

# Interaction of Insecticide and Media Moisture on Ambrosia Beetle (Coleoptera: Curculionidae) Attacks on Selected Ornamental Trees

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## Abstract

Exotic ambrosia beetles, particularly *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae) and *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), are among the most damaging pests of ornamental trees in nurseries. Growers have had few tactics besides insecticide applications to reduce ambrosia beetle attacks but recent research has shown that attacks may be reduced by maintaining media moisture below a 50% threshold thereby reducing flood stress. We compared the efficacy of managing media moisture and insecticide applications for reducing ambrosia beetle attacks on three ornamental tree species in North Carolina. During trials in spring 2013 and 2015, flooded *Cornus florida* and *Cornus kousa* were heavily attacked despite sprays with permethrin, but nonflooded *C. kousa* or *C. florida* were not attacked. In spring 2015 trials, both nonflooded and flooded *Styrax japonicus* were heavily attacked regardless of permethrin applications. Although ethanol emissions were not measured, the apparently healthy nonflooded *S. japonicus* trees may have been exposed to an unknown physiological stress, such as low temperature injury, the previous winter, which predisposed them to beetle attack. However, ethanol levels within host tissues were not measured as part of the current study. *X. crassiusculus* (75%), *Xyloborinus saxeseni* Ratzburg (13%), and *X. germanus* (9%) were the most abundant species collected in ethanol baited traps deployed in 2015, while *X. crassiusculus* (63%) and *X. germanus* (36%) were the predominant species reared from attacked trees. Results indicate that managing media moisture levels at or below 50%, and maximizing tree health overall, may provide significant protection against *Xylosandrus* spp. attacks in flood intolerant tree species.

**Key words:** *Xylosandrus crassiusculus*, *Xylosandrus germanus*, flood stress

Exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae), particularly *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford), are important pests of ornamental trees grown in nurseries (Adkins et al. 2010, Frank et al. 2013, Ranger et al. 2016b). Both *X. crassiusculus* and *X. germanus* are native to southeastern Asia and were first detected in the United States in 1974 and 1932, respectively (Felt 1932, Anderson 1974). *X. crassiusculus* and *X. germanus* have since become established in 29 and 32 states, respectively (Haack and Rabaglia 2013). *X. crassiusculus* tends to be more problematic in the mid-Atlantic and southern USA, while *X. germanus* is generally more abundant and problematic in the Midwest and Northeast.

*X. crassiusculus* and *X. germanus* are highly polyphagous with host ranges exceeding 120 and 200 species, respectively, including

popular ornamental trees such as dogwood (*Cornus* spp.), flowering cherry (*Prunus* sp.), Japanese snowbell (*Styrax japonicus* S. et Z.), magnolia (*Magnolia* spp.), and redbud (*Cercis canadensis* L.) (Schneider and Farrier 1969, Weber and McPherson 1983a). Female ambrosia beetles leave their overwintering host galleries in spring and bore into the heartwood of trees. Host galleries are then inoculated with a nutritional fungal symbiont on which the larvae and adults feed (Weber and McPherson 1983b, Harrington et al. 2014). Ambrosia beetle attacks weaken host trees, provide an entrance for secondary opportunistic pathogens, and ambrosia fungus can clog the vascular system of the tree (Buchanan 1941, Weber and McPherson 1985). Damaged trees typically die or become unmarketable (Frank et al. 2013).

Growers have limited Integrated Pest Management (IPM) strategies for dealing with *X. crassiusculus* and *X. germanus*. Bottle-traps

baited with ethanol are effective tools for monitoring the activity of these species (Reding et al. 2011, Frank and Ranger 2016). However, after *X. crassiusculus* and *X. germanus* become active, management entails preventive trunk applications of conventional insecticides every 2–4 wk (Ranger et al. 2016b). These preventive applications can reduce attacks and disrupt colonization success (Frank and Sadof 2011, Reding et al. 2013b, Ranger et al. 2016b), but larvae, pupae, and adults are generally protected from insecticides once inside their host galleries. Systemic insecticides have not exhibited efficacy at reducing attacks (Reding et al. 2013b), but the impact of systemics on disrupting colonization success and viability of established colonies within galleries is unknown. Since insecticide applications are expensive for growers, can drift to nontarget areas, cause secondary pest outbreaks, and do not fully prevent attacks (Frank and Sadof 2011, Reding et al. 2013b), development of cultural controls is needed to reduce tree susceptibility and attacks.

*X. crassiusculus* and *X. germanus* use volatile cues, primarily ethanol, to locate vulnerable host trees (Ranger et al. 2010, 2012, 2013a, 2015a, b). Ethanol is induced in response to a variety of physiological stressors, including flooding, drought, and low temperature injury (i.e., frost and freezing) (Kimmerer and Kozlowski 1982; Kimmerer and Macdonald 1987; Kelsey 2001; Ranger et al. 2013a, 2015a). Regarding flood stress, when tree roots are submerged in standing water they switch from aerobic to anaerobic respiration, the byproduct of which is ethanol (Tadage et al. 1999). Ethanol produced in tree roots is then transported to stem and leaf tissues and emitted from the epidermis (Drew 1997, Tadage et al. 1999). Trees growing in overly saturated media or flood-stress conditions emit higher concentrations of ethanol than trees in drier conditions and are thus more susceptible to ambrosia beetle attacks (Ranger et al. 2013a, 2015a; Frank and Ranger 2016). As a result, media moisture thresholds were tested and developed using dogwood (*Cornus florida* L.), a flood-intolerant species (Frank and Ranger 2016). Trees grown in media moisture above 50% incurred more ambrosia beetle attacks than trees maintained at a media moisture below this threshold, which received almost no attacks. Despite this, ornamental trees in nurseries regularly exceed the 50% media moisture threshold, often reaching media moisture levels between 70 and 90% (Frank and Ranger 2016). Multiple studies have documented that *Xylosandrus* spp. ambrosia beetles preferentially colonize trees that emit elevated levels of ethanol induced by flood-stress, so water management could complement or potentially replace preventive insecticides as a management strategy. Notably, beetles have not attacked trees not emitting ethanol during several studies (Ranger et al. 2010; Ranger et al. 2012, 2013a,b, 2015a,c; Frank and Sadof 2011; Reding et al. 2013b).

Nursery growers need more tactics for ambrosia beetle IPM. We believe that managing media moisture could be a cost-effective measure to minimize attacks by *Xylosandrus* spp. and reduce the need for insecticide applications in nurseries. However, the effect of flooding can vary depending on a particular species intolerance of flood-stress (Ranger et al. 2015c). Thus, even if reducing media moisture can eliminate attacks on some tree species, others may still require preventive insecticide treatments. To determine how water management affects the need for insecticide treatments, we compared beetle attacks on two dogwood species (*C. florida* and *Cornus kousa* Burg.) and Japanese snowbell (*S. japonicus*) grown in flooded and nonflooded (50% media moisture or below) conditions and either sprayed or nonsprayed with insecticide. Our specific objectives were to: 1) determine how tree species, media moisture, and insecticide application affect ambrosia beetle attacks, and 2) determine

if ambrosia beetle species composition and spring flight activity correspond with beetle species attacking trees. This research should advance water management as a cultural practice to strengthen IPM of exotic ambrosia beetles in nurseries.

## Materials and Methods

### Field Trapping

To assess ambrosia beetle flight activity and species composition, trapping experiments were conducted in the research nursery at the North Carolina State University Lake Wheeler Road Field Laboratory in Raleigh, NC (35°44'14.8"N 78°40'25.7"W) in the springs of 2013 and 2015. Three sides of the nursery are adjacent to woodlots (<30 m). The surrounding landscape consists of agricultural fields. The nursery has automated drip irrigation. The ground is covered with weed cloth.

In 2013, we deployed 15 ambrosia beetle traps each made from a 2-liter soda bottle connected to a 355 ml soda bottle with a threaded bottle connector (Reding et al. 2011) at the study site. Traps were deployed from 25 February 2013 until 30 April 2013 and were spaced at least 30 m apart along the edge of a wooded lot near the nursery. Traps were baited with an ethanol lure consisting of a cotton wick placed in a 15 ml glass vial filled with 15 ml of 90% ethanol. Propylene glycol was used as a killing and preserving agent in the traps. Ambrosia beetles were not identified to species as part of the 2013 trapping experiment.

In 2015, traps were made as in 2013 except lures were constructed from an 8 ml vial filled with 95% ethanol and covered with parafilm attached inside the 2-liter plastic bottle and refilled as needed. Five ethanol-baited traps were deployed from 20 February 2015 until 18 May 2015. Traps were positioned at least 30 m apart along a wooded edge adjacent to the experimental nursery at the Lake Wheeler Road Field Lab. Traps were checked every second day until the first ambrosia beetle was collected, after which traps were checked three times per week. At each visit, the collection bottles were replaced and beetles were transferred to vials of 95% ethanol. Specimens were later sorted and identified to species using the online Xyleborini Ambrosia Beetle key (Hulcr and Smith 2010) and the reference collection in the Frank Lab.

### Effect of Insecticide and Flooding on Ambrosia Beetle Attacks

To determine how flooding and insecticide applications affect ambrosia beetle attacks, we conducted factorial field experiments in 2013 and 2015. In 2013, we purchased 32 *C. kousa* (1–1.5 m tall and 1.5 cm diameter) in 18.9-liter pots with pine bark and sand potting media from a local nursery. On 26 February 2013, we randomly assigned eight *C. kousa* to each of two water treatments (flooded or nonflooded) crossed with two insecticide treatments (sprayed or nonsprayed; 32 trees total). Nonflooded trees were watered as needed, but media moisture levels were not measured. We used a pot-in-pot system to create the flooded treatment (Ranger et al. 2013a), whereby we placed each 18.9-liter pot containing a tree within another 18.9-liter pot lined with a plastic trash bag to prevent drainage. Flooded trees were watered each day to maintain standing water and media saturation. On 15 March 2013, after the first pest ambrosia beetle was collected in our traps, trunks of trees in the insecticide treatment were sprayed until runoff with permethrin (Martin's Permethrin SFR Termiticide/Insecticide, Control Solutions, Inc., Pasadena, TX) at the labeled rate (18.9 ml/liter). Applications were made using a CO<sub>2</sub> powered tank sprayer with nozzle at 30 psi.

A second application was made on 6 April 2013. We counted new attacks every 3–7 d from 5 March to 18 May 2013. During each tree inspection, new attacks were circled with a black indelible marker so they would not be recounted in subsequent weeks. Since the resulting data were nonnormal and the variance was not homogeneous, cumulative attacks per tree were compared among treatments using the nonparametric Kruskal–Wallis test ( $\alpha = 0.05$ ) (JMP, Version 12, SAS Institute Inc., Cary, NC).

In 2015, we tested how the effects of watering and insecticides on ambrosia beetle attacks may differ between tree species. Using a factorial design, we crossed two tree species (*C. florida* and *S. japonicus*), two insecticide treatments (sprayed and nonsprayed), and two water treatments (flooded and nonflooded). On 20 February 2015, before ambrosia beetles began their spring flight, six *C. florida* and six *S. japonicus* were assigned to each of the aforementioned four treatments for a total of 48 trees per species. *C. florida* and *S. japonicus* were chosen because they are common nursery trees prone to ambrosia beetle attacks (Frank et al. 2013). Trees were purchased from local nurseries and were ~1.5 m tall and 1.5–2 cm diameter. Each tree was planted in a 22.7-liter pot with pine bark and sand media. Trees were arranged in a complete random design along three irrigation rows spaced 0.61 m within rows and 1 m between rows. Trunks of trees in the insecticide treatments were sprayed with permethrin according to the aforementioned methods. Treatments were applied on 12 March 2015, just after the first pest ambrosia beetle species were collected in our bottle-traps, and then again on 16 April 2015.

Based on new media moisture thresholds, trees in the non-flooded treatments were maintained at media moisture between 30 and 50% (Frank and Ranger 2016). Flooded trees were maintained at media moisture between 70 and 100% using the pot-in-pot system above to reduce drainage. Trees were watered twice per day with an automated irrigation system to maintain water treatments. To ensure each tree remained in its designated water treatment, nonflooded or flooded, media moisture was measured with an SM150 Soil Moisture Sensor (Delta-T Devices, Cambridge, United Kingdom) three times per week. The probe was inserted into the surface of the media to measure the media moisture at the top of the pot. The probe was also inserted through the side of pots in the non-flooded treatments, 3–4 cm from the bottom, to measure percent media moisture at the bottom of the pot. Percent media moisture was not measured at the bottom of flooded treatments as it would puncture the lining used to achieve the flooded treatment, so these numbers were recorded as 100%. Percent media moisture at the top and bottom was measured three times in each pot; the mean top and bottom measurements were then used in analyses. The probe does not provide a reading for soil moisture over 85%, so these numbers were recorded as 90%. Water was adjusted accordingly to maintain each tree within its assigned treatment. We inspected each tree three times per week for 8 wk for new beetle attacks. Each attack was circled with a waterproof permanent marker at each visit to ensure no attack was recounted in following weeks. The experiment ended 18 May 2015 when trees were harvested to rear beetles (discussed in what follows).

We compared the top and bottom media moisture levels using repeated measures ANOVA in JMP to determine whether date, insecticide treatment, or their interaction affected media moisture and to confirm that experimental trees remained within their assigned percent moisture treatments (JMP Version 12). To test how tree species, flooding, insecticide applications, and their interaction affect ambrosia beetle attacks, we constructed a three-way ANOVA in SAS (Version 12; SAS Institute, Cary, NC) followed by LSD means

comparisons. To improve the distribution of residuals, we  $\log(x+1)$  transformed total beetle attacks.

## Rearing Beetles from Attacked Trees

Ethanol baited traps capture some ambrosia beetle species more effectively than other species. Our goal was to determine if species captured in traps represent species most likely to attack trees. In 2015, we reared ambrosia beetles from attacked *C. florida* and *S. japonicus* trees to test the hypothesis that species composition of trap captures would correspond with the species composition of beetles attacking trees. It was also of interest to determine whether tree species or flooding treatment affected the beetle species present in the attacked trees. On 18 May 2015, trees with attacks were cut at the soil surface and the stems from each tree were placed in separate 10 × 33 cm (diameter by length) cardboard craft tubes (Kraft Mailing Tube, Uline, Pleasant Prairie, WI) to rear any beetles within the stems. We made a 2.5 cm diameter hole in the plastic endcap to each tube and attached a 50 ml vial to collect emerging beetles. Rearing tubes with tree material were stored at room temperature in cardboard boxes in the laboratory and left undisturbed until the beetles emerged. After 60 d and substantial beetle emergence, all beetles were removed from tubes and identified to species (as described previously). We analyzed the frequency of each species that were collected in traps and reared from *C. florida* and *S. japonicus* stems using a Pearson chi-square test in JMP (Version 12).

## Results

### Field Trapping

In 2013, a total of 832 Scolytinae were collected using ethanol-baited traps. The number of ambrosia beetles in traps and the number of attacks on *C. kousa* trees peaked in mid-April.

In 2015, we collected 2,699 beetles representing eight Scolytinae species. Of these, ~75% were *X. crassiusculus*, ~13% were *Xyloborinus saxesenii* Ratzburg, ~9% were *X. germanus*, ~1% were *Xylosandrus mutilatus* (Blandford), and the remaining ~2% comprised the following genera: *Ambrosiodmus*, *Dryocoetes*, and *Euvallacea*. The first ambrosia beetle caught in the ethanol-baited traps, *X. crassiusculus*, was collected on 6 March 2015, 1 mo before experimental trees received attacks (Fig. 1).

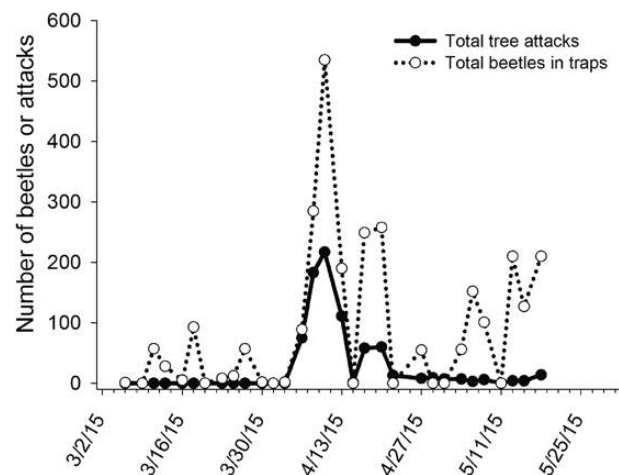


Fig. 1. Total number of scolytine beetles collected in bottle traps and total number of beetle attacks in 2015.

## Effect of Insecticide and Flooding on Ambrosia Beetle Attacks

In 2013, flooded *C. kousa* trees with no insecticide received a mean ( $\pm$ SEM) of  $46.8 \pm 10.5$  attacks per tree. This was significantly greater than flooded and insecticide treated trees that received  $0.6 \pm 0.6$  attacks per tree ( $\chi^2 = 12.3$ ;  $df = 1$ ;  $P < 0.001$ ). The number of attacks on flooded trees with no insecticide treatment was also significantly greater than attacks on nonflooded trees with and without insecticide treatment, both of which received  $0.0 \pm 0.0$  attacks per tree ( $\chi^2 = 12.9$ ;  $df = 1$ ;  $P < 0.001$ ).

In 2015, percent media moisture at the top and bottom portions of the pots were significantly affected by the flooding treatment (Top:  $F = 303.75$ ;  $df = 3, 4$ ;  $P < 0.001$ ; Bottom:  $F = 392.51$ ;  $df = 3, 4$ ;  $P < 0.001$ ) (Fig. 2). The insecticide treatment (Top:  $F = 0.04$ ;  $df = 1, 4$ ;  $P = 0.708$ ; Bottom:  $F = 0.03$ ;  $df = 1, 4$ ;  $P = 0.732$ ) and the interaction of media moisture and insecticides (Top:  $F = 0.01$ ;  $df = 1, 4$ ;  $P = 0.914$ ; Bottom:  $F = 0.1$ ;  $df = 1, 4$ ;  $P = 0.732$ ) did not affect percent media moisture (Fig. 2). There was a significant interaction of water treatment, insecticide treatment, and tree species on the mean number of ambrosia beetle attacks per tree ( $F = 5.41$ ;  $df = 1, 40$ ;  $P = 0.025$ ) (Table 1; Fig. 3). There was also a significant interaction between water treatment and tree species, and significant main effects of water treatment, insecticides, and tree species (Table 1). In particular, flooded and insecticide treated *C. florida* trees were attacked by ambrosia beetles, but there was no difference in cumulative attacks between these two treatments (Fig. 3). No attacks

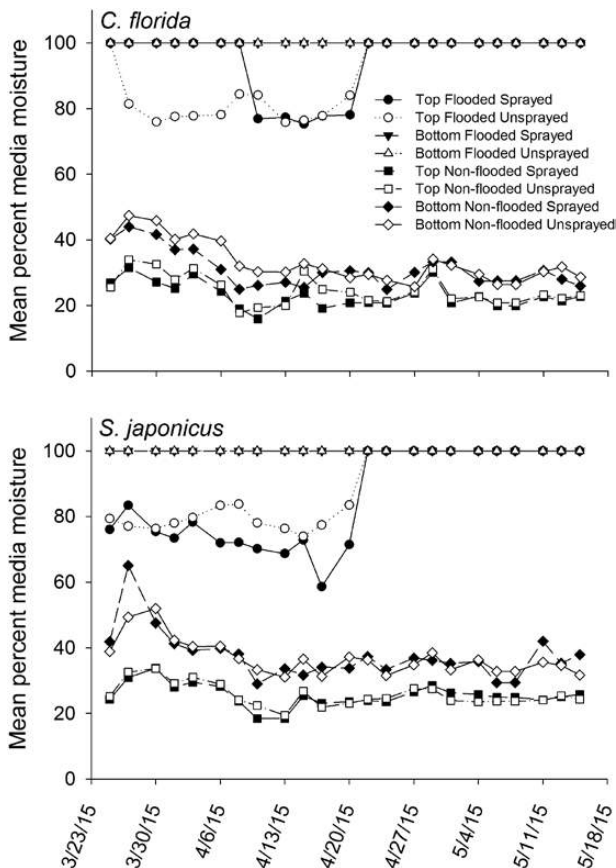


Fig. 2. Mean percent media moisture levels measured from the top and bottom regions of container-grown *C. florida* and *S. japonicus* trees that were either flooded or nonflooded and insecticide treated or untreated. Moisture levels were measured three times per week in the tree containers.

occurred on nonflooded *C. florida* trees (Fig. 3). Flooded and insecticide treated *S. japonicus* trees sustained fewer attacks than flooded and untreated *C. florida* trees (Fig. 3). Unlike nonflooded *C. florida*, nonflooded *S. japonicus* trees were also attacked by ambrosia beetles. Insecticide treatment reduced attacks on nonflooded *S. japonicus* compared with nonflooded and untreated trees, but insecticide treatment did not significantly reduce the number of attacks on flooded *S. japonicus* trees.

## Rearing Beetles from Attacked Trees

A total of 901 beetles were reared from attacked experimental trees deployed in 2015. *X. crassiusculus* and *X. germanus* represented the majority of the specimens, comprising ~63 and 36%, respectively (Fig. 4a-c). No other Scolytinae emerged from the infested stem sections. The frequency of *X. crassiusculus* and *X. germanus* reared from attacked trees was significantly different than expected from trap captures, such that *X. germanus* was reared from stems at a higher frequency than they were collected ( $\chi^2 = 304.7$ ;  $df = 1$ ;  $P < 0.001$ ) (Fig. 4a). The effect of tree species on the frequency of *X. crassiusculus* and *X. germanus* reared from trees was also significant, such that a greater proportion of beetles reared from *S. japonicus* were *X. germanus* than from *C. florida* ( $\chi^2 = 17.5$ ;  $df = 1$ ;  $P < 0.001$ ; Fig. 4b). Flood treatment did not have a significant effect on the frequency of *X. crassiusculus* and *X. germanus* reared from flooded and nonflooded *S. japonicus* ( $\chi^2 = 1.3$ ;  $df = 1$ ;  $P = 0.249$ ; Fig. 4c).

Table 1. Results of ANOVA on the effects of tree species (*C. florida* or *S. japonica*), water treatment (flooded or not), and insecticide treatment (sprayed or not) on total ambrosia beetle attacks

Effect (ndf, ddf)	F	P
Species (1, 40)	4.41	0.043
Water (1, 40)	22.00	<0.001
Insecticide (1, 40)	10.76	0.002
Insecticide $\times$ Water	0.01	0.913
Insecticide $\times$ Species (1, 40)	1.12	0.294
Species $\times$ Water (1, 40)	27.88	<0.001
Species $\times$ Water $\times$ Insecticide (1, 40)	5.43	0.025

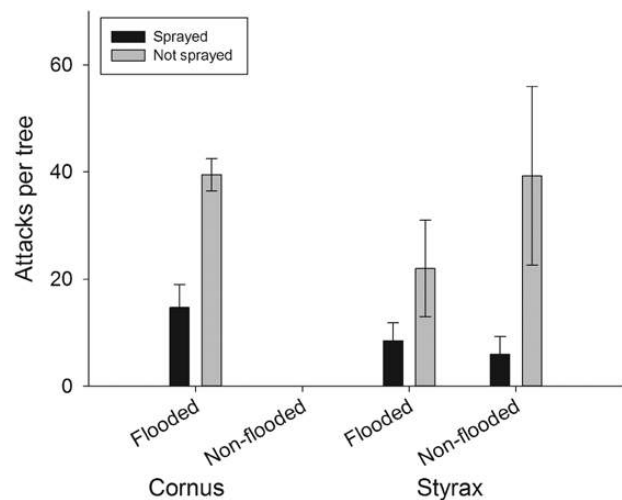


Fig. 3. Mean ( $\pm$ SEM) ambrosia beetle attacks per *C. florida* or *S. japonica* tree when flooded or nonflooded and sprayed with insecticide or nonsprayed. Note zero values for *Cornus* nonflooded, sprayed, and unsprayed. Bars with different letters are significantly different at  $P < 0.05$ .



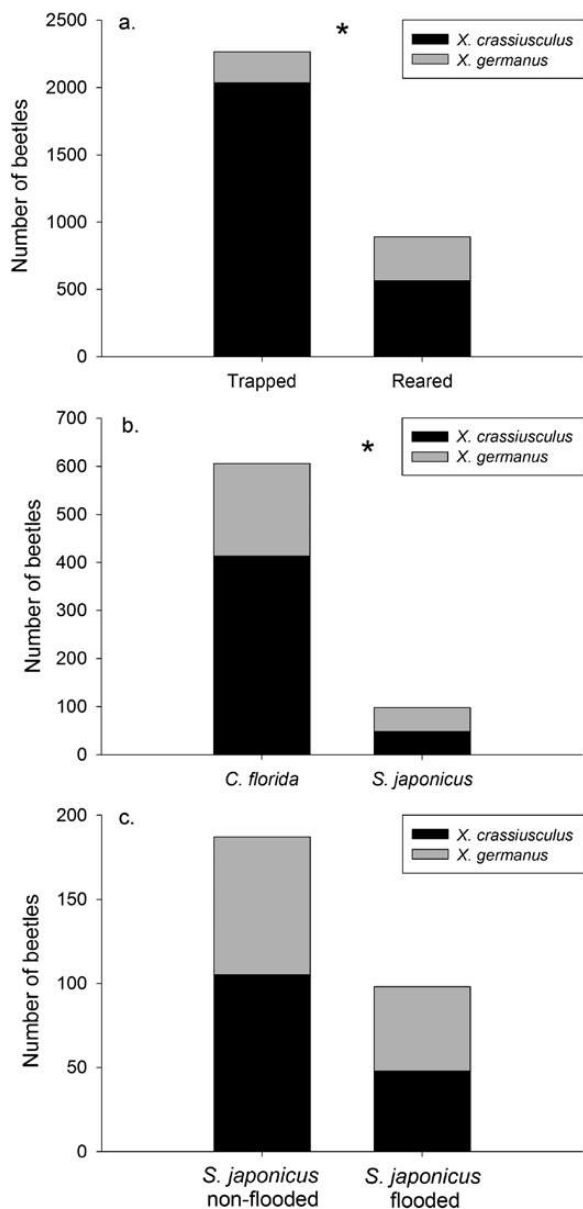


Fig. 4. Number of adult *X. crassiusculus* and *X. germanus* a) captured in ethanol-baited traps compared with specimens reared from attacked trees, b) reared from *C. florida* compared with *S. japonica*, and c) reared from flooded or nonflooded *S. japonica*. Asterisk indicates significant ( $P < 0.05$ ) differences from chi-square test.

## Discussion

Maintaining tree health is the primary foundation of an ambrosia beetle management plan, but few IPM tactics have been developed to aid in managing exotic ambrosia beetles (Adkins et al. 2010, Ranger et al. 2016b). Since flood-stress was found to induce ethanol emissions and predispose *C. florida* trees to attack by ambrosia beetles (Ranger et al. 2013a), subsequent research determined that container-grown *C. florida* trees should be maintained below a 50% media moisture threshold (Frank and Ranger 2016). Results from our current study further confirmed the benefits of maintaining media moisture below a 50% threshold for *C. kousa* in addition to *C. florida*. In particular, maintaining media moisture below 50% prevented ambrosia beetle attacks from occurring on *C. florida* and *C. kousa*. The 50% media moisture threshold was critical for these *Cornus* spp.

because, once flooded, permethrin applications did not prevent attacks. Tolerance for ambrosia beetle attacks is low among ornamental growers, so even trees with one attack are usually discarded. Our results contribute to growing evidence that water management can be the first line of defense against ambrosia beetle attacks.

Tree species differ in their physiological response to flooding (Joly and Crawford 1982, Kennedy et al. 1992), which in turn can influence their vulnerability to attack by ambrosia beetles. For instance, when ambrosia beetles were allowed to choose among flooded and nonflooded trees of varying intolerance to flooding, flood-stressed *C. florida*, *S. japonicus*, and *C. canadensis* were preferentially attacked in Ohio and flood-stressed *C. florida* was preferentially attacked in Virginia (Ranger et al. 2015c). In contrast, flood-stressed silver maple (*Acer saccharinum* L.), swamp white oak (*Quercus bicolor* Willd.), and river birch (*Betula nigra* L.), sustained few to no attacks in Ohio and Virginia trials. No attacks occurred on the nonflooded trees, with the exception of one attack on one redbud *C. canadensis* deployed in Virginia. Higher concentrations of ethanol were also detected in the vascular tissue of the flood-stressed and intolerant tree species compared with the flooded but moderately tolerant to tolerant species. Studies by Frank and Ranger (2016) also found flood-stressed red maples (*Acer rubrum* L.) were associated with low levels of ethanol and were rarely attacked even when flooded.

Unlike *C. florida* and *C. kousa*, container-grown *S. japonicus* maintained below and above the 50% media moisture threshold were attacked as part of the current study. Because *X. crassiusculus* and *X. germanus* are bio-indicators of stressed trees (Ranger et al. 2015a,c), we suspect the nonflooded *S. japonicus* trees were attacked due to the emission of ethanol in response to an undetermined physiological stressor such as low temperature stress the previous winter. *S. japonicus* is known to be intolerant of low temperature stress (Reed 2005). Low temperature stress also preceded attacks by *X. germanus* and other ambrosia beetles on *S. japonicus* and other intolerant species growing in ornamental nurseries, including *Acer palmatum* Thunb., *C. canadensis*, *Liriodendron tulipifera* L., and *Zelkova serrata* (Thunb.) (Ranger et al. 2015c). Although this is a possible cause for stress and attacks on *S. japonicus* trees, we did not measure ethanol production or winter damage and do not know why similar stress was not evident in *C. florida*. Permethrin also did not prevent attacks from occurring on *S. japonicus*, which further demonstrates the limitations of preventive insecticide applications for managing ambrosia beetles.

In our current study, the two dominant species collected in ethanol-baited traps and reared from infested stems of *C. florida* and *S. japonicus* were *X. crassiusculus* followed by *X. germanus*. These results indicate *X. crassiusculus* can be more abundant and problematic within this region, but also that *X. germanus* can be of concern. Previous studies have indicated that *X. germanus* tends to be dominant in the upper Midwest and northeastern USA, *X. crassiusculus* and *X. germanus* are somewhat co-dominant in the mid-Atlantic USA, and *X. crassiusculus* is dominant in the southeastern and southern USA (Oliver and Mannion 2001; Ranger et al. 2010, 2016b; Reding et al. 2011, 2013a). Differences in the proportions by which *X. crassiusculus* and *X. germanus* were collected in ethanol-baited traps versus reared from infested stems may be attributed to differences in attraction to ethanol or tree volatiles, establishment of the fungal symbiont, or colonization success or reproductive capabilities of the foundress' beetles. For instance, Oliver and Mannion (2001) found *X. saxesenii*, *X. crassiusculus*, and *Monarthrum fasciatum* Say were the dominant species collected in traps deployed in Tennessee, but the dominant species of progeny that emerged from galleries were

*X. germanus* (35.9%), *X. crassiusculus* (10.3%), *Hypothenemus* spp. (3.3%), and *X. saxesenii* (1.1%). Interestingly, *X. saxesenii* was captured in ethanol-baited traps but not reared from infested trees as part of our current study. Dissecting infested trees following the termination of a field experiment is ideal for assessing the dominant species at the time of attack, but rearing specimens from infested trees also provides important insight into the more prolific species.

Ornamental nurseries rely heavily on preventive insecticide applications for minimizing ambrosia beetle attacks, especially during spring. Systemic insecticides are not effective at preventing attacks and growers currently apply insecticides with large airblast sprayers (Frank and Sadof 2011). Since initial seasonal captures and peak flight activity coincide with a variety of flowering plants and trees (Reding et al. 2013a), these preventive applications could have a negative impact on insect pollinators. Knowing that tree health plays a critical role in tree attractiveness and vulnerability to ambrosia beetles, IPM tactics that maximize host vigor are crucial. A media moisture threshold has since been developed to minimize issues associated with overwatering and inadequate drainage (Frank and Ranger 2016), along with identifying trees intolerant of flooding and, therefore, prone to attack under such conditions (Ranger et al. 2016a). We predicted that insecticides would complement the cultural practices of water management on ornamental trees. However, results from our current study and previous ones demonstrate that preventive applications of conventional insecticides (Frank and Sadof 2011, Reding et al. 2013b, Ranger et al. 2016a), botanically based formulations (Ranger et al. 2011), and repellents (Ranger et al. 2013b) do not fully protect trees emitting ethanol from attack. Since untreated control trees have not been attacked in numerous aforementioned studies, preventive insecticide applications do not provide any added protection unless the tree is physiologically stressed and vulnerable to attack. Thus, by using proper water management tactics, insecticide applications on flood-intolerant species may be an unnecessary expense with potential negative effects like insecticide drift and secondary pest outbreaks (Frank and Sadof 2011).

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## References Cited

- Adkins, C., G. Armel, M. Chappell, J. C. Chong, S. D. Frank, A. Fulcher, F. Hale, W. E. Klingeman, K. Ivors, and A. Lebude. 2010. Pest management strategic plan for container and field produced nursery crops in GA, KY, NC, SC, TN. In A. Fulcher (ed.), Southern Region IPM Center. <http://www.ipmcenters.org/pmsp/pdf/GA-KY-NC-SC-TNnurserycropsPMSP.pdf>
- Anderson, D. 1974. First record of *Xyleborus semiopacus* in the continental United States (Coleoptera, Scolytidae). Cooperative Economic Insect Report 24: 863–864.
- Buchanan, W. 1941. Experiments with an ambrosia beetle, *Xylosandrus germanus* (Blfd.). J. Econ. Entomol. 34: 367–369.
- Drew, M. C. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. Annu. Rev. Plant Biol. 48: 223–250.
- Felt, E. 1932. A new pest in greenhouse grown grape stems. J. Econ. Entomol. 25: 418.
- Frank, S. D., and C. M. Ranger. 2016. Developing a media moisture threshold for nurseries to reduce tree stress and ambrosia beetle attacks. Environ. Entomol. 45: 1040–1048.
- Frank, S. D., and C. S. Sadof. 2011. Reducing insecticide volume and nontarget effects of ambrosia beetle management in nurseries. J. Econ. Entomol. 104: 1960–1968.
- Frank, S. D., W. E. Klingeman, S. A. White, and A. Fulcher. 2013. Biology, injury, and management of maple tree pests in nurseries and urban landscapes. J. Integr. Pest Manag. 4: 1–14.
- Haack, R. A., Rabaglia, R. J. and Peña, J. E., 2013. *Exotic bark and ambrosia beetles in the USA: potential and current invaders*. Potential invasive pests of agricultural crops. CAB International, Wallingford, pp 48–74.
- Harrington, T. C., D. McNew, C. Mayers, S. W. Fraedrich, and S. E. Reed. 2014. *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. Mycologia 106: 835–845.
- Hulcr, J., and S. Smith. 2010. *Xyleborini ambrosia* beetles: an identification tool to the world genera. <http://itp.lucidcentral.org/id/wbb/xyleborini/index.htm>
- Joly, C. A., and R. M. M. Crawford. 1982. Variation in tolerance and metabolic responses to flooding in some tropical trees. J. Exp. Bot. 33: 799–809.
- Kelsey, R. G. 2001. Chemical indicators of stress in trees: their ecological significance and implication for forestry in eastern Oregon and Washington. Northwest Sci. 75: 70–76.
- Kennedy, R. A., M. E. Rumpho, and T. C. Fox. 1992. Anaerobic metabolism in plants. Plant Physiol. 100: 1–6.
- Kimmerer, T. W., and T. T. Kozlowski. 1982. Ethylene, ethane, acetaldehyde, and ethanol-production by plants under stress. Plant Physiol. 69: 840–847.
- Kimmerer, T. W., and R. C. Macdonald. 1987. Acetaldehyde and ethanol biosynthesis in leaves of plants. Plant Physiol. 84: 1204–1209.
- Oliver, J. B., and C. M. Mannion. 2001. Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. Environ. Entomol. 30: 909–918.
- Ranger, C. M., M. E. Reding, A. B. Persad, and D. A. Herms. 2010. Ability of stress-related volatiles to attract and induce attacks by *Xylosandrus germanus* and other ambrosia beetles. Agric. For. Entomol. 12: 177–185.
- Ranger, C. M., M. E. Reding, J. B. Oliver, P. B. Schultz, J. J. Moyseenko, and N. Youssef. 2011. Comparative efficacy of plant-derived essential oils for managing ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) and their corresponding mass spectral characterization. J. Econ. Entomol. 104: 1665–1674.
- Ranger, C. M., M. E. Reding, P. B. Schultz, and J. B. Oliver. 2012. Ambrosia beetle (Coleoptera: Curculionidae) responses to volatile emissions associated with ethanol-injected *Magnolia virginiana*. Environ. Entomol. 41: 636–647.
- Ranger, C. M., M. E. Reding, P. B. Schultz, and J. B. Oliver. 2013a. Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate. Agric. For. Entomol. 15: 56–64.
- Ranger, C. M., P. C. Tobin, M. E. Reding, A. M. Bray, J. B. Oliver, P. B. Schultz, S. D. Frank, and A. B. Persad. 2013b. Interruption of the semiochemical-based attraction of ambrosia beetles to ethanol-baited traps and ethanol-injected trap trees by verbenone. Environ. Entomol. 42: 539–547.
- Ranger, C. M., P. C. Tobin, and M. E. Reding. 2015a. Ubiquitous volatile compound facilitates efficient host location by a non-native ambrosia beetle. Biol. Invasion. 17: 675–686.
- Ranger, C. M., P. B. Schultz, S. D. Frank, J. H. Chong, and M. E. Reding. 2015b. Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. PLoS One 10: e0131496.
- Ranger, C. M., P. B. Schultz, S. D. Frank, J. H. Chong, and M. E. Reding. 2015c. Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. PLoS One 10: e0131496.
- Ranger, C., P. Schultz, M. Reding, S. Frank, and D. Palmquist. 2016a. Flood stress as a technique to assess preventive insecticide and fungicide treatments for protecting trees against ambrosia beetles. Insects 7: 40.
- Ranger, C. M., M. E. Reding, P. B. Schultz, J. B. Oliver, S. D. Frank, K. M. Adesso, J. Hong Chong, B. Sampson, C. Werle, S. Gill, et al. 2016b. Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. J. Integr. Pest Manag. 7. doi:10.1093/jipm/pmw005

- Reding, M. E., P. B. Schultz, C. M. Ranger, and J. B. Oliver. 2011. Optimizing ethanol-baited traps for monitoring damaging ambrosia beetles (coleoptera: curculionidae, scolytinae) in ornamental nurseries. *J. Econ. Entomol.* 104: 2017–2024.
- Reding, M. E., C. M. Ranger, J. B. Oliver, and P. B. Schultz. 2013a. Monitoring attack and flight activity of xylosandrus spp. (coleoptera: curculionidae: scolytinae): the influence of temperature on activity. *J. Econ. Entomol.* 106: 1780–1787.
- Reding, M. E., J. B. Oliver, P. B. Schultz, C. M. Ranger, and N. N. Youssef. 2013b. Ethanol injection of ornamental trees facilitates testing insecticide efficacy against ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). *J. Econ. Entomol.* 106: 289–298.
- Reed, S. M. 2005. Japanese snowbell exhibits variability for time of vegetative bud-break and susceptibility to spring freeze damage. *HortScience* 40: 542–545.
- Schneider, I., and M. H. Farrier. 1969. New hosts, distribution, and biological notes on an imported ambrosia beetle, *Xylosandrus germanus* (Coleoptera: Scolytidae). *Can. Entomol.* 101: 412–415.
- Tadage, M., I. Dupuis, and C. Kuhlemeier. 1999. Ethanolic fermentation: new functions for an old pathway. *Trends Plant Sci.* 4: 320–325.
- Weber, B., and J. McPherson. 1983a. World list of host plants of *Xylosandrus germanus* (Blandford) (Coleoptera: Scolytidae). *Coleopt. Bull.* 37: 114–134.
- Weber, B., and J. McPherson. 1983b. Life history of the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). *Ann. Entomol. Soc. Am.* 76: 455–462.
- Weber, B. C., and J. E. McPherson. 1985. Relation between attack by *xylosandrus germanus* (Coleoptera, Scolytidae) and disease symptoms in black walnut. *Can. Entomol.* 117: 1275–1277.