Factors influencing transmission of Liberibacter bacteria by Asian citrus psyllid

By Kirsten S. Pelz-Stelinski and Lukasz L. Stelinski

The pathogen responsible for citrus greening disease, *Candidatus* Liberibacter asiaticus (Las), is transmitted by the Asian citrus psyllid (ACP). Las is primarily acquired when psyllids feed on the phloem, or "sap," of infected trees. Because Las is a phloem-limited bacterium, psyllids ingest it along with the plant juices while feeding. Acquisition of the pathogen may occur during the adult stage of the insect, but is more likely to occur during feeding by the immature or nymph stages. Psyllid nymphs that feed on leaf flush as they develop acquire Las very efficiently. The infection rate of newly emerged adult ACP that fed on Las-infected trees as nymphs can range from 40 percent to 100 percent during their 12 to 14 day development period, while the infection rate of psyllid adults exposed to Las-infected plants will reach only 40 percent after 30 days of feeding.

Many factors may contribute to the success of Las acquisition by psyllids. Over the past several years, we have been investigating a number of biotic and abiotic factors that play a role in this portion of the transmission process.

We conducted a series of experiments in temperature-controlled growth chambers to determine the optimal temperature range for Las acquisition by adult psyllids. Las-infected Valencia plants were placed in controlled chambers ranging in temperature between 65°F and 95°F. Adult psyllids were released onto caged plants for two-week feeding periods. The insects were then collected and tested for the presence of Las using real-time polymerase chain reaction (PCR) analysis. Our results indicated that the rate of Las acquisition by adult psyllids changes in response to temperature. At both high (95°F) and low (65°F) temperatures, fewer psyllids became infected with Las, compared with those psyllids that were exposed to moderate temperatures. The highest acquisition rate (45 percent) of Las occurred when temperatures were between 77°F and 86°F. Researchers in Brazil have reported that the titer of Las within plants is greatest when the daytime temperature is approximately 81°F. Therefore, higher acquisition rates are likely associated with a larger population of bacteria available to ACP within plants. It is also possible that psyllids are more active when temperatures are between 77°F–86°F. This may allow more feeding, resulting in more pathogen acquisition.

CULTIVAR MAY PLAY A ROLE

The cultivar may also play an important role in pathogen acquisition. Although psyllids can become infected with Las after feeding on all known citrus cultivars, we have been investigating how cultivar differences may affect the transmission process. To address this, we tested the ability of ACP to acquire the Las pathogen from a variety of common citrus cultivars. Rough lemon, Mexican lime, sweet orange (Valencia), sour orange and grapefruit (Ruby Red) plants of similar age were graft-inoculated with Las. After the plants became infected, we placed adult psyllids on plants with new leaf flush for two weeks. Analysis of the samples indicated that psyllids were able to acquire Las most easily from rough lemon plants (80 percent), followed by Mexican lime and Valencia oranges (40 percent). Very little acquisition, less than 5 percent, occurred by psyllids on sour orange or grapefruit plants. This occurred, despite roughly similar flush availability between the various cultivars tested.

OTHER TRANSMISSION METHODS

In addition to the typical mechanism of acquisition from plants, Las can also be transmitted from infected female psyllids to their offspring by a process known as transovarial transmission. In some cases, the pathogen can move to the reproductive organs of infected females where it can then pass to the unlaid eggs. Although this phenomenon occurs at a low frequency, with only 2 percent to 6 percent of psyllid offspring receiving the pathogen from an infected mother, it is an alternate pathway by which Las can be spread. Under high densities of psyllids, the small percentage of transovarial transmission could result in considerable spread of pathogen between mothers and offspring.

Recently, we have also explored sexual transmission of the pathogen during mating as another alternative mechanism by which the Las bacterium may be spread. Although rarely reported for insect-transmitted plant pathogens, sexual transmission of insect-associated bacteria between partners during mating has been described for aphids and mosquitos. We found that Las can be spread from infected males to uninfected females during mating at a frequency of approximately 4 percent. However, female to male or same-sex spread did not occur.

Our next question was whether Las could be detected in the reproductive organs of psyllids, as this would suggest a possible mechanism for transmission of Las during mating. Ovaries and testis from 34 and 32 female and male Las-infected psyllids, respectively, were dissected for Las detection. Of these, 13.5 percent were Las-positive, indicating that Las can invade both male and female reproductive organs. The presence of Las in male genitalia suggests that during mating, Las may be transferred to the female psyllid with the sperm and/or other seminal fluids. Similarly, presence of Las in ovaries is consistent with our previous observation of transovarial transmission. It is likely that Las is passed from the ovaries of an infected female to her unlaid eggs, resulting in infected offspring in the absence of infected plants.

We confirmed that it is possible for a female to acquire Las sexually from an infected male partner, and then transfer the bacterium to her offspring through the egg. Like transovarial transmission, sexual transmission of Las between male and female ACP may benefit the pathogen because it could persist within psylids during periods when citrus host plants are either in a sub-optimal stage for harboring the bacterium or in short supply.

Given that duration of feeding directly correlates with acquisition of the Las pathogen (more feeding results in greater probability of infection), it is important to minimize this feeding time for practical management of the disease. It is known that certain currently available insecticides (such as neonicotinoids and pyrethroids) can significantly reduce, and in some cases, eliminate the types of feeding behaviors that cause transmission of the Las bacterium from infected psyllids into plants. Such insecticides are likely also useful in preventing acquisition and subsequent spread of the bacteria if psyllids are killed before they acquire the pathogen and disperse with it to other plants. Since nymphs acquire the pathogen efficiently and feed for a long duration before emerging as mobile adults, it is important that nymphs (if present) are killed before they develop into adults.

In addition to reducing nymphs, minimizing psyllid movement to new flush by eliminating remaining adults is likely just as important to slow the spread of HLB. Because exposure to more Las bacteria may increase the chances for transmission, minimizing acquisition of the pathogen by the same psyllid multiple times and inoculation of the same tree by multiple ACP are also likely important factors for reducing spread of pathogen and tree decline. Effective insecticide use including large scale cooperation, rotation of modes of action, minimizing nearby unmanaged areas, and supplemental border row sprays are all tactics that should help decrease spread of the pathogen in light of the known pathogen transmission mechanisms.

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Using the Tools Available for Management of Citrus in an Era of HLB

By Harold Browning

The Annual Grower Meeting in Bonita Springs hosted by Florida Citrus Mutual provided an opportunity to review many of the tools available to respond to HLB, and to preview some of the likely next steps in the fight against this disease. During the educational seminar and all around the venue, discussions focused on implementing all of the available tools in an effort to minimize the impact of the disease. The overview of results in the Citrus Health Management Areas (CHMAs) presented during the educational session highlighted how CHMA-wide coordinated implementation of selected pesticidal modes of action and timing of application during critical times of the season can lead to overall reduced psyllid numbers. This is evidenced both by the Citrus Health Response Plan (CHRP) Asian citrus psyllid counts and ACP scouting conducted by growers, and the comparison of ACP incidence in CHMAs with broad participation versus those with less participation was striking. Managing HLB resets, new plantings and existing groves is made more difficult in CHMAs where ACP counts are high.

During the educational session, Tom Kirschner, production manager for a series of cooperative plantings in Southwest Florida, presented an overview of three properties he manages, including specifics of the HLB history, production practices, and utilization of tree removal, resets, and other tools to manage the groves. He also detailed ACP control protocols, and then presented costs and production figures over multi-year periods for each of the properties, indicating the numbers of trees infected, numbers of trees removed and reset, and other measures of disease impact.

His synopsis of the properties illustrated three different situations with varying HLB history, pressure from neighboring groves, and levels of HLB to manage. Evident was the fact that each property was being managed differently, in accordance with the situations present. Among the take-home messages was that tree removal and replacement remains a viable consideration under some scenarios, and not in others. Fundamental to management of HLB on all properties was aggressive ACP control and good cultural practices, including nutrition and weed control. Kirschner made the point that managing resets is confounded if weed control is not maintained, as soil-applied insecticides and other inputs cannot be fully utilized by the trees if applied over weed cover.

Other portions of the program highlighted progress toward other near-term solutions to HLB that are being investigated and for which the CRDF Commercial Product Delivery Committee has ongoing project oversight. Included in these topics was evaluation of antimicrobial materials that may provide therapy against HLB bacteria in infected trees. Laboratory, greenhouse and field experiments are under way or planned to address performance of the candidate materials, methods to introduce useful antimicrobials into citrus trees, and how use of antimicrobials might be integrated with other therapies, such as thermal treatment.

An overview of candidate rootstocks that have been observed to show potential HLB tolerance in field trials is encouraging plans to plant these candidate rootstocks under commercial-scale conditions to further assess their value.

Collectively, discussion around this meeting and the formal presentations provided a sense that tools currently available to respond to HLB need to be fully used, analyzing each situation and incorporating the appropriate elements. Those who take an aggressive approach are best positioned to incorporate new tools into HLB management as they emerge from the research.

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.