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Research

Pest Management

Developing a Media Moisture Threshold for Nurseries to Reduce Tree Stress and Ambrosia Beetle Attacks

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Abstract

Exotic ambrosia beetles are among the most damaging pests of trees grown in nurseries. The primary pests *Xylosandrus crassiusculus* Motschulsky and *Xylosandrus germanus* Blandford use ethanol to locate vulnerable trees. Research, primarily with *X. germanus*, has shown that flood-stressed trees emit ethanol and are preferentially attacked by ambrosia beetles. Our goal was to develop a media (also called potting soil) moisture threshold as an integrated pest management (IPM) tactic and assess grower practices that lead to ambrosia beetle attacks. Flooded *Cornus florida* L., *Cornus kousa* Burg., and *Magnolia grandiflora* L. trees incurred more attacks than unflooded trees that were not attacked. To determine optimal media moisture levels, we grew flood-tolerant *Acer rubrum* L. and flood-intolerant *C. florida* in containers with 10, 30, 50, 70, or 90% media moisture. No flooded or unflooded *A. rubrum* were attacked. However, *C. florida* grown in 70 or 90% moisture were attacked and died, whereas trees at 30 and 50% moisture were not attacked. Thus, we suggest an upper moisture threshold of 50% when growing *C. florida* and other flood-intolerant trees. However, during peak ambrosia beetle flight activity in spring 2013 and 2014, we found that media moisture levels in commercial nurseries were often between 50 and 90%. Implementing a media moisture threshold, as a new IPM tool, could reduce ambrosia beetle attacks and the need for insecticide applications, which is currently the only available management tactic. Future research should focus on how changes in substrates, irrigation, and other practices could help growers meet this threshold.

Key words: *Acer rubrum, Cornus florida, Magnolia grandiflora*, ethanol, flood-stress

Exotic ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are among the most damaging pests of trees grown in nurseries (Fulcher et al. 2012, Frank et al. 2013). Several species of ambrosia beetles attack nursery trees but the most common and economically important are *Xylosandrus crassiusculus* Motschulsky and *Xylosandrus germanus* Blandford (Frank et al. 2013, Ranger et al. 2016). *Xylosandrus crassiusculus* and *X. germanus* attack over 120 and 200 tree species, respectively, including some of the most popular and valuable trees grown in nurseries, such as cherry (*Prunus* spp.), crape myrtle (*Lagerstroemia* spp.), dogwood (*Cornus* spp.), elm (*Ulmus* spp.), Japanese snowbell (*Styrax* spp.),*Magnolia* spp., maple (*Acer* spp.), and redbud (*Cercis* spp.) (Schedl 1963, Schneider and Farrier 1969, Weber and McPherson 1983a, Fulcher et al. 2012, Ranger et al. 2015a).

Female beetles overwinter within galleries in host trees and become active in early spring (Oliver and Mannion 2001, Reding et al. 2013a). They subsequently bore into host tree trunks and excavate galleries in the heartwood and sapwood, where eggs are deposited and larvae develop (Weber and McPherson 1983b, Oliver and Mannion 2001). In addition to boring damage, *X. crassiusculus* and *X. germanus* inoculate trees with a symbiotic fungus on which the larvae and adults feed (Harrington et al. 2014, Mayers et al. 2015). Trees can also become infected with secondary pathogens (Kessler 1974, Anderson and Hoffard 1978, Weber and McPherson 1985, Dute et al. 2002). Infested plants die or become unmarketable from boring damage, branch dieback, or infection by pathogens (Frank et al. 2013, Ranger et al. 2016).

Unfortunately, growers do not have many integrated pest management (IPM) tools for ambrosia beetles (Frank et al. 2013). They can monitor ambrosia beetle flight activity with ethanol-baited traps and apply preventive contact insecticides to all susceptible trees once beetles are active (Frank et al. 2013, Ranger et al. 2016). However, this management tactic is expensive, can cause spider mite outbreaks by killing natural enemies, and still does not prevent all ambrosia beetle attacks (Frank and Sadof 2011, Ranger et al. 2011, Reding et al. 2013b). Maintaining tree health is the primary foundation of an ambrosia beetle management plan. As in other systems, IPM becomes more difficult when plants are stressed and more attractive or susceptible to pests. *Xylosandrus* spp. are particularly challenging because they are early colonizers of living but weakened trees that

are often asymptomatic and apparently healthy (Ranger et al. 2015a,b; 2016). Thus, growers are limited in their ability to monitor tree susceptibility.

Ethanol is the primary volatile cue used by many ambrosia beetles to locate vulnerable trees (Klimetzek et al. 1986; Ranger et al. 2010, 2012). Ethanol is produced by anaerobic respiration in tree roots and emitted by many tree species when their roots are submerged in water (Drew 1997, Tadage et al. 1999). Flooding and ethanol have been thought to induce ambrosia beetle attacks for decades. In 1941, Hoffman (1941) found heavy *X. germanus* infestations in elm trees that were partially submerged in a river. The same year, Buchanan (1941) reported that trees injected with ethanol were attacked by *X. germanus* more than uninjected trees. Recent studies have confirmed that injecting ethanol into living trees induces attacks by *X. crassiusculus*, *X. germanus*, and other ambrosia beetles (Ranger et al. 2010, 2012, 2015a; Frank and Sadof 2011; Reding et al. 2013b). Experimentally flood stressed trees also emit ethanol from their bark and are preferentially attacked by *X. crassiusculus*, *X. germanus*, and other ambrosia beetles (Ranger et al. 2013, 2015b; Reed et al. 2015).

Ambrosia beetles primarily attack nursery trees during their first spring generation, even though they are multivoltine and active throughout summer (Oliver and Mannion 2001). Potting media (a soilless mix of bark and sand sometimes called potting soil) is typically wetter in spring than summer, as cool temperatures reduce evaporation and trees do not have leaves and are thus not transpiring (Zhu et al. 2005). Based on these observations, our hypothesis was that potting media in nurseries is wet enough in spring to induce ethanol production in intolerant trees and subsequently lead to ambrosia beetle attacks. However, the media moisture level at which trees produce ethanol and attract ambrosia beetle attacks has not been characterized. Therefore, we cannot recommend an optimal media moisture level as a cultural management tactic. The overall goal of our current study was to understand how media moisture affects ambrosia beetle attacks and identify a threshold level that can be incorporated into cultural practices and used in conjunction with monitoring and insecticides in an IPM program. Our specific objectives were to determine 1) how flooding affects ethanol production and ambrosia beetle attacks on common nursery tree species in North Carolina, 2) optimal media moisture levels that reduce ethanol production and ambrosia beetle attacks, and 3) if trees in commercial nurseries are grown in media moisture levels that increase ambrosia beetle attacks.

Materials and Methods

Effects of Flooding on Ambrosia Beetle Attacks and Ethanol Production

Experiments were conducted in 2011 and 2013 to assess the role of flooding on predisposing trees to attack by ambrosia beetles. We conducted these experiments at the North Carolina State University Lake Wheeler Research Facility, where we have an experimental nursery (35° 44'14.5″ N 78° 40'25.8″ W). Three sides of the nursery are adjacent to woodlots (<30 m). The surrounding landscape consists of agricultural fields. The nursery has a 3-m deer fence and automated drip irrigation. The ground is covered with weed cloth.

In 2011, we conducted two experiments, each using a completely randomized design, to test the hypothesis that flooded trees would be attacked more than unflooded trees. For the first experiment, we purchased 36 *Magnolia grandiflora* L. from a local nursery. The trees were approximately 1.5-m-tall, 1.5–2.5 cm caliper, and planted in 11.4-liter pots with bark and sand potting media. Each tree was randomly assigned to one of three treatments: normal watering, flooded, or normal watering + ethanol injected. We arranged the trees 1 m apart in two rows. Positions within the rows were selected randomly with regard to treatment. Normal watering entailed watering trees as needed to keep the potting media moist. We did not measure media moisture levels as part of this experiment. We used a pot-in-pot system to create the flooded treatment (Ranger et al. 2013), whereby we placed each pot containing a tree within an empty 11.4-liter pot lined with a plastic trash bag to prevent drainage. Flooded trees were watered each day to maintain media saturation as indicated by water on top of the media. Trees in the ethanol injected treatment were watered normally and injected with approximately 100 ml of 90% ethanol using an Arborjet Tree I.V. Delivery System (Woburn, MA) based on Ranger et al. (2010).

We began flood treatments and injected trees on 15 March 2011 before we captured *X. crassiusculus* in traps at the research site. We counted the number of new attacks each week for 8 wk. Each week new attacks were circled with a black indelible marker, so they would not be recounted in subsequent weeks. As the resulting data were non-normal and the variance was not homogeneous, cumulative attacks per *M. grandiflora* tree were compared among treatments with Kruskal–Wallis tests ($\alpha = 0.05$).

We also conducted a concurrent experiment in 2011 using 12 *Cornus florida* L. trees purchased from a local nursery. The trees were 1-m-tall, 1.5–2.5 cm caliper, and grown in 18.9-liter pots with pine bark and sand media. Each tree was randomly assigned to normal or flooded treatments. Flooding was initiated on 15 March 2011 and attacks were monitored weekly as previously described. As the resulting data were non-normal and the variance was not homogeneous, cumulative attacks per *C. florida* tree were compared among treatments with Kruskal–Wallis tests ($\alpha = 0.05$).

In 2013, we conducted a complementary experiment, using a completely randomized design, to determine if flooding an intolerant tree species (*Cornus kousa* Burg.) would be associated with higher ethanol levels and ambrosia beetle attacks than a flood-tolerant tree species (*Acer rubrum* L.). *Acer rubrum* and *C. kousa* have been characterized as flood-tolerant and flood-intolerant, respectively (Carpenter and Mitchell 1980, Day et al. 2000). We purchased 16 Korean dogwoods, *C. kousa*, that were 1-1.5-m-tall and 1.5 cm caliper in 18.9-liter pots with pine bark and sand potting media from a local nursery. We also purchased 12 bare root red maples, *A. rubrum*, (4 each of var. 'Red Sunset', 'Marmo', and 'October Glory') that were 2-m-tall and 2.5 cm caliper. On 25 February 2013, we planted the maples in 37.9-liter pots with pine bark and sand media. On 26 February 2013, we randomly assigned six *A. rubrum* (two of each variety) and eight *C. kousa* to the flood treatment and the same number of trees to the normal water treatment. Flooding was then initiated as previously described. We counted new attacks every 3–7 d from 5 March to 18 May 2013. As the resulting data were non-normal and the variance was not homogeneous, cumulative attacks per *M. grandiflora* tree were compared among treatments with Kruskal–Wallis tests ($\alpha = 0.05$).

Ethanol concentrations associated with the flooded and unflooded *A. rubrum* and *C. kousa* trees were analyzed by solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) as described by Ranger et al. (2013, 2015a). On 10 April 2013, superficial stem tissue core samples (1 mm depth, 5 mm diameter) were taken from half of the trees within each treatment using an Osborne arch punch (C.S. Osborne & Co., Harrison, NJ). The remaining trees were sampled on 14 May 2013. Four tissue core samples were collected per tree. Tissue samples were placed in

Eppendorf tubes and immersed in dry ice immediately after sampling and then stored at −80 °C. Tissue samples were packed in a cooler with dry ice and shipped overnight to the USDA-ARS in Wooster, OH, where they were stored at −80 °C until analysis by SPME-GC-MS.

An SPME fiber coated with carboxen-polydimethylsiloxane (CAR/PDMS; 75 μm coating; Sigma-Aldrich, St. Louis, MO) was used to adsorb headspace ethanol associated with the tissue cores as described by Ranger et al. (2013, 2015a). Volatiles were thermally desorbed within the injection port of a gas chromatograph (Varian CP-3800; Varian Inc., Palo Alto, CA) equipped with a Merlin Microseal septum system (Sigma-Aldrich, St. Louis, MO). Fibers were held in the injection port for 2 min at 250 °C, which was operated in splitless mode from 0–2 min and then split at a ratio of 1:20 for the remainder of the analysis. A capillary nonpolar DB-5 column (0.25 μm × 30 m × 0.25 μm; cross-linked/surface bonded 5% phenyl, 95% methylpolysiloxane; Agilent J&W, Santa Clara, CA) was used according to the following program: 40–60 °C at 3 °C/min and 60–230 °C at 20 °C/min. A Varian 2200 mass spectral detector was operated in electron impact mode with a scan range of 14–415 *m/z*. Ethanol concentrations were determined using the external standard method and serial dilutions according to Ranger et al. (2013, 2015a).

Concentrations of ethanol associated with flooded and unflooded treatments were compared using a Kruskal–Wallis test ($\alpha = 0.05$). We also used a chi-square test ($\alpha = 0.05$) to analyze the frequency that trees with detectible ethanol concentrations were attacked relative to trees not associated with detectible concentrations of ethanol. As tissue collection date (10 April 2013 vs. 14 May 2013) did not have an effect on ethanol concentrations ($\chi^2 = 0.112$, df = 1, $P = 0.738$), we combined sample dates within a species for analysis, resulting in the following number of replicates per treatment and tree species: flooded *C. kousa* n = 7, unflooded *C. kousa* *n* = 8, flooded *A. rubrum* n = 5, and unflooded *A. rubrum* *n* = 6).

Determining Optimal Media Moisture Levels

We conducted experiments in 2013 and 2014 to determine optimal media moisture levels that minimize the production of ethanol and likelihood of ambrosia beetle attacks. These experiments were also conducted in our nursery at the Lake Wheeler Research Facility. In spring 2013, we purchased 30 *A. rubrum* (10 each of var. 'Red Sunset', 'Marmo', and 'October Glory') whips that were 2-m-tall and 2–3 cm caliper. We planted the trees in 37.9-liter pots with pine bark and sand media. To determine the amount of moisture that induced ethanol production and ambrosia beetle attacks, we assigned six *A. rubrum* trees, two of each cultivar, to treatments of 10, 30, 50, 70, or 90% media moisture. Trees were watered three times per day with automated drip irrigation to maintain media moisture treatments ranging from 10 to 90%. Each treatment required a different amount of water and thus a different irrigation line that could be adjusted to maintain the media moisture of that treatment. At the beginning of the experiment, each of five irrigation lines was randomly assigned to one of the five treatments. After 3 wk, the five irrigation lines were randomly assigned to new treatments and plants were moved. Irrigation lines were 1.5 m apart and plants were spaced 1.5 m apart in rows along each line. The media surface was covered with loose-fitting plastic shields made from inverted clear plastic water trays to reduce rain entering the pots.

We measured media moisture every 2–5 d and counted attacks each week from 26 March to 3 May 2013 using an ML3 ThetaProbe Soil Moisture Sensor (DeltaT Devices, London, UK). The Theta probe reports percent media moisture to a single decimal place. The Theta probe will not provide a reading when media moisture is over 85–90%; thus, we recorded these measurements as 100% moisture, as we generally observed standing water in these pots. We inserted the probe perpendicular to the surface of the media to measure the media moisture at the top of the pot. We also inserted the probe perpendicularly through the side of each pot at 3–4 cm from the bottom to measure percent moisture at the bottom of each pot. We measured the top and bottom percent moisture three times in each pot and calculated the mean top and mean bottom measurements to use in analyses. Data were analyzed with repeated-measures analysis of variance (ANOVA) with unstructured covariance structure using the MIXED procedure ($\alpha = 0.05$; SAS Institute 2014). Bottom percent moisture readings were $\log(x + 1)$ transformed before analysis to improve normality of residuals.

Stem tissue samples were collected on 10 April 2013 and 14 May 2013 and analyzed for ethanol as previously described. Ethanol concentrations among *A. rubrum* trees subjected to different media moisture concentrations were compared using a Kruskal–Wallis test ($\alpha = 0.05$). However, as ethanol concentrations on the first and second sampling dates were not significantly different from each other ($\chi^2 = 0.23$; df = 1; $P = 0.634$), within-treatment data for the two dates were combined for subsequent analyses.

We repeated this experiment in 2014 using *C. florida* trees purchased from a local nursery. Trees were 1–1.5-m-tall, 1.5-2 cm caliper, and planted in 11.4-liter pots with pine bark and sand media. We repotted the trees in 18.9-liter pots with pine bark and sand media. We assigned six *C. florida* trees to 10, 30, 50, 70, or 90% media moisture treatments, which were implemented on 20 March 2014. We measured media moisture every 2–5 d and counted attacks each week from 1 April 2014 to 12 May 2014. We stopped counting attacks and measuring media moisture levels on 12 May 2014 because trees in the 90% treatment started dying and few ambrosia beetles were captured in traps. However, we continued to monitor the trees each week until 4 June 2014 to record whether they were alive or dead. Trees were inspected for attacks a final time on 4 June 2014. We collected stem tissue samples for ethanol analysis on 20 May 2014 and analyzed as previously described.

Assessing Media Moisture Levels in Commercial Nurseries

In 2013, we recorded media moisture from *A. rubrum*, *C. florida*, and *Cercis canadensis* L. trees at six container nurseries in Johnston Co. and Wake Co. near Raleigh, NC. They are identified here as Nursery A, B, C, D, E, and F. We sampled from three to six different sites at each nursery. Each site was a single nursery pad covered with weed cloth or gravel and separated from other pads by dirt or gravel roads or cart paths. Sites within nurseries were considered independent experimental units, as they were separated by roads and could have different irrigation controls, elevation, slope, or container size. We consistently sampled one tree species at each site even if multiple species were present. At each site in 2013, we measured percent media moisture three times in the top and bottom of 10 randomly selected containers of *A. rubrum*, *C. florida* site, and *Ce. canadensis*. We used the mean of the three subsamples for analyses. We visited each nursery three times to sample moisture levels. However, as we could not visit every nursery in one day, sampling data were grouped into early-spring (25 February-8 March 2013), mid-spring (11–22 April 2013), and late-spring (10–13 May 2013) periods.

The early-spring group included: four sites at Nursery F (i.e., two *A. rubrum* sites, one *C. florida* site, and one *Ce. canadensis* site)

and five sites at Nursery E (i.e., one *A. rubrum* site, one *Ce. canadensis* site, and three *C. florida* sites) that were sampled on 25 February 2013; one site of each tree species at Nursery B and Nursery D on 5 March 2013; one site of each tree species at Nursery A on 8 March 2013; and two sites of each tree species at Nursery C on 8 March 2013. The total number of site replicates sampled during the early-spring group was n = 8 for *A. rubrum*, $n = 9$ for *C. florida*, and $n = 7$ for *Ce. canadensis*.

The mid-spring group contained four sites at Nursery F (i.e., two *A. rubrum*, one *C. florida*, and one *Ce. canadensis*), five sites at Nursery A (i.e., two *A. rubrum*, one *C. florida*, two *Ce. canadensis*), one site of each species at Nursery B and Nursery D, and two sites of each species at Nursery C sampled on 11 April 2013, and four sites at Nursery E (one *A. rubrum*, two *C. florida*, one *Ce. canadensis*) on 22 April. The total number of site replicates sampled during the mid-spring group was $n = 9$ for *A. rubrum*, $n = 8$ for *C. florida*, and $n = 8$ for *Ce. canadensis*.

The late-spring group consisted of the aforementioned sites that were sampled on 11 April 2013 were sampled again on 10 May 2013, and the aforementioned sites that were sampled on 22 April 2013 were sampled again on 13 May 2013. The total number of site replicates sampled during the late-spring group was $n = 9$ for *A. rubrum*, $n = 8$ for *C. florida*, and $n = 8$ for *Ce. canadensis*.

In 2014, we measured media moisture levels at the same six cooperating container nurseries as in 2013. As tree species did not affect media moisture in 2013, we only measured media moisture in *C. florida* pots in 2014. At each site in 2014, we measured percent media moisture three times in the top and bottom of 10 randomly selected *C. florida* containers. We used the mean of the three subsamples for analyses. We visited each nursery 12 times approximately weekly from 5 March 2014 to 3 June 2014 and once again grouped the sample dates according to early-spring, mid-spring, and late-spring periods (i.e., early-spring: 5, 11, 21, 27 March 2014; mid-spring: 3, 11, 18, 23 April 2014; late-spring 7, 14, 21 May 2014 and 3 June 2014). However, we did not sample Nursery B after 3 April 2014 because they sold all of their *C. florida* trees. In particular, we sampled from two sites at Nursery A, Nursery C, and Nursery E. Beginning 18 April 2014, we added a second site at Nursery F, as we stopped sampling at Nursery B. Thus, on every date, we sampled nine sites, except on 11 April 2014, when we sampled six due to rain.

We first analyzed 2013 media moisture data using a two-way ANOVA to determine if tree species, time of year, or their interaction affected media moisture; each of the three sampling periods (i.e., early-spring, mid-spring, and late-spring) and the three tree species (*A. rubrum*, *C. florida*, and *Ce. canadensis*) were separately analyzed. We then pooled all pots and species per nursery and analyzed the mean top, bottom, and average media moisture level per nursery (replicate) using repeated-measures ANOVA; differences among means were separated using Tukey HSD ($\alpha = 0.05$). Similarly, we analyzed 2014 media moisture levels using repeated-measures ANOVA to determine if site (some nurseries had multiple sites), date, or their interaction affected media moisture.

Monitoring Ambrosia Beetle Flight Activity

We deployed ambrosia beetle traps in 2013 and 2014 to assess how peak flight activity coincided with measurements of media moisture levels in cooperating nurseries. In 2013, 15 ambrosia beetle traps made from 2-liter soda bottles (Ranger et al. 2010, Reding et al. 2011) were spaced at least 30 m apart and deployed along the edge of a wooded lot at the NCSU Lake Wheeler Research Facility. We also installed two ethanol-baited bottle traps at all six of the aforementioned cooperating nurseries where media moisture levels were measured. Traps were monitored from 25 February 2013 until 31 April 2013 and again from 24 May 2013 to 31 May 2013. Propylene glycol was used as a killing and preserving agent in the traps. Traps at the NCSU Lake Wheeler Research Farm and the cooperating nurseries were visited every 3-4 d and 2-3 d, respectively, to count beetles and refill the ethanol dispenser consisting of a cotton wick placed in a 15-ml glass vial filled with 15 ml of 90% ethanol. Ambrosia beetles captured in traps were counted in the field and not identified to species.

In 2014, ethanol-baited traps were again deployed at the NCSU Lake Wheeler Research Farm and the six cooperating nurseries. Five traps were deployed at the research farm and two traps at each cooperating nursery. Traps were visited from 20 February 2014 until 21 May 2014 to collect the captured beetles. Scolytinae specimens were counted and identified to species.

Media moisture and trap data from each year were compared to determine if media moisture exceeded 50% and if this coincided with ambrosia beetle flight activity.

Results

Effects of Flooding on Ambrosia Beetle Attacks and Ethanol Production

In 2011, ethanol-injected *M. grandiflora* sustained significantly more cumulative attacks per tree (mean ± SE; 26.3 ± 5.3) than flooded *M. grandiflora* trees (3.1 ± 4.9), which sustained significantly more attacks than unflooded trees (0.0 ± 0.0; $\chi^2 = 19.49$; df = 2; $P < 0.001$). Similarly, flooded *C. florida* trees sustained significantly more cumulative attacks per tree (7.7 ± 5.6) than unflooded *C. florida* trees, which sustained no attacks ($\chi^2 = 5.25$; df = 1; $P = 0.022$).

In 2013, flooded *C. kousa* sustained significantly more cumulative attacks per tree (46.8 ± 10.5) than unflooded *C. kousa*, flooded *A. rubrum*, and unflooded *A. rubrum*, none of which was attacked ($\chi^2 = 26.02$; df = 3; $P < 0.001$). Furthermore, SPME-GC-MS analyses determined the flooded *C. kousa* trees had significantly higher ethanol concentrations per tree (1,071,541 ± 283,763) than flooded *A. rubrum* (184,543 ± 184,543), unflooded *C. kousa* (2,390 ± 2,390), and unflooded *A. rubrum* (which had no trees with detectible ethanol; $\chi^2 = 15.49$; df = 3; $P = 0.001$). The frequency of ambrosia beetle attacks was significantly associated with ethanol production such that 86% of flooded trees (six dogwoods) with detectible ethanol were attacked compared with 14% of flooded trees (one dogwood) without detectible ethanol that were attacked ($\chi^2 = 5.18$; df = 1; $P = 0.003$).

Determining Optimal Media Moisture Levels

In 2013, the top, bottom, and average percent media moisture levels were significantly affected by media moisture treatments ranging from 10% to 90%, sampling date, and their interaction (Table 1, Fig. 1). However, none of the flooded or unflooded *A. rubrum* trees was attacked by ambrosia beetles in 2013. Furthermore, ethanol production was not significantly different among *A. rubrum* trees maintained at 10, 30, 50, 70, or 90% media moisture levels ($\chi^2 = 2.43$; df = 1; $P = 0.657$).

In 2014, the top, bottom, and average media moisture were significantly affected by the interaction between media moisture treatment and date, media moisture treatment, and date (Table 1, Fig. 2). Furthermore, in 2014, *C. florida* trees maintained at 70 and 90% media moisture were attacked significantly more (11.5 ± 8.5 and

Table 1. Results of repeated-measures ANOVA on the effects of water treatment (10, 30, 50, 70, or 90% media moisture) and measurement date on media moisture of experimental trees measured at the top or bottom of nursery containers or the average (mean) of top and bottom in 2013 and 2014

Effect (ndf, ddf)	Top		Bottom		Average	
2013	*F*	*P*	*F*	*P*	*F*	*P*
Treatment (4, 325)	19.4	<0.001	15.2	<0.001	2888.6	<0.001
Date (12, 325)	5961.7	<0.001	847.5	<0.001	16.6	<0.001
Treatment × Date (48, 325)	8.5	<0.001	8.0	<0.001	9.0	<0.001
2014						
Treatment (4, 238)	411.4	<0.001	1400.3	<0.001	1207.1	<0.001
Date (9, 238)	2.8	<0.001	7.6	<0.001	8.7	<0.001
Treatment × Date (36, 238)	11.5	<0.001	5.6	<0.001	11.0	<0.001

29.3 ± 9.1, respectively) than trees at 30% and 50%, which were not attacked, and trees maintained at 10% (0.17 ± 0.17), wherein one tree was attacked one time ($\chi^2 = 19.8$; df = 4; $P < 0.001$). By 4 June 2014, all trees had died in the 90 and 70% moisture treatments, one tree died in the 10% treatment, and no trees died in the 30 and 50% treatments. No trees received additional attacks between 14 May and the final count 4 June. In 2014, no ethanol was detected by SPME-GC-MS in tissue samples from flooded or unflooded *C. florida* trees.

Assessing Media Moisture Levels in Commercial Nurseries and Corresponding Ambrosia Beetle Flight Activity

In 2013, tree species did not have a significant effect on top, bottom, or average media moisture levels during early, mid-, or late season (Table 2). When we analyzed the data by site and season (all species combined) with repeated-measures ANOVA, we found a significant interaction between site and season on media moisture measured at the top of pots, but not when measured at the bottom or when analyzing the average (Table 3). Furthermore, there were significant effects of site and season on top, bottom, and average media moisture (Table 3; Fig. 3).

In 2013, a total of 832 ambrosia beetles were collected. The number of ambrosia beetles in traps peaked in mid-April in 2013, which is also when media moisture tended to peak in nurseries (Fig. 4). Notably, when ambrosia beetle abundance peaked in mid-April, mean media moisture measured at the bottom of containers was over 50% at four out of six nurseries in 2013.

In 2014, there was a significant interaction between site and week on top, bottom, and average media moisture (Table 3). There were also significant effects of site and week on top, bottom, and average media moisture (Table 3, Fig. 5). There was no distinct peak in media moisture in 2014.

In 2014, a total of 2,829 pest ambrosia beetles were captured and used in analyses, which included *Cnestus mutilatus* (<1%), *Xyleborinus saxeseni* (14%), *Xylosandrus crassiusculus* (78%), and *X. germanus* (2%). When ambrosia beetle abundance peaked in mid-April and early May (Fig. 6), most nurseries had over 50% average media moisture and over 60% bottom media moisture.

Discussion

In the Southeastern United States, ambrosia beetles are among the most economically damaging pests and the pests for which growers apply the most insecticides (Fulcher et al. 2012, Frank et al. 2013, Ranger et al. 2016). Field observations by Frank and Ranger (personal observation) within ornamental nurseries and previous studies (Ranger et al. 2013, 2015b) have indicated that flooding–poor drainage induce ethanol production and predispose flood-intolerant trees to attack by *X. germanus* and other ambrosia beetle species. IPM is less tractable when plants are stressed, but growers do not have enough tools to manage inconspicuous tree stress in nurseries. Our goal was to understand how media moisture affects the preference behavior of *X. crassiusculus* and other ambrosia beetles and to develop guidelines to manage media moisture as part of an IPM program. We found that flooded *C. florida* and *M. grandiflora* trees, but not flood-tolerant *A. rubrum*, produced more ethanol and

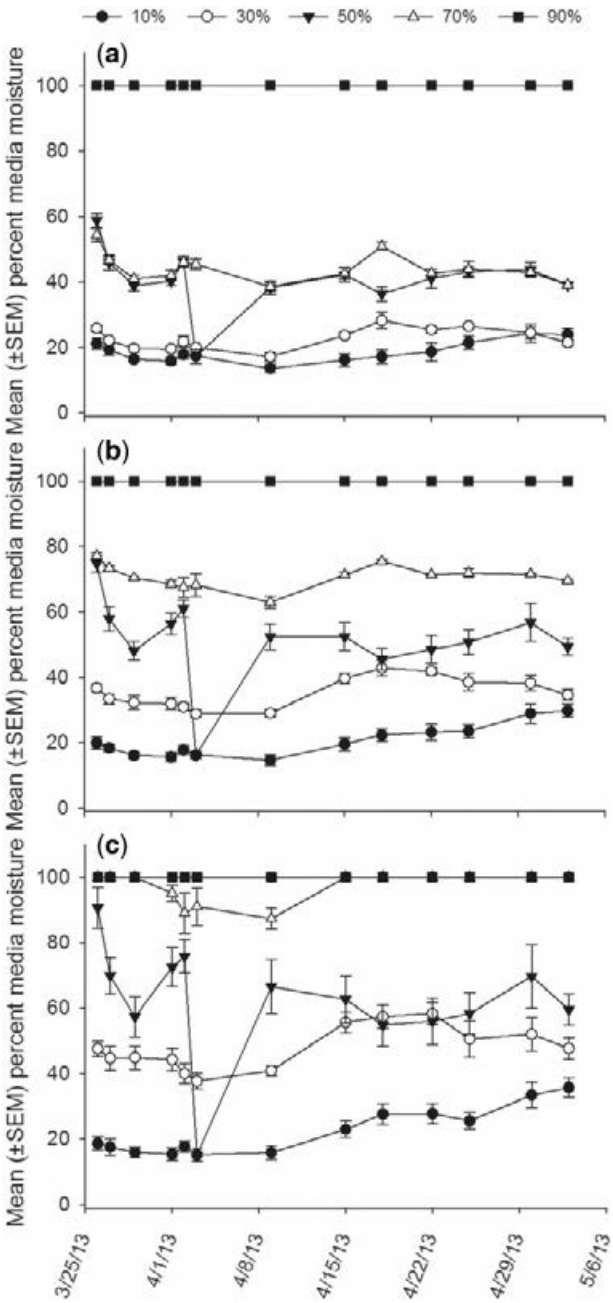

Fig. 1. Mean (±SE) percent media moisture in containers of trees assigned to 10, 30, 50, 70, and 90% media moisture treatments measured in the (a) top, (b) average of top and bottom, and c) bottom of containers in spring 2013.

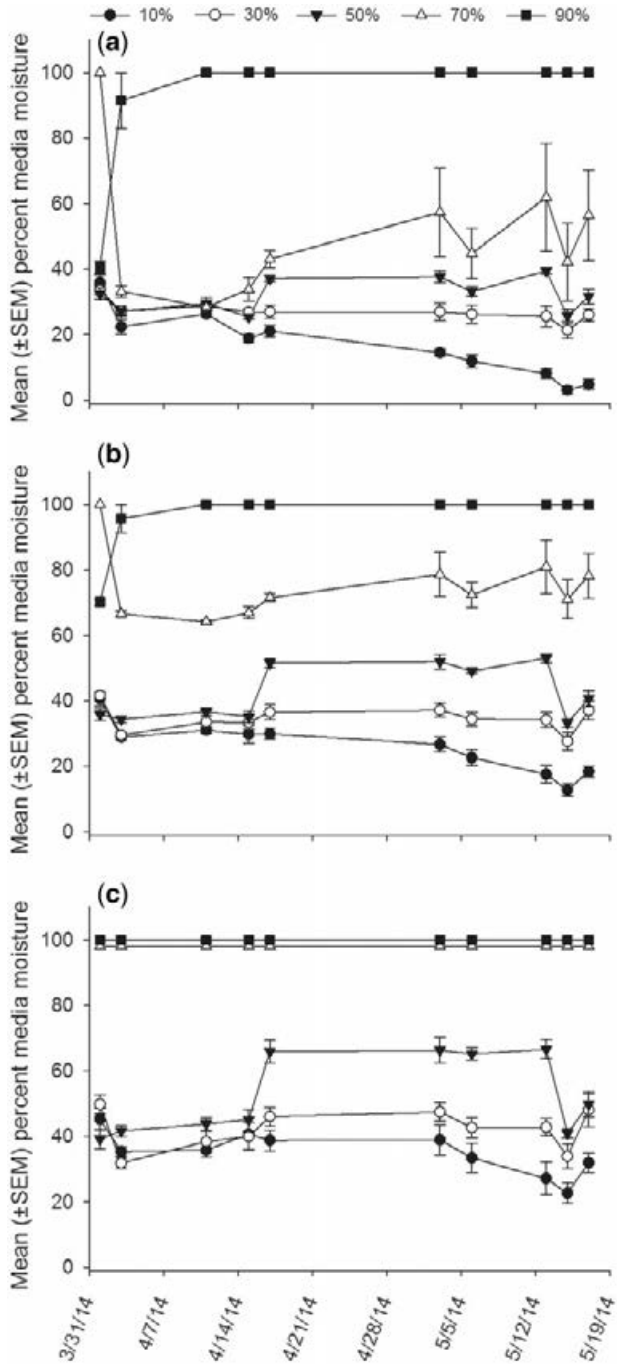

Fig. 2. Mean (±SE) percent media moisture in containers of trees assigned to 10, 30, 50, 70, and 90% media moisture treatments measured in the (a) top, (b) average of top and bottom, and c) bottom of containers in spring 2014.

sustained more attacks than unflooded trees. Our research in the Southeast, where *X. crassiusculus* is the primary ambrosia beetle pest, corroborates flood-stress research conducted in the Midwest with *X. germanus* (Ranger et al. 2013, 2015b). The most striking result may be that throughout our current study, and multiple studies with *X. germanus* (Ranger et al. 2010, 2011, 2012, 2013, 2015a,b), almost no unflooded or uninjected trees were attacked. This phenomenon shows the specificity with which ambrosia beetles target physiologically stressed trees (Ranger et al. 2015a,b) and suggests that reducing flood-stress and poor drainage is an effective IPM tactic.

Table 2. Results of ANOVA on the effect of tree species (*Acer rubrum, Cornus florida, Cercis canadensis*) on media moisture measured early, mid-, or late spring 2013 at the top or bottom of nursery containers or the average (mean) of top and bottom

Season	Top			Bottom			Average		
	F	ndf, ddf	*P*	*F*	ndf, ddf	*P*	*F*	ndf, ddf	*P*
Early	1.1	2, 16	0.362	1.0	2, 17	0.386	1.5	2, 16	0.262
Mid	1.0	2, 17	0.402	1.3	2, 16	0.308	1.21	2, 17	0.326
Late	0.7	2, 17	0.527	1.0	2, 17	0.389	0.5	2, 17	0.592

Table 3. Results of repeated-measures ANOVA on the effects of nursery site and season (early, mid-, or late spring) in 2013 and the effects of nursery site and week in 2014 on media moisture measured at the top or bottom of nursery containers or the average (mean) of top and bottom

Effect (ndf, ddf)	Top		Bottom		Average	
	F	*P*	*F*	*P*	*F*	*P*
2013						
Site (5, 54)	4.1	0.003	3.6	<0.001	22.0	<0.001
Season (2, 54)	2.5	0.092	5.3	0.008	5.4	0.007
Site × Season (10, 54)	2.5	0.015	1.2	0.328	1.2	0.064
2014						
Site (7, 720)	78.0	<0.001	139.5	<0.001	135.6	<0.001
Season (9, 720)	60.4	<0.001	59.2	<0.001	81.0	<0.001
Site × Season (63, 720)	10.2	<0.001	3.8	<0.001	5.3	<0.001

We grew *A. rubrum* and *C. florida* trees at five media moisture levels ranging from 10% to 90% to identify a threshold level that predisposes plants to ambrosia beetle attacks. No *A. rubrum* were attacked and media moisture was not related to ethanol production for this species. However, *C. florida* trees grown in 70 and 90% media moisture were heavily attacked. Trees grown in 30% and 50% media moisture were never attacked and only one tree received one attack in the 10% treatment. Thus, we propose a 50% media moisture threshold is appropriate for minimizing the likelihood of ethanol production and ambrosia beetle attacks on flood-intolerant tree species such as *C. florida*. By keeping media moisture below 50%, growers may also be able to reduce or even eliminate insecticide applications. However, in years where rain or other factors keep media above 50%, preventive insecticide applications will still be the primary management tactic. There is no evidence that plants in dry media are predisposed to attack and drought-stress has not been found to predispose trees to attack by *X. germanus* and *X. crassiusculus* (Ranger, personal observation). In addition, all trees in the 90% and 70% treatment died by mid-summer, whereas one tree in the 10% treatment died. Taken together, the risk of under watering *C. florida* in spring is less than that of overwatering.

Flooded *A. rubrum* trees were not attacked in either of our first experiments when potting media was fully saturated or when grown at a gradient of media moisture levels. However, in a previous study, *A. rubrum* that were injected with ethanol were heavily attacked by ambrosia beetles (Frank and Sadof 2011). The absence of attacks on *A. rubrum* and corresponding low concentrations of ethanol detected by SPME-GC-MS appears to result from flood-tolerant tree species generally not producing as much anaerobically-generated ethanol as flood-intolerant species (Joly and Crawford 1982, Kennedy et al. 1992, Ranger et al. 2015b). *Acer rubrum* are

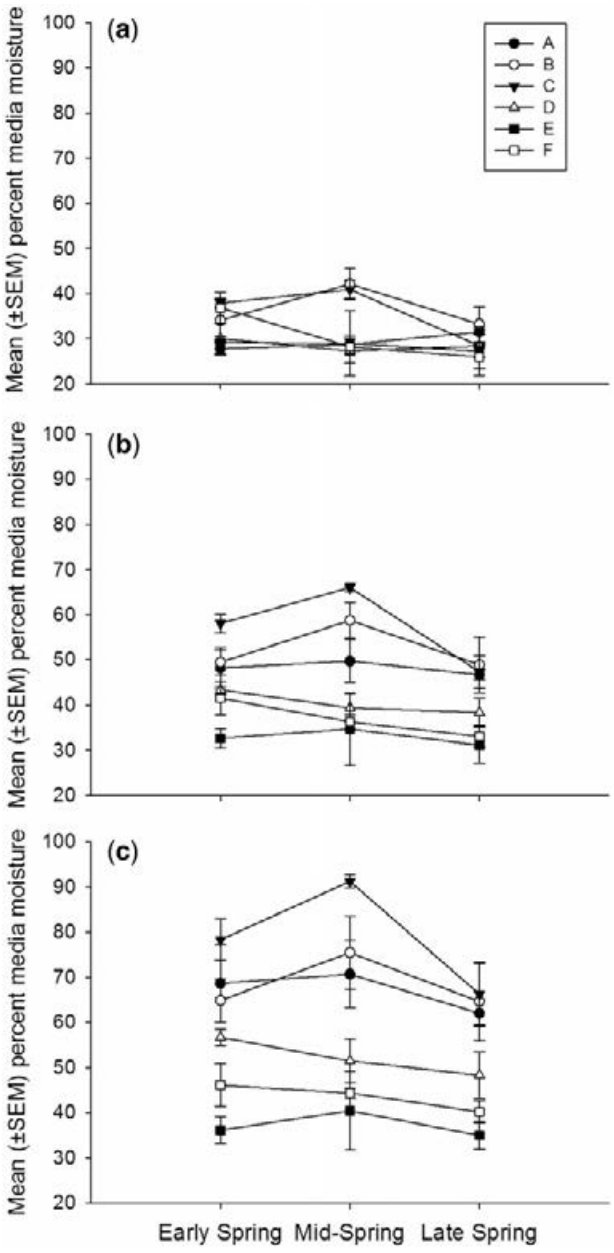

Fig. 3. Mean (±SE) percent media moisture in containers of trees in six commercial nurseries measured in the (a) top, (b) average of top and bottom, and (c) bottom of containers in early-spring (25 February–8 March 2013), mid-spring (11–22 April 2013), and late-spring (10–13 May 2013) 2013.

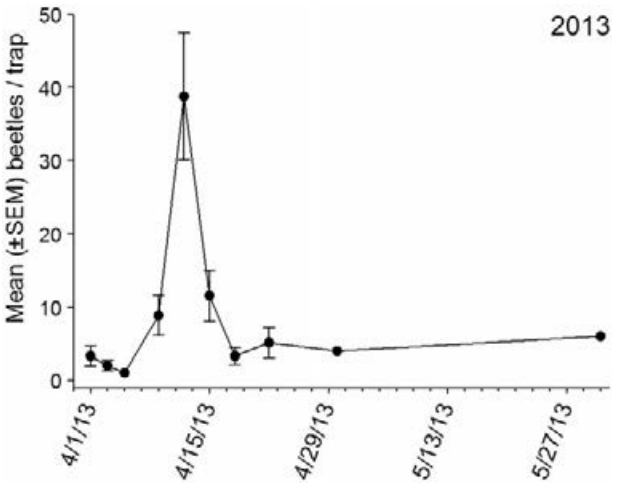

Fig. 4. Mean (±SE) ambrosia beetles captured per trap in spring 2013. Traps were deployed at the NCSU Lake Wheeler Research Farm and at six cooperating nurseries where media moisture levels were also measured. Data presented are the sum of beetles captured 7 d prior to and including each day we collected media moisture data.

evolutionarily adapted to grow in wet sites and thus presumably more tolerant of our flood treatments compared with flood-intolerant *C. florida* and *C. kousa*. The wetland indicator status of *A. rubrum* is "Facultative," meaning they can be found in wetland or non-wetland sites (Nesom 2000). In contrast, *C. florida* is adapted to growing in drier soil, has "Facultative Upland" indicator status, and is considered intolerant of flooding (Broadfoot and Williston 1973; Day et al. 2000; Wennerberg and Skinner 2004; Ranger et al. 2013, 2015b). This scenario is further supported by a recent study showing that flood-tolerant silver maple (*Acer saccharinum* L.) and swamp white oak (*Quercus bicolor* Willd.) were not attacked, unlike flood-ntolerant *C. florida*, *Styrax japonicus*, and *Ce. canadensis* (Ranger et al. 2015b). Future work should examine if flood intolerance predisposes other tree species to stress and ambrosia beetle attacks in saturated and poorly drained potting media. If so, growers could arrange plants in their nursery, so flood-intolerant plants are in the driest places and flood-tolerant species are in the wettest.

Both *X. crassiusculus* and *X. germanus* are known to attack stressed trees, but plant quality has not been investigated as a key factor influencing tree susceptibility until recently. It is clear from our current study and previous ones (Ranger et al. 2013, 2015b; Reed et al. 2015) that physiological stress induced by excess water is an important factor in predisposing trees to attack, and is a stressor documented to occur in container-based ornamental nurseries. In the present study, most trees in the nurseries we sampled were above 50% media moisture during spring months, when ambrosia beetles are most active. In particular, when ambrosia beetle abundance peaked in mid-April, mean media moisture measured at the bottom of containers was over 50% at four and five out of six nurseries in 2013 and 2014, respectively. Media moisture levels above 50% could then lead to the emission of ethanol from the bark and thereby signal tree vulnerability to ambrosia beetles; yet, such trees may appear "apparently healthy" to growers and perhaps even growing well the rest of the season if excessive moisture levels subside.

In spring, when *X. crassiusculus* become active, media moisture can be high in nursery containers because the weather is cool, which reduces evaporation, and trees have no or few leaves, which reduces transpiration. Weather and plant phenology are beyond growers' control, but even under these conditions, we observed growers irrigating during our nursery visits. Other factors that could contribute to overly saturated media include the composition and age of media and the drainage efficiency of pot-in-pot systems, which can become clogged. Additional factors contributing to overwatering in ornamental nurseries, and how to correct them, deserve further research.

Managing media moisture as an IPM tactic will also require development of optimal monitoring procedures. During our current study, moisture measurements from the media surface had much lower percent moisture levels than measurements from the bottom of containers. In the bottom of containers, measurements of the 70 and 90% treatments tended to converge at 100% due to water accumulation. The average of top and bottom measurements best reflected our percent moisture targets when we maintained trees at

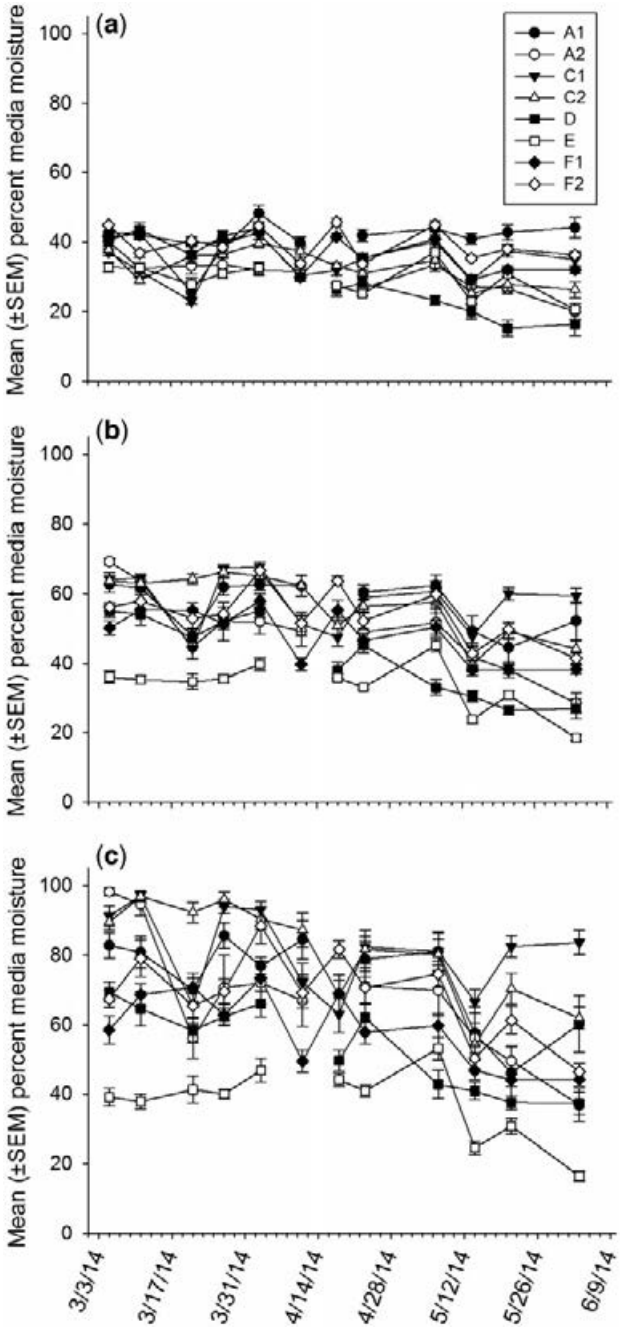

Fig. 5. Mean (±SE) percent media moisture in containers of trees in eight commercial nurseries measured in the (a) top, (b) average of top and bottom, and (c) bottom of containers in spring 2014.

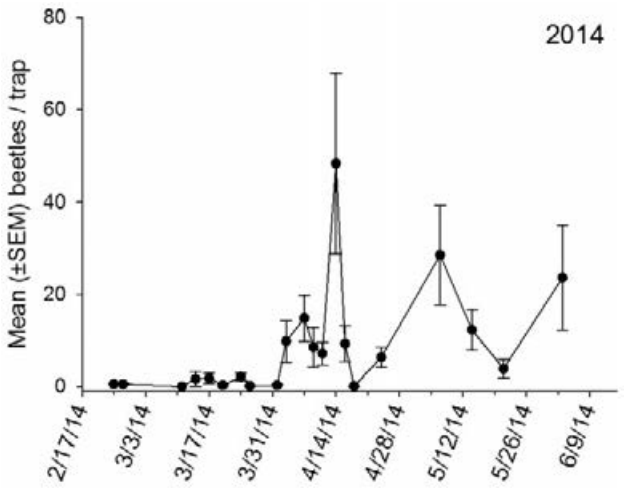

Fig. 6. Mean (±SE) ambrosia beetles captured per trap in spring 2014. Traps were deployed at the NCSU Lake Wheeler Research Farm and at six cooperating nurseries where media moisture levels were also measured. Data presented are the sum of beetles captured 7 d prior to and including each day we collected media moisture data.

10% moisture intervals. A moisture probe longer than 8 cm would allow growers to measure moisture in the middle or bottom of containers without lifting them from pot-in-pot sockets. A moisture monitoring program would require growers to purchase a probe. It would also require research to prescribe the frequency and number of containers that need to be monitored to make informed irrigation management decisions.

In conclusion, we have shown that flood stress induces *X. crassiusculus* attacks on flood-intolerant *C. florida* and *M. grandiflora*, while attacks were not observed on flood-tolerant *A. rubrum*. Based on the frequency that other tree species, such as *Ce. canadensis*, are attacked in nurseries, this interaction appears common, particularly among flood-intolerant species. We suggest a media moisture threshold of 50% when growing *C. florida* and other flood-intolerant trees. As media moisture levels in the majority of container nurseries we sampled were above 50%, future research should focus on how changes in substrates, irrigation, and other practices could help growers meet this threshold. Maintaining tree health is the primary foundation of an ambrosia beetle management plan in ornamental nurseries; thus, our new IPM tactic incorporating a moisture threshold could aid growers in minimizing the risk of ambrosia beetle attacks and the need for insecticide applications.

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