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Evaluation of Screen Barriers on Redbay Trees to Protect Them From *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae) and Distribution of Initial Attacks in Relation to Stem Moisture Content, Diameter, and Height

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ABSTRACT Fine mesh screen was used to create a physical barrier to prevent redbay ambrosia beetles, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), from accessing various parts of the boles of redbay trees, *Persea borbonia* (L.) Sprengel, and infecting them with the laurel wilt fungus, *Raffaelea lauricola* (T.C. Harrington, Fraedrich, & Aghayeva). Screen barriers prevented beetles from attacking boles of mature redbay trees from the ground to 1 or to 3 m and from 1 to 3 m above ground. Untreated control trees were sampled more extensively to determine how location of initial attacks varied with height, diameter, and moisture content of the wood. Screening did not affect tree survival, and all of the trees died within 243 d from the beginning of observation. Initial points of attack by *X. glabratus* varied from ground level to heights of at least 6.6 m. Trees showed characteristic laurel wilt symptoms with as few as two *X. glabratus* entry points. The number of attacks exhibited nonlinear relationships with diameter ($P = 0.0004$; $r^2 = 0.82$) and height ($P = 0.0013$; $r^2 = 0.69$) but were not correlated with moisture content. Attacks increased gradually with increasing stem diameter up to ≈10 cm after which the attacks became more numerous. *X. glabratus* attacks were most numerous on the tree bole near the ground. Attacks then declined as tree height reached 2–3 m. From 3 to 8 m, attacks were relatively consistent.

KEY WORDS laurel wilt, *Raffaelea lauricola*, *Persea borbonia*, redbay ambrosia beetle

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), and its associated fungus *Raffaelea lauricola* (T.C. Harrington, Fraedrich, & Aghayeva) are causing extensive mortality to redbay [*Persea borbonia* (L.) Sprengel] and are also killing sassafras [*Sassafras albidum* (Nuttall) Nees] and avocado (*Persea americana* Miller) trees (Crane et al. 2008, Fraedrich et al. 2008, Mayfield et al. 2008b), all of which are members of the Lauraceae. The beetle is an Asian species that was first detected in survey traps near Savannah, GA, in 2002 (Rabaglia et al. 2006). It was later linked to redbay trees that were exhibiting typical wilt disease symptoms and was found to be the vector of the laurel wilt fungus causing the disease (Fraedrich et al. 2008).

In its native range, *X. glabratus* appears to be a typical ambrosia beetle that tunnels and breeds within sapwood of dead or dying trees feeding exclusively on cultivated fungi known as ambrosia. However, unlike most other exotic ambrosia beetles here in the United States, *X. glabratus* bores into living healthy trees, inoculating them with *R. lauricola* (Fraedrich et al. 2008). The disease kills redbay trees within 9 wk, and a single artificial inoculation point is sufficient to kill apparently healthy trees in the field (Fraedrich et al. 2008, Mayfield et al. 2008a). Field observations suggest that a single beetle boring into a tree can also result in a lethal infection (S. Fraedrich, personal communication). As the fungus spreads and the tree begins to die, greater numbers of *X. glabratus* are attracted to the tree.

Many bark and ambrosia beetles exhibit specific attack patterns or distributions within host trees and different species prefer different parts of a tree. Roling and Kearby (1975) trapped beetles arriving to oak trees injected with ethanol. Some, like *Xyleborus ferrugineus* (F.) and *Xyleborus xylographus* (Say), were restricted primarily to traps below 2 m, whereas others like *Xyleborus saxeseni* (Ratzburg) were captured as high as 5.5 m. Reding et al. (2010) found that traps placed 0.5 m above ground accounted for nearly 80% of the trap catch for *Xylosandrus germanus* Blanford while *Xylosandrus crassiusculus* Motschulsky were more evenly distributed, although traps at or below 1.7 m caught more than those at 3 m. For both of these species, the average attack point on a tree is <30 cm

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from the ground, but not all bark and ambrosia beetles first attack at the base of a tree. Like *Xy. germanus*, *X. glabratus* are captured in traps near the ground (Hanula et al. 2011, Brar et al. 2012). Based on the height at which beetles are captured in traps and observations that the lower bole frequently seems to be the first part of a tree to be attacked, it was hypothesized that protecting the lower bole of a redbay tree may result in whole tree protection from *X. glabratus* and laurel wilt.

Wrapping trees in wire mesh screen to exclude beetles was one of the earliest methods of preventing bark beetle attack. Covering the lower 7.6 m of the bole with wire mesh screen was sufficient to protect lodgepole pines (*Pinus contorta* Douglas) from mountain pine beetle, *Dendroctonus ponderosae* Hopkins, in Crater Lake National Park (Miller and Keen 1960). Moeck et al. (1981) found that wire mesh screen wrapped around the boles of ponderosa pine effectively excluded *Dendroctonus brevicomis* (LeConte) and *Dendroctonus ponderosae* (Hopkins), but not the smaller *Gnathotrichus retusus* (LeConte) or *Ips latidens* (LeConte). Covering trees with screen is labor intensive, so even if effective it may be impractical to protect forest trees. However, physical barriers might give an indication of how high to spray an insecticidal barrier to prevent attack, and they might be useful for protecting high-value trees near homes or in parks. Effective physical protection using screen also has the advantage of potentially lasting several years compared with insecticides, which must be reapplied with much greater frequency.

To test whether protecting the lower bole of redbay trees might prevent the trees from being infected with laurel wilt, sections of mature redbay were wrapped in fine mesh screen to physically block beetles from boring into the trees. We examined barrier height and where initial attacks occurred in relation to height and stem diameter at the point of attack when no barrier was present. In addition, we examined the moisture content of the wood along the boles of trees to see whether it was correlated with the initial area of attack.

Materials and Methods

The study took place in Emanuel County, GA, ≈26 km due south of Swainsboro, GA (32° 39′07″ N, 82° 28′04″ W). A very sharp ecotone defined by ≈1-m elevation change separated an upland sand hill community from a riparian bay forest dominated by loblolly bay [*Gordonia lasianthus* (L.) Ellis] and redbay with an understory of *Itea*, *Smilax*, and *Lyonia* species. The bay forest was the site of the experiment.

A total of 45 mature healthy redbay trees, which ranged in size from 8.6 to 15.7 cm diameter at breast height [DBH], 1.4 m above ground), were selected in February 2011. Trees were randomly assigned to one of four treatments that consisted of 1) unprotected control trees; 2) fine mesh screen from ground level to ≈1 m; 3) screen from ground level to 3 m; or 4) screen from 1 to 3 m. Each treatment was replicated 10 times, but an additional five control trees were selected for studies to determine the initial point of attack. Boles of treated trees were wrapped with fine mesh (0.68-mm aperture) fiberglass screen that covered the appropriate parts of the boles (Fig. 1). Screen was wrapped around the tree bole, and the seams were then folded over and tightly stapled to prevent beetles from entering through them. These vertical seams were further sealed with 100% silicone caulk. Wire was twisted tightly around the tops (and bottoms of 1–3 m screened section) to seal them to the bark. Upper and lower edges (1–3 m treatment) of the screen were also sealed to the boles with Great Stuff foam insulation (DOW Chemical Corp., Midland, MI). For those trees covered to ground level, a 1-m^2 section of fine stainless steel screen (0.35-mm aperture) was cut so that it could be wrapped around the base of the tree but lie flat on the ground (Fig. 1). This was done to protect the root flare and intercept any beetles that fell to the ground after impact with the tree. The fiberglass screen was stapled to the stainless steel screen, and the seam was sealed further with silicone caulk. The seam of the steel screen where it was cut to fit around the tree was stapled to a piece of wood and sealed with foam insulation. Care was taken throughout the process to avoid wounding the tree boles or exposed root flares.

Fig. 1. Lower bole of redbay tree wrapped in fine mesh fiberglass screen with flare of stainless steel screen at base. Top is wrapped with wire and sealed with Great Stuff foam insulation.

Trees were wrapped on 17 February and were monitored for signs of laurel wilt, such as foliar discoloration, wilting in the crown, or apparent *X. glabratus* entry points and frass, on a monthly basis until 24 May, then on a weekly basis until the last tree died on 18 October. Trees with no obvious wilt were marked as healthy; those with some discoloration but not enough to confidently declare infection were noted; and those with obvious crown wilt were marked as infected. Because we were interested in determining the initial point of attack, trees that were showing obvious signs of wilt, i.e., those marked infected, were cut within 1 wk after infection was noticed. Infected trees were cut down, and all bark was scraped from the trunk up to a height of ≈9 m so that beetle attacks could be found easily. Aboveground portions of the stumps were also scraped. Attacks of *X. glabratus* were identified by gauging the diameter of gallery entrances using a standard map pin (≈1 mm in diameter). Attacks were counted, and heights of all attacks were measured from ground level. Cross sections of the tree 3–5 cm thick were then cut at ≈60-cm intervals throughout the length of the trunk until major branching occurred, usually 8–10 m from the ground. These sections were labeled and stored in plastic garbage bags during transport to the laboratory where diameter and wet weight of each section were determined. The sections were then placed into a drying oven for at least 4 d at 40°C. After drying, sections were again weighed, and percent moisture content was calculated as follows: (wet weight − dry weight/wet weight) × 100.

Statistical Analysis. Differences in time until tree death by treatment for all trees and for the subset of trees on which screens were effective were analyzed using the general linear models procedure of the SAS statistical package (PROC GLM, SAS institute 1985). Data for total attacks per tree were log transformed $[\log(x + 1)]$ based on results of the Shapiro–Wilk test for normality (Proc Univariate; SAS Institute 1985). A pooled *t*-test was used to compare whether screen failure affected time until tree death. Relationships between moisture content, height, stem diameter, and number of beetle attacks were analyzed by linear regression using PROC GLM (SAS institute 1985). For these analyses, each tree was broken into 60-cm sections, and values were expressed as attacks per section. Nonlinear relationships between number of attacks and stem diameter and height were determined using the regression wizard of SigmaPlot 10 (Systat Software Inc, Richmond, CA).

Results

All trees in the experiment died within 243 d after the beginning of the experiment, with an average of 165.9 ± 6.94 d until tree death. Time until tree death was not affected by screening treatments ($F_{3,39} = 1.92$; $P = 0.142$; Fig. 2). Screens were not totally effective, and at least 15 of the 30 trees wrapped with screen had at least one attack beneath the screen (Table 1). Whether or not the screen was fully effective did not impact the number of days until tree death within treatments (Table 1; screen at 0–1 m, $t = 1.22$, df = 8, $P = 0.26$; screen at 1–3 m, $t = 1.12$, df = 7, $P = 0.30$; screen at 0–3 m, $t = -0.33$, df = 7, $P = 0.74$), that is, trees died at approximately the same time regardless of whether or not beetles penetrated the screen. Trees screened from ground level

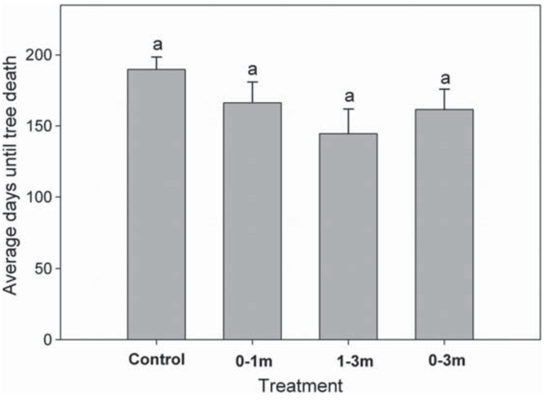

Fig. 2. Length of time redbay trees survived after being wrapped with screen to prevent redbay ambrosia beetle from attacking different areas of tree boles. Trees were 1) unwrapped as controls, 2) wrapped with screen from ground level to ≈1 m, 3) wrapped with screen from 1 to 3 m, or 4) wrapped with screen from ground level to 3 m. Columns with the same letter are not significantly different ($\alpha = 0.05$).

to 1 m died slightly more quickly than control trees when screens were successful, but there were no other significant differences among treatments. When screens failed, trees tended to have more attacks (Table 1), suggesting that screens failed because the trees were under heavy attack pressure. Greatest tree mortality was seen during the first week of August 2011 when nine trees died including at least one from each treatment.

Analysis relating to moisture content and attack height was performed only on the 15 control trees because attack height was affected by screened sections on treated trees. Number of attacks on control trees ranged from 3 to 193, with an average of 47.4 ± 14.5 attacks per tree. Percent moisture decreased with height (Fig. 3), but there was no correlation ($P = 0.4396$) between the moisture content of a tree at a particular location and the number of *X. glabratus* entry points.

The number of attacks per tree section was correlated with both stem diameter (Fig. 4a) and height (Fig. 4b). A nonlinear quadratic polynomial regression fit the attack frequency by diameter class data the best and showed that numbers of initial *X. glabratus* attacks were lowest on the smaller diameter class sections but increased rapidly when redbay stem diameters exceeded 10 cm (Fig. 4a).

The lowest attacks on the control trees ranged from 0 to 6.6 m, with the average for early attacks (1–2 wk after tree death) occurring at 2.38 ± 0.74 m. A nonlinear cubic polynomial regression fit the *X. glabratus* attacks by height class best (Fig. 4b). The numbers of attacks were highest near the base of the tree and declined rapidly until a height of ≈3 m. From 3 to 8 m, the number of attacks was relatively constant and then they declined at heights above 8 m. The highest attack was recorded at 10.6 m. The branches of trees were not

Table 1. Number of trees where attacks were detected within screened areas (failed) or not (successful), mean (±SE) days trees survived after treatment, and mean (±SE) number of *X. glabratus* attacks on the entire tree bole

Treatment	Screens successful			Screens failed		
	Number	Days survived[a]	Total attacks[a]	Number	Days survived[a]	Total attacks[a]
0–1 m	3	118 ± 44.1a	2.3 ± 0.33a	7	169 ± 20.3a	191.6 ± 58.3a
1–3 m[b]	5	143 ± 18.6ab	41.0 ± 22.9a	4	174 ± 20.2a	69.0 ± 41.1a
0–3 m[b]	5	169 ± 17.0ab	29.8 ± 10.6a	4	160 ± 24.4a	123.5 ± 104.1a
Control	15	191 ± 8.6b	47.4 ± 14.5a	NA	NA	NA

Screens were considered to have failed if one or more entrance holes were found beneath the wrapped area.

[a] Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$) according to the REGWQ multiple comparison test. Data for total attacks were log transformed prior to analyses, but untransformed data are presented in the table.

[b] Attack counts for one tree of each of these treatments were unknown.

closely examined, so some attacks could have occurred at greater heights.

Discussion

Trees were selected in February and were apparently healthy and uninfected at that time. On average, trees died 5–6 mo after the study was started, so it is unlikely they were infected before screens were placed on them. For example, Mayfield et al. (2008a) inoculated healthy trees in April that exhibited wilt symptoms on one third or more of their crown 1.5–3 mo later. However, almost all of their trees showed early, but obvious, symptoms of wilting, similar to those we looked for, within 1.5 mo (A. E. Mayfield, personal communication), so it is unlikely the trees we observed were infected before the start of the study.

Physical protection of trees by screening lower portions of the bole was not effective for *X. glabratus* because the beetles attacked from ground level up to 10 m on newly infected redbay trees. Some beetles were able to pass through the fiberglass screen, despite the mesh size being smaller than the diameter of an adult female. There were no differences in the timing of tree death between trees where the screens successfully kept beetles out and those where they did not. Beetles that made it beneath the screen may have either pushed apart the screen fibers or chewed directly through it. This was not anticipated, but during concurrent in vitro rearing experiments (Maner 2012), *X. glabratus* bored through the walls of plastic petri dishes (M. L. M., unpublished data), so it is not surprising that they may have also bored through fiberglass screen. The finer mesh stainless steel screen used to protect the roots of trees was too heavy and difficult to work with to allow use on the bole without risking damage to the bark and attracting beetles to the wound sites. Because of the very small size and the distribution of at least a few flying beetles to heights of up to 15 m (Hanula et al. 2011), any solution which

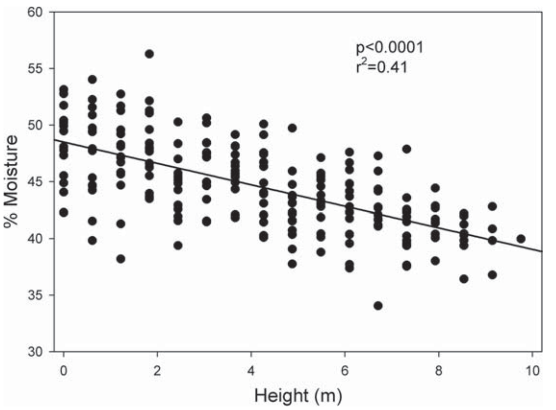

Fig. 3. Relationship between height and moisture content on unwrapped control trees that were in the early stages of laurel wilt disease.

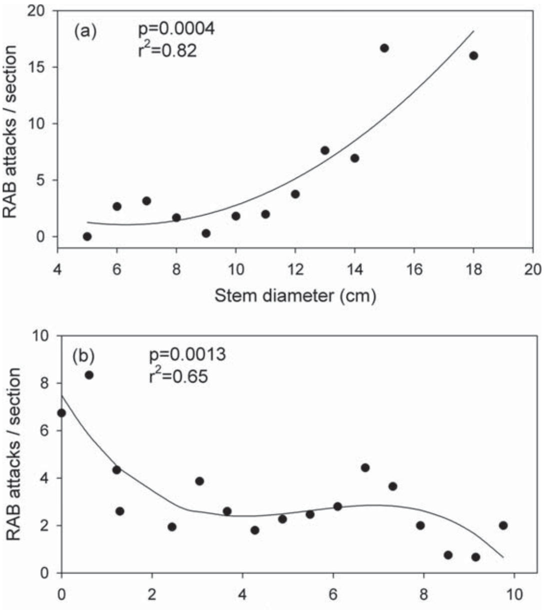

Fig. 4. (a) Relationship between rates of redbay ambrosia beetle attack and stem diameter ($y = 6.00 - 1.57x + 0.13x^2$). Values represent total number of attacks within a 1-cm stem diameter range divided by the number of sections that fell within that size range. (b) Relationship between rates of redbay ambrosia beetle attack and height of the tree where attacks occurred ($y = 7.746 - 0.0324x + 6.2762e^{-5}x^2 - 3.7907e^{-8}x^3$). Values represent total number of attacks within a 60-cm height range divided by the number of sections that fell within that height range.

protects only part of a host tree will likely prove ineffective for control of this pest.

Our results on the distribution of attacks were for initial attacks on trees cut within 1 wk of showing obvious wilt symptoms. Single inoculations of *R. lauricola* from cultures into larger diameter trees in forests, however, resulted in tree wilt symptoms 6–14 wk later (Fraedrich et al. 2008, Mayfield et al. 2008a), suggesting the trees we observed attacks on were inoculated weeks before they were cut. Despite this, wilting symptoms occurred with as little as two attacks, which strongly supports the idea that very few beetles or even a single beetle can infect and kill trees susceptible to laurel wilt. This makes prevention of laurel wilt even more difficult because, at least on redbay, control aimed at the beetle must be 100% effective to protect a tree from infection. Thus far, insecticidal treatments have not been able to achieve that level of success (Peña et al. 2011).

Although trees were cut within 1 wk of showing symptoms of laurel wilt, the numbers of attacks were often numerous and averaged nearly 50 attacks per tree, which allowed us to examine the distribution of initial beetle attacks on untreated control trees. Attacks were most numerous near ground level declining from that point to $\approx$2 m height. Numbers of attacks between 2 and 8 m were relatively constant and then attacks declined further above 8 m. This distribution of attacks is consistent with the height at which beetles are caught in traps, that is, 85% of the beetles that arrived at baited traps positioned from 1 to 15 m (Hanula et al. 2011) were captured at 1–2 m above ground, while Brar et al. (2012) found that traps 35–100 cm above ground were most effective among traps positioned from 15 to 330 cm.

The distribution of attacks by diameter (Fig. 4a) showed attacks were lowest in small (4.5–10 cm) diameter sections of the trees and then they increased with increasing stem diameters above 10 cm. Because larger diameter sections were near the base of the tree, these results are consistent with observations of beetle flight activity. In addition, *X. glabratus* are attracted to larger silhouettes (A. E. Mayfield, personal communication), and females that construct galleries in larger diameter redbay trees produce more progeny (Maner et al. 2013).

We found no evidence that moisture content of the wood influences where initial attacks by *X. glabratus* occur, even though females fly close to the ground (Hanula et al. 2011, Brar et al. 2012) and had higher initial attacks low on the tree bole where the moisture content of the tree is higher. Hoffman (1941) reported that wood moisture content near natural attacks by *Xy. germanus* were uniform and ranged from 51 to 53%, while moisture content of artificially infested material varied widely from 36 to 72%, suggesting that females selected the location for their galleries based on preferred wood moisture content. Although we found no association between attack position and moisture content of the wood, attacking lower on the tree where the wood is thicker and moister may slow the drying process after tree death and allow females to produce brood over a longer period of time.

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