Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate

Christopher M. Ranger*, Michael E. Reding*, Peter B. Schultz† and Jason B. Oliver‡

*USDA-Agricultural Research Service, Application Technology Research Unit, Horticultural Insects Research Laboratory, and Department of Entomology, The Ohio State University, Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, OH 44691, U.S.A., †Hampton Roads Agricultural Research and Extension Center, Virginia Tech., Virginia Beach, VA 23455, U.S.A., and ‡Otis L. Floyd Nursery Research Center, College of Agriculture, Human and Natural Sciences, Tennessee State University, McMinnville, TN 37110, U.S.A.

Abstract

1 Xylosandrus germanus (Blandford) is a key pest of ornamental nursery trees. Ethanol is the most attractive semiochemical known for X. germanus, and its emission from trees represents a primary host-selection cue. Ethanol production is induced by a variety of abiotic and biotic stressors, which could thereby predispose trees to attack by ethanol-responsive ambrosia beetles.

2 To better understand X. germanus host-selection behaviour within ornamental nurseries, a series of experiments examined the influence of flood-stress on the attractiveness and susceptibility of flowering dogwood Cornus florida L. Under field conditions, more X. germanus were attracted to experimentally flood-stressed dogwoods than neighbouring nonflooded controls in 2009, 2010 and 2011. Flood-stressed dogwoods were also preferentially attacked in 2009–2011, although no attacks occurred on any of the neighbouring nonflooded trees.

3 Solid-phase microextraction-gas chromatography-mass spectrometry detected ethanol in stem tissue from flooded dogwoods but not nonflooded trees. Acetaldehyde, acetic acid and ethanol were also emitted from the outer bark of flooded dogwoods but not nonflooded trees.

4 These results demonstrate that X. germanus preferentially lands on and attacks physiologically-stressed hosts, and further support the role of ethanol in mediating this interaction.

5 Attacks by X. germanus have previously been suspected to occur on trees viewed as ‘apparently-healthy’, although the possibility of such trees being in apparently-stressed at the time of attack cannot be ruled out given the results obtained in the present study. Minimizing the impact of stressors known to induce the production of ethanol should be the primary foundation of a management plan for X. germanus and other ethanol-responsive ambrosia beetles.

Keywords Ambrosia beetles, ethanol, flood-stress, Scolytinae, SPME-GC-MS.

Introduction

Exotic ambrosia beetles, including Xylosandrus germanus (Blandford), can be significant pests of forest stands (Grégoire et al., 2001), tree plantations (Weber & McPherson, 1984) and ornamental nursery stock (Oliver & Mannion, 2001; Reding et al., 2010; Frank & Sadof, 2011). Symptoms associated with colonization by these fungivorous wood-boring beetles can include aesthetic damage to the bark, wilting foliage, branch dieback and profuse basal sprouts. The host range of X. germanus includes more than 200 species worldwide, although deciduous hosts are preferred over coniferous species (Weber & McPherson, 1983). Despite a broad host range, the quality of the host appears to play a critical role in mediating host-selection (Weber & McPherson, 1984; Ranger et al., 2010, 2012). Previous studies have demonstrated that X. germanus exhibits an efficient olfactory mechanism for distinguishing among differing host qualities and locating hosts based on the
emission of stress-related volatiles (Weber & McPherson, 1984; Ranger et al., 2010, 2012).
A variety of abiotic and biotic stressors imposed on trees can lead to elevated emissions of low molecular weight volatile compounds, including acetaldehyde, acetic acid, acetone, ethane, ethanol, ethylene and methanol (Kimmerer & Kozlowski, 1982; Kimmerer & MacDonald, 1987; Holzinger et al., 2000; Rottenberger et al., 2008). In particular, ethanol represents a primary attractant for a variety of insects that target stressed, dying or dead hosts, including ‘secondary’ bark and ambrosia beetles (Kelsey, 2001). Ethanol is the most attractive semiochemical currently known for X. germanus and represents an important host-selection cue (Ranger et al., 2010, 2011a, 2012). Indeed, stem injections of ethanol into living trees induced attacks by X. germanus and other ambrosia beetles under field conditions, although neighbouring water-injected or uninjected trees were not attacked (Ranger et al., 2010, 2012). More attacks were also induced by injecting ethanol compared with other stress-related volatiles, such as acetaldehyde, acetone and methanol (Ranger et al., 2010). A positive correlation has also been demonstrated between the concentration of injected ethanol and cumulative ambrosia beetle attacks (Ranger et al., 2012).

Low amounts of ethanol can be found in the vascular tissue of healthy trees (MacDonald & Kimmerer, 1991), although much higher biotic and abiotic stressors are associated with trees subjected to girdling, freezing, pathogens, root and crown disturbance, and pollutants (Kimmerer & Kozlowski, 1982; Kimmerer & MacDonald, 1987; Sjödin et al., 1989; Joseph & Kelsey, 1997; Kelsey & Joseph, 1998, 2001; Kelsey, 2001). In the case of flooding, roots will switch from aerobic to anaerobic respiration when subjected to little or no oxygen (Tadage et al., 1999). Pyruvate formed during glycolysis will resolutely be converted into ethanol, which is then transported with the transpiration stream to stem and leaf tissues (Tadage et al., 1999). Ethanol is then re-metabolized by stepwise oxidation into acetaldehyde and acetate, although a portion of these stress-related volatiles are also emitted as the result of a ‘leak’ between their metabolic production and consumption (Kimmerer & Kozlowski, 1982; MacDonald & Kimmerer, 1993; Kreuzwieser et al., 1999; Rottenberger et al., 2008).

The frequency and intensity of extreme climatic events, including heavy precipitation, are generally predicted to increase over certain parts of the globe as a result of climate change (Cubasch et al., 1995; Zwiets & Kharin, 1998; Kunkel et al., 1999; Easterling et al., 2000; Kharin & Zwiets, 2005). Notably, the frequency of short duration (1–7 days) extreme precipitation events that are highly correlated with flooding has been increasing in the U.S.A. from the 1930s onward, especially for the Midwest and Great Lakes regions (Kunkel et al., 1999). As a result of an ability to induce ethanol production by trees, flood-stress has the potential to predispose trees in ornamental nurseries to attack by ambrosia beetles. For example, after an unusually wet June and July in north-eastern Ohio in 2006, intense rain and thunderstorms occurring on 27–28 July resulted in severe flooding in Lake County, which contains more than 100 licensed ornamental nurseries (Ebner et al., 2007; NOAA, 2012a). Extensive ambrosia beetle attacks were subsequently detected on field-grown flowering dogwoods (Cornus florida L.) that were subjected to standing water for 14 days at a commercial nursery (C. M. Ranger, personal observation). North-eastern Ohio also experienced above normal precipitation ranging from snow to rain during each month from February to May 2011, which contributed to Ohio experiencing its wettest year on record subsequent to 1895 (NOAA, 2012a). Extensive ambrosia beetle attacks were subsequently detected on field grown dogwoods (C. florida and Cornus florida × kousa) that were subjected to water logging and poor drainage at two commercial nurseries in north-eastern Ohio (C. M. Ranger, personal observation). Flowering dogwood is widely considered to be intolerant of flooding and compacted soils (Day et al., 2000).

A better understanding of the ability of stressors to predispose trees to attack by ambrosia beetles will aid with the development of an improved management strategy for reducing crop losses and insecticidal inputs. To further characterize the interaction between abiotic stress and ambrosia beetle host-selection, a series of experiments were conducted to assess the influence of flood-stress on the attractiveness and susceptibility of flowering dogwoods C. florida to these wood-boring pests. Solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) was also used to assess the association of selected stress-related volatiles with nonflooded and flood-stressed C. florida.

Materials and methods

Establishing flood-stress conditions

Flowering dogwood, C. florida, were aged 2–3 years old (height 0.9 m) and maintained in 19-L pots with a mixture of aged pine bark, peat and coarse sand (60:30:10, v/v). Cornus florida is native to eastern North America and ranges from southern Maine to northern Florida, and to eastern Kansas and south and eastern Texas. The native distribution of C. florida overlaps with that of the exotic species X. germanus (Solomon, 1995).

Flood-stress conditions were established using a pot-in-pot system, whereby a 26-L pot was first lined with a plastic waste bag of 3-mil (0.076 mm) thickness. A 19-L pot containing a single C. florida tree was then placed within the plastic lined pot. Flood-stress was initiated by irrigating the media within the internal 19-L pot until there was standing water around the base of the trees. Trees were checked daily to ensure that standing water was maintained in the selected pots throughout the duration of experiments. Excess plastic liner surrounding the edge of the flooded pot was also draped around the internal circumference of the pot up to the base of the stem to prevent beetles from landing in the standing water. As a control, plastic liner was also draped up to the base of the nonflooded trees.

Experiments 1 and 2: host attractiveness and susceptibility

Flood-stress of C. florida was established to assess its influence on host attractiveness and susceptibility to ambrosia beetles. We hypothesized that X. germanus and other ambrosia beetles...
would preferentially land on and colonize flood-stressed *C. florida* over nonflooded trees.

For Experiment 1, flood-stress conditions were established on 14 July 2009 and maintained until 5 August 2009. To measure host attractiveness, the main stems of a subset of flooded and nonflooded *C. florida* trees were treated with Tanglefoot® (The Tanglefoot Company, Grand Rapids, Michigan) up to 40 cm from the base because *X. germanus* mainly attacks the lower trunks of trees (Reding et al., 2010). Tanglefoot was applied on the same day that the experimental trees were deployed in the field and flood-stress conditions were established. Tanglefoot was used to entrap *X. germanus* landing on the stems of the experimental trees, which thereby determined whether beetles preferentially navigated towards and subsequently landed on flood-stressed trees over nonflooded trees. Only counting attacks on experimental trees would have provided insight into the effects of flood-stress on host susceptibility, and not necessarily host attractiveness. Ambrosia beetles were removed from the Tanglefoot every 3 days throughout the duration of the experiment. Ambrosia beetle attacks on the flood-stressed and nonflooded trees that were not treated with Tanglefoot were also tallied on the final day of the experiment. Six replicates were used for each treatment (i.e. nonflooded with and without Tanglefoot; flooded with and without Tanglefoot).

Experimental trees were arranged in six randomized complete blocks alongside a gravel service road within a woodlot in Wayne Co., Ohio. There was approximately 3 m between trees within each block and 10 m between blocks.

For Experiment 2, flood-stress conditions were established on 28 July 2010 and maintained until 10 August 2010. To confirm earlier observations associated with Experiment 1, a subset of the flooded and nonflooded *C. florida* trees were treated with Tanglefoot, whereas the remainder were left untreated. Ambrosia beetles were collected from the Tanglefoot-treated trees at the end of the experiment, and attacks were also quantified on the flooded and control trees that were not treated with Tanglefoot. Eight replicates were used for each treatment (i.e. nonflooded with and without Tanglefoot; flooded with and without Tanglefoot). A total of eight randomized complete blocks were arranged as described previously.

**Experiment 3: host attractiveness and stem tissue analysis**

Flood-stress of *C. florida* was established to assess its influence on host attractiveness and the localization of ethanol in trunk tissue core samples. We hypothesized that *X. germanus* and other ambrosia beetles would preferentially land on flood-stressed *C. florida* over nonflooded trees, and that higher amounts of ethanol would be associated with stem tissue samples from flooded trees.

Flood-stress conditions were initiated on 24 June 2011 and maintained until 15 July 2011. As in the previous experiments, a subset of the flooded and nonflooded *C. florida* trees used in 2011 were treated with Tanglefoot or left untreated. Entrapped ambrosia beetles were collected from the Tanglefoot at the end of the experiment. The experimental trees treated with Tanglefoot were also returned to our laboratory at the OARDC for dissection on the day that the field experiment was terminated. Pruning shears were used to carefully dissect the stem tissue and recover ambrosia beetles from galleries created in the experimental trees.

Stems of the remaining flooded and nonflooded trees that were not treated with Tanglefoot were wrapped with several layers of fine mesh from the base up to 40 cm to prevent *X. germanus* and other ambrosia beetles from attacking the trees. Beetles were prevented from attacking because it could have confounded tissue analyses for ethanol. The mesh was secured using twist ties and this effectively prevented ambrosia beetles from attacking the main stem, which is preferred for colonization (Reding et al., 2010). No attacks were observed on regions that were not covered by fine mesh throughout the experiment. Eight replicates were used for each treatment (i.e. nonflooded with and without protective mesh; flooded with and without protective mesh). A total of eight randomized complete blocks were arranged as described previously.

To assess the association of ethanol with flood-stressed and nonflooded *C. florida*, tissue core samples (depth 1 mm, diameter 5 mm) were taken on 15 July 2011 from tree trunks that were wrapped with protective mesh to prevent ambrosia beetle attacks from occurring. Four tissue core samples were taken at 10 cm above the base of individual flooded and nonflooded trees using an Osborne arch punch (C.S. Osborne & Co., Harrison, New Jersey). The superficial tissue core samples included the outer bark, phloem and vascular cambium, excluding the sapwood and heartwood. Tissue core samples were placed in 2-mL Eppendorf tubes immediately after sampling, flash frozen with liquid N$_2$ and temporarily stored on dry ice. Tissue samples were then stored at $-40$ °C until analysis. Eight flooded and nonflooded trees were sampled.

A razor blade was used to separate the outer bark from the phloem and vascular cambium. The four tissue core samples were immediately transferred to a 2-mL glass vial with a screw top cap and septum, which was then suspended in a water bath at 100 °C for 30 min. The tissue core samples were heated in the water bath to inactivate enzymes capable of degrading ethanol, in accordance with methods described by Kelsey and Joseph (1998). Vials were removed from the water bath and a syringe containing a retracted SPME fibre was immediately inserted through the septum in the cap of the vial. After securing the syringe holder in a clamp attached to a laboratory retort stand, the SPME fibre was extruded from the syringe tip and exposed to the headspace within the vial for 5 min. The fibre was retracted after sampling and the syringe was immediately capped with a sealed section of polytetrafluoroethylene microbore tubing (inner diameter 0.568 mm, outer diameter 1.07 mm; Cole-Parmer, Vernon Hills, Illinois) to prevent contamination of the fibre. A coating of divinylbenzenecarbonyl-polydimethylsiloxane (DVB/CAR/PDMS; 50/30 µm coating; Sigma-Aldrich, St Louis, Missouri) was used, which is ideal for a range of volatile and semivolatile compounds (Sigma-Aldrich, 2012).

Volatiles were thermally desorbed for analysis by GC-MS by exposing the fibre within the injection port of a gas chromatograph (Varian CP-3800; Varian Inc., Palo Alto, California) equipped with a Merlin Microseal septum system (Sigma-Aldrich). The injection port consisted of a deactivated glass liner with an inner diameter of 0.75 mm and a single tapered end specific for SPME applications. Fibres were held...
Experiment 4: bark volatile emissions

Flood-stress of *C. florida* was established and maintained accordingly to analyze for the emission of ethanol and other low molecular weight volatile compounds from the outer bark tissue of flooded and nonflooded trees. We hypothesized that higher amounts of ethanol would be emitted from the bark of flooded *C. florida* compared with nonflooded trees.

Flood-stress of *C. florida* trees was initiated accordingly on 10 August 2011 and maintained until 24 August 2011. Volatile emissions from the bark of *C. florida* trees subjected to flooding for 7 and 14 days were subsequently compared with nonflooded trees. Three flooded and nonflooded trees were analyzed. Volatiles emanating from the main trunk of *C. florida* were analyzed based on Ranger et al. (2012). In brief, volatile sampling chambers (length 6 cm, width 1 cm, height 7 cm), as described in Ranger et al. (2012), with an internal volume of 3 cm³ were constructed with polytetrafluoroethylene tubing and chromatography septa (Molded Thermogreen LB-2 Septa; Supelco, Bellefonte, Pennsylvania). Cables ties were used to snugly secure the volatile sampling chamber against the stem in parallel, thereby confining 6 cm² of bark tissue. The particularly smooth bark associated with *C. florida* allowed for close contact between the tissue and sampling chamber, which effectively sealed the chamber from passive air flow. The lower end of the chamber was positioned approximately 15 cm above the base of the trees.

Chambers were held in place for 30 min to allow for volatile equilibration. After sufficient equilibration, a SPME fibre was exposed for 4.5 h within the chamber as described by Ranger et al. (2012). The syringe was immediately capped after sampling, wrapped in a Teflon bag, and briefly held at −40 °C until analysis in accordance with the conditions noted above. For the purposes of this experiment, only compounds eluting within the first 2.5 min were considered. All identifications were confirmed by comparing mass spectral fragmentation patterns and retention times with authentic standards, and also by referencing the National Institute of Standards and Technology MS database. For determining relative quantities, serial dilutions of acetaldehyde (99.7% purity, Sigma-Aldrich), acetic acid (99.7% purity, Sigma Aldrich) and ethanol (99.5% purity; Acros Organics) ranging from 100 to 0.0001 g/L were individually made using distilled/deionized water. Standards were analyzed using the conditions described above and in accordance with Ranger et al. (2012). Standard concentration curves were used to determine the relative quantities of individual compounds. Because of differences in the affinities of SPME fibres for different compounds, relative quantitation by SPME does not the quantities of different compounds within the same sample to be compared (Bartelt, 1997; Romeo, 2009). Thus, only relative quantities of an individual compound were compared between 7 and 14 days after initiating flooding.

Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to initially test for within-subject and between-subject treatment × time effects associated with the time-course entrapment of *X. germanus* in Tanglefoot applied to *C. florida* as part of Experiment 1 (SAS Institute, 2001). Repeated measures ANOVA was used because repeated measurements were made on the same experimental units, and the number of entrapped beetles was cumulative and dependent in part on the previous number of captures (Littell et al., 1998). When a significant between-subject treatment × time effect was detected (*P* < 0.05), the number of entrapped beetles at each time point was compared between flood-stressed and control trees using an unpaired post-hoc *t*-test (*α* = 0.05; SAS Institute, 2001). An unpaired *t*-test was also used to compare entrapped beetles and cumulative attacks associated with flood-stressed and nonflooded trees for Experiments 2–4. A *t*-test was also used to compare the relative concentrations of individual volatile compounds at 7 and 14 days after initiating flood-stress as part of Experiment 4. A one-way ANOVA (*α* = 0.05; SAS Institute, 2001) was used to compare the number of ambrosia beetle specimens excavated from galleries of infested trees as part of Experiment 3, with differences between means being separated by Tukey’s studentized range (honestly significant difference) test.

Pearson’s correlation coefficient analysis was also used to determine the degree of correlation between cumulative ambrosia beetle attacks and days after initiating flooding as part...
respectively (Fig. 1). However, significantly more X. germanus d.f. = 10, \( P = 0.021 \), 13 days (3.8 ± 1.9 versus 0.4 ± 0.2; \( t = 2.70, \ d.f. = 10, \ P = 0.022 \)), 16 days (4.8 ± 2.8 versus 0.4 ± 0.2; \( t = 2.55, \ d.f. = 10, \ P = 0.029 \)), 19 days (5.0 ± 3.0 versus 0.4 ± 0.2; \( t = 2.53, \ d.f. = 10, \ P = 0.030 \)) and 22 days (5.3 ± 3.4 versus 0.4 ± 0.2; \( t = 2.50, \ d.f. = 10, \ P = 0.032 \)) (Fig. 1), respectively.

Repeated measures ANOVA detected a significant within-subject treatment \( \times \) time effect (\( F = 4.56, \ d.f. = 7, \ P = 0.0003 \)) for the mean cumulative number of X. germanus entrapped in the Tanglefoot from 1 to 22 days after initiating flooding. Pearson’s correlation coefficient analysis detected a significant positive correlation between the cumulative number of X. germanus entrapped in Tanglefoot on flood-stressed trees and days after initiating flood-stress (\( r = 0.48, \ P = 0.0005, \ n = 48 \)). However, a correlation was not detected between X. germanus entrapped in Tanglefoot on the trunks of nonflooded trees and days after initiating the experiment (\( r = 0.14, \ P = 0.34, \ n = 48 \)).

One of the six flood-stressed C. florida trees not treated with Tanglefoot sustained 16 cumulative attacks 22 days after initiating the experiment, although attacks did not occur on any of the remaining flooded or nonflooded trees not treated with Tanglefoot. Significant differences in cumulative attacks were not detected between treatments (\( t = 1, \ d.f. = 10, \ P = 0.341 \)).

As part of Experiment 2, a mean ± SE of 0.9 ± 0.3 cumulative X. germanus were entrapped in Tanglefoot applied to the trunks of flood-stressed C. florida 13 days after initiating the experiment, although no specimens were entrapped in Tanglefoot applied to the nonflooded controls (\( t = 3.20, \ d.f. = 14, \ P = 0.007 \)). Similarly, a mean ± SE of 2.4 ± 1.0 cumulative attacks occurred on flood-stressed C. florida 13 days after initiating the experiment, although no attacks occurred on the nonflooded controls (\( t = 3.11, \ d.f. = 14, \ P = 0.008 \)).

### Results

**Experiments 1 and 2: host attractiveness and susceptibility**

As part of Experiment 1, repeated measures ANOVA detected a significant between-subject treatment \( \times \) time effect (\( F = 6.18, \ d.f. = 1, 10, \ P = 0.032 \)) for the mean cumulative number of X. germanus entrapped in Tanglefoot applied to trunks of the flooded and nonflooded trees. No significant difference in the mean ± SE cumulative X. germanus entrapped in the Tanglefoot was detected between flooded versus nonflooded trees at 1 day (0.2 ± 0.2 versus 0.0 ± 0.0; \( t = 1, \ d.f. = 10, \ P = 0.341 \)), 4 days (1.0 ± 0.5 versus 0.4 ± 0.2; \( t = 1.02, \ d.f. = 10, \ P = 0.331 \)) and 7 days (1.2 ± 0.5 versus 0.4 ± 0.2; \( t = 1.52, \ d.f. = 10, \ P = 0.160 \)) after initiating flood-stress, respectively (Fig. 1). However, significantly more X. germanus were entrapped on flooded C. florida versus nonflooded trees at 10 days (2.2 ± 0.7 versus 0.4 ± 0.2; \( t = 2.73, \ d.f. = 10, \ P = 0.021 \)), 13 days (3.8 ± 1.9 versus 0.4 ± 0.2; \( t = 2.70, \ d.f. = 10, \ P = 0.022 \)), 16 days (4.8 ± 2.8 versus 0.4 ± 0.2; \( t = 2.55, \ d.f. = 10, \ P = 0.029 \)), 19 days (5.0 ± 3.0 versus 0.4 ± 0.2; \( t = 2.53, \ d.f. = 10, \ P = 0.030 \)) and 22 days (5.3 ± 3.4 versus 0.4 ± 0.2; \( t = 2.50, \ d.f. = 10, \ P = 0.032 \)) (Fig. 1), respectively.

### Experiment 3: host attractiveness and stem tissue analysis

A mean ± SE of 7.6 ± 3.3 cumulative X. germanus were entrapped in Tanglefoot applied to flood-stressed C. florida 21 days after initiating the experiment, which was significantly higher than the 0.4 ± 0.2 beetles associated with the nonflooded controls (\( t = 4.54, \ d.f. = 14, \ P = 0.0005 \)) (Table 1).

**Table 1** Species of ambrosia beetles entrapped in Tanglefoot adhesive applied to the stems of flood-stressed and nonflooded Cornus florida

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Xylosandrus germanus</th>
<th>Anisandrus sayi</th>
<th>Xyleborinus attenuatusa</th>
<th>Xylosandrus crassiusculus</th>
<th>Xylosandrus saxesenii</th>
<th>Monarthrum mali</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flood-stressed</td>
<td>7.6 ± 3.3</td>
<td>1.4 ± 0.7</td>
<td>1.3 ± 1.0</td>
<td>0.6 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 0.4</td>
</tr>
<tr>
<td>( t, \ P )</td>
<td>4.54, 0.0005</td>
<td>1.65, 0.12</td>
<td>1.26, 0.23</td>
<td>1.87, 0.082</td>
<td>1.97, 0.069</td>
<td>−0.91, 0.38</td>
</tr>
</tbody>
</table>

\( a \)Xylosandrus attenuatus = Xyloboirus ahi (Mokrzycki et al., 2011).

Unpaired t-test (\( \alpha = 0.05; \ d.f. = 14; \ n = 8 \) trees per treatment).

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Figure 2 (A) Mean ± SE cumulative number of ambrosia beetle attacks on flood-stressed and nonflooded Cornus florida L. (n = 8 replicates per treatment; unpaired t-test; α = 0.05). (B) Mean ± SE number of ambrosia beetle specimens excavated per infested flood-stressed C. florida tree (n = 8 replicates per treatment; one-way analysis of variance; Tukey’s honestly significant difference test; α = 0.05). As part of Experiment 3, flood-stress was initiated on 24 June 2011 and trees were held under field conditions until 15 July 2011.

Entrapped on the flooded trees, although significant differences were not detected compared with the nonflooded control trees (Table 1).

Despite the presence of Tanglefoot on the trunks, a mean ± SE of 8.1 ± 2.7 cumulative attacks per tree occurred on the flood-stressed C. florida below where the adhesive had been applied (Fig. 2A). No attacks occurred on the nonflooded controls, which were significantly different from the flooded trees (t = 5.52, d.f. = 14, P < 0.0001). A mean ± SE of 7.4 ± 2.8 X. germanus were recovered from dissected galleries per tree, which was significantly higher than the 0.6 ± 0.4 of X. crassiusculus and 0.1 ± 0.1 of A. sayi excavated per tree (F = 12.08, d.f. = 2, 21, P = 0.0003) (Fig. 2B). Xylosandrus germanus represented 91.4% of the specimens recovered from galleries of the infested trees, followed by X. crassiusculus (7.7%) and A. sayi (1.5%) (Fig. 2B).

The thawed tissue core samples associated with each tree replicate were weighed just before analysis by SPME-GC-MS, with a mean ± SE of 19.99 ± 1.50 and 16.61 ± 0.47 mg per tissue core for flood-stressed and nonflooded trees, respectively (t = −1.68, d.f. = 14, P = 0.114). SPME-GC-MS analysis detected ethanol associated with the superficial tissue core samples from stems of C. florida 14 days after imposing and maintaining flood-stress conditions. A mean ± SE of 612.38 ± 149.98 ng of ethanol g−1 tissue was associated with the flood-stressed trees. Ethanol was not detected in tissue samples from nonflooded trees.

Experiment 4: bark volatile emissions

SPME-GC-MS was successfully used to characterize selected volatile emissions from the outer bark of flooded and nonflooded C. florida. Acetaldehyde, ethanol and acetic acid were detected in bark emissions from flood-stressed C. florida at 7 and 14 days after initiating flooding but not from nonflooded trees (Fig. 3 and Table 2). Significant differences were detected for relative concentrations of ethanol between 7 and 14 days but were not detected for acetaldehyde and acetic acid (Table 2).

Concentrations of ethanol were highly variable among individuals 7 days after initiating flood-stress but were less variable at 14 days (Table 2). Concentrations of acetic acid showed considerable variability at both 7 and 14 days after initiating flooding (Table 2). A mean ± SE of 4.0 ± 1.5 cumulative attacks occurred on the flood-stressed C. florida trees at 14 days after initiating flooding, although there were no attacks on the nonflooded trees (t = 5.23, d.f. = 4, P = 0.006).
Table 2 Emission of selected volatiles from flood-stressed Cornus florida

<table>
<thead>
<tr>
<th>Peak</th>
<th>Compound</th>
<th>Mean ± SE relative quantity (μg/cm² bark tissue)</th>
<th>Days after initiating flood-stress</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetaldehyde</td>
<td>0.007 ± 0.003, 0.009 ± 0.002</td>
<td>7</td>
<td>-0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol</td>
<td>0.46 ± 0.12, 1.31 ± 0.26</td>
<td>14</td>
<td>-3.19</td>
<td>0.033</td>
</tr>
<tr>
<td>3</td>
<td>Acetic acid</td>
<td>4.85 ± 4.30, 25.48 ± 12.80</td>
<td>7</td>
<td>-1.90</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Peak numbers correspond to Fig. 3.
Unpaired t-test (α = 0.05; d.f. = 14; n = 3 trees per treatment).

Discussion

Flowering dogwood C. florida, a popular ornamental tree native to eastern North America, is widely considered to be intolerant of flooding and compacted soils (Day et al., 2000). Our findings show that more X. germanus were attracted to and landed on experimentally flood-stressed C. florida in 2009, 2010 and 2011 compared with the nonflooded controls. These data also support observations showing that X. germanus possesses an ability to orient towards and pinpoint the specific source of host-derived volatile cues, particularly ethanol (Ranger et al., 2010, 2012). Comparatively few specimens were entrapped on neighbouring nonflooded control trees, which provides further evidence that X. germanus has an efficient olfactory mechanism capable of assessing and distinguishing among differing host qualities, even when in-flight (Weber & McPherson, 1984; Ranger et al., 2010, 2012). Saint-Germain et al. (2007) found primary attraction functioned at a relatively large scale to pull Scolytinae into patches containing an attractant-baited tree, although random landing on adjacent unbaited trees then occurred at a finer spatial scale. The results of the present study suggest that random landing is less important for X. germanus at a fine spatial scale than primary attraction to a specific point source of volatile cue(s).

Our experimentally flood-stressed dogwoods were also preferentially attacked in 2009, 2010 and 2011, whereas no attacks occurred on neighbouring nonflooded control trees throughout the present study. Ambrosia beetle attacks have also not been observed on water-injected or uninjectected trees neighbouring attacked ethanol-injected trees (Ranger et al., 2010, 2012). Xylosandrus germanus was the most abundant species excavated from flood-stressed dogwoods in the present study, and was the most abundant species of ambrosia beetle emerging from infested nursery stock (Reding et al., 2010) and ethanol-injected trees (Ranger et al., 2010) in previous experiments in Ohio. The relatively low landing rates and attacks on flooded trees in certain experiments are likely attributed to the experiments being conducted after peak flight by X. germanus, which typically occurs in early to mid-May in Ohio (Reding et al., 2010).

Collectively, the comparatively low landing rates and absence of attacks on nonflooded trees by X. germanus further demonstrate a preference for (and ability to) efficiently locate physiologically-stressed hosts. Rather than randomly landing on and attacking ‘apparently healthy’ trees within ornamental nurseries, the results from these experiments and previous studies (Ranger et al., 2010, 2012) indicate that X. germanus targets specific trees based on the emission of stress-related volatiles, particularly ethanol. Considering the wide geographical distribution of X. germanus within North America, and a host range that includes > 200 species (Weber & McPherson, 1983), one would expect widespread attacks on a variety of species within ornamental nurseries and neighbourig woodlots if truly healthy trees were being targeted.

Attacks by X. germanus have been suspected to occur on trees viewed as ‘apparently-healthy’ (Weber & McPherson, 1984; Grégoire et al., 2001), although the possibility of such trees being inapparently-stressed at the time of attack cannot be ruled out given the results obtained in the present study, as well as previous studies (Ranger et al., 2010, 2012). Trees subjected to physiological stress may appear healthy but exhibit little or no growth (Schoeneweiss, 1978; Wargo, 1996). Visible symptoms resulting from physiological stress events may even take several years to become fully evident (Schoeneweiss, 1978; Wargo, 1996). Thus, characterizing a tree as ‘apparently healthy’ at the time of attack by secondary ambrosia beetles based solely on a visual assessment could be misleading, and inaccurately reflect the host-selection behaviour of X. germanus.

For example, black walnut (Juglans nigra L.) trees growing in a plantation in Alexander Co., Illinois were characterized as ‘apparently healthy’ at the time of attack by X. germanus in 1978, yet they exhibited slower growth rates in the previous year compared with uncolonized trees (Weber & McPherson, 1984). Although flood-stress probably does not explain every instance in which trees are colonized by X. germanus, it should be noted that above average precipitation was recorded within the county in 1973 (+30.09 cm), 1975 (+25.15 cm) and in the year that attacks were detected in 1978 (+28.09 cm) (Station: 111166/93809, CAIRO WSO CITY, Illinois; NOAA, 2012b). Alternatively, below average precipitation occurred in 1974 (−23.39 cm) and 1976 (−28.83 cm) (NOAA, 2012b), which also could have predisposed the trees to attack. Black walnut is considered intolerant of flooding (Broadfoot & Williston, 1973; Dudek et al., 1998) and drought (Loewenstein & Pallardy, 1998).

Similarly, recent studies indicate that beech (Fagus sylvatica L.) trees initially considered apparently-healthy at the time of attack by X. germanus and other ambrosia beetles were predisposed to attack by extreme frost events (Grégoire et al., 2001; La Spina et al., 2012). Host-selection by X. germanus in natural forested stands has not been well characterized, although it presumably involves physiologically-stressed trees being targeted.

Although physiologically-stressed trees may visually appear healthy, they could still emit ethanol and other stress-related volatiles that signify their suitability for colonization by secondary ambrosia beetles such as X. germanus. Results from our SPME-GC-MS analyses confirmed the association of ethanol with vascular tissue samples from stems of flood-stressed C. florida but not nonflooded trees. Acetaldehyde, acetic acid and ethanol were also detected by SPME-GC-MS in bark emissions from stems of flooded C. florida but not nonflooded trees. It is presently unclear whether acetaldehyde and acetic acid act additively or synergistically with ethanol to attract and induce
attacks by *X. germanus* and other ambrosia beetles. However, previous studies did not find acetaldehyde-baited traps to be nearly as attractive as ethanol to *X. germanus* and other Scolytinae (Montgomery & Wargo, 1983; Ranger et al., 2010), nor did acetaldehyde act synergistically when mixed with ethanol (Montgomery & Wargo, 1983). Stem injections of acetaldehyde also did not induce as many attacks as ethanol (Ranger et al., 2010). Nevertheless, additional studies assessing the antennal response of *X. germanus* to flood-stressed *C. florida* via gas chromatography-electroantennographic detection (GC-EAD) are warranted and are currently underway aiming to determine whether compounds in addition to ethanol function as important host-selection cues. *Xylosandrus germanus* exhibited an antennal response via GC-EAD to ethanol emitted from the bark of ethanol-injected *Magnolia virginiana* L. (Ranger et al., 2012).

In flooded trees, ethanol appears to diffuse from the transpiration stream to the vascular cambium, where it is either remobilized or emitted from the epidermis (MacDonald & Kimmerer, 1991). High variability among flood-stressed individuals in concentrations of acetaldehyde, acetic acid and ethanol has been observed for a wide variety of deciduous and coniferous tree species (MacDonald & Kimmerer, 1991; Rottenberger et al., 2008). Such variability appears to be attributed to intrinsic physiological differences among individuals in their ability to deal with hypoxic or anoxic conditions (MacDonald & Kimmerer, 1991). A high degree of variability among individuals in the relative concentration of ethanol was associated with stem tissue core samples from *C. florida* (see Experiment 3), and was also observed in emissions of acetaldehyde, acetic acid and ethanol from the bark tissue of *C. florida* (Table 2). Variability in the emission of stress-related volatiles from flood-stressed *C. florida*, particularly ethanol, may therefore account for the relatively high variability in *X. germanus* Tanglefoot captures and attacks per flooded tree within and between experiments. More extensive studies are currently underway to assess the interaction between variability in emissions of volatiles among flood-stressed trees and host-selection by *X. germanus* and other ambrosia beetles.

The present study has demonstrated the preference of *X. germanus* to colonize physiologically-stressed trees, and supports the ability of flood-stress to induce ethanol production and predispose trees to attack by ethanol-responsive ambrosia beetles. However, it should be emphasized that water stress probably does not account for every situation in which trees become attractive to *X. germanus*, particularly given the ability of a variety of abiotic and biotic stressors to induce ethanol production and/or ambrosia beetle attacks (Kelsey, 2001). A variety of such stressors can frequently occur in ornamental nurseries, including flood and drought stress, excessive heat and freezing, cultiver unsuitability for a particular growing zone, poor soil or site conditions, transplanting stress, excessive nutrients, girdling, pathogens, etc.

Because abiotic stressors are predicted to become more frequent as a result of climate change (Cubasch et al., 1995; Zwiers & Khairin, 1998; Kunkel et al., 1999; Easterling et al., 2000; Meehl & Tebaldi, 2004; Khairin & Zwiers, 2005), additional studies will aid in an understanding of their impact on the production of relevant stress-related volatiles and their ability to predispose trees within ornamental nurseries to attack by ambrosia beetles. Although synthetic and plant-derived essential oil insecticides can reduce ambrosia beetle attacks on vulnerable trees emitting ethanol (Frank & Sadof, 2011; Ranger et al., 2011b), the implications of the present study demonstrate that maintaining host vigour and minimizing stressors should be the primary foundation of a management plan for *X. germanus* and other ethanol-responsive ambrosia beetles.

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**References**


