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Abstract

Non-native ambrosia beetles, (Coleoptera: Curculionidae), especially Xylosandrus compactus (Eichhoff), Xylosandrus crassiusculus (Motschulsky), and Xylosandrus germanus (Blandford), are destructive wood-boring pests of trees in ornamental nurseries and tree fruit orchards. Previous studies have demonstrated the adults are repelled by verbenone and strongly attracted to ethanol. We tested a ‘push-pull’ semiochemical strategy in Ohio, Virginia, and Mississippi using verbenone emitters to ‘push’ beetles away from vulnerable trees and ethanol lures to ‘pull’ them into annihilative traps. Container-grown trees were flood-stressed to induce ambrosia beetle attacks and then deployed in the presence or absence of verbenone emitters and a perimeter of ethanol-baited interception traps to achieve the following treatment combinations: (1) untreated control, (2) verbenone only, (3) ethanol only, and (4) verbenone plus ethanol. Verbenone and ethanol did not interact to reduce attacks on the flooded trees, nor did verbenone alone reduce attacks. The ethanol-baited traps intercepted enough beetles to reduce attacks on trees deployed in Virginia and Mississippi in 2016, but not in 2017, or in Ohio in 2016. Xylosandrus germanus, X. crassiusculus, and both Hypothenemus dissimilis Zimmermann and X. crassiusculus were among the predominant species collected in ethanol-baited traps deployed in Ohio, Virginia, and Mississippi, respectively. Xylosandrus germanus and X. crassiusculus were also the predominant species dissected from trees deployed in Ohio and Virginia, respectively. While the ethanol-baited traps showed promise for helping to protect trees by intercepting ambrosia beetles, the repellent ‘push’ component (i.e. verbenone) and attractant ‘pull’ component (i.e. ethanol) will need to be further optimized in order to implement a ‘push-pull’ semiochemical strategy.

Keywords: stimulo-deterrent diversion, Scolytinae, ethanol, verbenone

1 | Introduction

Tree crops grown in ornamental nurseries and tree fruit orchards are threatened by several species of exotic ambrosia beetles, especially Xylosandus compactus (Eichhoff), Xylosandus crassiusculus (Motschulsky), and Xylosandus germanus (Blandford) (Coleoptera:

Curculionidae: Scolytinae) (Chong, Khan & Williamson, 2009; Agnello, Breth, Tee, Cox & Warren, 2014; Ranger et al., 2016a). 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 (French & Roeper, 1972; Biedermann & Taborsky, 2011). 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 e.g., *Fusarium* (Carrillo et al. 2014). Due to their wood-boring behavior and association with branch dieback and tree death, ambrosia beetles are often ranked among the most destructive insect pests of nursery trees (Oliver & Mannion, 2001; Fulcher et al., 2012; Ranger et al., 2016a). 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 et al., 2013a; Reding et al., 2015; Werle et al., 2015, 2017a). 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, Reid & Williamson, 2009; Ranger et al., 2016a). 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 et al., 2013b, 2015). 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 that predispose trees to beetle attack (Ranger et al., 2013b, 2015). During efficacy trials, flood stress has been used experimentally to promote ambrosia beetle attacks (Ranger et al., 2016b; Addesso et al., 2018).

Due to the preference of opportunistic ambrosia beetles for trees emitting ethanol, maintaining tree health is the primary foundation of a management plan. Conventional insecticides can be preventively applied to weakened and vulnerable trees, but they do not consistently reduce attacks below the low threshold ornamental growers have for ambrosia beetles (Frank & Sadof, 2011; Reding et al., 2013; Ranger et al., 2016b). Behaviorally manipulating host-seeking female beetles using a combination of repellents and attractants could be a sustainable alternative to conventional insecticides. First described by Pyke, Rice, Sabine & Zalucki (1987), and later formulated by Miller & Cowles (1990), a 'push-pull' or stimulo-deterrent strategy uses behavior-modifying stimuli (e.g., visual, chemical, and tactile cues) to manipulate the distribution of pests and/or natural enemies on host plants and within tree stands (Cook, Khan & Pickett, 2007). For instance, repellents could 'push' insects away from vulnerable crops, while attractants simultaneously 'pull' them into annihilative traps or trap crops.

Regarding a potential 'push' component for ambrosia beetles, the majority of studies conducted to date have assessed repellence associated with verbenone (4,6,6-trimethylbicyclo[3.1.1] hept-3-en-2-one). Verbenone was first identified from the hindgut of the southern pine beetle Dendroctonus frontalis Zimmerman and the western pine beetle Dendroctonus brevicomis LeConte (Renwick, 1967), and has since been demonstrated to act as an anti-aggregation pheromone for various bark beetles, including Dendroctonus spp. and Ips spp. (Coleoptera: Curculionidae) (Borden, Chong, Earle & Huber, 2003; Bentz, Kegley, Gibson, & Their, 2005; Gillette et al., 2006; Graves et al., 2008). Verbenone also reduces attraction of the ambrosia beetles X. compactus, X. crassiusculus, X. germanus, Xyleborinus saxesenii (Ratz.), and Xyleborus glabratus Eichhoff to ethanol-baited traps and/or ethanol-emitting trees (Dudley, Stein, Jones, & Gillette, 2006; Burbano et al., 2012; Van Der Laan & Ginzel, 2013; Ranger et al., 2013a; Hughes et al., 2017).

Regarding a 'pull' component, ethanol is the most efficacious compound for attracting a variety of opportunistic ambrosia beetles, including X. compactus, X. crassiusculus, and X. germanus (Miller & Rabaglia, 2009; Ranger, Reding, Persad & Herms, 2010). A strong positive correlation exists between ethanol emission and attraction of ambrosia beetles to ethanol-baited traps and ethanol-emitting trees (Klimetzek, Kohler, Vite, & Kohnle, 1986; Ranger, Reding, Schultz, & Oliver, 2012). Since ambrosia beetles disperse from woodlots into ornamental nurseries and the majority of individuals (~70−90%) are captured within 13 m of the nursery/forest interface (Ranger et al., 2013a; Reding et al., 2015; Werle et al., 2015, 2017a; Seo, Martini, Rivera & Stelinski, 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 behavior-manipulating stimuli by integrating the 'push' and 'pull' components (Pyke, Rice, Sabine, & Zalucki, 1987; Miller & Cowles, 1990; Cowles & Miller, 1992; Cook, Khan, & Pickett, 2007). 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 behavior 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: (1) no verbenone/no ethanol, (2) verbenone/no ethanol, (3) no verbenone/ethanol, and (4) verbenone/ethanol (Fig. 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 (Fig. 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) (Fig. 1). Two groupings of 3−4 flood-stressed trees were positioned within each of the two subplots in the presence or absence of a verbenone dispenser (see 'Push' Component) (Fig. 1). The flood-stressed trees were approximately 10−12 m from the edge of the previously described woodlots in Ohio, Virginia, and Mississippi.

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 (Fig. 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/d 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 (Fig. 1). This configuration resulted in 5 traps being in close proximity to the woodlot edge (~0 m), 2 traps at an intermediate distance (~10 m), and the remaining 5 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, Colorado). 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 were 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 center of each subplot (Fig. 1) were flood-stressed using a pot-in-pot protocol by Ranger et al. (2013b) 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 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 center 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 caliper, 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, Ohio). The media was also top dressed with Osmocote Plus 15−9−12 (Scotts Co., Marysville, Ohio) slow release fertilizer. Trees were fertilized with Jack's Classic All Purpose 20−20−20 (JR Peters, Inc., Allentown, Pennsylvania) 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 caliper, 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 (Koelreuteria paniculata Laxm.) were placed within each subplot (16 trees per plot, Fig. 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 caliper, 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.

2.3 | 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: (1) untreated control, (2) verbenone only, (3) ethanol only, and (4) verbenone plus ethanol. Since 3−4 flooded trees were used in each subplot (Fig. 1), the total number of attacks occurring per tree in the subplots were 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) (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 4B-C). Only the most predominant specimens 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 (Fig. 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 (Fig. 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, 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 and verbenone would 'push' 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, behavior-modifying semiochemicals are known for several of the most destructive species, and second, the dispersal of ambrosia beetles from woodlots into production areas favors 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 behavior-modifying effects of verbenone against ambrosia beetles (Dudley, Stein, Jones, & Gillette, 2006; Burbano et al. 2012; VanDerLaan & Ginzel, 2013; Ranger et al., 2013a, 2014), 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 et al., 2013a). A positive correlation occurred between attacks and distance from verbenone emitters, but the results were inconsistent (Ranger et al., 2013a). 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 behavior 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 et al. 2011). Since temperature plays a critical role in the emission of terpenoids (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 terpinolene (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 deterrent (Werle et al., 2017b).

Regarding the 'pull' component, ethanol is the most attractive compound known for several of the most destructive Xylosandrus spp. ambrosia beetles and is used a standard lure for monitoring programs (Miller & Rabaglia, 2009). Thirty non-native ambrosia beetles in the tribe Xyleborini are established in N. America (Gomez et al., 2018), and many of these species are likely to be attracted to ethanol. The exotic species X. germanus and X. crassiusculus were the predominant species collected in ethanol-baited traps deployed in Ohio and Virginia, respectively. Xylosandrus germanus and X. crassiusculus were also the predominate species dissected from attacked trees in Ohio and Virginia, respectively. Thus, the ethanol-based interception tactic effectively targeted the key species attacking vulnerable trees. Previous studies have demonstrated a correlation between concentration of ethanol emissions and attraction of opportunistic ambrosia beetles (Montgomery & Wargo, 1983; Klimetzek et al., 1986; Ranger et al., 2012). Increasing the number of lures per trap from one in 2016 to three in 2017 did not reduce the number of attacks on flood-stressed trees deployed in Virginia or Mississippi. Still, lures with considerably higher release rates compared to the 65 mg per day per lure tested in our current study should be evaluated further. The optimal release rate of ethanol needs to be determined since Montgomery & Wargo (1983) found a release rate of 2 g per day was more attractive than higher release rates. Ethanol-baited traps might also be enhanced by adding additional attractants, for instance, conophthorin (Van DerLaan & Ginzel, 2013; Ranger et al., 2014) or benzaldehyde (Yang, Kim, & Kim, 2018).

Different trap designs should also be evaluated for maximizing the interception of ambrosia beetles. Montgomery & Wargo (1983) found vane traps were more effective than sticky traps at capturing Scolytinae beetles. Similarly, Miller et al. (2018) demonstrated variability across geographic locations in the effectiveness of bottle traps vs. 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, Reding et al. (2010) demonstrated that traps 0.5 m above the ground captured more *X. germanus* than traps at 1.7 or 3.0 m, and taps 0.5 or 1.7 m above the ground captured more *X. crassiusculus* than traps at 3.0 m.

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, 2013b; Reding et al., 2015; Werle et al., 2015, 2017a). Werle et al. (2017a) 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, Khan, & Pickett (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 behavior 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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Author Contribution

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.

References

Addesso, K., Baysal-Gurel, F., Oliver, J., Ranger, C., & O'Neal, P. (2018). Interaction of a fungicide preventative treatment and root rot pathogen on ambrosia beetle attacks during a simulated flood event. Insects, 9. doi:10.3390/insects9030083.

Agnello, A., Breth, D., Tee, E., Cox, K. & Warren, H.R. (2014). Ambrosia beetle–an emergent apple pest. New York Fruit Quarterly, 23, 25−28.

Bentz, B. J., Kegley, S., Gibson, K. & Their, R. (2005). A test of high-dose verbenone for stand-level protection of lodgepole and whitebark pine from mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) attacks. Journal of Economic Entomology, 98, 1614−1621.

Biedermann, P. H. W. & Taborsky, M. (2011). Larval helpers and age polytheism in ambrosia beetles. Proceedings of the National Academy of Sciences USA, 108, 17064−17069.

Borden, J. H., Chong, L. J., Earle, T. J. & Huber, D. P. W. (2003). Protection of lodgepole pine from attack by the mountain pine beetle, Dendroctonus ponderosae (Coleoptera: Scolytidae) using high doses of verbenone in combination with nonhost bark volatiles. The Forestry Chronicle, 79, 685−691.

Burbano, E. G., Wright, M. G., Gillette, N. E., Mori, S., Dudley, N., Jones, T. & Kaufmann, M. (2012). Efficacy of traps, lures, and repellents for Xylosandrus compactus (Coleoptera: Curculionidae) and other ambrosia beetles on Coffea arabica plantations and Acacia koa nurseries in Hawaii. Environmental Entomology, 41, 133−140.

Burt, S. (2004) Essential oils: Their antimicrobial properties and potential applications in foods: A review. International Journal of Food Microbiology, 94, 223–253.

Carrillo, D., Duncan, R. E., Ploetz, J. N., Campbell, A. F., Ploetz, R. C. & Peña, J. E. (2014). Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathology, 63, 54−62.

Chong, J.H., Reid, L. & Williamson, M. (2009). Distribution, host plants, and damage of the black twig borer, Xylosandrus compactus (Eichhoff), in South Carolina. Journal of Agricultural and Urban Entomology, 26, 199−208.

Cook, S. M., Khan, Z. R. & Pickett, J. A. (2007). The use of push-pull strategies in IPM. Annual Review of Entomology, 52, 375−400.

Cowles, R. S. & Miller, J. R. (1992) Diverting Delia antiqua (Diptera: Anthomyiidae) oviposition with cull onions: field studies on planting depth and a greenhouse test of the stimulo-deterrent concept. Environmental Entomology, 21, 453–60.

Dodds, K. J. & Miller, D. R. (2010). Test of nonhost angiosperm volatiles and verbenone to protect trap trees for Sirex noctilio (Hymenoptera: Siricidae) from attacks by bark beetles (Coleoptera: Scolytidae) in the northeastern United States. Journal of Economic Entomology, 103, 2094−2099.

Dudley, N., Stein, J. D., Jones, T. & Gillette, N. E. (2006). Semiochemicals provide a deterrent to the black twig borer, Xylosandrus compactus (Coleoptera: Curculionidae, Scolytinae). In K. W. Gottschalk (Ed.), U.S.D.A. Interagency Research Forum on Gypsy Moth and other Invasive Species (p. 34). USDA Forest Service, Northern Research Station, Annapolis, MD.

Frank, S.D. & Sadof, C.S. (2011). Reducing insecticide volume and nontarget effects of ambrosia beetle management in nurseries. Journal Economic Entomology, 104, 1960−1968.

French, J. R. & Roeper, R. A. (1972). Interactions of the ambrosia beetle, Xyleborus dispar (Coleoptera: Scolytidae), with its symbiotic fungus Ambrosiella hartgii (Fungi Imperfecti). The Canadian Entomologist, 104, 1635−1641.

Fulcher, A., Klingeman, W. E., Chong, J. H., LeBude, A., Armel, G. R., Chappell, M., Frank, S., Hale, F., Neal, J., White, S., Williams-Woodard, J., Ivors, K., Adkins, C., Senesac, A. & Windham, A. (2012). Stakeholder vision of future direction and strategies for southeastern U. S. nursery pest research and extension programming. Journal of Integrated Pest Management, 3, 1−8.

Gillette, N. E., Stein, J. D., Owen, D. R., Webster, J. N., Fiddler, G. O., Mori, S. R. & Wood, D. L. (2006). Verbenone-releasing flakes protect individual Pinus contorta trees from attack by Dendroctonus ponderosae and Dendroctonus valens (Coleoptera: Curculionidae, Scolytinae). Agricultural and Forest Entomology, 8, 243−251.

Gomez, D. F., Rabaglia, R. J., Fairbanks, K. E. O., & J. Hulcr (2018). North American Xyleborini north of Mexico: a review and key to genera and species (Coleoptera, Curculionidae, Scolytinae). ZooKeys, 768, 19−68.

Graves, A. D., Holsten, E. H., Ascerno, M. E., Zogas, K. P., Hard, J. S., Huber, D. P. W., Blanchette, R. A. & Seybold, S. J. (2008). Protection of spruce from colonization by the bark beetle, Ips perturbatus, in Alaska. Forest Ecology and Management, 256, 1825−1839.

Grégoire, J.C., Piel, F., De Proft, M. & Gilbert, M. (2001). Spatial distribution of ambrosia-beetle catches: a possibly useful knowledge to improve mass-trapping. Integrated Pest Management Reviews, 6, 237–242.

Hughes, M. A., Martini, X., Kuhns, E., Colee, J., Mafra-Neto, A., Stelinski, L. L. & Smith, J. A. (2017). Evaluation of repellents for the redbay ambrosia beetle, Xyleborus glabratus, vector of the laurel wilt pathogen. Journal of Applied Entomology, 141, 653−664.

Klimetzek, D., Kohler, J., Vite, J. P. & Kohnle, U. (1986). Dosage response to ethanol mediates host selection by 'secondary' bark beetles. Naturwissenschaften, 73, 270−272.

Maleknia, S. D., Vail, T. M., Cody, R. B., Sparkman, D. O., Bell, T. L. & Adams, M. A. (2009). Temperature- dependent release of volatile organic compounds of eucalypts by direct analysis in real time (DART) mass spectrometry. Rapid Communications in Mass Spectrometry, 23, 2241−2246.

Miller, D.R. & Rabaglia, R.J. (2009). Ethanol and (−)-α-pinene: Attractant kairomones for bark and ambrosia beetles in the southeastern US. Journal of Chemical Ecology, 35, 435−448.

Miller, D. R., Crowe, C. M., Ginzel, M. D., Ranger, C. M. & Schultz, P. B. (2018). Comparison of baited bottle and multiple funnel traps for ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in Eastern United States. Journal of Entomological Science, 53, 347−360.

Miller, J. R. & Cowles, R. C. (1990). Stimulo-deterrent diversion: A concept and its possible application to onion maggot control. Journal of Chemical Ecology, 16, 3197−3212.

Montgomery, M. E. & Wargo, P. M. (1983). Ethanol and other host-derived volatiles as attractants to beetles that bore into hardwoods. Journal of Chemical Ecology, 9, 181−190.

Oliver, J. B. & Mannion, C. M. (2001). Ambrosia beetle (Coleoptera: Scolytidae) species attacking chestnut and captured in ethanol-baited traps in middle Tennessee. Environmental Entomology, 30, 909−918.

Pyke, B., Rice, M., Sabine, B. & Zalucki, M. P. (1987). The push-pull strategy - behavioural control of Heliothis. Australian Cotton Grower, 8, 7−9.

Ranger, C. M., Reding, M. E., Persad, A. B. & Herms, D. A. (2010). Ability of stress-related volatiles to attract and induce attacks by Xylosandrus germanus and other ambrosia beetles. Agricultural and Forest Entomology, 12, 177−185.

Ranger, C. M., Reding, M. E., Schultz, P. B. & Oliver, J. B. (2012). Ambrosia beetle (Coleoptera: Curculionidae) response to volatile emissions associated with ethanol-injected Magnolia virginiana. Environmental Entomology, 41, 636−647.

Ranger, C. M., Tobin, P. C., Reding, M. E., Bray, A. M., Oliver, J. B., Schultz, P. B., Frank, S. D. & Persad, A. B. (2013a). Interruption of semiochemical-based attraction of ambrosia beetles to ethanol-baited traps and ethanol-injected trap trees by verbenone. Environmental Entomology, 42, 539−547.

Ranger, C. M., Reding, M. E., Schultz, P. B. & Oliver, J. B. (2013b). Influence of flood-stress on ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) host selection and implications for their management in a changing climate. Agricultural and Forest Entomology, 15, 56−64.

Ranger, C. M., Gorzlancyk, A. G., Held, D. W., Addesso, K., Oliver, J. B., Reding, M. E. & Schultz, P. B. (2014). Conophthorin enhances the electroantennogram and field behavioral response of Xylosandrus germanus (Coleoptera: Curculionidae) to ethanol. Agricultural and Forest Entomology, 16, 327−334.

Ranger, C.M., Schultz, P.B., Frank, S.D., Chong, J.H. and Reding, M.E. (2015) Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. PLoS One, 10(7), p.e0131496.

Ranger, C. M., Reding, M. E., Schultz, P. B., Oliver, J. B., Frank, S. D., Addesso, K. M., Chong, J.-H., Sampson, B. J., Werle, C. T., Gill, S. & Krause, C. (2016a). Biology, ecology, and management of nonnative ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in ornamental plant nurseries. Journal of Integrated Pest Management, 7, 1−23.

Ranger, C.M., Schultz, P.B., Reding, M.E., Frank, S.D. & Palmquist, D.E. (2016b). Flood stress as a technique to assess preventive insecticide and fungicide treatments for protecting trees against ambrosia beetles. Insects, 7, 40; doi:10.3390/insects7030040

Ranger, C. M., Biedermann, P. H. W., Phuntumart, V., Beligala, G. U., Ghosh, S., Palmquist, D. E., Mueller, R., Barnett, J., Schultz, P. B., Reding, M. E. & Benz, J. P. (2018). Symbiont selection via alcohol benefits fungus-farming by ambrosia beetles. Proceedings of the National Academy of Sciences USA, 115, 4447−4452.

Reding, M., Oliver, J., Schultz, P., & Ranger, C. (2010). Monitoring flight activity of ambrosia beetles in ornamental nurseries with ethanol-baited traps: influence of trap height on captures. Journal of Environmental Horticulture, 28, 85−90.

Reding, M.E., Oliver, J.B., Schultz, P.B., Ranger, C.M. & Youssef, N.N. (2013). Ethanol injection of ornamental trees facilitates testing insecticide efficacy against ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Journal of Economic Entomology, 106, 289−298.

Reding, M. E., Ranger, C. M., Sampson, B. J., Werle, C. T., Oliver, J. B. & Schultz, P. B. (2015). Movement of Xylosandrus germanus (Coleoptera: Curculionidae) in ornamental nurseries and surrounding habitats. Journal of Economic Entomology, 108, 1947−1953.

Renwick, J. A. A. (1967). Identification of two oxygenated terpenes from the bark beetles Dendroctonus frontalis and Dendroctonus brevicomis. Contrib. Boyce Thompson Inst, 23, 355−360.

Rowan, D. D. (2011) Volatile metabolites. Metabolites, 1, 41−63.

SAS Institute (2001). SAS/STAT® User's Guide (release 8.2). Cary, NC.

Seo, M., Martini, X., Rivera, M. J. & Stelinski, L. L. (2017). Flight capacities and diurnal flight patterns of the ambrosia beetles, Xyleborus glabratus and Monarthrum mali (Coleoptera: Curculionidae). Environmental Entomology, 46, 729−734.

Van Der Laan, N. R. & Ginzel, M. D. (2013). The capacity of conophthorin to enhance the attraction of two Xylosandrus species (Coleoptera: Curculionidae: Scolytinae) to ethanol and the efficacy of verbenone as a deterrent. Agricultural and Forest Entomology, 15, 391−397.

Werle, C. T., Chong, J-H., Sampson, B. J., Reding, M. E. & Adamczyk, J. J. (2015). Seasonal and spatial dispersal patterns of select ambrosia beetles (Coleoptera: Curculionidae) from forest habitats into production nurseries. Florida Entomologist, 98, 884−891.

Werle, C. T., Sampson, B. J. & Reding, M. E. (2017a). A role for intercept traps in the ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) IPM strategy at ornamental nurseries. Midsouth Entomologist, 10, 14−23.

Werle, C. T., Addesso, K. M., Sampson, B. J., Oliver, J. B. & Adamczyk, J. J. (2017b). Integrating kaolin clay for ambrosia beetle management in ornamental crops of Eastern redbud. HortScience, 52, 94−98.

FIGURE 1 Plot design used to test a 'push-pull' strategy for protecting flood-stressed trees from attack by ambrosia beetles, whereby verbenone (V) dispensers were used as the 'push' component and ethanol-baited traps (X) were used as the 'pull' component. Within each plot, clusters 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 2A-E Mean (±SE) ambrosia beetle attacks per flood-stressed tree deployed in (A) Ohio 2016, (B) Virginia 2016, (C) Virginia 2017, (D) Mississippi 2016, and (E) Mississippi 2017. 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 (see Fig. 1). No significant difference was detected in a verbenone × ethanol interaction effect or a verbenone main effect, but a significant ethanol main effect was detected in (B) Virginia 2016 and (D) Mississippi 2016 (see Table 2).

FIGURE 3A-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 Fig. 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.

FIGURE 4A-E Mean (±SE) captures of Scolytinae per site in ethanol-baited interception traps as part of 'push-pull' experiments conducted in (A) Ohio in 2016, Virginia in (B) 2016 and (C) 2017, and Mississippi in (D) 2016 and (E) 2017. Different letters within a location and year indicate significant differences (one-way ANOVA; Tukey's HSD) (A) $F = 15.23$; df = 15, 48; $P < 0.0001$; (B) $F = 16.82$; df = 2,9; $P = 0.0009$; (C) $F = 9.94$; df = 3,12; $P = 0.0014$; (D) $F = 17.42$; df = 12, 26; $P < 0.0001$; (E) $F = 16.19$; df = 11, 24; $P < 0.0001$.

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.

	OH 2016	**VA 2016**	**VA 2017**	**MS 2016**	**MS 2017**
Source	**F, P**	**F, P**	**F, P**	**F, P**	**F, P**
Ethanol	0.97, 0.35	5.53, 0.04	0.81, 0.39	11.79, 0.01	0.33, 0.58
Verbenone	0.01, 0.93	0.36, 0.56	0.07, 0.80	1.73, 0.23	0.03, 0.87
Ethanol × Verbenone	0.44, 0.52	2.95, 0.11	0.11, 0.74	0.02, 0.88	0.03, 0.87

[a] See Fig. 2 for mean (±SE) values.

[b] 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.

Species	Mean (±SE) per Tree				F; P
	No Verbenone No Ethanol	**Verbenone No Ethanol**	**No Verbenone Ethanol**	**Verbenone Ethanol**	
Eggs	13.8 ± 6.4A	9.3 ± 3.7A	3.9 ± 2.9A	16.6 ± 11.1A	0.54, 0.45
A. maiche	0.33 ± 0.3Ab	0.1 ± 0.1Ab	0.0 ± 0.0Bb	0.0 ± 0.0Bb	4.42; 0.04
H. dissimilis	0.0 ± 0.0Ab	0.0 ± 0.0Ab	0.1 ± 0.1Ab	0.0 ± 0.0Ab	1.00; 0.32
X. crassiusculus	1.6 ± 1.2Ab	1.1 ± 1.0Ab	0.3 ± 0.3Ab	1.3 ± 0.6Ab	2.83; 0.1
X. germanus	23.1 ± 9.7Aa	19.3 ± 5.6Aa	11.8 ± 3.3Aa	17.3 ± 6.8Aa	0.22; 0.64
X. saxesenii	1.3 ± 0.7Ab	0.7 ± 0.4Ab	0.8 ± 0.4Ab	0.3 ± 0.2Ab	0.01; 0.92
F; P	12.12; 0.0004	17.03; <0.0001	22.07; <0.0001	34.59; <0.0001	

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).

TABLE 3. Specimens recovered from flood-stressed C. florida trees deployed in Virginia in 2016 during 'push-pull' field trials.

Species	Mean (±SE) per Tree				F; P
	No Verbenone No Ethanol	**Verbenone No Ethanol**	**No Verbenone Ethanol**	**Verbenone Ethanol**	
Eggs	89.3 ± 27.6A	112.3 ± 21.4A	95.2 ± 19.9A	128.8 ± 22.8A	0.00; 0.97
Larvae	247.2 ± 82.5A	222.4 ± 23.5A	262.6 ± 42.8A	246.3 ± 11.2A	0.05; 0.83
Pupae	17.6 ± 7.1A	7.9 ± 3.3A	15.9 ± 9.4A	4.8 ± 2.9A	0.27; 0.61
A. rubricollis	0.6 ± 0.4Abc	0.9 ± 0.1Ac	0.4 ± 0.1Ab	0.4 ± 0.2Ad	1.00; 0.34
X. compactus	3.4 ± 0.5Ab	5.6 ± 0.8Ab	2.3 ± 0.8Ab	3.6 ± 1.1Ab	0.07; 0.80
X. crassiusculus	33.8 ± 5.6Aa	29.1 ± 4.2Aa	28.5 ± 4.9Aa	27.6 ± 1.5Aa	0.19; 0.67
X. germanus	4.2 ± 2.1Ab	1.2 ± 0.4Ac	4.9 ± 3.6Ab	1.9 ± 0.3Abc	0.34; 0.57
C. mutilatus	2.3 ± 1.1Abc	2.2 ± 0.6Ac	1.4 ± 0.2Ab	0.8 ± 0.1Acd	0.63; 0.44
X. saxesenii	0.1 ± 0.1Ac	0.7 ± 0.5Ac	1.4 ± 0.8Ab	0.5 ± 0.4Acd	2.03; 0.18
F; P	19.66; <0.0001	42.44; <0.0001	11.43; <0.0001	45.23; <0.0001	

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).

TABLE 4. Specimens recovered from flood-stressed C. florida trees deployed in Virginia in 2017 during 'push-pull' field trials.

Species	Mean (±SE) per Tree				F; P
	No Verbenone No Ethanol	**Verbenone No Ethanol**	**No Verbenone Ethanol**	**Verbenone Ethanol**	
Eggs	89.8 ± 27.4A	112.6 ± 21.1A	95.2 ± 19.9A	128.8 ± 22.8A	0.00; 0.96
Larvae	264.4 ± 74.6A	239.2 ± 22.0A	262.6 ± 42.8A	246.3 ± 11.2A	0.02; 0.90
Pupae	19.8 ± 7.2A	9.9 ± 2.5A	15.9 ± 9.4A	4.8 ± 2.9A	0.64; 0.44
A. rubricollis	0.5 ± 0.5Ad	0.8 ± 0.2Ac	0.4 ± 0.1Ab	0.4 ± 0.2Ad	0.84; 0.38
X. compactus	1.9 ± 0.7Abcd	5.5 ± 0.8Ab	2.4 ± 0.8Ab	3.6 ± 1.1Ab	1.61; 0.23
X. crassiusculus	33.8 ± 5.6Aa	29.1 ± 4.2Aa	28.5 ± 4.9Aa	27.6 ± 1.5Aa	0.19; 0.67
X. germanus	4.5 ± 1.9Ab	1.2 ± 0.4Ac	4.9 ± 3.6Ab	1.9 ± 0.3Abc	0.87; 0.37
C. mutilatus	3.4 ± 1.0Abc	2.3 ± 0.6Abc	1.3 ± 0.3Ab	0.8 ± 0.1Acd	0.01; 0.92
X. saxesenii	0.5 ± 0.3Acd	0.7 ± 0.5Ac	1.4 ± 0.8Ab	0.5 ± 0.4Acd	0.77; 0.39
F; P	22.52; <0.0001	41.42; <0.0001	11.38; <0.0001	45.23; <0.0001	

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).

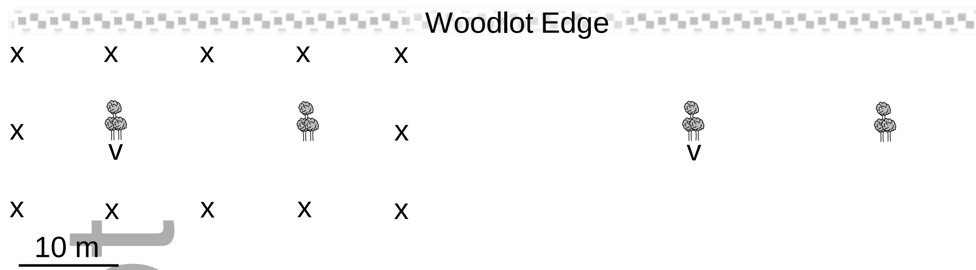

FIGURE 1

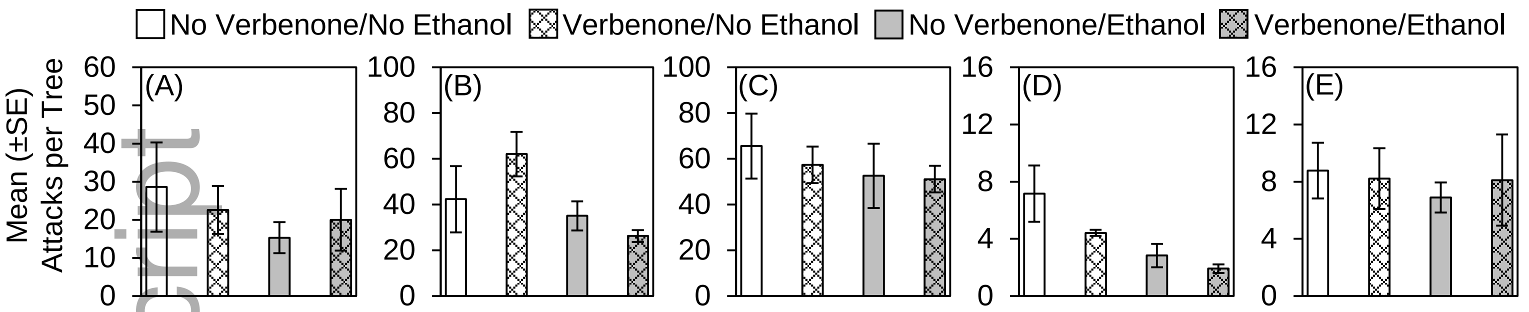
No Verbenone/No Ethanol
Verbenone/No Ethanol
No Verbenone/Ethanol
Verbenone/Ethanol
Mean (±SE)
Attacks per Tree
(A)
(B)
(C)
(D)
(E)
0
10
20
30
40
50
60
80
100
4
8
12
16

FIGURE 2

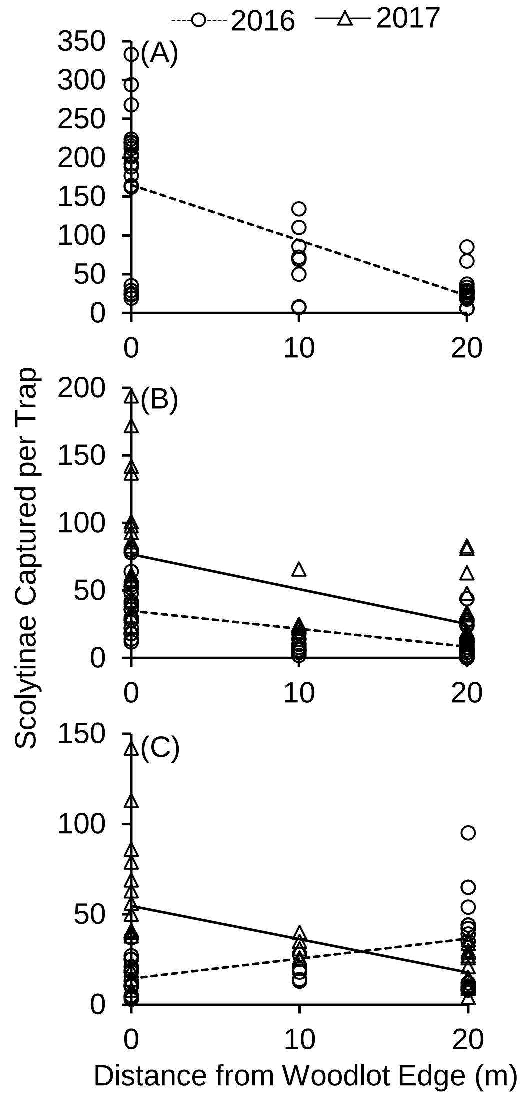
2016
2017
(A)
(B)
(C)
350
300
250
200
150
100
50
0
0
10
20
Scolytinae Captured per Trap
Distance from Woodlot Edge (m)

FIGURE 3

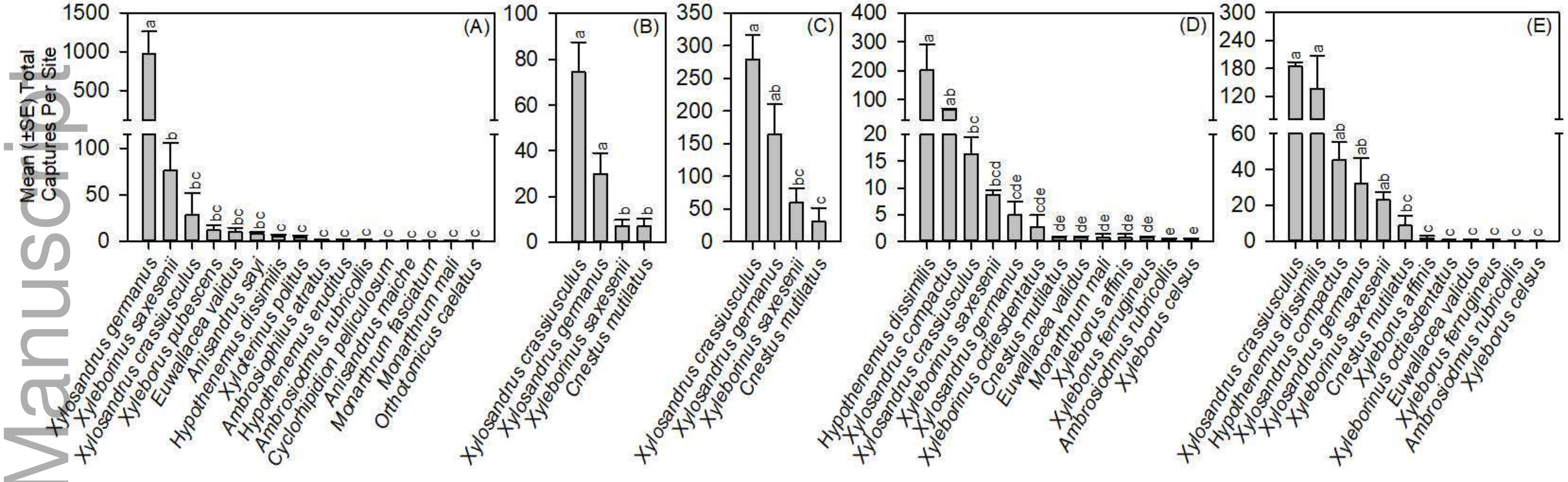
Mean (±SE) Total Captures Per Site
(A)
(B)
(C)
(D)
(E)
Xylosandrus germanus
Xyleborinus saxesenii
Xylosandrus crassiusculus
Xyleborus pubescens
Euwallacea validus
Anisandrus sayi
Hypothenemus dissimilis
Xyloterinus politus
Ambrosiophilus atratus
Hypothenemus eruditus
Ambrosiodmus rubricollis
Cyclorhipidion pelliculosum
Anisandrus maiche
Monarthrum fasciatum
Monarthrum mali
Orthotomicus caelatus
Cnestus mutilatus
Xylosandrus compactus
Xyleborinus octiesdentatus
Xyleborus affinis
Xyleborus ferrugineus
Xyleborus celsus

FIGURE 4