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## DIVERSITY OF SCOLYTINAE (COLEOPTERA: CURCULIONIDAE) ATTRACTED TO AVOCADO, LYCHEE, AND ESSENTIAL OIL LURES

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### ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring insect that vectors laurel wilt, a lethal vascular disease of trees in the Lauraceae, including avocado (*Persea americana*) and native *Persea* species (redbay, swampbay). As part of research to identify host-based attractants for *X. glabratus*, we discovered that a diverse array of non-target ambrosia beetles was attracted to the same substrates as *X. glabratus*. During Sep-Dec 2009, several field tests were conducted in north Florida (in woodlands with advanced stages of laurel wilt) with traps baited with commercial lures of the essential oils, manuka and phoebe, and with freshly-cut wood bolts of avocado (a known host) and lychee (*Litchi chinensis*, a non-host high in the sesquiterpene  $\alpha$ -copaene, a putative host attractant). In addition, manuka-baited traps were deployed in avocado groves in south Florida to monitor for potential spread of *X. glabratus*. The combined trapping results indicated that none of these substrates was specific in attraction of *X. glabratus*. Numerous non-target ambrosia beetles were captured, including 17 species representative of 4 tribes within the subfamily Scolytinae. This report provides photo-documentation and data on the species diversity and relative abundance for a group of poorly-studied beetles, the scolytine community in Florida *Persea* habitats.

Key Words: ambrosia beetles, *Persea americana*, *Litchi chinensis*, manuka oil, phoebe oil

### RESUMEN

El escarabajo de la ambrosía del laurel rojo (redbay), *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), es un insecto exótico barrenador de madera que transmite la marchitez del laurel, una enfermedad vascular mortal de árboles de la familia Lauraceae, los cuales incluyen el aguacate (*Persea americana*) y las especies nativas del género *Persea* (redbay, swampbay). Como parte de la investigación para identificar las sustancias químicas que atraen a *X. glabratus*, fue descubierto que un arsenal diverso de otros escarabajos de la ambrosía que no eran de interés económico fue atraído a las mismas sustancias. Entre septiembre y diciembre del 2009, se realizaron varias pruebas en el norte de la Florida (en arboledas con etapas avanzadas de la marchitez del laurel) usando trampas con cebos comerciales de los aceites esenciales manuka y de phoebe, y con madera de aguacate recién cortada (un huésped conocido) y del lychee (*Litchi chinensis*, que no es huésped, pero es alto en el sesquiterpeno  $\alpha$ -copaene, una sustancia química atractiva). Además, trampas con manuka fueron desplegadas en arboledas de aguacate en el sur de la Florida para supervisar la extensión potencial del *X. glabratus*. Los resultados de las capturas combinados indicaron que ninguna de estas sustancias eran específicas en la atracción del *X. glabratus*. Numerosos escarabajos de la ambrosía fueron capturados, incluyendo 17 especies que representan cuatro tribus dentro de la subfamilia Scolytinae. Este informe proporciona la foto-documentación y datos en la diversidad de la especie y la abundancia relativa para un grupo de escarabajos poco estudiado, la comunidad del Scolytinae en los hábitats de *Persea* de la Florida.

Translation provided by the authors.

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring insect that vectors laurel wilt, a lethal vascular disease of trees in the Lauraceae (Fraedrich et al. 2008). Haploid

males are flightless and remain within the host tree, while diploid females (typically sibling-mated) disperse to colonize new hosts. Unlike most ambrosia beetles, female *X. glabratus* are primary colonizers, capable of attacking healthy

unstressed trees. During gallery excavation, females introduce spores of a symbiotic fungus, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Harrington et al. 2008), carried in mycangial pouches located at the base of the mandibles (Fraedrich et al. 2008). The fungus provides food for both larvae and adults, but it also invades the host vascular system and results in systemic wilt and ultimately tree death. Native to southeastern Asia, *X. glabratus* was first detected in the U.S. in 2002 near Port Wentworth, Georgia (Rabaglia et al. 2006). Since then, the vector-disease complex has spread along the coastal plain into South Carolina and Florida, and has been reported from a single county in Mississippi (USDA-FS 2010). In northern Florida, high mortality has occurred in native *Persea* species, including redbay (*P. borbonia* (L.) Spreng.) and swampbay (*P. palustris* (Raf.) Sarg.), and the rapid southward spread of the pest complex currently threatens commercial groves of avocado (*P. americana* Mill.), a confirmed susceptible host (Mayfield et al. 2008). Florida's avocado production, centered in Miami-Dade County, is worth \$13 million annually (USDA-NASS 2010), and replacement costs of all avocado trees (commercial and backyard) in Miami-Dade, Broward, Palm Beach, and Lee Counties have been estimated at \$429 million (Evans & Crane 2008).

Due to the serious economic threat posed by *X. glabratus*, there is a critical need for effective attractants to detect, monitor, and control the spread of this invasive pest. Preliminary research provided no evidence of an aggregation pheromone and no strong attraction to its fungal symbiont, to its frass, or to ethanol (a standard attractant for ambrosia beetles); suggesting that host tree volatiles are the primary attractants for dispersing females (Hanula et al. 2008). Additional studies identified manuka and phoebe oils (essential oil extracts from the tea tree, *Leptospermum scoparium* Forst. & Forst., and the Brazilian walnut, *Phoebe porosa* Mez., respectively) as effective baits for field monitoring of *X. glabratus* in South Carolina (Hanula & Sullivan 2008). Based on comparisons of volatile chemicals emitted from chipped redbay wood, manuka oil, and phoebe oil, Hanula & Sullivan (2008) hypothesized that 2 sesquiterpenes,  $\alpha$ -copaene and calamenene, were likely the primary host attractants.

While conducting research to evaluate attraction of female *X. glabratus* to wood volatiles and essential oil lures, we discovered that a diverse number of non-target ambrosia beetles (both endemics and exotics established in Florida) were attracted to the same substrates as *X. glabratus*. Several field tests were conducted in north-central Florida (Alachua and Marion Counties) in natural stands of redbay and swampbay with known infestations of *X. glabratus* and visible signs of laurel wilt disease. We used 4-funnel

Lindgren traps and/or sticky panels baited with commercially available essential oil lures (manuka and phoebe) or with freshly-cut wood bolts of avocado (a confirmed host) and lychee (*Litchi chinensis* Sonn., a presumed non-host). Lychee is in the family Sapindaceae; it lacks the typical aromatic laurel volatiles, but it has a high content of  $\alpha$ -copaene (Niogret et al. unpublished). During that same period, monitoring traps (Lindgren traps baited with manuka lures) were deployed in avocado groves in south Florida (Miami-Dade County). This report summarizes and illustrates the ambrosia beetles captured over a 4-month period (Sep-Dec 2009) in Florida to (1) provide a tool for action agencies and field scientists to facilitate identification of non-target species captured in *X. glabratus* monitoring traps, (2) document the species diversity and relative abundance for the scolytine community in *Persea* habitats, and (3) identify potential secondary colonizers of *Persea* hosts subsequent to initial attack by *X. glabratus*.

#### MATERIALS AND METHODS

Field test 1 was conducted in Citra, Marion County, FL at the University of Florida Agricultural Experiment Station (PSREU). The back edge of the station bordered an upland wooded area dominated by mature live oak (*Quercus virginiana* Mill.) with an understory that included redbay trees symptomatic for laurel wilt. Test 1 was run from 27 Aug-22 Oct 2009 and consisted of 6 treatments: a commercial manuka lure (Synergy Semiochemicals, Burnaby, BC), wood bolts from 3 avocado cultivars representative of the 3 horticultural races ('Simmonds', West Indian race; 'Brooks Late', Guatemalan race; and 'Seedless Mexican', Mexican race), bolts from lychee (cv. 'Hanging Green'), and an unbaited control. Wood bolts were collected from the USDA-ARS germplasm collection at the Subtropical Horticulture Research Station (SHRS), Miami, FL 1 d prior to test deployment. The ends of the bolts were coated with wax to prevent desiccation, and then both ends re-cut when used as baits at the start of the test. All baits were deployed in four-funnel Lindgren traps (BioQuip, Rancho Dominguez, CA) with 300 mL of an aqueous solution of 10% propylene glycol (Low-Tox antifreeze; Prestone, Danbury, CT) added to the collection cup. For the manuka treatment, a single lure was hung from the trap lid by a wire twist tie. For the wood substrates, 2 freshly-cut bolts (5 cm diam  $\times$  15 cm length) were suspended with wire from the lid on opposite sides of the trap. Experimental design was a randomized complete block, with 5 replicate blocks arranged in a linear array along the fence at the back of the research station. Within a block, traps were spaced 10 m apart, 1.5 m above the ground, and spacing was 50 m between repli-

cate blocks. Traps were checked every 2 weeks for a total of 8 weeks. At each sampling date, the retention solutions (with insect captures) were collected, a thin layer was sawed from the lower end of each bolt (to “renew” release of wood volatiles), the collection cups were refilled, and trap positions were rotated sequentially within each block to minimize potential positional effects on beetle capture.

Field tests 2 and 3 were conducted in Cross Creek, Alachua County, FL at the Lochloosa Wildlife Conservation Area (St. John’s River Water Management District). The study site consisted of mesic flatwoods composed of an overstory of slash pine (*Pinus elliotii* Englem) with a mixed understory that included numerous swampbay trees exhibiting advanced stages of laurel wilt. Test 2 was conducted from 7 Oct-2 Dec 2009 and evaluated the same 6 treatments described above. Test 3 was conducted from 5 Nov-29 Dec (at a site adjacent to test 2) and contained a commercial phoebe lure (Synergy Semiochemicals) in addition to the 6 treatments above. However, with tests 2 and 3 there were differences in trap type and trap layout. The essential oil lures were still deployed in four-funnel Lindgren traps, but the wood bolts were paired and hung vertically with 2 white sticky panels (23 cm × 28 cm, Sentry wing trap bottoms; Great Lakes IPM, Vestaburg, MI) stapled back-to-back at the bottom of the bolts. Sticky panels were further secured with several binder clips around the edges. Tests 2 and 3 followed randomized complete block design, with 5 replicate blocks arranged in a rectangular grid. Each block consisted of a row of traps hung ~2 m high in non-host trees, with a minimum of 10 m spacing between adjacent traps in a row, and with 50 m spacing between rows. Both tests were 8 weeks in duration and checked every 2 weeks. At each check, the retention solutions and sticky panels were collected, a thin layer was sawed from the bottom of each bolt, the solutions/panels were replaced, and the trap positions were rotated sequentially within each row.

In addition to the field tests conducted in north Florida, monitoring/survey traps were deployed in several avocado groves in Miami-Dade County, FL during the fall of 2009. All monitoring traps consisted of four-funnel Lindgren traps baited with manuka lures, which were hung ~2 m above ground within the canopy of avocado trees. Traps were checked at 2-week intervals and sites included the SHRS avocado germplasm collection in Miami and 3 commercial groves in Homestead.

All sample collections (from monitoring traps and field tests) were sorted in the laboratory at SHRS, and scolytine species were counted, photographed, and stored in 70% ethanol. Specimens removed from sticky panels were soaked overnight in histological clearing agent (Histo-clear II; National Diagnostics, Atlanta, GA) prior to

storage in alcohol. Beetle identifications were confirmed at FDACS-DPI (Gainesville, FL) by K. E. Okins, and voucher specimens were deposited at both DPI and SHRS.

## RESULTS

The combined trapping results from field tests and monitoring traps totaled 659 ambrosia beetles, consisting of 17 species from 4 tribes within the subfamily Scolytinae (Table 1). More than 90% of the captures were from the tribe Xyleborini, and only 1 specimen (*Coccotrypes distinctus* (Motshulsky)) was representative of the tribe Dryocoetini. With the exception of *X. glabratus* (Fig. 1), most beetles were captured in fairly low numbers, with many species represented by only a single capture, despite significant trapping efforts with a variety of host-based attractants. *Xyleborus glabratus* comprised the majority of captures in north Florida, as expected due to its invasive pest status, but the percentages varied by site (15% in Marion County with test 1; 75.4% and 86.2% in Alachua County with tests 2 and 3, respectively). Four other species of *Xyleborus* were captured (Fig. 2), with *X. ferrugineus* (Fabricius) (Fig. 2A) and *X. affinis* (Eichhoff) (Fig. 2B) the 2 most abundant species after *X. glabratus*. There were several other representatives within the tribe Xyleborini (Fig. 3), with *Ambrosiodmus obliquus* (LeConte) (Fig. 3A) a dominant species at both the Alachua site and in the Miami-Dade avocado groves.

The tribe Cryphalini was represented by *Hypothenemus dissimilis* (Zimmerman) and several other *Hypothenemus* species difficult to discern to species level (Fig. 4). *Hypothenemus* beetles were major components at the Marion site and in Miami-Dade County. Within the tribe Corthylini, 5 species were captured, of which 4 are presented in Fig. 5. Two of those ambrosia beetles had distinctive morphological features. Females of *Corthylus papulans* Eichhoff (Fig. 5C) have greatly enlarged terminal antennal segments which bear several long, recurved setae (Fig. 5E); males of *C. papulans* lack the characteristic setae. In *Pityoborus comatus* (Zimmerman) (Fig. 5D), females are unique in that the mycangia are located on the pronotum, and consist of a pair of large shallow depressions covered with dense pubescence (Fig. 5F; Furniss et al. 1987).

## DISCUSSION

The subfamily Scolytinae contains 2 functionally distinct groups of beetles - bark beetles which feed on phloem from the inner bark of host trees, and ambrosia beetles which cultivate and feed on symbiotic fungi within the xylem layers (Rabaglia 2002). Among the bark beetles, there are major forest pests which have been well studied, includ-

TABLE 1. AMBROSIA BEETLES (CURCULIONIDAE: SCOLYTINAE) CAPTURED IN MARION, ALACHUA, AND MIAMI-DADE COUNTIES, FL FROM SEP-DEC 2009, ARRANGED ACCORDING TO LAWRENCE &amp; NEWTON (1995).

	Marion Co.		Alachua Co.		Miami-Dade Co
	Test 1 <sup>a</sup>	Test 2 <sup>b</sup>	Test 3 <sup>b</sup>	Monitoring <sup>c</sup>	
Tribe Dryocoetini					
<i>Coccotrypes distinctus</i> (Motschulsky)			1		
Tribe Xyleborini					
<i>Ambrosiodmus lecontei</i> Hopkins					1
<i>Ambrosiodmus obliquus</i> (LeConte)		6	14		22
<i>Premnobius cavipennis</i> Eichhoff					1
<i>Theoborus ricini</i> (Eggers)					1
<i>Xyleborus affinis</i> (Eichhoff)	2	11	4		
<i>Xyleborus californicus</i> Wood	2	1			
<i>Xyleborus ferrugineus</i> (Fabricius)	3	34	22		1
<i>Xyleborus glabratus</i> Eichhoff	3	193	287		
<i>Xyleborus volvulus</i> (Fabricius)		7	3		
Tribe Cryphalini					
<i>Hypothenemus dissimilis</i> (Zimmerman)	1	1			
<i>Hypothenemus</i> spp.	9	1	1		22
Tribe Corthylini					
Subtribe Corthylina					
<i>Corthylus papulans</i> Eichhoff					1
<i>Monarthrum mali</i> (Fitch)		1			
Subtribe Pityophthorina					
<i>Pityoborus comatus</i> (Zimmerman)			1		
<i>Pseudopityophthorus minutissimus</i> (Zimmerman)					1
<i>Pseudopityophthorus pruinus</i> (Eichhoff)		1			

<sup>a</sup>8-wk field test in redbay; Lindgren traps baited with wood bolts (avocado, lychee) or manuka oil lures.

<sup>b</sup>8-wk field test in swampbay; Sticky traps baited with wood bolts (avocado, lychee); Lindgren traps baited with manuka/phoebe oil lures.

<sup>c</sup>Monitoring in avocado groves; Lindgren traps baited with manuka oil lures.

ing the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Chellman & Wilkinson 1980), the western pine engraver, *Ips pini* (Say) (Kegley et al. 1997), and several other *Ips* spp. found in the southeastern U.S. (Conner & Wilkinson 1998). In contrast, the ambrosia beetles are generally not of economic importance and consequently have received less attention. They are minute beetles, spend the majority of their life concealed within host trees, and typically attack stressed or dying trees. Despite the large number of described species (e.g., >500 currently recognized *Xyleborus* spp. worldwide, Rabaglia et al. 2006), much is unknown regarding the basic biology, ecology, host range, fungal symbionts, and population dynamics of many endemic ambrosia beetles. Far less is known about exotic invasive species, which are not pests in their native lands but may acquire pest status when introduced into new environments, as is the case with *X. glabratus* in the U.S.

Although our research was focused on identification of attractants specifically for detection and control of *X. glabratus*, information was obtained

concurrently on the species diversity and relative abundance for the ambrosia beetles found in native *Persea* habitats in north-central Florida. In south Florida the trapping effort was less intensive, but preliminary data was also obtained for the species composition in avocado groves. This summary report identifies the species of Scolytinae most likely to be encountered while monitoring for *X. glabratus*, and the photo-documentation provides fellow researchers (non-taxonomists) and action agency personnel with a convenient tool for preliminary identification of non-target captures.

Some of the non-target beetles identified herein are species that may potentially function as secondary vectors of the laurel wilt pathogen. Once healthy trees are attacked by *X. glabratus*, the stressed trees are susceptible to further attack by secondary colonizers that can contribute to the rapid mortality seen in laurel hosts. Observations made on dead swampbay trees at the Lochloosa Conservation Area (Kendra et al. unpublished) indicated that multiple wood-boring species attacked those *Persea* trees, as evidenced

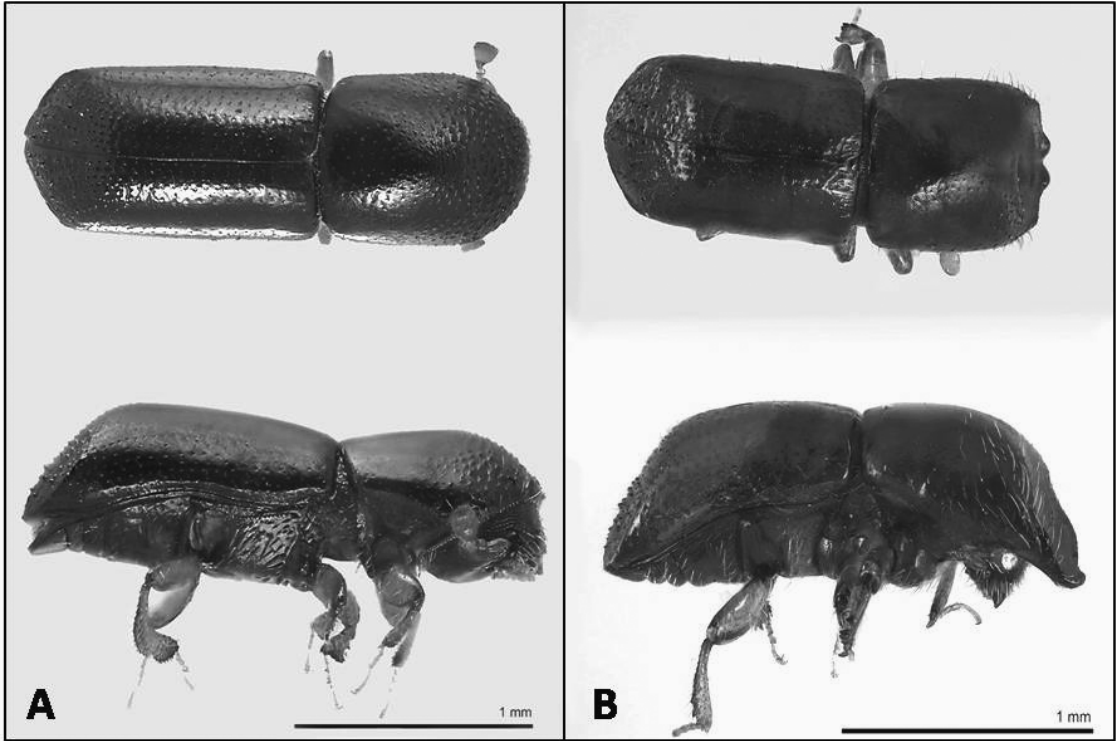


Fig. 1. The redbay ambrosia beetle *Xyleborus glabratus* Eichhoff, vector of a lethal wilt fungus (*Raffaellea lauricola*) causing high mortality of trees in the Lauraceae in the southeastern U.S. A. Female. B. Male. (Note: Males of *X. glabratus* are flightless; this specimen was obtained from host wood, not from a flight trap.)

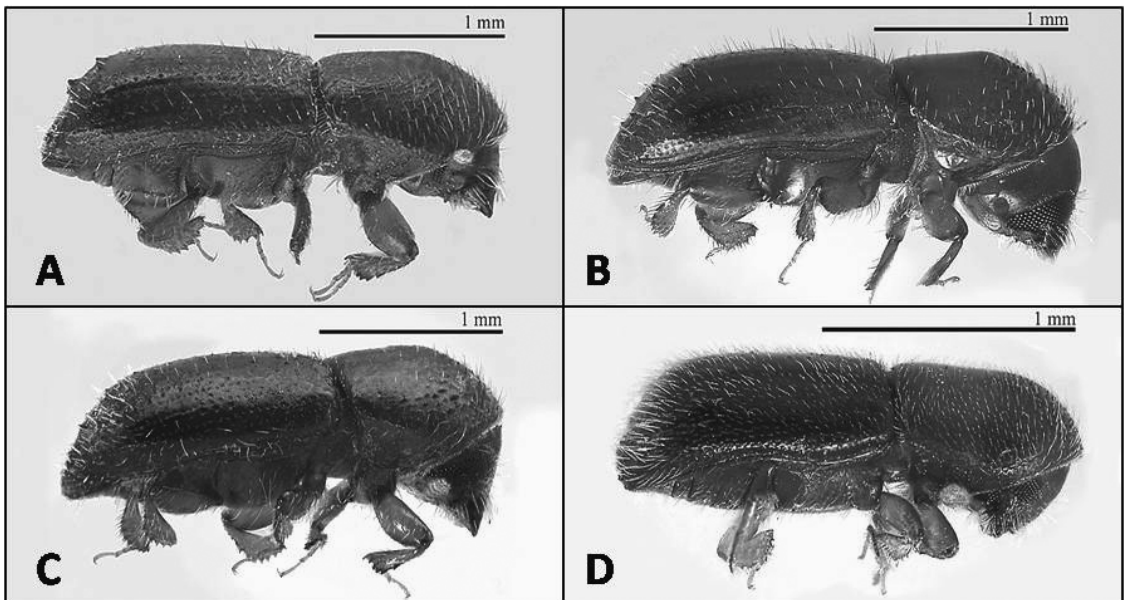


Fig. 2. Four species of *Xyleborus* not of economic importance. A. *X. ferrugineus* (Fabricius). B. *X. affinis* (Eichhoff). C. *X. volvulus* (Fabricius). D. *X. californicus* Wood. (All specimens female.)

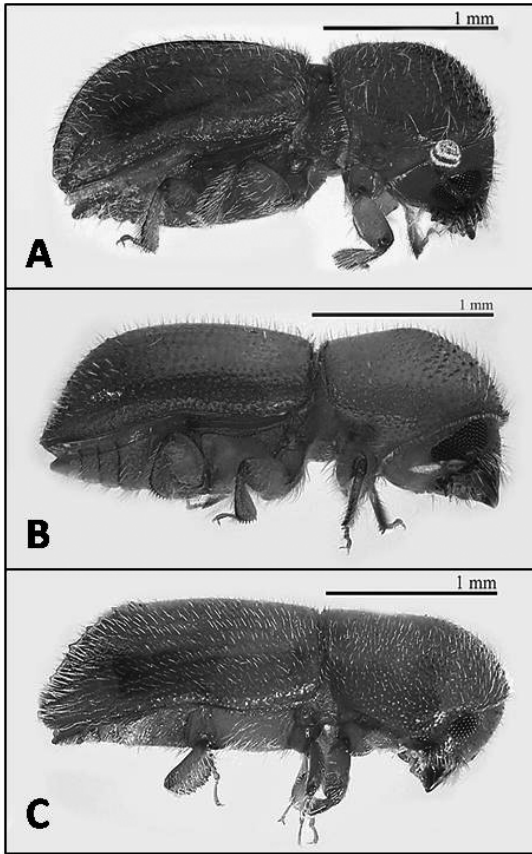


Fig. 3. Ambrosia beetles within the tribe Xyleborini. A. *Ambrosiodmus obliquus* (LeConte). B. *Theoborus ricini* (Eggers). C. *Premnobius cavipennis* Eichhoff. (All specimens female.)

by bore holes of various diameters. These secondary colonizers may potentially pick up *Raffaelea* from the host xylem and transport it to new trees, accelerating the spread of laurel wilt. In north Florida, *X. ferrugineus*, *X. affinis*, and *A. obliquus* were the most abundant species in native *Persea* habitats; in avocado *A. obliquus* and *Hypothenemus* spp. were dominant. In South Carolina, Hanula et al. (2008) found that *Xylosandrus crassiusculus* (Motschulsky) was attracted to wounded redbay trees. Further research should evaluate these additional beetle species to (a) determine if stressed (diseased) trees in the Lauraceae can serve as hosts, and if so, then (b) determine if *Raffaelea* spores can be recovered from the mycangia of ambrosia beetles that developed within *Raffaelea*-infected hosts. In other systems, there is evidence that exchange or "cross contamination" of symbiotic fungi may occur among ambrosia beetle species that occupy a common breeding site (Gebhardt et al. 2004). Alternatively, *Raffaelea* spores may potentially be transported pas-

sively by the setae and/or cuticular asperities (protuberances) commonly found on the anterior slope of the female pronotum, as has been demonstrated for *Hypothenemus hampei* (Ferrari) and spores of *Fusarium solani* (Martius) (Morales-Ramos et al. 2000).

The commercial lures currently available for *X. glabratus* are non-specific in attraction, so high numbers of non-target captures are likely to be encountered with the monitoring system (manuka-baited Lindgren traps) employed by the State of Florida. Manuka and phoebe oil lures were originally developed for field monitoring of another (phylogenetically distant) wood-boring beetle, the emerald ash borer *Agrius planipennis* Fairmaire (Coleoptera: Buprestidae) (Crook et al. 2008). Preliminary research (Kendra et al. unpublished) indicated that these essential oil lures were not only non-specific, but may have limited field life for attraction of *X. glabratus*. With the data set presented here, approximately 30% of the captures were non-target species of Scolytinae. Development of effective strategies for early detection and control (i.e., attract-and-kill systems) of *X. glabratus* is contingent on identification of specific attractants. In the absence of species-specific pheromones or food-based attractants for *X. glabratus* (Hanula et al. 2008), this will be a difficult challenge.

## CONCLUSIONS

Multiple trapping studies targeting the redbay ambrosia beetle, *Xyleborus glabratus*, effectively generated a survey of the overall scolytine community resident in Florida *Persea* habitats. These ambrosia beetle species that co-occur with *X. glabratus*, are attracted to the same host-based volatile chemicals; and they are the non-target species likely to be encountered in traps set out to monitor for *X. glabratus*. Those species that can function as secondary colonizers of *Persea* hosts should be evaluated as potential secondary vectors for transmission of the laurel wilt pathogen, *Raffaelea lauricola*.

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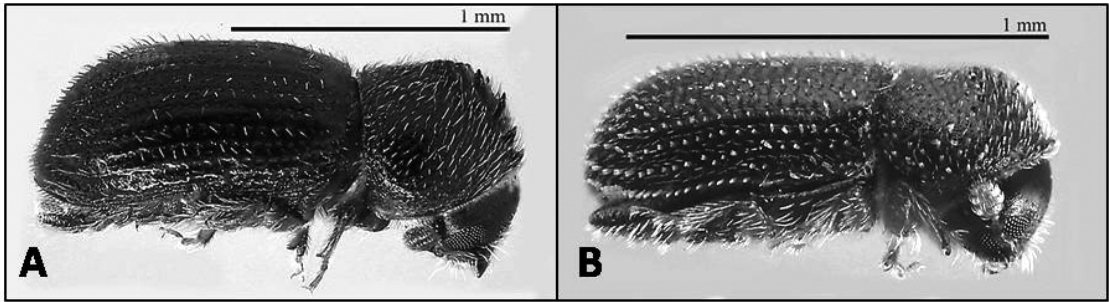


Fig. 4. Ambrosia beetles within the tribe Cryphalini. A. *Hypothenemus dissimilis* (Zimmerman). B. *Hypothenemus* sp. (Both specimens female.)

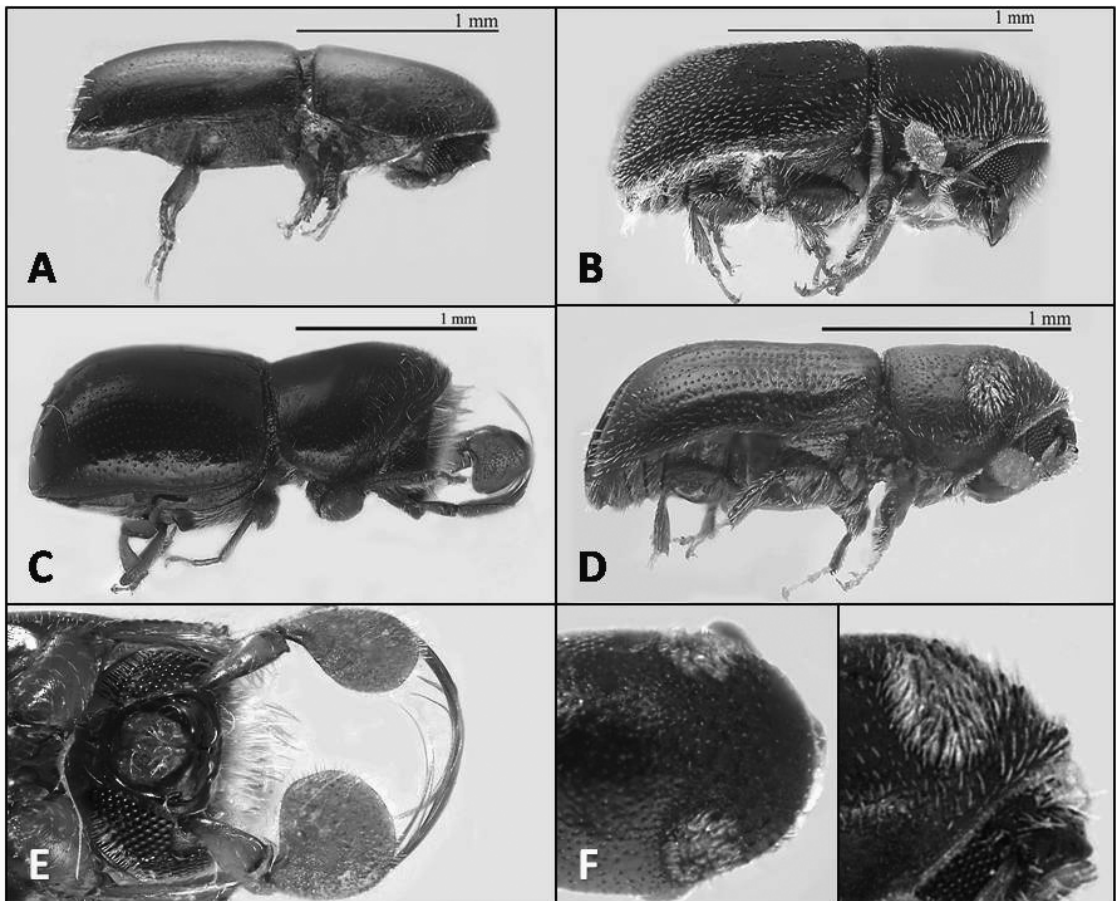


Fig. 5. Ambrosia beetles within the tribe Corthylini. A. *Monarthrum mali* (Fitch). B. *Pseudopityophthorus prunosus* (Eichhoff). C. *Corthylus papulans* Eichhoff. D. *Pityoborus comatus* (Zimmerman). E. Detail of *C. papulans* (anterior end; ventral view) showing enlarged terminal antennal segments bearing long recurved setae. F. Detail of *P. comatus* (anterior end; dorsal view on left, lateral view on right) showing pronotal mycangia (oval pits covered with dense setae), the storage site for symbiotic fungal spores. (All specimens female.)

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