California Melon Research Board

2018-19 Research List

Leader	Project Title	TOTAL APPROVED 3/1/2018
Gilbertson	Survey, Characterization and Biological Properties of a California Isolate of Squash Vein Yellowing Virus (SqVYV) and Associated Cucurbit-Infecting Viruses	**19,757**
Mauck	Combining a Plant Immune Activator with Newer Insecticides to Disrupt Vector Feeding and Virus Infection	**21,508**
Nansen	Repeatable, Rapid, and Cost-Effective Screening of Insecticide Performance	
Nansen/Vinchesi	Repeatable, Rapid, and Cost-Effective Screening of Insecticide Performance	**20,000**
Palumbo	Evaluation of Insecticide Alternatives for Whiteflies and CYSDV in Melons	**11,616**
Stoddard Bean	Evaluating Preplant and Post Plant Herbicide Programs for Weed Management in Transplanted LSL Melons	**14,150**
Turini	Evaluation of Plant Growth Regulator Application to Concentrate Fruit Set and Reduce the Period of Melon Harvest	**14,992**
Vinchesi	Assessment of Western Striped and Westerrn Spotted Cucumber Beetle Movement into Fresh-Market Melon Fields in the Sacramento Valley	
Wintermantel	Evaluation of RNAi Strategies for Reducing Whitefly Populations on Melons	**14,760**
CPS	Pledge to the Center for Produce Safety (CPS)	**25,000**
	TOTALS	**$ 141,783**

1 *Nansen & Vinchesi were asked to combine their two proposals into one project, not to exceed $20,000.*

2 *Stoddard was asked to include Lynn Sosnoski as a weed expert on this project, with "up to" an additional $1,592 if she accepts.*

3 *Turini was asked to revise to include additional cultivars/types and include a seed industry partner/breeder as a consultant.*

May 16, 2018

Interim Report to the California Melon Research Board

Project title: **Survey, characterization and biological properties of a California isolate of *Squash vein yellowing virus* (SqVYV) and associated cucurbit-infecting viruses**

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; **Bill Wintermantel**, Virologist, USDA-ARS, Salinas; Cooperators: **Monica Macedo**, Postdoctoral Research Associate, Department of Plant Pathology, UC Davis

In the fall 2014, the whitefly (*Bemisia tabaci*)-transmitted ipomovirus, *Squash vein yellowing virus* (SqVYV), was detected for the first time in the Imperial County, California. In Florida and, more recently, in Central America, SqVYV causes economics losses, especially in watermelon crops. Therefore, we have focused on two main objectives: 1) continued characterization of SqVYV from California (SqVYV-CA), and 2) surveying of melon production in areas with *B. tabaci* to assess the relative incidence and severity of SqVYV-CA.

We have now completed the analysis of the genome sequence of SqVYV-CA and this revealed 1) that it is a recombinant virus, with a portion of the genome more similar to that of an aphid-transmitted potyvirus, and 2) the rest of the genome was more similar to a SqVYV isolate from Israel (98% identity) than an isolate from Florida (SqVYV-FL, 84% identity). We also developed an infectious clone of SqVYV-CA, which allows us to assess the disease symptoms in different hosts and develop an approach for disease resistance screening. This year, we have assessed direct agroinoculation and mechanical (sap) inoculation in a range of cucurbits (cucumber, melon, pumpkin, and watermelon). Our results indicated that SqVYV-CA causes severe yellowing and mosaic in pumpkin and summer squash, vine decline in watermelon, symptomless infection in cucumber and no symptoms or infection in melons (varieties of honeydew and muskmelon). Furthermore, mechanical inoculation was considerably more efficient than direct agroinoculation. This suggests that, for resistance screening, susceptible plants can be agroinoculated to build-up inoculum, followed by mechanical inoculation for the screening of materials for resistance. The finding that the melons tested appear to be immune to SqVYV-CA may explain why this virus has not become economically important in southern California (unlike in Florida or Central America). Furthermore, because melons are susceptible to SqVYV-FL (although symptoms are relatively mild) we wanted to determine if the recombinant part of SqVYV-CA is responsible for this host range difference. We obtained a clone of this region from SqVYV-FL and used the Gibson assembly method to exchange it with the equivalent potyvirus region (P1a gene) in SqVYV-CA. We obtained the clones with the putative exchange (SqVYV-FL/CA) and sequencing confirmed that the exchange was successful and in-frame. We are now in the process of evaluating the infectivity and symptoms induced by SqVYV-FL/CA compared with SqLCV-CA.

Based upon information from stakeholders, whitefly populations in melon production areas in southern California have been low and virus diseases have not been a problem. Therefore, a survey of early-planted fields was not conducted. A survey of late-planted melon fields is planned.

CMRB Interim report - September 1, 2018

Project Director: Kerry Mauck, Assistant Professor of Vector Biology at University of California, Riverside. Phone: 951-827-5444. Email: kerry.mauck@ucr.edu

Project Title: Combining a plant immune activator with newer insecticides to disrupt vector feeding and virus infection

Original objectives:
Objective 1: Determine effects of acibenzolar-S-methyl alone, and in combination with dinotefuran, flupyradifurone, and cyantraniliprole, on melon resistance to CYSDV inoculation by whiteflies and subsequent disease progression.

Objective 2: Determine the effects of acibenzolar-S-methyl, dinotefuran, flupyradifurone, and cyantraniliprole, and their combinations, on whitefly feeding behaviors associated with successful CYSDV inoculation (to healthy hosts) and acquisition (from CYSDV-infected hosts).

Modifications:
Based on feedback received during review of the proposal at the 2018 CMRB board meeting, it was suggested that the project should focus not only on testing strategies to boost resistance to infection by CYSDV, but also mosaic viruses transmitted by aphids, such as *Cucumber mosaic virus* (CMV). Mosaic viruses are a problem in the Central Valley growing region, but new solutions for mitigating impacts have not been proposed for some time. As a result of the incorporation of an additional pathogen, we modified the objectives to focus on the plant immune elicitor alone and its effects on virus resistance and vector feeding behavior. Subsequently, we discovered that other researchers are pursuing studies on the impacts of the insecticides listed above on vector behavior using methods proposed in the original proposal. The Project Director will summarize and communicate these results to growers at the CMRB meeting in January 2019.

Revised Objective 1. Determine the effects of acibenzolar-S-methyl (Actigard) on melon resistance to CYSDV and CMV.
All work for this objective was performed in the greenhouse and a field trial conducted at the Agricultural Operations facility on the UCR campus. During preliminary experiments, we discovered that the label rate recommendation for use of the elicitor product “Actigard” in melons is phytotoxic to the plants and often lethal. This finding was confirmed by a paper that was published shortly after we began our experiments, in which the authors demonstrated yield reductions in melons due to application of Actigard at the label rate (Egel et al. 2018. Crop Protection 109: 136-141). We therefore performed a titration experiment to determine the maximum concentration of Actigard that results in no visible phytotoxicity for ‘Gold Express’ melons. We found that a 25 parts-per-million (ppm) concentration is well tolerated by the plant when applied as a foliar application. We also determined that a 25ppm soil application and 12.5ppm foliar application have positive effects on plant biomass, possibly through a growth-promotion effect. This finding and mechanisms underlying positive effects will be pursued in future experiments.

Using the 25ppm concentration, we performed two experiments to assess effects of a single application of Actigard on melon resistance to CMV and CYSDV. For CMV, Actigard significantly reduced symptom severity over the course of a four-week experiment. Plants with Actigard and CMV infection had equivalent biomass to control, virus-free plants with Actigard treatment, and control plants with no Actigard treatment. Virus titer in Actigard-treated plants was also significantly reduced relative to untreated plants. An identical experiment was recently completed for CYSDV disease progression and titer, symptom expression, and biomass results are currently being analyzed. Alongside more controlled greenhouse experiments, we also performed a factorial experiment as a small field trial to determine impacts of Actigard applications on melon growth and yield in the field. Foliar application of the 25ppm Actigard concentration had no negative effects on plant growth or melon yields. Virus and vector pressure were low throughout the season due to a cool spring, so we were not able to assess impacts on disease incidence or symptoms. There were no changes in the community of insect predators visiting plants as a result of Actigard application.

Revised Objective 2: Determine the effects of acibenzolar-S-methyl (Actigard) on melon resistance to aphid and whitefly vectors.

We performed several types of experiments to assess effects of Actigard on vector host preferences and feeding behavior. To determine if Actigard disrupts inoculation of viral pathogens by aphids, we treated plants with Actigard in 25ppm concentration and performed electrical penetration graphing analysis to measure probing and feeding behavior by a main aphid vector of CMV (*Aphis gossypii*). Our results indicate that Actigard reduces plant suitability for *A. gossypii* by making it more difficult for the aphid to ingest plant sap from the phloem. This is expected to reduce aphid performance in the field, leading to lower aphid populations. A downside of Actigard is that increased host resistance leads to more probing by the aphid, which is conducive to acquisition and inoculation of CMV and other non-persistently transmitted plant viruses. A second experiment is planned to determine if reductions in virus titer and symptom severity due to Actigard application offsets its effects on aphid behaviors conducive to CMV transmission. Preliminary results from aphid preference tests suggest that this may be the case. Aphids are attracted to untreated plants infected with CMV over healthy plants when given a choice, but when Actigard treatment is applied before CMV inoculation, symptoms are reduced and the attractive phenotype is eliminated. The consequences for this in more complex environments will be the target of future studies.

Experiments were also performed to assess effects of Actigard treatment and CYSDV infection on whitefly behaviors. Electrical penetration graphing data were collected for whiteflies feeding on untreated healthy melons, untreated melons infected with CYSDV, Actigard treated healthy melons, and Actigard treated melons inoculated with CYSDV. Data analysis of these recordings is in progress. We also performed behavioral assays to determine whitefly orientation and settling preferences for plants with the treatments listed above by presenting leaves of all treatments to groups of whiteflies simultaneously in a large arena. Data collection was completed within the last week, and these data will be analyzed alongside the EPG recordings to determine how Actigard influences whitefly behaviors associated with CYSDV transmission.

Project report – August 2018

REPEATABLE, RAPID, AND COST-EFFECTIVE SCREENING OF INSECTICIDE PERFORMANCE

Project team

Associate Professor Christian Nansen, Junior Specialist Rachel Purington, and Scientific Researcher II Alison Stewart, UC Davis, Department of Entomology and Nematology

Area Vegetable Crops Advisor Dr. Amber Vinchesi, Colusa, Sutter and Yuba counties. University of California Cooperative Extension

We wish to thank the Melon Board for giving us this opportunity to collaborate – the project is off to a very strong start, and the team is very excited about this collaboration – so thank you!

Update

Below, we provide a brief update for each of the project objectives.

Objective 1 - Establishment of colonies of western striped cucumber beetles to be used in bioassays. The Nansen Lab has collected both western spotted cucumber and western striped cucumber beetles and adults are currently maintained for bioassays (see objective 3).

Objective 2 - Through collaboration with the California Melon Research Board, obtain a list of the top 5-6 insecticides. Based on discussions with collaborative growers, we have decided to examine the following insecticides: bifenthrin, carbaryl, acetamiprid, lamba-cyhalothrin and zeta-cypermethrin.

Objective 3 - Use Ethovision XT to characterize and quantify the level of behavioral resistance/avoidance to the selected insecticides. Initial preparations have been made, and we are finalizing the design of the experimental setup.

When writing the project proposal, we were unaware that growers predominantly perform insecticide spray applications at night. At night, the relative humidity is generally high, and the intent was to obtain spray coverage data based on deployments of water sensitive spray cards. However, under high relative humidity these yellow water sensitive spray cards turn blue as they react with the humidity in the air. We attempted to collect deployed water sensitive spray cards at 4 am after a night spray, but this methods is being abandoned. Instead, we will proceed with an alternative and indirect method to quantify insecticide spray coverages. This method will constitute a combination of the original objectives 4 and 5.

New: Objective 4 - In commercial melon fields (honeydew and cantaloupe) in Sutter and Yuba counties, obtain quantitative insecticide spray coverage data based on bioassays of mortality of cucumber beetles. The day immediately before a night insecticide spray application, sweet potatoes will be placed in metal cages in the field with one cage on the ground and the other one on a raised platform to mimic the top of the canopy. The following morning, the sweet potatoes from the field will be brought back to the lab, and they will be referred to as "Pot_top" and "Pot_canopy". In the lab we will prepare a third set of sweet potatoes, "Pot_lab", which will be dipped (= 100% coverage) in an insecticide formulation that is identical (same insecticide and same concentration) as the one applied in the field. In separate bioassays, adults from both species of beetles will be transferred to sweet potatoes from the three treatments, and the mortality of adult beetles will be monitored daily for 1-7 days. The difference in mortality between the three treatments of sweet potatoes will provide a quantitative assessment of the importance of spray coverage.

(Originally Objective 6) Objective 5 - Obtain trapping data in and around melon fields in Sutter and Yuba counties. Based on extensive scouting and networking (Dr. Vinchesi), we have selected two field areas for collection of trapping data: River Garden in North Yolo County (5 honeydew fields) and Meridian in Sutter County (3 cantaloupe fields). We have obtained preliminary trapping data with traps with floral-based lures. Other trapping methods (emergence traps) have been tested but logistical challenges have occurred.

Evaluation of Insecticide Alternatives for Whiteflies and CYSDV in Melons

John Palumbo, University of Arizona, Yuma AZ

Research identifying alternatives for whiteflies and CYSDV management in melons has been very productive so far this season. We were able to develop useful information that should be helpful to desert growers. 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, Exirel, and Sivanto, provided good adult control and significant levels of CYSDV suppression. Other new registered products, Cormoran and Minecto Pro were not comparable to the standards. We have also been focused on two experimental products. PQZ (Pyrifluquinazon) was shown to be a very effective compound for both adult whitefly and CYSDV suppression. The compound should be registered for use in later 2018 or early 2019. Another numbered experimental compound, Sefina (Inscalis) also showed promise for whitefly control and virus suppression in foliar trials and will be available for use in 2019. Fall trials are currently underway to further evaluate soil and foliar insecticides on whitefly and CYSDV management. We are continuing our work with PQZ and Sefina and trying to determine their best fit in early season adult whitefly control. Whitefly populations have been moderate to date on the fall plantings, but we anticipate high virus incidence as the season progresses.

CALIFORNIA MELON RESEARCH BOARD
Progress Report Sept 9, 2018

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

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Summary to date: Trials were conducted at the UC Desert Research and Extension Center (Imperial County) and West Side Research and Extension Center (Fresno County) evaluating weed management and crop safety from various pre and post plant herbicides in transplanted cantaloupes. Cultivar “Figi” was used at both locations, and weed seed of various types was broadcast throughout the plot area. At DREC, Sandea 1 oz/A, Curbit 4 pts/A, and Prefar 6 qts/A were applied on April 3, 2018, half of the plots were sprinkler incorporated with about 1.5” of water. The other half received no incorporation. Plots were transplanted on April 4. Post plant treatments of Sandea 1 oz/A and clethodim 8 oz/A were made on May 3. All plots were irrigated using drip. Weed evaluations were performed at 2, 3, 4, 5, 6, and 8 weeks after transplanting. Harvest occurred on June 21. Fruit sizes were counted and weighed and Brix measured at this time. Plot size was 1 bed (80”) x 30 feet long, with 4 replications. In total, 12 treatments were evaluated. In general, all the pre plant incorporated treatments provided better weed control than where they were not sprinkler incorporated. No crop phytotoxicity was observed in any of the treatments.

At WSREC, drip tape was shanked into 80” beds in the spring and all beds were amended with 200 lbs/A of 10-52-0. Weed seed (a mixture of 12 different annual grass and broadleaf weeds) were broadcast and incorporated lightly. Sandea 1 oz/A, Curbit 4 pts/A, Prefar 6 qts/A, and Curbit+Prefar tank-mix were applied on May 31 to select plots 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. After transplanting, ½ of the plots were sprinkler irrigated. Post-plant applications of Sandea 1 oz/A, Clethodim 8 oz/A, and Poast 24 oz/A were made on June 26. In total, there were 20 treatments: 10 with sprinkler incorporation and 10 with mechanical incorporation of the herbicides. Weed and crop evaluations were made at 1, 3, 4, 6, 8, and 10 weeks after transplanting. A once-over harvest was performed on Aug 17 by counting all fruit by size in each plot. Brix readings were done on 1 sample fruit from each plot. Plot size was 1 bed by 30 feet long, replicated 4 times. Weed pressure from broadleaf weeds, especially nightshades, was very high at this location. Preplant incorporated herbicides provided good weed control, however, some phytotoxicity was observed in the Sandea treatments. Sprinkler incorporation did not provide adequate weed control, and in fact appeared to increase weed germination as compared to the mechanically incorporated plots. Yields were higher in the plots where weeds were suppressed. The lack of control in the sprinkler irrigated plots was probably due to insufficient water being applied. The sprinklers were on for only 4 hours, and the water applied was likely less than 0.5”

California Melon Research Board Progress Report

Evaluation of plant growth regulator application to concentrate fruit set and reduce the period of melon harvest

Jan 2018 through Dec 2018

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A harper type LSL melon, Caribbean King and a Western shipper, Olympic Gold, was be direct seeded into 80-inch beds on Panoche clay loam soil on 25 June at the University of California West Side Research and Extension Center. The first irrigation was applied through the sprinklers on 26 Jun and all irrigations were through sprinklers until 9 Jun, when the first water was applied through the trickle irrigation system buried at a depth of 10 inches at the center of the bed. On both varieties, the first applications of 300, 400, 500 ppm ethephon was on 16 Jul to plants with 3 to 4 true leaves. On 23 Jul, when the plants were at 8 to 11 true leaves plots were treated with 300, 400 or 500 ppm ethephon. By 24 July plants treated with the ethephon at early stages of plant development had fewer flowers than untreated. Twenty-four days after the ethephon applications, PoMaxa 2.4 fl oz per acre in the equivalent of 40 gallons per acre was applied. No other differences among treatments have been observed at this time. It is likely that harvest will begin on 18 Sep. At that time, yield and fruit size per harvest date, ^{o}brix levels and fruit firmness will also be evaluated.

The unusual challenge for melons grown at this location is that in early August, whitefly population densities reached levels higher than ideal. Insecticide applications were made with one application of Admire through the trickle irrigation system and three foliar applications through to present date. Nymph levels are currently low but adult levels are substantial .

Please find the plot map and treatment summary below.

Field map of plant growth regulator study at UC West Side Research and Extension Center 2018.

		Harper type melon Caribbean King									Western shipper Olympic Gold							
40 ft	R1		R1M2S4		R1M2S3		R1M2S1		R1M2S2		R1M2S3		R1M2S1		R1M2S2		R1M2S4	
35 ft			R1M1S3		R1M1S1		R1M1S2		R1M1S4		R1M1S2		R1M1S1		R1M1S4		R1M1S3	
35 ft	R2		R2M1S1		R2M1S3		R2M1S4		R2M1S2		R2M2S3		R2M2S4		R2M2S2		R2M2S1	
35 ft		buffer	R2M2S2	buffer	R2M2S1	buffer	R2M2S4	buffer	R2M2S3	buffer	R2M1S2	buffer	R2M1S1	buffer	R2M1S4	buffer	R2M1S3	buffer
35 ft	R3		R3M2S3		R3M2S2		R3M2S1		R3M2S4		R3M2S4		R3M2S3		R3M2S1		R3M2S2	
35 ft			R3M1S4		R3M1S2		R3M1S3		R3M1S1		R3M1S1		R3M1S2		R3M1S3		R3M1S4	
35 ft	R4		R4M1S4		R4M1S1		R4M1S2		R4M1S3		R4M2S3		R4M2S1		R4M2S2		R4M2S4	
40 ft			R4M2S1		R4M2S3		R4M2S4		R4M2S2		R4M1S4		R4M1S2		R4M1S3		R4M2S1	
		17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
			Harper type melon								Western shipper							

N

Split-Plot Design with Four Replications

Main-plot treatments – application timing

- M1 - First application at 3 true leaf stage of plant development
- M2 - First application at 9 true leaves

Sub-plot treatments – Florel rate (ethephon concentration) followed by PoMaxa 2.4 fl oz/a (25 days after Florel)

- S1 – 300 ppm ethephon
- S2 - 400 ppm ethephon
- S3 - 500 ppm ethephon
- S4 - untreated

Evaluation of RNAi strategies for reducing whitefly populations on melon

W.M. Wintermantel and N. Kaur
USDA-ARS, Salinas, CA

Objective 1: Evaluate dsRNA constructs for their ability to induce whitefly mortality on treated melon.

We developed over 35 novel DNA constructs designed to target genes unique to whitefly *Bemisia tabaci*, MEAM1 (biotype B), with the intent to kill whiteflies using a method known as RNA interference (RNAi). Results have shown the 4 highest performing constructs can yield mortality rates of 70 to 90% using *in vitro* experiments in which whiteflies feed on a sucrose solution containing the RNAi constructs.

Evaluations were performed with melon cuttings, in which detached melon leaves are sprayed with solution containing the 4 most high-performing RNAi constructs (based on *in vitro* tests), which then is absorbed into the leaf. Whitefly feeding on these treated leaves yielded mortality rates from 75-78% in melon for the two best constructs with control mortality at 24%.

Four new constructs were developed this summer focused on interference with virus transmission, and were based on results of comparative gene expression studies using whiteflies feeding on melon plants infected with CYSDV and healthy melon, as well as two additional virus-host systems (Kaur & Wintermantel, unpublished). Initial tests simply evaluated the rate of mortality induced by these constructs to establish a baseline, with results ranging from 47 to 66% mortality, although with higher than desired rates for a control construct as well (33%). Subsequent tests are beginning to evaluate impact on reducing virus transmission rates. The goal is to couple high mortality through one construct with reduced ability to transmit viruses by another.

Objective 2: Evaluate induced whitefly mortality on melon following treatment with cloned DNA constructs based on previously evaluated dsRNA

Four RNAi constructs were developed for expression of double-stranded RNA (dsRNA) and are being tested in both transient delivery assays in melon (and in parallel studies in tomato and cassava) and for plant transformation. The constructs were developed by the Wintermantel Lab for double-stranded RNA expression targeting whiteflies, and are based on the four highest-performing RNAi constructs from *in vitro* assays. Initial tests in cassava by collaborators in Africa showed similar ratios of whitefly mortality to results of *in vitro* assays and leaf feeding assays. Tests in melon are in progress.

Objective 3: Determine duration of RNAi-based control of whitefly in treated melon plants. This objective will follow establishment of reliable control methods.