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Research Article

A Role for Intercept Traps in the Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae) IPM Strategy at Ornamental Nurseries

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Abstract: Invasive ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) cause significant damage to ornamental nursery tree crops throughout the eastern United States. Depending on surrounding habitat, some nurseries can undergo large influxes of ambrosia beetles from the forest to susceptible nursery stock. Ethanol-baited intercept traps are highly effective as monitoring tools that can capture large numbers of dispersing ambrosia beetles. Beetle trap captures at varying distances within nursery interiors were determined across replicated transects that either included or lacked a row of edge intercept traps. Although nearly 90% of ambrosia beetle captures were from the edge intercept traps, there was no significant difference in nursery interior captures from replicates that were protected vs. unprotected by edge intercept traps. There may exist some benefit for integrating the intercept trap strategy with other control measures, but traps alone will not reliably protect vulnerable nursery stock.

Key words: dispersal, ecology, invasive, lure

Introduction

The U. S. ornamental nursery industry, estimated at $6.6 billion in annual sales, is being challenged by several species of exotic ambrosia beetles, including *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), *Xylosandrus compactus* (Eichhoff), and *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae: Scolytinae) (Mizell *et al.* 1994; USDA 2009). Due to their broad distribution throughout the eastern U. S., occasional severity of outbreaks, and expense of repeated insecticidal applications, ambrosia beetles were recently listed as the third-worst pest of southeastern nursery production (Oliver and Mannion 2001; Fulcher *et al.* 2012). Dispersing females enter tree nurseries from surrounding forested habitats and then tunnel into susceptible tree hosts, introducing symbiotic fungi, which are then maintained and consumed by both adults and larvae (Biedermann and Taborsky 2011; Reding *et al.* 2015; Werle *et al.* 2015). After the immature beetles complete development within their gallery, the newly-eclosed females will mate and disperse to a new host (Weber and McPherson 1984). While primary fungal symbionts are not always lethal to their host, attacked trees will typically die because of a combination of stresses including flooding, drought, disease, and insect injury (Weber and McPherson 1984; Kuhnholz *et al.* 2001).

It is during the critical dispersal period that ambrosia beetles are particularly vulnerable to control measures because before and after dispersal, these tree-infesting beetles are well protected from insecticide applications. While they do use visual cues in host selection, ambrosia beetles rely primarily on the plant stress volatile ethanol to locate hosts during their dispersal flights (Ranger *et al.* 2012, 2013, 2015; Werle *et al.* 2014). Standard management recommendations include monitoring spring flights using traps baited with ethanol lures in order to detect the optimal time to begin applying pyrethroid insecticides (Hudson and Mizell 1999; Ranger *et al.* 2010, 2012; Reding *et al.* 2010, 2011). Peak dispersal flights for female ambrosia beetles normally occur in spring, followed by a summer decline, but some regions report a second, smaller flight in late summer (Hudson and Mizell 1999; Oliver and Mannion 2001; Reding *et al.* 2010; Werle *et al.* 2012, 2015).

Although ambrosia beetle dispersal can cover a relatively long distance (≥200 m), the majority of individuals (~70%) can be captured within 13 m of the surrounding forest habitat (Reding *et al.* 2015; Werle *et al.* 2015). Thus, greater protection may be afforded to susceptible nursery crops that are placed ≥50 m from the nursery or forest edge. Because ethanol-baited traps will capture and kill a large proportion of dispersing females, a mass-trapping approach at the nursery or forest interface has potential as a stand-alone control measure, or may allow for reduced insecticidal inputs (Huber *et al.* 1979; Reding *et al.* 2015; Werle *et al.* 2015). Therefore, our research objectives include (1) determining the proportion of dispersing female ambrosia beetles that can be intercepted by a row of traps at the nursery edge and (2) verifying how far from the nursery or forest edge that nursery stock should be placed to avoid ambrosia beetle attacks.

Materials and Methods

Experimental locations. Two commercial nurseries were used as research sites, one in Tangipahoa Parish (30°47'30.39"N, 90°20'37.91"W), Louisiana and the other in Stone County (30°47'59.92"N, 89°15'21.64"W), Mississippi. The Louisiana site was surrounded by a combination of managed pine and natural mixed hardwood trees on three sides, with a road and residential area on the fourth side. The pine stand at the Louisiana site was subjected to a prescribed burn in February 2016, before the beginning of our study. The Mississippi site was bordered by managed pine forest on two sides, a barren sand or gravel pit on the third side, and a road with residential areas on the fourth side. The pine stand at the Mississippi site last received a prescribed burn in 2011.

Trapping methodology. 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) (Oliver *et al.* 2004; Ranger *et al.* 2010; Reding *et al.* 2011). The upper 2 L bottle had three rectangular openings (length 15 cm, width 6 cm) cut into the sides to allow beetle entry, while the 0.6 L collecting bottle below was partially filled with propylene glycol to kill and preserve insects. Traps were baited with slow-release (65 mg or day at 25° C) ethanol lures (AgBio, Westminster, CO) and suspended about 1 m above the ground with Japanese beetle trap stands (Tanglefoot, Grand Rapids, MI). Experimental design was randomized complete blocks (three replicates or site) with treatments consisting of (1) nursery interior traps placed at varying distances (13 m, 25 m, 50 m, and 100 m) from the nursery or forest edge and (2) presence or absence of a row of edge (0 m) intercept traps (n=7) separated from each other by 12.5 m and alternated among the blocks for each two-week collection period (Fig. 1). Research plots were separated from the lateral and distal nursery edges by at least 200 m.

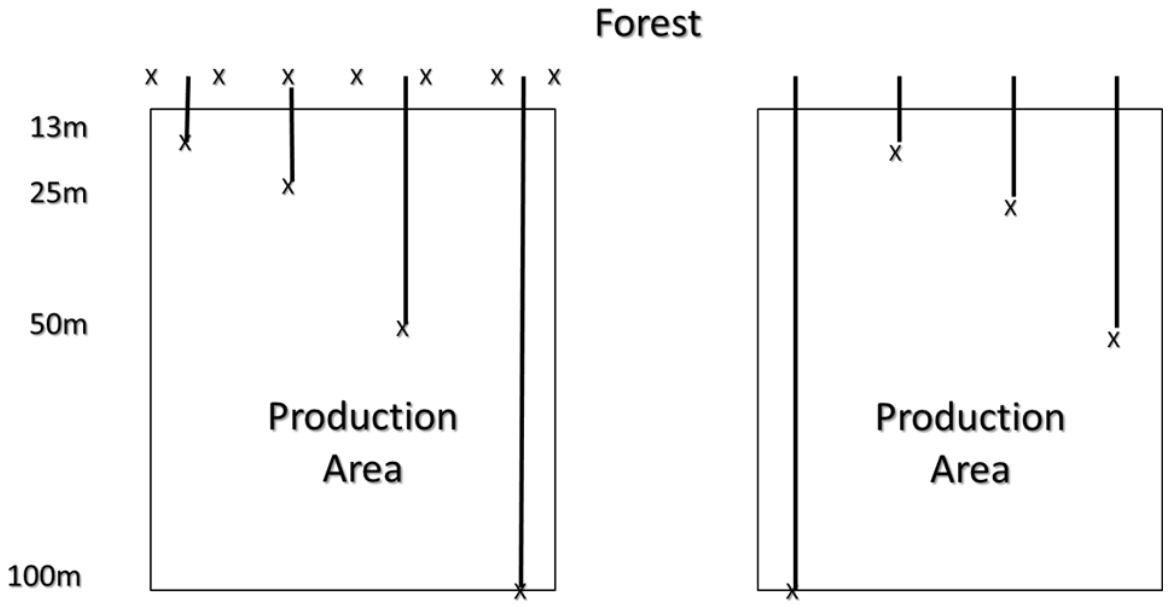

Fig. 1. Graphic representation of research plots, including edge intercept traps (n = 7) separated laterally by 12.5 m each, and nursery interior traps at four distance treatments separated laterally by 25 m each. Presence or absence of edge interceptors was alternated for each two-week collection period.

Traps were deployed in mid-March 2016, with collections made every 2 weeks from 5 April to 4 October 2016 (n = 13). Ambrosia beetles collected from individual traps were brought back to the laboratory for counts and determination of species using a standard dichotomous key (Rabaglia *et al.* 2006).

Statistical Analysis. Mean cumulative captures of the four most-problematic species of ambrosia beetles (*X. crassiusculus, X. germanus, X. compactus,* and *C. mutilatus*) were compared for each trap type (edge intercept traps vs. interior traps) and also for four-nursery interior trap distance treatments. Other ambrosia beetles captured were identified to species and counted, but were not used in analyses.

After preliminary analysis revealed no significant site*treatment interactions, data from the two collection sites were pooled and a log(x+1) transformation was used to meet assumptions of the F-test (Proc Univariate, SAS). Mean captures of four-target species were analyzed using analysis of variance (ANOVA) with two separate methods. The first method included only replicates that had edge intercept traps and compared captures from edge interceptors vs. interior traps (PROC GLM, SAS). The second method included all replicates and compared (a) captures from interior traps that were protected by edge interceptors vs. those that were unprotected, (b) captures from each of four distance treatments, and (c) captures from four distance treatments in protected vs. unprotected replicates.

Results

Nearly 1,700 specimens from four-target species were captured, with the most numerous being *X. compactus* (57%), followed by *X. crassiusculus* (41%), *C. mutilatus* (1%), and *X. germanus* (<1%) (Table 1). While more specimens were collected from the Mississippi site (F = 22.52; df = 1, 82; P < 0.001), the trap types performed similarly at each location (i.e., no site*trap-type interaction) (F = 2.02; df = 1, 82; P > 0.159). Nearly nine times more beetles were collected at the forest edge as compared with the nursery interior (edge versus interior traps, Fig. 2), representing a significant difference in capture (F = 80.76; df = 1, 82; P < 0.001) at both sites.

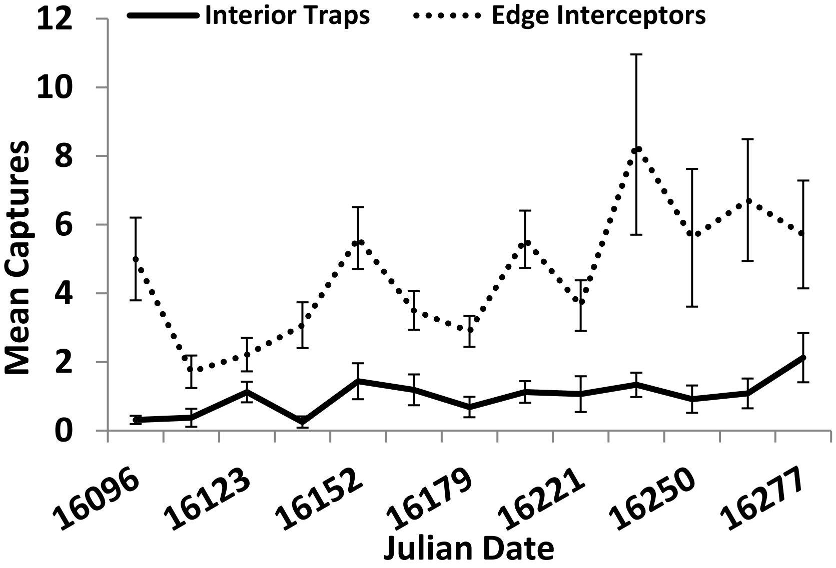

Fig. 2. Mean bi-weekly ambrosia beetle captures (± SE) from edge interceptor vs. nursery interior traps in replicates that only had both trap types. Significantly higher captures in edge intercept traps are evident from every collection period.

However, beetles still flew into the nursery interior at similar rates regardless of presence or absence of edge interceptors. When comparing replicates that included edge intercept traps against those lacking edge interceptors, we found no discernible effect on beetle capture (F = 0.61; df = 1, 301; P > 0.436) (Fig. 3) at any of four trapping distances (F = 1.02; df = 3, 301; P > 0.386) or replicate plots (F = 1.49; df = 2, 301; P > 0.228). Beetle captures did decline with trap distance from the woodland edge (F = 2.72; df = 3=3, 301;< 0.045).

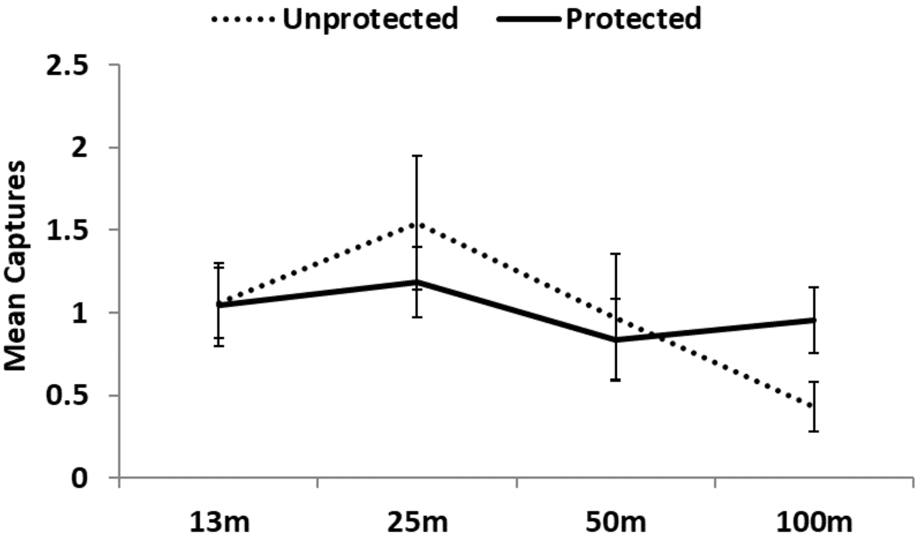

Fig. 3. Mean bi-weekly ambrosia beetle captures (± SE) from each of four nursery interior trap distances in replicates that were either protected or unprotected by edge intercept traps.

Table 1. Species composition of ambrosia beetles (Coleoptera: Curculionidae) captured in ethanol-baited bottle traps at ornamental tree nurseries in Louisiana and Mississippi in 2016.

Species	% total specimens	
	Tangipahoa Pa., LA	Stone Co., MS
Ambrosiodmus lecontei Hopkins	-	0.3
Ambrosiodmus rubricollis (Eichhoff)	0.20	-
Cnestus mutilatus (Blandford)	3.1	0.1
Cyclorhipidion bodoanum (Reitter)	-	0.1
Dryoxylon onoharaense (Murayama)	0.2	0.1
Euwallacea validus (Eichhoff)	-	0.2
Monarthrum mali (Fitch)	0.4	0.2
Xyleborinus octiesdentatus (Murayama)	0.2	0.7
Xyleborinus saxeseni (Ratzeburg)	6.7	2.4
Xyleborus affinis Eichhoff	2.8	1.6
Xyleborus celsus Eichhoff	0.4	0.1
Xyleborus ferrugineus (F.)	2.0	0.8
Xylosandrus compactus (Eichhoff)	8.6	69.2
Xylosandrus crassiusculus (Motschulsky)	75.0	23.5
Xylosandrus germanus (Blandford)	0.4	0.6

Discussion

While prior records exist for *X. germanus* in Mississippi (http: or or www.barkbeetles.info), these collections represent the first made in the course of our ambrosia beetle research over the past five years, and may indicate an expanding range of this exotic invasive pest (Werle *et al.* 2012, 2014, 2015, 2017). As the primary culprit of nursery losses due to ambrosia beetle attacks in northeastern and midwestern nurseries, the greater prevalence of *X. germanus* in southern Mississippi will be of great interest to regional ornamental production nurseries (Ranger *et al.* 2016).

Range expansions for *C. mutilatus* have been recently reported from Kentucky and Pennsylvania, but captures of this increasingly important pest remained relatively static from prior reports in southern Mississippi (Barringer 2016; Leavengood 2013; Werle *et al.* 2015). And while captures of *X. compactus* and *X. crassiusculus* continued to be most prevalent in this study, *X. compactus* has become the dominant species as compared with prior research in southern Mississippi (Werle *et al.* 2015).

Following summer decline in activity that has been previously reported from Mississippi and Louisiana populations, we observed a second peak in late summer that provides further evidence of a bivoltine ambrosia beetle life cycle in this region (Fig. 2). Reports of tree losses to ambrosia beetle attack are less common during this later flight, likely due to fewer abiotic tree stress factors such as frost or flood stress. Even so, awareness of second flight peak may encourage growers to continue a monitoring program using ethanol-baited traps throughout the summer months, providing advance warning for a possible resurgence in ambrosia beetle activity and the need for further preventative applications of insecticides to vulnerable crops.

A non-significant site*trap type interaction indicates that variability in captures by site was quantitative rather than qualitative and can be explained by relatively low captures from the Louisiana site. Similarly, to prior ambrosia beetle research conducted at this location, a February prescribed burn of surrounding forests appears to have destroyed much of the beetles' overwintering habitat (Werle *et al.* 2015). While this is not a practical management recommendation for many ornamental nurseries, a prescribed burn of peripheral forest habitat does seem likely to reduce ambrosia beetle populations at some sites.

With nearly 90% of ambrosia beetle captures coming from edge intercept traps, this study supports findings of prior research that indicated a decrease in captures as distance from the forest increased (Reding *et al.* 2015, Werle *et al.* 2015). Although presence of edge intercept traps consistently reduced the number of ambrosia beetles migrating into the nursery interior, the fact that they were not 100% effective may prevent mass-trapping from being recommended as a stand-alone control measure (Fig. 2). Even though ambrosia symbionts are not generally considered to be as virulent as some plant pathogens, there is evidence that even a small number of beetle attacks will reduce ornamental tree growth, hindering marketability and potentially leading to mortality in previously weakened trees (Oliver and Mannion 2001; Ploetz *et al.* 2013; Ranger *et al.* 2013). Ambrosia beetles are also known to vector other non-symbionts that are primary tree pathogens, including those that cause Dutch elm and laurel wilt diseases, so a preferred beetle management program would be one with extremely high efficacy (Batra 1966; Buchanan 1941; Carrillo *et al.* 2014; Weber and McPherson 1984).

Despite the high proportion of beetle captures from edge intercept traps, they did not significantly impact captures from nursery interior traps placed at varying distances into the nursery. In fact, at the distal 100 m traps, collections of ambrosia beetles were actually higher from replicates protected by edge intercept traps as compared with unprotected replicates. This trend was also reported from prior studies and likely indicates an influx of beetles from habitats at the opposite end of the nurseries (Reding *et al.* 2015; Werle *et al.* 2015).

Each nursery represents a unique habitat with soil conditions, forest vegetation, and land uses that can vary considerably even within a site. These contrasting environments can allow for significant differences in insect populations, and it is not difficult to identify certain 'hotspots' of ambrosia beetle activity within a large nursery. We hypothesize that high-density ambrosia beetle populations within certain replicates seemed to overwhelm the edge interceptors and disperse past them into the nursery interior, as compared with other replicates that had lower beetle pressure. This supposition is supported by previous observations of greater ambrosia beetle dispersal distances from larger source populations (Reding *et al.* 2015; Werle *et al.* 2015). Furthermore, it has been posited that, while smaller populations of invasive insects can be suppressed or even eradicated using a mass-trapping technique, larger or more established populations may be less vulnerable to this control strategy (Brockerhoff *et al.* 2010; El-Sayed *et al.* 2006).

In conclusion, a mass-trapping strategy can intercept large numbers of dispersing ambrosia beetles at nursery or forest interface before they have a chance to attack vulnerable nursery stock, but on its own, this method will not reliably protect the crop. There may still exist potential to combine intercept traps with a reduced pesticide input or another control measure. However, determining cost-effectiveness of integrating interception traps with other IPM controls will require further field trials. As a result, our future research objectives include a 'push-pull' strategy incorporating a ring of baited intercept traps (pull) surrounding trees treated with repellent semiochemicals (push), with the hope of finding a less insecticide-intensive management program that can protect ornamental tree crops.

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