Population Trends of the Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae): Does Utilization of Small Diameter Redbay Trees Allow Populations to Persist?

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POPULATION TRENDS OF THE REDBAY AMBROSIA BEETLE (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE): DOES UTILIZATION OF SMALL DIAMETER REDBAY TREES ALLOW POPULATIONS TO PERSIST?

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ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff, vectors laurel wilt, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva, that quickly kills all large diam (> 2.5cm) redbay trees [*Persea borbonia* (L.) Sprengel] in an area but smaller diam trees (< 2.5cm) survive for years. We measured densities of *X. glabratus* attacks on hanging bolts of freshly cut mature redbay annually for 5 yr (2007-2011) at 7 locations varying in age of infestation from newly infested in 2007 to those that were among the oldest infested areas (infested in 2002 or earlier), to determine if populations persist after mature host trees are gone. Attack densities on redbay bolts at the field sites varied from 10-25 *X. glabratus* attacks/100 cm^2 where large dying redbay trees were still present, to < 1 attack/100cm^2 at sites where few or no trees > 2.5 cm diam near ground level were still living at the beginning of the study in 2007. Despite having no large trees available, populations of the beetle persisted at 2 of the 3 oldest infested sites throughout the 5-year survey period (2007-2011). In 2012 we studied *X. glabratus* utilization of small diam redbay wood as a possible explanation of how populations might survive in these areas in the absence of larger trees. In laboratory trials, *X. glabratus* produced 27.8 ± 6.63 adults/gallery (± SE) from 18 galleries constructed in portions of redbay trees that averaged 3.2 ± 0.02 cm diam at the point of attack. The smallest stem section to support a successful gallery was 1.7 cm diam but it produced only 2 adults. In field trials no attacks occurred in portions of stems < 1.6 cm diam. Upon stem dissection, more brood was found in stem sections near the ground (0-10 cm) than in those over 80 cm above ground. In addition, brood abundance in10 cm long tree sections exhibited a positive non-linear relationship (cubic polynomial; R^2 = 0.21) with section diam. Our data suggest that *X. glabratus* can maintain low populations in areas devoid of large redbay by utilizing 2-3 cm diam portions of small trees primarily near ground level.

Key Words: Sassafras, avocado, exotic, invasive, laurel wilt, *Raffaelea lauricola*

RESUMEN

El escarabajo ambrosia de laurel rojo, *Xyleborus glabratus* Eichoff, es un vector de la marchitez del laurel, *Raffaelea lauricola* T.C. Harr., Fraedrich y Aghayeva, que mata con rapidez todos los árboles de laurel rojo [*Persea borbonia* (L.) Sprengel] de gran diámetro (> 2.5 cm) en un área pero los árboles de diámetros más pequeños (< 2.5 cm) sobreviven durante años. Se midió la densidad de los ataques de *X. glabratus* en los pernos de suspensión del laurel rojo maduro recién cortado durante 5 años (2007-2011) en 7 lugares que varían en el tiempo de estar infestado desde recién infestados en 2007 a los que se encuentran entre las áreas más antiguas infestadas (infestado en 2002 o antes) para determinar si las poblaciones persisten después de los árboles hospederos maduros se han ido. La densidad de ataque varia de 10-50 ataques de *X. glabratus*/100 cm2 donde grandes árboles moribundos de laurel rojo todavía estaban presentes, a < 1 ataque/100cm2 en lugares donde pocos o nada de árboles de > 2.5 cm de diámetro a nivel del suelo estaban vivos al principio del estudio en 2007. A pesar de no tener grandes árboles, las poblaciones persistieron en 2 de los 3 sitios infestados más antiguos por todo el período de estudio de 5 años. En el 2012, se estudió la utilización de *X. glabratus* de madera de laurel rojo de pequeño diámetro como una posible explicación de cómo las poblaciones pueden sobrevivir en estas áreas, en ausencia de los árboles más grandes. En ensayos de laboratorio, *X. glabratus* produjo 27.8 ± 6.63 adultos/galería (± SE) de 18 galerías construidas en partes de árboles laurel rojo con un promedio de 3.2 ± 0.02 cm de diámetro en el punto de ataque. La sección del tallo más pequeño para apoyar una galería con éxito fue de 1.7 cm de diámetro, pero sólo produjo 2 adultos. En pruebas de campo, no hubo ataques en porciones de tallos de < 1.6 cm de diámetro. Tras la disección del tallo, más crías fueron encontradas en las secciones de tallo cerca del suelo (0-10 cm) que en los más de 80 cm del suelo. Además, la abundancia de la cría en secciones de árboles de 10 cm de largo exhibieron una relación no lineal positiva con la sección de diámetro. Nuestros datos sugieren que *X. glabratus* puede mantener poblaciones bajas en las zonas desprovistas de gran laurel rojo mediante la utilización de porciones de 2-3 cm de diámetro de árboles pequeños principalmente cerca del nivel del suelo.

Palabras Clave: Sassafras, aguacate, exótico, invasor, marchitez del laurel, *Raffaelea lauricola*

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), and its associated fungus, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayev, are recent introductions to the southeastern United States responsible for rapid and widespread mortality of mature redbay, *Persea borbonia* (L.) Spreng., and swampbay, *P. palustris* (Raf.) Sargent, trees (Fraedrich et al. 2008). They also kill sassafras, *Sassafras albidum* Presl and avocado trees, *P. americana* Miller (Fraedrich et al. 2008; Mayfield et al. 2008; Smith et al. 2009a) and the fungus has been recovered from camphor tree, *Cinnamomum camphora* Presl, pondberry, *Lindera melissifolia* (Walter) Blume, and pondspice, *Litsea aestivalis* (L.) Fernald (Smith et al. 2009b; Fraedrich et al. 2011; Hughes et al. 2011), all of which are in the Lauraceae. Once *X. glabratus* and laurel wilt reach an area, larger diam redbay and swampbay are the first to be attacked and killed (Fraedrich et al. 2008; Shields et al. 2011). Smaller diam stems, however, often remain healthy and uninfected long after detection of *X. glabratus*. For example, Fraedrich et al. (2008) found that only 1 of 222 redbay with stems less than 2.5 cm DBH (diam at breast height or 1.4 m above ground) died from laurel wilt 2 yr after *X. glabratus* arrived at a study site on Fort George Island, Florida, despite over 90% mortality of all redbay > 2.5 cm DBH. One possible way *X. glabratus* might select trees is by randomly landing as they move through a forest. If that were the case, then the smallest trees would be less likely to be landed upon and bored into by the beetles because they are less apparent and represent much less of the overall surface area in a forest. However, landing sites may not be selected at random since *X. glabratus* are attracted to host volatiles (Hanula et al. 2008; Hanula & Sullivan 2008; Kendra et al. 2011) and Mayfield & Brownie (2013) found that silhouette diam was an important factor in determining captures of *X. glabratus* on essential oil baited traps, with larger silhouettes capturing more beetles. Bark beetles often combine visual with olfactory cues plus random landings to find host trees, all of which reduce the likelihood of a beetle landing on a small tree (Campbell & Borden 2006). It is unlikely *X. glabratus* is responding to greater quantities of essential oils that might be produced by larger trees since increasing release rates have no effect on trap captures of this beetle (Hanula & Sullivan 2008; Hanula et al. 2011). However, *X. glabratus* may have evolved a preference for larger diam tree silhouettes because they are more successful in larger trees (Maner et al. 2013) but *Xyleborus glabratus* performance in stems < 10 cm diam is unknown.

Hanula et al. (2008) measured attack densities of *X. glabratus* at 7 sites in Georgia and South Carolina that ranged from newly infested in 2007 to 3 sites that were among the first to show signs of redbay mortality in 2004 when surveys began (Bates et al. 2013). They found that attack densities were positively correlated with the numbers of dead and dying redbay trees in their plots. Plots located in the oldest infested areas with few or no large trees remaining had very low numbers of attacks. This raised the question - could *X. glabratus* populations survive after they eliminated all large redbay trees, since large sassafras are rare in the coastal plain where redbay grow and no other hosts outside of the Lauraceae have been found (Hanula et al. 2008; Mayfield & Hanula 2011; Kendra et al. 2013a; Mayfield et al. 2013)? Also, if they do persist, how are they sustaining their populations?

To answer these questions we continued monitoring attack densities annually during peak flight at the same sites utilized by Hanula et al. (2008) for 4 more yr to determine if *X. glabratus* populations survived in the absence of redbay > 2.5 cm DBH. In addition, in 2012 we tested the ability of beetles to infest and reproduce in small diam stems of both nursery grown redbay and sassafras trees, and in naturally growing redbay.

Methods and Materials

Population Trends

Populations of *X. glabratus* were monitored at 7 locations established by Hanula et al. (2008) for 4 additional yr (2008-2011) using the same methods and trapping positions established in 2007. Attack densities were measured by hanging freshly cut sections of healthy redbay trees ranging in size from 8-22 cm diam and 30-40 cm long. Sections were cut from healthy trees growing outside of the known range of *X. glabratus* at the time and were less than 48 hours old when hung vertically at the sampling locations. Redbay sections hung in the same manner were attractive for up to 70 days during Jul and Aug at Hunting Island, South Carolina (Hanula et al. 2008). At each location, 3 freshly cut, uninfested redbay bolts were hung ≈ 1.5 m above ground. Bolts were spaced at least 75 m apart and, to increase attraction, 2 strips of bark were removed (≈ 2.5 cm in width and the length of the bolt) on opposite sides of each bolt. A manuka oil lure (Synergy Semiochemical Co., Burnaby, British Columbia, Canada), which is attractive to *X. glabratus* (Hanula & Sullivan 2008), was attached near the center of each bolt to increase attraction. A white sticky trap (wing style trap bottom, Scentry Biologicals Inc., Billings, Montana) was also attached to one side of the bolt to capture some arriving beetles. The bolts were hung in early to mid-Aug, and then removed ≈ 30 days later and returned to the laboratory where they were examined for *X. glabratus* beetle gallery entrances. The field exposure period coincided with the beetle's peak flight period in the study area (Hanula et al. 2008, 2011). Gallery entrances were located by removing the bark from each bolt with a bark scraper and counting the entrances that were approximately the diam of a medium paper clip wire (0.85 mm). Very few ambrosia beetle attacks on redbay, other than *X. glabratus*, had galleries this size (Maner et al. 2013), and the density of attacks by other beetles in this size range were extremely low on redbay sections hung in a forest outside the range of *X. glabratus* (Hanula et al. 2008).

The locations used for the study and their condition in 2007 were: 1) Edisto Beach State Park, South Carolina, 1 yr post-invasion near the northern edge of the infestation; 2) Hunting Island State Park, South Carolina, a heavily infested area 2 yr post-invasion with many dead and dying redbay; 3) Newhall Audubon Preserve on Hilton Head Island, South Carolina, one of the oldest infested areas 5 yr post-invasion with no large standing redbay; 4) the North Ridge Tract on Hilton Head also an old infested area 5 yr post-invasion; 5) Colonels Island, Georgia, also 5 yr post-invasion with very few redbay left; 6) a portion of the Richmond Hill Wildlife Management Area near Eulonia, Georgia, a heavily infested area 2 yr post invasion; and 7) Jekyll Island, Georgia, 1 yr post-invasion near the southern edge of the infestation in 2007. The average numbers of live and recently killed redbay trees present at each location in 2007 were previously reported (Hanula et al. 2008).

Utilization of Small Diameter Trees

Laboratory Trial. In Jan 2012, twelve *P. borbonia* and 6 *S. albidum* trees were purchased (Superior Trees Inc., Lee, Florida) for testing. These trees were held outdoors beneath a lath shade house in Athens, Georgia for approximately 4 months where they were watered 3-5 times per week before beginning the experiment. On 14 May 2012 the trees were moved indoors to a small (≈30 m^2) windowless interior room where they were maintained at 24 °C and a 16:8 h L:D cycle. Trees were watered as needed and a humidifier was used throughout the experiment to increase humidity. All sassafras trees were 1.4-1.8 cm diam at ground level, and redbay ranged from 2.1-4.7 cm. There were 2 size classes of redbay, 6 smaller ones ranging from 2.1-2.5 cm diam at ground level and 6 larger ones ranging from 3.5-4.7 cm diam.

Initially *X. glabratus* females were introduced to the trees by removing a circular 5 mm diam section of bark using a size #0 cork borer. A single female was placed into one half of an empty gel capsule and the capsule was fitted into the hole in the bark. Later introductions of beetles were accomplished by using 200 µL pipet tips instead of empty gel capsules because the capsules were being dissolved by the tree's moisture. Each beetle introduction was assigned a number using numbered map pins, and the heights from the ground and the stem diam at the point of beetle introduction were measured. The gel capsule or pipet tip was removed and replaced with an emergence trap if beetles initiated and sustained boring (indicated by frass accumulation). Traps were made by attaching a 5 cm long section of 0.95 cm (3/8 in) inner diam Tygon® tubing to the tree stem using large (size 6-8) insect pins and capping the opened end of the tubing with an empty 20 mL plastic scintillation vial. The interface between tubing and stem was sealed using acrylic caulk. Vials were monitored weekly for beetle emergence and all emerging adult males and females were counted. A total of 140 beetles were introduced to redbay and 37 to sassafras, and 45 emergence traps placed over beetle gallery entrances on redbay trees, and 10 on sassafras trees. After emergence ceased, all redbay trees were cut into 10 cm sections, dissected using wood chisels, and all *X. glabratus* adults and immatures found within the stems were counted.

Field Trial. Beginning on 17 May 2012, 19 redbay trees were selected for a field study to determine the minimum stem diam required for reproduction of *X. glabratus*. The study site was located in Emanuel Co., Georgia and was a riparian forest composed primarily of redbay and loblolly bay (*Gordonia lasianthus* (L.) Ellis) with scattered remnant loblolly pine (*Pinus taeda* L.). Nearly all large redbay trees in the area had been killed by laurel wilt at the time the study began, but populations of *X. glabratus* were still high based on captures in essential oil baited traps (Hanula et al. 2013). The selected trees were located in close proximity to each other near the edge of the bay forest area of the site. All trees were observed to be healthy and no beetle attacks were present at the beginning of the experiment. Diameters of these trees at 1 m from ground level ranged from 1-6 cm. In order to attract *X. glabratus*, small sections of bark were scraped from the main stem of each tree at the beginning of the experiment, and additional bark was scraped every 2 weeks in order to keep fresh inner bark and sapwood exposed, which are highly attractive to *X. glabratus* (Hanula et al. 2008). Manuka oil lures were hung on 4 of the smallest trees which were unattacked beginning on the second week of the experiment to encourage beetle landings on these trees. The trees were monitored every other week and symptoms indicative of laurel wilt were recorded when they appeared. A single emergence trap identical to those described above was attached over beetle entry points. The contents of these traps were checked every other week and total numbers of adult male and female *X. glabratus* were recorded. On 3 Oct 2012 all trees involved in the experiment were harvested and transported back to the laboratory. The bark was scraped from all stems which were then cut into 10 cm sections, and the number of *X. glabratus*-sized entrance holes per section was counted. All sections were also dissected using wood chisels to split the sections into small pieces, and the number of *X. glabratus* eggs, larvae, pupae, adult males, and adult females that were exposed within each section was counted. The diameter of the galleries and the presence of living or dead *X. glabratus* adults in them insured that only that species was counted. We also noted if other species of similar sized ambrosia beetles were present.

Statistical Analyses

We calculated the mean number of beetles captured per sticky trap per day and the density of *X. glabratus* sized attacks on the sections of redbay hung at the various forest locations by years after initial invasion. Time of initial invasion was determined using the county level surveys conducted by state forestry organizations throughout the infested area (Bates et al. 2013). Because the surveys only started in 2004, two years after the first discovery of *X. glabratus* (Rabaglia et al. 2006), we assumed that the 3 oldest invaded sites were infested in 2002. This was a conservative estimate since tree mortality and the amount of decay in old dead redbay trees suggested that the invasion started earlier at these sites (JLH, personal observation).

Regression (PROC CORR, SAS Version 8, Cary, North Carolina) was used to analyze the relationships between numbers of adults that emerged and height or stem diam among successful galleries on trees in the laboratory. For trees in the field, linear regression was used to examine relationships between attack density versus stem diam. Non-linear relationships between variables were determined using the dynamic fit wizard of SigmaPlot 10 (Systat Software Inc., Richmond, California). Numbers of live *X. glabratus* recovered by dissection of field trees at different heights were analyzed with a one-way analysis of variance (Proc GLM, SAS 1985) and means were separated with the Ryan-Enoit-Gabriel-Welch Quotient (REGWQ) multiple comparison test ($\alpha = 0.05$).

Results

Population Trends

By 2011 all of the populations we monitored were below 2 attacks/100 cm^2 (Fig. 1). Three of the locations, Colonels Island and the 2 Hilton Head locations were in forests that had almost no large (> 2.5 cm DBH) redbay trees left at the beginning of the study in 2007 (Hanula et al. 2008). These 3 sites had very low beetle densities in 2007 (< 0.4 attacks/100 cm^2) and the populations continued to decline over time to < 0.1 attacks/100 cm^2 in 2011. No attacks were observed at Colonels Island in 2011, but 1 beetle was captured on a sticky trap attached to the redbay tree sections. Newhall Audubon Preserve on Hilton Head Island had no beetle captures in traps in 2010 and 2011, but several attacks of the appropriate size were found. Richmond Hill Wildlife Management Area and Hunting Island State Park were both in the middle phase of the infestation at the beginning of the study with high numbers of beetles and large numbers of dead and dying trees. Both of these sites declined from densities of 10-12 attacks/100 cm^2 in 2007 to 0.9 attacks/100 cm^2 or less in 2011. Edisto Beach and Jekyll Island were in the early stages of infestation in 2007 and had relatively low densities. Populations on Jekyll Island increased to 25 attacks/100 cm^2 in 2009 but Edisto Beach populations reached only 4.7 attacks/100 cm^2. Populations at both locations declined to < 1.3 attacks/100 cm^2 in 2011.

The average number of beetles captured and the number of *X. glabratus*-sized gallery entrance holes or attacks were ≈ 1 beetle/trap/day and 1 beetle attack/100 cm^2, respectively, for sites 1 yr after the initial invasion (Fig. 1). Both trap catch and attack density peaked 2-3 yrs after initial invasion and then they declined to low levels (<1 beetle captured or <1 attack/100 cm^2) 5 yr after invasion. By 8-9 yr after invasion the numbers of beetles captured or attacks on freshly cut redbay logs reached very low levels.

Utilization of Small Diameter Trees

Laboratory Trial. A total of 18 galleries in redbay trees successfully produced adult *X. glabratus*

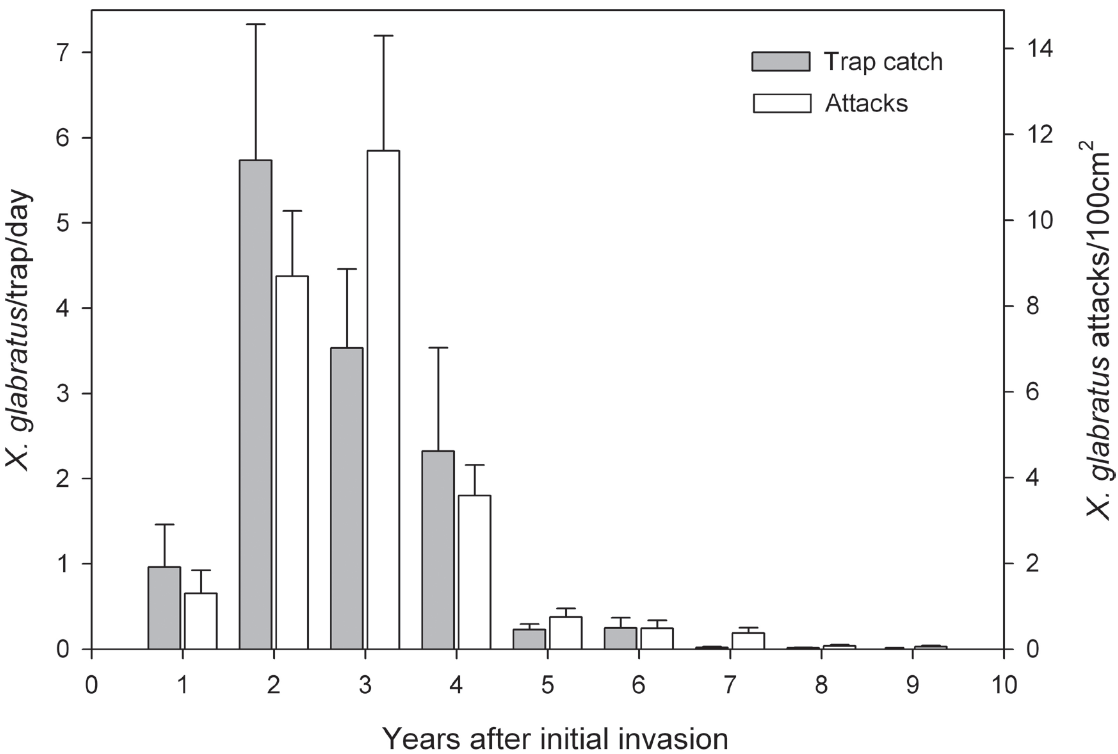

Fig. 1. Mean number (± SE) of *Xyleborus glabratus* captured on sticky traps and density of *X. glabratus*- sized entrance holes or attacks on freshly cut sections of healthy redbay trees ranging in size from 8-22 cm diam and 30-40 cm long. Three sections were exposed to attack at each of 7 locations each year for approximately one month during the peak flight period. Time of initial invasion was based on county level surveys conducted since 2004 (Bates et al. 2013).

and no successful galleries occurred on sassafras. Successful galleries occurred at an average height of 21.6 ± 3.34 cm (± SE) above ground on portions of stems 3.2 ± 0.02 cm diam, and they produced an average of 27.8 ± 6.63 adult beetles/gallery.

Beetles commonly initiated boring into even the smallest stems, but those galleries became inactive or the beetle re-emerged after a short time. The smallest stem diam to support a successful gallery was 1.7 cm but it produced only 2 adults. One gallery in a 1.8 cm diam stem produced 7 beetles, but 3 other successful galleries in stems < 2.5 cm also produced only 2 beetles/gallery. Successful galleries in stems 3-4.2 cm in diam (n = 14) produced an average of 34.8 beetles/gallery (SE = 7.53).

Initial adult emergence from successful galleries in stems < 2.5 cm (n = 4) took a long time; at least 4 months and as long as 6 months. Among all successful galleries there was a significant negative non-linear relationship between stem diam and number of days until first adult emergence (Fig. 2). Total number of adults produced per successful gallery was not correlated with either height (R^2 = 0.05, P = 0.3587, n = 18) or stem diam (R^2 = 0.16, P = 0.0970, n = 18).

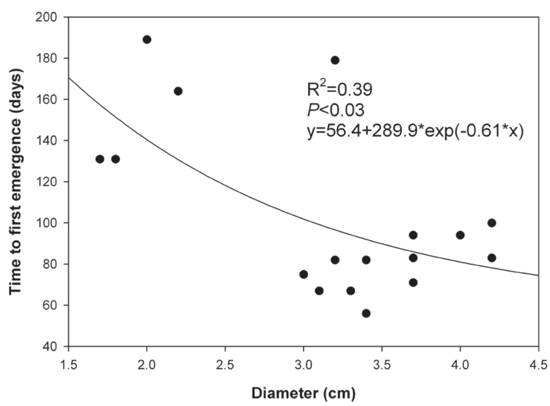

Fig. 2. Number of days until first *Xyleborus glabratus* adult emergence for successful (produced multiple adults) galleries in small diam redbay trees in the laboratory.

All emergence ceased by 10 Dec 2012, 203 days after foundress introduction to the last active gallery. Upon dissection, a total of 37 dead adult females, 9 live adult females, and 1 live male were found in the stems. No larvae or pupae were found.

In addition to the 45 locations where we caged *X. glabratus* females that we introduced, 110 galleries were initiated by other females at sites they chose. The most likely origin of these beetles was emergence from galleries created by some of the initial 95 beetle introductions that were not covered with emergence traps. Although an attempt was made to place emergence traps onto all galleries that showed obvious signs of frass extrusion, some may have been missed. The distribution of these 110 additional attacks was negatively correlated with height ($R^2 = 0.14$, $P < 0.0001$, $n = 174$ sections) and positively correlated with diam ($R^2 = 0.42$, $P < 0.0001$, $n = 174$ sections), i.e., attacks were more common lower on stems on larger diam sections.

Dissection of all redbay stems revealed galleries with considerable vertical (along wood grain) tunneling, frequent branching, and tunneling directly beneath the bark. The galleries differed from those observed by Brar et al. (2013) in larger diameter wood, but the combination of these attributes allowed *X. glabratus* to create tunnels of considerable length even in stems as small as 2.5 cm diam. No exit holes separate from gallery initiation points were seen, but rather when a gallery reached the far side of a stem the tunnel either stopped or abruptly turned before breaking through the bark.

Field Trial. Of the 19 trees wounded and monitored, 6 were never attacked. These 6 were the smallest trees selected, and all but one had diam < 2.0 cm at 1 m height. Only one tree with a diam < 2.0 cm at 1 m height was attacked, and the attack was at the very base of the tree into what appeared to be a canker, which resulted in a disproportionately large stem diam at that point.

By the end of the experiment on 3 Oct 2012, multiple *X. glabratus* adults were recovered from only 1 emergence trap attached to field attacked trees. This trap was attached over an *X. glabratus* gallery entrance at a height of 35 cm from ground level into a stem 2.7 cm in diam, and 3 adult females were collected.

After trees were fully wilted for several weeks, nearly all active galleries, judged by presence of exuded frass, were confined to the lower 30 cm of the trees. Upon stem dissection, greater numbers of brood were found in the sections near the ground (Fig. 3a). The number of brood found was significantly higher in sections from 0-10 cm above ground level than sections 80 cm or above except for sections at 110-120 cm. In addition, we observed a significant non-linear relationship between productivity or brood abundance within tree sections and the diam of the section (Fig. 3b). The regression model suggests no brood production in sections below 1.5 cm diam, very low levels of brood in sections 2-5 cm, and then increasing levels of productivity in larger stems.

A significant linear relationship was seen between the attack density (attacks/100 cm^2) and stem diam at the point of attack (Fig. 4) with larger stem sections sustaining greater attack densities. The model suggests attack density increased with increasing stem diam above 1.2 cm, although no attacks occurred in stems less than 1.6 cm diam. These results are similar to those reported by Kendra et al. (2013b) for swampbay, *P. palustris*.

Discussion

Xyleborus glabratus populations declined to very low levels over a period of 9 yr after their initial invasion. The rapid rise and decline of beetle populations within 5 yr of invasion was consistent with previous reports that 90 percent or more of large redbay trees died within 2 yr of beetle arrival to a new area (Fraedrich et al. 2008; Shields et al. 2011). The low *X. glabratus* population densities in areas where it and laurel wilt were present the longest suggest that it was not using other hosts for brood production or that the alternate hosts are unacceptable compared to redbay. Thus far *X. glabratus* has failed to produce brood in wood from trees outside the family Lauraceae (Mayfield & Hanula 2012; Mayfield et al. 2013) even when they are strongly attracted to it (Kendra et al. 2013a). However, some Lauraceae may not be acceptable hosts either. For example, Smith et al. (2009b) reported laurel wilt from camphor trees in Florida and Georgia, but tests of camphor wood as a host material for *X. glabratus* showed that numbers of beetles emerging from wood exposed to attack in the field were much lower than the initial attack densities, suggesting that the emerging beetles were females abandoning galleries and not their progeny (Mayfield & Hanula 2012).

The declining trend in populations where *X. glabratus* was present the longest (Colonels Island and Hilton Head) suggests that over time populations may go locally extinct. That process may take 10 yr or longer since beetles were still present at those locations 9 yr after the initial invasion. Whether they will be able to continue to persist is unclear although no beetle attacks occurred on Colonels Island in 2011 and attack densities at both sites on Hilton Head Island were very low (< 0.1 attacks/100 cm^2). In addition, no beetles were captured on sticky traps at Hilton Head's Newhall Preserve in 2010 and 2011 so it is possible that attacks at that site were by other small ambrosia beetles, although those are uncommon on redbay trees. For example, Hanula et al. (2008) found 0.4 *X. glabratus*-sized entrance holes/100 cm^2 in redbay bolts hung outside the known area of infestation in 2007 indicating very few similar sized beetles attack redbay. Likewise, Maner et al. (2013) reported only 4% of the galleries they caged were not *X. glabratus* and of those only 2% were *X. saxeseni* (Ratzeburg), ambrosia

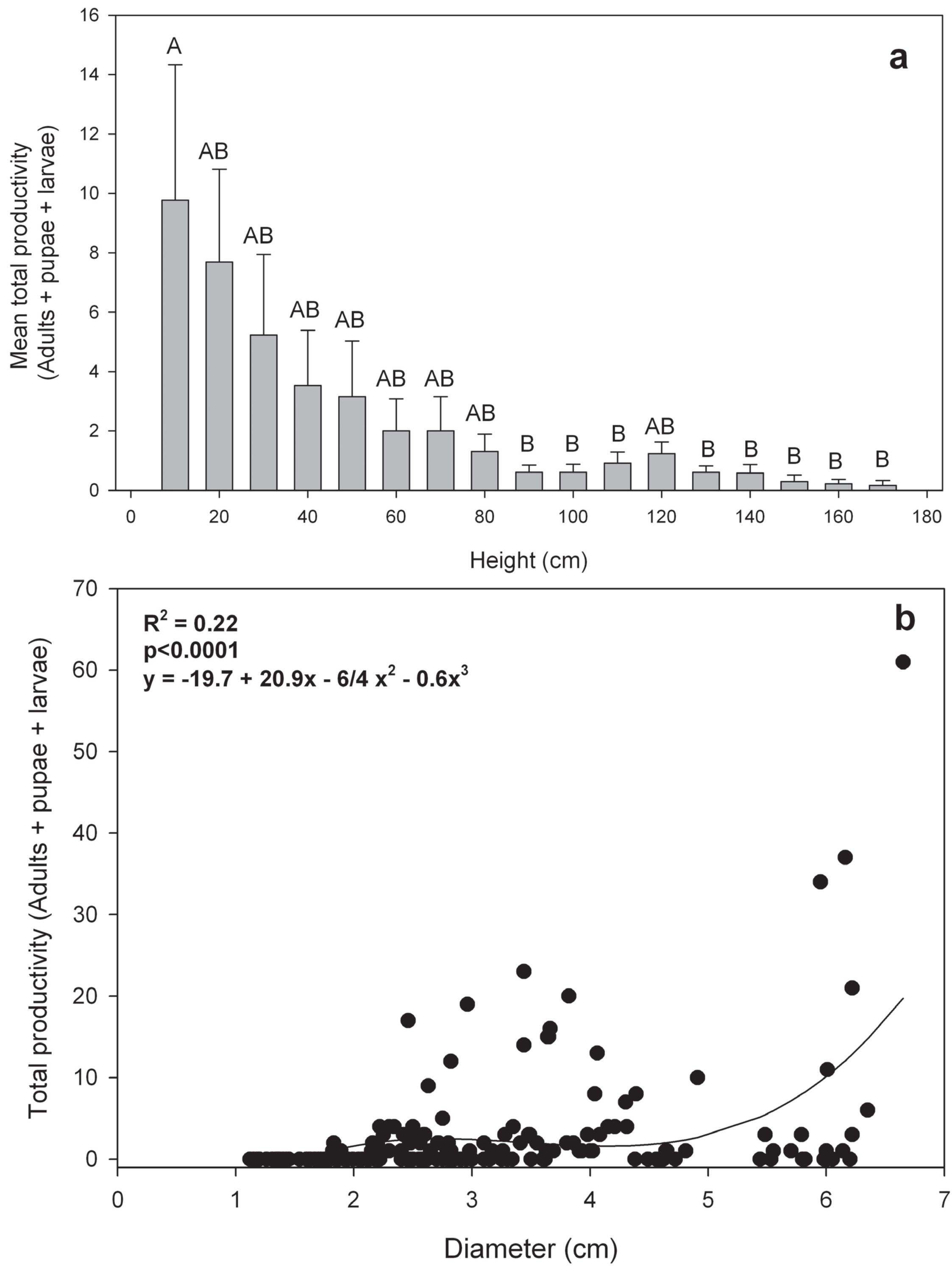

Fig. 3. Relationship of total number of *Xyleborus glabratus* brood (adults + pupae + larvae) recovered from 10 cm long sections of stems of small redbay trees in the field to: (a) height of the sections above ground level; and (b) stem diam.

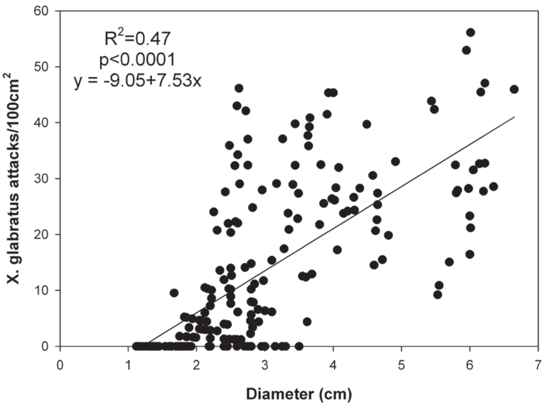

Fig. 4. Relationship between *Xyleborus glabratus* attack density and diam of 10 cm long sections cut from small diam redbay trees that were exposed to attack by *X. glabratus* in the field. Attack density was equalized as: attacks/cm^2 × 100.

beetles that produce holes very similar in size to *X. glabratus*. In that study, however, gallery diameter was estimated by sight rather than with a wire to avoid injuring females near the entrance, so a few larger diam holes were selected that would have been eliminated otherwise. The levels of attack observed on Hilton Head and Colonels Island were similar or lower than attack densities recorded outside the area of infestation in 2007 (Hanula et al. 2008) or by Maner et al. (2013) so the populations at those sites may be extremely low or locally extinct.

Trees < 2.5 cm were poor quality hosts for *X. glabratus*. While stems as small as 1.7 cm diam were able to support brood production, this was rare, took very long, and produced small numbers of beetles. Emergence from stems < 2.5 cm diam took more than 120 days while emergence times from stems ≥ 3 cm took from 50-100 days, consistent with summer development rates in large diam trees (10-26 cm) in the field (Maner et al. 2013). In the laboratory, stems >2.5 cm diam were adequate to support high levels of brood production. Successful galleries in sections of redbay trees 3-4.5 cm diam produced an average of 35 beetles/gallery, slightly higher than the 23 beetles/gallery produced from galleries in large diam trees in the field (Maner et al. 2013).

In contrast, in field studies 5 of 6 trees < 2 cm diam at 1 m above ground had no attacks and the one that did have an attack, the attack occurred where the tree was abnormally enlarged because of a canker, so it was > 2 cm at the point of attack. In the laboratory portion of the test, *X. glabratus* bored into stems 2.0 cm in diam and smaller when caged on them, but this did not happen in field trials even when the bark was freshly scraped and manuka oil lures were attached to encourage attacks. Small stems may not provide an adequate silhouette for *X. glabratus* to key in on (Mayfield & Brownie 2013), despite the added attractant, particularly when the stems are intermingled with non-host stems of similar size.

Very few beetles emerged from small field trees although there was evidence of brood production in them based on stem dissections. Although they may have needed more time to emerge, the experiment lasted 146 days and the galleries that were caged had no brood in them based on dissections, so it is likely that gallery success is very low in small trees in the field where temperatures were higher and humidity varied more than in the laboratory. Most successful galleries in small stems in the laboratory and most brood recovered from field trees were near ground level. This is likely because the lower portions of stems were able to retain moisture for ambrosia fungal growth over a longer period of time while the upper portions tended to dry out too quickly.

Our results show that *X. glabratus* does poorly once the initial phase of the infestation has killed all mature trees. Although population densities were very low, they persisted for at least 9 yr after the initial invasion even though all large redbay trees were dead. Our results on the use of small redbay stems by *X. glabratus* supports the idea that it is maintaining very low densities by attacking small trees and, despite being poor quality for brood production, they sustain low populations in them. These results are encouraging since they suggest that *X. glabratus* is unlikely to attack and spread naturally through small-stemmed pondberry populations, a federally listed endangered species (U.S. Fish and Wildlife Service 1993; DeVall et al. 2001). As redbay seedlings and saplings grow into the susceptible size class, however, it is likely that they will be attacked and support the low level residual population of *X. glabratus*.

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