FOREST ENTOMOLOGY

Effect of Chipping on Emergence of the Redbay Ambrosia Beetle (Coleoptera: Curculionidae: Scolytinae) and Recovery of the Laurel Wilt Pathogen From Infested Wood Chips

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ABSTRACT Significant mortality of redbay trees (*Persea borbonia* (L.) Spreng.) in the southeastern United States has been caused by *Raffaelea lauricola*, T.C. Harr., Fraedrich, & Aghayeva (Harrington et al. 2008), a fungal symbiont of the exotic redbay ambrosia beetle, *Xyleborus glabratus*, Eichhoff (Fraedrich et al. 2008). This pathogen causes laurel wilt, which is an irreversible disease that can kill mature trees within a few weeks in summer. *R. lauricola* has been shown to be lethal to most native species of Lauraceae and cultivated avocado (*Persea americana* Mill.) in the southeastern United States. In this study, we examined the survival of *X. glabratus* and *R. lauricola* in wood chips made from infested trees by using a standard tree chipper over a 10-wk period. After 2 wk, 14 *X. glabratus* were recovered from wood chips, whereas 339 *X. glabratus* emerged from nonchipped bolts. *R. lauricola* was not found 2 d postchipping from wood chips, indicating that the pathogen is not likely to survive for long inside wood chips. In contrast, *R. lauricola* persisted in dead, standing redbay trees for 14 mo. With large volumes of wood, the potential for infested logs to be moved between states or across U.S. borders is significant. Results demonstrated that chipping wood from laurel wilt-killed trees can significantly reduce the number of *X. glabratus* and limit the persistence of *R. lauricola*, which is important for sanitation strategies aimed at limiting the spread of this disease.

KEY WORDS *Raffaelea lauricola*, *Xyleborus glabratus*, avocado, redbay, phytosanitation

Laurel wilt is a vascular wilt disease that kills members of the Lauraceae plant family and has caused substantial mortality to native *Persea*, including redbay (*Persea borbonia* (L.) Spreng.), swampbay (*Persea palustris* (Raf.) Sarg.), sassafras (*Sassafras albidum* Nutt. Nees), and non-native avocado (*Persea americana* Mill.) (Fraedrich et al. 2008) in the southeastern United States. The fungal pathogen, *Raffaelea lauricola* T.C. Harr., Fraedrich, & Aghayeva, (Harrington et al. 2008), is transmitted to the sapwood of healthy host trees by the non-native redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Fraedrich et al. 2008), first detected near Savannah, GA, in 2002 (Rabaglia et al. 2006). The disease has killed >90% of the redbays (>2.5 cm diameter at breast height [DBH]) at Fort George Island, FL (Fraedrich et al. 2008), and 100% of the mature redbays in Etoniah Creek State Forest, FL (Shields et al. 2011), in <4 yr. Laurel wilt currently occurs from North Carolina, west to Mississippi, and south to southeastern Florida (Bates et al. 2012), and anthropogenic movement of the laurel wilt vector and pathogen is believed to have contributed to the rapid expansion of the disease range (Mayfield et al. 2009).

The movement of exotic organisms has increased dramatically over the past 200 yr, and although the impact is often limited, numerous exotic pest introductions have led to significant ecological and/or economic damage (Pimentel 1986, Liebhold et al. 1995). Specifically, wood-borne pests negatively impact forest productivity and wood quality (Pimentel 1986), urban arboriculture (Dreistadt et al. 1990), forest ecology (Liebhold et al. 1995), and agricultural crops, such as avocado (Evans et al. 2010). The primary means of movement of invasive plant pests has been through the importation of ornamental plants from other countries (Perrings et al. 2005) and in untreated wood products (Bridges 1995), which may have been how *X. glabratus* and *R. lauricola* arrived in the United States. At U.S. ports of entry alone, 6,788 individual exotic scolytine beetle interceptions from 49 different countries were made between 1985 and 2000 at inspection stations (representing 67 species), one of which was *X. glabratus* in 2002 (Haack 2001).

Common phytosanitary techniques for eliminating wood-borne pests include heat sterilization (Denlinger and Yocum 1998, Nzoko et al., 2008), fumigation (Cyr 2004), and the chipping of potentially infested

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material (Wang et al. 2000 McCullough et al. 2007). McCullough et al. (2007) found that chipping was more effective than grinding wood for elimination of the emerald ash borer (*Agrilus planipennis* Fairmaire). In addition, heating *A. planipennis*-infested wood bolts to between 60 and 65°C for 48 h was sufficient to kill the beetle in a laboratory setting (Denlinger and Yocum 1998, McCullough et al. 2007). In a similar study, Asian longhorn beetle (*Anoplophora glabripennis* (Motschulsky)) surrogates (plastic worms and gypsy moth larva) that were inserted into logs were damaged or killed after chipping (Wang et al. 2000).

This study explored the effectiveness of chipping trees as a means of sanitation for managing laurel wilt and evaluated the persistence of *R. lauricola* and *X. glabratus* in chipped wood. The objectives of the study were to: 1) evaluate the survival of *X. glabratus* after infested redbay wood is chipped, 2) determine how long *R. lauricola* remains viable in wood chips after chipping, 3) determine if small wood chips can provide an adequate environment for *X. glabratus* development, 4) determine if wood moisture and temperature are correlated with the occupation of intact bolts by *X. glabratus* and *R. lauricola*, and 5) determine how long *R. lauricola* can persist in standing dead redbay trees that were killed by laurel wilt.

Materials and Methods

Acquisition of Infested Wood. Infested redbay wood for the chipping experiments was collected at the University of Florida Austin Cary Forest (ACF), just north of Gainesville, FL, during 2010 and 2011. Redbay trees used for this study had a DBH (diameter at 1.4 m height) of ≥7 cm and had recently died from laurel wilt. Each tree had a completely wilted canopy, discolored sapwood, and evidence of *X. glabratus* beetle boring activity (entrance holes and boring dust). Approximately 30 and 20 dead infested trees were harvested in 2010 and 2011, respectively. Felled trees were chipped by using a Vermeer model BC 935 wood chipper (Vermeer Corporation, Pella, IA).

To confirm the presence of *R. lauricola* in the source material, wood plugs (≈13 by 1.5 mm) were taken from 20 logs in the prechipped infested woodpile with an increment bore hammer (Haglöf, Sweden). Wood cores were cut into smaller fragments (≈2 by 3 mm in size), surface sterilized for 60 s in 70% ethanol, and rinsed in ddH_2O for 60 s. When dry, the wood sections were plated on cycloheximide-streptomycin malt agar (CSMA), a semiselective medium for ophiostomatoid fungi (Harrington 1981), to which was added ampicillin sodium salt (350 mg L^{-1}) and rifampin (500 μl/liter of a 9 mg + 1,000 μl DMSO mixture) (Ploetz et al. 2012). Hereafter, we refer to this medium as CSMA++. All plates were kept at 25°C in the dark. Plates were evaluated for the presence of *R. lauricola* every 3 d.

Emergence of *X. glabratus* From Chipped and Nonchipped Bolts. Forty bolts of infested redbay wood, each 50 cm in length, were used to evaluate the effectiveness of chipping to eliminate *X. glabratus.* In both 2010 and 2011, 10 of 20 bolts were randomly selected and individually chipped onto a clean tarp and placed in individual piles (4.5 by 4.5 by 1.8 dm) on a cement floor in a nontemperature-controlled (although vented) garage at the ACF. The other 10 bolts were used as nonchipped controls and were set on end on the same cement floor. Both the wood chip piles and the intact bolts were covered with fine mesh (400-μm weave) netting, where the mesh was stapled to the inside of a wood frame that surrounded the sample. A plastic trough containing propylene glycol was placed around the perimeter of the wood chip piles and bolts. Beetles that emerged flew into the netting and fell into the collection trough. Insects that emerged from the wood chips and bolts were collected every 2 wk. In addition, one yellow sticky card (Seabright Laboratories, Emeryville, CA) was stapled to the interior top portion of each netted structure and was also collected every 2 wk to help assess the emergence of *X. glabratus.* Insects that emerged were placed into separate containers and returned to the laboratory for identification. Four additional sticky cards were placed around the interior of the garage next to windows to survey for the presence of any *X. glabratus* that escaped from the netted structures. To track the change in moisture of the outer sapwood of felled trees and wood chips, the moisture content was recorded at each sampling event by using a Protimeter Timbermaster (General Electric Corporation, Shannon, Ireland). Three weeks after chipping, a wood core was extracted from each of the nonchipped bolts to test for the presence of *R. lauricola* as previously described.

Persistence *R. lauricola* and *X. glabratus* in Wood Chip Piles. In 2011, experiments were conducted to evaluate the persistence of *R. lauricola* and *X. glabratus* in wood chip subjected to sunlight and tarp treatments. Infested redbay wood that was not used in the previously described emergence study was chipped onto a clean tarp and used to fill 336 mesh bags (150 cm^3). Bags were filled within 3 h of tree chipping (during the middle of the day). The remaining wood chips were used to fill bins and to create piles of wood chips in which the mesh bags would be placed. Because the volume of available redbay trees was insufficient to fill all the bins and piles, oak (*Quercus*) wood chips were purchased and mixed with the redbay wood chips to create the necessary volume to fill the bins and piles.

Eighteen 1-m^3 bins and 10 0.5-m^3 piles of wood chips were created and monitored over a 10-wk period. Nine of the 1-m^3 bins were placed in full sun (bin-sun), whereas the other nine were placed in constant shade (bin-shade). For the 0.5-m^3 piles of wood chips, a blue tarp was placed over all 10, with five placed in full sun (tarp-sun) and five in constant shade (tarp-shade). Tarps were held down with cement blocks and completely covered the entire wood chip pile. Six mesh bags containing wood chips derived from infested redbay wood were placed in the center and six on the top of each bin and pile. For the 0.5-m^3 wood chip piles, mesh bags were placed on top of the

wood chips but under the tarp. For mesh bags placed in the center of the bins, a 1-m-long string was tied to each bag to aid in their retrieval. Under each bin and pile, a sheet of plywood was positioned to isolate the wood chips from soil microorganisms.

Temperature probes (CAS Data Loggers, Chesterland, OH) were placed in the center of six bins and two piles for both the sun and shade treatments. Temperature probes placed at the center of the piles recorded the temperature of the wood chips every hour. To determine the temperature on the tops of the bins, we used a University of Florida weather station that was located 50 m away.

Mesh bags were extracted from the center and top of each bin and pile every other day after chipping for 1 mo and every 2 wk thereafter. The 0.5-m^3 tarp-sun and tarp-shade piles were tracked for 4 wk, whereas the 1-m^3 bin-sun and bin-shade were both followed for 10 wk. The mesh bags were taken back to the laboratory where the wood chips were placed on a white piece of paper to observe any beetles. Four wood chips from each mesh bag were set aside for evaluation of moisture content and presence of live *R. lauricola.* The rest of the wood chips were placed into a 226-cm^3 plastic insect-rearing chamber to monitor for the emergence of *X. glabratus.* Each chamber had five holes (covered with mesh) to relieve moisture build up. At the bottom, an inverted funnel allowed beetles to move into a clear collection jar. Rearing chambers were kept at room temperature (24°C) and were exposed to ≈12 h of light daily. Each collection jar was evaluated weekly for 90 d for the presence of *X. glabratus* and other wood-boring insects. After 90 d, wood chips in each rearing chamber were examined by using a dissecting microscope to determine if any *X. glabratus* remained inside the chambers. To test whether the rearing chambers provided an adequate environment for survival of *X. glabratus,* 10 small laurel wilt-infested redbay bolts (3 by 11 cm) were placed into the same rearing chambers. The bolts were monitored every 14 d for 6 mo.

Putative isolates of *R. lauricola* that were recovered from the four wood chips removed from each mesh bag were identified morphologically (Harrington et al. 2008). Suspect isolates from wood chips were then subcultured on CSMA++ for ≈10 d, and examined for diagnostic small subunit (18s) rDNA (Dreaden et al. 2008). DNA was extracted (Justesen et al. 2002) with the addition of a proteinase K digestion step. The small subunit (18s) rDNA was amplified via polymerase chain reaction (PCR) by using the primers NS1 (5′-GTAGTCATATGCTTGTCTC-3′) and NS4 (5′-CTTCCGTCAATTCCTTTAAG-3′) (White et al. 1990). A PCR master mix containing 12.5 μl of Amplitaq Gold (Applied BioSystems, Foster City, CA.), 9.5 μl of ddH2O, 1 μl of DNA, plus 1 μl of each primer was used. A MJ Research PTC-200 Peltier thermocycler was used for PCR with the following cycling conditions: 95°C for 6 min then 40 cycles of 95°C for 1 min., 48°C for 30 s, and 72°C for 2 min for DNA extension. ExoSAPit (Affymetrix, Inc., USB Products, Santa Clara, CA) was used to purify the amplicon, which was then sent to the University of Florida's Interdisciplinary Center for Biotechnology Research for Sanger sequencing. The sequenced amplicons were then compared with the GenBank library (http://www.ncbi.nlm.nih.gov/).

Beetle Presence Near the Study Site. Two weeks before chipping, five six-unit Lindgren multifunnel traps baited with manuka-oil (Synergy Semiochemical Corp., Burnaby, BC, Canada) were suspended from trees 2 m above the ground around the study site to attract *X. glabratus* (Kendra et al. 2011). Traps were checked weekly from July through mid-December, every 2 wk from mid-December through April, then weekly again through July 2011. The multifunnel traps were fitted with wet collection jars containing propylene glycol. Trap catches were collected and tabulated for scolytine catches only where the number of *X. glabratus* was recorded. Both *Xyleborus affinis* Eichhoff and *Xyleborus volvulus* (F.) were common around the site, but because of the time required to differentiate between the two, their numbers were combined.

Persistence of *R. lauricola* in Standing Trees. Fourteen redbay trees were monitored from January 2011 to July 2012 in Washington Oaks Garden State Park, Flagler County, FL. The park covers 425 acres on a barrier island in Flagler County, FL. The study trees occurred in a typical maritime hammock (Spence et al. 1998) dominated by live oak (*Quercus virginiana* Mill.), pignut hickory (*Carya glabra* Miller), southern magnolia (*Magnolia grandiflora* L.), southern red cedar (*Juniperus virginiana* L.), and redbay with an understory of yaupon holly (*Ilex vomitoria* Sol. ex Aiton). Study trees had DBH measurements that ranged from 6.4 to 24 cm. Trees in the study began to wilt in February 2011; by July, all trees were dead. Over the course of 18 mo, 11 trunk cores were taken from each of the 14 trees. Sapwood cores (13 by 1.5 mm) were collected by using an increment hammer to test for the presence of the pathogen at the start of the study. Two samples were collected at 2 m and two samples from 30 cm above natural grade, one from each side of the tree. The head of the increment hammer was rinsed with 70% ethanol between samples for each tree. Samples were placed in separate vials and were kept in a cooler for transport back to the laboratory. Sapwood core samples were cut into four or five smaller fragments (≈2 by 3 mm in size) and surface sterilized and plated as previously noted. Identifications of putative colonies were carried out by using the same methodology previously described.

Quantitative Analysis. A one-way analysis of variance was used to compare the emergence of *X. glabratus* from wood chips and nonchipped bolts (Microsoft 2010). A Student's *T*-test was used to compare the diameters and moisture of nonchipped and chipped bolts for both studies in 2010 and 2011 (Microsoft 2010). A significance level of $\alpha = 0.05$ was used for all tests. Linear regression analyses of *X. glabratus* emergence from nonchipped bolts versus daily temperature, moisture, and weeks since wood was chipped were conducted with Microsoft Excel 2010.

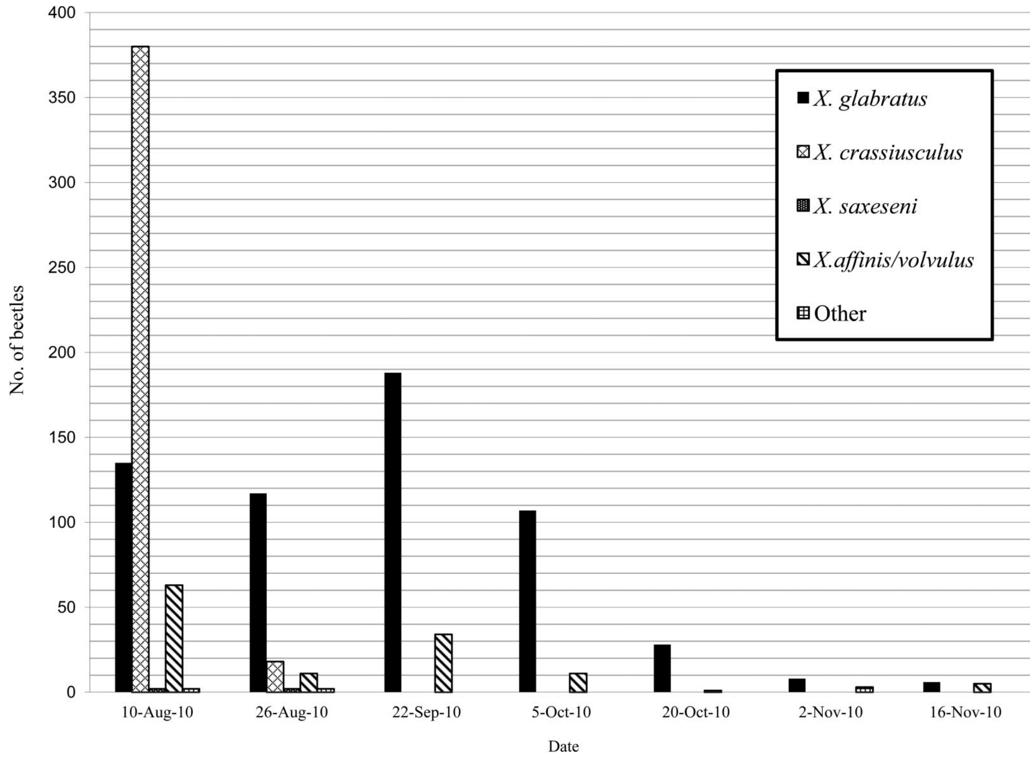

Fig. 1. Beetle emergence from nonchipped bolts from August 2010 to January 2011. Numbers of *Xyleborus affinis* and *Xyleborus volvulus* were combined.

Results

Emergence of *X. glabratus* From Chipped and Nonchipped Bolts. The differences in moisture of felled trees that were used for the chipping study ranged from 27.7 to 100%, with a mean of 60.4% and a standard deviation of 25%. A comparison of the moisture of 50 randomly selected wood chips taken 1 h after chipping ranged from 22 to 80%, with a mean of 37.9% and a SD of 11.9%.

When *X. glabratus* emergence from the nonchipped bolts was combined, there were 2,461 scolytine beetles that emerged over 10 mo. Over the first 2 wk, 1,200 scolytine beetles emerged, of which 339 were *X. glabratus.* Over the remaining 8.5 months, 1,261 scolytine beetles emerged, 1,034 of which were *X. glabratus* (Fig. 1). Over the same period, the emergence of *X. glabratus* from wood chips was significantly lower than from the intact, nonchipped bolts, with only 10 *X. glabratus* emerging (F (2,27) = 6.92; P = 0.0037). Over the 10-mo study, only one *X. glabratus* (and very few other scolytine beetles) was found on the sticky cards inside the garage (placed near windows), indicating that redbay ambrosia beetle escapes cannot explain the disparity in these data.

In 2010, an average of 25.2 beetles/bolt emerged from intact control logs compared with 0.3 beetles/bolt (or no beetles at all) from chipped bolts. Similarly, in 2011, an average of 18 and 0.4 beetles/bolt emerged from nonchipped and chipped logs, respectively (Table 1). After 3 wk, *R. lauricola* was recovered from all intact bolts used in both 2010 and 2011.

The outer sapwood moisture content of bolts used for the chipped versus nonchipped bolt emergence study was not significant (F (1,18) = 0.18; P = 0.676). In addition, diameters of the nonchipped bolts used for the emergence study did not differ

Table 1. Diameter, moisture content, and recovery of *Xyleborus glabratus* and *Raffaelea lauricola* from redbay bolts used in chipping studies in 2010 and 2011

Year	Treatment	No. bolts	Mean (SE)			No. bolts positive for *R. lauricola* prechipping
			Diameter (cm)	% moisture content, pretreatment	No. *X. glabratus* emerged per bolt	
2010	Chipped	10	13.9 (0.9)	92.7 (6.5)	0.3 (0.48)	10/10
	Nonchipped	10	13.4 (0.5)	91.7 (6.3)	25.2 (31.09)	10/10
2011	Chipped	10	12.4 (1.5)	91.3 (5.6)	0.4 (0.96)	10/10
	Nonchipped	10	13.5 (1.6)	92.9 (5.8)	18.0 (30.27)	10/10

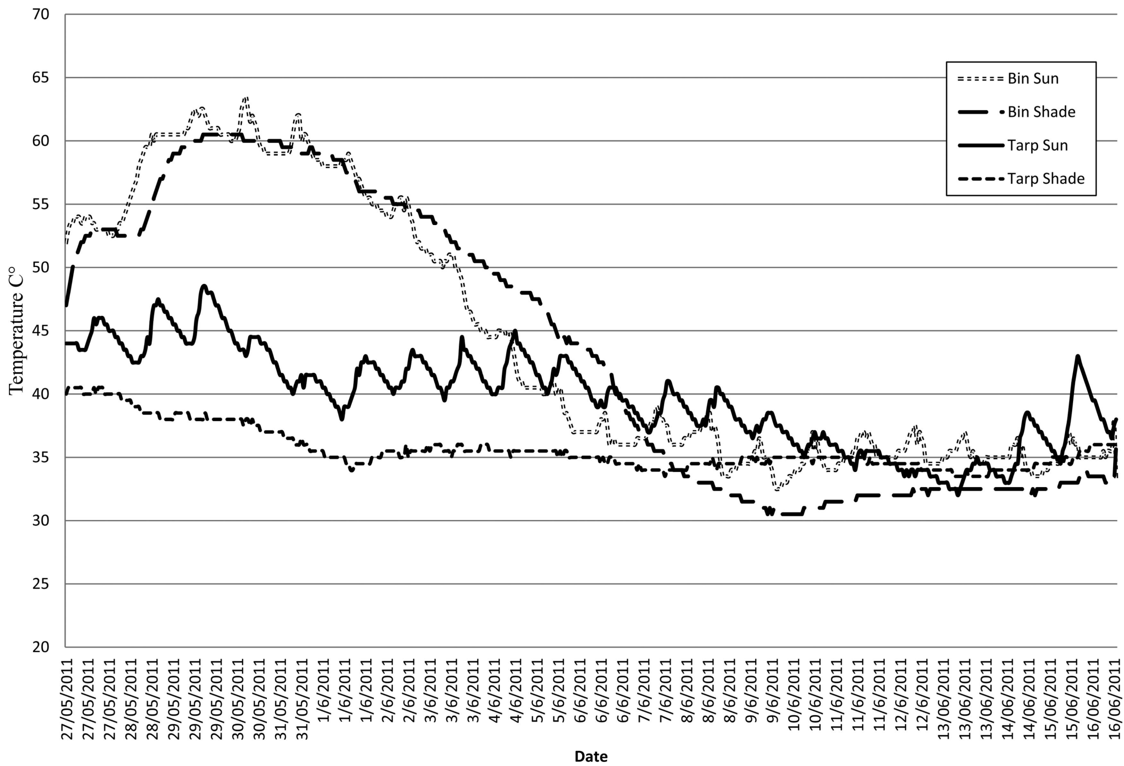

Fig. 2. Average daily maximum internal temperatures of wood chip in piles and bins, in full sun, shade, and under tarps, over the length of the study. Temperature probes were placed in the center of each treatment.

significantly between treatments (F (1,18) = 0.018; P = 0.895).

X. glabratus emergence was compared with nonchipped bolt moisture, daily outside temperature, and weeks since chipping. Daily outside temperature and moisture were not well correlated with *X. glabratus* emergence (r^2 = 0.123 and r^2 = 0.064, respectively). However, the emergence of *X. glabratus* was positively correlated (r^2 = 0.698) with the length of time that the study ran, from July 2010 through March 2011, with more beetles emerging toward the beginning of the study.

Persistence of *R. lauricola* and *X. glabratus* in Wood Chips. Over the course of the study, only 3 of 336 rearing chambers full of wood chips from the sun and shade treatments yielded *X. glabratus. X. glabratus* emerged into a collection jar from wood chips from the same rearing chamber at 2 d postchipping. These wood chips came from mesh bags that were on the top of a shade bin. Both beetles were female; one dark and one light yellow, indicating it recently eclosed. These were the only living beetles found in any of the rearing chambers. Through dissection of the wood chips, two intact dead *X. glabratus* were found inside two additional rearing chambers, which came from mesh bags from the top of a bin that was in sun. In addition to these two intact beetles, more than a dozen *X. glabratus* fragments were also found in mesh bags from both the top and center of the bins and piles.

In addition to the four beetles found in the rearing chambers, 10 redbay ambrosia beetles emerged from wood chips from the piles in the garage. From these piles, two beetles were recovered at 6 wk postchipping, whereas the other eight were all recovered within 2 wk.

No *X. glabratus* were found in the mesh bags from the tarp treatment. Other than *X. glabratus*, the species that were found in collection jars or by dissection were three *X. affinis*, five *Xylosandrus crassiusculus* (Motschulsky), and one *Xyleborinus saxeseni* (Ratz.). From the 10 small bolts of redbay that were placed inside the rearing chambers to test suitability of the rearing chambers, 24 *X. glabratus* emerged over a 6-mo period. No other species were found in those collection jars.

R. lauricola grew from wood chips from only four mesh bags, two from the top of the bin-sun treatment and two from the top of the bin-shade treatment. These four mesh bags were collected 2 d postchipping. *R. lauricola* was never recovered beyond 2 d postchipping. Each of the four isolates sequenced produced a 99 or 100%, match to voucher isolates of *R. lauricola* in GenBank.

Temperature of the Bins and Piles. The maximum temperatures of wood chips in the center of the bin-sun, bin-shade, tarp-sun, and tarp-shade piles were 63 (10.4), 60 (11.3), 48 (3.8), and 40°C (1.7°C), respectively. The average maximum daily internal temper-

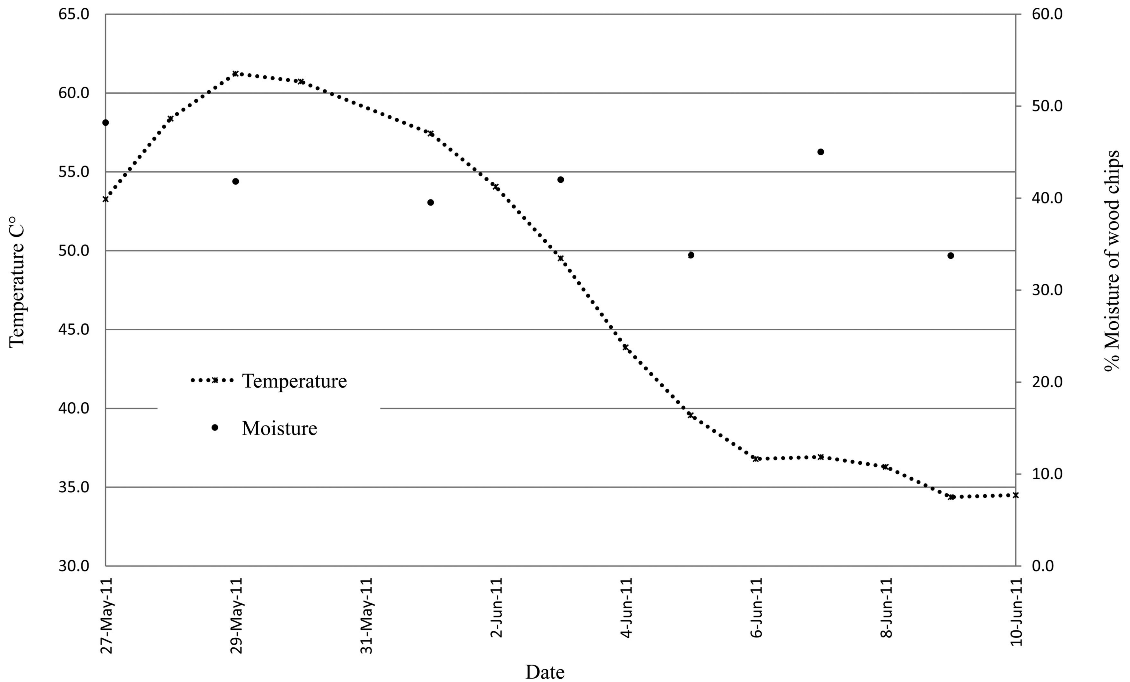

Fig. 3. Temperature and moisture of wood chips (inside bin measurements). The change in wood chip moisture in relation to the change in temperature at the center of the wood chip piles is represented. The temperature data presented are the averages of each treatment.

atures from each replicate are represented in Fig. 2. Temperatures recorded at the center of the 1-m^3 nontarped wood chip bins (both in the sun and shade) were higher than the temperature specified by the International Standards For Phytosanitary Measures No. 15 (ISPM 15) for heat treating wood packing material (56°C) (Animal and Plant Inspection Services [APHIS] 2004).

Moisture and Temperature Effects. The relative change in wood temperature and wood chip moisture was not correlated with the emergence of beetles from nonchipped bolts or survival of *R. lauricola* in wood chips. As *R. lauricola* was only found at 2 d postchipping, moisture and temperature could not have been the reason for its disappearance (Fig. 3).

To represent the temperature on the surface of the wood chip bins, temperatures from the nearby weather station were used over the course of the study (May through July). The maximum temperature reached at the ACF weather station was 35°C. The maximum daily temperature reached at Gainesville Regional Airport (≈9 km away) over the same period was 35.5°C.

Beetle Presence Near the Study Site. Beetles collected from five multifunnel traps monitored over a 12-mo period were: 706 *X. glabratus*, 572 *Xylosandrus crassiusculus*, 125 *Xyloborinus saxeseni*, 814 *X. affinis* + *X. volvulus* (these two species counted collectively), 16 *Monarthrum mali* (Fitch), 51 *Hypothenemus* spp., and 65 *Euplatypus* spp.

Persistence of *R. lauricola* in Standing Trees. Over the course of the 18-mo study, *R. lauricola* persisted in the outer sapwood in 2 of 14 trees for 15 mo. The shortest amount of time the pathogen persisted was 3 mo. This tree was cut down and dissected in August 2011 and was found to have *R. lauricola* in the inner sapwood, 7 cm beneath the bark. This indicates that *R. lauricola* can persist deep inside a dead tree for long periods in a standing dead tree.

Discussion

Data collected during this study clearly show that only a few *X. glabratus* survive the chipping process. Only 14 survived from chipping treatments (of which 10 were in a garage in a semicontrolled environment), whereas 1,373 emerged from the nonchipped bolts over the same period, a decline of 99%. In addition, *R. lauricola* did not persist in wood chips longer than 2 d.

More beetles emerged from the garage wood chips than from wood chips inside mesh bags, which may have been because of features of the outside environment (temperature, moisture, or fungal competition). The only beetles that survived chipping came from wood chips that were not covered with a tarp or were kept dry under netting in the garage. Because of this finding, it may be prudent to cover wood chips with a tarp for a week to ensure that any survivors are killed. Wood chips that came from the center of the bins and from under the tarps were wet from condensation and had been colonized by *Aspergillis fumigatus* Fresenius, *Syncephalastrum racemosum* Cohn, and *Rhizopus microporus* Tiegh, along with other unidentified oppor-

tunistic fungi. It is possible that these saprophytes could have out-competed *R. lauricola,* eliminating it from the wood. In this experiment, we used oak wood chips to create the volume necessary to fill the bins and make all the wood chips piles. This was necessary because there were not enough dead redbay trees to create the volume necessary to make all the replicates we needed. We do not believe that this was a factor that influenced our results.

Use of living, dead, dying, and decaying trees by ambrosia beetles is driven by a complex set of variables, one of which is wood moisture (Wagner et al. 1979). However, this study did not show a correlation between a change in outer sapwood moisture between nonchipped bolt moisture and *X. glabratus* emergence. *X. glabratus* emerged at a fairly constant rate for 8 wk from nonchipped bolts, whereas the other scolytine species emerged en mass over the first 2 wk. Neither the quick emergence by other scolytines nor the relatively steady emergence of *X. glabratus* was positively correlated to the outer sapwood moisture of the nonchipped bolts.

Because of the similar bore hole sizes between *X. glabratus* and *Xyleborinus saxeseni, X. affinis, Xyleborus ferrugineus* (F.), and *X. volvulus,* we determined that any beetle density calculations based on the number of bore holes would not be accurate. In addition, the small size of the beetles (2 mm in length) would also make it difficult to find all *X. glabratus* still inhabiting bolts of wood; therefore, no data are provided on the number of beetles that could potentially inhabit any given volume of wood.

With large numbers of killed Lauraceae across southeastern United States, there is the potential for wood processing facilities to convert these trees into mulch. Although not directly tested, this research indicates that *R. lauricola* would likely not move from wood chips to healthy trees if the wood chips were used in landscaping.

The persistence of *R. lauricola* in standing dead trees for at least a year suggests that standing trees serve as a significant reservoir for the pathogen. Although not investigated by this study, it is possible that the activity of *X. glabratus* may play a role in maintaining the fungus in dead trees.

In general, pests and pathogens are moved in intact wood, but we show that the potential of moving *X. glabratus* and *R. lauricola* in wood chips is low. Even if all or most Lauraceous species in urban and forest settings are killed, an active laurel wilt management program could be instituted in avocado fields to chip infested trees, which could aid in keeping this important crop from being decimated by this significant tree disease.

This investigation has demonstrated that chipping trees is an effective means of reducing both the laurel wilt pathogen, *R. lauricola,* and its beetle symbiont, *X. glabratus.* However, even with a population reduction of 99%, it only takes one female *X. glabratus* to start a new population.

Acknowledgments

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