California Melon Research Board
2017 Annual Report

I. Project title:

Evaluation of RNAi strategies for reducing whitefly populations on melon.

II. Principal investigator:

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IV. Cooperating personnel

None

V. Location(s) where work was performed

USDA-ARS, U.S. Agricultural Research Station, Salinas, California,

VI. Objectives

1. Evaluate existing and new RNA constructs for their ability to induce whitefly mortality on treated melon.
2. Determine duration of RNAi-based control of whitefly in treated melon plants.

VII. Results and Analysis

Confirmation of "Best Constructs" for Advanced Evaluations on Melon and Other Crops
Prior to the beginning of this project we evaluated 35 double stranded RNA (dsRNA) constructs from our laboratory using an *in vitro* system in which sweet potato whiteflies (*Bemisia tabaci* MEAM1; aka. Biotype B) feed on a sugar solution containing each dsRNA construct individually. Our colleagues in Charleston, SC did the same with their 35 constructs for a total of 70 evaluated using the *in vitro* system. From those experiments, ten constructs were selected for further evaluation based on the results of those preliminary tests. Six dsRNA constructs from Salinas were selected, along with four from Charleston to evaluate in a ring-test in which the Salinas and Charleston Labs, along with a collaborator in Dar es Salaam, Tanzania would conduct identical experiments to further validate construct effectiveness for killing whiteflies.

These experiments were conducted from late spring through mid-summer, 2017, with both the Salinas and Charleston Labs evaluating mortality of *B. tabaci* MEAM1 (biotype B common in California melon production areas) and Dar es Salaam evaluating for mortality of *B. tabaci* SSA1-SG3 (a whitefly that affects cassava and other plants in East Africa). All ten constructs were evaluated by all three labs; however, results of Charleston experiments showed reduced mortality across all experiments and did not match their previous results or results of the other two labs, and were therefore not included in further analyses. Results of comparative studies by the Wintermantel Lab (Salinas) and the African Lab (Dar es Salaam) were highly consistent, with both labs confirming the best performance by the same three constructs (WK1, WK2 and WH9) with the two different *B. tabaci* variants (MEAM1 and SSA1-SG3), although results varied in which was best depending on the whitefly type being targeted. Genes targeted by constructs are intentionally not mentioned here, and are replaced by coded acronyms in this report. Results demonstrated that two constructs, both developed and initially tested by the Wintermantel Lab (the second in conjunction with a collaborator) performed the best against both types of whiteflies; whereas a third was also effective to a lesser degree against both whiteflies (**Figure 1, Figure 2**). Therefore, we had great confidence that these two constructs should be developed further for whitefly control through experiments on both melon (this project), and for cassava and tomato.

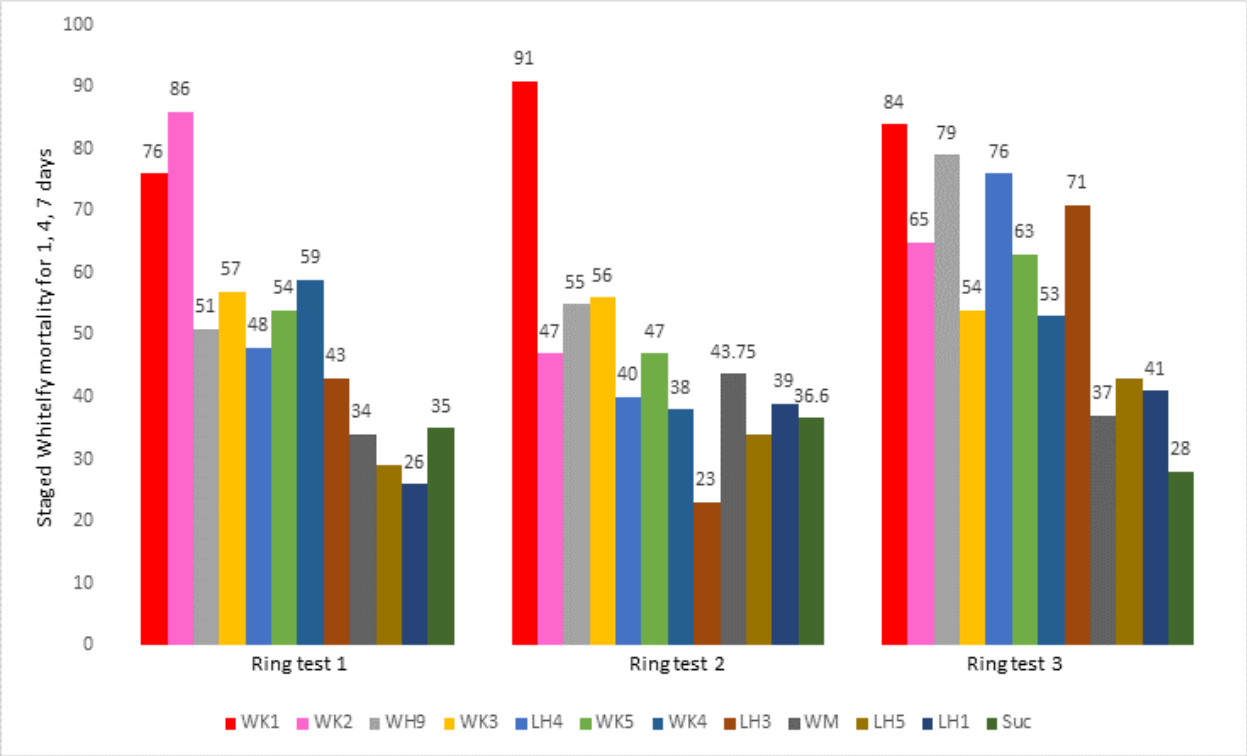

Figure 1: Sweet potato whitefly (*B. tabaci*, MEAM1) mortality induced by ten novel dsRNA constructs with feeding on 20% sucrose containing each dsRNA construct.

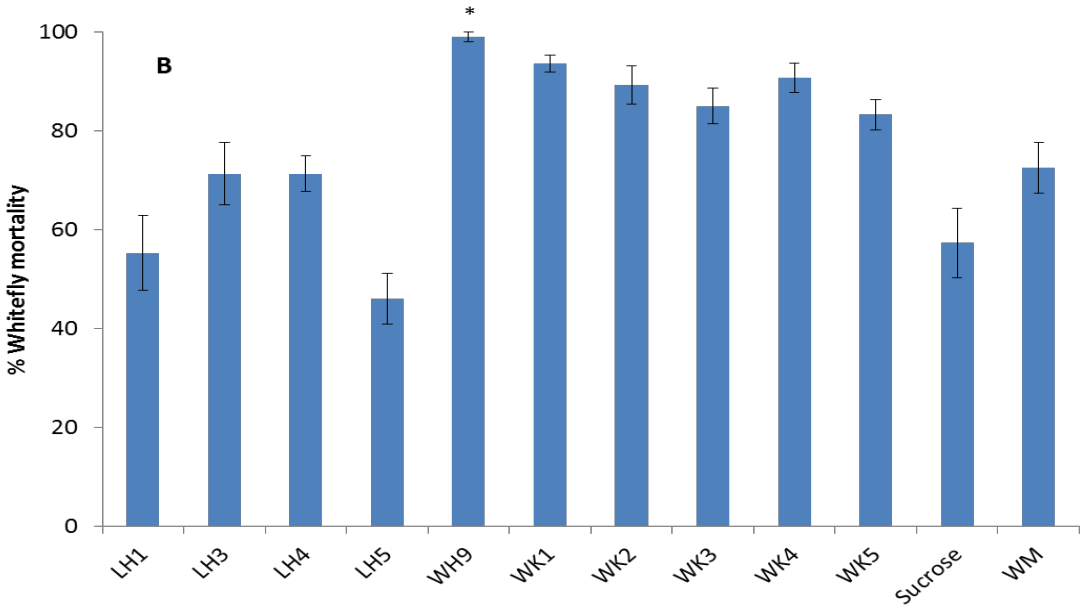

Figure 2: Average percentage of cassava *B. tabaci* (SSA1-SG3) mortality at 7 days after experiment initiation for ten dsRNAs and non-target controls (WM and sucrose only). DsRNAs with an asterisk (*) were significantly different from controls (WM and sucrose) (Tukey-Kramer test, P = 0.05).

Leaf Uptake Experiments

The three most effective constructs were all from the Wintermantel Lab, and were evaluated on melon using two methods: leaf uptake of dsRNA followed by exposure to whiteflies, and spray treatment of leaves with dsRNA followed by exposure to whiteflies. The first approach involved leaf uptake of dsRNA in an effort to determine appropriate concentrations for experiments. In this experiment, leaves of young, 2 true leaf stage melon plants were detached using a razor blade and petioles were placed in microcentrifuge tubes containing three different concentrations of dsRNA suspended in water. Leaves were allowed to absorb solution, after which petioles were placed in a sealed tube of water, allowed to set overnight, then 20 whiteflies were added to each leaf. This method was used for evaluation of each of the constructs. Numbers of dead whiteflies were recorded each day for 15 days. The initial experiment with this method was recently completed, and although some whitefly mortality did occur, the method was not as effective for inducing whitefly mortality whereas similar experiments in tomato had been successful (results not shown). We suspect melon may take longer periods of time to distribute the dsRNA throughout the leaf than tomato, or perhaps translocation of the dsRNA or its induced signal that causes gene silencing is not as efficient in melon as it is in tomato.

We do not plan to repeat this experiment as it was intended as a further method of vetting effective constructs, yet it was not useful for that purpose. Instead we focused on a spray method in which constructs are sprayed onto leaves, allowed to be absorbed into tissue, after which plants are exposed to whiteflies to determine whitefly mortality rates. This is an approach adapted for nursery or perhaps even field application to seedling melon plants. If the spray technique yields promising results on its own there will be no need to revisit the leaf uptake experiment.

Foliar Spray Experiments

Our second approach which has potential for functional application in melon production, involves direct treatment of leaves with dsRNA for induction of RNAi. In these experiments, each dsRNA construct to be tested is diluted in treatment buffer to a specific concentration and placed in a 10 ml spray bottle. Four leaf-stage melon seedlings are treated with the suspension, but prior to treatment all but the topmost leaf is removed, to limit surface area on which whiteflies can feed (easier to evaluate). The solution is then sprayed on the remaining leaf, and allowed to dry overnight. The next morning, each seedling is covered with a 4" diameter plexiglass vial containing a mesh on top with a cotton-plugged port through which whiteflies can be delivered to the vial (**Figure 3**), and 30 whiteflies are added to each vial. Whiteflies are allowed to feed on plants for 14 days (**Figure 4**), after which live and dead whiteflies are counted to determine mortality rate for each construct. Experiments were performed with five of the most effective constructs, and each construct was replicated five times per experiment. One construct was omitted due to limited amounts of available dsRNA. Results of the first replicate of this experiment are shown in **Table 1**. We had anticipated at least two replications by now, but had to wait until the conclusion of the multi-lab ring tests during the summer to finalize which constructs would be analyzed on melon in order to optimize the potential for success.

Fig. 3. Vials containing treated melon plants

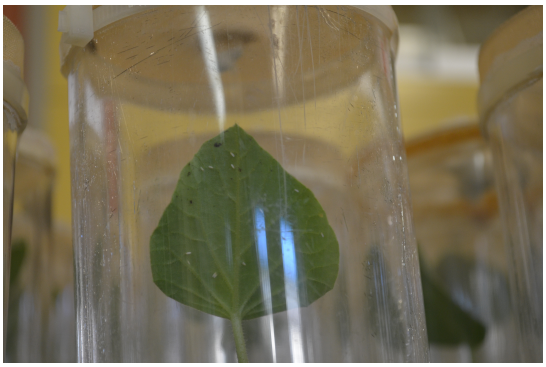

Fig. 4. Whiteflies feeding on melon leaf

Table 1. Melon leaf-spray assays

dsRNA construct	Rep1	Rep2	Rep3	Mortality (%)
WK1	23	28	20	78.9
WK3	24	24	23	78.9
WK2	24	23	23	77.8
WK4	28	29	23	88.9
WK5	22	30	20	80.0
WM	19	14	8	45.6
TNT2 only	24	16	26	73.3

Method: 1 leaf attached from 2-3 weeks old Top Mark melon was used for spray.
10ug of dsRNA per plant in 1 ml of 0.05% TNT2 spray solution
Dead whiteflies were counted after 14 days

Results of the initial spray experiment (Table 1), which involved three replications of the same treatments were unclear. The TNT2 control, in which leaves were sprayed only with the solution used to suspend dsRNA, resulted in about the same rate of mortality as test constructs, although a lower rate of mortality was observed with the construct targeting a gene unique to watermelon (negative control gene used because this gene is not present in the whitefly genome). Aside from the high mortality in the control solution treatment, results appear promising. However, we do not expect all of the constructs to work this well, based on previous studies, therefore results are suspect. An additional replication of this experiment is in progress and results will be presented in January. Hopefully this will clarify performance. Until we can see clear differences between control and test constructs, we cannot determine effectiveness of the strategy or the construct for use with this approach.

Duration of whitefly control (Objective 2)

Until methods are fully established and demonstrated to be reliable for induction of RNAi in melon, this objective is premature. We expect to complete experimentation by late spring 2018

and will then move into evaluations of duration of control using the most effective treatment method.

Further Studies

Recent studies have developed clones of select dsRNA constructs that can be used for transformation of cassava. We may also consider evaluation of these constructs using previously established methods to induce RNAi as a transient treatment (without transforming melon). This was not in the proposal for 2018, and will not add any further cost but will allow evaluation using a different delivery method.

Conclusions

RNA interference holds promise as one of the techniques with potential to control sap-sucking pests, such as the whitefly *Bemisia tabaci*, a major pest of agricultural and horticultural crops in the United States including melon. Methods to enhance whitefly control will improve performance of melon crops through reduced feeding pressure, through reducing the rate of spread of whitefly-transmitted viruses such as cucurbit yellow stunting disorder virus (CYSDV), as well as improving performance of resistant germplasm for control of virus and whitefly by reducing populations.