4.2 Zebra Complex and HLB: Seeking a Common Enemy?

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Zebra Complex (ZC), was first documented in potato fields in Mexico in 1994, and was first identified in the USA in 2000 in commercial potato fields in Texas. This disease has recently been associated with a new, fourth, pathogenic *Candidatus* Liberibacter species (called "psyllaurous") (Hansen 2008, Liefting 2008). *Candidatus* Liberibacter psyllaurous has also been associated with a similar disease in tomatoes and peppers. In all three cropping systems, the association of this pathogen with disease was originally missed because the traditional diagnostic method, PCR and QRT-PCR, did not amplify DNA from this fourth species. The bacterium is transmitted by the potato psyllid (*Bactericera cockerelli*), an insect that is prevalent in most growing areas of the US. While, the putative causal agent of ZC was unknown until just a few months ago, the potato psyllid's involvement on the disease was determined in 2006 (Goolsby 2007, Munyaneza 2007). When considering the epidemiology of ZC and the association with a potential HLB causal agent, it is important to consider the timeline of ZC history. First, the potato psyllid has been a minor problem in potato and tomato production for years. However, ZC first appeared in Mexico in 1994 then in the USA in 2000. Similarly, tomato and pepper disease pressure accelerated during this same time period. The foliar symptoms that define ZC, including stunting, chlorosis, swollen internodes of the upper growth, proliferation of axillary buds and aerial tubers, browning of the vascular system in belowground portions of stems, leaf scorching, and early plant decline (Munyaneza 2007), are all similar to the symptoms in Huanglongbing, HLB, affected citrus.

The interactions and associations between the cause of HLB and potato ZC is currently unknown. Reports have identified a Liberibacter related bacterium in the potato psyllid in two countries and in several states within the USA (Hansen 2008, Liefting 2008). There is a need to either confirm or eliminate this patho-system (Liberibacter-Potato-Psyllid) as to its real or perceived threat to US citrus industry. To resolve this issue we are applying molecular approaches using Liberibacter specific primers made to 5 locations in the genome. Samples of citrus, potato, and other potential Liberibacter host plants, plus both Asian citrus psyllid and potato psyllid, are being analyzed to confirm or deny the association between these two patho-systems. Preliminary trials have been conducted to determine if the potato psyllid can survive and transmit Liberibacter to other host plants. Additionally, we have determined that the potato psyllid can survive on citrus, thus demonstrating it could potentially transmit Liberibacter between cropping systems.

Materials and Methods

Asian citrus psyllids on tomato. Three groups of 20 adult Asian citrus psyllids, AsCP, *Diaphorina citri,* were caged on tomato seedlings, ~18cm tall.

Potato psyllids on Citrus. Three groups of 10 adult potato psyllids, *Bactericera cockerelli,* were caged on a three year old grapefruit tree for 10 days. As a control 10 adult potato psyllids were caged on pepper plant (a known acceptable host plant).

Detection of Can. Liberibacter sp. in potato psyllid populations. Potato psyllids were collected from potato fields in multiple locations across the US. DNA was extracted from groups of 5 psyllids using the DNeasy tissue extraction kit (Qiagen Inc., Valencia, CA). Detection of *Can.*

Liberibacter sp. by quantitative real time PCR was done using two 16S rDNA primer sets (Shatters 2008) and primers that have been found effected in use with the newly described *Can.* Liberibacter sp. (Liefting 2008). For all three primer sets an initial 3 minute melt at 94ºC, followed by 35 cycles of 95ºC for 15 sec, 58ºC or 54ºC for 30 sec, and 72ºC for 30 sec. Only positive values with a cycle time of less than 30 were considered possible positive samples using both primer sets. If a sample tested positive, the amplicon was sequenced and compared to known *Candidatus* Liberibacter species sequences.

Results

Asian citrus psyllids on tomato. The no choice test resulted in complete mortality within 48 hours, with many of the psyllids dead along the sides of the cages. AsCP did not respond to tomato as either a host, or transitory host plant. Preference of AsCP towards only citrus and its near relatives suggest that it would not be efficient at moving *Can.* Liberibacter asiaticus from citrus to tomato crops.

Potato psyllids on citrus. The no choice test where potato psyllids were exposed to citrus only resulted in 90% survival after 48 hours, 50% survival after 5 days, and <10% survival after 10 days. In comparison, 50% of the potato psyllids survived the 10 day period on the pepper plant. Potato psyllids did respond to citrus as a potential transitory host plant which suggests that it could be efficient at moving *Can.* Liberibacter sp. from tomato/potato crops to citrus. In this experiment, potato psyllids did not test positive for the presence of *Can.* Liberibacter sp. Therefore, vector capacity could not be evaluated.

Detection of Can. Liberibacter sp. in potato psyllid populations. Potato psyllids have tested positive for the presence of a *Can.* Liberibacter sp., however, we have not identified it to the species level yet.

Discussion

Potato psyllids are a potential bridge between the citrus and potato/tomato cropping systems and may be capable of moving *Can.* Liberibacter sp. in the process. In our screening of potato psyllid populations for the presence of *Can.* Liberibacter sp., we have routinely found the "psyllaurous" species. At this point we believe this presents a threat to the citrus industry, but further investigation is needed to determine the level of the threat.

References

Hansen, K., Trumble, J.T., Stouthamer, R., and Paine,T.D. 2008. New Huanglongbing (HLB) *Candidatus* species, "*C.* Liberibacter psyllaurous", found to infect tomato and potato is vectored by the psyllid *Bactericerca cockerelli* (Sulc). Appl. Environ. Microbiol. 74:5862-5865.

Liefting, L. W., Ward, L.I., Shiller, J.B., and Clover, G. 2008. A New '*Candidatus* Liberibacter' Species in *Solanum betaceum* (Tamarillo) and *Physalis peruviana* (Cape Gooseberry) in New Zealand. Plant Dis. 92: 1588.

Shatters, Jr., RG, W Hunter, D Hall, CL McKenzie, YP Duan. 2008. Optimizing a 16S rRNA-Based Double Stranded DNA Dye Binding Q-PCR Method for Detection of *Candidatus* Liberibacter Species Associated with Plants and Psyllids. Submitted to Molecular and Cellular Probes, September, 2008.

Goolsby, J.A., Bextine, B., Munyaneza, J.E., Sétamou, M., Adamczyk, J., and Bester, G. 2007. Seasonal abundance of sharpshooters, leafhoppers, and psyllids associated with potatoe saffected by zebra chip disorder. Subtrop. Plant Scie. 59: 15-23.

Munyaneza, J.E., Crosslin, J.M., and Upton, J.E. 2007. Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a New potato disease in Southwestern United States and Mexico. J. Econ. Entomol. 100:656-663.