Figure 1. A laurel wilt detector dog sits next to an avocado tree indicating to the handler that the tree is positive for laurel wilt. Innovative Detection Concepts (www.idetectionconcepts.com), the company that has trained the dogs, runs two dogs independently in each grove. Both dogs must hit on the same tree for it to be considered positive.

Progress Reported on Redbay Ambrosia Beetle & Laurel Wilt Issues

By Tim Spann
Research Program Director

On November 3 - 4, 2016, the University of Florida (UF), Tropical Research and Education Center hosted a two-day Avocado Laurel Wilt Summit in Homestead, Florida. The meeting consisted of a full day of research presentations by UF and United States Department of Agriculture (USDA) researchers followed by a day-long field tour.

The meeting was attended by about 150 people, mostly Florida avocado growers, but there was a sizeable delegation from Mexico, and California was well represented by

Figure 2. A screen capture of an aerial view of an avocado grove captured by a drone (inset). The yellow circle highlights a wilted limb in the upper part of the canopy of a laurel wilt affected tree.

Mary Lu Arpaia, Ben Faber (Ventura County), Mary Bianchi (San Luis Obispo County), Sonia Rios (Riverside and San Diego Counties), Akif Eskalen, Paul Rugman-Jones (from Richard Stouthamer's lab) and myself. I believe it is safe to say that we all came away with a sense of relief at the major progress that the Florida researchers have made in learning to manage this devastating pest and disease in avocados.

The Redbay Ambrosia Beetle (RAB; *Xyleborus glabratus*) is an invasive ambrosia beetle from Southeast Asia that was first found in Georgia in 2002, and was likely introduced through infested wood packing material. RAB's symbiotic fungal pathogen that causes laurel wilt, *Raffaelea lauricola*, is of unknown origin, but is believed to have arrived in the United States with the beetle. Since the first detection in Georgia, RAB and laurel wilt have moved quickly up and down the East Coast and across the Gulf Coast to eastern Texas. Unlike fusarium dieback and the shot hole borers we are currently dealing with in California, laurel wilt is a lethal, highly systemic pathogen, capable of killing a mature avocado tree within a couple of weeks after infection. Furthermore, RAB and laurel wilt are restricted to the Lauraceae, the laurel family that includes avocados.

Early Detection is Key

It was reported that about 25,000 commercial avocado trees (3 percent of the industry) have been lost to date in Florida, with an estimated value of $8.3 million (the industry includes about 8,300 acres). For commercial groves, a large effort has been placed on early detection of infected trees and grove sanitation practices. Early on, growers were trying to scout their groves from helicopters and by walking, but both methods proved impractical because of their cost and inability to detect very early symptoms, which is critical with this disease because of the speed with which it kills trees. In cooperation with forensic scientists from Florida International University, dogs have been trained to detect the laurel wilt pathogen and they can detect infected trees before any visual symptoms appear. At least one company is offering canine scouting services for about $150 per five acres.

Another scouting method being tested is the use of drones. Various entities are testing drones outfitted with cameras that can look at the visual, infrared and other spectra to try to detect the earliest symptoms. Drones are proving particularly helpful in old groves with large trees where it is difficult to see early wilt symptoms in the upper canopy from the ground. They also can be used in combination with the dogs to identify "hot spots" in groves and then have the dogs determine the extent of the infection around those hot spots.

Sanitation – immediate removal and destruction (burn or chip) of infected trees and prophylactic treatment of sur-

Figure 3. Drs. Daniel Carillo (UF TREC) and Paul Rugman-Jones (UC Riverside) have a discussion in front of a laurel wilt affected tree (left). The tree was just beginning to show wilt symptoms when the meeting organizers selected it about 10 days before the field tour; it was in full wilt by the time of our tour demonstrating the rapid decline caused by the disease. A branch on the tree showing the telltale sawdust "toothpicks" of beetle boring activity (upper right). A cut away of the branch showing a beetle entry hole (arrow) and dark staining from the laurel wilt fungus (center right). A cross section of the limb with beetle entry holes (arrow) and dark staining from the laurel wilt fungus under the bark (lower right).

rounding trees with fungicide — is proving to be the key to managing the spread of laurel wilt within commercial avocado groves. Anecdotal evidence suggests that the pathogen can readily move from tree to tree within a grove through root grafts, especially in old groves. When an infected tree is found and it is not immediately removed, including the roots, it is common to see the neighboring trees decline and the disease spread down the tree row. For this reason, the most aggressive growers are removing the infected tree and the neighboring trees on either side to ensure they get ahead of the spread, followed by treating a two-tree perimeter with fungicide.

Redbay Ambrosia Beetle and Other Ambrosia Beetles

A lot of research has focused on understanding the biology of RAB and potential control strategies. As California growers know from our own battle with shot hole borers (SHB), ambrosia beetles are notoriously difficult to control with pesticides since they spend so much of their life inside their host tree. Thus, pesticide treatments for laurel wilt are only recommended on neighboring trees following sanitation. Currently, Florida has a section 18 emergency exemption for the use of Hero® against RAB, and they are preparing another section 18 application for Cobalt® from Dow AgroSciences.

The unfortunate news in Florida is that RAB is not the only beetle spreading laurel wilt. Sixteen other ambrosia beetles — some native, some invasive — have picked up the pathogen and can spread it. It is not believed that these beetles have developed a symbiosis with *Raffaelea lauricola*, but they are simply becoming contaminated with the fungus when they bore into an infected tree. To date, there has not been a single documented case of laurel wilt spreading in avocados by RAB, it is all occurring from these other 16 species. In particular, *Xyleborus bispinatus* and *X. volvulus* are the biggest culprits; every specimen of these two species tested in South Florida has been contaminated with *Raffaelea lauricola*.

On the biological control front, there have been very few candidates found that have any real potential. Entomopathogenic fungi are proving to be the best potential option in Florida. However, because these fungi are relatively slow acting, RAB and other beetles can still infect and kill trees before dying. At this point, the available entomopathogens are viewed more as a strategy for broad population control and not a specific treatment for within groves.

Perhaps most exciting was work presented from Dr. Paul Kendra's lab, USDA Miami, on the chemical ecology of RAB. An interesting premise they started with is that since these beetles essentially have a "one and done" strategy — that is, they leave their host tree and fly once to find a new host — they must use some very reliable cues to find those hosts. However, they do not have pheromones and they are not attracted to volatiles from their symbiotic fungi over long distances, so how do they do it?

What Dr. Kendra's lab has discovered is that the beetles use a suite of cues to find their hosts. First are visual cues. These beetles see tree trunks and can home in on that silhouette. Second is a chemical called α-copaene. Copaenes are a class of chemicals known as terpenes that are produced by a number of different plant groups. α-copaene is attractive to RAB over relatively long distances and is likely a chemical cue the beetles use to find a host tree in the right family. Third is the use of quercivorol, the same lure we use in California for SHB. Quercivorol — a chemical component of the aggregation pheromone of the ambrosia beetle *Platypus quercivorus* — is believed to be a short-range attractant for RAB, allowing it to home in on a specific tree once it's in the general vicinity from using visual and α-copaene cues. When α-copaene and quercivorol were tested in combination on traps, the effect was synergistic, capturing more beetles than just the combination of either component alone.

Florida is home to another species of *Euwallacea* ambrosia beetle, the same cryptic group of beetles that includes the polyphagous and Kuroshio shot hole bores as well as the tea shot hole borer. In tests in Florida, the α-copaene was found to be attractive to their species of *Euwallacea* as well as to RAB. Thus, there may be potential for α-copaene to be used in California in conjunction with quercivorol for SHB monitoring or in an early detection program for RAB and laurel wilt.

Considerable progress also has been made in looking for repellants to prevent the beetles from finding avocado trees. Several researchers have found that fungal volatiles, not wood volatiles, drive beetle boring. This explains why it's been observed that initial beetle attacks are unsuccessful (the beetles do not create a gallery and lay eggs). However, these initial attacks serve to introduce the fungal symbiont to the host tree, which then attracts more beetles. By carefully analyzing the chemicals created during the period after initial fungal introduction the researchers found that the trees initially generate methyl salicylate (MS). MS is a near universal plant response to attack and is part of the systemic acquired resistance pathway in plants. The researchers found that MS production peaked three days after inoculation with the fungus and the trees repelled the beetles, but 10 days after inoculation MS production ceased and the trees became attractive to the beetles. It appears that MS is a general repellant to *Xyleborus* beetles and it is being tested against other genera. ISCA Technologies of Riverside is working to develop a commercial formulation of MS in combination with verbenone, another general beetle repellant, for use in Florida avocado groves, which may eventually be of use in California.

Raffaelea lauricola and the Development of Laurel Wilt

Plant pathologists characterize laurel wilt as a classic vascular wilt disease. That is, the tree responds to the presence of the pathogen by developing tyloses — outgrowths from the walls of xylem vessels used to wall-off injury or disease — which plug the xylem vessels and prevent the transport of water. In the case of laurel wilt, this process is very rapid and within three days of inoculation large portions of the xylem become non-functional.

Laurel wilt is unique in that it is the only systemic, lethal ambrosia beetle symbiont known. It is similar to Dutch elm disease and oak wilt and there are management lessons that can be learned from these diseases.

Figure 4. Three methods for treating trees with fungicide. Dr. Jonathan Crane demonstrates the passive root infusion (IV bag) method developed by UF TREC researchers (left), and two pressurized injection systems developed by growers (upper and lower right).

- Sanitation is critical — inoculum must be removed from the environment to help mitigate spread
- Fungicides are good only for high value trees — the labor and product costs involved make the wide use of fungicides impractical
- Trenching around trees to sever root grafts can reduce pathogen spread through this pathway
- Insecticides are seldom recommended due to poor efficacy, safety and cost
- There are no good conclusive data to support biocontrol strategies for these types of pests and diseases

Combined, Dutch elm disease and oak wilt provide us with 140 years of experience and one definitive conclusion can be drawn: **the most effective control strategy is a multipronged approach**.

Plant Physiology Affects Laurel Wilt

Perhaps some of the most interesting information presented during the meeting was about the tree itself. In general, ambrosia beetles are nature's cleanup crew, helping to break down dead and dying trees. The thing that makes RAB and the polyphagous and Kuroshio shot hole borers different is that they appear to attack "apparently healthy" trees. This has received some much-needed attention by the laurel wilt research team as "apparently healthy" may depend on your frame of reference.

There are many Lauraceous species that are valuable nursery tree crops in the southeastern United States and this has allowed for the study of tree attack under relatively controlled conditions. Researchers have found that even though nursery trees are "well-watered" there can be enough fluctuations in their water status to create transient periods of stress. By looking at physiological factors such as stomatal conductance — how much CO_2 and water vapor are moving in and out of a leaf's stomates — the researchers can document these periods of stress even though there are no visual signs of stress. It is during these periods of stress that the trees are attractive to the beetles and become susceptible. Thus, the researchers are working to develop recommendations — that may someday be applied to avocado groves — for minimizing tree stress and hopefully reduce laurel wilt incidence. This is a good lesson for us here in California as we enter our sixth year of drought. Although our trees may look "apparently healthy," there are likely stresses on the trees that cannot be seen with the naked eye.

Another group has focused on the apparent lower susceptibility of avocado to laurel wilt compared with native Lauraceous trees in South Florida (e.g., swamp bay), and the differences in susceptibility between avocado races. Initial investigations indicate that the West Indian race of avocados is more susceptible than Mexican and Guatemalan races. Early results indicate that this may be due to the rate of sap flow in the tree, with higher sap flow equating to more susceptibility. The reason for this is not yet known, but the researchers have documented variation in xylem vessel diameter among avocado races, and the West Indian race trees have the largest vessel diameters, which may allow the pathogen to spread more quickly.

Overall, I believe the tone was much more optimistic than it was when I last visited Florida two years ago. A lot of progress has been made in understanding how this pathogen works and about the biology of RAB and other ambrosia beetle carriers. But most importantly, significant progress has been made on early detection and management of the disease in commercial groves. It no longer appears that laurel wilt is an unmanageable disease, but the question remains whether it can be economically managed.