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Source: Florida Entomologist, 95(2) : 513-516

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.095.0244>

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## METHOD FOR COLLECTION OF LIVE REDBAY AMBROSIA BEETLES, *XYLEBORUS GLABRATUS* (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE)

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The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is the only confirmed vector of laurel wilt, a newly-described lethal disease of trees in the family Lauraceae (Fraedrich et al. 2008; Harrington et al. 2008). Unlike typical ambrosia beetles, *X. glabratus* attacks healthy unstressed trees, and preferred hosts in the U.S. are native *Persea* species (Laurales: Lauraceae), including redbay *P. borbonia* (L.) Spreng., swampbay *P. palustris* (Raf.) Sarg., and silkbay *P. humilis* Nash, but avocado *P. americana* Mill. is also susceptible (Hanula et al. 2008; Mayfield et al. 2008). First detected in the U.S. near Savannah, Georgia, *X. glabratus* has since spread to the Carolinas, Florida, Alabama, and Mississippi (USDA-FS 2012), and currently threatens commercial avocado groves in Miami-Dade County, FL (FDACS 2011). Curtailing further spread of laurel wilt is dependent on development of effective lures for early detection of the vector.

Initial research indicated that the primary semiochemicals used by dispersing females are host tree volatiles, and that manuka oil and phoebe oil are attractive baits (Hanula et al. 2008; Hanula & Sullivan 2008; Hanula et al. 2011). Progress has been made toward identification of host-based attractants through (1) comparative analysis of volatiles emitted from essential oils and known lauraceous hosts (Hanula and Sullivan 2008; Niogret et al. 2011a), and through (2) correlation of lure/host emissions with captures of *X. glabratus* in field tests (Kendra et al. 2011a, 2012). Based on these studies, the current hypothesis is that  $\alpha$ -copaene is the primary long-range attractant of *X. glabratus*, but that several other sesquiterpenes may also be involved in host location, including  $\alpha$ -humulene, cadinene,  $\beta$ -caryophyllene, and calamenene.

Confirmation of specific attractants could be greatly facilitated by electroantennography (EAG) or coupled EAG-gas chromatographic separation (electroantennal detection, EAD) of host volatiles. These methods could identify the individual chemicals detected by antennal receptors and quantify the olfactory responses from *X. glabratus*, as has been done with other

pests (e.g., Kendra et al. 2005, 2008; Niogret et al. 2011b). Development of EAG/EAD methods, as well as controlled laboratory bioassays, requires a reliable supply of female *X. glabratus*. As an alternative to establishing a laboratory colony of *X. glabratus*, and waiting potentially 1-2 mo for beetle emergence, we developed a method for field collection of live females that would be available for immediate use (and on an as-needed basis). The method was based on observations of *X. glabratus* in flight (presumably host-seeking), 1-1.5 m above ground, during the late afternoon and early evening h in the fall of 2010 (PK, WM – Highlands Co., FL; G. Brar, Univ. Florida – Alachua Co., Florida, personal communication).

To optimize captures of dispersing *X. glabratus*, we chose collection sites with ample *Persea* hosts, including both healthy trees and trees exhibiting early stages of laurel wilt. Beginning at ~1600 h (EDST), we spread a cotton sheet (over a tarp, if necessary) on the ground in a forest clearing, and then added host bait to the center (Fig. 1A). Bait consisted of cross-sectional branch disks (potential visual cues to complement olfactory cues), rasped branches, and sawdust from freshly-cut *Persea* spp. or from lychee (*Litchi chinensis* Sonn.; Sapindales: Sapindaceae), a presumed non-host high in  $\alpha$ -copaene (Niogret et al. 2011b) and highly attractive to *X. glabratus* in field tests (Kendra et al. 2011a). As beetles landed on the bait or surrounding sheet (Fig. 1B), they were collected by hand with a soft brush, and stored in plastic boxes containing slightly moist tissue paper (Fig. 1C). Alternatively, slow flying beetles and those hovering over the bait were gently batted down and collected. In addition, the attractive plume was sometimes supplemented with commercial lures of phoebe oil or manuka oil (each with a release rate of 50 mg oil/d; Synergy Semiochemicals, Burnaby, BC, Canada) suspended from a tripod 1 m above the sheet. To generate a pulsed release of host-based attractants, the lures were fanned and fresh wood was cut every 15-20 min. Collections were made up until dark, and no external light source was used to avoid attraction due to phototaxis. All scolytine beetles were sorted in the laboratory,



Fig. 1. Method for collection of live redbay ambrosia beetles, *Xyleborus glabratus*, using freshly-cut host wood as bait deployed in the late afternoon near trees symptomatic for laurel wilt. Branches from *Persea* spp. were cut into cross-sectional disks; cut wood and sawdust were placed in center of cotton sheet (A). Ambrosia beetles landing on the bait or surrounding sheet (B) were collected by hand with a soft brush and stored in plastic boxes containing moist tissue paper (C).

counted and identified according to Rabaglia et al. (2006), and voucher specimens deposited at SHRS (Miami, Florida) and at Archbold Biological Station (Lake Placid, Florida).

This collection method was replicated more than 25 times (Apr-Oct 2011) at several sites in Highlands County, supplying ample beetles for our experimental research. Although numbers captured each night varied with environmental conditions, several general patterns were evident. First, fresh wood bait consistently attracted 4 species of ambrosia beetles (mean  $\pm$  SE females/night): *Xyleborus glabratus* ( $58.4 \pm 8.2$ ), *Xyleborus affinis* (Eichhoff) ( $74.7 \pm 14.2$ ), *Xyleborus ferrugineus* (Fabricius) ( $5.5 \pm 1.1$ ), and *Xylosandrus crassiusculus* (Motschulsky) ( $3.2 \pm 0.8$ ) (Fig. 2A-D). The first 3 species were observed during all mo of collection, and have been documented previously as attracted to volatiles from avocado, lychee, and essential oils (Kendra et al. 2011b, 2012); *X. crassiusculus* was observed primarily from Apr through Jun, and is known to be attracted to redbay (Hanula et al. 2008) and essential oils (Kendra et al. 2012). A few other Scolytinae were occasionally collected, including *Hypothenemus* spp. and *Xyleborinus andrewesi* (Blandford) (Fig. 2E). The latter species was first detected in North America in 2010 (Okins & Thomas 2010),

and has been reported only from Lee, Highlands, and Duval Counties, Florida (K. Okins, FDACS-DPI, personal communication). Second, although multiple species of Scolytinae were attracted, *X. glabratus* was observed flying several h earlier than the others. All non-target species initiated flight just prior to sunset (1900-1930 h), but *X. glabratus* was seen as early as 1600 h, with ~80% of *X. glabratus* collections made before the appearance of non-targets, and with a sharp drop in *X. glabratus* collections at sunset (~2000 h, when non-target collections were highest). This temporal separation in flight provided a perfect window for selective capture of *X. glabratus*. Third, there were behavioral differences among species which also facilitated specific collection of *X. glabratus*, even under low light levels encountered late in the d. *Xyleborus glabratus* was much less active than the non-target species; once it had landed, it was reluctant to take to the wing, and it walked at a much slower rate than that observed with other Scolytinae.

#### SUMMARY

Development of improved lures for *Xyleborus glabratus* will be expedited through experimental research conducted with host-seeking females. As

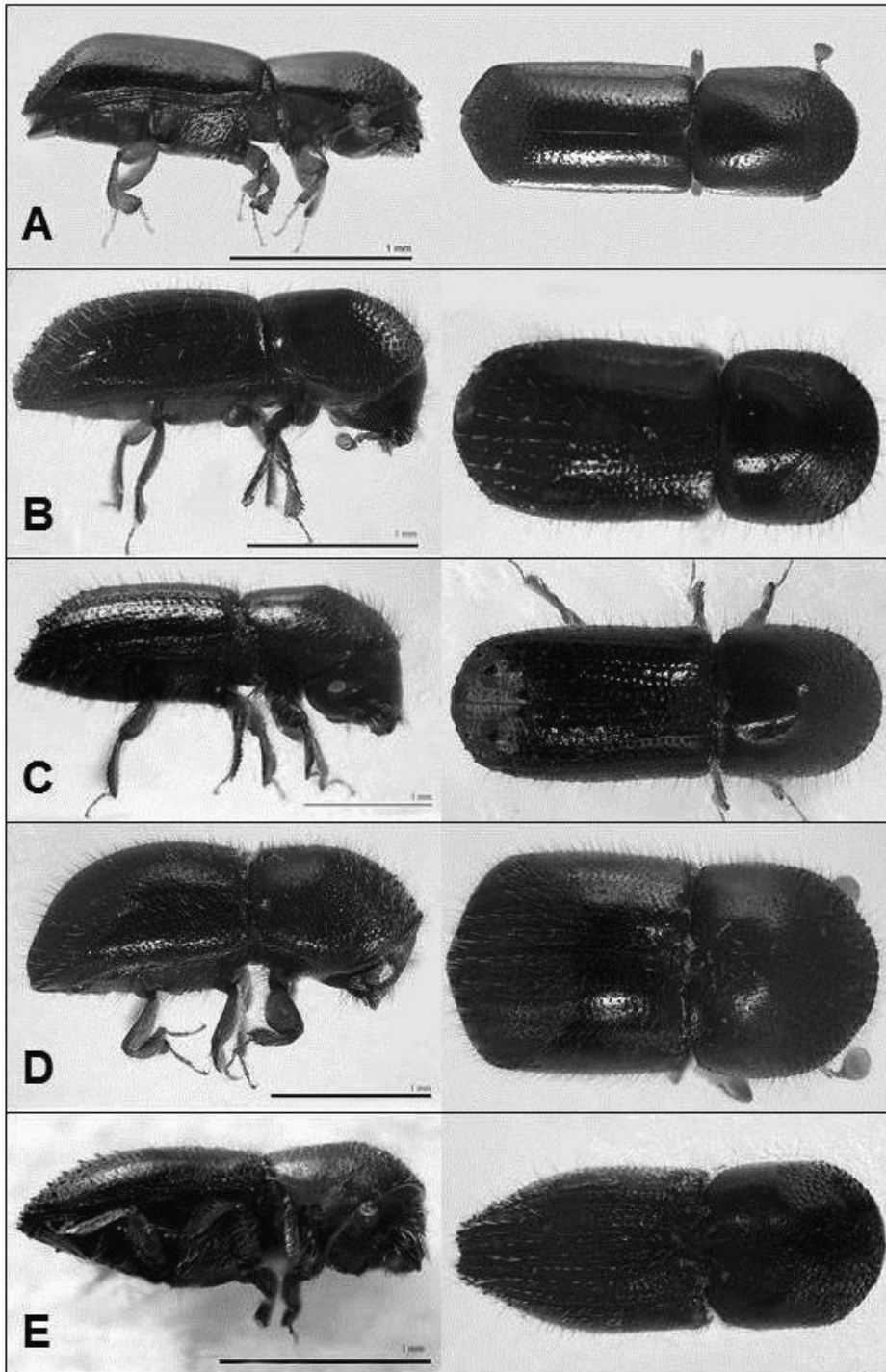


Fig. 2. Diversity of female Scolytinae attracted to host-based wood volatiles (lateral view on left, dorsal view on right): (A) *Xyleborus glabratus* Eichhoff, (B) *Xyleborus affinis* (Eichhoff), (C) *Xyleborus ferrugineus* (Fabricius), (D) *Xylosandrus crassiusculus* (Motschulsky), and (E) *Xyleborinus andrewesi* (Blandford). Despite attraction of multiple species to the host bait, *X. glabratus* was observed to fly several hours earlier than the non-target species, facilitating selective capture of *X. glabratus* for use in experimental research.



an alternative to laboratory rearing of *X. glabratus*, we developed a simple method for field collection of dispersing females using freshly-cut host wood as bait. Female *X. glabratus* collected with this method are behaviorally and physiologically in host-seeking mode, ideally suited for evaluation of host-based attractants in controlled laboratory tests.

#### ACKNOWLEDGMENTS

We thank Larissa Guillén Conde (Instituto de Ecología, Veracruz, Mexico), Daniel Carrillo (TREC, University of Florida, Homestead), and the journal referees for reviewing the manuscript; Hilary Swain for providing us with laboratory space at Archbold Biological Station (Lake Placid, FL); and Shane Belson (Florida Fish and Wildlife Conservation Commission; St. Cloud, Florida) for assistance in obtaining a special use permit for the Lake Wales Ridge Wildlife Management Area (Highlands County, Florida). This work was supported in part by the USDA-ARS National Plant Disease Recovery System, a NIFA Critical Issues Grant, and the Florida Avocado Administrative Committee. This report presents the results of research only; mention of a proprietary product does not constitute an endorsement by the USDA.

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