AGENDA
Diaprepes Task Force
Scientific Program
March 4, 2004

USDA-ARS Horticultural Research Laboratory
2001 South Rock Road
Fort Pierce, FL 34945

Convener: Steve Lapointe

9:00 a.m.	Welcome and Introductions	Garvie Hall, DTF Chair Constance Riherd, DTF Vice-Chair
	Resistant Rootstocks: Status and Prospects	
9:15	Rational and Strategies in Breeding Programs	James H. Graham, Pathologist
9:30	Status of Conventionally Bred Rootstocks	Kim Bowman, Research Geneticist
9:45	Status of Somatic Hybrids and Tetrazygs	Jude Grosser, Research Geneticist
10:00	*Bacillus thuringiensis* strains for use against *Diaprepes abbreviatus*	Allen Weathersbee, Entomologist
10:15	BREAK	
10:30	Genetic transformation to control *Diaprepes abbreviatus*	Randy Niedz, Research Geneticist
	Progress in Biological Control	
10:45	Status of Introduced Parasitoids	Jorge Pena, Entomologist
11:00	**Summary and General Discussion**	
12:00	**Lunch**	
1:00	**Poster Session**	
2:00-4:00	**Working Group Meetings** Host Plant Resistance Biological and Cultural Control Tactics	Conveners: Larry Duncan and Steve Lapointe

Resistant Rootstocks: Status and Prospects

Screening of Rootstock Resistance: Rationale and Strategies

J. H. Graham
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

Root feeding by larvae of *Diaprepes abbreviatus* predisposes the damaged tissues to infection by *Phytophthora* spp. Interaction between insect and fungus (termed the PD complex) causes greater damage to roots than either pest or pathogen alone, and accelerates the decline of trees on rootstocks susceptible to *P. palmivora* and *P. nicotianae*. The rationale for screening for resistance to *Phytophthora* spp. is based on the observation that no citrus species is resistant to root feeding, but under field conditions a *Phytophthora* resistant rootstock will show more tolerance to the PD complex. The screening process for seedlings and budded trees occurs in stages: 1) commercial rootstocks and new germplasm are screened in the greenhouse in tubs of marginal soils and native *Phytophthora* inoculum; 2) selected germplasm is challenged with Diaprepes larva, and tolerance evaluated as the ability to regenerate roots after short-term exposes; 3) 9-month to multi-year exposures of seedlings and budded trees under the same adverse soil, pest and pathogen conditions is used for rapidly assessing resistance to *Phytophthora* (populations measurements), Diaprepes feeding (visual root ratings) and resultant tree growth. Differential susceptibility of commercial rootstocks to the two *Phytophthora* spp. has shifted emphasis from trifoliate orange to sexual and somatic hybrids of mandarins and pummelos that exhibit some resistance to both *P. palmivora* and *P. nicotianae.* Greenhouse and field results confirm the value of screening at the early stages for resistance to *Phytophthora* spp. to select germplasm for tolerance to adverse soils and the PD complex.

New citrus rootstocks with tolerance to Diaprepes weevil and Phytophthora diseases

Kim D. Bowman
USHRL, ARS, USDA, Ft. Pierce, FL 34945

The USDA citrus rootstock program is focused on developing new rootstocks with improved effects on tree survival, productivity, and fruit quality. Important selective factors being targeted include adaptation to common Florida soils, and tolerance to *Diaprepes abbreviatus* weevil, *Phytophthora nicotianae*, and *Phytophthora palmivora*. Three new rootstocks in advanced testing have appeared promising for a combination of the desired traits. At one Indian River County site with heavy soil and a severe Diaprepes and Phytophthora infestation, young grapefruit trees on US-802, US-897, and US-942 are growing strongly, while trees on Swingle, Carrizo, and some other rootstocks are small and weak. Long-term trials with these three rootstocks in other locations without Diaprepes have indicated good potential for commercial use. New trials with these rootstocks have been established in other locations infested by Diaprepes to evaluate performance under a range of conditions. Other new rootstocks are being developed with sour orange parentage to combine resistance to CTV and good tolerance to Diaprepes and Phytophthora, with the best characteristics of sour orange rootstock. These “Supersour” rootstocks may offer provide the best opportunity to bring together the ideal combination of rootstock traits.

Development of Rootstocks Tolerant of the *Diaprepes/Phytophthora* Complex – Status of Somatic Hybrids and 'Tetrazygs'

J.W. Grosser, J.H. Graham, C.W. McCoy, A. Hoyte, H.M. Rubio, and J.L. Chandler
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

Our primary strategy for dealing with the *Diaprepes/Phytophthora* problem is to develop complex rootstock hybrids that have the capacity to tolerate mechanical damage caused by weevil feeding and then recovery by exhibiting vigorous root growth in challenging soils inoculated with both *Phytophthora nicotianae* and *P. palmivora*. Annual crosses are being made of superior allotetraploid somatic hybrid rootstocks and resulting seed are planted in a high pH calcareous 'Winder' soil inoculated with both *Phytophthora spp*. in greenhouse flats. Vigorous healthy "tetrazyg" seedlings are selected and propagated by grafting to vigorous rootstocks and subsequently rooted cuttings. Selected new mandarin + pummelo somatic hybrids (potential sour orange replacements) are also being included in the assays. Replicated *Diaprepes* force-feeding assays are conducted in conetainers®, and hybrids selected for reduced mechanical damage were replanted in a 'Winder'/*Phytophthora* mix to assess recovery potential. Several hybrids have shown excellent capacity for complete recovery in this greenhouse test and are now being propagated for more extensive field evaluation; these include hybrids of Nova+Hirado Buntan pummelo with Cleopatra+trifoliate orange and Cleo+sour orange. Two of the mandarin + pummelo somatic hybrids have also performed well. Other promising hybrids are being selected from crosses of Nova+HB pummelo with Cleo+Swingle (from 2002 crosses), and Succari+HB pummelo with Cleo x Carrizo (2003 crosses). Citrus rootstock breeding and selection at the tetraploid level maximizes genetic diversity and selection efficiency, and shows great promise for generating new rootstocks that can tolerate the *Diaprepes/Phytophthora* complex.

Bacillus thuringiensis* strains for use against *Diaprepes abbreviatus

Allen Weathersbee
USDA, ARS, USHRL, Fort Pierce, FL 34945

Bacterial entomopathogens have not been adequately investigated as potential microbial agents for control of *Diaprepes abbreviatus*. The bacterium, *Bacillus thuringiensis* is known to be pathogenic to many insect pests but relatively few strains of *B. thuringiensis* are active against coleoptera. Endotoxins produced by the bacterium are activated in the gut of susceptible hosts upon ingestion, and can cause feeding inhibition, infection of tissues, and eventual death. Researchers at the U.S. Horticultural Research Laboratory have assembled two collections of *B. thuringiensis* isolates that are plausibly active against *D. abbreviatus*. One collection contains isolates covered by industry patents that demonstrate activity against coleoptera. The other collection is comprised of isolates collected in Florida from diseased larvae of *D. abbreviatus* and associated soils. Isolates from both collections are being screened for activity against neonates and 3-4 week old larvae of Diaprepes root weevil. Effective rates are determined for isolates that demonstrate activity in initial screening experiments. Application strategies developed for particularly promising isolates may include transgenic approaches as deemed appropriate.

Genetic transformation to control *Diaprepes abbreviatus*

Randall P. Niedz
U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945-3030

Successfully controlling the *Diaprepes* root weevil by genetic transformation requires consideration of at least seven components. One, the control strategy must directly repel, inhibit the growth of, reduce the fecundity, kill the larval and/or adult stages of the insect, or indirectly result in an increase in the economic yield of the tree (e.g., *Phytophthora* resistance). Two, the control strategy is simple genetically and can be implemented via the insertion of one or two genes. Three, expression levels of the inserted gene(s) must be sufficient in the transgenic plants to control the insect. Four, the chosen plant type must be transformable. Five, suitable promoters are available to control the level, time, and location of gene expression. Six, the control strategy must be compatible with both the economical yield and environmental impact of the crop. Seven, deployment of these foreign genes must be consistent with insect resistance management (IRM) strategies. These seven components and their consideration in producing transgenic citrus trees resistant to the *Diaprepes* root weevil will be discussed.

Progress in Biological Control

Progress in biological control of *Diaprepes abbreviatus*

Jorge E. Peña
University of Florida/IFAS, TropicalREC, Homestead, FL 33031

The activities on biological control of the Diaprepes root weevil during 2003 were: 1) Surveys to detect the recovery of the egg parasitoids *A. vaquitarum* and *Q. haitiensi;* 2) Tests to detect constraints to parasitoid development, related to low winter temperatures and effect of chemicals; and 3) Two exploratory surveys in the island of Dominica, conducted by C. McCoy and J. E. Peña in May and June 2003. 1. Parasitoid Recovery. Levels of parasitism in citrus were low and erratic; the parasitoid *Q. haitiensis* was recovered once in citrus in Dade county, parasitizing *5%* of *Pachnaeus* eggs whereas *A. vaquitarum* was recovered once in St Lucie parasitizing 8% of *Diaprepes* weevil eggs in citrus groves in that area. However, levels of parasitism from both parasitoids fluctuated between 33 to 86% in ornamental plantings in Dade Co. , where the parasitoids are considered established. 2. Parasitoid biology, effect of chemicals and low temperatures on parasitoid development. Developmental time from egg to adult for *Q. haitiensis* decreased with increasing temperatures. Shortest parasitoid development was observed at 30°C and longest at 20°C. No development was observed at constant temperatures of 15°C or 5°C. *A. vaquitarum* emergence was affected on leaves treated the same day with Imidan or Sevin. Seven days later, parasitoid emergence continued to be reduced on Sevin XLR treated leaves compared to the untreated control or leaves treated with Imidan. There was no difference on parasitoid emerged between untreated leaves and those treated with Imidan or Sevin, 14 days after treatment. 3. Exploratory survey in Dominica. Two new egg parasitoids *Haeckeliania* n. sp., and *Fidobia* n. sp. were discovered in Dominica and introduced into UF/TREC quarantine facility. The parasitoids have been tested for specificity against several coccinellids, and native

Lepidoptera eggs. Tests are pending regarding specificity of the parasitoids on other curculionidae.

Poster Session

Ant predation on neonate larvae of the root weevil, *Diaprepes abbreviatus*: a manipulative field experiment

Robin J. Stuart and Clayton W. McCoy
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

The root weevil, *Diaprepes abbreviatus* (L.), continues to be a major pest of Florida citrus. When *D. abbreviatus* neonate larvae hatch from egg masses in the citrus canopy and drop to the soil surface before burrowing down to the roots for feeding, they are extremely vulnerable to ant predation. In Florida citrus groves, the red imported fire ant, *Solenopsis invicta* Buren, is one of the most common ant species, and previous research indicates that it is also one of the most important predators of *Diaprepes* neonates. We conducted a manipulative field experiment to examine the relationship between ant population levels and predation pressure on *Diaprepes* neonates. Granular ant baits were used to suppress ant populations in a young citrus grove. Ant populations were monitored by placing hamburger baits under the citrus canopy; and predation pressure on *Diaprepes* neonates was assessed by placing neonates in plastic dishes under the canopy. In this experiment, *S. invicta* was the major predator on *Diaprepes* neonates, and there was a significant correlation between ant population levels and predation rates on neonates. This research reinforces the view that red imported fire ants are major natural enemies in Florida citrus groves and important predators of *Diaprepes* neonates.

Comparisons of two different acoustic systems for detection of Diaprepes root weevil larvae in citrus groves

Richard Mankin and Stephen Lapointe,
USDA-ARS CMAVE, Gainesville and USDA-ARS-USHRL, Ft. Pierce

In recent field studies, two different acoustic detection systems (an accelerometer and the recently developed AED-2000) readily detected the presence or absence of insects in the root systems of small orange trees. However, the rate of sounds varied considerably at different times and at different positions around the trees. Potential reasons for such differences and their practical implications are discussed.

Differential susceptibility of larval instars of the citrus root weevil, *Diaprepes abbreviatus*, to the entomopathogenic nematode, *Steinernema riobrave*

Robin J. Stuart and Clayton W. McCoy
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

The root weevil, *Diaprepes abbreviatus* (L.), originated in the Caribbean and is a major pest of citrus and various other crops and ornamentals in Florida. Young larvae feed on fibrous roots, move to larger roots as they grow, and pupate in the soil after 9-11 instars. The application of entomopathogenic nematodes is one of the few tactics available to control *D. abbreviatus* larvae in Florida citrus groves. In a laboratory study, we examined the influence of larval age, weight, and instar on the susceptibility of *D. abbreviatus* larvae to the entomopathogenic nematode, *Steinernema riobrave* Cabanillas, Poinar and Raulston. *Diaprepes* larvae belonging to various age cohorts were weighed and their head capsules measured to determine larval instar. Larvae were placed in individual 25-dram snap-cap vials in Candler sand with 8% moisture by weight, and *S. riobrave* was applied at rates of 100 to 500 infective juveniles per container. Treatment containers were incubated at 24 °C and mortality was checked after 9-12 days. Mortality varied significantly among instars and decreased markedly in later instars. Within instars, mortality was not related to larval weight or age. The mechanisms responsible for differential susceptibility of larval instars are unknown but similar relationships have been demonstrated for other insects. Differential susceptibility and the relative vulnerability of smaller instars could have important implications for the timing of nematode applications for weevil control in Florida citrus.

Effect of chemically treated and untreated populations of *Diaprepes abbreviatus* on the growth, development and survival of 'Hamlin' orange trees budded to five rootstocks

C. W. Mc Coy, W. S. Castle, J. H. Graham, J. P. Syvertsen, A. W. Schumann, and R. J. Stuart.
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL. 33850

A pest management study was initiated in a bedded 2-year-old 'Hamlin' orange planting budded on five rootstocks. The experimental site was located within a mature grove harboring a high *Diaprepes* root weevil (DRW) population and with poorly drained fine-textured sandy soil. After 2 -years on a young tree care program that included insect and mite control, *Diaprepes* root weevil DRW was allowed to infest more than one-half of the new 'Hamlin' rootstock planting. In 2003, we compared the effect of foliar and soil-applied chemicals recommended for DRW to no pesticides in a seasonal control program that included monitoring adult emergence and tree canopy abundance. In the winter of 2003-2004, tree health was determined by measuring rate of tree trunk growth, and canopy volume, tree decline, and leaf injury for 'Hamlin' trees budded to different rootstocks. The incidence of *Phytophthora nicotianae* was monitored but not treated during the 3 years.

Foliar and soil-applied chemicals, timed according to adult DRW emergence and abundance on the tree, were effective in suppressing weevil populations. In addition, treated trees had a faster rate of growth, larger tree canopies, less foliar injury and less tree decline than untreated trees. 'Hamlin' trees on rootstocks such as C-32 and C-35 had higher growth rates and weevil population. In assessing overall root health, it appeared that DRW root injury was creating site(s) for infection and bark damage by *Phytophthora* based on the resistance of the rootstocks. Tree decline was most apparent among trees on Cleopatra mandarin, and was not reduced by DRW control; whereas, tree decline was lower with DRW control for certain *Phytophthora* resistant rootstocks (C-22, C-32).

External and internal morphology of the Diaprepes root weevil

Stephen L. Lapointe, Wayne B. Hunter, Rocco Alessandro, and Ute Albrecht
UDSA, ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL 34945

External gland openings and associated structures on elytra of teneral and mature Diaprepes root weevil were elucidated by scanning electron microscopy (SEM). Evidence is presented to document structures associated with the production of hydrocarbons and other compounds. Partial characterization by gas chromatography and mass spectrometry resulted in identification of more than 30 compounds in methylene chloride extracts. The morphology of the alimentary canal is presented in a series of cross sections obtained by microtome sectioning, staining, and observation by light microscopy.

Infection of *Diaprepes abbreviatus* with a unicellular green alga, *Helicosporidium* spp.

Verena-Ulrike Bläske and Drion G. Boucias
University of Florida, IFAS, Department of Entomology and Nematology, Gainesville, FL

Helicosporidium spp. is a unique invertebrate pathogen that, after its first description by Keilin in 1921, had long been considered to be either a protozoan or a fungus. Based on morphological studies and phylogenetic analyses conducted in our lab, however, this protist has recently been identified as a non-photosynthetic green alga (Chlorophyta). Several invertebrate hosts have been reported to support the growth and development of Helicosporidia in their hemolymph, including diverse groups of insects, as well as mites, crustaceans, and trematodes. The Helicosporidia are characterized by a distinct cyst stage that encloses three ovoid cells and a single elongate filamentous cell within a pellicle. The infectious cysts are transmitted per os, dehisce within the midgut lumen, and release the filaments, which potentially penetrate the midgut and enter the host's hemocoel. Within the hemocoel, vegetative cells develop, replicate massively and eventually form mature cysts. In order to examine the susceptibility of *Diaprepes abbreviatus* to helicosporidial infection, 4-week-old larvae were orally challenged with a cyst suspension at 2.5×10^5 cysts per larva. Three different *Helicosporidium* spp. isolates were tested, originating from an aquatic weevil, a collembolan, and a black fly host, respectively. High infection rates were diagnosed 3 week post-treatment ($88 \pm 10\%$, $91 \pm 8\%$, and $76 \pm 3\%$, respectively) and weights of infected larvae were significantly reduced compared with control larvae. No mortality occurred within this time frame.

Life cycle and development of *Quadrastichus haitiensis* (Hymenoptera: Eulophidae): A parasitoid of *Diaprepes abbreviatus*

José Castillo and J. E. Peña
University of Florida/IFAS, Tropical Research and Education Center, Homestead, FL 33031

An egg endoparasitoid, *Quadrastichus (Tetrastichus) haitiensis* (Gahan) (Hymenoptera: Eulophidae) was released during the 1970s in central (Apopka) and southeastern Florida (West Palm Beach), but failed to establish (Beavers and Selhime 1975). It was reintroduced into Florida from Puerto Rico by D. Hall, R. Nguyen and P. Stansly during 1998 and released in 2000. The parasitoid is currently established in Dade County, Florida but it has not established in other parts of Florida. The objective of this investigation was to determine the life cycle of *Quadrastichus haitiensis* as well as the influence of temperature and photoperiod on the development of this parasitoid. Developmental time from egg to adult for *Q. haitiensis* decreased with increasing temperatures. Shortest parasitoid development was observed at 30°C and longest at 20°C. No development was observed at constant temperatures of 15°C or 5°C. However, the emergence of *Q. haitiensis* was not reduced if parasitized egg masses at different stages of parasitoid development were exposed for 1 h to freezing temperatures (0°C). It is not known, if parasitoid development is reduced if freezing temperatures are longer than 1 hour. Developmental times in days, from egg to adult, were 12.75 to 18.75 d for 30°C, 16.75 to 29.95 d for 25°C and 39.37 to 46.37 d for 20°C. Percent successful parasitism was reduced at 15, 20, and 30°C compared to the highest parasitism observed at 25°C. Parasitoid development was not affected by different photoperiods at 30°C.

Recovery of egg parasitoids released for biological control of *Diaprepes abbreviatus* (Coleoptera:Curculionidae) in Florida

Jorge E. Peña,[1] D.G. Hall,[2] R. Nguyen,[3] C.W. McCoy,[4] S.L. Lapointe,[5] R. Duncan,[1] D.M. Amalin,[6] P.A. Stansly,[7] R.C. Adair,[8] and A.B. Hoyte[4]
[1]UF/IFAS, Department of Entomology, Tropical Research and Education Center, Homestead, FL 33031; [2]US Sugar Corporation, Clewiston, FL; [3]Florida Department of Agriculture and Consumer Services, Gainesville; [4]UF/IFAS, Department of Entomology, Citrus Research and Education Center, Lake Alfred, FL; [5]U.S. Horticultural Research Laboratory, USDA-ARS, Ft. Pierce, FL 34945 ; [6]USDA, Miami, FL; [7]UF/IFAS, Department of Entomology,Southwest Research and Education Center, Immokalee, FL; [8]Kerr Center

Releases of the egg parasitoid species have been conducted at multiple locations in Florida from 1998 until today. Results of surveys in 1998, 1999, 2000, 2001, and 2002 and 2003 showed that levels of parasitism of the egg parasitoids *Aprostocetus vaquitarum* and *Quadrastichus haitiensis* in citrus were low and erratic during 2003 in southeastern, central and southwestern Florida; For instance, the parasitoid *Q. haitiensis* was recovered once in citrus in Dade county, parasitizing *5%* of *Pachnaeus* eggs whereas *A. vaquitarum* was recovered once in St Lucie parasitizing 8% of *Diaprepes* weevil eggs. However, levels of parasitism from both parasitoids fluctuated between 33 to 86% in ornamental plantings in Dade Co., where the parasitoids are considered established contributing to 60% mortality of egg masses. The possible reasons for these different results in different areas and crops in Florida are discussed.

Soil and Flooding Factors Associated with Root Weevils and Citrus Tree Decline: A Case Study in Florida

Hong Li, Jim Syvertsen, Robin J. Stuart, Clay W. McCoy, Arnold W. Schumann, and William S. Castle
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

A study of soil characteristics, tree health and *Diaprepes* root weevil (DRW) distribution was conducted in a citrus grove in a poorly drained Alfisols in Osceola County, central Florida beginning in 2002. The trees were ‘Hamlin’ orange on Swingle citrumelo (*Citrus sinensis* L Osb.

X *Poncirus trifoliata* L.) rootstock, damaged by the root weevil, *Diaprepes* abbreviatus (L.). The objectives were to determine soil and environmental factors causing tree decline and spatial variability of DRW, and to delineate DRW management zones related to soil properties. Adult DRW populations were monitored using Tedders traps placed in a 34 x 24 m grid across the grove. Soil electrical conductivity (EC) was measured using an EM38, and soil organic matter, pH, P, K, Ca, Mg, and other properties were measured at each trap. Adult weevils appeared significantly greater in June than in other month ($P < 0.001$). DRW density was higher in areas that were low in Mg and Ca concentrations ($P < 0.05$). Three distribution zones of DRW were delineated based on the spatial patterns of soil EC and DRW. Semivariograms for DRW, EC, Mg and Ca ranged within 75-100 m, which was matched the DRW distribution zone limits. This match suggested management zones for DRW control. Soil EC, Mg, Ca, and Fe were correlated ($-0.38 < r < 0.61$). Tree decline was associated with high levels of Fe and soil flooding because of plant water stress and negative soil redox potential. It is suggested that soil and DRW relationships can be well described using distribution zoning. Flooding events, high level of Fe and soil liming, affecting soil Mg and Ca levels, could be factors linked to tree decline and DRW distribution patterns.

The relative status of peach and citrus as hosts of *Diaprepes abbreviatus*

Philip A. Stansly
University of Florida, IFAS, Southwest Florida Research and Education Center, Immokalee FL 34142

Peach cultivars are being evaluated for their adaptation to the conditions of the central region of Puerto Rico where the root weevil, *Diaprepes abbreviatus* (L.), is endemic. *Diaprepes abbreviatus* has been reported as a pest of peaches in Florida and so would naturally be expected to attack peaches in Puerto Rico. However, preliminary field observations showed only minimal damage to new peach plantings in two locations where *D. abbreviatus* is abundant. Therefore, we embarked on a study to evaluate the preference and performance of *D. abbreviatus* on peach compared to sweet orange. Adults fed significantly more on Navel orange leaf disks than on peach in both choice and no-choice tests. Oviposition occurred on both peach cultivars tested, but more egg masses were laid on Navel orange leaf strips in the no-choice test. However, given the choice, adults preferred to oviposit on peach leaf strips while fed on Navel orange leaf strips. In some replications this behavior was reversed. Larval feeding damage 90 days after infestation was severe on roots Cleopatra mandarin in 18.7 liter containers. Most of the cortex tissue on the primary root was removed and growth of roots and foliage was reduced. Larval feeding was also observed on peach roots, but there was no sign of growth reduction on foliage or the roots compared to the control. These preliminary results indicate that *D. abbreviatus* will probably not be a primary pest of peach in Puerto Rico.

Transgenic approach toward Diaprepes root weevil resistance in citrus rootstocks

Robert G. Shatters, Jr.,[1] Stephen L. Lapointe,[1], Charles A. Powell[2], and Dov Borovsky[3]
[1]USDA, ARS, USHRL, Fort Pierce, FL; [2]IRREC, IFAS, University of Florida, Fort Pierce, FL; [3]FMEL, IFAS, University of Florida, Vero Beach, FL

Because economically significant resistance to Diaprepes root weevil (DRW) larvae feeding has not been proven in citrus, alternative genetic sources of resistance are being sought. The idea is that a transgenic citrus plant expressing novel genes for DRW resistance can be used as a rootstock for commercially important citrus varieties. Disruption of nutritional uptake has been targeted as a point to block in the DRW metabolism, and to this end, genes that inhibit protein digestion in the gut are being studied. Transgenic plants expressing an inhibitor of gut proteinase synthesis normally synthesized in mosquitoes were produced and shown to be active against lepidopertan larvae; these are currently being tested using DRW larvae. Also, as part of a DRW genomics project, genes encoding gut proteinases were identified and characterized. Information from this work is being used to design multi-component inhibitor strategies to block DRW protein digestion and result in construction of DRW resistant citrus rootstocks.

Using insect viruses to control insect pests of citrus

Wayne Hunter,[1] Charles Powell,[2] and Stephen Lapointe[1]
[1]U.S. Horticultural Research Lab, Ft Pierce, FL and 2University of Florida IFAS, IRREC, Ft. Pierce, FL

Exotic insect pests continue to become established in Florida. These pests; brown citrus aphid, citrus psyllids, and citrus leafminers; have joined other endemic insects, citrus root weevils, citrus aphids, and whiteflies, to cause considerable damage to citrus and vegetable crops both by feeding and transmission of pathogens. Integrated control strategies are needed to control these pests. Biocontrol is an important component of this strategy. One potential group of biological control agents is the entomopathogenic viruses, such as the insect infecting Iridoviruses. We have isolated an insect iridoviruses, which was shown to be pathogenic to aphids and citrus root weevils. We propose to use these viruses as biological control agents against insect pests of citrus. Regions of the virus genome will be substituted with genes encoding products toxic to the insects. These modified viruses will be tested for their lethality on target pest insects. Further examination will evaluate the potential use of these altered viruses for efficacy as highly targeted, effective, and safe biological control candidates. The goal is to lead to the production of a product for use as a biological control agent to manage key insect pests of citrus.

Biological control of *Diaprepes abbreviatus* beneath citrus trees augmented with endemic or exotic entomopathogenic nematodes

Larry Duncan
University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850

We tested the hypothesis that net (cumulative) efficacy is positively related to persistence of entomopathogenic nematodes (EPN) used to augment endemic EPN communities. Plots beneath individual citrus trees were treated (25 IJ3 cm^{-2}) with *Steinernema riobrave* (Sr; short persistence), *S. diaprepesi* (Sd; long persistence), or no EPN in September and October 2002 and May and September 2003. Mortality of sentinel *Diaprepes abbreviatus* larvae was monitored six times between July and October 2003. The cumulative insect mortality from July through September was 61% and 81% greater (P = 0.05) in plots treated with Sd than in plots treated with Sr or no EPN, respectively. However, in October 2003 there was significantly less mortality in plots treated with Sr than in untreated plots and a similar non-significant trend occurred in plots treated with Sd. Cumulative prevalence of insect cadavers containing EPN throughout the experiment was 142% and 112% greater (P = 0.01) for plots augmented with Sd compared to untreated and Sr-treated plots, respectively. Cumulative prevalence of insect cadavers containing only free-living bactivorous nematodes was more than three-fold greater (P = 0.01) in Sr than in Sd-treated plots. The results support previous reports that augmentation with non-persistent EPN may reduce the prevalence of persistent endemic EPN, thereby mitigating net efficacy. Efficacy in October 2003 suggests that commercial laboratory adaptation by Sd may occur at the expense of traits needed for effective predation. The prominence of Sr (42% of EPN) in control plots not treated with Sr for more than two years suggests that Sr is adapting to Florida habitats. Improved methods to maintain wild traits in commercial EPN should result in increased efficacy of these products.