Host Range of Fusarium Dieback and Its Ambrosia Beetle (Coleoptera: Scolytinae) Vector in Southern California

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Abstract

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The polyphagous shot hole borer (PSHB) is an invasive ambrosia beetle that forms a symbiosis with a new, as-yet-undescribed *Fusarium* sp., together causing Fusarium dieback on avocado and other host plants in California and Israel. In California, PSHB was first reported on black locust in 2003 but there were no records of fungal damage until 2012, when a *Fusarium* sp. was recovered from the tissues of several backyard avocado trees infested with PSHB in Los Angeles County. The aim of this study was to determine the plant host range of the beetle–fungus complex in two heavily infested botanical gardens in Los Angeles County. Of the 335 tree species observed, 207 (62%), representing 58 plant families, showed signs and symptoms consistent with attack by PSHB. The *Fusarium* sp. was recovered from 54% of the plant species attacked by PSHB, indicated by the presence of the *Fusarium* sp. at least at the site of the entry hole. Trees attacked by PSHB included 11 species of California natives, 13 agriculturally important species, and many common street trees. Survey results also revealed 19 tree species that function as reproductive hosts for PSHB. Additionally, approximately a quarter of all tree individuals planted along the streets of southern California belong to a species classified as a reproductive host. These data suggest the beetle–disease complex potentially may establish in a variety of plant communities locally and worldwide.

Since the beginning of 2012, an emerging plant disease, referred to as Fusarium dieback (FD), has been detected on several host plants in Los Angeles and Orange Counties, CA (7). FD is caused by the combined effects of a tiny (<2.5 mm) invasive ambrosia beetle (Scolytinae) and its symbiotic fungal partner, a currently undescribed *Fusarium* sp. (7,15). Adult female beetles burrow into a tree, creating brood galleries beyond the cambium, which are concurrently inoculated with the *Fusarium* sp. The fungus colonizes the gallery walls and becomes the sole source of food for developing larvae and adult beetles (7,15). The fungus invades the vascular tissue of the tree, blocking water and nutrients from the roots to the rest of the tree, eventually causing branch dieback and tree death (Fig. 1) (7,15).

The specific identity of the beetle is currently unknown. It is morphologically indistinguishable from the tea shot hole borer (TSHB), *Euwallacea fornicatus*, a serious pest of tea (*Camellia sinensis*) in Sri Lanka and India (5) (Fig. 2A–C). However, there are significant differences in nuclear and mitochondrial DNA sequences between the California beetles and those from tea plantations in Sri Lanka and other Asian collection sites (P. F. Rugman-Jones and R. Stouthamer, *unpublished data*). This indicates that the California beetle is most likely a separate, congeneric species (representative mitochondrial sequences for the two species are available in GenBank [1], accession numbers JX912723-JX912725). Species within the genus *Euwallacea* have been subject to much revision and synonymization (28); therefore, establishing the exact identity of this beetle is likely to require extensive taxonomic research. Thus, for the purpose of this study, the beetle is simply referred to as *Euwallacea* sp. and the common name polyphagous shot hole borer (PSHB) is used.

In California, PSHB was first collected in 2003 from several tree species (20) but there were no reports of fungal damage before 2012. Preliminary survey data in 2012 showed that backyard avocado trees and many other woody plants in the urban landscape, especially castor bean (*Ricinus communis*), were heavily infested with this beetle–fungus disease complex in Los Angeles and Orange counties (A. Eskalen, *unpublished data*). Some of the most heavily infested areas in southern California are home to two of its biggest botanical gardens, the Los Angeles (LA) Arboretum and The Huntington Library, Art Collections and Botanical Gardens, which are relatively close to the original 2003 find of the beetle. These botanical gardens harbor a wide range of plant species that represent unique and common ecosystems from all over the world, and contain all the host species planted throughout urban forests in

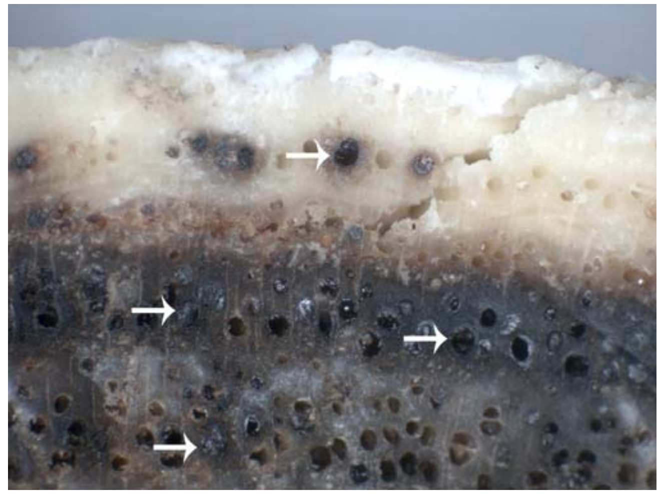

Fig. 1. Wood discoloration and xylem vessel plugging caused by *Fusarium* sp. on *Albizia julibrissin.*

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*The ***e*-X**tra logo stands for "electronic extra" and indicates that Figures 1 to 5, 7, and 8 appear in color in the online edition.

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southern California. In addition, the collections receive more consistent and even care as opposed to lands with multiple management strategies. Therefore, the botanical gardens provided a perfect natural laboratory to investigate the plant host range of the beetle–fungus complex. An understanding of the host range of the beetle–fungus complex is important in determining the present disease distribution and to make predictions about its future distribution.

At present, the beetle–fungus complex appears to be limited to the urban forest. In itself, this will likely present economic problems for households and cities that must bear the cost of removing dead or dying trees. However, the same disease complex was also found in Israel in 2009 in commercial avocado orchards, where it causes significant damage to avocado trees (15). Thus, the presence of FD in urban landscapes throughout southern California is a concern for the California avocado industry because it may eventually spread to commercial avocado groves. Similarly, the wide host range of this beetle may ultimately threaten native trees in the natural forests of southern California.

The objectives of this study were to (i) determine the plant host range of the PSHB and *Fusarium* sp. and (ii) characterize their symptoms on different host plants.

Materials and Methods

Host identification and symptom characterization. Trees were visited and identified to species at the LA Arboretum and The Huntington Library, Art Collections and Botanical Gardens in Los Angeles County; specific locations of sampled trees were noted using global positioning system equipment (Garmin 60CSx, 5- to 10-m accuracy). Each botanical garden covers approximately 48 ha and, in general, the species collections housed in each are different. Although the gardens contain a large number of plant species, the criteria in host plant selection included (i) common street trees found in southern California (4), (ii) agriculturally important trees and vines, (iii) species of tree families, and (iv) trees randomly encountered in the search for species outlined using the criteria i–iii.

Signs and symptoms of FD (presence or absence; see Results), severity of beetle colonization (0 = none, 1 = minor [1 to 10 entry holes], 2 = moderate [10 to 30 entry holes], 3= [>31 entry holes]), and branch dieback (0 = none, 1 = minor [1 to 25% of branches showed dieback], 2 = moderate [25 to 50%], and 3 = severe [>50%]) were also recorded. For each tree species showing symptoms characteristic of FD (one tree per species), tissue samples were collected by extracting symptomatic tissue from the trunk with a sterilized knife to a depth beyond the cambium. Samples were returned to the laboratory (University of California, Riverside) for morphological and molecular identification of the beetle, the fungus, or both. If visible, beetles were collected and put directly into 95% ethanol for later molecular identification (see below). The data were analyzed using JMP 9.0.0 statistical software (SAS 2012). Logistic regression was used to determine the presence and absence of *Fusarium* sp., gumming, and severity of beetle attack as contributing factors to branch dieback >50% at $\alpha = 0.05$.

The suitability of a tree species to act as a reproductive host for the PSHB was evaluated, where possible, by cutting into trees and confirming the presence of eggs, larvae, pupae, or teneral females or males in the galleries. Trees that did not show signs of heavy infestation by PSHB were not cut to examine and confirm as a reproductive host because species of these trees were limited in number within the botanical gardens' collections.

***Fusarium* sp. and PSHB confirmation.** To carry out fungal isolations, gross contamination was removed before bringing plant samples into a clean area by briefly flaming samples and removing the outer surface with a sterilized paring knife. All samples were then split in half and pieces excised from the leading margin of clean necrotic tissue were plated onto potato dextrose agar (Difco) amended with 100 μg of tetracycline (Fisher Scientific). Plates were incubated at 25°C, and fungal growth was subcultured for identification and long-term storage after 7 to 10 days.

Total genomic DNA was extracted from the mycelium of each isolate using the method described by Cenis (2). Portions of the following four gene fragments were chosen for multilocus sequence typing based on published genetic analyses (3,16–18,29): the internal transcribed spacer (ITS) region, translation elongation factor 1-α (EF1-α), the second-largest subunit of RNA polymerase (*RPB2*), and DNA-directed RNA polymerase subunit 1 (*RPB1*). Each 25-μl polymerase chain reaction (PCR) mixture contained 19.4 μl of PCR-grade water, 2.5 μl of standard Taq reaction buffer, 0.5 μl of dNTPs (10 mM), 0.125 μl of NEB *Taq* DNA polymerase (5,000 U/ml; New England BioLabs), 0.5 μl of each primer at 10 mM, and 1.5 μl of template DNA. PCR was carried out in a thermal cycler (Bio-Rad Laboratories, Inc.) using published cycling conditions (14,17,25). Amplification products were separated by electrophoresis in 1.5% agarose gels in 1.0× Tris-boric acid-EDTA buffer and visualized under UV light after staining with SYBR Green (Invitrogen). PCR products were purified using an Exo SAP-IT kit (Affymetrix). ITS, EF1-α, RPB1, and RPB2 regions were sequenced in both directions at the Institute for Integrative Genome Biology at the University of California, Riverside. Sequences of isolates from the present study were confirmed as *Fusarium* sp. after a BLAST search comparison in GenBank, which showed they were 100% homologous to known sequences of isolates of *Fusarium* sp. from Eskalen et al. and Mendel et al. (7,15). The ITS, EF1-α, RPB1, and RPB2 sequences used in this study were deposited into GenBank.

PSHB collection and confirmation. When present, live beetles were collected directly into 95% ETOH and returned to the laboratory for identification. In the field, beetles were collected from the surface of the trees using forceps. If they were in a gallery, the beetles were first coaxed to the entrance by spraying 95% ethanol in the gallery and then removed with the forceps (11).

In the laboratory, DNA was extracted from individual beetles by grinding two to three tibia in 2 μl of proteinase-K (>600 mAU ml^{-1}; Qiagen) and 35 μl of Chelex 100 resin (Bio-Rad Laboratories) followed by incubation for 1 h at 55°C, then for 10 min at 99°C. A section of the cytochrome c oxidase subunit I gene commonly used in barcoding studies (10) was amplified using the primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and

Fig. 2. Polyphagous shot hole borer (PSHB) (*Euwallacea* sp.): **A,** female; **B,** male; **C,** size comparison of both male (left) and female (right) on a penny.

Table 1. Hosts of the polyphagous shot hole borer and their susceptibility to Fusarium dieback (FD)[a]

Family, species[b]	Common name	*N*[c]	Crop[d]	Origin	FD[e]	S	P	G
Aceraceae s.s. (Sapindaceae s.l.)								
**Acer buergerianum*	Trident maple	4	…	East China, Taiwan	Y	…	…	…
A. davidii	Pere David's maple	…	…	China	…	Y	…	…
A. caudatifolium (syn. *A. kawakamii*)	Kawakami maple	…	…	Taiwan	…	Y	…	…
**A. macrophyllum*	Big leaf maple	…	…	Western North America	Y	Y	…	…
**A. negundo*	Box elder	…	…	North America	Y	Y	…	…
**A. palmatum* 'Bonfire'	Bonfire Japanese maple	…	…	East Asia	Y	…	…	…
**A. paxii*	Evergreen maple	3	…	Southwest China	Y	Y	…	…
A. × freemanii	Freeman maple	…	…	Eastern North America	…	Y	…	…
Alangiaceae s.s. (Cornaceae s.l.)								
Alangium chinensis	…	…	…	Tropical Africa to China	Y	Y	…	…
Anacardiaceae								
Pistacia chinensis	Chinese pistache	74	…	China	…	…	…	Y
Schinus terebinthifolius	Brazilian pepper tree	333	…	Brazil	Y	Y	…	…
Apocynaceae								
Thevetia thevetioides	Yellow oleander	…	…	Mexico	…	Y	…	…
Aquifoliaceae								
Ilex aquifolium	English holly	…	…	Western and Southern Europe, Northwest Africa, Southwest Asia	…	…	…	…
**I. cornuta*	Chinese holly	…	…	China and Korea	Y	Y	…	Y
I. latifolia	Tarajo holly	…	…	Japan and China	Y	Y	Y	…
Araliaceae								
Cussonia spicata	Spiked cabbage tree	…	…	South Africa	Y	Y	…	Y
Fatsia japonica	Japanese aralia	…	…	Japan	Y	Y	…	Y
Arecaceae								
Brahea armata	Mexican blue palm	1	…	Western Mexico	…	…	…	…
Butia capitata	Pindo palm	…	…	Brazil, Uruguay, Argentina	…	Y	…	Y
Livistona chinensis	Chinese fan palm	…	…	Australia, Southeast Asia	Y	…	…	Y
Washingtonia filifera	Desert fan palm	54	…	Southwest United States, Northwest Mexico	Y	…	…	Y
Asparagaceae								
Draceana draco	Canary islands dragon tree	…	…	Canary Islands, Cape Verde, Madeira, Morocco	…	Y	…	…
Asteraceae								
Verbesina gigantea	Crownbeard	…	…	Tropical America	…	…	…	Y
Betulaceae		…	…					
Alnus incana	Grey alder	…	…	Europe, Caucasus	…	Y	…	Y
A. rhombifolia	white alder	72	…	Western United States	…	Y	…	…
Betula pendula	Silver birch	12	…	Europe, Asia Minor	Y	…	…	…
Corylus colurna	Turkish hazelnut	…	Y	Southeast Europe, Asia Minor, Western Asia	Y	…	…	…
Bignoniaceae								
Catalpa speciosa	Northern catalpa	8	…	Central and Eastern United States	Y	Y	…	…
Handroanthus impetiginosus (syn. *Tabebuia impetiginosa*)	Brazilwood	…	…	Mexico to South America	…	…	Y	…
Bombacaceae s.s. (Malvaceae s.l.)								
Bombax ceiba	Cotton tree	…	…	India, China, Southeast Asia	…	…	…	Y
Ceiba speciosa	Silk floss tree	14	…	Brazil, Argentina	Y	Y	…	Y
Pseudobombax ellipticum	Shaving brush tree	…	…	Mexico and Central America	…	…	…	Y
Buddlejaceae								
Nuxia floribunda	Forest elder	…	…	Africa	…	…	…	…
Burseraceae								
Bursera hindsiana	…	…	…	Western Mexico	…	Y	…	…
Casuarinaceae								
Casuarina cunninghamiana	She-oak	…	…	Northeast Australia	…	Y	…	…
Cunoniaceae								
Cunonia capensis	Buttetspoon tree	…	…	South Africa	…	Y	…	…
Cornaceae								
Cornus controversa	Giant dogwood	…	…	Himalayas to Japan	Y	…	…	…
Cupressaceae								
Juniperus chinensis	Chinese juniper	26	…	Northeast Asia	Y	…	…	Y
J. virginiana	Eastern red-cedar	…	…	Eastern North America	…	…	…	Y
Ebenaceae								
Diospyros kaki	Japanese persimmon	2	Y	Asia	…	Y	…	…
D. lycidioides	…	…	Y	South Africa	Y	Y	…	…
Elaeocarpaceae								
Crinodendron patagua	Lily of the valley tree	…	…	Chile	…	Y	…	…
Ericaceae								
Arbutus unedo	Strawberry tree	…	…	Southwest Ireland to Asia Minor	…	Y	…	…

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[a] S = staining, P = powder, G = gumming, and Y = "yes".
[b] Asterisks: * indicates reproductive hosts and ** indicates critically endangered tree.
[c] Number of times planted along streets of City of Orange (4).
[d] Agriculturally important crop.
[e] FD susceptible.

Table 1. (*continued from preceding page*)

Family, species[b]	Common name	*N*[c]	Crop[d]	Origin	FD[e]	S	P	G
Euphorbiaceae								
Aleurites fordii	Tung oil tree	…	…	Asia	…	…	…	Y
Bischofia javanica	Bishop wood	…	…	Tropical Asia	Y	Y	…	…
Jatropha cf. *cinerea*	Limberbush	…	…	Arizona, Western Mexico	Y	…	…	…
Manihot esculenta	Cassava	…	…	Brazil	…	…	…	…
**Ricinus communis*	Castor bean	…	…	Mediterranean basin, East Africa, India	Y	Y	…	…
Sapium sebiferum	Chinese tallow tree	…	…	China, Japan	Y	Y	…	…
Fabaceae								
Acacia caven	Roman cassie	…	…	South America	…	Y	…	…
A. stenophylla	Shoestring acacia	…	…	Australia	…	…	…	Y
A. visco	Arca	…	…	Argentina	Y	…	…	…
Albizia kalkora	Kalkora mimosa	…	…	Southern Asia	…	Y	…	…
**A. julibrissin*	Silk tree	…	…	Southwest to east Asia	Y	Y	…	…
Bauhinia × *blakeana*	Orchid tree	1	…	China	Y	…	…	Y
B. galpinii	Red orchid bush	…	…	South Africa	…	Y	Y	…
Calpurnia aurea	…	…	…	Southern India, Africa	Y	Y	…	Y
Cassia brewsteri	Cassia pea	…	…	Northeast Australia	Y	Y	…	Y
**Castanospermum australe*	Black bean	…	…	Northeast Australia, New Caledonia	Y	Y	…	Y
**Cercidium floridum*	Blue palo verde	…	…	Southwest United States, Western Mexico	Y	Y	…	…
**C.* × *sonorae*	Brea	…	…	Northwest Mexico	Y	Y	…	…
Cladrastris sinensis	Chinese yellow wood	…	…	China	…	Y	…	Y
Erythrina folkersii	Coral tree	…	…	Central America	Y	…	Y	…
E. coralloides	Flame coral tree	…	…	Arizona and Mexico	…	Y	…	…
**Erythrina corallodendron*	Coral tree	…	…	West Indies	Y	Y	…	…
E. crista-galli	Ceibo	…	…	South America	Y	Y	…	Y
E. humeana	Dwarf coral tree	…	…	South Africa, Mozambique	Y	Y	Y	…
E. lysistemon	Common coral tree	…	…	South Africa	Y	Y	Y	…
E. × *sykesii*	Coral tree	…	…	Horticultural origin	Y	Y	…	…
Inga feuilleii	Pacay	…	…	South America	Y	Y	…	…
Lysiphylllum carronii	Queensland ebony	…	…	Australia	Y	…	…	Y
**Parkinsonia aculeata*	Palo verde	…	…	Tropical America	Y	Y	…	…
Pithecellobium sp.	…	…	…	…	…	…	…	Y
Schotia brachypetala	Huilboerboon	…	…	Southern Africa	Y	Y	…	Y
Senna racemosa	Limestone senna	…	…	Central and South America	…	Y	…	…
Senna racemosa var. *liebmanni*	…	…	…	Central and South America	Y	Y	…	…
S. spectabilis var. *spectabilis*	…	…	…	…	…	Y	Y	Y
Styphnolobium japonicum (syn. *Sophora japonica*)	Pagoda tree	…	…	East Asia	…	…	…	Y
Tipuana tipu	Tipu tree	1	…	Brazil, Argentina, Bolivia	…	Y	…	…
Wisteria sinensis	Chinese wisteria	…	…	China	Y	…	…	…
W. floribunda 'Macrobotyrus'	Japanese wisteria	…	…	Japan	Y	Y	…	…
Zenia insignis	…	…	…	South China and Vietnam	…	Y	…	…
Fagaceae								
Fagus sylvatica	European beech	…	…	Central Europe to Caucasus	Y	Y	…	…
F. sylvatica 'Riversii'	Riversii European Beech	…	…	Horticultural origin	…	…	Y	…
Quercus acutissima	Sawtooth oak	…	…	China, Korea and Japan	…	…	…	…
**Q. agrifolia*	Coast live oak	13	…	California, Baja California	Y	Y	…	…
Q. alba	White oak	…	…	Eastern North America	…	…	…	…
Q. chrysolepis	Canyon live oak	…	…	Western North America	Y	Y	…	…
Q. engelmannii	Engelmann Oak	…	…	Southwest California	…	Y	…	…
Q. ilex	Holly oak	306	…	Mediterranean	Y	Y	…	…
Q. lobata	Valley oak	…	…	California	Y	Y	…	…
Q. macrocarpa	Bur oak	…	…	Central and Eastern North America	Y	Y	…	…
Q. mexicana	Cozahuatl	…	…	Mexico	Y	Y	…	Y
**Q. robur*	English oak	…	…	Europe, Anatolia, and North Africa	Y	Y	…	…
Q. rubra	Northern red oak	7	…	Eastern North America	…	Y	Y	Y
Q. suber	Cork oak	…	…	Southwest Europe and Northwest Africa	Y	Y	…	…
Q. virginiana	Southern live oak	…	…	Southeastern United States	Y	Y	…	…
Hamamelidaceae s.l. (Altingiaceae s.s.)								
Liquidambar formosana	Chinese sweet gum	12	…	South China, Taiwan	Y	Y	…	…
**L. styraciflua*	American sweetgum	4,916	…	Eastern North America	Y	Y	…	…
Hydrophyllaceae s.s. (Boraginaceae s.l.)								
Wigandia urens	…	…	…	Peru	…	Y	…	…
Juglandaceae								
Carya illinoinensis	Hickory	36	…	South-Central North America	Y	Y	…	Y
Jugans mandshurica (syn. *Juglans formosana*)	Hayata	…	…	China, Taiwan	…	…	Y	…
J. nigra	Eastern black walnut	5	Y	Eastern North America	…	Y	…	…
Pterocarya sp.	Wingnut tree	…	…	Asia	…	Y	…	Y

(*continued on next page*)

Table 1. (*continued from preceding page*)

Family, species[b]	Common name	*N*[c]	Crop[d]	Origin	FD[e]	S	P	G
Lauraceae								
Cinnamomum glanduliferum	False camphor tree	…	…	China	…	…	…	…
C. camphora	Camphor tree	690	…	Japan to Tropical Asia	Y	Y	Y	…
Nothaphoebe cavalieri	…	…	…	South China	…	Y	…	…
**Persea americana*	Avocado	70	Y	Mexico, Guatamala, West Indies	Y	Y	Y	…
Machilus thunbergii (syn. *Persea thunbergii*)	Asian avocado	…	…	East Asia	Y	Y	…	…
Umbellularia californica	California bay laurel	…	…	Western North America	Y	Y	…	…
Magnoliaceae								
Magnolia 'Columbus'	Columbus magnolia	…	…	Horticultural origin	…	Y	…	…
M. delavayi	Chinese evergreen magnolia	…	…	Southwest China	…	Y	…	…
M. doltsopa	Sweet michelia	…	…	Southwest China, Himalayas	…	Y	…	…
M. grandiflora	Southern magnolia	2,174	…	Southeast United States	Y	Y	…	…
M. guatemalensis	Mamey	…	…	Central America	…	Y	…	Y
M. × *soulangeana*	Saucer magnolia	8	…	Horticultural origin	…	Y	…	Y
M. × *veitchii*	Veitch's magnolia	…	…	Horticultural origin	Y	Y	…	…
Malvaceae								
Dombeya cacuminum	Strawberry tree	…	…	Madagascar	Y	…	Y	…
Brachychiton acerifolius	Illawarra flame tree	46	…	Northeast Australia	Y	Y	…	Y
B. australis	Kurrajong	…	…	Northern Australia	Y	Y	…	…
B. discolor	lacebark tree	…	…	Northeast Australia	Y	…	…	…
B. rupestris	Queensland bottle tree	…	…	Northeast Australia	Y	…	…	Y
Chiranthodendron pentadactylon	Devil's hand tree	…	…	Mexico and Central America	…	Y	…	Y
Firmiana simplex	Chinese parasol tree	…	…	East Asia	Y	…	…	…
Meliaceae								
Melia azedarach	Cape lilac	11	…	India	…	Y	…	…
Swietenia chickrassa (syn. *Chukrasia tabularis*)	…	…	…	India and China	…	…	…	Y
Melianthaceae								
Melianthus major	Giant honey flower	…	…	South Africa	Y	…	…	…
Menispermaceae								
Cocculus laurifolius	Laurel-leaved snail tree	…	…	Himalayas to Japan	Y	Y	…	…
C. orbiculatus (syn. *C. trilobus*)	Korean moonseed	…	…	East and Southeast Asia	Y	Y	…	Y
Monimiaceae								
Peumus boldus	Boldo	…	…	Chile	…	Y	…	…
Moraceae								
Broussonetia papyrifera	Paper mulberry	…	…	Eastern Asia	…	Y	…	…
Ficus benjamina	Weeping fig	1,182	…	Southeast Asia and Australia	…	Y	…	…
F. macrophylla	Moreton Bay fig	2	…	Northeast Australia	Y	Y	…	Y
F. maxima	Fig	…	…	Tropical America	…	…	…	…
F. platypoda	Desert rock fig	…	…	Australia and Indonesia	Y	Y	…	Y
Morus alba	White mulberry	43	Y	China	Y	Y	…	…
Myrtaceae								
Callistemon salignus	Willow bottlebrush	…	…	Australia	…	…	…	…
C. viminalis	Weeping bottlebrush	91	…	Australia	…	Y	…	…
Eucalyptus camaldulensis	River red gum	2	…	Australia	…	…	…	…
E. cinerea	Argyle apple	…	…	Southeast Australia	…	Y	…	…
E. ficifolia	Red flowering gum	33	…	Western Australia	…	Y	…	Y
E. froggattii	…	…	…	Southeast Australia	…	Y	…	Y
E. kitsoniana	Gippsland mallee	…	…	Southeast Australia	…	Y	…	…
E. perriniana	Spinning gum	…	…	Southeast Australia	…	Y	…	Y
E. polyanthemos	Red box	6	…	Southeast Australia	Y	Y	…	…
E. torquata	Coral gum	…	…	Western Australia	Y	Y	…	…
Nyssaceae s.s. (Cornaceae s.l.)								
Camptotheca acuminata	Happy tree	…	…	China and Tibet	Y	Y	…	…
Oleaceae								
Chionanthus retusus	Chinese fringetree	…	…	East Asia	Y	Y	…	…
Fraxinus sp.	Ash	…	…		…	Y	…	…
F. uhdei	Shamel ash	48	…	Central America	Y	Y	…	Y
F. velutina	Velvet ash	57	…	Southwestern North America	…	Y	…	
Olea europaea	Olive	29	Y	Mediterranean, Asia and Africa	Y	Y	…	Y
Osmanthus fragrans	Sweet osmanthus	…	…	Himalayas to Japan	…	Y	Y	…
Onagraceae								
Hauya microcerata	…	…	…	Central America	…	…	…	…
Pinaceae								
Cedrus atlantica	Atlas cedar	1	…	Algeria and Morocco	…	Y	…	Y
Keteleeria delavayi (syn. *K. evelyniana*)	…	…	…	South China, Laos and Vietnam	…	Y	…	…
Pinus densiflora	Japanese red pine	…	…	Japan, Korea, China	…	Y	…	Y
P. douglasiana	…	…	…	Western Mexico	…	…	…	Y
Pittosporaceae								
Hymenosporum flavum	Native frangipani	20	…	Australia and New Guinea	Y	…	…	Y
Pittosporum undulatum	Victorian box	…	…	Eastern Australia	Y	Y	…	Y

(*continued on next page*)

HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (8). PCR and sequencing was performed as described by Rugman-Jones et al. (23). Sequences were aligned manually using BioEdit, version 7.0.9.0 (9). Primers were removed and each sequence was translated (http://www.ebi.ac.uk/Tools/emboss/transeq/index.html) to confirm the absence of nuclear pseudogenes (26). All specimens were confirmed as PSHB (and not TSHB) by comparison with GenBank accession numbers JX912723–JX912725.

Table 1. (*continued from preceding page*)

Family, species[b]	Common name	*N*[c]	Crop[d]	Origin	FD[e]	S	P	G
Platanaceae								
Platanus mexicana	Mexican sycamore	…	…	Northeast Mexico	Y	Y	…	…
P. occidentalis	American sycamore	19	…	Eastern North America	Y	Y	…	…
**P. racemosa*	California sycamore	68	…	California, Baja California	Y	Y	…	…
P. wrightii	Arizona sycamore	…	…	Southwest United States, Northwest Mexico	Y	…	…	…
Poaceae								
Bambusa sp.	Bamboo	…	…	…	…	Y	…	…
Podocarpaceae								
Afrocarpus gracilior (syn. *Podocarpus gracilior, Decussocarpus gracilior*)	Fern pine	…	…	East Africa	…	…	…	…
Proteaceae								
Banksia saxicola	Grampians banksia	…	…	Southeast Australia	Y	Y	…	…
Macadamia integrifolia	Macadamia nut	…	Y	Eastern Australia	Y	Y	…	…
Rhamnaceae								
Rhamnus californica	Coffee berry	…	…	Western North America	…	…	…	Y
Ziziphus jujuba	Chinese date	…	…	Temperate Asia	Y	Y	…	…
Rosaceae								
Eriobotrya japonica	Loquat	4	Y	China, Japan	Y	…	…	…
Malus floribunda	Japanese crabapple	2	Y	East Asia	…	Y	…	Y
Prunus cerasoides	Wild Himalayan cherry	…	…	South Asia	…	…	…	Y
P. caroliniana	Wild mock orange	…	…	Southeastern United States	…	Y	…	Y
P. mume	Chinese plum	…	…	Southern Japan	Y	…	…	Y
P. persica	Peach	4	Y	China	Y	Y	…	Y
P. serrulata	Japanese cherry	…	…	Japan, Korea and China	Y	…	…	Y
Chaenomeles sinensis (syn. *Cydonia sinensis*)	Chinese quince	…	…	China	…	…	…	…
Pyrus kawakamii	Evergreen pear	156	…	China, Taiwan	Y	Y	…	…
Rutaceae								
Citrus sinensis	Orange	6	Y	Southeast Asia	Y	Y	…	Y
Salicaceae								
Salix babylonica	Babylon willow	1	…	Asia	…	Y	Y	Y
Salix sp.	…	…	…	…	Y	Y	Y	Y
Xylosma congestum	Dense logwood	…	…	China	Y	Y	…	…
Sapindaceae								
Alectryon excelsus	Tītoki	…	…	New Zealand	Y	Y	…	Y
Harpullia arborea	Tulip-wood tree	…	…	Tropical Southeast Asia	Y	Y	Y	
H. pendula	Tulipwood	…	…	Northeast Australia, Tropical Southeast Asia	…	Y	Y	Y
Koelreuteria bipinnata	Chinese flame tree	30	…	Southwest China	…	Y	…	Y
K. elegans	Chinese rain tree	…	…	Taiwan and Southern China	…	Y	…	Y
K. elegans subsp. *formosana* (syn. *K. henryi*)	…	…	…	Taiwan	…	Y	…	Y
Ungnadia speciosa	Mexican buckeye	…	…	South United States, North Mexico	Y	Y	…	…
Simaroubaceae								
Ailanthus altissima	Tree of heaven	2	…	China and Taiwan	…	…	…	Y
Taxodiaceae s.s. (Cupressaceae s.l.)								
*Metasequoia glyptostroboides***	Dawn redwood	…	…	Western China	Y	…	…	Y
Theaceae								
Camellia japonica	Japanese camellia	…	…	Japan, Korea and China	…	…	Y	…
Camellia 'Apple Blossom'	Apple Blossom camellia	…	…	Horticultural origin	…	Y	…	…
Camellia 'Pink Sparkle'	Pink Sparkle camellia	…	…	Horticultural origin	…	Y	…	…
C. reticulata	Camellia	…	…	Southwest China	Y	Y	…	…
**C. semiserrata*	Camellia	…	…	…	Y	…	…	…
Cleyera japonica	Sakaki	…	…	East Asia	Y	Y	…	…
Tiliaceae s.s. (Malvaceae s.l.)								
Heliocarpus donnellsmithii	…	…	…	Mexico and Central America	Y	Y	…	…
Luehea divaricata	Açoita-cavalo	…	…	Brazil	Y	Y	Y	Y
Tilia americana	Basswood	1	…	Central and Eastern North America	Y	Y	Y	…
Ulmaceace								
Ulmus alata	Winged elm	…	…	Central and Eastern United States	…	Y	…	…
U. americana	American elm	1	…	Eastern North America	Y	…	…	…
U. parvifolia	Chinese elm	183	…	East Asia	Y	Y	…	…
Zelkova serrata	Ju shu	1	…	East Asia	Y	…	…	…
Urticaceae								
Pipturus argenteus	…	…	…	Australia, Tropical Asia	Y	Y	Y	…
Verbenaceae								
Aloysia virgate	Sweet almond bush	…	…	South America	…	…	…	…
Vitaceae								
Vitis vinifera	Grapevine	…	Y	Mediterranean, Europe	Y	…	…	Y

Table 2. Apparent nonhosts of polyphagous shot hole borer

Family, species	Common name	Origin	*N* planted[a]
Aceraceae s.s. (Sapindaceae s.l.)			
Acer rubrum	Red maple	Eastern North America	1
Adoxaceae			
Sambucus mexicana	Mexican elderberry	Western North America	…
Sambucus nigra	Black elderberry	Europe, North Africa, Southwest Asia	…
Agavaceae s.s. (Asparagaceae s.l.)			
Beaucarnea recurvata (syn. *Nolina recurvata*)	…	Mexico	…
Aloeaceae s.s. (Xanthorrhoeaceae s.l.)			
Aloe bainesii	…	Southern Africa	…
Aloe dichotoma	…	Southern Africa	…
Anacardiaceae			
Rhus lancea	African sumac	South Africa	18
Apocynaceae			
Acokanthera oppositifolia	…	Tropical and Southern Africa	…
Nerium oleander	Oleander	Mediterranean, Middle and Central Asia	68
Arecaceae			
Archontophoenix cunninghamiana	King palm	Eastern Australia	15
Syagrus romanzoffianum (syn. *Arecastrum romanzoffianum*)	Queen palm	South America	426
Chamaerops humilis	Mediterranean fan palm	Mediterranean	2
Phoenix canariensis	Canary Island date palm	Canary Islands	34
P. dactylifera	Date palm	Unknown origin	46
P. reclinata	Senegal date palm	Tropical Africa	…
Trachycarpus fortunei	Chinese windmill palm	Asia	80
Asteraceae			
Montanoa guatemalensis	…	Central America	…
Betulaceae			
Betula nigra ‘Cully’	Heritage river birch	Horticultural origin	…
Carpinus caroliniana	America hornbeam	Eastern North America, Mexico, Central America	…
Ostrya virginiana	American hophornbeam	Eastern North America	…
Bignoniaceae			
Jacaranda mimosifolia	Jacaranda	South America	753
Handroanthus chrysotrichus (syn. *Tabebuia chrysotricha*)	Golden trumpet tree	Brazil	1
Boraginaceae			
Ehretia latifolia (syn. *E. austin-smithii*)	…	Mexico, Central America	…
Burseraceae			
Bursera odorata	…	Mexico	…
Cactaceae			
Pereskia grandiflora	…	Brazil	…
Caprifoliaceae			
Weigela coraeensis	Japanese weigela	Eastern Asia	…
Celastraceae			
Euonymus bungeanus	…	China, Korea	…
Maytenus boaria	Mayten	Chile	5
Cercidiphyllaceae			
Cercidiphyllum japonicum	Katsura tree	China, Japan	…
Clethraceae			
Clethra macrophylla	Mexican clethra	Mexico	…
Corynocarpaceae			
Corynocarpus laevigatus	Karaka	New Zealand	…
Cupressaceae			
Calocedrus decurrens	California incense cedar	Western North America	22
Juniperus bermudiana	Bermuda juniper	Bermuda	…
Ericaceae			
Erica × *darleyensis*	…	Horticultural origin	…
Fabaceae			
Ceratonia siliqua	Carob	Mediterranean, North Africa, Middle East and Western Asia	15
Millettia reticulata	Evergreen wisteria	South China, Taiwan	…
Pithecellobium dulce	Huamuchil	Mexico, Central America	…
Sophora secundiflora	Mountain laurel	Southwest United States, Mexico	…
Fagaceae			
Lithocarpus glaber	…	Japan, East China	…
Quercus emoryi	Emory oak	Southwest United States, Mexico	…
Q. variabilis	Chinese cork oak	China, Japan, Korea	…
Fouquieriaceae			
Fouquieria macdougalii	Mexican ocotillo	Northwest Mexico	…
Garryaceae			
Garrya wrightii	…	Southwest United States, Northwest Mexico	…
Ginkgoaceae			
Ginkgo biloba	Maidenhair tree	China	231

(*continued on next page*)

[a] Number of times planted along streets of City of Orange (4).

Table 2. (*continued from preceding page*)

Family, species	Common name	Origin	*N* planted[a]
Hernandiaceae			
Hernandia bivalvus	Grease nut	Australia	…
Hippocastanaceae s.s. (Sapindaceae s.l.)			
Aesculus hippocastanum	Horse chestnut	Southeast Europe	2
Aesculus × *carnea*	Red horse chestnut	Horticultural origin	…
Iteaceae			
Itea yunnanensis	…	Southwest China	…
Lamiaceae			
Vitex agnus-castus	Vitex	Southern Europe	…
Lauraceae			
Cryptocarya rubra	…	Chile	…
Lythraceae			
Lagerstroemia indica	Crape myrtle	Asia	1,979
L. subcostata		Asia	…
Punica granatum	Pomegranate	Eastern Mediterranean to Himalayas	1
Magnoliaceae			
Liriodendron tulipifera	Tulip tree	Eastern North America	232
Magnolia figo	Banana shrub	China	…
Malvaceae			
Brachychiton populneus	Bottle tree tree	Northeast Australia	129
Lagunaria patersonii	Cow itch tree	Northeast Australia, South Pacific	1
Moraceae			
Ficus carica	Edible fig	Mediterranean to Near East	2
Myrtaceae			
Acmena smithii	Lilly pilly	Eastern Australia	1
Agonis flexuosis	Willow myrtle	Western Australia	…
Angophora costata	Smooth-bark apple	Eastern Australia	…
Callistemon citrinus	Lemon bottlebrush	Southeast Australia	433
Eucalyptus cladocalyx	Sugar gum	South Australia	8
E. deglupta	Rainbow gum	New Guinea to Philippines	…
E. erythrocorys	Red cap gum	Western Australia	5
E. globulus	Blue gum	Southeast Australia, Tasmania	43
E. maculata	Spotted gum	Eastern Australia	…
E. microtheca	Coolibah	Australia	…
E. robusta	Swamp mahogany	Northeast Australia	…
E. saligna	Sydney blue gum	Eastern Australia	…
E. sideroxylon	Red ironbark	Eastern Australia	9
E. torelliana	Cadagah	Northeast Australia	…
Acca sellowiana (syn. *Feijoa sellowiana*)	Pineapple guava	Brazil, Argentina	…
Lophostemon confertus	Brush box	Eastern Australia	92
Melaleuca lanceolata	Moonah	South Australia	…
M. linariifolia	Snow-in-summer	Northeast Australia	…
M. quinquenervia	Cajeput	Eastern Australia	53
Metrosideros excelsa	New Zealand Christmas tree	New Zealand	…
Syzygium buxifolium	…	Chile	…
S. paniculatum	Brush cherry	Northeast Australia	…
Nyctaginaceae			
Bougainvillea	Bougainvillea	…	…
Nyssaceae s.s. (Cornaceae s.l.)			
Nyssa sylvatica	Sour gum	Eastern North America	…
Oleaceae			
Ligustrum lucidum	Chinese privet	East Asia	100
Papaveraceae			
Bocconia arborea	Tree poppy	Central America	…
Pinaceae			
Pinus canariensis	Canary Islands pine	Canary Islands	724
P. halepensis	Aleppo pine	Mediterranean	35
P. pinea	Italian stone pine	Mediterranean	11
P. thunbergiana	Japanese black pine	Japan, Korea	42
P. torreyana	Torrey pine	California	1
Pittosporaceae			
Pittosporum tobira	Japanese pittosporum	China, Japan	3
Poaceae			
Phyllostachys aurea	Golden bamboo	Southeast China	…
Podocarpaceae			
Podocarpus elatus	Rockingham podocarp	Eastern Australia	…
P. macrophyllus	Bigleaf podocarp	South China, Japan	2
Nageia nagi (syn. *Podocarpus nagi*)	Nagi	China, Taiwan, Japan	7
P. totara	Totara	New Zealand	…
Polygalaceae			
Polygala apopetala	Dwarf milkwort	Baja California	…
Proteaceae			
Grevillea robusta	Silky oak	Eastern Australia	9
Stenocarpus sinuatus	Firewheel tree	Eastern Australia	…

(*continued on next page*)

Table 2. (*continued from preceding page*)

Family, species	Common name	Origin	*N* planted[a]
Rhamnaceae			
Hovenia dulcis	Raisin tree	East Asia	…
Ziziphus spina-christi	…	East Africa, Middle and Near East	…
Rosaceae			
Crataegus laevigata	Midland hawthorn	Europe, North Africa, India	…
Eriobotrya deflexa	Bronze loquat	China, Taiwan	…
Malus syvestris	Apple	Central Europe	1
Photinia × *fraseri*	Fraser's photinia	Horticultural origin	14
Pyrus calleryana	Ornamental pear	East Asia	2173
Rutaceae			
Calodendrum capense	Cape chestnut	South Africa	12
Casimiroa edulis	Cochitzapotl	Mexico and Central America	…
Citrus limon	Lemon	Unknown origin	5
C. sinensis 'Moro'	Moro blood orange	Horticultural origin	…
*Tetradium danieli (*syn. *Evodia danieli)*		China, Korea	…
Geijera parviflora	Australian willow	Australia	147
Melicope elleryana	Pink flowered soughwood	Australia	…
× *Citrofortunella*	…	Horticultural origin	…
Salicaceae			
Dovyalis hebecarpa	Ketembilla	Sri Lanka, Southern India	…
Olmediella betschleriana	Guatemalan holly	Mexico, Central America	…
Oncoba spinosa	Snuff-box tree	Africa, Arabia	…
Sciadopityaceae			
Sciadopitys verticillata	Japanese umbrella-pine	Japan	…
Scrophulariaceae			
Eremophila bignoniflora	Emu bush	Australia	…
Myoporum laetum	Myoporum	New Zealand	1
Solonaceae			
Brugmansia 'Charles Grimaldi'	Charles Grimaldi angel's trumpet	Horticultural origin	…
Lycianthes rantonnei	Blue potato bush	Argentina, Paraguay	1
Taxodiaceae s.s. (Cupressaceae s.l.)			
Sequoia sempervirens	Coast redwood	Western North America	…
Taxodium mucronatum	Mexican swamp cypress	Mexico	…
Theaceae			
Camellia hiemalis 'Kanjiro'	Kanjiro camellia	Horticultural origin	…
C. sinensis	Tea plant	Western China	…
Gordonia axillaris	…	Southeast Asia	…
Ulmaceae			
Ulmus parvifolia	Chinese elm	East Asia	175
Verbenaceae			
Lippia umbellata (syn. *L. torresii*)	…	Mexico, Central America	…

Table 3. Polyphagous shot hole borer (PSHB)–*Fusarium* sp. complex collected from tree species found in two botanical gardens located in a heavily infested part of Los Angeles County

Symptoms	Number of tree species	Fraction of all species (%)	Fraction of attacked species (%)
Total number	335	…	…
Attacked by PSHB	207	62	…
Host for *Fusarium* sp.	112	33	54
Weeping spots on bark	147	44	71
Powder depositions	22	7	11
Gumming on bark	69	20	33
Reproductive hosts for the beetle	19	6	9

Results

Host identification. Of 335 tree species (in 83 families) examined (Tables 1 and 2), 207 (62%) had symptoms consistent with attack by PSHB (small entry holes in tree bark, wet spots, gumming, white powdery exudate, and frass; Tables 1 and 3; Figs. 3A–K and 4A–C). The 207 species belonged to 58 families, with origins representing all the continents except Antarctica. Among the attacked species were 11 species native to California, and 13 that are agriculturally important crops in California (see below). There were 128 tree species in 58 families without obvious signs of attack by PSHB (Table 2). Thirty-three plant families examined contained species that were both attacked and not attacked by PSHB.

Fungal DNA was extracted from *Fusarium* sp. isolates recovered from symptomatic tissues of 113 of the 207 tree species (54%) attacked by PSHB. In all cases, sequences of the ITS, EF1-α, RPB1, and RPB2 genes of the fungus were identical to the respective known sequences of isolates of *Fusarium* sp. from Mendel et al. (15) (GenBank accessions JQ038007 to -038034) and Eskalen et al. (7) (GenBank accessions JQ723753 to -723756 and JQ723760 to -723763). These findings indicate the susceptibility of the host tree to the growth of *Fusarium* sp., at least at the site of the entry hole. Fungal sequences generated in the current study have been deposited in GenBank under the following accession numbers: ITS, JX891673 to -JX891784; EF1-α, JX891785 to -JX891896; RPB1, JX891897 to -JX892008; and RPB2, JX892009 to -JX892120. *Fusarium* sp. was isolated from 113 tree species in 40 families. Families with the highest number of infected species were the Aceraceae, Fabaceae, and Fagaceae.

Of the tree species susceptible to varying degrees of attack by PSHB, 19 were suitable for beetle reproduction. Suitability for reproduction was confirmed by either the presence of eggs, larvae, pupae, or teneral females or the presence of males in the galleries (Table 3; Fig. 5). This number might be an underestimate, because

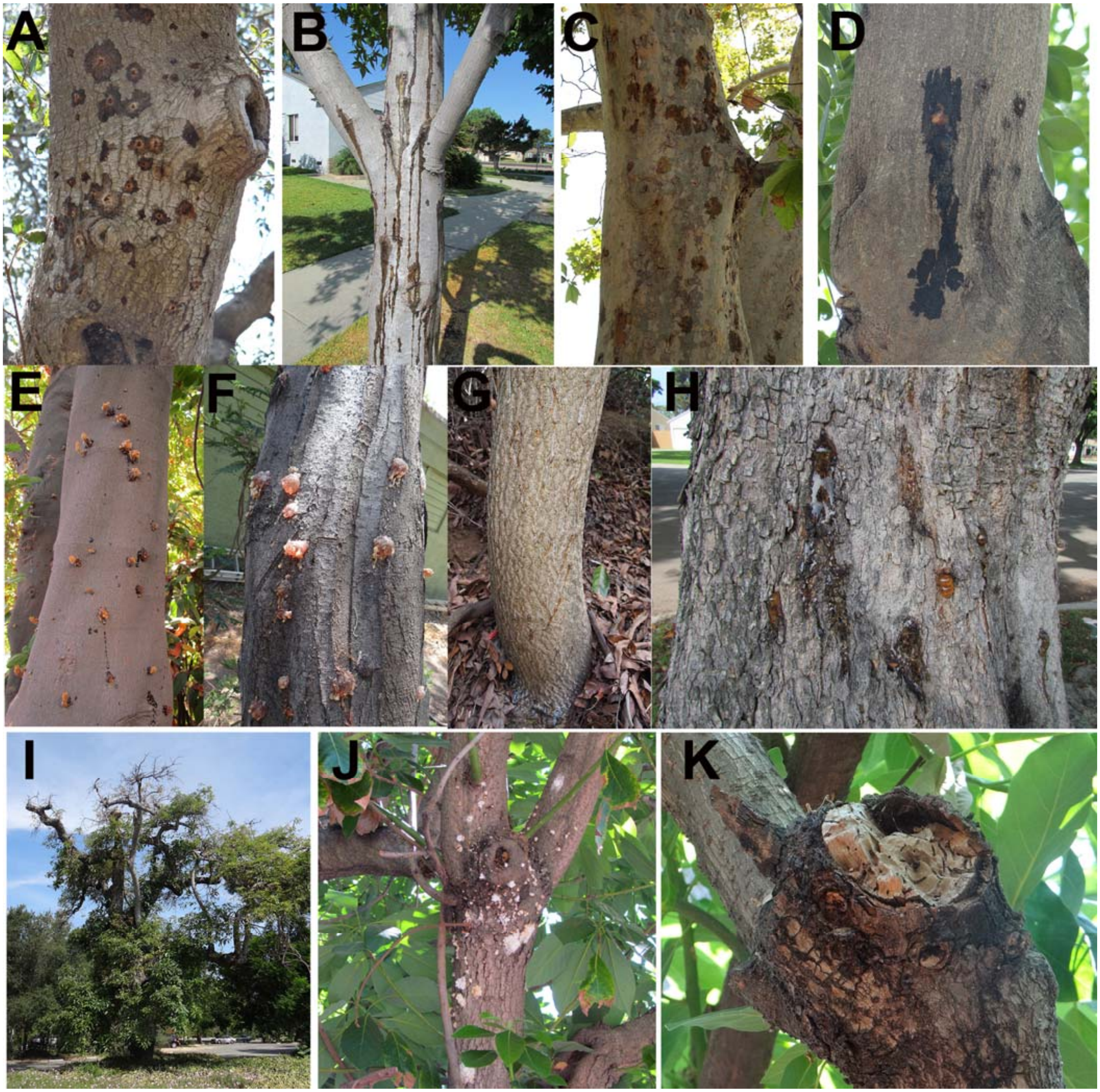

Fig. 3. A–D, External symptoms of staining; **E–H,** gumming; and **I–K,** branch dieback for polyphagous shot hole borer (PSHB)–Fusarium dieback disease on **A,** *Quercus agrifolia*; **B,** *Liquidambar styraciflua*; **C,** *Platanus racemosa*; **D,** *Senna specatibilis* var. *excelsa* (syn. *Cassia excelsa*); **E,** *Alectryon excelsus*; **F,** *Acacia melanoxylon*; **G,** *Alianthus altissima*; **H,** *Koelreuteria elegans*; and **I–K,** *Persea americana*.

confirmation requires cutting into trees, which was not logistically possible at the time due to the limited number of trees that represent each species within the botanical gardens' collection.

All tree species considered to be reproductive hosts had severe branch dieback (Fig. 3I–K), and the death of mature reproductive hosts that were infested with FD was observed for *Quercus robur, Acer negundo*, and *R. communis. A. negundo* and *Q. robur* also exhibited leaf wilting and discoloration on the branches prior to dieback and tree death.

Symptom characterization. Symptoms surrounding the beetle entry hole included staining (71% of hosts), gum deposition (33% of hosts), and white powder-like exudates (11% of hosts) (Figs. 3, 4, and 6, Tables 1 and 3). Staining was characterized by a moist discoloration of the outer bark that oozed out of the entry holes in various amounts, depending on the host (Fig. 3A–D). Discoloration caused by fungal colonization was observed underneath the inner bark layer when the beetle entry hole penetrated through the cambium and into the xylem (Figs. 7 and 8). On various hosts, PSHB attack resulted in the deposition of an amber colored gum that exuded through the entry holes and varied in consistency between spongy and pliable to solid and hard, depending on the host (Table 1, Fig. 3E–H). After peeling back the outer bark beneath the gum deposition, tissue discoloration was observed and subsequent recovery of *Fusarium* sp. occurred on 46% of the tree species showing a gumming response (Table 2). Otherwise, the beetle was found embedded within the gum deposit, and there was no discoloration of the plant tissue. A white powdery exudate was observed on some species (Table 1). On avocado, it characteristically emerged from entry holes and formed a white "sugar volcano" approximately 1 to 15 cm in diameter and 0.5 to 5 cm in height (Fig. 4A). Frass was occasionally observed, particularly on trees in the Aceraceae (Fig. 4B).

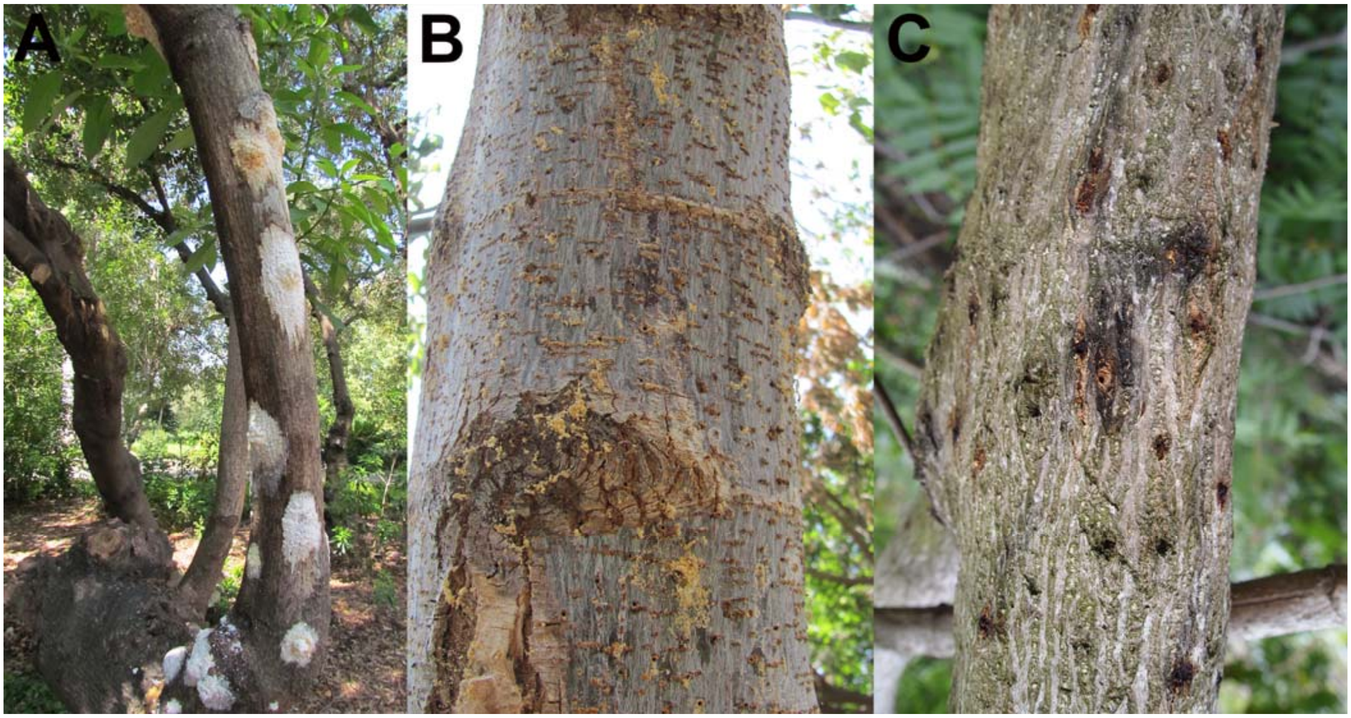

Fig. 4. A, External symptoms of polyphagous shot hole borer and Fusarium dieback disease that are characteristic for *Persea americana* (white powder exudate/sugar volcano); **B,** *Acer negundo*, (abundant entry holes associated with frass); and **C,** *Ricinus communis* (staining).

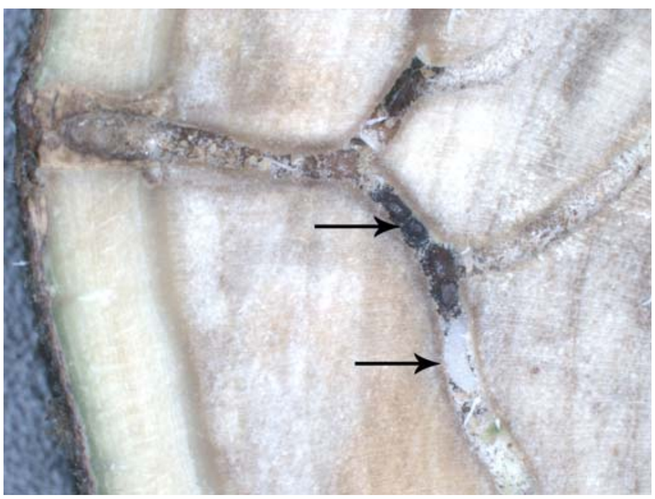

Fig. 5. Offspring (top arrow) and larvae (bottom arrow) in beetle galleries confirm *Acer negundo* as a reproductive host of polyphagous shot hole borer.

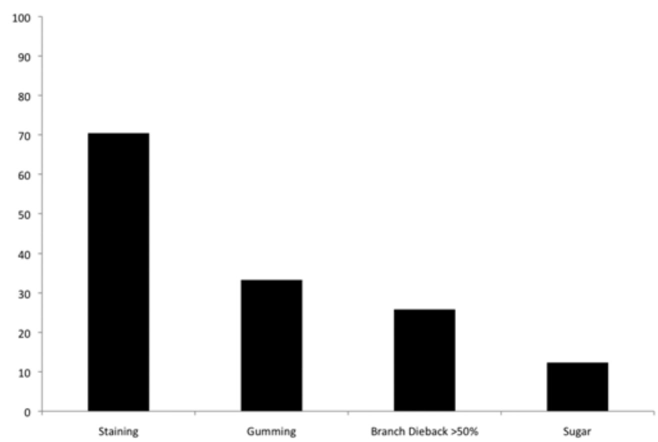

Fig. 6. Proportion of trees prone to polyphagous shot hole borer attack with respective symptoms.

In total, 26% of the identified hosts of FD had branch dieback on over 50% of the tree. Symptoms of branch dieback were typically observed on primary branches where areas of weakness caused by beetle and fungal colonization were observed at the branch nodes (Figs. 3I–K and 8A–F). For trees attacked by PSHB, the likelihood of a higher than 50% branch dieback was positively correlated with presence of *Fusarium* sp. and increasing severity of PSHB attack, and negatively correlated with symptoms of gumming (Table 4).

Estimated frequency of trees affected by PSHB–*Fusarium* sp. **complex in the urban forest.** The botanical garden survey was used to determine the susceptibility of 103 common street tree species (Table 5). In all, 53 species were attacked by PSHB and, of these, *Fusarium* sp. was isolated from 36. The beetle was able to reproduce in six of these species. Relating these numbers to the relative abundance of the different trees in the urban environment of southern California (4) reveals that, of the individual street trees, over half (56%) belong to species that are attacked by the PSHB, 48% belong to species that are hosts to the *Fusarium* sp., and 26% belong to species that are reproductive hosts of PSHB (Table 5).

FD complex in agriculturally important plants. In this survey, 13 agriculturally important crops displayed signs and symptoms consistent with PSHB attack (Table 1). Of these, growth of the *Fusarium* sp. was confirmed on avocado, eastern mulberry, olive, macadamia, Turkish hazelnut, loquat, peach, grapevine, and citrus (although the latter does not appear to be commonly attacked). Avocado was the only species confirmed as a reproductive host for PSHB. Symptoms consistent with attack by PSHB were detected on cassava, Japanese persimmon, Japanese crabapple, and eastern black walnut but the *Fusarium* sp. was not detected growing on these species. The following agricultural crops showed no evidence of PSHB attack: date palm, pomegranate, edible fig, apple, and most citrus tree varieties (see above; Table 2).

Discussion

Host range, symptoms, and plant responses. In this study, we present the host range of an emerging generalist pest–pathogen complex in California. The behavior of PSHB was consistent with

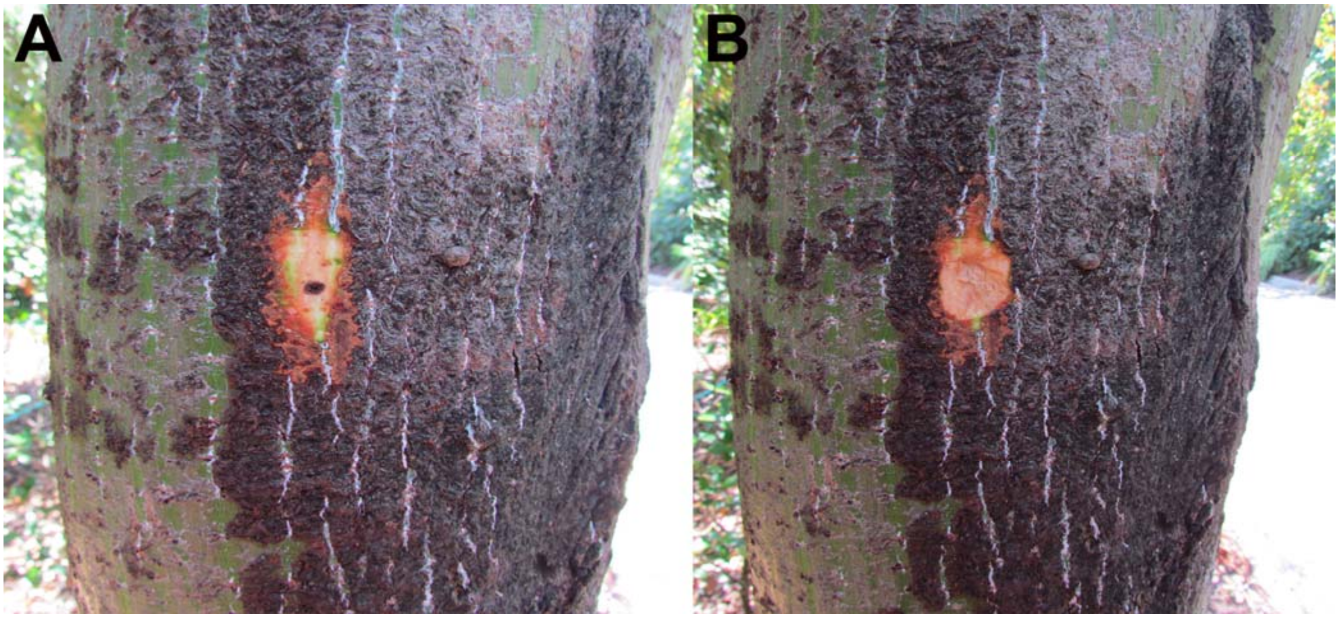

Fig. 7. Evidence of an unsuccessful attempt by polyphagous shot hole borer to establish a gallery in *Acer pectinatum* subsp. *laxiflorum*. **A,** Depth of penetration by the beetle is less than 1 mm. **B,** No sign of fungal colonization beneath the cambium.

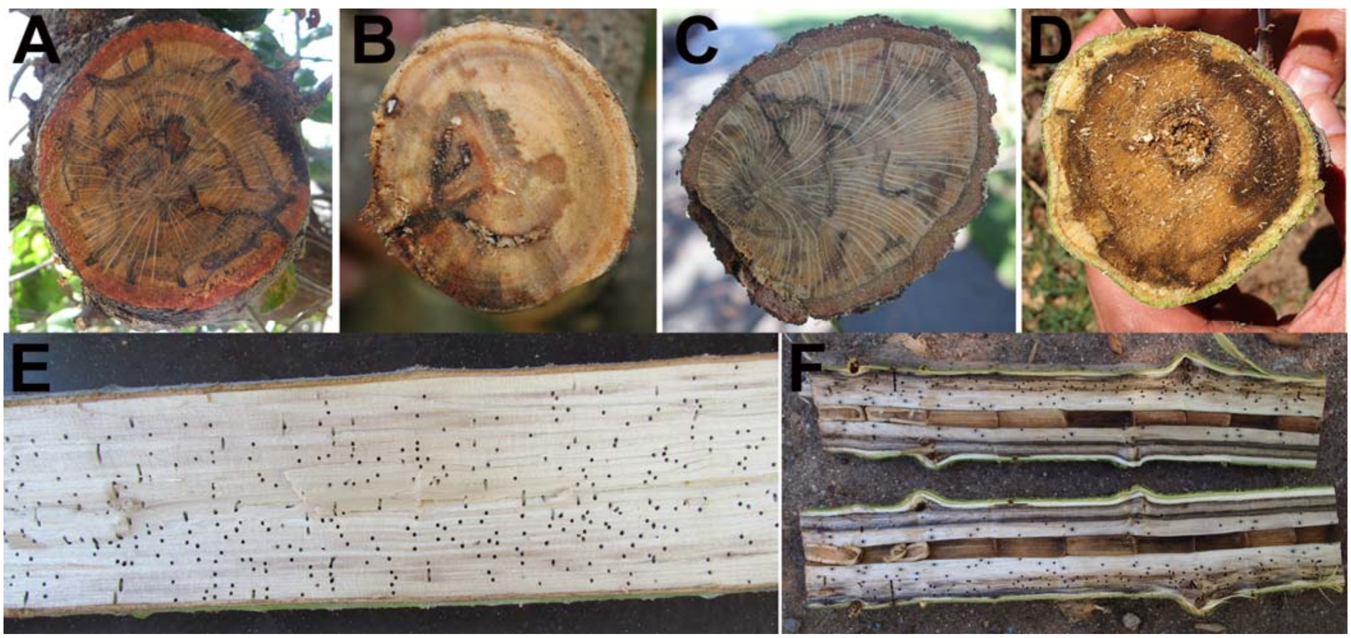

Fig. 8. A, Internal symptoms of polyphagous shot hole borer or Fusarium dieback disease showing gallery formation discoloration caused by fungal colonization on *Quercus agrifolia*; **B,** *Persea americana*; **C,** *Quercus robur*; and **D,** *Ricinus communis* in cross section; and **E,** *Acer negundo* and **F,** *R. communis* in longitudinal section.

the ecological group of first-degree primary polyphagous borers that invade healthy trees in many different plant families (5,12,22,24). Plant species attacked by the FD disease complex have worldwide origins, and include species such as the critically endangered dawn redwood (*Metasequoia glyptostroboides*) from China. In purely economic terms, however, the threat posed by FD to important agricultural crops, ornamental trees used for landscaping, and native forest tree species in California is a major concern. The wide host range of PSHB described in our southern California study was consistent with literature reports for the "tea shot hole borer" (5). However, with the present uncertainty about the species status of individuals morphologically recognized as *E. fornicatus*, the host range reported by Danthanarayana (5) may actually refer to the hosts of two (or more) cryptic species, each with a more limited host range.

Currently, it is difficult to estimate the longevity of trees infested by either the beetle or fungus alone, or trees that are reproductive hosts. Even though a tree species is not a reproductive host, an attempted PSHB attack may serve as an infection site for *Fusarium* sp. In some cases, *Fusarium* sp. was not able to infect the tissue. Even on known hosts of the fungus, tree infection required penetration into at least the cambium layer, which generally varied in depth below the outer bark between species. Given that 82% of species susceptible to FD in the present study are not reproductive hosts, we conclude that infection is most likely due to susceptibility of the tree to the fungus if the beetle is able to penetrate into or through this critical layer of tissue. Based on the previous observations of *Fusarium* sp. clogging conductive tissue within the tree (7,15) and dead or dying reproductive hosts infested with FD, and given that there is a correlation between severity of the beetle attack (which thereby increases severity of infection by *Fusarium* sp.) and the observed dieback, we may predict that, over time, infection by *Fusarium* sp. may lead to tree death in some species.

Although gumming was present on some tree species from which *Fusarium* sp. was also recovered, severe branch dieback was more likely in trees that did not exhibit gumming. This suggests that trees with gumming may be more resistant to beetle (12) and fungal attack. Most trees with gumming did not have necrotic tissues beneath the bark because the gum provided a physical barrier for beetle and fungal entry into the plant. This pattern is consistent with attack to trees by the TSHB in southern Asia, although there is no mention of resistance to fungal infection (5). Host response and resistance to disease or beetle infestation of this particular complex needs to be studied further to control disease establishment and spread.

Potential threat to California landscapes, agriculture, and global plant communities. The research and understanding of the distribution and impact of the FD complex are in the very early stages. However, the ability of this complex to attack multiple tree species in a large number of families suggests that it may become established across a range of plant communities on both a local and global scale. *Phytophthora ramorum* is an example of a generalist pathogen within California that also has a global distribution. It is the cause of sudden oak death, or ramorum dieback. This pathogen has killed thousands of oaks, tanoaks, and other woody hosts in California plant communities (6,13,19,21). However, the number of families susceptible to natural attack by *Fusarium* sp. in this study is five times higher than that of *P. ramorum* (6).

Reproductive hosts are negatively impacted when infected with FD and perpetuate healthy populations of PSHB, which facilitates long-term disease establishment and spread within a system. This study revealed that 5 of the 11 California native plant species surveyed were reproductive hosts of PSHB. This suggests that native plant communities and landscapes with these host plants are in danger of attack. Natural plant communities that are particularly vulnerable in southern California because reproductive hosts of PSHB are dominant members of the plant community include mixed evergreen forests, oak woodlands, foothill woodlands, and riparian habitats. Also noteworthy was that each continent on the globe had at least one reproductive host in this study, excluding Antarctica. This indicates a potential for FD establishment on a global scale. Studies on climatic conditions and other factors that influence PSHB distribution need to be implemented to determine the degree to which areas in other parts of the world are at risk for establishment by PSHB and *Fusarium* sp.

The juxtaposition of agriculture, urban landscapes, and forests in southern California harboring reproductive host species allows for potential rapid disease spread over a large geographic area that would otherwise be limited to islands of suitable habitat for PSHB and *Fusarium* sp. As an example, the urban forest in southern California could exacerbate the spread of FD, given that 48% of the street trees in southern California (4) belong to species that were confirmed as susceptible to FD. Six of these tree species are also reproductive hosts for PSHB, including American sweetgum, which is one of the most commonly planted street trees. Additionally, castor bean, a reproductive host and weed with a global distribution, is prevalent in urban, agriculturally important, and riparian areas and in the interface between these landscapes in southern California (27). The potential costs associated with the infestation of the urban forest may become substantial.

The FD complex is of special concern to the California avocado industry. It is already established and causing significant losses in commercial avocado groves in Israel (15). Given the high frequency and broad distribution of reproductive hosts and other host species throughout the region, disease spread and widespread establishment into commercial avocado groves in southern California is almost inevitable. At the time of writing, symptoms of FD have not been observed in commercial citrus groves or vineyards but have been confirmed in two commercial avocado groves in Los Angeles County. Disease distribution throughout southern California is currently being investigated.

Although studies are underway to determine host resistance, disease distribution, and impact, the results from this study suggest that plants confirmed as nonhosts, or hosts that display symptoms of resistance to PSHB, be given priority in future landscape plantings.

Table 4. Parameter estimate, odds ratio, and *P* values for the logistic regression model describing factors contributing to the likelihood of branch dieback >50% on trees attacked by the polyphagous shot hole borer (PSHB) (whole model $P > \chi^2$ 0.0035 and 0.0003)

Variable	Estimate	Odds ratio	$P > \chi^2$
Fusarium sp.	0.5269	2.869	0.0065
Gumming	–0.4048	2.610	0.0202
PSHB severity	0.9553	3.4147–6.9055	0.0004

Table 5. Number of tree species and abundance of tree specimens susceptible to the polyphagous shot hole borer (PSHB)–*Fusarium* sp. complex in the southern Californian urban forest[a]

Variable	Number of species	Abundance (%)[b]
Total number	103	…
Attacked by PSHB	53	56
Host for *Fusarium* sp.	36	48
Reproductive hosts for the beetle	6	26

[a] Estimations were made using a representative tree species list (4).
[b] Percentage of all specimens in the urban forest.

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