Citrus Research and Development Foundation, Inc.

Huanglongbing Research and Management in São Paulo, Brazil

A report of a Citrus Research and Development Foundation Visit to Brazil in October 2013

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PURPOSE OF THE TRAVEL TO BRAZIL

At its April, 2013 Board meeting, the Citrus Research and Development Foundation (CRDF) board discussed the need to become more connected with Brazil's efforts in managing Huanglongbing (HLB) or citrus greening, and make sure that everything is being done to coordinate between Florida and Brazil with regard to solutions to manage HLB. An example is field trials in Brazil that are evaluating the effects of 2-4, D and other plant growth regulators on HLB infection and fruit retention. Florida growers are already applying 2-4 D, as well as various nutrient treatments, and want to learn more. CRDF is supporting experiments on how best to use these and other materials. Following the discussion of HLB research in Brazil, the Board recommended that plans be made for a visit to Brazil to get an update on research, field trials, and HLB management tools being implemented by growers and how we can coordinate more closely with Fundecitrus (Fundo de Defesa da Citricultura), with Brazilian researchers, and with growers.

Much has changed in both Brazil and Florida in recent years with to HLB, and with regard to measures being developed and evaluated to stem disease advance. In Florida, more growers are realizing that HLB is impacting their crop in a significant way, and are more interested than ever in replanting. The degree of confidence in the availability of tools to manage young trees in the presence of HLB determines growers' willingness to replant.

CRDF therefore approved support for a small group trip to Brazil to meet with relevant entities and to observe experiments underway in the field that are addressing these and other issues:

- Asian citrus psyllid control
- Scouting and removal of HLB infected trees
- Strategies to manage infection pressure from neighboring citrus groves
- Evaluation of treatments applied to promote tree health in the presence of HLB
- Effects of plant growth regulator treatments in limiting fruit drop
- Status of other citrus diseases in relation to HLB

The HLB study trip was conducted by Harold Browning, CRDF's Chief Operations Officer, Bobby Barben, Citrus Grower and Chair of the CRDF Research Management Committee, and Ben McLean, Citrus Grower and Chair of the CRDF Commercial Product Delivery Committee. Two University of Florida, IFAS Researchers were invited to participate in this activity. However, schedules did not allow their participation in this itinerary, so they will be making plans for a follow-up visit to Brazil.

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ACKNOWLEDGEMENTS

Travel support for the trip was provided by CRDF, and this was complemented by considerable on-site support and hospitality from Fundecitrus, from research institutions, and from numerous growers in Brazil. Special recognition is extended to Mr. Juliano Ayres and his staff at Fundecitrus, who organized the itinerary, provided much of the local arrangements and transportation, and accompanied the team on virtually every aspect of the itinerary. Many of the individuals and groups involved in this exchange are identified within the report and in the appendices at the end of the report. While it is difficult to formally recognize all those who contributed to the success of such an activity, we express our thanks to all who made this possible. In addition, we would like to thank Dr. Jim Graham of UF, IFAS, who reviewed the report and contributed his observations from a recent trip with similar objectives.

BASIS FOR THIS REPORT

This report is compiled from number of resources including the following:

- Formal presentations given by Fundecitrus, researchers, grower groups and others. In some cases, copies of the PowerPoint presentations or summary written reports were provided
- Many hours of grove visits with open discussion and information presentation while in the groves
- Laboratory and greenhouse visits and demonstrations
- Extensive tour of a commercial citrus nursery operation
- Discussions and information exchange throughout the week, both planned and those which occurred while travelling and over meals

The report was constructed from the above resources, using the materials provided, our notes, photos and other information recalled from the visit. Misinterpretations are not uncommon in these kinds of intensive visits, and we apologize in advance for any errors of fact or interpretation associated with this report. Sources are cited where possible within the report.

TRAVEL ITINERARY

October 26	Travel to San Paulo, Brazil
October 27	Travel São Paulo to São Jose do Rio Preto; overnight in Rio Preto
October 28	Morning Visit to Citrosol Citrus Nursery; travel to Araraquara; discussion of research projects; Area-wide HLB management is theme for farm visits, as well as collaborative research; Cambuhy Farm visit and research in area.
October 29	Meet with Fundecitrus Director and staff; Cutrale Farm and research site visits
October 30	Presentations at Fundecitrus; USP, Piracicaba – Entomology program research overview
October 31	Citrosuco Farm visit; Fundecitrus discussion and tour of facilities
November 1	GCONCI Group Presentation and Tour of Physiological Treatments; Valim Group Farm; Agricola São Jose Farm; To São Paulo for Overnight
November 2	Travel to Florida

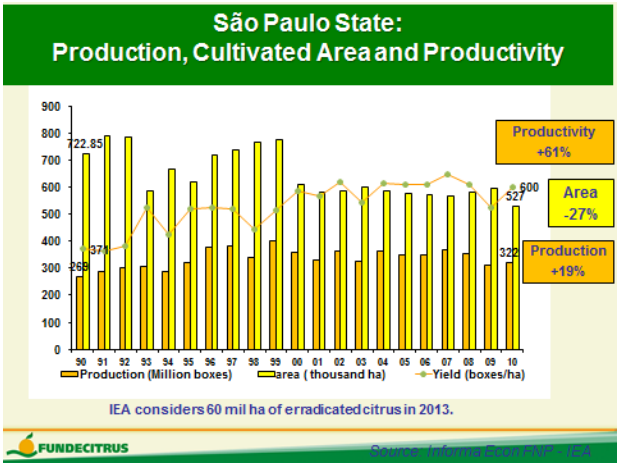
BACKGROUND ON BRAZILIAN CITRUS IN 2013

The majority of citrus in Brazil is concentrated in the state of São Paulo, as indicated in this slide provided by Fundecitrus Citrus is also produced in a number of other states as shown on the map.

Source: Juliano Ayres, Fundecitrus, Oct. 2013

São Paulo State

Citrus is the crop in the region with the highest risk (particularly with HLB) and highest potential economic return. The São Paulo orange crop in 2012-13 was ca. 430 million 90 lb-equivalent boxes, and unofficial estimates indicate this year's crop will be in the range of 270-280 million boxes. While there has been a dramatic reduction in the number of hectares in citrus, the per-hectare yields (efficiency) have increased. This is in part due to removal of low-producing groves and improved production practices, as indicated on the chart.



Fundecitrus also reports that two years ago there were approximately 628,000 ha of citrus in São Paulo State. Approximately 100,000–150,000 hectares have been removed, since, bringing citrus plantings to around 500,000 hectares. It is anticipated that another 50-70,000 ha will be removed over next two years.

Due to high crop size last year, ca. 30 million boxes of early oranges (Hamlin) were not harvested, and some growers experienced unsold oranges left hanging on trees. The large annual flux in crop size is mainly due to weather variation, since almost 80% of the citrus acreage is not irrigated, and variable weather around bloom drives variable crop set and fruit retention.

Utilization: Overview of Processing and Fresh

In discussing the marketing of Brazilian citrus products, and the needs to address future issues like GMO citrus, Juliano Ayres described the emergence of "Uni-Citrus", an association similar to Florida Citrus Mutual that now represents 20 million trees. Uni-Citrus is a new organization that was spurred by the fruit crop size and value fluctuations described above. This organization will be involved in marketing as well as funding issues associated with keeping Brazilian citrus economically viable and productive.

The need for this organization and consideration of marketing is that, with larger crops in recent years, prices to growers are low, and due to inventory, prices this year to growers also are low. This makes HLB management costs prohibitive for some growers, particularly those who were not able to sell their entire crop last year. At the same time, orange juice is more expensive in Brazil than either Europe or the U.S. The needs include marketing of larger (and varying) crops of oranges profitably for growers and processors. In addition, building a marketing message that explains why orange juice is good will help expand markets.

The opportunity to combine forces in global marketing of orange juice has encouraged communication between U.S. and Brazilian officials, and this might be fostered through emergence of Uni-Citrus.

Soils and growing conditions (rainfall, irrigation, cultural practices)

Soils in the state of São Paulo are typically fertile clay-based soils with 15-50% clay. Occasionally, soils are somewhat sandy (considered poorer soils), but most are clay-based. Soil pH is often around 5.5, and for citrus production, is increased to up to pH 6.5 through application of dolomite, gypsum, or phosphite. We were told that virtually all soils in the state (and in Brazil) are acidic. Dr. Graham indicates that this may point to the scenario that bicarbonate stress is not as serious in Florida soils and water as indicated here for Brazil. Other resources are available to characterize Brazil's soils in more detail. Details are embedded in grove visit accounts in this report as they were provided during site visits.

Scions and rootstocks

The São Paulo citrus industry is largely devoted to orange production, but specialty varieties of fresh fruit, including lemons also, are cultivated. The orange crop is used for processing for orange juice, also fresh sales of oranges provide for domestic use in juicing at home. Like Florida, Brazil grows a range of

orange scions to fill harvest timelines, and also employs a range of rootstocks that are adapted to the varying growing conditions and pest/disease threats in the region. Throughout the report, rootstock/scion combinations are mentioned and described as appropriate to the topic.

Citrus Nurseries

There are a number of large citrus nurseries in São Paulo State which supply reset and replanting needs through the region. Many are associated with large farms, and production in some nurseries is several million trees per cycle. All citrus nurseries in São Paulo are required to be enclosed and are inspected for pests and diseases, including citrus canker, Citrus black spot, and HLB.

We visited Citrosol Citrus Nursery near São Jose do Rio Preto as one example of a modern citrus nursery. This nursery was established by Juliano Ayres in 1997 with 33,000 tree capacity. A field nursery was managed previously but had to be treated weekly for sharpshooters to prevent CVC problems. This nursery site has no history of citrus canker. The land is isolated from other citrus and has a well-developed sanitation program. The nursery employs 150 workers, and over time, has employed over 1900 people.

Figure 1. *Citrosol citrus nursery visit by Bobby Barben, Ben McLean with Juliano Ayres (left) and interior view of one of the production houses (right).*

Nursery Operations: Employees are assigned to work in one greenhouse, and change into work uniforms and boots upon start of day. Laundry on site cleans all uniforms daily and if an employee has to work in more than one house in a given day, a shower and clothing change is required in between. No food or other materials are allowed to be brought on site. The company provides food and transportation for employees on site.

Seed House: Seed are planted in 6" tubes containing coconut media. Figure 2 shows liner planted in May (6 months old). The nursery has 2 seed houses, with a combined capacity of 1.5 million liners per 4 months, or 4.5 million per year. An entire seed house is planted at once, so all liners are same aged, and moved to propagation houses. In this manner, the seedling house is vacated and cleaned between crops and this maintains sanitation. Note that seed are extracted from fruit in a separate facility on the property which is reached from a separate entry to the property which prevents employees from going

between seed extraction and the greenhouses. Seed are treated with heat and also seed coats manually removed (soaked in material to facilitate removal). There is a dedicated facility for seed storage on site.

Production Houses: Each house is 100 m x 40 m, accommodating 150,000–180,000 trees per cycle. Depending upon the customer preference, production in pots or bags occurs with either coconut fiber or pine bark media. Irrigation supply for the nursery is accomplished through deep wells, which reduces Phytophthora and other issues, and well water is treated as necessary for use. The total cycle time for citrus trees produced in this nursery from seed planting to tree delivery is generally 12 months. When scion/rootstock incompatibility (e.g. 'pera' on 'swingle') requires budding into an inter-stock, the timeframe is extended for 2-3 months.

Figure 2. *Rootstock seedling house (l); and production house (r) at Citrosol Nursery.*

Finished plants are moved to a transport staging house to be packaged for transport to the field. This separate house allows all staging to be accomplished in one spot, and also allows plants which are produced in citrus pots to be transferred to a sock which is then transported to the field and the tree planted directly in the gauze sock. Trees finished in plastic bags are transported to field in bag. Trees sell for $R5.50 +/- ($US~2.50), and this is standard across the citrus industry, so competition is tight. Delivering a quality tree does not necessarily garner a premium sale price, as the growers are conditioned to this price.

Citrosol Nursery also produces rubber plants for field planting, and markets these to rubber plantations. The production is a combination of field and covered growing situations, and the nursery has been producing these plants for 6-7 years. Plants are started in bags planted in the field, and budded in field. One stage under greenhouse cover (rainout) is necessary during the time the liner is cut off to avoid fungal growth associated with wet plants. Rootstock and scion sources are on site. The nursery produces 1.5 million trees on a 12-18 month cycle and they sell for ca. $US~3. Most rubber plantations are 50-100 ha, so are considered relatively small plantings.

Figure 3. *Production of citrus liner (left), budded plant (middle) and finished nursery tree (right) in Citrosol Nursery*

AN OVERVIEW OF CROP ALTERNATIVES TO CITRUS IN SÃO PAULO STATE

Sugarcane

Sugarcane is a profitable agricultural enterprise at present, due in part to high demand for ethanol production. Nationwide, Brazilian gasoline has 20-25% ethanol with an average of 22% countrywide. In most of the state, sugarcane can be grown for a plant season and 5 rattoon seasons before removal and replanting. This makes sugarcane a desirable multi-year alternative crop for citrus growers who are removing blocks and are reluctant to replant in citrus In cases where citrus growers are ready to consider alternative land use for some period, they can rent their land out to sugarcane farmers, who will remove trees and prepare the land to plant sugarcane.

Coffee

In addition to citrus, we saw considerable coffee on the Valim Group Farm near Limera, with blocks of various ages interspersed with citrus blocks. This farm originated as a coffee plantation, and continues to grow coffee, having the infrastructure and open land. In fact, much of the state of São Paulo formerly was planted to coffee. A coffee planting has a productive lifespan of over 20 years in this area, particularly if it is pruned back (either moderately or through "buck-horning") to rejuvenate once the original plants begin to show decline. Coffee can be harvested 2-3 years after planting, and most coffee in this region is harvested mechanically (Fig. 4). We were told that, on this farm, the cost of production was estimated at $R300 per 60 kg of beans harvested. Current market prices of around $R230 per 60kg mean that this farm is experiencing a loss on coffee. We heard from others that the area planted to coffee in São Paulo state is decreasing due to sustained lower prices, and more coffee is produced at higher elevation where quality and therefore prices are better.

Eucalyptus plantings were located around the Valim Group farm. These trees are used as wood to fuel the coffee dryers, as they no longer sun-dry coffee beans. Eucalyptus does not appear to be a standalone crop of interest, although we saw plantings in various locations.

Figure 4. *Coffee Production as an alternative to citrus, showing mature producing field (left) and mechanical harvesting equipment (right).*

Rubber

Rubber plantations are an option for São Paulo agriculture which provides good returns, but production of the first crop follows 7-8 years of establishment. Latex harvest can proceed for 25 years after the plants mature. Yields of 12 kg of latex per tree are average and are sold at around $R1.50 per kg.

Figure 5. *Rubber plantations as alternatives to citrus groves. Two-year old planting (left) and producing stand of trees (right).*

Soybean

We also saw soybean plantings around the Valim Group Farm, on land that was formerly planted to citrus. Specifically, we saw a long block planted to soybean where a 'Murcott' block had failed. This was due primarily to *Alternaria* infection in a poor site choice (low and longer periods of wetting) for 'Murcott'. Soybeans offer a short-term rotation when immediate replanting of citrus is not planned. The same is true for corn, which we saw here and on other farms in this area.

Figure 6. *Soybean planting in former citrus grove site, with newly planted citrus in the background.*

OVERVIEW OF CITRUS DISEASES

Whereas, the primary objective of this visit to Brazil was to evaluate HLB management, we also observed and discussed the role of other diseases that are a threat to Brazilian citrus production and for which Florida shares concern. Details of the prevalence and importance of selected disease is provided in this section, and where discussed, ongoing research is highlighted. Other comments and discussion of these citrus diseases are incorporated into the discussion of site visits as they occurred, and thus are scattered throughout the report.

Citrus canker

Source: Notes from Citrus Canker discussion with Franklin Behlau, Fundecitrus. Citrus canker is a disease that is still under legislative (mandatory phytosanitary) control. As it continues to spread, as it is doing, there is more difficulty in suppressing canker, and the impact of the disease is increasing. For many growers, they have effectively limited the disease, and we heard from several that, when detected, they are eliminating whole blocks that contain the disease. This is the law, but it is becoming more difficult to enforce as more blocks are infected and as HLB and other diseases are on the rise. Dr. Graham, an expert in citrus canker, indicated that whether citrus canker suppression by tree removal continues is site-specific, depending on how widespread the infestation is when first detected.

Our discussion included how impactful the disease is, including the loss to fruit drop. As with the situation in Florida, canker intensity is variable across the state and among growers. Locations where it has become established see increases in infection and fruit drop in subsequent years as rainfall promotes, while others remain canker-free. Most growers manage citrus canker through exclusionary and preventative practices, as they do with HLB.

We discussed the approaches to canker management, and Brazil still has rigorous phytosanitary practices in place that we saw in many locations, including the following:

- Disinfection sites at farm entry. At larger farms, this includes the standard spray entry, with the addition of a pressure wash for the whole vehicle exterior, and personal decontamination wash/walk-throughs for all coming onto the farm. Several farms sprayed the vehicle interior after we disembarked for personal decontamination.
- Scouting and removal of canker infection (whole blocks to trees)
- Clipping of infected plant parts from infected trees in areas where complete eradication is no longer practiced.
- Use of roadside fruit load transfer from grove equipment to on-road equipment is still in use on some farms. These transfer sites are equipped with bins for transfer storage and also some have equipment for washing of fruit for disinfection
- Tarping of on-road trailers to prevent transfer of leaves, etc.
- While it is the law, it is increasingly difficult to enforce these phytosanitary practices

Treatment for canker in groves remains effectively the application of copper sprays, in addition to the decontamination of vehicles and equipment entering and leaving. Like Florida, growers in Brazil are interested in non-copper tools, but Fundecitrus reported lack of alternatives and little ongoing evaluation of those that are available. We discussed that increasing the treatments for citrus black spot has the added advantage of canker suppression, as it is used numerous times (7-8) per season and often includes copper. Jim Graham observed that more proactive companies are considering planting of *Corymbia* windbreaks in areas with highly susceptible varieties and wind exposed conditions (e.g., in river valleys).

Citrus Black Spot

Source: Discussion with Geraldo José Silva Jr., Fundecitrus. The Citrus Black Spot (CBS) situation in Brazil continues to change as the disease spreads in the state. We observed CBS in many groves across the state, and in general, growers thought that they were doing a pretty good job of managing the disease, despite our ability to see symptomatic fruit on trees and in some cases, on the ground. In discussion with Dr. Silva, we learned that they feel pretty comfortable with their understanding of the seasonal epidemiology of the disease, especially the timing of vulnerability for infection. As with canker, groves with history of CBS are watched more closely and will support local inoculum for infection in the next season. They are aware of the role of infected foliage litter and promote cultural practices which hasten the breakdown of leaf litter. Since most groves are not irrrigated, growers cannot manipulate litter decomposition through irrigation. However, many growers direct row middle mowing debris into the tree row to add organic matter, for the purpose of hastening leaf decomposition.

Figure 7. *Citrus black spot symptoms on Valencia oranges, as witnessed on several farms. Many growers direct mowing debris into tree rows to add organic matter to accelerate decomposition of fallen citrus leaves.*

Dr. Silva indicated that they have fine-tuned the timing and materials associated with protection against CBS at critical times, and most growers feel that this disease is managable at present to prevent excessive fruit loss. The tools include copper and strobilurin fungicides. These are used in sequence as well as combined. They feel that the combination provides better suppression, but there is the issue of resistance development if strobilurins are used too frequently. Dr. Silva did not mention any ongoing efforts to monitor for resistance development. The standard program is 7-8 applications during the season of susceptibility.

In discussing CBS in Florida, Drs. Silva and Behlau felt that this disease should be managable, and agreed that understanding the seasonal epidemiology will facilitate timing of treatments. They strongly endorsed the widely practices use of tarping fruit trailers to prevent spread with leaf litter during harvest and transport.

Citrus Variegated Chlorosis (CVC)

We did not spend significant time in discussion of CVC, but suffice it to say that this disease has decreased in importance with the onset of HLB infection in the state. Infection remains across the citrus growing areas, and vector (sharpshooters) control is important as it is for HLB (ACP) and Citrus Tristeza Virus (brown citrus aphid). We learned from several sources that in general, the use of pesticidal treatments to suppress ACP populations is having the added advantage of suppressing populations of the other vector insects of importance.

Citrus Leprosis

As stated elsewhere, growers and Fundecitrus staff indicated that this disease is managed within reason by application of miticides to minimize spread by the vector *Brevipalpus* mites. Some indicated a single well-timed miticide application per year suffices, while others indicated that 2 sprays per year may be necessary for vector control.

Post-Bloom Fruit Drop

This disease was evident across many farms, with varying degrees of lost fruit set. Disease outbreaks are sporadic in Brazil, as in Florida, by virtue of weather conditions coincident with bloom. We did not observe severe fruit loss, but generally low incidence of fruitlets lost.

Sudden Death

Figure 8. *Post-bloom fruit drop evidence on orange trees, observed in several citrus groves around São Paulo.*

This disease, which emerged in northern areas of São Paulo State, is most important in these warmer northern zones of citrus production. Spread of the disease did not progress southerly as some expected. Although it remains a disease of concern, like other citrus diseases, it has been reduced in importance by the spread of HLB.

In characterizing the net effects of these disease threats, we asked various participants to rank what they considered the most important diseases after HLB, which most felt was of greatest importance. Many indicated that perhaps CBS was contributing more to costs and losses than was canker, making them 2nd and 3rd in order. However, there was general consensus that the importance of citrus canker is rising, and will likely take on more importance as inoculum levels increase and infection outstrips phytosanitary measures. Some indicated that epidemiology of canker under Brazilian conditions and the limited control options make it likely to eclipse CBS in importance.

HLB IN SÃO PAULO STATE

Discussion with Juliano Ayres indicated that HLB incidence is about 7% in São Paulo State. Estimates report as many as 2-3% of citrus trees show advanced HLB symptoms, including fruit loss across the state. This lower percentage is due to aggressive psyllid control and removal of infected trees. For this reason, the total impact of HLB in the state is still relatively light, particularly when you are assessing measures such as pre-harvest fruit drop and smaller fruit size. It is felt that in 3-4 years, there will be groves that will be as heavily diseased and symptomatic as those in Florida. With low returns on citrus sales (lower prices to growers), some growers are doing less to control ACP and HLB.

Bobby and Ben: More integrative synopsis comments could be added here

APPROACHES TO CITRUS PRODUCTION IN SÃO PAULO STATE IN THE PRESENCE OF HLB

Source: Juliano Ayres comments on industry, Fundecitrus origins and involvement in HLB research 10-31-13

Fundecitrus originated in 1977 in response to the canker eradication campaign. Citrus growers were working with the government and funding efforts to manage canker, but felt an industry support organization was in order. (See History of Fundecitrus, appendix 2 for more details).

Juliano Ayres commented on the distinction that is necessary in characterizing HLB impacts and the industry as a whole. He described the measures used often in discussing the industry as production – the gross measure of fruit production of a block, or across sectors of the industry. Production is a measure that varies widely from year to year (e.g. reported 430 million boxes of oranges in the 2011-2012 season vs. estimated 275 million boxes in 2012-2013). The second measure that he finds useful is productivity – the production of fruit per hectare. Thus, this is a measure of effectiveness of inputs compared to outputs. He commented that production, despite its variability, is likely to decline as HLB affects more groves, especially those farms of growers who are unable to invest sufficiently to offset infection or to treat ACP effectively. This relates to the reported 150,000 hectares of citrus estimated removed over the recent 4-5 years. Productivity, however, is increasing, in part to the gradual attenuation of lower productive groves, and replacement with more intensive, higher density new plantings. This, he says, is important to the future of the industry and to the economics of producing citrus in Brazil under increasingly high costs.

On the subject of pesticide use and honeybee issues, Mr. Ayres commented that many farmers allow beekeepers to place hives on their lands. Bees forage on the forested portions of the farm to produce high quality honey. This practice and increases in ACP control applications have the potential to raise issues similar to those in the U.S. between farming and honeybee husbandry.

Mr. Ayres offered that there are a number of tools available to apply to HLB management, summarized as:

- Tools to suppress vector ACP populations and limit pathogen spread
- Tools used to identify and eliminate HLB-infected trees
- Tools used to maintain tree health and respond to HLB-related stress
- Tools used to eliminate tree stress beyond the stress caused by HLB
- Tools and tactics to successfully manage HLB in new plantings

The discussions held during grove visits helped clarify the current status of these tools and how they are currently being used to manage HLB in São Paulo citrus groves. Details of those discussions are provided in the accounts provided for each field and lab visit.

SUMMARY OF GROVE VISITS, FIELD TRIALS, LAB TOURS, AND RELATED DISCUSSIONS

Cambuhy Farms Visit, 10-28-2013

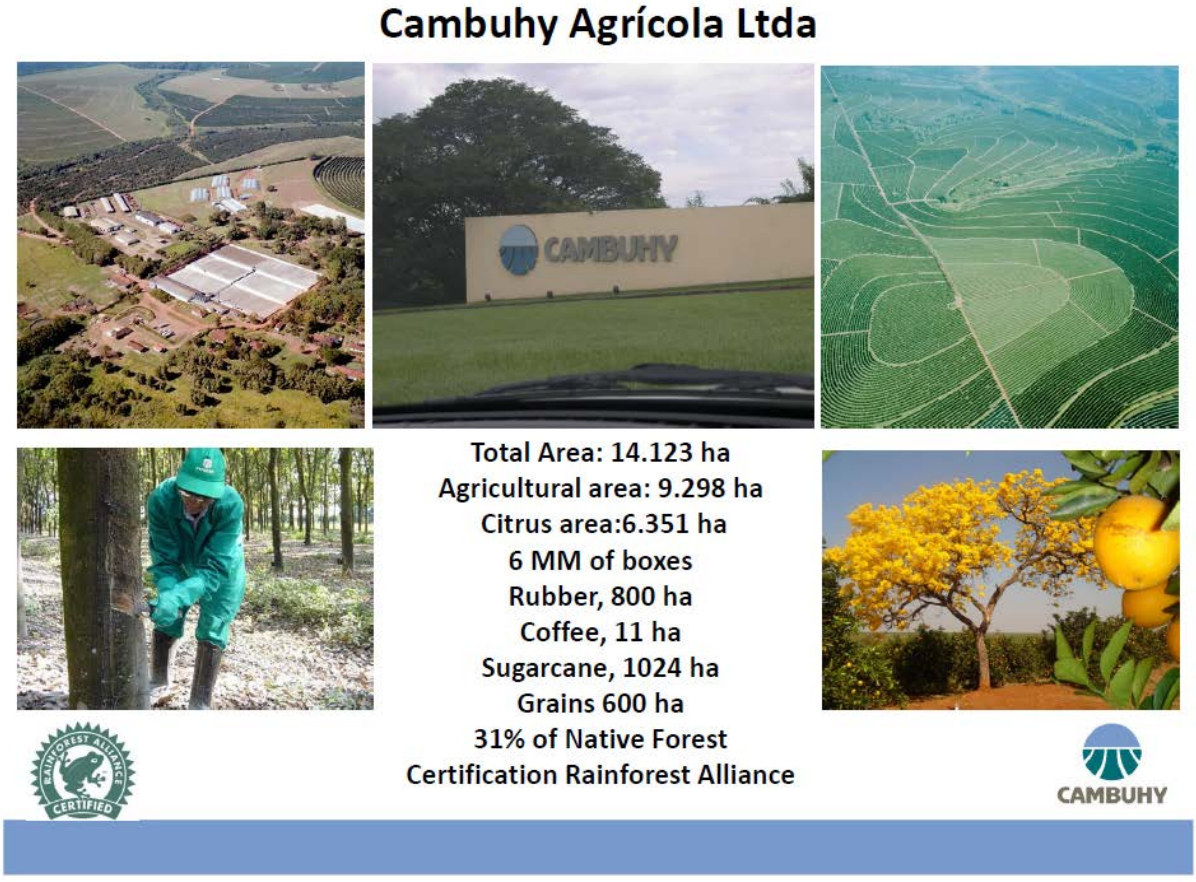
It was reported to us that this company has a long-term agreement with Citrosuco and Fisher (now merged) for fruit disposition, so have good prospects for future sale of fruit and returns in uncertain markets. "Growers like Cambuhy are succeeding and will succeed because they are: 1) big; and 2) smart. (Juliano Ayres)".

Participants in this farm visit:
Alexandre Tachibana, Chief Executive Officer of Cambuhy Agricola, is also formerly a Fundecitrus staff.

Ivan Brandimarte, Production Manager at Cambuhy. Formerly with Fundecitrus, then Citrosuco, Ivan is now at Cambuhy. Mr. Brandimarte received an MS through the Fundecitrus program.

Renata Maria Lanza, Agronomist at Cambuhy, is also working on the Fundecitrus MS degree.

A brief overview was provided by Cambuhy staff, showing the Cambuhy Agricola Ltda. property near Araraquara.



Source: Cambuhy Overview presentation October 2013

We were briefed on the importance and complication of neighbors and its effects on HLB spread. Cambuhy Agricola Ltda is diligently removing all infected trees property-wide, and showed numerous examples of how the edge effect presents challenges on their property. Five years ago, they had over 100 neighbors who were growing citrus, and now that has been reduced to 77, with many growers removing citrus groves and converting to sugarcane. We discussed the importance of borders and how they manage their own borders and also the neighbors. Cambuhy Agricola designs budgets for ACP management on their groves, as well as on neighboring groves, and claims that the investment in monitoring and treating neighbor farms reduces cost of ACP and HLB control on their property, leading to a net gain. More on this topic is presented in the section on neighbors.

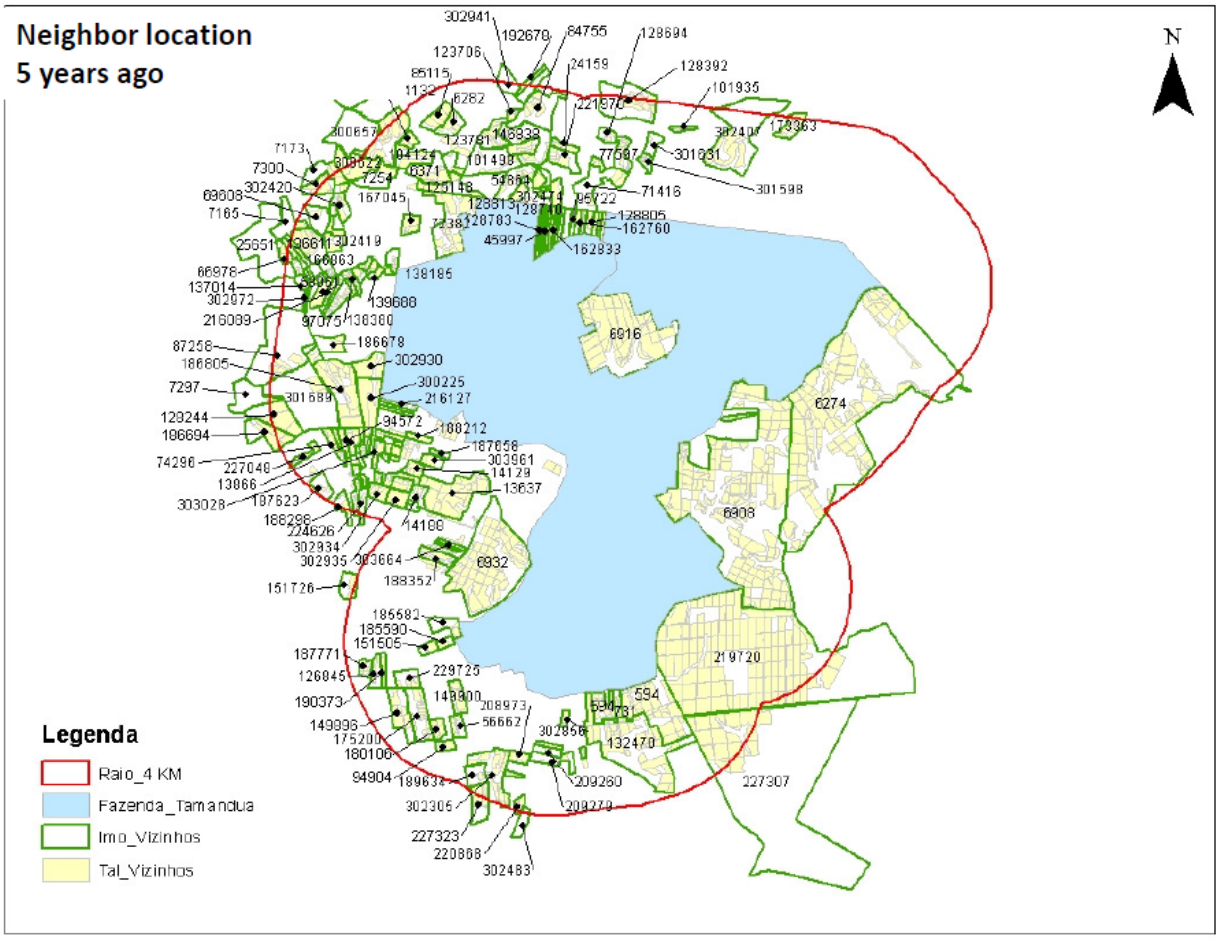


Figure 9. *Location of neighboring citrus farms surrounding the Cambuhy Maringa Farm (shaded in light blue) in 2008. This represents over 100 neighbors, and has been reduced to about 77 in 2013.*

Other notes relating to our discussion at Cambuhy Farms include the observation that 'Swingle 4475' is the best adapted rootstock to their region.

A pdf copy of the presentation was provided to CRDF for reference.

Lunch was provided at Cambuhy House with Mr. Tachibana as host. General discussion was held over lunch and then the group went to the field.

Disease considerations:

- Black Spot: Good control in 'Valencia' block, sprayed six times, starting at petal fall with copper only, then next five applications with copper and strobilurins combined. The 'Pera' block only required 5 applications and 'Hamlins' only 4 applications this season, in part due to lower inoculum available and earlier harvest of these two cultivars that removes the infected fruit before symptoms occur.
- Citrus Canker: Alexandre Tachibana indicated that across this entire property, they have only detected canker once in 2012, and in that instance, they eliminated two blocks that showed infection. No further infection has been detected on the property. They practice sanitation at entrances to the property, but not a lot between blocks within the farm. Decontamination was not practiced as we visited neighboring property blocks.
- Citrus Leprosis: The Cambuhy group indicated that they are not as concerned about Leprosis as they are for other diseases, as long as an application of miticide occurs once +/- per year. No Leprosis damage was seen in the blocks we visited.
- Citrus Variegated Chlorosis (CVC): Also not viewed as a big issue at this time in relation to HLB, and also due to extensive ACP treatments which also are presumed effective against sharpshooter vectors of the CVC pathogen, *Xylella*.

Cambuhy Grove Details:

- Eight year old 'Valencia americana', an early Valencia. A non-irrigated block next to a newly planted 'natal' on 'Sunki' rootstock. They are using a lot of 'Sunki' due to disease resistance. Trees in an 8-year old higher density planting were heavily cropped this season. Several blooms were evident and a lot of cluster fruit. This planting had some trees removed over time, but not as apparent in the higher density planting.

Figure 10. *Eight-year old Valencia block at Cambuhy Farms with solid fruit set (left) with adjacent newly planted 'natal' orange block.*

- The "border effect" discussed earlier was apparent on the farm along many margins that were adjacent to neighboring farms. Most noticeable was the high number of replaced trees, since all HLB+ trees are removed and rapidly replaced. To maintain buffer integrity and function,

resetting is scheduled more frequently than in the recent past (Jim Graham comment). Borders are being treated more aggressively to contain neighboring effects to edges, and this aggressive border management was also referred to as use of a "sacrificial border".

- A large number of rubber trees in solid blocks are interspersed among groves, as are large blocks of sugarcane, mostly in "mid-season" of growth. Ivan and Alexandre expressed concern over the adjacent edges of sugarcane and citrus blocks interacting, especially with regard to herbicide spray drift from sugarcane to citrus
- Overall, citrus plantings on the property look really good. Some older blocks show stress-related dieback (e.g., CVC), some blight, and other stresses. HLB-infected trees are not being removed in these older blocks. In susceptible rootstocks, blight is a larger issue than HLB in these older blocks.

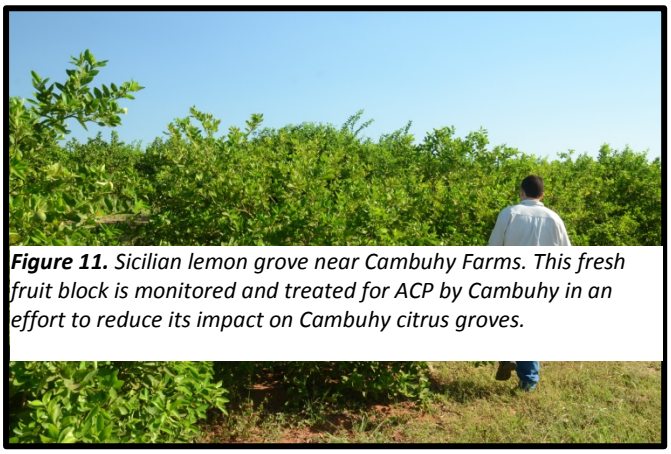


Figure 11. *Sicilian lemon grove near Cambuhy Farms. This fresh fruit block is monitored and treated for ACP by Cambuhy in an effort to reduce its impact on Cambuhy citrus groves.*

Cambuhy Neighbors

Neighboring groves located near Cambuhy Farm were visited, where we stopped and entered two of the groves surrounding the farm. The first block was a lemon grove being grown for fresh fruit sales ($US20 per box). This block was not well managed and looked more or less abandoned. Under agreement with grower, the groves are monitored by Cambuhy via sticky traps which are checked weekly (1 Cambuhy employee on motorcycle). The neighbor block is treated (via ground application by Cambuhy) when any ACP is detected in the sticky traps, or otherwise monthly. This is a scheduled expense for Cambuhy operations, as indicated earlier. This is an example where the larger farm (Cambuhy) assumes total responsibility for reducing ACP pressure on neighboring farms. We observed other models of how neighboring blocks are managed but this was the most aggressive management system we observed during the trip.

Visit to Cutrale Farm10-29-13:

The Cutrale staff hosting us on the visit were A. Ricardo Violante, Technical Department and several colleagues. Luis Scandelai, Agronomist from Fundecitrus also participated in this activity, and in the afternoon, Juliano Ayres joined the visit.

The first site visited was a new planting about 1 year old that was planted in a relatively isolated area. The site formerly was planted to citrus, but the entire area is newly planted (ca. 500 ha) following removal of previous blocks of citrus on the site. Planting is higher density, and is the site of larger scale plastic greenhouse cloth mulch application, which occurred prior to planting. The observations on this mulch use indicate that transmission is less, due to lower number of ACP in the plots (Figure 12). However, unlike findings of Dr. Phil Stansly, after 1 year, there is no evidence of greater plant growth under the plastic mulch as opposed to adjacent rows without mulch. Under this set of conditions, it may

take a longer period to determine the influence of this treatment. In addition, Richardo indicated that they are seeking substitute mulch materials, as the material used in this trial is quite expensive. The material is commonly used in greenhouses to provide overhead shade, and is quite durable woven plastic cloth. This is much heavier that material used in Florida trials by Dr. Stansly.

Figure 12. *Young citrus planting on Cutrale Farm about one year old. Installation of plastic reflective mulch on a portion of one grove to discourage Asian citrus psyllid colonization (right).*

Nutritional Trials and the Field Site at Cutrale Santa Maria Farm:

In the afternoon, we visited Santa Maria Farm, and began the visit with presentations by Dr. Jose Antonio Quaggio and Dr. Dirceu Mattos, Jr. on the nutritional experiments located on the São Jose Farm. This trial is established to evaluate nutrient management versus HLB. Dr. Quaggio is a collaborator with Fundecitrus and Cutrale from the oldest University in Brazil, Instituto Agronomico. Dr. Mattos works at IAC Centro de Citriculturas, Sylvio Moreira, another branch of the Instituto Agronomico. Dr. Mattos completed his PhD at UF with Dr. Ashok Alva and Dr. Jim Syvertsen.

The trial designers provided an overview of the experimental details and the rationale behind the trails. The Valencia experiment has been in the ground for 3 years, and the harvest underway will be the third harvest. This trial evaluates the nutritional status of test trees responding to varying nutritional treatments.

Dr. Jose Bove and a group of scientists contributed to design of this nutritional experiment, incorporating information from Maury Boyd's reports and from other research ideas emerging in Brazil. The nutritional studies were contemplated +/- ACP control, but the Santa Maria trial includes ACP control in almost all treatments. The goal was interaction between nutrition status, vector interaction, and physiological tree responses.

Details from the trials:

Field Experiments were started in November 2010. Treatments included 4 groups of foliar macro- and micro- nutrients +/- SARs, leading to 8 separate treatments as defined below in Table 1. Treatments to plots in the Valencia orange experiments were begun in 2010 and were set up in 10 rows x 160 trees, with 4 replicates for each of the 8 treatments. Tables 1 and 2 provide details of the individual treatment

combinations. At the beginning of the trials, there was HLB infection in the block approaching 10%, and the emphasis was on symptomatic trees with low, P, K and micronutrient levels

Table 1. *Characterization of citrus plots used in nutritional treatment evaluations on the Cutrale Santa Maria Farm.*

Block	Variety	Planting	Spacing (m)	Irrigation	ACP Control
103	Valencia/Rangpur	July 2002	6.8 x 3.4	no	yes
104	Valencia/Rangpur	July 2002	6.8 x 3.4	no	yes
105	Valencia/Rangpur	Sept/ 2002	6.8 x 3.4	no	yes
110	Natal/Rangpur	July 2002	6.8 x 3.4	no	no

These are the 8 treatments that are being evaluated in the Cutrale Santa Maria Farm nutrition trial, now in its 3rd year. Details were provided by Drs. Quaggio and Mattos and the Cutrale cooperators, and are depicted in Table 2.

Table 2. *Treatment details for nutritional evaluation experiments on the Cutrale Santa Maria Farm.*

Treatment	Nutrients	Amount	# Applications
T_0 (control)	NPK solid		3x
T_1 (T_0 + 'SP' micronutrients)	T0		
	Boric acid	1.0 kg/ha	2x
	Boric acid foliar	3.0 kg/2000L	4x
	Zinc sulfate (20%)	7.5 kg/2000L	4x
	Manganese sulfate (31%)	3.2 kg/2000L	4x
T_2 (T_1 + KNO_3)	T_1		
	Potassium nitrate	7.0 kg/2000L	4x
T_3 (T_0 + 'FL' micronutrients)	T_0		
	Boric acid	2.0 kg/ha	2x
	Boric acid foliar	3.0 kg/2000L	4x
	Zinc sulfate (20%)	7.5 kg/2000L	4x
	Manganese sulfate (31%)	12.0 kg/2000L	4x
T_4 (T_1 + phosphite)	T_1		
	phosphite 00-28-26	5.0 L/2000L	4x
T_5 (T_1 + salicilate)	T_1		
	Salicilic acid	0.1 kg/2000l	4x
T_6 (T_3 + KNO_3 + phosphite + salicilate) 'Complete'	T_3		
	Potassium nitrate	7.0 kg/2000L	4x
	Phosphite 00-28-26	5.0 L/2000L	4x
	Salicilic acid	0.1 kg/2000L	4x
T_7 (T_0 + 'cocktail')	T_O		
	'Cocktail'	According to mfg.	4x

Treatments included consideration of Florida (Boyd and Premier Citrus approaches) compared to Brazilian recommendations.

The Zn/Mn ratio was cited as an integral part of the nutritional picture. Experimental treatment ratios in this trial are higher (0.4) than regularly used in Brazil citrus production (details highlighted in yellow in Table 2 above).

Following initiation of the treatments, the following measures have been recorded:

- Soil chemical nutrient analyses for symptomatic and asymptomatic trees
- Leaf chemical nutrient analyses as above
- Leaf area and dry mass analyses
- Chemical analysis of phloem (sap). The hypothesis for testing phloem is that this is more sensitive than leaf or other tissue, as nutrients are sensitive to phloem flux and the measures may be more responsive to treatments. Souza (2008) reports work by an Italian scientist who has developed these methods, and has worked with Quaggio in Brazil and with Tim Spann in Florida.
- PCR test for *CLas*, *Clam* and phytoplasma presence

Results thus far:

- Treatments of infected trees did not restore measured nutrition to the level associated with healthy trees
- Nitrogen metabolism was altered among treatments
- PCR Evaluation of the plots showed the following results averaged across all treatments

Table 3. *Santa Maria Farm Nutritional Trial HLB infection evaluations (PCR), 2012.*

Sample Date	PCR value symptomatic (Ct value)	PCR Asymptomatic (Ct value)
April 2012	22.8	36.7
November 2012	24.8	undetectable

Figure 13. *Nutritional experiments on Valencia orange plots on Cutrale's Santa Maria Farm.*

Preliminary conclusions:

- Mineral nutrition status of symptomatic trees is less desirable compared to asymptomatic trees

- Treatments are not restoring full nutritional status in symptomatic trees
- Seasonal variation in response to nutrition has been observed
- This experiment and its interpretation are supported by Renato Bassanesi's damage and yield data
    - HLB incidence has ranged from 2-12% from December 2010 to Jan 2013
    - Disease incidence has not increased a lot in the last year (no data specifically presented)
    - Factors such as strong ACP control and good rainfall are affecting this trial (tree health benefit)
- Root systems are more affected in infected than uninfected trees

(Ben/Bobby: you can expand on this detail and conclusions, including your thoughts on the plot maps and info on treatments that they provided through Ben)

Other comments regarding HLB in general and the nutritional trials:

- Juliano Ayres commented that at Santa Maria, production averaging 3.5 boxes per tree this season. It demonstrates that high yields can be retained despite dramatic effect of HLB on yield, given that the incidence of HLB is less than 10% in the groves.
- Dr. Quaggio commented that, in Brazil, fruit drop associated with HLB occurred within 4-5 months after initial infection. Interestingly, he noted in Florida fruit drop appeared not to begin until 5 years after infection (is this true?) Dr. Graham responded to this observation by Dr. Quaggio that trees with few or no foliar symptoms experienced fruit drop in the first year in Florida. Hence much sooner after infection, the tree is losing roots and is dropping fruit as a consequence. Bobby Barben agrees with Dr. Graham's assessment of Florida fruit drop.
- Dr. Quaggio commented on the morphology of leaves in relation to nutrition: Calcium nitrate versus ammonium nitrate fertilizer form affects leaf morphology, as indicated by microscopy of leaf cross-sections:
    - Better organization and integrity of epidermis when calcium nitrate is the source, as well as palisade layers and other leaf components. This appears to be true in high and low nitrogen rate applications, with more clear evidence in the higher rates.
    - Higher N rate, the thicker the leaf, true in both calcium and ammonium sources

Following the seminar, there was a site visit to the 'Valencia' block experimental plots, with Drs. Quaggio and Mattos participating along with the Cutrale Production Managers and Fundecitrus. The trees in this trial are now 15 years old, and were 12 years old when the trial was initiated.

Data from the first 2 years of this trial presented during the preview indicated that there were no significant differences between the treatments in HLB incidence or severity, and there also were no treatment differences in yield or fruit quality in this trial after 2 years. However, the appearance of trees in the various plots showed visible differences as follows:

- Foliage condition, including leaf size, leaf thickness, leaf color and shape

- Canopy density appeared greater in some treatments compared to the “normal nutritional treatments”, and this was quite noticeable in some treatments.
- Bloom was variable, as was the residual fruit maturing on the trees. The harvest of Valencia oranges had begun in this trial, but there were fruit from “off-blooms” present in the plots. This was reportedly due to weather conditions during flowering and fruit set, and appeared more prevalent in some plots than others.
- Individual treatments that showed moderate HLB symptoms appeared also to have the more sporadic bloom and fruit size variation.
- All who viewed the plots felt that there likely will be significant differences in treatment effects characterized by the 3rd season data, and if not, in the 4th and 5th years. These field observations point to the need to consider longer-term measures to best understand the effects of nutritional and other tree health treatments. The delayed separation of effects among treatments speaks to the potential short term value of these treatments as opposed to longer-term impact yet to be confirmed. (Bobby and Ben: Your comments encouraged here, particularly about waiting 4-5 years to see treatment effects manifested.)

A similar trial has been set up on ‘Hamlin’ oranges on site, but this trial is in its first year (about 9 months ago), and thus no results are available. This trial has similar design with fewer trees per plot (still large plots). The treatment regime and data collection protocols will be followed in this trial as they are in the Valencia trail.

Cutrale Neighbors and Border Effects:

We visited an area on the farm that represents bordering blocks with neighbors, in some cases immediately adjacent blocks owned and managed by others. Some of these blocks were clearly more highly infected than are blocks of Cutrale, even though Cutrale has blocks where they do not necessarily remove infected trees. It was stated that on some of Cutrale farms, they remove HLB-infected trees from new plantings, and once the trees reach 7 years of age, they continue to remove trees but don’t reset with new trees. This also was a feature of discussions at other owners’ farms, where infected trees removed are reset in blocks from seven to ten years of age. Driving along the border areas between Cutrale blocks and those of neighbors, the border effect was quite noticeable (Figure 14). At one particularly obvious border area with neighboring blocks immediately adjacent, we evaluated the intensity of both HLB infection and depth of penetration of HLB pressure from the adjacent grove into a mature Cutrale block, as measured by the number and age of resets. The incidence of resets was highest on the outside row, and was most visible for up to 5 rows in (Figure 14). There appeared to be a drop-off of incidence of trees diagnosed, removed and reset due to HLB infection after about 5 rows.

Figure 14. *Border row effects of HLB infection coming from neighboring groves. Border rows are consistently scouted and trees found infected removed and replanted.*

This indicated the impact of border effect, and with tree removal and resetting being aggressively followed, these practices appeared to be limiting the impact on new plantings of adjacent, more heavily infected blocks. Discussion indicated that Cutrale generally treats the border every 1-2 weeks for ACP, and the interior of the farm on a monthly interval. Worthy of note was that this strategy appears effective even when there are sources of infection in mature blocks within the farm that is surrounded by the border. In this case, Cutrale has mature blocks that are highly enough infected that they are not removing infected trees. Blocks like this may be affected by other stresses like leprosis and blight, and thus, it is an economic decision on how to manage HLB and other diseases in these older blocks. Like other growers, Cutrale plans to completely remove entire blocks as their productivity declines due to a mix of stresses and age.

Unlike what we observed with Cambuhy, Cutrale does not monitor and treat neighboring blocks, but on this and other Cutrale farms, they are quite aware of the border effects. In discussing this, we bounced ideas about how wide the border of one owner's farm needs to be in terms of increased monitoring and treatment, and also for consideration of more intense scouting and infected tree removal, and even the idea of row configuration and higher density sets in "border" areas. A general refrain was that borders can be affected up to 100 meters (ca. 10+ rows) deep, and if the farm size allows, that is considered the first approximation of an effective border area. This concept was consistent with discussion we had with other owners of larger farms, and is probably shared among large growers and to/from them via Fundecitrus.

<u>Citrosuco Farms</u>

An introductory presentation was provided by Rene de Sousa Lima, Manager of the Central Region Farm, known as Maringa Farm. We were joined by Helton Carlos de Leao, who is Technical Manager for all regions.

General Information:

Citrosuco has farms which grow citrus in 4 regions of São Paula, including South, Central, North, and Northwest. There are a total of nearly 40 farms in this operation, since the merger between Citrosuco and Fisher, and the size is near 40,000 hectares planted to citrus, close to that of Cutrale Farms.

On the Central Region Farm (Maringa), the entire planting is relatively new, with the following history:

- A large number of blocks were removed since 2007, particularly over the 2-3 year period 2007-2009.
- Blocks with citrus removed were planted to sugarcane, as there were a lot of surrounding groves with high incidence of HLB.
- They planned to wait until neighboring groves "went away" to replant citrus (estimated 2 years)
- Before planting, Citrosuco chose locations on this farm to replant citrus where neighbors had the least influence (farthest from neighboring inoculum)
- 36 blocks of citrus were planted 4-6 years ago, mostly with 'Pera', a few blocks of 'Hamlin' and one block of 'Rio' oranges
- This new planting is 330,000 trees comprising 634 ha (1,566 acres)
- The entire farm is unirrigated

HLB Management and results at Maringa Farm

HLB Management is based on 3 tenets:

1. Inspect for ACP
2. Apply suppression of ACP
3. Inspect for HLB and eradicate infected plants

Maringa Farm sprays 3 times per month for ACP due to the young age of this farm's groves. Other farms of Citrosuco are sprayed less frequently. They utilize ground air-blast and aerial application, but predominantly ground. There is no (or little) use of helicopters in aerial sprays in São Paulo.

1. ACP Inspection and monitoring:
   Sticky traps are the primary monitoring tools, and are placed along the border of the farm and within interior blocks. All sticky traps are monitored weekly. Around the border (ca. 100 m depth) traps are located every 50 m. Within blocks, traps are located one per 500 M. This results in 125 border traps and 25 internal traps across the farm. When any ACP is detected in any trap, they apply pesticide to entire farm.
   In addition to sticky traps, staff members inspect the farm visually for ACP and other pests:
   - ACP visual inspection, 1% of trees in each block, out to edges, looking at 3 flushes per tree. This is presence/absence sampling
   - Citrus rust mite visual inspection 2 times per month, sampling .5 % of trees
   - Citrus Leprosis (*Brevipalpus* mites) visual inspection once per month

2. ACP Control Applications:
   Ground applications are variable by tree age

- 0-2 years old, once per week
- Older 2-4 times per month
- Mature groves 1-3 times per month
- They use calendar scheduling of treatments, but, if either trapping or visual monitoring detects ACP, they treat immediately, even if it is not yet time to treat according to the schedule. This generally leads to only 15% extra sprays.

Ground materials include pyrethroids, organophosphates (dimethoate) and neonicotinoids. They indicated that they did not have some of the classes of compounds that are available in the U.S. for ACP.

Aerial Application against ACP: Talstar (pyrethroid) is applied on trees 0-5 years old by air at 5 L per hectare with an 18 m swath width. 2-3 L spray oil is added to balance water (reduce evaporative loss of droplets). During dry season they do not spray during mid-day due to low humidity and droplet shrinkage.

The coverage time of aerial treatments across the whole farm is 1 day, while it takes 1 week to cover the farm with ground spray equipment. Generally the cost is similar, but Citrosuco owns the airplane, so aerial applications slightly cheaper.

Soil Drenches with insecticides: In addition to the ACP treatments mentioned above, young plantings are treated every 60 days with systemic insecticides (imidacloprid). The effectiveness of this treatment has not yet been proven. The limit on applications of this treatment is the cost, as both materials and labor for application are high.

There is variable involvement in helping neighbors with sprays, as some neighbors are infected at high enough rates that they don't want to prolong the survival of the blocks. Nonetheless, often neighbors participate in cooperative spray programs similar to our CHMA programs.

3. Inspection for HLB:
Each farm inspects entire blocks visually for HLB, every tree being evaluated at the prescribed interval. Inspection is done by ground in young trees, and when trees are older than two years, they use ground and platforms mixed. Their goal is seven inspections per year, and their goal is to remove HLB+ trees within seven days of inspection. Most often, infected trees are removed the day following detection.

Figure 15. *Platform-based HLB scouting as observed on Citrosuco Farms.*

Results of HLB management programs on Maringa Farms:

Success in managing ACP is measured by mapping the number of times ACP (minimum 1) is found in each block by both monitoring methods. This mapping effort has demonstrated the higher incidence along borders (edge effect) and less on edges adjacent to another Citrosuco block under similar management.

The Citrosuco group presented data maps and a lot of additional information, reinforcing the information and trends that we observed and discussed. Occurrence of ACP in 2012-2013 was reported as 80% of blocks with at least one incident of ACP, while February-June monitoring yielded less than 20% of blocks infected once.

HLB Monitoring and eradication was reported as follows for the 4-6 year old plantings:

- 2010/2011: 0.1% of trees infected and removed (about 1,000 trees)
- 2011/2012: 0.8 % of trees removed (over 2,000 trees)
- 2012/2013: 1% trees removed and replaced
- The cumulative loss to HLB eradication since planting is 1.9%, considered very low for this region, which is viewed as among the most heavily infected region of São Paulo.

Field visit to Maringa Farm plots (photos)

1. 'Rio' Early oranges: 2010 planting on 6.5 x 2.2 m spacing. This scion produces small trees, better fruit quality than 'Hamlin', with higher brix/acid ratio and color scores than 'Hamlin'. Yields are slightly lower than Hamlin trees of similar age.
   This plot (and other blocks on this farm) was treated with zinc and manganese monthly except during the dry season, using sulfate rather than chloride formulation in most cases. In addition, urea is applied during bloom, and magnesium is supplemented as needed. Boric acid applications provide soil boron supplement.

2. 'Pera' mid-season oranges: 2009 planting on 7 x 3 m spacing and on 'Sunki' rootstock. Note: Trees on 'Sunki' have intermediary resistance to drought, fruit size and maturity, and productivity between 'Cleopatra' and 'Rangpur' lime. They are resistant to citrus blight, tristeza, citrus sudden death, but very susceptible to foot and root rot. 'Sunki' has been a rootstock choice for 'Pera' orange in areas with high incidence of sudden death or citrus blight, or used as a second rootstock on 'Pera' trees budded on 'Rangpur' lime (from Citrolima

Figure 16. *Ground-based pesticide application typically used to delivery ACP suppression, as n well as materials to suppress other pests and diseases.*

Nursery webpage). After seeing performance of this block, Citrosuco now is planting at 6.5 x 2.5 m spacing since the trees are growing in more slowly. This block is treated 3 +/- times per year for phytophthora in the young trees. Other treatments in this and other blocks on site include citrus black spot and ACP spray, using dimethoate for ACP. We observed an application of this treatment in the Pera block that was applied at 1,500 l/ha and a speed of 4.4 km/h. This set of parameters is used to achieve better coverage needed for CBS control (Figure 16). When insecticides only are applied in blocks of this size, typically a volume of 800 – 1,000 l is applied at 7 km/h.

3. 'Hamlin' on 'Swingle' that is now 6 years old on 7.3 x 2.9 m spacing. This block produced 1,100 boxes per hectare last year at 5 years old.

Figure 17. *Young citrus trees on CItrosuco's Maringa Farm (left); and in-grove discussions of production practices and HLB management (right).*

Drought impact on blocks where no irrigation is available:

Although the most of this citrus region generally receives favorable rainfall during the growing season, drought periodically impacts citrus plantings in this area. It was stated that 1 or 2 times since the new plantings were made there has been inadequate rainfall which has led to drought conditions, including some leaf-drop and fruit loss. This is particularly troublesome when the dry weather occurs during bloom and fruit set. In the case of 'Pera' oranges, trees may recover within the season by responding with the next fruit cycle (multiple blooms are common), but with other scions, it may not be until the next season that recovery from drought occurs.

<u>Citrosuco Nursery on site at Maringa Farms:</u>

Citrosuco has a citrus nursery on site that produces plants for their farms state-wide. Its location is somewhat isolated from the citrus plantings. One reason for the nursery location at Maringa is that the

property has never had canker. The site has 20 greenhouses with a capacity in excess of 2 million trees per cycle. Citrosuco does not sell trees outside of the company.

Citrosuco Neighbors:

Nearest neighbors around the Maringa Farm are other large farms (Cutrale, Cambuhy and a Citrosuco stockholder's private farm). These large farms also are replanting with similar goals, after having experienced high HLB infection on this site. This neighbor situation has changed from infection risk to a positive neighbor situation to the extent that the large companies continue their rigorous HLB and ACP management programs.

Other citrus plantings around Maringa farm are smaller farms, and with some abandoned groves nearby. These farmers have an exit strategy from citrus by renting their citrus blocks to sugarcane growers. The sugarcane growers clear the property and plant sugarcane under a rental agreement. This arrangement may continue through the cycle of the sugarcane crop, often 5-6 seasons. Then the grower can determine if it is time to replant citrus

Catapani Farms – Discussion with Luis Fernando Catapani, citrus grower at Fundecitrus office 10-31-13

History with HLB: HLB was found on their farms in 2004, and they started pushing trees. On a second farm that was isolated by Eucalyptus and no citrus for 5 miles, they also scouted for HLB, controlled ACP, and eradicated infected trees during the initial years of HLB spread in the region.

Ten Years Later:

In the Catapani farm with isolation from other citrus, they have experienced very low infection (only a few trees). They attribute this to isolation, and through their management (ACP control and infected tree removal), they have not allowed the few infections to spread internally.

On the second Catapani farm, near Araraquara ("highest incidence region"), HLB pressure reached 70%. They stopped removing trees in 2009 when the incidence was 50%. Production was decreasing so the entire farm is being cleared of citrus. The use of nutritionals allowed trees to look better, and fruit drop decreased to some degree, compared to the control area not so treated. But this management was not enough, since the economics were not there. In the ensuing period, 14 of 15 neighboring citrus farms have removed all citrus, with the only remaining farm with citrus belonging to Cutrale.

Today, Catapani is planting the citrus back in the Araraquara area farm to the extent of 1,000 ha. They are planning the installation of a "circular" oriented border to allow improved border maintenance and have planned a 6 x 1 m spacing (double density) to increase the border impact from the beginning. The block interior will be planted on 6 x 2 m spacing, and will be sprayed once monthly. The three blocks being planted with this border architecture will have border ACP spray weekly, with aggressive tree removal and replacement, particularly in the border areas. The calculation of the amount of border that is involved in a given block depends on the width of the border (assume 100m) and how large the block is. For a 20 ha block, the border would comprise approximately 20% of the area, and a greater

percentage of the trees, given the higher density in the border. As the contiguous size of new plantings increases, the percentage invested in managed borders is progressively less.

GCONCI Consultants – Physiological Treatments for HLB Management 11-1-2013:

GCONCI is the Group of Consultants of Citrus, a company with 17 partners who provide technical support and management to citrus growers. Each partner has a separate business and specialty, and engages the group when collaboration benefits the individuals and the group. They have clients throughout the state of São Paulo, and also in other citrus areas in Brazil. Their clients are large and small, and the range of services offered by partners independently or as part of GCONCI varies widely. Our primary contacts for this field day were GCONCI partners Gilberto Tozatti, Hamilton F. de Carvalho, and Camilo Lazaro Medina.

The purpose of this day-long discussion and grove visit was to learn about the field evaluation of 2, 4-D products and associated treatments that have been communicated to Florida from Brazilian colleagues. These trials have been in place for some time, and early success in slowing spread and retaining tree health also has been communicated to Florida growers.

During conversations while driving, through formal presentation by Dr. Medina, and through site visits to farms using the physiological treatments, we gained impressions about what is and is not known about these treatments, and the general sense of what they are contributing to overall citrus management in the presence of HLB. The day in the field was conducted around the Limera area, in south-Central São Paulo State. We visited two companies who own several farms each, and saw numerous groves treated with the products over recent years for varying durations. We were told that there are 10 or more other farms that also are applying the physiological treatments scattered around the state.

The products and materials being tested:

The products being evaluated and marketed are a combination of nutrients, SARs PGRs and perhaps other materials that have been designed by Camilo Medina and his colleagues at Conplant, a company separate from GCONCI, and run by Camilo. We learned the following:

- Inspiration for the products came from groves visited in the Limera area which were doing well in presence of HLB
- Incorporation of information from Florida (Maury Boyd and others) who were attempting to treat infected trees to restore health
- Fundamentals of plant physiology – Camillo and two other PhDs in Conplant are Plant Physiologist, including Eng. Agr. Ondino Cleante Bataglia – Partner in ConPlant who joineds us for part of the day. Camilo presented a short version of the seminar that he gave at UF, IFAS CREC, Lake Alfred and at UF, IFAS, SWFREC, Immokalee in August, 2013.
    - Dr. Medina's opinion is that water pressure in the citrus vascular system ("in phloem") is vital for healthy plants. While he has no data, he hypothesizes that reduced water

pressure in HLB infected plants leads to reduced flow in the phloem, an important feature leading to disease symptoms.

- Camilo also presented his hypotheses that plant defense pathways (especially SA) are important in disease development and perhaps treatment responses. For this reason, they are including SAR materials in the physiological treatments.
- Components of the physiological treatments were described earlier in this report, and were not clarified to any extent in this presentation/discussion

The products that are now being manufactured and sold in Brazil via license from Conplant to a single manufacturer are comprised of two separate products formulated in a manner consistent with their pH requirements.

- Medici (sp?) is a combination of foliar fertilizer components
- Domini (sp?) contains SARs, PGRs, etc.

These two components are used together in tank mixes to achieve the goal of "Physiological Treatment". The rationale for two products is dissimilar chemical characteristics among the components which don't allow for stable formulation in one product. When diluted in spray tanks, the products are compatible and are used together.

The trials that we visited were blocks with variable application history of these two products together, along with other details as provided in the following site descriptions.

For cases where tree health decline is severe, Camilo has developed an additional material that he proposes will restore health of infected trees and is beginning to field test. This, he indicated can be used where phloem disruption is severe enough not to allow nutrient movement, which limits effectiveness of his other products. We saw one example of trees buck-horned and treated with the experimental material but no time had passed since the treatment (2 months). Again, this does not mean reduction in HLB bacteria but tree response. He did not indicate that the components of this treatment might be or how long recovery might take.

Figure 18. *HLB-infected tree that has been buck-horned and treated with experimental recovery treatment, observed by Bobby Barben.*

<u>Valim Group – Physiological Treatments:</u>

Fazenda Giriva: Farm of Valim Group near Limera: This farm is a former coffee plantation that is largely converted to other crops including citrus. The Valim Group also has another farm named Mato Blosso. Collectively, this group has 100,000 head of cattle, 17,000 ha soybean scattered around the state. Fazenda Giriva is 1,500 ha with numerous citrus blocks comprising 200 ha. Among these citrus blocks are about 100 ha of mature producing trees and 100 ha of young trees (both resets and replants). The farm produces approximately 90,000 boxes of fruit per season.

San Jose Farm

- A ‘Natal’ orange block 18 years old on ‘trifoliate’ rootstock. This block was first infected with HLB in 2005. During the period 2005-2009, the percent infection of trees in the block (visual scouting) was 0-19% annual rate. In 2009 this block was the first on this farm to begin physiological treatment, as it was the worst infected spot on the farm. Tree removal was being practiced at the time, as it was on other blocks at this farm. Other blocks on the farm continued with conventional treatment in 2009.

  The HLB situation following 2009 initiation of the treatment:
  - 2009: new infected trees removed and treatments begun
  - 2010: no new infected trees identified. This is interesting, since at this time, there should have been asymptomatic newly infected trees in the block.
  - 2011: one newly infected tree detected. At this point, all blocks on the farm were added to the physiological treatment regime.
  - Yield in the ‘natal’ block has averaged 1,200 to 1,300 boxes of fruit per hectare.

Figure 19. *Citrus grove visit to observe physiological treatment being applied to these blocks. Dr. Camilo Medina describes application to trees with low incidence of HLB (left); and application to a block that has already experienced high levels of HLB infection and disease symptom development (right).*

- Neighbor block immediately adjacent to the ‘Natal’ block. This block is planted to ‘Folia Murcha’ and was 5 years old. This block is heavily infected with HLB, having been the first block infected in 2005. Following detection, this block increased to 2 percent infection, then 5 percent, then two years ago, physiological treatments began. In addition, resets in this block began 3 years ago, but resets were standard ‘Valencia’, as ‘Folia Murcha’ plants were not available. The production manager and Hamilton Carvalho believe that the susceptibility of ‘Folia Murcha’ and ‘Valencia’ is similar, while Gilberto Tozzati indicated that the flush phenology (folded leaves) mean that ‘Folia Murcha’ is less vulnerable than ‘Valencia’. He attributes this to less ACP colonization of the folded leaves earlier in their development and emergence.

- We observed a lot of HLB evidence in this block, as in the adjacent 'natal' block, but with no previous personal experience in the block, it was difficult to see how its appearance and infection had changed in recent years since treatment.

- A new planting was visited that is 18 months old and extends across a hilltop. The block is large and has established well. This block has been treated with the physiological treatment program since it was planted.
  - According to the GCONCI folks, there is no detection of HLB in this block at this point (perhaps not surprising for a block only 18 months old).

Figure 20. *Eighteen-month old citrus trees on São Jose Farm under experimental trial of physiological treatment*

- Next to this block is a 13-year-old 'Valencia' block that has experienced "high incidence" of HLB. This block began treatment with the physiological treatment program two years ago.
  - Since treatments began there is no new HLB detection and no disease expansion. It wasn't clear if there are data that support this statement, since they did not discuss in detail how HLB monitoring is conducted and or show any data.

Figure 21. *Healthy appearance of 13-year old Valencia trees that has been treated for two years with the physiological treatment.*

- In this same area was a 3-year-old planting that also has received the physiological treatment since it was one year old.
  - Camilo Medina commented in response to the question that maybe one or two trees have become HLB infected in this block.

- Another block we drove through as we left the farm was a 'Valencia' on 'Swingle' block 3 years old. This block also has received the physiological treatment since it was one year old
    - The block has a crop that is estimated at 80kg (2 boxes) per tree at this time, not yet harvested.
    - This block is reported to have less than 0.5 % infection with HLB.

Figure 22. *Three-year old citrus planting which is subject to physiological treatment.*

Santa Maria Farm of Valim Group

- This farm has a significant new planting strategy that was planted 3 years ago. It began with traditional production inputs, including the use of insecticidal drenches for ACP. After one year, physiological treatments were initiated across the whole farm, and at this time, soil drench treatments were discontinued.

Figure 23. *Three-year old Valencia trees on Valim Farm which have been subjected to physiological treatment trials for two years.*

- The owner indicated that this farm is relatively isolated, and we viewed the landscape around this farm. Mr. Valim indicated that the nearest citrus is 2-3 km away, with a lot of non-citrus land use in between.

- There are a number of blocks of roughly the same age with the following among them:
    - 'Valencia' on 'Trifoliate'
    - 'Pera' on 'Rangpur'
    - 'Hamlin' on 'Swingle'

We drove around the farm, looking at newer blocks. We observed *Phytophthora* infection and tree decline on the lower end of a hillside block where flooding had allowed accumulation of water.

- Observation of the 3 year old 'Valencia' block indicated strong grove establishment, good structure of the trees, and a considerable crop set. There was no evidence of infection in this block or others that we observed closely. No evidence of ACP was observed, but presence of small numbers of brown citrus aphids on two plants was observed.

- Mr. Luis Valim, owner of the farm, indicated that they are spraying ACP monthly and borders weekly in addition to the physiological treatments. Mr. Valim indicated that they are not and do not intend to remove HLB infected trees.

Neighbors of Valim Group

As we departed the San Jose Farm, we visited a neighbor grove that is planted to lemon for fresh fruit utilization. The block is 30,000 plants and is not treated regularly for ACP. It has a high incidence of HLB and we observed large numbers of ACP on terminals, both adults and nymphs.

Agricola São Jose Farm – Physiological Treatments

Overview of Fazenda São Jose, another of the 10 or so farms that are including physiological treatments in their production practices to manage HLB.

Fazenda São Jose is a family farm (Agricola São Jose) that has been in business for 180 years. The farm is comprised of 7,000 hectares devoted to the following:

- 3,000 hectares of citrus, including oranges ('Hamlin', 'Valencia', 'natal' and 'Pera') and is the 2nd largest Sicilian fresh lemon grower in the Brazil with 350 ha. The farm also maintains a citrus nursery with capacity of 150,000 plants per cycle for internal use. They are planning 850 additional ha of citrus plantings.
- 1,600 of sugarcane
- 300 ha rubber plantations
- They also breed race horses on the farm, and have full facilities for this business

Dr. Andre Creste of Agricola São Jose described impressions of physiological treatments used on their farm. Dr. Creste is a plant physiologist and colleague of Dr. Medina. Like the Valim Group, they have adopted the physiological treatment program on all of their citrus groves in the past 2-3 years. Their comments:

- The program is maintaining productivity at profitable levels. Production is good, reaching a high of 2,000 boxes per hectare in the best producing grove, and averaging 900 boxes per hectare. The farm has approximately 1 million citrus trees, of which 45% are young trees (not defined).
- Their plan over the next 4 years is to remove 1,200 hectares of old grove and replant with either citrus or sugarcane.

- Their policy with regard to HLB is to scout and remove infected citrus trees. In blocks less than ten years old, infected trees are removed and reset. In groves older that ten years, trees are removed but not replanted.
- Scouting for ACP is constant, with full-time scouting program.
- Blocks are scouted once per week and borders are sprayed every 10 days. All blocks are treated every 2 weeks for ACP.
- HLB incidence in the farm varies widely, with the average being 8-10%.
    - Up to 35% in worst (oldest) blocks
    - 3-4 % incidence in young blocks. It was indicated that they are not finding symptoms of HLB. It begs the question if the treatments might mask symptoms, making it difficult to assess infection levels.
- Physiological treatments are applied 12 times per year, once per month. This is different from what we heard at Valim Group, who indicated they treated monthly, but not during the dry season. They claimed treatments were made only 7-8 times per year.

Grove Visits with Agricola São Jose

- 'Pera' on 'Rangpur' 18 months old. This block receives the following management treatments:
    - ACP management as described above for this farm. This includes neonicotinoid soil drenches
    - Standard nutrient treatments plus addition of composted chicken manure.
    - Physiological treatments were begun on this block at time of planting.
    - Those present reported no HLB in this block.
- 5-6 year old block with high incidence of HLB. This block has received physiological treatment for two years.
    - The yield was 620 boxes per hectare last crop year, with a higher yield the previous season.
- We drove through the farm and looked at several other blocks that are now being treated with the physiological treatment. The grower indicated satisfaction with the health of blocks, and now that they are all being treated with physiological treatments, they are confident that they are managing well in the presence of HLB.

The grower with us in the grove indicated that they spend in the range of $R4,500 per hectare per year, which he claims is about one half of what other growers are spending. The grower also indicated that their current grove density is 450 trees per hectare, and they are targeting 750 trees per hectare on new plantings. We did not learn what rootstock would be used in these high density plantings.

Figure 24. *Five to six-year old citrus trees being treated with physiological treatments.*

The Agricola São Jose Farm hosted a nice barbecue for us on site, which allowed U.S.to visit further with the GCONCI group and the growers.

Luis Vasconsuelus Consultant

Ben, can you summarize the conversation about costs, management tools, and challenges of small growers who don't have the resource base to invest heavily in HLB management when fruit harvest and returns are so variable?

TOPICAL OVERVIEW OF HLB MANAGEMENT STRATEGIES: RESEARCH PERSPECTIVE

During this trip to Brazil, we had discussions and presentations on the science behind various aspects of HLB management strategies, and more limited discussions about other citrus disease management. We had discussions and tours with Fundecitrus scientists, and also spent one day at the University of São Paulo, Piracicaba with the Entomology and Acarology Department faculty and staff. We had an opportunity to discuss numerous research projects during field visits as well.

Fundecitrus Research Program Overview

Fundecitrus Funding and Research Support: Fundecitrus manages about 100 research projects, 60 within Fundecitrus and 40 outside. Their budget is in the neighborhood of $7 million per year, generated 50% by growers and 50% by processing industry funds. In addition, the processing industry has provided support for the GMO Project being conducted at Fundecitrus. In addition to supporting its internal programs, Fundecitrus provides approximately $1.5 million to outside research projects, such as those we visited at ESALQ at Piracicaba. Fundecitrus has a staff of approximately 80, including scientists, agronomists who provide technical support to growers, and those involved in inspection, training, information, and other goals.

Fundecitrus has recently been approved to offer an applied Master's degree to interested parties. This program emerged when the country reorganized its accreditation of colleges and universities, and allowed other institutions to apply to deliver approved curriculum leading to a Master's degrees. This is

a “Professional Master Degree”. In this program, those enrolled attend classroom instruction weekly over an extended period, and these classes are taught by Fundecitrus scientists and guest lecturers. In addition, those enrolled in the program conduct a supervised research project, often a field evaluation. Many of the people we met working with citrus farms have received this degree or are currently enrolled.

Overview of ACP research in Brazil – Presented by Marcelo Pedreira de Miranda of Fundecitrus – The presentation PowerPoint was provided by Juliano Ayres. This presentation was made at the Cambuhy Farm office prior to their presentation and field visits on 10-28-13.

ACP Trap Construction: ACP control is applied about once per month, so trapping is used to monitor ACP populations. Their evidence of effectiveness in young trees indicates that yellow sticky traps are more effective (sensitive) than taps, and are used pretty widely. Other slides show lack of difference between the two methods, and some confusing results on which is best. High variability in results of evaluating ACP monitoring leads to no significant differences in many measures, including trap comparisons. In general if ACP is found in a trap, treatment is applied immediately. If not, then once per month is the schedule of treatment.

Control with Insecticides: Pesticide application remains an important element of the fight against HLB. As with Florida, systemic materials are the main tools for young trees, and foliar insecticides are used on older trees. The primary classes of materials used are neonicotinoids, organophosphates, pyrethroids, juvenile hormone analogs, and other organic materials. Emphasis on insecticidal control includes reducing volume of applications and also the possibility of reducing rates of active ingredients. Field trials of insecticides are evaluated using caged psyllids, which can be installed prior to the application or after the materials dry. These are generally read 1 day post-treatment. Researchers have found that caged psyllids one week post-treatment provide highly variable results, “due to weather conditions”. Work with petroleum oils follows previous efforts of Andrew Beattie in China and Southeast Asia. There is a lot of effort to evaluate the effectiveness of aerial applications, since they are fast and efficient to apply.

Marcelo also presented slides on probing/feeding behavior of ACP, similar to work done by Michael Rogers at UF, IFAS. More on this topic is presented in the Piracicaba research report. Reflective mulch treatments also are being evaluated, and these field trials were viewed at one of the Cutrale farms as described earlier.

Asian Citrus Psyllid (ACP)

Fabio Santos presented information on ACP management, building on the presentation by Marcelo Miranda made earlier in the week.

Marcelo Lopes and Andre (Graduate Student) from Fundecitrus gave an overview of the work on attractants and repellents (volatiles), indicating that we would visit the lab later in the week. They have worked out a system to categorize tree phonological state in relation to volatile production and attractiveness to ACP. The system uses a scale of vegetative status for use in trapping and relating to

ACP response, with v_1 representing emerging feather leaves to the other extreme phenology v_7 where no new leaves are present on the plant. A block is rated as to proportion of trees in each of the 7 vegetation stages. The results are displayed in a histogram.

Figure 25. *Tour of ACP attractant and repellent research lab at Fundecitrus, displaying methodology used in isolating and testing plant and insect-based volatile chemicals.*

Fundecitrus has adapted a CHMA-like system for reporting ACP trapping results to growers, and uses mapping to display mean numbers of ACP per site. Traps used in monitoring are geo-referenced, allowing for analysis of regional trends and historic reference.

HLB Detection:

The unique situation in Brazil requires that detection be conducted to include *Candidatus Liberibacter asiaticus, Candidatus Liberibacter americanum*, and the phytoplasm which causes similar symptoms in citrus Efforts have led to development of a multiplex PCR reaction that can detect all three of the pathogens. The Fundecitrus Diagnostic Lab processes 25,000-30,000 samples per year, with the majority being research samples. The cost of the triplex diagnostic is estimated at $R8 per sample. This assay was developed in part in cooperation with Dr. Dawson at UF, IFAS, CREC, with the visitation of Dr. Diva Teixeira.

Genomic Sequencing:

The bacterial genome from *Clam* has been completed from samples originating in periwinkle. The bacteria were transmitted from citrus to periwinkle with dodder. Studies focus on the comparison of *CLas* and Clam, and how the two pathogens have changed over time in their prevalence in Brazilian citrus *CLas* is more heat tolerant, and expresses higher titer in citrus The two species have similar acquisition characteristics, but in *Murraya paniculata*, *CLas* successfully colonizes, while *CLam* apparently fails to colonize. Genomic studies indicate that *Clam* lacks the lipopolysaccharide biosynthesis genes that *CLas* and *CL solanacearum* have.

Sequencing results of the phytoplasma genome from citrus in Brazil places it in group IX of phytoplasmas, the same group as the phytoplasma found in sun hemp (*Crotalaria* sp.). There is some evidence that these two phytoplasmas may have a close relationship, and some suspect that hemp is

the original source of the citrus phytoplasma. Dr. Graham added that is likely, as citrus to citrus transmission does not appear to be occurring in the field.

Testing SARS:

A new project has been initiated to evaluate the role of SARs, particularly Actigard against HLB under greenhouse conditions. Actigard appears active, and Fundecitrus is using graft and ACP transmission to test activity, and some growers are field testing the material. This work referenced previous research by Dr. Graham at UF, IFAS, CREC who evaluated Actigard against citrus canker. Nelson Wulff indicated in his report that, following pruning of plants treated with Actigard, the effects against HLB are lost. Dr. Graham is communicating with Dr. Wulff regarding research on SARs.

Research to Incorporate HLB Resistance Genes into Citrus :

Presented by Nelson Wolff on behalf of the Fundecitrus research team. Nelson Wulff, in cooperation with Vivianae Marques, has been working since 2009 on GMO citrus to combat HLB. Since the community has not yet discovered plant targets for HLB suppression, his work focuses on Asian citrus psylild interference. The program has two goals:

1. Lower ACP transmission by incorporating guava-based volatiles which repel ACP. Candidate volatiles are sesqueterpenes, and 'Pera' and 'Valencia' scions are being transformed. This work is in cooperation with Leandro Pena of IVIA, Spain, who is providing leadership on mature citrus transformation. The Fundecitrus group also is working with Rui Liete from Parana State, who has been investigating GMO citrus for canker resistance. The work is targeting mitochondrial chloroplasts for transformation, and 165 constructs have been made. Resulting plants will be screened for HLB titer and the team will evaluate ACP interference with olfactory assays.

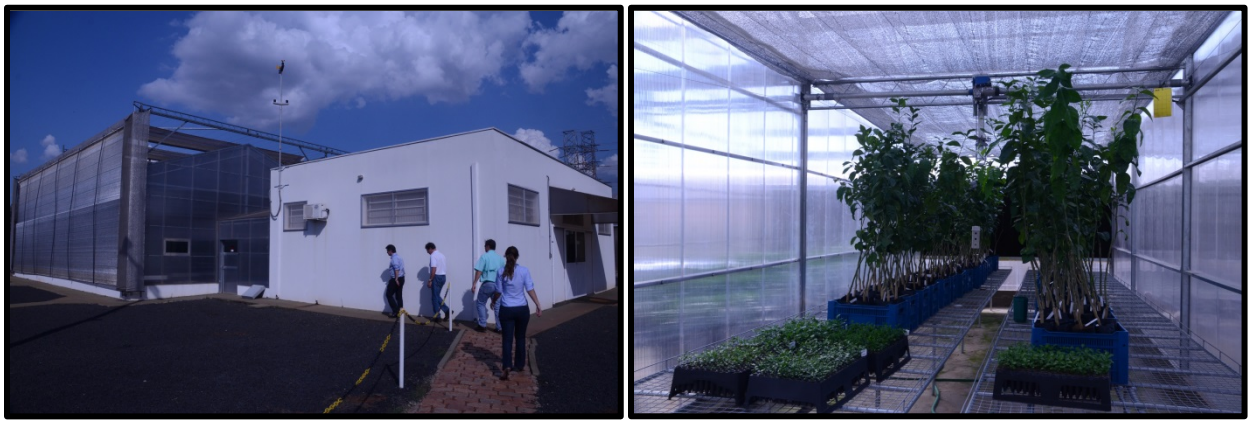


Figure 26. *Exterior view of new facilities at Fundecitrus in which experimental plants are engineered to incorporate various strategies to fight HLB (left); Citrus plants grown in this mature transformation facility in preparation for testing against ACP and HLB (right).*

2. Transform *Murraya paniculata*. The goal is to develop attract and kill strategies in *Murraya* instead of in citrus This would provide opportunities beyond those associated with GMO citrus.

HLB Pathology and Epidemiology Research:

Silvio Lopes and Renato Bassanesi are the leads on this research presented by Nelson Wulff on behalf of the research team.

Research conducted by Silvio Lopes:

- Effects of high temperatures in the field on multiplication of *CLas* and ACP. Also inoculation of citrus by ACP under varying conditions
- Regional differences in transmission around São Paulo State
- Seasonal conditions and how they affect symptom development
- HLB and lemon interaction; symptomology differences in infected lemon versus other citrus genotypes
- Effects of rootstocks (those commonly in use) on scion symptom development. Dr. Graham indicates that the IFAS team of Dr. Graham and Dr. Evans are coordinating their investigations of rootstocks with Silvio.

Research conducted by Renato Bassanesi – Epidemiology

- Timing of vector control and infected tree elimination
- Young groves
    - Effects of ACP treatments on disease development
    - Effects of Inoculum reduction on disease spread
- Nutritional evaluation field trial – Cutrale's Santa Maria Farm – epidemiological evaluation
- Evaluation of ACP management
- Cost/Benefit Analysis of HLB management

We met a scientist named Dr. Diva Teixiera who is collaborating with Bill Dawson and Gowda at UF in building a CTV vector based on a Brazilian isolate of CTV.

ESALQ, PIRACICABA, UNIVERSITY OF SÃO PAULO CITRUS ENTOMOLOGY PROGRAMS

Visit to University of São Paulo, Piracicaba (ESALQ), Department of Entomology 10-30-2013.

Piracicaba is a traditional College of Agriculture started in 1902, and it joined the University of São Paulo (USP) in 1934. USP has over 85,000 students at its many campuses across the state, and this campus has both undergraduate and graduate studies, with several thousand students. It has a history of research in agriculture across a lot of cropping systems and forestry.

Department of Entomology and Acarology:

There are 12 faculty in the department, with 8 conducting research on citrus and ACP. A rough estimate of their level of effort is approximately 50%, with some more and others less. They rely heavily on

graduate student projects to move their research objectives forward. Among those working on citrus in cooperation with Fundecitrus are:

- Dr. Jose Roberto Parra
- Dr. Jose Mauricio Simoes Bento
- Dr. Itala Delalibena
- Dr. Wesley Godoy
- Dr. Fernando Consoli
- Dr. João Lopes
- Dr. Celso Omoto
- Dr. Pedro Takao Yamomoto

Mass Rearing of Biological Control Organisms:

An overview of the mass rearing operations on ACP and *Tamarixia radiata* were provided by Dr. Parra, and later in the day, we visited the lab and greenhouse facilities associated with this research program. Dr. Parra has extensive experience with a broad number of natural enemies of agricultural pests, and conducts research to complement rearing procedures. He presented data on the following elements of ACP biology that are pertinent to rearing of both the psyllid and its natural enemies:

- ACP biology/development on various rootstocks and citrus relatives
- Evaluation of citrus shoot size for ACP rearing
- Day Degree model for ACP stage-wise development at various temperatures

Dr. Parra described the cost of setting up a facility to rear 100,000 *T. radiata* parasitoids per month to be in the range of $R150,000. He also indicated that the cost of producing the parasitoids was $R0.09 for each individual produced. This includes cost of rearing the plants, the ACP, and subsequently rearing and collection of the parasitoids. Of interest in this story is that *Tamarixia radiata* was discovered in Brazil in 2005, predating the intentional introduction of this parasitoid into the country.
As an aside, Dr. Parra reported that *T. radiata are* more aggressive if they contain the endosymbiont *Wolbachia*. Another program in the department is evaluating *Wolbachia* as an endosymbiont of ACP.

Evaluation of the results of release of *T. radiata* in Brazil indicates that parasitism in the first 3 years following mass release was from 20% down to 5%. However, since that time, parasitism has decreased substantially in the presence of increasing ACP pesticidal applications. Releases at this point are targeted to areas that are less likely to be treated with pesticides for ACP.

This work has been developed in cooperation with Dr. Flores at the USDA, APHIS rearing facility in Mission, Texas, who is a cooperator on the CRDF NIFA nuPsyllid project. Dr. Parra also has cooperated with Dr. Marjorie Hoy at the University of Florida, Department of Entomology in rearing of ACP and *T. radiata*. They also cooperated with Dr. Hoy's lab in the introduction of *Ageniaspis citricola* into Brazil for CLM control. The ACP rearing program was scaled at 250 cages of 6 *Murraya* plants each that provide the host insect material on which 100,000 *T. radiata* are raised each month (4,000-5,000 per day).

Plants of appropriate phenology following pruning are moved to ACP production greenhouse where they are inoculated with ACP (300 ACP/6 plants in one cage). Ten days later, plants containing ACP nymphs are moved to parasitization cages for oviposition by *T. radiata*, and ten days later, the stems are cut and placed in parasitoid emergence cages, where light is used to attract (collect) adult parasitoids into clear containers. The rearing cost (in addition to the amount cited during the morning presentation for establishing the rearing system facilities) is estimated at $R5,000-10,000 per month.

Later in the day we conducted a tour of Dr. Parra's lab and greenhouse facilities where he is rearing both hosts and natural enemies. He has a major emphasis on rearing of Lepidoptera pest species on artificial diet as a means to mass produce egg and larval parasitoids. This is the basis of his overall program.

The tour focused on the steps required to rear large numbers of *Tamarixia radiata*, as he presented earlier in the day. We were shown host plant rearing, ACP nymphal culture on the plants, and finally, the *T. radiata* parasitoid rearing and collection steps.

Insect Semiochemicals:

Dr. Jose Mauricio Simoes Bento provided a lab tour of the new facilities dedicated to research on attractants and repellents, which focuses on insect-based chemicals as well as plant-based chemicals. We were shown the equipment used to extract and evaluate volatiles from various sources. Methods for extraction and GCMS identification were explained, and we were shown the various assay systems used to evaluate behavioral response of insects to volatiles. Y-tube and other apparatus were demonstrated.

Transmission Biology and Ecology/Behavior of ACP:

Work conducted in the lab of Joao Lopes includes the following elements:

- ACP acquisition of CLas is a major area, with research focused on psyllid tissues and the activities leading to acquisition from plant tissues. Timetables for acquisition under varying conditions are a component of this research. EPG studies indicate that most ACP individuals have acquired within 90 minutes of feeding. Bonani (2010) reported on the description of ACP feeding as measured by EPG. This is a Piracicaba/UF interaction, as the work was part of a graduate study at UF, IFAS, CREC.
- Persistence of *CLas* within the vector ACP is the next area of research. The route of *CLas* within the insect and details of the increase and movement of bacteria within psyllid tissues is the greatest focus.
- Transmission efficiency is the third step in vector biology, and the research program is looking to understand the relative efficiency of nymphs versus adults in transmitting. The time required for transmission is being evaluated, and the latest information indicates an average of about 17 days from acquisition to first transmission.
- Effects of ACP infection by *CLas* on the insect's fitness and longevity are being evaluated
- ACP dispersion from a single point of release is being evaluated using marked individuals. This is a PhD project being conducted by Arturo. They have demonstrated that ACP will move across

corn fields, weeds, and other non-citrus host patches. This work has not yet been published, but they have demonstrated movement of 150m within 6 hours of ACP release. These results may indicate the value of trap plants around citrus and non-citrus landscapes.

Note: In this discussion it was apparent that there is limited interaction and connection between this lab and the entomology research labs in Florida (Stelinski, Rogers, LaPointe, Hall). Although the work is greatly overlapping with work in Florida, Dr. Lopes indicated that he is not fully aware of the work in Florida. We discussed the opportunity to increase the communication, in an effort for harmonization to accelerate results. Much of the fundamental work on *CLas* acquisition and transmission by ACP in Florida has been published and thus should serve to pinpoint follow-up work in Brazil. Likewise, publications from this lab in the area of pathogen transmission could advance the work in Florida, so it would be beneficial to both sides. We recognized the connection between UF and USP labs in the EPG evaluation of ACP feeding and ACP acquisition.

Ecological Modeling in Citrus Infestations by *Diaphorina citri* from Time Series and Binomial Data**:**

This project is overseen by Wesley Godoy, who provided a written summary of his work, which is included here as a summary.

Modeling of population dynamics is an essential part of both research and management of citrus pest insects. Analytical tools regularly used in statistical and mathematical models have been employed in order to model infestations by *D. citri* in citrus orchards in Brazil. Three different types of models have been used to model *D. citri* populations. The first one includes time series tools such as autocorrelation, partial autocorrelation and spectral analysis. This type of analysis has been employed for monitoring of *D. citri* populations in long time series, in order to investigate density dependence between successive time steps, cycles and to estimate new occurrences of peaks. Another ecological approach is alert zone models, which allow one to know ecological patterns of oscillations that precede outbreaks. This tool has also been applied to better understand the oscillation patterns in *D. citri*. Finally, binomial models have been employed to evaluate how explanatory variables influence probabilities of presence of absence of D. citri in orchards.

Effects of Agricultural pesticides on pests and natural enemies:

Research by Pedro Yamomoto. A handout was provided that summarizes research in this program as follows:

- Impact of pesticides on natural enemies of pests – studies on lethal and sub-lethal effects of agricultural chemicals on natural enemies.
- Influence of insecticides on secondary pest outbreaks – determine causes of outbreaks of spider mites after application of pyrethroid and neonicotinoid pesticides.
- Influence of regional ACP management on populations and the incidence of HLB in citrus groves in the region around Piracicaba.
- Influence of citrus varieties and combinations of rootstocks/scions on feeding, development and biology of ACP.

- Selection of pesticides for control of *D. citri* – screening of insecticides for vector control.

This work includes effects of pesticides on honeybees, parasitoids and predators, as well as the following:

- Pesticidal influence on secondary pests, indicating the range of activity for key materials.
- Use of pesticides in area-wide management, including incidence of use of materials around the São Paulo State citrus groves
- Evaluation of effectiveness of insecticides – screening materials for efficacy and persistence
- Rootstock and scion effects on behavior and biology of ACP

<u>Resistance Management of Diaphoria citrus to Insecticides:</u>

Celso Omoto, Alex Sandro Poltronieri, Fernando Amaral, and Vitor Stella. A printed copy of a powerpoint presentation was provided and is summarized here:

Major Objectives:

- To understand geographical and temporal variability in the susceptibility of D. citri to insecticides (neonicotinoids, pyrethroids and organophosphates)
    - 25 locations spread across São Paulo were sampled and evaluated during 2010-2012
    - Populations of D. citri from these sites were then treated with diagnostic concentrations of imidacloprid, deltamethrin and dimethoate
    - Conclusions:
        - There were no differences in survival (resistance) to the insecticides tested on D. citri populations collected from field locations with different treatment regimes
        - Highest survival of insects at diagnostic concentrations was observed in 2010 (4.7%-24.0% imidacloprid; 0.9% to 11.8% for deltamethrin; and 5.2%-13.0% for dimethoate)
        - There were no significant increases in survival of *D. citri* on monitoring conducted in 2011 or 2012
- To evaluate the impact of the mixture of imidacloprid with other insecticides/fungicides for managing *D. citri* resistance to imidacloprid
    - Eight materials were included in this evaluation at field rates
    - Residual contact assays were used for adult assessment and direct contact and residual assays were used to test nymphs
    - Conclusions:
        - Additive effects on adult mortality of *D. citri* were detected with mixtures of imidacloprid and deltamethrin or dimethoate
        - Additive effect on nymphal mortality of *D. citri* was detected with the mixture of imidacloprid with buprofezin or pyriproxyfen

        - The biological activity at field rates of imidacloprid and buprofezin as well as the mixtures of these insecticides showed a similar degradation in activity to control *D. citri* under field and greenhouse conditions
        - Evaluation of the persistence of the mixture of pyriproxyfen and imidacloprid indicated that pyriproxyfen degradation was faster than imidacloprid
        - The mixture of LC_{25} of imidacloprid to *D. citri* with fungicides (field rates of benzimidazole, strobilurin, and triazole) did not affect the performance of imidacloprid.
- To evaluate imidacloprid toxicity and its association with *Tamarixia radiata* on the demography of *D. citri*.
    - Conclusions:
        - A significant reduction in the instantaneous rate of increase of *D. citri* was observed when exposed to different residue ages of imidacloprid sprayed at concentration of 40ug/ml, even when exposed to 56-day old residues
        - The exposure to CL5, CL10, LC25 and LC50 of imidacloprid to *D. citri* did not cause their extinction. However, these concentrations of imidacloprid affected the rate of increase of the parasitoid, causing their extinction at LC50.

<u>Insect Endosymbionts for ACP Control:</u>

Fernando L. Consoli, Entomologist: This program focuses on insect interactions, particularly with symbionts. Dr. Consoli works with *Wolbachia*, including studies of the diversity of *Wolbachia* species in Brazil, and has collections of ACP from São Paulo State and several other states. He is evaluating the presence of *Wolbachia* via markers, and can distinguish presence of more than once species nondestructively (hemolymph samples). He is using the MultiLocus Sequence Typing (MLST) approach to differentiate species, even those closely related. His results indicated the following in ACP from samples covering several states:

- 99% of samples contained one species
- Two other *Wolbachia* species appeared in samples, but have not been successfully recollected from the field
- No samples of individual ACP contained more than one species, and no samples were without *Wolbachia*. According to Dr. Consoli, no one he is aware of around the world has found ACP without *Wolbachia*. He indicates everyone is finding 100% *Wolbachia* infection.

His project also has made progress on partial bacteriome of the two bacterial species that are primary endosymbionts of ACP. These are *Carsonella* and *Profatella amatra* (Ben, can you confirm this name?). You were doing google searches of these primary endosymbionts). There are cytoplasmic incompatability issues associated with *Wolbachia* endosymbionts of ACP, and this program has attemped to eliminate *Wolbachia* from psyllids differentially while not eliminating the primary endosymbionts. Heat and antibiotics have been used, but both affect the primaries as well. Currently Dr. Consoli is attempting to microinject probiotics to achieve his goals.

Dr. Consoli also is working with medfly, and particularly *Wolbachia*-free medfly. He is cooperating with a Greek scientist on the 'Vienna' strain of medfly inoculated with the V-8 strain of *Walbacia*. Following a female-terminal treatment, males infected have cytoplasmic incompatibility due to the foreign *Wolbachia*.

Insect Pathology Program:

Dr. Italo Delalibera Jr. has been studying insect pathogens for 10 years, including during his MS degree at Wisconsin and PhD at Cornell. His current project related to ACP/HLB is the development of the insect pathogenic fungus *Isaria fumosorosea (*IFR) for ACP suppression. The department is renowned for its efforts to commercialize agricultural pest products containing *Trichoderma* and *Metarrhizium anisoplaea*. The latter is currently used on 4 million acres of crops in Brazil, including some reliance on *M.anisoplaea* strains isolated at the ESALQ Piracicaba lab. Over the past 3 years, they have amassed a collection of 2,500 isolates of entomopathogens in the department.

IFR Strain development project:

Rationale for this project:

1. Present development of pesticide resistance
2. IFR can be combined with lower-dose pesticides (pyrethroids) making the treatment cheaper
3. Use alone for control, creating more sustained tools for ACP management.

These positives may be offset by limitations imposed by fungicidal treatments for citrus fungal diseases.

Currently Dr. Delalibera is screening isolates of IFR collected from Brazil.

- He has identified successful strains
- Now the project is evaluating treatment in large scale trials
- They also are looking at the range of activity of the IFR strains of interest (e.g., may also control aphids)

IFR 1267 is an isolate of interest and is among those being considered for commercialization, along with *Beauvaria bassiana*.

- Currently testing susceptibility of nymphs, which are more sensitive to treatment than are adults
- It is established that these treatments will not control eggs
- Target is 2.4-5 x 10^6 spores per ml of solution as an effective spore density
- This project is using conidia (aerial dispersed), whereas, the U.S. product of IFR (from Florida) is using blastospores, which Dr. Delalibera indicated is likely to be less effective, since it is not the infective stage.
- Field trials involve air-blast sprayer applications at 2,000 l/ha
- The field assay uses bagged ACP on leaves which are put in place prior to treatment and checked for survival after treatment.

- Dr. Deliabera indicates that honeybees are insensitive to IFR, another advantage.

While we were visiting Piracicaba, a meeting was being held with potential commercialization partners regarding the IFR that is being tested. Representatives of ESALQ, Fundecitrus and the commercial partner met to discuss the business partnership necessary to move this forward. A press release was issued (copy below) that reported this discussion:

PRESS RELEASE: November 1st, 2013, from www.freshfruitportal.com

Brazilian citrus producers may soon have access to their first biological pesticide to fight citrus greening, Fundecitrus reported. The product derived from entomopathogenic fungi is currently in the testing phase, scheduled to finish in the first half of 2014. Researchers from Fundecitrus have teamed up with Agricultural College Luiz de Queiroz (Esalq/USP) and Koppert Biological Systems to develop the biopesticide. The product would be the first of its kind from Brazil directed at naturally controlling populations of psyllid *Diaphorina citri*, the cause of citrus greening.

Industry director of Koppert Brazil, Danilo Pedrazzoli, explained that the goal of the initiative is to find better solutions to control citrus greening. "A goal of these three institutions is to bring to the field the knowledge obtained in research conducted by ESALQ and Fundecitrus Koppert is developing the final technology so that this product arrives to citrus producers," he said. "Apart from effectiveness in controlling pests, you can ration the use of insecticides and avoid insect resistance."

WHAT ARE THE IMPORTANT OUTCOMES OF THE BRAZIL VISIT?

Transfer of Information:

We encourage readers to consider the information provided in this report and contemplate how current practices used to counter HLB in Brazil can be incorporated into the Florida situation. Further, we encourage you to join with us in bringing the knowledge of HLB and ACP from Brazilian growers, researchers and citrus organizations together with similar groups in Florida so that both can overcome the current threates of HLB in each country. The following are our thoughts from the visit.

Research Perspective – Harold to complete

Grower Perspective – Bobby and Ben to complete

Steps Forward – Cooperation and Coordination Opportunities:

Grower to Grower

- Plant bigger trees to overcome/shorten vulnerable period following planting. Brazilian trees are in general quite a bit larger caliper when they are planted. The climate allows for shorter growing time for nursery stock, as well as faster growth under field conditions. Thus, they already benefit from a shorter "pre-harvest production period" following planting. It would make sense from an exposure perspective to consider how to plant larger nursery trees in Florida to accelerate their growth into producing trees and avoidance of the accumulation of disease and other issues at such a young age.
- Expand on current thinking to make the border management phenomenon more effective in both Florida and Brazil:
    - Current elements
        - Consider much higher density in borders vs. core of block (Citrosuco reported doubling the density to make the borders more impervious to ACP, disease)
        - Plant border in to encircle the main block, for efficiency in ACP and other control and to eliminate head-row air flow deep into groves (moving ACP)
        - Monitor and treat borders more aggressively than core of block
        - Consider treatment of adjacent neighbor tree rows
        - <u>Regularly</u> scout, eliminate, and replant infected plants in border
    - Potential additional elements to consider
        - Dwarfing rootstocks in border rows to retain ability to use soil neonicotinoids
        - Incorporate tolerant/resistant rootstocks into borders
        - Use pheromones, other volatile technologies along borders
        - Consider the concept of building or managing "borders" in adjacent blocks, particularly those close by which are already infested. Management of this adjacent border could slow migration of ACP from the blocks of concern, and reduce the pressure on new blocks. This would be most effective if you were planting a new block on property where you have existing mature trees and want to manage both to optimal gain.

- In association with the above concept of borders, encourage group design of the "best new Florida planting", using all available tools and ideas, and encourage aggressive management of such a new block to evaluate the collective value of all tools incorporated. Again, we heard that some of the tools used in Brazil are not as size-dependent as it may appear, and we won't know the benefits without testing. Encouragement of Fundecitrus participation in discussion of the tools and design might help translate the Brazil experience to Florida. Once envisioned and deisgned, find an innovative Florida grower who is willing to install and manage this "best available tools grove". It could be argued that Brazilian growers are already doing this with new plantings, although not all are using the same approaches.

<u>Scientist to Scientist</u>

It has been suggested that a future International Research Conference on HLB (IRCHLB) be held in Brazil to encourage scientists outside of Brazil to become more aware of what is going on in research. Dr. Graham, in supporting this idea indicates that his visits to São Paulo and Parana States continue to be a great asset to his knowledge of HLB and other diseases.

Learning from Field Trials

Joint Field Implementation Projects

- E.g., IFR evaluation, joint testing to better understand nutritionals

Develop pathways to try new tools – e.g., physiological treatments, ISR

We observed citrus growers using a relatively new "enhanced nutrition program" that they call "physiological treatments". This treatment is composed of materials similar to those being used and tested by Florida growers. We did not see any replicated field evaluations, as apparently there are none. Thus, it is difficult to evaluate whether this is a technology that has merit in combatting HLB in Florida. Without a clearer understanding of the components of the treatment, claims and even field observations are difficult to interpret. The opportunity raises the question if this warrants research or is it a case where commercial product access should allow growers to conduct their own field trials.

Co-Funding Opportunities

We discussed the possibility of collaborating on a joint FAPESP (Government) grant that would need to be very topical (e.g., volatiles for ACP management). With international collaboration, the likelihood of funding success would increase. This would not likely lead to funding flow to Florida from Brazil, but could strengthen necessary research in Brazil. This funded work could then be coordinated with Florida-based research on the same topic.

Communication

- Enhanced Communication: It was apparent from the visits, discussions and presentations that additional communication would be productive among and between many of the following groups

    - Sharing among scientists – occurs but could be enhanced
    - More sharing across growers – how others can learn from growers who have observed, visited?
    - Associations could work more closely in planning research, demonstration, and commercialization. It is obvious that this will need to be in place when either industry is ready to move forward with a GMO plant, for example.

- Dr. Graham offered that he has encountered few barriers to exchanges between scientists to scientists or growers to growers in his numerous trips to Brazil since 2000. He elaborates that there remain misconceptions among scientists and growers regarding the ultimate goals for citrus grown for juice

APPENDICES

Appendix 1. Contacts Associated with this Brazil Visit

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Appendix 2. History of FUNDECITRUS – from Fundecitrus website http://www.fundecitrus.com.br/

Translation to English was provided by Dr. Katia F. Rodrigues, Biological Scientist, University of Florida, IFAS, Citrus Research and Education Center.

Fundecitrus (Citrus Defense Fund) is a non-profit institution dedicated to the phytosanitary defense and research. It was created in 1977 by growers and industry, and throughout this time was able to establish a collaborative relationship between private and public sectors, and has become one of the most respected research institutions in the world.

Currently, they have developed more than 80 projects on pests and diseases in partnership with national and international institutions. In the last decade, it has developed important research such as the genome of *Xyllela fastidiosa* (causal agent of the CVC), the synthesis of the pheromone of the citrus fruit borer (*Ecdytolopha aurantiana*), the identification of greening and sudden death, among other relevant studies for citriculture.

The institution operates in more than 500 municipalities in the states of São Paulo, Minas Gerais and Paraná, including the following areas: Financial Administration, Science and Technology. Their professionals are dedicated to awareness and phytosanitary education services and training for growers.

The research profile began to take shape in 1986 when the Deliberative Council approved the establishment of a Research Department for scientific assistance within the institution.

In the mid-80's, it also participated actively on the planning and viability of two big campaigns, Canecc (National Campaign to Prevent Citrus Canker) and Ceprecc (State Campaign to Prevent Citrus Canker). In the first year, more than 10,000 growers received technical assistance and guidance on the effective management of the disease.

The proposal to disseminate knowledge and contribute effectively to the health of orchards, ensuring competitiveness in the sector, gained momentum in 1985 when Fundecitrus launched its first newspaper and opened its own headquarters in the city of Araraquara / SP. Three years later, it was responsible for the first census of citrus, conducted in partnership with the State Department of Agriculture, in order to detect and eradicate outbreaks of citrus canker. Sixty-six million plants were inspected in two years.

In the 90's, Fundecitrus took important steps towards its consolidation as a key sector institution in Brazil and abroad. It launched the Global Management in Citriculture, combining two technologies to maintain the health of plants and reduce production costs; it was accredited by the Ministry of Agriculture for plant defense; absorbed the structure of Procitrus (Foundation for the Development of Citrus in Brazil), a fusion that ensured greater agility of the research, and was invited to expand their work for the state of Minas Gerais.

Between 1996 and 1998, it developed a simple and inexpensive method for diagnosing CVC (Citrus Variegated Chlorosis) by means of optical microscopy; it financed and coordinated the first lines of research on citrus black spot disease by creating a diagnostic system that produces results in 12 hours, and succeeded, with the support of other research institutions, to isolate the sex pheromone of the citrus fruit borer. The product was approved by the Ministry of Agriculture and made available to the market in 2001 as a low cost tool with high efficiency in monitoring and controlling the pest.

Also at the end of 1990, it participated in the launch of the Genome Project, with the aim of sequencing the bacterium that causes CVC. In 2001, its researchers were able to obtain a mutant of the bacterium, opening new avenues of action against the yellowing disease. A year later, Fundecitrus coordinated a project that involved 32 researchers and 15 institutions that resulted in scientific evidence that the MSC (Citrus Sudden Death) is a disease caused by an infectious agent that can be transmitted. It also proved that the damage from the disease can be reduced at the time of inarching (approach grafting).

Fundecitrus also had a key role in identifying and determining control measures for greening between the years 2003 and 2005. With the support of state and federal governments, it conceived and executed a national campaign for information and awareness, including TV commercials, explanatory leaflets, manuals and stickers. It confirmed disease transmission by psyllids, *Diaphorina citri,* and that the incidence of greening can decrease by up to 15% when disease management is done at a regional level.

The Fundecitrus institution always believed that no knowledge can be applied effectively if the information does not reach the field quickly and clearly, and since the early 2000s has invested in improving their communication channels. The institution offers the industry a bimonthly magazine, annual technical manuals, specific brochures on prevention, campaigns, a computerized library, lectures and meetings with international representatives, a website and free hotline through 0800-112155.

There has been more than three decades solidifying an institution that is always attentive to the needs of the world market, focused on sustainable production methods that are also lucrative to the grower, protects the environment, creates jobs and promotes hundreds of communities involved in this important agribusiness.

Appendix 3. Fundecitrus Story Posted to their website November 5, 2013

Translation to English was provided by Dr. Katia F. Rodrigues, Biological Scientist, University of Florida, IFAS, Citrus Research and Education Center.

American producers say that São Paulo has better management of HLB than Florida and they want to bring together the research of both states.

Members of the Citrus Research and Development Foundation, Inc., (CRDF) visited São Paulo state for an update on research and techniques used in the management of HLB/greening.

The operational chief of CRDF, Harold Browning, and the citrus growers Robert Barben and Ben McLean visited groves, Universities, nurseries and the laboratories of Fundecitrus. The aim was to become familiar with the control measures of HLB used by the state of São Paulo. "We want to see what is good regarding management and nutrition that can be incorporated by our growers in Florida in order to save time and resources" said Browning, who was the director of the Lake Alfred campus of the University of Florida for 12 years. He said he observed two different situations in the groves in São Paulo: "I saw growers who pulled infected trees and are renewing their groves and are being successful; and I saw those who are not pulling infected plants, and their groves are declining as are the groves in Florida," he said.

Barben was very impressed with the laboratories and the quality of the researchers, but also noted the decrease of producers. "I was here six years ago and now I see that São Paulo is ahead of Florida in control, but I had not expected that so many people had given up citrus because of HLB," he said.

Both believe that it is possible to improve the synergy between Florida and São Paulo so there is no duplication of research projects and results may come faster.

The CRDF is a private research institute for citrus. They have a budget of US$ 19.3 million, in which 95% of the research is focused on HLB, and the remaining is destined for citrus black spot, scab and leprosis.

Appendix 4.

Bayer CropScience and Brazil's Fundecitrus have finalized a five-year, €3 million (US$4 million) investment directed at combating citrus greening or Huanglongbing (HLB).

The so-called "Citrus Unidos" agreement will focU.S.on new methods to control HLB and prevent *Diaphorina citri*, the psyllid behind this devastating disease.

One sustainable control method will be the propagation of *Tamaraxia radiata*, a natural enemy of the psyllid. The team will also explore new diagnostic methods and organize trainings to keep producers up-to-date with scientific advances.

Bayer CropScience Brazil said the partnership with Fundecitrus would bring a new perspective to citrus management through advanced research and educational techniques, explained crop protection director Gerhard Bohne.

"We are developing new products and technologies to control disease and insects, like the psyllid *Diaphorina citri*, to provide farmers innovative tools that contribute to better production," Bohne said.

"This partnership will help the Brazilian agricultural sector to maintain a worldwide leadership position in citrus This will be beneficial, however, not only to Brazil but also to other citrus-producing countries."

Fundecitrus president, Lourival do Carmo Monaco, said the agreement will speed up the technological response to sector demands.

www.freshfruitportal.com