



# California Melon Research Board

## 2019-20 Research Interim Report List

Leader Project Title		TOTAL APPROVED as of 3/1/2019
<b>Gilbertson</b>	Monitoring an Outbreak of <i>B. Tabaci</i> Whiteflies in Melons in 2018 and Continued Development of Vector-Independent Screening for Whitefly-Transmitted Viruses Infecting Melons	<b>17,757</b>
<b>Mauck</b>	SEED GRANT: Assembling and Synthesizing Information on Melon Pollinator Health to Develop Best Management Practices	<b>4,840</b>
<b>Mauck</b>	Discovery and Validation of Elicitor Products for Control of Aphid and Whitefly-Transmitted Viruses in Melons	<b>13,670</b>
<b>Palumbo</b>	Evaluation of Insecticide Alternatives for Whiteflies and CYSDV in Melons	<b>16,698</b>
<b>Sidhu/Nunez</b>	Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes in Melons	<b>16,000</b>
<b>Stoddard Bean</b>	Evaluating Preplant and Post Plant Herbicide Programs for Weed Management in Transplanted LSL Melons - Year 2	<b>12,800</b>
<b>Swett</b>	Characterizing and Assessing Risk of Emerging Fungal and Bacterial Pathogens of Melons (& other cucurbit crops) Across the Nursery-Field Production Continuum	<b>10,000</b>
<b>Vinchesi</b>	Management of Spotted and Striped Cucumber Beetle in Melon Production	<b>16,500</b>
<b>Wintermantel</b>	Development of Rapid Detection Methods for Evaluation of Germplasm for Resistance and Determination of the California Host Range of Cucurbit Chlorotic Yellows Virus and Squash Vein Yellowing Virus	<b>14,632</b>
<b>TOTALS</b>		<b>\$ 122,897</b>

## Interim Report to the California Melon Research Board

Project title: **Monitoring an outbreak of *B. tabaci* whiteflies in melons in 2018 and continued development of vector-independent screening for whitefly-transmitted viruses infecting melons**

Principal investigator (PI): **Robert L. Gilbertson**, Department of Plant Pathology, UC Davis

Co-PIs: **Tom Turini**, University of California Cooperative Extension, Farm Advisor, Fresno County; **William Wintermantel**, Virologist, USDA-ARS, Salinas; Cooperators: **Marcela Vasquez-Mayorga**, Graduate research assistant, Department of Plant Pathology, UC Davis.

There were two main goals for this project: 1) monitoring melon production in the Central Valley for outbreaks of whiteflies and use molecular methods to identify the whiteflies involved and 2) to continue to develop and test vector-independent methods for infecting melons with two economically important whitefly-transmitted RNA viruses: *Squash vein yellowing virus* (SqVYV) and *Cucurbit yellow stunting disorder virus* (CYSDV).

**Monitoring for whitefly outbreaks in the Central Valley.** We conducted two surveys in Fresno County: an early season survey (June 18, 2019) and a later season survey (August 23, 2019). In the early season survey, a honeydew and cantaloupe field were surveyed at early green fruit stage and no adult whiteflies were observed at the four locations checked by the leaf-turn method. In the late season survey, three cantaloupe fields were surveyed, and none or trace populations of adult whiteflies were observed. These results reveal a very different situation from last year when late season whitefly outbreaks were observed in numerous melon fields. This likely reflects the cool wet spring conditions, which may have delayed or interfered with the presumed migration of adult whiteflies from southern locations, e.g., the Imperial Valley. This may indicate that a predictive temperature model (e.g., degree day) could be developed to predict the possibility of whitefly outbreaks in the Central Valley.

**Development of vector-independent inoculation and screening methods for SqVYV and CYSDV.** In the year 2014, SqVYV, a whitefly (*Bemisia tabaci*) transmitted Ipomovirus, was detected for the first time in California, from pumpkins with yellowing symptoms in the Imperial Valley. The virus causes economically losses to watermelon and to a lesser extent, melon, production in Florida and Central America.

A previously developed SqVYV agroclone (an infectious clone delivered by *Agrobacterium tumefaciens*) was infectious in squash and watermelon, but did not provide high rates of infection. Although this virus is efficiently mechanically transmitted, there are advantages to having an agroclone (long-term storage, genetic studies and improved germplasm screening). Therefore, we decided to generate a new clone from the original sample collected in 2014 (and stored at -80 C) and to use a new assembly approach (Hi-Fi, NEB) for the cloning. We were unable to amplify a full-length copy of the SqVYV genome from the frozen material. Next, pumpkin plants (cv. Big Max) were rub-inoculated with sap prepared from the original samples. After several attempts, one plant developed characteristic SqVYV symptoms (vein yellowing and mosaic) was obtained and confirmed to be infected with SqVYV by RT-PCR with primers that target the capsid protein gene. From this plant, RNA was extracted and cDNA with specific primers for SqVYV was synthesized.

The PCR amplification was optimized to a higher efficiency and putative full-length DNA fragment was obtained (~10000 bp). The amplified band was excised from the gel and cleaned for improved assembly into the binary vector pJL89. The resulting plasmids from two clones were partially sequenced and identity of 99% obtained when compared with the previous clone. These two SqVYV clones were transformed into *Agrobacterium tumefaciens* C58 cells. The resulting *A. tumefaciens* colonies were screened for the presence of the SqVYV-pJL89 clones. Two-week-old pumpkin plants were agroinoculated with the newly developed clone. Currently the plants are being monitored for symptoms of infection (at 14, 21 and 28 dpi), and will be evaluated by PCR for the presence of the virus. So far, preliminary tests indicate that these plants are not infected with SqVYV. If this continues to be the case, we will produce and screen more clones and test for infectivity.

CYSDV-infected melon leaves were obtained from the laboratory of our collaborator Dr. Willam Wintermantel (USDA) and total RNA was extracted and used as a template for cDNA production with specific primers for each of the two RNAs (RNA-1 and RNA-2) that compose the genome of CYSDV. Putative full-length fragments of both genomic RNAs were obtained and assembled into the binary vector pJL89. The presence of RNA-1 and RNA-2 clones in the constructs was confirmed by sequencing. Melon plants were co-agroinoculated with the RNA1 and RNA2. Symptoms were evaluated for 14 dpi, but no infection has been observed to date. Therefore, a second attempt is underway to obtain additional clones. The new RNA1 clone has been completed and we are currently working on the construction of the RNA2 new clone.

## CALIFORNIA MELON RESEARCH BOARD

Progress report September 2, 2019

**PROJECT: Discovery and validation of elicitor products for control of aphid and whitefly-transmitted viruses in melons**

### Principal Investigator contact information.

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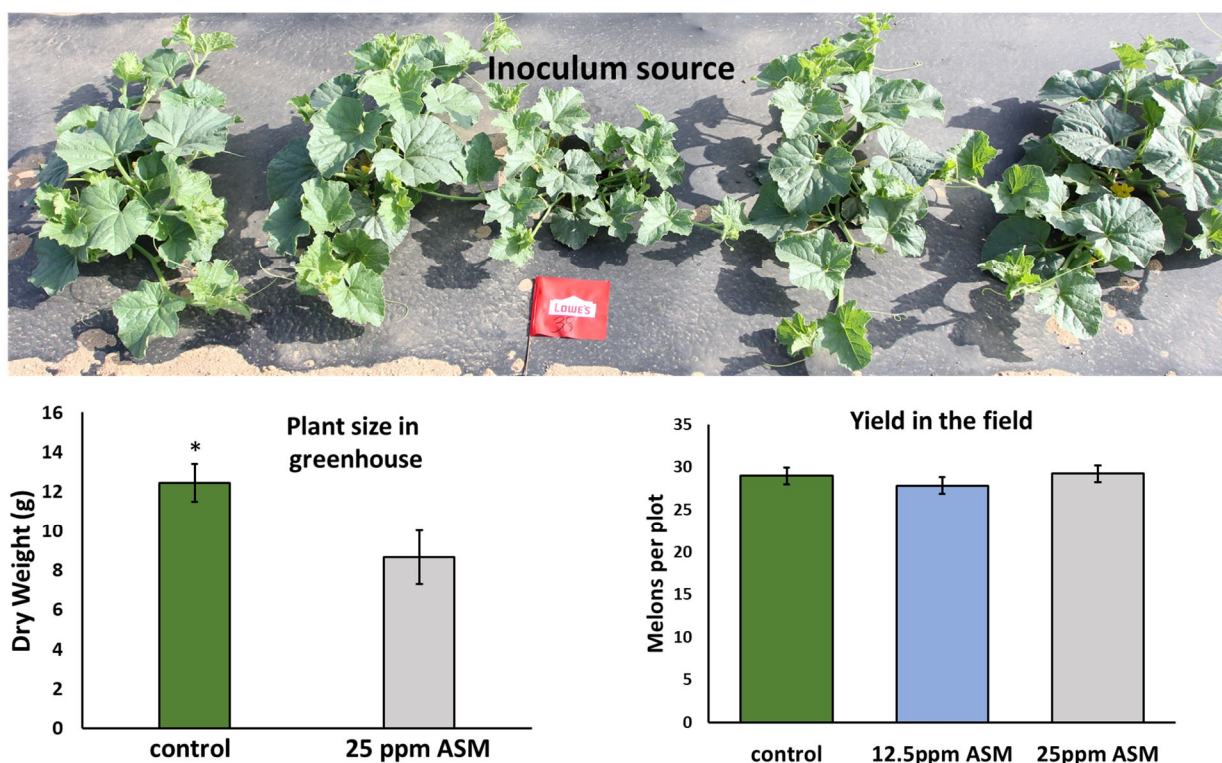
### F. Cooperating Personnel

<p>Dr. Hailing Jin Professor, &amp; Cy Mouradick Endowed Chair Department of Microbiology and Plant Pathology Center for Plant Cell Biology Institute for Integrative Genome Biology University of California, Riverside</p> <p>3234B Genomics Bldg. University of California, Riverside Riverside, CA 92521 hailingj@ucr.edu</p>	<p>Dr. Clare Casteel Assistant Professor Department of Plant Pathology University of California, Davis</p> <p>258 Hutchison Bldg. University of California, Davis Davis, CA 95616 ccasteel@ucdavis.edu</p>
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**Summary:** We conducted field trials of one of the most promising elicitor products for protection of plants against virus infection (acibenzolar-S-methyl, ASM) to determine phytopathological effects, protective effects against virus inoculation from an inoculum source, and effects on melon yield and quality (size) using a popular western shipper cultivar (Gold Express). The experiment involved two doses of the product, one at 25ppm and one at 12.5ppm, applied as one foliar spray application to transplants approximately one week after establishment in the field (May). Trials were drip irrigated with standard fertilizer and watering regimes. Plant health was monitored over the course of the experiment by visual surveys and individual photographs of over 260 plants. Overall, we did not detect a negative effect of ASM application to transplants (at either rate) on plant health or yield in the field experiment. In the greenhouse, we did detect slight negative effects on plant size when using the 25ppm dose applied as a foliar spray. Additionally, a grower trial with seeded melons of several varieties suggests that later application is required. Some varieties (Cayucas, a unique ESL variety) were unusually susceptible to phytotoxic effects (stunting) which were worse in combination with Sandea herbicide.

Overall, these trials indicate that later application (four leaf stage) is best, in the absence of herbicide. Additionally, testing on more varieties is needed before recommending the product to growers.

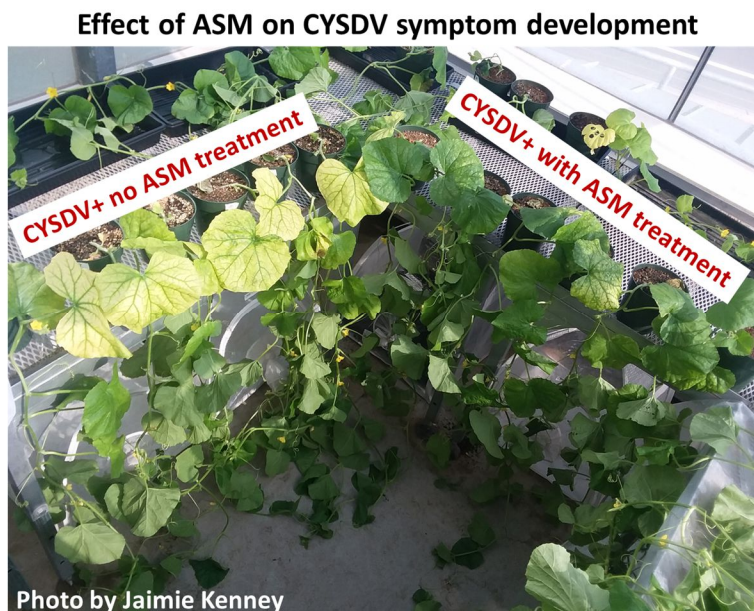
We collected tissue from treated plants at three time points throughout the experiment and this will be used to determine virus infection status of the plants. Overall, virus pressure was relatively low despite use of inoculum sources (a mild strain of cucumber mosaic virus collected locally). This may be due to aphid population fluctuations diverging from normal phenology as a result of an unusually wet winter and spring. Whiteflies were present in the field. We collected tissue from a sub-set of plots for un-targeted virus discovery via next-generation sequencing to determine how ASM treatment affects the overall virus community in melons. This approach will identify viruses transmitted by all possible vectors (beetles, whiteflies, thrips, and aphids).



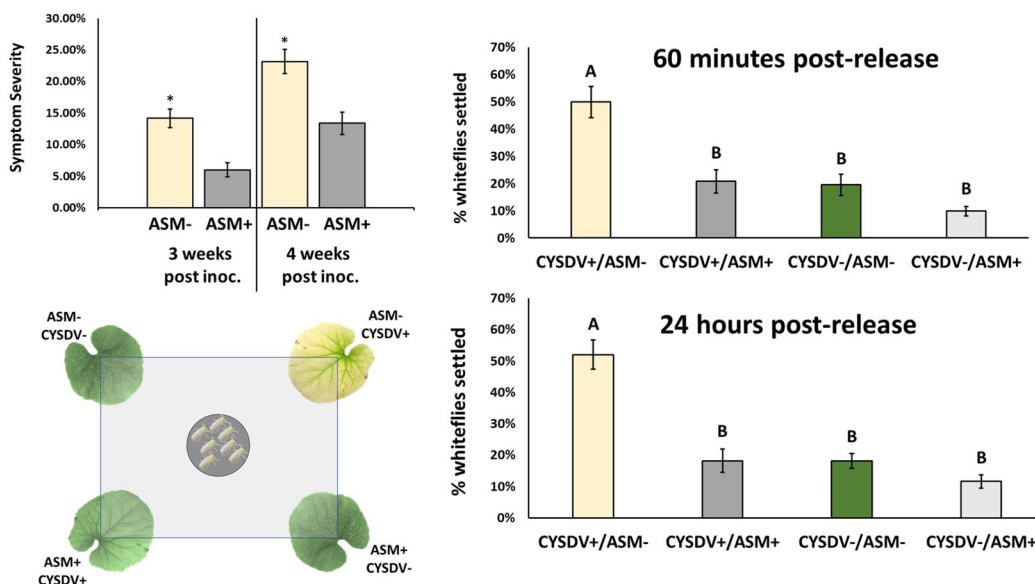
**Figure 1: A plot of test plants with center inoculum source (above) and effects of ASM on plant size and productivity.**

Experiments performed as part of the prior funded work were repeated at different times of the year to confirm initial findings regarding ASM protection against CMV and CYSDV. With further repetitions, we established that ASM has clear protective effects against both CMV and CYSDV, delaying infection symptoms by up to 2 weeks with a single application (Figure 2). Effects against CYSDV are most evident when conditions are conducive to symptom development – i.e., long day length of natural sunlight available in the greenhouse. Attenuation of symptoms is associated with titer reductions, especially at earlier stages of infection. This may reduce the chances of vectors acquiring virus from the plants. Behavioral assays indicate that whitefly attraction to CYSDV-infected plants is disrupted by ASM treatment. ASM itself also tends to make plants less palatable and attractive for whiteflies. Protective

effects may therefore extend to repellency as well as direct attenuation of symptoms should virus be inoculated. Similar assays with CMV did not show a strong effect on vector preferences, as CMV infection does not have strong effects on vector preferences even in the absence of treatment. However, ASM treatment still attenuates symptoms and improves plant health under CMV infection. Future experiments are testing the effects of multiple applications on symptom development and the effects of ASM on leaf morphology and toughness as a measure of possible off-target effects on plant resistance to other pests (e.g., cucumber beetles).



**Figure 2: CYSDV symptom attenuation by ASM treatment in greenhouse experiments.**



**Figure 3: ASM treatment effects on whitefly vector preferences among infected and healthy hosts.**

We also planted a trial to test two additional elicitor products. Although we originally proposed to examine the effects of ethylene-manipulating compounds on virus infection, we opted instead to pursue two compounds that are already registered for use in melons, and which may be useful for organic growers. The first product is Regalia (Marrone Biologicals), which consists of a plant extract with both priming and plant growth-promoting activity. The product is marketed for control of fungal and bacterial pathogens. The other product is called Venerate (Marrone), which consists of heat-killed *Burkholderia* bacteria. The bacteria contain toxins with insecticidal activity, as well as bacteria-produced molecules that can prime plant defenses against pathogens following detection. Both products were trialed in the field and produced no phytotoxic effects when applied to Gold Express transplants. Regalia produced plants with darker leaves and a fuller canopy. We are presently testing these products in the greenhouse under more controlled conditions. Regalia-treated plants are consistently larger and healthier. Effects on viruses (CMV and CYSDV) will be tested this fall now that we have confirmed a lack of phytotoxicity at the label rates. Along with these registered products, we are also testing for protective effects of antimicrobial peptides derived from disease-resistant citrus. These peptides are in development for protection of citrus against the citrus greening pathogen, but have activity in other hosts, where they strongly prime pathogen defenses. Two concentrations of the peptides were tested as foliar sprays with an added adjuvant and no phytopathological effects were found. These peptides will be tested for protective effects against CMV and CYSDV in the fall alongside Regalia and Venerate.



## CALIFORNIA MELON RESEARCH BOARD

Progress report September 2, 2019

**PROJECT: Assembling and synthesizing information on melon pollinator health to develop best management practices.**

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**Summary:** This project combines field collections with information synthesis to provide growers with information on the pollinator communities in melon crops, floral biology of melons, and strategies to conserve pollinators and pollination services from managed and wild bees. Field collections were performed in a melon field free of insecticide treatment at the UC Agricultural Operations facility. The sampling strategy involved observations and collections across multiple time points to understand what pollinators are visiting flowers and how honeybee activity compares to non-managed visitors. Honeybee hives are present on site within half a mile of the melon field.

The following protocol was followed:

Overall sampling period: During peak bloom period (3 weeks), sample melon visitors 3 times per week, aiming for different time periods each time you sample (for example: 7-8 AM on one day, 8-9 AM the second day, 9-10 AM the third day)

Sampling/observation protocol:

- Round 1A: Have one collector walk along around the perimeter of the area, counting the number of honey bees (using a clicker). You may have to stop and count within, for example, a 3-ft length of the row, then move forward to the next 3-ft length and count, and so on. You can modify this part of the protocol - the goal is to count all the honey bees and not re-count anyone
- Round 1B: Next, the collector walks along the perimeter and collects any insects they see

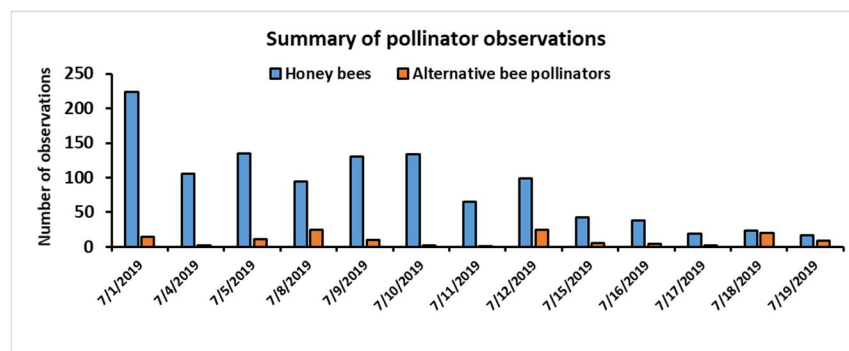


other than honey bees and cucumber beetles. Only collect insects observed in contact with reproductive parts of flowers. Collect using vials or a bug vacuum to avoid destroying flowers.

- Next, wait at least 20 minutes until proceeding to 2A and 2B.
- Round 2A: The other collector performs the same steps as in round 1A.
- Round 2B: The other collector performs the same steps as in round 1B.

Collect insects into dry vials on ice in a cooler then kill in the -20 in the lab. Keep in the -20 until ready to pin (no longer than 1 week).

Results of the field collection indicate that alternative pollinators are present during most sampling dates. The percentage of total observations made up by these alternative bee visitors ranges from about 5-50% of all pollinator observations for a sampling date. Variation in the ratio of alternative bee pollinators to honey bee pollinators likely reflects differences in activity due to temperature. The data will be further explored to identify these relationships. A key finding is that bumble bees are using melons as pollen and nectar resources. We observed a threatened bumble bee species visiting flowers during early morning observation periods (8-9am) before elevated temperatures occurred later in the day. Honey bees were generally the most abundant visitors, followed by cucumber beetles (not shown). Honey bee visitation dropped off toward the latter portion of our observations, but was robust during the initial observations (mid bloom period). Overall, our results suggest that honey bees are the most common visitors, but that alternative pollinators are common enough to potentially contribute to overall fruit set. The efficiency of these alternative pollinators could be explored experimentally, or as part of the literature synthesis.



During the remaining months of the project, the students will assemble a database of literature (peer-reviewed scientific papers and specific recommendations for melons). These efforts will consider alternative pollinator species identified in the surveys, their current distributions, and likelihood to be present in melon-producing regions based on crop use patterns and physiological tolerance. We will then develop an initial draft of management guidelines for conserving pollination services in melons. These guidelines will attempt to incorporate regional differences in key production regions in California.

## **Evaluation of Insecticide Alternatives for Whiteflies and CYSDV in Melons**

John Palumbo, University of Arizona, Yuma AZ

Research identifying insecticide alternatives for whiteflies and CYSDV management in melons was the focus of our project in 2019. We were able to develop new information that should be helpful to desert growers. Research was initiated this year to investigate use of foliar and soil insecticides with cantaloupe transplants. In our spring trials, we examined transplant tray drenches and their efficacy against whitefly adults. The most revealing results from that work showed that drenching trays with Venom prior to transplanting provided the most consistent control of whiteflies. In contrast, Sivanto caused unacceptable crop injury and excessive plant mortality within a few days of transplanting. Another study examined the foliar application of PQZ and Sefina to plants within trays just prior to transplanting and compared with foliar sprays of transplants immediately following transplanting. Both application methods resulted in significantly reduced whitefly numbers compared with the standard spray approaches. Unfortunately, we were not able to take yields and virus ratings due to canopy collapse in many of the treatments due to high temperatures in early July. The trials were planted on a block with very sandy soil and our drip irrigation could not keep up with the heat stress.

In direct seeded melons, several new and existing insecticide alternatives were evaluated for management of whitefly adults/CYSDV in melons. Growing conditions were good, as were whitefly population numbers in our spring/summer trials. Because we planted these trials in late April, CYSDV incidence was high and we were able to determine differences in infection rates among treatments. We saw for a second season that among the newer conventionally available products registered on melons, PQZ and Sefina provided good adult control and significant levels of CYSDV suppression when used in a spray program. Other new registered products, Cormoran and Minecto Pro were not comparable to the standards.

Fall trials are currently underway (planted Aug 22) and we are continuing our work with PQZ and Sefina and trying to determine their best fit in early season adult whitefly / CYSDV management. Our focus is to further evaluate transplant drenches (Venom and others), and transplant tray spray applications with PQZ and Sefina prior to transplanting under heavy whitefly and CYSDV conditions. Whitefly populations have been low-moderate to date on the fall plantings, but we anticipate high virus incidence as the season progresses.

## California Melon Research Board Mid-season report January 2019 to December 2019

**Project Title:** Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes in melons

**Project Leader:** Jaspreet Sidhu and Joe Nunez

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**Cooperating personnel:** Antoon Ploeg, Department of Nematology, UC Riverside, E-mail: [antoon.ploeg@ucr.edu](mailto:antoon.ploeg@ucr.edu)

This study is being conducted as small plot field trials on our nematode nursery at the Shafter research farm, Shafter. A western Shipper type melon variety ‘Durango’ was direct seeded INTO 60- inch beds on July 19, 2019 using a jab type planter. There are four replications and six treatments in this trial (Table 1). One of the controls (Treatment 2) was destroyed by gophers and was abandoned. Each plot is 60 inches wide and 20 feet in length. Treatments were applied either as a pre-plant or post-plant application as shown in table 1. The plots were maintained using standard agronomic practices. Miracle grow fertilizer was applied on August 8 and August 30. One application of insecticide Admire was made on August 30 through drip system.

Before applying the treatments, soil samples were collected (from each plot) and submitted to nematology lab at UC Riverside (Antoon Ploeg) to determine the RKN count. Soil samples will be collected and analyzed for nematodes again at harvest. Melon roots will be evaluated for galling at mid-season and at harvest. Data on nematode counts and root galling will be analyzed using SAS (statistical analysis software).

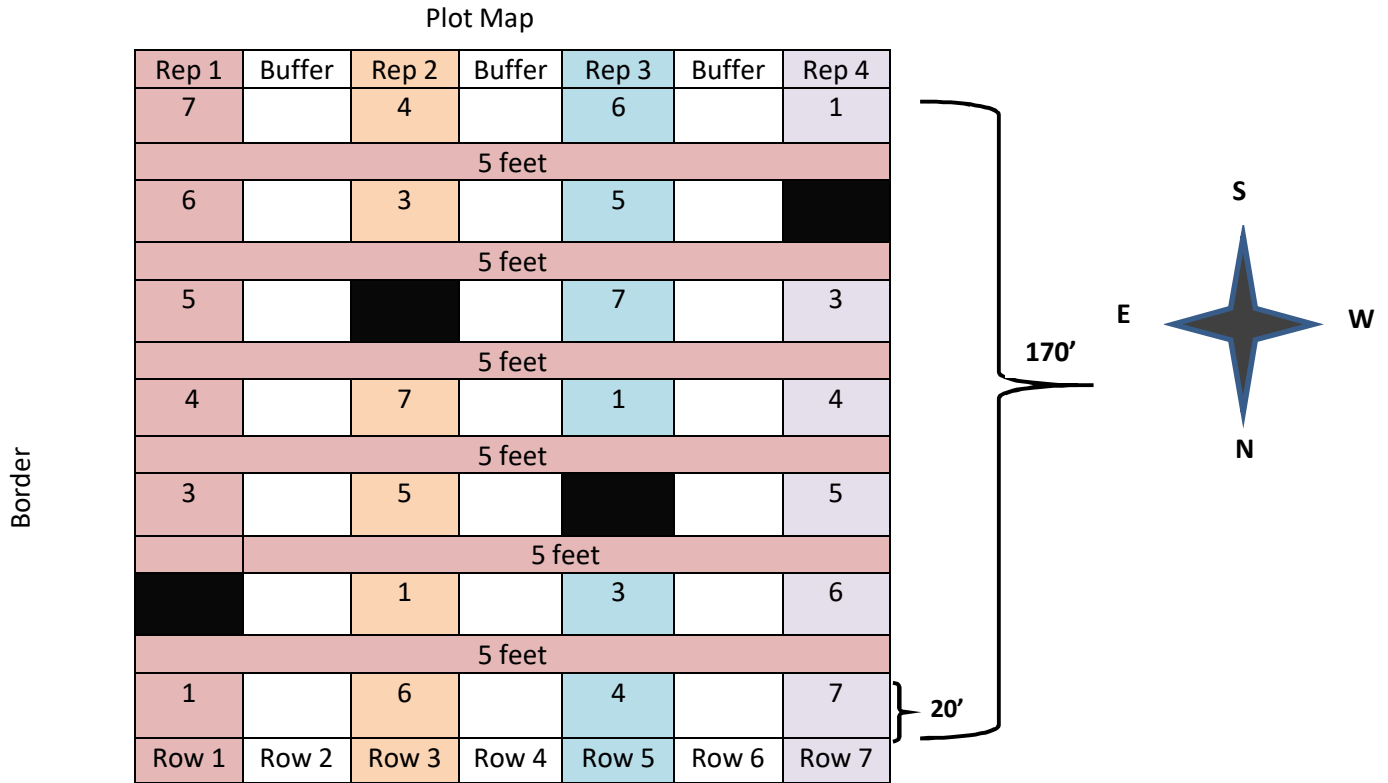
Table 1. Treatments, rate and application methods and timings

Treatment	Trt	Date of application	Rate	Method of application
Untreated Control	1			Buried Drip
Vydate (Standard)	3	19 July, 5 August	5pt/A	Soil Drench incorporated
Nimitz (pre plant)	4	3 July	5pt/A	Buried drip (12’')
Nimitz (pre plant)	5	3 July	5pt/A	Soil drench incorporated
Salibro (pre&post plant)	6	12 July, 15 August	30.7fl oz/A	Buried drip (12’')
Salibro (pre&post plant)	7	12 July, 15 August	30.7fl oz/A	Soil drench incorporated

### Challenges so far:

1. Slow germination, Gophers destroyed one of the control (Treatment 2), which is now abandoned and data will not be collected for treatment 2.
2. Birds took out seeds and ate them, so after one week we had to plant more seeds and then covered the plot with netting to save from birds. Some young seedlings were also pulled out.

Please find plot map, and pictures of the field layout and drip system below.



5 feet buffer in between plots. Gophers destroyed two plots in treatment 2. Data will not be taken from treatment 2, which was a surface drench control.







## CALIFORNIA MELON RESEARCH BOARD

Progress Report Aug 23, 2019

### **PROJECT. Evaluating preplant and post plant herbicide programs for weed management in transplanted LSL melons, year 2.**

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**Summary to date:** Trials were conducted at the UC Desert Research and Extension Center (DREC Imperial County) and UC West Side Research and Extension Center ((WSREC Fresno County) evaluating weed management and crop safety from various pre plant incorporated (PPI) and post plant herbicides in transplanted cantaloupes. Cultivar “Fiji” (LSL) was used at WSREC, and “Cayucos Beach” (ESL) at DREC. At both locations, various pre-plant herbicides were applied before transplanting and either mechanically or water incorporated.

Cayucos Beach ESL cantaloupe transplants were planted at the University of California Desert Research and Extension Center in Holtville, CA, on 3 April 2019. Ten treatments were applied using a randomized complete block design with four replications on 3 April 2019 (unless otherwise noted) and included 1) Curbit pre-plant incorporated (PPI) 4 pints/A, 2) Curbit 4 pints/A + Prefar 6 quarts/A (Tank Mix PPI), 3) Devrinol PPI 10 pints/A, 4) Dual Magnum PPI 1 pint/A, 5) Prowl PPI 3 pints/A, 6) Prefar PPI 6 quarts/A, 7) Sandea PPI 1 oz/A, 8) Dacthal POST 4 lbs/A (banded, 14 days after transplanting), 9) Sandea POST 1 oz/A (over-the-top or banded, 14 days after transplanting), 10) Untreated Control (weedy). The field was divided into two sections, where the experiment was duplicated and treatments received either sprinkler or mechanical incorporation. All plots were drip irrigated for the remainder of the experiment. Weed control and crop safety were evaluated May 9, 17, 24, and June 4. Melons were harvested from June 13 to 18, separated into cull and marketable melons, and grouped by size for weight and brix measurements. Data analysis is in progress. No major issues were experienced during the experiment.

At WSREC, the same beds from the 2018 season were utilized. All beds were amended with 200 lbs/A of 10-52-0 one month before planting. Treatments were the same as at DREC (10 herbicide treatments and 2 incorporation methods), with the addition of a hand weeded check plot. Statistical design was a randomized complete block design with 4 replications; plots were 1 bed wide x 30 ft long. Pre-plant treatments were applied on 30-May-2019 and mechanically incorporated using a rotary power mulcher to a depth of about 2”. The plots were then transplanted using mechanical finger planters on a 24” spacing on 31-May-2019. After transplanting, ½ of the plots were sprinkler irrigated two times for a target of 1” applied water, however actual applied water ranged from 0.5 – 2” depending on location. All plots were drip irrigated for the remainder of the experiment. Post-plant applications of Sandea 1 oz/A, and Dacthal 10 pts/A were made on 10-June. All plots were mechanically cultivated 1 month after transplanting to remove emerged weeds outside of the plant row; no in-row cultivation was performed except in the hand weeded plots. Weed and crop evaluations were made at 10, 30, 48, and 66 days after transplanting. A once-over harvest was performed on 20-Aug by counting all fruit by size in each plot. Brix readings were done on 1 sample fruit from each plot. Weed pressure from broadleaf weeds, especially groundcherry (nightshade family), was very high at this location. Preplant incorporated herbicides provided good weed control, however, crop phytotoxicity was observed in the Prefar+Curbit, Devrinol, Prowl, and Sandea PPI



treatments. Crop injury was exasperated by water stress in the drip-only treatments. As in 2018, sprinkler incorporation did not provide adequate weed control, and in fact increased weed germination as compared to the mechanically incorporated plots. Only Sandea at 1 oz/A PPI maintained good weed control throughout the season. Yields were higher in the plots where weeds were suppressed. Data analysis is pending.

At WSREC, the initial sprinkler irrigation missed many plots due to wind, and therefore a second application was made the following week. One major pitfall at the WSREC site location was the loss of plants in the mechanical incorporated plots to lack of water. The trial was planted on a Friday and the plots were not adequately irrigated to sub up water from the drip tape. As a result, the root ball dried out before more water could be applied the following week.



## CALIFORNIA MELON RESEARCH BOARD-Interim report

### Characterizing and assessing risk of emerging fungal and bacterial pathogens of melons (and other cucurbit crops) across the nursery-field production continuum

#### IMMEDIATE OBJECTIVES AND STATUS

1. Evaluate *Fusarium falciforme* as a potentially new crown rot pathogen of melon and other cucurbit crops in the California Central Valley, conduct cucurbit crown rot surveys, and use information to improve crown rot diagnostics

##### *A. Proof of pathogenicity trials.*

This trial was planted at the Plant Pathology Field Research Station on June 6. We are evaluating pathogenicity of *Fusarium falciforme* on cantaloupe melon, cucumber and pumpkin using two inoculation methods. For the first, we dip inoculated seedlings in fungal spores ( $10^6$  spores/ml, one minute) the day before transplant; non-inoculated plants were the control. For the second, we placed a colonized agar plug on top of a stem wound on mature plants; controls consisted of plants with a sterile plug placed on the wound. We continue to monitor for symptoms and will evaluate plants for stem and crown rot in early October. Thus far, no clear symptoms have developed.

##### *B. Cucurbit crown rot surveys in the Central Valley.*

In collaboration with farm advisors, we have received a range of cucurbit submissions thus far including seedling damping off leaf spots, root rot, and stem rot. Thus far we have not recovered *F. falciforme* from any cucurbit crop. As crown rots are late season diseases, we anticipate receiving more as the season progresses; many counties had late plantings due to late spring rains which has likely delayed the onset of late season disease development.

2. Conduct a hazard assessment to identify critical control points (HACCP) for bacterial and fungal transplant pathogens in greenhouses

We conducted HACCP assessments at the two proposed vegetable transplant houses in Fresno as well as an additional transplant house in Winters. To evaluate efficacy of sanitation practices we evaluated microbial (bacterial and fungal) loads on trays and benches pre and post treatment and tested potting media and water as potential pathogen sources. Across all three facilities, propagation trays had high bacterial loads ( $>250$  colony forming units /  $5\text{ cm}^2$  area) after steam sterilization ( $82^\circ\text{C}$ ). Bench disinfection methods (application of sanitizing solutions) did not appear effective in reducing microbial loads (both bacteria and fungi). There was a high prevalence of standing water in the ground, at a close distance (15 cm) from the raised propagation benches, increasing the likelihood of water-splashed contamination with soil-borne pathogens. Media also contained high bacterial and fungal loads. Both a *Botrytis* species (causing leaf blight) and *Thielaviopsis basicola* (causing black root rot) were recovered from symptomatic seedlings at high incidences, despite a rigorous fungicide regime, suggesting potential development of fungicide resistance to these pathogens. Based on this assessment, targets for improved disease management are: (1) improved sanitation of re-used trays and benches, (2) alteration of benches to reduce humidity and water dispersal of pathogens, and (3) assessment of fungicide resistance and potential introduction of additional chemistries.

3. Melon disease outreach

Updates to the UC IPM cucurbits diseases are underway and include new information about seedling disease management.

**Objective 1. Evaluate insecticides and insecticide seed treatments for management of cucumber beetles in fresh-market melons.**

We based treatments on what is commonly used commercially plus newer insecticides untested in melons on cucumber beetles. We direct-seeded single lines of honeydews on 60-inch beds and at a high density on June 19, 2019 and then thinned to 12 in. spacing around the four-five leaf stage. Once cucumber beetles were observed in the field, treatments were applied on July 22 using a backpack CO<sub>2</sub> sprayer at 20 GPA. Plots were treated again on August 15 and August 29. Beetles were counted on plants in center rows 3, 7, 14 and 21 days after the first application (DAT). They were counted 4 and 11 DAT2, and will be counted 4, 10, 11 DAT3. An additional application will be made if necessary. Weeds were managed with a pre-emergent herbicide followed by hand weeding and fertilizer was injected through the drip. We will assess scarring damage in each plot in several weeks.

Populations of cucumber beetles were initially low when plants were emerging, so damage was extremely low early. We therefore weren't able to assess "early-season" management. Spotted cucumber beetle were the only species present at first, but striped cucumber beetle populations have built.

Trt#	Insecticide	Active ingredient(s)	MOA
1	Untreated check	--	--
2	Sevin	carbaryl	1A
3	Cruiser	thiamethoxam (seed trt only)	4A
4	Cruiser, Capture+Assail	thiamethoxam (seed) & bifenthrin + acetamiprid	4A/3A/4A
5	Voliam Flexi	thiamethoxam + chlorantraniliprole	4A/28
6	Harvanta	cyclaniliprole	28
7	Assail 70WP	acetamiprid	4A
8	Exirel	cyantraniliprole	28
9	Brigadier	bifenthrin+ imidacloprid	3A/4A
10	Capture	bifenthrin	3A
11	Entrust SC, Surround	Spinosad + kaolin clay (Organic)	5
12	Celite, Surround	diatomaceous earth + kaolin clay (Organic)	--



Spotted cucumber beetle on young melon plant

**Objective 2. Test different trap designs with floral lures to refine and improve monitoring of WStrCB and WSpCB.**

Based on trap availability, our three traps consisted of Japanese beetle traps (with sticky card inside), yellow sticky card traps, and yellow sticky card traps on top of large yellow corrugated plastic board to increase visual stimulus. Floral lures were attached to each trap. Three of each type of trap were placed in two conventional fresh-market melon fields in Sutter County and three organic melon fields in Yolo County.

One challenge we encountered was the attractiveness of the floral lures to bees. Due to significant bee kill in the first week, Japanese beetle traps were removed. To solve this problem, wire mesh was placed at the top of the Japanese beetle traps so that bees would not be able to get into the trap, but cucumber

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beetles could fit through. The floral lure was also placed further above the sticky traps. We will continue to check and replace traps through melon harvest.



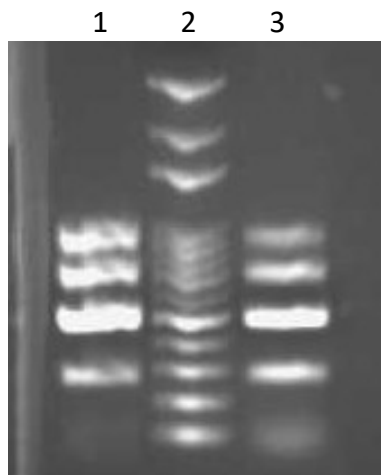
**Mid-Year Report to the California Melon Research Board**  
**William M. Wintermantel**

**Project Title: Development of rapid detection methods for evaluation of germplasm for resistance and determination of the California host range of *Cucurbit chlorotic yellows virus* and *Squash vein yellowing virus*.**

**Objective 1.** *Develop standard multiplexed RT-PCR primers for simultaneous detection of CCYV, CYSDV, CABYV, and SqVYV from plants and insect vectors.*

DNA Primers were designed to portions of the genome sequence of each of the four RNA viruses targeted: Cucurbit chlorotic yellows virus (CCYV), Cucurbit yellow stunting disorder virus (CYSDV), Squash vein yellowing virus (SqVYV), and the aphid transmitted virus Cucurbit aphid-borne yellows virus (CABYV) which causes symptoms nearly identical to those of CYSDV and CCYV. Primers were developed such that each virus-specific primer set amplifies a different size fragment, making it easy to see which viruses are present in a sample and which are not, when analyzed by electrophoresis on agarose gels (standard methods for evaluating results of RT-PCR). A method was developed in which primers for the RNA viruses (CCYV, CYSDV, SqVYV, CABYV) are combined into a single reaction mix and used to test for the presence of each of the viruses from a single sample. The method has been validated and reliably detects each virus based on analysis of laboratory (**Fig. 1**) and field samples. A parallel amplification has been developed for detection of the DNA virus, CuLCrV, using PCR.

**Figure 1.** 1% Agarose gel showing how RT-PCR products differ in size to allow determination of which viruses are present during multiplex RT-PCR. Lane 1) 100 ng RNA containing all 4 viruses; Lane 2) DNA size marker; Lane 3) 10 ng RNA containing all 4 viruses.



**Band sizes**

953 bp CCYV  
723 bp SqVYV  
492 bp CYSDV  
277 bp CABYV

**Objective 2.** *Develop multiplexed real time (quantitative) RT-qPCR primers for simultaneous determination of titers of CCYV, CYSDV, CABYV, and SqVYV from plants that may be infected by one or more of these viruses simultaneously.*

DNA probes (modified primers for use in real-time/quantitative RT-PCR) for use in TaqMan assays were designed to quantify the amount of each of the four RNA viruses in melons and other host plants. Probes for each virus have been validated against each target virus and against RNA samples containing RNA of one to four of the different viruses together. To date all probes have been designed and tested. We have confirmed optimized performance of probes for measuring differential levels of CCYV and CYSDV but are still optimizing those for SqVYV and CABYV. There was an issue with the fluorescent marker for SqVYV being too similar to that for another fluorescent marker, and therefore we had to reorder that probe with a different fluorophore. Evaluation of the latter two are in progress and should be completed soon. Quantitative PCR for detection of CuLCrV has been developed as well and is highly efficient.

**Objective 3.** *Use primers to begin to evaluate germplasm for presence and/or levels of each virus from field or greenhouse samples, and to determine the California host range of newly introduced virus, CCYV.*

Primers for standard multiplex RT-PCR were used in combination with primers for CuLCrV on samples at the end of the spring season (June) from melon breeding plots at DREC in the Imperial Valley. All primers performed well and determined the presence of CCYV and CYSDV, as well as a large amount of SqVYV in the breeding plots from the spring trial. CuLCrV and CABYV were not detected from the field samples. Fall breeding plots at DREC will be sampled the week of Sept. 23, 2019, as will commercial fields in California and Arizona. These samples will be evaluated with both standard and quantitative methods developed in Objectives 1 and Objective 2.