Comparison of Baited Bottle and Multiple-Funnel Traps for Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) in Eastern United States[1]

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Comparison of Baited Bottle and Multiple-Funnel Traps for Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) in Eastern United States[1]

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Abstract We compared bottle traps to 4-unit multiple-funnel traps (both baited with ethanol and conophthorin) for relative efficacy in catching ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) at four locations in the eastern United States. Our results were geographically inconsistent for three target species. Catches of *Xylosandrus germanus* (Blandford) in Ohio were greater in bottle traps than in funnel traps while the opposite occurred in Virginia, with no difference in Indiana. Catches of *Xyleborinus saxesenii* (Ratzeburg) were greater in funnel traps than in bottle traps in Georgia, Indiana, and Virginia but no different in Ohio. Similarly, catches of *Xylosandrus crassiusculus* (Motschulsky) were greater in funnel traps than in bottle traps in Georgia and Virginia but not in Indiana. Bottle traps caught more *Anisandrus maiche* Stark in Ohio and *Anisandrus sayi* (Hopkins) in Indiana whereas more of the following species were caught in funnel traps: *Ambrosiophilus atratus* (Eichhoff) in Virginia, *Cyclorhipidion bodoanum* (Reitter) and *Dryoxylon onoharaense* (Murayama) in Georgia, *Euwallacea validus* (Eichhoff) in Ohio, and *Cyclorhipidion pelliculosum* (Eichhoff) and *Monarthrum fasciatum* (Say) in Indiana. Catches of *Cnestus mutilatus* (Blandford) in Georgia and *Monarthrum mali* (Fitch) in Indiana were unaffected by trap type. Differences in trap height, bottle size, and forest composition may have contributed to between-site variability in trap type preferences, thereby requiring further research to resolve these issues.

Key Words ambrosia beetles, kairomones, ethanol, trap type, geographical variation

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are an important target group in detection programs for exotic insects. To date, 58 species of ambrosia beetles have invaded the United States (Haack and Rabaglia 2013). Several species such as *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus germanus* (Blandford) cause economic damage in fruit orchards and horticultural nurseries (Agnello et al. 2015, 2017; Kovach and Gorsuch 1985; Ranger et al. 2015, 2016). Early detection programs targeting nonnative, potentially

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invasive species of ambrosia beetles use traps baited with attractants such as ethanol (Jackson et al. 2010; Rabaglia et al. 2008). Ethanol is broadly attractive to ambrosia beetles (Coyle et al. 2005; Kelsey et al. 2013; Miller and Rabaglia 2009; Oliver and Mannion 2001; Ranger et al. 2010, 2014; Reding et al. 2011).

We found variation in responses of ambrosia beetles to traps baited with ethanol and conophthorin (7-methyl-1,6-dioxaspirol[4.5]decane) (Dodds and Miller 2010; Miller et al. 2015; Ranger et al. 2014; VanDerlaan and Ginzel 2013). Conophthorin is a pheromone for some species of bark beetles, such as the white pine cone beetle *Conophthorus coniperda* (Schwarz), (Birgersson et al. 1995; de Groot and DeBarr 2000) and is found in the bark of several genera of angiosperms (Huber et al. 1999). Catches of *X. germanus* were enhanced by conophthorin in New York with funnel traps (Dodds and Miller 2010) and in Ohio with bottle traps (Ranger et al. 2014) but not in Indiana with bottle traps or in Georgia, Michigan, Oregon, and New Hampshire with funnel traps (Miller et al. 2015; VanDerLaan and Ginzel 2013). For *X. crassiusculus*, conophthorin increased catches in ethanol-baited bottle traps in Indiana (VanDerLaan and Ginzel 2013) but not in Georgia with funnel traps or in Ohio with bottle traps (Miller et al. 2015; Ranger et al. 2014; VanDerLaan and Ginzel 2013). Conophthorin increased catches of *Xyleborinu saxesenii* in ethanol-baited funnel traps in Georgia, New Hampshire, and Oregon (Miller et al. 2015) but not in Ohio with bottle traps (Miller et al. 2015; Ranger et al. 2014).

Multiple-funnel traps are commonly used in detection programs (Jackson et al. 2010; Rabaglia et al. 2008) whereas managers of orchards and horticultural nurseries typically employ plastic bottle traps (Mazón et al. 2013; Oliver et al. 2004; Ranger et al. 2014, 2016; Reding et al. 2011; Steininger et al. 2015). Bottle traps are often preferred because they are easy to make and considerably cheaper than multiple-funnel traps. Our objective in this study was to compare these two trap types for relative efficacy in trapping three common species of nonnative ambrosia beetles in the eastern United States: *X. crassiusculus*, *X. germanus*, and *X. saxesenii* (Ratzeburg). In particular, we sought to determine the contribution of trap type on the observed variation in responses of ambrosia beetles to conophthorin using the same field methods for each location as previously used (Miller et al. 2015; Ranger et al. 2014; VanDerLaan and Ginzel 2013).

Materials and Methods

In 2015, we conducted the same field experiment at four locations in eastern United States (Table 1). Catches of ambrosia beetles in 4-unit multiple funnel traps (Synergy Semiochemicals Corp., Burnaby BC) were compared to those in clear plastic bottle traps. At each location we used trapping protocols, regarding size of bottle traps and trap height, that corresponded to those used in previous publications (Miller et al. 2015; Ranger et al. 2014; VanDerLaan and Ginzel 2013). We used 4-unit funnel traps instead of 10-unit traps as used in Miller et al. (2015) in order to allow height placement to be the same for funnel traps as for bottle traps (Fig. 1). Funnel traps were modified by increasing the center hole of each funnel from 5 cm to 12 cm, allowing placement of all lures within the trap (Miller et al. 2013). As described in Ranger et al. (2014, 2016), bottle traps consisted of two clear plastic bottles (1 L top and 0.5 L bottom in Indiana and Ohio;

Table 1. Locations, coordinates, dominant tree species, and trapping dates for each of four experiments on flight responses of ambrosia beetles to bottle and multiple-funnel traps baited with ethanol and conophthorin in the eastern United States.

Exp	Location	Coordinates	Tree Species	Trapping Dates
1	Oconee National Forest, Greene Co., GA	N 33.750°, W 83.261°	*Pinus taeda* L., *P. echinata* Miller, *Liquidambar styraciflua* L.	1 April–23 June 2015
2	Richard G. Lugar Forestry Farm, Tippecanoe Co., IN	N 40.422°, W 86.966°	*Acer saccharum* Marshall, *Carya ovata* Miller, *Prunus serotina* Ehrhart, *Sassafras albidium* Nees, *Liriodendron tulipifera* L.	5 May–28 July 2015
3	Ohio State University, Wayne Co., OH	N 40.760°, W 81.854°	*Quercus rubra* L., *Q. velutina* Lamark, *P. serotina*	14 May–8 July 2015
4	Hampton Roads AREC, Virginia Beach, VA	N 36.888°, W 76.171°	*Quercus alba* L., *Carya tomentosa* Nuttall	1 April–27 May 2015

2 L top and 0.3 L bottom in Georgia and Virginia) connected by tornado tube connectors (Steve Spangler Science, Englewood, CO). In Indiana and Ohio, each top bottle had two vents (6 × 12.5 cm in Indiana, 7.5 × 11 cm in Ohio) on opposite sides whereas top bottles in Georgia and Virginia had three vents (each 6 × 14 cm) spread evenly around the circumference of the bottle.

At each location, we deployed 10 bottle and 10 funnel traps in 10 replicate blocks of two traps (one bottle and one funnel) per block. Traps were spaced ≥8 m apart and suspended above ground at a height (to bottom of collection cup or bottom bottle) of 1.3–1.5 m in Georgia, 0.2–0.3 m in Ohio, and 0.5–1.0 m in Indiana and Virginia (Fig. 1). Each trap was baited with ethanol and conophthorin lures from Contech Enterprises (Victoria, BC), releasing at 0.25 g/d and 0.5 mg/d, respectively. Lures were tied inside the top funnel of all funnel traps and inside the top of the top bottle in bottle traps, except in Indiana where an ethanol lure was adjacent to the bottle trap (Fig 1B). Collection cups of funnel traps and bottom bottles of bottle traps contained 100–150 ml of a solution of propylene glycol (Miller and Duerr 2008). Splash RV & Marine Antifreeze (Fox Packaging Inc., St. Paul, MN) was used in Georgia, Indiana, and Virginia whereas Prestone RV Waterline Antifreeze (Prestone Products Corp., Danbury, CT) was used in Ohio. Vouchers were deposited in the University of Georgia Collection of Arthropods, Athens, GA.

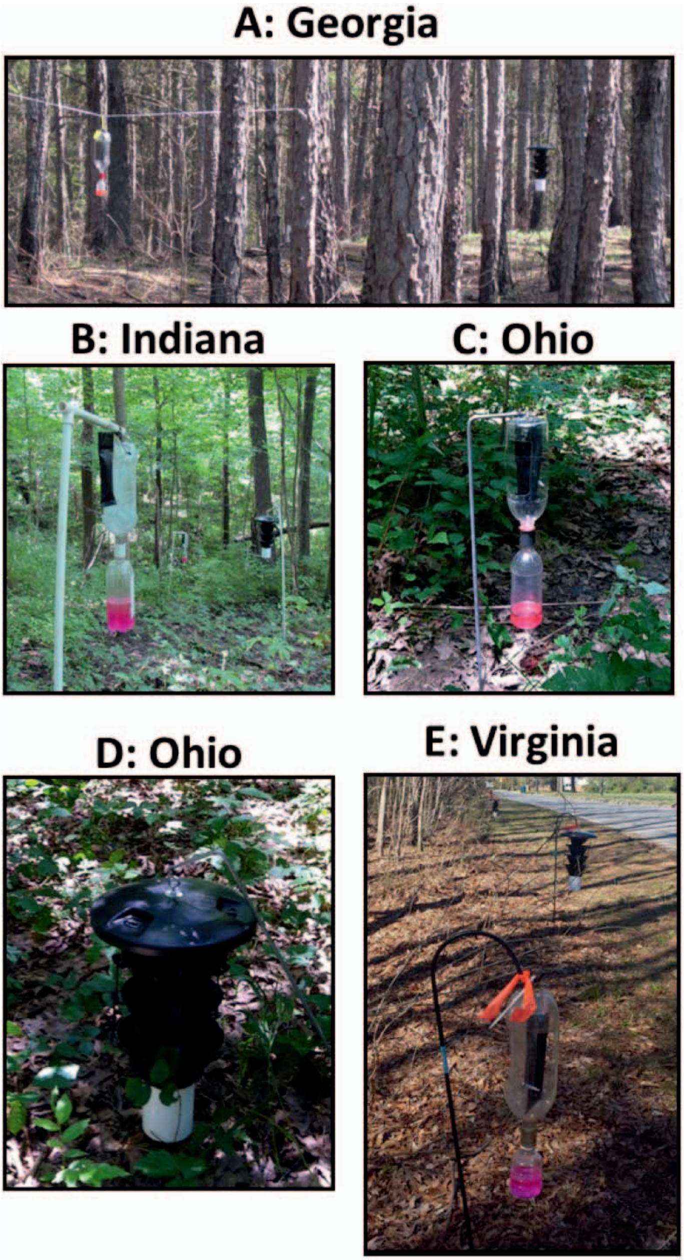

Fig. 1. Trapping sites used in comparing bottle and multiple-funnel traps for catching ambrosia beetles in Georgia (A), Indiana (B), Ohio (C,D) and Virginia (E) in 2015.

Using the SYSTAT (ver. 13) statistical package (SYSTAT Software Inc., Point Richmond, CA), data on species caught at more than one location (*X. saxesenii*, *X. crassiusculus*, and *X. germanus*) in sufficient numbers for analysis ($N \geq 30$) were analyzed by general linear model (GLM) analysis of variance (ANOVA) using the following model factors: (a) replicate nested within location; (b) location; (c) treatment; and (d) location $\times$ treatment. The same model was used to analyze data on the number of scolytine species caught per trap. At each location, using the SigmaStat (ver. 3.01) statistical package (SYSTAT Software Inc.), we conducted two-tailed paired *t*-tests for each species caught in sufficient numbers ($N \geq 30$) as well as on total number of species caught in traps. Normality was verified using the Kolmogorov-Smirnov test.

Results

We caught a total of 36,125 ambrosia beetles in our study, representing 16 common species or taxa (Table 2). Species composition and relative abundance varied among the four locations. The most abundant species in Georgia was *X. crassiusculus* whereas *X. saxesenii* and *X. crassiusculus* were equally abundant in Virginia. The most abundant species in Indiana and Ohio was *X. germanus*. Trap treatment had significant effects across locations on catches of *X. saxesenii* ($F = 92.51$; df $= 1, 36$; $P < 0.001$), *X. crassiusculus* ($F = 13.63$; df $= 1, 27$; $P = 0.001$), and *X. germanus* ($F = 27.35$; df $= 1, 27$; $P < 0.001$). There were significant interactions between location and treatment on catches of the three species ($F = 23.09$; df $= 3, 36$; $P < 0.001$; $F = 6.32$; df $= 2, 27$; $P = 0.006$; and $F = 24.39$; df $= 2, 27$; $P < 0.001$, respectively).

In Georgia, greater numbers of *Cyclorhipidion bodoanum* (Reitter), *Dryoxylon onoharaense* (Murayama), *X. saxesenii*, and *X. crassiusculus* were caught in funnel traps than in bottle traps (Fig. 2). Trap type had no effect on catches of *Cnestus mutilatus* (Blandford). In Indiana, funnel traps caught more *Cyclorhipidion pelliculosum* (Eichhoff), *Monarthrum fasciatum* (Say), and *X. saxesenii* than did bottle traps whereas more *Anisandrus sayi* (Hopkins) were caught in bottle traps than in funnel traps (Fig. 3). Trap type had no effect on catches of *Monarthrum mali* (Fitch), *X. crassiusculus*, and *X. germanus*. In Ohio, greater numbers of *X. germanus* and *Anisandrus maiche* were caught in bottle traps than in funnel traps whereas more *Euwallacea validus* (Eichhoff) were caught in funnel traps than in bottle traps (Fig. 4). Trap type had no effect on catches of *X. saxesenii*. In Virginia, funnel traps caught more of the following species than did bottle traps: *A. atratus*, *X. saxesenii*, *X. crassiusculus*, and *X. germanus* (Fig. 5).

A few species of beetles, often associated with bark and ambrosia beetles, were also caught in our study. In Indiana and Georgia, more *Stenoscellis brevis* (Boheman) (Cossoninae: Curculionidae) were caught in funnel traps than in bottle traps (Table 3). More of the bark beetle predator, *Enoclerus nigripes* (Say) (Cleridae), were caught in funnel traps than in bottle traps in Indiana but not in Ohio. The two longhorn woodborers, *Cyrtophorus verrucosus* (Olivier) and *Gaurotes cyanipennis* (Say) (Cerambycidae), were caught in greater numbers in funnel traps than in bottle traps in Georgia and Indiana, respectively. In contrast, bottle traps caught more *Agrilus* spp. (Buprestidae) than did funnel traps in Indiana.

Table 2. Total catches of ambrosia beetles (Coleoptera) in bottle and multiple-funnel traps baited with ethanol and conophthorin in Georgia, Indiana, Ohio, and Virginia.

Species	Location			
	GA	IN	OH	VA
Ambrosiodmus obliquus (LeConte)	–	–	–	15
Ambrosiophilus atratus (Eichhoff)	–	6	17	146
Anisandrus maiche Stark	–	–	245	–
Anisandrus sayi (Hopkins)	–	76	7	–
Cnestus mutilatus (Blandford)	49	–	–	1
Cyclorhipidion bodoanum (Reitter)	42	–	–	–
Cyclorhipidion pelliculosum Eichhoff	–	87	16	–
Dryoxylon onoharaense (Murayama)	66	–	–	–
Euwallacea validus (Eichhoff)	–	–	42	23
Gnathotrichus materiarius (Fitch)	–	–	–	–
Monarthrum fasciatum (Say)	–	127	–	–
Monarthrum mali (Fitch)	–	53	8	–
Xyleborinus saxesenii (Ratzeburg)	145	1,356	171	1,581
Xyleborus spp.	23	3	–	19
Xylosandrus crassiusculus (Motschulsky)	1,725	623	17	1,347
Xylosandrus germanus (Blandford)	3	12,820	15,220	46
Total number of beetles	2,053	15,151	15,743	3,178

There was a significant effect of location ($F = 7.25$; df = 3, 36; $P = 0.001$) and treatment ($F = 34.07$; df = 1, 36; $P < 0.001$) on the number of scolytine species detected in traps. However, there was no significant interaction between location and treatment ($F = 1.26$; df = 3, 36; $P = 0.301$). Mean species diversity in trap catches was greater in funnel traps than in bottle traps in Georgia, Indiana, and Virginia (Fig. 6). A treatment effect on mean number of scolytine species found in traps was not detected with the Holm-Sidak test in Ohio.

Discussion

In summary, catches of *X. germanus* were greater in bottle traps than in funnel traps in Ohio while the opposite was true for Virginia, and no difference was observed in Indiana (Figs. 2–5). Catches of *X. saxesenii* were greater in funnel traps than in bottle traps in Georgia, Indiana, and Virginia but no difference occurred

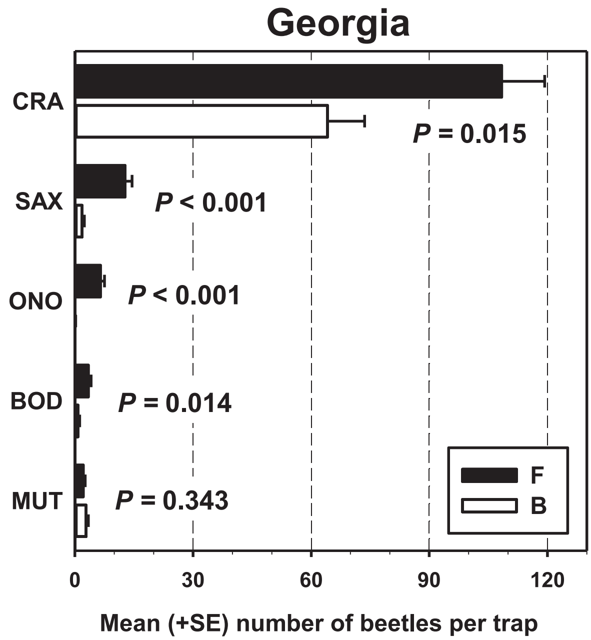

Fig. 2. Mean (+SE) catches of *Cnestus mutilatus* (MUT), *Cyclorhipidion bodoanum* (BOD), *Dryoxylon onoharaense* (ONO), *Xyleborinus saxesenii* (SAX), and *Xylosandrus crassiusculus* (CRA) in bottle (B) and multiple-funnel (F) traps in Georgia. *P* values for two-tailed paired *t*-test (df = 9).

in Ohio. Similarly, catches of *X. crassiusculus* were greater in funnel than bottle traps in Georgia and Virginia but not Indiana. In addition to geographic location and differences in species composition of trees, the sites also varied by height of traps above ground (lowest in Ohio and highest in Georgia) and size of bottle traps (1-L top bottles in Ohio and Indiana versus 2-L bottles in Georgia and Virginia). Very little is known about the flight and landing behaviors of ambrosia beetles, making it difficult to interpret our results with two different types of traps. We can only speculate about some of the potential causes for the observed variation in responses for our three target species.

Multiple-funnel traps were designed as intercept traps for three native species of ambrosia beetles: *Trypodendron lineatum* (Olivier), *Gnathotrichus sulcatus* (LeConte), and *Gnathotrichus retusus* (LeConte) (Lindgren 1983). Males and females of all three species fly with both sexes responding to known pheromones and kairomones. When baited with these attractants, multiple-funnel traps caught weekly catches in the thousands (Lindgren and Borden 1983). Observations of the beetles found that most would fly into the trap, hit the underside of a funnel, and tumble down into the collection cup (Lindgren 1983), similar to beetle interactions with window flight traps (Chapman and Kinghorn 1955).

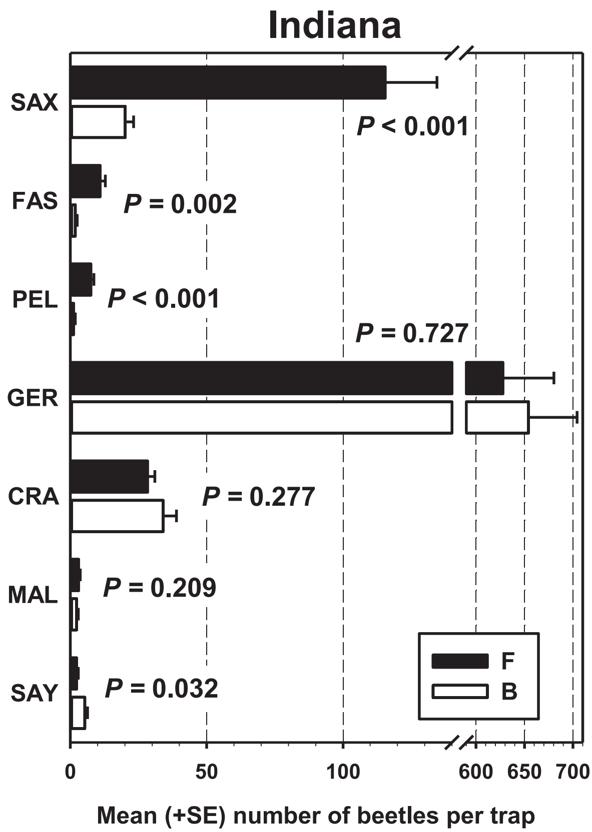

Fig. 3. Mean (+SE) catches of *Anisandrus sayi* (SAY), *Cyclorhipidion pelliculosum* (PEL), *Monarthrum fasciatum* (FAS), *M. mali* (MAL), *Xyleborinus saxesenii* (SAX), *Xylosandrus crassiusculus* (CRA), and *X. germanus* (GER) in bottle (B) and multiple-funnel (F) traps in Indiana. *P* values for two-tailed paired *t*-test (df = 9).

Our results in Georgia are consistent with this type of dispersal flight behavior with catches of two target species (*X. saxesenii* and *X. crassiusculus*) greater in funnel traps than in bottle traps (Fig. 2). This response would be expected given the larger and darker silhouette of a funnel trap compared to bottle traps. By placing lures within the funnel trap, volatiles are emitted from all funnels, activating the entire silhouette (Lindgren 1983). This same pattern was observed in Virginia for all three target species *X. germanus*, *X. crassiusculus*, and *X. saxesenii* (Fig. 5). In Virginia, traps were placed outside a forest edge and adjacent to a roadway, typically considered flight corridors for insects in general, especially when a tail wind is present. Klingeman et al. (2017) also caught more *X. saxesenii* in Lindgren traps than in bottle traps but the bottle traps were more effective for *C. mutilatus* than were the Lindgren traps.

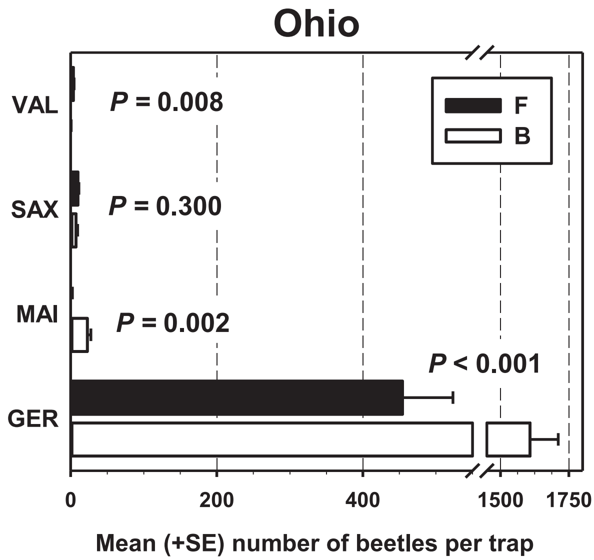

Fig. 4. Mean (+SE) catches of *Anisandrus maiche* (MAI), *Euwallacea validus* (VAL), *Xyleborinus saxesenii* (SAY), and *Xylosandrus germanus* (GER) in bottle (B) and multiple-funnel (F) traps in Ohio. *P* values for two-tailed paired *t*-test (df = 9).

In contrast, *X. germanus* were caught in greater numbers in bottle traps in Ohio where traps were located close to the ground (Fig. 4). It is possible that beetles employ a different behavior as they attempt to land on a host close to the ground and that bottle traps are more effective than funnel traps in trapping beetles that are attempting to land. Ambrosia beetles typically infest downed woody material as well as the bases of standing trees (Oliver and Mannion 2001; Reding et al. 2010). This landing behavior would likely occur at a slower velocity than foraging flight behavior. Funnel traps have more landing surfaces than do bottle traps, allowing more beetles to land and fly away and not be captured. A landing-type of flight behavior might also explain higher trap catches of *Anisandrus maiche* in Ohio and *A. sayi* in Indiana in bottle traps than in funnel traps (Figs. 3–4).

Height of bottle traps above ground level is known to affect catches of *X. crassiusculus*, *X. germanus*, and *X. saxesenii* (Klingeman et al. 2017; Reding et al. 2010; Turnbow and Franklin 1980; Weber and McPherson 1991). Using window traps in black walnut plantations in Illinois and North Carolina, Weber and McPherson (1991) found that more species of ambrosia beetles were caught in traps 1 m above ground than in traps 2–7 m above ground. The one exception was *X. saxesenii*, which was caught in significant numbers at all trap heights. In contrast, Klingeman et al. (2017) found that catches of most species of ambrosia beetles, including *X. saxesenii*, in prism traps deployed under urban walnut trees in Tennessee were greatest in traps suspended 1.5–3 m above ground compared to heights of 3–15 m.

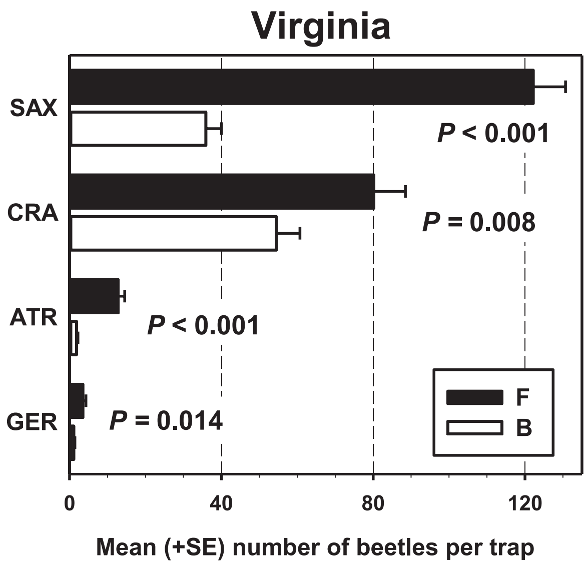

Fig. 5. Mean (+SE) catches of *Ambrosiophilus atratus* (ATR), *Xyleborinus saxesenii* (SAX), *Xylosandrus crassiusculus* (CRA), and *X. germanus* (GER) in bottle (B) and multiple-funnel (F) traps in Virginia. *P* values for two-tailed paired *t*-test (df = 9).

Table. 3. Mean (±SE) trap catches of *Stenoscellis brevis* (Boheman) (Curculionidae), *Enoclerus nigripes* (Say) (Cleridae), *Agrilus* spp. (Buprestidae), *Cyrtophorus verrucosus* (Olivier), and *Gaurotes cyanipennis* (Say) (Cerambycidae) in multiple-funnel and bottle traps baited with ethanol and conophthorin. *P* values for two-tailed paired *t*-test (df = 9).

Species	State	Bottle Trap	Funnel Trap	*P*
S. brevis	Indiana	0.6 ± 0.2	7.7 ± 1.7	0.003
	Virginia	0.3 ± 0.2	3.2 ± 0.7	0.004
E. nigripes	Indiana	0.4 ± 0.2	3.1 ± 0.8	0.006
	Ohio	1.7 ± 0.8	2.2 ± 0.6	0.244
Agrilus spp.	Indiana	5.4 ± 1.2	0.1 ± 0.1	<0.001
C. verrucosus	Georgia	0 ± 0	3.1 ± 0.8	0.004
G. cyanipennis	Indiana	2.8 ± 0.8	7.1 ± 1.3	0.037

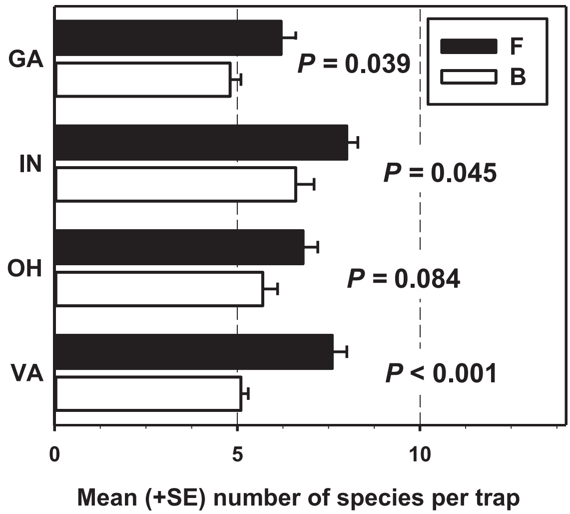

Fig. 6. Mean (+SE) number of scolytine species detected in bottle (B) and multiple-funnel (F) traps in Georgia, Indiana, Ohio, and Virginia. *P* values for two-tailed paired *t*-test (df = 9).

Using window traps, Roling and Kearby (1975) in Missouri and Turnbow and Franklin (1980) in Georgia found similar results for *X. saxesenii* from 0.5 to 8 m above ground level, although highest catches seemed to be around 1–2 m. In Ohio, Tennessee, and Virginia, Reding et al. (2010) found that greater numbers of *X. germanus* were caught in bottle traps placed 0.5 m above ground than in traps placed 1.7 and 3 m above ground. Similarly, greater numbers of *X. crassiusculus* were caught in traps placed either 0.5 or 1.7 m above ground than in traps placed 3 m above ground. Using opaque prism traps, Klingeman et al. (2017) caught more *D. onoharaense*, *Xyleborus affinis*, *Xyleborus ferrugineus*, *X. crassiusculus*, and *X. saxesenii* in traps positioned 1.5–3 m above ground than in traps 3–6 m and 9–15 m above ground level. These results might be a consequence of greater numbers of beetles flying close to the ground, which could be to optimize their detection of ethanol because it is heavier than air and would settle to ground level (C.M. Ranger unpubl. data). Bottle traps near the substrate might have trapped beetles attempting to land at the base of a host whereas traps positioned higher might have trapped beetles in flight dispersal mode, particularly as the traps were colocated on the same pole. Reding et al. (2010) found that 90% of attacks on standing trees occurred with 1 m of the ground. It is possible that bottle traps might be more effective at catching landing beetles than beetles in dispersal flight.

Bottle traps used in Ohio and Indiana were smaller than those used in Georgia and Virginia (1 L versus 2 L). The ethanol pouch would have occupied much of the volume within the smaller bottles, possibly making it more difficult for beetles to escape in Ohio. The lure was attached adjacent to the bottles in Indiana, possibly explaining the difference with results in Ohio for *X. germanus* (Figs. 3–4). Additionally, our results suggest that not all species of ambrosia beetles behave in the same way in similar circumstances, possibly due to differences in preferred hosts. The four locations differed significantly in the composition of tree species. Our speculations regarding the variation in our results in comparing bottle and multiple-funnel traps need to be examined further while keeping parameters such as bottle trap design, trap height, forest composition, and trap placement (edge versus within forest) consistent among trapping sites. There are many factors that could be at play, requiring further studies on the flight and landing behaviors of ambrosia beetles.

Managers of fruit orchards and horticultural nurseries should make their own assessments of the different trap types under their specific situation and expectations for specific pest species of ambrosia beetles. Multiple-funnel traps should be considered by managers of detection programs that target a broad range of species due to the higher diversity generally detected by multiple-funnel traps compared to bottle traps (Fig. 6). Alternatively, managers could deploy multiple bottle traps in place of a single funnel trap to increase diversity, as all species were detected in both types of traps. Detection programs should also consider placing traps along edges of wooded habitats to maximize trap catch diversity.

Acknowledgments

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