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SJVASC Update

January 2017

Research Highlight

Developing friendly strains of Citrus tristeza virus in the fight against serious citrus pathogens.

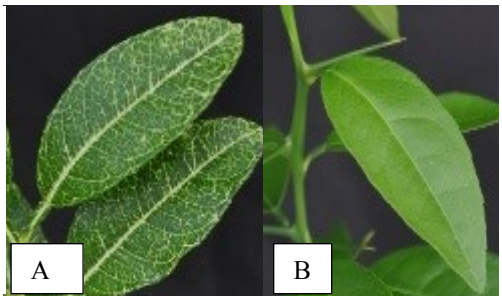
Quick decline in field

Tristeza is a citrus disease caused by *Citrus tristeza virus* (CTV). CTV is the most destructive virus of citrus, and it is transmitted by aphids. CTV-induced decline on sour orange rootstock killed millions of citrus trees in the early 1900's in Australia, Brazil, South Africa, Spain, Venezuela, and California. Through a combination of the use of alternative rootstocks and scion cultivars with tolerance to CTV, economical citrus production was restored. CTV moves and replicates in phloem, and exotic strains exist that induce severe stem pitting (SP) in the scion regardless of rootstock which destroys citrus quality and production. In California, CTV is controlled by planting certified virus-free citrus trees, regulations to prevent introduction of citrus from uncertified sources, and removal of citrus trees harboring potentially virulent strains of CTV.

Research in Parlier by Dr. Ray Yokomi has shown that CTV incidence in Central California is low and indigenous aphids are not efficient in transmitting CTV compared to the exotic brown citrus aphid (BrCA). The BrCA is widely distributed on citrus in tropical and subtropical regions. Moreover, the vast majority of CTV isolates examined are mild and symptomless in commercial citrus planting. Dr. Yokomi's research focuses on molecular strain differentiation of CTV, and it supports a grower-funded CTV control program implemented by the Central California Tristeza Eradication Agency in Tulare, CA. Recent efforts in Parlier have been directed toward developing friendly strains of CTV to combat severe SP strains of CTV by mild strain cross-protection and also recombinant technology to manage huanglongbing (HLB) (aka citrus greening) and its vector, the Asian citrus psyllid (ACP), by RNA interference (RNAi).

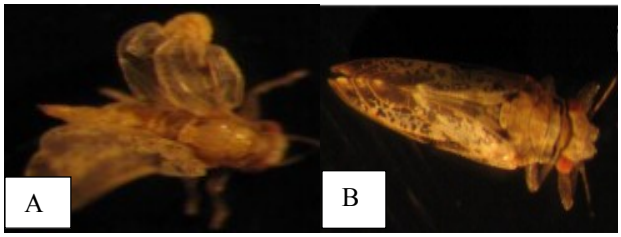
CTV strains in virus index

Cross-protection is a natural antiviral defense system by which citrus excludes infection of a severe strain of CTV by preinoculation of a mild isolate of the same virus strain. This is a strategy used for citrus production in Brazil, Peru, and South Africa where severe strains of CTV and the BrCA are endemic. Genomic sequence similarities between several known mild cross-protective strains from Peru and several mild CTV strains from California suggest that some local CTV strains may be able to cross-protect against some SP-CTV strains. Experiments are underway in an ARS quarantine facility in Beltsville, MD, to examine this interaction.



A. Photo bleaching in citrus leaves resulting from VIGS and expression of rCTV containing tPDS. B. Symptomless citrus inoculated by a mild CTV isolate.

RNAi is also a natural defense system of living organisms, and it is being developed as a potential strategy to help manage HLB and ACP. Specifically, a recombinant CTV (rCTV) strain is used to vector and express RNAi in citrus phloem tissue. As proof of concept, a truncated citrus phytoene desaturase (tPDS) gene was inserted into an rCTV vector and inoculated into citrus. The tPDS gene is expressed in citrus phloem as the rCTV replicates. Through a process called Virus Induced Gene Silencing (VIGS), silencing of the PDS pathway results in inhibition of carotenoid biosynthesis and leads to photo bleaching.



A. Adult ACP with malformed wings developed from nymphs fed on citrus inoculated with rCTV containing *Awd* gene sequences. B. Healthy control ACP adult with normal wing development.

In the case of the ACP, sequences of its abnormal wing disc (*Awd*) gene were engineered into the rCTV vector and inoculated in citrus which resulted in *Awd* gene expression in phloem. When ACP nymphs fed on treated plants, malformed-winged adults were produced along with increased adult mortality as a result of RNAi. Another use of the rCTV vector is to deliver and express antimicrobial peptides directed against the bacterial pathogen presumed to be the causal agent of HLB. Research is now underway in Parlier to develop a California mild rCTV vector to battle serious citrus pests without dependence on transgenic plants or chemical pesticides.

Retirement of Dr. Jim Gerik

Dr. Jim Gerik retired on 22 January 2017 after 20 years with ARS as a Research Plant Pathologist conducting research to better understand soil health and to find alternatives to the use of methyl bromide, particularly for growing floriculture, strawberry, perennial, and nursery crops. His research on soil-borne fungi broadened our understanding of the process of root colonization. He isolated a strain of tomato bushy stunt virus, which led to the discovery that appropriate soil fumigation could control the disease, and he was the first researcher to show that *Fusarium oxysporum* was the causal agent of a new wilt disease of lettuce. He demonstrated that alternative fumigants and other chemicals are efficacious for disease and weed control in cut flower crops including calla lily, snapdragon, *Liatris*, Dutch iris, *Freesia*, myrtle, stock, *Rhamnus*, and *Gypsophila*. The research demonstrated that the use of alternative chemicals may be feasible for floriculture crop production. His research provided evidence necessary for granting flower growers a critical use exemption for use of methyl bromide, which allowed additional time to transition to the phase-out of methyl bromide. He also demonstrated that drip-applied methyl bromide alternatives for the production of rhizomes in calla lily nurseries provided superior disease control compared with the standard application of methyl bromide/chloropicrin applied by shank application. The drip-applied materials provide superior disease and weed control resulting in greater returns to the grower. His studies of alternatives to the use of methyl bromide to control soil-borne pathogens and weeds were invaluable to the floriculture industry, which depends greatly on finding ways to grow their crops in ecologically smart ways.


Research Updates


Key factors, soil N processes, and nitrite accumulation affecting nitrous oxide emissions

Submitted to: Soil Science Society of America Journal

Authors: Z. Cai, S. Gao, A. Hendratna, Y. Duan, M. Xu, B. Hanson

Agricultural soil is a significant source of nitrous oxide (N_2O) emissions contributing to global warming, and mitigation strategies depend on better understanding of the environmental factors and processes affecting N_2O production. This study examined the dynamics of both N_2O emission and N transformation processes from urea application by conducting a series of laboratory soil incubation experiments under varying conditions of application rate, soil moisture, temperature, incorporation of biochar, and the use of nitrogen transformation inhibitors (fertilizer stabilizers). Soil water content was found to be the most important environmental factor impacting N_2O emissions. Higher emissions and total gaseous N loss were found in soil above water holding capacity (WHC) than those below. This research also showed that nitrite ($NO2^-$) was highly correlated with N_2O emission but within two distinct water content ranges (above or below WHC). Biochar and the inhibitors reduced total N_2O emissions >70%, and the inhibitors also significantly reduced total gaseous N loss. This information can be used to guide development of practices for effective N management and minimizing losses.

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Post-harvest development of *Candidatus* Liberibacter solanacearum and its impact on the late-season infected potato tubers, with emphasis on cold storage procedures

Submitted to: Phytopathology

Authors: A. Rashed, N. Olsen, C. Wallis, L. Paetzold, L. Woodell, M. Rashidi, F. Workneh, C. Rush

Tubers infected with '*Candidatus* Liberibacter solanacearum' (Lso), the causal agent of zebra chip disease, just prior to harvest appear asymptomatic but may develop undesirable zebra chip disease symptoms while in storage. This study characterized tuber Lso titers, frying quality, and associated changes in tuber phenolic chemistry when potato plants were inoculated with Lso 4, 10, or 14 days before harvest. In all cases, as storage time increased infected tubers exhibited greater Lso titers and associated phenolic accumulation. Plants inoculated with Lso 10 or 14 days before harvest had decreased tuber frying quality at the end of the storage period, whereas plants inoculated 4 days before harvest had no such reductions in quality. These results suggest that greater screening for Lso in tubers prior to placement in storage is needed, and confirm recommendations to control psyllid vectors up to at least a week before harvest.

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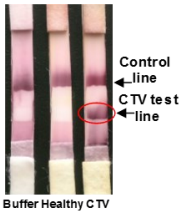

On-site detection of *Citrus tristeza virus* (CTV) by lateral flow immunoassay using polyclonal antisera derived from virions produced by a recombinant CTV

Submitted to: Phytoparasitica

Authors: Y. Maheshwari, V. Selvaraj, S. Hajeri, C. Ramadugu, M. Keremane, R. Yokomi

Citrus tristeza virus (CTV) is graft- and aphid-transmissible, and it is a regulated pathogen in California. Rapid detection and elimination of CTV in budwood is mandatory in commercial citrus nurseries. A 10-minute, lateral flow immunoassay (LFI) was developed using CTV antigens produced in tobacco plants infected with a recombinant CTV isolate. The LFI was validated by comparison with Enzyme-Linked Immuno Assay (ELISA) and Reverse Transcription-quantitative PCR (RT-qPCR) from 52 field trees from several commercial citrus orchards. CTV detection by the immunostrip agreed 100% with ELISA and RT-qPCR results. Since no specialized equipment is needed, the LFI can be used to test for CTV in the field or nursery by growers.

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Orientation of navel orangeworm larvae and adults (*Amyelois transitella*: Lepidoptera: Pyralidae) toward *Aspergillus flavus*

Submitted to: Environmental Entomology

Authors: D. Bush, A. Lawrance, J. Siegel, M. Berenbaum

The navel orangeworm is an important pest of tree nuts in California, and this insect is often associated with the fungus *Aspergillus flavus*. Navel orangeworm larvae grow faster and survive better on diets containing this fungus, and we investigated whether larvae respond to chemical cues indicating fungal presence and plant damage. In our assays we found a strong response to three compounds, two associated with damaged plants (1-octen-3-ol and 2-phenylethanol) and one which is an inhibitor of fungal growth (ethyl benzoate). More eggs were laid on diet containing the fungus than on plain diet. Interestingly, there were twice as many fertilized eggs laid on the fungus diet than on the plain diet. We investigated whether navel orangeworm females had a strategy to lay more unfertilized eggs on poor diet to help feed the larvae that hatched, and found that larvae lived 2.5X as long when provided with eggs compared to larvae that did not have eggs as a supplement to their diet. Although this could be an artifact of our experimental protocol, there are numerous insects that lay unfertilized eggs as a dietary supplement. Further research is needed to determine if this truly is a strategy used by navel orangeworm. We clearly demonstrated that larvae reacted strongly to three compounds that were either associated with damaged plants or had antifungal properties, and this information could be used to develop attractants or repellents to help manage this pest.

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Combination phenyl propionate/ pheromone traps for monitoring navel orangeworm (Lepidoptera: Pyralidae) in almonds in the vicinity of mating disruption

Submitted to: Journal of Economic Entomology

Author: C. Burks

Mating disruption for navel orangeworm, used in an increasing proportion of almond and pistachio orchards, is a least-toxic pest management alternative that decreases populations of and damage from this pest, but also makes detection with pheromone lures more difficult in both the treated block and neighboring blocks. Previous studies showed that a combination phenyl propionate/ pheromone lure attracts similar numbers of adults compared to pheromone-only lures in the absence of mating disruption, and more adults than either lure type alone in the presence of mating disruption. Data from the current study demonstrate a field life of 6 weeks for phenyl propionate dispensers, and indicate that the number of adults captured was similar across different intensities of mating disruption in experimental plots. Data from monitoring sites 0.6 to 2.8 miles from large commercial mating disruption blocks indicated that phenyl propionate/pheromone combination traps detected navel orangeworm under conditions in which traps baited only with pheromone were partially or completely suppressed. These findings indicate that phenyl propionate/pheromone combination traps will be useful for monitoring for navel orangeworm, the key insect pest of the $7.8 billion almond and pistachio industries, in areas in which mating disruption is commonly used.

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Impact of pesticide resistance on toxicity and tolerance of hostplant phytochemicals in *Amyelois transitella* (Lepidoptera: Pyralidae)

Submitted to: Journal of Insect Science

Authors: B. Vikram, J. Siegel, M. Demkovich, L. Zehr, M. Berenbaum

In California, crops from the old and new world are grown close together, and a pest's ability to switch crops can have a major economic impact. The factors that enable this to occur are not well described and we used the navel orangeworm, *Amyelois transitella*, an important pest of almonds, pistachios, walnuts, and figs in California to explore this question. Resistance of navel orangeworm to the pyrethroid family of insecticides has been recently described in Kern County, California, and we thought resistant insects could attack new crops. We report the variability in the response to pyrethroids of three lines of navel orangeworm, a lab strain (CPQ, unexposed to pyrethroids) and two strains isolated from the field in almonds (R347, resistant to pyrethroid) and figs (FIG). We chal-

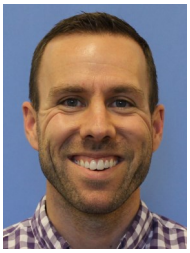
lenged larvae with a pyrethroid insecticide, three chemicals that plants use for defense against insects (allelochemicals) and two novel hosts that occur in California but are not used by navel orangeworm (mesquite and wisteria). As expected, R347 was more tolerant to pyrethroids than CPQ, but, surprisingly, FIG was equally as tolerant as R347, even though pyrethroids are not used in figs. The field strains were also more tolerant to allelochemicals than CPQ. Surprisingly, R347 did not do well on wisteria while FIG did the best on this plant. We had expected R347 to do well in all challenges because of its insecticide resistance, but FIG outperformed it. FIG may have a superior ability to detoxify chemicals because its host contains high levels of allelochemicals. R347 may have sacrificed its ability to handle the defensive chemicals of wisteria when it became resistant to pyrethroids.

Contact: Joel Siegel, Joel.Siegel@ars.usda.gov, (559) 596-2735

New Visiting Scientist

Dr. Raul Narciso C. Guedes will be doing a 1.5-year sabbatical with Dr. Spencer Walse in the Commodity Protection and Quality Research Unit. He received his PhD in Entomology in 1997 from Kansas State University. He is a professor at the Federal University of Viçosa in Brazil with broad interests in insecticide ecotoxicology and insect stress biology. He has published 224 research articles in international peer-reviewed journals. He is a Fellow of the Royal Entomological Society, senior research scholar of the Brazilian National Council of Scientific and Technological Development (CNPq), and member of the Applied Biology panel of the Research Foundation – Flanders (FWO, Belgium). He was Chair of the Entomology Graduate Program at the Federal University of Viçosa (UFV) and Scientific Director of the Arthur Bernardes Foundation (FUNARBE), the supporting foundation of UFV. He is also an editorial board member of the scientific journals PLoS ONE, Pest Management Science, Journal of Stored Products Research, and Journal of Economic Entomology. He has spent research leaves at Leicester University (UK), USDA-ARS Center for Grain and Animal Health Research (Manhattan KS), and Carleton University (Canada).

New Employee



Dr. Christopher Van Horn is a postdoctoral scientist working with Dr. Jianchi Chen in the Crop Diseases, Pests and Genetics Research Unit. He is originally from Yakima, WA, where his family grows apples and cherries. He received his B.S. in Cell Biology and Genetics from Washington State University in 2010 and his PhD. in Cell and Molecular Biology from Colorado State University in 2016. In Colorado, he worked in the Weed Research Lab investigating the molecular basis for glyphosate resistance in giant ragweed. He has recently started exploring the world of next-generation sequencing with a transcriptomic experiment from his PhD work and now genomic analysis of *Xylella fastidiosa* in Parlier.

USDA 2015 Technology Transfer Report

For a compilation of USDA Technology Transfer activities for Fiscal Year 2015, go to https://www.ars.usda.gov/ARSUserFiles/ott/FY15-TT_9-30.pdf.

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