



Twelfth Annual Report

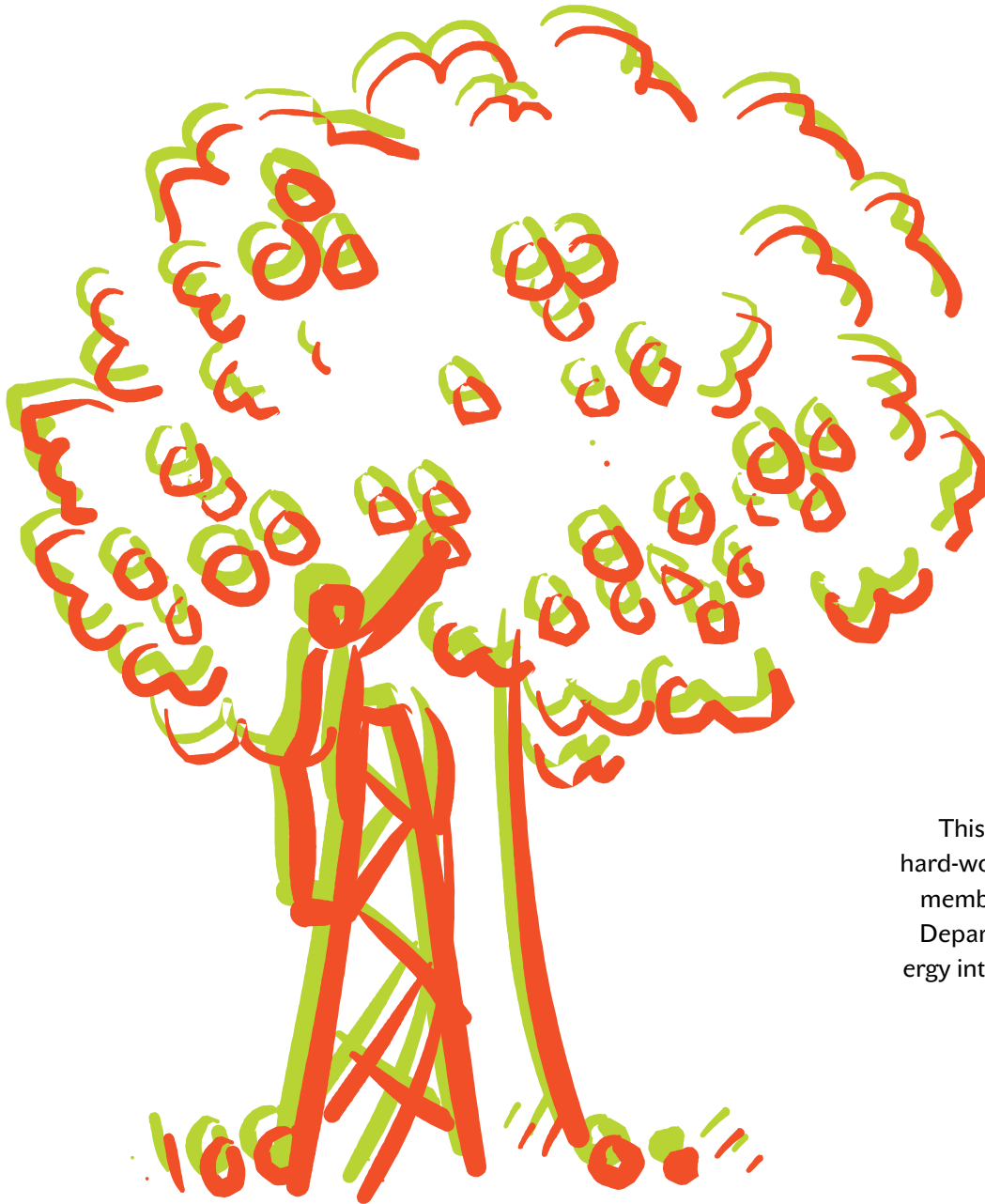
2003 to 2004



Florida Citrus Production Research Advisory Council

Twelfth Annual Report

July 1, 2003 to June 30, 2004



This document is dedicated to the many hard-working researchers, FCPRAC Council members and the CREC Word Processing Department who invest their time and energy into serving the Florida citrus industry.

Administered by
Charles H. Bronson, Commissioner
The Florida Department of Agriculture and Consumer Services

Florida Citrus Production Research Advisory Council

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Florida Citrus Production Research Advisory Council



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Presented in this Twelfth Annual Report is information about research conducted during Fiscal year 2003–2004 by the Florida Citrus Production Research Advisory Council. The Council operates under the Florida Citrus Production Research Marketing Order. This research support program was established under the more general Florida Marketing Act, which enables Florida Citrus Growers to tax themselves to provide funding to support essential research needs. Growers approved the marketing order by referendum in 1991 and again recently in 2004 for another 6-year term. The Council began its work in 1992.

Introduc

Financial Summary for Fiscal Year 2003–2004

Balance Forward – 7/1/2003	\$1,783,417
Collections – 7/1/03 to 6/30/04	2,151,137
Refunds	5,165
Total Cash	\$3,939,719
Disbursements	
Research Projects	\$2,208,296
Administrative Costs	61,580
Total Disbursements	2,269,876
Available 2004-2005	\$1,669,843

TABLE 1. FCPRAC financial summary for Fiscal Year 2003–2004. Amounts are represented in total dollars.

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TABLE 2. FCPRAC funding by years for each category. Smaller numbers in parentheses are numbers of projects (new and continued). Larger numbers in each cell represent funding in dollars.

	Entomology	Manage/Phys.	Pathology	Improvement	Totals
FY 93 - 97	(10) 306,702	(10) 314,792	(12) 304,818	(3) 127,398	(25) 1,053,710
FY 97 - 98	(13) 258,814	(14) 372,064	(10) 363,354	(3) 135,000	(40) 1,129,232
FY 98 - 99	(16) 349,248	(17) 462,291	(14) 453,379	(4) 177,000	(51) 1,441,918
FY 99 - 00	(13) 250,035	(7) 163,965	(9) 300,513	(9) 338,722	(38) 1,053,235
FY 00 - 01	(10) 239,070	(10) 221,810	(10) 351,057	(8) 360,500	(38) 1,172,437
FY 01 - 02	(13) 326,154	(13) 333,500	(12) 516,251	(3) 578,000	(41) 1,753,905
FY 02 - 03	(10) 523,870	(11) 372,500	(10) 640,579	(4) 687,965	(35) 2,224,914
FY 03 - 04	(7) 493,340	(12) 459,700	(8) 552,292	(3) 703,000	(30) 2,208,332
FY 04 - 05	(6) 201,375	(13) 407,000	(7) 567,240	(3) 843,000	(29) 2,018,615
Totals	(97) 2,948,608	(107) 3,107,622	(92) 4,049,483	(40) 3,950,585	(327) 14,056,298



Message from the Scientific Coordinator

Dr. Larry Jackson without fanfare spent the last eleven years of a highly productive career as the Scientific Coordinator of the Florida Citrus Production Research Advisory Council. Dr. Jackson was hired into this position based on his broad range of knowledge and experience in both the practical and scientific aspects of citrus production. However, June 2004 came and the FCPRAC saw the retirement of Dr. Jackson from this valuable industry position.

It is thus with great respect for the precedent established by Dr. Jackson that I took over the responsibilities of the Scientific Coordinator in June 2004. In applying for and interviewing for this position, I conducted my own thorough investigation into the FCPRAC program, the people who established and operate it and how the program was managed. I am proud to say that I came into an extraordinarily well-managed and efficient program. Having served now as Scientific Coordinator for about 8 months, I am hard-pressed to find anything that does not contribute directly to a well-honed machine in which all parts serve directly to get good, practical, scientifically-based technology into the field for the benefit of the growers.

With such a well-run organization, I was baffled at first if I would be able to contribute much to the solid infrastructure established by Dr. Jackson. Nevertheless, I persevered and soon found my “niche” – automation and information design. It was clear that the policies and procedures developed by Dr. Jackson were well-founded, so we hardly changed anything at all regarding the procedural aspects of the program. But we developed an approach to managing FCPRAC information that has allowed us to improve

efficiency in acquiring and managing the information flow: a new web site, technologies to automate reporting and proposal submissions and a unique new way for researchers to share their results in real-time with the citrus industry – the FCPRAC News Server. As with any new approach, there were a few “kinks” that had to be worked out, but overall, I believe the new technologies have added value and efficiency to the FCPRAC program. This year, we are also trying a new approach to the Annual Report – a new design and organization that will hopefully encourage everyone in the industry to keep and return to this report well into the future. In spite of all the things accomplished this year, it is only the first half of the first year of my first term, so there are a few more surprises waiting in the wings that I hope to unveil in the next year or two.

All this said, it has been an honor and a privilege to serve as the new Scientific Coordinator. The Council members are dedicated individuals who contribute their time and energy reviewing proposals, ranking candidate projects and later meeting with researchers to explore what are the best research and funding plans for the industry. The Council receives no remuneration for their efforts, so I view one of my important roles as organizing FCPRAC information in a way that is pleasing and informative to work on by the Council members.

There are few greater thrills than to participate in the development of new science and technology and then helping facilitate that new knowledge in getting applied in the field. It is thus with a sense of great anticipation that I look forward to the coming season working with the Council and the researchers.

– **Steven Rogers**, Highland City, FL, December 2004

Wow! What a year. Hurricanes, wars, and tsunamis prove that most of what we do is merely trivial pursuit. I would like to send a prayer of hope to all of those that suffered tragic losses this year.

This is the Twelfth Annual Report of the Florida Citrus Production Research and Advisory Council (FCPRAC). Here we show how we have spent funds collected from the Florida Citrus Growers via this marketing order. In this report, the researchers describe their projects along with their results to date.

In January 2004, this program was voted on once again. The Florida Department of Agriculture and Consumer Services (FDACS) mailed out referendum ballots to approximately 8,500 citrus growers in January 2004. The measure passed overwhelmingly. It is testimony to the gritty fortitude of growers under economic duress that they voluntarily bear this burden; it demonstrates an optimism towards the future. It is also confirmation that the research community is forwarding a worthwhile product. The tax currently is $\frac{3}{4}$ of a cent per box, which is collected for citrus production research. Staff and overhead consume less than 3% of funds, resulting in over 97% of the funds collected being spent on research.

Dr. Steven Rogers is our new Scientific Coordinator. Steve hails from local Polk County citrus stock, went to a great school here, Florida Southern College, and also one of those little schools out on the west coast somewhere. Steve has hit the ground running, applying his Berkeley-silicon-valley-savvy to the great playbook he inherited from Dr. Larry Jackson. Steve has accomplished a lot in a short period of time, working to automate and improve the flow of information. In this annual report you can get a small taste of his organizational and artistic talent. Steve is also an accomplished researcher in his own right, and is a valuable resource to Council members in evaluating some of the more complex

scientific techniques that are utilized today. We are proud to have Steve on board.

Mirroring the plight of the growers, this year will provide some challenges for the research community and the Council. We will have less funding for this year, and most likely less next year as well. Therefore we must be extra diligent in the husbandry of these funds to make sure that we aim our scarce dollars at the most promising and meaningful research proposals. Our industry is in a state of great flux; there is no way to know what new configuration it will take. It will probably be a much different industry in ten years than it is today. However, I believe that the research we are funding will still be valuable to the industry going forward. We have funded a mix of short- and long-term research proposals that will be useful no matter what form the industry takes. Research naturally builds upon itself, like stepping stones, and once a truth is discovered, it becomes part of the foundation that is used by researchers, and more importantly, growers, on into the future.

Thanks to Drs. Calvin Arnold at USDA and Harold Browning at UF-IFAS for their leadership within the scientific community. Thanks to the many researchers and their staff for the hard work they put into these projects. Thanks to the Council members for the time they take to study the proposals and winnow out the very best ones for funding. Commissioner Bronson and the staff at FDACS administer the referendum and the administration and accounting for the tax dollars and are thus invaluable to the program. It is a good team effort all the way around.

I'd like to ask growers to please discuss any concerns or ideas with Council members or Dr. Steve Rogers. Feedback from the taxpayer to help keep us on track is a healthy part of the process.

- Peter McClure, Immokalee, FL, December 2004



Message from the Council Chairman

Overview

The Florida Citrus Research Advisory Council views the monies invested through this program to be contributed by growers to enhance existing research efforts. The Council monitors overall citrus production research expenditures to ensure growers that their tax monies are in fact enhancing programs.

This section provides a summary of information received from IFAS and USDA-ARS regarding their research expenditures. The Council has not verified this information and may see some adjustments in the future since definitions have not been established for each aspect of research.

Most of this information has not been previously presented in this form before and can only be provided because of the candid disclosures of the research institutions. The Council appreciates their openness and cooperation.

TABLE 3. Florida citrus production research overview for the University of Florida, Institute of Food and Agricultural Sciences (IFAS). This table provides a summary of information received from IFAS regarding their research expenditures. Council monies shown reflect actual expenditures and do not reflect balance of grants held in reserve.

	Total (1)	Third-Party (2)	State/Federal (3)	FCPRAC Grants (4)
1992-1997	\$ 95,300,000	\$ 6,900,000	\$ 1,800,000	\$ 700,000
1997-1998	98,900,000	8,500,000	1,800,000	1,000,000
1998-1999	98,100,000	8,800,000	2,900,000	1,200,000
1999-2000	111,000,000	10,900,000	4,300,000	800,000
2000-2001	114,500,000	10,800,000	5,200,000	1,000,000
2001-2002	124,800,000	12,100,000	4,500,000	1,500,000
2002-2003	124,200,000	11,400,000	7,100,000	1,800,000
2003-2004				

(1) Total all institutional research expenditures, including citrus and all others; (2) Other third-party grants in citrus production research; (3) State and Federal citrus production research expenditures; and, (4) FCPRAC Council grants.

TABLE 4. Florida citrus production research overview for the United States Department of Agriculture, Agricultural Research Service (USDA). This table provides a summary of information received from USDA regarding their research expenditures. Council monies shown reflect actual expenditures and do not reflect balance of grants held in reserve.

	Total (1)	Third-Party (2)	State/Federal (3)	FCPRAC Grants (4)
1992-1997	\$ 4,800,000	n/a	\$ 3,400,000	\$ 200,000
1997-1998	5,600,000	n/a	3,900,000	80,000
1998-1999	6,200,000	n/a	4,100,000	100,000
1999-2000	6,600,000	n/a	4,200,000	100,000
2000-2001	6,700,000	n/a	4,500,000	110,000
2001-2002	7,600,000	n/a	4,700,000	150,000
2002-2003	8,600,000	n/a	4,900,000	400,000
2003-2004	9,200,000	n/a	5,100,000	500,000

(1) Total all institutional research expenditures, including citrus and all others; (2) Other third-party grants in citrus production research (n/a represents data not available); (3) State and Federal citrus production research expenditures; and, (4) FCPRAC Council grants.

of Florida Citrus Research

Approved Projects

FY 2004–2005

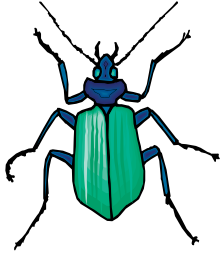
TABLE 5. FCPRAC funding for approved FY 2004–2005 projects by category. Total for all projects is shown in the lower right corner.

	Totals
Entomology	\$ 201,375
Improve/Other	843,000
Manage/Phys.	407,000
Pathology	567,240
	\$2,018,615

TABLE 6. FCPRAC funding for approved FY 2004–2005 projects by principle investigator. Investigators are organized by last name. This list also represents the list of Annual Reports that will be required in June 2005.

Name	Project Title	Number	Amount	Location
Albrigo	Decision Information System for Citrus (DISC) - Further Evolution of the System and Integration of Production Practices, Record Keeping with Bookkeeping Systems	042-13M	\$46,500	IFAS
Albrigo	Foliar Application of NPK: Comparison of Urea Source, Value of P in Foliar Applications and Comparison of Time and Rate of Application on Fruit Set and Yield of Florida Citrus	041-06M	\$30,000	IFAS
Bowman	BLOCK GRANT: Development of Promising New Rootstocks and Scions for Florida Citrus	025-02I	\$338,000	USDA/IFAS
Browning	Provision of Office, Computer & Clerical Support for Dr. Steven Rogers, Scientific Coordinator for FCPRAC	041-03O	\$5,000	IFAS
Castle	Rootstock Performance in Relation to Soils in Flatwoods Citrus and the Development of a Case-Based Expert System to Select Rootstocks	043-05M	\$78,000	IFAS
Chung	Field Trials of Hormone Inhibitors on Yield Production of Citrus Associated with Postbloom Fruit Drop (PFD) and Fungal Pathogenicity of <i>Colletotrichum acutatum</i>	033-03P	\$48,464	IFAS
Chung	Identification of Effective Compounds from Antagonisms for Controlling Foliar Fungal Pathogens of Citrus	033-02P	\$35,464	IFAS
Dawson	BLOCK GRANT: Management of Citrus Tristeza Diseases in Florida	025-01P	\$250,000	IFAS/USDA/DPI
Derrick	Studies to Determine the Cause and Develop Strategies to Control Citrus Blight	003-04P	\$56,822	IFAS

Name	Project Title	Number	Amount	Location
Duncan	Use of Endemic Entomopathogenic Nematodes to Increase Efficacy Against Citrus Root Weevil: The Role of Adaptation	042-10E	\$30,000	IFAS
Futch	Tree Thinning Studies to Improve the Production and Harvesting of Florida Oranges	005-04M	\$10,000	IFAS
Gmitter	BLOCK GRANT: A Comprehensive Program for the Genetic Improvement of Florida Citrus Scion and Rootstock Varieties	0110-03I	\$500,000	IFAS
Graham	BLOCK GRANT: Young Tree Replant Failure	013-99M	\$55,000	IFAS/USDA
Graham	Control of Citrus Canker with Novel Chemical Compounds	042-01P	\$60,990	IFAS/USDA/DPI
Grosser	Rapid Efficient Clean-up of CTV-Infected Field Sources	042-02P	\$17,500	IFAS/DPI
Lee	Citrus Yield Mapping System Using Machine Vision	032-03M	\$35,000	IFAS
McCoy	Assessing Different Management Tactics for the Suppression of <i>Diaprepes</i> Root Weevil Larvae and Adults with Emphasis on Different Rootstocks	045-11E	\$50,000	IFAS
McCoy	Ants, Pesticides and Biological Control of <i>Diaprepes abbreviatus</i>	025-01E	\$39,075	IFAS
Parsons	Effects of Irrigation Rate on Hamlin Orange (Name Change from 2003-04)	034-08M	\$15,000	IFAS
Peña	Classical Biological Control of <i>Diaprepes abbreviatus</i>	043-02E	\$14,000	IFAS
Schneider	Mass Production and Rearing Improvement of <i>Diaprepes abbreviatus</i> to Meet Essential Needs of Florida Researchers	043-05E	\$33,300	DPI
Schumann	Soil Amendments to Improve Tree Performance on Florida Citrus Soils	034-10M	\$33,000	IFAS
Schumann	Implementation of Precision Agriculture Technology to Improve Profitability of Florida Citrus	032-02M	\$42,000	IFAS
Stansly	Biological Control of Citrus Leafminer with the Parasitic Wasp <i>Citrostichus phyllocnistoides</i>	032-05E	\$22,500	IFAS
Stover	Competition Between Shoot Growth and Fruit Set	041-11M	\$10,000	IFAS
Stover	Understanding and Controlling Flushing of Citrus Initial Study on Potted Trees	041-12M	\$15,000	IFAS
Stover	Optimizing Winter GA Application Parameters for Increasing Grapefruit Size	042-10M	\$15,000	IFAS
Timmer	Management Programs for Fungal Diseases of Fruit and Foliage	043-07P	\$98,000	IFAS
Toreki	Polymeric Enhancement of Fruit Fly Bait Sprays to Increase Field Longevity, Decrease Cost and Reduce Pesticide Use	041-03E	\$35,000	Analytical Research



Entomology

2004 Annual Progress Report

Project No. 032-01E

Year 4/4



Assessment of Petroleum Oil Only Programs in Four Sweet Orange Blocks for Process Fruit

Carl C. Childers (UF-IFAS-CREC, Lake Alfred, FL)

Florida citrus growers need cost-effective pest control programs that minimize foliar spray applications while providing optimal returns in fruit quality and yield. This research focuses on several objectives to assess the effectiveness of HMO treatments over 4 consecutive seasons. Impacts on pest mites, greasy spot, fruit set, non-target arthropods, and beneficial mites will be assessed.

Entomology	
Funding Sources	
FCPRAC	\$38000
Agency	31000
Other	0
Total	\$69000

Objectives

1. Four test sites including 2 Hamlin and 2 Valencia blocks were selected. Each test block was compared with an identical block of trees that remained on copper + non-HMO miticides + HMOs.
2. Compare pest mite numbers, rind blemish damage by rust mites, greasy spot control, fruit yields, and secondary pest development.
3. Document re-establishment of beneficial mite complex.
4. Evaluate selected pesticides as potential added products that could be used in this program.

Accomplishments

There were no differences in pest mite populations or rind blemish damage between treatments in any of the 4 sites during 2003. There were slight differences in the number of leaves infected with trace levels of greasy spot in the test blocks versus the controls. However, there were no significant differences in the numbers of leaves remaining on the trees in either the test (HMO only treatments) or control blocks. There were no differences in yields during 2003 after 4 consecutive years on HMO only spray programs. None of the strobilurin fungicides appear to have any negative effects on pest mite increase.

2004 Annual Progress Report

Project No. 971-21E

Year 5/5



Classical Biological Control of Citrus Psylla and Pink Mealybug

Marjorie A. Hoy, Lucy Skelley, Clint Mcfarland, Jeyaprakash R. Wilcox (UF-Department of Entomology and Nematology, Gainesville, FL) and Ru Nguyen (FDACS-DPI, Gainesville, FL)

When this project began in 1998, neither the pink mealbug or the Asian citrus psylla were present in Florida. Both were suitable targets for classical biological control programs because effective natural enemies were known. We were able to respond rapidly to the invasion of the Asian citrus psylla. Host-specific parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, were imported from Thailand and Taiwan and evaluated in quarantine. Permits ere obtained from state and federal authorities to release these exotic insects in a classical biological control program. Rearing methods were developed and parasitoids were released throughout the state in citrus groves and *Murraya* hedges as the pest spread throughout the citrus-growing areas in Florida. To obtain release permits, we had to develop a new molecular test to 'prove a negative', namely that our parasitoids were NOT infected with the bacterial disease called citrus greening. Field surveys conducted over several years throughout the state indicate that *Tamarixia* has established, spread, overwintered successfully, and multiplied. Few specimens of *D. aligarhensis* have been recovered, for unknown reasons. At this point, relationships between the parasitoids, native natural enemies, and the Asian citrus psylla require additional study. We know that the red imported fire ant, *Solenopsis invicta*, is a predator of psyllids parasitized by *Tamarixia* but not of psyllids that are healthy; this predation could have negative effects on *Tamarixia* populations. Parasitoids of the pink mealybug have been imported, released, and are being monitored by Ru Nguyen, my collaborator on this project, and other scientists (including USDA personnel). The parasitoids seem to be establishing, multiplying and are spreading with the pink mealybug. The ladybird beetle (mealybug destroyer) called *Cryptolaemus* is also an important natural enemy of the pink mealybug. It appears that the pink mealybug will not become a pest of commercial citrus due to the effectiveness of these natural enemies, although the pink mealybug is still in the colonization process, so it is too early to be certain.

Entomology	
Funding Sources	
FCPRAC	\$32000
Agency	45000
Other	0
Total	\$77000

Objectives

1. Obtain permits to import parasitoids of the citrus psylla and pink mealybug.
2. Develop rearing methods.
3. Obtain parasitoids and evaluate them in quarantine.
4. Write environmental assessments.
5. Obtain permits to release them in Florida Mass rear and release parasitoids.
6. Evaluate overwintering, dispersal, and impact of parasitoids on pests.

Accomplishments

The project achieved it objectives with the Asian citrus psylla, with one exception. We did import, rear, release, and monitor establishment, overwintering and spread of the two parasitoid species. One, *Tamarixia*, is more common than the other. However, we did not adequately have the time to evaluate impact of these natural enemies on Asian citrus psyllid populations because it takes time for pest and natural enemies to achieve a stable relationship. We can conclude the major steps were achieved but the IMPACT of the establishment should be evaluated in the future. The pink mealybug project has been successful, to date, because of the joint efforts of Ru Nguyen and USDA scientists. This project is still in progress, so it is too early to resolve how effective the parasitoids will be in suppressing pest populations. However, because the project began promptly after the pink mealybug was discovered in Florida, the pest did not spread into commercial citrus groves [pink mealybugs attack citrus primarily when their populations explode on their preferred host plants and citrus is not a preferred host]. The chances are good, therefore, that this pest will not be a serious pest of citrus in Florida.

2004 Annual Progress Report

Project No. 031-10E

Year 3/3



Block Grant: Integrated Control of the *Diaprepes* Root Weevil

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control. It should be noted that accomplishments for Federal researchers are limited this year because their funding for research was delayed for a significant period of time. The following research activities were conducted at the U.S. Horticultural Research Lab using funds provided by FCPRAC for the first *Diaprepes* Block Grant. Diverse approaches to control were explored including aspects of basic biology, plant resistance, viral pathogens, bacterial pathogens, cultural methods, and foliar sprays. Interactions between activities developed over the year. For example, a bioassay developed to study the effect of botanical compounds on neonate larvae is now being used to test newly acquired isolates of *B. thuringiensis*. Kaolin sprays are also being used for field evaluation of rootstocks (2a) to create a split plot (infested vs. non-infested) design.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

The accomplishments of the researchers in their various projects are presented in the following Pages 5 through 15.

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Project No. 031-10E

Year 3/3

Diaprepes Block Grant: Section 1 - Biology and Ecology: Reproductive Biology of the *Diaprepes* Root Weevil

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology

Funding Sources

FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Reproductive Biology of the *Diaprepes* Root Weevil (Sirot, Brockmann and Lapointe).

We investigated seminal proteins in DRW that, together with sperm, comprise the ejaculate. In other insects, such proteins have profound effects on female reproductive behavior including delayed remating, increased egg laying, and decreased longevity. Research into these proteins may lead to new methods for controlling reproduction. In DRW, significant levels of seminal proteins were transferred to females at the beginning of mating. Through radioisotope labeling, we demonstrated that male proteins are found throughout mated females, implying uptake by the hemolymph. They were found primarily in ovaries and eggs, suggesting a role in oviposition behavior and embryo nutrition.

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Project No. 031-10E

Year 3/3

***Diaprepes* Block Grant: Section 1 - Biology and Ecology: Soil Characteristics and *Diaprepes* Management Zones at the Field Scale**

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Soil Characteristics and *Diaprepes* Management Zones at the Field Scale (Syvertsen and Li). *Diaprepes* root weevil management requires an understanding of variations in soil properties in space and time. Starting in 2002, we conducted a study in a citrus grove of Hamlin orange on Swingle citrumelo rootstock in a poorly drained Alfisol near Poinciana, Osceola County. The objectives were to assess the spatial variability of *Diaprepes* populations in relation to soil characteristics and to determine *Diaprepes* management zones related to spatial patterns of soil characteristics. Soil electrical conductivity (EC) was measured using throughout the grove. *Diaprepes* adult populations were monitored using Tedders traps and soil organic matter, pH, P, K, Ca, Mg, and other properties were measured at each Tedders trap. A total of 1400 *Diaprepes* adults were captured in 2002. *Diaprepes* weevil distribution varied with space and time. Peaks of *Diaprepes* adults were measured in the non-flooded high-elevation areas and adults appeared in significantly higher numbers in June (450 weevils) than the other months (24-236 weevils). Three biological zones for *Diaprepes* populations were delineated based on the spatial patterns of soil EC. Over the entire field, *Diaprepes* frequency was significantly high in areas low in Mg and Ca. Semivariograms for *Diaprepes*, Mg and Ca ranged within 100-175 m, which was matched with the *Diaprepes* biological zone limits. The semivariogram ranges of soil variables with the *Diaprepes* zone limits suggested management zones for integrated weevil control. Root weevil management could be related to site elevation, flooding and soil liming practices, but further study is necessary. More precise *Diaprepes* trap monitoring and soil sampling are needed to study detailed soil variability in space and time. We plan to establish a trapping network in different sites to better capture variability of soil, *Diaprepes* population, and landscape position for improved management.

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Project No. 031-10E

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Diaprepes Block Grant: Section 1 - Biology and Ecology: Citrus Rootstock Growth Subjected to Flooding and *Diaprepes* Feeding

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Citrus Rootstock Growth Subjected to Flooding and *Diaprepes* Feeding (Syvertsen and Li).

Flooding events and *Diaprepes* root feeding may have long-term negative impacts on citrus rootstock growth. We conducted a greenhouse study to determine the effects of different flooding durations on soil redox potential, citrus seedling growth, and plant water relations. We studied interactions between previous flooding and *Diaprepes* larval root weevil feeding on leaf stomatal conductance and root damage. The experimental design was completely randomized with two rootstocks (Swingle citrumelo (SWI) and Smooth Flat Seville (SFS)), four flooding durations (0, 10, 20, and 30 days), and larval feeding for 42 days. Plants were flooded, then drained for a week, and five neonate larvae per seedling were introduced onto the soils. Flooding significantly reduced redox potential. Flooding treatment, rootstock variety, and their interaction significantly affected leaf stomatal conductance ($P < 0.001$). Swingle appeared to be more tolerant of flooding stress than SFS. Survival of *Diaprepes* larvae was higher in flooded treatments than non-flooded treatments ($P < 0.05$). Flood damaged seedlings were more susceptible to root injury by larval feeding than non-flooded seedlings. Treatments flooded for 10 days had higher stomatal conductance and lower root injury from larval feeding than those flooded for 30 days. We conclude that a negative soil redox potential and a decrease of leaf stomatal conductance might be useful as early indicators of plant water stress from flooding and root damage from weevil larvae. The potential associations of flooding, root feeding by *Diaprepes* larvae, and infections by *Phytophthora* or other pathogens should also be studied.

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Project No. 031-10E

Year 3/3



Diaprepes Block Grant: Section 1 - Biology and Ecology: Effect of Different Control Strategies for Citrus Root Weevils and *Phytophthora* on Weevil Abundance, Fibrous Root Density, and Yield

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Effect of Different Control Strategies for Citrus Root Weevils and *Phytophthora* on Weevil Abundance, Fibrous Root Density, and Yield (McCoy, Graham, Duncan, and Stuart).

The seasonal control of different life stages of *Diaprepes abbreviatus* and *Pachnaeus litus* using different foliar and soil treatments was compared to no control for 3 consecutive years in a commercial orange grove near Fort Pierce, FL. Seasonal control of *Phytophthora* spp. was also evaluated with and without fungicide in a strip plot design. Treatment effects were determined by measuring weevil abundance via Tedder traps, fibrous root density, and fruit yield. *Pachnaeus litus* was more abundant in the grove than *D. abbreviatus* throughout the study. Seasonal abundance of adults was highest from March through June. Total adult weevils trapped seasonally varied within plots and between replicates, thereby masking treatment effects. Two foliar sprays applied during peak adult abundance had no significant effect on total adult weevils captured annually. One or two soil applications of a chemical or biological pesticide respectively had no significant effect on total *Diaprepes* trapped within a year, however, there was a tendency for lower numbers. This trend was also seen where multiple applications to foliage and soil were applied against *Diaprepes*. In the case of *P. litus*, no significant differences were found between treatments and control. Fibrous root density was significantly higher in all treatments receiving two applications of fungicide annually, however, no treatment effects were found for yield in years 1-3.

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Project No. 031-10E

Year 3/3



Diaprepes* Block Grant: Section 2 - Plant Resistance: (1) Field Comparison of New and Commercially Available Rootstocks for Resistance to DRW; (2) Assessing Rootstocks and Management Tactics on Suppression of *Diaprepes

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
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3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Field Comparison of New and Commercially Available Rootstocks for Resistance to DRW (Lapointe)

Three rootstock trials containing standard rootstocks and selected promising new hybrids were planted with cooperators in *Diaprepes*-infested sites in Indian River County in late 2002 and early 2003. Young tree performance on these sites is being monitored, and additional trials in other *Diaprepes*-infested areas are planned.

Assessing Rootstocks and Management Tactics on Suppression of *Diaprepes* (McCoy and Castle).

A long-term study is underway to determine the seasonal effect of high DRW populations on the growth and development of reset Hamlin orange budded on different non-resistant and *Phytophthora* resistant rootstocks in clay loam soil with and without pest control. After 2 years of optimum care and regular pest control that included suppression of *Diaprepes* with foliar sprays on a monthly basis, trees have grown uniformly throughout the study site. According to soil sampling, *Phytophthora* spp. levels were too low for regular treatment. According to trunk growth measurements taken for all rootstocks after 2 years post-plant, tree growth was significantly greater for C-35 citrange. C-32 citrange, Swingle citrumelo, and Cleopatra mandarin, in that order, were similar in growth. C-22 citrange was the slowest growing rootstock. In 2003, DRW was allowed to infest the whole planting and season long weevil control initiated in trees representing one-half the plot. Control decisions were based on visual and trap counting of adult and larval insects.

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Project No. 031-10E

Year 3/3



***Diaprepes* Block Grant: Section 3 - Biological Control: (1) A Lethal Male Strategy for Management of *Diaprepes*; (2) Development of Bacterial Entomopathogens**

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
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Accomplishments

A Lethal Male Strategy for Management of *Diaprepes* (Hunter and Lapointe).

Infection of *Diaprepes* by an insect virus (IIV6) was confirmed by scanning electron microscopy. Infection was covert and transovarial transmission was demonstrated. To facilitate identification of infected tissues, a complete histological description of the reproductive and alimentary systems was completed. This atlas of the internal morphology and histology of *Diaprepes* will be made available to other *Diaprepes* researchers.

Development of Bacterial Entomopathogens (Weathersbee, McKenzie, and Lapointe).

A technician was hired and identification of bacterial isolates collected from *Diaprepes* cadavers was initiated at USHRL. Twenty-five isolates of *Bacillus thuringiensis* were obtained from a USDA collection including several beetle-active isolates. These isolates are currently being characterized by bioassay against *Diaprepes* larvae, including neonates using the bioassay developed for screening of botanical compounds.

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Project No. 031-10E

Year 3/3



Diaprepes Block Grant: Section 3 - Biological Control: Ants, Pesticides, and Biological Control of *Diaprepes abbreviatus*

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

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Entomology	
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Accomplishments

Ants, Pesticides, and Biological Control of *Diaprepes abbreviatus* (Stuart and McCoy). Two manipulative field studies are under way to test how ant communities impact *Diaprepes* predation pressure. In one study, granular ant baits are being applied at different rates to determine their impacts on ant communities and predation. Such applications tend to reduce the abundance of red imported fire ants but enhance diversity of the ant community. We want to determine whether this process will have a positive or negative impact on *Diaprepes* predation pressure. We are also using a tree-banding technique to exclude ground-nesting ants from citrus canopies to test how the presence or absence of these ants influences predation pressure in the canopy, and the abundance and distribution of other pests such as aphids, scales, and psyllids. Ants tend to be common on the ground and canopy of Florida citrus groves and undoubtedly have a major impact on various ecological interactions. However, they can have positive and negative effects as they prey on certain insect pests (e.g., *Diaprepes*, leaf miners) but protect and potentially enhance others (e.g., aphids, scales, psyllids). These studies will help us unravel the positive and negative impacts of ants within groves and to determine to what extent ants should be conserved for their beneficial impacts. Our first year with the granular ant bait study indicates that reductions in fire ant populations by about 50% (measured using hamburger baits) results in a similar reduction in predation on *Diaprepes* neonates (measured using laboratory reared neonates placed in the field). Moreover, across treatments in which ant populations were reduced to varying degrees, the response of ants to the hamburger baits was highly correlated with predation pressure on *Diaprepes* neonates ($r = 0.623$, $df = 26$, $P = 0.0004$). The granular ant bait study is continuing, but the results reinforce the view that ants are major predators on *Diaprepes* neonates on the soil surface.

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Project No. 031-10E

Year 3/3



Diaprepes Block Grant: Section 3 - Biological Control: Classical Biological Control of Diaprepes with Egg Parasites

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
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Agency	635000
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5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Classical Biological Control of *Diaprepes* with Egg Parasites (Peña).

A total of 2,710,300 *Q. haitiensis* and 402,309 *A. vaquitarum* have been released in Florida until December 2002. A 14% recovery has been recorded on citrus groves in Indian River County for *Q. haitiensis* whereas 43%, 17%, and 14% recoveries of *A. vaquitarum* have been registered in citrus groves located in Miami-Dade, St. Lucie, and Polk Counties, respectively. *Q. haitiensis* appears to be affected by temperatures lower than 59 F, perhaps reducing its effectiveness during winter months. Two exploratory trips were undertaken in May and June 2003, in the island of Dominica. Parasitism of *Diaprepes* eggs in this island fluctuated between 35 to 63%. These trips resulted in the introduction of two additional strains of *Q. haitiensis* and *A. vaquitarum* collected from higher altitudes than the previous strains and perhaps less susceptible to Florida winter temperatures. The exploratory trips resulted in the discovery and introduction of two additional egg parasitoids, i.e., *Haeckeliana* sp. (Hymenoptera: Trichogrammatidae) and a Platygasteridae. All specimens are being successfully reared at TREC under quarantine conditions. The parasitoids will be released after specificity tests are performed and permits obtained.

2004 Annual Progress Report

Project No. 031-10E

Year 3/3



Diaprepes Block Grant: Section 3 - Biological Control: Foliar Sprays of Kaolin-Based Particle Films

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Foliar Sprays of Kaolin-Based Particle Films (Lapointe).

Field trials on the effect of Surround, a kaolin-based particle film, are now in their third and final year. Suppression of oviposition by DRW continues to be documented along with a highly significant effect on tree growth. A new trial was established to examine possible interactions between soil fertility and growth enhancement by Surround. One trial at an infested commercial grove was terminated due to sale of the property. A new trial was established in a commercial grove in central Florida. While data confirm the effect of deterrence by Surround to oviposition by *Diaprepes*, the effect is diluted by the growth enhancement we have observed on heavy soils in St. Lucie County. Surround-sprayed trees were more vigorous and produced flush at a greater rate resulting in greater adult feeding on unprotected foliage that emerged between spray dates. Frequent applications of Surround also appeared to induce outbreaks of scale insects.

2004 Annual Progress Report

Project No. 031-10E

Year 3/3



Diaprepes Block Grant: Section 4 - Cultural Control: A Survey of Potential Cover Crops for Toxicity to DRW Larvae

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

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Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

A Survey of Potential Cover Crops for Toxicity to DRW Larvae (Lapointe).

Species of legumes with potential as cover or companion crops in citrus were examined for repellency and toxicity to *Diaprepes* adults and larvae. Species such as *Arachis pintoi* (perennial peanut), *Crotalaria pallida* (rattlebox), and *Cajanus cajan* (pigeon pea) were shown to be excellent hosts for larval development and therefore not appropriate for use with citrus (Lapointe 2003, Florida Entomologist 86:80-85). Toxicity to larvae and antifeedant activity towards adults were demonstrated for the legume *Tephrosia candida* (Lapointe et al., 2003, J. Economic Entomology 96:811-816). A new high-throughput bioassay involving use of neonate larvae was developed. Roots of *T. candida* were toxic to neonate and 3-week-old larvae. There was significant antifeedant activity towards neonates not previously observed for older larvae. Activity was isolated in biochemically separated fractions and these are now being analyzed to identify active compounds.

2004 Annual Progress Report

Project No. 031-10E

Year 3/3



***Diaprepes* Block Grant: Section 5 - Chemical Control: Effect of Thiamethoxam on Neonate *Diaprepes abbreviatus* in the Laboratory and Greenhouse**

Clayton W. McCoy (UF-IFAS-CREC, Lake Alfred, FL), Stephen L. Lapointe (USDA-ARS, Fort Pierce, FL), George H. Schneider III, Suzanne Fraser, Reed E. Burns, Jr. (FDACS-DPI, Gainesville, FL), James P. Syvertsen, Hong Li, Larry W. Duncan, Jim H. Graham, Robin J. Stuart, Jorge Peña, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL), Jane Brockmann, Laura K. Sirot (UF-CALS, Gainesville, FL), Wayne Hunter, Cindy McKenzie, and Albert A. Weathersbee, III (USDA-ARS, Ft. Pierce, FL)

This progress report for FY 03-04 relates to specific projects of research teams developing pest management (IPM) components for controlling *Diaprepes* root weevil (DRW). The goal of the Block Grant team composed of State and Federal scientists is to implement control strategies, based on sound pest biology, that will increase citrus productivity through reduced crop loss. The content of this progress report includes both new research and continuation of work began in 2001-2002. Contributions from scientists are summarized as research accomplishments in the following block grant thrust areas: 1) biology/ecology, 2) plant resistance, 3) biological control, 4) cultural control, and 5) chemical control.

Entomology	
Funding Sources	
FCPRAC	\$330000
Agency	635000
Other	230000
Total	\$1195000

Objectives

1. Investigate both basic and field research on the biology, behavior, and ecology of all life stages of DRW in relationship to abiotic and biotic factors using sampling or forecasting methods.
2. Investigate root stock performance under different edaphic conditions in groves with and without *Phytophthora* spp. but under seasonal population pressure by DRW life stages.
3. Investigate the role of exotic and indigenous natural enemies of all DRW life stages as biological control agents.
4. Investigate the use of chemical and biological insecticides as foliar and soil treatments to control all DRW life stages with strong consideration to environmental effects.
5. Investigate other non-conventional cultural and mechanical methods for controlling all DRW life stages that are user-friendly.

Accomplishments

Effect of Thiamethoxam on Neonate *Diaprepes abbreviatus* in the Laboratory and Greenhouse (McCoy).

Thiamethoxam, a second-generation neonicotinoid insecticide, is toxic to a wide range of foliar and soil inhabiting insects that include whiteflies, aphids, Colorado potato beetle, and wireworms. In soil column bioassays, thiamethoxam at two rates was highly effective as a contact insecticide against neonate *Diaprepes abbreviatus* after 144 hr exposure. In a greenhouse trial where four rates of thiamethoxam were tested as a soil drench for controlling neonate larvae in container-grown citrus, all rates gave virtually 100% control and total plant protection at 6 weeks post-treatment. In a similar residual trial, thiamethoxam gave excellent larval mortality and plant protection up to 80 days post-treatment. Efficacy at low rates and long residual favor field testing against citrus root weevils.

2004 Annual Progress Report

Project No. 013-11E

Year 3/3



Maximization of Bait/Pesticide Combinations for Caribbean Fruit Fly Control in Florida Citrus

H. N. Nigg (UF-IFAS-CREC, Lake Alfred, FL)

The attractiveness of NuLure7/malathion and other bait-pesticides to Caribbean fruit fly has never been assessed. Also, the quantity consumed of a bait/pesticide for Caribbean fruit fly has not been determined. Consumption is an important consideration because we, and the public, believe that the flies consume the bait/pesticide combination used to control these flies. This is an important point because in a toxicological test flies are simply counted as alive or dead. But why did those flies, which survived, survive? Were they physiologically resistant or did they consume less pesticide than the dead flies? How does pesticide concentration affect the consumption of a bait/pesticide combination? Can we reduce the pesticide concentration in a bait by increasing consumption of the bait and maintain effectiveness? The objective of this project is to produce a maximally consumed, effective bait-pesticide that contains a minimal, but effective pesticide concentration.

Entomology	
Funding Sources	
FCPRAC	\$40940
Agency	95000
Other	10000
Total	\$145940

Objectives

1. Compare food consumption techniques with Caribbean fruit fly (completed).
2. Compare the consumption of commercial lures by Caribbean fruit fly (completed).
3. Compare the consumption components of commercial lures, where known by Caribbean fruit fly (completed).
4. Compare the attraction of the Caribbean fruit fly to host volatiles and other volatiles (completed).
5. Compare the attraction of the Caribbean fruit fly to bait/pesticide with pesticide concentration (in progress).
6. Compare the consumption of the Caribbean fruit fly of bait/pesticide with pesticide concentration (in progress).
7. Test the best combination in the greenhouse (in progress).
8. Field test our final product (spring 2004).

Accomplishments

We have completed Objectives 1-4. We have determined that sucrose is the appropriate sugar for Caribbean fruit fly at 0.2M concentration, approximately 7% sucrose. Most commercial baits contain no sugar or, in the case of Spinosad, 14% sugar. We have determined that there is no toxic interaction between malathion and fluorescein, allowing progress with the ingestion of bait pesticides. We have tested and developed a greenhouse procedure for comparison of baits and are proceeding with greenhouse development and testing. We have developed a maximally consumed simple bait for Caribbean fruit fly. We are proceeding with testing of lures and lure/bait combinations. These objectives (6&7) will be completed without additional funding.

2004 Annual Progress Report

Project No. 013-14E

Year 3/3



Mass Production and Rearing Improvement of *Diaprepes abbreviatus* to Provide Essential Needs of Florida Researchers

George H. Schneider III, Suzanne Fraser and Reed E. Burns (FDACS-DPI, Gainesville, FL)

Numerous researchers are investigating a variety of control measures to counter the ever increasing threat of the Diaprepes root weevil against the Florida citrus industry. This project for mass rearing and improvement of those specialized techniques will ensure that the demand for various Diaprepes life stages will continue to be met so that researchers can forge ahead to find effective control measures against this serious pest of citrus. Specific objectives are outlined below.

Entomology	
Funding Sources	
FCPRAC	\$22800
Agency	60415
Other	0
Total	\$83215

Objectives

1. Provide a continuous supply of any Diaprepes life stage to various researchers in both the government and private sectors. 2. Continuously evaluate and improve upon the mass rearing procedures for Diaprepes to make production more efficient and economical.

Accomplishments

Multiple diet cups were infested with approximately 274,000 neonates, about 74,000 were transferred to single cups, from which 47,000 pupated and 43,000 emerged as adults. This minimal 9% increase in neonate infestation correlated with a 19% increase in grub transfers and 53% increase in pupae and adult production. The use of irradiated larval diet material, the Büchner funnel/vacuum pump infestation method for neonates, and more experienced technicians all contributed to the increased production by aiding in reducing microbial contamination. Shipments included 266,000 eggs, 25,000 neonates, 4,100 grubs and 39,000 adults to twelve different researchers developing a wide range of control strategies against this agricultural pest. This is an increase of 13%, 20% and 46% for neonates, grubs and adults, respectively, over the previous fiscal year. Another trial was run to see if the larval development time interval could be shortened by transferring 120-day-old larvae out of their larval diet and into either vermiculite or perlite media. Unfortunately there were no significant differences between the regular diet and either alternative so the additional transfer step that would be involved is not merited. However, another way to label diet cups is being investigated to save time and labor while minimizing data errors.

2004 Annual Progress Report

Project No. 022-07E

Year 2/2



Improving Efficacy of Sterile Insect Technique for Mediterranean and Other Tephritid Fruit Flies with Hormone Supplement Therapy

Peter Teal (USDA-ARS Center for Medical, Agricultural and Veterinary Entomology), Yeudiel Gomez-Simuta (Moscamed Program, Tapachula, Mexico) and Timothy C. Holler (USDA-APHIS-PPQ, Gainesville, FL)

The Sterile Insect Technique (SIT) is a proven method to eradicate Mediterranean and Mexican fruit flies. The goal of our research is to develop a facile method for incorporating Juvenile Hormone therapeutic techniques into mass rearing of sterile Tephritid fruit flies and demonstrate that the technique results in production of sterile male flies that are far superior in their abilities to mate with wild females than sterile males currently in use.

Entomology	
Funding Sources	
FCPRAC	\$16064
Agency	25000
Other	21000
Total	\$62064

Objectives

1. Study the effects of juvenile hormone (JH) supplement therapy on reproductive development, sexual signaling, and mating in the irradiated flies.
2. Determine the optimal age and stage of development for application of hormone supplement therapy and most effective dose of hormone for maximum acceleration of reproductive development and sexual signaling.
3. Compare the mating competitiveness of hormone supplement therapy treated males to untreated males.
4. Develop a method for application of hormone supplement therapy in mass rearing systems.
5. Provide documentation of improved mating competence to and transfer the technology to managers of factories that produce sterile flies for use in SIT programs.

Accomplishments

Year 2: 1. Demonstrated that sexually mature wild females were more likely to be attracted to 6-day-old sterile laboratory reared males fed hormone supplements than to wild males 16 or more days old fed the optimal rearing diet containing a 3:1 ratio of sugar and hydrolyzed yeast. Thus, hormone treatment results in production of flies that are more competitive than wild males in attracting wild females and are sexually mature at least five days earlier than the standard laboratory strain. 2. Documented that just adding 10% protein to the sugar/agar diet used to feed adults prior to release resulted accelerated reproductive development compared to sterile flies fed only the sugar/agar diet. Thus, protein increases reproductive efficiency of reproduction. 3. Documented that incorporation of hormone into the sugar/agar diet containing protein results in accelerated reproductive development as compared with sterile males fed the sugar agar diet containing protein as evidenced by production of more pheromone and capture of more females. Therefore, hormone supplements have a dramatic effect on reproductive development of flies. 4. In field cage studies in which we assessed the mating performance of 5-day-old sterile Mexflies treated with hormone against wild males and 12-day-old sterile males that received no hormone and compared the results we found that the 5-day-old hormone treated males were as competitive as 12-day-old steriles and effectively mated with wild females. We are currently assessing the efficacy of our hormone therapy system using the eclosion tower system used by APHIS in Texas and Florida. The success of this will show that the technology can be applied to mass rearing.

2004 Annual Progress Report

Project No. 032-05E

Year 1/2



Biological Control of Citrus Leafminer with the Parasitic Wasp *Citrostichus phyllonistoides*

Philip Stansly (UF-IFAS-SWFREC, Immokalee FL)

Biological control is a viable option for control of citrus leafminer (CLM) that has not yet been fully exploited in Florida. *Ageniaspis citricola* was widely released and established throughout the region but has not provided sufficient control. CLM is still a serious pest, ravaging young trees and greatly increasing the risk of canker that has already caused the demise of more than 2 million productive trees in Florida. CLM opens wounds in young foliage increasing susceptibility to canker, and producing multiple infection sites that further augment inoculum and rate of spread. CLM was once a serious pest in Spain requiring multiple sprays, but incidence and damage were greatly reduced by *Citrostichus phyllocnistoides*, a parasitic wasp released for biological control. Given the success of this species in Spain and elsewhere, *C. phyllocnistoides* offers our best prospects for improving biological control of this important pest, thereby reducing the risk of canker throughout the state.

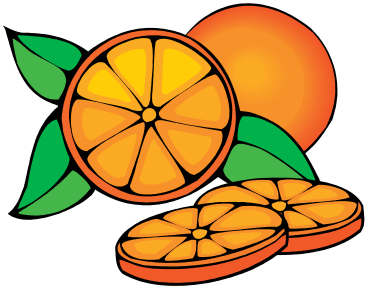
Entomology	
Funding Sources	
FCPRAC	\$22500
Agency	15000
Other	0
Total	\$37500

Objectives

1. Import and permit *C. phyllocnistoides* for release. 2. Ascertain compatibility of *C. phyllocnistoides* with existing biological control agents of CLM. 3. Establish *C. phyllocnistoides* in the field and evaluate effectiveness against CLM.

Accomplishments

1. The permit from USDA-APHIS-PPQ to import *C. phyllocnistis* into quarantine was received 8 January 2003. However, parasitoids were not received at DPI until 17 October due low populations of CLM before that. Since then the wasps have been reared continuously in quarantine inside plexiglass cylinders on CLM itself reared on seedlings of sour orange. Life cycle is 10-12 days at 78 to 85 °F, 60-70% relative humidity and 16:8 hour (light:dark) photoperiod. 2. The cage experiments planned for last year in Spain could not be conducted due to loss of the CLM colony due to excessive heat in the greenhouse. The colony was reinitiated and both *C. phyllocnisoides* and *A. citricola* sent to Spain to restart their colonies of these parasitoids. Nevertheless, as of August 2004 the experiments have not been completed. A contingency plan has been formulated to conduct the planned research at the new quarantine facility in Ft. Pierce in the event that results are not obtained from Spain this year. 3. Federal and State release permits have not been applied for due to lack of information from the experiments planned for Spain, so no post-release fieldwork could be conducted. Nevertheless, baseline data was obtained from a field study conducted in Immokalee in which biotic mortality factors were identified and quantified. The results of these studies concurred with similar studies we conducted from 1994-1996 and showed that predation primarily by ants was the principal mortality factor, providing about 60% mortality with parasitization, primarily by *A. citricola*, providing about 20% mortality. Thus, there is plenty of room for contributions from additional parasitoid releases.



Plant Improvement

and Other Projects

2004 Annual Progress Report

Project No. 025-021

Year 2/8



Development of Promising New Rootstocks and Scions for Florida Citrus

Kim D. Bowman, Greg McCollum, Randall Niedz, Jose Chaparro (USDA-ARS-USHRL, Ft. Pierce, FL) and Jim Graham (UF-IFAS-CREC, Lake Alfred, FL)

Growers do not make a good profit from trees that decline from disease, bear poor quality fruit, never produce good yields, or become too large for efficient management and harvest. These problems that reduce production efficiency and profitability can be eliminated by planting better scion cultivars on improved rootstocks. The proposed research will help develop new rootstocks and scions with superior field performance for use by the industry.

Plant Improvement Funding Sources	
FCPRAC	\$223000
Agency	1800000
Other	0
Total	\$2023000

Objectives

Candidate rootstock and scion cultivars will be created by hybridization, mutation, and transformation. The most promising new cultivars will be identified through use of biological and molecular screens, and entered into long-term field trials at multiple sites. Field performance will be evaluated for numerous traits, including tree size, survival, disease resistance, and fruit yield, quality, and storage problems. Release of new rootstock and scion cultivars will be based on performance information from these trials over multiple years.

Accomplishments

New crosses were completed to create a "supersour" hybrid rootstock, including 87 different parental combinations in 2004. About 1200 candidate supersour hybrids from crosses in previous years are undergoing screening and testing to identify the most promising individuals for widespread replicated field trials. Crosses for improved tangerine, orange, and grapefruit scions were completed and more than 2000 hybrid seed collected and planted. Budwood of high quality, but seedy, scion varieties was irradiated and about 1250 trees planted for selection of seedless mutations. Methods were refined and evaluations conducted to determine rootstock and/or scion resistance to a number of important horticultural problems, including CTV, Phytophthora, flooding, salinity, scab, and canker. Performance information on new rootstocks and scions was collected from more than 20 bearing trials, and 8 new rootstock trials were established at additional sites. Results from trials indicate that some new USDA rootstocks and scions under evaluation may be superior to existing cultivars for some common production situations. Hybrid rootstocks US-802, US-897, and US-942 continue to appear especially promising for ridge and flatwoods areas, including sites with Diaprepes. Detailed information on progress in the USDA breeding program was reported at several meetings attended by growers, including the Florida State Horticulture Society Annual Meeting, Indian River Citrus Seminar, Florida Citrus Nurserymen's Meeting, a field day in Osceola County held jointly with the UF Citrus Breeding Team, Diaprepes Task Force Seminar, and growers group meetings in Lake, Hillsborough, St. Lucie, and Collier Counties.

2004 Annual Progress Report

Project No. 0110-03I

Year 4/10



SECTION 1: Scion Improvement: A Comprehensive Program for the Genetic Improvement of Florida Citrus Scion and Rootstock Varieties

Fred G. Gmitter Jr., Jude W. Grosser, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL) and Gloria A. Moore (UF Extension Horticultural Sciences, Gainesville, FL)

Florida faces many challenges in the production and marketing of citrus products. To insure that the Florida citrus industry can have the most genetically advanced varieties to maintain a competitive advantage, an integrated and comprehensive program for the genetic improvement of scion and rootstock varieties has been established. The overall goal of this project is to develop, test, and release new rootstock and scion varieties for Florida growers through the application of appropriate genetic tools and strategies. Additionally, research focused on the development of basic information and improved tools for breeding and selection will be conducted. Particular objectives are given below. In-kind contributions for this project total to \$7,600,000.

Plant Improvement Funding Sources	
FCPRAC	\$475000
Agency	600000
Other	515000
Total	\$1590000

Objectives

1. Resistance to devastating diseases and pests. 2. Adaptation to diverse soils, growing conditions, and environmental stresses. 3. Improved product marketability through enhanced fruit and juice quality and extension of the harvest season. 4. Lower input costs, along with increased yields and profits. 5. High quality, seedless, easy-peeling mandarins.

Accomplishments

SECTION 1 – SCION IMPROVEMENT Sexual Hybridization: Approximately 4000 embryos were rescued from interploid crosses made in spring 2003 and plants were regenerated through tissue culture and micrografting. Crosses to produce seedless triploids were increased during 2003. Triploid families from 2002 were planted in the field. Crosses designed to move canker resistance from kumquat into mandarin-hybrid scions and for studies on genetic resistance to canker were continued. **Evaluation and Selection:** Data from several sweet orange trials (including Valencia somaclones, irradiated Midsweets, and seedling introductions) continued to be collected. Testing of juice from promising sweet orange and grapefruit clones continued; hundreds of clones were sampled from various plant families throughout the season, and run through the CREC juice analysis facilities. Sensory panel evaluations comparing new processing sweet orange selections continued, and clones with superior flavor were identified. With DPI collaboration, CTV was removed from 20 advanced mandarin selections. Nine new 2nd generation budlines of low-seeded Murcott were identified. **Released Materials:** The patent application on LB8-g tangelo is nearly completed (planned 2005 release), and adequate data have been collected to prepare an application for a superior Valencia somaclone that matures in January. **Somaclonal Variation:** Continued preparation of new sweet orange somaclones for field planting, and evaluation of 2nd generation advanced Valencia selections for early and late maturity, color and flavor. **Somatic Hybridization:** Produced new tetraploid breeding parents of Hamlin sweet orange + LB8-g tangelo, and Page + Dancy. Produced cybrid plants of LB8-g tangelo, and putative cybrid plantlets of Murcott, containing the Satsuma mitochondrial genome for potential seedlessness.

2004 Annual Progress Report

Project No. 0110-031

Year 4/10



SECTION 2: Rootstock Improvement: A Comprehensive Program for the Genetic Improvement of Florida Citrus Scion and Rootstock Varieties

Fred G. Gmitter Jr., Jude W. Grosser, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL) and Gloria A. Moore (UF Extension Horticultural Sciences, Gainesville, FL)

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Plant Improvement	
Funding Sources	
FCPRAC	\$475000
Agency	600000
Other	515000
Total	\$1590000

Objectives

1. Resistance to devastating diseases and pests. 2. Adaptation to diverse soils, growing conditions, and environmental stresses. 3. Improved product marketability through enhanced fruit and juice quality and extension of the harvest season. 4. Lower input costs, along with increased yields and profits. 5. High quality, seedless, easy-peeling mandarins.

Accomplishments

SECTION 2 – ROOTSTOCK IMPROVEMENT Sexual Hybridization/Selection: Several new crosses (at diploid and tetraploid levels) were made in spring 2003, to combine genes for multiple disease resistance, soil adaptations, tree size control, and superior nursery and field performance characteristics. Seed from diploid crosses made in spring 2003 were collected and sown. Hybrid individuals were selected and grown off for field planting and primary evaluation and selection. Evaluations of previously produced citranges for tree health and vigor, fruitfulness, seediness, and the degree of nucellar embryony were continued; 32 were selected, along with other hybrids and parents, for screens for tolerance of high pH heavy soil and resistance against two species of Phytophthora, resistance to CTV-induced quick-decline, tolerance to iron chlorosis, and flooding tolerance (Screening Core). Continued greenhouse screening of 'tetrazyg' hybrids for tolerance of Phytophthora/Diaprepes complex (in cooperation with Jim Graham and Clay McCoy) identified several tolerant hybrids. Somatic Hybridization: Seven new hybrids were produced including 6 mandarin + pummelo combinations (combining pre-selected parents) in efforts to replace sour orange. Field Trials: Among 36 active and 18 inactive field trials, fruit and yield data were collected and tree status records maintained. Data were collected from Murcott rootstock trials (Benton was the top performer). Several field trials were planted, including the large Silver Strand trial in Collier County. Propagation continued for cooperative rotation trials and the large Lykes-Basinger trial for planting during 2005. The blight field-screening program was expanded to two new field sites, and 30 new rootstock candidates were entered into the program.

2004 Annual Progress Report

Project No. 0110-031

Year 4/10



SECTION 3: Plant Molecular Genetics and Transformation: A Comprehensive Program for the Genetic Improvement of Florida Citrus Scion and Rootstock Varieties

Fred G. Gmitter Jr., Jude W. Grosser, William S. Castle (UF-IFAS-CREC, Lake Alfred, FL) and Gloria A. Moore (UF Extension Horticultural Sciences, Gainesville, FL)

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Plant Improvement Funding Sources	
FCPRAC	\$475000
Agency	600000
Other	515000
Total	\$1590000

Objectives

1. Resistance to devastating diseases and pests.
2. Adaptation to diverse soils, growing conditions, and environmental stresses.
3. Improved product marketability through enhanced fruit and juice quality and extension of the harvest season.
4. Lower input costs, along with increased yields and profits.
5. High quality, seedless, easy-peeling mandarins.

Accomplishments

SECTION 3 – PLANT MOLECULAR GENETICS AND TRANSFORMATION Gene Cloning: Three genes upregulated in blighted plants were identified. Transgenic plants containing antisense constructs of chalcone synthase and isomerase were determined to have consistently decreased levels of naringin in leaves. Poncirus and grapefruit genes (CBF) analogous to an Arabidopsis gene for cold acclimation were cloned. Several sequences from citrus, highly similar to the rice Xa21 gene for Xanthomonas resistance were isolated. These are being used to recover similar genes from canker resistant plants, for developing canker resistant commercial varieties. Sequence comparisons between R and S genomic regions revealed the most likely candidates for CTV resistance. New markers for nematode resistance have been developed, based on cloned disease resistance gene candidate sequences, allowing for precise selection for resistance. Transformation: Over 320 transgenic orange and grapefruit plants have been produced containing the most likely CTV resistance candidate genes cloned from Poncirus; CTV challenge assays are underway. A population of transgenic Hamlin plants containing the intron-free cDNA of Xa21 was produced using our protoplast/GFP transformation system. Core Citrus Transformation Facility (CCTF): The CCTF continued to function as a service lab for producing transgenic citrus plants, processing a steady flow of orders. Transgenic plants produced included: 74 of four different grapefruit cultivars (A392' plants for potential CTV resistance); two grapefruit and 4 orange plants that contain an AC Oxidase construct; 31 plants of Valencia and Marsh grapefruit with a Lipid Transfer Protein gene; and nearly 80 grapefruit plants containing prospective CTV resistance gene candidates from Poncirus.

2004 Annual Progress Report

Project No. 0110-11O

Year 1/1



Provision of Office, Computer and Clerical Support for Dr. Steven Rogers, Scientific Coordinator for FCPRAC

Harold W. Browning (UF-IFAS-CREC, Lake Alfred, FL)

The FCPRAC provides significant financial support and direction to citrus production research conducted by IFAS, the USDA, ARS, and other research organizations in Florida and beyond. The grower-driven process has brought greater interaction between the growers and production managers and the research agencies, leading to improved progress towards solving industry challenges related to production. A great deal of this improvement is due to enhanced communication and coordination of efforts, and every opportunity should be recognized to enhance this communication. The CREC, as a major recipient of FCPRAC funding, has the infrastructure in place to support the Research, Extension, and Teaching Programs of the Center and provides access to these facilities to Emeritus Faculty and Visiting Scientists as a matter of course. Thus, the offering of space and support to the FCPRAC Coordinator is in keeping with established policy, and the funding request is to provide for the expendable supplies and to aid in deferring the personnel costs associated with the work load.

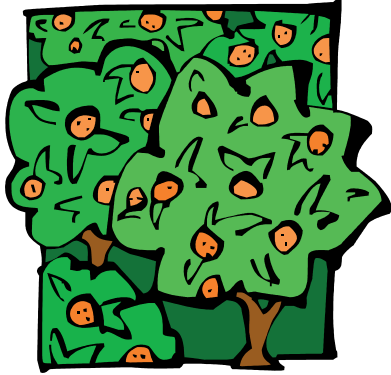
Other	
Funding Sources	
FCPRAC	\$5000
Agency	0
Other	0
Total	\$5000

Objectives

1. Provide office, computing, and clerical support to FCPRAC Scientific Coordinator at UF, IFAS, CREC.

Accomplishments

Provision of office, computing, and clerical support was provided at UF-IFAS-CREC during 2003-2004 as planned. Office space in building 7110 was made available and internet- and network-linked computing was available. Clerical support was available to assist with calls for pre-proposals, proposals, processing and managements of proposals, and preparation of the annual report. The UF-IFAS-CREC website housed some documentation for FCPRAC, and the calls for proposals, instructions for pre-proposers, and annual reports were updated for broad access. Timely management of the FCPRAC documentation and grant processing by Dr. Rogers was facilitated through the support provided by UF-IFAS-CREC.



Management & Physiology

2004 Annual Progress Report

Project No. 013-02M

Year 3/3



Nutrient Management for Optimum Production Efficiency

Joseph P. Albano, Kim Bowman, Michael Bausher (USDA-ARS-USHRL, Ft. Pierce, FL), Jeff Kuehny (LSU), Thomas Obreza (UF-IFAS-SWFREC, Immokalee, FL) and William Henriques (CDC-ATSDR)

Citrus trees express episodic growth patterns of alternating shoot and root growth. In episodic plants, nitrogen uptake by roots is greatest during an episode of root growth, decreasing as an episode of shoot growth begins. There is little information documenting uptake and partitioning patterns for other essential nutrients during episodes of root and shoot growth, and the effects of foliar applied nutrients on the rhythm of root and shoot growth (flush) in citrus is unknown. Studies will be conducted under controlled conditions (laboratory), and in the field. Ruby Red grapefruit and/or Valencia orange grafted on Swingle, Cleopatra, and Volkamer (less than 3 years old) will be used in laboratory studies, and mature (greater than 3 years old), established Ruby Red grapefruit and/or Valencia orange on Swingle and Volkamer will be used in field studies. These studies will provide information to develop nutrient management plans for grapefruit, and to support or revise Best Management Practices.

Manage/Physiology	
Funding Sources	
FCPRAC	\$36000
Agency	89200
Other	0
Total	\$125200

- Objectives**
1. Determine the nutrient uptake patterns during episodes of root and shoot growth.
 2. Determine the effects of foliar applied nitrogen on the rhythm of root and shoot growth and nutrient uptake.
- Accomplishments**
- To understand the effects of foliar nitrogen (urea) on shoot flush, research was conducted during the reporting period with 'Ruby Red' grapefruit grafted on Volkamer or Swingle rootstock cultivars. Plants were grown in a peat-based media in a greenhouse. Data from the study is currently being evaluated, but initial results from gene expression using citrus gene arrays indicates that the method of fertilization/plant nutrition is influenced substantially by the rootstock/scion interaction. The study is currently being repeated.

2004 Annual Progress Report

Project No. 022-01M

Year 2/2



Grower Testing of Florida's DISC (Decision Information System for Citrus): Version 1.0

L. Gene Albrigo, William S. Castle, Ronald P. Muraro, L.W. Timmer, T. Adair Wheaton (UF-IFAS-CREC, Lake Alfred, FL), Howard W. Beck, James J. Ferguson, J. David Martsolf and Fedro S. Zazueta (UF-IFAS, Gainesville, FL)

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 (i.e., Copper Spray Schedule System and a Production Practices Module) for the eventual integrated decision support system for citrus were developed to the stage of functioning prototypes. A phenology model for timing production practices is needed and the record keeping program for applied production practices requires improvements. The first phase of the phenology program, a flowering intensity and bloom date expert system, was first tested during the 2001-2002 winter-spring period and needs further evaluation.

Manage/Physiology Funding Sources	
FCPRAC	\$40000
Agency	44500
Other	20000
Total	\$104500

Objectives

1. Continue to test and refine the Citrus Management System (CMS), which is driven by a flower intensity and bloom date expert system (done) with links to drive the growth events for the fruit and vegetative cycles by citrus type. Irrigation and fertilization should be linked to the growth events, with specialized and pest control timings incorporated for fresh fruit decision-making with. All systems should be auto-linked to a weather data service.
2. Integrate IPM into the CMS for processing and fresh fruit protection.
3. Develop and integrate a Comprehensive Nutrient Management Plan into the CMS.
4. Modify Pre-plant Decision System to incorporate soil specific relationships to rootstocks.

Accomplishments

The Flowering Expert System was tested with an expanded group of growers for a third year. The program was successful and predicted heavy flowering in 2 waves (occurred). Bloom dates predicted in January deviated from the actual bloom dates by less than 7 days. Several operational problems were identified and corrected over the test period. The FAWN weather network interface was improved and more reliable. Some progress was made in allowing AgriLink weather access for growers with that service. A fruit growth model is under development and when added to the flowering model gives a complete reproductive growth program for timing production practices. Another year's data of cultivar fruit growth curves was collected for this model. The Production Practices Scheduling System was improved and further developed through interaction with 4 grove production operations. Irrigation, fertilization and some fruit development management programs are under development. A PDA based scouting software program was integrated with the record keeping system to allow data storage for short- and long-term evaluation. Grower requirements for record keeping are good enough to complement some accounting systems and a workable system is used by one cooperator. More growers were recruited to cooperate in the integration of production practices, development of appropriate record keeping and improvement of the user interface for accounting purposes.

2004 Annual Progress Report

Project No. 013-03M

Year 3/3



Foliar Application of NPK: Comparison of Urea Source, Value of P in the Foliar Application and Time and Rate of Application on Fruit Set and Yield of Florida Citrus

L. Gene Albrigo (UF-IFAS-CREC, Lake Alfred, FL)

Previous work has shown that winter urea sprays can enhance flowering, and bloom and post-bloom application of NPK can increase set and yield. This study will provided comparative data on using various combinations of the 3 seasonal timings at 2 rates, and whether P sources provide any benefit since the amount of P in the fruit is small compared to N and K. In other studies in Valencias, biuret toxicity and possible yield reduction from 2 NPK sprays containing 0.8% biuret (w/w) urea, are being compared to using <0.5% biuret urea. The proposed work would look at these issues and provide more information for grower decisions on use of foliar NPK nutrition. This information would become part of a comprehensive nutrient management plan.

Manage/Physiology Funding Sources	
FCPRAC	\$30000
Agency	35000
Other	2000
Total	\$67000

Objectives

1. Determine effect of various winter timings of flower enhancing urea sprays. 2. Determine flowering, fruit set and yield response of a 3 spray (winter urea (1), bloom (2) and post-bloom(3)) in all combinations within each of 2 rates vs no foliar NPK. 3. Determine if PO₃ or PO₄ in foliar sprays are beneficial in increasing fruit set and yield. Compare these NPK results to a spray containing only N and K. 4. Evaluate if <0.8% (w/w) biuret in the urea in a 2 spray program results in more toxicity and a significant yield reduction compared to a <0.5% biuret urea source. 5. Evaluate if PO₃ sprays of different concentrations up to 2x the recommended rate result in any leaf burn or leaf drop.

Accomplishments

Foliar NPK sprays (14-7-7 at bloom and post-bloom) using either 0.4 or 0.8% biuret urea showed no toxicity symptoms. It appears that 0.8% biuret urea can be used up to twice each year in a foliar spray program. Yields from plots in good ridge and bedded groves were collected from the following: Valencia (2), Hamlin (2), Flame grapefruit and navel orange (1 each) blocks treated with 2 rates of NPK (2-1-1 ratios). Rates of 8 or 16 lb N/A were applied at both bloom or post-bloom or just at one or the other timing with or without a winter urea spray. Other Valencia and Hamlin blocks were treated with NK sprays at bloom or postbloom with or without PO₄ or with PO₃ and with or without urea or PO₃ in the winter. In this second year, again some treatments had yield increases that were more than a box per tree. Also, for a second year there did not appear to be a consistent trend in which treatments gave the higher yields. Flower and fruitlet counts for the third year were made in all plots on spring and summer flush. These counts were similar to the previous year. No further phytotoxicity symptoms from bloom sprays of PO₃ occurred after the first year in any of the Valencia blocks. After 2 years of data collection, early spring foliar nutrition does not appear beneficial on well-cared-for groves.

2004 Annual Progress Report

Project No. 002-01M

Year 4/4



Rootstock Performance in Relation to Soils in Flatwoods Citrus and the Development of a Case-Based Expert System to Select Rootstocks

Bill Castle (UF-IFAS-CREC, Lake Alfred, FL), Brian Boman and Tom Obreza (UF-IFAS-IRREC, Ft. Pierce, FL)

Attempts to maximize grove performance are compromised by two key factors: soil variation and the lack of proven rootstocks for specific site conditions. We initiated a project with two parts designed to learn how trees vary in behavior according to site conditions and the soils and to identify conditions that may relate to the decline of trees on Swingle citrumelo rootstock. The goal of this research is to provide citrus growers with the information and methodology to decide which rootstocks to plant across a landscape based on specific soil properties that can be easily measured.

Funding Sources	
FCPRAC	\$51000
Agency	8000
Other	14000
Total	\$73000

Objectives

1. To identify and characterize soils that are apparently suitable or unsuitable for certain rootstocks, then combine the information into a rootstock-soil catalog that will form a part of the DISC preplant expert system. 2. Conduct a representative survey of Indian River groves to locate old, and poor- and well-performing trees on Swingle and other rootstocks, then correlate soil and site factors to tree behavior.

Accomplishments

One part of the project is basically a series of field trials involving 'Valencia' orange trees on 7 rootstocks planted in replicated areas of 5 soils. We routinely measure root system development, leaf nutrient concentrations, yield, and tree growth. Tree growth and yield so far reflect mostly known rootstock differences. This past year, emphasis in part of the project shifted away from a rootstock-soil catalog concept to producing a case-based reasoning expert system. About 40 cases have been submitted to our collaborator, Howard Beck. A complete draft of the associated field guide to soil identification was assembled with publication expected in 2004. Data collection (tree growth and water table fluctuations) continued in several previously established field trials involving bedding types, soil enrichment, and soil characterization. Grapefruit trees on Swingle citrumelo rootstock in 20-gallon containers of reconstituted parts of the Riviera sand soil profile have grown well with quarterly measurements of growth and annual measurement of root development. A survey was conducted and 74 sites of trees on Swingle and other rootstocks have been located. Tree status in each site was evaluated and the soil series identified.

2004 Annual Progress Report

Project No. 005-04M

Year 6/5



Tree Thinning Studies to Improve the Production of Harvesting of Florida Oranges

Stephen H. Futch (UF-IFAS-CREC, Lake Alfred, FL)

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 as 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. Tree thinning treatments have been under investigation to maintain or improve production and improve manual harvesting rates. The effects of many of these treatments on fruit yield and quality in Florida are not known. Two experiments were established to determine the affect of tree thinning on fruit yield, fruit quality, tree growth and profitability. The two separate blocks consist of trees originally planted in 1990 at a tree spacing of 8' x 24' (227 trees per acre) on two-row beds. The blocks consist of Hamlin with tree thinning initiated in December 1998 and Valencia initiated in May of 2000. Current tree densities consist of 227, 151, and 113 trees per acre and planted on Carrizo citrange rootstock.

Manage/Physiology	
Funding Sources	
FCPRAC	\$10000
Agency	15000
Other	0
Total	\$25000

Objectives 1. Determine the effects of tree thinning on subsequent fruit yields and quality. 2. Determine the effects of tree thinning on tree growth.

Accomplishments Hamlin and Valencia tree thinning experiment is in its 14th year of growth, the sixth crop for the Hamlins and fifth for the Valencias after thinning. The average yields for the 2003-04 for Hamlins was 998, 938, and 997 (boxes/acre) where the trees were unthinned, every second or third tree removed. However, cumulative yield for the sixth season after tree removal has significantly suppressed the Hamlin yield by 20.7% and 13.2%, respectively, where every second or third tree was removed as compared to the unthinned hedgerow. Corresponding pound solids per acre followed similar trends. The average 2003-04 yields for Valencia was 691, 516, and 588 (boxes/acre) where the trees were unthinned, every second or third tree removed. Cumulative Valencia yields for the five years after thinning have also been significantly reduced where trees were thinned with a reduction of 33.9% and 23.2%, respectively, where every second or third tree was removed as compared to the unthinned hedgerow. Corresponding pound solids per acre followed similar trends. Historically, alternate bearing has been a problem with a hedging program being implemented where a severe cut on each fourth side has been implemented. This hedging program has not dramatically reduced the alternate bearing. The differences in yields between thinning treatments for both Hamlins and Valencias is decreasing as the trees fill in the allotted space. Changes in tree growth over time, as determined by trunk diameter, was greatest where every other tree was removed and the least for the unthinned hedgerow.

2004 Annual Progress Report

Project No. 013-12M

Year 3/3



Biology and Control of Vines and Other Difficult-to-Control Weeds in Florida Citrus

Megh Singh and Samunder Singh (UF-IFAS-CREC, Lake Alfred, FL)

Cost of weed control is the largest single component of the production cost in Florida citrus. Chemical weed control is the most common and economic method used in citrus, however, certain weeds are either partially controlled or not controlled with the existing herbicides. Common examples are milkweed vine, balsam apple vine, Brazil pusley, dayflower, goatweed, doveweed, ragweed, spanish needle, prickly sida and teaweed. Higher rates of herbicides or their repeated use is commonly used to control these weeds. Higher rates not only increase the cost, but they are environmentally un-sustainable. Often even higher rates fail to control the tolerant weeds. Consequently these weeds grow more luxuriantly since the competition from other weeds has been eliminated by chemical weed control, thereby potentially increasing losses to citrus in yield and also in quality while increasing production costs. Regeneration in many weeds species is a warning signal for the future. Therefore, an integrated approach using knowledge of weed biology with herbicide rotations or their tank mix or sequential applications and other tools are required for effective control of these hardy weed species.

Manage/Physiology	
Funding Sources	
FCPRAC	\$40000
Agency	0
Other	0
Total	\$40000

Objectives 1. The project emphasized on studying the bioecology of major weeds of citrus, including evaluation of new herbicides for difficult-to-control weeds. 2. Integrate bio-ecological factors with herbicides for sustainable weed management.

Accomplishments Field and glasshouse experiments were conducted on several herbicide combinations, their sequential application (POST and PRE) and integrating bioecological information of weeds for improved weed control efficacy. Water stress resulted in reduced germination and growth of Brazil pusley and improved herbicide efficacy of POST herbicides. Tank mix application of glyphosate with trifloxysulfuron was more effective than their alone applications against yellow nutsedge and Brazil pusley. Trifloxysulfuron had no phototoxicity to young citrus trees with 3x rate, but its alone application was not effective on Brazil pusley, common ragweed, teaweed and grassy weeds. Three way mixture of glyphosate with trifloxysulfuron and butafenacil or flumioxazin had some antagonistic effect under glasshouse conditions; sequential application of glyphosate with flumioxazin provided excellent control of Brazil pusley. Carfentrazone was more effective than glyphosate against dayflower, but not against Brazil pusley. Persistence of diuron and simazine under glasshouse conditions inhibited germination and growth of several vines and broadleaf weeds for 90 days; higher rates of these herbicides were required to inhibit germination for up to 180 days. Glyphosate alone was not able to provide effective control, but sequential application with diuron plus simazine or diuron plus norflurazon provided effective control up to 150 days for several weed species. Weed control in diuron alone treatment was reduced after 90 days, but follow up treatment of diuron plus simazine or diuron plus norflurazon provided more than 90% control of weeds. Repeat applications of rimsulfuron with glyphosate or bromacil plus diuron (Krovar 1), provided better control of hard-to-kill weeds.

2004 Annual Progress Report

Project No. 022-08M

Year 2/2



Refining Management Practices to Enhance Citrus Cropping and Improve Fruit Size

Ed Stover (UF-IFAS-IRREC, Ft. Pierce, FL)

Larger grapefruit receive a premium price early in the season. Large tangerines also receive a substantial premium, and alternate bearing can damage trees and compromise subsequent crops. Techniques for enhancing fruit size and cropping are being explored to provide good recommendations. In 3 previous trials, GA applied late in flower induction has greatly enhanced fruit size. Although hedging and topping around physiological drop did not enhance fruit size in earlier experiments, growers suggest that skirting and topping prior to bloom will be effective. Use of foliar K to enhance fruit size is common and we don't know whether the effect is additive with other methods. Chemical thinning with NAA has been advantageous in many trials with alternate bearing varieties, but further work is needed to determine how best to use NAA thinning.

Manage/Physiology	
Funding Sources	
FCPRAC	\$36000
Agency	45000
Other	5000
Total	\$86000

Objectives 1. Compare effects of NAA, skirting/topping, and winter GA treatments on fruit size, fruit numbers, and yield. 2. Determine potential additive effects between K treatments and other fruit size enhancement methods. 3. Determine cumulative effects of NAA thinning strategies. 4. Conduct commercial scale trials of NAA thinning. 5. Develop strong recommendations to help enhance profitability of Florida citrus production.

Accomplishments Topped trees flushed and flowering earlier than controls; percentage flower removal exceeded percentage canopy removal; winter GA in 2003 reduced flowering by 80-90%. In 2003 grapefruit block 1: topping or GA increased fruit size with no yield decrease; potassium sprays increased fruit numbers with 60% increase in total cropping. In 2003 grapefruit block 2: single GA reduced total yield by 35% and increased mean fruit size, topping reduced yield by 40% with no fruit size increase; and K sprays had no effect. 'Murcott' topping and January GA increased larger fruit with no reduction in yield. 'Sunburst', GA increased fruit size with no reduction in yield; topping reduced yield with no size increase. TPA and K had no effect in either mandarin. Five diverse Murcott blocks were NAA thinned with 30% cropload reduction; 3x more of the 100+ size fruit; 90% fewer small fruit; 10% reduction in yield and 10% increase in fruit value. Thinned trees averaged 3x increase in value in year following thinning. Commercial scale thinning trials in 3 Murcott sites. NAA reduced cropload and increased fruit size in all, with more NAA thinning in Ridge. Higher temperature helped thinning in all blocks and in the IR blocks, ten days without irrigation during drop reduced cropload and increased fruit size better than NAA. Multiple levels and times of topping were tested in another 'Murcott' trial and resulted in transient improvements in stem water potential, but no treatments influenced fruit size. Final yield data to be collected in the winter of 2004-05.

2004 Annual Progress Report

Project No. 013-99M

Year 3/5



Young Tree Replant Failure

Jim Graham, Steve Futch, Larry Duncan (UF-IFAS-CREC, Lake Alfred, FL), Chris Wilson, Tom Obreza (UF Soil & Water Science Department, Gainesville, FL), Kim Bowman, Joseph Albano, Dan Chellemi and Steve Lapointe (USDA-ARS-USHRL, Ft. Pierce, FL)

Citrus reset/replant problems described by production managers as “failure to thrive” are becoming increasingly widespread in Florida citrus, and associated economic losses are considered highly significant. A survey based on mailings to the Florida Citrus Production Managers and an insert in Citrus Industry Magazine has been completed that identifies and characterizes the possible sources of reset/replant problems. Survey results provide key information for in-depth field studies under development to characterize problems and to find possible solutions. Multi-disciplinary teams have been assembled to address the remaining research objectives. Collaborative field-experiments will be conducted in selected production systems in representative regions of the citrus industry. The goal is to develop recommendations that increase young tree performance, while reducing management costs, to sustain earlier tree profitability.

Manage/Physiology	
Funding Sources	
FCPRAC	\$90000
Agency	30000
Other	0
Total	\$120000

Objectives 1. Conduct survey of production managers to evaluate the extent and severity of problems with young tree performance. 2. Conduct site surveys for detailed evaluation of abiotic, biotic, and management problems. 3. Evaluate treatments that may improve young tree growth. 4. Conduct research on specific sources of young tree problems.

Accomplishments Research studies focus on specific problems associated with young tree performance. Sites are established in major production areas (ridge, flatwoods southwest, and flatwoods east coast) to compare pest, pathogen and tree responses to varying soil types, rootstocks, and to clipping vs. pushing of trees before replanting. In both ridge and flatwoods location, higher populations of Phytophthora occurred in sites where trees were clipped than in the push sites, but tree performance was comparable between tree removal treatments. In LaBelle, young Valencia trees on Swingle supported damaging Phytophthora in soils with texture that promoted excessively wet or dry conditions. In Ft. Pierce, the interaction of rootstock and soil type was evaluated for trees on 6 rootstocks after 4 years. Phytophthora was the cause of early mortality of trees on Sun Chu Sha and Cleopatra rootstocks. In this same area in 2002, Hamlin trees on Cleopatra, Smooth Flat Seville, and Swingle rootstocks were planted to compare young tree performance on marginal versus good sites. Mulch of urban plant waste was applied in 2004 as subplots to monitor benefits and economic sustainability. In central Florida, Diaprepes larval feeding predisposed 3-yr-old trees on Phytophthora susceptible rootstocks to tree mortality. Tree decline was reduced by chemical treatments that controlled weevil damage, and was lower for Phytophthora resistant rootstocks (also see FCPRAC 045-11E report).

2004 Annual Progress Report

Project No. 032-03M

Year 1/2



Citrus Yield Mapping System Using Machine Vision

Wonsuk "Daniel" Lee and Thomas F. Burks, (UF-Agricultural and Biological Engineering, Gainesville, FL) and John K. Schueller (UF-Mechanical and Aerospace Engineering, Gainesville, FL)

The proposed research is to develop a yield mapping system using machine vision. Current Goat yield mapping system requires hand harvesting beforehand to create a yield map. The proposed research doesn't need any hand harvesting to create a yield map. The machine vision system will be mounted on a pickup truck, be driven around inside a citrus grove, and take non overlapping and successive pictures of citrus trees with a GPS receiver when the fruits turn to orange color or near harvesting season. The system will identify citrus fruits from images using color information in real time. The number of fruits in a given image will be counted automatically and be used to estimate yield at a given location. Unlike the Goat system, this proposed machine vision system can estimate yield from individual trees. The overall goal of this project is to develop a real time machine vision system for citrus yield mapping to manage grove site-specifically and to increase profit ultimately.

Manage/Physiology	
Funding Sources	
FCPRAC	\$25000
Agency	0
Other	0
Total	\$25000

Objectives 1. Design a machine vision based yield mapping system. 2. Develop a computer vision algorithm to identify fruits in an image for a real time detection of fruits.

Accomplishments A machine vision system utilizing color vision was investigated as a means to identify citrus fruits and to estimate yield information of the citrus grove in real-time. Images were acquired for 98 citrus trees in a commercial grove located near Orlando, Florida. The trees were distributed over 48 plots evenly. Images were taken in stationary mode using a machine vision system consisting of a color analog camera, a DGPS receiver, and an encoder. Non-overlapping images of the citrus trees were taken by determining the field of view of the camera and using an encoder to measure the traveled distance to locate the next position for acquiring an image. The threshold of segmentation of the images to recognize citrus fruits was estimated from the pixel distribution in the HSI color plane. A computer vision algorithm to enhance and extract information from the images was developed. The total time for processing an image was 119.5 ms, excluding image acquisition time. The image processing algorithm was tested on 329 validation images and the R-square value between the number of fruits counted by the fruit counting algorithm and the average number of fruits counted manually was 0.79. The 44 plots were divided into calibration and validation sets and a model was developed for citrus yield. The R-square value between the number of fruits/plot counted by the yield prediction model and the number of fruits/plot counted by hand harvesting for the validation data set was 0.53.

2004 Annual Progress Report

Project No. 034-08M

Year 1/5



Effects of Irrigation Management on Sweet Orange

Larry R. Parsons, Kelly Morgan (UF-IFAS-CREC, Lake Alfred, FL), Brian Boman (UF-IFAS-IRREC, Ft. Pierce, FL) and T. Adair Wheaton (UF-IFAS-CREC, Lake Alfred, FL)

Periodic droughts and increasing energy costs have made economical management of citrus irrigation more critical than ever before. At the same time, recent restrictions on water use permits have made water less available to growers. Less water will potentially be available for irrigation, and we do not have a great deal of quantitative data on the effects of reduced annual irrigation rates on microsprinkler-irrigated groves. This project will study the effect of several different seasonal water application levels on citrus plantings. Fruit yield, Brix, acid, and ratio will be monitored. Additionally, the project will determine the economic advantages or disadvantages of applying higher and lower than normal annual irrigation amounts on citrus production. This project will determine how much savings can be obtained by improved irrigation management. This study will show the impact of irrigation restrictions on citrus productivity and help provide the basis for more reasonable regulations.

Manage/Physiology	
Funding Sources	
FCPRAC	\$27500
Agency	5000
Other	25000
Total	\$57500

Objectives

1. Establish an experiment with Hamlin oranges at a ridge site that provides several irrigation levels.
2. Determine effects of different seasonal irrigation application levels on yield and profit.

Accomplishments

The irrigation system in a mature Hamlin grove at the Water Conserv II ridge site was modified to allow different application rates to selected trees. Poly tubing, flow meters, and underground PVC lines were installed in the fall of 2003. Six irrigation treatments were started in the spring of 2004. Treatments are arranged in a randomized complete block design experiment with seven blocks covering approximately 5 acres. Using a water budget model, different amounts of water were applied to the treatments. Soil moisture (Echo) capacitance probes were installed at different depths under selected treatment trees to monitor soil water content and depth of water movement. Treatments received irrigation in the spring and fall of 2004 at different depletion levels ranging from 25 to 100% depletion. The wettest treatment was irrigated at 25% depletion in both spring and fall, and the driest treatment received no irrigation (100% depletion) and is surviving on rainfall. Data on fruit production and quality in the different irrigation treatments will be taken in fall, 2004. Brix will be monitored to see the relationship between fruit sugar level and yield of trees stressed in the spring or fall. Planning was started for a similar irrigation study on the flatwoods. Economics of water application will be determined to see how higher and lower than normal irrigation rates affect profitability.

2004 Annual Progress Report

Project No. 032-02M

Year 1/2



Implementation of Precision Agriculture Technology to Improve Profitability of Florida Citrus

A.W. Schumann, W.M. Miller, and M. Salyani (UF-IFAS-CREC, Lake Alfred, FL), J.K. Schueller and W. Lee (UF-IFAS, Gainesville, FL)

Recent studies showed that soil variability is a dominant factor affecting in-field spatial variability and profitability of citrus. Over the past year we have identified some specific soil factors responsible such as water tables, organic matter content and iron deficiency, by using precision agriculture techniques. Maps of fruit yield, quality, and canopies along with topography, soil characteristics, available water, fertility, leaf analyses, and pest populations are the basis for varying application rates of water, nutrient, and pesticides in citrus production. We propose to conduct further research into causal soil factors in different representative areas of the citrus industry, and also to develop and test suitable site-specific remedies, rates of amendments and cultural practices for these weak soils. After this information has been mapped and the required cultural practices determined, variable rate, control and sensing equipment will be used to apply the required remedy or cultural practices as needed in the site-specific areas.

Manage/Physiology	
Funding Sources	
FCPRAC	\$42000
Agency	65000
Other	120000
Total	\$227000

Objectives

1. Evaluate strategies for linking spatial yield variability with measurable grove variability (identify cause and effect relationships).
2. Improve profitability by i) varying inputs and management strategies to optimize returns from both highly productive and less productive areas of a grove, ii) develop custom remedies for under-performing soils to improve productivity, iii) remove the least profitable portions of groves out of production in order to curtail losses.
3. Evaluate costs and returns of precision agriculture with interested citrus growers.

Accomplishments

An on-farm experiment was installed at Southern Farms to test the efficacy of supplementary calcium nitrate fertilization to enhance fruit set during the main bloom period of Valencia. Also at Southern Farms, a sewage sludge rate experiment (0-4000 lb/ac/yr) was established to evaluate higher-than-normal rates of biosolids on weak sandy soils. We initiated a comparison of variable rate (VRT) versus fixed rate granular fertilization of a spatially variable Valencia block on a Ridge site with Gapway Groves, and on a soil-limited Hamlin/Valencia block on a Flatwoods site with Cargill. Objectives of these experiments are to conserve fertilizer (up to 40%), improve yields and fruit quality by better fertilizer supply-demand matching, and reduce nitrate leaching by avoiding excess N in the root zone. Thirty-six suction lysimeters were installed in the first experiment and 20 in the second, to monitor soil solution nitrate levels exiting the root zone. Performance of the spreader with tree-to-tree variable rates is being assessed from the as-applied rate maps collected during fertilization and the yield is being measured by yield mapping. We developed new Windows software for the Durand-Wayland ultrasonic rig that now allows real-time measurement of tree canopy volume and height for entire groves. We developed and built three fully automated sensor-based yield monitoring loggers for mounting on commercial goat harvesters. These new loggers eliminate the substantial errors introduced by operators using manual yield monitors.

2004 Annual Progress Report

Project No. 034-10M

Year 1/4



Microbial Soil Amendments to Improve Tree Performance on Florida Citrus Soils

A.W. Schumann, J.P. Syvertsen, L.G. Albrigo, J.H. Graham (UF-IFAS-CREC, Lake Alfred, FL) and B. Boman (UF-IFAS-IRREC, Ft. Pierce, FL)

The profitability of Florida citrus groves is often limited by soil conditions, mostly by weak soils interspersed with better soils in production blocks. Research shows that the weak soils often have depleted soil organic matter content and generally weakened biological activities. There are many advertised amendments that claim to "condition" unproductive soils by improving the balance of beneficial microbes. Because there is no known research demonstrating the effectiveness and profitability of these products on citrus, we will undertake to test a selection of these in a potted greenhouse experiment and in the field. Four products will be screened in the greenhouse for tree growth and nutrient uptake response, soil water potential, and nutrient leaching. The best two candidates from the greenhouse tests will be included in three field-scale experiments at three locations (Southern flatwoods, Indian River and Ridge). At the end of the 4-year study, we expect to establish the validity of recommending and profitability of using microbial soil conditioners for Florida citrus.

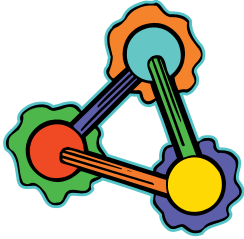
Manage/Physiology	
Funding Sources	
FCPRAC	\$32100
Agency	30000
Other	10000
Total	\$72100

Objectives

1. Conduct a potted tree experiment to determine if any of the available microbial products improve seedling growth and nutrient content in a ridge sand.
2. Use two products selected from Objective (1) in field trials to determine if tree growth and yields respond to microbial products with or without surface bio-solid additions in three major growing areas.

Accomplishments

A greenhouse experiment with potted seedlings was conducted in summer of 2003 to screen the effectiveness of four commercial microbial products for potential future field testing. Three rates of each product were evaluated using Carrizo citrange seedlings growing in unsterilized Candler sand from the field. Seedling growth, nutrient leaching and leaf nutrient levels were monitored. Plant growth analyses showed that two microbial products significantly enhanced total plant dry weight above that of the non-amended controls while two products had no effect. A replicated field experiment was established with the two best products in each of the Ridge, Indian River and Southern flatwoods citrus growing areas during winter of 2003/04. The first season was used to establish baseline yield, leaf nutrients and soil properties. Microbial amendment treatments were applied in spring of 2004 (the two microbial products at recommended rates and a non-amended control, with or without 1000 lb/ac of bio-solids). All other management practices were identical. Response measurements will include canopy growth, yield, leaf nutrient concentration, leaf color, and soil moisture profiles annually. Soil Phytophthora status, microbe/active organic matter assessments and root density will be measured after treatments have been applied for 2 years.



Pathology

2004 Annual Progress Report

Project No. 013-07P

Year 3/3



Control of Citrus Canker with Novel Chemical Compounds

Jim Graham (UF-IFAS-CREC, Lake Alfred, FL) and Tim Gottwald (USDA-ARS-USHRL, Ft. Pierce, FL)

Two existing major outbreaks of Asiatic citrus canker caused *Xanthomonas axonopodis* pv. *citri* (Xac), one in Miami Metro and a second in Manatee Co., and several other minor outbreaks scattered throughout the southern portion of Florida, raise serious concerns regarding the spread of the disease to commercial citrus in Florida. Control of canker on susceptible varieties is limited with copper bactericides. Although copper diminishes infection to an extent by acting on surfaces of tissues to reduce bacterial populations, the effectiveness is minimal once rains with wind introduce bacteria into tissues. Other contact bactericides tested are not as effective as copper because of their inability to persist on the leaf surface due wash-off or breakdown on the leaf surface. A novel class of compounds that act through induced systemic resistance (ISR) might increase resistance of susceptible citrus tissues to citrus and complement the activity of copper that controls leaf surface bacteria.

Plant Pathology	
Funding Sources	
FCPRAC	\$164785
Agency	360000
Other	40000
Total	\$564785

Objectives 1. Screen new compounds for citrus canker control in the greenhouse using the citrus bacterial spot disease surrogate, *Xanthomonas axonopodis* pv. *citrumelo*. 2. Evaluate promising compounds from greenhouse tests in field situations utilizing existing nursery infestations of CBS for evaluation. 3. Test promising compounds resulting from the first two steps on citrus canker-infested trees under containment in greenhouse and field quarantine facilities in Florida and in Brazil.

Accomplishments Induced Systemic Resistance (ISR) compounds, Actigard (acibenzolar-s-methyl, Syngenta) and Messenger (harpin protein, Eden Bioscience), were screened in the greenhouse against *Xanthomonas axonopodis* pv. *citrumelo*, the cause of citrus bacterial spot (CBS), and *X. axonopodis* pv. *citri*, the cause of Asiatic citrus canker (ACC). Greenhouse sprays of Actigard and Messenger reduced lesions when either bacterium was injection-infiltrated into Swingle *citrumelo* leaves. ISR activity was further evaluated in 2 years of field trials under disease endemic conditions Brazil in spray programs with and without copper oxychloride (COC) and copper hydroxide (CuOH) on 3- to 4-yr-old sweet orange trees with low to moderate ACC pressure. Six sprays of COC and CuOH were moderately to highly effective for reducing foliar disease, fruit infection and premature fruit drop. ISR compounds with and without Cu formulations did not further reduce citrus canker incidence on foliage, fruit or reduce fruit drop compared to Cu alone. Up to six sprays of formulations of contact materials, oxolinic acid (Valent So208) and DBNPA (Dow 120192) that showed promise for control of *Xanthomonas* diseases in other fruit crops failed to control ACC in the field probably due to their high solubility and lack of residual activity. Evaluation of novel chemical compounds so far indicates that: 1) contact materials must be rain-fast to be effective, and 2) induced resistance compounds and other contact materials must be combined with copper to achieve disease control.

2004 Annual Progress Report

Project No. 013-05P

Year 3/3



Keeping Citrus Propagation Increase Blocks Free of Severe Citrus Tristeza Virus

Susan Halbert, Tim Schubert (FDACS-DPI, Gainesville, FL) and Tim Gottwald (USDA-ARS-USHRL, Ft. Pierce, FL)

The establishment of brown citrus aphid has caused a significant increase in both incidence and severity of citrus tristeza virus (CTV) infection in Florida. The best way to ensure that Florida continues to have quality healthy citrus groves is to keep planting stock clean. Current Division of Plant Industry regulations require that citrus scion source trees be tested for CTV each year. If scion source trees are found positive for severe CTV, they are no longer allowed to be used as sources for budwood. Growers may choose to propagate via increase blocks that can remain in use for 24 months after establishment before additional CTV tests are required. The 24-month time period was never documented by data, but was considered a "best guess" at the time the regulations were written. Now that brown citrus aphid is well established, and CTV transmission is increasing, it is time to determine whether earlier testing of increase blocks may be necessary to prevent distribution of plants infected with severe CTV.

Entomology	
Funding Sources	
FCPRAC	\$15480
Agency	22500
Other	0
Total	\$37980

Objectives

1. Establish insecticide treated and untreated increase blocks under commercial management.
2. Test the increase blocks periodically for severe CTV.
3. Monitor presence of brown citrus aphids.

Accomplishments

In July 2001, we began sampling an increase block established near Immokalee in April 2000. We collected leaves from all plants (about 15,400). 17.7% of the samples (10 plants/sample) were positive for severe CTV. In the second and third sets of samples (December 2001 and April 2002), rows 6 and 12 were sampled. In row 6, 10.6% of the plants tested positive for severe CTV in December. No severe CTV was ever found in row 12. The pattern of severe CTV in the field suggested a combination of contaminated budwood and aphid transmission. This happened in spite of the fact that the grower followed the DPI regulations, indicating that the 2-yr time frame for increase blocks may be optimistic for outdoor nurseries, even with minimal aphid pressure. A second increase block in a modified greenhouse was established in Winter Haven in June 2002. Liners, budwood, and four sets of subsequent samples (October 2002, April 2003, August 2003, and December 2003) were tested. All samples were negative. Brown citrus aphids were collected in the trap outside, but not inside the enclosure. Most plants sampled in the neighboring grove had severe CTV. The protection afforded by the enclosure and the clean start may account for the major difference in severe CTV incidence. Our results indicate that in order to maintain an increase block free of severe CTV for 2 years, it is necessary to start with clean liners and scions. A screened enclosure also is necessary.

2004 Annual Progress Report

Project No. 013-16P

Year 3/3



Biology and Control of Fungal Diseases of Fruit and Foliage

L. W. Timmer, K.-R. Chung (UF-IFAS-CREC, Lake Alfred, FL) and T. L. Peever (Washington State University, Pullman, WA)

Fungal diseases cause significant losses in external quality of fresh fruit and yield of all citrus. New fungicides, such as Abound, Headline, and Enable, and products which induce systemic resistance (SIR) promise to improve disease control. We propose to determine the baseline sensitivities of citrus pathogens to better manage resistance and to assess SIR products to determine their efficacy in disease management. Means of using the epiphytic growth on leaves to better time fungicide applications for greasy spot control will be investigated. We will evaluate thresholds for use of the Alter-Rater for different varieties and situations to better utilize the model. We propose to determine the origin of the postbloom fruit drop and Alternaria brown spot problems by using molecular methods to study population dynamics of these pathogens.

Plant Pathology	
Funding Sources	
FCPRAC	\$149000
Agency	60000
Other	10000
Total	\$219000

Objectives

1. Disease control - determine the baseline sensitivities of foliar fungal pathogens to Abound, Headline, and Enable; assess activity of resistance-inducing compounds for control of foliar fungal diseases.
2. Improve timing of fungicide applications - develop means to measure epiphytic growth of Mycosphaerella and assess its use for timing of greasy spot sprays; determine appropriate thresholds for the Alter-Rater for brown spot control.
3. Investigate the origin of fungal pathogens - the relationship of Colletotrichum acutatum isolates from postbloom fruit drop and lime anthracnose and the origin of PFD; determine whether non-pathogenic strains of Alternaria or pathogens of rough lemon carry genes for the toxin for tangerines and have the potential to attack new varieties.

Accomplishments

The 50% effective doses (ED₅₀) for all 5 foliar pathogens to Abound, Headline, and Enable have been determined and the range of activity of 60 isolates of each pathogen from statewide collections have been assessed. Methods are now available to detect resistance if control problems occur. Thresholds for use of the Alter-Rater predictive system for Alternaria brown spot have been determined and published. Conditions for pseudothecial development, ascospore release, and dispersal have been determined for Mycosphaerella citri, the cause of greasy spot. Peak periods for ascospore release and the development of the epiphytic growth have been determined and the recommendations for best control of greasy spot modified accordingly. Applications of lime, extra irrigations, or urea reduce inoculum production of greasy spot. RAPD analyses and DNA sequencing indicated that PFD isolates from all over the Americas are clonal and the fungus has probably spread from country to country. Molecular studies indicate that Alternaria isolates from Minneola tangelo from Florida and Colombia form one group; those from Turkey, Israel, and South Africa a second; and those from Australia, a third. Molecular analyses indicate that the tangerine pathotype, the rough lemon type, black rot isolates, and saprophytes all belong to the same species, A. alternaria.

2004 Annual Progress Report

Project No. 033-02P

Year 1/3



Identification of Effective Compounds from Antagonisms for Controlling Foliar Fungal Pathogens of Citrus

Kuang-Ren Chung (UF-IFAS-CREC, Lake Alfred, FL)

Microorganisms, mainly fungi and bacteria, produce many kinds of toxic compounds inhibitory to the growth and development of other organisms, representing enormous but largely untapped natural resources for disease controls. The goals of this project are to identify and characterize the microorganisms, specifically their secondary products with antagonistic activity against the major citrus foliar fungal pathogens such as *Alternaria*, *Diaporthe*, *Elsinoe* and *Mycosphaerella*, to investigate the antagonistic mechanisms, and to evaluate the inhibitory compounds for disease control in citrus.

Plant Pathology	
Funding Sources	
FCPRAC	\$25000
Agency	0
Other	0
Total	\$25000

Objectives

1. Isolation of antagonists from citrus phyllospheres, and characterization of antagonistic activities against foliar fungal pathogens, including *Alternaria*, *Mycosphaerella*, *Elsinoe*, and *Diaporthe*. 2. Investigation of antagonistic mechanisms (antibiosis, toxicity, mycoparasitism, or competition for resources), and identification of antagonistic compounds. 3. Initiation of greenhouse trials for disease controls.

Accomplishments

A simple, effective bioassay has been established to screen antagonistic microorganisms from citrus groves. Four fungal isolates (designated LAL₅, 11, 13 and 16) and one bacterial strain (designated LAL₄₀) exhibiting strong growth inhibition to *Diaporthe* and/or *Alternaria* in cultures have been identified. Both LAL₅ and 11 isolates were identified as *Cladosporium cladosporioides*, and LAL₁₃ and 16 isolates were *Fusarium rigidiusculum*. The identity of LAL₄₀ remains uncertain. The cultural filtrates from LAL₅ and LAL₁₁ reduced *Diaporthe* growth by 90-95%, whereas reduced *Alternaria* growth by 40-50%. In contrast, the cultural filtrates from LAL₁₃ reduced *Alternaria* and *Diaporthe* growth by 60-75%. The cultural filtrate from LAL₁₆ exhibited a strong growth inhibition to *Alternaria* (by reducing 75% growth), but had little effect on the growth of *Diaporthe* (by reducing 3% growth only). Interestingly, heating the cultural filtrate of LAL₁₆ greatly increase the growth inhibition to *Diaporthe* (up to 40%). The cultural filtrates from LAL₅, 11 and 13 boiled at 95C for 15 min slightly reduced the ability for growth inhibition of both *Diaporthe* and *Alternaria*, indicating that the inhibitory compounds are heat stable. The feature of these toxic compounds requires further determination. The information generated from this research may have great potential on identifying and applying the microorganisms and active bio-products to the field through fermentation and/or genetic engineering manipulation.

2004 Annual Progress Report

Project No. 033-03P

Year 1/3



Field Trials of Hormone Inhibitors on Yield Production of Citrus Associated with Postbloom Fruit Drop (PFD) and Fungal Pathogenicity of *Colletotrichum acutatum*

Kuang-Ren Chung, Jacqueline K. Burns and L. W. Timmer (UF-IFAS-CREC, Lake Alfred, FL)

Postbloom fruit drop (PFD) of citrus is caused by the fungus *Colletotrichum acutatum*, usually resulting in fruit drop, production of persistent buttons, and leaf distortion. In this proposal, we determined the levels of hormones or growth regulators (IAA, ethylene, ABA, Jasmonic acid, and salicylic acid) in response to fungal infection. Efficacy of hormone inhibitors in preventing yield losses was also evaluated. A KLP₁ (Key Lime Pathogenicity) gene involved in fungal infection was cloned from *C. acutatum*. Functional analysis using a gene disruption is being conducted to verify the involvement of KLP₁ in symptom development. The proposal intends to provide valuable information for future decision making on PFD management. Specific objectives are outlined below.

Plant Pathology	
Funding Sources	
FCPRAC	\$45000
Agency	0
Other	0
Total	\$45000

Objectives

1. To evaluate the effectiveness of hormone inhibitors for preventing yield loss due to PFD. 2. To determine the effect of hormone inhibitors on gene expression and hormone accumulation. 3. To isolate and characterize the full-length KLP₁ gene potentially involved in fungal pathogenesis. 4. To conduct gene disruption to confirm gene function associated with fungal pathogenesis.

Accomplishments

Colletotrichum acutatum infects citrus petals, and induces premature fruit drop and the formation of persistent calyces. After fungal infection, ethylene evolution increased threefold and IAA accumulation was as much as 140 times. ABA levels showed no significant response. Both trans- and cis-12-oxo-phytodienoic acid increased eight- to tenfold. No significant difference of trans-jasmonic acid (JA) was observed in citrus flower petals or pistils. However, a fivefold increase of cis-JA was detected. The amount of salicylic acid (SA) was elevated twofold in affected petals, but not in pistils. Northern-blot analyses revealed that the genes encoding ACC oxidase or ACC synthase, and 12-oxo-phytodienoic acid (12-oxo-PDA) reductase were highly expressed in affected flowers. The genes encoding auxin-related proteins were also up-regulated. Application of clofibrate (a putative auxin inhibitor), 2,3,5-triiodobenzolic acid (TIBA, an auxin transport inhibitor), or SA after inoculation significantly decreased the accumulation of the gene transcripts of auxin-responsive, GH3-like protein and 12-oxo-PDA reductase, but resulted in higher percentages of young fruit retention. The results indicate that imbalance of IAA, ethylene and JA in *C. acutatum*-infected flowers may be involved in symptom development and young fruit drop. To investigate the factors required for fungal infection, we have clone the first gene, named KLP₁ (Key Lime Pathogenicity) gene from *C. acutatum* KLA isolate. The KLP₁ gene encodes a fungal transcription activator, and is involved in fungal pathogenesis. Gene replacement is being used to confirm its function. The funding from FCPRAC to this project has resulted in 4 publications.

2004 Annual Progress Report

Project No. 025-01P

Year 3/5



Management of Citrus Tristeza Diseases in Florida

Bill Dawson, Steve Garnsey, Richard Lee (UF-IFAS-CREC, Lake Alfred, FL), Tim Gottwald, Mark Hilf, Scott Adkins (USDA-ARS-USHRL, Ft. Pierce, FL) and Peggy Sieburth (FDACS-DPI, Winter Haven, FL)

Citrus tristeza virus (CTV) is widespread throughout Florida. Florida growers face several CTV-induced problems. First, the increasing distribution of decline isolates has removed sour orange as a rootstock choice. Growers are forced to use less well-adapted rootstocks and hence suffer increased production losses from other problems. Secondly, the hazard of introduction and spread of new severe isolates, particularly those causing stem pitting, is increasing. The introduction of these severe stem pitting strains would seriously affect citrus production. Increasing spread of the decline strain into budwood increase blocks has hampered nursery production because there is a lack of detection procedures to differentiate severe exotic strains from the decline strain that already is endemic in commercial citrus. We are making a unified, coordinated effort to manage the present situation and to be prepared to manage more severe CTV diseases should they become a problem in Florida.

Plant Pathology	
Funding Sources	
FCPRAC	\$250000
Agency	40000
Other	164000
Total	\$454000

Objectives 1. Categorize worldwide CTV isolates based on disease and sequences. 2. Develop and evaluate procedures capable of detecting new severe strains. 3. Adapt new procedures for large scale screening by DPI. 4. Engineer "ideal" cross protecting isolates of CTV. 5. Evaluate new potential cross protecting isolates.

Accomplishments Genetic analysis of 350 exotic isolates of CTV found three easily distinguished groups, with isolates in each group showing wide variation of disease severity. Based on these groupings, sensitive RNA-based diagnostic techniques were adapted for high-output screening by DPI to identify CTV isolates with a high probability of causing stem pitting. Unexpectedly, these procedures identified from field trees a series of closely related isolates identified to a CTV group (VT) that contains mostly stem-pitting isolates. A survey throughout commercial citrus in Florida has found low incidence in Collier and St. Lucie Counties and some groves with high incidence in Polk County, suggesting that the virus is efficiently spread by aphids. These isolates induce mild to moderate degrees of stem pitting in sweet orange in the greenhouse. We are developing methods to protect trees from decline or stem pitting by mapping disease determinants in the virus for creation of protecting isolates. We already are evaluating some engineered mild strains for protection against severe strains. We have disarmed the ability of the decline isolate to induce seedling yellows, which often correlates with decline, and it is possible that we have disarmed its ability to cause decline on sour orange. Tests are underway. We also have ongoing experiments in the quarantine greenhouse in Beltsville, MD, to reproduce and dissect a stem-pitting protection system that is effective in Peru. As a by-product, we have developed CTV into a tool to test products for control of citrus canker or greening.

2004 Annual Progress Report

Project No. 971-43P

Year 2/3



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

Kenneth Derrick, Gary Barthe and Julia Beretta (UF-IFAS-CREC, Lake Alfred, FL)

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.

Plant Pathology	
Funding Sources	
FCPRAC	\$20000
Agency	2000
Other	10
Total	\$22010

Objectives 1. To develop rapid detection methods for citrus psorosis virus (CPV). 2. To develop a virus vector for expressing foreign genes in citrus.

Accomplishments Several strains of the psorosis virus and some additional unknown infectious agents have been isolated from grove trees. Efforts are being made to characterize these unknowns using procedures similar to those that were used to characterize citrus psorosis virus. The sequence of the viral genome segment that encodes for the coat protein has been determined for isolate CRSV-4 and was used to make various RNA transcripts for potential use as a virus-based gene vector. Hamlin orange and grapefruit were transformed with the coat protein gene or CPV. Some of these plants have been challenged by graft inoculations and appear to be resistant to the virus. Reliable PCR based detection methods for several isolates of the virus that causes citrus psorosis were developed in this project. In response to call outs from growers to examine citrus trees showing bark scaling, we are now making assays using the PCR methods for citrus psorosis virus and by biological indexing.

2004 Annual Progress Report

Project No. 003-04P

Year 2/3



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

Kenneth Derrick, Gary Barthe and Julia Beretta (UF-IFAS-CREC, Lake Alfred, FL)

The Florida citrus industry loses more than 600,000 bearing trees per year to citrus blight. There are no reliable methods for controlling blight and the cause of the disease is unknown. The symptoms associated with citrus blight can be reproduced by root graft inoculations, which indicates the disease is caused by a pathogen. A major effort to determine the cause of blight using subtraction hybridization techniques is ongoing. Blight associated protein p12 is always present in trees with blight and appears to be involved in cell growth. Research is in progress to determine if p12 produced by the tree in an effort to resist the disease and will transgenic expression of the p12 gene in scions and rootstocks provide resistance to blight. In addition, using cDNA subtraction methods, we have found several additional genes that are either up or down regulated in blight affected trees.

Plant Pathology	
Funding Sources	
FCPRAC	\$56822
Agency	2000
Other	40000
Total	\$98822

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.

Accomplishments The gene for the citrus blight associated protein, p12, was used to produce transgenic rootstocks (rough lemon, Carrizo citrange) expressing both sense (p12 producing) and antisense (blocking p12 production) directions. Some of the transgenic plants have multiple copies as shown by Southern blots. Further evaluations of these plants are in progress. Selected plants have been increased to produce trees for field trails for resistance to blight. Three subtracted libraries of cDNA from dsRNA from blighted and healthy trees were made. Approximately 130 clones from these libraries have been sequenced. Clones from various strains of citrus tristeza virus, strains of two known viruses that are not reported to infect citrus and numerous unknowns were identified. Although present in very low concentrations and very unevenly distributed in roots of trees with blight, some of these virus sequences appear to be associated with the disease.

This document is the Twelfth Annual Report of the Florida Citrus Production Research Advisory Council. The purpose of this document is to present a review and summary of the previous year's research projects and to present the current financial status of the program.

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Florida Citrus Production Research Advisory Council

Administered by
The Florida Department of Agriculture and Consumer Services
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