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Original Contribution

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Ambrosia Beetles (Coleoptera: Curculionidae)**

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36 **Abstract**

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

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

59 **1 | Introduction**

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

63 Curculionidae: Scolytinae) (Chong, Khan & Williamson, 2009; Agnello, Breth, Tee, Cox &
64 Warren, 2014; Ranger et al., 2016a). Adult females tunnel into the stems and branches of trees
65 to cultivate gardens of their fungal symbiont on which the larvae and adults must feed to
66 properly develop and reproduce (French & Roeper, 1972; Biedermann & Taborsky, 2011).
67 Ambrosia beetle fungal symbionts are rarely pathogenic, but a variety of secondary
68 microorganisms can be passively introduced to trees, some of which are tree pathogens e.g.,
69 *Fusarium* (Carrillo et al. 2014). Due to their wood-boring behavior and association with
70 branch dieback and tree death, ambrosia beetles are often ranked among the most destructive
71 insect pests of nursery trees (Oliver & Mannion, 2001; Fulcher et al., 2012; Ranger et al.,
72 2016a). Even small numbers of ambrosia beetle attacks can lead to economic losses for
73 nurseries due to reduced tree marketability.

74 After leaving their overwintering sites within host tree galleries, adult female ambrosia
75 beetles disperse from wooded habitats into ornamental nurseries in search of a new host tree
76 (Ranger et al., 2013a; Reding et al., 2015; Werle et al., 2015, 2017a). Opportunistic species
77 such as *X. compactus*, *X. crassiusculus*, and *X. germanus* attack a broad range of trees with an
78 apparent preference for thin-barked deciduous species (Chong, Reid & Williamson, 2009;
79 Ranger et al., 2016a). Despite a broad host range, host quality plays an important role during
80 tree selection by opportunistic ambrosia beetles. Physiologically-stressed trees can emit
81 ethanol, a volatile compound used by female beetles as a chemical indicator of weakened trees
82 (Ranger et al., 2013b, 2015). The presence of ethanol within host tree tissues also promotes
83 the growth of their fungal symbionts and inhibits fungal competitors, thereby improving the
84 colonization success of ambrosia beetles (Ranger et al., 2018). A variety of abiotic and biotic
85 factors induce the emission of ethanol, but water stress (i.e., flooding) and low temperature
86 stress (i.e. freezing and frost) are among the key stressors in ornamental nurseries that
87 predispose trees to beetle attack (Ranger et al., 2013b, 2015). During efficacy trials, flood
88 stress has been used experimentally to promote ambrosia beetle attacks (Ranger et al., 2016b;
89 Adesso et al., 2018).

90 Due to the preference of opportunistic ambrosia beetles for trees emitting ethanol,
91 maintaining tree health is the primary foundation of a management plan. Conventional
92 insecticides can be preventively applied to weakened and vulnerable trees, but they do not
93 consistently reduce attacks below the low threshold ornamental growers have for ambrosia

94 beetles (Frank & Sadof, 2011; Reding et al., 2013; Ranger et al., 2016b). Behaviorally
95 manipulating host-seeking female beetles using a combination of repellents and attractants
96 could be a sustainable alternative to conventional insecticides. First described by Pyke, Rice,
97 Sabine & Zalucki (1987), and later formulated by Miller & Cowles (1990), a ‘push-pull’ or
98 stimulo-deterrent strategy uses behavior-modifying stimuli (e.g., visual, chemical, and tactile
99 cues) to manipulate the distribution of pests and/or natural enemies on host plants and within
100 tree stands (Cook, Khan & Pickett, 2007). For instance, repellents could ‘push’ insects away
101 from vulnerable crops, while attractants simultaneously ‘pull’ them into annihilative traps or
102 trap crops.

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

115 Regarding a ‘pull’ component, ethanol is the most efficacious compound for attracting a
116 variety of opportunistic ambrosia beetles, including *X. compactus*, *X. crassiusculus*, and *X.*
117 *germanus* (Miller & Rabaglia, 2009; Ranger, Reding, Persad & Herms, 2010). A strong
118 positive correlation exists between ethanol emission and attraction of ambrosia beetles to
119 ethanol-baited traps and ethanol-emitting trees (Klimetzek, Kohler, Vite, & Kohnle, 1986;
120 Ranger, Reding, Schultz, & Oliver, 2012). Since ambrosia beetles disperse from woodlots into
121 ornamental nurseries and the majority of individuals (~70–90%) are captured within 13 m of
122 the nursery/forest interface (Ranger et al., 2013a; Reding et al., 2015; Werle et al., 2015,
123 2017a; Seo, Martini, Rivera & Stelinski, 2017), ethanol-baited traps could potentially be used
124 to intercept host-seeking ambrosia beetles.

125 Several studies have indicated that additive or synergistic effects can enhance the
126 effectiveness of behavior-manipulating stimuli by integrating the ‘push’ and ‘pull’
127 components (Pyke, Rice, Sabine, & Zalucki, 1987; Miller & Cowles, 1990; Cowles & Miller,
128 1992; Cook, Khan, & Pickett, 2007). An additive effect occurs when the combined effect is
129 equal to the sum of the individual effects, while a synergistic effect occurs when the effect of
130 the combined compounds is greater than the sum of their individual effects (Burt, 2004).
131 Since previous studies have demonstrated verbenone and ethanol influence the behavior of
132 ambrosia beetles, we hypothesized that additivity or synergy between verbenone (i.e. push
133 component) and ethanol (i.e. pull component) would function to minimize attacks by
134 ambrosia beetles on vulnerable trees. The overall objective of our current study was to test the
135 efficacy of verbenone and ethanol individually and combined for protecting flood-stressed
136 trees from attack by opportunistic ambrosia beetles.

137

138 **2 | Materials and Methods**

139 2.1 | Plot Design

140 Experiments were conducted at three different geographic locations (Ohio, Virginia, and
141 Mississippi) to target populations of key species, particularly *X. compactus*, *X. crassiusculus*,
142 and *X. germanus*. Plots were arranged in Mississippi, Ohio, and Virginia to test the integration
143 of verbenone (i.e., push component) and ethanol (i.e., pull component) for protecting flood-
144 stressed trees from attack by ambrosia beetles. The plot design included the following ‘push-
145 pull’ treatments: (1) no verbenone/no ethanol, (2) verbenone/no ethanol, (3) no
146 verbenone/ethanol, and (4) verbenone/ethanol (Fig. 1).

147 Each field plot consisted of two 40 × 20 m subplots that were adjacent to the edge of
148 woodlots supporting natural populations of non-native and native ambrosia beetles (Fig. 1).
149 The field plots used in Ohio, Virginia, and Mississippi were grass-dominated and recently
150 mowed prior to initiating experiments. The woodlots adjacent to the field plots used in Ohio,
151 Virginia, and Mississippi were dominated by mature deciduous trees with a few coniferous
152 trees interspersed throughout. One of the 40 × 20 m subplots included a perimeter of ethanol-
153 baited traps spaced 10 m apart, whereas the other 40 × 20 m subplot lacked a perimeter of
154 ethanol-baited traps (see ‘Pull’ Component) (Fig. 1). Two groupings of 3–4 flood-stressed
155 trees were positioned within each of the two subplots in the presence or absence of a

156 verbenone dispenser (see 'Push' Component) (Fig. 1). The flood-stressed trees were
157 approximately 10–12 m from the edge of the previously described woodlots in Ohio, Virginia,
158 and Mississippi.

159 Four replicated plots were established in Wayne Co., Ohio (40°46'21"N, 81°56'02"W),
160 (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).

161 Four replicated plots were established in York County, Virginia (37°17'17.8"N,
162 76°38'59.1"W). Three replicated plots were established in Mississippi with two replicates in
163 Pearl River Co., Mississippi (30°39'34.36"N, 89°38'06.46"W) and a third replicate in
164 Hancock Co., Mississippi (30°21'09.17"N, 89°38'29.99"W). Field trials were conducted in
165 Ohio from 25 May 2016 to 31 May 2016; Virginia from 11 April 2016 to 2 May 2016 and 5
166 April 2017 to 1 May 2017; and Mississippi from 7 April 2016 to 2 June 2016 and 6 April
167 2017 to 8 May 2017.

168

169 2.1.1 | 'Push' Component

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

176

177 2.1.2 | 'Pull' Component

178 Ethanol-baited traps were deployed at 10 m intervals around the perimeter of one of the two
179 subplots (Fig. 1). This configuration resulted in 5 traps being in close proximity to the
180 woodlot edge (~0 m), 2 traps at an intermediate distance (~10 m), and the remaining 5 traps
181 being the furthest from the woodlot edge (~20 m). Traps were constructed using two recycled
182 soda bottles (~0.6 L and 2 L sizes) attached with a Tornado Tube (Steve Spangler Science,
183 Englewood, CO) (Ranger et al., 2010). The upper 2 L bottle had three rectangular openings
184 (length 15 cm, width 6 cm) cut into the sides for beetle entry, while the lower 0.6 L bottle was
185 partially filled with propylene glycol to collect and preserve insects. Traps were suspended 1
186 m above the ground using metal rods and baited with an ethanol sachet lure (65 mg/day at

187 25°C; AgBio, Inc., Westminster, Colorado). One ethanol lure was used in each trap in Ohio,
188 Virginia, and Mississippi in 2016, while three lures were used per trap in Mississippi and
189 Virginia in 2017. Since a positive concentration response exists between ambrosia beetles and
190 ethanol emissions (Klimetzek et al., 1986), the number of lures per interception trap were
191 increased in 2017 to assess if higher ethanol emission corresponded with decreased attacks on
192 the flood-stressed trees. Field experiments were not conducted in Ohio in 2017. Trap contents
193 were periodically collected throughout the duration of each experiment at each location, with
194 specimens returned to the laboratory and identified to species. All specimens collected in
195 Ohio and Mississippi were identified to species and quantified, while only the most
196 predominant specimens were identified to species and quantified in Virginia in 2016 and
197 2017.

198

199 2.1.3 | Imposing Flood-Stress

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

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

214 In the Virginia 2016 and 2017 trials, four flood-stressed dogwood trees (*C. florida*) were
215 placed in each subplot (16 trees per plot). Flood-stressed *C. florida* trees used in the Virginia
216 experiments were 4 years old, 3.8 cm caliper, and growing in 28 L pots containing a mixture

217 of 92:8 aged pine bark:coarse sand, and dolomitic lime to stabilize pH. The media was top
218 dressed with Osmocote Plus 15–9–12 (Scotts Co., Marysville, Ohio) slow release fertilizer.

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

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

234 235 2.3 | Statistical Analysis

236 A two-way ANOVA was used to test the interaction of the ‘push’ and ‘pull’ components,
237 along with the two main effects, on cumulative ambrosia beetle attacks on the flood-stressed
238 trees (SAS Institute, 2001). Tukey's HSD test ($\alpha = 0.05$) was used to separate differences
239 among treatments in the number of attacks occurring on trees subjected to one of the
240 following four treatments: (1) untreated control, (2) verbenone only, (3) ethanol only, and (4)
241 verbenone plus ethanol. Since 3–4 flooded trees were used in each subplot (Fig. 1), the total
242 number of attacks occurring per tree in the subplots were considered subsamples and therefore
243 averaged prior to analysis. Regression analysis was used to test for a correlation between trap
244 distance from the woodlot edge and ambrosia beetle captures. Data were $\log(x+1)$ transformed
245 prior to analysis, but untransformed data are presented.

246 247 3 | Results

248 3.1 | Efficacy of ‘Push-Pull’ Strategy

249 The repellent effect of verbenone and the attractant effect of ethanol did not significantly
250 interact as part of a ‘push-pull’ strategy to reduce or prevent attacks on flood-stressed trees
251 during field experiments conducted in Ohio (2016), Virginia (2016–2017), or Mississippi
252 (2016–2017) (Fig. 2A-E, Table 1). The verbenone-based ‘push’ component was also not
253 associated with a significant main effect at reducing attacks on the flood-stressed trees in any
254 location or year (Fig. 2A-E, Table 1). By contrast, the ethanol-based ‘pull’ component
255 exhibited a significant main effect at reducing attacks on the flood-stressed trees deployed in
256 Mississippi and Virginia in 2016, but not Ohio in 2016 or Mississippi and Virginia in 2017
257 (Fig. 2A-E, Table 1). While the perimeter of ethanol-baited traps reduced attacks on the flood-
258 stressed trees deployed in Mississippi and Virginia in 2016, the traps did not completely
259 prevent attacks from occurring.

260

261 3.2 | Dispersal of Ambrosia Beetles

262 A negative correlation was observed between Scolytinae trap captures and distance of the
263 ethanol-baited traps from the edge of the woodlot (Fig. 3A-C), such that beetle captures
264 decreased with an increasing distance from the woodlot edge for Ohio in 2016 ($r^2 = 0.51$; $F =$
265 47.42 ; $df = 1, 46$; $P < 0.0001$), Virginia in 2016 ($r^2 = 0.36$; $F = 26.02$; $df = 1, 46$; $P < 0.0001$)
266 and 2017 ($r^2 = 0.31$; $F = 20.72$; $df = 1, 46$; $P < 0.0001$), and Mississippi in 2017 ($r^2 = 0.31$; $F =$
267 15.75 ; $df = 1, 34$; $P = 0.0004$). A positive correlation instead of a negative correlation was
268 observed in Mississippi in 2016 between Scolytinae trap captures and distance from the edge
269 of the woodlot ($r^2 = 0.25$; $F = 11.31$; $df = 1, 34$; $P = 0.002$).

270

271 3.3 | Scolytinae Abundance and Distribution

272 The perimeter of ethanol-baited traps positioned around the flood-stressed trees captured a
273 total of 4,491 Scolytinae specimens in Ohio in 2016, consisting of 16 species (Fig. 4A).

274 *Xylosandrus germanus* was the most predominant species collected in ethanol-baited traps
275 deployed in Ohio in 2016, representing 86.5% (3,889 specimens) of the total trap captures.

276 Ethanol-baited traps caught 475 and 2,136 Scolytinae specimens in Virginia in 2016 and
277 2017, respectively (Fig. 4B-C). Only the most predominant specimens were identified to
278 species in Virginia in 2016 and 2017. *Xylosandrus crassiusculus* and *X. germanus* were the

279 two most predominant species collected in Virginia in 2016 and represented 62.7% (298
280 specimens) and 25.3% (120 specimens) of the total trap captures, respectively. Similarly, *X.*
281 *crassiusculus* and *X. germanus* were the two most predominant species collected in Virginia
282 in 2017 and represented 52.2% (1,115 specimens) and 30.8% (658 specimens) of the total trap
283 captures, respectively.

284 In Mississippi in 2016 and 2017, 917 and 1,304 Scolytinae specimens were collected,
285 respectively (Fig. 4D-E). *Hypothenemus dissimilis* (Zimmermann) and *X. compactus* were the
286 most predominant species collected in Mississippi in 2016, representing 66.0% (605
287 specimens) and 22.0% (202 specimens) of the total trap captures. In 2017, *X. crassiusculus*,
288 *H. dissimilis* and *X. compactus* were the most predominant species collected in Mississippi,
289 representing 42.3% (552 specimens), 31.4% (410 specimens), and 10.4% (136 specimens) of
290 the total trap captures, respectively. Notably, *X. crassiusculus*, *X. germanus*, and *X. saxesenii*
291 were the three non-native species collected in all three states (Fig. 4A-E).

292

293 3.4 | Scolytinae Attacking Flood-Stressed Trees

294 In Ohio in 2016, 952 specimens representing five Scolytinae species were recovered from
295 flood-stressed *C. florida* trees, namely, *X. germanus*, *X. crassiusculus*, *X. saxesenii*,
296 *Anisandrus maiche* Stark, and *H. dissimilis* (Table 2). Similar to the ethanol-baited traps, *X.*
297 *germanus* was the most predominant species recovered from flood-stressed *C. florida* trees
298 deployed in Ohio in 2016 (Table 2) representing 90.0% of the total specimens. Relatively few
299 specimens of other Scolytinae were recovered from the dissected trees, including *X.*
300 *crassiusculus* as 5.5%, *X. saxesenii* as 3.8%, *A. maiche* as 0.5%, and *H. dissimilis* as 0.1% of
301 total specimens (Table 2). Fewer *A. maiche* were recovered from flood-stressed trees
302 protected by the perimeter of ethanol-baited traps compared to trees without the perimeter of
303 traps (Table 2). However, this effect was not detected for the remaining species. In addition to
304 the adult specimens, eggs were recovered from Scolytinae galleries created in the flood-
305 stressed *C. florida* trees. The presence or absence of the verbenone emitters or the ethanol-
306 baited traps did not have an effect on the number of eggs dissected per tree (Table 2).

307 A total of 3,383 Scolytinae specimens were recovered from flood-stressed *C. florida* trees
308 deployed in Virginia in 2016. The five most common species were *X. crassiusculus*, *X.*
309 *germanus*, *X. compactus*, *Ambrosiodmus rubricollis* (Eichhoff), and *X. saxesenii*. Similar to

310 the ethanol-baited traps, *X. crassiusculus* was the most predominant species recovered from
311 flood-stressed *C. florida* trees deployed in Virginia in 2016, representing 56.3% of the total
312 specimens (Table 3). *Xylosandrus compactus* represented 7.1%, *X. germanus* represented
313 5.8%, *C. mutilatus* represented 3.2%, *X. saxesenii* represented 1.3%, and *A. rubricollis*
314 represented 1.1% of total specimens recovered from flood-stressed *C. florida* trees deployed
315 in Virginia in 2016. Scolytinae eggs, larvae, and pupae were recovered from galleries created
316 in the flood-stressed trees, but there was no effect by the presence or absence of verbenone
317 emitters and the ethanol-baited traps (Table 3).

318 A total of 3,466 Scolytinae specimens were recovered from flood-stressed *C. florida* trees
319 deployed in Virginia in 2017. *Xylosandrus crassiusculus* was the most predominant species
320 recovered from flood-stressed *C. florida* trees deployed in Virginia in 2017, representing
321 55.0% of the total specimens, followed by *X. compactus* as 6.2%, *X. germanus* as 5.8%, *C.*
322 *mutilatus* as 3.6%, *X. saxesenii* as 1.4%, and *A. rubricollis* as 1.0% (Table 4). There was no
323 effect of the presence or absence of the verbenone emitters or the ethanol-baited traps on the
324 recovery of the aforementioned species from the flood-stressed trees (Table 4). Scolytinae
325 eggs, larvae, and pupae were recovered from the flood-stressed *C. florida* trees deployed in
326 Virginia in 2017, but there was no effect by the presence or absence of verbenone emitters and
327 the ethanol-baited traps (Table 4).

328

329 4 | Discussion

330 As part of multistate trials, the verbenone-based ‘push’ component did not provide an
331 acceptable level of protection against ambrosia beetle attacks on the flood-stressed trees. In
332 some instances, the ethanol-based ‘pull’ component intercepted enough ambrosia beetles to
333 reduce attacks on the flood-stressed trees, but the effect was variable across locations and
334 years. There were no indications of an additive or synergistic effect between verbenone and
335 ethanol. The results obtained as part of our current study did not meet the expectations of our
336 original hypothesis that ethanol would ‘pull’ beetles and verbenone would ‘push’ beetles
337 away from stressed trees. Still, two factors suggest a ‘push-pull’ management strategy has
338 utility for protecting trees against ambrosia beetles in ornamental nurseries and tree fruit
339 orchards; first, behavior-modifying semiochemicals are known for several of the most
340 destructive species, and second, the dispersal of ambrosia beetles from woodlots into

341 production areas favors a semiochemical-based interception tactic. The repellent and attractant
342 semiochemical components will need to be further optimized to implement a viable ‘push-
343 pull’ management strategy. Additional studies should assess a higher verbenone release rate or
344 release mechanism for the ‘push’ component, along with evaluating other potential repellents.
345 Applying a repellent, reduced-risk, or conventional insecticide directly to vulnerable trees
346 should also be evaluated. A higher release rate of ethanol as part of the ‘pull’ component
347 should also be assessed, along with comparing the efficacy of various trap designs for
348 maximizing captures of the most destructive Scolytinae species. These factors are discussed in
349 greater detail below.

350 Because previous studies have demonstrated the behavior-modifying effects of verbenone
351 against ambrosia beetles (Dudley, Stein, Jones, & Gillette, 2006; Burbano et al. 2012;
352 VanDerLaan & Ginzler, 2013; Ranger et al., 2013a, 2014), the lack of effect as part of our
353 current study was unexpected. Notably, verbenone reduced attacks by *X germanus* on
354 herbicide-injected *Pinus resinosa* Aiton trees, but it did not completely prevent them from
355 occurring (Dodds & Miller 2010). Similarly, verbenone reduced captures of *X. germanus* in
356 ethanol-baited traps by >95% compared to ethanol alone (Ranger et al., 2013a). A positive
357 correlation occurred between attacks and distance from verbenone emitters, but the results
358 were inconsistent (Ranger et al., 2013a). Since the verbenone emitters were placed in close
359 proximity to the flood-stressed trees as part of our current study, but did not reduce attacks,
360 the attractiveness of the stressed trees perhaps overpowered the repellence of the verbenone
361 emitters. For instance, the higher volatility of ethanol compared to verbenone might result in
362 ethanol influencing ambrosia beetle behavior at long and short ranges while verbenone would
363 be active at a shorter range. Notably, ethanol has a lower molecular weight (46.07 g/mol) and
364 boiling point (78°C) compared to the molecular weight (150.21 g/mol) and boiling point
365 (227–228°C) of verbenone (Rowan 2011; Zhao et al. 2011). Since temperature plays a critical
366 role in the emission of terpenoids (Maleknia et al. 2009; Zhao et al. 2011), emission of
367 verbenone from the emitters used as part of our current study might not have been high
368 enough to strongly repel ambrosia beetles during their peak spring flight activity.

369 Increasing the release rate or release mechanism of verbenone might aid in reducing
370 attacks on trees. Gillette et al. (2006) proposed that verbenone dispensing strategies could
371 influence efficacy, and the deployment of many small, point-source releasers, such as

372 verbenone-releasing flakes, could be an improvement over plastic pouches or bubblecap
373 dispensers. Screening for a more effective repellent is also warranted; previous studies have
374 demonstrated terpinolene (Ranger et al., 2014) and methyl salicylate (Hughes et al., 2017)
375 repel ambrosia beetles. Application of kaolin clay to stems was also demonstrated to reduce
376 attacks, perhaps by acting as a settling deterrent (Werle et al., 2017b).

377 Regarding the ‘pull’ component, ethanol is the most attractive compound known for
378 several of the most destructive *Xylosandrus* spp. ambrosia beetles and is used a standard lure
379 for monitoring programs (Miller & Rabaglia, 2009). Thirty non-native ambrosia beetles in the
380 tribe Xyleborini are established in N. America (Gomez et al., 2018), and many of these
381 species are likely to be attracted to ethanol. The exotic species *X. germanus* and *X.*
382 *crassiusculus* were the predominant species collected in ethanol-baited traps deployed in Ohio
383 and Virginia, respectively. *Xylosandrus germanus* and *X. crassiusculus* were also the
384 predominate species dissected from attacked trees in Ohio and Virginia, respectively. Thus,
385 the ethanol-based interception tactic effectively targeted the key species attacking vulnerable
386 trees. Previous studies have demonstrated a correlation between concentration of ethanol
387 emissions and attraction of opportunistic ambrosia beetles (Montgomery & Wargo, 1983;
388 Klimetzek et al., 1986; Ranger et al., 2012). Increasing the number of lures per trap from one
389 in 2016 to three in 2017 did not reduce the number of attacks on flood-stressed trees deployed
390 in Virginia or Mississippi. Still, lures with considerably higher release rates compared to the
391 65 mg per day per lure tested in our current study should be evaluated further. The optimal
392 release rate of ethanol needs to be determined since Montgomery & Wargo (1983) found a
393 release rate of 2 g per day was more attractive than higher release rates. Ethanol-baited traps
394 might also be enhanced by adding additional attractants, for instance, conophthorin (Van
395 DerLaan & Ginzl, 2013; Ranger et al., 2014) or benzaldehyde (Yang, Kim, & Kim, 2018).

396 Different trap designs should also be evaluated for maximizing the interception of
397 ambrosia beetles. Montgomery & Wargo (1983) found vane traps were more effective than
398 sticky traps at capturing Scolytinae beetles. Similarly, Miller et al. (2018) demonstrated
399 variability across geographic locations in the effectiveness of bottle traps vs. funnel traps for
400 capturing key species, such as *A. maiche*, *X. crassiusculus*, and *X. germanus*, thereby
401 warranting additional studies to characterize the basis for discrepancies. Since trap density did
402 not substantially impact mass-trapping of *X. germanus* (Grégoire, Piel, De Proft & Gilbert,

403 2001), it is unlikely that spacing traps any closer than a 10 m distance between traps would be
404 beneficial or economically feasible. Trap height is also an important factor for intercepting
405 certain ambrosia beetles. For instance, Reding et al. (2010) demonstrated that traps 0.5 m
406 above the ground captured more *X. germanus* than traps at 1.7 or 3.0 m, and traps 0.5 or 1.7 m
407 above the ground captured more *X. crassiusculus* than traps at 3.0 m.

408 Our current study further supports that the ideal placement of traps for *X. crassiusculus*
409 and *X. germanus* is at the interface of wooded habitats and tree production areas (Ranger et
410 al., 2010, 2013b; Reding et al., 2015; Werle et al., 2015, 2017a). Werle et al. (2017a)
411 determined nearly 90% of ambrosia beetle captures occurred in a row of ethanol-baited
412 intercept traps placed along a nursery/forest interface. Scolytinae trap captures from Ohio in
413 2016, Virginia in 2016-2017, and Mississippi in 2017 provide further support that trap
414 captures decrease with increasing distance from the edge of woodlots. The opposite scenario
415 observed in Mississippi in 2016 is likely attributed to an unexpected source of beetles that
416 emerged from infested crape myrtle (*Lagerstroemia indica* L.) stems that were inadvertently
417 left in a pile on the side of the research plots opposite of the woodlot edge.

418 Cook, Khan, & Pickett (2007) noted that a ‘push-pull’ strategy has considerable potential
419 in horticulture due to the unique production areas and high crop value, but the strategy has not
420 yet been widely adopted. Results from our current study did not find that integrating
421 verbenone and ethanol semiochemicals as part of a ‘push-pull’ management strategy
422 effectively suppressed ambrosia beetle attacks on vulnerable trees. Still, a ‘push-pull’ strategy
423 seems appropriate for ambrosia beetles attacking tree crops, especially since their behavior
424 can be modified through semiochemicals and the dispersal of overwintered adults lends itself
425 to interception. Optimizing the ‘push’ and ‘pull’ components as previously described might
426 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.

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621 **FIGURE 1** Plot design used to test a ‘push-pull’ strategy for protecting flood-stressed trees
622 from attack by ambrosia beetles, whereby verbenone (V) dispensers were used as the ‘push’
623 component and ethanol-baited traps (X) were used as the ‘pull’ component. Within each plot,
624 clusters of 3–4 flood-stressed trees were subjected to the following four treatments: (1) no
625 verbenone/no ethanol, (2) verbenone/no ethanol, (3) no verbenone/ethanol, and (4)
626 verbenone/ethanol.

627

628 **FIGURE 2A-E** Mean (\pm SE) ambrosia beetle attacks per flood-stressed tree deployed in (A)
629 Ohio 2016, (B) Virginia 2016, (C) Virginia 2017, (D) Mississippi 2016, and (E) Mississippi
630 2017. Flood-stressed trees were subjected to the following four treatments: (1) no
631 verbenone/no ethanol, (2) verbenone/no ethanol, (3) no verbenone/ethanol, and (4)
632 verbenone/ethanol (see Fig. 1). No significant difference was detected in a verbenone \times
633 ethanol interaction effect or a verbenone main effect, but a significant ethanol main effect was
634 detected in (B) Virginia 2016 and (D) Mississippi 2016 (see Table 2).

635

636 **FIGURE 3A-C** Correlation between distance of ethanol-baited traps from the woodlot edge
637 and ambrosia beetle captures as part of ‘push-pull’ experiments conducted in (A) Ohio, (B)
638 Virginia, and (C) Mississippi (see Fig. 1 for layout of traps in relation to edge of woodlot)
639 (Dashed lines are fitted to 2016 data while solid lines are fitted to 2017 data). Experiments
640 were conducted in 2016 in Ohio, and 2016 and 2017 in Virginia and Mississippi. Trap
641 captures generally decreased with decreasing proximity from the edge.

642

643 **FIGURE 4A-E** Mean (\pm SE) captures of Scolytinae per site in ethanol-baited interception
644 traps as part of 'push-pull' experiments conducted in (A) Ohio in 2016, Virginia in (B) 2016
645 and (C) 2017, and Mississippi in (D) 2016 and (E) 2017. Different letters within a location
646 and year indicate significant differences (one-way ANOVA; Tukey's HSD) (A) $F = 15.23$; df
647 $= 15, 48$; $P < 0.0001$; (B) $F = 16.82$; $df = 2, 9$; $P = 0.0009$; (C) $F = 9.94$; $df = 3, 12$; $P = 0.0014$;
648 (D) $F = 17.42$; $df = 12, 26$; $P < 0.0001$; (E) $F = 16.19$; $df = 11, 24$; $P < 0.0001$.

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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.

	OH	VA	VA	MS	MS
	2016	2016	2017	2016	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 (\pm SE) values.

^b df = 1 for all analyses.

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TABLE 2 Specimens recovered from flood-stressed *C. florida* trees deployed in Ohio in 2016 during ‘push-pull’ field trials.

Species	Mean (\pm SE) per Tree				F; P
	No Verbenone	Verbenone	No Verbenone	Verbenone	
	No Ethanol	No Ethanol	Ethanol	Ethanol	
Eggs	13.8 \pm 6.4A	9.3 \pm 3.7A	3.9 \pm 2.9A	16.6 \pm 11.1A	0.54, 0.45
<i>A. maiche</i>	0.33 \pm 0.3Ab	0.1 \pm 0.1Ab	0.0 \pm 0.0Bb	0.0 \pm 0.0Bb	4.42; 0.04
<i>H. dissimilis</i>	0.0 \pm 0.0Ab	0.0 \pm 0.0Ab	0.1 \pm 0.1Ab	0.0 \pm 0.0Ab	1.00; 0.32
<i>X. crassiusculus</i>	1.6 \pm 1.2Ab	1.1 \pm 1.0Ab	0.3 \pm 0.3Ab	1.3 \pm 0.6Ab	2.83; 0.1
<i>X. germanus</i>	23.1 \pm 9.7Aa	19.3 \pm 5.6Aa	11.8 \pm 3.3Aa	17.3 \pm 6.8Aa	0.22; 0.64
<i>X. saxeseni</i>	1.3 \pm 0.7Ab	0.7 \pm 0.4Ab	0.8 \pm 0.4Ab	0.3 \pm 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 (\pm SE) per Tree				F; P
	No Verbenone	Verbenone	No Verbenone	Verbenone	
	No Ethanol	No Ethanol	Ethanol	Ethanol	
Eggs	89.3 \pm 27.6A	112.3 \pm 21.4A	95.2 \pm 19.9A	128.8 \pm 22.8A	0.00; 0.97
Larvae	247.2 \pm 82.5A	222.4 \pm 23.5A	262.6 \pm 42.8A	246.3 \pm 11.2A	0.05; 0.83
Pupae	17.6 \pm 7.1A	7.9 \pm 3.3A	15.9 \pm 9.4A	4.8 \pm 2.9A	0.27; 0.61
<i>A. rubricollis</i>	0.6 \pm 0.4Abc	0.9 \pm 0.1Ac	0.4 \pm 0.1Ab	0.4 \pm 0.2Ad	1.00; 0.34
<i>X. compactus</i>	3.4 \pm 0.5Ab	5.6 \pm 0.8Ab	2.3 \pm 0.8Ab	3.6 \pm 1.1Ab	0.07; 0.80
<i>X. crassiusculus</i>	33.8 \pm 5.6Aa	29.1 \pm 4.2Aa	28.5 \pm 4.9Aa	27.6 \pm 1.5Aa	0.19; 0.67
<i>X. germanus</i>	4.2 \pm 2.1Ab	1.2 \pm 0.4Ac	4.9 \pm 3.6Ab	1.9 \pm 0.3Abc	0.34; 0.57
<i>C. mutilatus</i>	2.3 \pm 1.1Abc	2.2 \pm 0.6Ac	1.4 \pm 0.2Ab	0.8 \pm 0.1Acd	0.63; 0.44
<i>X. saxesenii</i>	0.1 \pm 0.1Ac	0.7 \pm 0.5Ac	1.4 \pm 0.8Ab	0.5 \pm 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 (\pm SE) per Tree				F; P
	No Verbenone	Verbenone	No Verbenone	Verbenone	
	No Ethanol	No Ethanol	Ethanol	Ethanol	
Eggs	89.8 \pm 27.4A	112.6 \pm 21.1A	95.2 \pm 19.9A	128.8 \pm 22.8A	0.00; 0.96
Larvae	264.4 \pm 74.6A	239.2 \pm 22.0A	262.6 \pm 42.8A	246.3 \pm 11.2A	0.02; 0.90
Pupae	19.8 \pm 7.2A	9.9 \pm 2.5A	15.9 \pm 9.4A	4.8 \pm 2.9A	0.64; 0.44
<i>A. rubricollis</i>	0.5 \pm 0.5Ad	0.8 \pm 0.2Ac	0.4 \pm 0.1Ab	0.4 \pm 0.2Ad	0.84; 0.38
<i>X. compactus</i>	1.9 \pm 0.7Abcd	5.5 \pm 0.8Ab	2.4 \pm 0.8Ab	3.6 \pm 1.1Ab	1.61; 0.23
<i>X. crassiusculus</i>	33.8 \pm 5.6Aa	29.1 \pm 4.2Aa	28.5 \pm 4.9Aa	27.6 \pm 1.5Aa	0.19; 0.67
<i>X. germanus</i>	4.5 \pm 1.9Ab	1.2 \pm 0.4Ac	4.9 \pm 3.6Ab	1.9 \pm 0.3Abc	0.87; 0.37
<i>C. mutilatus</i>	3.4 \pm 1.0Abc	2.3 \pm 0.6Abc	1.3 \pm 0.3Ab	0.8 \pm 0.1Acd	0.01; 0.92
<i>X. saxesenii</i>	0.5 \pm 0.3Acd	0.7 \pm 0.5Ac	1.4 \pm 0.8Ab	0.5 \pm 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).

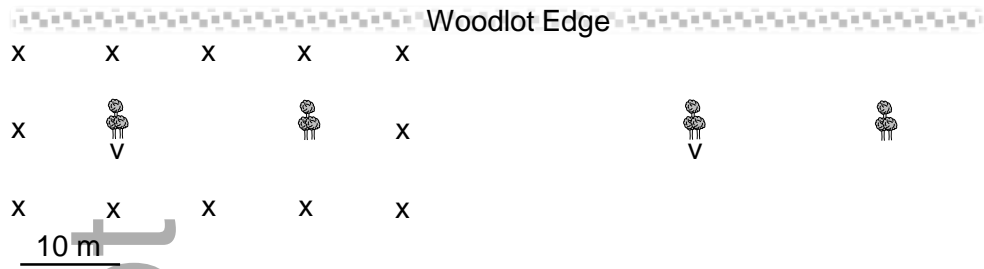


FIGURE 1

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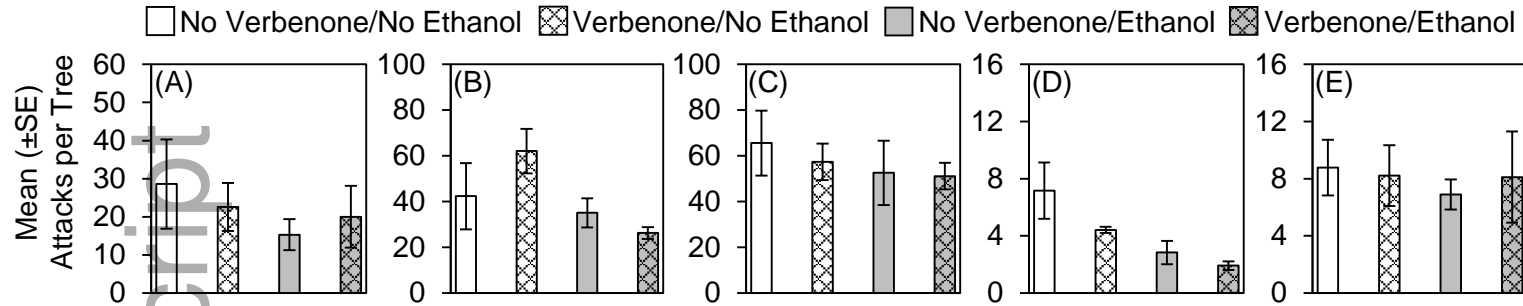


FIGURE 2

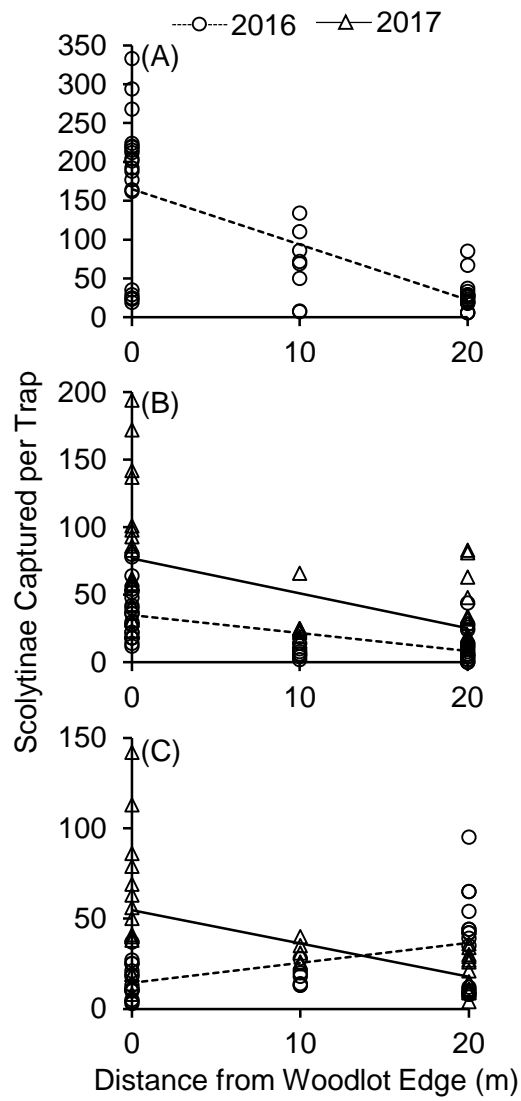


FIGURE 3

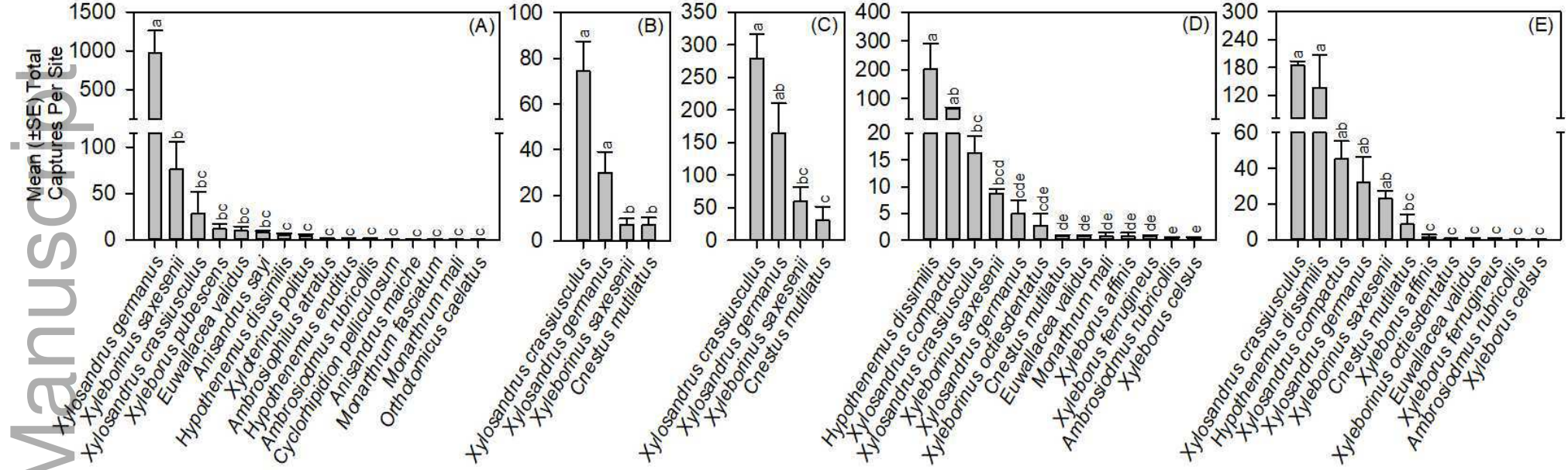


FIGURE 4