2nd Annual Citrus Health Research Forum
October 4-6, 2011
Abstracts

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Speaker Abstracts

ECOLOGY OF THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI*

Hans Alborn[3], Rocco Alessandro[1], Jared Ali[2], Lisa Forster[4], David Hall[1], Stephen Lapointe[1], Rajinder Mann[2], Joseph Patt[5], Anand Ray[4], Matthew Richardson[1], Paul Robbins[1], Mamoudou Setamou[6], Robert Shatters[1], Lukasz Stelinski[2], and Abigail Walter[1]

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Host selection by psyllids in general appears to involve taste rather than olfaction. Adults are often less discriminating than nymphs. *A priori*, there is good reason to doubt that ACP uses a long-distance sex pheromone or that ACP orients to host plant volatiles over large (m) distances. Research to discover pheromones or kairomones must accept the potential for "failure", i.e., disproving the hypothesis that long-range attractants exist. Nevertheless, the urgency of the problem and the potentially high return on investment if semiochemicals were to be identified justify the efforts underway to discover attractants or repellants that may be useful in control or management of this pest. Our approaches attempt by various means to gain insight into the mechanisms by which ACP locates its preferred feeding site and conspecifics for mating. These include characterization of adult preference for host plants in lab and field trials; characterization of host plant volatiles from preferred and non-preferred genotypes; lab and field evaluations of common plant volatiles identified from flush of susceptible species; collection and analysis of databases of cuticular hydrocarbons collected from male and female ACP; electrophysiological studies including antennograms and single-cell recordings; and attempts to disrupt feeding and formation of stylet sheaths. To date, no highly attractive compounds have been identified. However, consistent results have been obtained that point the way forward. Recent results from our various efforts will be presented and discussed.

THE SALIVARY GLAND AND ALIMENTARY CANAL AS TRANSMISSION BARRIERS TO *CANDIDATUS* LIBERIBACTER ASIATICUS IN THE ASIAN CITRUS PSYLLID, VECTOR OF CITRUS HUANGLONGBING DISEASE

El-Desouky Ammar, Robert Shatters and David Hall

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Candidatus Liberibacter asiaticus (Las), associated with huanglongbing disease, is transmitted by the Asian citrus psyllid (ACP, *Diaphorina citri*) in a persistent manner. For a persistent pathogen to be transmitted, it has to be ingested, pass through the alimentary canal wall, move through hemolymph to the salivary glands, and pass into the salivary secretions, so that it can be inoculated into host plants during feeding. We used quantitative PCR (qPCR) and fluorescent *in situ* hybridization (FISH) for localization of Las in dissected organs of ACP that fed on Las-infected citrus. Las was detected in the alimentary canal, hemolymph, salivary glands, and other organs/tissues of ACP. The relative titer of Las compared to psyllid genomic DNA, using qPCR, was significantly higher in both the salivary gland and alimentary canal compared to that in other tissues, indicating that Las replicates or accumulates in both organs. Using FISH, the proportion of Las-infected salivary glands (21.2%) was significantly lower than that of infected alimentary canals (44.4%) or other organs/tissues (41.8%). Using qPCR in combination with a 'detached leaf assay' method, only 4-5% of the psyllids were able to inoculate Las into detached citrus leaves, although 46-78% of these psyllids were Las-infected. Our results show the near systemic infection of ACP by Las and suggest that both the alimentary canal and salivary gland constitute infection and/or transmission barriers to Las in this vector. Further research on these transmission barriers, and possible receptors associated with them, can potentially help in devising innovative control methods aimed at blocking Las transmission by ACP.

EARLY RNA AND METABOLIC CHANGES IN CITRUS INFECTED WITH *CANDIDATUS* LIBERIBACTER ASIATICUS

Bowman, K.[1], Albrecht, U.[1], Skogerson, K.[2], and Fiehn, O.[2]

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We have conducted several transcriptomic and metabolomic experiments with sweet oranges (*Citrus sinensis*), mandarins (*C. reticulata*), and HLB-tolerant *Poncirus trifoliata* hybrids infected and non-infected with *Candidatus* Liberibacter asiaticus (Las). These studies should reveal biomarkers useful for early detection of Las infection, as well as yield valuable information about plant disease response that may be used for engineering resistance to HLB disease. Transcriptional analyses using the Affymetrix GeneChip citrus genome array discovered many genes whose expression in leaves was associated with the symptomatic stage of the disease. But only a few genes, such as those for several glucose-6-phosphate/phosphate transporters, were strongly induced at the pre-symptomatic stage of the disease and may be potential biomarkers for early detection of Las infection. Additional gene expression experiments using quantitative RT-PCR analysis confirmed the findings of the microarray experiments. Metabolomic experiments applying GC/TOF-MS analysis discovered several organic compounds in leaves of sweet orange plants which were related to the symptomatic stage of the disease. Some of these compounds are known to enhance plant tolerance to other environmental stresses. In an additional experiment, volatile organic compound (VOC) profiling was conducted on sweet orange plants over a time period from five to 21 weeks after inoculation with Las. Results indicated that emission profiles are more strongly affected by plant development and leaf age than by infection with Las. Though several compounds were induced in response to Las, no consistency was observed throughout the study, and VOC profiling did not appear promising as a Las disease diagnostic.

PREPARING A SUCCESSFUL JOINT CITRUS PROPOSAL FOR NIFA

Kitty F. Cardwell

National Program Leader, Plant Pathology, USDA - NIFA, Washington, DC

In general, the most successful proposals have four qualities: (1) the work is novel and of high consequence; (2) the work is doable given the time allotted and other resources; (3) the team is credible, and (4) results are measurable. Large proposals are challenging because of their scope and complexity. Still, large proposals must demonstrate a clear strategic understanding of component projects, so that the path leading to outcomes of the program are clear and logical. If a project is integrated (research, education, extension), everyone needs to be at the table early on. Integrated components added ex post facto are easy for reviewers to spot, and most often result in projects that are poorly integrated. Large projects can benefit greatly from including a program manager role on the team (separate from project directors (PDs)).

Develop a core team of the best writers, not necessarily your usual collaborators. Don't forget to include stakeholders in some meaningful way. Delay proposal writing until a clear, tightly integrated "storyboard" has been created. When writing begins, make sure that all the RFA's evaluation criteria have been adequately addressed. Think like a reviewer/panelist. What makes their job easier and makes your proposal easier to read and understand (and yes, a joy to read)? Well-designed and professional graphics not only look good, but they convey concepts clearly and concisely for reviewers. A Gantt chart (or similar timeline of activities) of who is doing what and when, is very informative. Consider including a logic model to illustrate your project. Proposals should have good balance and flow, and present a compelling argument.

Finally, allow time to have non-team reviewers read and critique the proposal. The final proposal should read as though it was written by a single individual presenting a well-coordinated agricultural project, not a patchwork of different PDs, each with a pet research project that needs funding. Contact your sponsored programs offices early and identify resources they have that might be useful to you, e.g., grant writers, budget preparation, training etc. Also, work with them to identify deadlines, and work backward from those dates to ensure an on-time submission. Do not underestimate the time required to get signatures and budgets from multiple collaborating institutions!

WHOLE GENOMIC AND SMALL MOLECULE ANALYSIS OF *PLASMODIUM FALCIPARUM*: POTENTIAL APPROACHES TO THE PATHOBIOLOGY OF CITRUS-LIBERIBACTER

Johanna P. Daily

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There are many similarities of malaria infection of humans to HLB of citrus. These include the critical role of a vector, a susceptible host and a pathogen. Employment of whole genome and small molecule analysis to the problem of HLB may be central to inform a pathogenesis model and identify biomarkers of infection. Thus we will present our experience with use of genomic and small molecule investigations to address the clinical questions involved in *Plasmodium falciparum* infection. We have identified novel malaria biology in parasites derived directly from patient blood samples. This suggests a previously unknown parasite physiology which may have a bearing on patient disease outcomes. We will also present host transcriptional profiling derived from children with severe malaria who go on to a second mild infection to begin to identify effective immune responses that are developed over time and may inform vaccine development strategies. Our work has also involved the use of small molecule analysis. Small molecules comprise low molecular weight organic compounds that have a variety of important functions. Often genetic analysis will not identify these molecules as they can be the product of multiple gene products, and thus need to be characterized directly. To identify plasma markers of malaria infection in adults residing in an endemic area we carried out a broad analysis of small molecules in a group of malaria smear positive and malaria smear negative individuals residing in Senegal. We identified a plant hormone, traumatin as the most abundantly differential small molecules in patients with malaria. Finally an overview of malaria control strategies will be reviewed. Potential similar control strategies and scientific approaches can be aimed at Liberibacter to reduce the citrus disease burden now facing the US.

NEW TOOLS, APPROACHES, AND CONSTRUCTS FOR TRANSGENIC RESISTANCE TO HLB/ACP IN CITRUS

Erik Mirkov[1] and Ed Stover[2]

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The challenge of HLB has stimulated numerous research efforts to develop resistance in citrus using transgenics. The most-tested strategies targeting Liberibacter include expression of plant defensins, antimicrobial peptides, SAR-related proteins, and bacteriophages. Increased understanding of the relationships between host/pathogen, pathogen/vector, and host vector is opening up an array of new opportunities. Extensive genomic information is providing strategies specific to the HLB pathosystem. This discussion will survey the status of research on developing transgenics with resistance to HLB and ACP, covering not only selection of transgenes, but also promoters and other construct components. It is anticipated that participants will share and brainstorm over emerging opportunities, possibly contributing to identification of new strategies and initiating new teams for grant development.

VECTOR PATHOGEN INTERACTIONS: TRANSMISSION OF *CANDIDATUS* LIBERIBACTER ASIATICUS AND ITS EFFECT ON ASIAN CITRUS PSYLLID LIFE HISTORY

Kirsten S. Pelz-Stelinski[1], Michael Rogers[1], and Ron Brlansky[2]

[1]Entomology and Nematology Department, University of Florida/IFAS, [2]Citrus Research and Education Center, Lake Alfred, FL, 33850, USA

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, which transmits the bacteria *Candidatus* Liberibacter asiaticus (Las), is responsible for the spread of citrus huanglongbing (HLB), throughout most of the world's citrus-producing regions. Understanding the relationship between *D. citri* and Las is critical for the development of psyllid and HLB management programs. As part of a comprehensive study on this vector-pathogen interaction, we have conducted a series of studies investigating psyllid fitness, Las transmission, and replication of Las within the psyllid. Our results suggested that Las infection plays an important role in the fitness of *D. citri*, resulting in greater reproductive output from infected psyllids. Furthermore, nymph development time was shorter when *D. citri* fed on infected plants compared to healthy citrus. Decreased survival was observed in association with feeding on infected plants, suggesting that a trade-off may exist between these life-history traits in response to Las infection. Our findings from acquisition and inoculation assays indicated that transmission of Las by Florida *D. citri* populations occurs at a lower rate than previously reported for other populations of *D. citri* and for the African citrus psyllid, *Trioza erytreae*, and that transmission may be mediated by temperature. Acquisition of Las was greatest in nymphs reared on infected plants, while a smaller percentage of psyllids acquired the bacterium during adult feeding. Inoculation experiments indicated that approximately 10% of citrus plants developed HLB within one year after exposure to a single infected psyllid.

A SUMMARY OF FLORIDA CITRUS NUTRITIONAL SUPPLEMENT FIELD TRIALS FROM A CITRUS RESEARCH AND DEVELOPMENT PUBLIC HEARING AND PANEL MEETING HELD AUGUST 24th, 2011

Tom Turpen

The Research Management Committee (RMC) of the CRDF invited its Scientific Advisory Board Chair, Dr. George Bruening and citrus stress physiology expert, Dr. Jim Syvertsen to join them for a committee meeting and public hearing to assess field trials on citrus nutritional supplements in Lake Alfred on Aug. 24th, 2011. Judging from the crowd of growers and members of the public in attendance, there is great interest in optimizing citrus nutrition. Individual researchers presented results from 10 different field trials with a wide variety of experimental designs and the panel noted several important open questions for future research emphasis. The RMC took immediate actions to add an additional field trial with a younger planting, to attempt to develop a useful assay for phloem-health and to study the feasibility of creating a system for growers to benefit from sharing their own production experience. There will be a great deal of risk and continued uncertainty for some time. Optimizing citrus nutrition is subject to enormous variability in yield that is both site and season dependent. Additionally, the rate of decline of HLB-infected groves is highly dependent on the age of the trees at the time of infection and overall condition of the grove, including genotype, environmental interactions, soil, salinity, root disease and the overall level of abiotic and biotic stress already imposed on a specific site. Therefore, it is prudent for production managers to maintain stringent psyllid control and to monitor nutritional status with accurate tissue and soil sampling to look at the effects of staying toward the high side of the traditionally recommended ranges for nutritional supplements in their groves especially if they choose not to remove infected trees.

Poster Abstracts

A NEW 'DETACHED LEAF ASSAY' METHOD TO TEST THE INOCULATIVITY OF PSYLLIDS WITH *CANDIDADUS* LIBERIBACTER ASIATICUS ASSOCIATED WITH CITRUS HUANGLONGBING DISEASE

El-Desouky Ammar, Abigail J. Walter and David G. Hall

Subtropical Insects Res. Unit, USDA-ARS, USHRL, Fort Pierce, FL

To test the inoculativity of the Asian citrus psyllid (ACP, ***Diaphorina citri*)** with ***Candidatus*** Liberibacter asiaticus (Las), associated with huanglongbing (HLB) or citrus greening disease, psyllids are usually fed singly or in small groups on citrus seedlings. These seedlings are then tested by PCR normally 3-12 months later because of the long incubation period of Las\HLB in whole citrus plants. Here, we developed a new 'detached leaf assay' method in which ACP adults from a Las/HLB-infected colony were fed for one week on detached healthy sweet orange leaves placed in 50-ml polypropylene tubes (as described in Ammar and Hall, 2011, Florida Entomologist 94:340-342). One week later, these leaves were processed by quantitative PCR (qPCR) using two Las primers (Li and LJ900). In two tests, when feeding 10, 5 or 1 adult/ leaf/week, the percentages of Las-positive leaves averaged 28, 15.4 and 5%, respectively using Li primers, and 48, 28.9 and 12% respectively using LJ900 primers. Ct values for qPCR using LJ900 primers were much lower than those using Li primers. Our results, using the more commonly used but less sensitive Li primers, are largely comparable to those obtained by previous workers using whole citrus seedlings for Las-inoculation by ACP. However, using more sensitive primers can increase the usefulness of this method. We suggest that this new 'detached leaf assay' method can potentially speed up inoculativity tests on ACP from 3-12 months to only 2-3 weeks, which can greatly enhance vector relation studies on Las and probably other bacteria associated with HLB.

DESIGNING CITRUS TRANSGENICS: COMPARISON OF CONSTITUTIVE AND VASCULAR-SPECIFIC PROMOTERS DRIVING BETA-GLUCURONIDASE IN TRIFOLIATE HYBRIDE ROOTSTOCK US-802

Benyon, L.S., Stover E., Bowman K., and Niedz, R.

US Horticultural Research Laboratory, USDA-ARS, Ft. Pierce, FL

Transgenic solutions are being widely explored to develop HLB resistance in citrus and a critical component of the introduced transgenic construct is the promoter, which determines tissue and level of target gene expression. Five promoters were tested for their characteristics in driving the beta-glucuronidase (GUS) reporter gene in the trifoliate hybrid rootstock US-802. Two selected promoters direct high levels of constitutive transgene expression in other dicotyledonous plants, the tandem repeat promoter of cauliflower mosaic virus 35S (2X35S) gene and *bul*409S a truncation of the potato polyubiquitin promoter. Because *Candidatus* Liberibacter, the gram negative bacterium associated with HLB, infects only the citrus phloem tissue, it may be advantageous to limit transgene expression to the vascular tissue and reduce expression in the fruit. Thus we chose three promoters that demonstrate vascular specificity when transformed and expressed in other plants, WDV from wheat dwarf geminivirus, AtSuc2 the sucrose-H+ symporter gene promoter from Arabidopsis, and CsSUS the sucrose synthase promoter from citrus. X-gluc staining occurred throughout leaf and stem tissues for the constitutive promoters while the three vascular promoters largely showed expected tissue-specific staining. There were individual transformants with promoters CsSUS and WDV whose expression appeared leaky with some laminar tissue staining. Relative quantification of RT-qPCR data revealed a wide range of mRNA transcription levels from transgenics using all five promoters. Fluorometry also revealed the translation of GUS under each promoter varied but the two data sets did not correlate indicating possible post-transcriptional modification.

DEVELOPMENT OF A PATHOGEN DISPENSER TO CONTROL ASIAN CITRUS PSYLLID IN RESIDENTIAL CITRUS

Andrew Chow[1], William Meikle[1], Mark Jackson[2], Christopher Dunlap[2], Robert Mangan[1], John Adamczyk[1], and Joseph Patt[1]

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ACP from residential citrus may stymie the effectiveness of area-wide management programs aimed at containing the spread of HLB in commercial citrus. However, control measures for ACP in residential citrus lag behind the insecticide-based strategies used in commercial orchards. ACP is susceptible to several native pathogens, some of which show potential to be microbial control agents for ACP and can be mass produced. We are attempting to develop an autodisseminator ('dispenser') of pathogen spores to help suppress ACP populations in residential citrus. Proof-of-concept tests designed to measure infection levels from primary infection and horizontal transmission were performed in a greenhouse. Free-flying ACP were exposed to dispensers coated with the blastospores of a strain of *Isaria fumosorosea* (*Ifr* 3581), originally isolated from sweet potato whitefly in southern Texas. Psyllids exposed to the dispensers were recovered from potted sour orange or orange jasmine placed near the dispensers. In the primary infection tests (n = 3), a mean of 55% of the recovered adults were infected following contact with the dispensers. In the horizontal transmission tests (n = 2), a mean of 30% of the nymphs infesting the potted orange jasmine plants placed near dispensers became infected following exposure to free-flying adults. Subsequent tests with non-infected nymph populations (n = 2) showed a ca. 30% decline in adult emergence from infected nymph clusters. Ongoing observations will determine whether production of conidia from mycosed nymphs will lead to further propagation of the pathogen within the ACP population.

STUDIES ON HUANGLONGBING ASSOCIATED '*CANDIDATUS* LIBERIBACTER ASIATICUS' IN PSYLLID AND CITRUS HOSTS IN PAKISTAN

Muhammad Fakhar-ud-Razi[1], Chandrika Ramadugu[2], K. L. Manjunath[3], Mikeal L. Roose[2], Iqrar Ahmad Khan[1], and R. F. Lee[3]

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In Pakistan, occurrence of citrus huanglongbing and the associated psyllid vector, *Diaphorina citri* Kuwayama was first documented by Husain and Nath in 1927. The disease has been ignored in Pakistan; systematic and molecular studies of the associated vector and pathogen are lacking. In this study we monitored psyllid populations in twelve orchards from Sargodha region using yellow sticky traps. Populations peaked in April-May and October-November. Presence of HLB-associated *Candidatus* Liberibacter asiaticus (LAS) in psyllids and plant samples (Kinnow mandarin) was tested by qPCR for the 16s rRNA region. During hot months, June and July, psyllids were negative for LAS and plant samples showed very low titers of LAS. Approximately 20% of the symptomatic plants collected from Sargodha, Kot Momin, Lalian and Shamasabad regions tested positive for LAS indicating the widespread nature of the disease. Results of qPCRs using primers designed to multiple regions of the corresponding LAS genome showed amplification products with most primer pairs indicating the high level of similarity between the Florida strain and the Pakistan strain. Analysis of regions known to have high variability in different Liberibacter strains showed sequence differences between the Florida strain and the Pakistan strain and indicated the presence of at least two strains of Liberibacters in Pakistan. This study sheds light on survival of Las in hot temperatures, and variability of Las in different regions of the world.

RNA INTERFERENCE (RNAi) APPROACHES FOR CONTROLLING HEMIPTERAN VECTORS OF CITRUS PATHOGENS

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RNA interference (RNAi) is a natural gene regulation and antiviral defense system of eukaryotes. RNAi functions as a "gene silencing" mechanism by targeting specific RNA sequences for degradation resulting in eliminating or reducing expression of the RNA target. RNAi has rapidly become an intensely studied research area with great potential for both fundamental and practical biology. RNAi-based strategies recently have been studied for a number of insect systems but whether or not they will be useful for controlling Hemipteran vectors and/or their associated plant diseases is currently unknown. We are taking new approaches to target two important Hemipteran vectors of citrus pathogens. These are *Homalodisca vitripennis*, the sharpshooter vector of *Xylella fastidiosa* (causal agent of Citrus variegated chlorosis), and the psyllid, *Bactericerca cockerelli* (the tomato/potato psyllid) as a model for the Asian citrus psyllid, *Diaphorina citri*, the vector of the causal agent of citrus greening or Huanglongbing. We can induce RNAi effects in psyllids and sharpshooters by injection, and in psyllids by oral acquisition of specific effector double-stranded RNAs (dsRNAs), and even by siRNAs. RNAi effects are seen by molecular hallmarks of RNAi activity and phenotypic effects (death) in recipient target insects. Specific sequences induce different effects, as well as local vs. systemic effects. We are taking transcriptome and small RNA-based sequencing efforts as a means to identify new important RNAi targets, and we are evaluating using insect-infecting viruses as a means to induce systemic RNAi effects in Hemipteran populations, thereby obviating the need for transgenic citrus plants.

EARLY ACTIVATION OF DEFENSE GENES IN KUMQUAT BY THE CITRUS CANKER PATHOGEN

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Citrus canker, caused by Xanthomonas citri subsp. citri (Xcc), is an economically important disease that affects various citrus types around the world. During previous work we showed that kumquat [Fortunella margarita (Lour.) Swingle] was resistant to citrus canker, the result of an active defense response. In this study we compared the induction in kumquat and susceptible grapefruit (Citrus paradisi Macf.) of a number of defense genes associated with PAMP-triggered immunity (PTI, or basal defense) and effector triggered immunity (ETI, or R-mediated resistance), the latter often leading to systemic acquired resistance (SAR). Our results showed that, generally, defense genes were induced earlier and to higher levels in the resistant kumquat after inoculation with Xcc. In addition, three genes belonging to the NPR1 family were differentially expressed between the two citrus types. This is important because in other species these genes are central in the induction of SAR and function either as positive or negative regulators of a number of downstream genes. We also compared the response of the two citrus species when the peptide flg22 from Xcc (a PAMP) was used instead of the pathogen. The response to this treatment was less intense than the response observed after Xcc inoculation. Our results indicate that an earlier and enhanced activation of defense genes may play an important role in the defense mechanism of resistance to citrus canker observed in kumquat.

EVALUATION OF ENTOMOPATHOGENIC FUNGI ISOLATES AGAINST *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) IN MEXICO

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As Huanglongbing, the lethal disease of citrus worldwide, has reached the main producing areas of Mexican lime in the Pacific coast of Mexico, growers have initiated an intensive use of insecticides in order to control populations of the vector, the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Presently, costs of insecticides and side effects of their use are the main concerns because they could halt the strategy against the vector; thus, ecological and economic alternatives to conventional insecticides are required in the short term. The goal of this study was to evaluate different isolates of diverse entomopathogenic fungal species with potential for nymph and/or adult control of *D. citri*. There were evaluated 14 isolates of *Beauveria bassiana* (Vuill.), seven isolates of *Isaria fumosorosea* Wize, and one isolate of *Hirsutella citriformis* Speare and *Metarhizium anisopliae* (Metsch). Treatments were incubated during 72 hours at 26±1°C, 60% R.H. and 16:8 h L:D. The results showed that the isolates HIB2, HIB3, HIB4 and HIB5 of *B. bassiana* produced 100% mortality in nymphs of *D. citri*; meanwhile, in the controls (untreated and Tween 80 0.1%) the mortality was 13%. In adults of *D. citri*, the isolates Asdel 139 and A-48 of *B. bassiana* produced 89 and 90% mortality, respectively (mortality in the untreated control= 13%). The isolates of *I. fumosorosea* Pfr-114 and Pfr-612 caused 100 and 92% mortality; in the untreated control the mortality was 20%. The results have implications for the use of entomopathogenic fungi in the management of *D. citri* in Mexico.

COMPARATIVE ANALYSIS OF VARIOUS SHORT-TERM STORAGE METHODS FOR PRESERVING '*CANDIDATUS* LIBERIBACTER ASIATICUS' IN ITS VECTOR *DIAPHORINA CITRI*

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Huanglongbing (HLB) is a devastating citrus disease recently introduced in the United States. Where it occurs in the U.S. it has been associated with '*Candidatus* Liberibacter asiaticus' (CLas), which is transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri*, Kuwayama. Quantitative polymerase chain reaction (Q-PCR), targeting a region of the CLas 16S ribosomal gene, is the accepted method of testing for the presence of the putative pathogen by regulatory and non-regulatory entities, and is used for both citrus and psyllid samples. Because of the documented instability of CLas DNA in ACP removed from sticky traps in Florida (Irey *et al.*, 2011), concerns have been raised about the stability of the microbial DNA in ACP. There is very little in the literature (Bisanti *et al.*, 2008; Stoeckle *et al.*, 2010) that directly addresses the preservation of insect samples for DNA extraction. Consequently, a study was conducted to evaluate several ambient and cold storage options using ACP from a CLas infected colony maintained by USDA-ARS in Fort Pierce, Florida. The six treatments consisted of 95% ethanol at –25°C, no fluid at –25°C and each of the following under ambient conditions: 95% ethanol, 70% ethanol, propylene glycol and DNAgard (Biomatrica, Inc., San Diego, CA). The samples were evaluated by Q-PCR for detection of both the insect and pathogen DNA at 3, 17, 38 and 59 days. Results showed no significant differences in percentage of ACP positive for CLas in any of the treatments except no fluid at –25°C at 59 days.

MANAGEMENT OF HUANGLONGBING (HLB) AND ITS VECTOR *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) IN THE YUCATAN CITRUS INDUSTRY

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The objective is generate information and technology for the management of HLB-*Diaphorina citri* complex. **Population fluctuation**. Are studied in 8 orchards of orange, 5 Persian lime, 2 lemon, 2 tangerine and 1 grapefruit, the largest population of adult occurs in the period from February to July, coinciding with periods of increased emission of vegetative shoots. **Biological control**. Sampled 95 commercial citrus orchards were found two species (*Olla v-nigrum* and *Cycloneda sanguinea* and several species of the genus *Chrysoperla* (*C. rufilabris*) and *Ceraeochrysa* (*C. valida, C. Claverii, C. cubana, C. sp. nr. cincta, C. everes*). Parasitism of *D. citri*. Citrus orchards of parasitism was very low, with 2 to 8%. In backyard gardens, the level of parasitism is also low in citrus, but not in *Murraya paniculata* where the % parasitism ranged from 2 to 31% and this was constant as was observed in all localities. **Chemical control.** Evaluation of Dimetoato, Clorpirifos and Imidacloprid. All products and doses had a high level of control of psyllid with mortality above 90%. Evaluation of mixtures of insecticides. The three mixtures of pesticides have high mortality of the vector. Evaluation of biorational insecticides. The best treatments in the three evaluations were (BioDie 1 ml/liter of water), (Cimax 5 ml) and (BioDie 1.5 ml).

USE OF TRANSBACTER FOR TRANSFORMATION OF JUVENILE AND MATURE CITRUS

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Transformation of Arabidopsis, tobacco and rice using non-*Agrobacterium* "Transbacter" strains (e.g., *Rhizobium sp.*, *Mesorhizobium loti*, and *Sinorhizobium meliloti*) carrying binary Ti plasmids was first reported by Broothaerts et al. (2005). More recently, "Transbacter" strains were used or reportedly used to transform potato, soybean, canola, corn and tetraploid cotton species that were difficult to transform with *Agrobacterium* (Wendt et al., 2011). In these studies, strain-specific optimizations were necessary for using "Transbacter" strains to transform plant tissues. Although transformation of some cultivars of juvenile citrus by current methods using *Agrobacterium* is at low-moderate frequency, transformation of mature citrus has proven difficult and also requires considerable optimization. We report here the use of "Transbacter" (*Sinorhizobium meliloti* strain 1021) carrying the hypervirulence plasmid pTiWB3 (strain obtained from CAMBIA, Canberra, Australia) to transform juvenile Carrizo rootstock (self-rooted) as well as a mature Hamlin scion (grafted onto nontransgenic rootstock), at frequencies of 1.5% to 5 % for Carrizo, and (in one experiment) 0.6% for the mature Hamlin. By comparison, *Agrobacterium tumefaciens* was used to transform juvenile Carrizo at frequencies up to 10%, and mature Hamlin, Valencia and Mid-Sweet scions (grafted onto nontransgenic rootstocks) at frequencies ranging from 3% to 7%. With optimization for strain and cultivar, "Transbacter" may therefore be useful for transformation of citrus at frequencies comparable to those obtained through the use of *Agrobacterium*.

SMALL RNA PROFILING REVEALS ONE IMPORTANT FACTOR OF PATHOGENESIS OF HUANGLONGBING (HLB) DISEASE - PHOSPHORUS DEFICIENCY

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Citrus greening or Huanglongbing (HLB), caused by bacteria *Candidatus* Liberibacter (*Ca*. L.), is one of the most devastating diseases of citrus. However, the pathogenesis of this disease is hardly understood. HLB has been rapidly spreading due to the lack of sensitive early diagnosis markers and effective disease-control reagents. Low accumulation and uneven distribution of this bacterium in the hosts make it difficult to diagnose at early stage. Some host small RNAs are rapidly and specifically induced by pathogens, which makes them attractive markers for early diagnosis. We profiled endogenous small RNAs from *Ca*. L. asiaticus-infected and uninfected *Citrus sinensis* (*C. sinensis*) by high-throughput sequencing. We found that several endogenous small RNAs were induced by HLB but not by *Spiroplasma citri* (*S. citri*), the causing agent for citrus stubborn disease with a similar disease symptom to HLB. Low basal expression level and early specific induction nature of two small RNAs by *Ca*. L. asiaticus make them ideal candidates for early diagnosis markers. Furthermore, the induction of one miRNA by *Ca*. L. asiaticus suggests Phosphorus (P) deficiency in HLB-positive plants. Indeed, the P content in HLB-positive plants was only 60-70% of that in wild type plants. Application of inorganic phosphate (Pi) clearly reduced HLB symptoms and significantly increased fruit yield. Thus, our research reveals a crucial factor of pathogenesis of HLB disease - phosphate deficiency.

A TOMATO MODEL SYSTEM FOR STUDYING CITRUS HUANGLONGBING

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Citrus greening disease (huanglongbing, HLB) is one of the most serious diseases of citrus world-wide. Progress in devising experiments to better understand the dynamics of HLB that can be useful for disease mitigation has been slow because of the biological complexity of citrus/HLB. HLB-associated *Candidatus* Liberibacter asiaticus (LAS) is in low titer both in the plant and the psyllid vector. Infected plants exhibit prolonged latency, disease progression is slow, and seasonality of infection is observed. A closely related bacterium, *Candidatus* Liberibacter psyllaurous (LPS), causes "psyllid yellows" symptoms in tomatoes. This disease has been graft and/or insect transmitted to healthy tomatoes easily with symptoms showing within 2–3 weeks. We observed that symptom expression in tomatoes is temperature dependent. Symptoms expressed well at about 25°C, and the bacterial titer decreased transiently when the plants were maintained at 36°/30°C (16h/8h) for a week. Host range studies using several members of Solanaceae with both graft and insect transmission of LPS revealed both resistant and susceptible varieties. The suitability of the tomato/psyllid yellows model system to screen a large number of chemicals, antibiotics and transgenes for developing strategies for potential control of citrus HLB will be discussed. In an effort to sequence the genome of LPS, we constructed a BAC library from infected tomato psyllids, and we have generated genomic sequence information on LPS using a metagenomic approach. LPS genome is closely associated with LAS and the tomato/psyllid yellows system offers the opportunity to conduct thorough controlled pathogenesis-related experiments. The knowledge gained from the tomato/LPS disease complex will be very useful to better understand the citrus HLB disease.

AGROBACTERIUM-MEDIATED TRANSFORMATION OF MATURE INTERNODE EXPLANTS OF SWEET ORANGE, GRAPEFRUIT, CITRON, AND A TRIFOLIATE ORANGE HYBRID

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Background: Citrus has an extended juvenile phase and trees can take 2-20 years to transition to the adult reproductive phase and produce fruit. For citrus variety development this substantially prolongs the time before adult traits, such as fruit yield and quality, can be evaluated. Methods to transform tissue from mature citrus trees would shorten the evaluation period via the direct production of adult phase transgenic citrus trees directly.
Methodology/Principal Findings: Factors important for promoting shoot regeneration from internode explants from adult phase citrus trees were identified and included a dark incubation period and the use of the cytokinin zeatin riboside. Transgenic trees were produced from four citrus types including sweet orange, citron, grapefruit, and a trifoliate orange hybrid using the identified factors.
Significance: The critical importance of a dark incubation period for shoot regeneration was established. These results confirm previous reports on the feasibility of transforming mature tissue from sweet orange and are the first to document the transformation of mature tissue from grapefruit, citron, and a trifoliate orange hybrid.

IDENTIFYING THE PROTEIN EXPRESSION PROFILES OF CITRUS SPECIFICALLY RESPONDING TO "*CANDIDATUS* LIBERIBACTER ASIATICUS" INFECTION

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Huanglongbing (HLB) is a destructive citrus disease associated with the phloem-limited bacterium, "*Candidatus* Liberibacter spp". All cultivated citrus are susceptible or highly susceptible to the disease. Despite the economic importance of citrus HLB disease, information regarding molecular details of host response to "*Ca.* Liberibacter asiaticus" (Las) infection and metabolic pathways leading to HLB development is lacking. In this study, we employed 2-DE and mass spectrometry analyses to elucidate the global protein expression profiles in leaves of grapefruit and lemon plants in response Las infection at early asymptomatic and symptomatic stages. Our preliminary studies have detected 210 and 75 protein spots that were differentially expressed in leaves of grapefruit and lemon plants, respectively. Protein mass spectrometry analysis further demonstrated that a subset of proteins that were specifically expressed in response to Las occurred at a very early infection stage. Our initial results revealed that up-regulated chitinase proteins in leaves of asymptomatic Las-infected plants may be involved in host defense response to the infection. Interestingly, we also observed a strong up-regulation of a protein homologous to a granule-bound starch synthase in both asymptomatic recently Las-infected grapefruit and lemon plants. This study thus, provides insights into novel biochemical mechanisms for better understanding cellular events involved in the host response to Las infection. We anticipate that information derived from this study will also facilitate identification of host proteins that are expressed specifically and systemically to the Las infection which can be used to develop immunoassay-based diagnostics tools for early detection and management of citrus HLB disease.

TAMARIXIA RADIATA POSES LOW RISK TO NATIVE CALIFORNIA PSYLLIDS

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Introduction of *Tamarixia radiata*, a host specific parasitoid of Asian Citrus psyllid (ACP), *Diaphorina citri*, is one possible pest management strategy for areas where pesticide use is undesired. 'Punjab (Pakistan) strain' of this parasitoid is of interest for use in California because of high climate match of this region with major citrus production areas in the Central Valley of California. Studies in Quarantine were conducted to determine if introduction of *Tamarixia radiata* could adversely impact non-target native California psyllid populations. Four native California psyllids, *Calophya californica, Dichlidophlebia fremontiae, Heteropsylla texana,* and *Heteropsylla* sp. found respectively on native host plants *Rhus ovata, Fremontodendron californicum, Prosopis glandulosa,* and *Acacia farnesiana* were tested. The native pestiferous potato psyllid, *Bactericera cockerelli*, the invasive pest olive psyllid *Euphyllura olivina*, and the beneficial psyllid *Arytainilla spartiophylla* attacking noxious weed 'scotch broom' were also tested. *Tamarixia radiata* did not parasitize any psyllid species tested except the pestiferous potato psyllid which was attacked at low rates, <5% of tested nymphs were parasitized with subsequent emergence of adult parasitoids. It is concluded from these quarantine studies that introduction of *T. radiata* for the classical biological control of ACP has low risk to native California psyllids and other non-target psyllid species in California.
These data are being used to prepare an Environment Assessment Report for USDA-APHIS, NAPPO, and the CDFA. Field studies will be initiated once permission to mass rear and release the Punjab strain of *T. radiata* for classical biological control of ACP in California is granted.

MULTIMODAL CUES DRIVE HOST-PLANT ASSESSMENT IN ASIAN CITRUS PSYLLID

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We measured behavioral responses of ACP to combinations of visual, olfactory, and gustatory stimuli in test arenas. Stimuli were presented to the psyllids in droplets or lines of an emulsified wax formulation (SPLAT ™) in two different arena types in no-choice tests. First, when placed on a colored ring situated halfway between the center and perimeter of a Petri dish, ACP spent more time on yellow v. gray rings; however, this response disappeared when either gray or yellow wax droplets were applied. When the psyllids were presented with droplets scented with terpenes, the response to both scent and color was strong. The addition of a dilute (ca. 0.1 M) sucrose solution to the wax droplets increased the magnitude of ACP responses. Next, groups of ACP were placed on plastic laboratory film covering a sucrose solution, to mimic a leaf surface. Test stimuli were presented via two 'midribs' made from lines of emulsified wax formulation. Probing levels were measured as a function of color saturation and scent composition, and concentration. The test scents were based on qualitatively major volatiles emitted by orange jasmine, Mexican lime, and sour orange. The highest probing response was observed on the middle concentration (20 µl scent/10 ml wax formulation) of the Mexican lime-scented wax lines. Results indicate that there are interactive effects between the different sensory modalities in directing host-plant assessment behavior.

THE NORTHROP GRUMMAN VIABLE BIOAEROSOL COLLECTOR (VBC)

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Northrop Grumman has developed an advanced aerosol collection system called the Viable Bioaerosol Collector or VBC. The VBC can be used in both field and laboratory applications. In the field it is suitable for air monitoring in industrial, residential, and agricultural environments. In the lab the VBC can be used as a reference collector for aerosol studies. The core of the VBC is an innovative Wetted Wall Cyclone designed to provide highly efficient wet collection of aerosols. The VBC has undergone extensive testing in lab and field environments, and has been proven to deliver excellent sample viability and collection efficiency, and low consumable usage over a wide operational temperature range. The VBC design is also scalable to a wide range of air flow rates and particle size cut points down to the nanoparticle regime. The basic system is portable and will operate using normal 15A/120VAC power, or batteries. Northrop Grumman will present the system's theory of operation, summarize recent results from independent government and academic studies that demonstrate some of the VBC's characteristics in the area of organism viability, collection efficiency, and scalability, and touch on future areas of research. The system described in this abstract is addressed in patent application # 20110039679.

AUGMENTATION OF *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE) TO REDUCE INCIDENCE OF ASIAN CITRUS PSYLLID *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) AND SPREAD OF HUANGLONGBING IN FLORIDA

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The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama vectors *Candidatus* Liberibacter asiaticus, a bacterium which causes huanglongbing (HLB) or citrus greening disease now wide-spread in Florida. HLB is a devastating disease and poses a serious threat to the citrus industry. *Tamarixia radiata* Waterston, a species-specific ectoparasitoid of *D. citri* nymphs, has been effective in providing significant reduction in ACP populations on the islands of Réunion, Guadaloup and Puerto Rico. *T. radiata* was imported from Taiwan and South Vietnam and released in Florida 1999-2001. Our studies during 2006-2007 showed that the parasitoid was established throughout the citrus growing region of the state, although parasitism rates were variable, averaging <20% and lowest during spring and summer when psyllid populations are high. Therefore, we initiated a mass rearing and release program using the already established *T. radiata* originally brought in from Taiwan and South Vietnam and new colonies from Pakistan, South China and North Vietnam. We mass rear the Florida colony at UF-Southwest Florida Research and Education Center, Immokalee, FL and the other colonies are currently housed at FDACS-DPI in Gainesville, FL. More than 350,000 adults of *T. radiata* from all 4 colonies were released during 2009-2011. We have observed two to three times more parasitism at release sites during spring and summer compared to pre-release levels or non-release sites, showing that mass release of *T. radiata* can potentially increase incidence of parasitism in the field. Cooperative efforts with citrus producer Orange Co, the Division of Plant Industry (DPI), and USDA-ARS are aimed at establishing large-scale mass rearing and release facilities to increase ACP mortality and reduce the incidence of HLB, particularly during spring when young shoots and psyllids are abundant and beneficial insects active and therefore vulnerable to insecticides.

COMBINING PENETRANTS AND ANTIMICROBIALS IN A NOVEL TRUNK APPLICATION METHOD TO CONTROL HLB

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Citrus HLB disease is recalcitrant to control through chemical applications because *Candidatus* Liberibacter asiaticus (CLas), the causal agent of HLB, is phloem limited and it is inherently difficulty and/or costly getting antimicrobial chemicals into the phloem. We have been exploring novel control strategies that involve trunk application of combinations of penetrants and antimicrobials in an attempt to get better phloem penetration and better HLB control. The application method also involves the localized application to the citrus tree trunk from the crown up some distance dependant on the size of the tree. Experiments have included greenhouse studies where HLB–positive sweet orange citrus trees (inoculated with HLB using a bark patch grafting method) were treated monthly and monitored for changes in CLas titer and HLB symptoms, and monthly treated field-located uninnolculated trees that were monitored for the development of HLB symptoms and detectable CLas titer. In HLB-positive greenhouse trees, the penetrant mixture alone appeared to cause at least a transient reduction in CLas titer and when one antimicrobial mixture was combined with the penetrant mixture, a sustained reduction in CLas titer was observed over a one year period. In field treated trees, two antimicrobial/penetrant combinations appear to delay detection of CLas titer. Although one treatment resulted in a prevention of any HLB symptoms over a one year period, none of the treatments completely inhibited development of CLas titer in all treated plants. Our results show that with a combined application of penetrants and antimicrobial products, a trunk application method may be an economical and more environmentally sound method of treating citrus to reduce HLB symptoms and CLas titer.

SCREENING TRANSGENIC SWEET ORANGE FOR RESISTANCE TO HUANGLONGBING

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Thirty-three D35S::D4E1transformant lines of 'Hamlin', five of 'Valencia', four of midseason oranges, and three non-transformed 'Hamlin were propagated on C-35. Two trees of each line were assigned by size and date of ACP caging into each of four blocks. Each tree was individually caged 1-4 weeks with 20 Asian citrus psyllids (ACP) selected from colonies on HLB symptomatic plants. Initial *Candidatus* Liberibacter asiaticus (CLas) levels in ACPs were 39% with Ct<36 and these averaged Ct= 30. At the end of the feeding period, cages were removed and ACP released within the greenhouse for sustained feeding. Highly HLB symptomatic CLas-source trees were introduced to elevate levels of CLas in ACP. CLas was assessed on an 80 ACP sample at three and five months after initial tree exposure: 7.5 and 8.8% had Ct<36 which averaged Cts of 31 and 29. Leaf samples and stem diameters were collected at six week intervals beginning eight weeks after initial ACP exposure. Five months after initiation, data on mean percentage increase in stem cross-sectional area (SCSA) formed a continuous distribution containing most lines. Trees from four transgenic lines were outliers with significantly greater %SCSA increases than control lines, and well above the range of other lines. These promising lines and controls are being tested for CLas levels and extra plants of each are being tested for susceptibility to canker. The experiment will have six months total of ACP exposure, six more months of ACP-free greenhouse data, followed by additional field-testing of promising lines and controls.

PRELIMINARY ANALYSIS OF THE DRAFT GENOME OF *CANDIDATUS* LIBERIBACTER ASIATICUS STRAIN SÃO PAULO

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A draft genomic DNA sequence of *Ca*. L. americanus (Lam) strain "São Paulo" isolated from infected periwinkle (Vinca) in Brazil has been assembled. A total of 1,743,660 shotgun library reads of 1.2 kb average insert size were assembled into 6,293 contigs of avg. size > 2.8 kb. Following gap closure by PCR, the Lam genome now has a total of 1,240,227 bps in 30 contigs. The average length of the contigs is 29,956 bps, with the largest being 332,541 bps. All contigs have been extensively confirmed by PCR. The average GC content of Lam is 30% (Min: 21%, Max: 36%). Approximately 80% of this DNA encodes genes, and 1,096 total proteins have been predicted. The overall gene organization and structure of the Lam genome is more similar to Lso than to Las. There are 845 genes common to Lam, Lso and Las, 26 genes found in Lam and Lso but not Las, and only 6 genes common to Lam and Las but not found in Lso. The latter included a DNA uptake competence gene. Lam phage sequences corresponding to both SC1 and SC2 of Las were found, although SC1 (and not SC2 as in Las) appears to replicate as an integrative plasmid prophage. We previously reported a potential anti-bacterial protein, colicin IA, and potential cognate colicin immunity protein on Las SC1 and SC2, respectively. No such genes were found in Lam, indicating that Lam should be sensitive to Las colicin, and may explain why Lam is disappearing in Brazil.

REPORT OF A NEW POTENTIAL PSYLLID VECTOR OF HUANGLONGBING

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The Asian citrus psyllid (ACP), Diaphorina citri Kuwayama, has long been regarded as the sole insect vector of Asian huanglongbing (HLB). Our recent surveys in China revealed another potential psyllid vector of the disease. Cacopsylla (Psylla) citricola (pending final taxonomical confirmation) Yang & Li, were collected from huanglongbing (HLB) symptomatic lemon trees, Citrus limon Burm, in Yunnan Province, China. The psyllids and lemon leaves were extracted, DNA isolated, and standardized protocols followed to detect Candidatus Liberibacter asiaticus (Las) in group and in individuals by using Nested-PCR. The result showed that 23 out of the 52 lemon trees were Las positive. Psyllid nymphs collected from 7 out of 23 Las-positive lemon plants also tested Las-positive. Individual nymphs (3rd, 4th and 5th instars) from the HLB symptomatic trees were then run through PCR trials. Six out of 22 psyllids tested Las positive. All nymphs collected from Las-negative lemon trees were also Las-negative. This result coupled with the facts that no ACP has ever been found in the high mountain areas (1200 m above sea level) and that the observed HLB-infected lemons are mature plants, strongly suggest that Cacopsylla (Psylla) citricola is likely to be another insect vector of Asian form HLB. Further transmission studies are underway for final confirmation that Las can be transmitted to the insect from Las positive lemon and vice versa.

CHINESE HUANGLONGBING LITERATURE COLLECTION AND REVIEW

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Huanglongbing (HLB, also "citrus greening") has been extensively studied in China since the 1930s. A collection and subject review of Chinese literature on the disease complex was initiated in 2010. A total of 765 Chinese publications on HLB and the Asian citrus psyllid (ACP), Diaphorina citri Kuwayama, have been collected and categorized. This collection is now accessible online, by request, to research counterparts. Most of the complete, original text of the reference is available electronically. Critical publications have been verbatim translated into English; secondary publications have been summarized and are available for translation in their entirety by request. Additionally, US counterparts have requested specific projects supporting specific challenges to HLB management in Florida and other citrus production states. This led to specific efforts including: 1) collection, review, and survey of Chinese literature and research on nutritional approaches for HLB management, 2) collection, review, and survey of research in critical areas such as shoot (flush) control using chemicals; 3) review of Chinese literature and surveys related to impacts of elevated temperature on HLB and heat-treatment/thermotherapy for producing HLB-free scions.

The URL for the collection is: http://cipmtest.ent.ncsu.edu/invasive/literature/. Please contact yulu_xia@ncsu.edu for login information.

DEVELOPING A HEAT-BASED APPROACH FOR MANAGING HUANGLONGBING (CITRUS GREENING) DISEASE IN THE FIELD: REVIEW AND SURVEY RESULTS

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The Asian form Huanglongbing (HLB, or citrus greening), which is caused by Candidatus Liberibacter asiaticus (Las), is known to be heat-tolerant, similarly for the insect vector, the Asian citrus psyllid (ACP), Diaphorina citri Kuwayama. However, our review and analysis suggest that high summer temperatures appear to be a factor restricting HLB distribution and occurrence. Studies indicated that high summer temperature resulted in low titers of Las in affected citrus plants and ACP. HLB worldwide distribution and severity also appear to be impacted by high temperature – HLB is most severe in subtropical and tropical regions with moderate summer temperature maxima. Florida in US, Sao Paulo in Brazil, Guangdong in China, and other well-known HLB occurrence regions share this climatological characteristic. The disease is either absent or moderate in tropical or subtropical regions with high summer temperature maxima. High temperature maxima may be responsible for slow disease spread or have decreased impacts in regions such as Texas and California of US, the Middle East, and the Mediterranean Basin. We therefore advanced testable hypotheses and proposed and initiated two novel heat-based approaches for managing HLB infected groves – foliar spray of heat-inducing agents and plastic sheeting. Preliminary greenhouse data look promising.

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