FINAL REPORT - Fiscal Year 2018

A. Project Title

Combining a plant immune activator with newer insecticides to disrupt vector feeding and virus infection.

B. Research Priority Area

#12 (Understanding of the basic biology of disease/vector relationships and how to reduce losses from viruses)

C. Principal Investigator contact information.

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D. Location where work was performed.

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E. Project Overview

Melons in California routinely suffer losses from viral pathogens transmitted by aphids and whiteflies. These insect vectors can also reach extremely high population sizes, both within melon crops and at a landscape level across multiple crop species that serve as suitable hosts. Because the virus-vector association poses a dual threat, management of these pests presents a unique challenge for growers. Cultivars with resistance to both viruses and vectors are not available. Insecticides often do not act fast enough to prevent inoculation of viral pathogens, and in some cases, the viruses themselves modify plant traits in ways that make them more attractive or palatable to insect vectors. Virus effects on plant hosts also disrupt physiological processes in ways that modify fruit output and quality. Even if a crop is produced, the flavor may be altered due to virus infection, rendering the crop unmarketable. The symptoms of virus infection that alter both fruit quality and host-vector interactions occur because the plant is not able to resist or attenuate the effects of the pathogen.

The goal of this project was to test the efficacy of a plant immune boosting chemical (elicitor) as priming agent to prepare the plant immune system for virus attack before it is ever inoculated. We focused on acibenzolar-S-methyl (ASM), a commercially available elicitor that mimics the activity of a key signaling hormone in plant defense against diverse pathogens. To determine the effects of ASM on plant resistance to virus infection we used two pathogens that routinely plague melon growers in different regions: *Cucumber mosaic virus* (CMV) and *Cucurbit yellow stunting disorder virus* (CYSDV). CMV is problematic in the Central Valley. It has a wide host range and is transmitted by over 80 vectors (creating many opportunities for introduction to melon fields). *Cucurbit yellow stunting disorder virus* (CYSDV) is a whitefly-transmitted virus prevalent in Imperial and Riverside counties. CYSDV has had devastating effects on melon production during the fall in these areas. We evaluated ASM as a possible component of integrated pest management strategies through the following objectives:

<u>Objective 1. Determine the effects of ASM (Actigard) on melon resistance to CYSDV and CMV.</u>

<u>Objective 2:</u> <u>Determine the effects of ASM (Actigard) on melon resistance to aphid and whitefly vectors.</u>

F. Results & Analysis

<u>Objective 1. Determine the effects of ASM (Actigard) on melon resistance to CYSDV and CMV.</u>

All work for this objective was performed in the greenhouse and through 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 ASM product “Actigard” (Syngenta) 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 ASM (Actigard, Syngenta) at the label rate (Egel, Kleczewski, Mumtaz, &

Foster, 2018/7). We therefore performed an experiment to determine the maximum concentration of ASM 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 (Fig. 1). We also determined that a 25 ppm soil application and 12.5 ppm foliar application have positive effects on plant biomass, possibly through a growth-promotion effect (Fig. 1).

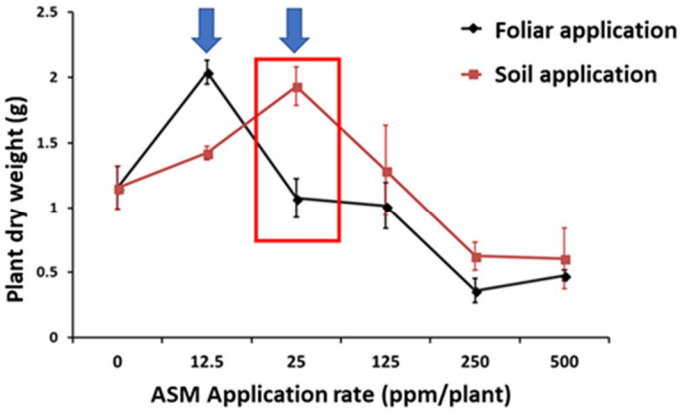
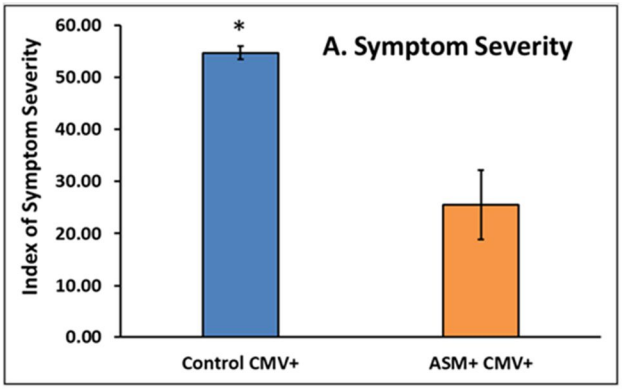
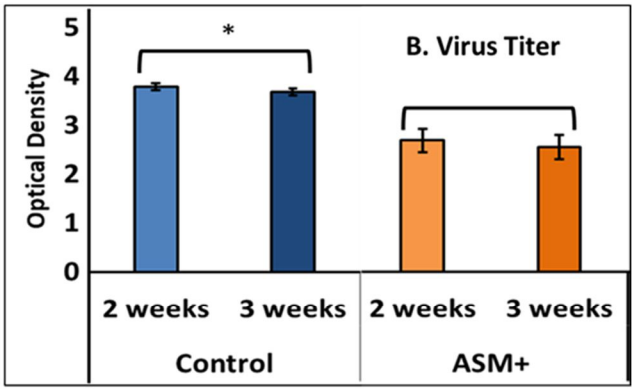


Figure 1. Effects of various ASM concentrations and application methods on plant growth (4 weeks). 25ppm/plant was chosen for use in subsequent experiments. Arrows indicate growth promotion effects of certain concentrations and application methods.

Using the 25ppm concentration, we performed experiments to assess effects of a single application of ASM on melon resistance to CMV and CYSDV. For CMV, ASM significantly reduced symptom severity over the course of a four-week experiment (Fig. 2). Plants with ASM and CMV infection had equivalent biomass to control, virus-free plants with ASM treatment, and control plants with no ASM treatment (Fig. 2). Virus titer in ASM-treated plants was also significantly reduced relative to untreated plants (Fig. 2). An identical experiment was completed for CYSDV disease progression and titer, symptom expression, and biomass. However, we found little effect of ASM on CYSDV infection rates or disease progression (Fig. 3). This experiment is being repeated to confirm that ASM is not effective as a treatment for CYSDV.





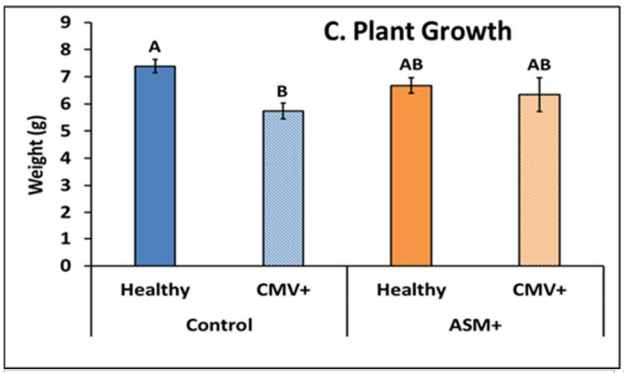
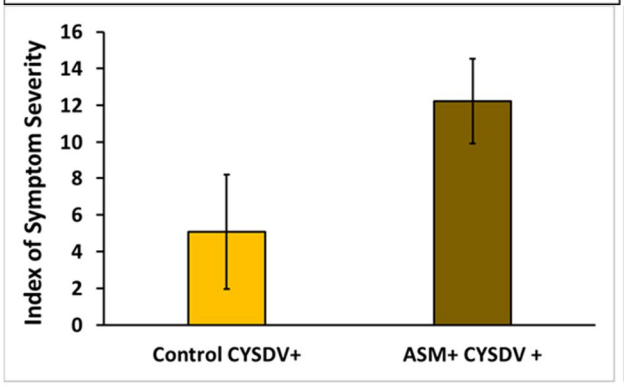
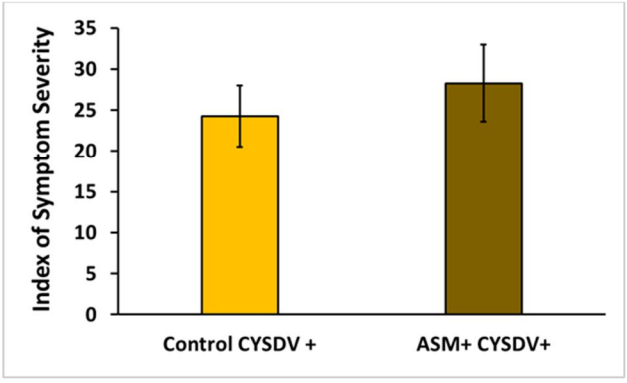


Figure 2. Effects of ASM treatment on symptom expression, virus titers, plant growth, and aphid preferences. ASM attenuates CMV symptoms (A), reduces virus titer (B), and reduces negative effects of CMV on plant size (C). N = 15 plants per treatment. * and different letters indicate significant differences at P<0.05.





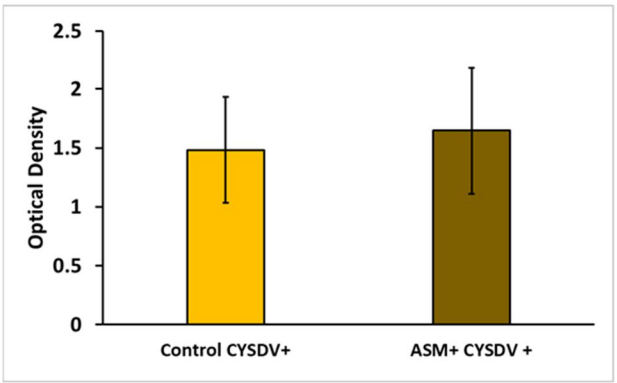


Figure 3. Effects of ASM treatment on CYSDV symptom severity during two separate trials (graph A = trial 1, graph B = trial 2). Virus titer for CYSDV+ plants in trial 1 is shown in graph C. ASM treatment had neutral to negative effects on CYSDV symptoms, with no effects on virus titer. N = 3-7 plants per treatment. Samples from trial 2 are being processed for virus titer measurements and dry mass data from both experiments will be quantified in the near future.

Alongside more controlled greenhouse experiments, we also performed a factorial experiment as a small field trial to determine impacts of ASM applications on melon growth and yield in the field. Two foliar applications of the 25ppm ASM concentration (one pre-planting and one post-planting) had no negative effects on plant growth or total

fruit production (harvestable and immature fruit) (Fig. 4). 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. However, there were no changes in the community of insect predators visiting plants as a result of ASM application. We will use controlled inoculations of a local isolate of CMV in future field experiments to determine efficacy of ASM under standard growing conditions.

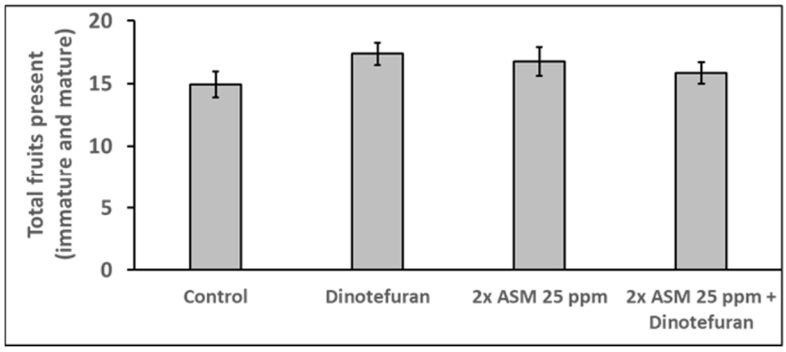


Figure 4. Fruit production by melon plants treated with insecticide alone (Dinotefuran – preplant soil application at recommended rates), 2 applications of ASM at 25 ppm, or ASM plus Dinotefuran. Bars show mean +/- SE, with N of 23-27 plants per treatment. There are no statistically significant differences among treatments.

Objective 2: Determine the effects of ASM (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 feeding by aphids, we treated plants with Actigard in 25ppm concentration and performed electrical penetration graphing (EPG) analysis to measure probing and feeding behavior by a main aphid vector of CMV (*Aphis gossypii*). The EPG technique permits fine-scale detection of whitefly vector mouthpart positions within specific plant cells, and enables measurement of phloem penetration, salivation duration, and ingestion duration - all of which are behaviors that are responsible for the inoculation and acquisition of CYSDV by whiteflies. EPG is routinely used to identify mechanisms of host plant resistance and understand virus transmission mechanisms. Our results indicate that ASM treatment reduces uninfected plant suitability for *A. gossypii* by making it more difficult for the aphid to ingest plant sap from the phloem (Fig. 5). This is expected to reduce aphid performance in the field, leading to lower aphid populations, but the consequences for virus spread are not yet known. This will be explored in future experiments. Electrical penetration graphing data were recently collected for whiteflies feeding on untreated healthy melons, untreated melons infected with CYSDV, ASM treated healthy melons, and ASM treated melons inoculated with CYSDV. Data analysis of these recordings is in progress and additional recordings are being collected. Analysis will be completed by February 2019.

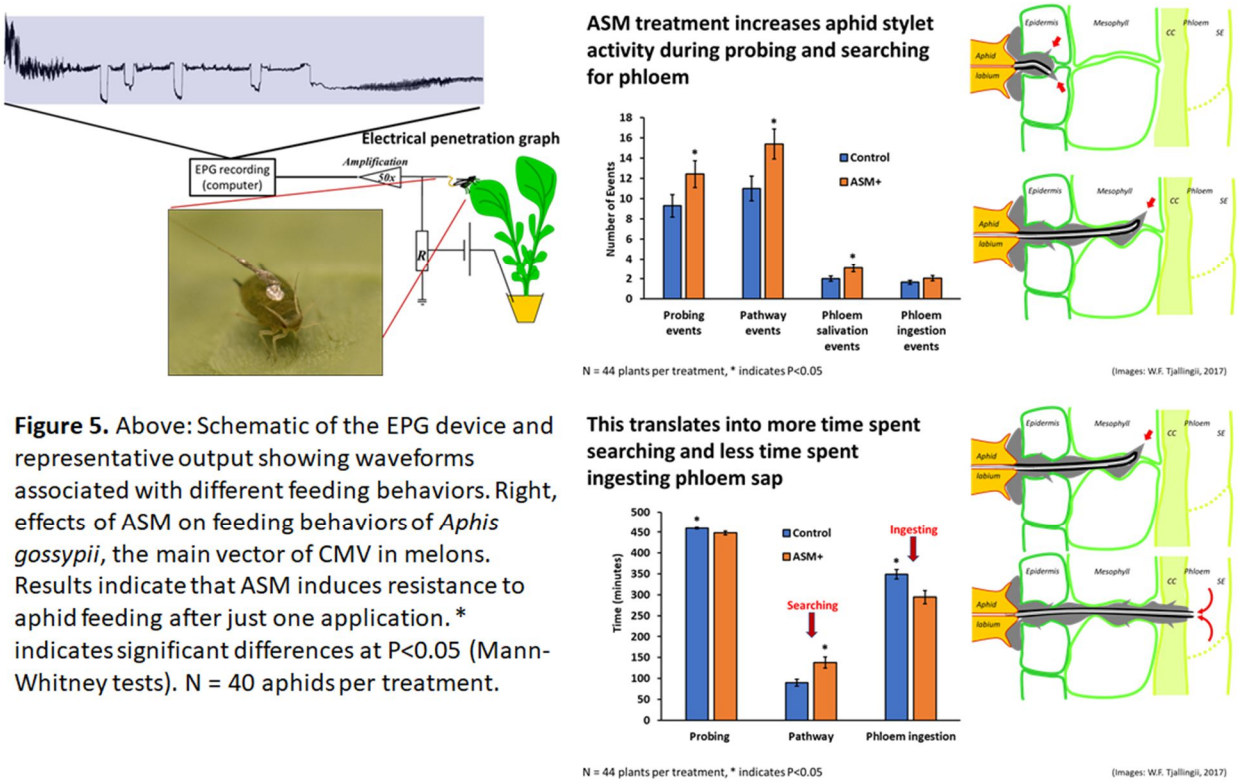


Figure 5. Above: Schematic of the EPG device and representative output showing waveforms associated with different feeding behaviors. Right, effects of ASM on feeding behaviors of *Aphis gossypii*, the main vector of CMV in melons. Results indicate that ASM induces resistance to aphid feeding after just one application. * indicates significant differences at P<0.05 (Mann-Whitney tests). N = 40 aphids per treatment.

Complicating control in both aphid and whitefly virus pathosystems are the effects of the viruses themselves on host appearance and suitability for vector feeding. We previously found that *Cucumber mosaic virus* (CMV) increases the attractiveness of infected cucurbit hosts to aphid vectors by altering appearance and emissions of plant odor compounds (K. E. Mauck, De Moraes, & Mescher, 2014; Kerry E. Mauck, De Moraes, & Mescher, 2010, 2014). This same virus also reduces palatability, stimulating aphids to disperse to new hosts after they acquire virions (K. E. Mauck et al., 2014; Kerry E. Mauck et al., 2010, 2014). Through the activities under this objective, we recently found that the effects of CYSDV on melons are even more dramatic - whiteflies are strongly attracted to CYSDV-infected plants, more rapidly reach the phloem when feeding on these plants, and ingest more phloem sap - the medium which contains CYSDV virions (Fig. 6). Numerous mathematical models demonstrate that vector preferences of this nature will lead to more rapid and extensive virus spread in monoculture environments (Roosien et al., 2013; Shaw, Peace, Power, & Bosque-Pérez, 2017; Sisterson, 2008). Disruption of these effects for major viral diseases of melons, such as CMV and CYSDV, is therefore expected to mitigate virus spread and delay epidemics.

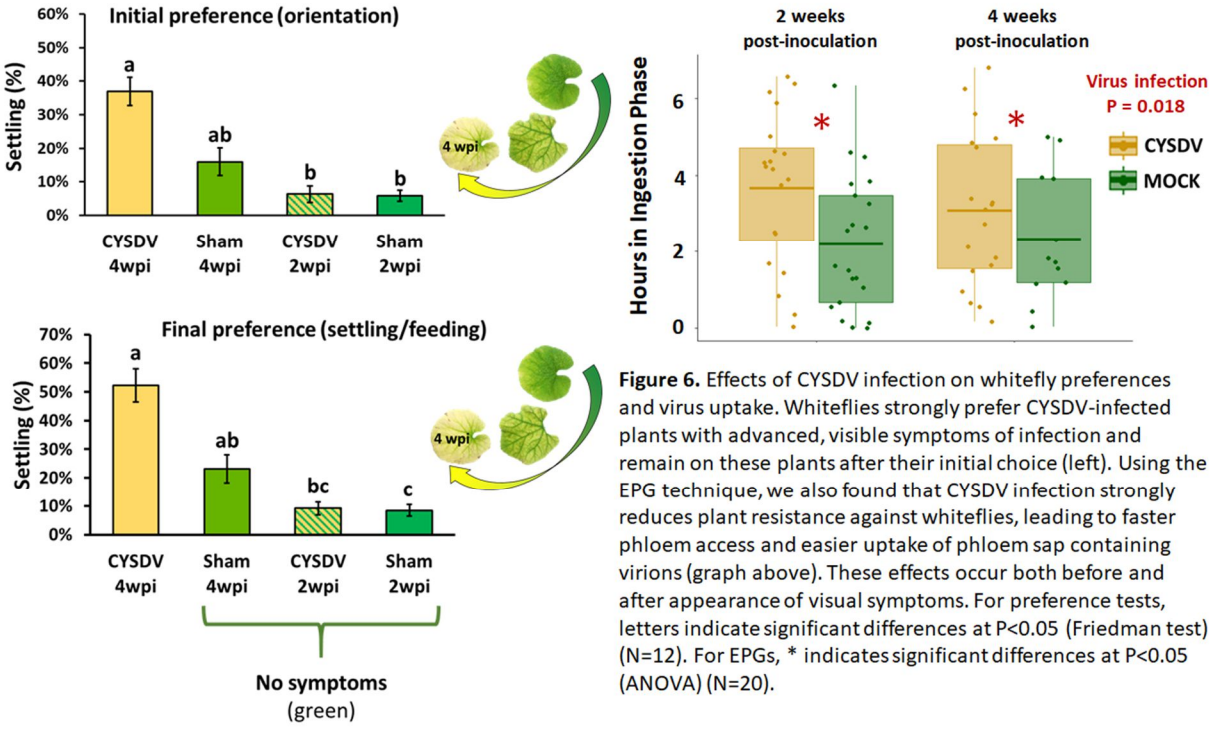


Figure 6. Effects of CYSDV infection on whitefly preferences and virus uptake. Whiteflies strongly prefer CYSDV-infected plants with advanced, visible symptoms of infection and remain on these plants after their initial choice (left). Using the EPG technique, we also found that CYSDV infection strongly reduces plant resistance against whiteflies, leading to faster phloem access and easier uptake of phloem sap containing virions (graph above). These effects occur both before and after appearance of visual symptoms. For preference tests, letters indicate significant differences at $P<0.05$ (Friedman test) (N=12). For EPGs, * indicates significant differences at $P<0.05$ (ANOVA) (N=20).

Our experiments with CYSDV also provide evidence that the more severe the infection, the more pronounced the effects on vectors, leading to whole cohorts of insects engaging in ideal behaviors for virus transmission among melon hosts. These same virus effects on host physiology are also responsible for reductions in melon quality and yield. Thus, any tactics we might employ to disrupt the severity of infection and symptoms responsible for enhancing virus spread by vectors should have the added benefit of mitigating negative effects of viruses on yield and fruit quality. For example, we found that the intense yellowing produced by CYSDV is highly attractive to whitefly vectors (Fig. 6). This phenotype is also associated with reductions in sugar content in melon fruits produced by plants with advanced stages of disease progression following early inoculation with CYSDV. Similar reductions in fruit quality and appearance due to CMV infection are also common and become pronounced with more severe and advanced infections, most of which are the product of transmission occurring during the early stages of plant growth.

To determine if reductions in virus titer and symptom severity due to ASM application offsets its effects on vector behaviors conducive to virus transmission, we explored the behavior of aphids and whiteflies in response to infected and healthy plants, with and without ASM treatment. Results from aphid preference tests suggest that ASM can disrupt vector preferences for infected plants (Fig. 7). Aphids are attracted to untreated plants infected with CMV over healthy plants when given a choice, but when ASM treatment is applied before CMV inoculation, symptoms are reduced and the attractive phenotype is eliminated (Fig. 7). The consequences for this in more complex environments will be the target of future studies. 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. In contrast to effects on CMV and aphid behavior, we found that ASM treatment does not disrupt attraction to CYSDV-infected plants (Fig. 7). Based on these results, we are pursuing other options for disrupting CYSDV symptoms that act via different plant defense pathways.

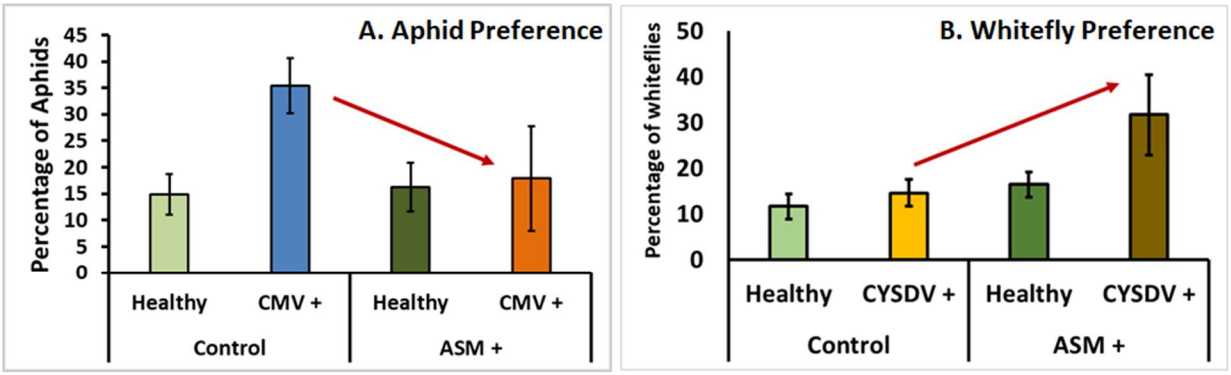


Figure 7. Effects of ASM treatment on aphid (graph A) and whitefly (graph B) preferences. In four-way choice tests, aphid preferred CMV-infected plants *without* ASM treatment over all other treatments. But whiteflies preferred CYSDV-infected plants with ASM treatments relative to all other treatments. Experiments are in progress to repeat these preference tests and confirm results.

G. Conclusions & Future Work

We found evidence that CMV effects on host attractiveness and palatability to vectors can be disrupted by application of a commercially available plant immune elicitor - acibenzolar-S-methyl (ASM). These effects are linked to other positive benefits such as attenuation of symptoms and elimination of negative effects on host growth. We further documented that the label rate for the ASM product (Actigard, Syngenta) is not suitable for effective disease control in melons (instead having phytotoxic effects) and we determined the correct application rate based on plant growth experiments. The ASM product also deters aphid vector feeding directly, which will help reduce vector populations by impeding ingestion of plant resources. And at certain concentrations, ASM has plant growth-promoting capabilities. Based on these activities, we expect that ASM applied through drip irrigation or foliar sprays at the newly tested rate established in our experiments could be a viable treatment option for protecting melon plants against aphid-transmitted CMV. In follow-up activities, we will determine the best dosage rates to protect plants during the most vulnerable periods and perform further field testing of protective effects with controlled CMV inoculations.

Surprisingly, we do not have strong evidence that ASM provides protection against CYSDV or whiteflies. Data collection and analysis are still ongoing for the second iteration of an experiment testing effects of ASM on inoculation success, symptom progression, virus titer, plant size, and vector behavior. But preliminary results show no reductions in symptoms or titer, with some evidence of detrimental effects on transmission-conducive behaviors of whiteflies. This suggests that infection by CYSDV occurs via different pathways relative to CMV. The ASM product acts on the plant

immune system by mimicking the activity of a conserved signalling hormone - salicylic acid - production of which stimulates the plant to resist infection by various plant pathogens. Priming this pathway prior to inoculation reduces the probability of pathogen establishment. However, if primed salicylic acid mediated defenses are not active against a given pathogen, then using ASM will not help prevent or attenuate infection.

Ethylene is another plant hormone has been implicated as an important part of symptom induction and expression of transmission-enhancing traits during the virus-host interaction (Casteel et al., 2015). In experiments with *Turnip mosaic virus*, the PI's collaborator (Dr. Clare Casteel) demonstrated that a short disruption of ethylene production was sufficient to attenuate the effects of this virus on host physiology and vector transmission (Casteel et al., 2015). As a next step, we will test the effects of a commercially available ethylene disruptor (Retain) on plant resistance to CYSDV infection and symptom progression following inoculation. This product is also being explored as a possible tool for bloom concentration in melons using CMRB funding, so its effects on plant susceptibility or resistance to viral pathogens are important to establish. Additionally, new products are in development for plant priming purposes within the PI's laboratory, including antimicrobial proteins that show priming activity in various model hosts (i.e. Solanaceae, Rutaceae). These proteins, which are in development for treatment of citrus greening, strongly prime the plant immune system in addition to directly inhibiting the propagation of bacterial pathogens. They are also safe, stable in the environment, and easy to produce. EPA registration of these peptides is expected within the next 2-3 years, and we will test their efficacy against CYSDV to determine if they could be labeled for disease control in melons. Alongside these activities, we will use chemical analytical techniques to better characterize the effects of CYSDV infection on plant immunity in order to develop new strategies for boosting immune response and breeding cultivars with robust defenses.

H. References

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