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Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles

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Different ambrosia beetle species can coexist in tree trunks, where their immature stages feed upon symbiotic fungi. Although most ambrosia beetles are not primary pests and their fungal symbionts are not pathogenic to the host tree, exceptional situations exist. Notably, *Xyleborus glabratus* carries a phytopathogenic symbiont, *Raffaelea lauricola*, which causes laurel wilt, a lethal disease of some Lauraceae species. Both *X. glabratus* and *R. lauricola* are natives of Asia that recently invaded much of the coastal plain of the southeastern USA. This study examined ambrosia beetles that breed in susceptible trees in Florida (USA), including avocado (*Persea americana*), redbay (*P. borbonia*) and swampbay (*P. palustris*). *Raffaelea lauricola* was recovered from six of eight ambrosia beetle species that emerged from laurel wilt-affected swampbay trees, in addition to *X. glabratus*. Controlled infestations with cohorts of the six species other than *X. glabratus* revealed that each could transmit the pathogen to healthy redbay trees and two could transmit the pathogen to healthy avocado trees; laurel wilt developed in five and one of the respective beetle × host interactions. These results indicate flexibility in the lateral transfer of a non-native ambrosial fungus to other ambrosia beetles, and for the first time documents the transmission of a laterally transferred phytopathogenic symbiont by new ambrosia beetle species. Additional work is needed to determine whether, or to what extent, the new beetle × *R. lauricola* combinations play a role in spreading laurel wilt.

Keywords: beetle–fungus symbiosis, invasive species, lateral transfer, laurel wilt, *Raffaelea lauricola*

Introduction

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae) are fungus farmers (Farrell *et al.*, 2001). They have obligate nutritional relationships with fungi that grow in their natal galleries in host tree xylem (Six, 2012). Their larval stages feed upon these fungi until they reach the adult stage, which in turn carries the fungal symbionts from tree to tree in specialized structures called mycangia. Mycangia are found in either or both sexes in the platypodinae ambrosia beetles, but occur only in females of the scolytinae ambrosia beetles. The scolytinae females start new colonies/broods and are responsible for gallery establishment and maintenance (Six, 2012).

A given beetle species is usually associated with one or more primary fungal symbionts that are transmitted vertically from one generation to the next (Baker & Norris, 1968; Kolařík & Hulcr, 2009; Gibson & Hunter, 2010; Harrington & Fraedrich, 2010). However, symbionts are not transmitted directly from mother to offspring; rather, they grow independently in natal galleries from which offspring obtain symbionts via feeding. This period of independent growth represents a weak link in the transmission process and provides an opportunity for horizontal transmission of symbionts (Six, 2012). Multiple species of ambrosia beetles can coexist in a single host plant, in which a beetle species could interact with another beetle's brood gallery and its associated fungi (Kendra *et al.*, 2011). Although specific symbionts have been found in two or more beetle species (Batra, 1967; Gebhardt *et al.*, 2004), the lateral movement of a fungal symbiont from one ambrosia beetle species to another is not well studied.

Typically, ambrosia beetles infest dead or stressed trees, and their fungal symbionts are saprobes that colonize the lining of natal galleries and surrounding tissues; they are usually not phytopathogens. However, an increasing number of phytopathogens have been identified among these usually benign fungi (Hulcr & Dunn, 2011). For example, *Raffaelea lauricola*, a symbiont of the invasive Asian species *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), causes laurel wilt, a new disease that has decimated vast areas of native trees in the Lauraceae in the southeastern USA (Fraedrich *et al.*, 2008) and threatens the avocado industry in south Florida (Ploetz *et al.*, 2011a). Although *X. glabratus* clearly plays a significant role as this pathogen's vector (Hanula *et al.*, 2008; Harrington & Fraedrich, 2010), not much is known about the interaction of *R. lauricola* with other ambrosia beetle species or their potential role in spreading laurel wilt.

Lateral transfer of *R. lauricola* to scolytinae other than *X. glabratus* has previously been noted in four species (*Xyleborinus* (*Xi.*) *saxeseni* (Coleoptera: Curculionidae: Scolytinae), *Xyleborus affinis*, *Xylosandrus* (*Xa.*)

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crassiusculus and *Xyleborus ferrugineus*) (Harrington & Fraedrich, 2010; Harrington *et al*., 2010; Ploetz *et al*., 2011a). However, information on the frequency and prevalence of these associations, or on their potential significance, has not been published. In Florida, at least 14 ambrosia beetle species have emerged from laurel wilt-affected (*R. lauricola*-infected) redbay and avocado. In a recent study, *X. glabratus* was abundant in redbay, but seldom recovered from avocado (Carrillo *et al*., 2012).

Given the rarity of *X. glabratus* in laurel wilt-affected avocado trees and the detection of *R. lauricola* in other scolytinae that infest avocado and redbay trees, it can be hypothesized that beetles other than *X. glabratus* could be vectors of *R. lauricola*. To test this hypothesis, this study aimed to: (i) determine the presence and prevalence of *R. lauricola* in scolytinae species that emerged from laurel wilt-affected swampbay, (ii) assay transmission of the pathogen by the different scolytinae species to healthy avocado and redbay trees, and (iii) assess the development of laurel wilt in these trees.

Materials and methods

Wood samples (>10 cm in diameter × 50 cm long) were collected from laurel wilt–affected swampbay trees in a natural area in Miami Dade County, Florida, USA (25°43′37·96″N 80°28′36·16″W), where previous work had documented the presence of *X. glabratus* and *R. lauricola*. Wood was placed inside emergence chambers (166 L Brute container 2643–60, Rubbermaid®, with Mason jars attached to collect the emerging beetles) and held at 14-/10-h light: darkness, 80% relative humidity (RH) and 25°C in the Containment Facility of the University of Florida, Tropical Research and Education Center (TREC), Homestead, Florida, USA. The presence of *R. lauricola* in wood was determined with a semiselective medium (CSMA+) as described previously (Ploetz *et al*., 2012).

Ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) that emerged from *R. lauricola*-infected wood were collected daily and identified. Identities of a subset of these beetles were confirmed by either Drs M. Thomas or K. Okins (Florida Department of Agriculture and Consumer Services (FDACS), Division of Plant Industry, Gainesville, Florida, USA). Nine ambrosia beetle species were assayed for *R. lauricola*: *Xyleborus volvulus*, *X. glabratus*, *X. affinis*, *X. ferrugineus*, *Ambrosiodmus devexulus*, *Ambrosiodmus lecontei*, *Xyleborinus gracilis*, *Xi. saxeseni* and *Xa. crassiusculus*. Voucher specimens for each species were deposited in the Florida State Collection of Arthropods, FDACS, Gainesville, Florida, USA.

Recovery of *R. lauricola* from ambrosia beetles

Beetles were surface disinfested for 15 s in 70% ethanol and washed three times in sterile deionized water before the part of the body that contained mycangia was separated from the rest of the body and macerated in sterile glass tissue grinders (Pyrex no. 7727-07). For *Xi. saxeseni* and *Xa. crassiusculus,* which have metanotal and mesonotal mycangia, the abdomen and the whole body were tested, respectively, whereas assays of the remaining beetles, which have mandibular mycangia, used only the heads. The macerate was serially diluted one, 10 and 100 times, and aliquots of the dilutions were plated on CSMA+ medium. After 7–10 days under ambient light in the laboratory, colonies of *R. lauricola* were putatively identified based on their characteristic appearance on this medium (Harrington *et al*., 2010); numbers of colony-forming units (CFU) of the fungus were then calculated for individual beetles. The identity of a subset of single-conidium isolates from these colonies was confirmed as *R. lauricola* with two diagnostic microsatellite markers, CHK and IFW, which were amplified in PCR-based assays (Fig. 1). Each locus was shown previously to distinguish *R. lauricola* from 21 closely related species of *Raffaelea* and *Ambrosiella*, and the assay has been used to identify *R. lauricola* and to diagnose laurel wilt in four independent laboratories (T. Dreaden & J. Smith, University of Florida, Gainesville, USA, personal communication). Vouchers of representative isolates from different beetle species and trees affected by laurel wilt in these experiments were deposited at the Centraalbureau voor Schimmelcultures (CBS Fungal Biodiversity Centre, Utrecht, The Netherlands).

Transmission of *R. lauricola* to, and the development of laurel wilt in, healthy avocado and redbay trees

Cohorts of the seven beetle species from which *R. lauricola* was recovered were used to infest healthy trees of the laurel wilt-susceptible avocado cv. Simmonds (clonal scions grafted on seedling rootstocks, 3–4 years' old, 2 m tall, 4 cm diameter at the trunk base) and redbay (3-year-old seedlings, 1·5 m tall, 3 cm diameter at the trunk base) under glasshouse conditions (80 ± 15% RH, 25 ± 4°C, drip irrigation). For each beetle species, a total of five avocado and five redbay trees were infested over a period of 2 months (i.e. as sufficient numbers of beetles became available). A total of 80 trees were tested, comprising 10 trees infested with each of the seven beetle species and 10 non-infested trees used as control treatments.

Forty females of a given species were released in 25 × 15 cm sleeves of white cotton fabric affixed to trunks. The ends of sleeves were tied around trunks with 5 mm thick tagging tape. At 15 cm and 65 cm from the base of the trees, additional strips of tagging tape, impregnated with Tangle-foot®, were used to prevent entry of crawling arthropods into sleeves. Velcro strips, sewn into the sleeves, enabled access to the enclosed stem and, after closure, containment of the released beetles.

Trees were inspected for the development of laurel wilt on a weekly basis, as described previously (Ploetz *et al*., 2011b, 2012). Wilted trees were monitored until leaves had dried and the trunk started to show basipetal necrosis, at which time they were inspected in detail. Wilted trees were initially inspected with a hand lens to ensure that no beetle entry holes were present outside the sleeves. No evidence for this was found and subsequent evaluations were restricted to the area that was enclosed in the sleeve. The portion of the trunk that was enclosed by the sleeve was then removed, inspected for beetle boring, and dissected to determine how many boring attempts resulted in penetration of the bark and resulted in gallery construction in the xylem. Gallery length and life stages of the beetles that were found in galleries were recorded, as were the numbers of dead beetles in the sleeve. After 3 months, non-infested control trees and trees that did not wilt after infestation with a given beetle species were inspected and dissected as described above. Wood samples from the portion of the trunk that was enclosed by the sleeve were plated on CSMA+ medium. The identity of a subset of single-conidium isolates from these colonies was confirmed as *R. lauricola* with microsatellite markers as above. Trees infested with *X. glabratus* were considered positive controls and non-infested trees were considered negative controls.

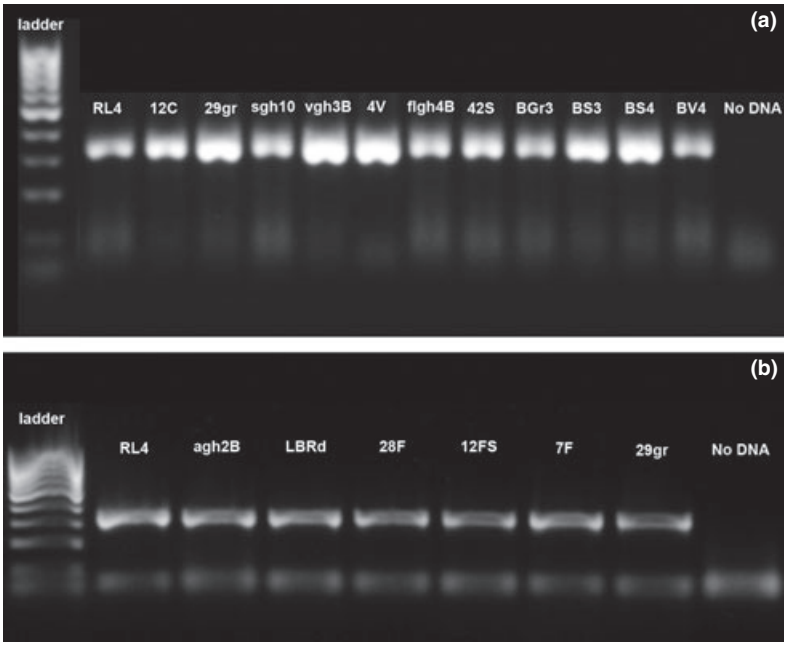

Figure 1 Molecular confirmation of representative isolates from individual beetles and trees as *Raffaelea lauricola*, based on amplification of the (a) IFW and (b) CHK microsatellite loci (T. Dreaden & J. Smith, University of Florida, Gainesville, USA, personal communication). PCR products were separated on 1·5% agarose gels at 75 V for 60 min. Ladder: New England Biologicals low DNA mass ladder; RL4: reference isolate of *R. lauricola* CBS 127 349, Centraalbureau voor Schimmelcultures (CBS Fungal Biodiversity Centre, Utrecht, The Netherlands). (a): 12C (CBS 133 549) recovered from *Xylosandrus crassiusculus*; 29gr (CBS 133 548) from *Xyleborinus gracilis*; sgh10 and 42S (CBS 133551) from *Xyleborinus saxeseni*; vgh3B and 4V from *Xyleborus volvulus*; flgh4B from *Xyleborus ferrugineus*; BGr3 from a redbay tree infested with *Xi. gracilis*; BS3 and BS4 from redbay infested with *Xi. saxeseni*; BV4 from redbay infested with *X. volvulus*. (b): agh2B from *Xyleborus affinis*; LBRd from *Ambrosiodmus lecontei* (a specimen examined in another study); 28F, 12FS and 7F (CBS 133 546) from *X. ferrugineus*; 29gr from *Xi. gracilis*. The IFW and CHK amplicons were generated for all isolates (top intense bands). The faint band in these and the 'No DNA' lanes are non-diagnostic primer-dimer bands.

Statistical analysis

The PROC GLIMMIX procedure (SAS v. 9.3) was used to assess differences in the probabilities that individuals of the tested beetle species carried *R. lauricola*, and the Steel–Dwass method (SAS v. 9.3) was used for non-parametric paired comparisons of mean CFU of *R. lauricola* in the different beetle species. Due to variance heterogeneity and non-normality of data, boring attempts, gallery formation, average gallery length, recovery of *R. lauricola* and development of laurel wilt symptoms in avocado and redbay trees infested with the different ambrosia beetle species were analysed with Kruskal–Wallis tests (SAS v. 9.3).

Results

Recovery of *R. lauricola* from ambrosia beetles

A total of 473 adult females of the nine scolytinae species that were recovered from the swampbay samples (25 to 118 individuals for a given species) were assayed for *R. lauricola*. *Raffaelea lauricola* was not detected in *A. devexulus* and *A. lecontei*, but was isolated from at least one individual of each of the seven other beetle species that were assayed (Table 1). The identity of a subset of single-conidium isolates was confirmed with the two microsatellite markers, CHK and IFW (Table 2). Great variation was observed in the proportion of individuals that carried the fungus and the CFU detected in each (Table 1). The probability of carrying *R. lauricola* was significantly higher for *X. glabratus* than for *X. ferrugineus*, *X. volvulus* and *Xi. gracilis* which, in turn, were significantly more likely to carry the pathogen than *X. affinis*, *Xi. saxeseni* and *Xa. crassiusculus* (d.f. = 6,401; $F = 12{\cdot}92$; $P < 0{\cdot}0001$; Table 1). The mean number of *R. lauricola* CFUs per beetle was more than one order of magnitude higher in *X. glabratus* than in the other beetle species, whereas CFUs detected from *X. ferrugineus, X. volvulus* and *Xi. gracilis* were significantly greater than those found in *X. affinis*, *Xi. saxeseni* and *Xa. crassiusculus* (d.f. = 6,401; $F = 12{\cdot}92$; $P < 0{\cdot}0001$; Table 1).

Transmission of *R. lauricola* to, and the development of laurel wilt in, avocado and redbay trees

No differences in the mean number of boring attempts or the number and length of galleries were detected in redbay versus avocado trees infested with *X. glabratus* (Table 3). All trees that were infested with *X. glabratus* were infected by *R. lauricola* and developed symptoms of laurel wilt (Fig. 2). *Xyleborus affinis* bored a similar number of times in redbay and avocado, but the numbers and lengths of its galleries were significantly greater

Table 1 Recovery of *Raffaelea lauricola* from ambrosia beetles reared from laurel wilt-affected swampbay bolts

Species[a]	*n*	No. beetles carrying *R. lauricola*	Probability of a beetle carrying *R. lauricola*	CFU mean ± SEM	CFU range
Xyleborus glabratus	50	43	0·86a	2783·3 ± 281·9a	0–7800
Xyleborus affinis	41	5	0·12c	1 ± 0·6c	0–20
Xyleborus volvulus	39	20	0·51b	28·4 ± 10·6b	0–100
Xyleborus ferrugineus	118	70	0·59b	33 ± 7·4b	0–118
Ambrosiodmus devexulus	25	0			
Ambrosiodmus lecontei	41	0			
Xyleborinus gracilis	52	26	0·50b	100·6 ± 34b	0–1240
Xyleborinus saxeseni	68	2	0·03c	1·5 ± 1c	0–60
Xylosandrus crassiusculus	39	1	0·03c	2·6 ± 2·6c	0–100

n: number of individuals tested; CFU: colony-forming units; SEM: standard error of the mean.
Means followed by the same letter within columns are not significantly different at $P < 0{\cdot}05$. The PROC GLIMMIX procedure (SAS v. 9.3 2012) was used to assess differences in the probability values, and the Steel–Dwass method (SAS v. 9.3 2012) was used for non-parametric paired comparisons of mean CFU of *R. lauricola* in the different beetle species.
[a]Upon emergence adult beetles were surface disinfested in 70% ethanol and plated on CSMA+ semiselective medium. The identity of a subset of single-conidium isolates from the fungal colonies was confirmed as *R. lauricola* with two diagnostic microsatellite markers (Fig. 1; T. Dreaden & J. Smith, University of Florida, Gainesville, USA, personal communication).

Table 2 Confirmation of representative isolates from ambrosia beetles and redbay and avocado trees as *Raffaelea lauricola* with species-specific microsatellite markers, CHK and IFW[a]

Beetle species	CBS accession no.[b]	No. isolates tested	No. isolates positive		Beetle-infested plant species	CBS accession no.[b]	No. isolates tested	No. isolates positive	
			CHK	IFW				CHK	IFW
Xyleborus glabratus	133545	3	3	3	Redbay		1	1	1
Xyleborus affinis	133547	3	3	3	Redbay	133553	3	3	3
Xyleborus volvulus	133550	3	3	3	Redbay		1	1	1
					Avocado		1	1	1
Xyleborus ferrugineus	133546	4	4	4	Redbay		1	1	1
					Avocado	133552	1	1	1
Xyleborinus gracilis	133548	3	3	3	Redbay	133554	2	2	2
Xyleborinus saxeseni	133551	3	3	3	Redbay		2	2	2
Xylosandrus crassiusculus	133549	1	1	1	Redbay		2	1	1

[a]Representative isolates from different individuals (beetles or trees) were assayed with a species-specific pair of microsatellite DNA markers (T. Dreaden & J. Smith, University of Florida, Gainesville, USA, personal communication).
[b]A single representative isolate from each beetle species, as well as single representative isolates from an avocado tree infested with *X. ferrugineus*, and redbay trees infested with *X. affinis* or *Xi. gracilis*, were deposited at the Centraal Bureau voor Schimmelcultures (CBS Fungal Biodiversity Centre, Utrecht, The Netherlands).

in redbay (Table 3). After infestation with *X. affinis*, three of the five tested redbay trees were infected by *R. lauricola* and developed laurel wilt, but none of the five avocado trees were infected or developed the disease (Fig. 2). *Xyleborus volvulus* also bored a similar number of times in redbay and avocado, with significantly greater numbers and lengths of its galleries in redbay (Table 3). All of the redbay trees that were infested with *X. volvulus* were infected by *R. lauricola* and developed laurel wilt, whereas three of the five tested avocado trees were infected, two of which developed laurel wilt (Fig. 2). Similar numbers of boring attempts, and numbers and lengths of galleries were observed on redbay and avocado trees that were infested with *X. ferrugineus* (Table 3). Four of five redbay trees infested with *X. ferrugineus* were infected with *R. lauricola*, three of which developed laurel wilt, whereas one symptomless avocado tree was infected with *R. lauricola* (Fig. 2). For both *Xi. gracilis* and *Xi. saxeseni*, significantly greater boring attempts, galleries, and gallery lengths were observed in redbay than in avocado (Table 3). Both *Xi. gracilis* and *Xi. saxeseni* transmitted *R. lauricola* to redbay, but not to avocado. All redbay trees infested with *Xi. gracilis* were infected by *R. lauricola* and developed laurel wilt, whereas four redbay trees infested with *Xi. saxeseni* were infected by *R. lauricola*, three of which developed laurel wilt (Fig. 2). Finally, on *Xa. crassiusculus*-infested trees more boring attempts, gallery numbers, and gallery lengths were observed on redbay than on avocado (Table 3). In contrast to observations with the other beetles, the pathogen was recovered from only one redbay, but three of these trees wilted. However, typical

Table 3 Behaviour of ambrosia beetle species that attacked healthy avocado and redbay trees under no-choice conditions[a]

Species	Avocado	Redbay	χ^2 (1, n = 10)	P
Xyleborus glabratus				
No. boring attempts	14·4 ± 2·6a	14·8 ± 3·4a	0·01	0·91
No. of galleries	10·0 ± 1·4a	13·8 ± 3·8a	0·51	0·463
Gallery length (mm)	11·4 ± 1·3a	9·9 ± 1·8a	0·88	0·347
Xyleborus affinis				
No. boring attempts	2·0 ± 0·7a	2·4 ± 0·9a	0·1	0·751
No. of galleries	0·2 ± 0·2b	2·2 ± 0·9a	4·05	0·044
Gallery length (mm)	0·8 ± 0·8b	19·9 ± 6·1a	4·51	0·034
Xyleborus volvulus				
No. boring attempts	8·4 ± 2·8a	8·0 ± 1·4a	0·01	0·916
No. of galleries	2·0 ± 0·9b	6·8 ± 1·6a	4·03	0·045
Gallery length (mm)	3·5 ± 0·9b	13·5 ± 2·6a	6·86	0·008
Xyleborus ferrugineus				
No. boring attempts	3·0 ± 1·1a	1·8 ± 0·7a	0·74	0·39
No. of galleries	0·6 ± 0·4a	1·6 ± 0·6a	1·72	0·189
Gallery length (mm)	3·7 ± 2·8a	22·5 ± 7·4a	2·97	0·085
Xyleborinus gracilis				
No. boring attempts	11·0 ± 1·8a	6·4 ± 1·5b	3·66	0·05
No. of galleries	0·2 ± 0·2b	5·6 ± 1·4a	7·33	0·006
Gallery length (mm)	0·8 ± 0·8 b	9·4 ± 0·8a	7·25	0·007
Xyleborinus saxeseni				
No. boring attempts	3·6 ± 0·8b	6·4 ± 0·9a	3·6	0·05
No. of galleries	0 b	5·6 ± 0·9a	7·75	0·005
Gallery length (mm)	0 b	9·3 ± 1·4a	7·76	0·005
Xylosandrus crassiusculus				
No. boring attempts	2·6 ± 1·0a	9·6 ± 4·9a	2·99	0·083
No. of galleries	2·0 ± 0·4b	9·0 ± 4·8a	6·48	0·01
Gallery length (mm)	2·2 ± 1·4b	8·6 ± 2·4a	4·03	0·045

[a]Mean ± standard error of the mean (SEM). Means ± SEM followed by the same letter within rows are not significantly different at $P < 0·05$ in paired Kruskal–Wallis tests.

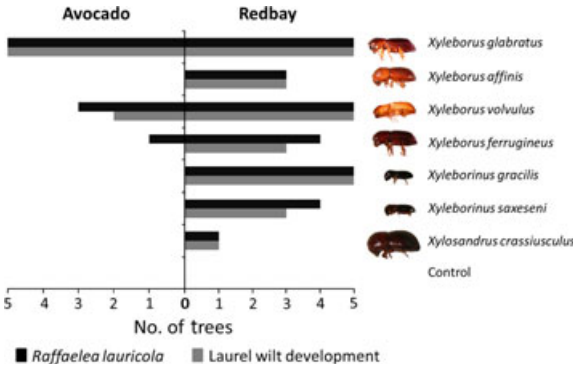

Figure 2 Transmission of *Raffaelea lauricola* to, and the development of laurel wilt in, healthy avocado and redbay trees infested with seven species of ambrosia beetles under no choice conditions. A total of five avocado and five redbay trees were infested for each beetle species, or were not infested (non-infested control).

symptoms of laurel wilt were not observed in the latter plants and it is probable that they succumbed to the numerous, large galleries that were excavated by this relatively large ambrosia beetle (*Xa. crassiusculus* is *c.* 50% larger than other species in this study). Avocado trees infested with *Xa. crassiusculus* were neither infected by *R. lauricola* nor did they wilt (Fig. 2).

All beetle species attempted to bore into all avocado and redbay trees that were tested, and the total numbers of attempts were not significantly different between the two tree species (χ^2 (1, n = 80) = 0·24, P = 0·62). However, the number of boring attempts that resulted in gallery formation and the average lengths of galleries were significantly higher in redbay than in avocado (χ^2 (1, n = 80) = 20·22, P = 0·0051; χ^2 (1, n = 80) = 18·31, $P < 0·0001$, respectively). Compared to avocado, more redbay trees were infected by *R. lauricola* and developed laurel wilt (χ^2 (1, n = 80) = 16·12, $P < 0·0001$). In general, all trees that were infected by *R. lauricola* exhibited laurel wilt symptoms 4–7 weeks after infestation and were dead after 2–3 months. No beetle boring, disease or infection by *R. lauricola* was observed in non-infested control trees.

Discussion

The recent introduction of *X. glabratus* and *R. lauricola* into the eastern USA has resulted in lateral transfer of this phytopathogenic fungus to several other species of ambrosia beetles. Although lateral movement of phytopathogenic symbionts is known to occur in the related bark beetles (Gibbs, 1978; Massoumi Alamouti *et al.*, 2009), the present study is the first to provide detailed information on this phenomenon in ambrosia beetles.

Before its detection in America, *X. glabratus* was associated with relatively few plant species in Asia (Bonin, Burma, Assam, Bengal, Bangladesh, India, Japan, Myanmar and Taiwan) (Wood, 1982; Rabaglia *et al.*, 2006; Table 4). Compared to *X. glabratus*, the other beetle species in this study are highly polyphagous. Six of the species studied are considered to be New World endemics (Table 4): *X. affinis* and *X. ferrugineus* are native to tropical America (Wood, 1982; Rabaglia *et al.*, 2006) and have continuous distributions throughout South America, Central America, the Gulf Coast and southeastern USA (Atkinson & Peck, 1994); *X. volvulus* is distributed throughout South and Central America, the Caribbean and south Florida (Wood, 1982); *Xi. gracilis* is distributed in tropical and subtropical areas in the Caribbean and South, Central and North America (Atkinson & Peck, 1994); and both *A. lecontei* and *A. devexulus* are restricted to the Caribbean and southeastern USA. In contrast, *Xa. crassiusculus* and *Xi. saxeseni* are Eurasian species which immigrated to the New World (Wood, 1982). *Xyleborinus saxeseni* has a worldwide distribution and is thought to be one of the first non-native scolytids introduced to America (Rabaglia *et al.*, 2006), whereas *Xa. crassiusculus* is distributed in Africa and Asia and was introduced to North America (Rabaglia *et al.*, 2006).

In this study, evidence for the acquisition of *R. lauricola* by six of the above species is presented, presumably

Table 4 Distribution and host range of ambrosia beetle species examined in this study

Ambrosia beetle species	Distribution[a]	Hosts[b]
Xyleborus glabratus	Asia (N), NA (I)	Lauraceae (6), Dipterocarpaceae (1), Fagaceae (1), Fabaceae (1)
Xyleborinus saxeseni	NA (I), SA (I), Europe (I), Oceania (I), Africa, Asia (N)	Aceraceae (3), Anacardiaceae (1), Annonaceae (1), Apocynaceae (1), Betulaceae (3), Cornaceae (1), Ericaceae (1), Fagaceae (2), Juglandaceae (2), Magnoliaceae (6), Rosaceae (5), Salicaceae (1), Taxodiaceae (1), Tiliaceae (1), Ulmaceae (1)
Xyleborus affinis	NA (N), SA (N), CA (N), Caribbean (N), Africa (I), Asia (I), Europe (I), Oceania (I)	Agavaceae (1), Anacardiaceae (7), Annonaceae (2), Arecaceae (1), Betulaceae (1), Bignoniaceae (3), Burseraceae (2), Caesalpinaceae (3), Clusiaceae (2), Combretaceae (2), Cupressaceae (1), Cyrillaceae (1), Eleaocarpaceae (1), Euphorbiaceae (2), Fabaceae (3), Fagaceae (9), Hamamelidaceae (2), Juglandaceae (2), Lauraceae (3), Lecythidaceae (1), Melastomataceae (1), Meliaceae (2), Mimosaceae (8), Moraceae (2), Myricaceae (3), Palmaceae (1), Pinaceae (3), Poaceae (1), Rosaceae (1), Rubiaceae (1), Rutaceae (2), Sapindaceae (1), Sapotaceae (3), Sterculiaceae (1), Taxodiaceae (2), Ulmaceae (2), Verbenaceae (1)
Xyleborus ferrugineus	NA (N), SA (N), CA (N), Caribbean (N), Africa (I), Oceania (I)	Aceraceae (1), Agavaceae (1), Anacardiaceae (4), Apocynaceae (1), Araliacae (1), Araliaceae (1), Bignoniaceae (3), Burseraceae (6), Caesalpinaceae (5), Clusiaceae (1), Combretaceae (1), Eleaocarpaceae (1), Fabaceae (7), Fagaceae (8), Lauraceae (3), Lecythidaceae (1), Leguminosae (1), Melastomataceae (1), Meliaceae (3), Mimosaceae (3), Moraceae (8), Musaceae (1), Nyctaginacae (1), Nyssaceae (1), Pinaceae (4), Rutaceae (2), Sapindaceae (2), Sapotaceae (3), Sterculiaceae (1), Tiliaceae (3), Urticaceae (1), Verbenaceae (1)
Xyleborinus gracilis	NA, SA, CA, Caribbean, Africa	Araliaceae (1), Burseraceae (1), Clusiaceae (1), Combretaceae (1), Fagaceae (1), Lauraceae (1), Melastomataceae (2), Mimosaceae (1), Moraceae (1), Sapotaceae (1), Sterculiaceae (1)
Xyleborus volvulus	SA, CA, NA, Caribbean, Africa, Asia, Oceania	Anacardiaceae (12), Apocynaceae (1), Araliacae (1), Arecaceae (1), Burseraceae (18), Caesalpinaceae (5), Caricaeae (1), Casuarinaceae (1), Combretaceae (2), Eleaocarpaceae (1), Euphorbiaceae (3), Fabaceae (6), Lauraceae (2), Leguminosae (1), Meliaceae (2), Mimosaceae (5), Moraceae (9), Myricaceae (1), Palmaceae (1), Rubiaceae (1), Sapindaceae (2), Sterculiaceae (2), Taxodiaceae (1), Tiliaceae (1), Verbenaceae (1)
Xylosandrus crassiusculus	NA (I), Africa, Asia	Annonaceae (1), Apocynaceae (1), Convolvulaceae (2), Cornaceae (1), Ebenaceae (1), Fabaceae (1), Fagaceae (1), Hamamelidaceae (3), Juglandaceae (3), Lauraceae (1), Magnoliaceae (2), Melastomataceae (1), Moraceae (5), Rosaceae (17), Sapindaceae (3), Sapotaceae (1), Ulmaceae (2), Verbenaceae (2), Vochysiaceae (1)
Ambrosiodmus lecontei	NA (N), Caribbean (N)	Anacardiaceae (2), Aquifoliaceae (1), Burseraceae (1), Caesalpinaceae (3), Combretaceae (1), Compositae (1), Fabaceae (1), Juglandaceae (1), Lauraceae (2), Meliaceae (1), Mimosaceae (2), Phyllanthaceae (1), Pinaceae (2), Rosaceae (1), Rutaceae (2)
Ambrosiodmus devexulus	NA (N), Caribbean (N)	Anacardiaceae (1), Caesalpinaceae (1), Fagaceae (1), Hamamelidaceae (1), Lauraceae (1)

[a]Distributions are from Wood (1982), Atkinson & Peck (1994), Rabaglia *et al.* (2006) and Atkinson (2012). NA: North America; CA: Central America; SA: South America; (I): introduced; (N): native.
[b]Host ranges are from Atkinson (2012). Numbers in parentheses are numbers of host species reported in a family.

after *X. glabratus* was introduced to the USA in 2002. Although lateral movement of symbionts among ambrosia beetles has been recognized for at least four decades (Batra, 1967), such a dramatic increase in the numbers of beetle species that have acquired a 'new' symbiont appears to be unprecedented, as there are no similar reports in the literature. Why this has occurred is not clear.

Although there may be a phylogenetic relationship between the different beetle species and the probability that they carry *R. lauricola* (three of the four highest probabilities in the study are for *Xyleborus* spp.), *R. lauricola* was not detected in species in the next most closely related genus (Cognato *et al.*, 2011), *Ambrosiodmus*. The systemic colonization of host trees by *R. lauricola* may play a role in its relatively rapid acquisition by multiple species. Contact with another ambrosial fungus would occur when one beetle species came in contact with galleries of another beetle species. However, *R. lauricola* is not restricted to the galleries of *X. glabratus*; rather, it colonizes much of the host tree xylem and can be recovered from these tissues for more than a year after a tree dies (Spence, 2012). Thus, the probability that ambrosia beetles other than *X. glabratus* would come into contact with *R. lauricola* should be far greater than in typical situations in which an ambrosial fungus has a restricted distribution in a tree.

Although an increase in the species and numbers of beetles that are vectors of *R. lauricola* might affect the

spread of laurel wilt, it is clear that more data are needed. For example, information on the efficiency of the new vectors and their attraction to healthy hosts in natural environments would affect this outcome but is not available. In addition, the proportion of a beetle population in which *R. lauricola* is found and the number of pathogen propagules that individual beetles carry should influence host infection and the development of laurel wilt, as observed in the present study. Although the present assays examined only mycangia, propagules on the exoskeleton of the beetles (phoretic transmission) may also play an important role in the lateral movement of these fungi, as has been shown for some bark beetles (Webber & Gibbs, 1989; Webber, 2004). In the present study, low numbers of *R. lauricola* CFUs were detected in surface-disinfested bodies (mycangia) of *X. affinis* and *Xi. saxeseni*, yet, when redbay plants were confronted with non-disinfested cohorts of these species, relatively high rates of pathogen transmission and laurel wilt development occurred. Additional research is warranted on these topics.

Several outcomes are possible with new symbiont × ambrosia beetle associations. The fungus could benefit from transport to new host trees, especially if its primary vector is rare, and/or if the new vector attacks plants that the primary vector cannot. *Xyleborus glabratus* is known to attack mostly lauraceous plants, but it has also been reported from a few other species in the Fagaceae, Fabaceae and Dipterocarpaceae (Rabaglia *et al.*, 2006; Fraedrich *et al.*, 2008; Peña *et al.*, 2012; Table 4). In contrast, the other ambrosia beetles in the present study are polyphagous and can breed in a wide variety of hosts (Atkinson & Peck, 1994; Table 4). Thus, *R. lauricola* could benefit from the expanded host range afforded by the new, potential vectors described in this study. The recent recovery of *R. lauricola* from live oak (*Quercus virginiana*) in Florida in which *X. glabratus* was not detected (J. Smith, Forestry & Conservation Department, University of Florida, USA, personal communication), suggests that vector-mediated host range expansion for *R. lauricola* may already occur.

In these situations, laterally transferred fungi could have a range of relationships with new beetles. Host range expansion, as described above, might benefit the fungus but not affect the beetle (i.e. they could have a commensalistic relationship), or the new relationship could benefit both partners (mutualistic) if the new fungus provided enhanced nutrition for the beetle or if the fungus were pathogenic on the new host trees and, thereby, gave the beetle a competitive advantage over other ambrosia beetle species (i.e. provided greater opportunities for brood development). Alternatively, the fungus could negatively affect the beetle either by displacing nutritionally superior symbionts or by being directly antagonistic to the beetle. Little is known about these possibilities because of the inherent difficulties in working with obligate nutritional symbioses. For example, although six *Raffaelea* spp. have been recovered from the mycangia of *X. glabratus*, nothing is known about their relative importance or roles as a food sources for this or other ambrosia beetles (Harrington & Fraedrich, 2010). Greater understanding is needed for how and why different ambrosia beetles obtain new symbionts.

Leach (1940) established four principles to determine whether an insect is a vector of a phytopathogen. The first states that the insect must be closely associated with diseased plants. Based on a prior study (Carrillo *et al.*, 2012) and the results of the present study, the scolytinae species examined were consistently associated with laurel wilt-affected trees in Florida.

Leach's second principle states that a vector must interact with healthy plants. Most ambrosia beetles are thought to colonize dead, stressed or dying trees, and occasionally attack live trees (Kühnholz *et al.*, 2001). Some, most often exotic, species are known to attack healthy living trees (Hulcr & Dunn, 2011). Ambrosia beetles of the genus *Xyleborus* typically infest trees of low vigour, or which have been cut, damaged or windthrown (Roeper *et al.*, 1980). However, Rabaglia *et al.* (2006) reported that *X. affinis* and *X. ferrugineus* can cause economic damage in moist, lowland areas of the neotropics. There are reports of *X. affinis* attacking healthy sugarcane and *Dracaena massangeana* (Merkl & Tusnádi, 1992; Granda Giro, 2003), and of *X. ferrugineus* boring into healthy pecan trunks and twigs (Aguilar-Pérez *et al.*, 2007). *Xyleborinus saxeseni* is regarded as one of the most damaging species in the tribe Xyleborini (Rabaglia *et al.*, 2006), and there are reports of it attacking healthy chestnut (Oliver & Mannion, 2001) and stressed peach trees (Kovach & Gorsuch, 1985). Lastly, *Xa. crassiusculus* can attack over 200 tree species and is an important pest of nursery grown trees (Frank & Sadof, 2011). In summary, despite the usual preference of ambrosia beetles for unhealthy or dead trees, several of the species that were examined in the present study can interact with healthy trees.

Because the present experiments were conducted under no-choice conditions, it is not possible to conclude that the new beetles from which *R. lauricola* was recovered are effective vectors in nature. However, under these conditions all beetle species attempted to bore into trees and there was a marked difference in host suitability, in that gallery number and length were significantly higher in redbay than avocado. More research is needed to determine the ability of these ambrosia beetles to attack healthy plants under natural conditions.

Leach's third principle indicates that the pathogen must be associated with the insect. In this study, *R. lauricola* was consistently associated with four species (i.e. $\geq$50% of the assayed individuals) and was found less commonly in three others. Given the way in which these beetles were processed, it is presumed that the fungus was located in their mycangia. Whether or not phoretic, non-mycangial associations of *R. lauricola* occur with these insects should be examined.

Leach's final principle states that disease should develop in healthy plants after they interact with

pathogen-infested insects. In this study, *R. lauricola* was transmitted to avocado by only two species besides *X. glabratus*, but it was transmitted to at least one redbay tree by all of the species that were tested. Laurel wilt developed in most of the avocado and redbay plants that were infected by *R. lauricola*. However, disease did not develop in single infected avocado trees that were infested with *X. volvulus* and *X. ferrugineus*, and in single infected redbay trees that were infested with *Xa. crassiusculus*, *X. ferrugineus* and *Xi. saxeseni*. Although it is possible to kill both avocado and redbay plants with inoculum containing as few as 100 conidia, there is considerable variation in disease development at such low inoculum levels (i.e. some inoculated trees develop little or no disease) (M. Hughes, University of Florida, Gainesville, USA, personal communication). Considering the low numbers of *R. lauricola* CFUs that were detected in the above species, it is probable that insufficient levels of the pathogen were present in the infected, symptomless plants to cause disease.

In conclusion, this present study demonstrated that lateral transfer of the primary symbiont of *X. glabratus*, *R. lauricola*, has occurred in six additional invasive or native species of ambrosia beetle in Florida. Based on results from no-choice tests, some of these beetles can transmit this pathogen to avocado and/or redbay. However, more research is needed to determine whether these beetles attack healthy trees in natural and agroecosystems in Florida. New vectors could expand the host range of this pathogen and may influence the development of laurel wilt, especially on hosts that support little or no reproduction of *X. glabratus*.

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