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

Progress report – after year 1, Dec 2018

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Project alignment

This project addresses three of the highlighted research priorities by the California Melon Research Board: 1) Control strategies for western striped and spotted cucumber beetles in the melon production regions of the San Joaquin and Sacramento Valleys. 2) Insect pest management studies with new insecticides.

Background

In the Sacramento Valley, both the western striped cucumber beetle (*Acalymma trivittatum*), and the western spotted cucumber beetle (*Diabrotica undecimpunctata undecimpunctata*) have been serious pests of fresh-market melons since the 1980s. The severity of pest pressure changes every year, but both pest species occur annually. It is difficult to control cucumber beetle adults with insecticide sprays, because they are protected underneath the fruit. However, applications of pesticides continue to be the most successful (almost exclusive) approach to management of insect pests in melon crops. Although melon growers follow application instructions regarding operational spray settings, concentrations of active ingredients in spray formulations, and recommended spray volumes, there is growing evidence and concern about the high inconsistency of spray applications under field/commercial conditions. The topic of insecticide spray coverage and its implications short-term (application efficacy) and long-term (risk of target pests developing resistance) is an important component of the research portfolio in the Nansen lab (http://chrnansen.wixsite.com/nansen2/pesticide-performance). The reality is therefore that growers often apply pesticides with very low spray coverages, and that should be considered a major concern to both the melon industry but also to pesticide manufacturers, as there is growing evidence of how low and inconsistent spray coverage creates a selection pressure leading to behavioral resistance/avoidance (Martini et al., 2012; Nansen et al., 2016). It is therefore paramount to develop screening methods and procedures to quantify not only levels of resistance but also to quantify or characterize spray coverages during commercial field applications.

Project goal and objectives

The long-term goal by Nansen and Vinchesi is to develop a highly synergistic collaboration in which both applied and more fundamental pest biology questions are being addressed in the context of providing sustainable solutions to melon growers in California and elsewhere. Moreover long-term objectives intend to address the following: 1) how to optimize the performance of pesticide applications, 2) how to minimize the risk of target pest populations developing resistance to available/registered active ingredients, 3) how to optimize detection and sampling of insect pests, and 4) increase our current understanding of pest movements (i.e. from neighboring vegetation into melon fields) in agricultural landscapes. Below, we have listed the original six project objectives:

Objective 1 - Establishment of colonies of western striped cucumber beetles to be used in bioassays.

Objective 2 - Through collaboration with the California Melon Research Board, obtain a list of the top 5-6 insecticides (below referred to as "selected insecticides") being applied by commercial melon growers for control cucumber beetles.

Objective 3 - Use Ethovision XT to characterize and quantify the level of behavioral resistance/avoidance to the selected insecticides.

Objective 4 - In commercial melon fields (honeydew and cantaloupe) in Sutter and Yuba counties, obtain quantitative insecticide spray coverage data.

Objective 5 - Bioassay mortality of western striped cucumber beetles on melons.

Objective 6 - Obtain trapping data in and around melon fields in Sutter and Yuba counties.

Modifications of original 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, and establishment of lab colonies was attempted many times without success. We contacted colleagues and it was confirmed that establishment and maintenance of these colonies is non-trivial (very difficult to get the beetles to lay eggs). Consequently, bioassays (Objective 3) were performed with field-collected adults. Thus for 2019, we have decided to discontinue this objective

Objective 2 - Through collaboration with the California Melon Research Board, obtain a list of the top 5-6 insecticides. Insecticide information was obtained from Matt Lagorio, Pest Control Advisor, Growers Ag (The Tremont Group), and we have decided to examine the following insecticides: bifenthrin, acetamiprid, carbaryl, lamba-cyhalothrin and zeta-cypermethrin. Preliminary data from bioassays with bifenthrin and acetamiprid have already been collected.

Objective 3 - Use Ethovision XT to characterize and quantify the level of behavioral resistance/avoidance to the selected insecticides. As rather in-depth data collection has been performed, we decided to include appendix A in this proposal, in which the results are presented and interpreted.

When writing the original 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 – see below for details.

<u>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.</u> 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.

As this methodology had been developed, we were informed that melon growers had completed this year’s insecticide sprays, so we were unable to obtain field data in the 2018 growing season. However, we have conducted lab bioassays with sweet potatoes dipped in insecticide formulations to simulate 100% coverage, and these data are presented and interpreted in appendix A to this proposal.

<u>(Originally Objective 6) New Objective 5 - Obtain trapping data in and around melon fields in Sutter and Yuba counties</u>. Despite intensive efforts, field trapping of cucumber beetles was unsuccessful. A detailed report of the trapping efforts is included as appendix B in this proposal.

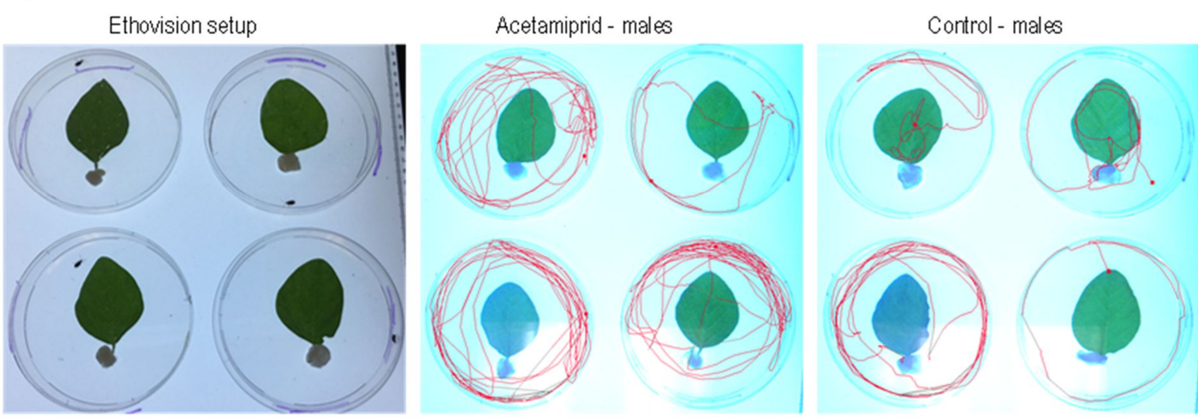


Fig. 1. Movement of cucumber beetles in response to insecticide treatments

Cucumber beetle bioassays

<u>Behavioral bioassay</u>. Similar to studies published by Nansen (Martini et al., 2012; Nansen et al., 2016), Ethovision XT (a behavioral software program) is being used to quantify movement by individual beetles inside an “arena” containing a soybean leaf, which is either untreated (control) or treated with one of the selected insecticides (Fig. 1).

The red lines inside each petri dish represent the total movement tracks by individual cucumber beetles within a 10 min time period. It is seen that male cucumber beetles were generally more “agitated” (moved more) when exposed to acetamiprid-treated leaves than when exposed to untreated control leaves. It is also seen that the adult cucumber beetles were more likely to avoid the leaf altogether when treated with acetamiprid.

In these Ethovision bioassays, we decided to use soybean leaves, as the large size of midvein and high density of leaf trichomes of melons make them difficult to use in bioassays with Ethovision XT. For each combination of cucumber beetle gender and insecticide and we have tested the highest labeled rates (based on: Bifenthrin (Brigade) = 0.1 lb / 100 gal, Acetamiprid (Assail) = 2.3 oz / 100 gal). Individual soybean leaves are dipped in the insecticide formulation to obtain 100% coverage, and the leaves are allowed to dry for one hour prior to being used in bioassays. The movement of individual beetles inside the arena is monitored for 10 min, and Ethovision XT is used to quantify the following variables:

1) Total distance traveled within the arena (distance): It was assumed that treatment of leaf with an insecticide could affect the beetles’ willingness to move, so that the distance moved would either be significantly higher or lower than that of beetles exposed to control leaves.

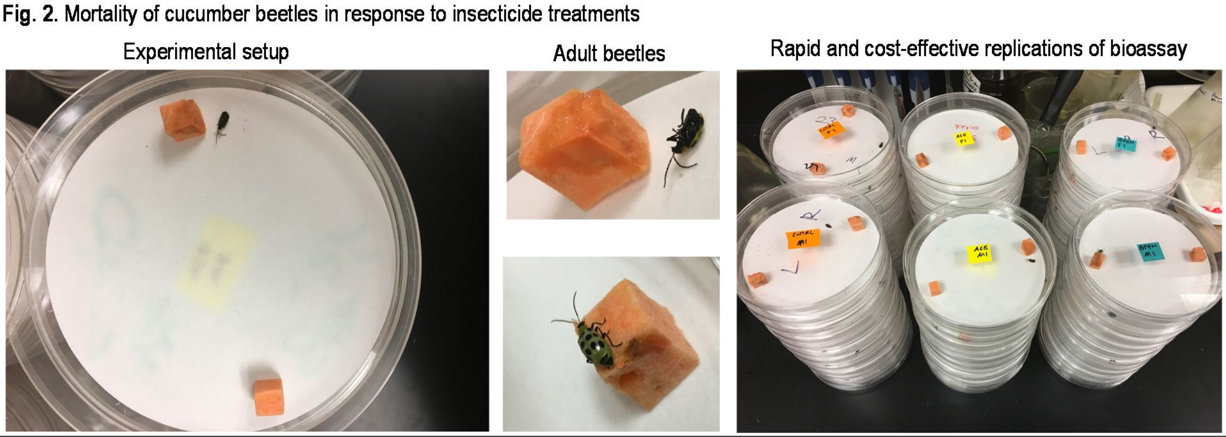
2) Average speed (speed): This is a different indicator of beetles’ willingness to move in response to insecticide treatment of leaves.

3) How frequently the beetle moved compared to being still (mobility): This is also an indicator of beetles’ willingness to move in response to insecticide treatment of leaves.

4) How often the beetle turned more than 20^{O} to either left or right. It was assumed that treatment of leaf with an insecticide could significantly affect the beetles’ ability to control their movement (as several of the insecticides affect their central nervous system).

Each combination of beetle gender and insecticide is replicated 16 times with replications performed on multiple days.

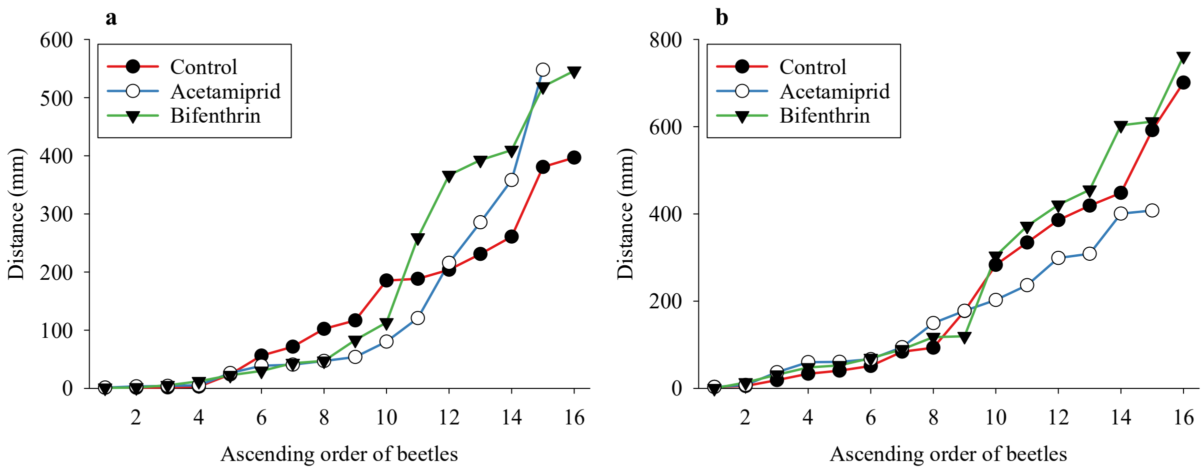
Fig. 2. Mortality of cucumber beetles in response to insecticide treatments



Mortality bioassay. Using the same insecticides and also rates as described for the behavioral response bioassays, standardized pieces of sweet potato, using a French fry cutter and cutting each fry into 2 cm pieces, which were dipped in water (control) or high rates of either bifenthrin or acetamiprid to obtain 100% coverage. Control treatment consisted of dipping sweet potato pieces in water. Two pieces of sweet potato (both treated the same way) are placed inside a petri dish with a single beetle (Fig. 2). We assessed the mortality of individual beetles after 24 h, 48 h, 72 h, and one week.

Results

Fig. 3. Movement of female and male cucumber beetles in response to insecticide treatments



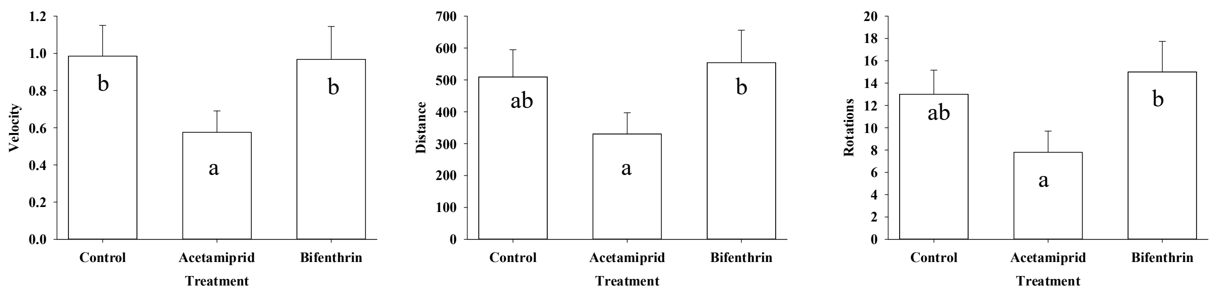
Behavioral bioassay. As the beetles are field collected, their age and mating status are unknown and can vary markedly (adult beetles can live for several months). The variance in distance across all treatments (control and insecticides combined) is illustrated in Fig. 3, and it is seen that both individual females (Fig. 3a) and males (Fig. 3b) varied greatly in their total movement. That is, based on the 45 individuals of each genders (15 for each of the three treatments), we found that: 1) females moved less than males, and 2) for both genders, a large proportion of individuals moved very little, while others were highly active. With such variance in behavioral response, it is near-impossible to demonstrate statistical difference among treatments, unless the observations are either transformed or grouped into intervals. We decided to group the 15 observations for each combination of treatment and beetle gender into three groups: low, medium, and high (five observations in each group).

Table 1. Analyses of behavioral responses to insecticides

Sex	Variable	Low	Medium	High
Female	Velocity	N.S.	N.S.	N.S.
Female	Distance	N.S.	N.S.	N.S.
Female	Rotations	N.S.	N.S.	N.S.
Female	Mobility	N.S.	N.S.	N.S.
Male	Velocity	N.S.	N.S.	*
Male	Distance	N.S.	N.S.	*
Male	Rotations	N.S.	N.S.	*
Male	Mobility	N.S.	N.S.	N.S.

The results are presented in Table 1, and it is seen that female beetles did not show significant behavioral responses, and only male beetles with high activity showed a significant response. The behavioral responses in terms of velocity, distance, and rotation of adult male beetles with high activity are shown in Fig. 4, and it is seen that the only acetamiprid caused a significant reduction in total distance traveled. Overall, the many non-significant (N.S.) results from this detailed analysis of behavioral response by both male and female beetles suggest that the beetle populations are highly resistant to the examined insecticides.

Fig. 4. Behavioral response by male beetles with high activity



Mortality bioassay. Table 2 shows preliminary data regarding mortality of individual female and male beetles after 72 hours of feeding on treated pieces of sweet potato, and it is seen that: 1) none of the control beetles died, 2) that females were more resistant than males, and 3) mortality was higher in bioassays involving bifenthrin compared to those involving acetamiprid.

Table 2. Mortality after 72 hours

Treatment	**Female**	**Male**
Control	0.00	0.00
Acetamiprid	0.17	0.45
Bifenthrin	0.83	0.67

Trapping of cucumber beetles

Floral-based lures (AgBio Inc.) have shown high western striped cucumber beetle capture rates when used in studies in the Midwest. These lures will be attached to traps to be placed along gradient inwards into commercial melon fields in Sutter and Yuba counties. In addition, emergence traps will be placed along the same gradient to capture newly emerged western striped cucumber beetle adults. We will collect seasonal trapping data from a minimum of two commercial melon fields, in which data on insecticide spraying events will also be recorded (as insecticide spraying may likely affect both overall abundance but also spatial distribution of western striped cucumber beetles. As recently reported regarding aggregations of insect pests in commercial fields (Nguyen & Nansen, 2018), we predict that the spatial distribution of western striped cucumber beetles will follow a strong "edge-effect" with highest densities near field edges. Information about the spatial distribution of insect pests, and how the spatial distribution patterns change during the growing season is of critical importance for the development of effective and sustainable pest management strategies (Nguyen & Nansen, 2018).

Two field areas were used: River Garden Farms is located in North Yolo County—5 honeydew fields were planted here. Three cantaloupe fields were located east of Meridian, CA in Sutter County.

Three traps with floral-based lures were placed along the edges of eight fresh-market melon fields, for a total of 24 traps. They were not placed within the field because they would interfere with field operations and equipment. These traps were made from gallon jugs painted yellow with small holes punched in two sides. The floral-based lure (AgBio, Inc.) was hung underneath the lid of the jug and an insecticide-treated net (AgBio, Inc.) was placed at the bottom (UM-IPM 2016; Piñero 2018).

Emergence traps were not placed within the fields because during bloom they exclude pollinators necessary to set fruit. Three emergence traps were placed in one newly planted field prior to flowering, but were removed when bloom began. The traps were placed over young plants with cucumber beetle feeding damage, but no cucumber beetles emerged under the traps over the 3-week period. Also, due to the heavy smoke in the area, the field remained saturated with dew until 12pm for weeks and two of the traps collapsed because of the moisture.

Various pitfalls and challenges were encountered during this field season. Floral-based lure traps only captured 3 spotted cucumber beetles among 24 traps in 5 weeks, 2 from traps in honeydew fields, and 1 from cantaloupe fields. No striped cucumber beetles were trapped. The trap design, based off of a study at Lincoln University in Missouri (UM-IPM 2016; Piñero 2018), did not achieve the same capture rate in large commercial melon fields as it did in small squash fields in the Midwest.

The lack of beetle capture is likely due to many factors.

1. There were not enough floral-based lure traps placed around each field to attract cucumber beetles, though the floral-lures successfully attracted honey bees. The Missouri study featured a much smaller area with a higher number of traps.

2. The diversity of agriculture and large monocultures surrounding the melon fields (vineseed, tomato, corn, alfalfa, weedy field edges), provided many resources for cucumber beetles.

3. Fresh-market melons may be more attractive to cucumber beetles than the floral-lure. A higher density of these traps is needed to accurately test whether this trap design works in large-scale commercial melon fields in the Sacramento Valley.

4. The fresh-market melon field sizes were ~75-100x larger than the fields used in the Midwest and may not be suitable for attract and kill traps. Using the same trap densities per acreage as in the Missouri study would mean 1,000-1,500 traps in one melon field. This would be impractical until the traps can be tested for effectiveness in California at a smaller scale.

From a preliminary survey in 2017, sticky traps without lures captured more cucumber beetles (both striped and spotted) than the jug traps with lures. Though sticky cards were still not effective at predicting or monitoring beetle levels in the field, it may be worthwhile to attach a lure to yellow sticky cards and test beetle capture.

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