INTRODUCTION

Tree crops grown in ornamental nurseries and tree fruit orchards are threatened by several species of exotic ambrosia beetles, especially *Xylosandrus compactus* (Eichhoff), *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae: Scolytinae; Chong, Reid, & Williamson, 2009; Agnello, Breth, Tee, Cox, & Warren, 2014; Ranger, Reding, et al., 2016). Adult females tunnel into...
the stems and branches of trees to cultivate gardens of their fungal symbiont on which the larvae and adults must feed to properly develop and reproduce (Biedermann & Taborsky, 2011; French & Roeppe, 1972). Ambrosia beetle fungal symbionts are rarely pathogenic, but a variety of secondary microorganisms can be passively introduced to trees, some of which are tree pathogens, for example, Fusarium (Carrillo et al., 2014). Due to their wood-boring behaviour and association with branch dieback and tree death, ambrosia beetles are often ranked among the most destructive insect pests of nursery trees (Fulcher et al., 2012; Oliver & Mannion, 2001; Ranger, Reding, et al., 2016). Even small numbers of ambrosia beetle attacks can lead to economic losses for nurseries due to reduced tree marketability.

After leaving their overwintering sites within host tree galleries, adult female ambrosia beetles disperse from wooded habitats into ornamental nurseries in search of a new host tree (Ranger, Tobin, et al., 2013; Reding et al., 2015; Werle, Chong, Sampson, Reding, & Adamczyk, 2015; Werle, Sampson, & Reding, 2017). Opportunistic species such as X. compactus, X. crassiusculus and X. germanus attack a broad range of trees with an apparent preference for thin-barked deciduous species (Chong et al., 2009; Ranger, Reding, et al., 2016). Despite a broad host range, host quality plays an important role during tree selection by opportunistic ambrosia beetles. Physiologically stressed trees can emit ethanol, a volatile compound used by female beetles as a chemical indicator of weakened trees (Ranger, Schultz, & Oliver, 2012). The presence of ethanol within host tree tissues also promotes the growth of their fungal symbionts and inhibits fungal competitors, thereby improving the colonization success of ambrosia beetles (Ranger et al., 2018). A variety of abiotic and biotic factors induce the emission of ethanol, but water stress (i.e., flooding) and low temperature stress (i.e., freezing and frost) are among the key stressors in ornamental nurseries and the majority of individuals (~70%–90%) are captured within 13 m of the nursery/forest interface (Ranger, Tobin, et al., 2013; Reding et al., 2015; Seo, Martini, Rivera, & Stelinski, 2017; Werle et al., 2015; Werle, Sampson, et al., 2017), ethanol-baited traps could potentially be used to intercept host-seeking ambrosia beetles.

Several studies have indicated that additive or synergistic effects can enhance the effectiveness of behaviour-manipulating stimuli by integrating the “push” and “pull” components (Cook et al., 2007; Cowles & Miller, 1992; Miller & Cowles, 1990; Pyke et al., 1987). An additive effect occurs when the combined effect is equal to the sum of the individual effects, while a synergistic effect occurs when the effect of the combined compounds is greater than the sum of their individual effects (Burt, 2004). Since previous studies have demonstrated verbenone and ethanol influence the behaviour of ambrosia beetles, we hypothesized that additivity or synergy between verbenone (i.e., push component) and ethanol (i.e., pull component) would function to minimize attacks by ambrosia beetles on vulnerable trees. The overall objective of our current study was to test the efficacy of verbenone and ethanol individually and combined for protecting flood-stressed trees from attack by opportunistic ambrosia beetles.

2 | MATERIALS AND METHODS

2.1 | Plot design

Experiments were conducted at three different geographic locations (Ohio, Virginia and Mississippi) to target populations of key species, particularly X. compactus, X. crassiusculus and X. germanus.
Plots were arranged in Mississippi, Ohio and Virginia to test the integration of verbenone (i.e., push component) and ethanol (i.e., pull component) for protecting flood-stressed trees from attack by ambrosia beetles. The plot design included the following "push–pull" treatments: (a) no verbenone/no ethanol, (b) verbenone/no ethanol, (c) no verbenone/ethanol, and (d) verbenone/ethanol (Figure 1).

Each field plot consisted of two 40 × 20 m subplots that were adjacent to the edge of woodlots supporting natural populations of non-native and native ambrosia beetles (Figure 1). The field plots used in Ohio, Virginia and Mississippi were grass-dominated and recently mowed prior to initiating experiments. The woodlots adjacent to the field plots used in Ohio, Virginia and Mississippi were dominated by mature deciduous trees with a few coniferous trees interspersed throughout. One of the 40 × 20 m subplots included a perimeter of ethanol-baited traps spaced 10 m apart, whereas the other 40 × 20 m subplot lacked a perimeter of ethanol-baited traps (see “Pull” Component; Figure 1). Two groupings of 3–4 flood-stressed trees were subjected to the following four treatments: (1) no verbenone/no ethanol, (2) verbenone/no ethanol, (3) no verbenone/ethanol, and (4) verbenone/ethanol (Figure 1).

Four replicated plots were established in Wayne Co., Ohio (40°46'21"N, 81°56'02"W), (40°45'42"N, 81°54'38"W), (40°46'04"N, 81°53'35"W) and (40°51'53"N, 82°03'06"W). Four replicated plots were established in York County, Virginia (37°17'17.8"N, 76°38'59.1"W). Three replicated plots were established in Mississippi with two replicates in Pearl River Co., Mississippi (30°39'34.36"N, 89°38'06.46"W), and a third replicate in Hancock Co., Mississippi (30°21'09.17"N, 89°38'29.99"W). Field trials were conducted in Ohio from 25 May 2016 to 31 May 2016; Virginia from 11 April 2016 to 2 May 2016 and 5 April 2017 to 1 May 2017; and Mississippi from 7 April 2016 to 2 June 2016 and 6 April 2017 to 8 May 2017.

2.1.1 “Push” component

A verbenone emitter was placed among one of the two clusters of flood-stressed trees within each subplot (Figure 1); the other cluster without the verbenone served as a control. Verbenone dispensers consisted of a heat-sealed, permeable membrane pouch containing 92% verbenone (BeetleBlock-Verbenone; 50 mg/day at 25°C; AgBio, Inc., Westminster, CO). Verbenone emitters were attached to a metal rod and suspended 1 m above the ground and within 30–60 cm of the cluster of flood-stressed trees.

2.1.2 “Pull” component

Ethanol-baited traps were deployed at 10-m intervals around the perimeter of one of the two subplots (Figure 1). This configuration resulted in five traps being in close proximity to the woodlot edge (~0 m), two traps at an intermediate distance (~10 m) and the remaining five traps being the furthest from the woodlot edge (~20 m). Traps were constructed using two recycled soda bottles (~0.6 L and 2 L sizes) attached with a Tornado Tube (Steve Spangler Science, Englewood, CO; Ranger et al., 2010). The upper 2 L bottle had three rectangular openings (length 15 cm, width 6 cm) cut into the sides for beetle entry, while the lower 0.6 L bottle was partially filled with propylene glycol to collect and preserve insects. Traps were suspended 1 m above the ground using metal rods and baited with an ethanol sachet lure (65 mg/day at 25°C; AgBio, Inc., Westminster, CO). One ethanol lure was used in each trap in Ohio, Virginia and Mississippi in 2016, while three lures were used per trap in Mississippi and Virginia in 2017. Since a positive concentration response exists between ambrosia beetles and ethanol emissions (Klimetzek et al., 1986), the number of lures per interception trap was increased in 2017 to assess if higher ethanol emission corresponded with decreased attacks on the flood-stressed trees. Field experiments were not conducted in Ohio in 2017. Trap contents were periodically collected throughout the duration of each experiment at each location, with specimens returned to the laboratory and identified to species. All specimens collected in Ohio and Mississippi were identified to species and quantified, while only the most predominant specimens were identified to species and quantified in Virginia in 2016 and 2017.

2.1.3 Imposing flood stress

Trees placed in the centre of each subplot (Figure 1) were flood-stressed using a pot-in-pot protocol by Ranger, Reding, et al. (2013)
to induce emission of ethanol and promote attacks by ambrosia beetles. The three to four flood-stressed trees were arranged in a triangle or square pattern, respectively, with about 30 cm between adjacent pots. Flood stress was initiated on the day the trees were placed within each plot, and flooding was maintained for the duration of the experiment.

In the Ohio 2016 trial, three flowering dogwood trees (Cornus florida L.) were placed in the centre of each subplot (12 trees per plot). Flood-stressed C. florida trees used in the Ohio experiments were 4 years old, 2.5–3.8 cm calliper and growing in 26.5 L pots containing a mixture of 90:10 pine bark and sphagnum peat moss, along with lime and Micromax Micronutrients (Scotts Co., Marysville, OH). The media was also top dressed with Osmocote Plus 15:9-12 (Scotts Co.) slow release fertilizer. Trees were fertilized with Jack’s Classic All Purpose 20-20-20 (JR Peters, Inc., Allentown, PA) with water soluble plant food with micronutrients in late March before using in experiments.

In the Virginia 2016 and 2017 trials, four flood-stressed dogwood trees (C. florida) were placed in each subplot (16 trees per plot). Flood-stressed C. florida trees used in the Virginia experiments were 4 years old, 3.8 cm calliper and growing in 28 L pots containing a mixture of 92:8 aged pine bark:coarse sand, and dolomitic lime to stabilize pH. The media was top dressed with Osmocote Plus 15–9-12 (Scotts Co., Marysville, Ohio) slow release fertilizer.

In the Mississippi 2016 trial, two groupings of four flood-stressed golden rain trees (Koelerieta paniculata Laxm.) were placed within each subplot (16 trees per plot, Figure 1). In the Mississippi 2017 trial, two groupings of three redbud trees (Cercis canadensis L.) were placed within each subplot (12 trees per plot). Flood-stressed K. paniculata and C. canadensis trees used in the Mississippi experiments were 2–3 years old, 2.5–3.8 cm calliper and growing in 23 L pots containing a mixture of pine bark, sand and peat moss. The media was top dressed with Osmocote Plus 15–9-12 (Scotts Co., Marysville, Ohio) slow release fertilizer.

Flood stress was initiated on the day trees were placed within each plot, and flooding was maintained for the duration of the experiment. New attacks were monitored every 2–4 days throughout the experiment and circled with a wax pencil or Sharpie pen. Trees were cut at the base at the end of the experiments in Ohio 2016 and Virginia 2016–2017 and temporarily stored at 5°C. Stems and ambrosia beetle galleries were carefully dissected using pruning shears and examined under a stereomicroscope. Adult foundresses were tallied and identified to species, with additional counts of eggs, larvae and pupae made within each gallery. Specimens were preserved in 70% ethanol.

### 2.2 Statistical analysis

A two-way ANOVA was used to test the interaction of the “push” and “pull” components, along with the two main effects, on cumulative ambrosia beetle attacks on the flood-stressed trees (SAS Institute, 2001). Tukey’s HSD test ($\alpha = 0.05$) was used to separate differences among treatments in the number of attacks occurring on trees subjected to one of the following four treatments: (a) untreated control, (b) verbenone only, (c) ethanol only and (d) verbenone plus ethanol. Since 3–4 flooded trees were used in each subplot (Figure 1), the total number of attacks occurring per tree in the subplots was considered subsamples and therefore averaged prior to analysis. Regression analysis was used to test for a correlation between trap distance from the woodlot edge and ambrosia beetle captures. Data were log($x + 1$) transformed prior to analysis, but untransformed data are presented.

### 3 RESULTS

#### 3.1 Efficacy of “push–pull” strategy

The repellent effect of verbenone and the attractant effect of ethanol did not significantly interact as part of a “push–pull” strategy to reduce or prevent attacks on flood-stressed trees during field experiments conducted in Ohio (2016), Virginia (2016–2017) or Mississippi (2016–2017; Figure 2a–e, Table 1). The verbenone-based “push” component was also not associated with a significant main effect at reducing attacks on the flood-stressed trees in any location or year (Figure 2a–e, Table 1). By contrast, the ethanol-based “pull” component exhibited a significant main effect at reducing attacks on the flood-stressed trees deployed in Mississippi and Virginia.
in 2016, but not Ohio in 2016 or Mississippi and Virginia in 2017 (Figure 2a–e, Table 1). While the perimeter of ethanol-baited traps reduced attacks on the flood-stressed trees deployed in Mississippi and Virginia in 2016, the traps did not completely prevent attacks from occurring.

### 3.2 Dispersal of ambrosia beetles

A negative correlation was observed between Scolytinae trap captures and distance of the ethanol-baited traps from the edge of the woodlot (Figure 3a–c), such that beetle captures decreased with an increasing distance from the woodlot edge for Ohio in 2016 ($r^2 = 0.51$; $F = 47.42$; $df = 1, 46$; $p < 0.0001$), Virginia in 2016 ($r^2 = 0.36$; $F = 26.02$; $df = 1, 46$; $p < 0.0001$) and 2017 ($r^2 = 0.31$; $F = 20.72$; $df = 1, 46$; $p < 0.0001$), and Mississippi in 2017 ($r^2 = 0.31$; $F = 15.75$; $df = 1, 34$; $p = 0.0004$). A positive correlation instead of a negative correlation was observed in Mississippi in 2016 between Scolytinae trap captures and distance from the edge of the woodlot ($r^2 = 0.25$; $F = 11.31$; $df = 1, 34$; $p = 0.002$).

### 3.3 Scolytinae abundance and distribution

The perimeter of ethanol-baited traps positioned around the flood-stressed trees captured a total of 4,491 Scolytinae specimens in Ohio in 2016, consisting of 16 species (Figure 4a). *Xylosandrus germanus* was the most predominant species collected in ethanol-baited traps deployed in Ohio in 2016, representing 86.5% (3,889 specimens) of the total trap captures.

Ethanol-baited traps caught 475 and 2,136 Scolytinae specimens in Virginia in 2016 and 2017, respectively (Figure 4b,c). Only the most predominant species were identified to species in Virginia in 2016 and 2017. *Xylosandrus crassiusculus* and *X. germanus* were the two most predominant species collected in Virginia in 2016 and represented 62.7% (298 specimens) and 25.3% (120 specimens) of the total trap captures, respectively. Similarly, *X. crassiusculus* and *X. germanus* were the two most predominant species collected in Virginia in 2017 and represented 52.2% (1,115 specimens) and 30.8% (658 specimens) of the total trap captures, respectively.

In Mississippi in 2016 and 2017, 917 and 1,304 Scolytinae specimens were collected, respectively (Figure 4d–e). *Hypothenemus dissimilis* (Zimmermann) and *X. compactus* were the most predominant species collected in Mississippi in 2016, representing 66.0% (605 specimens) and 22.0% (202 specimens) of the total trap captures. In 2017, *X. crassiusculus*, *H. dissimilis* and *X. compactus* were the most predominant species collected in Mississippi, representing 42.3% (552 specimens), 31.4% (410 specimens) and 10.4% (136 specimens) of the total trap captures, respectively. Notably, *X. crassiusculus*, *X. germanus* and *X. saxesenii* were the three non-native species collected in all three states (Figure 4a–e).

### 3.4 Scolytinae attacking flood-stressed trees

In Ohio in 2016, 952 specimens representing five Scolytinae species were recovered from flood-stressed *C. florida* trees, namely

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**Table 1** Two-way ANOVA testing the interaction and main effects of verbenone and ethanol for reducing attacks on trees as part of “push–pull” field experiments conducted in Ohio, Virginia and Mississippi.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>OH 2016</th>
<th>VA 2016</th>
<th>VA 2017</th>
<th>MS 2016</th>
<th>MS 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source</td>
<td>F, P</td>
<td>F, P</td>
<td>F, P</td>
<td>F, P</td>
<td>F, P</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.97, 0.35</td>
<td>5.53, 0.04</td>
<td>0.81, 0.39</td>
<td>11.79, 0.01</td>
<td>0.33, 0.58</td>
<td></td>
</tr>
<tr>
<td>Verbenone</td>
<td>0.01, 0.93</td>
<td>0.36, 0.56</td>
<td>0.07, 0.80</td>
<td>1.73, 0.23</td>
<td>0.03, 0.87</td>
<td></td>
</tr>
<tr>
<td>Ethanol × Verbenone</td>
<td>0.44, 0.52</td>
<td>2.95, 0.11</td>
<td>0.11, 0.74</td>
<td>0.02, 0.88</td>
<td>0.03, 0.87</td>
<td></td>
</tr>
</tbody>
</table>

*See Figure 2 for mean (±SE) values. df = 1 for all analyses.*

**Table 2** Specimens recovered from flood-stressed *C. florida* trees deployed in Ohio in 2016 during “push–pull” field trials.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean (±SE) per Tree</th>
<th>No Verbenone No Ethanol</th>
<th>Verbenone No Ethanol</th>
<th>No Verbenone Ethanol</th>
<th>Verbenone Ethanol</th>
<th>F, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>13.8 ± 6.4A</td>
<td>9.3 ± 3.7A</td>
<td>3.9 ± 2.9A</td>
<td>16.6 ± 11.1A</td>
<td>0.54, 0.45</td>
<td></td>
</tr>
<tr>
<td>A. maiche</td>
<td>0.33 ± 0.3Ab</td>
<td>0.1 ± 0.1Ab</td>
<td>0.0 ± 0.08b</td>
<td>0.0 ± 0.08b</td>
<td>4.42, 0.04</td>
<td></td>
</tr>
<tr>
<td>H. dissimilis</td>
<td>0.0 ± 0.0Ab</td>
<td>0.0 ± 0.0Ab</td>
<td>0.1 ± 0.1Ab</td>
<td>0.0 ± 0.0Ab</td>
<td>1.00, 0.32</td>
<td></td>
</tr>
<tr>
<td>X. crassiusculus</td>
<td>1.6 ± 1.2Ab</td>
<td>1.1 ± 1.0Ab</td>
<td>0.3 ± 0.3Ab</td>
<td>1.3 ± 0.6Ab</td>
<td>2.83, 0.1</td>
<td></td>
</tr>
<tr>
<td>X. germanus</td>
<td>23.1 ± 9.7A</td>
<td>19.3 ± 5.6A</td>
<td>11.8 ± 3.3A</td>
<td>17.3 ± 6.8A</td>
<td>0.22, 0.64</td>
<td></td>
</tr>
<tr>
<td>X. saxesenii</td>
<td>1.3 ± 0.7Ab</td>
<td>0.7 ± 0.4Ab</td>
<td>0.8 ± 0.4Ab</td>
<td>0.3 ± 0.2Ab</td>
<td>0.01; 0.92</td>
<td></td>
</tr>
<tr>
<td>F, P</td>
<td>12.12; &lt;0.0001</td>
<td>17.03; &lt;0.0001</td>
<td>22.07; &lt;0.0001</td>
<td>34.59; &lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. Means with different uppercase letters within a row indicate significant differences among treatments (two-way ANOVA; Tukey’s HSD; df = 1 for all comparisons). Means with different lowercase letters within a column indicate significant differences among Scolytinae species within a treatment (one-way ANOVA; Tukey’s HSD; df = 4, 15 for all comparisons).
FIGURE 3  (a–c) Correlation between distance of ethanol-baited traps from the woodlot edge and ambrosia beetle captures as part of “push–pull” experiments conducted in (a) Ohio, (b) Virginia and (c) Mississippi (see Figure 1 for layout of traps in relation to edge of woodlot; Dashed lines are fitted to 2016 data while solid lines are fitted to 2017 data). Experiments were conducted in 2016 in Ohio, and 2016 and 2017 in Virginia and Mississippi. Trap captures generally decreased with decreasing proximity from the edge

X. germanus, X. crassiusculus, X. saxesenii, Anisandrus maiche Stark and H. dissimilis (Table 2). Similar to the ethanol-baited traps, X. germanus was the most predominant species recovered from flood-stressed C. florida trees deployed in Ohio in 2016 (Table 2) representing 90.0% of the total specimens. Relatively few specimens of other Scolytinae were recovered from the dissected trees, including X. crassiusculus as 5.5%, X. saxesenii as 3.8%, A. maiche as 0.5% and H. dissimilis as 0.1% of total specimens (Table 2). Fewer A. maiche were recovered from flood-stressed trees protected by the perimeter of ethanol-baited traps compared to trees without the perimeter of traps (Table 2). However, this effect was not detected for the remaining species. In addition to the adult specimens, eggs were recovered from Scolytinae galleries created in the flood-stressed C. florida trees. The presence or absence of the verbenone emitters or the ethanol-baited traps did not have an effect on the number of eggs dissected per tree (Table 2).

A total of 3,383 Scolytinae specimens were recovered from flood-stressed C. florida trees deployed in Virginia in 2016. The five most common species were X. crassiusculus, X. germanus, X. compactus, Ambrosiodmus rubricollis (Eichhoff) and X. saxesenii. Similar to the ethanol-baited traps, X. crassiusculus was the most predominant species recovered from flood-stressed C. florida trees deployed in Virginia in 2016, representing 56.3% of the total specimens (Table 3). Xylosandrus compactus represented 7.1%, X. germanus represented 5.8%, C. mutilatus represented 3.2%, X. saxesenii represented 1.3%, and A. rubricollis represented 1.1% of total specimens recovered from flood-stressed C. florida trees deployed in Virginia in 2016. Scolytinae eggs, larvae and pupae were recovered from galleries created in the flood-stressed trees, but there was no effect by the presence or absence of verbenone emitters and the ethanol-baited traps (Table 3).

A total of 3,466 Scolytinae specimens were recovered from flood-stressed C. florida trees deployed in Virginia in 2017. Xylosandrus crassiusculus was the most predominant species recovered from flood-stressed C. florida trees deployed in Virginia in 2017, representing 55.0% of the total specimens, followed by X. compactus as 6.2%, X. germanus as 5.8%, C. mutilatus as 3.6%, X. saxesenii as 1.4% and A. rubricollis as 1.0% (Table 4). There was no effect of the presence or absence of the verbenone emitters or the ethanol-baited traps on the recovery of the aforementioned species from the flood-stressed trees (Table 4). Scolytinae eggs, larvae and pupae were recovered from the flood-stressed C. florida trees deployed in Virginia in 2017, but there was no effect by the presence or absence of verbenone emitters and the ethanol-baited traps (Table 4).

4 | DISCUSSION

As part of multistate trials, the verbenone-based “push” component did not provide an acceptable level of protection against ambrosia beetle attacks on the flood-stressed trees. In some instances, the ethanol-based “pull” component intercepted enough ambrosia beetles to reduce attacks on the flood-stressed trees, but the effect was variable across locations and years. There were no indications of an additive or synergistic effect between verbenone and ethanol. The results obtained as part of our current study did not meet the expectations of our original hypothesis that ethanol would “pull” beetles away from stressed trees. Still, two factors suggest a “push–pull” management strategy has utility for protecting trees against ambrosia beetles in ornamental nurseries and tree fruit orchards; first, behaviour-modifying semiochemicals are known for several of the most destructive species, and second, the dispersal of ambrosia beetles from woodlots into production areas favours a semiochemical-based interception tactic. The repellent and attractant semiochemical components will need to be further optimized to implement a viable “push–pull” management
strategy. Additional studies should assess a higher verbenone release rate or release mechanism for the "push" component, along with evaluating other potential repellents. Applying a repellent, reduced-risk or conventional insecticide directly to vulnerable trees should also be evaluated. A higher release rate of ethanol as part of the "pull" component should also be assessed, along with comparing the efficacy of various trap designs for maximizing captures of the most destructive Scolytinae species. These factors are discussed in greater detail below.

Because previous studies have demonstrated the behaviour-modifying effects of verbenone against ambrosia beetles (Burbano et al., 2012; Dudley et al., 2006; Ranger et al., 2014; Ranger, Tobin, et al., 2013; Van Der Laan & Ginzel, 2013), the lack of effect as part of our current study was unexpected. Notably, verbenone reduced attacks by X. germanus on herbicide-injected Pinus resinosa Aiton trees, but it did not completely prevent them from occurring (Dodds & Miller, 2010). Similarly, verbenone reduced captures of X. germanus in ethanol-baited traps by >95% compared to ethanol alone (Ranger, Tobin, et al., 2013). A positive correlation occurred between attacks and distance from verbenone emitters, but the results were inconsistent (Ranger, Tobin, et al., 2013). Since the verbenone emitters were placed in close proximity to the flood-stressed trees as part of our current study, but did not reduce attacks, the attractiveness of the stressed trees perhaps overpowered the repellence of the verbenone emitters. For instance, the higher volatility of ethanol compared to verbenone might result in ethanol influencing ambrosia beetle behaviour at long and short ranges while verbenone would be active at a shorter range. Notably, ethanol has a lower molecular weight (46.07 g/mol) and boiling point (78°C) compared to the molecular weight (150.21 g/mol) and boiling point (227–228°C) of verbenone (Rowan, 2011; Zhao, Shu, Wang, Wang, & Tian, 2011). Since temperature plays a critical role in the emission of terpenoids.
TABLE 4  
C. flora trees deployed in Virginia in 2017 during “push–pull” field trials

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean (±SE) per Tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Verbenone No Ethanol</td>
</tr>
<tr>
<td>Eggs</td>
<td>89.8 ± 27.4A</td>
</tr>
<tr>
<td>Larvae</td>
<td>264.4 ± 74.6A</td>
</tr>
<tr>
<td>Pupae</td>
<td>19.8 ± 7.2A</td>
</tr>
<tr>
<td>A. rubricollis</td>
<td>0.5 ± 0.5Ad</td>
</tr>
<tr>
<td>X. compactus</td>
<td>1.9 ± 0.7Abcd</td>
</tr>
<tr>
<td>X. crassiusculus</td>
<td>33.8 ± 5.6Aa</td>
</tr>
<tr>
<td>X. germanus</td>
<td>4.5 ± 1.9Ab</td>
</tr>
<tr>
<td>C. mutilatus</td>
<td>3.4 ± 1.0Abc</td>
</tr>
<tr>
<td>X. saxesenii</td>
<td>0.5 ± 0.3AcD</td>
</tr>
<tr>
<td>F; P</td>
<td>22.52; &lt;0.0001</td>
</tr>
</tbody>
</table>

Note. Means with different uppercase letters within a row indicate significant differences among treatments (two-way ANOVA; Tukey’s HSD; df = 1 for all comparisons). Means with different lowercase letters within a column indicate significant differences among Scolytinae species within a treatment (one-way ANOVA; Tukey’s HSD; df = 5, 18 for all comparisons).

(Maleknia et al., 2009; Zhao et al., 2011), emission of verbenone from the emitters used as part of our current study might not have been high enough to strongly repel ambrosia beetles during their peak spring flight activity.

Increasing the release rate or release mechanism of verbenone might aid in reducing attacks on trees. Gillette et al. (2006) proposed that verbenone dispensing strategies could influence efficacy, and the deployment of many small, point-source releasers, such as verbenone-releasing flakes, could be an improvement over plastic pouches or bubblecap dispensers. Screening for a more effective repellent is also warranted; previous studies have demonstrated terpenoids or bubblecap dispensers. Screening for a more effective repellent is also warranted; previous studies have demonstrated terpenols (Ranger et al., 2014) and methyl salicylate (Hughes et al., 2017) repel ambrosia beetles. Application of kaolin clay to stems was also demonstrated to reduce attacks, perhaps by acting as a settling cation in the effectiveness of bottle traps versus funnel traps for capturing key species, such as A. maiche, X. crassiusculus and X. germanus, thereby warranting additional studies to characterize the basis for discrepancies. Since trap density did not substantially impact mass-trapping of X. germanus (Grégoire, Piel, De Proft, & Gilbert, 2001), it is unlikely that spacing traps any closer than a 10 m distance between traps would be beneficial or economically feasible. Trap height is also an important factor for intercepting certain ambrosia beetles. For instance, conophthorin (Ranger et al., 2014; Van Der Laan & Ginzel, 2013) or benzaldehyde (Yang, Kim, & Kim, 2018).

Different trap designs should also be evaluated for maximizing the interception of ambrosia beetles. Montgomery and Wargo (1983) found vane traps were more effective than sticky traps at capturing Scolytinae beetles. Similarly, Miller, Crowe, Ginzel, Ranger, and Schultz (2018) demonstrated variability across geographic locations in the effectiveness of bottle traps versus funnel traps for capturing key species, such as A. maiche, X. crassiusculus and X. germanus, thereby warranting additional studies to characterize the basis for discrepancies. Since trap density did not substantially impact mass-trapping of X. germanus (Grégoire, Piel, De Proft, & Gilbert, 2001), it is unlikely that spacing traps any closer than a 10 m distance between traps would be beneficial or economically feasible. Trap height is also an important factor for intercepting certain ambrosia beetles. For instance, conophthorin (Ranger et al., 2014; Van Der Laan & Ginzel, 2013) or benzaldehyde (Yang, Kim, & Kim, 2018).

Our current study further supports that the ideal placement of traps for X. crassiusculus and X. germanus is at the interface of wooded habitats and tree production areas (Ranger et al., 2010; Ranger, Reding, et al., 2013; Reding et al., 2015; Werle et al., 2015; Werle, Sampson, et al., 2017). Werle, Sampson, et al. (2017) determined nearly 90% of ambrosia beetle captures occurred in a row of ethanol-baited intercept traps placed along a nursery/forest interface. Scolytinae trap captures from Ohio in 2016, Virginia in 2016–2017 and Mississippi in 2017...
provide further support that trap captures decrease with increasing distance from the edge of woodlots. The opposite scenario observed in Mississippi in 2016 is likely attributed to an unexpected source of beetles that emerged from infested crape myrtle (Lagerstroemia indica L.) stems that were inadvertently left in a pile on the side of the research plots opposite of the woodlot edge.

Cook et al. (2007) noted that a “push–pull” strategy has considerable potential in horticulture due to the unique production areas and high crop value, but the strategy has not yet been widely adopted. Results from our current study did not find that integrating verbenone and ethanol semiochemicals as part of a “push–pull” management strategy effectively suppressed ambrosia beetle attacks on vulnerable trees. Still, a “push–pull” strategy seems appropriate for ambrosia beetles attacking tree crops, especially since their behaviour can be modified through semiochemicals and the dispersal of overwintered adults lends itself to interception. Optimizing the “push” and “pull” components as previously described might facilitate implementing the strategy for management purposes.

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AUTHORS’ CONTRIBUTIONS

CTW, CMR, PBS, MR, KMA and JBO conceived the research. CTW, CMR, PBS, MR, KMA and JBO conducted experiments and statistical analyses. CTW and CMR contributed equally to writing the manuscript. CMR, PBS, MR, KMA, JBO and BS secured funding. All authors read and approved the manuscript.

ORCID

Christopher M. Ranger

https://orcid.org/0000-0002-2012-6984

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