Laurel wilt working group
Meeting Notes

Date: Monday, November 18, 2013
Time: 10:00 AM – 2:30 PM
Location: TREC Classroom (teaching building)
18905 SW 280 St., Homestead, FL 33031-3314

Opening remarks/welcome……………Jonathan Crane/Richard Gaskalla

Trapping results/trees positive for LW…..Richard Gaskalla/Wayne Dixon (FDACS-DPI)

Dr. Dixon presented an updated map which showed laurel wilt (LW) continued to spread into new counties in Florida; 8 new counties in 2013 for a total of 48 counties positive for LW to date. He then showed a map of all the RAB trapping sites and LW sentinel tree sites located in the Miami-Dade's urban, urban-natural boundary and agricultural area; there are 47 DPI RAB trap sites, 28 with sticky cards. Wayne then showed a table of 16 beetle trapping sites, the tree species it is hung in (native or avocado tree) and the number of RAB captured. The five highest RAB traps have captured 292, 287, 78, 75, and 73 RAB (number trapped somewhat dependent on start time of the trap); all located between SW 137 Ave. on the east, SW 8th St. on the north, SW 56 St. on the south and SW 187 Ave. on the west.

Current industry LW survey updates and control strategy ...Don Pybas (Avo. Adm. Com.) /Jonathan Crane (UF-TREC)

Dr. Crane reviewed the dates and number of LW suspect trees identified from the June, July, August, and October aerial surveys conducted by Mr. Pybas, Laurel Wilt Coordinator. The number of LW suspect avocado trees identified ranged from zero on 6 June, SE quadrant, to 64 on 7 October, NE quadrant. During 2013 the trend in the number of molecular LW samples taken increased from January to March, peaked in May, was lower and flat during June through October. The number of positive LW samples identified molecularly also increased from January to March, peaked at March-May, and declined during June through October. This sampling data may however, not reflect the true number of suspect trees because if multiple trees are identified at a specific grove not every symptomatic tree may be sampled. In addition, since the LW pathogen is not evenly distributed in the trees, is at a low titer inside the trees, and sampling error may miss wood sites with the pathogen, the number of LW suspects and detections may be higher than reported. In summary, since September 2011 (to November 2013) the UF/IFAS TREC Diagnostic Clinic has processed 351 LW samples and 58% of those have been positive for LW. However, due to the spread of LW through root grafted avocado trees the number of trees destroyed (either positively identified with LW or symptomatic) has been in excess of 2,500 (~0.36% of the estimated total number of avocado trees in the industry).

Dr. Crane reported that the number of cultivars where mature avocado trees have been documented to succumb to LW is 25. He then briefly re-iterated the current RAB-LW control strategy and cited some of the industry's future research needs: RAB trap/kill system, more rapid identification of LW suspect trees from the air and trees nonsymptomatic but infested with LW, RAB repellents, determination of the extent of non-RAB LW infested beetles attacking transmitting LW, development of a entomopathogen system, improved fungicide application methodology and efficacy, and new fungicides and/or formulations.

Research updates and plans

Dr. Evans (UF-TREC): Economic analysis of early detection and suppression program

Dr. Evans presented a preliminary economic analysis comparing the economic and biological consequences of not implementing, incompletely implementing and completely implementing the LW early detection, suppression, and tree destruction program (called sanitation program). The analysis was designed to estimate the benefits of the sanitation program; the benefit in this case is avoiding the cost of biological and economic damage. Three costs were estimated, the cost of doing nothing (i.e., no management), the cost of doing something (i.e., the cost of the control program), and the amount of economic damage occurring in spite of doing something (i.e., the control program).

Benefits = (costs of doing nothing) – (cost of control program + damage in spite of program)

A cost-effective program would be one where the cost of the program is greater than the costs of the control program. One of the major components of the analysis is the epidemiology or “natural” spread rate of LW which encompasses the ambrosia beetle vectors and the pathogen movement through root grafting to adjacent trees. Unfortunately the spread rate data is limited, extremely complicated and in-progress so only a preliminary analysis with the data in-hand could be performed. The analysis assumes 1 tree is initially succumbs to LW. The preliminary results of the analysis suggest that doing nothing could result in a 425% spread rate and rapid decline in avocado trees within a two-year time frame. In contrast, implementing the sanitation program imperfectly (allowing some infested wood material to remain intact) would result in a spread rate of about 125% and implementing the sanitation program perfectly a spread rate of 10%; both with substantially less tree loss. In reality over the last 21 months (i.e., since the first LW positive commercial avocado tree was confirmed) about 2,500 trees have been lost. Theoretically in order to reach a loss of 100,000 trees would take about 2-3 years with no sanitation program, 4-6 years with a program leaving infested wood, and 40+ years with a well-run sanitation program.

Dr. Evans went on to analyze the additional costs and benefits of spot treating trees or completely treating an entire grove with fungicide using the current fungicide and technology available and concluded that in the near-term implementing the sanitation program and spot treatment approach was the best option. He cautioned however that additional control procedures and data are needed in order to successfully manage LW in the long-term.

<u>Drs. Kuhns and Stelinski (Dr. Peña) (UF-CREC): Update on the RAB attractants</u>

Dr. Peña presented the work from Dr. Kuhn and Dr. Stelinski's laboratory. *Xyleborus glabratus* (RAB) was shown to be attracted to its fungal symbiont *Raffaelea lauricola* (LW pathogen) and an *Ambrosiozyma* species which have ethyl acetate and isoamyl acetate volatiles in common. A synthetic blend consisting of these components plus ethanol and isoamyl alcohol (called fungus lure) was tested and compared to fungus lure + manuka oil, manuka oil alone, and blank control in a natural area infested with RAB using four different lure devices (prototypes). After two weeks the number of RAB and other beetles captured was determined. The fungus lure + manuka oil was superior to all other treatments and device-D with the fungus lure + manuka oil was best of the four lure devices tested. The odors from *Raffaelea lauricola* appeared to be synergistic with manuka oil. In addition, the longevity of the fungus lure + manuka oil device-D was superior to all the other devices tested, i.e., lasting 35+ days.

In an analysis of wood volatiles from redbay and three avocado cultivars ('Peterson' – WI; 'Lula' – GxWI and; Booth – GxWI) Dr. Kuhn showed twelve common constituents including eucalyptol, cubebene, α-copaene, and β-caryophyllene. However, the eucalyptol content of avocado compared to redbay is miniscule and may account for why avocado is not as preferred a host as redbay to RAB. Host eucalyptol content may have potential as a predictor of host susceptibility.

<u>Drs. Kendra, Niogret, and Epsky and Mr. Montgomery and Mrs. Schnell: Update on RAB research from the USDA-ARS</u>

Terpenoids function as attractants for RAB and Dr. Kendra reported on the terpenoid distribution within RAB host trees. They found a gradient from high to low concentration of α-cubebene and α-copaene from the trunk>proximal branches>distal branches>leaf petioles>leaf blades. The found a reverse gradient for monoterpenes and β-caryophyllene.

Dr. Kendra discussed their current hypothesis that:

- Long range: RAB is attracted in-flight to the odor plume of terpenoids emitted by host trees
- Mid-range: RAB incorporates visual cues to find a host of suitable diameter
- Short-range: RAB detects chemical gradients to identify the best site for boring (α-copaene and α-cubebene)
- On contact with the host: RAB 'taste' and 'feel' the wood to confirm suitability of host before committing to boring

Paul then reported on work in collaboration with Dr. Mayfield which demonstrates that California bay laurel (*Umbellularia californica*) is not only susceptible to the laurel wilt pathogen (reported earlier by Dr. Fraedrich) but to RAB attack and inoculation with the pathogen and successful brood rearing. He then showed RAB trapping data on the relative host attraction of six Lauraceae, they are: silkbay=camphor>>California bay laurel = sassafras = swampbay>>>spicebush=blank control. He also showed a higher percentage of female RAB boring and less time-to-commence boring when RAB was exposed to silkbay=California bay laurel>sassafras>spicebush=camphor. Paul also reported the reproduction potential of RAB in silkbay, sassafras, and California bay laurel was similar. He concluded that these findings on the host status of California bay laurel pose a threat to that species and if RAB-LW became established in the natural areas of California it could pose a threat to their avocado industry.

Dr. Kender's lab also reported on the RAB and LW host status of lychee work in collaboration with Dr. Ploetz. They found 35% and 44% of the RAB exposed to bolts of 'Mauritius' and 'Brewster' bored into the wood. Using live trees, they reported *Raffaelea lauricola* was not recovered from any galleries where RAB bored into live trees, no recovery of the pathogen from trees artificially inoculated, and no symptoms of laurel wilt in artificially inoculated with the pathogen or infested with RAB.

Dr. Kender then reported on the comparison of seven essential oils for RAB attraction. Previously his lab and Dr. Hanula's lab reported cubeb oil was potentially a good RAB attractant. In several efficacy and longevity trials cubeb bubble lure captured three to six times more RAB than the manuka lure and non-baited control over an 8 week period. The manuka lure was only efficacious for two-weeks and even during this period captured fewer RAB than the cubeb bubble lure.

<u>Dr. Peña and Dr. Carrillo (UF-TREC): Ambrosia beetles research update</u>

Dr. Carrillo reported on the RAB control results after injecting avocado trees with emamectin benzoate, imidacloprid, cyazypyr, or sulfoxaflor. Trees were injected with the insecticides and bolts were sampled at 20, 50 and 70 cm from the injection site and exposed to RAB under laboratory conditions. One month later the number and length of beetle galleries, number of live and dead beetles inside galleries, and number of immature (eggs, larvae, pupae) were counted. They found those trees injected with emamectin benzoate had the shortest galleries, significantly more dead beetles, and significantly fewer live beetles at all sampling heights. No immature beetle stages were found in those bolts treated with emamectin benzoate compared to all other treatments. More sampling of the same trees treated previously will commence in the near future to determine the longevity of the responses to insecticide injection.

Daniel then reported that a trial testing two formulations of *Beauveria* when applied to bark and wood chips for biocontrol of RAB were in progress. He then described the results of sampling LW symptomatic avocado trees which is part of the spectral aerial survey project. He reported on the number of and species of ambrosia beetle species emerging from the wood samples they have collected and their *Raffaelea lauricola* (*Rl*) status. To date 13 of 14 trees that were sampled were found positive for LW. Fourteen different ambrosia species were identified and the number of beetles to emerge from the samples ranged from 63 to 5,720. Of the 129 beetles assayed for *Rl* status, 9 were found positive for *Rl*. The five most common beetle species to emerge from the samples (highest to lowest) were *Xyleborus volvulus, X. affinis, Xylosandrus crassiusculus, Xyloborinus saxeseni, Xyleborus ferrugineus,* and *X. bispinatus.* No *Xyleborus glabratus* (redbay ambrosia beetles) have been found.

Dr. Carrillo then reported on their ambrosia trapping investigation in 8 commercial avocado groves comparing six different lures plus a no-lure control. The data from the first 3 weeks of the trial include: (1) from 130 to 1,104 beetles have been captured at different sites; (2) fourteen ambrosia species have been identified and; (3) the five most common captured so far are *Xyleborus volvulus, X. affinis, X. crassiusculus, X. saxeseni*, and a *Hypotheneus* spp. Again, no *X. glabratus* have been captured. To date only six RAB have been captured in the avocado production area during the past 2.5 years. In contrast, up to 50 RAB have been captured per week in natural swampbay habitats. This information along with previous research which found the number of RAB to develop inside avocado wood is miniscule compared to natural hosts (e.g., redbay, swampbay) suggests that avocado is not a good host for RAB. This also strongly suggests other ambrosia beetles are the potential LW vectors in the avocado production area.

Daniel then reported on the field results from their investigation to develop and test ambrosia beetle repellents. Previously, Dr. Peña's lab reported two cyclic ketones (CK) had been found to repel RAB under laboratory conditions. Current field testing using avocado bolts found the two formulations of CK had significantly fewer beetle entry holes 7 and 15 days after treatment than non-CK treatments.

<u>Dr. Russ Mizell (UF-NFREC): Results from a comparison of lure types in North Florida</u>

Dr. Mizell reported lure test results from comparing the RAB attractiveness of AgBio-1, AgBio-2, AgBio-3 lures (α-cubebene), AlphaScent lure, Manuka oil lure, Synergy lure and non-treated control over a 2 month period. RAB captures were highest during October and Abio-1 and Abio-2 attracted more RAB than all other treatments. Preliminary results from an on-going field test designed to compare AgBio alone, AgBio+Synergy, AgBio+AlphaScent, AgBio+Manuka oil, and non-treated control, after about 30 days, more RAB have been captured with AgBio+Manuka oil than other treatments.

<u>Dr. Ploetz (UF-TREC): Pathology update on LW research</u>

Dr. Ploetz reviewed previous findings: triazole fungicides are effective against the LW pathogen, macroinfusion of propiconazole protects trees for at least 11 months; very little fungicide residue is found in the fruit; soil drenches and topical bark applications of propiconazole are not efficacious and; treatment of “hot spots” (LW focal points) may be a way to prevent root movement of the LW pathogen to adjacent trees. He then outlined objectives of field trials either in-progress or about to commence. These include: determining if location of infusion or injection affects fungicide efficacy; determining lateral distribution of fungicide after application to root flares or major limbs; does volume of fungicide solution affect efficacy (macroinfusion of large volumes of dilute fungicide vs. microinjection of undiluted product); determining the effect of application devices (pressurized macroinfusion, passive macroinfusion, injection); effectiveness of different propiconazole and tebuconazole formulations; comparing the xylem movement of different formulations of propiconazole and; incorporation of systemic insecticides with systemic fungicides.

Randy then described two on-going field trials designed to determine efficacious “hot spot” treatments; these are treatments of healthy avocado trees adjacent to LW infested trees. In trial 1, treatments include non-treated controls, Tilt infused to 1 tier of trees adjacent to a LW symptomatic tree; Tilt infused to 2 tiers; tebuconazole#1 infused to 1 tier and tebuconazole#1 infused to 2 tiers. In trial 2, treatments include non-treated controls, Tilt plus emamectin benzoate and tebuconazole#1 plus emamectin benzoate. Dr. Ploetz discussed the problem with some tebuconazole formulations inability to stay in solution which makes infusion difficult. Dr. Ploetz reported the field testing of avocado germplasm for LW tolerance is in progress in cooperation with Drs. Kuhn, Gutierrez, and Stover, USDA-ARS in Ft. Pierce and Miami.

Dr. Ploetz reported the results from the collaboration with Dr. Peña's lab on the recovery of the LW pathogen from eight ambrosia beetle species that emerged from swampbay and avocado wood samples. *Raffaelea lauricola* was recovered from seven ambrosia beetle species emerging from swampbay including *Xyleborus glabratus, X. affinis, X. ferrugineus, X. volvulus, Xyleborinus gracilis, X. saxeseni,* and *Xylosandrus crassiusculus*. In contrast, on avocado *R. lauricola* was only detected on *X. volvulus* and *X. ferrugineus* and no *X. glabratus* (RAB) beetles emerged from the wood samples. The number of colony forming units (CFUs) ranged from zero to 7800 CFUs/beetle on beetles recovered from swampbay trees. The number of CFUs recovered from *X. volvulus* and *X. ferrugineus* emerging from avocado ranged from 0-1140 and 4-6, respectively. Interestingly, four ambrosia beetle species emerged from infested avocado wood samples in Ft. Pierce (*Xyleborus glabratus, X. affinis, X. volvulus,* and *X. crassiusculus)* and *R. lauricola* was only detected on *X. glabratus*. In a no-choice greenhouse test, *X. volvulus* was successful in transmitting *R. lauricola* to young avocado trees. He also reported his lab has determined that about 100 conidia of *R. lauricola* are sufficient to kill avocado and redbay trees.

<u>Dr. Ehsani (UF-CREC): An update on applications of spectroscopy and mulit-band imaging techniques for detection of laurel wilt</u>

Drs. Ehsani and Castro Megias reported on the aerial spectral detection of LW results. The objectives were to develop a remote sensing technique for rapid detection of LW, to evaluate spectral features of various biotic (e.g., LW and Phytophthora root rot) and abiotic stresses (e.g., freezing, flooding, heavy crop load), identify the most effective spectral bands for LW detection, develop algorithms that could differentiate among plants infested with LW and other stresses, and compare multi-band imaging with visual sensing for LW detection.

The spectral features of avocado leaves from plants healthy plants, plants inoculated with the LW pathogen, plants exposed to freezing, Phytophthora root rot, salinity, nitrogen and iron deficiency under greenhouse conditions were compared. Fourteen days after plants were inoculated with LW there was a difference in the spectral signature of leaves from healthy plants and plants exposed to freeze damage, Phytophthora root rot, LW, salinity, iron and nitrogen deficiency. There were significant differences in the spectral signature of leaves in the green and red and near infrared bands among stresses at 14 days and this became more apparent at 28 days. More light filters and algorithms will be evaluated in the future to improve the detection capability. Reza reported that the Tetracam Mini MCA-6 camera was more accurate and suitable for the spectral detection of LW than the modified Cannon SX260-NDVI camera. The selection of the best algorithm, wavebands, and vegetative indices is still in progress.

<u>Mr. Monterroso and Jose Perez-Martinez (Brooks Tropicals): New findings on Tilt applications (residue and analysis) on avocado trees</u>

Mr. Monterroso presented results comparing Tilt wood residue concentrations after one injection or repeated applications by bark-directed spray or soil drenching.

Over a 12 month period the propiconazole wood residue concentrations were higher after one injection (about 1.5-7.5 ppm) than those from repeated bark directed sprays (about 0.2-0.4 ppm) and soil drenches (0.1-0.2 ppm). He then showed photographs of avocado trunk cross-sectional areas after blue dye was injected into base or flare roots of avocado trees which suggested the dye distributed laterally to some extent; suggesting that injection of fungicide may have some lateral distribution inside the tree. Armando then reported propiconazole residue concentrations from wood at 6 inches, 3 ft, and 6-8 ft above injection points after 31, 215, and 365 days. Residue concentrations were higher at 6 inches above the injection point (1542-15791 ppm) than at 3 ft above (1.2-4.6 ppm) and 6 ft above (0.254-1.8 ppm) over the 215-day period.

Mr. Monterroso then described a trial designed to determine Tilt wood residue concentrations above and below injection sites along large diameter limbs. Treatments were 70 ml Tilt/tree plus 30 ml water/tree, Tilt plus KeyPlex 1000 (30 ml/tree) and Tilt plus phosphonate (30 ml/tree). Limbs were injected at 3-4 ft above the base of the tree and wood Tilt (propiconazole) residue concentrations were determined 1 ft and 2 ft above injection ports. At 29 days after injection (DAI) with Tilt + phosphonate or Tilt + water, propiconazole concentrations were about 228 ppm and 1232 ppm, respectively. At 60 DAI, wood propiconazole residue concentrations declined for all treatments and were 36, 47, and 56 ppm for Tilt + KeyPlex 1000, Tilt + phosphonate, and Tilt + water, respectively. Tilt residue concentrations from wood samples from these same treatments but 6 inches and 12 inches below injection points 29 and 60 DAI were higher than those above the injection points. At 29 and 60 DAI Tilt + phosphonate concentrations were 1519 ppm and 1049 ppm, respectively. Tilt residue concentrations 29 and 60 DAI Tilt + water were 1673 ppm and 922 ppm, respectively, whereas 60 DAI Tilt + KeyPlex 1000 concentrations were 756 ppm. Thus it appears propiconazole does move downward in the tree at least directly below injection sites 29 and 60 DAI. In another trial, three trees were injected at the base of the tree and three months later roots were sampled for propiconazole residue concentrations. Root propiconazole residue concentrations 2-3 ft from the base of the trees were low and variable (0.28 ppm – 1.9 ppm). From an additional root sample 2 ft directly below the injection site 4.7 ppm detected.

Mr. Monterroso then showed slides of the mobile injection system Brooks Tropicals is employing to inject large numbers of trees. The system includes an ATV mounted with a 30 gallon tank, pump to keep the fungicide solution mixed and the system pressurized and two long hoses with ArborSystems Wedgle devices attached. A field crew is employed to drill and set re-usable (up to 40 re-uses) injection ports along the base of the tree and at about 3-5 ft height along major limbs followed by two crew members tasked with injecting the calculated amount of fungicide solution into each injection port (and tree). The set-up can work two rows of trees at a time. Several days later another crew returns to remove the re-usable injection ports. Armando concluded that their work suggests Tilt is not phytotoxic to avocado trees, Tilt can be injected without mixing with water, Tilt does move above and below the injection site, Tilt does not move rapidly in the tree, Tilt can be mixed with micronutrients and phosphonate, and that the cost to inject trees is commercially viable.

Dr. Mills, Dr. Furton and Dr. Gebelein (FIU): Disease, dogs and drones: early detection of the laurel wilt pathogen

Dr. Mills outlined the objectives of their program was to improve the early detection of laurel wilt so trees could be treated before they became non-treatable and to use canines along with spectral imaging (perhaps drones) in the future. Drones have the potential of flying more often than helicopters and at lower altitude.

Dr. Mills reported two female canines were imported from a Mexican source and are currently being vetted as potential LW detectors. She indicated the canines from this source were better adapted to field conditions found in south Florida than other canine sources. In a preliminary test using several dogs they found that female dogs were less distracted by grove environment than male dogs (just too many trees to mark) and that dogs unused to outdoor conditions (e.g., money and/or drug detecting dogs used to working inside air conditioned buildings) would not have the stamina to work for long periods out of doors. She then described the process to find and make scented canine training aides which include extraction and identification of key volatile compounds specific to either or the pathogen, the insect vector and/or infected trees, and then their use in creating efficacious, mobile, and safe training aids for the canines. After the training aid is developed it can be used as a universal calibration device to train the canines.

Dr. Mills then discussed potential additional biochemical and genetic investigations of the laurel wilt pathogen, the potential effect plant nutrients may play in ambrosia beetle attraction, pathogen virulence, and plant pathogen mutagenesis. Dee informed the group that several grant applications had been submitted and a third has been funded and slated to start in 2014.

Outreach………………Denise Feiber/Jonathan Crane

Denise informed the group that visits to the FDACS-DPI website has been good as well as response to their tweets about LW and wood movement. Jonathan provided a handout of the various extension and research activities he has participated in during the last 6 months

(c://ext/programs/2013/RAB-LW/LW working group/Minutes from LW Working Group 11-18-13.doc)