Update on insecticide resistance in ACP

By Lukasz L. Stelinski

The Asian citrus psyllid (ACP), *Diaphorina citri*, is perhaps the most destructive citrus pest, because it is a vector for the putative causal agent of huanglongbing (HLB), *Candidatus* Liberibacter asiaticus. Currently, the main way to limit the spread of the disease is by managing the vector through multiple applications of insecticides. Intense insecticide use has led to the development of varying levels of insecticide resistance in populations of ACP in Florida. Although new technologies are on the horizon, preserving the effectiveness of insecticides as valuable tools should remain a priority.

THE CURRENT SITUATION

We began monitoring insecticide resistance for ACP in Florida in 2008. Our findings from 2009-2011 indicate that ACP populations in Florida have progressively developed varying levels of resistance to several insecticide chemistries. Baseline susceptibility data for both adult and immature ACP to commonly used insecticides were obtained in 2009 and 2010. These data were collected for five ACP populations from various parts of Florida.

In 2009, the highest level of resistance (35 fold) was found with imidacloprid for adult ACP, as compared with the laboratory susceptible population. This was followed by chlorpyriphos (7-18 fold resistance), thiamethoxam (15 fold resistance), malathion (5 fold resistance) and fenpropathrin (5 fold resistance). By 2010, we determined that various populations of ACP from across Florida showed some level of decreased sensitivity (as compared with the laboratory susceptible population) to virtually every insecticide that was being used for ACP control.

By 2011, this dynamic situation changed for the better in some cases, but for the worse in others. For example, resistance did not get any worse for the neonicotinoid insecticides, an important mode of action because formulations of these insecticides are systemic and cause several weeks of continuous ACP control. This group of insecticides is particularly important for young tree protection, because of the insecticides' systemic activity and associated long duration of protection. Unfortunately, we found that resistance levels further increased in 2011 for organophosphate and pyrethroid insecticides.

Despite these increases in resistance observed in field populations across Florida, it is important to remember that our baseline comparison is a laboratory susceptible culture of ACP that has not been previously exposed to insecticides. Therefore, we have a very sensitive method for determining changes in insecticide susceptibility. Although the changes in susceptibility that we have documented for field populations from commercial groves indicate that the potential for resistance development is real and population changes are occurring, we have not yet observed a product failure in a commercial grove that could be correlated with resistance development. So, it is important to remember that things are not at a crisis level and this research is being conducted proactively, such that product failures hopefully never occur in commercial production due to insecticide resistance.

UNDERLYING MECHANISMS CAUSING RESISTANCE

We have conducted a significant amount of research with the goal of understanding the mechanisms or underlying causes of insecticide resistance development in ACP. It is important to determine these underlying reasons, because this information can guide us in developing strategies for rotating insecticides most effectively.

An understanding of how resistance develops gives us insights into the potential for something called "cross-resistance." This is the phenomenon where an insect develops resistance to one insecticide, and by doing so, also becomes simultaneously resistant to another insecticide. This phenomenon occurs when there are multiple different insecticides that exert the same mode of action. Commonly, if an insect develops resistance to one insecticide within a particular mode of action, it simultaneously develops some level of resistance to all of the insecticides in that same mode of action. Examples of insecticide modes of action used for ACP management include the organophosphates, pyrethroids and neonicotinoids, among others. However, there is also the potential for some level of "multiple-resistance" to develop between different modes of action, depending on the specific mechanisms involved. Therefore, we have been investigating these underlying mechanisms to get a better understanding of how to manage potential problems.

To date, we have found no evidence that the target sites (where the insecticide binds/targets) of ACP populations that exhibit some levels of insecticide resistance have changed or mutated. Instead, we have found that ACP showing some level of insecticide resistance express higher levels of detoxifying enzymes that break down the insecticides after contact or ingestion. These enzymes are present in insects naturally and their function is to break down toxic chemicals.

For example, insects must detoxify or break down natural plant chemicals, which plants use to ward off insects as a natural form of defense. These enzymes, however, are able to break down and detoxify a diversity of toxic chemicals to various degrees, including insecticides. We found that in populations of ACP that are less susceptible to insecticides, the levels of these detoxifying enzymes are much higher. More enzymes leads to greater capability of breaking down encountered insecticide(s) and therefore a greater probability of survival of those ACP that produce more of these enzymes.

What is the significance of knowing that resistance appears to be due to increased production of detoxifying enzymes? First, it may mean that it is a progressive phenomenon that slowly increases and decreases over time, rather than an all-or-nothing switch. This is a good thing, because if we notice resistance creeping up to unacceptable levels, we can make a strong push to decrease the so-called "selection pressure" and levels of susceptibility should return. For example, in this case, the insecticide to which resistance is developing should be used more sparingly, until the population becomes susceptible to it again. We plan on investigating this process in detail in the future. But, unfortunately, it also means that there may be some level of multiple-resistance between insecticides of different modes of action, depending on the breadth of activity of the particular detoxifying enzymes that are expressed at higher levels. This is one reason that we are trying to develop optimized rotation schedules, because we might find that rotating insecticide "A" with insecticide "C" is more effective than rotating "A" with "B," for example.

Also, once we understood that there was an enzymatic basis for this resistance, we asked: Can we identify the genes that are "turned on" to cause ACP to produce more of these detoxifying enzymes? We figured that we might be able to use this information for possible novel control strategies. Pursuing this line of reasoning, we have been able to identify at least some of the genes that are "turned on" to a greater degree in more resistant ACP and cause these psyllids to produce greater amounts of the detoxifying enzymes. These genes are within the so-called "CYP4" family and are known to cause production of detoxifying enzymes in insects.

A Simple Math Equation to Describe HLB Research Direction

By Harold Browning

Questions continue to arise regarding what areas of research should be pursued in seeking solutions to HLB. The simplest answer is "any direction that may lead to solutions." Pursuing short-term, temporary maintenance of tree health is critical, as it is the current tree inventory which will provide fruit production until longer term solutions like resistant trees are developed, tested and approved for use. Between these two timetables falls the development of alternatives to reduce Asian citrus psyllid (ACP), and potential "therapies" that may change the dynamics of disease onset in infected trees. With so many research project areas and approaches being investigated, how do they interact?

A simple consideration of the three elements that come together to define HLB disease may help explain why all of these approaches need to be pursued to achieve success, and why it is advantageous to pursue them in parallel. We can offer a math equation which might help illustrate the interactions, with the principal terms being:

- Transmission by vector populations: the numbers of psyllids in citrus groves
- Inoculum: amount of Candidatus Liberibacter asiaticus (CLas) bacteria present
- Susceptibility of citrus rootstocks and scions

Simply stated, **HLB intensity = Transmission x Inoculum x Citrus Susceptibility**

From 1998 until 2004, Florida had moderate populations of ACP and susceptible citrus trees, but little or no inoculum, so there was little or no disease. In 2005, infected trees were detected, but the numbers of infected trees were low, and thus HLB intensity was low. Progressively, the infection spread and HLB intensity increased dramatically. ACP populations were high, inoculum grew, and citrus plants remained susceptible to the disease.

The goal of HLB research must be to impact HLB intensity by reducing all three of the principal elements.

Incremental success with ACP suppression. This will be accomplished through improvements in use of pesticides, combined with biological control tools, use of attractants and repellents, and novel disruption to ACP populations that build on genetics and understanding biological processes.

Reducing Inoculum. CLas inoculum is high in Florida citrus as a result of increasing percentage of infected trees and groves. Research is attempting to deliver methods to reduce the inoculum pressure by developing therapies based on anti-bacterial materials and chemicals, genetic approaches to interfere with CLas growth and success, and to elicit increased tree defense.

Reducing susceptibility. Citrus susceptibility to CLas leads to broad incidence of disease, and will determine long-term tree response to infection. Through traditional citrus breeding and also search for CLas resistance or tolerance from non-citrus sources, researchers are developing and testing candidates that could lead to rootstocks and scions being less susceptible.

Combined, the three terms of the HLB disease equation describe our current situation. Progress in one or more of these areas will contribute to lower disease severity in the equation above. Some solutions are further out, but the implementation of results will progressively drive disease severity down.

Harold Browning is Chief Operations Officer of CRDF. The foundation is charged with funding citrus research and getting the results of that research to use in the grove.

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POTENTIAL NOVEL APPROACH TO COMBAT RESISTANCE

The primary and most straightforward method to prevent the development of insecticide resistance and even possibly reverse it, should it develop, is rotating insecticide modes of action. Resistance develops when one mode of action is applied repeatedly, which exerts a "selection pressure" on a population of insects where the susceptible ones are killed off, but the resistant ones survive and continue to breed. With each generation of breeding, there is potential for that "selected" population to become more and more resistant with each successive generation. Given that ACP have a short generation time (can be approximately three weeks from egg to adult), this breeding for resistance can occur rapidly. We have found that 100-fold resistance to imidacloprid can develop in the laboratory after only five generations of continuous exposure and constant selection. Therefore, rotation of insecticide modes of action is critical. If a population develops that is resistant to insecticide mode of action "A," there is a good chance that it can be effectively killed by application of insecticide mode of action "B." This is the basis for why rotating modes of action is critical. But, there may be additional approaches to combat resistance to complement rotation of modes of action.

Since we identified the genes that are likely responsible for activating production of the enzymes that break down certain insecticides in ACP, we decided to investigate whether we can shut down the expression of these genes, essentially "cutting the problem off at the pass." For example, we found that sub-lethal exposure alone of ACP to imidacloprid turns these genes on and their expression is elevated in populations of psyllids that are less sensitive to this insecticide. We therefore explored the possibility of using a technique called RNA interference, or RNAi for short, to shut down expression of these genes and therefore shut down expression of the detoxifying enzymes. This is essentially a method of silencing what genes normally express — in this case, the proteins or enzymes that degrade insecticides like imidacloprid.

In the laboratory, we have been able to demonstrate that we can successfully silence the expression of imidacloprid-degrading enzymes with RNAi. Importantly, we were able to do this by "topical application" method — in other words, by treating the insect externally rather than having to inject it with something or relying on it to feed on something. This suggests that in the future, we might be able to "spray" psyllids with a solution that causes this type of gene silencing and renders ACP more susceptible to insecticides, like imidacloprid.

This research has been a collaborative effort at the Citrus Research and Education Center and included the input of molecular biologists, plant pathologists and entomologists. It may be a useful additional tool to more traditional methods of resistance management, like effective rotation of modes of action. It is something we will be exploring in our future research.

CONCLUSIONS

Current status of ACP insecticide resistance in Florida:

- Not due to target site insensitivity.
- Appears to be due to elevated expression of detoxifying enzymes.
- 100-fold resistance to imidacloprid can be observed after five generations of constant exposure/selection in the laboratory.

Current obstacles and ongoing management efforts:

- Cost of sprays and limited number of modes of action can make effective rotation difficult; but it is important to rotate despite the challenges.
- Influx of "hot" (carriers of pathogen) vectors from non-managed, often abandoned sites, makes effective control difficult and may cause the need for additional sprays.
- Insecticide rotation is still our current best strategy.
- Innovation/new technologies such as RNAi are medium- to long-term possible tools, and may prove to be effective supplemental resistance management tools in the future.

Lukasz L. Stelinski is an assistant professor in the University of Florida-IFAS's Entomology and Nematology Department at the Citrus Research and Education Center in Lake Alfred.