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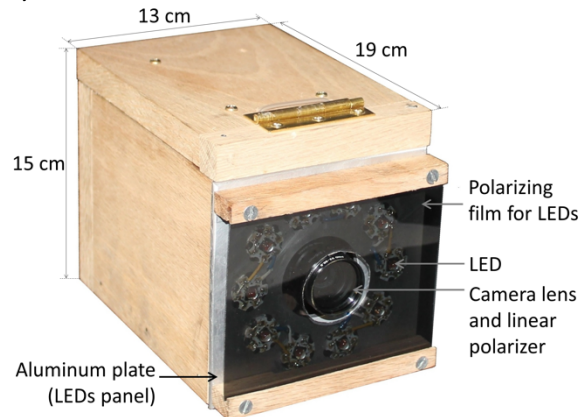
REAL-TIME SENSOR FOR EARLY DETECTION OF CITRUS HUANGLONGBING (HLB)

Alireza Pourreza, Kearney Research and Extension Center, University of California Cooperative Extension.

California is the major producer of fresh market citrus in the U.S., a \$2 billion industry that is threatened by a devastating disease called citrus Huanglongbing (HLB). Unfortunately, there is no cure for this disease and if a tree gets infected, it will die in a few years. In Florida, HLB was first seen in 2005, but after a few years the entire state of Florida got infected. Today, about 60% of Florida citrus has gone, mostly because there was no efficient HLB monitoring practice. HLB diagnosis using laboratory-based methods required manual sampling and they were time and effort consuming. An efficient HLB management requires high spatial and temporal resolution monitoring and eradication of infected trees. Therefore, a diagnosis sensor is needed for detecting HLB infected canopies before the development of symptoms. For high resolution monitoring, the sensor should also be able to conduct rapid and inexpensive inspection with high accuracy.

Starch accumulation in HLB infected leaves is an early indication of the disease. Starch has an optical characteristic of rotating the polarization plane of light. We employed this characteristic of starch to develop an early detection methodology in which the sensing system was very sensitive to the rotation in polarization plane of light. The sensor has a customized illumination system including 10

high-power and narrow band LEDs at 591 nm and a polarizing film. The sensor also has a monochrome camera equipped with a linear polarizing filter that is set in a perpendicular direction to the polarizing film of the illumination system.



The early detection sensor prototype

Starch accumulation in an HLB infected leaf generates blotchy mottle in an asymmetrical yellowing pattern. Deficiency of certain nutrients such as Mg and Zn causes symptoms similar to HLB.



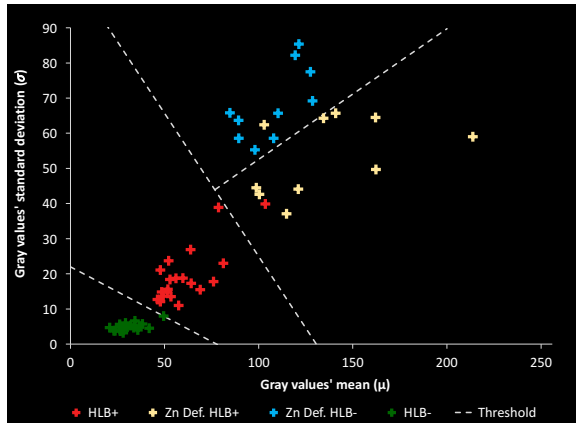
Leaf symptom of HLB, and Zn deficiency

The sensor was mounted on a gator vehicle and was tested in a citrus grove in Florida. The polarized images acquired from healthy, HLB, and Zn deficient canopies were further analyzed for diagnosis purpose.



Polarized imaged from healthy, HLB, and HLB with Zn deficiency

HLB samples were accurately identified from healthy and Zn deficient samples. Also, the sensor was able to detect HLB within Zn deficient samples.



Four classes of samples scatter-plotted based on image brightness (gray values' mean) and contrast (gray values' standard deviation)

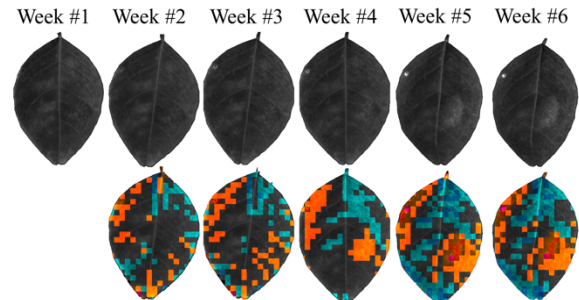
The polarized imaging methodology was adopted in two separate studies at the University of Florida to investigate the earliest time HLB can be diagnosed by polarized imaging technique after infection. In one study, two-year old Valencia orange plants were inoculated using disk-graft method.



A citrus leaf graft-inoculated by a disk tissue from a HLB infected leaf

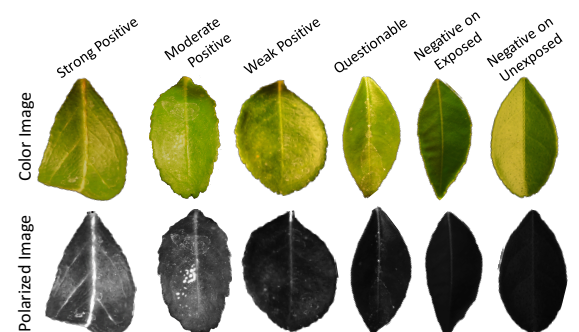
Time-lapse polarized images of leaves from inoculated citrus plants were acquired on a weekly basis. HLB symptoms (as starch

accumulation) started to become visible in the polarized images five weeks after inoculation, while the plants were still in asymptomatic stage.



Time-lapse images of a leaf from HLB infected plant acquired on a weekly basis after infection

In another study, the polarized imaging methodology was employed to detect HLB in insect inoculated citrus seedlings while in asymptomatic stage. Citrus seedlings were exposed to intensive HLB-positive Asian Citrus Psyllid (ACP) feeding. Polarized images were acquired two times; once after one month after inoculation and again two months after inoculation. As well as HLB detection, the level of infection was obtained for different leaf samples. Polymerase chain reaction (PCR) tests were conducted to validate the HLB status and the level of infection in each leaf sample.



Color and polarized images of healthy and HLB infected leaf samples in asymptomatic stage with different infection level

Currently, we focus on improving the accuracy and early detection performance of the polarized imaging sensor and developing a commercialized product for practical in-field diagnosis. This affordable tool can help the California citrus growers to protect their groves from HLB.

I would like to express my great appreciation to Drs. Won Suk Lee, John Schueller, Reza Ehsani, Ed Etxeberria, Bill Gurley, and Arunava Banerjee at the University of Florida (UF). Also, I would like to thank Dr. Eran Raveh at ARO Gilat Research Center, Negev, Israel, for his assistance during this project.

THE 2016 INTERNATIONAL CITRUS CONFERENCE

Ben Faber, UCCE Farm Advisor in Ventura and Santa Barbara Counties

The International Citrus Conference was held at Iguazu Falls (Iguassu or Iguazu depending on what country or side of the falls you are on) in southern Brazil from September 18-23. The falls are truly amazing and so was the conference. This is the world's largest waterfall system and in 3 minutes the equivalent of all the water used in Ventura agriculture in a year takes a dive on the Iguazu River. The amount of information that flowed in the conference was almost the same. There were about 1,500 from around the world, growers but mainly researchers. And there were folks from California there – two growers, a Citrus Research Board representative, a journalist, a Pest Control Advisor, six folks from UCR and their graduate students and all the spouses associated with these people.

In 18 separate sessions, from marketing to pests to pathosystems (interactions of plants/arthropods/disease) to genetics to

breeding to biotechnology to cultural practices to it seemed to go on forever, the information flowed. The sessions were actually separated into topics as well-defined and distinct as Breeding from Scion and Rootstock Varieties. Some of the information was a continuation of studies from those presented at the Spanish meeting four years ago. Some was totally new. Some was like a test balloon without a lot of supporting material. It was sort of like, it worked in this situation on this other crop and might work in citrus. Some of the presentations were by chemical representatives, but the bulk were made by researchers from various institutes and universities. It is good to have the chemical reps there to get some insight into the products they are working on. So the conference is a lot of new ideas and new products. Some may work and many won't.

I can say that thankfully we do not have a lot of the problems that the rest of the citrus world has or at least that we don't have yet. There are problems like Greasy Spot that are high humidity pathology problems, but there are others like Citrus Longhorn Beetle and Citrus Canker that could become problems here in California. They just aren't here yet. This conference and others like it, like the American Society of Horticultural Sciences and the American Psychopathological Society act like early warning exchanges. Problems are described along with the biology of the problem and then people can determine whether it might be likely in their situation.

Some of the highlights of the meeting for me were the presentations on various aspects of Huanglongbing and Asian Citrus Psyllid and tree interactions. It turns out that there are some major physiological changes that occur in the tree with not just the disease but also the

infestation by the insect. The tree can put up various defenses and internal “communications” in response to feeding by the insect. Some of this can be “managed” and how this can be done is being studied. Some scion varieties and rootstocks are more responsive than others and will become a basis for building other commercial selections of citrus. This will be through traditional breeding practices and through new enhanced breeding methods that do not introduce non-Citrus genetics into the resulting tree. There are currently a number of projects around the world looking at new breeds of citrus and these will be tested for their resistance/tolerance/immunity to HLB. There are some very promising trials going on.

Full commercial application of these findings is still years away. If an ideal combination of rootstock/scion combination came along tomorrow, it would still take years to gear up for replacing the world’s trees in the ground. In the meantime, the Brazilians and others have shown how to manage the disease on the ground through chemical treatments, monitoring, roguing and replanting with disease-free material. There really is a lot of activity and success at finding new and faster techniques for finding diseased trees. This is key in keeping inoculum levels low, so that there is reduced likelihood of disease spread. There is a lot of good reading and introspection in the abstracts from the conference. If you read them, don’t be put off by some of the terminology. Some is highly technical, as only some professions can speak, but there is still a lot of information that a grower can garner from a reading.

The abstracts can be viewed at: http://www.icc2016.com/images/icc2016/downloads/Abstract_Book_ICC_2016.pdf. Soon the full papers will be available in a Proceedings. I’m

not sure when that will be available, but also will be available online.

UC RIVERSIDE SCIENTISTS EVALUATE TRUNK INJECTIONS OF PESTICIDES FOR THE MANAGEMENT OF AMBROSIA BEETLES IN CALIFORNIA AVOCADOS

Frank Byrne, Akif Eskalen and Joseph Morse, University of California, Riverside

Two closely related Ambrosia beetles (*Euwallacea* sp.) have been identified in commercial avocado groves in California. The polyphagous shot hole borer (PSHB), detected in Los Angeles, Orange counties and recently in Ventura county, and the Kuroshio shot hole borer (KSHB), detected in San Diego and recently in Orange and Santa Barbara counties, are morphologically indistinguishable, but genetically distinct. Already widespread in a variety of reproductive host trees common in the urban landscape (including box elder, willow, several maples, oak and sycamore species), the beetles represent a significant threat to trees in both landscape and agricultural settings. Adult females construct galleries in the xylem system of host trees, where they cultivate symbiotic fungi (*Fusarium*, *Paracremonium* and *Graphium* spp.) as a food source for their developing young. The fungi are taken up by progeny females in specialized organs within their mouthparts, and transported to other sites within the same tree, where new colonies are established, or to newly colonized hosts. The galleries compromise the structural integrity of infested trees, which can represent a serious safety hazard in urban environments, and disrupt the flow of water and essential nutrients within the xylem. In addition to the physical damage, the fungi extract nutrients from the xylem system, further depriving the tree of nutrients essential for healthy growth and fruit production.

An effective biological control agent is not yet available to manage the SHB in California, and so management for now must rely on the use of chemical pesticides. The control of Ambrosia beetles and their associated fungi using



Fig.1 Wood core sampling from avocado using increment borer.

chemical pesticides is complicated because of their location inside the host trees. The application of insecticides to the external surfaces of trees, where the beetles must first alight prior to boring, has the potential to kill beetles by contact activity, and they may also have the potential to control emerging young adults before they can re-infest the trees.

The drawback of surface treatments is that multiple applications are often required because of the relatively short duration of efficacy. In addition, once the beetle burrows inside the tree, surface treatments are become ineffective. One possible solution to this problem may be the use of systemic pesticides, and scientists at UC Riverside are evaluating the use of both systemic insecticides and fungicides in a 2-pronged attack against the symbiotic system.



Fig.1 Wood core sampling from avocado using increment borer.

Systemic pesticides are mobile within the xylem system of plants, and the fungicides could potentially target the fungi growing in the xylem and deprive the beetle larvae of a food source. The insecticides would prevent the beetle from establishing galleries within susceptible tree hosts, and prevent the survival of beetles and their offspring already present within trees. The big problem with systemic pesticides is getting sufficient concentrations of chemicals to the areas within the trees where the beetle and fungus occur. Although there are exceptions, most systemic treatments are administered to the soil for uptake through the roots. However, in mature avocado groves, the high organic matter content of the soil can prevent effective absorption by roots because the pesticide becomes bound to organic components within the soil. Trunk injection of pesticides directly into the vascular system of trees eliminates the

potential for binding of pesticides within the soil, and increases the amount of active ingredient inside the tree available to impact the beetle/fungal system. Systemic pesticides must be formulated for trunk injection and so careful evaluation is needed to ensure optimal efficacy. Trials are being conducted with the assistance of avocado industry and grower collaborators in areas where the SHB has been recorded. The chemicals are injected into the trees using commercially available equipment, and the movement of the active ingredients is then monitored over time in wood core samples taken at different heights of the trees. Two methods are being used to confirm the presence of the chemicals. Insecticides are being quantified using ELISAs that are specific for the active ingredients under investigation. Wood cores taken from trees treated with fungicides are placed in direct proximity to the fungal pathogens growing on agar plates to determine if growth of the fungus is inhibited.

The investigations are still at an early stage, but the researchers are optimistic that they will develop effective control strategies for the SHB that growers can incorporate into their overall pest management programs. Laboratory based bioassays have been used to identify several pesticides that are toxic to the beetle and fungi. The objective of the field trials is to determine whether these chemicals can be utilized as trunk injection agents for the protection of avocado trees. Anyone interested in finding out more about the SHB should go to the web site maintained by Dr. Akif Eskalen at:

<http://eskalenlab.ucr.edu/avocado.html>

AN OVERVIEW OF MANGO

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Mango (*Mangifera indica*) is a major fruit tree crop of the tropics and subtropics, particularly in Asia, where it is among the most economically important crops and considered by many to be the “king of fruits”. Mango currently ranks 5th in global production of major fruit crops after Bananas, Citrus, Grapes, and Apples. Mango has very limited production in temperate Mediterranean climates and currently the only US states producing the fruit are California, Florida, and Hawaii. In California, mango grows in a geographical range similar to avocado, including the southern coastal part of the state. However, unlike avocado, commercial production of mango in the Coachella desert is possible (Figure 1) despite several challenges.

Production of mangos continues to grow globally, from 21M tons in 1994 to 43M tons in 2013. Much of this production occurs in India, which alone accounts for about 30% of mango production worldwide. Other major producers of mango include (in ranked order) China, Thailand, Mexico, Indonesia, Pakistan, and Brazil (FAOSTAT 2013 data). Mangoes are a vital component of the diet in many of the less developed countries in the tropics and subtropics. Most mangoes are eaten fresh and only about 1% of fruit produced globally is destined for processing. Generally, there is little waste with mango production as the seeds are used for starch, peels used as a source for anacardic acid, and wood used for low quality lumber.



Figure 1: Mango can be grown in the California desert.

The mango's origin is believed to be in the tropical rainforests of South and South-East Asia. The tree is a tropical evergreen, with simple, alternate leaves ranging from 12-38 cm. Leaf shape is highly variable depending on the cultivar and are produced in whorls. Fully mature trees can reach heights of 40m and survive for hundreds of years. Typically, the juvenile period lasts from 3-7 years. The root system consists of a large central taproot and many shallow feeder roots. Mango flowers are produced on widely-branched panicles that reach up to 30cm in length. The inflorescence is very dense, covered with hundreds of small flowers about 5-10 mm in diameter. Flowers are largely fly pollinated, but may also be pollinated by other insects and/or by wind.

Mango fruits are large, fleshy drupes containing carbohydrates, proteins, minerals, vitamins, and amino, organic, and fatty acids. While ripening, mangos are very acidic and contain high levels of vitamin C. Fully ripe mangos contain moderate levels of vitamin C and are rich in provitamin A and vitamins B₁ and B₂. Sucrose is the primary sugar of fully ripened mangos and a ripe mango contains about 11%-20% sucrose, or 15%-20% of the total soluble solids.



Figure 2: Evaluation of cover crops (left: sudan grass –right: alfalfa) in a California desert mango production to improve soil fertility and mitigate tree nutrient unbalance.

While annual cereal and legume crops benefitted greatly from the agronomic improvements of the Green Revolution, the improvement of tree crops has lagged behind. This is due to their complex genetics (polyploidy, heterogeneity), long life cycles, and high cost of maintenance. Although tree crop improvement technology is progressing rapidly, most fruit cultivars today tend to come from ancient selections. Thus, mango trees continue to face serious problems including alternate bearing, frost intolerance, lack of disease resistance, and low yields.

The desert of Southern California's Coachella Valley presents a unique set of challenges for

mango production, especially in an organic certified orchard. The alkaline (pH 8.1) calcareous sandy loam soil can cause tree nutrient unbalance. High day temperatures in the summer (115°F) cause high nutrient turnover and leaf/fruit burn while low night temperatures in the winter (29°F) can cause irreversible frost damage. To address these challenges, our research group at UC Riverside has been working since 2014 towards using a combination of acidified irrigation water, cover crops (Figure 2), and soil amendments to enhance the soil fertility and ultimately improve mango yields in this organic system.

WATER-BASED LATEX PAINT AS A MEANS TO TRACK AMBROSIA BEETLE ACTIVITY ON INFESTED TREES

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Ambrosia beetles are known to cultivate their symbiotic fungi as a nutrient source inside the various woody hosts they bore into and colonize. In their natural tropical environment, the beetles serve an ecological purpose by accelerating degradation of decaying wood. However, when introduced to a non-native environment, the beetles can cause significant damage in living hosts (Hulcr and Dunn, 2011). Since 2012, in southern California, a disease called Fusarium Dieback has been causing significant damage to landscape trees, native tree species, and agriculturally important crops, such as avocado (Eskalen et al., 2013). This disease is the result of activity from two closely related invasive ambrosia beetles and their associated mutualistic fungi. These two Shot-Hole Borers (SHB) are

morphologically indistinguishable, but have been found to be genetically distinct along with the fungal symbionts they cultivate. Currently there are limited options to treat affected trees which too often result in complete removal of infested trees.



Figure 2: Shows a female shot-hole borer blocking the entry hole with her abdomen (A) and in cross section (B).

To establish inside a host, a single female beetle bores through the bark to initiate gallery construction in the wood. Soon after the initial attack, the female beetle inoculates the wall of the galleries with fungal spores of their associated symbiotic fungi (*Fusarium* spp., *Graphium* spp., *Paracremonium pembeum*) which they use as a food source (Lynch et al., 2016, Freeman et al., 2015). After the female has completed constructing and inoculating the gallery, and laying eggs, the female beetles were observed blocking the entry point of the gallery with their abdomen. If the entry hole is obstructed by a foreign object, then the beetles will clear the entrance of the obstruction and continue to block the entry point of the gallery with their abdomen which indicates that the beetle is alive and active. However, upon close inspection of individual entry holes, it is apparent that beetles are not present or active in *all* entry holes inspected. In this study, we took advantage of the blocking behavior of the beetle to determine if galleries are active in order to determine the efficacy of pesticides

applied to affected trees in an attempt to manage this pest.



Figure 3: 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

We are currently testing various pesticides along with evaluation methods to accurately assess the efficacy of applied treatments, with the ultimate goal of managing these pests and their symbiotic fungi. When conducting field trials to investigate pesticide efficacy on reducing SHB activity, it can be difficult to determine which galleries are active or inactive. Close inspection can help determine if a given entry hole is active, but in heavily infested trees there can be over 100 entry holes to inspect in a square foot, which can be time consuming and increase the possibility of miscounting. To easily distinguish between whether the beetle is alive or dead in the gallery, we have developed a method using water-based latex paint to cover a designated area on an infested tree and compare activity between

areas within the same tree and also between different trees. This method is particularly useful to compare pesticide treatments from one treated tree to another in order to quantify the efficacy of experimental treatments on SHB activity.



Figure 4: Progression of beetle reemergence after paint application. After paint application has begun to dry (A), the female beetle will begin to re-establish the entry point with her abdomen (B), break through the paint obstruction (C), and continue to maintain the gallery (D).

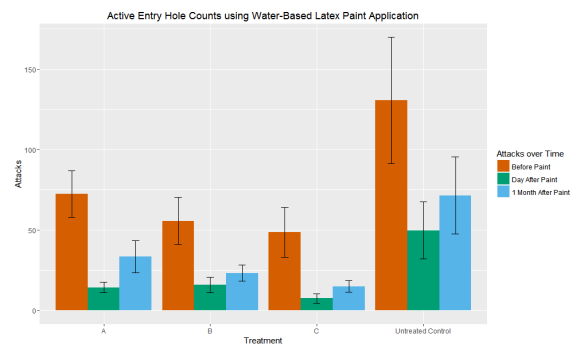


Figure 1: Counts of SHB attacks over a one-month period treated with various applied chemicals after application of water-based latex paint.

The method was as follows: An 8" x 11.5" (size of an A4 paper sheet) area was outlined in vertical orientation with a paint marker at each cardinal direction, on the trunk, at a consistent height, for a total of four replications on each tree per treatment. Before painting the marked area, entry holes were counted. After initial counting, the off-white water based latex paint was applied with a paint brush. The painted areas were revisited and counted again the next day to look for visible entry holes; the areas that have holes visible after the paint application has dried are indicative of active entry holes (Figure 2D). These painted areas were revisited the following month to track the activity. If new entry holes were visible on the painted area, this indicates an active beetle gallery. In our trial, we compared three chemical treatments to an untreated control to observe effects on beetle activity. In Figure 1, there is a significant decrease in SHB attacks from "Before Paint" to "Day After Paint" since all holes counted initially are not truly active. Treatments B and C were shown to have a statistically significant lower increase in active entry holes within a month's time when compared to treatment A and the untreated control. This method is a useful evaluation method to monitor beetle activity as a response over time to applied treatments in field trials.

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