MANAGEMENT/PHYSIOLOGY

Continuing Projects

Use of Host-specific Pathogens as Bioherbicides to Manage Weeds in Florida Citrus
Project No. 942-33

Length of Project: 4 years

Investigators: R. Charudattan, Megh Singh
IFAS - Gainesville/ Lake Alfred

Abstract

We are developing a bioherbicide consisting of a mixture of three fungi to control several grasses that are problematic weeds in citrus. The fungi, a Drechslera sp. and two Exserohilum spp., isolated respectively from large crabgrass, crowfootgrass, and johnsongrass, are native to Florida. They have been field tested in two locations during the past three years with successful results. The fungi were tested against eight grasses transplanted in experimental plots at Lake Alfred. At Ft. Pierce, they were tested in natural populations of guineagrass. A formulation containing an emulsified inoculum was highly effective compared to inoculum containing a humectant or water. A technique was developed for mass production and multiple harvesting of these fungi. The fungi were first shake-cultured in 1000 ml of V8 broth for 2-3 days at 25°C. The mycelium and broth were mixed with 10 ml of an antibiotic solution (3.7 mg/ml streptomycin and 2.5 mg/ml chloramphenicol) and blended in a Waring blender. Five hundred ml of this suspension was then poured onto a 500-ml layer of V8 agar containing antibiotics (as above) in aluminum-foil-lined trays and exposed to alternating, 12-h light and dark cycles at 25°C. The spores developed within 24 h and were collected by gently scraping off with a rubber spatula into sterile water. The remaining spores were then rinsed-off the agar surface with sterile water. The spore suspensions were pooled and the spores were allowed to settle down. The excess supernatant was decanted and the spores were resuspended in 250 ml sterile water. The trays were reincubated under light as before and subsequent crops of spores were harvested twice at 24 h and 48 h. The spore yields for D. gigantea and E. rostratum averaged 2.05x10^5, 3.43x10^5, and 1.89x10^5, and 2.44x10^5, 2.16x10^5, and 1.00x10^5 spores/ml/harvest, respectively. Thus, it is feasible to mass produce these fungi by solid-substrate culturing and multiple spore harvests.

Objectives

1) Develop methods for inoculum production and application of agents.
2) Determine weed control efficacy of agents in miniplot trials and in growers' fields.
3) Determine the ability to combine several pathogens in a tank-mix, post-emergent application to control several weeds.

Summary of Accomplishments

We have determined that it is possible to control several grassy weeds with three pathogens: a Drechslera sp. and two Exserohilum spp. from large crabgrass, crowfootgrass, and johnsongrass, respectively. In greenhouse and field trials, these fungi have been determined to be safe and effective biocontrol agents for bermudagrass, large crabgrass, crowfootgrass, guineagrass, johnsongrass, southern sandbur, Texas panicum, and yellow foxtail. The pathogens were field tested at two locations: at Lake Alfred, 4-wk-old seedlings of large crabgrass, crowfootgrass, johnsongrass, guineagrass, southern sandbur, Texas panicum and yellow foxtail, transplanted within each 1 sq.m plot, were killed with spore suspensions of each pathogen or a mixture of all three pathogens (1:1:1 by vol). The fungi were applied as foliar sprays (5x10^5 spores per ml) in water, 0.5% aqueous Metamucil, or an emulsion, using appropriate controls. At Ft. Pierce, control of natural populations of guineagrass was field-tested. The fungi were applied as in the Lake Alfred study, using appropriate controls. A second application was done at 2 wk after the first inoculation. Individual pathogens and the pathogen mixture were effective in controlling guineagrass. Thus, it is possible to control several grasses using an emulsion-based inoculum.

Funding:

FCPRAC Grants $ 30,000
IFAS Contribution 67,000
Florida Department of Transportation 30,200
Total $127,200

Modification of Nitrogen Applications to Citrus to Prevent Pollution of Groundwater with Nitrates
Project No. 942-42

Abstract

At 5 locations NO$_3$-N, pH, total dissolved solids, P, K, Na, and Cl were determined every 30 days in water samples from 16 wells, 10 - 40 ft deep, and 14 shallow "seepage" wells. The response to cutting N application in half at 4 locations to 100 - 123 lbs/acre varied throughout the year, but average levels were lower (4 - 8 ppm vs. 19 - 29 ppm) at 2 locations, there was no difference in 2 locations (4 and 18 ppm) and in Dade City, with equal N application, 1 block had 0 ppm NO$_3$-N, the other 6 ppm. The main factor determining response to changes in fertilization in groundwater nitrate appears to be depth to water table, with no response at deep (> 15 ft) water table locations. Maximum NO$_3$-N in seepage and drainage ditch water exceeded 5 ppm only in 1 location where seepage emerges above the general water table. Low N regimes decreased fruit production from 6 to 21 percent in 3 places.

Objectives

1. Document the extent, duration, and frequency of high groundwater nitrate levels in groves on various soil types in the principal citrus production areas of Florida.
2. Determine the effects of changes in fertilization on groundwater nitrate levels.
3. Record total soluble solids, pH, phosphorus, potassium, sodium, and chloride in groundwater.
4. Continue to investigate factors which influence ground-water nitrate.
5. Monitor NO$_3$-N, P, K, Na, and Cl in drainage and other waters close to citrus groves.

Summary of Accomplishments

In St. Cloud, with 2 high-N (222 lbs/acre) and 2 low N (111 lbs/acre) plots, 3 of the plots rarely had more than 10 ppm NO$_3$-N in the groundwater, but an erroneous over application of N to 1 of the high-N plots in June 1997 resulted in 40 ppm NO$_3$-N in the groundwater in August and higher levels than in the other wells persisted for 9 months until April 1998. In Lake Wales, in spite of differential N applications (237 lbs/acre high N, 134 lbs/acre low N), groundwater NO$_3$-N remained the same (18) ppm in both plots throughout the year. In Frostproof, with 3 levels of N (150, 215, and 270 lbs/acre) applied, the corresponding groundwater NO$_3$-N levels were 7, 16, and 29 ppm. Both Lake Wales and Frostproof are deep-sand Ridge locations; the difference is depth to water table: 20-21 ft in Lake Wales, 6-8 ft in Frostproof. In Manatee County, N application in 1997 was 200 lbs/acre to both plots (a mistake) but reverted to 200 lbs/acre for the high N plot and 100 lbs/acre for the low N plot in 1998. The result was very high groundwater NO$_3$-N (68 ppm) in the high-N plot, but the levels in both plots were variable (12-month averages: high N 19 ppm, low N 8 ppm). The Dade City, with 240 lbs/acre applied to both groves, the uphill grove had 0 ppm NO$_3$-N in the groundwater, the grove at the bottom of the slope had 6 ppm. There were problems getting yield records, Valencias in St. Cloud produced 6% less with low N, in Manatee County 9% less, and in Hamilns in Lake Wales 21% less. The only place where there was nitrate-N in excess of 5 ppm in seepage or drainage water was in Frostproof where water seeps out of grove on a slope well above the general water table. There the seepage water has the same NO$_3$-N level as the well water in the grove, indicating that depth to water table is a key factor determining ground water nitrate levels.

Funding:

FCPRAC Grant $ 31,626.00
USDA Contribution 40,000.00
Total $ 71,626.00

With the latest USDA salary adjustments, the entire grant is used to pay the salary of a GS-6 technician, Mr. Mark Bierman. The USDA contributes 20% of a GS-15 salary (the principal investigator's), 15% of a GS-7 salary (a laboratory technician's), motor vehicle use and equipment to collect samples, laboratory facilities and chemicals for analyses, and computer and fax use for recording and distributing data.

Tree Pruning Studies to Improve the Production and Harvesting of Florida Oranges

Project No. 951-23

Length of Project: 3-5 years

Investigators: J. D. Whitney , T. A. Wheaton , W. S. Castle , D. P. H. Tucker , J. K Burns , IFAS- Lake Alfred ; R. E. Rouse , IFAS - Immokalee

Abstract


11/21/2008
Controlling tree size and maintaining good fruit production have been a problem in some orange plantings, particularly in cases where vigorous scion/rootstock combinations have been planted at close tree spacings. Fruit production is usually high early in the life of these plantings, and then becomes marginal after the trees have reached containment size. Low fruit production not only reduces gross returns but further reduces net returns because the per box harvesting costs can be higher.

In recent years, tree skirting has become a more common practice, particularly in fruit destined for the fresh market. In the last 4 seasons, tree skirting has been necessary to accommodate new shake-catch mechanical harvesting systems. The effects of skirting on fruit yields and quality in Florida are not known.

**Objectives**

1. Determine the effects of hedging, topping, nutritional, irrigation, and skirting treatments on the subsequent fruit yields, fruit characteristics, and conventional harvesting rates of mature hedgerow orange trees with declining production levels due to close spacing and excessive vigor.

2. Determine the effects of skirting treatments for fresh fruit purposes and mechanical harvesting on the subsequent fruit yields and fruit characteristics of hedgerow orange trees which are approximately 10 ft tall.

**Summary of Accomplishments**

During this third year, the Hamlin/Sour hedging/topping/nutrition demonstration experiment showed no treatment effects on leaf analyses and subjective crop load and bloom ratings.

In the hedging/topping/skirting experiment in 15 ft tall Rohde Red Valencia/Carrizo trees, flat topping in the spring (as compared to the fall) increased juice brix and pound solids/box; flat topping at 12 ft reduced fruit juice content as compared to flat topping at 15 ft. Hedging and skirting effects on fruit yield were insignificant.

In the adjacent nutrition (nitrogen)/irrigation experiment in the same Valencia grove, reduced irrigation compared to the grower's irrigation program resulted in a 40% reduction in boxes and pound solids/acre, and lowered the 1998 bloom rating. Nitrogen rates (130 to 220 lb/acre) affected fruit juice content and pound solids/box with the 190 lb rate being superior.

Manual harvesting rates in the Valencia experiments above averaged 8.5 boxes/hour and increased 0.5 to 2 boxes/hour for every 100 box/acre increase in yield.

In the skirting experiment in 10-12 ft tall Valencia/Swingle trees, average fruit yields were not affected by skirting. The skirt fruit were smaller and had higher incidence of fresh fruit blemishes than fruit from the skirted trees.

**Funding:**

FCPRAC Grants $ 21,865  
IFAS Contribution: 25,000  
Orange Service Co. Pruning in-kind donation 8,500  
Total $ 55,365

**Effect of Adjuvants on Weed Control Efficacy of Glyphosate**

**Project No. 961-31**

Length of the Project: 3 years  
Investigators: Megh Singh IFAS - Lake Alfred

**Abstract**

Glyphosate has been used extensively for postemergence weed control in citrus for several years. The study investigates the improvement of glyphosate efficacy on hard to control weeds through the use of spray adjuvants. To date ten adjuvants have been evaluated in greenhouse and laboratory. Optima, Kinetic, and AgriDex greatly increased glyphosate efficacy at 0.5 lb/A in the tested weed species. Physico-chemical studies indicated that silicone-based adjuvants had a lower surface tension and a contact angle. No linear relationship existed between the physico-
chemical properties and herbicide efficacy.

**Objectives**

1. To study the effect of various new adjuvants on glyphosate efficacy.
2. To study the effect of adjuvants on glyphosate efficacy in difficult to control weed species.
3. To select and test several promising combinations of adjuvants with glyphosate in citrus grove based on the greenhouse results.

**Summary of Accomplishments**

Excellent progress was made in the past two years on the effect of adjuvants in weed control efficacy of glyphosate. To date, ten adjuvants have been evaluated in greenhouse and laboratory experiments. These included LI-700, Freeway, X-77, Impact, Agri-Dex, Dyne-Amic, Kinetic, Induce, Optima, and Silwet L-77. Two weed species, barnyardgrass and redroot pigweed were used in the experiment to represent grass and broadleaf weed species. All adjuvants except Silwet L-77 and Induce significantly improved glyphosate efficacy. Optima was found to be the most effective herbicide for enhancing the efficacy of glyphosate. Laboratory studies indicated that silicone based adjuvants had a lower surface tension and contact angle. Adjuvants reduced the surface tension and contact angle of the herbicide solution as well thereby enhancing their wetting properties. Field experiments will be conducted in the following year and additional adjuvants will also be tested during the next fiscal year. Recommendation to the citrus grower will be developed by the end of this project.

**Funding:**

FCPRAC Grant $34,000
IFAS/USDA 20,000
Other 6,000
Total $60,000

**Effects of Foliar and Broadcast P and K on Size and Quality of Grapefruit**

**Project No. 961-33**

Length of Project: 3 years

Investigator: B.J. Boman

IFAS - Fort Pierce

**Abstract**

A field experiment was initiated during the 1996/97 production year to investigate effects of increased N:K and N:P ratios with ground-applied fertilizers. The experiment is in bedded 'Marsh' grapefruit on sour orange rootstock block in Martin County with 4 replicates of each of 9 N-P-K. All plots receive 120 lb per acre of N and K₂O. Additional fertilizer in the form of muriate of potash and triple superphosphate is broadcast on selected plots to achieve P₂O₅ rates of 24, 104, or 184 lb per acre and K₂O rates of 120, 224 or 328 lb per acre. The effects of the fertilization programs on the trees is evaluated by fruit yields and fruit size, shape, and juice quality factors.

A second component was added in 1997/98 production season to evaluate the effects of supplemental phosphorous and potassium foliar applications on fruit size, shape, and internal quality for fresh citrus production. Foliar sprays of calcium nitrate, potassium nitrate, and monopotassium phosphate are being compared to untreated trees in commercial grapefruit blocks. Foliar applications are made at critical times (pre-bloom, post-bloom, and summer) to determine the role of timing on fruit response. Treatment differences are measured by leaf mineral levels, yield, juice quality, peel thickness, and fruit size and shape.

**Objectives**

1. Determine the effects of higher rates of ground-applied P and K on the size and internal quality of grapefruit grown for the fresh market in a modern citrus production system.

2. Evaluate the effects of supplemental phosphorous and potassium foliar applications on fruit size, shape, and internal quality for fresh fruit production.

Summary of Accomplishments

Additional soil-applied triple superphosphate had no effect on raising leaf P levels during 1996/97. In 1997/98, trees with the higher P rates and low K rate had higher leaf P. Leaf K was increased in 1996/97 with the additional KCl applications, averaging 1.11% for the 120 lb/ac/year rate and 1.25% for the higher K rates. In 1997/98, leaf K was directly related with the amount of K applied. In both seasons, leaf N was highest in the trees receiving the medium P rate. Little differences in any of the other leaf mineral concentrations were noted with respect to fertilization treatment. Peel thickness was least for the fruit receiving the low rates of both K and P.

Fruit from the high K plots had a slightly lower Brix:acid ratio. Little effect of fertilization rates has been evident on the juice content, Brix, acid content, or total solids. Highest yields have been on the medium P - high K and high P - medium K treatments. Fruit from trees that received medium or high K rate tended to have a higher percentage of fruit that were size 36 and larger.

Orlando tangelo trees that received 3 foliar applications of KNO₃ had a greater number of fruit that were > 64 mm in diameter (30%) compared to non-treated control trees (24%) during 1997/98. Valencia orange trees receiving 3 MKP applications resulted in a larger average diameter than control fruit. Trees with 3 KNO₃ or MKP applications had greater numbers of fruit and greater yield and solids than control trees in 1997/98.

Funding:

FCPRAC Grant $22,500
IFAS Contribution 27,000
Other grants 0
Total $49,500

Irrigation Best Management Practices for Florida Citrus on Vulnerable Soils

Project No. 961-36

Length of Project: 3 years

Investigators: L. Parsons, T. Adair Wheaton, IFAS - Lake Alfred

Abstract

The importance of irrigating a large area under a citrus tree canopy was demonstrated during the extended drought of April-June of 1998. Record heat accompanied a drought in which less than 1/4 inch of rain occurred in a 50-day period during May and June, 1998. Trees with full irrigation coverage under the canopy remained in good condition, but trees with only one quarter of the canopy area irrigated went into a permanent wilt. Several types of soil moisture sensors documented that water extraction from the soil was more rapid with the quarter coverage treatment. A computer program for scheduling irrigation is being developed and will be field tested during the coming year. Improved irrigation scheduling using either soil moisture sensors or the water budget program will reduce costs, conserve water, and reduce the potential for environmental degradation.

Objectives

1. Improve irrigation scheduling and reduce soluble nutrient leaching due to over-irrigation.
2. Determine effects of partial irrigation coverage on tree water uptake.
3. Measure water extraction from wetted and non-wetted zones under the tree.

Summary of Accomplishments

A replicated experiment involving partial coverage irrigation with microsprinklers demonstrated the importance of wetted area during the severe spring/summer drought of 1998. Treatments included wetting one quarter, one half, or the entire area under the canopy. Although water extraction...
rates were higher with partial coverage irrigation, trees with the smallest wetted area reached permanent wilt even with frequent irrigation. A small wetted area may suffice during periods with occasional rainfall that maintains some moisture in the non-wetted area. However, during an extended drought it is difficult to supply enough water to the tree when only a fraction of the canopy area is irrigated.

Tensiometers, resistance blocks, and capacitance sensors all provided a satisfactory basis for scheduling irrigation. The capacitance probe gives good detail on soil water movement and has verified models showing rate of movement to different depths. Both switching and transducer tensiometers were satisfactory for automatically scheduling irrigation. Soil moisture release curves were completed at several locations. These curves along with visual observations indicated that one should irrigate on the ridge at 8-10 cb during critical growth periods (flowering and fruit set), and at 15 cb during less critical periods. A computer program for scheduling irrigation by the water budget method is being developed and will be field tested during the coming year. Trees were planted in weighing lysimeters to accurately measure water use by young trees to validate the rate of evapotranspiration.

Funding:

FCPRAC Grants $48,395
IFAS Contribution 30,000
Other Grants --
Total $78,395

Enhance Activity of Pre-emergence Herbicides with Adjuvants

Project No. 961-37

Length of the Project: 3 years

Investigators: Megh Singh , R. S. Chandran , IFAS - Lake Alfred

Abstract

Effective preemergence herbicides are suspected to leach into groundwater because of their high soil mobility. Previous research indicated that adjuvants decreased the spread ability of these herbicides in soil. Forty-five commercially available adjuvants were screened for their efficacy to reduce herbicide leaching. The effective adjuvants, Monazoline-T, Monazoline-O, and E-17-2 were also studied for their effect on weed control upon tank mixing with the herbicide. Results indicate that these adjuvants reduced herbicide leaching significantly and that weed control was not affected as a result of tank mixing the adjuvant with the herbicide solution.

Objectives

1. Screen commercially available adjuvants to determine their efficacy to decrease leaching potential of the preemergence herbicides.

2. Determine the effect of tank mixing adjuvants with herbicide solution on weed control.

3. Optimize the use rate of adjuvants to reduce application costs while maintaining reduced herbicide leaching.

Summary of Accomplishments

Our research indicated that certain adjuvants, when added to a herbicide solution, resulted in reduced leaching of a herbicide in the soil. We tested forty-five commercially available herbicides for their relative efficacy to decrease herbicide leaching. Leaching columns filled with top four-feet of a typical sandy soil, were used to study herbicide (norflurazon) leaching as affected by adjuvants using two bio-indicator species (sugarbeet or barnyard grass). Of the adjuvants tested, Monazoline-T, Monazoline-O, and E-17-2 were chosen to carry out further tests based on the significant ability to reduce herbicide leaching. E-17-2 was found to be reduce herbicide leaching by approximately 50% even at a low rate use of 6.25% (vol/vol) of this adjuvant in the mixture. Higher rates of this adjuvant further reduced herbicide leaching but are yet to be tested in the field for application efficacy. Weed control was not affected even when the adjuvants were mixed with the herbicide in a 1:1 ratio. The top adjuvants will be field-tested during the next fiscal year to determine their effectiveness under field conditions.

Funding:

FCPRAC Grants $ 46,000
IFAS/USDA 25,000
Other 5,000
Total $ 76,000

Evaluation of Wild Perennial Peanuts for Use as Cover Crops in Citrus Groves

Project No. 971-03

Length of Project: 3 years


Abstract

Wild peanuts assimilate nitrogen from soil atmosphere and can deposit it into the soil through mowed foliage and from root dying and sloughing. It is estimated that from 50 to 150 lb/A/yr of N would be freed. Additionally, the most competitive and persistent species may permit reduction of the number of summer mowings which is a major management cost. Thirty five accessions (four replications) were planted and are being evaluated in each of five newly planted citrus groves, one on a ridge soil. Data collected include rapidity of plant spread, foliage density, and nut production; a larger bed area in three-year-old trees was harvested commercially. Some of the accessions manifest excellent attributes as well as drought and water tolerance. With proper nut seeding, bed coverage can be complete during one growing season.

Objectives

1. Evaluate 35 accessions of wild peanuts to select one or more that will provide the best persistence and weed competition.

2. Determine persistence, rapidity of plant spread, density of foliage, nut production, and N content.

3. Determine the optimum seeding rate to obtain complete coverage in one growing season.

4. Estimate the potential annual N released to compare with conventional management costs.

5. Estimate competitiveness and if mowings can be reduced.

6. Maximize nut harvesting procedures in young citrus plantings.

Summary of Accomplishments

At present, about 35 wild peanut accessions with four replications each, have been established (in rows perpendicular to tree rows) in five double bed recently planted citrus groves; one each in Martin and Highlands, and three in St. Lucie County. Soils range from low organic matter sand to finer textured soil to ridge soil (Highlands). One clean cultivated experiment was terminated and a similar one begun at the IRREC. Soil pH ranged from 5.8 to 8.2, and phosphorous, potassium, calcium, and magnesium levels ranged from 12-103, 2-82, 152->3765, and 4-200 ppm, respectively. A spacing experiment was begun to determine the minimum time for complete plot coverage when nuts were planted at 2 or 4 ft apart in a grid pattern.

Data obtained include nut production, plant establishment, plant spread, foliage density, and drought tolerance. Nut production ranged from 0 to 486 per 2 by 2 ft square quadrant. Natural hybrids produce few or no nuts. Twenty two Arachis Pintoi (pinto peanut) accessions produced between 38 and 486 nuts, while A. stenosperma and A. Kretschmeri produce from 126 to 166 (sandhill cranes attacked these plots eating many of the nuts produced). Another set of these data will be obtained in the fall of 1998. In four groves, peanut plant spread (perpendicular to original planted row) was as high as 18 ft (from 16 June 1995 to 7 March 97, or about 1.3 years); 11 ft (from 8 Aug 95 to 5 Sep 96, 1.1 years); 5 ft (from 8 May 97 to 1 Dec 97, 0.6 years; and 5 ft (30 July 97 to 13 Nov 97, 0.3 years). Most rapid spread occurred with A. stenosperma and several pinto accessions; while foliage density of the former decreased in the fall and winter pinto types maintained excellent foliage density.

Nuts were mechanically harvested in April 1997 from three beds (planted in 1995) with tree rows 20 ft apart. Yield was about 600 lb/A for the 0.4A of harvested area. After harvesting a light rain caused excellent germination of remaining nuts, with seedlings observed to be 2 to 4 inches apart. In spite of the continuing drought for the next month, an excellent plant population remained (July).
Funding:

FCPRAC Grant $ 5,764
IFAS Contribution 32,500
Other - 0 - Total $ 38,264

**Yield Reduction of Citrus from Overcrowding-Economic Evaluation and Management Strategies**

**Project No. 971-52**

Length of Project: 2 years

Investigators: R. P. Muraro, IFAS-Lake Alfred
T. H. Spreen, P. Koukos (Graduate Student), IFAS-Gainesville
F. M. Roka, IFAS-Immokalee

**Abstract**

At least one-half of Florida's current 853,000 acres was planted since 1980 with an average tree density of approximately 150 trees per acre. Much of this acreage has produced well; however, yield in some blocks has declined substantially as trees matured and hedging and topping were begun to control tree size. We do not know how extensive this yield decline is nor how it is related to tree spacing, tree age, scion/rootstock combination and management practices.

Growers have raised concern with the hedgerow yield reduction problem and what will be the impact on future production and revenues. Some questions being asked are "what is the extent of hedgerow yield reduction problem within the citrus industry" and "how can we evaluate the economic impact of specific corrective management strategies that can be employed". Currently, Florida's citrus industry does not have sufficient data to evaluate either of these questions.

**Objectives**

1. To establish the relationship between tree age, rootstock, scion and tree spacing on per acre yields.
2. To use the information from Objective #1 to improve projections/forecasts of future citrus production in Florida.
3. To develop a computerized decision-aid to improve decision making in the management of high density planted citrus groves.

**Summary of Accomplishments**

After a review of the literature on high density, hedgerow plantings, growers were contacted to provide yield data. Hamlin and Valencia oranges were targeted for initial data collection from hedgerow groves planted from the 1980s. Swingle and Carrizo are the two primary rootstocks; however, several blocks on Volkameriana, Rough Lemon and Cleopatra were also selected. To date, yield data from 54 blocks have been compiled with data from another 27 blocks being provided by grower participants. The goal is to have yield data from 100 blocks by the fall of 1998. Currently twenty citrus growers/firms are providing data for this study.

Initial grower interviews/discussions, have indicated that preplant evaluation of scion/rootstock combination along with soil type on which the trees are to be planted is most important and can greatly affect tree growth and yield potential. With the exception of very close spacings, the initial information collected indicates that tree spacing between rows may be more important than spacing down the rows on delaying the effects of tree crowding on reduce yields. Although most of the tree densities of the blocks range between 140 to 150 trees per acre, several of the blocks have tree densities exceeding 200 trees per acre.

Along with the collection of yield data, work has begun on developing computer decision models for growers to evaluate high density plantings. The goal is to provide growers with computer "spreadsheet" analyses so they can evaluate "what if" scenarios on tree removal and/or replanting strategies to maintain a high production level.

**Funding:**


11/21/2008
Development of Decision Information Systems for Florida Citrus

Project No. 971-55

Length of Project: 2 years

Investigators: IFAS-Gainesville IFAS-Lake Alfred J. D. Martsolf L. G. Albrigo R. M. Peart W. S. Castle

Abstract

Making decisions about the best production practices, their timing and integration of options, has become very complex. Growers can use help-guides to effectively consider all of the options. Expert systems tied together on the basis of vegetative and fruit development stage can help to clarify and suggest timely options, provide production practices record keeping and free up time for other management decisions. Several units for the eventual integrated decision support system for citrus were developed to the stage of functioning prototypes during this first year of work.

Objectives

1. Develop and demonstrate a Preplant (rootstock selection) Decision System.
2. Develop and demonstrate a Melanose and Greasy Spot Control Decision System.
3. Develop a framework for the determination of timing and options among production practices in relation to citrus growth events to facilitate management decisions.
4. Develop a tree size and yield diagnostic system to be used as a baseline for comparing tree growth and yield.
5. Integrate these various applications within a user-friendly operating system.
6. Collaborate in the production of DISC CD-ROM Version 1.0 using graphics design to improve the interface.

Summary of Accomplishments

First or second versions of a preplant decision module for rootstock selection and a Cu residue decay system for disease control were developed. These will be ready for grower testing this coming year. A prototype for the production decision framework based on stages of vegetative and reproductive growth was developed. It includes components of tree development, production practices and will integrate climatic effects on time of bloom and flowering intensity. The prototype can operate by grove and block for record keeping and brings in GPS mapping. This prototype should be ready for testing by the end of the second year. Several growers are cooperating in the integration of production practices, appropriate record keeping and user-friendly requirements. Data collection for the tree size and yield graphs by rootstock, scion, age and district is underway. Version 1.0 of a CD-ROM has been printed, but more work is necessary prior to full release.

Funding:

FCPRAC Grant $35,000.00
Agency* Contributions 41,000.00
Grower Contributions-Time 10,000.00 Other Grants

- 0 - Total $86,000.00

* Ten percent of 1 faculty, five percent of 3 faculty and 1 percent of 4 faculty members plus 5 percent of 4 growers time. Other infrastructure support was supplied at two locations of the University of Florida.

MANAGEMENT/PHYSIOLOGY

Towards Improving the Efficacy of Nutrient and Water Use Under Fertigation for Young Citrus Trees in Deep Ridge Soils

Project No. 928-33

Length of Project: 5 years (Fifth and Final Year)

Investigators: A. K. Alva, IFAS - Lake Alfred

Abstract

Improving the efficacy of nutrient and water use by young citrus trees is aimed to attain optimal tree growth and fruit yield and minimize NO₃ leaching below the rootzone. This project is part of the major research program of developing N Best Management Practice (N BMP) for orange and grapefruit trees.

Objectives

1. To evaluate the effects of various N sources and rates on growth, and leaf mineral concentrations of young orange trees in a sandy soil under optimal irrigation regime.

2. To evaluate the effects of two N rates, as fertigation, with three different wetting areas on growth, and leaf mineral concentrations of young orange trees.

3. To evaluate the potential leaching of NO₃-N below the rooting depth under various N rates, sources, and wetting areas.

4. To improve the precision of irrigation scheduling for citrus using capacitance based soil moisture sensing probes.

5. To develop soil water budget using on-site soil water measurements and to calculate evapo-transpiration.

Summary of Accomplishments

Two field experiments were conducted with 4-year-old Hamlin orange trees on Swingle citrumelo rootstock in a Candler fine sand to evaluate the tree response, and potential leaching of N below the rooting depth under various N management practices with optimal irrigation scheduling. Since the differential N management programs began from the year of planting, it is critical to evaluate the effects of these treatments on 3 to 4 years of fruit production. At a given N rate fruit production was greater with fertigation compared to the other sources. Fruit production increased with an increase in N rate: (i) from 70 to 120 lb N/ac/year for all sources, (ii) from 120 to 170 lb N/ac/year only for fertigation. Further increase in N rate to 220 lb N/ac/year decreased the fruit production for all sources. In the second experiment, fruit yield increased with an increase in emitter wetting area diameter from 5 to 10 feet/tree, but decreased at 15 feet wetting area diameter. An increased leaching of water and nutrients below the rooting zone at the 5 feet wetting area and application of water and nutrients outside the area of maximum rooting appear to be responsible for reduced fruit yield. The data from continuous monitoring of soil moisture content (capacitance probes) were used to develop soil water mass balance (1997; with 57.8 inch annual rain and 16.8 inch irrigation). The cumulative ET for that year was 37.3 inch while the cumulative transport of water below the top 12 inch rooting zone of the soil was 35.0 inch. The daily ET varied from 0.4 mm/d in January to 5 mm/d in July and August.

Funding:

FCPRAC Grants $30,774

UF/IFAS Contribution* $13,100

Total $43,874

*Accounts for 10% and 15% of time by the PI and Technician, respectively, and percentage of time for analytical services in the PI's laboratory.

Foliar Urea, Phosphorus and Potassium Applications in Citrus Production: Their Role in Flowering and Fruit Set

Project No. 961-42

Length of Project: 2 years

Investigators: L. G. Albrigo, J. P. Syvertsen, IFAS - Lake Alfred

Abstract

The overall objective of this research is to continue investigations of potential benefits from the winter application of foliar urea or phosphorous acid on flowering and spring foliar additions of phosphorus and potassium to urea on fruit set in Florida citrus production. Multiple year applications are required to determine if alternate bearing or off-years occur from continuous use of foliar nutritional sprays.

Objectives

1. Determine uptake and stress responses of different urea N sources and potential P and K sources for foliar uptake.
2. Study further the role of winter foliar urea and phosphorous acid sprays in enhancing winter time flower induction and yields.
3. Determine if foliar sprays at bloom time of urea (N source), P and K can increase fruit set and yields of citrus.

Summary of Accomplishments

Foliar applications of urea and phosphorous acid in winter increased yields of Valencias in 3 of 4 years as did N, P, K applications at bloom plus postbloom. These treatments did not increase alternate bearing tendencies in Valencia. The highest response was a 25% increase in boxes of Hamlin oranges receiving 25, 15 and 15 lbs N/ac as urea in winter, bloom and postbloom with 7.5 lbs/ac of P and K in each of the last two sprays. Leaf N and K increased, but P did not. P as polyphosphate (form used) either was not a suitable source for foliar application or P is mobilized out of the leaves rapidly. Based on fruit levels of N, P and K, the P requirements during this competition period may be fairly low compared to N and K. Ambersweet did not respond to bloom or to postbloom foliar N, P, K. Phytotoxicity from foliar nutritional sprays was minimal unless tank-mixes with additional chemicals were applied. Biuret toxicity was observed, particularly by growers, several times over the past 4 years. Better spray grade urea labelling is necessary. Funding has been obtained to study acceptable biuret levels and uptake of P from various chemicals. These FCPRAC supported studies have convinced many growers to institute N, P, K foliar sprays as a management practice for increased yields.

Funding:

FCPRAC Grants $ 13,140.00

Agency* Contribution 19,000.00

Other Grants 10,000.00

Total $ 42,140.00

* Twenty percent of 1 faculty and one technician position

How to use a sprayer for different volume rates?

Project No. 971-32

Length of Project: one year

Investigator: M. Salyani, IFAS - Lake Alfred

Abstract

The purpose of the project was to identify the most efficient way for changing the volume rate of the citrus sprays. The research involved 33 treatments, using different combinations of nozzle disc and core sizes, number of nozzles, and ground speed to obtain volume rates of 66-546 gpa. Spray deposition was sampled at 2 heights and 4 distances from the sprayer. The amount of deposit was determined by colorimetry. It was concluded that low volume rates should be achieved by reducing the number of nozzles and using small nozzle disc and core sizes, rather than spraying at high ground speeds. On the other hand, high volume rates should be achieved by increasing the number of nozzles and spraying at


11/21/2008
higher ground speeds, instead of using large disc and/or core sizes. Medium volumes were not sensitive to any of the variables.

Objectives

1. Determine the effects of nozzle size (disc and core), number of nozzles, and ground speed on deposition efficiency and examine the interactions.

2. Identify the best way to change the spray volume rate of citrus sprays in airblast applications.

Summary of Accomplishments

An airblast sprayer was operated with 1, 2, or 3 nozzle sets (6, 12, or 18 nozzles), 13 disc-core combinations, different operating pressures, and ground speeds of 1, 2, or 3 mph, to obtain volume rates of 66-546 gpa. The project involved 33 sprayer treatments with 4 replications. Spray mixtures, containing Kocide DF (40% Cu), were applied to citrus tree shoots positioned at 6 and 12 ft heights and 4 distances of 5, 9, 13, and 17 ft from the centerline of the sprayer. Four to 6 leaf samples were collected from each target shoot at each sample location. Copper deposit was quantified by colorimetry, and the leaf area was measured with an Area-meter. The treatments were compared in terms of deposition efficiency (DE), i.e., g/cm² of copper deposit per mg of copper discharged during one cm of sprayer travel. The project involved the following experiments:

1. Effects of the nozzle disc size, number of nozzles, and ground speed on deposition efficiency (9 treatments). With small nozzles, DE decreased as number of nozzles and ground speed increased. The trend was reversed for large nozzles, and significantly higher DE was observed with higher number of nozzles at higher ground speeds. Deposition efficiency was not affected significantly with mid-size nozzles.

2. Effects of the nozzle disc size, core size, and number of nozzles on deposition efficiency (6 treatments). With the small disc size, increasing the core size and reducing the number of nozzles gave better DE compared to increasing the number of nozzles and using smaller core sizes. With mid-size discs, comparable DEs were achieved for all combinations.

3. Effects of the nozzle disc-core combination and sprayer ground speed on deposition efficiency (18 treatments). Mid-size discs gave higher DEs compared to larger discs. Increasing the core size decreased the DE, while increasing the ground speed increased the deposition. The effect was more pronounced at high volume rates and at locations nearer to the sprayer. From the above it was concluded that low volume rates should be achieved by reducing the number of nozzles and using small nozzle disc and core sizes, rather than spraying at high ground speeds. On the other hand, high-volume rates should be achieved by increasing the number of nozzles and spraying at higher ground speeds, instead of using large disc and core sizes. Deposition efficiencies of medium volumes were not sensitive to any of the variables.

Funding:

FCPRAC Grants $ 12,000
IFAS Contribution 22,000
Other -0-
Total $ 34,000

Weathering Of Copper Fungicides Used for Greasy Spot Control

Project No. 971-54

Length of Project: 1 year

Investigators: L. G. Albrigo, L. W. Timmer, K. Townsend, IFAS - Lake Alfred

Abstract

Copper fungicides used for melanose and greasy spot control and some other diseases of citrus are often applied in excessive amounts but control may still be inadequate. This project is designed to evaluate safe levels and frequency of Cu application on fruit and leaves for control of greasy spot. The desired result is to maintain adequate Cu residues on leaves and on fruit during dilution from fruit growth and weathering (rain and wind) without excessive use of Cu fungicides that can cause phytotoxicity and soil contamination.

Objectives
1. Determine typical deposits and distribution on fully expanded leaves and medium expanded grapefruit for summer greasy spot control. Two or 4 lb Cu/ac at 25 or 125 gal/ac will be compared with oil at 5 or 1 gal/ac with the low and high Cu rates, respectively.

2. Determine weathering losses of summer applied Cu deposits on grapefruit fruit and leaves and orange leaves.

3. Use a spectrophotometric method (field portable) to determine uniformity of Cu deposits on fruit (front versus back) and leaves (top vs bottom) and to determine the period of time that these deposits are adequate for fungal control.

4. Determine fruit grade and degree blemishes in Cu test blocks are accentuated or caused by excessive Cu.

Summary of Accomplishments

The use of 2 or 4 lbs/ac of Cu product with oil at 5 or 1 gal/ac, respectively, sprayed in 25-30 or 125 gal/ac for greasy spot control minimized spray burn at 2 locations over 2 years of tests. Growers still report spray burn for the early summer spray timing when oil rates are increased with higher Cu rates. In experimental tests, Melanose and greasy spot ratings were good. Export grade fruit ranged from 21% for 4 lbs Cu at 25 gal spray volume in one grove to 53% for 4 lbs at 125 gal in the second grove. Poor export grade at 25 gal/ac reflected slight spray burn. Export plus #1 grade fruit averaged 81.5% for the 125 gal/ac sprays and 78.5% for the 25 gal/ac sprays. Cu deposits decreased primarily due to fruit expansion with only about 30-40% total loss due to weathering. Cu residues can be monitored in the field with a portable spectrophotometer. Data has been summarized and reported in two 1997-98 publications. This data was used to develop a Cu residue decay model for the DISC project.

Funding:

FCPRAC Grants $11,000.00

Agency* Contribution 19,000.00

Other Grants 6,000.00

Total $ 46,000.00

* Twenty percent of 1 faculty and one technician position

PATHOLOGY

Continuing Projects

Development of Methods to Manage Citrus Tristeza Virus

Project No. 928-74

Length of Project: 5 years

Investigators: W. O. Dawson ,S. Gowda ,D. J. Lewandowski , IFAS - Lake Alfred

Abstract

Some citrus tristeza virus (CTV) isolates cause severe disease while others cause no disease. We now know that CTV isolates in Florida are complex mixtures of CTV-like viruses and defective RNAs. It is important to determine which one is the "enemy" that citrus must be protected against. The overall goal of this project is to develop a genetic system that will allow the determination of what components of CTV isolates are responsible for decline and stem pitting diseases. This information will allow development of "ideal" mild strains to use to cross protect existing citrus planting and production of genetic engineered plants resistant to CTV for the next generations of trees. This is the fifth year of the first phase of this project.

Objectives

Year 1 -- Cooperate with other laboratories to obtain cDNA clones and sequence the entire genome of CTV strain T-36. (completed)

Year 2 -- Assemble CTV cDNA clones into a series of full-genomic cDNAs from which infectious virus can be recreated. (completed)
Year 3 -- Development methods to infect plants from CTV cDNA clones.

Years 4 and 5 -- Determine how CTV causes disease in citrus by creating and examining hybrids between severe and mild isolates and mapping disease causing sequences.

**Summary of Accomplishments**

In the first phase of this project, we developed the necessary genetic systems to allow the research to develop methods to manage citrus tristeza diseases. We completed sequencing the entire genome of the major sequences of a decline CTV isolate (T-36) and a mild CTV isolate (T-30). Both have been assembled a series of full-genomic cDNAs from which RNA transcripts can be produced in the test tube to infect protoplasts. This completes the most difficult component of our project. This now allows us to identify sequences of the virus that cause disease and cross protection. We are making sequence exchanges between severe and mild sequences in an effort to create "ideal" cross protecting strains. We are racing to have protecting isolates available to protect against any new severe isolates that begin threatening Florida citrus and to allow use of the sour orange rootstock wherever needed.

At the same time, we are characterizing the makeup of CTV populations. We found that CTV isolates are complex mixtures of CTV-like viruses and defective RNAs. Strategies to manage CTV diseases must be directed against specific parts of the population. Thus it is necessary that we understand which parts of the population cause diseases. We have accomplished all of the initial requirements to custom design cross-protecting isolates of CTV in the next five years to manage any CTV diseases that threaten Florida and to allow the use of sour orange rootstocks, even in the presence of decline isolates of CTV.

**Funding:**

FCPRAC Grants $ 44,930

Agency Contribution 54,000

Other Grants 97,000

Total $ 195,930

**Determination of Inoculum Levels and Environmental Factors to Predict Severity and Improve Control of Fungal Diseases of Citrus Fruit and Foliage**

**Project No. 961-04**

Length of Project: 3 years

Investigators: L. W. Timmer, J. H. Graham IFAS-Lake Alfred; T. R. Gottwald, USDA - Orlando

**Abstract**

Fungal diseases of fruit and foliage reduce yields and the external quality of fresh market fruit. We proposed to develop methods to assess inoculum levels for greasy spot, Alternaria brown spot, Phytophthora brown rot, citrus scab, and melanose prior to the infection period to determine the potential for disease development. The effect of environment on spore production, spore release and dispersal, and infection of the plant are being determined. These data will be used to develop predictive models for disease incidence and severity. This information will be useful in modifying cultural practices to reduce disease severity and as a basis to eliminate unnecessary fungicide applications and to improve the timing and effectiveness of applications made.

**Objectives**

1. Develop methods to quantify inoculum levels and determine the conditions under which inoculum is produced.

2. Determine the environmental conditions under which spores are released and dispersed. Determine the environmental conditions necessary for infection and disease development. Develop models which incorporate environmental factors and inoculum level to predict disease and determine the need for and proper timing of fungicide applications and develop cultural methods to reduce inoculum production and improve disease control.

**Summary of Accomplishments**

With greasy spot, infection of trap plants and numbers of ascospores peaked in Mar-Apr following freeze-induced leaf drop in January 1997 in...
In Immokalee, high levels of infection occurred in Jul-Aug despite low numbers of ascospores. Infection decreased in Oct, but increased with high rainfall in Nov-Dec despite cool temperatures. Ascospore numbers were closely related to amounts of leaf litter.

With Alternaria brown spot, a preliminary model has been developed based on laboratory studies of temperature and leaf wetness. Infection of trap plants placed in the field weekly demonstrated the seasonal patterns, but was not strongly related to spore numbers, rainfall, leaf wetness, or temperature. Infection of trap plants placed in the field daily was related to rainfall totals and hours of leaf wetness, but not to temperature.

With Phytophthora brown rot, the optimum temperature for infection of fruit was 80-86°F and less than 3 hr of wetness were needed. In contrast, the optimum temperature for sporangium production was 70-75°F. Up to 24-36 hr were needed for optimum sporangium production.

Twigs affected by melanose were cut each month and suspended in mesh bags in the tree canopy. Dead twigs began to produce spores in 2-3 months and continued to produce for 8-10 months depending on temperature and rainfall. The optimum temperature for scab infection was 75-80°F. Periods of wetness less than 2 hr produced some infection, and severity increased up to 24 hr of wetness. A model is being developed based on laboratory data.

**Funding:**

FCPRAC Grants: $ 49,000

Agency Contribution: 85,000

Other Grants: -- Total $134,000

**Studies to Determine the Cause and Develop Strategies to Control Citrus Blight**

**Project No. 961-17**

Investigator: K. S. Derrick, CREC - Lake Alfred

**Abstract**

The goal of this project is to develop strategies to control citrus blight. Some groves are devastated by blight while others remain free of the disease for many years. This indicates that if the cause and methods of spread of blight were known procedures could be developed to maintain groves relatively free of the disease. Research will continue on the characterization of the blight protein p12, which is the protein used for the monoclonal antibody assay for blight detection. The gene that produces p12 has been isolated and sequenced. We propose to determine the effect the protein has on the expression of blight symptoms. For example, does p12 help the tree resist blight or does it induce symptoms? A complex of microorganisms can be detected in citrus trees by analysis of genomic fingerprints using PCR with arbitrary and consensus tRNA or rRNA primers. We have experiments in progress to determine if one of these microorganisms could be the cause of blight and if beneficial microorganisms are present in old-line trees making them more resistant to blight in comparison to trees from nucellar budwood.

**Objectives**

1. To determine the cause of citrus blight.

2. To determine the function of the blight protein p12.

3. To develop strategies to control citrus blight.

**Summary of Accomplishments**

The experiments to determine the effect of old-line budwood on the incidence of citrus blight were expanded by additional propagations of trees using old line, nucellar and shoot-tip grafted Valencia budwood on rough lemon rootstock. Experiments to identify microorganisms in blighted, healthy and old-line budwood trees using molecular techniques were continued; we have obtained the cDNA sequence of tRNA and 16s RNA of various bacteria found in trees with blight and are attempting to determine if any of these bacteria are associated with blight. Studies on the function of blight associated protein p12 were continued. We have shown that p12 is similar to expansins, which are proteins involved in cell enlargement, but the function of p12 in trees with blight is not known. Transgenic plants of tobacco and rough lemon citrus were made to produce p12 and to block the expression of the gene. These plants will be evaluated to determine the effect that p12 has on the physiology and stress resistance of the plants.

**Funding:**

Studies on a Virus Isolated from Trees with Citrus Blight

Project No. 961-18

Length of the Project: 3 years

Investigators: R. H. Brlansky, IFAS - Lake Alfred

Abstract

Citrus blight continues to be one of the most economically important diseases of Florida citrus. The cause of blight appears to be caused by a graft transmissible agent. The identity of the agent is essential since control measures will ultimately depend on the detection of the pathogen in diseased citrus materials. We have been studying a rod-shaped virus isolated from blight-affected trees. This project investigates this virus and its presence in blight and healthy citrus trees in Florida. The main objectives of this project are:

Objectives

1. Continue isolation of the virus from blighted trees and characterize it. (Year 1, 2)

2. Prepare and test antibodies for detection from blight-affected trees. Accumulate detection data to determine if a relationship exists between the presence of this virus and citrus blight. (Year 1, 2, 3).

3. Prepare cDNA probes, sequence the viral nucleic acid and develop a PCR detection procedure. Compare the PCR data with the serological detection data for efficacy and sensitivity and correlate with the blight protein test. (Year 1, 2, 3). Inoculate citrus trees to determine if this is the cause of citrus blight. Continue to test other plants as alternative hosts. (Year 1, 2).

Summary of Accomplishments

During the second year of this project we have continued successfully isolating a virus from blight-affected trees. Isolations have been from both roots and shoots of blighted trees. In the past we isolated only from large roots. Virus also was isolated from several healthy appearing trees that tested negatively for blight. Some of these trees have recently developed blight. We continue to observe the other trees. The virus was successfully used to make antibodies in rabbits. Antibodies, produced in warm blooded animals in response to viruses are useful for detection of viruses in plants. The antibodies were shown to be specific for the virus (did not react to citrus tristeza virus). These antibodies are being used in ELISA test to detect the virus in diseased trees.

More inoculation tests with the virus were done this year using healthy 3½-4 year old Hamlin/Rough lemon trees. To date no symptoms have appeared. The Hamlin/Carrizo trees inoculated in the first year of this work continue to die back, flush and then die back again. Dieback is most evident on flush that is produced after older growth is cut back. These trees are currently being tested with the ELISA test to determine if the virus is present. The expected results from this work are to generate data on the identification of this unknown virus, to develop methods to detect it and to determine its relationship to the cause of citrus blight. The key to controlling citrus blight is identification of the causal agent.

Funding:

FCPRAC Grants: $ 37,600
Agency Contribution 42,510
Other Grants --
Total $ 80,110
Development of a Rapid Method to Determine the Decline-inducing Potential of Tristeza (CTV) and Other Virus-like Diseases.

Project No. 961-28

Length of the project: 3 years

Investigators: M. Bausher, R. Niedz, S. Garnsey, USDA-Orlando

Abstract

Why and how certain CTV strains cause citrus grafted onto sour orange to decline is a problem that has not yet been answered. Biological indicators such as Key lime tests can give an indication of the presence of strains of the virus both mild and severe, no current technology can predict that a particular strain of CTV will, in fact, cause severe decline in a tree. This is particularly true in field situations. An efficient biological test will help determine the potential of current and new strains of CTV to cause physiological decline due to the incompatibility of the scion with the rootstock.

Objectives

1. Produce small trees and inoculate immediately to greatly reduce the time until symptom expression.

2. Compare and determine the most advantageous conditioning for initiation of severe decline.

3. Identify decline-based metabolites which may be the agents of the incompatibility of sour orange to scions affected by CTV.

4. Use the above information to identify scions and rootstocks which are resistant to CTV and facilitate the possible reestablishment of rootstocks which are susceptible to CTV decline.

Summary of Accomplishments

We have made progress in the collection of the phloem constituents and are in the process of determining their identities. Currently we are collecting phloem constituents from healthy, mild, and severe isolates of CTV. Preliminary experiments that utilize crude plant extracts for the recovery of the enzyme polygalacturonase (PG) to determine the system's suitability for recovery of possible active components which were enzymatic. We successfully recovered enzymatic activity and were able to identify this enzyme from its sequence. The recovery of the individual components from phloem material is quite low, so the use of trapping agents which utilize reverse phase bonding have greatly increased the concentration of the collected components.

One problem with testing of field grown material is the presence of contaminating microorganisms which can cause graft failure. By using a series of biocides, contamination has been nearly eliminated, thereby allowing microbudding of field grown material and further testing of the micrografting technique. A simple test for the presence of CTV severity has evolved from these experiments. By excising the dormant buds from citrus and placing them in a tissue culture vessel the ensuing growth can differentiate between healthy, mild, and severe isolates during the winter periods.

Inducing the quick decline in citrus by varying the environmental condition was disappointing because of the failure to maintain temperature in the environmental chambers due to mechanical failure. These chambers have been repaired and we will attempt these experiments in the near future.

Funding:

FCPRAC Grants $ 30,000

IFAS Contribution 150,000

Other Grants -0-

Total $ 180,000

Mild Strain Cross Protection to Protect Against Citrus Tristeza Virus for Florida Citrus

Project No. 961-47

Investigators: R. F. Lee, IFAS- Lake Alfred

Abstract

Mild strain cross protection (MSCP) is defined as the ability of a mild strain of a virus to prevent the expression of a second, more severe strain of the same virus in the same plant. MSCP will be an important management strategy for tristeza (CTV) now that the brown citrus aphid (BrCA) is in Florida. The overall objective of this project is to improve MSCP for citrus in Florida. The interactions of CTV “populations” and strain interactions between protecting and non-protecting strains will be studied. A concentrated effort will be made to improve MSCP in grapefruit. Information developed from this project should enable better application of MSCP for control of CTV and permit quicker selection of mild strains for use for MSCP against new severe, stem pitting strains of CTV which will arise and spread in Florida in the next decade due to the presence of the BrCA.

Objectives

1. Determine the role of CTV “populations” in mild strain cross protection (MSCP). Define the population variation within severe strains of CTV which can cause damage and mild strains used for cross protection.

2. Study strain interactions between cross protecting mild strains, non-cross protecting mild strains, and severe challenge strains of CTV.

3. Continue to characterize, screen and evaluate additional mild strains of CTV for MSCP with emphasis on mild strains for use in grapefruit.

Summary of Accomplishments

Excellent progress has been made during the first two years of this three year project. From the many dozens of CTV isolates collected for evaluation for mild strain cross protection (MSCP) potential, 19 isolates remain which look promising based on preliminary biological tests as well as my probing with several molecular procedures to determine presence of mild and/or severe strains. Seven isolates have been selected for further evaluation in replicated MSCP tests. The isolates were chosen based on demonstrated potential in preliminary trials, considering the desire to find isolates for MSCP in grapefruit which have originated from grapefruit, and locations in the state. The MSCP tests include Val/Swingle, Hamlin/smooth flat Seville, and grapefruit seedlings; challenge isolates include Meyer lemon isolates and other stem pitting isolates which have been found in Florida. Population specific probes are being prepared for region near the 5’ end of these selected mild and severe isolates. Interestingly, four of the seven mild isolates selected for further evaluation were collected from trees which survived the Florida freezes of 1885-96. Work is continuing on sequential single aphid transmission (SSAT) of all 19 isolates which show some promise for MSCP, and field collection on new isolates will continue. Evaluation of sub-isolates resulting from SSAT indicate segregation of CTV isolates which differ in biological and molecular characteristics. The data the evaluation of MSCP experiments established in Beltsville suggests that rootstock usage may influence efficiency of MSCP whereby rootstocks which are good hosts for CTV to not enable MSCP while rootstocks such as sour orange or citranges enhance MSCP.

Funding:

FCPRAC Grants $ 56,000

IFAS Contribution 60,000

Other Grants 41,000

Total $157,000

Evaluation of Decline and Stunting Problems in Citrus Trees on Swingle Rootstocks

Project No. 971-11

Projected length of Project: 4 yr.

Investigators: S. M. Garnsey, H. K. Wutscher, K. D. Bowman, USDA - Orlando

Abstract

Some citrus trees on Swingle rootstocks have failed to grow normally, and others have declined following initial normal growth. The causes for these problems are unknown, and they could be associated with use of off-type or misidentified rootstocks, budunion incompatibilities, or infection by viruses or viroids. The objectives of this project are to investigate the possible causes of stunting and decline syndromes in trees on...
Swingle and determine if these problems are associated with virus or virus-like pathogens or have non-infectious causes. The information developed will help growers evaluate the current problems on Swingle and avoid or control them in the future. FCPRAC funding provides technical help and supplies essential for this investigation.

**Objectives**

1. Determine location, extent, and distribution of trees on Swingle with stunting or decline problems.

2. Determine associations with specific soil conditions, possible use of off-type or misidentified rootstocks, or with specific scions or budwood sources.

3. Determine if a graft-transmissible agent is involved and look for new virus and virus-like pathogens that may cause stunting or decline in Swingle.

4. Determine more precisely the effects of known viruses and viroids in trees grafted on Swingle.

**Summary of Accomplishments**

Contacts were made with growers, production managers, extension personnel and scientists to locate groves with problems. We visited 18 different locations and the problems observed can be grouped into three general categories: 1) trees declining with a budunion incompatibility, 2) trees that are stunted, and 3) trees that are declining from undetermined causes. We also investigated a newly reported bark scaling problem in grapefruit that occurs on several rootstocks, including Swingle. Tissue samples from over 50 trees were collected and established in the greenhouse. Tests were run for viroids, tristeza, and tatterleaf on selected samples. We did not find tatterleaf in any samples tested, and could not correlate viroid or CTV infection specifically with the stunting, decline, or bark scaling problems encountered. A decline of trees of Roble sweet orange on Swingle was observed in multiple locations which is associated with a genetic incompatibility that appears from 4 to 10 yr. after planting. Analysis for 14 elements in plant tissue and 10 elements in soil extracts made at 10 locations did not show a consistent correlation between stunting and soil or nutrition factors. Some, but not all, grapefruit trees with bark scaling had high levels of chlorides in the wood. Blight was found in two locations, but several decline situations remain undetermined. A paper describing the budunion incompatibility between Roble and Swingle is in preparation. Talks on decline and stunting problems will be given at the Citrus Expo in Ft. Myers and the FACTS Conference in Lakeland.

**Funding:**

FCPRAC Grants $20,000

USDA Contribution 27,500

Total $47,500

**Development of Detection Methods for Citrus Psorosis Virus and Use of the Virus as a Vector to Express Foreign Genes in Citrus**

**Project Number: 971-43**

**Length of Project: 3 years**

**Investigator: K. S. Derrick ,CREC - Lake Alfred**

**Abstract**

There is a need for methods for rapid indexing of citrus budwood for psorosis. Indexing for psorosis is now done by graft inoculation of citrus seedlings and observing leaf symptoms, which can be transitory and very mild. Also, there are some isolates that apparently do not induce any leaf symptoms, and bioindexing for psorosis is time consuming and subject to considerable error. Psorosis is one of the few remaining viruslike pathogens of citrus that cannot be indexed by nonbiological methods. We characterized an unusual spiral shaped virus, referred to as a spirovirus, that is associated with psorosis. We have had numerous requests from regulatory agencies and diagnostic laboratories for nonbiological methods for detecting this virus for use in citrus clean stock programs. This project is designed to meet that need. In addition, the virus associated with psorosis has some unusual properties that make it an ideal choice for use as a vector for expressing foreign genes in citrus. The most obvious advantage of using a virus vector for citrus is that foreign genes can be expressed in existing trees.

**Objectives**
1. To develop rapid detection methods for citrus psorosis virus (CPV).

2. To develop a virus vector for expressing foreign genes in citrus.

Summary of Accomplishments

The spiroviruses (SV) found in citrus and associated with citrus psorosis are serologically and biologically diverse. In addition, the uneven distribution and often low concentration of SV in citrus makes their detection difficult. We have had little success in detecting SV in grove trees by serology, but assays based on RT-PCR were found to detect most Florida isolates. Currently, we are using primers designed from the sequence of the coat protein gene of a Florida isolate (CRSV-4) to amplify a 600 bp product. In an effort to build some confidence in the PCR assays, grove samples are biologically indexed by side grafting into sweet orange or grapefruit plants to confirm the PCR results. We are building a database of coat protein sequences of various SV isolates that will be used to design consensus primers to expand the range of detection. The isolates that have been sequenced vary from 71-98% (nucleic acid) and 97-99% (amino acid) in comparison to CRSV-4. The complete sequence of the viral genome segment that encodes for the coat protein has been determined for isolate CRSV-4. We are currently making and testing various RNA transcripts for infectivity that can be used as a virus-based gene vector.

Funding:

FCPRAC Grant for OE $20,000

IFAS provides salary for PI and one technician.

PATHOLOGY

Completed Projects

Further Development and Field Testing of Sensitive, Specific and Rapid Methods and Probes for the Detection and Differentiation of Strains of Citrus Tristeza Virus.

Project No. 928-68

Length of Project: 1 year

Investigators: C. L. Niblett, IFAS - Gainesville; R. F. Lee, IFAS - Lake Alfred

The overall objective of this research was to field test the methods developed for CTV detection and differentiation of specific strains. The specific objectives for this project were:

1. Assay both citrus trees and BrCA from the Broward/Dade County area for CTV by biological assays and by BD/PCR and the specific probes.

2. Prepare specific probes capable of detecting and differentiating the individual mild, QD, and SP strains of CTV which are found.

3. Develop methods to use BD/PCR and the CTV strain specific probes to measure strain interactions in cross protected plants and to more rapidly select effective cross protecting mild strains.

4. Utilize and field test BD/PCR and the CTV strain specific probes in the Florida Citrus Budwood Certification Program to ensure that the budwood is either uninfected or infected with the desired mild strain(s), but not inadvertently infected with innocuous or severe strains of CTV. Quality control will provide assurance and confidence in and for the new Quality Tree Program.

Summary of Accomplishments

We have made excellent progress during this research. We have not completed each of the original objectives, but we have made progress in other and more important areas.

1. Most importantly, we have prepared two new CTV probes and labeled them with different fluorogenic dyes. This enabled us to enhance the sensitivity of CTV detection in composite samples to 1 infected tree in 100. This is a much greater sensitivity than the 1 in 5 or 1 in 10 currently possible with ELISA, and the costs per assay are now comparable. This will greatly assist the Florida Quality Tree Program in testing large numbers of trees, as is now necessary.

2. We have demonstrated that BD/PCR, although useful in detecting mixtures of CTV strains is not useful on a large scale basis because of cost and its inability to indicate the specific strains present. These needs are met and with greater sensitivity with the current biotin labeled probes. Successive reprobing of the same membrane with the different probes revealed that some citrus trees are infected by as many as four different strains. Double infections are very common, usually with a mild and a decline strain.

3. Citrus trees in the Broward/Dade County area and in the Immokalee area, which were previously CTV-free, were found to be infected with decline and/or stem pitting strains, probably as a result of the high populations of the brown citrus aphid in both areas.

4. A workshop was held the week of April 20, 1998. for the demonstration and training of these techniques. It was attended by 10 research and regulatory personnel.

5. We have not yet applied these techniques to cross protection situations to monitor both mild and severe strains, nor have we yet tested them in the selection of mild cross protecting strains.

This research will immediately benefit the Florida Quality Tree Program and the Florida Department of Agriculture and Consumer Services by enabling the more efficient detection of specific strains of CTV in larger composite samples from budwood source trees and their elimination from the budwood stream. It also should speed up the identification and selection of mild strains of CTV which are capable of cross protecting against severe strains, and ensure that only those desirable strains of CTV are present in "protected" budwood. The patenting and widespread use of such probes will generate revenues for additional citrus research.

**Funding:**

(Totals For Both Investigator's Programs)

FCPRAC Grants $ 58,002

IFAS Contributions 100,000

USDA and CBAG Grants 50,000

Total $208,002

**A Survey for Severe Isolates of Citrus Tristeza Virus That are Not Detected by Standard Antibodies**

**Project No. 971-36**

Length of Project: One Year

Investigations: C. A. Powell ,IFAS- Ft. Pierce ;S. M. Garnsey ,USDA - Orlando

**Abstract**

The purpose of this project was to conduct a survey of citrus trees in Florida to determine the prevalence of severe isolates of citrus tristeza virus that are not detected by standard antibody diagnosis. One hundred and thirty-nine samples were collected from eight different sites and analyzed for CTV using 17G11 and MCA 13 monoclonal antibodies. Ninety-seven sources of CTV that reacted negatively with the MCA13 antibody were identified and partially characterized by budwood indexing. One source of the virus that did not react with MCA 13, but indexed as severe on Mexican lime was identified. These results indicate that testing budwood sources for severe CTV in Florida using MCA13 antibody is 99% effective.

**Objectives**

Determine the prevalence of CTV isolates in Florida that do not react with MCA13 antibody, but cause severe reactions in indicator trees.

**Summary Of Accomplishments**

The results indicate that testing budwood sources and grove trees for severe forms of CTV using MCA13 is highly effective in Florida, with a 99% success rate. However, one severe isolate was discovered from grapefruit that did not react with MCA13. This suggests that there is a potential to select CTV by budwood certification that relies only on this antibody. Further indexing will continue to determine the distribution of this isolate.

Establishment and use of overhead sprinkler system for studies in managing Colletotrichum acutatum and Alternaria citri.

Project No. 971-50

Length of project: 1 year

Investigator: R. M. Sonoda, B. A. Boman, IFAS - Ft. Pierce

Abstract

The greatest obstacle in obtaining information needed to manage Post Bloom Fruit Drop of citrus (PFD) is the unpredictable nature of rainfall occurrence. In the past, experiments established to collect data on effectiveness of various management techniques, chemical and biological fungicides, etc. have depended upon PFD disease pressure enhanced by or brought on by natural rainfall. Often little or no data has been obtained because of insufficient rainfall or incidences of rainfall during the bloom period. The objective of this proposal is to establish an experimental site where PFD and other citrus foliage diseases will be induced with a computer-controlled overhead misting system. Once established, the overhead-irrigated site will be used to study effects of new chemicals, new techniques, already available chemicals, or techniques used in new ways to manage these diseases. Once established the system can be used for many experiments over many years.

Summary of Accomplishments

Previously established single row beds in an approximately 4.5 acre area were reconstructed. Nine rows of navel oranges and two rows of Minneola tangelo were planted 43 trees/row, 15 ft apart. Keylime trees were interplanted in four rows of navel oranges. A well was installed. Construction of an overhead water-delivery system with mist-producing nozzles, one for each tree, was completed in late spring 1998. A datalogger-managed system was installed to provide up to 54 seven tree replicate plots of navel orange for PFD studies next spring. Sets of replicate plots of seven trees each can be misted at the same time. Microjet sprinklers were also installed. About 10 of the 378 navel orange trees bloomed in spring 1998. The key lime trees were inoculated with key lime anthracnose and will be used for tests in late summer or fall 1998. A separate datalogger system was established for replicate studies on Alternaria in the two rows of Minneola tangelo.

Funding:

FCPRAC Grant $ 16,000.00
IFAS Contribution 21,000.00
Other Grants 4,500.00
Total $ 41,000.00

ENTOMOLOGY

Continuing Projects

Biological and Chemical Controls for Diaprepes abbreviatus Larvae and Adults

Project No. 942-18

Length of project: 3 years

Investigators: C. W. McCoy, S. R. Krueger, E. D. Quintela, L. W. Duncan, IFAS - Lake Alfred

Abstract

Currently, *Diaprepes abbreviatus* is found in all commercial citrus regions of Florida infesting an estimated 160,000 acres. Research shows that larval injury to the roots can be a precursor to invasion by soil-borne *Phytophthora* spp. common to flatwoods soils. This insect/disease combination can be devastating to a citrus grove and control of both organisms may be necessary.

This research addressed the use of biological and chemical control agents for larvae and adults and their effect on non-target insects in both central and coastal groves. In 1997, research focused on the efficacy of 2 species of nematodes for larval control in flatwoods and ridge soils and the effectiveness of the fungus, *Beauveria bassiana* (Mycotrol) with and without Admire at sublethal dosages as a soil barrier treatment. In addition, the pyrethroid, Brigade, was evaluated as a soil surface treatment for larvae. Specific objectives are listed as follows:

1. Determine the effect of nematodes applied to flatwoods soil via herbicide delivery in the spring on *Diaprepes* larval mortality and root protection based on a destructive tree-soil sampling procedure.

2. Determine the effect of soil barrier treatments with fungi, nematodes and chemicals applied to central Florida soil via herbicide delivery on *Diaprepes* larval mortality and root protection using artificial larval infestation and a destructive tree-soil sampling procedure.

Summary of Accomplishments

Research has demonstrated that the chemicals, Admire, Brigade and the fungal biopesticide Mycotrol with and without Admire at optimal rates, control neonate *Diaprepes* invading container-grown citrus. Field studies conducted in 1996-97 in central Florida tested the above agencies as soil barrier treatments against neonates. Results based on soil bioassays and/or root health diagnosis were: 1) Mycotrol alone at 1.8×10^4 CFU's/g soil had no effect on larval survival and root protection, 2) Admire alone at 1.0 µg ai/g soil reduced larval survival and provided moderate root protection, 3) combination of Admire and Mycotrol provided the best control of larvae and root protection at 1.0µg and 4.8 × 10^4 conidia/g soil, but for only 8-10 days and 4) Brigade at 0.05 g/ft^2 or at split rate of 0.025g/ft reduced larval survival 60-80% and persisted in the upper 2" soil about 4 wk.

Biological and technique-related discoveries made relating to the field efficacy were: 1) artificial soil infestation of 3-year-old citrus trees with neonates was ineffective in establishing a larval population on the roots, 2) ant predation was an important natural control of neonates at the soil surface, 3) Brigade, Admire and Admire/Mycotrol as larval control agents appeared to disrupt ant predation in the field while Mycotrol alone and BioVector had no negative effect and 4) a mechanical soil shaker was developed for processing large volumes of soil for weevil larval recovery.

Studies show that *S. riobravis* has a short survival time in soils (<2 wk), but can kill approximately 90% of the larvae within the citrus tree rhizosphere. Field studies conducted on flatwoods soil in 1997 with *S. riobravis* and *Heterorhabditis bacteriophora* suggest that both nematodes reduce larval populations after 4 wk; however, and the later species was efficacious at a lower rate/acre.

Funding:

FCPRAC Grant $42,678

Field Evaluation of Attractant Traps for *Diaprepes abbreviatus*

Project No. 942-56

Length of project: 3 years

Investigators: H. N. Nigg , IFAS-Lake Alfred, S. E. Simpson, FDACS-Winter Haven

Abstract

The thrust of this project is the early detection of *Diaprepes abbreviatus* through attractants and trapping of adults and stimulation of female egg laying in artificial media.

Objectives

Overall objective: To develop an attractant trap for adults and for egg laying by the female.
1. Estimate trap efficiency in the field.

2. Determine adult attractants

3. Laboratory and field testing of oviposition attractants

**Summary of Accomplishments**

1. Trap efficiency. We have completed the field experiment, data analyses and manuscript preparation phase of this objective. In a nutshell, the Tedders trap and cup trap, regardless of whether they are placed in, under or between trees, were very inefficient for detecting this weevil.

   Part of this discussion depends on how you view efficiency. For a citrus grower it seems to us that efficient should mean detection of a pest 100% of the time when the pest is actually present and in a manner which allows a population estimation so treatment decisions can be made.

   In a 65 week experiment there were 10 weeks when no weevils were detected with any method including umbrella beating. There were 46 weeks in which one trap or another caught a weevil so in 9 weeks weevils were detected with the umbrella, but none were caught in a trap.

   Please understand that every one of the 80 trees in this experiment had an associated trap. When weevils were present the cup trap in the tree caught 50.9% of the time (28 of 55 weeks), Tedders under the dripline 45.4% (25 weeks), Tedders between trees 23.6% (13 weeks), cup under the dripline 16.3% (9 weeks) and cup trap between trees 3.6% (2 weeks).

   In our estimation, this is a dismal record for a citrus grower to depend on for making management decisions.

   We also ran a separate experiment in which we released weevils at set distances and positions from the Tedders trap. The overall efficiency of the Tedders trap was 28% in this experiment. This sounds high, but consider that 3 of 4 weevils were not caught. What if one weevil at a time emerges? Would we catch any? And it makes a huge difference if the weevil has the trap between it and the tree or is between the trap and the tree. With the trap between the weevil and the tree the catch was 40%, with the weevil between the trap and tree, 25%.

2. Attractants. The most important factor for attractants is a laboratory bioassay. All past efforts in this respect have failed. We believe we have a quantifiable, consistent laboratory bioassay for attractants for *Diaprepes* adults. We also are beginning to understand what determines the hosts they 'prefer'. The development of a bioassay took considerable time this year.

   The oviposition work is continuing. We have a laboratory bioassay, but no significant hits on attractant chemicals.

**Funding:**

FCPRAC Grants $12,500

Agency* Contribution 46,000

Other Grants -----

Total $58,500

*UF and FDACS

**Suppression of Caribfly Egg Laying**

**Project No. 971-12**

Length of project: 3 years

Investigators: H. N. Nigg ,IFAS - Lake Alfred ;S. E. Simpson ,FDACS-Winter Haven

**Objectives**

Overall objective: To provide substitutes for the fly free zone program for Florida grapefruit growers.

1. To determine the LC50 of candidate
pesticides

2. To determine if 'low' pesticide doses suppress or obliterate egg laying or result in nonviable eggs.

3. To develop a Calomondin bioassay for semi-field testing.

Summary of Accomplishments

Table 1. General Toxicity Data

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>100 ppm Egg Approx. effect</th>
<th>LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinosad</td>
<td>100% in under 72 hr test</td>
<td>25 ppm</td>
</tr>
<tr>
<td>Lorsban</td>
<td>100% in + 25 ppm 48 hr</td>
<td></td>
</tr>
<tr>
<td>Supracide</td>
<td>90% in + 30 ppm 72 hr</td>
<td></td>
</tr>
<tr>
<td>Guthion</td>
<td>100% in + &lt;10 ppm 96 hr</td>
<td></td>
</tr>
<tr>
<td>Baythroid</td>
<td>100% in + 10 ppm 120 hr</td>
<td></td>
</tr>
<tr>
<td>Monitor</td>
<td>100% in N/A &lt;10 ppm 24 hr</td>
<td></td>
</tr>
<tr>
<td>Agrimek</td>
<td>100% in + &lt;10 ppm 48 hr</td>
<td></td>
</tr>
<tr>
<td>Bay NTN</td>
<td>100% in under 72 hr test</td>
<td></td>
</tr>
<tr>
<td>Fipronil</td>
<td>100% in under 96 hr test</td>
<td></td>
</tr>
<tr>
<td>Fulfill</td>
<td>8% in N/A 168 hr</td>
<td></td>
</tr>
<tr>
<td>Proclaim</td>
<td>100% in + 25 ppm 48 hr</td>
<td></td>
</tr>
<tr>
<td>Alert</td>
<td>100% under under 72 hr test</td>
<td>60% under 168 hr test</td>
</tr>
<tr>
<td>Trilogy</td>
<td>0% in N/A -- 168 hr</td>
<td></td>
</tr>
<tr>
<td>Fenoxycarb</td>
<td>0% in N/A -- 168 hr</td>
<td></td>
</tr>
<tr>
<td>Nexter</td>
<td>24% in N/A -- 168 hr --</td>
<td></td>
</tr>
<tr>
<td>Micromite</td>
<td>0% in N/A -- 168 hr</td>
<td></td>
</tr>
</tbody>
</table>

The data in Table 1 shows clearly that malathion probably can be replaced in the fly free zone program. The LC50 of malathion which we previously published is about 30 ppm.

Four organophosphates were effective and Monitor (an organophosphate) was one of the most toxic compounds. Organophosphates may not be accepted in the fly free zone program for political and environmental reasons. Nonetheless if any effective compound (above) were substituted at 100 ppm that is 2000 times less than the 20% malathion (20,000 ppm) in the present bait.

We have noted compounds which suppress eggs with a plus (+) in Table 1. It is important to note that toxic compounds also suppress egg laying.


11/21/2008
This is a new finding. For the fly free zone and eradication programs these data indicate that doses which do not kill 100% suppress egg laying in survivors. The length of this effect needs to be calculated and/or determined.

For the Calomodin bioassay we have the plants, but have no progress to report on this facet of our Caribfly project.

We have four other compounds (recently received) to be included in these tests and two compounds on the way from chemical companies.

**Funding:**

FCPRAC Grants $22,000

Agency Contribution 66,000

Other Grants --

Total $88,000

**Suppression of Egg Laying of *Diaprepes abbreviatus***

**Project No. 971-14**

Length of project: 2 years

Investigators: H. N. Nigg IFAS - Lake Alfred ; S.E. Simpson, FDACS - Winter Haven

**Objectives**

Overall objective: To provide pesticidal management tools for *Diaprepes abbreviatus*.

1. To bioassay pesticides for death of *Diaprepes*.

2. To bioassay pesticides for egg laying suppression of *Diaprepes*.

3. To determine the length of any egg laying suppression effect.

**Summary of Accomplishments**

Insecticides were received from chemical companies and tested at 100 ppm by spraying flush bouquets.

**Table 1. Summary of Toxicity Experiment**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
<th>100 ppm deaths/treated Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proclaim</td>
<td>2/6</td>
<td>168 hr</td>
</tr>
<tr>
<td>Fulfill</td>
<td>0/6</td>
<td>168 hr</td>
</tr>
<tr>
<td>Agrimek</td>
<td>2/6</td>
<td>168 hr</td>
</tr>
<tr>
<td>Supracide</td>
<td>5/6</td>
<td>24 hr</td>
</tr>
<tr>
<td>6/6</td>
<td>48 hr</td>
<td></td>
</tr>
<tr>
<td>Guthion</td>
<td>2/6</td>
<td>24 hr</td>
</tr>
<tr>
<td>6/6</td>
<td>48 hr</td>
<td></td>
</tr>
</tbody>
</table>

None of the controls died.

Each of the surviving weevils in these assays was followed for 7 days after treatment to determine if egg laying was suppressed. The egg laying results were equivocal because none of these weevils laid eggs, including controls. Due to this result we are determining the egg laying pattern of *Diaprepes* in order to know how long a female must be followed to determine if a pesticide treatment actually has affected her egg laying ability.

As soon as we know the pattern of egg laying, the LC50 work will begin.

Nonetheless, it is obvious that there are a number of compounds which will control adult *Diaprepes*.

Note that only field collected weevils were used for these assays.

**Funding:**

FCPRAC Grants $ 9,500

Agency Contribution 41,000

Other Grants: -- Total $ 50,500
Field Evaluation of Natural Enemies of Brown Citrus Aphid

Project No. 971-16

Length of Project: 2 years

Investigators: H.W. Browning, J.P. Michaud, IFAS - UF - CREC

Abstract

The research mandate of the primary investigator (J. P. Michaud) has been to develop a biologically-based management strategy for the brown citrus aphid, *Toxoptera citricida* (Kirkaldy), (BrCA) in Florida citrus. The project was begun in Puerto Rico in January, 1996 and studies were initiated in Florida in June, 1996. A grant from FCPRAC was acquired for FY 97-98 and was used to extend our efforts to these ends. A list of our objectives during this period is outlined below.

Objectives

1. To monitor the population dynamics of the BrCA in Florida.

2. To collect, identify and list the insect species found attacking the BrCA in the major citrus-growing regions of the state.

3. To evaluate, and quantify the impact of, the primary natural enemy species on BrCA populations in the field.

4. To assess the effectiveness of exotic parasitoid species released against the BrCA, *i.e.* *Lysiphlebia japonica*.

5. To assess the performance (survival and development) of key predators and parasites on BrCA.

6. To assess the potential role of alternate prey or host species in recruitment and/or retention of predators and parasites within citrus groves.

Summary of Accomplishments

Substantial progress has been towards achieving all of the objectives outlined above. In the interest of disseminating information to citrus growers, the primary investigator J. P. Michaud has given several educational seminars to various growers associations upon invitation, and will provide an update on the BrCA situation in Florida at the FACTS conference this October. The details of our specific accomplishments will be discussed in the same order as the objectives listed above.

1. Substantial infestations of BrCA developed in many areas of southwestern Florida and the Indian River district throughout the fall of 1996 and the spring and fall of 1997. The latter season also saw the first large-scale infestations developing in the ridge district north of Highlands County. Small over-wintering populations, typically confined to isolated resets and rootsprouts, were detected in virtually all groves that experienced fall infestations.

The spring of 1998 saw exceptionally large populations of ladybeetles (Coccinellidae) state-wide which were sufficient to decimate BrCA populations before they could expand into full-fledged infestations. Whether or not beetle populations will continue to provide this level of control in spring flush will require subsequent years of observation to ascertain.

Our observations indicate that the high temperatures that prevail through most citrus-growing regions in mid-summer are sufficient to decimate BrCA populations on an area-wide basis, although isolated colonies inevitably survive in protected locations, typically on adventitious shoots in the interior of mature trees.

2. The following is a list of the natural enemies of the BrCA in Florida recorded to date.

**Coleoptera: Coccinellidae.**


**Diptera: Syrphidae**


11/21/2008
Allograpta obliqua (Say), Ocyptamus antiphates (Walker), Ocyptamus fuscipennis (Say), Paragus tibialis (Say), Pseudodorus clavatus (F.), Syrphus citrinum (Brunetti), (one sp. unidentified)

Diptera: Chamaemyiidae

Leucopis sp.

Hemiptera: Anthocoridae

(one sp. unidentified)

Hymenoptera: Aphidiidae

Lysiphlebus testaceipes Cresson

Neuroptera: Chrysopidae

Cereaochrysa lineaticornis (Fitch), Chrysoperla rufilabris (Burneister)

Neuroptera: Hemerobiidae

Micromus posticus (Walker)

3. We have completed a detailed study of 16 different cohorts of the BrCA during FY 97-98,12 in Florida and 4 in Puerto Rico. From these data we were able to determine: (1) The proportion of colonies surviving to maturity (producing alates that spread CTV); (2) the incidence of different natural enemies at BrCA colonies and; (3) the cumulative impact of different natural enemies. The following is a point-form summary of the most important findings:

1. The proportion of newly formed BrCA colonies that survive to maturity ranges from 0 - 17%.

2. Coccinellids (primarily C. sanguinea and H. axyridis) were the most important predators of BrCA colonies in spring, coccinellids and syrphids almost equally in early summer, and syrphids (primarily P. clavatus) almost exclusively in late summer and fall.

3. Predatory larvae experienced significant mortality during hot weather, at temperatures lower than those necessary to cause aphid mortality.

4. Fungal diseases were not observed in Florida, as they were in Puerto Rico.

5. The exotic parasitoid, L. japonica, was released at approximately 30 different locations in Florida over the past year. To date, only a single recovery has been from the Ft. Pierce area about 2 weeks after a release. No subsequent recoveries have been reported. We do not consider L. japonica to be suited for survival in Florida.

5. Our extensive field observations provided sufficient evidence that all of the primary predator species can successfully complete their life cycles on a diet of BrCA. In lieu of this work, a laboratory study was performed to examine in detail the settling behavior of the migratory alatae. We discovered that (1) there is a large amount of variation between trees of a given citrus variety, and even between shoots on a single tree, in terms of their attractiveness to the BrCA; (2) alates have a strongly gregarious tendency which leads to many colonies being initiated by multiple foundresses (this presumably increases colony growth rates and improves colony survivability upon discovery by predators).

6. We identified a number of weed species that either harbored non-pest aphids serving as alternate prey, or served as sources of floral nectar for adult natural enemies. These included Ambrosia artemisiifolia, Bidens pilosa, Chenopodium botrys, Lantana camera, Morremia odorata, Ocnothera laciniata, Parthinium sp. Richardia scabra, Solanum americanum, Taraxacum sp. We concluded that plant diversity within the citrus ecosystem is an important factor influencing natural enemy populations and is therefore deserving of recognition and cultural consideration. However, we doubt that active manipulation of understory plant species would be an effective strategy for influencing or altering the seasonal abundance of natural enemy species.
Funding:

FCPRAC Grants $10,000.00
USDA APHIS $48,000.00
Total $58,000.00

Classical Biological Control of Citrus Psylla and Pink Mealybug

Project No. 971-21

Length of Project: 4 years

Investigators: Marjorie A. Hoy , IFAS - Gainesville; Ru Nguyen , FDACS-DPI-Gainesville

Abstract

This project was proposed as a "proactive" classical biological control program for two pests that were expected to invade Florida "soon". I expected the pink mealybug to invade Florida first because it was already found in multiple Caribbean islands. Surprisingly, the Asian citrus psyllid invaded Florida first and was discovered in several counties in southeastern Florida in June 1998. Both pests are good targets for classical biological control programs because effective natural enemies are known for them. Now that the psylla is in Florida, we have switched to an action mode. We expect to import two parasitoids of Asian citrus psyllid soon. In addition, we are preparing host plant material for the expected invasion of the pink mealybug so that we can rear and release parasitoids of this pest. We hope to discover the pink mealybug while it is still in a relatively small area in Florida so that we can release parasitoids immediately.

Objectives

1. Obtain permits to import parasitoids of the citrus psylla and the pink hibiscus mealybug.
2. Obtain host plants and develop rearing methods for both pests and their parasitoids.
3. Obtain parasitoids and evaluate them in quarantine; write environmental assessments.
4. Obtain permits from state and federal regulatory agencies to release them in Florida.
5. Mass rear and release parasitoids in Florida citrus groves.
6. Evaluate overwintering, dispersal, and impact of parasitoids on pests.

Summary of Accomplishments

When this proposal was written neither the citrus psylla or the pink mealybug were present in Florida, but both were the targets of classical biological control programs because effective natural enemies are known for both. This project officially began January 1, 1998.

The Asian citrus psylla was found in Southeast Florida in June 1998, and our "proactive" classical biocontrol project is off and running! We have identified sources of parasitoids of the psylla in Taiwan, Thailand, and Viet Nam. We have permits to import them, and have purchased 2400 citrus jasmine plants (Murraya paniculata) for rearing psyllids in quarantine. We have permits to bring psyllids from southeastern Florida to Gainesville to rear them in quarantine as hosts of the parasitoids we plan to import. We are developing molecular tests (polymerase chain reaction) to confirm the absence of greening disease in the psyllid colony and will use the same test to confirm that the parasitoids imported from Asia are free of this disease. We have obtained the assistance of taxonomists at the British National Museum who will confirm the parasitoids' identity. We hope to obtain shipments of parasitoids from Thailand and Viet Nam soon. Because we are allowed to import only adult parasitoids, we may have difficulty getting live parasitoids in, so M. A. Hoy is making plans to travel to Asia in October or November to collect parasitoids and hand carry them back to Florida. A draft environmental assessment document is being developed that will be required to remove parasitoids from quarantine for release into the field.

In June 1998, we attended a workshop on the pink mealybug in St. John, the US Virgin Islands, and observed the damage caused by this pest. I learned rearing and identification methods and obtained the assurance from 3 different agencies that they would provide starter cultures of the...
parasitoids once the pest was found in Florida.

We planted Japanese pumpkins (needed for rearing the pink mealybug in quarantine) and processed 1400 lbs of potatoes from the UF-Hastings Research Center that have not been treated with sprout inhibitors. Both the potatoes and pumpkins are in cold storage and will serve as hosts for the pink mealybug once we discover this pest in Florida.

We have enlisted cooperative extension agents, the general public via news releases, and master gardeners to search for the pink mealybug so that we can find the pest as quickly as possible. The sooner we discover it and release parasitoids, the less damage it will do.

**Funding:**

FCRAC Grant $ 15,893

**Optimal Timing and Placement of Entomophagous Nematodes for Control of *Diaprepes Abbreviatus***

**Project No. 971-27**

Investigators: P. A. Stansly, L. W. Duncan, IFAS - Immokalee/ Lake Alfred

**Abstract**

Root injury caused by *Diaprepes* weevils is an insidious but expanding problem in the Florida citrus industry. Nematodes such as *Steinernema riobravis* and *Herterorhabditis* spp., represent the only means now and in the foreseeable future to control the damaging larval stage. Experimental results with perishable formulations of *S. riobravis* at high rates and under ideal conditions have been successful, but results under commercial conditions have not been as positive.

**Objectives**

The objectives of this project are to maximize impact on *Diaprepes* by:

1. Evaluating microirrigation injection of commercially available formulations of *S. riobravis* and comparing with alternative methods of application.

2. Comparing efficacy of the two most promising nematodes on the market today, *S. riobravis* and *H. indicus*.

Experiments are being conducted in the Ft. Denaud area of Hendry County on two commercial citrus groves. Experimental blocks are approximately 40 acres in size with individual plots measuring almost 1 acre. *S. riobravis* is being applied once in mid-summer by injection through 2 types of microirrigation equipment (drip and microsprinkler) at the labeled rate or in two split applications of half the labeled rate in summer and early fall. These results will be compared to a split application of *S. riobravis* and *H. indicus* (a native Florida nematode) applied with a herbicide boom in mid summer and early fall. Emitters are sampled during application to ascertain concentrations of viable nematodes. Soil is sampled after application and at weekly intervals. Viable nematodes are extracted and counted and soil is bioassayed with *Diaprepes* larvae to simulate the effect on larvae in the field. Tedders traps are used to monitor emergence of *Diaprepes* adults and to compare treatment effects in the field.

**Summary of Accomplishments**

The project has been hampered by a number of factors beyond our control: unavailability of nematodes, unusually dry weather this summer and management changes in the groves, and equipment failures. Yet, we continue to gain experience with large scale nematode applications, and were able to put out two, full scale experiments this summer, one in a drip-irrigated grove (Kinzer) and the other in a micro-jet irrigated grove (Eagle Point). Both locations represent new, experimental sites where cooperation is excellent and *Diaprepes* populations high. Although in a previous study we found that most nematodes fell out of suspension ¾ of the way down a 240-foot line in a vegetable drip system, this time at the Kinzer grove we observed nematodes in all but the last emitter of a 400 ft run. We were able to obtain the dry formulation of BioVector 350 for the test and did not have problems with dispersion that we experienced last year. However, given continued difficulty obtaining this material and the presence of *Heterorhabditis* nematodes on the market from Integrated Biocontrol Systems, we have included the most promising of these, *H. indicus* in our trials. This native Florida nematode arrived in excellent condition in damp sponges and was applied with a herbicide boom in the first of 2 split applications at Kinzer and Wheeler's where it will be compared with BioVector applied the same way.

Current and pending support for the research: None in addition to present project for Stansly.

**Funding:**

Diaprepes Damage and Interactions with Phytophthora

Project No. 971-45

Length of Project: 3 years

Investigators: J. H. Graham, C. W. McCoy, IFAS - UF - CREC

Abstract

Larvae of Diaprepes abbreviatus are causing devastating tree losses in about 30,000 acres of the citrus industry and the weevil's spread is accelerating rapidly. Due to the lack of early detection methods and highly effective control options for the weevil, citrus over much of this acreage is in decline or out of production. Phytophthora spp. and form a complex that causes severe structural root damage, especially in the presence of a newly discovered root pathogen, P. palmivora. This Phytophthora pathogen is of greater concern than P. nicotianae because it breaks resistance of the rootstock, Swingle citrumelo.

Objectives

1. To survey weevil-infested groves for Phytophthora spp. and damage to large structural roots of trees on susceptible and resistant rootstocks to fungal infection with emphasis on P. palmivora and the associated tree decline in the East Coast region of the citrus industry.

2. To quantify the contribution of Phytophthora spp. to decline of young trees on susceptible and resistant rootstocks through selective control of the weevil and the fungus with the biocontrol nematode, Steinernema riobravis, and the fungicide, Ridomil Gold.

3. To evaluate the efficacy of Ridomil Gold for control of structural root infections in groves under IPM management for Diaprepes.

Summary of Accomplishments

Phytophthora spp. commonly infect the margin of Diaprepes larval etchings in the root bark and cause bark collapse that girdles structural roots. Structural root attack by Phytophthora spp. and tree decline is greatest on Phytophthora-susceptible rootstocks in poorly-drained, heavier soils in flatwoods groves, but also occurs in ridge soils when structural root damage is severe. Swingle citrumelo rootstock maintains resistance to P. nicotianae after larval feeding because root etchings rapidly heal. However, in the Vero Beach area P. palmivora, an aggressive root pathogen, breaks resistance of Swingle citrumelo rootstock. In this case, P. palmivora causes more damage to the structural roots than the larvae alone.

In greenhouse studies, the greater the larval feeding damage established on the roots, the higher the susceptibility of roots to Phytophthora infection and development of root rot. Although initial levels of weevil damage on Cleopatra mandarin and trifoliate orange are similar, subsequent damage by P. nicotianae increases with severity of feeding damage on Phytophthora-susceptible Cleopatra but did not on resistant trifoliate orange. For Cleopatra but not trifoliate, Phytophthora infection is correlated with the increased leakage of wound exudates from roots when a damage threshold is exceeded.

In the first season at Vero Beach, ground spray application of Ridomil Gold to Red grapefruit trees on Swingle citrumelo affected by the complex of P. palmivora/P. nicotianae and Diaprepes increased root density and canopy flushing activity 30-40% compared to non-treated trees. Yield and fruit size was increased by 10%. This rapid response to fungicide control is indicative of the potential for Phytophthora management to augment a Diaprepes IPM program.

Funding:

FCPRAC Grants: $22,150

Agency Contribution 50,000

Other grants: 26,000

Total $98,150
Assessment of the Potential Impact of Citrus Rust Mite Dispersal on Management Programs and Field Validation of a Trap-Based Monitoring System

Project No. 971-51

Length of Project : 1 year

Investigators: J. C. Bergh, C. W. McCoy, IFAS - Lake Alfred

Abstract

Aerially-dispersing citrus rust mites can be captured in sticky traps placed within or near citrus groves. Trap-catch data can provide information about the potential for dispersing mites to affect mite management programs in groves downwind of infested groves. As well, if the relationship between the number of mites captured and the population density of mites on fruit is sufficiently strong and consistent, sticky traps might prove to be a useful and cost-effective mite monitoring tool. This project investigates the potential impact of citrus rust mite dispersal on the initiation of infestations and seeks to characterize the relationship between trap-catch and mite population density on fruit. This information will enhance the ability to optimize mite control and will lead to conclusions about the utility of trapping for monitoring mite populations.

Objectives

1. Estimate the percentage of dispersing citrus rust mites that arrive at an adjacent grove.
2. Characterize the relationship between mite trap-catch and population density in groves under different management programs.
3. Compare the relationship between trap-catch and population density generated by unidirectional and omnidirectional traps.
4. Determine the minimum number of traps required to provide an accurate estimate of mite populations.

Summary of accomplishments

At 2 sites with commercial citrus groves separated by a clearing (320 - 335'), regression analyses of trap-catch data estimated that 13 - 22% of mites leaving the upwind grove arrived at the downwind location. At a third site with groves separated by a road (45'), more mites were captured next to the downwind grove than next to the grove upwind. At two locations chosen to examine the effect of dispersal on the initiation of rust mite infestations, mite populations remained so low throughout the 1997 growing season that this experiment could not be initiated. With continued support from the FCPRAC for FY 98-99, this test will be performed in 1998.

Sixteen unidirectional and/or 8 omnidirectional traps were deployed in 5 commercial groves. At 3 sites with both traps, omnidirectional traps yielded a stronger relationship between trap-catch and population density on fruit than unidirectional traps. The relationship generated by omnidirectional traps was strong at 2 sites, moderately strong at 2 sites and non-existent at 1 site. Actual numbers of mites on fruit were a better predictor of trap-catch than was the percentage of infested fruit. Preliminary data suggested that 0.5" diameter omnidirectional traps are as efficient as the 1.0" diameter omnidirectional traps used, and that the smaller traps take much less time to process. In FY 98-99, 1" and 0.5" traps will be compared, and the effects of trap placement and trapping interval will be investigated. The remaining objectives of this proposal will be based upon the outcome of the 1998 research.

FCPRAC Grants $25,000.00
Agency Contribution 10,000.00
(IFAS infrastructure)
Other Grants:
Merck - 15,000.00
BASF - 15,000.00
UniRoyal- 6,000.00 Valent - 2,500.00
Total $73,000.00
Abstract

The spread of the citrus root weevil, Diaprepes abbreviatus, to an estimated 160,000 acres throughout Florida and virtually all commercial citrus growing regions, has precipitated the need for weevil control in many areas. Both adult and larval control is best achieved when population density is known. Since larvae in the soil are extremely difficult to sample, researchers have used adult emergence traps to estimate time of movement from the soil to the tree. Since cone-type ground traps have a limited range and are not grower friendly, research has focused on the Tedders trap as a tool to determine seasonal trends in adult emergence.

This proposal addressed trap positioning in the grove and trap color for maximum attraction. The objectives of our 3-year study were to:

1. Determine the range of attractancy for the Tedders trap to adult Diaprepes abbreviatus, the blue green citrus root weevil, Fuller rose beetle and little leaf notcher.

2. Determine seasonal adult emergence (population dynamics) of the above species of citrus root weevils using Tedders trap compared to ground traps.

3. Test the validity of using Tedders trap as monitoring method for determining efficacy of different control measures applied to the soil.

Summary of Accomplishments

Different scientists involved in citrus research determined the utility of the Tedders pyramidal ground trap as a tool to monitor the seasonal abundance of adult Diaprepes in the field. The following summarizes their findings:

1. The Tedders trap had attractancy for the following citrus root weevils: Diaprepes abbreviatus, D. famelicus, Asynonychus godamni, Pachnaeus spp., Tanymechus lacaena and Artipus floridanus.

2. Traps positioned beneath the tree canopy caught more adult weevils than those in row.

3. Sixty-one percent of the weevils caught in Tedders traps had fed previously on citrus foliage.

4. Fewer females were caught than males.

5. Traps with reflective color (blue, white, yellow) caught fewer Diaprepes than dark color traps (red, black, brown, green).

6. Within grove differences in adult emergence of Diaprepes were detected using Tedders traps.

7. Adult emergence of Diaprepes abbreviatus in central and coastal Florida citrus groves over a 3-year period was continuous with a distinct peak in April/May according to weekly Tedders and cone trap catches.

8. Tedders trap catches were generally higher compared to cone traps, probably because their range of attractancy exceeds that of the cone trap.

9. Tedders traps appear to be effective as a research device that can be used to measure changing weevil population densities in time in large plot...
Limitations to Pollination of Citrus Hybrids Under Florida Conditions

Project No. 961-10

Length of Project: 2 years

Investigators: L. G. Albrigo, R. Rouseff, R. V. Russ, IFAS - Lake Alfred

Abstract

There are over 60,000 acres of mandarins (specialty fruit) in Florida requiring some cross pollination to achieve adequate crop loads. Many blocks are not meeting desired production levels and bees appear not to prefer these cultivars. Understanding the reasons for this reduced activity and bee behavior in citrus may improve pollination procedures.

Objectives

1. Characterize the nectar of common citrus hybrids, oranges and grapefruit cultivars. Measure nectar volume, sugar and volatile aromatic contents.

2. Determine flower preference of honey bees for various hybrids compared to orange or grapefruit cultivars in controlled exposures.

3. Determine activity of honey bees in field conditions with large and small blocks of hybrids.

4. Determine bee movement when hives are placed in different locations or at different times in hybrid blocks during the bloom period or if placement of other cultivars in the hybrid block improves bee activity.

Summary of Accomplishments

In controlled exposure of bees from a mini-hive to replicated sets of flowers from various cultivars, bees preferred large flowered cultivars which have more nectar (4.4 to 7.8 uL/flower) than the non-preferred flowers (0.2 to 4.1 uL/flower). Sugar contents were nearly equal across cultivars but levels were nearly double in the second year. Volatile levels were lower in the hybrids compared to the preferred flowers per fresh weight and presumably even lower per each smaller flower. At the end of the flowering period, flower thrips built up and further decreased the nectar of all the late flowering hybrids. In year 2, Ambersweet was intermediate in preference and nectar quality, but was still very low in pollen count per anther and most of the pollen would not germinate. In the field, Valencia is not a suitable cross pollinator for Ambersweet or other hybrids, while Orlando and Minneola appear good. They have a long bloom period and good overlap with most hybrids. Hamlin appears suitable for Ambersweet also. Murcott blooms too late to serve as a cross pollinator. Grove hedgerows limit direction of bee activity. Greater distribution of hives and bee placement internally in the block just as the flowering is beginning is advisable although more costly. Early hive location for large flowered cultivars should be changed with placement closer to the later blooming small flowered cultivars.

Funding:

FCPRAC Grants $ 7,800.00

Agency* Contribution 19,000.00 Other Grants - 0 -

Total $ 26,800.00

* Twenty percent of 1 faculty and one technician position
Reevaluating Methods to Identify Sting Nematodes and Coffee Lesion Nematodes Attacking Florida Citrus

Project No. 961-24

Length of Project: 2 years

Investigators: L. W. Duncan ,IFAS - Lake Alfred ;D. T. Kaplan USDA - Orlando ;R. N. Inserra ,FDACS-Gainesville

Objectives

We are investigating heterogeneity among populations of sting nematodes and coffee lesion nematodes to clarify their status as major citrus pathogens. Our preliminary research indicates that numerous populations of putative coffee lesion nematodes from native and ornamental plants in Florida do not infect citrus. Detection of these nematodes currently results in nursery de-certification. Similarly, there is evidence that some populations of sting nematodes, known only from a restricted area of Florida, are more virulent to young citrus trees than most commonly encountered populations of this nematode. These nematodes are being spread from nurseries into groves on infected rootstocks. The results of these studies will serve as a basis to clarify the regulatory status of each of these nematode groups, to better serve the interests of both nurserymen and growers. Specific objectives include:

1. Characterize the genetic diversity among coffee lesion and sting nematode populations using DNA-based analysis. Identify polymorphic DNA associated with the ability to parasitize citrus.

2. Identify discriminating genetic morphometric and morphological characters, using RAPD-based methods, light and electron microscopy.

3. Determine the status of citrus as a host plant for each of the various types of coffee lesion nematode and sting nematode populations that occur in Florida.

4. Reevaluate the taxonomic relationships of the above populations, based on information obtained in objectives 1-3.

5. Provide technical information to the FDACS-DPI for use in the rootstock certification program with respect to management of sting and coffee lesion nematodes.

Summary of Accomplishments

Coffee lesion nematodes: By studying the morphology, host ranges and molecular genetics of 28 isolates of putative Pratylenchus coffeae collected from various crops, worldwide, we clarified the regulatory status of all known populations of these nematodes in Florida by determining that:

1. Pratylenchus coffeae does not infect citrus (the lesion nematode on citrus in Florida, currently referred to as P. coffeae, is closely related to P. musicola, but is likely an unnamed species).

2. Pratylenchus coffeae was only detected among isolates from coffee.

3. All isolates from native plants in Florida, previously considered to be P. coffeae, are P. pseudocoffeae and an unnamed species, and do not infect citrus.

4. Isolates that infect citrus in Florida and Oman are the same species, but isolates that infect citrus in Brazil are a different, unnamed species.

5. Isolates from yam, aglaonema, and several other plants may be the same species as the isolates from Florida citrus. If this is true, most of these isolates are different races and do not infect citrus.

6. All isolates from banana are P. musicola, a valid species, formerly synonomized with P. coffeae.

Having resolved the taxonomic relationships among these nematodes, we are positioned to develop molecular-based diagnostic methods.

Sting nematodes: Morphological and host range studies indicate that:

1. All isolates designated from morphology as putatively virulent to citrus are virulent in greenhouse tests.

2. Some isolates designated from morphology as putatively avirulent to citrus are avirulent, while others are virulent to various degrees.
Our results do not resolve whether there is a need to regulate the movement of sting nematodes from nurseries. While some virulent populations can be identified based on morphology, others cannot. DNA relationships among the populations are not available due to technical problems that have been resolved. When those data become available, we will attempt to develop molecular-based diagnostic methods.

**Funding:**

FCPRAC Grants $ 60,300

Agency Contribution* 98,000

Other Grants:

Total $158,300

*Partial salaries of three scientists and three technicians.

**Classical Biological Control of the Citrus Leafminer**

**Project No. 9310-06**

Length of Project: 3.5 years

Investigators: M. A. Hoy , IFAS - Gainesville ; Ru Nguyen , FDACS-DPI-Gainesville

**Abstract**

The citrus leafminer is a serious pest of young citrus trees and trees in flush and became the target of a classical biological control program shortly after this pest invaded Florida in 1993. The project officially terminated on Dec. 31, 1997 but I will continue additional studies (funded by another source); we are evaluating another biotype of the highly-effective parasite *Ageniaspis citricola*. In addition, we will attempt to import one more parasitoid for evaluation in quarantine.

*Ageniaspis citricola* was first released on May 1, 1994. Since then, we have reared it in Gainesville and released it throughout the state with the assistance of Phil Stansly, Robert Bullock, David Hall, Joe Knapp, Harold Browning and Jorge Pena. This effective natural enemy has established, multiplied, and spread throughout the state. It is now the most abundant natural enemy of the leafminer, which is also attacked by native predators (ants, spiders, lacewings) and parasitoids. Without doubt, it has contributed to the decline of citrus leafminer populations throughout the state.

**Objectives**

Identify source of appropriate parasitoids

Obtain permits for importation from Australia and Asia (Taiwan and Thailand)

Collect parasitoids from Australia and Asia

Import parasitoids into quarantine

Develop rearing methods

Write environmental assessment

Apply for permission to release parasitoids

Mass rear and release parasitoids throughout Florida

Evaluate establishment, overwintering, and distribution of parasitoids

**Summary of Accomplishments**

This project ended in December 1997 with all goals met. *Ageniaspis citricola*, a host-specific parasitoid of the citrus leafminer was imported from Australia and Asia and has become established throughout the state. It has successfully overwintered and spread so that it is the dominant parasitoid of the leafminer. citrus leafminer populations declined about the same time that this parasitoid was establishing, spreading, and increasing in density. While detailed experiments should be conducted to confirm scientifically the importance of *A. citricola* in this population decline (and there is no doubt that spiders and ants are important predators), this parasitoid clearly is a very important natural enemy of this pest. Most growers with mature citrus groves report that they no longer spray for the leafminer. If we assume that this classical biological control project eliminated the need for one spray (oil @ $16/acre X 860,000 acres), then the annual savings to the industry could be calculated as ca. $13.7 million per year. The costs of the project supported by the FCPRAC totaled approximately $115,000, or a return on the investment of ca. $119 per year for each $1 invested. Since this natural enemy should continue to reduce leafminer populations indefinitely, this one-time cost provides an exceptionally high return on the investment by FCPRAC.

**Funding:**

FCPRAC Grant (1997) $15,993

IFAS Contribution ca. 33,000

Total $48,993

**PLANT IMPROVEMENT**

**Continuing Projects**

**Development and Evaluation of Improved Citrus Rootstocks**

*Project No. 928-27*

Length of Project: 20 years

Investigators: J. W. Grosser, F. G. Gmitter, Jr., W. S. Castle, IFAS - Lake Alfred

**Abstract**

The goal of this project is to develop the improved rootstocks that the Florida industry will rely on to remain competitive and profitable in the future, and to provide growers with information necessary to make the best rootstock choice for any situation. When new rootstock germplasm is developed and reaches an advanced stage of evaluation, the plant material goes into commercial trial, and appropriate data entered into formalized decision-making models. New improved rootstocks will help Florida growers to compete internationally. Tree loss and associated production losses, and tree replacement costs will be minimized. Citriculture will have a reduced impact on the environment through the biological control of diseases, thereby reducing chemical applications. New, widely adapted rootstocks will allow citriculture to expand into marginal areas. Overall, growers can expect savings in production costs, more continuous and higher productivity, and increased tree longevity.

**Objectives**

1. Develop improved rootstocks that when budded with a scion, produce long-lived, compatible trees that yield large quantities of high quality fruit and juice, and tolerate the diseases, pests and environmental stresses that occur in Florida.

2. Improve the characteristics of existing selected commercial rootstocks.

3. Develop and employ appropriate rootstock evaluation procedures and up-to-date techniques for comparative financial analyses of evaluation data and expert system models in order to give growers sophisticated approaches to rootstock selection.

**Summary of Accomplishments**

Sexual Hybridization Program:

1) Made 38 crosses for rootstock development in spring 1997 and harvested seed from 17 crosses. Seeds were planted and hybrids are being grown for field-planting.

2) Made 7rootstock crosses in 1998 (crossing inhibited by poor weather and erratic flowering).
3) Planted >50 new citranges from previous crosses, all preselected with markers for the CTV resistance gene.

4) Selected and began propagation of 6 hybrids (Palestine sweet lime x *C. ichangensis*) for the new lime roostock trial in Homestead.

Somatic Hybridization Program: Planted phase E of the SandRidge rootstock trial (86 trees of Valencia budded onto 3 somatic hybrid, 3autotetraploid, and 3 control rootstock selections). Propagated 3 new somatic hybrids and x639 for the final phase F of the SandRidge trial. Budded a new grapefruit rootstock trial to be planted for the Indian River area in cooperation with Dr. Ed Stover (>500 Flame and RioRed trees on 27 rootstocks including 17 somatic hybrids). Produced 5 new somatic hybrids, including sour orange + trifoliate orange 50-7 and sour orange + Benton.

Blight Escapes: Provided seeds of 18 rough lemon blight escapes to Lykes Bros. for advanced testing.

Continuing Field Trials:

1) Conserve II: there was little effect of rootstock on scion fruit quality in the mandarin scion/rootstock trial, and the trees on Swingle have been generally the most productive.

2) At an east coast navel trial, trees on citrumelo 80-5 and 80-18 and large trees on C-32 and x639 [Cleo x TF] have been yielding well with Marsh, Ambersweet, and Valencia.

3) Financial analysis of a long-term Hamlin/rootstock trial showed that Benton and Carrizo had the highest internal rates of return.

4) New experiments include a Valencia trial involving 7 rootstocks planted across 5 soil series; and a second generation sweet orange trial involving several promising scions selected after 10 years of evaluation at St. Cloud and propagated on Benton citrange.

**Funding:**

FCPRAC Grants $ 65,000

IFAS Contribution 270,000

Other Grants --

Total $335,000

**Utilization of Gene-Mapping and Marker-Assisted Selection for Citrus Variety Improvement**

**Project No. 942-27**

Length of Project: 3 years

Investigators: F.G. Gmitter, Jr. , IFAS - Lake Alfred ; G. A. Moore , IFAS - Gainesville

**Abstract**

The greatest impediment to development of genetically-improved citrus varieties is the lack of simple, inexpensive, and effective screening and selection procedures for most important traits. New genetic technology based on molecular markers provides a method to select for desired traits indirectly, by selecting for linked markers. Once markers have been identified, the use of costly, ineffective, and time-consuming selection procedures can be minimized. More hybrids can be evaluated per year as young seedlings, and the development of improved varieties can be hastened.

**Objectives**

1. Markers developed previously for CTV and citrus nematode resistance, and tolerance of salinity and cold, will be utilized for selection of improved rootstock hybrids for advanced tests.

2. Markers for other important traits, possibly including resistance to fungal diseases (Phytophthora, Alternaria, and scab) and fruit quality traits (e.g., color, size, season of maturity, shape, and ease of peeling) will be developed.

3. Genes involved in the carotenoid (i.e., color) and flavonoid (i.e., compounds affecting fruit bitterness and processed OJ "cloud") biosynthetic pathways will be mapped and tagged.

**Summary of Accomplishments**

1. Genetic markers developed previously for CTV-immunity and citrus nematode resistance have been used to pre-select resistant hybrids from crosses made for rootstock improvement.

2. Resistance to *Alternaria* in mandarin hybrids, and resistance to *Phytophthora* spp. in *Citrus x Poncirus* hybrids, has been characterized by inoculation with pathogens. *P. nicotianae* and *P. palmivora* have been used on *Poncirus* hybrids by soil inoculation and *in vitro* testing methods. Nine putative markers have been identified for *Alternaria* resistance susceptibility. Eighteen putative markers have been identified for *Phytophthora* resistance, including 4 linked to resistance to both *Phytophthora* species.

3. Mapping of QTLs for cold tolerance continues. The freezing and recovery studies for one family of 500 seedlings are completed. A linkage map has been constructed for this family using RAPD markers and areas of the genome involved in cold tolerance have been identified. Current efforts are toward producing easy to use, PCR-based, co-dominant markers that can be used to link the maps for salinity tolerance traits and cold tolerance traits, as well as other maps that have been produced. When possible, the markers are based on known sequences for citrus genes, including genes involved in the carotenoid and flavonoid biosynthetic pathways. Markers derived from RAPD products (SCAR markers) are also being tested. The ultimate goal is to learn whether the same genomic regions are involved in tolerance to these two types of environmental stress.

**Funding:**

FCPRAC Grants $49,000

$20,000

Agency Contribution $50,000

Other Grants $205,000

Total $219,000

* Funds from this granted project, 942-27.

* Funds allocated to Dr. Gmitter from FCPRAC -- granted project for rootstock cultivar improvement, 928-27; this project is indirectly related to 942-27.

* Estimated IFAS contribution based on salaries of PIs, technical support staff, and graduate students; percentage of time allocated to the project; and research support funds. This figure does not include overhead, which is substantially greater than the figure given.