

Effects of timing infection by *Cucurbit yellow stunting disorder virus* (CYSDV) on melon growth and yield

September 2008 Progress Report by Principal Investigator Bryce W. Falk

My project is way behind schedule. I have had problems getting efficient inoculations, and all inoculations must be done in the contained research facility, so it is taking much longer than I thought. I will complete the project with the funds that were originally allocated, but it will take some extra time. I should have one run done at least by the January meeting. I am using Laredo as the cultivar of choice, which was suggested by Milas Russell, Jr.

Please give my regrets for taking longer than anticipated, I will get it done and still give a full report when finished. Let me know if you have any thoughts or questions.

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Investigation of Fungicide Activity against Powdery Mildew of Muskmelon

September 2008 Progress Report by Principal Investigator Thomas A. Turini

Location where work will be performed: Westside Research and Extension Center

The powdery mildew project is in progress. There have been evaluations and disease is currently present, but the data have not yet been entered.

Casaba melon, cv. 'Golden Beauty' seed were sown and irrigated in early June at West Side Research and Extension Center. The experimental design is a four replication randomized complete block and there are seventeen treatments. The treatments are as follows: Inspire Super 14 fl oz/A, 20 fl oz/A, Quadris 15.5 fl oz/A, Quadris 15.5 fl oz/A + KTS 1.0 gal/A, Quadris 15.5 fl oz/A + KBS 2.0 gal/A, Procure 480SC 8 fl oz, Procure 480SC 4fl oz + Quintec 250SC 4 fl oz, Procure 480SC 8fl oz (1,3) rotated with Quintec 250SC 6fl oz (2), Procure 480SC 8fl oz (1,3) rotated with Quintec 250SC 6fl oz (2), Sovran 4.8 oz, Sovran 4.8 oz + Endura 6.5 oz, Quintec 250SC 6fl oz, Rally 40 5.0 oz, Endura 6.5 oz, Microthiol 80 5lbs (1, 3), Topsin M (2), Microthiol 80 5 lbs, Pinpoint (V10118) 9.37 fl oz/A.

Materials were applied on 26 Aug and 3 Sep, and an additional application is scheduled for 12 Sep. The percentage of the upper and lower leaf surface covered with signs of the disease is being estimated for each of ten leaves per plot. Disease evaluations have occurred one to two days before each application.

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Examining the effects of Surround® to control aphids and viruses in commercial honeydew fields

September 2008 Progress Report by Principal Investigator W. D. Gubler

We were able to get four field sites lined up and they currently are being treated with product. We set these up on large scale and are collecting insects weekly for aphid flights and will check fields for virus before each is harvested.

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Improving the Understanding and Management of Cucumber Beetles

September 2008 Progress Report by Principal Investigator Peter B. Goodell

This funding of this project was contingent on the appearance of beetle populations in the Fresno/Merced County growing area. As no substantial problems arose, no field trials were conducted and no funding was requested from reserves.

The identification guide to cucumber beetles was printed in limited numbers and provided to John LeBoeuf for distribution to the Board.

My primary interest has been focused on Western striped Cucumber Beetle. An attempt at developing greenhouse cultures was made but populations failed to establish. An early infestation of Western Striped Cucumber Beetle in the Nees/Oxford area of Fresno was identified by Pat Romero and beetle populations from the field were collected and placed onto melon plants. The expected outcome was to be able to develop lab cultures and conduct lure and feeding trials throughout the year. The colony failed to carry beyond the initial population. No further field populations were reported.

The dozen specimens that were in the laboratory cage were tested against two lures from APTIV, Western Striped and Western Spotted Cucumber Beetle. APTIV informed me that there was no difference in the lure constituents (floral attractants) but neither really seemed to get the attention of the 12 beetles in the cage.

Andrew Pedersen, the Masters Student with Larry Godfrey, UC Davis Dept of Entomology, has prepared a draft of his proposed studies. Much of the work in which the Melon Research Board was interested is contained in his proposal. I am participating with his committee in the process. No funding will be requested in 2008. A renewal proposal, possibly linked to supporting Andrew Pederson's work out of Davis, will be submitted in 2009.

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Gene expression profiling in developing melons: linking aroma to genomic traits.

September 2008 Progress Report by Principal Investigator Florence Negre-Zakharov

Honeydew melon aroma profiling: Honeydew melons (cultivars Saturno, Santa Fe, Vanessa, Emerald and Summer Dew) were grown in Woodland at the Seminis trial fields with kind permission of Joe King and under the supervision of Paul Chung. Melons were direct seeded on raised beds and grown with standard cultural practices. Aroma volatiles were sampled over fruit development until fruits had reached commercial maturity, as measured by a minimum Soluble Solids Content of 10%. Five fruits per cultivar per stage were harvested and analyzed for volatiles immediately. Samples will now be analyzed by Gas Chromatography Mass Spectrometry.

Melon genomic analysis: Melon microarray chips were purchased through the Center for Gene Expression Profiling at Boyce Thompson Institute (Ithaca, NY). RNA was extracted from melon fruit samples using standard procedures and used as probes for hybridization to the microarray chips. Analysis will be performed this Fall with the help of the Bioinformatics group within the Genome Center.

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Melon tolerance and weed control with new herbicides

September 2008 Progress Report by Principal Investigator Tom Lanini

Only Prefar and Curbit are currently registered for preemergence weed control in melons. These herbicides fail to control many weeds, which then must be controlled by other means or allowed to compete with the melons. Herbicides evaluated in 2008 for preemergence weed control in melons included clomazone (Command), rimsulfuron (Matrix), metolachlor (Dual Magnum), flumioxazin (Chateau), sulfentrazone (Spartan), pendimethalin (Prowl H₂O) pyriithobac (Staple), halosulfuron (Sanda) and fomesafen (Reflex). Honeydew, cantaloupe and watermelon were tested for tolerance and weed control with these new herbicides. The melon varieties were: Cantaloupe - Oro Rico and Esteem; honeydew melon - Saturno; and watermelon - Paradise. Applications were made immediately after planting, and cultivated with a lilliston rolling cultivator to move the herbicide into the soil. Furrow irrigation was used throughout the study. Crop tolerance was very good with all herbicides except Spartan which caused only slight injury to all melon types. Weed control was good to excellent and hand weeding time was reduced with all the herbicides compared to the untreated control. Preliminary observations are that yield has not been affected by any treatment. Melons are still being harvested.

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Evaluation of New Approaches for Whitefly and Cucurbit Yellow Stunt Disorder Virus Management in Desert Melons

September 2008 Progress Report by Principal Investigator John Palumbo

Research projects in 2008 continued to examine new insecticide chemistries for soil insects, flea beetles, whiteflies and CYSDV. During the early spring, several seed treatments and insecticides were tested for control of seed corn maggot (SCM) on cantaloupes. Results showed that the Cruiser (thiamethoxam) seed treatments provided the best protection from SCM comparable to the standards Diazinon and Capture. Other in furrow treatments of Alverde, Venom and Lannate were not as effective.

Several spring whitefly trials were conducted to identify candidates for suppression of CYSDV based primarily on adult control. Melons were planted in mid-May to increase the chance of CYSDV infection. Soil uses of Venom and a Venom+Coragen combination provided very consistent control of both adults and immature. Although CYSDV symptoms were light, these two treatments appeared to suppress CYSDV compared to the untreated melons. Foliar trials identified several insecticides that provided good knockdown and in some cases residual control of adults. Among the currently available products these included Fulfill+ Thionex , Venom + Capture and Capture+Thionex combinations, and among the experimental products included Pyrifluquinazon and Cyazapyr. At the early netting stage, CYSDV symptoms were reduced by >50% in the Fulfill+Thionex and Pyrifluquinazon treatments, and ~40% in the Cyazapyr and Capture+Venom treatments. Because of the voluntary disk-down of melons in Yuma (July 10), we were unable to evaluate CYSDV incidence beyond this point.

Fall trials are presently underway and include testing of seed treatment. Again the Cruiser seed treatments provided significant control of flea beetles and leafminers. In addition, a number of studies are in progress to evaluate whitefly efficacy and CYSDV management. Among the tactics being evaluated are various combinations of soil insecticides, foliar spray regimes, soil and foliar fertility programs, row covers and commercial variety susceptibility to CYSDV. As of September 6, CYSDV symptoms have been recorded in some treatments (1-2% of plants) at the early bloom stage.

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Evaluation of cantaloupe and honeydew varieties for nutrient composition and content

September 2008 Progress Report by Principal Investigator Dr. Li Tian

The objective for Year 2008 is to evaluate the nutrient composition and content of three cantaloupe and three honeydew varieties that are supplied by the California melon growers, together with three melon varieties grown offshore that are collected from the market place. Pro-vitamin A and vitamin C are the two major phytonutrients in melons and are therefore selected as the targets of our analysis. Other classes of phytonutrients (such as vitamin E and Vitamin B6) are present in melons only in trace amount. They do not significantly impact melon nutritional values and will not be analyzed.

To date, with the assistance of Mr. John LeBoeuf, we have received three California-grown honeydew varieties: Saturno, Emerald and Vanessa, and one cantaloupe variety: Oro Rico. We expect to obtain the two late-season cantaloupe varieties in late September or early October, and the offshore honeydew and cantaloupe melons in November. (Durango melons will be collected in late September.)

To minimize the variation in nutrient content within the same melon variety (caused by growth location, light exposure etc.), six melon samples from each variety were collected for nutrient analysis. The pro-vitamin A and vitamin C content will be presented as the average value of the six samples. After the seeds and rinds were removed, the central portion of melon flesh was cut into small cubes and frozen immediately in liquid nitrogen, ground into fine powder and stored at -80°C until analysis.

In the mean time, we have optimized the extraction procedures for pro-vitamin A (carotenoid) and vitamin C from melon fruits. In addition, we have established the analytical methods (using High Performance Liquid Chromatography, HPLC) for pro-vitamin A and vitamin C separation and quantification in my laboratory. We are currently collecting HPLC data on the melon samples that are available in my laboratory. Additional melon samples will be processed and analyzed when they are received. Pro-vitamin A and vitamin C contents in different California- and offshore- grown cantaloupe and honeydew varieties will be compared and summarized. The research results will be reported to the Melon Research Board at the end of this year.

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Assessment of Rapid Pathogen Detection Kits for Preharvest Surveillance and Test and Hold Programs for Melons - Objective 1

September 2008 Progress Report by Principal Investigator Trevor Suslow

Research staff involved in project: Paula Martins de Freitas – technical coordinator, Adrian Sbodio, and Dr. Carol D'lima

Current Objective 1:

Evaluate the specificity (false-positive rate) and sensitivity (false-negative rate) of rapid test kits currently used for real-time testing and Test and Hold protocols on cantaloupes and honeydew.

Salmonella detection platforms used to date:

- Pathatrix- combined PCR detection and culture confirmation
- Qualicon BAX Biocontrol GDS
- Rapid Chek® SDI Reveal® Neogen

Bacteria used: a cocktail of Salmonella Poona PTVS 28 (Rif resistant), Salmonella Newport PTVS 83 (RIF resistant and GFP) and Salmonella Montevideo PTVS 45 (RIF resistant).

Preliminary experiment 1:

Commercial melons, (3 honeydew and 3 cantaloupes) were peeled and placed into enrichment media, then the *Salmonella* inoculum was added to achieve 11 CFU (colony-forming-units) per sample. One negative control for honeydew and one for cantaloupe was established for each detection methodology.

Results: Cantaloupe: BAX GDS, Pathatrix, Neogene and SDI were all positive for inoculated samples and negative for the uninoculated control.

Honeydew: Results were similar to cantaloupe, with the exception of Neogen AcuScan III (digital imaging reader) assigning samples as negative while visual inspection by trained operator rated inoculated samples as positive.

Preliminary experiment 2: Honeydew and cantaloupes were inoculated with cocktail of *Salmonella*, as above, with a target of 36 CFU per 4cm² marked area on rind surface. Melons were stored at 5C (41F) for 5 days. Then melons were peeled assayed with detection methods as above; 3 replicates of 25g rind tissue were used per sample plus one negative control for each melon type.

Results: Cantaloupe: BAX detected all inoculated samples as positive, Pathatrix detected two of three, SDI one of three and Neogen and GDS resulted in 0/3. Uninoculated controls were negative in all test formats.

Honeydew: All test results were negative using this inoculum level.

Field Harvested Melons 1: Melons from commercial plantings of 3 different cooperating growers were harvested at maturity. A total of 10 cantaloupes and 20 honeydews were evaluated per detection system. From each field, 8 fruits were inoculated with

Salmonella, as above, at 36 CFU per sample and 2 were used as negative control. After inoculation, melons were stored at 5C for 5 days and processed as above.

Results: Detection efficiency was highly variable across all melons and test methods and a decision was made to increase the inoculum level in subsequent tests.

Field Harvested Melons 2: Melons from commercial plantings of 4 different cooperating growers were harvested at maturity. A total of 20 cantaloupes and 20 honeydews were used per detection system. From each field, 8 fruits were inoculated with 100 CFU per sample and 2 were used as negative control. SDI test kits were not available for this evaluation. In addition, 20 cantaloupes and 20 honeydews were also inoculated to test the ability to detect *Salmonella* by culture confirmation methods after refrigerated storage; 5C for 5 days.

Results: All 20 cantaloupes were positive while all of the 20 honeydew were negative for *Salmonella*. Detection kit performance was variable across those evaluated. Final comparisons of culture outcomes are needed to differentiate efficacy.

Field Harvested Melons 3- not inoculated: Melons from commercial plantings of 8 different cooperating growers were harvested at maturity. Five melons were taken per location per detection methodology. A total of 40 melons were assayed per detection methodology.

Results: All samples were negative for GDS and Neogen while 2 out of 40 were initially positive with BAX. Further tests are being conducted to determine if this outcome for BAX represents a “false positive” outcome. Thus far indications from secondary tests have not confirmed cultureable *Salmonella* from these two samples.

Field Harvested Melons 4- not inoculated: Melons from commercial plantings of 8 different cooperating growers were harvested at maturity. Five melons were taken per location per detection methodology. A total of 40 samples were done per detection methodology.

Results: All samples were negative for GDS and Neogen, and 1 out of 40 was positive with the BAX test kit. Further tests are been done to determine confirm this outcome by culturing methods and complimentary PCR detection methods.

Continuing Efforts: Fields and cooperators have been identified to complete this project. Additional test kit platforms may be evaluated, if available this season by donation from commercial suppliers.

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Assessment of Rapid Pathogen Detection Kits for Preharvest Surveillance and Test and Hold Programs for Melons - Objective 2

September 2008 Progress Report by Principal Investigator Trevor Suslow

Research staff involved in project: – Adrian Sbodio as technical coordinator, Sharyn Maeda, and Paula Martins de Freitas

Current Objective 2: Establish a seasonal baseline of indicator generic *E. coli* in diverse irrigation water sources associated with production

Activities to Date: Three sampling dates (June to August)
30 samples with 3 reps/ sample (volume per site ranged from 3 to 9 liters)
Sites are located across 3 counties including primary and secondary irrigation district canals as well as some on-farm irrigation ditches.

Data Collected On-site: Water temperature at sampling time

Data Determined in: turbidity, pH

Quanti-Tray 2000 (MPN method for thermotolerant coliform (aka fecal coliform) and generic *E. coli*, 3 replicates of 100 ml)

GDS (real time PCR screening for *Salmonella* and *E. coli* O157:H7; filtering 3-4.5L per irrigation source site prior to enrichment and detection).

Results: Will be presented in final report

Continuing Efforts: Public access locations and some on-farm cooperators have been identified to complete this project. Additional on-farm irrigation samples would be beneficial but not essential.

Assessment of preharvest attachment and internalization of pathogenic bacteria into melons from irrigation water

September 2008 Progress Report by Principal Investigator Trevor Suslow

Research Staff Involved in Project: Dr. Carol D'lima; project leader and Kin Hup Tan

Current Objective:

1. Evaluate preharvest survival and potential for uptake of bacteria within vines from irrigation water in growth chamber and greenhouse trials

Experiment 1

Cantaloupe seeds were planted in standard horticultural soil mix in a growth chamber at 20C (68F). The seedlings were inoculated on day 9 with log 8 CFU (approx. 100 million colony-forming units) fluorescent marked pathogenic *E. coli* by injecting under the soil surface with a syringe. Samples were watered daily.

Part I

Samples were processed on day 14 by cutting the vine with a sterile blade just above the soil. Seedlings were divided into hypocotyl (seedling stem) and epicotyl (growing point above cotyledons) and ground separately in extraction buffer and detection by culturing on selective media was attempted. All the uninoculated control samples were negative for *E. coli* O157:H7. With the inoculated seedling samples, the hypocotyl had an average of more than 100 CFU/sample. No recovery was observed from the epicotyl tissue.

Conclusions: This method of inoculation with very high levels of bacteria under the soil showed no signs of internalization through the root system and short-term systemic movement within a seedling plant, under test conditions. Some transference from soil mix to the above soil hypocotyls during handling was detected.

Experiment II

Cantaloupe seeds were planted and germinated as above. The soil was inoculated by irrigating with water inoculated to contain log 8 CFU fluorescent marked pathogenic *E. coli* on day 1. Samples were watered daily to maintain uniform moisture.

Part I

Samples were processed on day 14 by cutting with a sterile blade just above the soil. Seedlings were divided into hypocotyl and epicotyl and ground separately with extraction buffer and detection by culturing on selective media was attempted. Some of the seed coats were still attached to the cotyledon or epicotyls. All the uninoculated control samples were negative for *E. coli* O157:H7. With the inoculated seedling samples, the hypocotyl had an average of more than 60 CFU/sample and more than 600 CFU/sample on the epicotyl.

Part II

Samples were processed on day 19 by cutting with a sterile blade just above the soil. The sample was divided into hypocotyl and epicotyl. Hypocotyl was further divided into “top” and “bottom”. Each sample was ground separately with buffer and plated, as above. Of the 11 replicate samples tested, the “top” of the hypocotyl had an average recovery of 0.5 CFU/sample, the “bottom” of the hypocotyl had an average recovery exceeding 30 CFU/sample. The epicotyl had an average recovery exceeding 700 CFU/sample. Separate plating of detached seed coats showed high levels of inoculated bacteria remaining.

Conclusions: This method of inoculation showed that the seed coats were responsible for transference of inoculum to above soil tissues and this was responsible for the high counts in the epicotyl. The recovery in the hypocotyl region was possibly due to contamination during emergence from the soil and that is why when the hypocotyl was divided into “top” and “bottom”, the recovery was only seen in the “bottom” half of the hypocotyl.

Continuing Work: greenhouse trials to evaluate longer timelines of melon vine growth are in progress.

Emergence of *Cucurbit yellow stunting disorder virus (CYSDV)* in the desert Southwest: Assessment of the threat to melon production and development of an integrated disease management strategy

September 2008 Progress Report by Principal Investigator Bob Gilbertson

No report submitted as of September 23, 2008.

Request For Proposals For 2009 Funding

The California Melon Research Board will consider proposals for new and continuing melon research projects for the coming calendar year, 2009. The call for proposals was sent out on September 18, 2008 by Dr. Michael Stanghellini. To be considered for funding by the Melon Research Board, each proposal should be received no later than October 20, 2008.

Please review the following research priorities and contact John LeBoeuf, Research Coordinator at 559-431-2360 as soon as possible if you have a new topic that you would like to add to the list. A search could then be done for an appropriate researcher.

2009 Top Research Priorities: Finding practical solutions to problems of immediate and serious concern to producers of cantaloupes, honeydews, and mixed melons in California.

- 1) Control strategies for western striped and spotted cucumber beetles in the melon production regions of the San Joaquin and Sacramento Valleys are needed. Evaluate use of Cidetrak attractant from Trece, Surround kaolin clay, and dusting sulfur as alternatives to traditional pesticides. Beetle biology studies are also encouraged along with possible monitoring techniques for beetle adults and larvae.
- 2) Soil-borne disease research is needed that includes pathogen biology and control strategies for *Macrophomina*, *Phytophthora*, *Pythium*, and races of *Verticillium* and *Fusarium* that are found in Central California melon regions. In the desert melon regions, work on *Monosporascus cannonballus* and any other pathogen associated with vine decline remains a priority. Studies that evaluate how to prevent and manage plant disease in both drip and furrow irrigated fields are encouraged.
- 3) Insect pest management studies with new insecticides remains a priority.
- 4) Evaluate nematode control strategies, offer recommendations for preplant, at planting, and after planting scenarios. Evaluate oxamyl (Vydate) and other nematicides for best time and rates to apply.
- 5) Pre-emergence and also post emergence control techniques and products are needed for the following broadleaf weed species: black nightshade, field bindweed, yellow nutsedge, common purslane, and pigweed. Evaluate potential new herbicides including DPX 438.
- 6) Evaluate control of soil pests that damage melons at harvest time, such as earwigs, so-called pinworms, and cutworms. Offer control strategies with alternatives with very low pre-harvest intervals to rotate with registered organophosphates and carbamates.

- 7) Evaluate carbamate alternatives for use in baits, substrate materials to mix insecticides with, and offer control strategies for darkling ground beetles, flea beetles, seed-corn maggots and other soil pests. Evaluate bifenthrin (Capture) as an alternative at seeding and contact manufacturer with results for possible label amendment. Evaluate alternatives to Diazinon for control of soil-borne pest complex
- 8) Identify races of powdery mildew from the various melon producing regions. Offer guidance on what fungicides may develop problems with pest resistance.
- 9) Evaluate efficacy of Raynox for sunburn control, Surround (tm) for aphid repellency and melon size increases.
- 10) Identify nutritional status of various cultivars of cantaloupes and honeydews grown in California for comparison with melons grown in other regions of the United States and from off-shore production.
- 11) Virus biology research is needed for all viruses that can inflict losses in melon production. The understanding of the basic biology of disease/vector relationships is critical to the industry, especially for learning how to avoid and reduce losses from viruses.
- 12) Evaluate plant growth regulators (PGRs) of registered materials; identify optimum rates and timing of applications.

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Research Cooperators

Many thanks to the grower/shipper cooperators who have allowed melon research teams to access their fields for collection of melon and irrigation water samples as well as to cooperate with field studies. Thanks to all board members who answered my calls during 2008 for help in getting our projects up and running. Many of our currently funded projects rely on cooperators to provide melons fields for research sites or for access for sampling procedures. In addition, several of our funded projects have relied upon delivery of cantaloupe and honeydew seeds from seed companies and various pesticides from ag-chem companies. Without this continued cooperation, research projects would take much longer to get results, have higher costs associated with the projects, or not be able to be undertaken at university research facilities. I appreciate all of the support by the members of the board of directors in helping make the research program a success.

Respectfully submitted,

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