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Source: Florida Entomologist, 95(3) : 573-579

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.095.0306

AMBROSIA BEETLES (COLEOPTERA: CURCULIONIDAE: SCOLYTINAE) THAT BREED IN AVOCADO WOOD IN FLORIDA

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Abstract

Laurel wilt is a destructive disease caused by the fungus *Raffaelea lauricola*, which is transmitted by the invasive redbay ambrosia beetle, *Xyleborus glabratus*. Here we document ambrosia beetles that emerged from wilted avocado trees throughout Florida. In addition, the ambrosia beetle fauna associated with wilted swampbay trees in Miami-Dade was studied. Fourteen species of scolytine beetles were found associated with avocado wood from different parts of Florida. Multiple species of ambrosia beetles were found breeding in avocado and swampbay wood infected by *R. lauricola* with or without the presence of its primary vector, *X. glabratus*. Work is under way to determine whether other ambrosia beetle species can carry *R. lauricola* and transmit this pathogen to healthy avocado and swampbay trees.

Key Words: *Euwallacea fornicatus*, *Xyleborus glabratus*, *Raffaelea lauricola*, *Persea americana*, *Persea palustris*, laurel wilt

Resumen

La marchitez del laurel es una enfermedad destructiva causada por el hongo *Raffaelea lauricola,* el cual es transmitido por el escarabajo de ambrosia, *Xyleborus glabratus.* Se presenta un registro de las especies de escarabajos de ambrosia que emergieron de árboles de aguacate marchitos, colectados en diferentes partes de Florida. También se estudio la fauna de escarabajos de ambrosia asociados a *Persea palustris* en el condado de Miami-Dade. Catorce especies de escarabajos de ambrosia fueron encontradas reproduciéndose en madera de aguacate en diferentes partes de Florida. Varias especies de escarabajos de ambrosia se encontraron en árboles infectados con *R. lauricola*. Sin embargo, su principal vector, *X. glabratus,* no se encontró en todos los árboles infectados. Se está trabajando para determinar si otras especies de escarabajos de ambrosia pueden transmitir este patógeno a plantas sanas de aguacate y *P. palustris*.

Palabras Clave: *Euwallacea fornicatus, Xyleborus glabratus*, *Raffaelea lauricola*, *Persea americana*, *Persea palustris*, marchitez del laurel

Multiple species of ambrosia beetles can coexist in tree trunks, where their immature stages develop by feeding upon symbiotic fungi. In general, ambrosia beetles are not primary pests and their symbiotic fungi are not pathogenic. An exception is the invasive species *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), which carries a phytopathogenic fungus, *Raffaelea lauricola* T.C. Harr. (Ophistomatales: Ophiostomataceae), that causes laurel wilt, a lethal disease of several plants within the Lauraceae (Fraedrich et al. 2008; Hanula et at. 2008; Harrington et al. 2008; Gramling 2010; Peña et al. 2012). The introduction of *X. glabratus* and *R. lauricola* into ecological niches occupied by other ambrosia beetle species raises the possibility that other Scolytinae species could facilitate the spread of this disease. For instance, Harrington and Fraedrich (2010) recovered *R. lauricola* from *Xyleborinus saxeseni* (Ratzeburg) (Coleoptera: Curculionidae: Scolytinae), an ambrosia beetle species that was found on naturally infested pondberry, *Lindera melissifolia* (Walter) Blume. As background to determine whether other ambrosia beetles can transmit *R. lauricola*, surveys were conducted to identify the ambrosia beetles that share their ecological niche with *X. glabratus* and *R. lauricola*.

The plants most affected by laurel wilt in areas recently invaded by *X. glabratus* are redbay, *Persea borbonia* (L.) Spreng, and swampbay, *Persea palustris* (Raf.) Sarg., which are abundant in swampy wood and coastal hammock ecosystems throughout the southeastern USA. In addition, avocado, *Persea americana*

Mill., an important fruit crop in Florida, is also susceptible to this disease. Avocados are grown across the state of Florida, with scattered trees grown by tropical fruit enthusiasts in the northern and central areas of the state. The commercial production of avocados, however, is concentrated in the southern portion of the state. Field evidence of the susceptibility of avocados to laurel wilt was first observed in a backyard-grown avocado in Jacksonville, Florida in 2007 (Mayfield et al. 2008). Then, in October 2008 laurel wilt symptoms and *X. glabratus* infestations were found in 1 to 3 yr-old avocado trees in a Malabar neighborhood (Brevard County, Florida); by Feb 2009 all of the avocado cultivars at that site were infested by both organisms (J. E. Peña et al., unpublished). A similar rapid progression of this disease was observed in 2010 by J. E. Peña and J. H. Crane (unpublished) in scattered avocado trees in Highlands County, Florida. Infestations of *X. glabratus* in avocado were also detected by the authors in other counties, i.e., Saint Lucie and Indian River. *Xyleborus glabratus* has continued to move south, and early in 2011, a large infestation of swamp bay trees was detected in Miami-Dade County, 10 miles north of Florida's main avocado production area in Homestead, Florida. This Miami-Dade County site is presumed to be the source of infestation for the first avocado tree diagnosed with *R. lauricola* in the Homestead area in May 2012 (FDACS, 2012). Here we document cases of avocado trees observed wilting throughout Florida and the ambrosia beetles that have emerged from wood samples from those trees. In addition, the ambrosia beetle fauna associated with naturally infested swampbay trees in Miami-Dade was studied.

Materials and Methods

Wood samples were collected from wilted avocados. Wilt can be caused by *R. lauricola* or other pathogens, but also by non-pathogenic causes. Before the presence of *R. lauricola* in the US, wilting of Florida avocados was caused by various pathogenic (e.g. Verticillium wilt and Phytophthora root rot) (Cook 1975; Ploetz et al. 2002) and non-pathogenic factors (e.g. lighting strikes). Therefore, our samples included laurel wilt-positive and negative trees (i.e., those from which *R. lauricola* was or was not isolated).

Wood samples from avocado trees affected by laurel wilt were collected from 4 sites in central Florida from 2009 to 2011 (Fig. 1). Trees were cut, measured, and wood over 3-4 cm in diameter was placed in emergence chambers (44 gal. [167 L] Brute container 2643-60, Rubbermaid® with a 1-quart [0.946 L] Mason jar with a 3 in [76 mm] diam mouth placed in a hole on one of each chamber's sides to collect emerging beetles) at the University of Florida, Medical Entomology Laboratory, (200-9 St. SE, Vero Beach, Florida). Wood samples were also collected from: 6 wilted avocado trees in commercial groves in Homestead (Miami-Dade County, Florida) during 2010 - 2011, 1 yr before laurel wilt was detected in the county; 17 swampbay trees affected by laurel wilt in Miami-Dade County near the commercial avocado growing region; and a wood sample from the first avocado tree known to be affected by laurel wilt in the Homestead area (May 2012; FDACS, 2012). The wood samples from Miami-Dade County were held in emergence chambers at the Containment Facility of the University of Florida, Tropical Research and Education Center (TREC), Homestead, Florida. The location and dates of all samples are presented in Table 1.

Fig. 1. Sample sites for avocado (1-11) and swampbay (12) trees that were infected with *Raffaelea lauricola*, cause of laurel wilt. The names of the localities and the collection dates are provided in Table 1.

Ambrosia beetles were collected at least biweekly and identified by R. E. Duncan. The identity of species was confirmed with representative samples of each that were sent to Drs. Michael Thomas or K. Okins at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville, Florida. The presence of *R. lauricola* in wood samples was determined by growth of the pathogen on a selective media on malt extract agar as described by Ploetz et al. 2011. These determinations were conducted by plant pathologists at either TREC or the Florida Department of Plant Industry.

Results

TABLE 1. LOCALITIES AND DATES WHEN AVOCADO AND SWAMPBAY WOOD SAMPLES WERE COLLECTED FOR DETERMINATION OF LAUREL WILT AND PRESENCE OF WOOD-INFESTING BEETLES.

Tree No.[a]	Locality, County	Address Coordinates	Plant	Sampling date	samples held (months)	Infected by *R. lauricola*
1	Malabar, Brevard Co.	28°0'13.07"N-80°33'56.19"W	Avocado	2/10/2010	5	yes
2	Vero Beach, Indian River Co.	27°38'19.11"N -80°23'50.19"W	Avocado	5/18/2010	5.5	yes
3	Palm Bay, Brevard Co.	28°2'4.06"N-80°35'19.19"W	Avocado	11/4/2010	5.5	yes
4	Fort Pierce, Saint Lucie Co	27°32'7.62"N-80°23'6.39"W	Avocado	2/8/2011	6	yes
5	Homestead, Miami-Dade Co.	25°29'28.68"N-80°29'13.92"W	Avocado	2/27/2011	7	no
6	Homestead, Miami-Dade Co.	25°30'21.62"N-80°31'35.39"W	Avocado	7/8/2010	5	no
7	Homestead, Miami-Dade Co.	25°33'2.29"N-80°29'37.80"W	Avocado	7/8/2010	5	no
8	Homestead, Miami-Dade Co.	25°25'33.13"N-80°30'33.89"W	Avocado	7/26/2010	5	no
9	Homestead, Miami-Dade Co.	25°29'28.68"N-80°29'13.92"W	Avocado	2/27/2011	7	no
10	Homestead, Miami-Dade Co.	25°31'28.60"N-80°31'20.60"W	Avocado	2/28/2011	7	no
11	Homestead, Miami-Dade Co.**	25°35'40.50"N-80°28'20.56"W	Avocado	2/10/2012	4	yes
12	Miami, Miami-Dade Co.	25°43'37.96"N-80°28'36.16"W	Swampbay	1/20/2012	4	yes

**This locality is in unincorporated Miami-Dade County but is treated here as Homestead, because it is in the northern part of the Homestead avocado growing region.

Fourteen species of scolytine beetles were reared from avocado wood from different parts of Florida (Table 2). From a given sample a range of 2 to 8 species and 38 to 6,730 adults were recovered. Only one ambrosia beetle species, *X. saxeseni,* was found in all avocado trees that were sampled, and it was the most abundant beetle in 3 trees that tested positive for *R. lauricola.* Two of these infected avocado trees were in Brevard County (Sites 1 & 3), and one was in Miami-Dade County (Site 11). *Xyleborus volvulus* was found in 10 of the 11 avocado trees that were sampled, and it was the most abundant beetle in 3 trees (sites 6, 8 and 10) in Homestead, all of which were negative for *R. lauricola*. However, it was also found in 3 trees that were positive for the fungus (sites 2, 3 and 4). *Xyleborus ferrugineus* was present in 8 trees and was the most abundant beetle in *R. lauricola*-positive avocado wood collected from Fort Pierce, Florida (Site 4). *Xyleborus affinis* and *X. crassiusculus* were present in 8 sites including *R. lauricola*-infected and non-infected trees; *X. affinis* was highly abundant in 2 non-infected trees from commercial avocado groves in Homestead (Sites 5 and 9), and *X. crassiusculus* was the most abundant species in the *R. lauricola*-infected tree from Vero Beach. Following in abundance were *Hypothenemus* sp., *X. gracilis* and *A. lecontei* which were present in relatively low numbers at sites 6, 4 and 3, respectively. The species *Premnobius cavipennis*, *Ambrosiodmus devexulus, Corthylus papulans, Euwallacea fornicatus* and *Theoborus ricini* were seldom found and in low numbers. Interestingly, although *R. lauricola* was recovered from 6 avocado trees, *X. glabratus* was recovered only from 2 of these and in very low numbers.

The beetle complex associated with swampbay trees was composed of 9 species (Table 3). The number of ambrosia beetle species emerging from a sample ranged from 4 to 8 and the total number of ambrosia beetles ranged from 63 to 19,311 individuals. All sampled swampbay trees were infested with *X. glabratus* and infected by *R. lauricola*. The total numbers of *X. glabratus* individuals that were recovered from these trees ranged from 48 to 11,589, and it was the most abundant species in 11 of the 17 trees. *Xyleborus volvulus* was also present in all of the sampled trees and in relatively high numbers. *Xyleborinus gracilis* and *X. affinis* were both found in 16 trees, and they were the most abundant species in 3 and 1 of the samples, respectively. *Xyleborus ferrugineus* was found in 14 swampbay trees and *X. saxeseni* in 11. *Xylosandrus crassiusculus* was found only in 7 samples but it was the most abundant ambrosia beetle in 2 of those. Finally, both *Ambrosiodmus lecontei* and *Ambrosiodmus devexulus* were found at low numbers and in few samples.

TABLE 2. RELATIVE ABUNDANCE (% OF TOTAL) OF AMBROSIA BEETLES ASSOCIATED WITH *PERSEA AMERICANA*, AVOCADO.

	Tree number										
	1*	2*	3*	4*	5	6	7	8	9	10	11*
Ambrosia beetle species	n = 38	n = 3663	n = 117	n = 363	n = 2903	n = 944	n = 201	n = 211	n = 2940	n = 92	n = 6730
Ambrosiodmus lecontei Hopkins	—	—	—	—	—	0.3	0.5	—	—	2.2	—
Ambrosiodmus devexulus (Wood)	—	—	—	—	—	—	—	—	0.5	—	—
Corthylus papulans Eichhoff	—	24.1	—	—	—	—	—	—	—		—
Euwallacea fornicatus (Eichhoff)	—	—	—	—	—	—	0.5	—	—	—	0.06
Hypothenemus sp.	—	—	—	4.2	0.9	0.1	0.0	0.5	0.9	5.4	—
Premnobius cavipennis Eichhoff	—	0.5	—	—	—	—	3.5	—	0.0	—	0.01
Theoborus ricini (Eggers)	—	—	—	—	—	—	9.5	—	—	—	—
Xyleborinus gracilis (Eichhoff)	5.3	—	—	—	4.1	2.0	—	—	4.0	—	—
Xyleborinus saxeseni (Ratzeburg)	76.3	28.3	79.8	12.8	6.1	18.5	44.8	11.8	6.0	2.2	84
Xyleborus affinis (Eichhoff)	—	8.4	10.1	16.3	71.5	20.6	—	1.9	70.6	—	0.5
Xyleborus ferrugineus (Fabricius)	5.3	0.5	—	54.6	5.1	0.6	1.5	1.4	5.0	—	—
Xyleborus volvulus (Fabricius)	—	2.3	3.3	12.1	11.9	50.2	13.4	71.6	11.7	44.6	2.8
Xylosandrus crassiusculus (M.)	10.5	35.9	6.8	—	—	6.7	26.4	5.2	—	33.7	12.02
Xyleborus glabratus Eichhoff	2.6	—	8.4	—	—	—	—	—	—	—	

*indicates that *R. lauricola* was recovered from the wood sample from that tree.

Table 3. Relative abundance (% of total) of ambrosia beetles associated with *Persea palustris*, swampbay.

Species	Tree number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Total beetles n=	4897	1857	2772	19311	3459	2202	2186	770	385	63	2374	7138	1209	124	255	327	4650
A. lecontei	—	13.8	—	—	—	—	—	—	—	—	0.2	2.2	—	—	—	—	0.2
A. devexulus	—	6.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
X. gracilis	22.0	0.5	4.3	3.9	7.3	14.1	21.9	0.3	—	12.7	30.6	39.9	7.9	8.1	4.3	22.9	28.4
X. saxeseni	0.1	0.2	2.2	0.1	—	—	0.4	—	—	—	—	3.2	0.2	3.2	1.6	3.7	1.6
X. affinis	3.4	4.9	22.4	8.4	11.9	3.2	3.8	2.1	1.8	3.2	9.5	6.5	5.6	2.4	—	6.5	11.6
X. ferrugineus	2.3	26.7	21.9	7.9	2.5	1.7	2.0	23.0	22.3	3.2	5.2	0.5	8.2	—	—	—	1.6
X. volvulus	14.0	9.0	22.2	18.9	19.9	2.9	7.5	1.3	1.8	7.9	23.9	21.6	18.8	4.8	2.7	4.6	27.2
X. crassiusculus	—	—	8.3	—	—	—	—	—	—	—	—	0.1	1.5	66.9	35.7	36.7	20.0
X. glabratus	**58.2**	**38.0**	**18.5**	**60.8**	**58.5**	**78.2**	**64.5**	**73.4**	**74.0**	**73.0**	**30.5**	**26.0**	**57.6**	**14.5**	**55.7**	**25.7**	**9.4**

Discussion

Our data suggest that the presence of *X. glabratus* and *R. lauricola* does not have a major effect on the ambrosia beetle communities that are found in Florida avocado trees. Most ambrosia beetle species that were found in avocado trees infested by *X. glabratus* and/or infected by *R. lauricola* were also present in trees in which the beetle and/or pathogen were not present. Thus, beetles that were present before *X. glabratus* and *R. lauricola* had arrived in the Western Hemisphere can coexist with these invasive species in avocado. All ambrosia beetle species found associated with avocado trees were also found in swampbay trees. However, avocados were sampled from multiple sites in central and south Florida, while swampbays were surveyed at only one site. The greater number of avocado sample sites could explain why more species were found associated with avocado than with the one-site swampbay sample. In general, each tree, either avocado or swampbay, had one dominant species with several others present at lower levels. Differences in community composition and species abundance may be due to beetle colonization and seasonality patterns, when the tree was cut, and other tree species that were present in a given area. The species *X. saxeseni, X. affinis, X. ferrugineus, X. volvulus, X. gracilis* and *X. crassiusculus* were consistently found in relatively high numbers in avocado and swampbay trees. Among these, *X. crassiusculus* and *X. saxeseni* are invasive, whereas the rest are widely distributed in the neotropics (Atkinson & Peck 1994; Rabaglia et al. 2006). All of these species are generalists that can breed in a wide variety of hosts (Atkinson & Peck 1994). With the exception of *X. crassiusculus,* which can attack living plants, the remaining species are known to breed mostly in wood of stressed or dead plants (Atkinson & Peck 1994).

Besides the ambrosia beetles reported here, the species *Platypus parallelus* (Fabricius), *Theoborus solitariceps* (Schedl) and *Coccotryceps* sp. have also been recorded emerging from avocado wood in Homestead Florida (J. E. Peña, unpublished data). *Xylosandrus compactus* (Eichhoff) was found associated with avocados in a botanical garden in Gainesville, Florida (M. Thomas, pers. comm.). In addition, Kendra et al. (2011) captured 8 species of ambrosia beetles in Lindgren traps baited with manuka oil lures in avocado groves in Miami-Dade County. These include 2 species that are not reported here, *Pseudopityophthorus minutissimus* (Zimmerman) and *Ambrosiodmus obliquus* (LeConte); it is possible that these species also breed in avocado wood.

The life history of the invasive species, *X. glabratus,* has important differences from the histories of the other ambrosia beetle species found in this study. The host range of *X. glabratus* is mostly restricted to Lauraceous plants and it can attack healthy plants. However, a marked difference in the abundance of *X. glabratus* in avocado and swampbay was observed; although the beetle was uncommon in avocado trees, it was abundant in all examined swampbay trees. Moreover, all swampbay trees were infected by *R. lauricola* and developed laurel wilt, whereas this occurred in only 5 of 11 avocado trees; interestingly, *X. glabratus* was detected in only 2 of the avocado trees and at very low densities. Wilting in the remaining 6 avocado trees that were assayed was presumably due to factors other than laurel wilt as these trees were not infected by *R. lauricola*.

The available evidence suggests that swampbays are more suitable hosts for *X. glabratus* than avocados. Moreover, *X. glabratus* was not recovered from avocado trees affected by laurel wilt/ infected by *R. lauricola* in Miami-Dade County. It is unclear whether detection of *X. glabratus* is simply more difficult in avocado than in swampbay, or if species other than *X. glabratus* are vectors of *R. lauricola*. Work is under way to investigate the latter possibility in healthy avocado and swampbay trees.

Another important finding of this study is the first report of *E. fornicatus* breeding in avocado wood in Florida. This species is known to carry a fungus, *Fusarium* sp., that causes a dieback of avocado in Israel and California (Mendel et al. 2012; Eskalen & Stouthamer 2012). However, preliminary DNA analyses comparing *E. fornicatus* specimens from Florida and California revealed differences that suggest they could be different species (Stouthamer, pers. comm.). More research is needed to understand the potential effects of ambrosia beetles vectoring pathogenic fungi to avocados in Florida and other parts of the New World.

Acknowledgments

We thank Drs. M. Thomas and K. Okins (Florida Department of Agriculture and Consumer Services) for insect identification, Drs. Randy Ploetz, Aaron Palmateer and the Florida Department of Plant Industry for diagnosis of *R. lauricola* in the tree samples, Drs. J. Capinera and J. H. Frank (University of Florida, Entomology and Nematology Department, Gainesville) for suggestions to improve the manuscript and Dr. L. P. Lounibus (University of Florida, Medical Entomology Laboratory, Vero Beach, Florida) for his help. We thank Jose Alegria, Ana Vargas, Katia Santos, for their help. This research was partially funded by a CSREES grant and a Florida Avocado Committee grant to Jorge E. Peña.

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