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Suitability of California bay laurel and other species as hosts for the non-native redbay ambrosia beetle and granulate ambrosia beetle

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Abstract

1. The redbay ambrosia beetle *Xyleborus glabratus* Eichhoff is a non-native vector of the pathogen that causes laurel wilt, a deadly disease of trees in the family Lauraceae in the southeastern U.S.A.
2. Concern exists that *X. glabratus* and its fungal symbiont could be transported to the western U.S.A. and cause damage to California bay laurel *Umbellularia californica* (Hook. & Arn.) Nutt. in California and Washington.
3. The present study evaluated in-flight attraction, attack density and emergence of *X. glabratus* and another invasive ambrosia beetle *Xylosandrus crassiusculus* (Motschulsky) on cut bolts of California bay laurel and eight related tree species in an infested forest in South Carolina. *Xylosandrus crassiusculus* is not a vector of the laurel wilt pathogen but is a pest of nursery and ornamental trees.
4. Mean catch of *X. glabratus* on California bay laurel bolts was not significantly different from catches on bolts of known *X. glabratus* hosts sassafras *Sassafras albidum* (Nutt.) Nees and swampbay *Persea palustris* (Raf.) Sarg. Mean attack density and adult emergence of both beetle species from California bay laurel was equal to or greater than all other tree species tested. Both beetle species readily produced brood in California bay laurel bolts.
5. The results obtained in the present study suggest that California bay laurel may be negatively impacted by both of these invasive ambrosia beetles if they become established in the tree's native range.

Keywords Attraction, Curculionidae, invasive species, Laurel wilt, Scolytinae, *Umbellularia californica*, wood borer, *Xyleborus glabratus*, *Xylosandrus crassiusculus*.

Introduction

Bark- and wood-boring beetles are some of the most commonly transported insects in international trade, and dozens of exotic species in this group have become established in the U.S.A. in recent decades (Haack, 2006). Because these beetles spend much of their life cycle within the xylem or phloem of their hosts, they are easily introduced into new ecosystems through the intra- and intercontinental movement of untreated logs, lumber and solid wood packing material. Bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) have close symbiotic relationships with wood-inhabiting, ascomycetous fungi (order Ophiostomatales), which are carried by the beetles into host trees where they are cultivated as food or function in other roles beneficial to the beetles (Farrell *et al*., 2001). Although rarely serious pests of live trees in their native ranges,

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bark and ambrosia beetles and their associated fungi are being increasingly recognized as substantial threats to forest health when these symbiotic complexes are introduced to novel hosts and environments (Hulcr & Dunn, 2011).

The redbay ambrosia beetle *Xyleborus glabratus* Eichhoff is a non-native invasive forest pest in the Atlantic and Gulf Coastal Plain regions of the southeastern U.S.A. The insect is native to southeastern Asia, where it has been reported from hosts in several plant families (including Lauraceae, Dipterocarpaceae, Fagaceae and Fabaceae) but is not considered a pest of live trees (Rabaglia *et al*., 2006). In North America, *X. glabratus* was first detected in a survey trap near Port Wentworth, Georgia, in 2002 (Rabaglia *et al*., 2006) and has subsequently expanded its distribution into five additional states, from eastern North Carolina south to Florida and west to Mississippi (Bates *et al*., 2012). *Xyleborus glabratus* carries several species of symbiont fungi in its mycangia (Harrington *et al*., 2008, 2010) one of which, *Raffaelea lauricola* Harrington, Fraedrich and Aghayeva, causes a deadly vascular disease known as laurel wilt in redbay *Persea borbonia* (L.) Spreng. and several other tree species in the family Lauraceae (Fraedrich *et al*., 2008). Laurel wilt occurs when female *X. glabratus* attack healthy host trees, bore into the sapwood and inoculate the xylem with *R. lauricola*. In redbay, the pathogen moves rapidly through the xylem, causing the outer sapwood to discolor and the tree to wilt in a matter of weeks to a few months (Fraedrich *et al*., 2008). Upon tree death, *X. glabratus* females successfully create galleries in the sapwood and produce brood. Haploid, flightless males hatch from unfertilized eggs and mate with parent or sibling females to produce additional females (Wood, 1982). Peak flight in South Carolina and Georgia occurs in late summer to early autumn, although there are multiple generations of beetles per year and females can be collected in baited flight traps throughout the year (Hanula *et al*., 2008, 2011).

Widespread mortality of redbay and the closely-related species swampbay *Persea palustris* (Raf.) Sarg. and silkbay *Persea humilis* Nash has occurred as a result of laurel wilt throughout the introduced range of *X. glabratus* and *R. lauricola*. In stands where redbay is abundant, > 90% of tree-sized (diameter > 10 cm) redbays are killed by laurel wilt within the first 2–5 years after initial disease detection, whereas redbay seedlings and small saplings are much less affected (Fraedrich *et al*., 2008; Shields *et al*., 2011). Sassafras *Sassafras albidum* (Nutt.) Nees is being killed by laurel wilt in the Atlantic Coastal Plain (Smith *et al*., 2009a; Cameron *et al*., 2010), although the pathogen and vector have yet to spread into the more upland and northern portions of the sassafras range where the tree is more abundant. Cut bolts of avocado *Persea americana* Mill. are attractive to *X. glabratus* (Hanula *et al*., 2008; Kendra *et al*., 2011) and laurel wilt has killed certain cultivars of avocado in landscapes and experimental trials (Mayfield *et al*., 2008a,b), prompting concern from the commercial avocado industry (Crane *et al*., 2008). The laurel wilt pathogen *R. lauricola* has also been recovered from camphortree *Cinnamomum camphora* (L.) J. Presl and the rare native shrubs pondberry *Lindera melissifolia* (Walter) Blume and pondspice *Litsea aestivalis* (L.) Fernald (Fraedrich *et al*., 2008, 2011; Smith *et al*., 2009b; Hughes *et al*., 2011).

The granulate ambrosia beetle *Xylosandrus crassiusculus* (Motschulsky), another invasive wood-borer of Asian origin, has been widely introduced around the globe, including in North America, Africa, India, Europe, Indonesia and the South Pacific (Atkinson *et al*., 1988; Pennacchio *et al*., 2003). It is a pest of nursery stock, ornamentals and fruit trees, and is reported to attack > 120 nonconiferous woody plant species in > 40 families worldwide. Similar to other ambrosia beetles, *X. crassiusculus* bores into the sapwood of its hosts and cultivates symbiotic ambrosia fungi in its galleries (Atkinson *et al*., 1988). Infestations are found on stressed trees and occasionally on apparently healthy hosts (Atkinson *et al*., 1988; Horn & Horn, 2006) but, unlike *X. glabratus*, it does not vector a wilt pathogen that kills the host tree. In the continental U.S.A., *X. crassiusculus* was first reported in South Carolina (Anderson, 1974), has established in several southeastern states in the lower Piedmont and Coastal Plain regions (Atkinson *et al*., 1988), and has also been reported from Indiana, Oklahoma, Missouri and Kansas (Cote, 2008). The only reported occurrence of *X. crassiusculus* in the western U.S.A. was a localized infestation in Oregon that was aggressively eradicated because of the perceived threat to the state's agricultural industry (LaBonte, 2010).

California bay laurel *Umbellularia californica* (Hook. & Arn.) Nutt. is an aromatic, broadleaved, evergreen tree native to the Pacific coastal regions of southwestern Oregon and western California and in the foothills of the Cascade Range and Sierra Nevada mountains (Stein, 1990). It grows as a shrub or tree (attaining 12–24 m in height) on a variety of topographical locations and soil types, and occurs both in pure stands and with a diversity of other species in mixed stands. Its attractive wood is used for cabinetry, furniture, veneer and other specialty wood products, and various parts of the tree have long been used for culinary and medicinal purposes. The fruit, which matures in autumn, is a thinly-hulled drupe containing a single, nutlike seed and is a food source for squirrels, jays and other animals (Stein, 1990). California bay laurel is a foliar host of *Phytophthora ramorum* (S. Werres, A. W. A. M. deCock & W. A. Man in't Veld), a non-native pathogen that causes a disease known as sudden oak death in tanoak *Lithocarpus densiflorus* (Hook. & Arn.) Rehder, coast live oak *Quercus agrifolia* Née and several other oak species in central coastal California and southwestern Oregon (Goheen *et al*., 2002; Rizzo *et al*., 2002). Foliar lesions caused by *P. ramorum* have little impact on California bay laurel, although prolific sporulation of the pathogen on these trees plays an important role in the epidemiology of sudden oak death and the development of tree-killing forest infestations (Davidson *et al*., 2002; Swiecki & Bernhardt, 2008).

California bay laurel seedlings maintained in growth chambers and artificially inoculated with the laurel wilt pathogen *R. lauricola* exhibited sapwood discolouration with wilting and dieback of a few to most branches but not rapid wilting of the entire crown as seen in redbay (Fraedrich, 2008). This suggests that *U. californica* is susceptible to laurel wilt, although the actual threat of the disease to *U. californica* is still uncertain because trees growing in their native environment have not been exposed to the pathogen or its principal vector *X. glabratus*. Furthermore, if California bay laurel is not attractive to *X. glabratus* or suitable for colonization and breeding by the beetle, the risk of infection to live trees and the progression of laurel wilt disease in *U. californica* stands may be low. Nonetheless, concern exists that *X. glabratus* and *R. lauricola* could be introduced to the western U.S.A. through human transport of infested host material (e.g. firewood), and more information on the susceptibility of California bay laurel to these organisms is needed to help anticipate their impact and formulate management responses should they become introduced in the range of *U. californica*.

The primary objective of the present study was to evaluate the suitability of California bay laurel as a potential host for the redbay ambrosia beetle and granulate ambrosia beetle. A secondary objective was to compare the host suitability of several additional eastern U.S. tree species, including several known hosts of *X. glabratus* and *X. crassiusculus*, as well as related tree species of unknown suitability to these beetles. To address these objectives, we compared attraction, attack density and emergence of these ambrosia beetle species on cut bolts of California bay laurel and eight other related tree species in an infested forest stand in the southeastern U.S.A.

Materials and methods

Test bolts

As noted above, California bay laurel is a foliar host of the sudden oak death pathogen *P. ramorum*. The potential threat posed by *P. ramorum* to oak-dominated forests in eastern North America was recognized early in the sudden oak death epidemic in California and Oregon, and resulted in many state, federal and international quarantines and regulations restricting movement of host materials (Frankel, 2008). Therefore, before moving cut bolts of California bay laurel from its native range to the eastern U.S.A. for exposure to redbay ambrosia beetle, two precautions were taken to ensure that *P. ramorum* was not transported with the wood. First, California bay laurel trees on the Stanislaus National Forest, Tuolumne County, California (37.88200°N, −119.96907°W), located well outside the known distribution and quarantine zone for *P. ramorum*, were selected for use in the present study. Second, a bioassay was performed on a preliminary set of 16 California bay laurel bolts to test for presence of *P. ramorum* on the bark after a tap water wash (the treatment applied to bolts shipped to the eastern U.S.A.), and *P. ramorum* was not detected on the material (see Supporting information, Doc S1).

Ten additional California bay laurel bolts used for field exposure to redbay ambrosia beetle populations were cut on 1 August 2011 from the location noted above. Using a standard garden hose and spray nozzle, each bolt was thoroughly sprayed with tap water on all sides as a coarse-level treatment to remove debris, soil and potential soil-borne pathogens or other unwanted organisms. Cut ends were wrapped in Parafilm® (Pechiney Plastic Packaging Company, Chicago, Illinois) and shipped overnight in insulated coolers to Asheville, North Carolina. Ten fresh bolts of each of eight species were also obtained for comparison with California bay laurel in the field trial: swampbay, silkbay, sassafras, camphortree, northern spicebush *Lindera benzoin* (L.) Blume, sweetbay *Magnolia virginiana* L., Fraser magnolia *Magnolia fraseri* Walter and cucumbertree *Magnolia acuminata* (L.) L. The first five of these species were selected because they are members of the Lauraceae, the only plant family in which laurel wilt disease has been reported. The latter three *Magnolia* species were chosen because they are in the same subclass of plants (Magnoliidae) as the Lauraceae and sometimes co-occur in southeastern U.S. forests with either *Persea* bays or sassafras. Bolts were obtained from several locations in 2011: camphortree from Bok Tower Gardens, Lake Wales, Florida on 28 July; silkbay from the Archbold Biological Station, Lake Placid, Florida on 29 July; sassafras, Fraser magnolia, cucumbertree and northern spicebush from the Bent Creek Experimental Forest, Asheville, North Carolina on 2 August; and swampbay and sweetbay from the Francis Marion National Forest, Cordesville, South Carolina, on 3 August. All bolts were approximately 30 cm long and 9–15 cm in diameter. The cut ends of each bolt were coated with melted paraffin to reduce desiccation and a screw hook was inserted onto one cut end. To enhance release of host volatiles, a 2-cm wide wood bit attached to a handheld drill was used to remove a shallow, circular patch of outer bark and expose the phloem at six systematically spaced spots on the surface of each bolt.

Ambrosia beetle attraction

Bolts were deployed on 4 August 2011 in a floodplain stand of mixed deciduous and pine species that paralleled the headwaters of Alligator Creek on the Francis Marion National Forest near Cordesville, South Carolina (33.16707°N, −79.87013°W). Common tree species included swamp tupelo *Nyssa sylvatica* var. *biflora* (Walter) Sarg., swampbay, red maple *Acer rubrum* L., sweetgum *Liquidambar styraciflua* L., baldcypress *Taxodium distichum* (L.) Rich., laurel oak *Quercus laurifolia* Michx., loblolly pine *Pinus taeda* L. and longleaf pine *P. palustris* Mill. Each trap consisted of one bolt hanging from a hook on a rope suspended between two trees (1.2–1.8 m above the ground). Two white sticky cards (23 × 14 cm, half of a wing-style trap bottom, Scentry Biologicals, Inc., Billings, Montana), facing out in opposite directions, were stapled to the bottom 2 cm of the hanging bolt. No bolt was hung within 3 m of a swampbay tree. Bolts were arranged in a randomized complete block design with seven replicate blocks of 10 traps. Treatments included one of each of the test bolt species, plus one dry pine landscape timber of similar size as an unattractive control (Mayfield & Hanula, 2012). Bolts were spaced at least 10 m apart within blocks; blocks were spaced at least 20 m apart and were arranged in a linear pattern to stay within the mixed floodplain forest that was bounded by pine stands on either side. All bolts and sticky cards were collected after 21 days. Numbers of adult female *X. glabratus* and *X. crassiusculus* captured on the cards were counted and expressed as beetles per trap per day.

Ambrosia beetle attack density and adult emergence

After removal from the field, each bolt was placed in a ventilated plastic rearing container constructed from a 19-L plastic bucket as described previously (Mayfield and Hanula, 2012). The collection jar attached to each container was filled with a small amount of propylene glycol antifreeze to kill and preserve insects entering the jar. Containers were suspended by their handles on wooden racks and kept at room temperature. A light-emitting diode was positioned directly beneath each collection jar and left on continuously in an otherwise dark room. Approximately every 2 weeks, all adult beetles in the jars were collected and preserved, and all *X. glabratus* and *X. crassiusculus* were counted. Pine landscape timber bolts were not placed in rearing containers because they were not attractive to ambrosia beetles in the field and were impervious to wood-boring insects. After 8 months, bolts were removed from rearing containers, all bark was removed with a draw knife and ambrosia beetle entrance holes on the surface of the sapwood and the cut ends were counted. A distinction was made between holes of the size typically made by *X. glabratus* (roughly equal to the diameter of a No. 1 size paper clip, 0.8 mm in diameter) and those made by the larger *X. crassiusculus*. Ambrosia beetles other than *X. glabratus* or *X. crassiusculus* rarely emerged and represented less than 1% of the total beetles emerged. The numbers of ambrosia beetles and entrance holes were expressed per 100 cm^2 of bolt surface area.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test the null hypothesis of no significant difference between treatment means for the variables: (i) number of ambrosia beetles caught per trap per day; (ii) number of entrance holes per 100 cm^2 of bolt surface; and (iii) number of ambrosia beetles emerged per 100 cm^2 of bolt surface. Means for both *X. glabratus* and *X. crassiusculus* were analyzed. In all tests, response variables were log-transformed ($\log_{10}[x+1]$) to reduce heteroscedasticity but actual means are reported in figures. Treatments with no mean or variance were excluded from ANOVA. Means were considered significant at the $\alpha = 0.05$ level and mean comparisons were made using Tukey's honestly significant difference. Data were analyzed using Statistica version 9.1 (Statsoft, 2008).

Results

Ambrosia beetle attraction

Tree species had a significant effect on the mean number of *X. glabratus* trapped per day on sticky cards attached to cut bolts ($F = 46.0$; d.f. = 1,9; $P < 0.001$). Camphortree and silkbay bolts attracted the most *X. glabratus* (> 11 beetles/trap/day) but not significantly more than sassafras and swampbay (8.4 and 5.8 beetles/trap/day, respectively) (Fig. 1). Mean attraction of *X. glabratus* to California bay laurel (4.3 beetles/trap/day) did not differ significantly from known *X. glabratus* hosts swampbay and sassafras. Mean *X. glabratus* catch on Fraser magnolia (3.2 beetles/trap/day) did not differ significantly from catches on California bay laurel and swampbay. Spicebush, sweetbay, cucumbertree and control bolts were relatively unattractive, catching fewer than 1 beetle/trap/day (Fig. 1).

Tree species also had a significant effect on the mean number of *X. crassiusculus* trapped per day ($F = 12.6$; d.f. = 1,8; $P < 0.001$). Camphortree and California bay laurel were most attractive and trapped similar numbers of *X. crassiusculus* (1.4 and 1.7 beetles/trap/day, respectively) (Fig. 1). Catch on cucumbertree was notably low (0.3 beetles/trap/day) but not significantly different from catches on camphortree. All other species tested were relatively unattractive and no *X. crassiusculus* were trapped on sweetbay (Fig. 1).

Ambrosia beetle attack density and adult emergence

Tree species had a significant effect on the mean number of *X. glabratus* attacks (entrance holes) per unit surface area

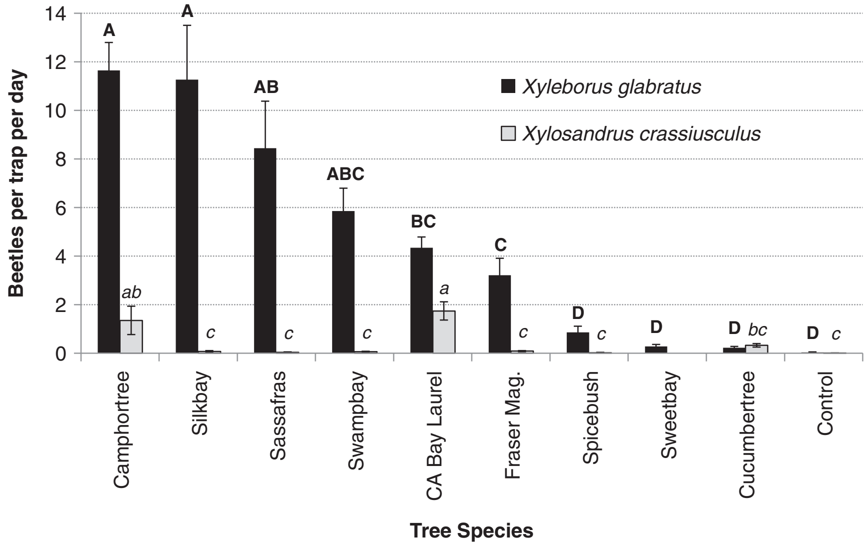

Figure 1 Mean ± SE number of *Xyleborus glabratus* and *Xylosandrus crassiusculus* adults captured per trap per day on sticky cards attached to cut bolts of various tree species hung near Cordesville, South Carolina, in August 2011. Bars labelled with the same letter and case are not significantly different ($\alpha = 0.05$).

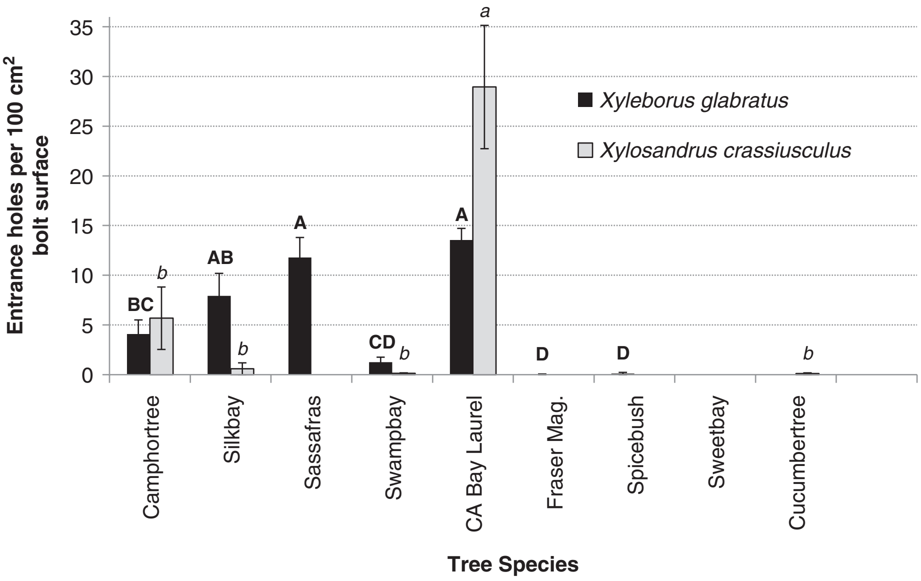

Figure 2 Mean ± SE number of *Xyleborus glabratus* and *Xylosandrus crassiusculus* entrance holes per 100 cm^2 on bolts of various tree species hung near Cordesville, South Carolina, in August 2011. Bars labelled with the same letter and case are not significantly different ($\alpha = 0.05$). Tree species with mean and variance of zero for the response variable were excluded from the analysis of variance.

($F = 27.0$; d.f. = 1,6; $P < 0.001$). Mean *X. glabratus* entrance hole density was highest on California bay laurel, sassafras and silkbay (13.5, 11.8 and 7.9 holes per 100 cm^2, respectively) (Fig. 2). Mean attack density on camphortree (4.1 holes per 100 cm^2) did not differ significantly from that of silkbay or swampbay (1.2 holes per 100 cm^2). *Xyleborus glabratus* holes were rare on swampbay, spicebush and Fraser magnolia and absent from all sweetbay and cucumbertree bolts (Fig. 2).

Mean attack density of *X. crassiusculus* was more than five times higher on California bay laurel than on camphortree (28.9 and 5.7 holes per 100 cm^2, respectively) ($F = 14.8$; d.f. = 1,4; $P < 0.001$) (Fig. 2). Few *X. crassiusculus* entrance holes were observed on silkbay, swampbay and cucumbertree, and none were observed on sassafras, spicebush, Fraser magnolia or sweetbay (Fig. 2).

Across tree species, patterns of ambrosia beetle emergence (Fig. 3) were consistent with patterns of entrance hole density (Fig. 2). Mean *X. glabratus* emergence was highest from California bay laurel, sassafras and silkbay (22.9, 12.9 and 11.9 beetles per 100 cm^2, respectively) ($F = 4.5$; d.f. = 1,4; $P = 0.006$) (Fig. 3). Mean *X. glabratus* emergence from swampbay and camphortree (3.1 and 2.7 beetles per 100 cm^2, respectively) was relatively low and no *X. glabratus* emerged from sweetbay and cucumbertree (Fig. 3). Only two female *X. glabratus* emerged from spicebush (one bolt), and one female emerged from Fraser magnolia. Flightless males were recovered in emergence containers from each of the five species with the greatest *X. glabratus* emergence, indicating brood production within the bolts (Table 1).

Mean emergence of *Xylosandrus crassiusculus* was more than 20-fold higher from California bay laurel (113.3 beetles per 100 cm^2) than from camphortree or silkbay (5.0 and 2.2 beetles per 100 cm^2, respectively) ($F = 13.3$; d.f. = 1,2; $P < 0.001$) (Fig. 3). Flightless males of *X. crassiusculus* were recovered from all three of these tree species, indicating brood production within the bolts (Table 1). No *X. crassiusculus* emerged from sassafras, swampbay, Fraser magnolia, spicebush, sweetbay or cucumbertree (Fig. 3).

Discussion

California bay laurel bolts were attractive to both the redbay ambrosia beetle and the granulate ambrosia beetle. Mean trap catch of *X. glabratus* on California bay laurel (approximately 4 beetles/trap/day) was similar to values observed on known *X. glabratus* hosts sassafras and swampbay in the present study, as well as to trap catch values observed on swampbay in previous studies using similar types of bolt traps (Mayfield & Hanula, 2012). Field studies of *X. glabratus* by Hanula *et al*. (2008) found no evidence for an aggregation pheromone or long-range attraction to beetle frass or fungal symbionts, suggesting that host-produced volatile compounds are the principal cues used by dispersing *X. glabratus* females during host location. Hanula and Sullivan (2008) analyzed the volatile chemical composition of redbay wood, manuka oil (extract of *Leptospermum scoparium* Forst. and Forst., family Myrtaceae) and phoebe oil (extract of *Phoebe porosa* Mez., family Lauraceae) and suggested that α-copaene may be a primary, host-produced attractant. Subsequent trap studies and comparative analyses of volatile emission from multiple Lauraceous species, lychee *Litchi chinensis* Sonn. (Sapindaceae) and essential lure oils support the importance of α-copaene as an attractant, and suggest that β-caryophyllene and α-humulene may be additional semiochemicals for *X. glabratus* (Kendra *et al*., 2011; Niogret *et al*., 2011). Current studies designed to correlate the sesquiterpene content of California bay laurel with trap captures of *X. glabratus* (P. E. Kendra, unpublished data) should help confirm which

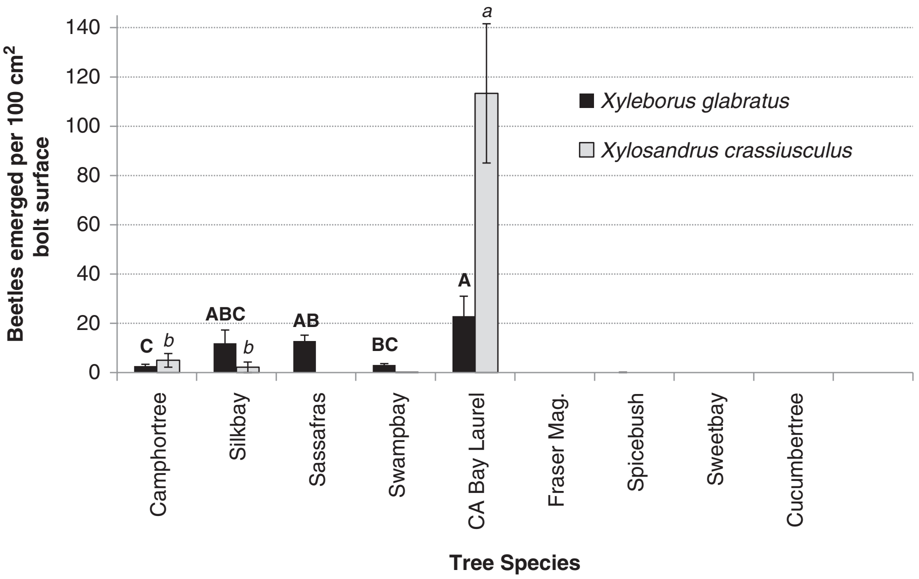

Figure 3 Mean ± SE number of adult *Xyleborus glabratus* and *Xylosandrus crassiusculus* emerged per 100 cm^2 from bolts of various tree species hung near Cordesville, South Carolina, in August 2011. Bars labelled with the same letter and case are not significantly different ($\alpha = 0.05$). Tree species with mean and variance of zero for the response variable were excluded from the analysis of variance.

Table 1 Total number of adult female and male *Xyleborus glabratus* and *Xylosandrus crassiusculus* that emerged from containerized bolts of various tree species hung in the field near Cordesville, South Carolina, in August 2011

	Xyleborus glabratus			*Xylosandrus crassiusculus*		
Tree species	Females	Males	Percentage males	Females	Males	Percentage males
California bay laurel	710	92	11.5	3550	464	11.6
Silkbay	521	74	12.4	117	8	6.4
Sassafras	453	35	7.2	0	0	0.0
Swampbay	98	11	10.1	0	0	0.0
Camphortree	125	3	2.3	248	6	2.4

volatile compounds emitted by *U. californica* are attractive to *X. glabratus*.

Although the results derived from cut bolts may not be directly representative of insect responses to standing live trees, the relative attractiveness of California bay laurel bolts compared with that of other known *X. glabratus* hosts suggests that *U. californica* is at risk of attack by *X. glabratus* if the beetle becomes established in the tree's native range. The potential impact of laurel wilt disease on California bay laurel and other novel hosts, however, will depend on the tree's interaction with both the beetle and the pathogen. In the epidemiology of laurel wilt on redbay, it is hypothesized that initial attacks of *X. glabratus* on healthy hosts (which are cryptic and difficult to locate) do not result in successful colonization and egg-laying but, instead, serve to infect the sapwood with the pathogen, *R. lauricola*. Trees become suitable for brood production only after the pathogen has moved systemically through the xylem and trees are physiologically weakened or killed (Fraedrich *et al*., 2008). The results of the present study indicate that California bay laurel is highly suitable for *X. glabratus* brood production, and that the tree species would likely perpetuate the laurel wilt disease cycle if *R. lauricola* is sufficiently virulent to kill mature California bay laurel trees. Artificial inoculations of California bay laurel seedlings with *R. lauricola* in a controlled environment resulted in sapwood discolouration and varying degrees of branch wilt and dieback (Fraedrich, 2008), although the response of mature California bay laurel trees to infection is not known.

Xylosandrus crassiusculus was remarkably prolific in California bay laurel. Despite catching fewer *X. crassiusculus* than *X. glabratus* on attached sticky cards, California bay laurel bolts exhibited twice as many entrance holes and produced five times as many emerging beetles of *X. crassiusculus* compared with *X. glabratus* (Figs 2 and 3; Table 1). The high *X. crassiusculus* attack density and emergence relative to trap catch may have resulted from some beetles emerging in the containers, re-entering the bolts and producing additional brood. Although *X. crassiusculus* is a common pest of nursery stock, small-diameter ornamentals and orchard trees (Atkinson *et al*., 1988) it is not generally known in the U.S.A. as a killer of trees in natural forest settings. Still, the demonstrated success of *X. crassiusculus* in the California bay laurel bolts tested in this study suggest it could become a significant pest of ornamental *U. californica* if introduced in the western U.S.A.

An unexpected result of this study was the relative attractiveness and suitability of sassafras and swampbay bolts to *X. glabratus*. Although sassafras is a known host of laurel wilt and *X. glabratus* in field (Smith *et al*., 2009a; Cameron *et al*., 2010), cut bolts of sassafras were significantly less attractive than redbay and swampbay in previous trap trials and produced fewer beetles than swampbay when containerized

(Hanula *et al*., 2008; Mayfield & Hanula, 2012). By contrast, *X. glabratus* trap catch and beetle emergence from sassafras bolts did not differ significantly from swampbay and silkbay in the present study (Figs 1 and 3), and attack density was higher on sassafras than on swampbay (Fig. 2). One possible explanation for this difference is that bolts in this study were end-sealed with melted paraffin wax and then bark wounded, whereas, in the previous studies, bolts were either unsealed or end-sealed with a wax-and-water emulsion (Mayfield & Hanula, 2012). The various end treatments may have affected the relative moisture content, attractiveness or wood suitability of sassafras and swampbay in different ways. For example, application of wax-and-water emulsion to cut ends of bolts decreased *X. glabratus* entrance hole density on swampbay but not on sassafras (Mayfield & Hanula, 2012). Additional studies directly comparing the effect of different wax treatments (e.g. paraffin versus wax-water emulsion) on beetle response would help clarify this question.

Another possible explanation for the different relative attractiveness of swampbay and sassafras bolts across studies is that there may be considerable genetic- or environmentally-based differences in chemical composition or moisture content among different trees of the same species. It is noteworthy that the swampbay trees used for bolts were among the few trees of this species still alive in the study area as a result of widespread mortality caused by laurel wilt. These trees may have survived because *X. glabratus* had not yet encountered them, or possibly because beetles determined them to be unsuitable for attack for some reason. Swampbay is normally a very suitable host for both *X. glabratus* and *X. crassiusculus* (Mayfield & Hanula, 2012) but, in the present study, relatively few *X. glabratus* and no *X. crassiusculus* emerged from swampbay (Fig. 3 and Table 1). These unexpected results suggest that additional comparative evaluation of swampbay and sassafras may be warranted, and perhaps that a wider diversity of source trees should be used when acquiring replicate bolts for studies of ambrosia beetle attraction and colonization.

Camphortree had the highest mean *X. glabratus* trap catch of any species tested in this study, although relatively few adult beetles emerged from bolts of this species. This result is consistent with a previous evaluation of camphortree bolts in Florida (Mayfield & Hanula, 2012). The laurel wilt pathogen *R. lauricola* has been isolated from wilting camphortrees in the southeastern U.S.A., although rapid wilting of the entire crown is not characteristic of the disease in this species (Smith *et al*., 2009b). Because it is a species of Asian origin, camphortree may have coevolutionary history with the *X. glabratus/R. lauricola* complex and thus some level of resistance to or tolerance of these organisms, although this possibility has not been formally examined.

Northern spicebush was not very attractive to *X. glabratus* and only two female beetles emerged from it. Although this Lauraceous shrub native to eastern North America is widely distributed and very aromatic, it is unlikely to be attacked by *X. glabratus* or to serve as a source of beetle brood that could perpetuate the laurel wilt disease cycle. Similarly, none of the *Magnolia* species evaluated in the present study sustained many attacks or produced any brood of *X. glabratus* or *X. crassiusculus*, although Fraser magnolia attracted significantly more *X. glabratus* than the other *Magnolia* species (Fig. 1). Evaluation of volatile emissions from these magnolia species might reveal the presence of a compound in Fraser magnolia that is common to the Lauraceous species but is absent or reduced in sweetbay or cucumbertree; such a compound might be an important semiochemical for *X. glabratus*.

In summary, the present study provides evidence that California bay laurel has an attractiveness and suitability to the redbay ambrosia beetle similar to that of other known Lauraceous hosts native to the U.S.A. This tree species may be negatively affected if *X. glabratus* becomes established in coastal California or Oregon and the pathogen *R. lauricola* proves to be virulent in mature California bay laurel trees. California bay laurel is also an attractive and suitable host for *X. crassiusculus*, which is not yet established in western U.S.A. but could negatively affect nursery production or ornamental use of *U. californica* if introduced. In addition, our data suggest that northern spicebush, Fraser magnolia, sweetbay and cucumbertree are not suitable hosts for *X. crassiusculus* or *X. glabratus*, and are unlikely to contribute to the spread of the redbay ambrosia beetle and laurel wilt disease in eastern North America.

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Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference:
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Doc. S1. Bioassay to test for presence of *Phytophthoraramorum* on the bark of a preliminary set of California bay laurel bolts.

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