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Gram-negative bacterial pathogens, like the Huanglongbing (HLB)-associated pathogen, *Candidatus* Liberibacter asiaticus (CLas), possess secretion systems that deliver virulence proteins into the host. These "effector" proteins can aid infection by manipulating plant physiology and subverting host immunity, thereby promoting bacterial colonization and disease progression. We predicted Sec-delivered effectors (SDEs) from the genome sequence of the CLas isolate psy62 and analyzed their expression in infected citrus tissues. One effector, named SDE1, has 40-folds higher expression in citrus relative to in psyllid, implicating a possible role in HLB disease progression. We then performed yeast two-hybrid (Y2H) screening using a citrus cDNA library to identify the interacting proteins of SDE1, and found that SDE1 directly interacts with several members belonging to a specific family of proteases, known as papain-like cysteine proteases (PLCPs). PLCPs are upregulated during CLas infection; homologs of PLCPs in other plant-pathogen systems have also been shown to contribute to defense against microbial pathogens. Our current hypothesis is that SDE1 affects the activity of these proteases in order to promote CLas infection. We will discuss recent progress on the characterization of SDE1 virulence function and the role of PLCPs in disease resistance.

Streptomycin and oxytetracycline-resistance profiles in Liberibacter crescens suggests a management strategy HLB on citrus

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Emergency use of 2.23 million pounds of oxytetracycline and streptomycin on 388,534 acres of citrus has been approved for the control of HLB in Florida in 2016. As a result, future use of these two antimicrobials for HLB control will require management strategies that reduce the frequency of resistance. As a first approximation of the resistance problem, the frequency of resistance to both antimicrobials, singly and together, was determined in the closest cultured relative of the HLB pathogen, *Liberibacter crescens*. Spontaneous streptomycin resistance was easily obtained in the lab at a frequency of <1 in 10 million cells. All nine of the streptomycin resistant mutants obtained were also resistant to oxytetracycline despite having no past exposure to oxytetracycline, suggesting a common resistance mechanism. In contrast, all attempts to date to generate spontaneous mutants to oxytetracycline have failed when exposing *L. crescens* to oxytetracycline alone. Attempts to obtain spontaneously resistant mutants to both antimicrobials by treating a large number of cells with both was successful at about the same frequency as spontaneous streptomycin mutants. This also agrees with the hypothesis that spontaneous resistance to streptomycin also confers spontaneous resistance to oxytetracycline by the same resistance mechanism. These results suggest that treating citrus with streptomycin greatly increases the prospects of resistance to oxytetracyline as well. Treatment with oxytetracycline alone induces resistance at much lower levels, if at all.

The discovery of citrate as an important carbon source for culturing *Liberibacter crescens* suggests an HLB management strategy

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The inability to culture *Candidatus* Liberibacter asiaticus, the causal agent of HLB, limits our understanding of the molecular bases of this disease and further improve treatment methods. *Liberibacter crescens* is the only species from the genus *Liberibacter* that has been cultured to date, and is used as a surrogate for culturing methods and the identification of antimicrobials suitable for testing against HLB. The only medium available to date for the culturing slow-growing *L. crescens* is the complex medium BM7, making it difficult to understand its metabolism. Here, a chemically defined medium is described, M12, which includes the known components present in BM7 medium plus other compounds. With the availability of M12, two carbon sources, α-ketoglutarate and citrate, were identified as each being able to substitute for all other carbohydrates in the medium. Citrate provides longer sustained growth while α-ketoglutarate allows faster growth early in the growth phase. In contrast, other organic acids, including malate, succinate, and fumarate, could not substitute for other carbohydrates. Citrate, but not α-ketoglutarate, is present in mM levels in citrus phloem. The genes for citrate metabolism are identical between *L. crescens* and *Ca*. L. asiaticus. Thus, these results are expected to be applicable to the pathogen. This discovery allows for the simplification of a defined medium for *L. crescens*. Further simplification will come from similar experiments with other classes of compounds including amino acids and vitamins. A simplification of the medium will increase our understanding of the growth requirements of *Liberibacter*. In addition, the importance of organic acids for the growth of *Liberibacter* suggests that nutritional approaches that discourage phloem loading of organic acids in plants may starve *Ca*. L. asiaticus in citrus phloem leading to suppression of HLB symptoms.

Huanglongbing in Texas 2012-2017 – an update

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Citrus Huanglongbing (HLB) was confirmed in Texas in January 2012, 11 years after the first report of its vector, the Asian citrus psyllid (ACP) in the state. Texas has put in place a number of steps to counter the threat, including regulations requiring the screening of citrus nurseries, the establishment of an area wide psyllid management (AWM) program, improved orchard care, surveys to determine the spread of the disease by qPCR tests of both psyllids and suspect trees, and, where possible, tree removal. Since 2009, over 80,000 tree and 74,000 ACP samples have been tested, with 4.8% tree and 1.9% ACP testing positive. Recently, incidence appears to be increasing more in residential sites where ACP control is less aggressive. The disease has spread to all three counties in the commercial citrus area of South Texas, and to two counties around Houston. There has, thus far, been little evidence of severe tree decline except in some residential sites where tree care is minimal, and in orchards where other issues such as *Phytophthora* infections are impacting tree health. The voluntary AWM of psyllid combining two dormant sprays and aggressive ACP management during the active growing season implemented in > 92% of citrus acreage has resulted in significant vector population suppression which may be slowing HLB spread in Texas. Other factors which may be affecting the slower development of HLB in Texas include the discrete flush cycles that are not allowing continuous availability of resources for ACP reproduction, and the long (4 to 5 months), dry and hot summers with mean daily temperatures exceeding 90 ºF from May to September.

A deeper look into the causes of off-flavor in orange juice affected by huanglongbing (HLB)

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Huanglongbing (HLB) compromises citrus fruit and juice flavor resulting in reduced quality for citrus producers and the food industry. Stress caused by HLB induces the production of secondary metabolites associated with bitter and astringent tastes in orange juice. The objective of this study was to identify which compounds, aside from limonin and nomilin, are responsible for the bitterness and astringency in juice made with oranges harvested from trees symptomatic for HLB. Alcohol soluble compounds were extracted from healthy and symptomatic orange juice and fractionated using chromatography into five fractions. The experiment was performed with food grade equipment and the organic solvents were eliminated before tasting. Three subjects tasted each fraction and documented their observations. Harsh, bitter and astringent tastes were identified in the first two fractions. Both of these fractions included hydroxycinnamates, polymethoxylated flavones, flavanone rutinosides, limonin and nomilin. These fractions were further separated by HPLC into 13 and 11 sub-fractions, respectively. Each was tasted and the majority of the compound groups contained in the sub-

fractions were identified. In general, the sub-fractions of healthy orange juice presented descriptors of a high quality juice, such as: flavors of citrus, caramel, honey, fruity, floral etc. On the other hand, the sub-fractions of juice made from oranges harvested from trees symptomatic for HLB presented descriptors such as: acrid, strong bitterness, smoky, astringent aftertaste and lingering irritation in the back of the throat.

Quantifying *Diaphorina citri* invasion dynamics in Southern California citrus groves

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For the first three years following the initial detection of *Diaphorina citri* in Southern California, it was limited exclusively to urban and suburban environments. However, starting in 2011, the first *D. citri* detections occurred in citrus groves and since then it has become prevalent in commercial citrus throughout region. We leveraged a statewide monitoring database to conduct a spatial analysis of the factors contributing to *D. citri* invasion into commercial citrus. Specifically, using a survival analysis framework, we modeled the effects of select grove characteristics (i.e. area, amount of edge, degree of perforation), surrounding landscape characteristics (i.e. proximity to major roadways, urbanization intensity), and neighbor effects (i.e. proximity to residential detections or other grove detections) on the rate at which individual citrus blocks became invaded. Invasion rate depended on grove characteristics, with larger and more convoluted-shaped blocks being at higher risk, and landscape context. But, the strongest predictors of invasion rate were proximity to residential detections early on, and then proximity to other invaded groves in more recent years. These results suggest that there is a strong linkage between urban infestations and spillover into commercial groves. Yet, they also suggest that, as more groves became invaded by *D. citri*, these infestations drove the majority of new grove invasions. This “tipping point” phenomenon may have implications for how to prioritize the various elements of a larger disease management strategy.

ELISA detection for HLB using a pathogen-secreted protein as the biomarker

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We have identified detection markers from the HLB-associated bacterial pathogen, *Candidatus* Liberibacter asiaticus (CLas), and developed an enzyme-linked immunosorbent assay (ELISA) using CLas-specific antibodies. The antibodies specifically recognize epitopes from a Sec-secreted protein of CLas (called SDE1), which is presumably present in the phloem of infected trees. As such, this biomarker may have a greater distribution in the trees than the bacterial DNA, and thereby allow for more robust HLB detection. Using this antibody, we have developed a competitive indirect ELISA protocol, which is based on a competition for the HLB-specific antibody, between the purified antigen coated on the ELISA plate and the CLas biomarker presumably present in the HLB-infected citrus tissues. The protocol has been applied for HLB detection using freeze-dried field samples from Texas and Florida, which were simultaneously analyzed by qPCR. Our data showed that the ELISA protocol gave comparable results with qPCR and in some cases could provide more definite diagnosis, e.g. for samples with qPCR results higher than the established threshold (Ct value>38). Our work demonstrates that competitive ELISA is a valuable tool for detecting CLas secreted proteins and thus HLB diagnosis.

Asian citrus psyllid predator *Ceraeochrysa valida* (Chrysopidae) tolerant to insecticides. Production, release and effectiveness in field.

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The trash-carrying larvae of *Ceraeochrysa valida* (Banks) (Neuroptera: Chrysopidae) have been found preying on the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, in citrus grove of Central America even after pesticides application. There are different efforts of citrus growers to control populations of *D. citri*, as it vectors *Candidatus* Liberibacter spp., a disease that is present in Costa Rica science 2011. The objective of this study is to provide the Costa Rica citrus industry with a new biological control agent of the vector. *C. valida* is produced in Ticofrut science February 2014 with 200.000 eggs average by month. We present a form of eggs production and release; evaluation of four dosages: 0, 400, 800 and 1.000 eggs by hectare, its effectivity and the effect of pesticides on larval stage in field. For the production, *C. valida* larvae are rear on *Sitotroga cerealella* eggs and adults with pollen, honey bee and yeast in controlled conditions (25±2ºC, 80-85% RH, 12:12h). A pot of production lined with paper consist in 50 adults with 7:3 female-male proportion, water and food; than is changed daily to collect the eggs. Each pot produce eggs for 45 days, with 6.82$\pm$2 eggs in average by female. The paper fragments of 20 eggs with three days after oviposition caused 84% mortality in ACP population 15 days after field application; which was better than larval and adult stages (gl: 4; p=0.000). In 100% of infestation with ACP eggs and 1-3 nymph was not significant differences between the different evaluated dosages 15 days after application (gl: 3; p=0.0518); but 21 days after, dosage of 800 eggs was better (gl: 3; p=0.000). Similar results were found in presence of leaf miner, scale bugs and mites. In presence of aphids, the ACP control by *C. valida* varied and the higher dosage was better (83%). Thanks to its trash, *C. valida* larvae after three days of emerged are 78% tolerant to Zeta-cypermethrin, 60% to dimethoate and was not affected by systemic insecticides (Thiamethoxam). This study presents a new tool for the integrated management of ACP compatible with the actual practices.

Characterization of a ribonuclease of the causing agent of HLB *Liberibacter asiaticus*

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Liberibacter asiaticus, the causative agent of HLB, is an endogenous and seive-stricted Gram-negative bacterium, transmitted from tree to tree by citrus psyllid insect vector *Diaphorina citri*. In absence of laboratory conditions that allow the propagation of *L. asiaticus*, the functional characterization of proteins with putative essential activities is a priority for the development of effective management practices. YbeY has been reported as a specific endoribonuclease and its homologs are present in almost all sequenced bacteria. In *Sinorhizobium meliloti*, YbeY is involved in the regulation of small RNAs, as well as in the ribosome quality control. YbeY was also shown to play a critical role in the processing and maturation of the three rRNAs in *V. cholerae* and in *E. coli*, and to be required for expression of various virulence genes in *Yersinia enterocolitica*. The aim of this work is to analyze the role of YbeY homolog in *L. asiaticus*. Preliminary results showed that the heterologous expression of $YbeY_{Las}$ in the *E. coli* BW25113Δ*ybeY* genetic background partially restores the defective growth of this strain at 43°C. *In vitro* assays, aimed to test the RNAse activity of YbeY on ribosomal RNA, showed full degradation of the substrate, suggesting that $YbeY_{Las}$ may have exonuclease activity. Since YbeY has been described as an endoribonuclease involved in maturation of 5’ end of 16S and 23S rRNA, the 5’ ends of 16S and 23S rRNA from *L. asiaticus* were synthesized and assessed for YbeY’s enzymatic activity. Our results suggest that YbeY may perform a similar activity as reported in other Gram-negative bacteria, and may be essential for *L. asiaticus* growth within the host, which makes it a suitable target for the development of new management strategies.

Citrus cropping systems under the huanglongbing expansion in Bhutan

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Citrus industry in Bhutan enjoys many advantages over the other domestic crops. It is one of the major export commodities that fetch foreign revenue and has a well established marketing chain that provides growers with assured export and domestic market. However, Huanglongbing (HLB) is currently a major threat to citrus cultivation in Bhutan. We assessed change in the directives and the citrus cultivation