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From the **Grove**

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Severity of *Fusarium* Dieback – Shot Hole Borers Analyzed

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Introduction

The emergent *Fusarium* dieback – Shot Hole Borers (FD-SHB) pest-disease complex has been a source of concern to the avocado industry since its discovery on a backyard avocado tree in the Los Angeles basin in 2012. The dieback is caused by the combined effects of two ambrosia beetles, polyphagous shot hole borer (PSHB), *Euwallacea whitfordiodendrus* and Kuroshio shot hole borer (KSHB), *Euwallacea kuroshio* and the specific fungal pathogens each beetle carries (*Fusarium euwallaceae* and *Fusarium kuroshium*). In addition to their fusaria symbionts, each beetle carries *Graphium euwallaceae* and *G. kuroshium*, respectively, and *Paracremonium pembeum*. The adult female tunnels galleries into a wide variety of host trees, where it lays its eggs and grows their symbiotic fungi.

Although PSHB rapidly spread throughout urban-wildland forests in the Los Angeles basin, it was not detected in commercial avocado groves until after a KSHB attack was confirmed in commercial groves shortly after its detection in San Diego County in late 2013. As KSHB became well established in avocado groves in San Diego County, PSHB subsequently spread into Ventura County and was detected on trees in a handful of groves in late 2015. Given that San Diego and Ventura counties produce 70 percent of the U.S. domestic

avocado crop, it has become critical to understand patterns of spread and severity of attack to inform best management practices of the problem. The need for recommendations guiding the long-term management response to the threat FD presents is recognized as a top priority for a variety of land managers and agencies throughout the state. The cur-

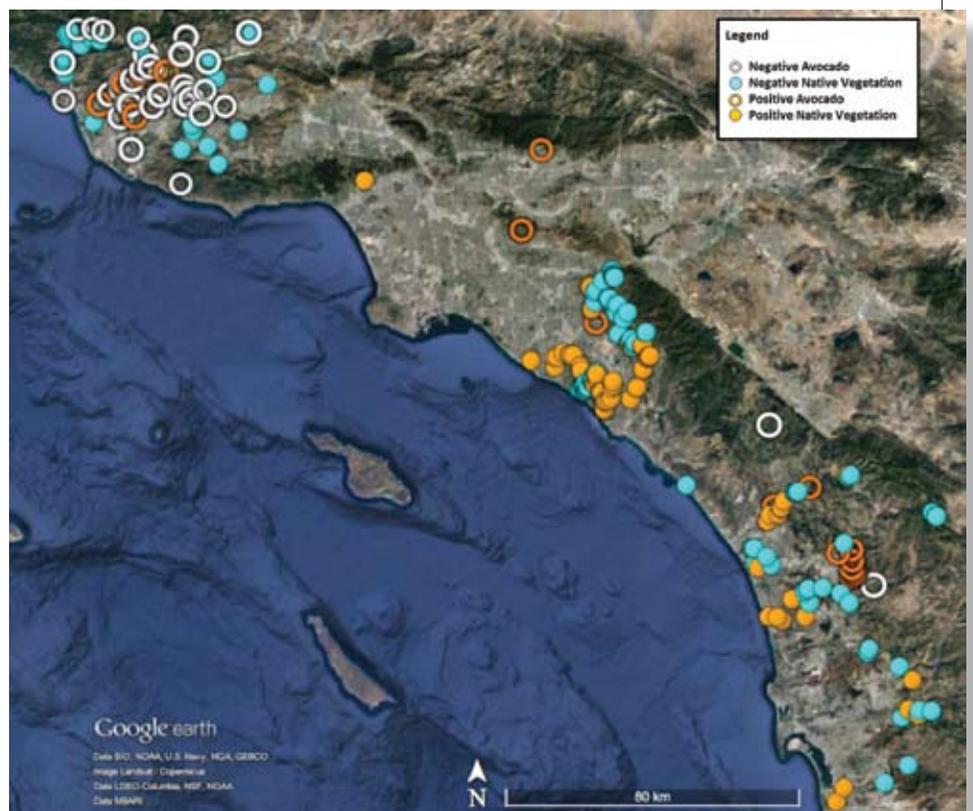


Figure 1. *Fusarium* dieback – shot hole borer monitoring plots established throughout San Diego, Orange and Ventura counties, including those in native vegetation.

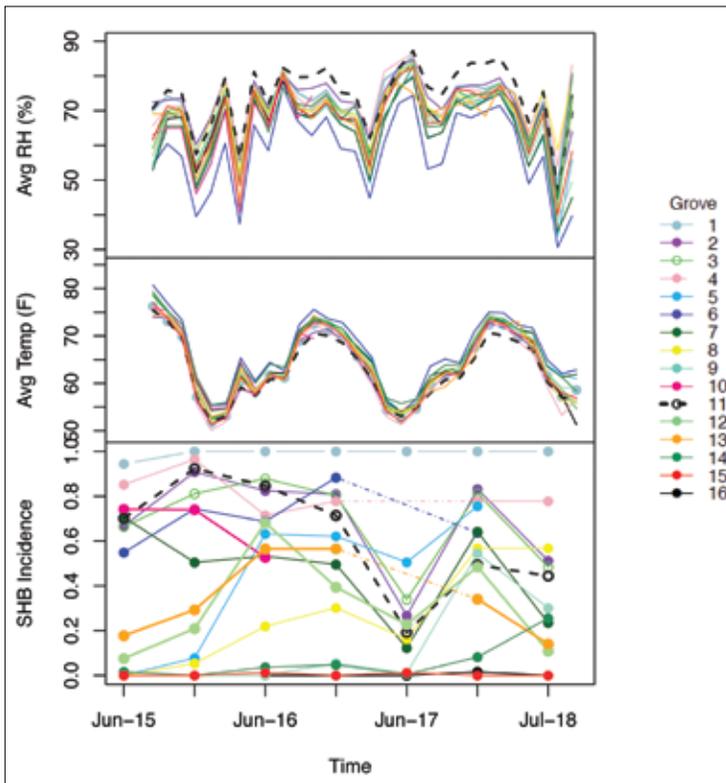


Figure 2. Average relative humidity (A) and average temperature (B) and SHB incidence (fraction of infested trees per grove, between 0 – 1) (C) for sixteen groves surveyed in San Diego County between 2015 and 2018.

rent project herein is the initiation of a multi-phased effort to develop essential building blocks for Integrative Pest Management (IPM) to address the FD threat, which involves identifying the scope of the problem throughout the avocado growing range in California and the development of a policy and management response in line with the magnitude of the identified problem.

Our objectives were to 1) Determine patterns of spread of SHB on avocado trees and across groves; 2) Identify potential environmental factors that contribute to the distribution, establishment, and spread of SHB; and 3) Provide initial management guidelines in light of ongoing experiments and monitoring studies.

Methods

In June and August 2015, we established 16 one-hectare monitoring plots in 13 infested and five non-infested groves in San Diego and Orange counties (Fig. 1). Plots were visited every six months for a total of eight visits. Each plot contains a Hobo data logger that measures temperature and relative humidity every 30 minutes. For each of the 3,346 trees surveyed, we assessed their health, overall SHB severity, attack severity by plant part, and recorded the presence of other diseases (e.g. bacterial canker, botryosphaeria canker, black streak, phytophthora root rot). The proportion of groves infested increased from 70 percent to 87 percent by the fourth

visit and did not change in the two subsequent surveys. Interestingly, the proportion of trees infested (SHB incidence) fluctuated over time (Fig. 2 C), with a striking drop in incidence in five of the heavily infested plots in June 2017 (20 – 88 percent decrease), followed by a spike in December 2017. Multiple logistic regression analyses revealed that incidence trends were not associated with microclimate (temperature and relative humidity, Fig. 2 A – C), tree density, or tree size, suggesting that spread is mostly driven by host availability in this early phase of the epidemic. However, an assessment of whether these factors influence *Fusarium* dieback spread over a broader geographic area – combined with long term monitoring and landscape considerations – will likely reveal a clearer picture as to what is driving these dynamics. To that end, with additional funding granted by CDFA and the Farm Bill we established an additional 36 plots in avocado groves in Ventura County and 5 plots in San Diego County (Fig. 1) in June 2017. Five of the 36 plots in Ventura (14 percent) were infested in locations in proximity to the Santa Clara River, with an incidence ranging from 2 – 20 percent (Fig. 4). In addition, we established 200 plots in infested and non-infested native vegetation throughout the infested range from July – October 2018 (Fig. 1). All 260+ plots, including avocado and native vegetation, are being monitored to address our broader questions concerning which locations are most vulnerable to *Fusarium* dieback-shot hole borer establishment and most important in its spread.

Trapping data reflect the number of beetles caught in funnel traps equipped with the attractant lure Quercivorol. The beetles often show peak in-flight activity – and with that, trap catch numbers – in winter and spring that reflect specific temperature ranges in the afternoon. The ability of beetles

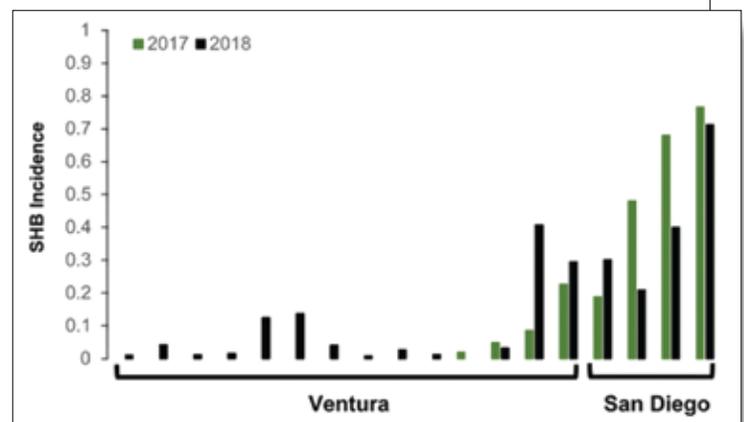


Figure 3. SHB incidence (the fraction of trees infested in each plot, between 0 – 1) for 14 of the 36 avocado groves that were infested in Ventura County and four of the five groves that were infested in San Diego County in 2017 and 2018 (green and black respectively).

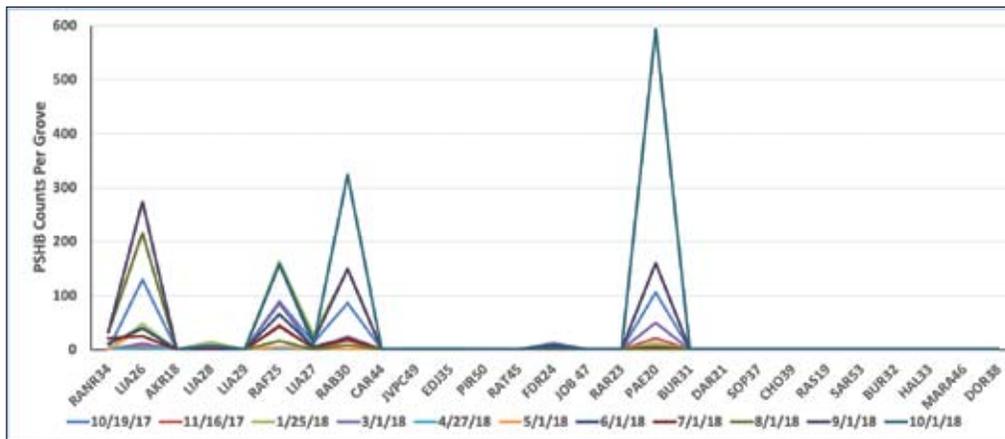


Figure 4. Number of beetles trapped over time in Ventura County avocado groves.

to develop depends on the temperature. If the temperature is above 59°F and below 90°F, the larval and pupal stages of the beetle can develop. However, adult beetles can only fly if the afternoon temperature is above 68°F. In winter and spring the peaks in trap catches can be explained by the fact that many beetles develop and become adults in the tree and accumulate, waiting to emerge and fly when temperatures are adequate. When a warm spell arrives, the “back log” of adult beetles will start to fly resulting in a peak in trap catches during these seasons.

Branch Assessment

To understand attack progress and impact of SHB on individual trees, we assessed 1,995 branches on 660 avocado trees in San Diego, Orange, and Los Angeles counties between 2015-2018. For each tree, we counted the number of entry holes for beetle establishments that were and were not successful on different tree parts including the trunk, primary, secondary and tertiary branches, and twigs. The beetle was able to establish on 1,013 of these branches (50 percent). Significantly more established hole counts were observed on smaller, tertiary branches ranging from 4-6 inches in diameter (Fig. 5; general linear model $p = 0.01386$). We did not observe tree death on established plots.

Strategies for Management of SHB in Avocado Groves

Pruning is a common management practice in commercial avocado groves as it prevents breakage from upward growth, reduces cost of harvesting and increases sunlight penetration to the lower canopy. Pruning also may serve as a potential strategy to remove SHB infested branches from the tree in an attempt to reduce beetle populations and further colonization of the tree. Data from our survey study show that beetle galleries are most common in the vicinity of branch collar regions on avocado (Fig. 8). Given that pruning infested branches — in combination with spray treatments to protect the branch collar from further attack — is a promising management

strategy to control the new beetle attack, we conducted a field trial to test a commercially available *Bacillus subtilis* (Serenade®) with/without a surfactant against *Fusarium dieback* associated with SHB. We randomly selected five eight-year old trees (Hass cv.) in an infested avocado grove for each treatment. Spray treatments included: *Bacillus subtilis* (Serenade®) at max field rate (1.5 percent), Serenade® (1.5 percent) with a surfactant (Pentra-Bark®) (1.5 percent), and a water control treatment. The treatments were applied once, directly to the branch collar, with a standard spray bottle immediately after pruning the branch. Beetle activity was quantified by

counting new entry holes in monthly intervals after branch removal on the pruned surface. All treatments were compared to the control for efficacy over the six-month period.

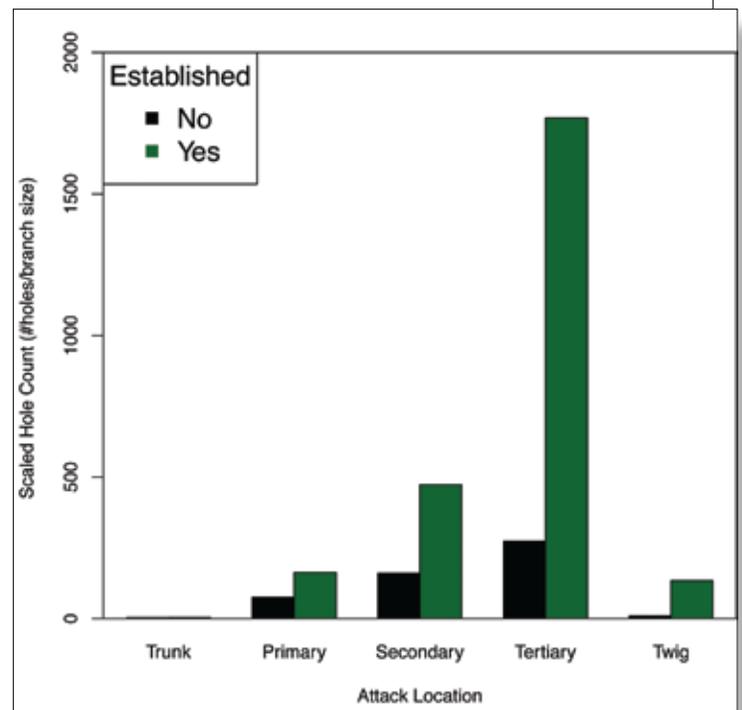


Figure 5. Attack frequency of SHB on infested trees by plant part.

After the six-month period, water-based latex paint was applied over the pruning surface and branch collar to evaluate which beetle holes were active. Using a method developed by our lab (<https://ucanr.edu/sites/eskalenlab/files/292756.pdf>), the beetle entry holes were covered with the paint, allowed to dry and then evaluated the next day to see if the beetles

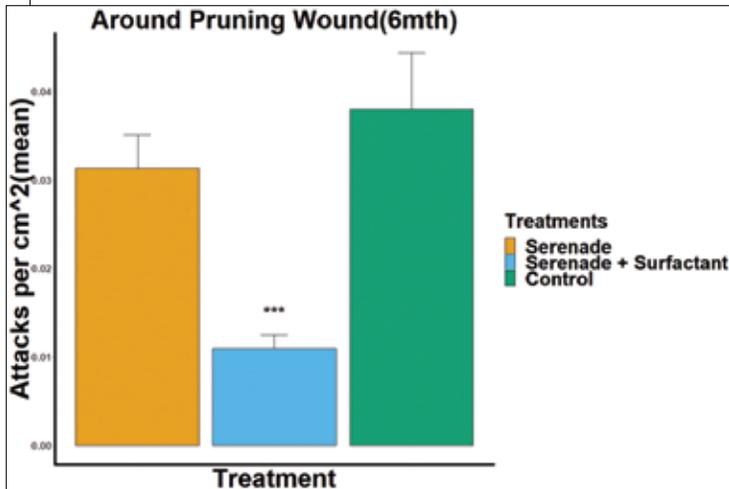


Figure 6. Six-month average count data per cm² of entry holes from the 30-cm area around the site where the infested branches were removed. Serenade® and surfactant mix was shown to be significantly effective compared to the control treatment ($P < 0.001$).

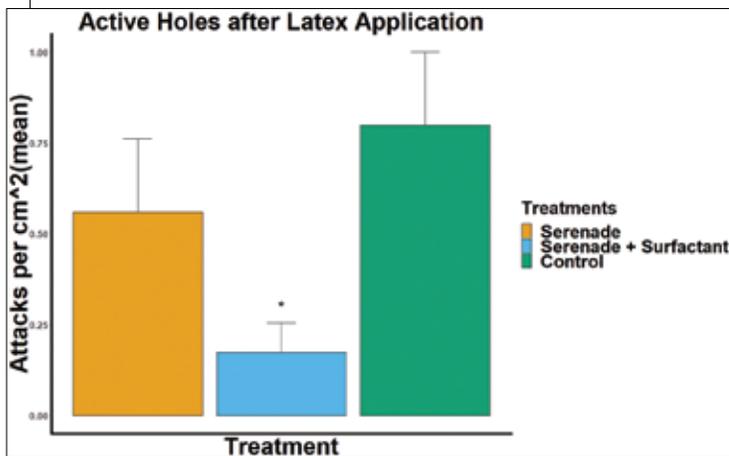


Figure 7. Six-month counts per cm² after water based latex paint application onto treated areas. Serenade® and surfactant mix was shown to be significantly different compared to the control treatment ($P < 0.05$).

opened the entry of the gallery. This allowed us to determine which galleries were active in the treated areas. Results revealed that pruning wounds treated with Serenade® plus surfactant had approximately four-fold fewer beetle attacks than either Serenade® by itself or the water control (Fig. 6).

Summary and Guidelines

Monitoring and experimental results show that SHB-FD is causing less damage to avocado trees compared to other hosts in native vegetation (e.g., box elder, willows, sycamores). Given that incidence fluctuates between sampling periods,

long term monitoring is essential to understand which factors are driving the disease dynamics. The beetle seems to prefer primarily the branches of avocado — in particular the tertiary branches. Hence, removal of infested branches and orchard sanitation is essential to control the population size of the beetle. Below are the revised best management practices that we recommend for avocado growers in California.

What to Do

1. Inspect branches and branch collar for signs of SHBs and FD symptoms in avocado.
2. If the beetle has not colonized (produced a gallery), there is no need to make pruning cuts.
3. If the beetle has colonized, or a cluster of attack is observed on branches, remove the entire branch.
4. If the beetle/fungus has colonized on the branch collar, remove the infested branch, including the branch collar. According to beetle flight activity (Fig 4.), the winter months (Dec-Jan) are the best time for pruning in SHB infested groves.
5. Spray pruning wound with *Bacillus subtilis* (1.5 percent Serenade ASO®) plus surfactant.
6. Chip and then solarize pruned wood using a clear plastic tarp for several months.

Pruned Plant Debris

1. To avoid beetle flight during pruning and reduce population pressure, conduct activities during the winter months (December and January) when the beetle population is building up in trees.
2. Do not move infested plant material without chipping the material.
3. Chip infested wood on site to a size of one inch or smaller.
4. If the branch is too large to chip, solarize using a clear plastic tarp on site for several months. (Winter — at least 3 months; Summer — 6 weeks.)
5. After plant material is chipped, it may be safe for using as mulch in the grove.

Equipment Disinfecting

1. Sanitizing equipment after pruning each tree will reduce the spread of fungal pathogens.
2. Prior to cutting/pruning, remove organic debris from equipment used for cutting (e.g., hand and power tools such as pruning shears, chainsaws), then spray or wipe with either Lysol® or 70 percent ethanol. Clorox® bleach diluted to 5 percent may be used.
3. Never use disinfectants on pruning wounds, as they could be phytotoxic. 🍌

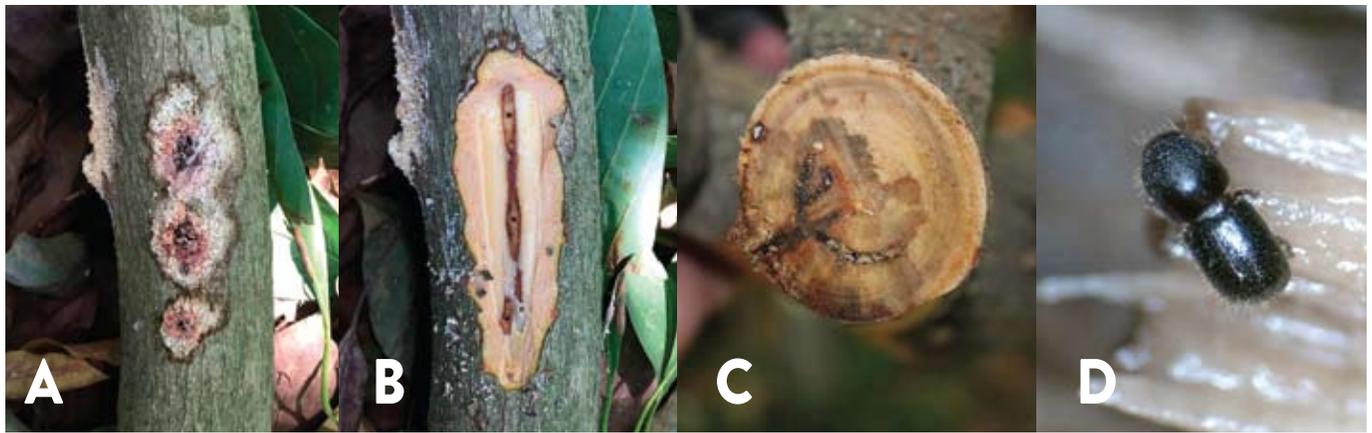


Figure 8. White ring of sugar exudate surrounds the beetle entry/exit hole (A), wood discoloration caused by *Fusarium euwallaceae* (B), beetle gallery formation (C) and female PSHB (D).

SIGNS AND SYMPTOMS:

The combined effects of tunneling and fungal growth cause *Fusarium* dieback (FD) disease, which interrupts the transport of water and nutrients in more than 64 tree species that are suitable for beetle reproduction. Once the beetle/fungal complex has killed the host tree, pregnant females fly in search of a new host.

Attack symptoms, which are a host tree's visible response to stress, vary among host species. Staining, sugary exudate (Fig. 8A), gumming, and/or frass may be noticeable before the tiny beetles are observed (females are typically 1.8-2.5 mm long) (Fig. 8D). Beneath or near these symptoms, you also may see the beetle's entry/exit holes, which are ~0.85 mm in diameter. The abdomen of the female beetle can sometimes be seen sticking out of the hole. Sugary exudate on trunks or branches may indicate a PSHB attack, but also could be the host response to tree injury. Note that exudate may be washed off after rain events and therefore may not always be present on a heavily infested branch. *Fusarium* dieback pathogens cause brown to black discoloration in infected wood (Fig. 8C). Scraping away bark over the entry/exit hole reveals dark staining around the gallery, and cross

sections of cut branches show the extent of infection (Fig. 8B). Advanced infections eventually lead to branch dieback (Fig. 9) and death of the tree.

HOSTS:

SHBs attack hundreds of tree species, but they can only reproduce in certain hosts. These include: avocado, box elder, California sycamore, coast live oak, cottonwood and willows. Visit <http://pshb.org> for the full list of susceptible tree species.



Figure 9. Branch dieback caused by Polyphagous shot hole borer-*Fusarium* on a Hass avocado tree in Azusa, CA.