d post-deployment, significantly more numbers of total ambrosia beetles and adult *X. crassiusculus* were collected for the Trécé treatment than for the LR treatment, followed by the HR treatment. There was no clear significant pattern in the number of adult *X. germanus* observed between bait treatments throughout the post-deployment periods (Supplementary Table 3; Fig. 6D).

Discussion

Because early detection of ambrosia beetles is critical for pest management decisions, it is important to compare monitoring tools for

Table 2. Two-way analysis of variance of ambrosia beetles captured to determine the effects of treatment (ethanol baits: LR, HR, and Trécé), sampling date, and their interactions in ornamental nurseries in the spring of 2022, 2023, and 2024

	2022			2023			2024		
Variable	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
All beetles									
Treatment	12.0	3,79	<0.001	39.9	3,219	<0.001	17.5	2,55	<0.001
Sampling date	46.5	3,79	<0.001	12.4	10,219	<0.001	325.0	3,55	<0.001
Treatment × Sampling date	8.7	9,79	<0.001	2.3	30,219	<0.001	17.6	6,55	<0.001
X. crassiusculus									
Treatment	24.5	3,79	<0.001	31.9	3,219	<0.001	22.7	2,55	<0.001
Sampling date	42.6	3,79	<0.001	10.7	10,219	<0.001	306.6	3,55	<0.001
Treatment × Sampling date	5.7	9,79	<0.001	2.0	30,219	0.002	16.6	6,55	<0.001
X. germanus									
Treatment	6.1	3,79	<0.001	2.5	3,219	0.055	1.2	2,55	0.325
Sampling date	6.9	3,79	<0.001	1.1	10,219	0.331	14.7	3,55	<0.001
Treatment × Sampling date	2.3	9,79	0.022	0.5	30,219	0.982	8.5	6,55	<0.001

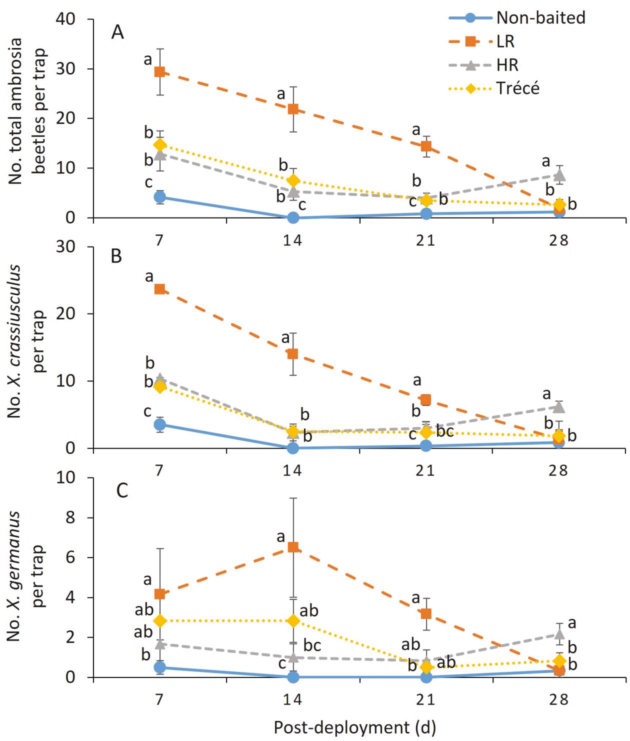

Fig. 4. Mean ± (SE) number of (A) total ambrosia beetles, (B) *X. crassiusculus*, and (C) *X. germanus* adults captured in bottle traps using bait treatments in an ornamental nursery in 2022. The same letters among the treatments for each sampling date are not significantly different using Tukey–Kramer test ($\alpha = 0.05$).

the detection of early flight of ambrosia beetles, especially adult *X. crassiusculus* in the spring. Although ethanol baits in pouches are commercially available, no study directly assessed ethanol release patterns from these baits or compared captures of adult *X. crassiusculus* and *X. germanus* using these baits. This is the first study that measured ethanol release rates and compared captures of adult *X. crassiusculus* and *X. germanus* using 3 commercial ethanol baits in bottle traps. A gravimetric approach was adopted to understand the release rates of ethanol from ethanol bait pouches under constant and sequential temperatures in controlled environmental chambers. The amounts of ethanol loaded in these 3 baits varied; thus, the ethanol released also varied. When temperatures increased from 15.6 to 32.2 °C, ethanol release rates steadily increased for LR bait, whereas for HR and Trécé baits, release rates were similar at 15.6, 21.1, and 26.7 °C. When exposed to high temperatures (32.2 °C), the rate of ethanol released from HR bait was ~8 × more than that released from Trécé bait. In field experiments of the current study, adults of *X. crassiusculus* were consistently trapped using all 3 ethanol baits (Figs. 4–6). Ethanol release patterns from the 3 baits were relatively stable under constant or sequential temperatures. However, many biotic (such as combinations of host tree species, their health, and *X. crassiusculus* population size in woodlots, etc.) and abiotic (such as temperature, wind velocity and direction, relative humidity, and rain, etc.) factors could influence the initiation of mass flight activity and effective interception of adult *X. crassiusculus* and *X. germanus* in traps with ethanol bait pouches. Thus, more thorough research is warranted where biotic and abiotic factors are combined through modeling to characterize adult *X. crassiusculus* and *X. germanus* captures in the field.

The sequential temperature experiment was conducted to determine the effects of fluctuating temperatures on release rates of commercial ethanol baits during the late winter and early spring in Georgia, USA. When exposed to a variable sequence of temperatures, release rates from all 3 baits were steady for up to 30 d. Moreover, when the average release rates under constant temperatures (15.6, 21.1, and 26.7 °C) were compared to rates of ethanol release under a variable sequence of temperatures, we observed a similar pattern (Fig. 3). The amount of ethanol released from the HR bait was much higher than from the LR and Trécé baits as indicated by the manufacturer. The LR collected more *X. crassiusculus* adults in the field than HR or Trécé baits in 2022 and 2023. This suggests that *X. crassiusculus* adults can detect low to moderate amounts of released ethanol and lure beetles into traps, which can be used for pest management decisions. The ethanol baits tested in the current study had concentrations of 83 to 95% in a pouch (Fig. 1A). Perhaps, lower ethanol concentrations (< 83%) with similar or higher rates of release as used in the current study may capture specific pest species of ambrosia beetles, such as adult *X. crassiusculus* and *X. germanus*, which warrants more research. These findings will determine the best combination between ethanol concentration and release rate for early captures of adult *X. crassiusculus* and *X. germanus* in field conditions.

Results showed that adults of *X. crassiusculus* and *X. germanus* were collected in bottle traps using all 3 bait pouches for 3 yr. In

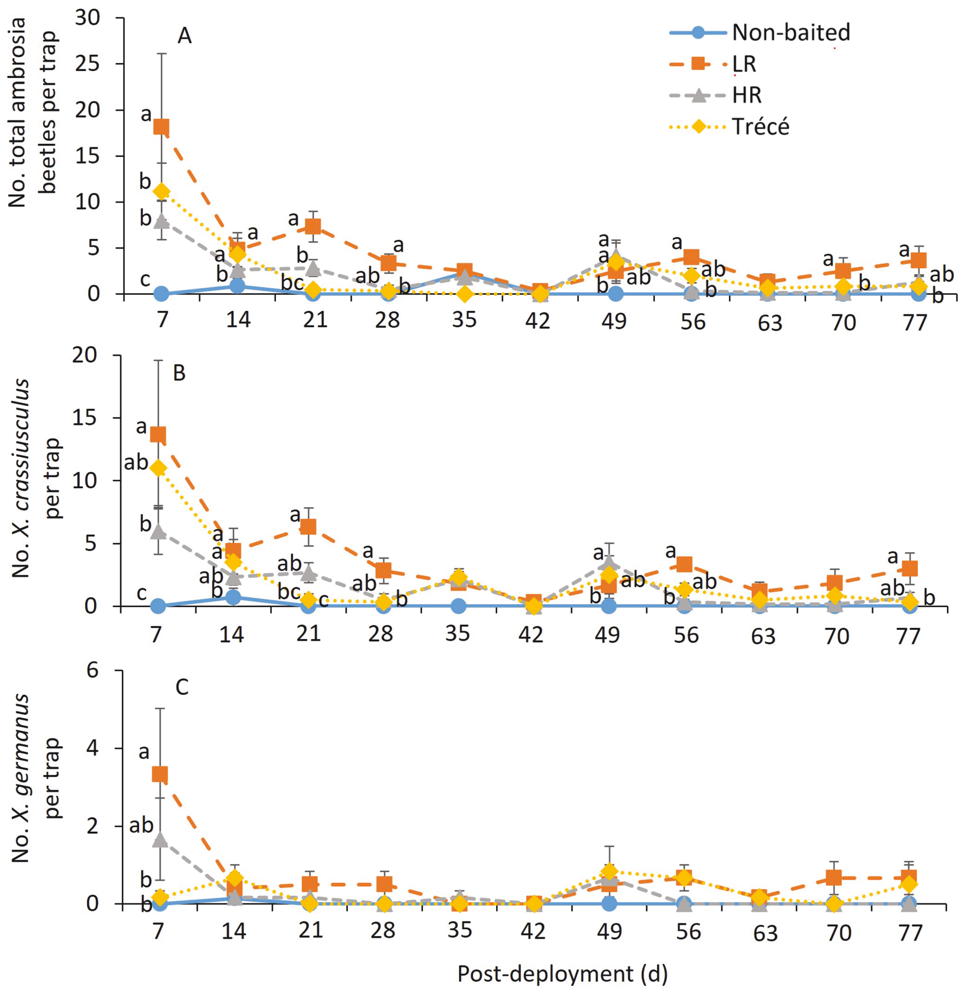

Fig. 5. Mean ± (SE) number of (A) total ambrosia beetles, (B) *X. crassiusculus,* and (C) *X. germanus* adults captured in bottle traps using bait treatments in an ornamental nursery in 2023. The same letters among the treatments for each sampling date are not significantly different using Tukey–Kramer test (α = 0.05). Where no differences were observed, no letters are given.

2022 and 2023, the LR bait collected more *X. crassiusculus* adults than HR or Trécé baits, although the temperatures were highly variable in all 3 yr(Supplementary Fig. 1). The LR bait was previously evaluated as a monitoring tool for adult *X. crassiusculus* and *X. germanus* (Monterrosa et al. 2021, 2022). The LR bait effectively captured adult *X. crassiusculus* for up to 8 wk in field conditions (Monterrosa et al. 2021). Conversely, the HR-baited bottle traps collected more adult *X. crassiusculus* and *X. germanus* than LR-baited traps in most sites (Govindaraju et al. 2024). Each LR bait is 3.7 times cheaper than HR bait. Moreover, the LR and Trécé baits are relatively smaller pouches than the HR bait and were easier to suspend inside a 1.7 L bottle than the HR bait (Fig. 1). In 2024, however, adult *X. crassiusculus* captures were shifted, and more captures were observed in Trécé bait, especially during the latter 2 sampling dates. Although the exact reasons for these results are unclear, it is possibly related to higher counts of adult flight activity in the spring of 2024 than in previous years. Also, perhaps a more conclusive pattern would have emerged if the trapping had continued for 4 more weeks. Thus, all 3 ethanol pouches are suitable for early spring detection of adult *X. crassiusculus*. Although captures of ambrosia beetles could be influenced by the release rate of ethanol (Cavaletto et al. 2023, Tobin and Ginzel 2023), adults of *X. crassiusculus* were the dominant ambrosia beetle species collected in bottle traps in the current and past studies for ornamental sites in central Georgia (Monterrosa et al. 2021, 2022). However, the abundance of adult *X. crassiusculus* captures varied between years regardless of release rates from baits in bottle traps in the field settings.

In summary, the current study showed that LR, HR, and Trécé baits released a steady rate of ethanol under constant temperatures. The Trécé bait released lesser amounts of ethanol at the highest temperature (32.2 °C), whereas the LR and HR baits released more ethanol at the highest temperature. We did not study the influence of other abiotic factors, such as relative humidity, on ethanol release and evaporation rate using various commercial baits. This suggests that more research that integrates multiple layers of factors on ethanol release patterns is warranted. *Xylosandrus crassiusculus* is the major ambrosia beetle species in the Georgia nursery system (Monterrosa et al. 2021, 2022). More numbers of adult *X. crassiusculus* were collected in the LR baited bottle traps than using HR or Trécé lure in the trap in 2022 and 2023, suggesting that the increased release rates of ethanol may not necessarily elicit enhanced attractive responses to flying adult *X. crassiusculus*. Conversely, more adults of *X. crassiusculus* were captured in traps baited with Trécé lure when the *X. crassiusculus* adult activity was greater than in previous years. The results clearly show that bottle traps with either one of the ethanol baits can be used to detect *X. crassiusculus* in the spring. A recent study showed that LR-baited clear sticky cards collected high numbers of *X. crassiusculus* and *X. germanus* (Tobin

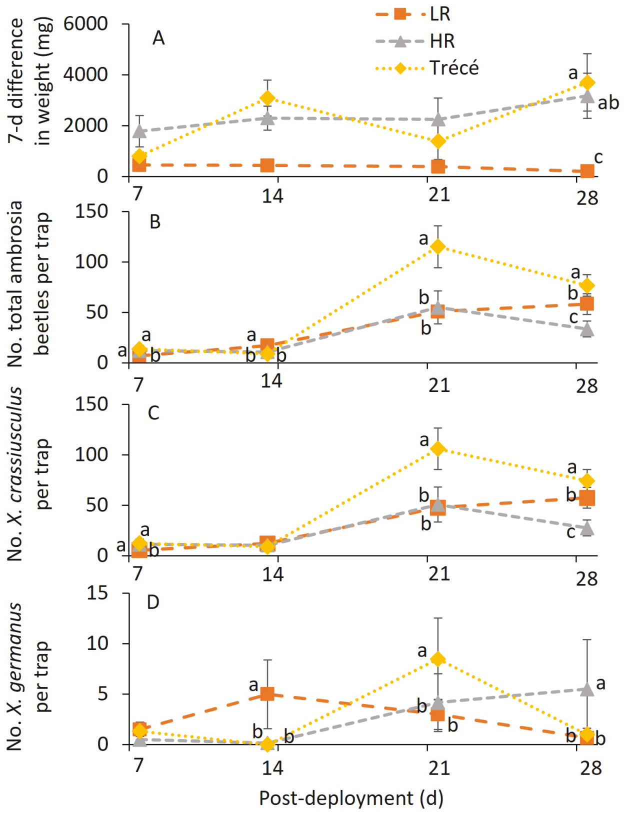

Fig. 6. (A) 7-d-difference weights at 7 d intervals and mean ± (SE) number of (B) total ambrosia beetles, (C) *X. crassiusculus,* and (D) *X. germanus* adults captured in bottle traps using bait treatments in an ornamental nursery in 2024. The same letters among the treatments for each sampling date are not significantly different using Tukey–Kramer test (α = 0.05). Where no differences were observed, no letters are given.

et al. 2024). Because adult *X. crassiusculus* and *X. germanus* attacks are likely during 5 wk during or after bud break in the early spring (Monterrosa et al. 2021, 2022), a monitoring tool is essential to determine the application timing of pyrethroids. Ethanol baits, regardless of release rates, are critical elements of traps for the detection of adult *X. crassiusculus* in nursery settings.

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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 [equal], Writing—review & editing [lead]), and Ramkumar Govindaraju (Investigation [equal], Project administration [lead], Writing—original draft [equal])

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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Conflicts of interest

The authors declare no conflicts of interest.

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