# FINAL REPORT

## PROJECT TITLE: Risk, spread, and control of Fusarium dieback – shot hole borers throughout native plant communities in San Diego County

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## INTRODUCTION

The viability of native riparian, oak woodland, and mixed evergreen plant communities in southern California is threatened by the exotic pest complex Fusarium dieback (FD) (Eskalen et al., 2012; Mendel et al., 2012), which is formed by two invasive shot hole borer beetle species (ISHB– polyphagous and Kuroshio shot hole borers, *Euwallacea fornicatus* and *E. kuroshio*), each associated with specific fungal pathogen species (*Fusarium euwallaceae* and *F. kuroshium* respectively) (Freeman et al., 2013; O'Donnell, 2014; Lynch et al., 2016; Na et al. 2018; Smith et al. 2019). The broad range of alternative hosts has fostered rapid spread throughout urban-wildland forests and commercial avocado groves in Los Angeles, Orange, San Diego, and most recently Riverside and Ventura Counties (Eskalen et al. 2013; Eskalen et al. *pers. obs.*). These observations confirm our previous predictions that the aforementioned native plant communities in Southern California are particularly susceptible to invasion and mortality by FD-ISHB (Eskalen et al., 2013).

The devastating effects of FD-KSHB on native riparian habitat in the Tijuana River Valley is of great concern to land managers in San Diego County, especially as many new infested sites continue to be identified throughout the region. These plant communities serve as critical breeding habitat for species such as the endangered least bell's vireo (*Vireo bellii pusillus*; LBVI) (Kus 2002) and southwestern willow flycatcher (*Epidomax traillii extimus*; SWFL) (Craig and Williams 1998). Our work points to a path forward to make informed decisions on best approaches to management in the short- and long- term. In this project, our objectives were to 1) determine KSHB distribution with respect to key least Bell's vireo and southwestern willow flycatcher habitat; 2) develop a predictive model for which specific native habitats are most vulnerable to FD-SHB invasion and impacts, based on an understanding of the evolutionary ecology of the fungus and beetles, beneficial endophyte distribution, a landscape analysis of vegetation and surrounding avocado groves, and environmental conditions; 3) evaluate biological control potential of beneficial endophytic fungi and bacteria; 4) assess the efficacy of application methods of entomopathogens suppressive to ISHB; 5) evaluate water-based latex paint as a means to track ambrosia beetle activity on infested trees; 6) evaluate efficacy of pesticides in suppressing beetle populations on critical host species; and 7) evaluate pesticide residues on critical host tissues.

The results and tools developed through these efforts will provide the necessary elements to develop best protocols for integrated pest management of FD-ISHB in riparian habitats and oak woodlands of San Diego County. To that end, our final objective was to 8) develop and train land managers on best protocols for IPM in native vegetation. Originally, our fifth objective was to evaluate mechanical barrier options that inhibit beetle dispersal from reproductive hosts, but because materials were to be imported from southeastern Asia, we had to redirect this research due to COVID.

## OBJECTIVES AND RESULTS

### Objective 1: Determine KSHB distribution with respect to key least Bell's vireo and southwestern willow flycatcher habitat.

#### Methods and Results

In response to SWFL and LBVI concerns articulated by USFWS and USGS, we identified 30 kilometers of highest priority habitat along the San Luis Rey watershed where intensive monitoring will be targeted. We placed one sticky panel monitoring trap every 500 m throughout the 30 km of greatest concern (60 traps total). This area covered locations below Lake Henshaw, Bonsall area, Gregory Canyon and Couser Canyons, and the lower San Luis Rey west of I-15 to College Blvd. For the remainder of the watershed (35 km), we deployed monitoring traps every two kilometers (18 traps total).

We checked all 78 traps on a monthly basis. Any beetles captured in traps were returned to the lab for counting and morphological identification. To screen for potential PSHB detections in San Diego County, we used molecular techniques on a random subsample of collected beetles to identify beetle and fungal species in collaboration with Dr. Richard Stouthamer in the Entomology Department at UCR. PSHB was never detected in our monitoring traps. Beetle activity was not detected in traps beyond Pauma Valley (red arrow) (Fig. 1).

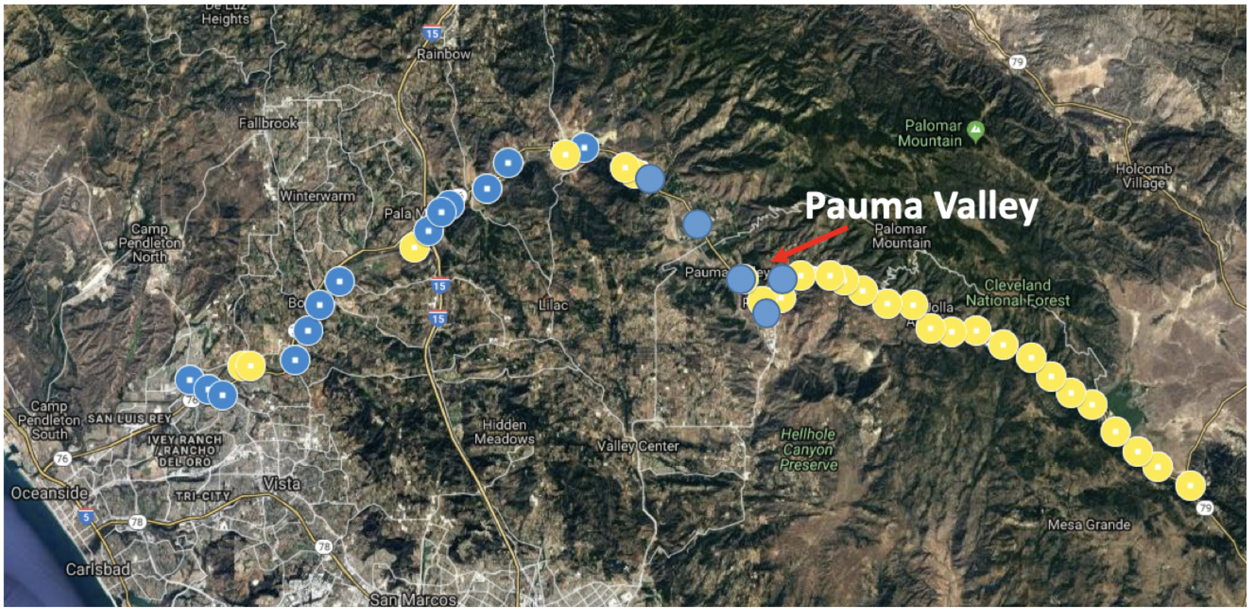


**Figure 1.** San Luis Rey monitoring trap locations. Blue circles represent KSHB-positive detections and yellow circles represent no KSHB detections. The red arrow represents recent findings as of October 2021.

### Objective 2: Predictive Model Development

In the early stages of the FD-ISHB epidemic, we observed variation in the severity of the impact of the pest-pathogen complex on hosts, where some hosts showed just branch dieback and others were killed when attacked (Eskalen et al. 2013; Fig. 2; Table SI). Moreover, when the beetle tried to attack a tree, it could not establish a gallery and successfully colonize some species. On other species, the beetle could not establish a gallery but the pathogen could colonize the tree. Finally, the beetle could bore into the trunk on other species, produce a gallery, establish a fungal garden for food, make its brood, and then spread to a new host. Given that each of those steps have the potential to differ among susceptibility in host species, and those host species are distributed across diverse systems (e.g., wildland, urban, and peri-urban forests and avocado groves), we first aimed to see if we could predict *which* tree species are attacked and colonized, and support reproduction or are killed. Previous experimental and observational work has shown a phylogenetic signal in pest-pathogen host ranges. That is to say, there is a predictable nature in pathogen host ranges where closely related hosts are more likely to share a pathogen than those distantly related (Gilbert & Webb, 2007; Parker et al. 2015). Therefore, we used host evolutionary relationships as a basis for our analysis. We then aimed to use that understanding of host range and climate data to predict FD-ISHB spread and impact across sites.

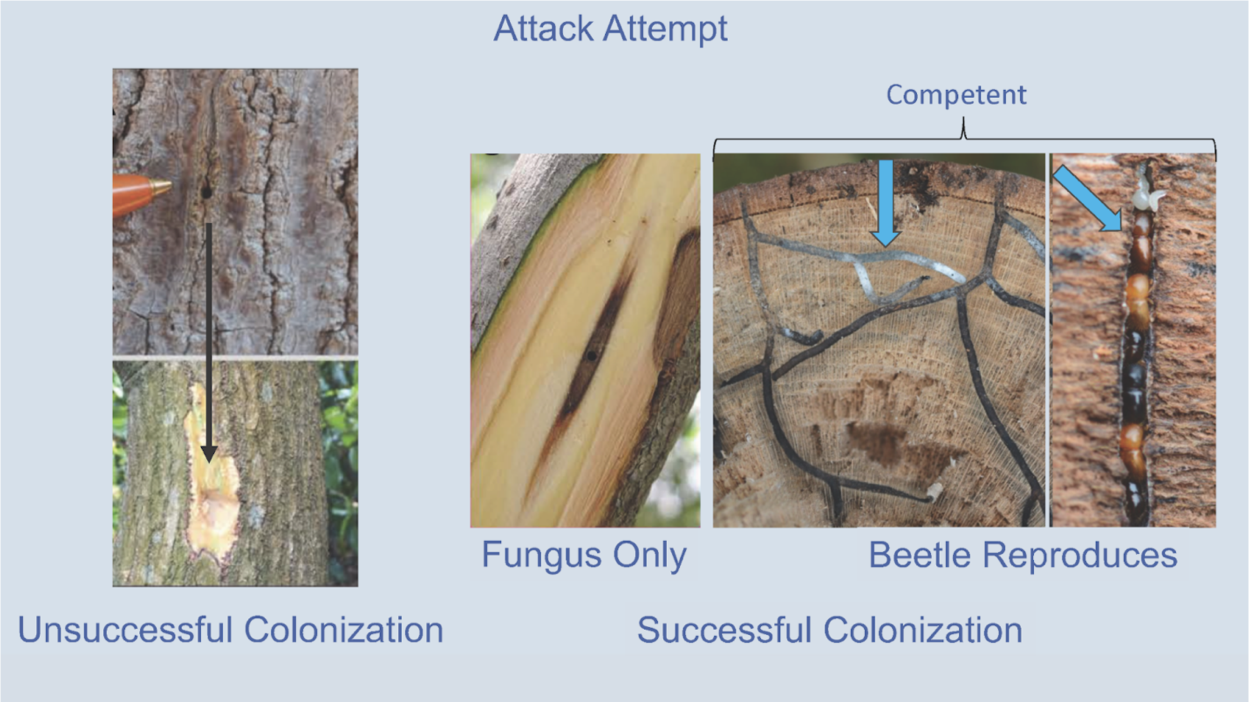


**Figure 2.** Differences in susceptibility among host species where neither the beetle nor fungus could successfully colonize an attacked tree (left), just the fungus colonizes the host (middle), or the beetle colonizes the host, farms the fungus, and produces offspring (right). Tree species that support reproduction of the beetle and pathogen are competent hosts.

## Methods and Results

### *Predicting FD-ISHB impacts based on host evolutionary relationships*

To test for phylogenetic signal in host types, we built a phylogenetic tree of over 2,700 tree and shrub species trees in California that the pest-pathogen could encounter that are either attacked or ignored. From there, we calculated the phylogenetic distances between all species pairs and then looked at the distribution of those distances within host categories from the most inclusive to the least inclusive (Lynch et al. 2021; Fig. 3). Our analysis showed that the phylogenetic distances of all attacked tree species were shorter (at 135 million years) than all available tree species that the beetle-pathogen could encounter (at 170 million years), indicating that attacked tree species are more closely related to one another than expected by chance. In contrast, the phylogenetic distances for the ignored tree species were longer than all available tree species (at 185 million years), indicating that these non-host tree species are less closely related. These results suggest there is something special about those groups of species that allows the beetles to attack them that is related to their evolutionary history (i.e., there is a phylogenetic signal).

Looking further into the signal, *Fusarium* spp. can colonize a subset of those attacked species, but the distances are the same on average. However, the phylogenetic distances for competent host species (those that support reproduction of the beetle and pathogen) were 48 million years shorter, and the phylogenetic distances for the even narrower subset of very closely related species that are rapidly killed by the beetle and pathogen were shorter by an additional 45 million years. Therefore, once the beetle-pathogen can attack one species, chances are they can also affect close relatives with similar traits because they are more likely to behave in the same way. Together, we found that hosts are a non-random, closely related subset of all available tree species. This phylogenetic signal is more pronounced for each of the nested interaction outcomes between the host, beetle, and pathogen. As such, these competent host species that support beetle-pathogen reproduction are important epidemiologically and have a phylogenetic structure. This tool has proven useful in understanding how the beetle-pathogen will behave when they encounter a new set of possible hosts in a novel location so decision-makers can respond appropriately. For example, PSHB has recently established in South Africa (2017) and western Australia (September 2021), where colleagues are using this tool to prioritize which tree species to monitor so response efforts are targeted and efficient at these initial stages of the epidemic.

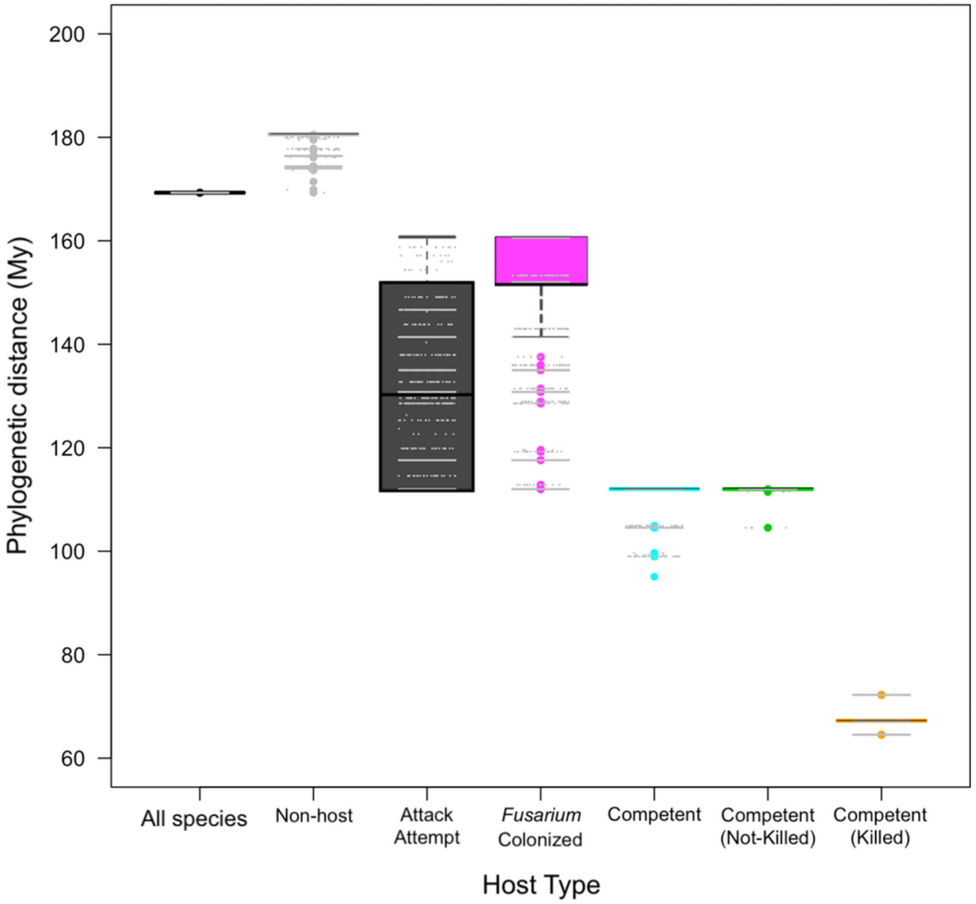


**Figure 3**. Phylogenetic distances (in millions of years) between all tree and shrub species in California compared to non- FD-ISHB host species and species within each host type. Competent hosts support reproduction of the beetle and pathogen. Adapted from Lynch et al. (2021).

### *Predicting risk of FD-ISHB establishment across sites*

The phylogenetic structure of the FD-ISHB host range describes the relationships among host types and, as a first pass, tells us which species will be affected and to what extent in novel locations. Given that FD-ISHB hosts are distributed over a complex landscape, where some sites have one species (e.g., an avocado grove), and others have many species (e.g., a riparian forest), we used this evolutionary understanding of host relationships to predict in which locations the beetle-pathogen is most likely to establish and cause damage in San Diego

County and beyond. As microclimate strongly influences beetle development, we further integrated microclimate into our model to refine our predictions.

***Study sites and monitoring:*** In 2017, we established 82 0.25-ha permanent monitoring plots throughout infested and non-infested avocado groves and native vegetation in San Diego county (Fig. 4; Table I; Table SII). These monitoring sites are part of a larger network of 260 plots in southern California (San Diego, Orange, and Ventura counties), spanning 300 km across forest types. Sites in San Diego County include locations along the San Luis Rey, the Vista Canal between Lake Wohlford and Valley Center Road, along Escondido Creek and surrounding Escondido, along the San Dieguito and Mission Valley Rivers, along Sloane Canyon and the Otay River Valley, and in the Tijuana River Valley (Table SII). Each plot contains at least 50 geo-referenced trees varying in species composition (Table SII) and a data logger to monitor microclimate. We surveyed 7,152 trees across plots and 3,441 trees across infested plots. We recorded attack severity, counted entry holes, and visited each tree in 2017, 2018, and 2020 to follow attack progression.

The proportion of infested plots in 2017 and 2020 increased from 36% to 50% in native vegetation and 81% to 94% in avocado groves (Table I). The proportion of infested trees across infested plots in 2017 and 2020 increased from 19% to 42% in native vegetation and 67% to 71% in avocado groves. Habitat alliance, basal area, tree density, and ISHB-attacked tree density each year for each plot are listed in Supplementary Table II.

**Table I**. Total number of infested plots and trees within infested plots in native vegetation and avocado groves in San Diego County in 2017, 2018, and 2020.

| | Number of Infested Plots | | | | Number of Infested Trees Within Infested Plots | | | |
|---|---|---|---|---|---|---|---|---|
| Plot Type | 2017 | 2018 | 2020 | Total Plots | 2017 | 2018 | 2020 | Total Trees |
| Native Vegetation | 24 | 29 | 33 | 66 | 349 | 599 | 756 | 1798 |
| Avocado Groves | 13 | 13 | 15 | 16 | 1108 | 1139 | 1166 | 1643 |

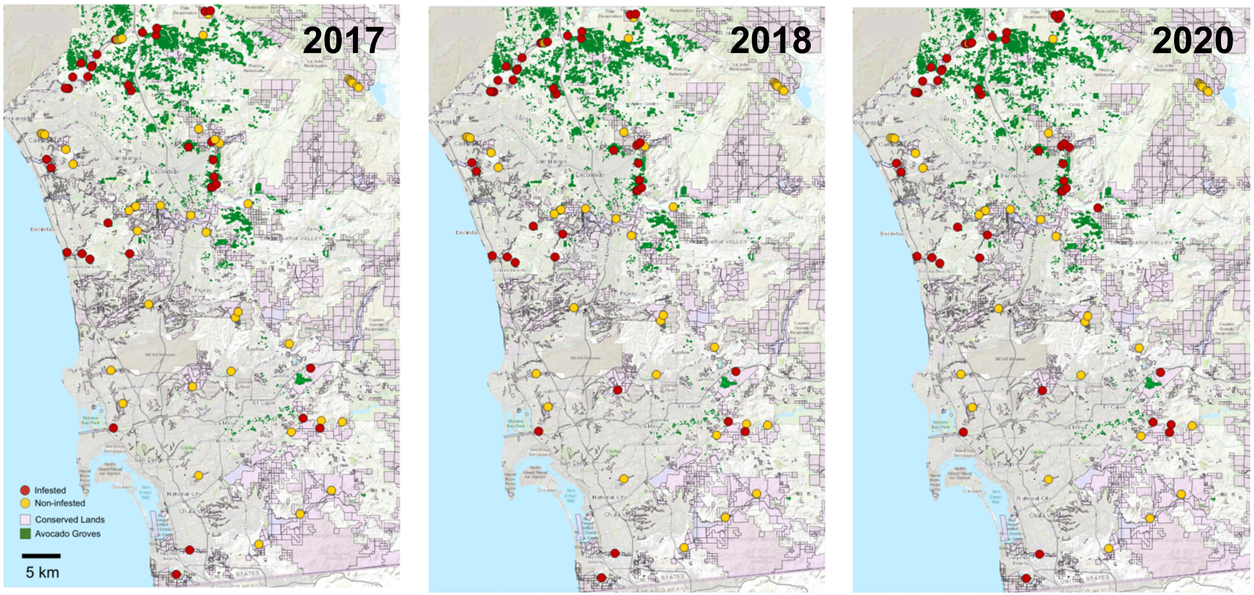


**Figure 5.** Permanent monitoring plot network across FD-ISHB -infested and non-infested sites across conserved lands and avocado groves in San Diego county in 2017, 2018, and 2020.

***Predictive Model Analysis:*** Host composition, relative abundances of beneficial endophytes (see below), biomass, distance to suitable habitat patches (based on GIS data), ground

***Predictive model development:*** Previous experimental work established that disease pressure on an *individual* plant is driven by the context of the surrounding plant community, which includes the abundance of each species in the local community and how closely related those plant species are to the focal individual (Parker et al. 2015). To estimate *site* susceptibility ($wpS_k$) based on the interaction between phylogenetic structure and host abundance we added those estimates for each species in a plot ($wpS_i$). First, we used the phylogenetic distances (PD) between each species (*i*) in a plot (*k*) and each competent host (*j*) and calculated the probability that each resident species (*i*) does not share FD-ISHB with each competent host (*j*).

$$1.\quad p(nH)_{ij} = \text{antilogit}(3.4 - 3.7*(\log_{10}(PD_{ij} + 1))$$

We then calculated the probability that a resident species (*i*) is a host by taking the complement of the product of the probability of not sharing FD-ISHB with each competent host.

$$2.\quad p(H)_i = 1 - \Pi(1 - p(nH)_{ij})$$

We weighted that probability by the abundance (*A*) of each resident species in a plot to get an estimate that each species is susceptible ($wpS_j$)

$$3.\quad wpS_i = p(H)_i * A_i$$

and then added those weighted probabilities for each species at a site (*k*) to get an estimate of site susceptibility based on the community context.

$$4.\quad wpS_k = \sum wpS_i$$

We then used those estimates of site susceptibility in a logistic regression to find the probability a site is infested and compared those probabilities to the observed (Fig. 5a). We found a highly significant effect in which the probability a site is infested increases with increasing phylogenetically weighted host abundance ($P \leq 0.001$). The distribution of the number of infested and non-infested plots across those wpS estimates (Fig. 5b) revealed a skew where predicted infested plots are actually infested, and most of the actual non-infested plots are predicted to be non-infested. These results suggest that although it does not capture everything, the community context alone is a good predictor of FD-ISHB establishment.

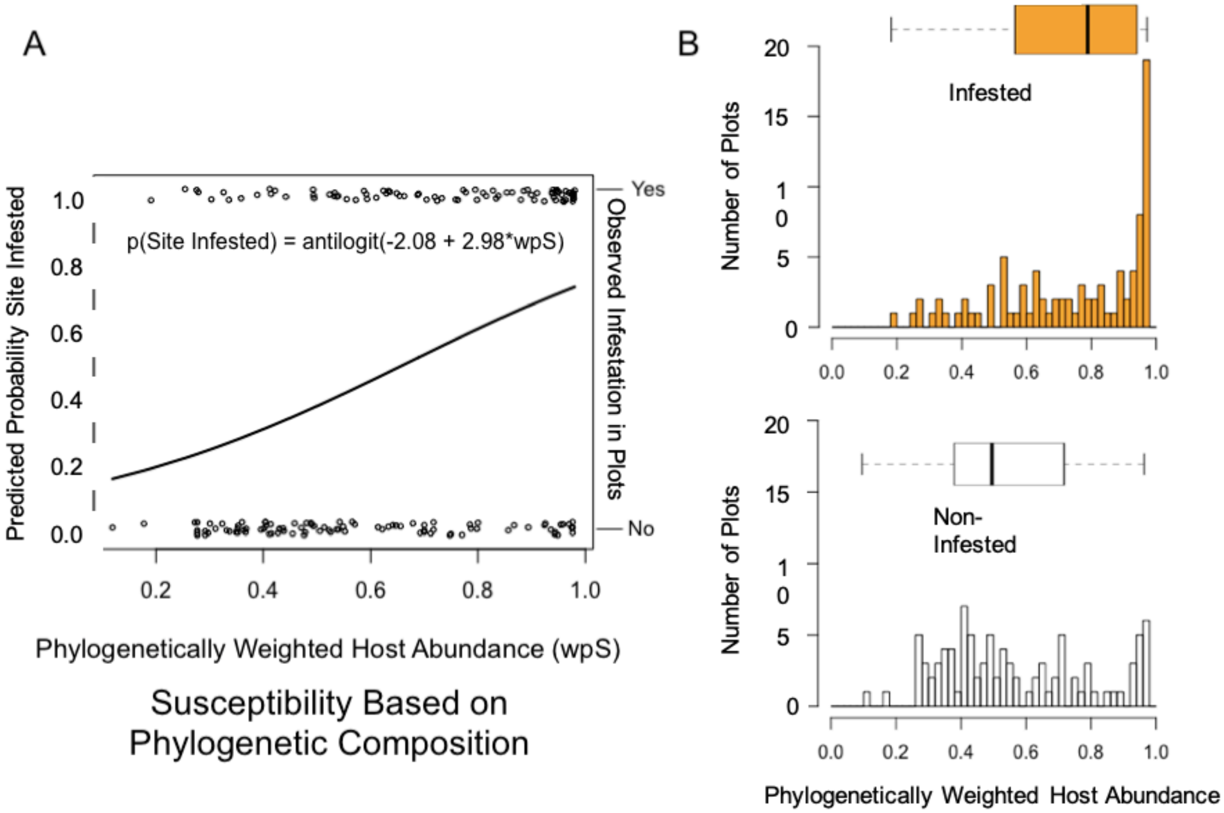


**Figure 5**. (a) Logistic regression analysis of FD-ISHB establishment as a function of site susceptibility based on phylogenetic composition of the resident community, and (b) histograms of the number of infested and non-infested plots across site susceptibility estimates.

Previous work established that the beetles produce more generations per year with warmer conditions. We used parameters from previous experimental work on PSHB and KSHB (Umeda & Paine 2018; Dodge & Stouthamer 2020) and our microclimate data in a degree day model to estimate the number of beetle generations per year for each site. This measure of climate suitability and potential propagule pressure for each site was added to the previous model in addition to the interaction between each effect (Fig. 6).

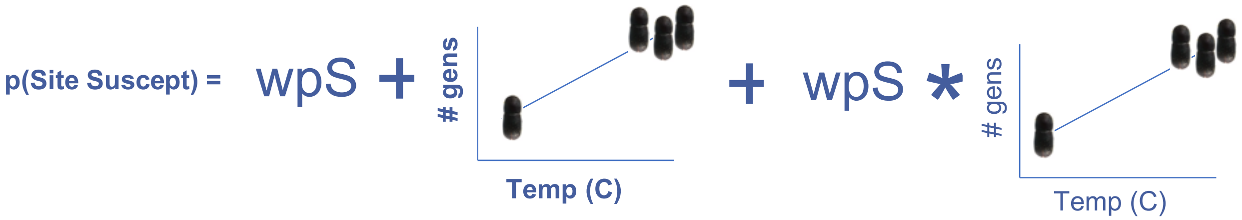


**Figure 6**. Risk model representation incorporating phylogenetic structure of resident plant community and climate effects on beetle development. The model includes both effects individually and the interaction between them.

Logistic regression analysis revealed highly significant effects for all three terms ($P \leq 0.001$). Therefore, we conducted a sensitivity analysis to explore the interaction effects of phylogenetic community composition and microclimate on FD-ISHB establishment under different climate conditions (Fig. 7). Sensitivity analysis showed that non-susceptible sites just based on species composition had a higher chance of FD-ISHB establishment if local climate conditions could produce more beetle generations (e.g., two or three generations versus one generation; Fig. 7). In contrast, the community structure had more of an influence on FD-ISHB establishment than climate conditions with higher estimates of phylogenetically weighted host abundance. Results suggest there are different effects of temperature on sites depending on the community context in which warmer places that can produce more generations of beetles allow infestation of sites that are otherwise unfavorable.

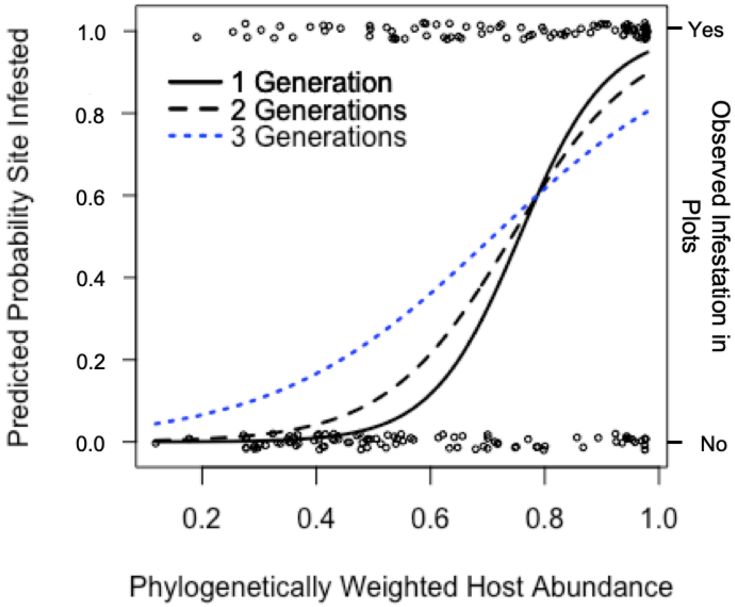


**Figure 7**. Sensitivity analysis exploring the interaction effects of phylogenetic community composition and microclimate on FD-ISHB establishment under different climate conditions. Warmer temperatures can produce more beetle generations.

## Management Applications

Supplementary Figure 1 shows a map of our monitoring network in San Diego County with the probability of FD-ISHB establishment over each plot to illustrate where monitoring and response efforts could be. Sites at 0.30 and above should be monitored regularly as we continue to validate and improve the model. This adaptive model is currently being used to make statewide predictions that are integrated into economic models to inform management decisions relevant to specific sites. Landscape considerations are additionally being integrated into the model to account for connectivity between sites. Continuous monitoring over time will be crucial to validate and improve the model.

## Objective 3: Evaluate biological control activity of beneficial endophytes.

### Recovery of endophytes from monitoring plots and *in vitro* screening

Endophytes (beneficial fungi and bacteria living inside the trees) may also play a role in the establishment and spread of the pest-disease complex. Preliminary data suggest that endophytes found in non-infested avocado and native sycamore trees in a disease hot spot provide promise as preventative biocontrol measures. The preliminary results additionally show that *Fusarium* spp. cannot colonize young avocado and sycamore plants inoculated with beneficial endophytes, indicating a need for further screening and field trials. Endophytes are known to play a role in preventing the spread of pathogens in grapevine, cacao, and grasses.

We sampled a subset of infested and non-infested native trees within 48 of the 82 plots throughout the San Diego County to identify which endophytes could be biocontrol candidates for consideration as a management response (Table SII). The infection status of each sampled tree was either infested (+), non- infested (-), recovered, or became infested over time. We selected representative tree species of eight critical hosts within 50 of the 265 established plots (*Alnus rhombifolia, Platanus racemosa*, *Populus fremontii*, *P. trichocarpa*, *Quercus agrifolia*, *Salix gooddingii, S. laevigata*, and *S. lasiolepis*). For each plot, we collected 5 mm diameter xylem core samples from a representative sample of up to two individuals per species – status pair in 2020 to explore *in vitro* interactions between cultural microbes and ISHBs – fusaria pathogens. Fungal and bacterial endophytes were isolated from wood tissues plated onto general agar media and identified by morphotype.

Antagonistic endophytes may inhibit the ability for the beetle to cultivate its fungal food source and to produce offspring, increasing the health of the host and limiting the spread of the beetle. To test for antagonism between culturable endophytes and *Fusarium* spp. pathogens, we conducted bioassays using ten replicates of each morphotype. Our initial screening assays are depicted in Figure 8.

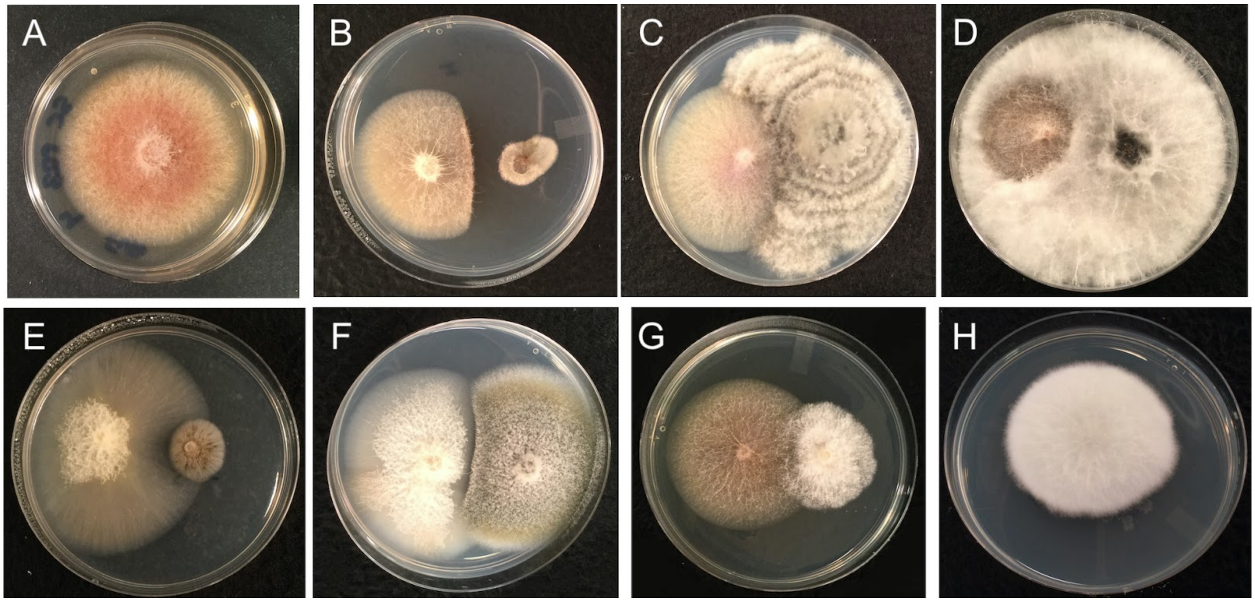


**Figure 8.** Representative outcomes of interactions between *Fusarium* spp. (left) and endophytes (right) observed in vitro. A) Fusarium colony in the absence of a contending endophyte. B) Antibiotic inhibition of *Fusarium* caused by *Pithomyces chartarum*. Outcomes of antagonism by competition include C-D) partial replacement (e.g., *Neofusicoccum parvum*), E) mycelial thinning (e.g., *Aureobasidium* sp.), or F) mutual inhibition made evident by the presence of a barrage between deadlocked colonies (e.g., *Alternaria infectoria*). In contrast, coexistence was characterized by G) mutual intermingling of hyphae between colonies after physical contact (e.g., *Clonostachys* sp.), or when colony vigor of either microbe was unaffected at a distance. H) Commensal interactions in particular resulted in enhanced filamentation and branching of *Fusarium* hyphae after contact with *Paenibacillus* sp.

We identified several fungi and bacteria that show promise as biocontrol agents. Specifically, three previously undescribed bacteria species of (*Pseudomonas, Pantoea, Variovorax*) and four fungal species (*Aureobasidium pullulans*, *Pithomyces chartarum*, *Acremonium* sp.*, Alternaria alternata*) exhibited inhibition of the fusaria pathogens (Tables II-III). Six additional fungi (*Cladosporium cladosporioides, Clonostachys solani, Neosetophoma* sp., *Stemphylium* sp., *Epicoccum nigrum, Neofusicoccum parvum*) appeared to suppress growth of the fusaria pathogens through competition for resources, the latter two of which are known plant pathogens. These results point to the potential of using these endophytes as an option for control. Although microbial interactions are complex, the patterns we observe from the culture data also affirm our planned next steps to identify a microbial fingerprint for diseased trees using high throughput next generation sequencing, and further investigate microbial interactions in diseased and non-diseased trees. Our findings in this study will further guide policy towards management of the problem.

**Table II.** *In vitro* interaction outcomes between endophytic fungi and *Fusarium* pathogens. “Dominant Inhibitor” refers to which microbe “won” in the interaction. “Dominant Mechanism” refers to the type of interaction (i.e., antibiosis, competition, coexistence). Values indicate the average (± standard deviation) number of cases among replicate strains in which a given outcome was observed between endophyte and pathogen.

| | | | Dominant Inhibitor | | | Dominant Mechanism | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Competition | | Coexistence | |
| Fungal Endophyte | *n* Strains | Isolate Abundance | Endophyte | Pathogen | Mutual Inhibition[1] | Antibiosis | Mycelial Thinning[2] | Partial Replacemet[3] | Mutual Intermingling | No Contact |
| *Aspergillus* sp. | 1 | 19 | 1 | | | 1 | | | | |
| *Cladosporium cladosporioides* | 1 | 3 | 1 | | | 1 | | | | |
| *Didymocyrtis brachylaenae* | 1 | 33 | 1 | | | 1 | | | | |
| *Epicoccum nigrum* | 2 | 4 | 1 ± 0 | | | 1 ± 0 | | | | |
| *Leptoxyphium kurandae* | 1 | 1 | 1 | | | 1 | | | | |
| *Pithomyces chartarum* | 2 | 2 | 1 ± 0 | | | 1 ± 0 | | | | |
| *Penicillium nalgiovense* | 4 | 32 | 0.94 ± 0.13 | | | 0.94 ± 0.13 | | | 0.06 ± 0.13 | |
| *Ascochyta phacae* | 1 | 3 | 0.67 | | 0.33 | 0.67 | | | | |
| *Phaeoacremonium* sp. | 2 | 7 | 0.75 ± 0.35 | | | 0.75 ± 0.35 | | | 0.25 ± 0.35 | |
| Dothideomycetes | 1 | 1 | 1 | | | 0.50 | 0.50 | | | |
| *Botryosphaeria parva* | 8 | 11 | 1 ± 0 | | | | | 1 ± 0 | | |
| *Trichoderma harzianum* | 2 | 9 | 1 ± 0 | | | | | 1 ± 0 | | |
| *Botryosphaeria iberica* | 1 | 2 | 1 | | | | | 1 | | |
| *Botryosphaeria obtusa* | 1 | 3 | 1 | | | | | 1 | | |
| *Fusarium brachygibbosum* | 1 | 1 | 1 | | | | | 1 | | |
| *Lasiodiplodia gilanensis* | 1 | 2 | 1 | | | | | 1 | | |
| *Neocucurbitaria salicis-albae* | 1 | 4 | 1 | | | | | 1 | | |
| *Ulocladium* sp. | 1 | 3 | 1 | | | 0.33 | | 0.67 | | |
| *Cladosporium* Group 5 | 5 | 56 | 0.80 ± 0.45 | | 0.03 ± 0.07 | | 0.78 ± 0.36 | 0.80 ± 0.45 | 0.17 ± 0.37 | |
| *Botryosphaeria* sp. | 1 | 3 | 0.67 | | 0.33 | | | 0.67 | | |
| *Cladosporium* Group 1 | 3 | 57 | 0.67 ± 0.58 | 0.11 ± 0.19 | | 0.33 ± 0.58 | 0.33 ± 0.38 | 0.53 ± 0.41 | 0.22 ± 0.38 | |
| *Neosetophoma italica* | 2 | 7 | 0.63 ± 0.18 | | 0.13 ± 0.18 | 0.50 ± 0 | 0.38 ± 0.53 | | 0.25 ± 0.35 | |
| *Aureobasidium* sp. 4b | 3 | 7 | 0.56 ± 0.10 | | 0.39 ± 0.10 | | 0.80 ± 0.05 | | 0.06 ± 0.10 | |
| *Querciphoma carteri* | 2 | 8 | 0.50 ± 0.35 | | 0.25 ± 0.35 | | 0.75 ± 0 | | 0.25 ± 0 | |
| *Cladosporium* sp. | 4 | 86 | 0.50 ± 0.43 | 0.08 ± 0.17 | 0.33 ± 0.47 | 0.06 ± 0.13 | 0.75 ± 0.32 | 0.17 ± 0.19 | 0.08 ± 0.17 | |
| *Fusicoccum vitifusiforme* | 1 | 2 | | 1 | | 1 | | | | |

[1] Evident through the formation of a barrage between deadlocked colonies, with the exception of *Cladosporium* Group 10 and *Querciphoma carteri*

[2] Effect on pathogen

[3] Effect on endophyte or pathogen

|  |  |  | Dominant Inhibitor |  |  | Dominant Mechanism |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  | Competition |  | Coexistence |  |
| Fungal Endophyte | *n* Strains | Isolate Abundance | Endophyte | Pathogen | Mutual Inhibition[1] | Antibiosis | Mycelial Thinning[2] | Partial Replacemet[3] | Mutual Intermingling | No Contact |
| *Cladosporium aphidis* | 1 | 5 |  | 1 |  |  |  | 1 |  |  |
| *Hormonema carpetanum* | 1 | 6 |  | 1 |  |  |  | 1 |  |  |
| *Aureobasidium melanogenum* | 2 | 4 | 0.25 ± 0.35 | 0.63 ± 0.53 | 0.13 ± 0.18 |  | 0.38 ± 0.18 | 0.63 ± 0.53 |  |  |
| *Aureobasidium* sp. 3 | 4 | 8 | 0.19 ± 0.24 | 0.48 ± 0.34 | 0.33 ± 0.12 |  | 0.46 ± 0.22 | 0.56 ± 0.43 |  |  |
| *Aureobasidium pullulans* | 6 | 43 | 0.35 ± 0.27 | 0.42 ± 0.49 | 0.23 ± 0.26 | 0.24 ± 0.28 | 0.33 ± 0.38 | 0.42 ± 0.49 |  |  |
| *Alternaria multiformis* | 1 | 2 |  |  | 1 |  |  |  |  |  |
| *Aureobasidium* sp. 4a | 1 | 2 |  |  | 1 |  | 1 |  |  |  |
| *Cladosporium* Group 10 | 1 | 7 |  |  | 1 |  | 1 |  |  |  |
| *Phialemonium* sp. | 1 | 2 |  |  | 1 |  | 0.50 |  |  |  |
| *Libertasomyces myopori* | 1 | 1 |  |  | 1 |  | 0.25 |  |  |  |
| *Cladosporium* Group 8 | 1 | 2 |  |  | 1 |  |  |  |  |  |
| *Diaporthe* sp. | 1 | 2 |  |  | 1 |  |  |  |  |  |
| *Paraconiothyrium brasiliense* | 1 | 3 |  |  | 1 |  |  |  |  |  |
| *Pleiochaeta carotae* | 1 | 1 |  |  | 1 |  |  |  |  |  |
| *Alternaria* sp. | 3 | 15 | 0.08 ± 0.14 |  | 0.91 ± 0.14 |  | 0.25 ± 0.25 | 0.08 ± 0.14 |  |  |
| *Alternaria alternata* | 17 | 50 | 0.01 ± 0.06 |  | 0.89 ± 0.21 |  | 0.20 ± 0.21 | 0.11 ± 0.22 | 0.09 ± 0.17 |  |
| *Alternaria infectoria* | 6 | 31 | 0.04 ± 0.10 |  | 0.88 ± 0.21 |  | 0.08 ± 0.13 |  | 0.08 ± 0.20 |  |
| *Acrostalagmus luteoalbus* | 4 | 7 |  | 0.13 ± 0.25 | 0.83 ± 0.24 | 0.13 ± 0.14 | 0.17 ± 0.24 | 0.06 ± 0.13 | 0.04 ± 0.08 |  |
| *Stemphylium* sp. | 1 | 2 |  |  | 0.75 |  | 0.25 |  | 0.25 |  |
| *Aureobasidium* sp. 2 | 2 | 4 |  | 0.38 ± 0.53 | 0.63 ± 0.53 |  | 0.25 ± 0.35 | 0.38 ± 0.53 |  |  |
| *Pyricularia caricis* | 1 | 1 | 0.25 |  | 0.50 |  | 0.75 |  | 0.25 |  |
| *Pseudocamarosporium* | 1 | 6 | 0.25 |  | 0.50 | 0.25 | 0.50 |  | 0.25 |  |
| *Hermatomyces* sp. | 1 | 1 |  |  | 0.50 |  | 0.50 |  | 0.50 |  |
| *Alternaria atra* | 1 | 1 |  |  | 0.50 |  |  |  | 0.50 |  |
| *Cystobasidium slooffiae* | 1 | 1 |  | 0.50 |  |  |  | 0.50 |  | 0.50 |
| *Sarocladium* sp. nov. | 2 | 18 |  | 0.38 ± 0.53 | 0.50 ± 0.35 | 0.13 ± 0.18 | 0.25 ± 0 | 0.38 ± 0.53 | 0.13 ± 0.18 |  |
| *Brachysporiella navarrica* | 1 | 1 |  |  |  |  |  |  |  | 1 |
| *Didymosphaeria variabile* | 1 | 7 |  |  |  |  |  |  | 1 |  |
| *Phragmocamarosporium hederae* | 2 | 11 |  |  |  |  |  |  |  | 1 ± 0 |

[1] Evident through the formation of a barrage between deadlocked colonies, with the exception of *Cladosporium* Group 10 and *Querciphoma carteri*

[2] Effect on pathogen

[3] Effect on endophyte or pathogen

|  |  |  | Dominant Inhibitor |  |  | Dominant Mechanism |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  | Competition |  | Coexistence |  |
| Fungal Endophyte | *n* Strains | Isolate Abundance | Endophyte | Pathogen | Mutual Inhibition[1] | Antibiosis | Mycelial Thinning[2] | Partial Replacemet[3] | Mutual Intermingling | No Contact |
| *Clonostachys sp.* | 3 | 4 |  |  | 0.17 ± 0.14 |  | 0.17 ± 0.14 |  | 0.83 ± 0.14 |  |
| *Populocrescentia forlicesenensis* | 1 | 1 | 0.25 |  |  |  | 0.25 |  |  | 0.75 |
| *Pleurostoma richardsiae* | 1 | 1 | 0.33 |  |  |  | 0.33 |  | 0.67 |  |
| *Aureobasidium subglaciale* | 2 | 3 |  | 0.10 ± 0.14 | 0.35 ± 0.21 |  | 0.42 ± 0.12 | 0.17 ± 0.24 | 0.55 ± 0.07 |  |
| *Pleomassaria* sp. nov | 1 | 3 |  |  | 0.50 |  |  |  | 0.50 |  |

[1] Evident through the formation of a barrage between deadlocked colonies, with the exception of *Cladosporium* Group 10 and *Querciphoma carteri*

[2] Effect on pathogen

[3] Effect on endophyte or pathogen

**Table III.** *In vitro* interaction outcomes between endophytic bacteria (*E*) and *Fusarium* pathogens (*P*). "Dominant Mechanism" refers to interaction type (i.e., antibiosis, competition). Values are the average (± standard deviation) number of times a given outcome observed between *E* and *P* among replicate strains.

| | | | Dominant Mechanism | | Other Interaction | | |
|---|---|---|---|---|---|---|---|
| **Bacterial Endophyte** | **n Strains** | **Isolate Abundance** | **Antibiosis** | **Competition** | **Neutral** | **Commensal** | **Observation** |
| *Bacillus* sp. | 4 | 18 | 1 ± 0 | | | | Inhibited fungal growth |
| *Raoultella terrigena* | 1 | 2 | 1 | | | | Inhibited fungal growth |
| *Pantoea agglomerans* | 5 | 6 | 0.75 ± 0.31 | | 0.25 | | Inhibited fungal growth |
| *Pantoea* sp.[1] | 10 | 55 | 0.50 | 0.22 | 0.27 ± 0.36 | | Inhibited fungal growth, mycelial thinning |
| *Acidovorax* sp. | 1 | 2 | | 1 | | | Mycelial thinning |
| *Acinetobacter johnsonii* | 1 | 1 | | 1 | | | Stunted radial growth, enhanced filamentation |
| *Brenneria* sp. | 1 | 12 | | 1 | | | Mycelial thinning |
| *Gibbsiella quercinecans* | 1 | 2 | | 1 | | | Mycelial thinning |
| *Lysinibacillus fusiformis* | 1 | 2 | | 1 | | | Stunted radial growth, enhanced filamentation |
| *Novosphingobium resinovorum* | 1 | 1 | | 1 | | | Stunted radial growth, mycelial thinning |
| *Pantoea ananatis* | 1 | 1 | | 1 | | | Mechanical blocking, reduced growth or enhanced filamentation<br>•Enhanced bacterial biofilm |
| *Stenotrophomonas* sp. | 4 | 5 | | 0.88 ± 0.25 | 0.13 | | Mycelial thinning |
| *Pseudomonas aeruginosa* | 1 | 1 | | 0.83 | 0.17 | | Mycelial thinning |
| *Arthrobacter* sp. | 1 | 5 | | 0.75 | | | Mycelial thinning |
| *Flavobacterium* sp. | 3 | 3 | | 0.69 ± 0.05 | | | Mycelial thinning |
| *Pseudomonas fluorescens*[3] | 14 | 273 | 0.07 | 0.70 | 0.23 ± 0.32 | | Mycelial thinning, mechanical blocking |
| *Erwinia* sp.[4] | 3 | 6 | 0.27 | 0.64 | 0.13 | | Mycelial thinning, mechanical blocking, reduced fungal growth<br>• Enhanced bacterial biofilm |
| *Paenibacillus* sp.[2] | 8 | 47 | | | | 1 ± 0 | Enhanced filamentation |
| *Brenneria populi* | 1 | 2 | | 0.50 | | 0.50 | Reduced (*F. euwallacea*) and enhanced (*F. kuroshium*) filamentation |

[1] 0.68 ± 0.35 Antibiosis and competition combined

[2] Effect on *Fusarium* spp. potentially commensal

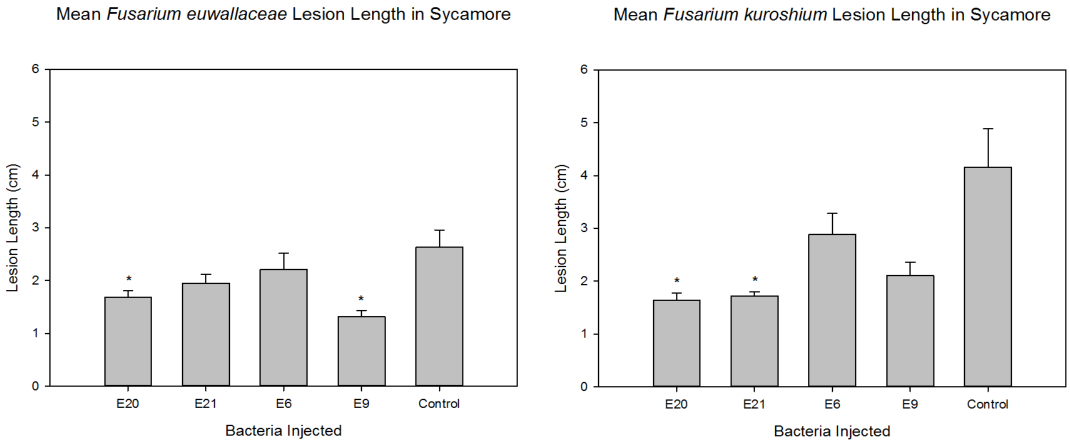
[3] 0.77 ± 0.32 Antibiosis and competition combined

[4] 0.92 ± 0.14 Antibiosis and competition combined

### **Greenhouse trials to test viability of endophytes within cuttings**:

A greenhouse study with sycamore plants was conducted using methods described by Eastwell et al. (2006). We selected four the best-performing isolates of *Bacillus* spp. obtained from sycamore (E6, E9) and avocado (E20, E21) to test the efficacy of *Fusarium* spp. establishment on treated sycamore saplings. Ten plants each were inoculated with *F. euwallaceae* or *F. kuroshium* seven days after inoculation and establishment of the beneficial bacteria. The treated plant stems were collected from the greenhouse four weeks after the fungal inoculation and destructively sampled four weeks later for laboratory testing.

Three of the four beneficial bacteria inoculated into sycamore significantly reduced severity of the xylem staining caused by *F. euwallaceae* (E9 and E20) and *F. kuroshium* (E20 and E21) (Fig. 9).



Mean *Fusarium euwallaceae* Lesion Length in Sycamore

Mean *Fusarium kuroshium* Lesion Length in Sycamore

**Figure 9**. Mean lengths of *F. euwallaceae* and *F. kuroshium* lesions in xylem tissues of sycamore treated with endophytic bacterial injections or sterile water. Lesion lengths were measured 30 days after pathogen inoculation. Vertical lines represent standard error of mean. Statistical analysis was performed using Tukey's honest significant difference (HSD) test at α = 0.05. Asterisks denote significant reduction of lesion length of control

### Management Applications

These results suggest that some beneficial bacteria could be applied broadly (e.g., E20) and supplemented by other beneficial bacteria (e.g., E9 and E21) to protect plants from pathogen impacts by reducing the severity of the symptoms caused by *F. euwallaceae* and *F. kuroshium* associated with PSHB and KSHB in sycamore trees. For restoration projects, we are currently developing fermentation protocols to introduce beneficial bacteria into plants at large scale.

## Objective 4: Assess the efficacy of application methods of entomopathogens suppressive to ISHB

Entomopathogens are fungi that infect and kill insects. The entomopathogen *Beauvaria bassiana* is currently being used commercially with some success on avocado in Florida to control the redbay ambrosia beetle (*Xyleborus glabratus*), the vector of laurel wilt disease (Daniel Carillo *personal communication*) and coffee berry borer (*Hypothenemus hampei)* in Hawaii. It is uncertain whether the fungi will remain viable over time in California, where conditions are drier. If successful, this treatment could be applied to selected heavily infested reproductive hosts to kill emerging beetles and aid in slowing disease spread.

### Methods and Results

Adult female beetles were collected from infested box elder (*Acer negundo*) branches located at the Huntington Botanical Gardens (San Marino, CA) and stored in vials for use in *in-vitro* studies.

Spore suspensions of three fungal strains (*Metarhizium brunneum*, *Beauveria bassiana,* and *Isaria* sp.) provided by Angela Payne (USDA-ARS) were diluted to a final concentration of ~$10^8$ spores/ml and applied to 10 collected beetles per strain in petri dishes using an atomizer. Control beetles were misted with sterile 0.01% TritonX-100 solution. Following treatment application, beetles were transferred to sterile Petri dishes and placed in an incubator at 25°C in the dark and beetle survival/mortality was monitored for 11 days.

The *in vitro* application of *Beauveria bassiana, Metarhizium brunneum,* and *Isaria* sp. showed that the beetles sprayed with *M. brunneum* exhibited the highest mortality rate. The onset of mortality for beetles sprayed with *M. brunneum* was the earliest, with the first death being observed 2 days after treatment (Fig 11). While the mortality rate in *M. brunneum*-treated beetles remained uniform throughout the 11-day period, *B. bassiana*-treated beetles exhibited a sharp increase in mortality in one of the replicates toward the end of the experiment period. *M.*

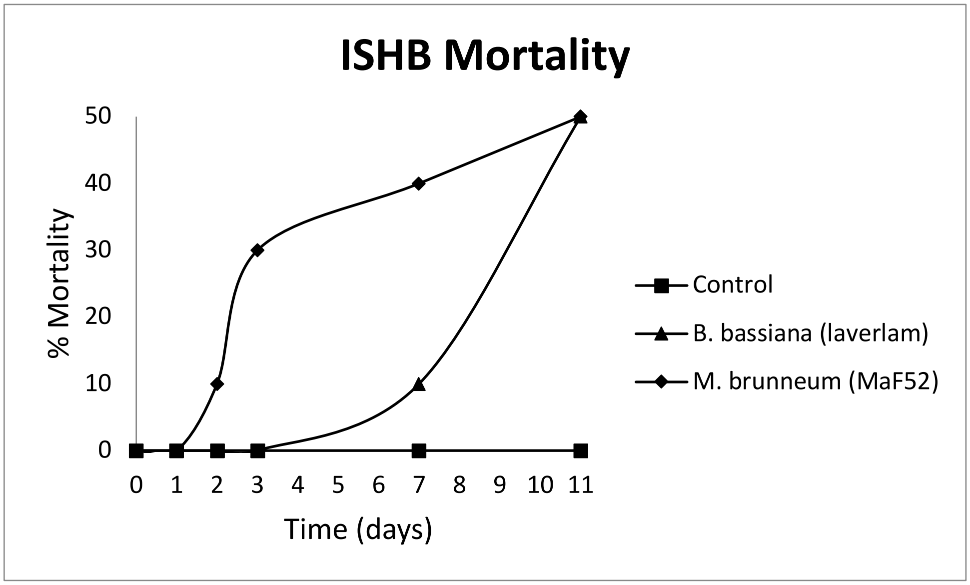


**Figure 11.** ISHB mortality over time after spray application with *Beuveria bassiana*, *Metarhyzium brunneum*, or 0.01% Triton-X100 solution (control) in laboratory conditions.

*brunneum*-treated and *B. bassiana*-treated groups both had 5 surviving beetles each 11 days after treatment. *Isaria* sp. was not effective at killing the beetles.

The remainder of this objective is integrated into Objective 6, where we further tested the efficacy of commercially available biopesticides (*B. bassiana* and *Bacilus subtilis*) and synthetic pesticides in killing ISHB beetles and their associated fungi *in vitro* using sawdust rearing media and *in planta* using cut bolts and live avocado plants.

### Objective 5: Evaluate water-based latex paint as a means to track ambrosia beetle activity on infested trees

Ambrosia beetles are known to cultivate their symbiotic fungi as a nutrient source inside the various woody hosts they bore into and colonize. To establish inside a host, a single female beetle bores through the bark to initiate gallery construction in the wood. Female beetles block the entry point of the gallery with their abdomen after laying eggs within their gallery. If the entry hole is obstructed by a foreign object, then the beetles will clear the entrance of the obstruction gallery and continue to block the entry point of the gallery, indicating that the beetle is alive and active. Given that beetles or activity is not apparent in *all* entry inspected entry holes (Fig 12), we aimed develop a way to track ambrosia beetle activity on infested trees. A robust method to track ambrosia beetle activity is essential to determine the efficacy of treatment options.

**Figure 12.** Blocking of the entry hole by a female invasive shot hole borer.

#### Methods

An 8” x 11.5” (size of an A4 paper sheet) area was outlined in vertical orientation with a paint marker at a consistent height on four sides at cardinal directions on ten sycamore trees. We applied an off-white water based latex paint using a paint brush after counting the number of entry holes within each area (Fig. 13). The painted areas were revisited and counted again the next day to look for visible entry holes to count; the areas with visible holes after the paint application has dried are indicative of active entry holes (Figs. 13-14). These painted areas were revisited the following month to track the activity.

**Figure 13**. A-D shows the process of counting the initial area for beetle attacks (A), applying water-based latex paint with brush (B), All the holes covered by the paint application (C), and resurgence of active beetles a week after application (D).

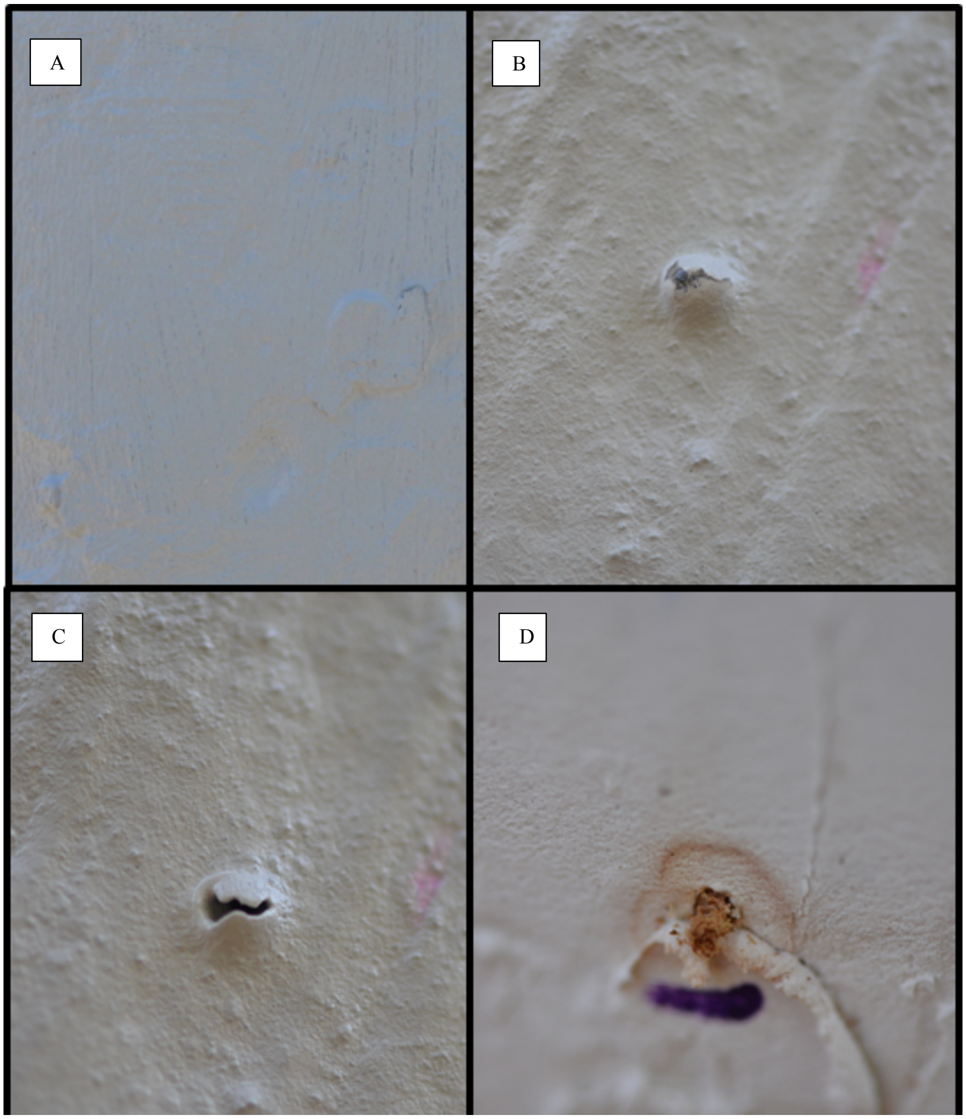


**Figure 14**. Progression of beetle reemergence in after paint application. After paint application has begun to dry (A), the female beetle will begin re-establish the entry point with her abdomen (B), break through the paint obstruction (C), and continue to maintain the gallery (D).

## Results and Management Applications

We observed open entry holes after covering those suspect holes with latex paint, demonstrating that the female within each gallery is alive and active. This proof of concept study illustrates the validity of using a simple, water-based latex paint to assess beetle activity on attacked trees and evaluate the efficacy of control treatments.

## Objective 6: Evaluate the efficacy of pesticides in suppressing beetle populations on critical host species

The management of ambrosia beetles is particularly challenging since these beetles spend most of their life within their hosts, emerging only to colonize nearby hosts. This life history trait drastically limits the exposure of these beetles to contact insecticides. Furthermore, xylomycetophagous ambrosia beetles do not consume the wood of host trees as bark beetles do (Batra 1966; Beaver 1989; Francke-Grosmann 1967), making it unclear how much contact occurs between beetle and insecticide during injections. Cooperband et al. (2016) observed that PSHB adults develop within 22 days at 24°C and produce an average of 32 viable female offspring and it is thought that adult beetles and larvae are present throughout the year suggesting that beetle reproduction takes place year-round (Eatough-Jones and Paine 2015). The high and constant reproductive output of ISHB suggests that multiple spray applications of contact insecticides would likely need to be made throughout the year as no clear window of beetle emergence can be accurately predicted in a given area. Thus, the use of injectable systemic insecticides may be more appropriate for management of this vector to circumvent these issues. Several studies have shown the efficacy of various insecticides in the management of various bark and ambrosia beetles in avocado (Peña et al. 2011), oak (Svira et al. 2004), and elms (Pajares and Lanier 1989). However, the association of these beetles with symbiotic and auxiliary fungi presents another challenge as the ISHB symbionts are known to be pathogenic on their hosts (Lynch et al. 2016; Mendel et al. 2012; Na et al. 2017). This highlights the need for two levels of management— one to directly manage vector populations and a second level which aims to manage the fungal populations established by ISHB once a host has been invaded.

The symbiosis between ISHB and their associated fungi is essential to the survival and persistence of these ambrosia beetles as they feed exclusively on the symbiotic mutualists. In addition to *F. euwallaceae*, the primary symbiont of PSHB, which is known to be essential for the beetle to complete its full life cycle and is likely required for gallery establishment in new hosts (Freeman et al. 2013; Freeman et al. 2016), PSHB is also associated with two other fungi: *Graphium euwallaceae* and *Paracremonium pembeum* (Lynch et al. 2016). *Graphium euwallaceae* likely serves as the primary food source for immature beetle stages, but may also play a prominent role during initial gallery formation. *Paracremonium pembeum* is not required as a source of food for any stage of beetle development nor is it currently considered a symbiont, but it could play a role as a fungal antagonist towards contaminating fungi within beetle galleries (Freeman et al. 2016, Lynch et. al. 2016). Similarly, KSHB is known to associate with two symbiotic fungi: *Fusarium kuroshium* and *Graphium kuroshium*, both of which presumably serve the same function in KSHB as *F. euwallaceae* and *G. euwalla*ceae serve in ISHB. This complex obligate association between beetle and fungi highlights an intrinsic vulnerability of this system whereby suppression of fungal symbionts and associates could have severe impacts on beetle establishment and development. Exploiting this obligatory relationship between ISHB and their associated fungi could be useful in the management of ISHB-FD as fungicides could be used to restrict the growth of fungal populations within tree hosts which in turn would impact the endurance of ISHB.

Here, we investigate the efficacy of various chemical fungicides, two insecticides (one systemic and one contact), and biological control agents of the fungi and beetle in controlling the FD-ISHB epidemic. Specifically, we 1) identified which synthetic fungicides inhibit the growth of ISHB symbionts *in vitro* using agar media; 2) investigated pesticide effects on ISHB and fungal fecundity *in vitro* using sawdust rearing media; 3) assess the efficacy of synthetic and biopesticides in reducing ISHB populations *in planta* in the field.

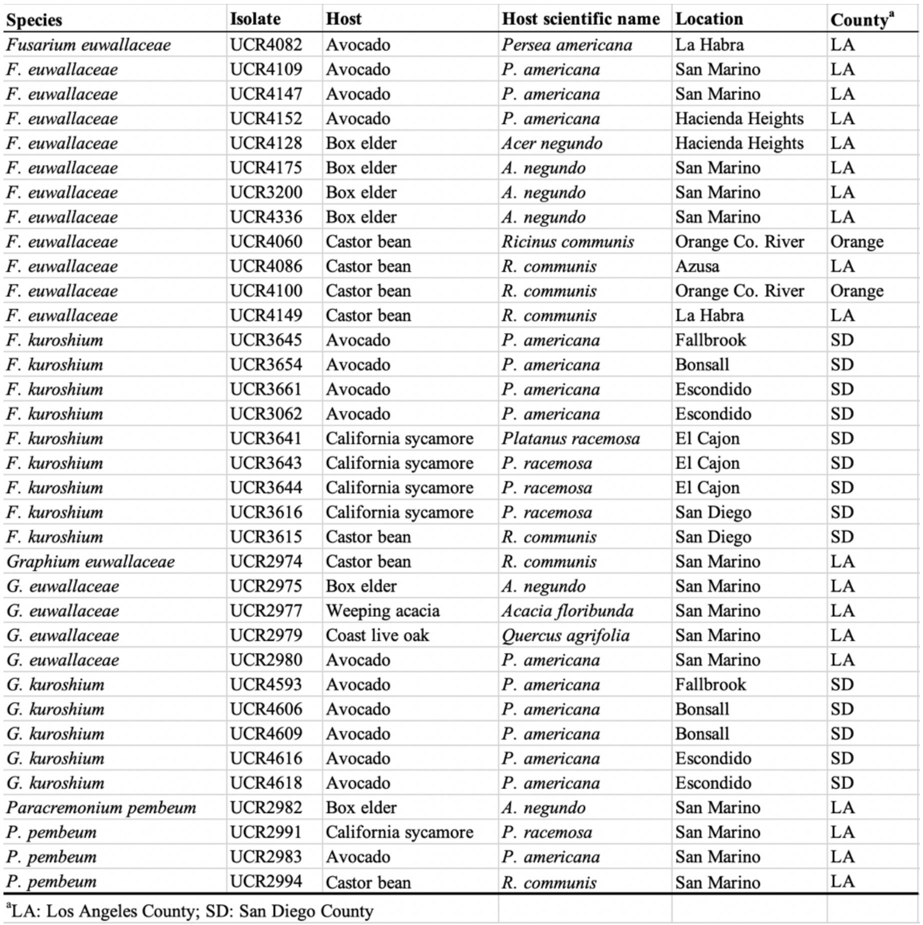
### 1. *In vitro* fungicide screening

**Fungal isolates.** Twelve isolates of *Fusarium euwallaceae*, nine isolates of *Fusarium kuroshium*, five isolates of both *Graphium euwallaceae* and *Graphium kuroshium*, and four isolates of *Paracremonium pembeum* were used in the *in vitro* fungicide screening experiment. These isolates were recovered from samples collected from box elder (*Acer negundo*), castor bean (*Ricinus communis*), California sycamore (*Platanus racemosa*), weeping acacia (*Acacia floribunda*), and coast live oak (*Quercus agrifolia*) showing symptoms of FD in Southern California (Table IV).

***In vitro* fungicide screening methods*.*** We screened 13 fungicides belonging to different chemical families (Table V) on agar media *in vitro* to determine the effective concentration that reduces 50% of mycelial growth (EC50 values) of the 35 fungal isolates using a spiral gradient dilution (SGD) method (Förster et al., 2004).

**Results from *In vitro* fungicide screening**. Pyrimethanil had the highest EC50 against *F. euwallaceae* and Fluopyram had the highest EC50 value against *F. kuroshium* (Table V). Pyrimethanil did not inhibit growth *Fusarium kuroshium*. Fluxapyroxad, trifloxystrobin, and triadimefon showed no inhibition of *Fusarium* spp. at the concentrations tested. Triflumizole had highest EC50 against *Graphium* spp. Myclobutanil, fluxapyroxad, and triadimefon was not effective at inhibiting growth of *Graphium* spp. and *P. pembeum* and Fluopyram did not inhibit growth of *G. euwallaceae*. Thiabendazole had the highest EC50 against *P. pembeum* isolates. Results suggest that a combination of fungicides (i.e., Pyrimethanil, Fluopyram, Triflumizole, Thiabendazole) is needed to inhibit growth of all ISHB fungal symbionts, which in turn would inhibit ISHB-establishment.

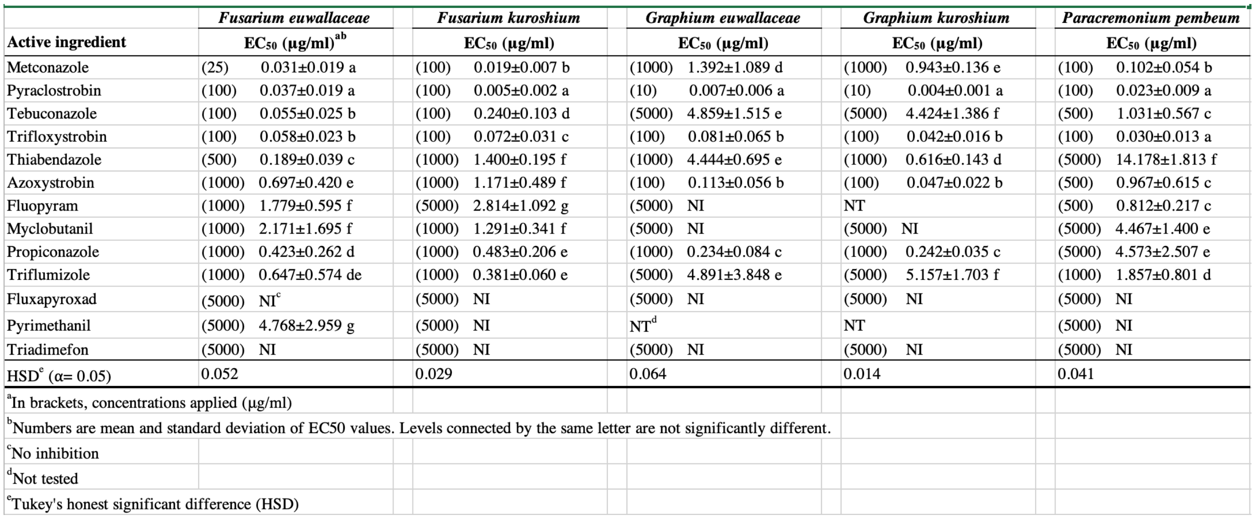
**Table IV**. Fungal isolates used in the *in vitro* fungicide screening experiment



| Species | Isolate | Host | Host scientific name | Location | County[a] |
|---|---|---|---|---|---|
| *Fusarium euwallaceae* | UCR4082 | Avocado | *Persea americana* | La Habra | LA |
| *F. euwallaceae* | UCR4109 | Avocado | *P. americana* | San Marino | LA |
| *F. euwallaceae* | UCR4147 | Avocado | *P. americana* | San Marino | LA |
| *F. euwallaceae* | UCR4152 | Avocado | *P. americana* | Hacienda Heights | LA |
| *F. euwallaceae* | UCR4128 | Box elder | *Acer negundo* | Hacienda Heights | LA |
| *F. euwallaceae* | UCR4175 | Box elder | *A. negundo* | San Marino | LA |
| *F. euwallaceae* | UCR3200 | Box elder | *A. negundo* | San Marino | LA |
| *F. euwallaceae* | UCR4336 | Box elder | *A. negundo* | San Marino | LA |
| *F. euwallaceae* | UCR4060 | Castor bean | *Ricinus communis* | Orange Co. River | Orange |
| *F. euwallaceae* | UCR4086 | Castor bean | *R. communis* | Azusa | LA |
| *F. euwallaceae* | UCR4100 | Castor bean | *R. communis* | Orange Co. River | Orange |
| *F. euwallaceae* | UCR4149 | Castor bean | *R. communis* | La Habra | LA |
| *F. kuroshium* | UCR3645 | Avocado | *P. americana* | Fallbrook | SD |
| *F. kuroshium* | UCR3654 | Avocado | *P. americana* | Bonsall | SD |
| *F. kuroshium* | UCR3661 | Avocado | *P. americana* | Escondido | SD |
| *F. kuroshium* | UCR3062 | Avocado | *P. americana* | Escondido | SD |
| *F. kuroshium* | UCR3641 | California sycamore | *Platanus racemosa* | El Cajon | SD |
| *F. kuroshium* | UCR3643 | California sycamore | *P. racemosa* | El Cajon | SD |
| *F. kuroshium* | UCR3644 | California sycamore | *P. racemosa* | El Cajon | SD |
| *F. kuroshium* | UCR3616 | California sycamore | *P. racemosa* | San Diego | SD |
| *F. kuroshium* | UCR3615 | Castor bean | *R. communis* | San Diego | SD |
| *Graphium euwallaceae* | UCR2974 | Castor bean | *R. communis* | San Marino | LA |
| *G. euwallaceae* | UCR2975 | Box elder | *A. negundo* | San Marino | LA |
| *G. euwallaceae* | UCR2977 | Weeping acacia | *Acacia floribunda* | San Marino | LA |
| *G. euwallaceae* | UCR2979 | Coast live oak | *Quercus agrifolia* | San Marino | LA |
| *G. euwallaceae* | UCR2980 | Avocado | *P. americana* | San Marino | LA |
| *G. kuroshium* | UCR4593 | Avocado | *P. americana* | Fallbrook | SD |
| *G. kuroshium* | UCR4606 | Avocado | *P. americana* | Bonsall | SD |
| *G. kuroshium* | UCR4609 | Avocado | *P. americana* | Bonsall | SD |
| *G. kuroshium* | UCR4616 | Avocado | *P. americana* | Escondido | SD |
| *G. kuroshium* | UCR4618 | Avocado | *P. americana* | Escondido | SD |
| *Paracremonium pembeum* | UCR2982 | Box elder | *A. negundo* | San Marino | LA |
| *P. pembeum* | UCR2991 | California sycamore | *P. racemosa* | San Marino | LA |
| *P. pembeum* | UCR2983 | Avocado | *P. americana* | San Marino | LA |
| *P. pembeum* | UCR2994 | Castor bean | *R. communis* | San Marino | LA |

[a] LA: Los Angeles County; SD: San Diego County

**Table V**. Mean and standard deviation of EC$_{50}$ values for synthetic fungicides used in this study.



| | *Fusarium euwallaceae* | *Fusarium kuroshium* | *Graphium euwallaceae* | *Graphium kuroshium* | *Paracremonium pembeum* |
|---|---|---|---|---|---|
| **Active ingredient** | **EC$_{50}$ (µg/ml)[ab]** | **EC$_{50}$ (µg/ml)** | **EC$_{50}$ (µg/ml)** | **EC$_{50}$ (µg/ml)** | **EC$_{50}$ (µg/ml)** |
| Metconazole | (25) 0.031±0.019 a | (100) 0.019±0.007 b | (1000) 1.392±1.089 d | (1000) 0.943±0.136 e | (100) 0.102±0.054 b |
| Pyraclostrobin | (100) 0.037±0.019 a | (100) 0.005±0.002 a | (10) 0.007±0.006 a | (10) 0.004±0.001 a | (100) 0.023±0.009 a |
| Tebuconazole | (100) 0.055±0.025 b | (100) 0.240±0.103 d | (5000) 4.859±1.515 e | (5000) 4.424±1.386 f | (500) 1.031±0.567 c |
| Trifloxystrobin | (100) 0.058±0.023 b | (100) 0.072±0.031 c | (100) 0.081±0.065 b | (100) 0.042±0.016 b | (100) 0.030±0.013 a |
| Thiabendazole | (500) 0.189±0.039 c | (1000) 1.400±0.195 f | (1000) 4.444±0.695 e | (1000) 0.616±0.143 d | (5000) 14.178±1.813 f |
| Azoxystrobin | (1000) 0.697±0.420 e | (1000) 1.171±0.489 f | (100) 0.113±0.056 b | (100) 0.047±0.022 b | (500) 0.967±0.615 c |
| Fluopyram | (1000) 1.779±0.595 f | (5000) 2.814±1.092 g | (5000) NI | NT | (500) 0.812±0.217 c |
| Myclobutanil | (1000) 2.171±1.695 f | (1000) 1.291±0.341 f | (5000) NI | (5000) NI | (5000) 4.467±1.400 e |
| Propiconazole | (1000) 0.423±0.262 d | (1000) 0.483±0.206 e | (1000) 0.234±0.084 c | (1000) 0.242±0.035 c | (5000) 4.573±2.507 e |
| Triflumizole | (1000) 0.647±0.574 de | (1000) 0.381±0.060 e | (5000) 4.891±3.848 e | (5000) 5.157±1.703 f | (1000) 1.857±0.801 d |
| Fluxapyroxad | (5000) NI[c] | (5000) NI | (5000) NI | (5000) NI | (5000) NI |
| Pyrimethanil | (5000) 4.768±2.959 g | (5000) NI | NT[d] | NT | (5000) NI |
| Triadimefon | (5000) NI | (5000) NI | (5000) NI | (5000) NI | (5000) NI |
| HSD[e] ($\alpha$= 0.05) | 0.052 | 0.029 | 0.064 | 0.014 | 0.041 |

[a] In brackets, concentrations applied (µg/ml)

[b] Numbers are mean and standard deviation of EC50 values. Levels connected by the same letter are not significantly different.

[c] No inhibition

[d] Not tested

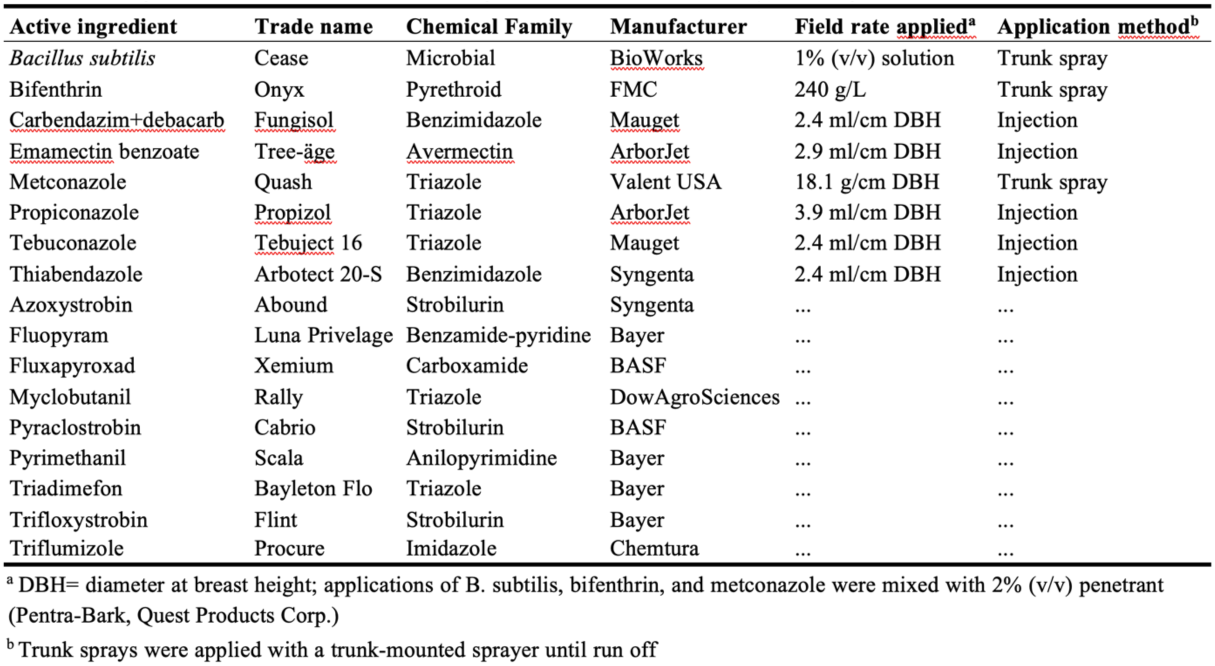
[e] Tukey's honest significant difference (HSD)

### 2. ***In vitro* screening in sawdust rearing media.**

Sawdust rearing media was used to introduce ISHB directly to the various pesticide treatments mixed in the media. All sawdust rearing media treatments were amended with streptomycin (0.35g/L) prior to autoclaving to prevent bacterial contamination, except for the *Bacillus subtilis* without streptomycin treatment. Synthetic and biopesticides (including *B. subtilis* with and without streptomycin) (Table VI) and spores of *Beauvaria bassiana* for the entomopathogen treatment were added after the media had cooled to 60°C and mixed with a sterilized paint mixer attached to a standard power drill. The insecticides used in this study included Bifenthrin (contact) and Emamectin benzoate (systemic). We introduced one inseminated ISHB female into the rearing media containing ten replicates of each treatment and total offspring were counted after 40 days.

Any females present from surviving treatments were sampled with the offspring. We used a sterile toothpick to sample the gallery wall for fungi. The macerated female samples were suspended in 1 ml of sterile 0.01% triton (adult female bodies suspended in 200 ul) and spread plated onto two petri dishes containing PDA amended with tetracycline hydrochloride (0.01%). The PDA plates were incubated at 25°C for one week and colony forming units (CFU) of *Fusarium* spp. and other ISHB fungal associates (*Graphium euwallaceae*, and *Paracremonium pembeum*) were counted.

**Table VI**. Pesticides tested in the *in vitro* sawdust media screening and the first field trial for their ability to manage Fusarium Dieback.



| Active ingredient | Trade name | Chemical Family | Manufacturer | Field rate applied[a] | Application method[b] |
|---|---|---|---|---|---|
| *Bacillus subtilis* | Cease | Microbial | BioWorks | 1% (v/v) solution | Trunk spray |
| Bifenthrin | Onyx | Pyrethroid | FMC | 240 g/L | Trunk spray |
| Carbendazim+debacarb | Fungisol | Benzimidazole | Mauget | 2.4 ml/cm DBH | Injection |
| Emamectin benzoate | Tree-äge | Avermectin | ArborJet | 2.9 ml/cm DBH | Injection |
| Metconazole | Quash | Triazole | Valent USA | 18.1 g/cm DBH | Trunk spray |
| Propiconazole | Propizol | Triazole | ArborJet | 3.9 ml/cm DBH | Injection |
| Tebuconazole | Tebuject 16 | Triazole | Mauget | 2.4 ml/cm DBH | Injection |
| Thiabendazole | Arbotect 20-S | Benzimidazole | Syngenta | 2.4 ml/cm DBH | Injection |
| Azoxystrobin | Abound | Strobilurin | Syngenta | ... | ... |
| Fluopyram | Luna Privelage | Benzamide-pyridine | Bayer | ... | ... |
| Fluxapyroxad | Xemium | Carboxamide | BASF | ... | ... |
| Myclobutanil | Rally | Triazole | DowAgroSciences | ... | ... |
| Pyraclostrobin | Cabrio | Strobilurin | BASF | ... | ... |
| Pyrimethanil | Scala | Anilopyrimidine | Bayer | ... | ... |
| Triadimefon | Bayleton Flo | Triazole | Bayer | ... | ... |
| Trifloxystrobin | Flint | Strobilurin | Bayer | ... | ... |
| Triflumizole | Procure | Imidazole | Chemtura | ... | ... |

[a] DBH= diameter at breast height; applications of B. subtilis, bifenthrin, and metconazole were mixed with 2% (v/v) penetrant (Pentra-Bark, Quest Products Corp.)

[b] Trunk sprays were applied with a trunk-mounted sprayer until run off

**Results from *in vitro* screening in sawdust rearing media.**

***ISHB fecundity.*** Sawdust media amended with all treatments produced significantly less offspring than the untreated control, with bifentrhrin, *B. bassiana,* and *B. subtilis* with streptomycin producing no offspring (Fig 15). Mycosed beetles (beetles colonized by the entomopathogen) were observed in *B. bassiana* treatments. When offspring were separated into female, male, and larvae groups, females were dominant in the the azoxystrobin and *B. subtilis* without streptomycin treatments, which produced offspring. (Fig. 16).

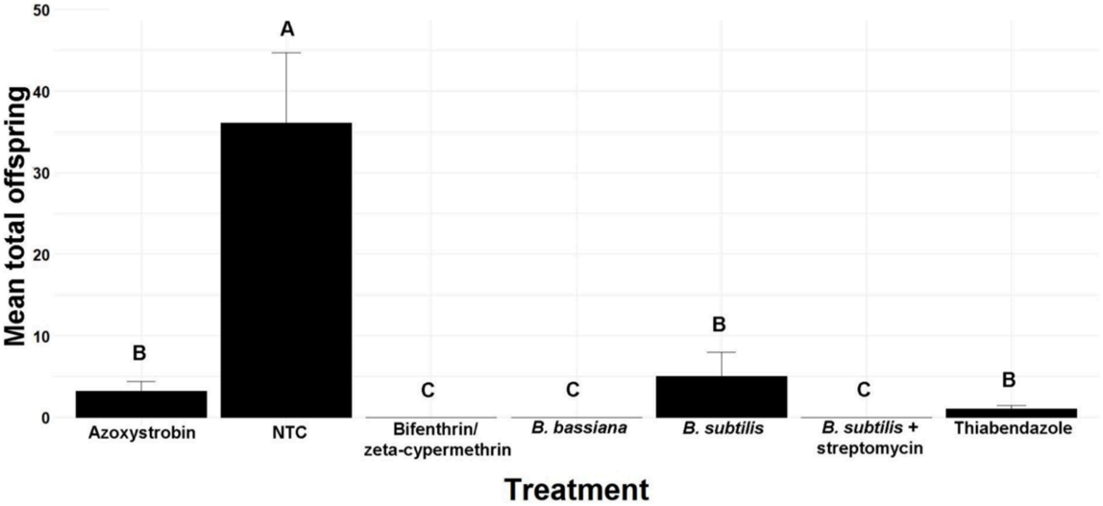


**Figure 15.** Total offspring from sawdust media amended with pesticide treatments with NTC representing the non-treated control. Letters indicate significance from EMM analysis at α=0.05 comparing treatments to each other.

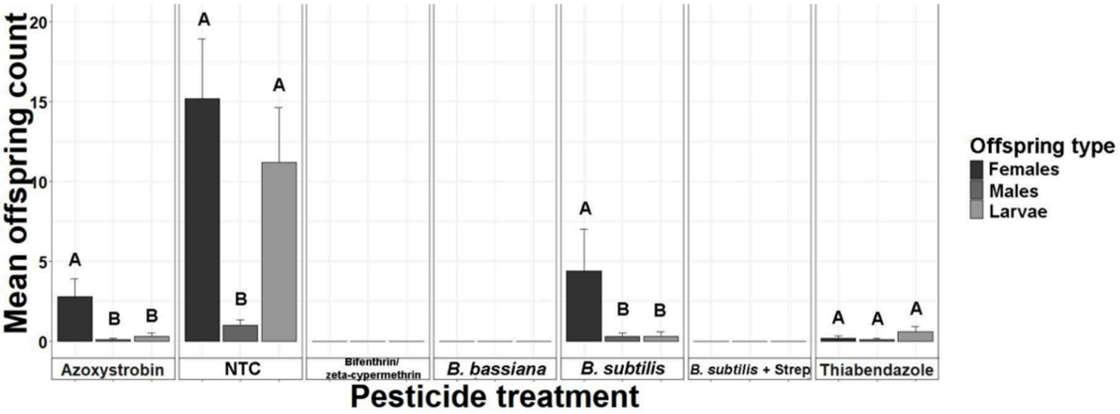


**Figure 16.** Counts of offspring from different sample types from sawdust media amended with pesticide treatments. Letters indicate significance from EMM analysis at α=0.05 comparing offspring types to each other within each treatment.

***Fungal inhibition.*** Media amended with the maximum label rate of autoclaved *B. subtilis* with streptomycin and *B. bassiana* both completely inhibited growth of all symbionts (Fig. 17). It should be noted that max rate of the insecticide bifenthrin/ zeta- cypermethrin (Hero®, 262 ppm

AI) was tested but was not included in the analysis due to lack of inhibition. Across all pesticide treatments that produced offspring, female heads contained significantly more *F. euwallaceae* compared to the other symbionts except those with thiabendazole, where CFUs of *P. pembeum* were not different from those of *F. euwallaceae*. In contrast to the *in vitro* screening in agar, thiabendazole was not effective at inhibiting *P. pembeum* on the female body, head, or within the gallery.

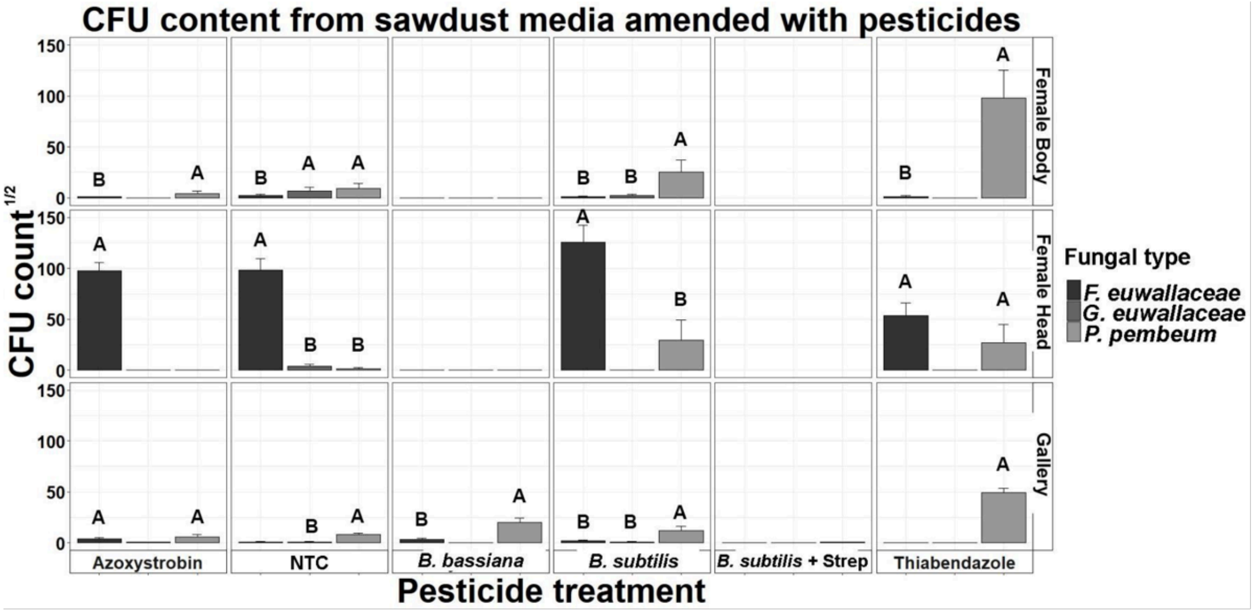


**Figure 17**. CFU content recovered from female head (mycangia), bodies (guts), and gallery samples from sawdust media amended with pesticide treatments. CFU values were transformed by square root and letters indicate significance from EMM analysis at α=0.05 comparing fungal species to each other within each facet.

### 3. *In planta* field experiments

We conducted two separate field experiments on sycamore trees and chose sites based on two criteria: availability of ISHB hosts and level of infestation. The first experiment assessed at post-application attack rates on trees while the second experiment examined beetle entry ratios and gallery success by using 3-D printed traps to artificially introduce. The second experiment tested a smaller set of synthetic pesticides, but included the entomopathogen *B. bassiana.*

### First experiment

***Methods.*** Field trials were established at three county regional parks throughout Orange County California: Yorba Regional Park (33°52'14.4" N, 117°45'45.2" W); Carbon Canyon Regional Park (33°55'19.9" N, 117°50'13.2" W); and Ted Craig Regional Park (33°54'10.2" N, 117°53'04.7" W). The most common host trees available at all three sites included: *Platanus racemosa, Platanus x acerifolia*, *Alnus rhombifolia*, *Liquidambar styraciflua*, *Quercus agrifolia, Salix laevigata*, and *Populus fremontii*, with *P. racemosa* being the most abundant host tree available (Arbor Access Tree Inventory, West Coast Arborists). To evaluate level of infestation, trees were classified by number of beetle entry holes on trunks and dieback presence according to the following criteria: low infestation (<30 entry holes and no dieback); moderate infestation (≥30 entry holes and no dieback); and heavy infestation (≥30 entry holes and dieback present).

Trees from all three sites ranged from 18 to 71 cm diameter at breast height (DBH) and levels of infestation were as follows: Yorba Regional Park (low infestation), Ted Craig Regional Park (moderate infestation), and Carbon Canyon Regional Park (high infestation). Based on these criteria, 80 sycamore trees from each park were randomly selected for inclusion into the pesticide trial using a randomized complete block design with each park functioning as a block. Each of the 80 eighty trees were randomly assigned to one of 10 pesticide treatments for a total of 8 trees per treatment (Table VI). Assigned trees were sorted by DBH and initial entry holes to check for similar DBH and entry holes across all treatments using ANOVA; no significant differences ($P > 0.05$) in initial entry holes were detected between treatments. Trees were treated with the pesticides and rates in Table VI. Trees treated with thiabendazole, emamectin benzoate, propiconazole, and emamectin benzoate + propiconazole were injected using the Arborjet QUIK-jet Air injector (Arborjet, Woburn, MA); trees treated with carbendazim + debacarb and tebuconazole were injected using Chemjet tree injectors (Queensland Plastics, Australia); and trees treated with *Bacillus subtilis* (strain QST713), metconazole, and bifenthrin were mixed with a bark penetrant (Pentra-Bark®) at a rate of 2.9 ml/cm DBH and applied with a spray rig until run-off.

Trees were evaluated every month (approximately 28 days), for 12 months, following pesticide application by counting the number of beetle entry holes on the bole as described above. Loose bark from trees was removed with a plastic putty knife. Oil-based paint pens (Diagraph MSP) were used to count entry holes by dotting to the right of beetle entry holes and a unique color pen was used for every month.

***Results from first experiment.*** Mean attacks/m$^2$ of both treated and untreated control trees increased over time (Fig. 18). Significant differences in attacks were observed when comparing parks, therefore each park was analyzed individually. With the exception of propiconazole and thiabendazole, treatments in Yorba Regional Park (low infestation) did not decrease attack rates compared to untreated control trees (Fig. 18). Tebuconazole and bifenthrin were the most effective at reducing attacks in Craig Regional park (moderate infestation). Metconazole was the most effective at reducing attack rates in Carbon Canyon Regional Park (high infestation), followed by carbendazim + debacarb, emamectin benzoate, and emamectin benzoate + propiconazole (Fig. 18). Tebuconazole was highly effective initially in Carbon Canyon, but attacks spiked in months 11 and 12, suggesting that reapplication is necessary after ten months.

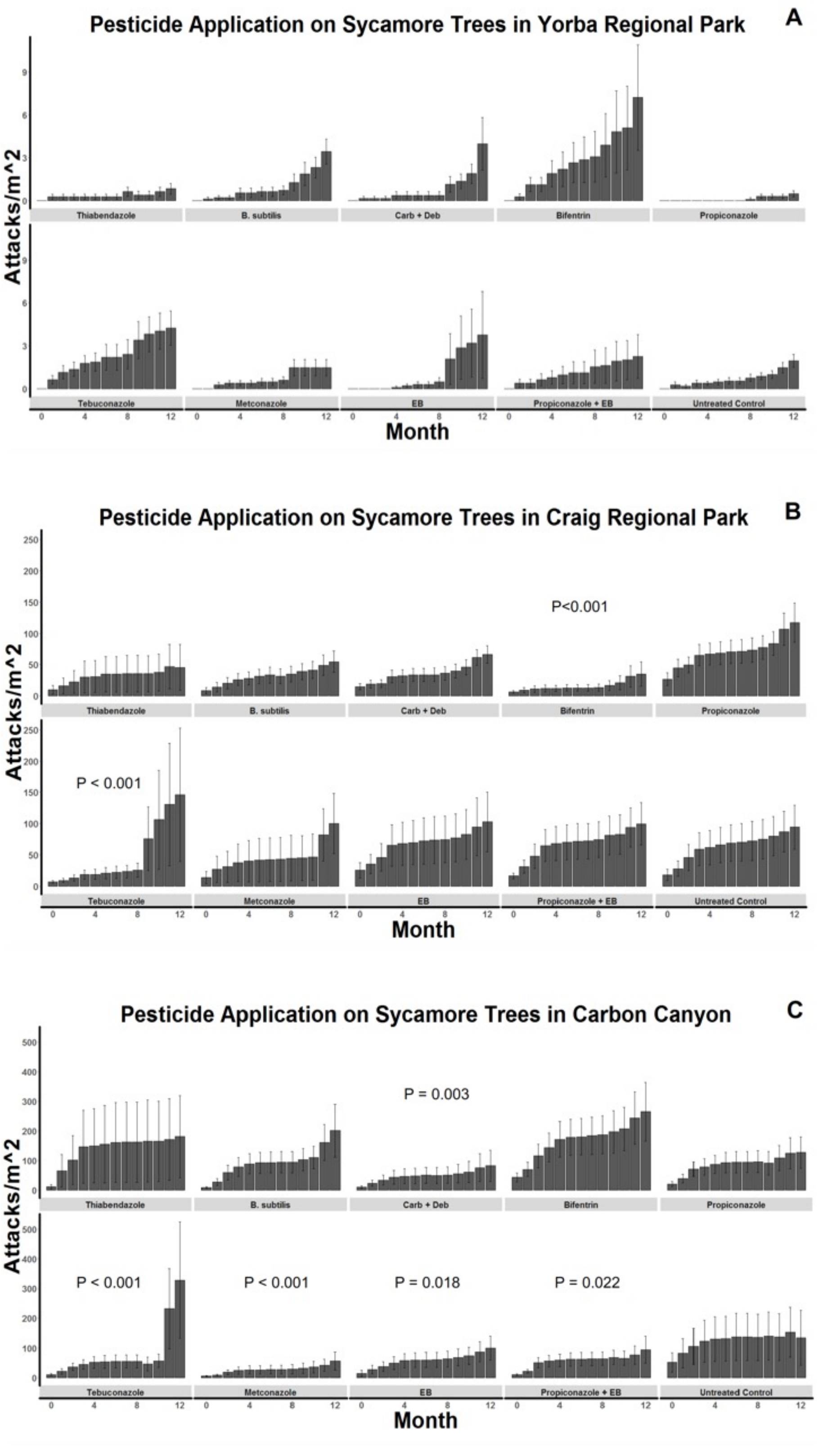


**Figure 18.** Mean accumulated shot hole borer attacks per square meter for pesticide treated trees at A). Yorba Regional Park, B) Craig Regional Park, and C) Carbon Canyon Regional Park. Vertical bars represent standard error of means. P values are derived from comparison between individual treatments and untreated controls at $\alpha$=0.05.

### Second Experiment

***Methods.*** Locations in Riverside (UCR Agricultural Operations) and Pomona (Cal Poly Pomona, CPP) were being used to introduce live lab-reared inseminated ISHB females into treated host tree branches. The experimental design was done by RCBD with each treatment being randomized on branches within each tree (block) on five trees per location over two trials. Selected branches were spray treated until runoff with either 2700 ppm azoxystrobin (Abound®), 50ppm thiabendazole (Arbortect 20S®), 262ppm bifenthrin/ zeta-cypermethrin (Hero®), 30 percent (v/v) *B. subtilis* (Serenade® ASO), a solution of 9x1010 spores/ml *B. bassiana* (Mycotrol WPO®), and an untreated control (Table VII). Twenty-four hours post-application, four beetles were introduced using 3D printed traps (Figure 16D) (2 beetles per branch) and fastened to the tree with steel screws to secure the traps. After 40 days the infested wood was removed to evaluate the entry ratio of the 4 beetles, as well as the gallery success if tunnels were greater than 10 mm in length.

**Table VII**. Pesticides used in the second field trial.

| Active ingredient | Chemical family | Trade Name | Manufacturer | Rate(s) Applied[a] |
|---|---|---|---|---|
| *Bacillus subtilis* | Biological | Serenade ASO | Bayer | 30% (v/v) solution |
| *Beauvaria bassiana* | Biological | Mycotrol WPO | BioWorks | 9x$10^{10}$ spores/ml |
| Azoxystrobin | Strobilurin | Abound | Syngenta | 2700 ppm |
| Thiabendazole | Benzimidazole | Arbotect-20S | Syngenta | 50 ppm |
| Bifenthrin/Zeta-Cypermethrin | Pyrethroid | Hero | FMC | 262, 100, 50, 25, 10 ppm |

[a] Multiple rates were only used for Bifenthrin/Zeta-Cypermethrin in one portion of the study

***Results second experiment***. The UCR and CPP field sites were analyzed separately due to the difference in cultivar and age of the trees although there was no significant difference ($P < 0.05$) between the two data sets. In the UCR field trial, entry ratios for both bifenthrin/ zeta-cypermethrin and *B. bassiana* were lower than the control but were not significant ($P > 0.05$) (Fig. 19a). However, gallery success was significantly lower ($P < 0.05$) in bifenthrin/ zeta-cypermethrin compared to all other groups including the control (Fig. 19b). At the CPP site, bifenthrin/ zeta-cypermethrin had the lowest entry ratio compared to all other groups. Measuring gallery success, bifenthrin/ zeta-cypermethrin was the only treatment significantly lower ($P < 0.05$) than the control. Gallery success in azoxystrobin treatments were lower than the control but this interaction was not significant ($P > 0.05$).

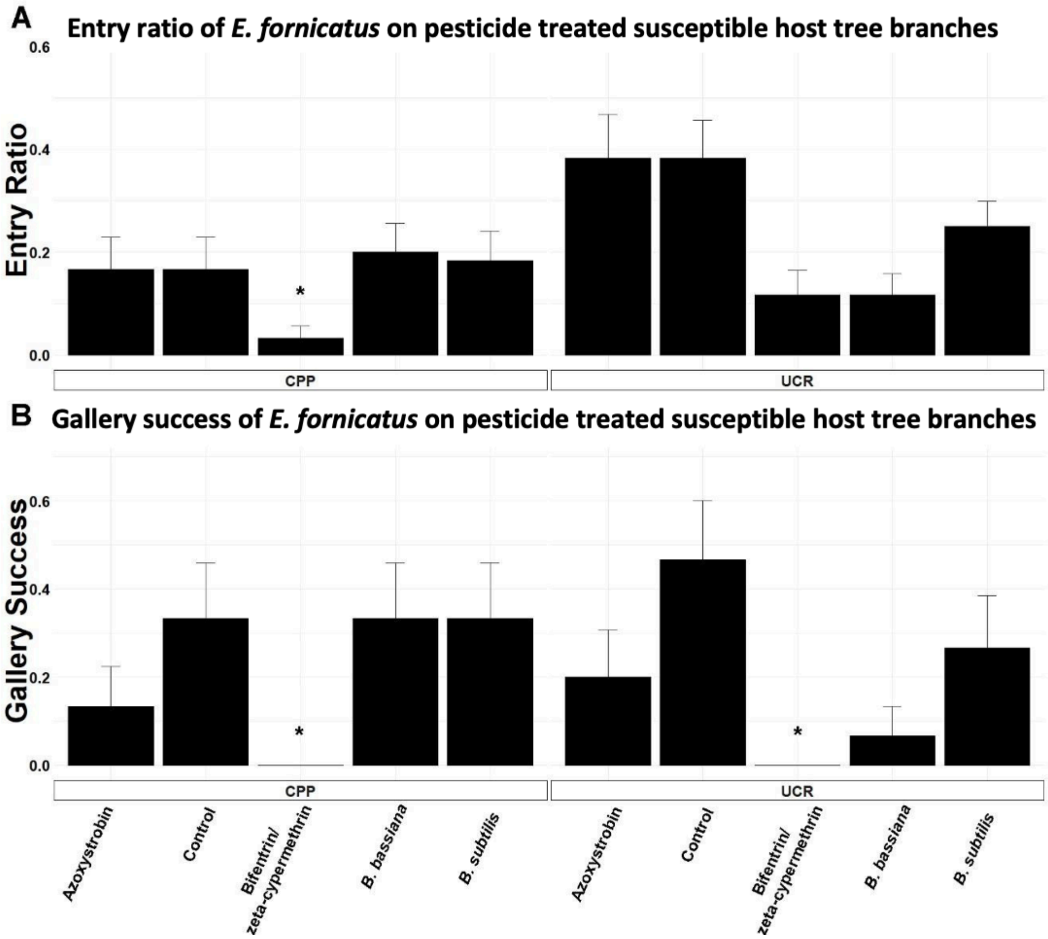


**Figure 19**. (a) Entry ratios for PSHB introduced via 3D traps in pesticide-treated avocado wood recovered from field trials. (b) Gallery success for PSHB introduced via 3D trapsin pesticide-treated avocado wood recovered from field trials. Asterisks indicate significance from EMM analysis at α=0.05 comparing fungal species to the non-treatedcontrol (NTC) within each plot.

## Applications for Management

Please refer to discussion below.

## Objective 7: Evaluate pesticide retention on host tissues

Systemic pesticide residues within plant tissue organs (e.g. leaves, seeds, and other reproductive organs) may adversely affect native insects and animals, leading to unintended trophic alterations that result from bioaccumulation. Here, we determined pesticide retention in field injected trees to evaluate potential movement of pesticide residues into peripheral plant organs on selected injected trees.

## Methods

Bioassays were conducted in our first field experiment (Objective 6) according to the method of Mayfield et al. (2008) to determine pesticide retention in field injected trees. At 12 months post-injection, tree cores were taken from all treated trees at Yorba Regional Park for all treatments except bifenthrin and emamectin benzoate treatments as these pesticides are insecticides. Briefly, wood cores (approximately 1 cm x 8 cm, each) were removed from pesticide treated and untreated trees using an increment borer in four cardinal directions at approximately 1.2 m above the soil line. Bark was removed and remaining xylem cores were placed onto PDA-t seeded with spores (1 × 106 ml) of *F. euwallaceae* (UCR4082) which were applied using an atomizer. Plates were incubated for six days and scored (ratings 0-3) using a modified rating-scale from Stennes and French (1987) as follows: 0, fungal growth over entire plug; 1, fungal growth on part of the plug; 2, no fungal growth on plug; 3, no fungal growth on plug + presence of zone of inhibition (ZOI) in agar medium. Scores ≥1 are considered to show inhibition. The number of trees showing inhibition was calculated from the presence of at least one core with a score ≥1 and percent inhibition was calculated as the percent of plugs per tree with scores ≥1.

## Results

Results from the bioassay study showed that contact (metconazole) and systemic (tebuconazole, carbendazim, propiconazole) pesticides showed little retention after 12 months post application (Table VI), suggesting these pesticides do not rapidly move systemically through the plant. All the thiabendazole treated tree samples exhibited retention in the bioassay but it was not effective in reducing attack of ISHB on California sycamore in moderate to heavily infested sites.

Table VI. Pesticide retention bioassay from treated California sycamores

| | | | % of samples per tree exhibiting inhibition | | | % of samples per tree exhibiting ZOI[a] in agar | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **No. of trees** | **No. of trees with samples exhibiting inhibition** | **Mean** | **Range** | **P-value[b]** | **Mean** | **Range** |
| Thiabendazole | 8 | 8 | 47 | 25-75 | < 0.0001 | 16 | 25-50 |
| Propiconazole | 8 | 5 | 50 | 25-100 | 0.0001 | 0 | 0 |
| Propiconazole + emamectin benzoate | 8 | 5 | 45 | 25-75 | 0.0004 | 0 | 0 |
| Carbendazim + Debacarb | 8 | 5 | 25 | 25 | 0.0127 | 0 | 0 |
| Tebuconazole | 8 | 5 | 25 | 25 | 0.0127 | 0 | 0 |
| Metconazole | 7 | 1 | 25 | 25 | > 0.05 | 0 | 0 |
| *B. subtilis* | 8 | 0 | ... | ... | ... | ... | ... |
| Untreated | 8 | 0 | ... | ... | ... | ... | ... |

[a] Zone of inhibition

[b] p-values for treatments are in comparison to untreated controls using independent contrast

### Applications for Management

After conducting the pesticide experiments, we determined that the pesticides did not move through the plant or were retained enough to be detected in the more distal leaf and fruit organs.

## SYNTHESIS AND FUTURE STUDIES

The use of insecticides and/or fungicides is promising for the management of ISHB-FD in southern California especially when used as part of an IPM strategy for this pest-disease complex. It should be noted that the labels should be checked for their registration status for each tree, and professionals must be consulted prior to application. The commercially available fungicide azoxystrobin in Florida, which is already registered to use to control other common diseases (Schaffer et al 2013), didn't show any inhibition to the growth of the fungal symbionts *in vitro*. Because the high application rate achieves such a mild effect, this fungicide may not be the best approach for this pest. The variation in efficacy of synthetic pesticides depending on infestation levels at different sites suggests that treatments may not be broadly effective and need to be tailored to the appropriate conditions at each site. For example, thiabendazole may be more appropriate for use in lightly infested sites, bifenthrin in moderately infested sites, and metconazole in heavily infested sites. It has been suggested that products with longer retention times (e.g., thiabendazole) may reduce fungitoxicity. The timing of pesticide application should be further investigated to develop an integrated management plan to effectively reduce populations of the pest while reducing the volume of chemical pesticide applied. This may be possible by targeting the pests in the colder winter months since their activity has been previously shown to decrease in these times (Lynch et al. 2018; Mayorquin et al. 2018). Therefore, further chemical studies using other hosts of ISHB-FD and longer trial times (> 4 years) will be necessary to determine application intervals and pesticide effectiveness over time.

Current management suggestions in other hosts (Eatough-Jones et al. 2015) as well as avocado (Mendel et al. 2017) have suggested removal of infested material to reduce localized populations. However, when infested branches are pruned away, beetles may still remain in areas that cannot be pruned away. The best approach is to manage FD-ISHB at this time is through a combination of cultural practices such as pruning, removing infested material coupled with application of chemical control strategies, when necessary, to treat remaining populations of ISHB that cannot be pruned away. Our investigation into pesticides for use in infested hosts indicate some candidates with significant effectiveness against ISHB and their fungal symbionts from *in vitro* and in field trials. An IPM strategy of pruning and different synthetic pesticides at appropriate times can aid in reducing local ISHB populations and help reduce disease pressure and prevent further spread to other areas.

Pesticide use in native vegetation can be limited and cause some difficulty in management decisions of certain foliar plant diseases (Van Bruggen and Finckh 2016). Organic biological control agents such as *B. subtilis* have been of interest because of their anti-fungal properties (Fiddaman and Rossal 1993) and effectiveness against *F. euwallaceae* and *F. kuroshium*. We observed *B. subtilis* to be effective in reducing the mycelial growth of all ISHB fungal symbionts *in vitro* on agar and amended sawdust. Interestingly, when the commercial product was amended to media with streptomycin and autoclaved, inhibition of all symbiotic fungi and prevention of offspring production from ISHB was achieved indicating the bacteria may be producing heat stable antifungal agent(s), which has been reported previously in *B. subtilis*. (Munimbazi and Bullerman 1998; Walker et al. 1998). However, field application of the product was less effective, which is likely due to application requirements by the label. At this time, commercially available *B. subtilis* cannot be injected into trees. As such, the local *B. subtilis* strains and other endophytes we recovered from trees in this project may be better candidates for application into host wood tissues, which have been shown to have similar inhibitory effect to QST713 against *F. euwallaceae* and *F. kuroshium* in vitro and in planta experiments on avocado (Na 2016). We are currently developing methods to introduce the local endophytes we isolated from this project in San Diego county (including species of *Bacillus*) into trees at a large scale. Future studies testing these methods in the field will be needed, in addition to testing whether different combinations of endophytes have a greater effect at inhibiting beetle establishment.

The entomopathogen *B. bassiana* used in this project was also very effective at reducing ISHB offspring when deposited on sawdust media and moderately effective in field trials. The reduction in effectiveness on wood application may be due to the lack of optimal conditions for the entomopathogenic fungi to proliferate on the wood as these treatments were applied toward the end of spring and again in the warm summer months. High humidity in riparian areas may provide better conditions for this fungus to persist in absence of the target insect host. It has been reported that conidia of *B. bassiana* can be sensitive to other environmental factors such as solar radiation which was greatly improved when applied with 10% humic acid (Kaiser et al. 2018). Using our risk model, we have identified candidate target sites in native vegetation to conduct experiments on best *B. bassiana* delivery methods. Our preliminary investigation into landscape considerations suggests these sites may be an inoculum reservoir, preserving the regional beetle population during unfavorable conditions, and serving as a source from which beetles may spread when conditions are favorable. Controlling beetle populations at these sites may reduce risk of spread to other more susceptible and vulnerable sites. In the meantime, non-infested sites with a high probability of beetle establishment should be monitored regularly to elicit a rapid response.

### Objective 8: Develop and train land managers on Best Protocols for IPM in Native Vegetation.

We gave several field and indoor workshops since the inception of this project (Fig. 20) and developed many handouts in collaboration with our partners based on the research that came out of this project (Appendix). SANDAG is acknowledged in these handouts.

**Figure 20.** Research update workshop with Land managers in San Diego

**Placeholder supplementary figure map**

**Placeholder supplementary map**

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**Supplementary Table I.** Native range and infested distribution (California—CA or South Africa—ZA) of tree and shrub ISHB host species organized phylogenetically from the most to least ancestral lineages. Non-competent hosts are either attacked by the beetle species alone (A) or attacked by beetles and colonized by *Fusarium* spp. (F). Competent hosts support beetle-pathogen reproduction and are killed (K) or not killed (NK) when attacked. This table was published in Lynch et al. (2021).

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Pinales | Cupressaceae | *Juniperus chinensis* | F | | | CA |
| Pinales | Cupressaceae | *Juniperus virginiana* | A | | | CA |
| Pinales | Cupressaceae | *Metasequoia glyptostroboides* | F | | | CA |
| Pinales | Cupressaceae | *Taxodium distichum* | F | | | CA & ZA |
| Pinales | Podocarpaceae | *Afrocarpus falcatus* | F | | ZA | ZA |
| Pinales | Podocarpaceae | *Afrocarpus gracilior* | A | | | CA |
| Pinales | Podocarpaceae | *Podocarpus henkelii* | F | | ZA | CA & ZA |
| Pinales | Pinaceae | *Cedrus atlantica* | A | | | CA |
| Pinales | Pinaceae | *Keteleeria evelyniana* | A | | | CA |
| Pinales | Pinaceae | *Pinus densiflora* | A | | | CA |
| Pinales | Pinaceae | *Pinus douglasiana* | A | | | CA |
| Laurales | Lauraceae | *Beilschmiedia miersii* | A | | | CA |
| Laurales | Lauraceae | *Cinnamomum camphora* | F | | | CA & ZA |
| Laurales | Lauraceae | *Cinnamomum glanduliferum* | A | | | CA |
| Laurales | Lauraceae | *Machilus thunbergii* | F | | | CA |
| Laurales | Lauraceae | *Nothaphoebe sp.* | A | | | CA |
| Laurales | Lauraceae | *Persea americana* | | NK | | CA & ZA |
| Laurales | Lauraceae | *Umbellularia californica* | F | | CA | CA |
| Laurales | Monimiaceae | *Peumus boldus* | A | | | CA |
| Magnoliales | Magnoliaceae | *Liriodendron tulipifera* | F | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia delavayi* | A | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia doltsopa* | A | | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Magnoliales | Magnoliaceae | *Magnolia grandiflora* | | NK | | CA & ZA |
| Magnoliales | Magnoliaceae | *Magnolia guatemalensis* | A | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia soulangeana* | A | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia* sp. | A | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia veitchii* | F | | | CA |
| Magnoliales | Magnoliaceae | *Magnolia virginiana* | | NK | | CA |
| Poales | Poaceae | *Bambusa oldhamii* | A | | | CA |
| Poales | Poaceae | *Bambusa* sp. | A | | | CA |
| Arecales | Arecaceae | *Archontophoenix cunninghamiana* | | NK | | CA |
| Arecales | Arecaceae | *Butia capitata* | A | | | CA |
| Arecales | Arecaceae | *Chamaedorea elegans* | F | | | CA |
| Arecales | Arecaceae | *Howea forsteriana* | | NK | | CA |
| Arecales | Arecaceae | *Livistona chinensis* | F | | | CA |
| Arecales | Arecaceae | *Ptychosperma elegans* | | NK | | CA |
| Arecales | Arecaceae | *Washingtonia filifera* | F | | CA | CA |
| Arecales | Arecaceae | *Washingtonia robusta* | A | | | CA |
| Asparagales | Asparagaceae | *Dracaena draco* | A | | | CA |
| Ranunculales | Menispermaceae | *Cocculus laurifolius* | | NK | | CA |
| Ranunculales | Menispermaceae | *Cocculus orbiculatus* | F | | | CA |
| Ranunculales | Papaveraceae | *Bocconia arborea* | A | | | CA |
| Proteales | Proteaceae | *Banksia saxicola* | F | | | CA |
| Proteales | Proteaceae | *Macadamia integrifolia* | F | | | CA & ZA |
| Proteales | Proteaceae | *Protea mundii* | F | | ZA | ZA |
| Proteales | Platanaceae | *Platanus acerifolia* | | K | | CA & ZA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Proteales | Platanaceae | *Platanus hispanica* | | K | | CA |
| Proteales | Platanaceae | *Platanus mexicana* | | NK | | CA |
| Proteales | Platanaceae | *Platanus occidentalis* | F | | | CA & ZA |
| Proteales | Platanaceae | *Platanus racemosa* | | K | CA | CA & ZA |
| Proteales | Platanaceae | *Platanus wrightii* | F | | | CA |
| Caryophyllales | Tamaricaceae | *Tamarix ramosissima* | | NK | | CA |
| Cornales | Cornaceae | *Alangium sp.* | A | | | CA |
| Cornales | Cornaceae | *Camptotheca acuminata* | F | | | CA |
| Cornales | Cornaceae | *Cornus controversa* | F | | | CA |
| Ericales | Pentaphylacaceae | *Cleyera japonica* | F | | | CA |
| Ericales | Ebenaceae | *Diospyros dichrophylla* | F | | ZA | ZA |
| Ericales | Ebenaceae | *Diospyros kaki* | A | | | CA |
| Ericales | Ebenaceae | *Diospyros lycioides* | F | | ZA | ZA |
| Ericales | Primulaceae | *Rapanea melanophloeos* | F | | ZA | ZA |
| Ericales | Theaceae | *Camellia japonica* | F | | | CA & ZA |
| Ericales | Theaceae | *Camellia reticulata* | F | | | CA |
| Ericales | Theaceae | *Camellia semiserrata* | | NK | | CA |
| Ericales | Ericaceae | *Arbutus unedo* | A | | | CA |
| Aquifoliales | Aquifoliaceae | *Ilex cornuta* | | NK | | CA |
| Aquifoliales | Aquifoliaceae | *Ilex latifolia* | F | | | CA |
| Asterales | Asteraceae | *Baccharis pilularis* | | NK | | CA |
| Asterales | Asteraceae | *Baccharis salicina* | | NK | CA | CA |
| Asterales | Asteraceae | *Verbesina gigantea* | A | | | CA |
| Dipsacales | Adoxaceae | *Viburnum sinensis* | F | | | ZA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Apiales | Pittosporaceae | *Hymenosporum flavum* | F | | | CA |
| Apiales | Pittosporaceae | *Pittosporum undulatum* | F | | | CA |
| Apiales | Araliaceae | *Cussonia spicata* | F | | ZA | CA & ZA |
| Apiales | Araliaceae | *Fatsia japonica* | F | | | CA |
| Boraginales | Boraginaceae | *Cordia caffra* | F | | ZA | ZA |
| Boraginales | Boraginaceae | *Wigandia urens* | A | | | CA |
| Lamiales | Oleaceae | *Chionanthus retusus* | F | | | CA |
| Lamiales | Oleaceae | *Fraxinus excelsior* | F | | | CA & ZA |
| Lamiales | Oleaceae | *Fraxinus uhdei* | F | | | CA |
| Lamiales | Oleaceae | *Fraxinus velutina* | A | | CA | CA |
| Lamiales | Oleaceae | *Olea europaea* | F | | | CA & ZA |
| Lamiales | Oleaceae | *Olea sp.* | F | | ZA | ZA |
| Lamiales | Oleaceae | *Osmanthus fragrans* | A | | | CA |
| Lamiales | Scrophulariaceae | *Buddleja saligna* | F | | ZA | ZA |
| Lamiales | Scrophulariaceae | *Myoporum laetum* | F | | | CA |
| Lamiales | Stilbaceae | *Halleria lucida* | F | | ZA | ZA |
| Lamiales | Stilbaceae | *Nuxia floribunda* | F | | ZA | CA & ZA |
| Lamiales | Bignoniaceae | *Catalpa speciosa* | F | | | CA |
| Lamiales | Bignoniaceae | *Handroanthus impetiginosus* | A | | | CA |
| Lamiales | Bignoniaceae | *Jacaranda mimosifolia* | | NK | | CA & ZA |
| Lamiales | Bignoniaceae | *Markhamia lutea* | A | | | CA |
| Lamiales | Bignoniaceae | *Spathodea campanulata* | | NK | | CA |
| Lamiales | Verbenaceae | *Aloysia sp.* | F | | | CA |
| Gentianales | Apocynaceae | *Cascabela thevetioides* | A | | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Gentianales | Apocynaceae | *Plumeria rubra* | F | | | CA & ZA |
| Saxifragales | Altingiaceae | *Liquidambar formosana* | F | | | CA |
| Saxifragales | Altingiaceae | *Liquidambar styraciflua* | | K | | CA & ZA |
| Vitales | Vitaceae | *Vitis vinifera* | F | | | CA & ZA |
| Celastrales | Celastraceae | *Gymnosporia buxifolia* | F | | ZA | ZA |
| Oxalidales | Cunoniaceae | *Cunonia capensis* | A | | | CA & ZA |
| Oxalidales | Elaeocarpaceae | *Crinodendron patagua* | A | | | CA |
| Malpighiales | Phyllanthaceae | *Bischofia javanica* | F | | | CA |
| Malpighiales | Salicaceae | *Dovyalis caffra* | F | | | CA & ZA |
| Malpighiales | Salicaceae | *Populus fremontii* | | K | CA | CA |
| Malpighiales | Salicaceae | *Populus nigra* | | K | | CA & ZA |
| Malpighiales | Salicaceae | *Populus tremuloides* | | NK | CA | CA |
| Malpighiales | Salicaceae | *Populus trichocarpa* | | K | CA | CA |
| Malpighiales | Salicaceae | *Salix alba* | | NK | | ZA |
| Malpighiales | Salicaceae | *Salix babylonica* | | NK | | CA |
| Malpighiales | Salicaceae | *Salix exigua* | F | | CA | CA |
| Malpighiales | Salicaceae | *Salix gooddingii* | | K | CA | CA |
| Malpighiales | Salicaceae | *Salix laevigata* | | K | CA | CA |
| Malpighiales | Salicaceae | *Salix lasiolepis* | | K | CA | CA |
| Malpighiales | Salicaceae | *Salix mucronata* | | NK | ZA | ZA |
| Malpighiales | Salicaceae | *Xylosma congesta* | | NK | | CA |
| Malpighiales | Euphorbiaceae | *Jatropha cinerea* | F | | | CA |
| Malpighiales | Euphorbiaceae | *Manihot esculenta* | A | | | CA |
| Malpighiales | Euphorbiaceae | *Ricinus communis* | | K | | CA & ZA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Malpighiales | Euphorbiaceae | *Triadica sebifera* | A | | | CA |
| Malpighiales | Euphorbiaceae | *Vernicia fordii* | A | | | CA |
| Rosales | Rosaceae | *Chaenomeles sinensis* | A | | | CA |
| Rosales | Rosaceae | *Eriobotrya japonica* | F | | | CA & ZA |
| Rosales | Rosaceae | *Malus floribunda* | A | | | CA |
| Rosales | Rosaceae | *Prunus africana* | F | | ZA | ZA |
| Rosales | Rosaceae | *Prunus caroliniana* | A | | | CA |
| Rosales | Rosaceae | *Prunus cerasoides* | A | | | CA |
| Rosales | Rosaceae | *Prunus mume* | F | | | CA |
| Rosales | Rosaceae | *Prunus nigra* | F | | | ZA |
| Rosales | Rosaceae | *Prunus persica* | F | | | CA & ZA |
| Rosales | Rosaceae | *Prunus serrulata* | F | | | CA |
| Rosales | Rosaceae | *Pyrus calleryana* | F | | | CA |
| Rosales | Rosaceae | *Pyrus kawakamii* | F | | | CA |
| Rosales | Ulmaceae | *Ulmus alata* | F | | | CA |
| Rosales | Ulmaceae | *Ulmus americana* | F | | | CA |
| Rosales | Ulmaceae | *Ulmus minor* | F | | | CA & ZA |
| Rosales | Ulmaceae | *Ulmus parvifolia* | F | | | CA & ZA |
| Rosales | Ulmaceae | *Zelkova serrata* | F | | | CA |
| Rosales | Rhamnaceae | *Frangula californica* | A | | CA | CA |
| Rosales | Rhamnaceae | *Ziziphus jujuba* | F | | | CA |
| Rosales | Moraceae | *Broussonetia papyrifera* | A | | | CA |
| Rosales | Moraceae | *Ficus altissima* | | NK | | CA |
| Rosales | Moraceae | *Ficus benjamina* | A | | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Rosales | Moraceae | *Ficus carica* |  | NK |  | CA & ZA |
| Rosales | Moraceae | *Ficus macrophylla* | F |  |  | CA |
| Rosales | Moraceae | *Ficus maxima* | A |  |  | CA |
| Rosales | Moraceae | *Ficus natalensis* | F |  | ZA | ZA |
| Rosales | Moraceae | *Ficus platypoda* | F |  |  | CA |
| Rosales | Moraceae | *Morus alba* | F |  |  | CA & ZA |
| Rosales | Urticaceae | *Pipturus argenteus* | F |  |  | CA |
| Fagales | Fagaceae | *Fagus crenata* |  | NK |  | CA |
| Fagales | Fagaceae | *Fagus sylvatica* | F |  |  | CA |
| Fagales | Fagaceae | *Quercus acutidens* | A |  | CA | CA |
| Fagales | Fagaceae | *Quercus acutissima* | A |  |  | CA |
| Fagales | Fagaceae | *Quercus agrifolia* |  | NK | CA | CA |
| Fagales | Fagaceae | *Quercus alba* | A |  |  | CA |
| Fagales | Fagaceae | *Quercus buckleyi* | A |  |  | CA |
| Fagales | Fagaceae | *Quercus chrysolepis* |  | NK | CA | CA |
| Fagales | Fagaceae | *Quercus coccinea* | A |  |  | CA |
| Fagales | Fagaceae | *Quercus engelmannii* |  | NK | CA | CA |
| Fagales | Fagaceae | *Quercus ilex* | F |  |  | CA |
| Fagales | Fagaceae | *Quercus lobata* |  | K | CA | CA |
| Fagales | Fagaceae | *Quercus macrocarpa* |  | NK |  | CA |
| Fagales | Fagaceae | *Quercus mexicana* | F |  |  | CA |
| Fagales | Fagaceae | *Quercus palustris* | F |  |  | CA & ZA |
| Fagales | Fagaceae | *Quercus polymorpha* | A |  |  | CA |
| Fagales | Fagaceae | *Quercus robur* |  | K |  | CA & ZA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Fagales | Fagaceae | *Quercus rubra* | A | | | CA |
| Fagales | Fagaceae | *Quercus rugosa* | A | | | CA |
| Fagales | Fagaceae | *Quercus shumardii* | F | | | CA |
| Fagales | Fagaceae | *Quercus suber* | | NK | | CA |
| Fagales | Fagaceae | *Quercus virginiana* | F | | | CA |
| Fagales | Fagaceae | *Quercus wislizeni* | A | | CA | CA |
| Fagales | Juglandaceae | *Carya illinoinensis* | F | | | CA & ZA |
| Fagales | Juglandaceae | *Juglans mandshurica* | A | | | CA |
| Fagales | Juglandaceae | *Juglans nigra* | A | | | CA |
| Fagales | Juglandaceae | *Pterocarya sp.* | A | | | CA |
| Fagales | Juglandaceae | *Pterocarya stenoptera* | | NK | | CA |
| Fagales | Betulaceae | *Alnus incana* | A | | CA | CA |
| Fagales | Betulaceae | *Alnus rhombifolia* | | NK | CA | CA |
| Fagales | Betulaceae | *Betula pendula* | F | | ZA | CA & ZA |
| Fagales | Betulaceae | *Corylus colurna* | F | | | CA |
| Fagales | Casuarinaceae | *Casuarina cunninghamiana* | F | | | CA |
| Fagales | Casuarinaceae | *Casuarina equisetifolia* | | NK | | CA |
| Fabales | Fabaceae | *Acacia aneura* | F | | | CA |
| Fabales | Fabaceae | *Acacia baileyana* | F | | | CA |
| Fabales | Fabaceae | *Acacia floribunda* | A | | | CA |
| Fabales | Fabaceae | *Acacia mearnsii* | | NK | | ZA |
| Fabales | Fabaceae | *Acacia melanoxylon* | | NK | | CA & ZA |
| Fabales | Fabaceae | *Acacia saligna* | A | | | CA |
| Fabales | Fabaceae | *Acacia sp.* | | NK | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Fabales | Fabaceae | *Acacia stenophylla* | A | | | CA |
| Fabales | Fabaceae | *Acacia victoriae* | A | | | CA |
| Fabales | Fabaceae | *Albizia gummifera* | A | | | CA |
| Fabales | Fabaceae | *Albizia julibrissin* | | NK | | CA |
| Fabales | Fabaceae | *Albizia kalkora* | A | | | CA |
| Fabales | Fabaceae | *Bauhinia blakeana* | F | | | CA |
| Fabales | Fabaceae | *Bauhinia galpinii* | F | | ZA | CA & ZA |
| Fabales | Fabaceae | *Bauhinia petersiana* | A | | | CA & ZA |
| Fabales | Fabaceae | *Bauhinia purpurea* | F | | | ZA |
| Fabales | Fabaceae | *Bauhinia variegata* | | NK | | CA |
| Fabales | Fabaceae | *Caesalpinia cacalaco* | A | | | CA |
| Fabales | Fabaceae | *Calpurnia aurea* | F | | ZA | CA & ZA |
| Fabales | Fabaceae | *Cassia abbreviata* | A | | ZA | CA & ZA |
| Fabales | Fabaceae | *Cassia brewsteri* | F | | | CA |
| Fabales | Fabaceae | *Cassia fistula* | A | | | CA |
| Fabales | Fabaceae | *Cassia leptophylla* | F | | | CA |
| Fabales | Fabaceae | *Castanospermum australe* | | NK | | CA |
| Fabales | Fabaceae | *Ceratonia siliqua* | F | | | CA |
| Fabales | Fabaceae | *Cercidium floridum subsp. floridum* | | NK | CA | CA |
| Fabales | Fabaceae | *Cercidium microphyllum* | A | | CA | CA |
| Fabales | Fabaceae | *Cercidium sonorae* | | NK | | CA |
| Fabales | Fabaceae | *Cercidium* sp. 1 | A | | | CA |
| Fabales | Fabaceae | *Cladrastis sinensis* | A | | | CA |
| Fabales | Fabaceae | *Dalbergia sissoo* | F | | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Fabales | Fabaceae | *Erythrina abyssinica* | A | | | CA |
| Fabales | Fabaceae | *Erythrina caffra* | | NK | ZA | CA & ZA |
| Fabales | Fabaceae | *Erythrina coralloides* | | NK | | CA |
| Fabales | Fabaceae | *Erythrina crista-galli* | F | | | CA |
| Fabales | Fabaceae | *Erythrina falcata* | | NK | | CA |
| Fabales | Fabaceae | *Erythrina folkersii* | F | | | CA |
| Fabales | Fabaceae | *Erythrina humeana* | F | | | CA & ZA |
| Fabales | Fabaceae | *Erythrina livingstoniana* | F | | | ZA |
| Fabales | Fabaceae | *Erythrina lysistemon* | F | | ZA | CA & ZA |
| Fabales | Fabaceae | *Erythrina sykesii* | A | | | CA |
| Fabales | Fabaceae | *Gleditsia triacanthos* | | NK | | CA & ZA |
| Fabales | Fabaceae | *Inga edulis* | F | | | CA |
| Fabales | Fabaceae | *Inga feuilleei* | A | | | CA |
| Fabales | Fabaceae | *Lysiphyllum carronii* | F | | | CA |
| Fabales | Fabaceae | *Olneya tesota* | A | | CA | CA |
| Fabales | Fabaceae | *Parkinsonia aculeata* | | K | | CA |
| Fabales | Fabaceae | *Pithecellobium sp.* | A | | | CA |
| Fabales | Fabaceae | *Podalyria calyptrata* | | NK | ZA | ZA |
| Fabales | Fabaceae | *Prosopis articulata* | | NK | | CA |
| Fabales | Fabaceae | *Prosopis chilensis* | F | | | CA |
| Fabales | Fabaceae | *Prosopis glandulosa* | F | | | CA |
| Fabales | Fabaceae | *Prosopis velutina* | F | | | CA |
| Fabales | Fabaceae | *Psoralea pinnata* | | NK | ZA | ZA |
| Fabales | Fabaceae | *Schotia brachypetala* | F | | ZA | CA & ZA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Fabales | Fabaceae | *Senegalia caffra* | A | | | CA & ZA |
| Fabales | Fabaceae | *Senegalia galpinii* | F | | ZA | ZA |
| Fabales | Fabaceae | *Senegalia visco* | F | | | CA |
| Fabales | Fabaceae | *Senna racemosa* | F | | | CA |
| Fabales | Fabaceae | *Senna spectabilis* | A | | | CA |
| Fabales | Fabaceae | *Senna splendida* | F | | | CA |
| Fabales | Fabaceae | *Styphnolobium japonicum* | A | | | CA |
| Fabales | Fabaceae | *Tipuana tipu* | A | | | CA |
| Fabales | Fabaceae | *Vachellia caven* | A | | | CA |
| Fabales | Fabaceae | *Vachellia cochliacantha* | A | | | CA |
| Fabales | Fabaceae | *Vachellia etbaica* | A | | | CA |
| Fabales | Fabaceae | *Vachellia farnesiana* | F | | | CA |
| Fabales | Fabaceae | *Vachellia karroo* | F | | ZA | ZA |
| Fabales | Fabaceae | *Vachellia sieberiana* | F | | ZA | ZA |
| Fabales | Fabaceae | *Virgilia divaricata* | F | | ZA | ZA |
| Fabales | Fabaceae | *Virgilia oroboides* | | NK | ZA | ZA |
| Fabales | Fabaceae | *Wisteria floribunda* | | NK | | CA |
| Fabales | Fabaceae | *Wisteria sinensis* | F | | | CA |
| Fabales | Fabaceae | *Zenia insignis* | A | | | CA |
| Geraniales | Melianthaceae | *Melianthus major* | F | | ZA | CA & ZA |
| Myrtales | Combretaceae | *Combretum erythrophyllum* | F | | ZA | ZA |
| Myrtales | Combretaceae | *Combretum kraussii* | | NK | ZA | ZA |
| Myrtales | Onagraceae | *Hauya elegans* | A | | | CA |
| Myrtales | Myrtaceae | *Callistemon salignus* | A | | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Myrtales | Myrtaceae | *Callistemon viminalis* | A | | | CA |
| Myrtales | Myrtaceae | *Corymbia ficifolia* | | NK | | CA |
| Myrtales | Myrtaceae | *Eucalyptus camaldulensis* | F | | | CA & ZA |
| Myrtales | Myrtaceae | *Eucalyptus cinerea* | A | | | CA |
| Myrtales | Myrtaceae | *Eucalyptus froggattii* | A | | | CA |
| Myrtales | Myrtaceae | *Eucalyptus kitsoniana* | A | | | CA |
| Myrtales | Myrtaceae | *Eucalyptus perriniana* | A | | | CA |
| Myrtales | Myrtaceae | *Eucalyptus polyanthemos* | F | | | CA |
| Myrtales | Myrtaceae | *Eucalyptus torquata* | F | | | CA |
| Myrtales | Myrtaceae | *Psidium guajava* | F | | | CA & ZA |
| Sapindales | Anacardiaceae | *Harpephyllum caffrum* | F | | ZA | CA & ZA |
| Sapindales | Anacardiaceae | *Pistacia chinensis* | A | | | CA |
| Sapindales | Anacardiaceae | *Schinus molle* | F | | | CA & ZA |
| Sapindales | Anacardiaceae | *Schinus polygama* | F | | | ZA |
| Sapindales | Anacardiaceae | *Schinus terebinthifolia* | F | | | CA |
| Sapindales | Burseraceae | *Bursera hindsiana* | A | | | CA |
| Sapindales | Sapindaceae | *Acer buergerianum* | | K | | CA & ZA |
| Sapindales | Sapindaceae | *Acer caudatifolium* | A | | | CA |
| Sapindales | Sapindaceae | *Acer davidii* | A | | | CA |
| Sapindales | Sapindaceae | *Acer freemanii* | A | | | CA |
| Sapindales | Sapindaceae | *Acer macrophyllum* | | K | CA | CA |
| Sapindales | Sapindaceae | *Acer negundo* | | K | CA | CA & ZA |
| Sapindales | Sapindaceae | *Acer palmatum* | | K | | CA & ZA |
| Sapindales | Sapindaceae | *Acer paxii* | | NK | | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Sapindales | Sapindaceae | *Acer pectinatum* | F |  |  | CA |
| Sapindales | Sapindaceae | *Aesculus californica* |  | NK | CA | CA |
| Sapindales | Sapindaceae | *Alectryon excelsus* |  | NK |  | CA |
| Sapindales | Sapindaceae | *Cupaniopsis anacardioides* |  | NK |  | CA |
| Sapindales | Sapindaceae | *Harpullia arborea* | F |  |  | CA |
| Sapindales | Sapindaceae | *Harpullia pendula* |  | NK |  | CA |
| Sapindales | Sapindaceae | *Koelreuteria bipinnata* |  | NK |  | CA |
| Sapindales | Sapindaceae | *Koelreuteria elegans* | F |  |  | CA |
| Sapindales | Sapindaceae | *Koelreuteria paniculata* | F |  |  | CA |
| Sapindales | Sapindaceae | *Ungnadia speciosa* | F |  |  | CA |
| Sapindales | Meliaceae | *Aglaia odorata* | A |  |  | CA |
| Sapindales | Meliaceae | *Ekebergia capensis* | F |  | ZA | ZA |
| Sapindales | Meliaceae | *Melia azedarach* | F |  |  | CA & ZA |
| Sapindales | Meliaceae | *Swietenia chickrassa* | A |  |  | CA |
| Sapindales | Rutaceae | *Calodendrum capense* | F |  | ZA | ZA |
| Sapindales | Rutaceae | *Citrus limon* | F |  |  | CA & ZA |
| Sapindales | Rutaceae | *Citrus sinensis* | F |  |  | CA & ZA |
| Sapindales | Simaroubaceae | *Ailanthus altissima* |  | NK |  | CA |
| Malvales | Malvaceae | *Bombax ceiba* | A |  |  | CA |
| Malvales | Malvaceae | *Brachychiton acerifolius* | F |  |  | CA |
| Malvales | Malvaceae | *Brachychiton australis* | F |  |  | CA |
| Malvales | Malvaceae | *Brachychiton discolor* | F |  |  | CA & ZA |
| Malvales | Malvaceae | *Brachychiton populneus* |  | NK |  | CA |
| Malvales | Malvaceae | *Brachychiton rupestris* | F |  |  | CA |

Supplementary Table I. Continued.

| Order | Family | Species | Non-Competent | Competent | Native Range | Infested Distribution |
|---|---|---|---|---|---|---|
| Malvales | Malvaceae | *Ceiba pentandra* | F | | | ZA |
| Malvales | Malvaceae | *Ceiba speciosa* | F | | | CA |
| Malvales | Malvaceae | *Chiranthodendron pentadactylon* | A | | | CA |
| Malvales | Malvaceae | *Dombeya cacuminum* | | NK | | CA |
| Malvales | Malvaceae | *Dombeya wallichii* | A | | | CA |
| Malvales | Malvaceae | *Firmiana simplex* | F | | | CA |
| Malvales | Malvaceae | *Grewia occidentalis* | F | | ZA | ZA |
| Malvales | Malvaceae | *Heliocarpus sp.* | A | | | CA |
| Malvales | Malvaceae | *Luehea divaricata* | F | | | CA |
| Malvales | Malvaceae | *Pseudobombax ellipticum* | A | | | CA |
| Malvales | Malvaceae | *Tilia americana* | F | | | CA |

**Supplementary Table II**. Habitat alliance, tree basal area, tree density, and attacked tree density in 2017, 2018, and 2020 across sampling sites San Diego county. Sites are presented in order from northwest to southeast.

| | | | | ISHB Attack Density ($ha^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | Habitat Alliance | Basal Area ($m^2$/ha) | Density ($ha^{-1}$) | 2017 | 2018 | 2020 | Species Richness | Latitude[1] | Longitude[1] |
| HA13 | Avocado | 20.8 | 117 | 0 | 0 | 6 | 1 | 33.48 | -117.26 |
| WGP107[2] | Sycamore-Oak Riparian | 4.9 | 54.3 | 1.7 | 1.7 | 1.7 | 3 | 33.353243 | -117.031194 |
| WGP106 | Sycamore-Oak Riparian | 3.2 | 73.2 | 1.2 | 1.2 | 1.2 | 6 | 33.352471 | -117.04015 |
| WGP105 | Sycamore-Oak Woodland | 33.1 | 221.7 | 0 | 0 | 13 | 3 | 33.348055 | -117.034876 |
| HA58[2] | Avocado | 31 | 117.6 | 80 | 80 | 80 | 1 | 33.33 | -117.12 |
| DRA5 | Avocado | 17.4 | 132 | 110 | 114 | 114 | 1 | 33.32 | -117.15 |
| HA57[2] | Avocado | 64.2 | 228 | 0 | 0 | 0 | 1 | 33.32 | -117.04 |
| HA56[2] | Avocado | 76.2 | 212 | 40 | 64 | 80 | 1 | 33.32 | -117.12 |
| CALT128[2,3] | Mixed Riparian | 21.8 | 816 | 0 | 8 | 8 | 5 | 33.314081 | -117.180371 |
| CALT127[2,3] | Mixed Riparian | 14.6 | 272.7 | 0 | 0 | 14 | 4 | 33.313209 | -117.183121 |
| CALT126[3] | Mixed Riparian | 12.3 | 135.6 | 0 | 0 | 0 | 6 | 33.31258 | -117.185934 |
| CALT125[3] | Mixed Riparian | 11 | 158.1 | 0 | 19.4 | 19.4 | 4 | 33.312414 | -117.18837 |
| CALT124[3] | Mixed Riparian | 10.6 | 215.1 | 7.5 | 22.6 | 22.6 | 4 | 33.312316 | -117.191368 |
| CALT138[2] | Willow Riparian | 19.3 | 759.2 | 195.9 | 453.1 | 453.1 | 6 | 33.291701 | -117.222963 |
| HA4[3] | Avocado | 34.8 | 120 | 120 | 120 | 120 | 1 | 33.28 | -117.25 |
| CDFW119[2] | Mixed Riparian | 7.8 | 160.8 | 7 | 11.7 | 25.6 | 5 | 33.276011 | -117.230468 |
| CDFW123 | Mixed Riparian | 26.4 | 644 | 32 | 80 | 152 | 4 | 33.274171 | -117.232068 |
| CALT137[2] | Mixed Riparian | 14 | 243.2 | 59.5 | 132.4 | 132.4 | 5 | 33.260272 | -117.238243 |
| CNLM118[2] | Willow Riparian | 10.3 | 215.2 | 81.1 | 123.5 | 123.5 | 5 | 33.25949 | -117.263498 |
| CNF129[2] | Oak Riparian | 119.7 | 1128.4 | 0 | 0 | 0 | 4 | 33.256735 | -116.797467 |
| CNF130 | Mixed Riparian | 44.6 | 424 | 0 | 0 | 0 | 6 | 33.255125 | -116.795277 |
| CNF131 | Mixed Riparian | 53.4 | 197.9 | 0 | 0 | 0 | 5 | 33.25423 | -116.792954 |
| CNF132 | Mixed Riparian | 44.8 | 181.2 | 0 | 0 | 0 | 8 | 33.25207 | -116.79349 |
| CNF133[2] | Sycamore-Oak Riparian | 32.5 | 143.8 | 0 | 0 | 0 | 6 | 33.251218 | -116.791261 |
| HA55[2] | Avocado | 69.3 | 380 | 292 | 292 | 292 | 1 | 33.25 | -117.17 |
| CNF134 | Mixed Riparian | 20.9 | 197.3 | 0 | 0 | 0 | 6 | 33.24643 | -116.783629 |
| CNF135[2] | Mixed Riparian | 43.5 | 315.1 | 0 | 0 | 0 | 5 | 33.244721 | -116.781169 |
| GRP110 | Willow Riparian | 20.7 | 244 | 4 | 76 | 104 | 5 | 33.243851 | -117.276185 |
| GRP109[2] | Willow Riparian | 29.5 | 776 | 12 | 72 | 348 | 3 | 33.243553 | -117.273148 |
| GRP108[2] | Mixed Riparian | 15.1 | 400 | 12 | 56 | 56 | 7 | 33.243452 | -117.270912 |
| HA54[2] | Avocado | 69.5 | 508 | 248 | 248 | 248 | 1 | 33.24 | -117.17 |
| DAR153 | Oak Riparian | 14.9 | 109.5 | 0 | 0 | 0 | 5 | 33.186493 | -117.05081 |
| CDFW113[2] | Willow Riparian | 15.1 | 158 | 0 | 0 | 0 | 3 | 33.179562 | -117.314499 |

**Supplementary Table II**. Habitat alliance, tree basal area, tree density, and attacked tree density in 2017, 2018, and 2020 across sampling sites San Diego county. Sites are presented in order from northwest to southeast.

| | | | | ISHB Attack Density (ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | Habitat Alliance | Basal Area ($m^2$/ha) | Density (ha$^{-1}$) | 2017 | 2018 | 2020 | Species Richness | Latitude[1] | Longitude[1] |
| CDFW112[2] | Willow Riparian | 14.4 | 217 | 0 | 0 | 0 | 5 | 33.179502 | -117.317238 |
| CDFW114[2] | Willow Riparian | 18.5 | 214.7 | 0 | 0 | 0 | 3 | 33.178532 | -117.310761 |
| LWO206 | Oak Woodland | 34.1 | 260 | 0 | 4 | 4 | 7 | 33.171504 | -117.023828 |
| HA16 | Avocado | 23.2 | 317 | 154 | 154 | 154 | 1 | 33.17 | -117.03 |
| LWO205 | Mixed Riparian | 34.5 | 371.2 | 0 | 0 | 16.7 | 8 | 33.166318 | -117.015792 |
| CA14[2] | Avocado | 4.4 | 51 | 21 | 23 | 23 | 1 | 33.16 | -117.07 |
| CDFW152 | Oak Riparian | 17.8 | 180 | 0 | 0 | 0 | 3 | 33.157736 | -117.275478 |
| CDFW151[2] | Willow Riparian | 15.7 | 180 | 4 | 120 | 164 | 2 | 33.143613 | -117.307916 |
| HA8[2] | Avocado | 23.1 | 168 | 150 | 169 | 169 | 1 | 33.14 | -117.03 |
| CNLM156 | Oak Riparian | 13.1 | 225.9 | 0 | 0 | 0 | 5 | 33.136718 | -117.263039 |
| CNLM150[2] | Willow Riparian | 422.8 | 237.7 | 82.7 | 139.5 | 258.4 | 5 | 33.131579 | -117.300064 |
| DS12[2] | Avocado | 18.2 | 157 | 105 | 105 | 105 | 1 | 33.12 | -117.03 |
| HA3[2] | Avocado | 33.6 | 104 | 104 | 104 | 104 | 1 | 33.11 | -117.02 |
| HA11[2,3] | Avocado | 26.1 | 97 | 37 | 37 | 37 | 1 | 33.1 | -117.03 |
| HA1[2] | Avocado | 14.2 | 172 | 128 | 128 | 128 | 1 | 33.1 | -117.03 |
| HA2[2] | Avocado | 22.8 | 156 | 0 | 0 | 17 | 1 | 33.08 | -116.97 |
| SDRP176[2] | Sycamore-Oak Riparian | 49.2 | 135.5 | 0 | 0 | 0 | 7 | 33.07851 | -117.115402 |
| TECC121 | Sycamore-Oak Riparian | 42.4 | 122.5 | 0 | 0 | 0 | 5 | 33.077062 | -117.157094 |
| TECC120[2] | Sycamore-Oak Riparian | 34.6 | 256.7 | 0 | 0 | 0 | 5 | 33.076355 | -117.159615 |
| TECC149 | Mixed Riparian | 43.8 | 292 | 0 | 0 | 0 | 8 | 33.071027 | -117.168441 |
| SDRP175[2] | Willow Riparian | 16 | 612 | 0 | 0 | 0 | 4 | 33.064094 | -117.064334 |
| TECC122[2] | Mixed Riparian | 15.8 | 174.8 | 17.5 | 24.5 | 45.5 | 6 | 33.05355 | -117.204286 |
| SDRP173[2] | Willow Riparian | 11.3 | 360.4 | 0 | 4.5 | 4.5 | 7 | 33.042541 | -117.154207 |
| SDRP174 | Mixed Riparian | 24.9 | 254.9 | 0 | 0 | 0 | 7 | 33.040351 | -117.038133 |
| SEER111[2] | Willow Riparian | 19.5 | 204 | 152 | 172 | 172 | 3 | 33.012032 | -117.273317 |
| SEER157 | Mixed Riparian | 11.3 | 163.5 | 4 | 4 | 4 | 5 | 33.010153 | -117.247933 |
| LCOP172[2] | Willow Riparian | 13.1 | 357.4 | 25.5 | 17 | 34 | 3 | 33.010024 | -117.167398 |
| SDCP171[2] | Sycamore-Willow Riparian | 19.4 | 175.3 | 80.6 | 73.6 | 80.6 | 9 | 33.002351 | -117.234756 |
| LPCP214 | Mixed Riparian | 17.7 | 273.1 | 0 | 0 | 0 | 7 | 32.938322 | -117.135242 |
| GRSCP162 | Sycamore-Oak Riparian | 24.6 | 311.4 | 0 | 0 | 0 | 5 | 32.92777 | -116.984625 |
| GRSCP161 | Sycamore-Oak Riparian | 12.4 | 112.2 | 0 | 0 | 0 | 5 | 32.919567 | -116.989192 |
| LSCP163 | Sycamore-Oak Riparian | 41.2 | 184.5 | 0 | 0 | 0 | 8 | 32.88234 | -116.898076 |
| FSCP164[2] | Sycamore-Oak Riparian | 52.2 | 229.5 | 42.4 | 42.4 | 60 | 6 | 32.847837 | -116.861699 |

**Supplementary Table II**. Habitat alliance, tree basal area, tree density, and attacked tree density in 2017, 2018, and 2020 across sampling sites San Diego county. Sites are presented in order from northwest to southeast.

| | | | | ISHB Attack Density (ha$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | Habitat Alliance | Basal Area (m$^2$/ha) | Density (ha$^{-1}$) | 2017 | 2018 | 2020 | Species Richness | Latitude[1] | Longitude[1] |
| MBNP207[2] | Sycamore-Oak Riparian | 43.7 | 484.6 | 0 | 0 | 0 | 5 | 32.845069 | -117.199031 |
| MPDC211 | Mixed Riparian | 33.6 | 555.6 | 0 | 0 | 0 | 7 | 32.843768 | -116.995897 |
| MTRP212 | Mixed Riparian | 16.3 | 200 | 0 | 3.8 | 3.8 | 6 | 32.821961 | -117.061285 |
| TCNP208[2] | Mixed Riparian | 28 | 381 | 0 | 0 | 0 | 6 | 32.798004 | -117.179396 |
| SR115[2] | Mixed Riparian | 32 | 145.4 | 101.5 | 101.5 | 101.5 | 8 | 32.777135 | -116.874573 |
| SR116 | Mixed Riparian | 19.3 | 62.6 | 0 | 0 | 1.2 | 5 | 32.773663 | -116.844132 |
| CDFW155[2] | Mixed Riparian | 13.2 | 362.7 | 0 | 0 | 0 | 6 | 32.771991 | -116.808345 |
| MVP209 | Mixed Riparian | 23.5 | 244 | 12 | 12 | 12 | 6 | 32.763205 | -117.194981 |
| SR117[2] | Sycamore-Oak Woodland | 33.5 | 114.8 | 32.4 | 44.2 | 44.2 | 4 | 32.762775 | -116.846311 |
| CDFW154 | Mixed Riparian | 20.6 | 416 | 0 | 0 | 0 | 5 | 32.75762 | -116.894623 |
| PCOS213[2] | Sycamore-Willow Riparian | 2.5 | 71.8 | 0 | 0 | 0 | 8 | 32.695786 | -117.051046 |
| HCWA210 | Mixed Riparian | 16.7 | 40 | 0 | 0 | 0 | 5 | 32.674769 | -116.826575 |
| CDFW158[2] | Sycamore-Willow Riparian | 15.9 | 311.6 | 0 | 0 | 0 | 6 | 32.640867 | -116.879603 |
| OVRP165[2] | Willow Riparian | 13.1 | 220 | 0 | 0 | 0 | 4 | 32.597954 | -116.949348 |
| OVRP160[2] | Sycamore-Willow Riparian | 15.6 | 140.8 | 56.3 | 87.3 | 87.3 | 7 | 32.589843 | -117.066133 |
| TRVRP159[2] | Willow Riparian | 19.1 | 351.4 | 281.1 | 335.1 | 335.1 | 3 | 32.555394 | -117.088553 |

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[1] Avocado locations are accurate to one km to maintain privacy

[2] Sampled for endophytes

[3] Burned in Lilac Fire (12/7/2017-12/16/2017)

**APPENDIX.** Management and educational handouts developed from the research in this project and other collaborations.

## ISHB-FD management matrix for infested urban and peri-urban forest.

This matrix was developed by Beatriz Nobua-Behrmann (UC ANR), Monica Dimson (UCLA), Shannon C. Lynch (UCD), John Kabashima (UC ANR), and Akif Eskalen (UCD)

| | | | ISHB Infestation Level & Management Options | | | | |
|---|---|---|---|---|---|---|---|
| | Host Type | Hazard Level[1] | No Infestation | Low | Moderate | Heavy | Severe |
| HIGH VALUE TREES[1] | Reproductive Host | Low | Monitor | Monitor & Spot Inject | Monitor[2]<br>Remove Actively Infested Branches | Monitor[2]<br>Remove Actively Infested Branches | Remove Actively Infested Tree[2] & Stump |
| | | High | Monitor | Monitor & Remove Hazard Branches | Monitor[2]<br>Remove Hazard Branches | Remove Hazard Branches, or Remove Tree & Stump | Remove Tree[2] & Stump |
| | Non-Reproductive Host | Low | Monitor | Monitor | Notify UC ANR; consult with ISHB-FD experts to determine if species is a new reproductive host | | |
| | | High | Monitor | Monitor | | | |

| | | | ISHB Infestation Level & Management Options | | | | |
|---|---|---|---|---|---|---|---|
| | Host Type | Hazard Level[1] | No Infestation | Low | Moderate | Heavy | Severe |
| LOW VALUE TREES[1] | Reproductive Host | Low | Monitor | Treat/Remove Infested Branches[3] | Treat/Remove Actively Infested Branches[3] | Treat/Remove Actively Infested Branches[2,3] | Remove Actively Infested Tree[2] & Stump |
| | | High | Monitor | Treat/Remove Hazard Branches[3] | Treat/Remove Hazard Branches[3] | Remove Infested Branches, or Tree[2] & Stump | Remove Tree[2] & Stump |
| | Non-Reproductive Host | Low | Monitor | Monitor | Notify UC ANR; consult with ISHB-FD experts to determine if species is a new reproductive host | | |
| | | High | Monitor | Monitor | | | |

**Definitions:**

| **Tree Value[1]** | |
|---|---|
| Low | Species of low economic value; smaller and/or younger trees; trees with undesirable form, structural issues (e.g., codominant branches), or other issues (e.g., other pests) |
| High | Species of high economic or cultural value (e.g., heritage trees); larger and/or older trees |

| **Host type** | |
|---|---|
| Reproductive Host | Plant species suitable for beetle reproduction and growth of *Fusarium euwallaceae* or *F. kuroshium* (see www.ishb.org for updated list of ISHB-FD reproductive hosts) |
| Non-Reproductive Host | Plant species that have not yet proved suitable for beetle reproduction; however, these species might be susceptible to *Fusarium euwallaceae* or *F. kuroshium* |

| **Hazard Level[1]** | |
|---|---|
| Low | Trees that pose a low risk to people or property |
| High | Trees that might pose a high risk to people or property (e.g., trees adjacent to walkways, playgrounds, high-use lawns, parking lots) |

| **Infestation Level** | **Attacks (number of entry holes observed)** |
|---|---|
| Low | < 50 |
| Moderate | ≥ 50 and < 150 |
| Heavy | ≥ 150 |
| Severe | ≥ 150 + ISHB-related dieback |

[1] Definitions for tree value and hazard level vary. Classification must be determined by site and site use (e.g., economic or cultural value and risk to people or property).

[2] Confirm if beetle is actively reproducing in galleries by painting over select entry holes with water-based latex; gallery is active if entry hole is re-opened on painted area.

[3] If ISHB attack is confined to the branches of host tree, prune affected branches immediately to prevent advancement to the trunk. Prune hazardous branches on high-value hosts and treat pruning wounds to prevent re-infestations.

# Invasive Shot-Hole Borers + Fusarium Dieback
# Prioritizing Management Efforts

## HOW TO USE THIS CHART

This chart is intended to help inform ISHB (Polyphagous and Kuroshio Shot-Hole Borers) management decisions. Consider potential safety hazards, tree value (economic and ecological), available resources, and other factors unique to each situation when using this tool.

## REPRODUCTIVE HOSTS

A reproductive host is a species that supports 1) ISHB reproduction and 2) growth and development of the beetle's symbiotic fungi. These species are currently the priority for control efforts as they can produce more beetles that may spread the infestation. Some of the more susceptible reproductive hosts appear to be box elder, castor bean, valley oak, Engelmann oak, coral, and several species of sycamore, willow, and cottonwood.

Visit pshb.org for the full host list.

## LIMB FAILURE HAZARD

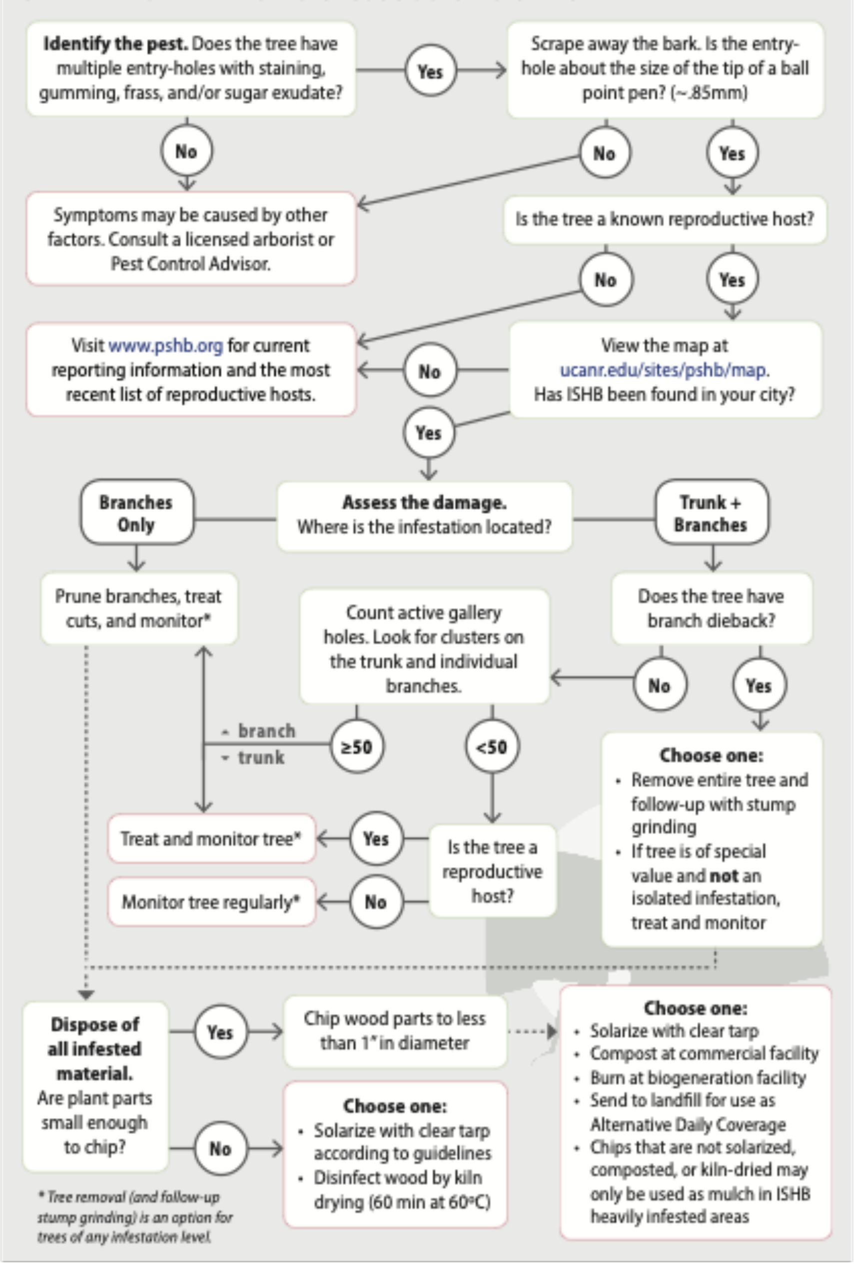
The point of attachment between a tree branch and the main stem is called the branch collar. ISHB infestation in this area poses a serious safety hazard: a weakened collar may not be able to support the weight of the branch, creating potential for limb failure.

Infested trees—including those that have been treated or pruned—must be regularly monitored so that hazards can be identified and removed. When monitoring, consider beetle attacks in the branch collar as part of the branch.

## AUTHORS

Akif Eskalen[1], Ph.D; Monica Dimson[2]; John Kabashima[2], Ph.D; Shannon C. Lynch[1]; Michele Eatough-Jones[2], Ph.D; Tim Paine[2], Ph.D. [1]UC Davis [2]UC Riverside [3]UC Cooperative Extension

## IDENTIFYING + MANAGING ISHB: SUGGESTED STEPS



**Identify the pest.** Does the tree have multiple entry-holes with staining, gumming, frass, and/or sugar exudate?

- Yes → Scrape away the bark. Is the entry-hole about the size of the tip of a ball point pen? (~.85mm)
- No → Symptoms may be caused by other factors. Consult a licensed arborist or Pest Control Advisor.

Scrape away the bark. Is the entry-hole about the size of the tip of a ball point pen? (~.85mm)

- No → Symptoms may be caused by other factors. Consult a licensed arborist or Pest Control Advisor.
- Yes → Is the tree a known reproductive host?

Is the tree a known reproductive host?

- No → Visit www.pshb.org for current reporting information and the most recent list of reproductive hosts.
- Yes → View the map at ucanr.edu/sites/pshb/map. Has ISHB been found in your city?

View the map at ucanr.edu/sites/pshb/map. Has ISHB been found in your city?

- No → Visit www.pshb.org for current reporting information and the most recent list of reproductive hosts.
- Yes → **Assess the damage.** Where is the infestation located?

**Assess the damage.** Where is the infestation located?

- **Branches Only** → Prune branches, treat cuts, and monitor*
- **Trunk + Branches** → Does the tree have branch dieback?

Does the tree have branch dieback?

- No → Count active gallery holes. Look for clusters on the trunk and individual branches.
- Yes → **Choose one:**
  - Remove entire tree and follow-up with stump grinding
  - If tree is of special value and **not** an isolated infestation, treat and monitor

Count active gallery holes. Look for clusters on the trunk and individual branches.

- ≥50: branch → Prune branches, treat cuts, and monitor*; trunk → Treat and monitor tree*
- <50 → Is the tree a reproductive host?

Is the tree a reproductive host?

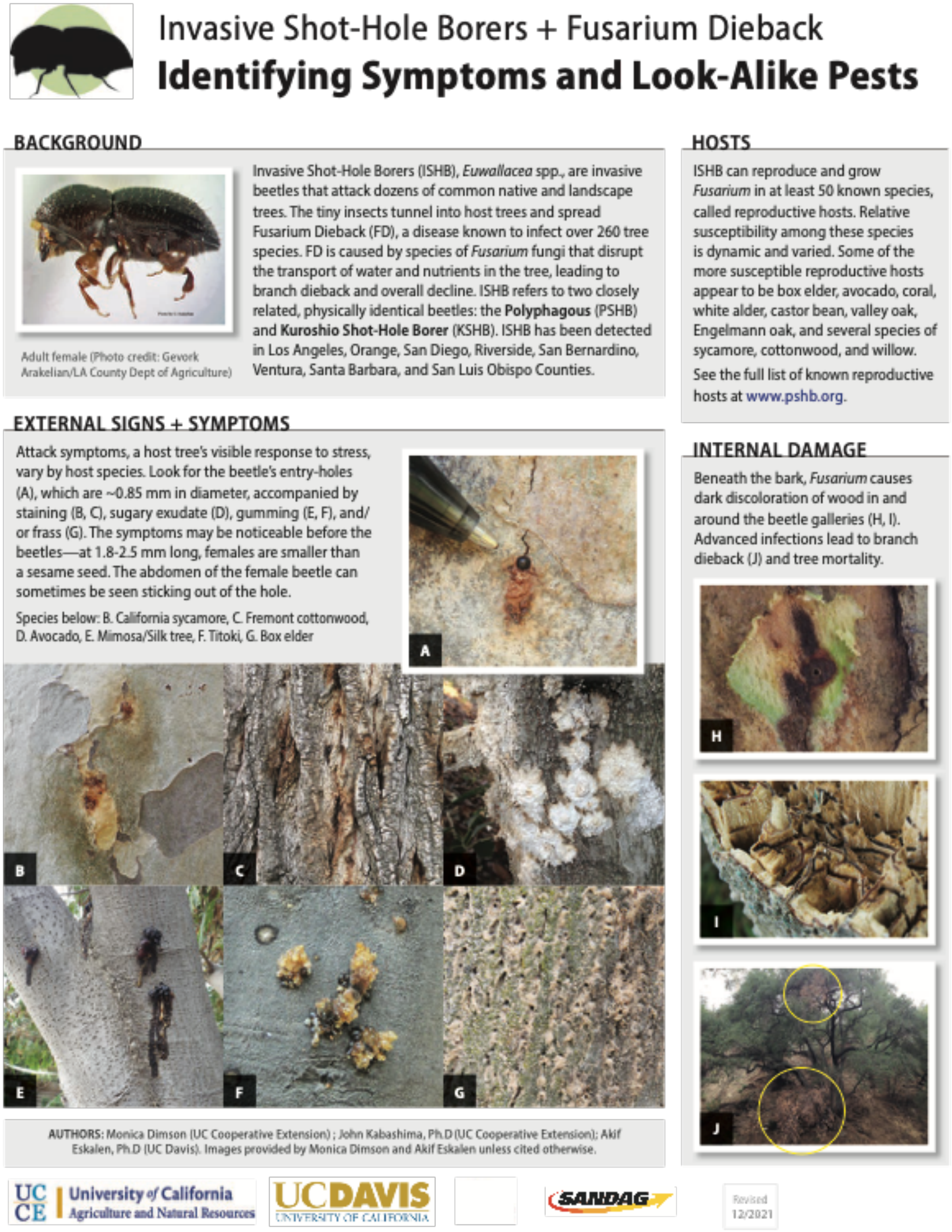
- Yes → Treat and monitor tree*
- No → Monitor tree regularly*

**Dispose of all infested material.** Are plant parts small enough to chip?

- Yes → Chip wood parts to less than 1" in diameter → **Choose one:**
  - Solarize with clear tarp
  - Compost at commercial facility
  - Burn at biogeneration facility
  - Send to landfill for use as Alternative Daily Coverage
  - Chips that are not solarized, composted, or kiln-dried may only be used as mulch in ISHB heavily infested areas
- No → **Choose one:**
  - Solarize with clear tarp according to guidelines
  - Disinfect wood by kiln drying (60 min at 60ºC)

** Tree removal (and follow-up stump grinding) is an option for trees of any infestation level.*

Revised 03/2021



# Invasive Shot-Hole Borers + Fusarium Dieback

# Identifying Symptoms and Look-Alike Pests

## BACKGROUND

Adult female (Photo credit: Gevork Arakelian/LA County Dept of Agriculture)

Invasive Shot-Hole Borers (ISHB), *Euwallacea* spp., are invasive beetles that attack dozens of common native and landscape trees. The tiny insects tunnel into host trees and spread Fusarium Dieback (FD), a disease known to infect over 260 tree species. FD is caused by species of *Fusarium* fungi that disrupt the transport of water and nutrients in the tree, leading to branch dieback and overall decline. ISHB refers to two closely related, physically identical beetles: the **Polyphagous** (PSHB) and **Kuroshio Shot-Hole Borer** (KSHB). ISHB has been detected in Los Angeles, Orange, San Diego, Riverside, San Bernardino, Ventura, Santa Barbara, and San Luis Obispo Counties.

## EXTERNAL SIGNS + SYMPTOMS

Attack symptoms, a host tree's visible response to stress, vary by host species. Look for the beetle's entry-holes (A), which are ~0.85 mm in diameter, accompanied by staining (B, C), sugary exudate (D), gumming (E, F), and/or frass (G). The symptoms may be noticeable before the beetles—at 1.8-2.5 mm long, females are smaller than a sesame seed. The abdomen of the female beetle can sometimes be seen sticking out of the hole.

Species below: B. California sycamore, C. Fremont cottonwood, D. Avocado, E. Mimosa/Silk tree, F. Titoki, G. Box elder

## HOSTS

ISHB can reproduce and grow *Fusarium* in at least 50 known species, called reproductive hosts. Relative susceptibility among these species is dynamic and varied. Some of the more susceptible reproductive hosts appear to be box elder, avocado, coral, white alder, castor bean, valley oak, Engelmann oak, and several species of sycamore, cottonwood, and willow.

See the full list of known reproductive hosts at www.pshb.org.

## INTERNAL DAMAGE

Beneath the bark, *Fusarium* causes dark discoloration of wood in and around the beetle galleries (H, I). Advanced infections lead to branch dieback (J) and tree mortality.

**AUTHORS:** Monica Dimson (UC Cooperative Extension) ; John Kabashima, Ph.D (UC Cooperative Extension); Akif Eskalen, Ph.D (UC Davis). Images provided by Monica Dimson and Akif Eskalen unless cited otherwise.

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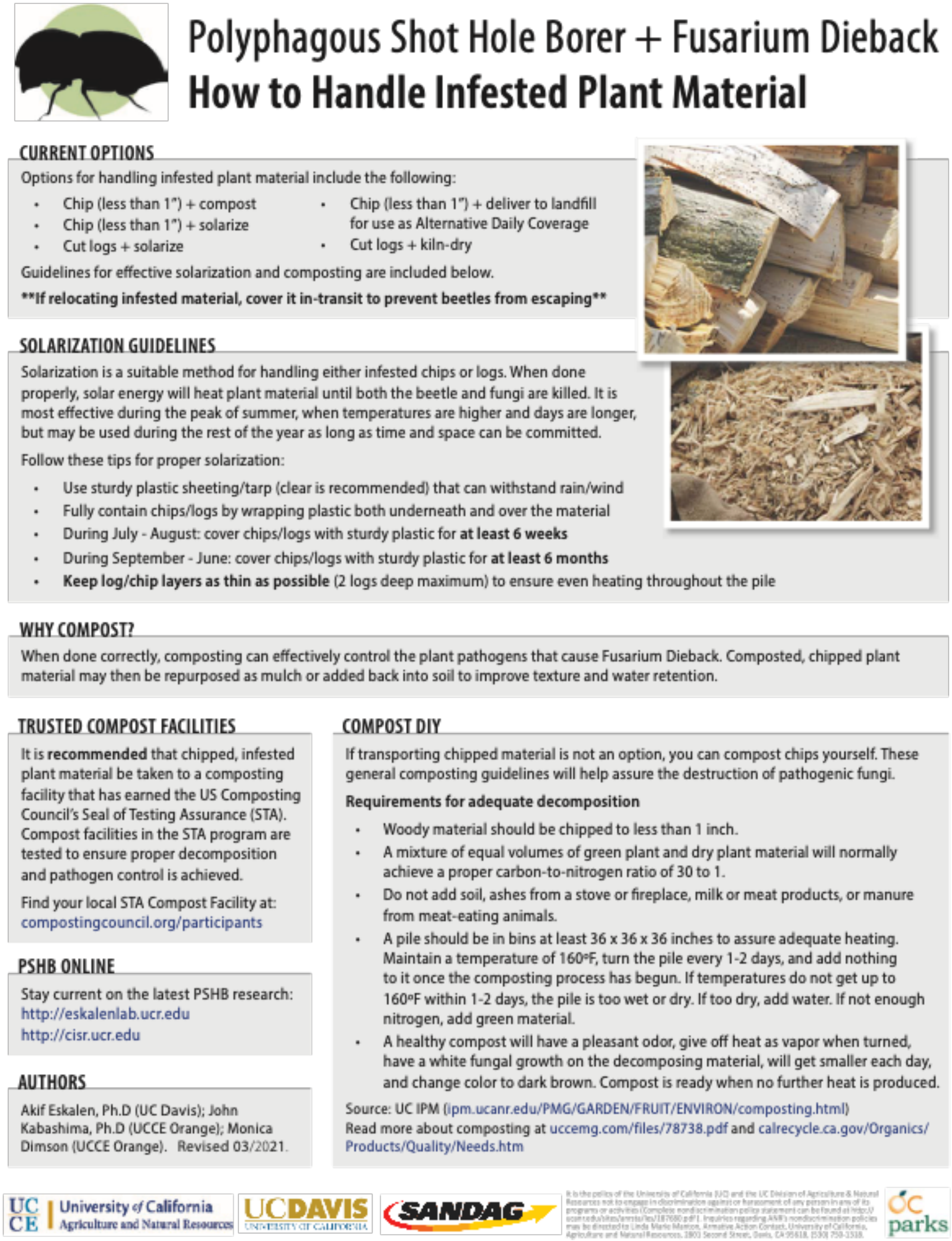
SANDAG

Revised 12/2021



# Polyphagous Shot Hole Borer + Fusarium Dieback
# How to Handle Infested Plant Material

## CURRENT OPTIONS

Options for handling infested plant material include the following:

- Chip (less than 1") + compost
- Chip (less than 1") + solarize
- Cut logs + solarize
- Chip (less than 1") + deliver to landfill for use as Alternative Daily Coverage
- Cut logs + kiln-dry

Guidelines for effective solarization and composting are included below.

****If relocating infested material, cover it in-transit to prevent beetles from escaping****

## SOLARIZATION GUIDELINES

Solarization is a suitable method for handling either infested chips or logs. When done properly, solar energy will heat plant material until both the beetle and fungi are killed. It is most effective during the peak of summer, when temperatures are higher and days are longer, but may be used during the rest of the year as long as time and space can be committed.

Follow these tips for proper solarization:

- Use sturdy plastic sheeting/tarp (clear is recommended) that can withstand rain/wind
- Fully contain chips/logs by wrapping plastic both underneath and over the material
- During July - August: cover chips/logs with sturdy plastic for **at least 6 weeks**
- During September - June: cover chips/logs with sturdy plastic for **at least 6 months**
- **Keep log/chip layers as thin as possible** (2 logs deep maximum) to ensure even heating throughout the pile

## WHY COMPOST?

When done correctly, composting can effectively control the plant pathogens that cause Fusarium Dieback. Composted, chipped plant material may then be repurposed as mulch or added back into soil to improve texture and water retention.

## TRUSTED COMPOST FACILITIES

It is **recommended** that chipped, infested plant material be taken to a composting facility that has earned the US Composting Council's Seal of Testing Assurance (STA). Compost facilities in the STA program are tested to ensure proper decomposition and pathogen control is achieved.

Find your local STA Compost Facility at: compostingcouncil.org/participants

## PSHB ONLINE

Stay current on the latest PSHB research:
http://eskalenlab.ucr.edu
http://cisr.ucr.edu

## AUTHORS

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## COMPOST DIY

If transporting chipped material is not an option, you can compost chips yourself. These general composting guidelines will help assure the destruction of pathogenic fungi.

**Requirements for adequate decomposition**

- Woody material should be chipped to less than 1 inch.
- A mixture of equal volumes of green plant and dry plant material will normally achieve a proper carbon-to-nitrogen ratio of 30 to 1.
- Do not add soil, ashes from a stove or fireplace, milk or meat products, or manure from meat-eating animals.
- A pile should be in bins at least 36 x 36 x 36 inches to assure adequate heating. Maintain a temperature of 160ºF, turn the pile every 1-2 days, and add nothing to it once the composting process has begun. If temperatures do not get up to 160ºF within 1-2 days, the pile is too wet or dry. If too dry, add water. If not enough nitrogen, add green material.
- A healthy compost will have a pleasant odor, give off heat as vapor when turned, have a white fungal growth on the decomposing material, will get smaller each day, and change color to dark brown. Compost is ready when no further heat is produced.

Source: UC IPM (ipm.ucanr.edu/PMG/GARDEN/FRUIT/ENVIRON/composting.html)
Read more about composting at uccemg.com/files/78738.pdf and calrecycle.ca.gov/Organics/Products/Quality/Needs.htm

## Invasive Shot-Hole Borer + Fusarium Dieback

# Identifying Symptoms and Look-Alike Pests on Willow

Various willow species (*Salix* spp.) are severely impacted by the emergent Invasive Shot-Hole Borers – Fusarium Dieback pest-disease complex (ISHB-FD). Other pests produce symptoms that appear to be indistinguishable from ISHB attack. This form was developed to accurately identify ISHB-FD and other pests exhibiting similar symptoms on willow.

### ISHB – Fusarium dieback

### American horned moth (*Sesia tibialis*)

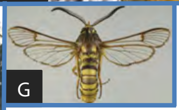
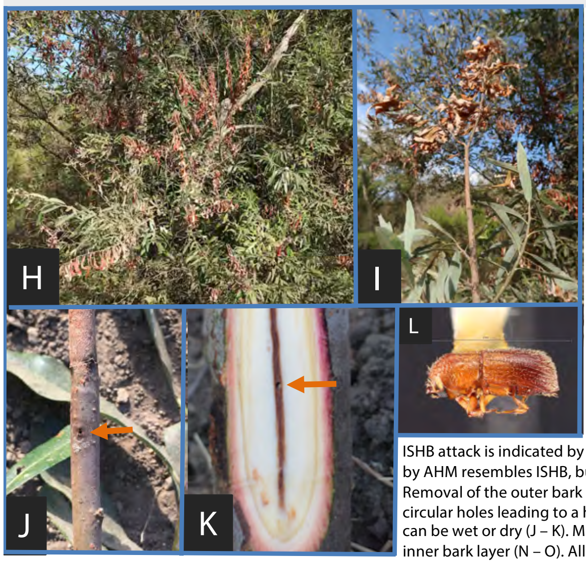


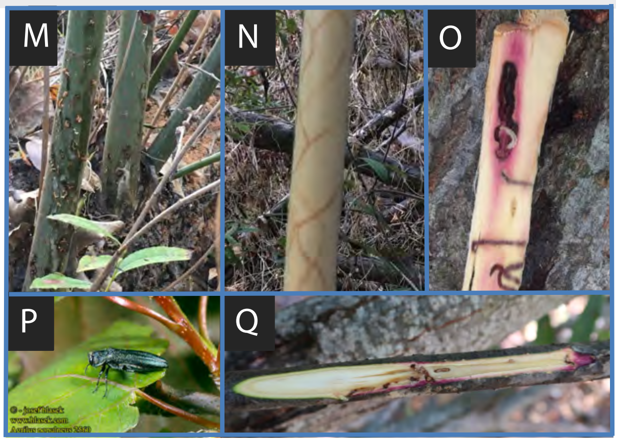
Photo Credit:
A – C; E – F.; H – O; Q: A. Eskalen & S. Lynch;
D: K. Moreno;
G: Kipling Will;
P: Jose Hlasek

Photo: Kipling Will

### Twig beetle (*Micracis swainei*)



### Metallic wood-boring beetle (*Agrilus populenus*)



ISHB attack is indicated by 0.85 mm entry holes and associated galleries in the wood tissue (A – B). Advanced attack by AHM resembles ISHB, but is distinguished by symptoms of sunken and swollen portions of the wood (C & E). Removal of the outer bark reveals a vertical slit and associated cankered tissues that leak water (D), or 3 -5 mm circular holes leading to a hollowed stem (F). Twig beetles produce 0. 5 mm entry holes on 0.5 – 3 cm stems that can be wet or dry (J – K). Metallic Wood – Boring Beetles are restricted to the cambium at the outer wood layer and inner bark layer (N – O). All pests can occur on a tree simultaneously depending on location.

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**Authors: S. Lynch[1,2]; A. Eskalen, [2] ([1]UC Santa Cruz, [2]UC Davis)**

Identities of Non-ISHB pests were confirmed by Drs. Richard Stouthamer, Paul Rugman-Jones (UC Riverside), Jiri Hulcr (University of Florida), & Robert Rabaglia USDA-FS

# Invasive Shot-Hole Borers + Fusarium Dieback

# Sycamore Assessment

Various sycamore species (*Platanus* spp.) are severely impacted by the emergent Invasive Shot-Hole Borers – Fusarium Dieback pest-disease complex (ISHB-FD). These trees are widespread throughout urban and wildland forests in Southern California. With support by the Los Angeles Center for Urban Natural Resources and Sustainability, this form was developed to determine ISHB-FD damage on sycamore trees for comparable and systematic assessments.

A **American Sycamore**
**Leaves**: Toothed, pointed margins, broader than long
**Fruit:** In singles
**Bark**: Dark, scaly older

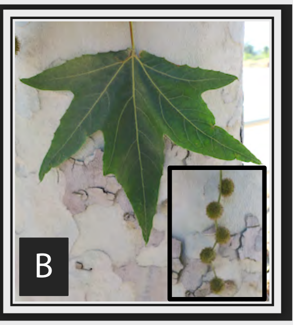
B **CA Sycamore**
**Leaves:** Finely serrated to smooth margins
**Fruit:** In chains of 4-7
**Bark:** White, tawny beige bark

C **London Plane**
**Leaves:** Toothed, pointed margins, as long as wide
**Fruits:** In pairs
**Bark:** Flaky, blotchy older bark

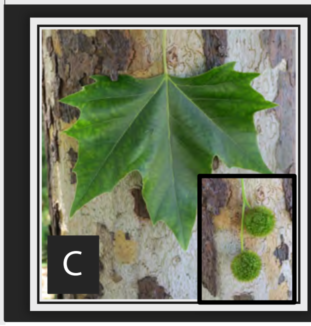
D **Mexican Sycamore**
**Leaves**: Toothed, pointed margins, underside "silvery" smooth
**Fruits**: In chains of 3-5
**Bark**: Gray white

**American Sycamore**
***P. occidentalis***

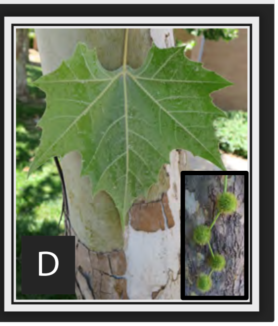
**CA Sycamore**
***P. racemosa***



**London Plane**
***P. x acerifolia***



**Mexican Sycamore**
***P. mexicana***



## Crown Condition:

1: 0-10% Dieback

2: 10-40% Dieback

3: >40% Dieback

**Authors**: S. Lynch[1,2]; A. Eskalen, [2]; J. Mayorquin,[2] G. Gilbert[1] ([1]UC Santa Cruz, [2]UC Riverside) All images by authors.

Contact: www.eskalenlab.ucr.edu

# Invasive Shot-Hole Borers + Fusarium Dieback
# Monitoring Trap Guidelines

## WHEN TO TRAP

Monitoring for Invasive Shot-Hole Borer (ISHB), *Euwallacea* spp., and Fusarium Dieback can be challenging: the invasive pest complex has attacked over 260 different species, including common native, landscape, and agricultural trees.

Visual surveys are effective for identifying ISHB symptoms on individual trees, but may not be practical for several acres of inaccessible forest. In this case, monitoring traps can be installed to detect ISHB presence. A lure called quercivorol helps attract beetles to the trap. This document describes trap options and the process of trap installation and maintenance.

An adult female beetle[†]
is 1.8-2.5 mm (0.07-0.1 inches) long

## VISUAL SURVEYS

Whenever possible, visual tree surveys are preferred over monitoring traps. Trapping is a passive detection method that is useful for large or inaccessible areas. However, regular inspections of individual trees are recommended for homes or managed landscapes. If time and resources allow, this is a more accurate and precise way of detecting ISHB.

Visit www.pshb.org for the full ISHB host list and photos of symptoms on a variety of tree species.

## TRAP OPTIONS

| Trap Type | Lindgren/Funnel Trap (A) | Panel Trap (B) | Bottle Trap (C) |
|---|---|---|---|
| How it Works | Insects encounter trap, tumble down through funnels, and fall into cup of preservative* | Insects that fly into trap become stuck on the sticky surface of the panel | Insects encounter upper bottle and tumble down into lower bottle of preservative* |
| Pros | • Lasts for multiple field seasons<br>• Easy to service and maintain<br>• Insects will be easier to identify | • Less expensive than Lindgren/Funnel trap<br>• Less frequent service required, as beetles are preserved well on surface | • Less expensive than Lindgren/Funnel trap |
| Cons | • More expensive than other traps<br>• More frequent service required to prevent trap cup from overflowing or drying out<br>• Requires regular cleaning (dust, spider webs)<br>• Possibility of vandalism | • Samples need to be individually removed from trap surface<br>• Insects are sticky and harder to identify<br>• Traps are not re-usable (good for ~1 field season) | • Lower trap catches expected compared to other trap options<br>• Requires assembly and multiple parts |

***see Trap Preservatives section on reverse side**

**Authors:** Akif Eskalen, Ph.D.[1]; John Kabashima, Ph.D.[2]; Monica Dimson[2]; Jan Gonzales[2]; Stacy Hishinuma, Ph.D.[3]; Richard Stouthamer, Ph.D.[1] **Photographs:** female beetle[†] - Gevork Arekalian/LA County Dept of Agriculture; bottle trap - Benjamin DiAnna[2]; all other images - Monica Dimson[2]

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Revised
12/2021