An update on research funded by FCPRAC

By Megan Dewdney

In the last few years, the Florida Citrus Production Research Advisory Council (FCPRAC) has dedicated an unprecedented amount of money to citrus research. Much of the money has been reserved for research toward solutions for two diseases — citrus canker and Huanglongbing (HLB; citrus greening). This article will report on some of the plant pathology research that is under way.

CANKER RESEARCH

One of the major outcomes from canker research published this year is the change to the interstate export regulations. The change will eliminate the need for packinghouse inspection and for each lot of fruit to be free of visible canker lesions for interstate export, as well as allow for the transportation of fruit to states with commercial citrus industries. There is still a requirement for any fruit shipped from quarantine areas to be packed in commercial pack- inghouses with compliance agreements and treated with an approved disinfectant.

Findings that contributed to the regulation change were:

1) low numbers of lesion-causing bacteria were found on fruit, even from symptomatic fruit;

2) the population of bacteria decreased even further when the fruit were in cold storage and

3) if detergent was used before the chlorine treatment on the packing line, the number of bacteria from those fruit was reduced compared to untreated fruit.

These findings were interpreted to mean that Florida fruit present a very low risk of spreading the canker-causing bacterium, *Xanthomonas citri* subsp. *citri*, to citrus industries that do not have the disease.

Other areas of funded canker research included control strategies, biological questions and ways to reduce the number of lots with canker in packinghouses. Some of the control strategies include improved timing of copper applications which allowed for a revision to the 2009 Florida Citrus Pest Management Guide and new product testing.

A study has been initiated to look at the effects of windbreaks, leafminer control and copper applications and the interactions among the treatments to design improved control measures. The plots for the project have been planted and windbreak establishment is under way, but experimental results are not available at this time.

Some of the biological questions under investigation are whether there is a potential for copper resistance to occur in the *X. citri* subsp. *citri* populations of Florida and what factors contribute to the survival of *X. citri* subsp. *citri* on fruit surfaces. The development of copper-resistant populations of *X. citri* subsp. *citri* would limit our canker control options. Knowing how bacteria survive on fruit will help in the design of ways to further mitigate movement of the bacterium to places without a history of canker.

HLB

The most pressing concern of the citrus industry for several years has been the discovery and management of HLB. Huanglongbing research can be placed into several categories: Sequencing and genome based research, culturing *Ca*. Liberibacter asiaticus, symptomatology and the causes of symptoms, detection of *Ca*. L. asiaticus, epidemiology of HLB, HLB control and miscellaneous projects. *Ca*. L. asiaticus is the bacterium that is presumed to cause HLB in Florida, Brazil and much of Asia. Many research projects could be classified into several categories, but each has a main focus.

A major breakthrough in HLB research this year is the completion of the *Ca*. L. asiaticus sequencing project. This work allows genetic comparisons of *Ca*. L. asiaticus to other organisms, thereby leading to development of an understanding of some of the basic biology of the bacterium and development of ideas for working with this organism in laboratories. For example, the genome can give us hints as to what the bacterium requires for growth and thus aid the culturing process. It also has the potential to allow us to understand how *Ca*. L. asiaticus interacts with citrus trees to cause what we see as the disease HLB.

In addition to sequencing *Ca*. L. asiaticus, there is an effort to sequence *Ca*. L. americanus. Having the two genomes to compare will allow researchers to evaluate what common features between the two bacteria contribute to disease.

Further projects involving the genome include identification of candidate genes that may contribute to the disease process and then confirmation of the suspected gene function. It is important to confirm gene function because the predicted function is not always correct. The genome also allows the determination of whether there is more than one genetic isolate of *Ca*. L. asiaticus in Florida and how isolates vary. This knowledge could contribute to understanding how the bacterium spread through Florida so quickly.

Finally, so that the important discoveries are shared among researchers quickly, a database has been developed to collect and share all genome-based research in a timely and easily accessible manner so that researchers can build on each other's findings.

Culturing of *Ca*. Liberibacter spp. has eluded scientists for many decades. If we could culture any of the *Ca*. Liberibacter spp., we could confirm definitively that these bacteria cause HLB. Until they are cultured and re-inoculated into citrus and shown to produce comparable symptoms to what is seen in the field, we are not completely certain that the *Ca*. Liberibacter spp. cause the disease despite mounting evidence that the bacteria are the cause of HLB. Earlier this year there was a claim of culturing success, but no one to date has been able to reproduce the results found in this report. For a culturing technique to be useful, it must be reproducible and able to be done with relative ease. Work on this front is ongoing. It is possible that *Ca*. Liberibacter spp. may need to be cultured in insect cell lines. Similar techniques have been employed in related organisms, *Rickettsia* spp., the cause of human diseases such as Rocky Mountain Spotted Fever.

Screening of citrus germplasm material for the range of HLB symptoms has been undertaken in greenhouse experiments for a couple of years. There has been a wide range of

symptoms expressed in these studies, from extremely mild to near collapse of the tree. One finding that researchers have made is that those plants with the mildest symptoms are not necessarily free of *Ca*. L. asiaticus. In fact, many of these trees had more bacteria in them than trees with severe symptoms. This is a sober reminder that we understand very little about how this disease occurs and how the bacterium causes disease. Further work has confirmed earlier findings that there is phloem plugging by plant proteins caused by the disease and the presence of *Ca*. L. asiaticus. There is also an effort to find which genes in the bacterium or plant are responsible for symptom development.

Identification of infected trees in Florida is done with PCR detection of *Ca*. L. asiaticus mainly in leaf tissue. For the number of samples that are sent in for testing, PCR is relatively slow and expensive. There are several attempts to develop alternative means for detecting *Ca*. L. asiaticus in plant tissue. One is to develop blotting techniques based on *Ca*. L. asiaticus DNA that would not require extensive sample preparation. Another is to develop antibodies against *Ca*. L. asiaticus so that they could be deployed in a dipstick detection kit that could be used in the field. Both of the projects have good potential, but there are difficulties. The most critical issue is whether the diagnostic tests can be made specific enough that they detect only *Ca*. L. asiaticus and not other non-HLB causing organisms.

Some of the epidemiological projects under way are attempting to address some very important questions. They are interested in the question of how long the period is between infection of a tree with the bacterium until it begins to show symptoms. This period of time is called the latent period. One of the critical questions is how long after citrus is infected are Asian citrus psylla able to acquire *Ca*. L. asiaticus and whether this can occur before the end of the latent period (before symptoms appear).

Another question is how tree age affects disease progress. There is much anecdotal evidence that young trees show symptoms faster than older trees, but so far no one has been able to quantify this phenomenon. Further subjects of study are whether psyllid control is sufficient to stop the spread of disease and how necessary tree removal is for grove viability. Early results indicate that both tactics are important and that there is a lot of long-distance spread of the bacterium which emphasizes the importance of area-wide control of psylla.

HLB CONTROL STRATEGIES

There are several projects under way exploring HLB control strategies. Plots have been established to explore the feasibility of interplanting with guava to deter psylla, but it is too soon to have any results of this project. There has been screening of chemicals in vitro to see if there is disease control. Some antibiotics were found to be effective, but this is similar to the work done in the 1980s where antibiotics were injected into trees, but not found to be practical on a large scale.

The development of resistant trees is being actively pursued. One method of rapidly deploying an antibacterial protein into the phloem under investigation is the use of Citrus Tristeza Virus to insert proteins. The idea is that the antibacterial proteins would kill the bacterium in the phloem before disease development. Young trees are the most important to protect from infection. Since imidacloprid induces an SAR response in citrus at high rates, researchers are investigating whether it can also control HLB after graft inoculation of trees as well as reduce psyllid pressure.

Finally, researchers are attempting to transform citrus to produce more salicylic acid, which is part of the SAR pathway, to improve ability of the citrus tree to overcome HLB.

There were a few projects that do not fit into any categories. The first is seed transmission of *Ca*. Liberibacter spp. from infected tree to seedling. So far the results from several studies are inconclusive and scientists are still attempting to understand the varying results that have been obtained. It is well known that *Murraya paniculata* is an alternate host for both the bacterium and the Asian citrus psyllid, but it is not known how many other citrus relatives could also harbor the disease. It is important that these are identified so that the ornamental industry can be involved in HLB management strategies, but so far results are not available.

Finally, a project to investigate the microbial community in citrus phloem is under way. Researchers are hoping to find microbes that are antagonistic to *Ca*. Liberibacter spp. that could help with HLB control in the future.

This article was a very brief summary of projects that have been funded in the last year by FCPRAC. If you wish further details on any of the projects, please consult the FCPRAC Web site http://research.fcprac.com/reports/.

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