CALIFORNIA LEAFY GREENS RESEARCH BOARD
Annual Research Report
April 01, 2019 – March 31, 2020

Project title:
Insecticide resistance monitoring and evaluation of efficacy of current chemical tactics for managing aphids and thrips in lettuce

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Abstract:
We proposed to investigate insecticide efficacy and insecticide resistance for aphids and thrips in lettuce in the Salinas Valley. We conducted ten insecticide efficacy field trials using insecticides registered to control aphids and thrips. The goal of this work was to create a publicly available database documenting the efficacy of different insecticides on aphids and thrips in lettuce. Growers and PCAs will be able to use these data to select the best management tactics and to have the necessary efficacy data to rotate modes of action. To evaluate current levels of insecticide resistance and gather baseline data on susceptibility to insecticides, we collected aphids and thrips from four locations to assess, in the laboratory with bioassays, the presence of any insecticide resistance to our insecticide treatments. Our combination of efficacy trials and insecticide resistance monitoring will help growers and PCAs strengthen their current IPM programs for lettuce.

Objectives:
1. Evaluate conventional and organic-certified insecticides for efficacy against aphids and thrips on lettuce.

2. Assay field-collected aphids and thrips for insecticide resistance to selected insecticides using laboratory bioassays.

3. Provide periodic and descriptive updates of results from Obj. 1 and Obj. 2 to grower and PCA clientele through web-based blogs and meetings.

Procedures:

<u>Objective 1</u>

For our aphid field experiments, trials were located in 1) USDA Spence Farm Research Station, Salinas (3 blocks, Romaine lettuce, 40-inch beds, 2 seedlines), 2) Castroville (2 blocks, Romaine lettuce, 80-inch beds, 5 seedlines), and 3) Gonzales (3 blocks, Iceberg lettuce, 40-inch beds, 2 seedlines). For our thrips experiments, trials were located in 1) USDA Spence Farm, Salinas (1 block, Romaine lettuce, 40-inch beds, 2 seedlines), and 2) Gonzales (1 block, Iceberg lettuce, 40-inch beds, 2 seedlines). Planting dates for all trials varied, ranging from May to August. When trials were located in commercial fields, standard grower practices were followed.

All field experiments were arranged in a randomized complete block design with four replications (r = 4). Each experimental plot was either two 40-inch beds or one 80-inch bed wide by 35 ft long. There were seven different insecticide treatments plus an untreated control and a 'grower standard'. 'Grower standard' encompassed experimental plots sprayed by our grower collaborators, using their own insecticide selections. Insecticides sprayed in these experiments (trade names, active ingredients, and rate/acre) are listed in Table 1. All insecticide applications were made in combination with an adjuvant, following label rates. We initiated spraying in field trials at least a week after thinning, or when populations of aphids and thrips exceed ten individuals/plant. All insecticide applications were made using a 3-gal CO_2 backpack sprayer, with two- (40-inch beds) or four-nozzles (80-inch beds) per bed, and between 50-60 gal water/acre. We collected insect samples one day before spraying the experimental treatments, and three, seven, and ten days after treatment application. To assess aphid and thrips populations, two randomly selected plants per plot were cut, individually bagged, and transported to the UCCE Entomology laboratory in Salinas for quantification and species identification of aphids and thrips. The number of adults and immatures of aphids and thrips per lettuce plant were recorded.

Table1. Experimental treatments, including insecticide trade names, active ingredients, rates and target pests.

Active ingredient	**Chemical class**	**Trade name**	**IRAC group**	**Rate (per acre)**	**Target pest**	
					Aphid	**Thrips**
Control - Untreated	N/A	Control	N/A	N/A	N/A	N/A
Imidacloprid	Neonicotinoid	Admire-Pro	4A	1.3 fl oz	X	
Flonicamid	Flonicamid	Beleaf	29	2.8 oz	X	
Beauveria bassiana	Entomopathogen	BoteGHA	UNF	1 qt		X
Garlic + hot pepper	Botanical	Captiva Prime	UNE	2 pints		X
Chlorantraniloprole	Diamides	Exirel	28	13.5 fl oz		X
Pymetrozine	Pyridine	Fulfill	9B	2.75 oz	X	
Spirotetramat	Tetronic acid	Movento	23	5 fl oz	X	
Oils	Plant-based biostimulant	Pure Crop 1	UNE	0.39 gal		X
Sulfoxaflor	Sulfoximine	Sequoia	4C	5.75 fl oz	X	
Lambda-cyhalothrin	Pyrethroid	Silencer, Warrior	3A	3.64 fl oz	X	X
Flupyradifurone	Butenolide	Sivanto	4D	14 fl oz	X	
Rosemary extracts	Botanical	SNS 209	UNE	2 qt		X
Spinetoram	Spinosyn	Radiant	5	10 fl oz		X

Objective 2

Throughout this project, we assayed for 'practical insecticide resistance' in different populations of aphids and thrips. Evaluating for practical insecticide resistance was based on exposing individuals from each field-collected population to our selected insecticides under controlled conditions, using the maximum allowable label rate per acre diluted at the average standard volume sprayed per acre (50 gal / acre).

We collected at least 500 aphids and 500 thrips individuals from five different locations, resulting in five different insect pest populations (four aphid populations and one thrips population). Specimens were transported to the UCCE Entomology greenhouse and caged on greenhouse-grown and insecticide-free Romaine lettuce plants. The day before setting up the laboratory bioassays, only the adult aphids and thrips to be used in the bioassays were moved from our greenhouse cages, and kept separated without food.

For aphids and thrips, we tested for susceptibility to our experimental insecticides using modifications of the Insecticide Resistance Action Committee (IRAC) Susceptibility Test Method 019 and Test Method 010, respectively. In brief, our experimental unit consisted of a closed feeding arena with ten adults (either aphids or thrips) enclosed in a 1-oz plastic cup containing an insecticide-treated lettuce leaf disk. The leaf disks were previously dipped into a solution containing each of our insecticide treatments. Each experimental insecticide had three repetitions (r = 3) across different dates. We used distilled water on leaf disks as a negative control. Treatments within each repetition were arranged as a randomized complete block design with four blocks per repetition. We recorded adult mortality rates at 24, 48, 72, 96, and 120 hours after assay set-up.

Objective 3

We analyzed the data from this project and created powerpoint presentations. The goal was to share results from this study, as invited speakers, during grower meetings, and other Continuing Education meetings.

Statistical analysis

Data from Obj. 1 was partitioned by days after treatment (3, 7, and 10 DAT), log-transformed to comply with the assumption of normality, and then subjected to ANOVA. Mean separations were done using Tukey's HSD at $\alpha = 0.05$. The performance of each experimental treatment was ranked based on aphid densities per plant. A low number on the ranking represented treatments with the highest mortality rates.

Data from Obj. 2 were analyzed by location. Repetitions were pooled together. Averages were calculated for each evaluation time point.

Results:

The lettuce aphid, *Nasonovia ribisnigri*, was the predominant species of aphid across samples from all our trials, accounting for 95% of individuals. Other aphid species identified from our samples (5% of the samples) were the potato aphid, *Macrosiphum euphorbiae* and the green peach aphid, *Myzus persicae*. All of our thrips samples were identified as the western flower thrips, *Frankiniella occidentalis*.

Objective 1 – Insecticide field efficacy for aphids

All seven tested insecticides significantly reduced aphid densities per plant when compared to the untreated control 3, 7, and 10 days after treatment (DAT; df = 8, 27; $P < 0.05$ for all dates). For instance, in one trial in Castroville, CA, aphid (adults + nymphs) densities were highest in the untreated control plots at 3 DAT and then increased to far above any of the insecticide treatments at 7 and 10 DAT (Fig. 1). From the same trial location, the experimental plots with the 'grower standard' treatment had the lowest aphid densities across the whole duration of the trial (Fig. 1). Lastly, insecticide performance, expressed as aphid density per plant, varied among tested insecticides and across trials. Our overall ranking for insecticide performance showed three main groupings for our insecticide treatments (Table 2). Admire Pro (imidacloprid), Beleaf (flonicamid), Movento (spirotetramat), and Sequoia (sulfoxaflor) had a better ranking (lower ranking number representing high mortality rates) in 60% or more of the trials. Sivanto Prime (flupyradifurone) had a high ranking in 50% of the locations. And Fulfill (pymetrozine) and Silencer (lambda-cyhalothrin) had a low performance ranking in 50% or more of the locations.

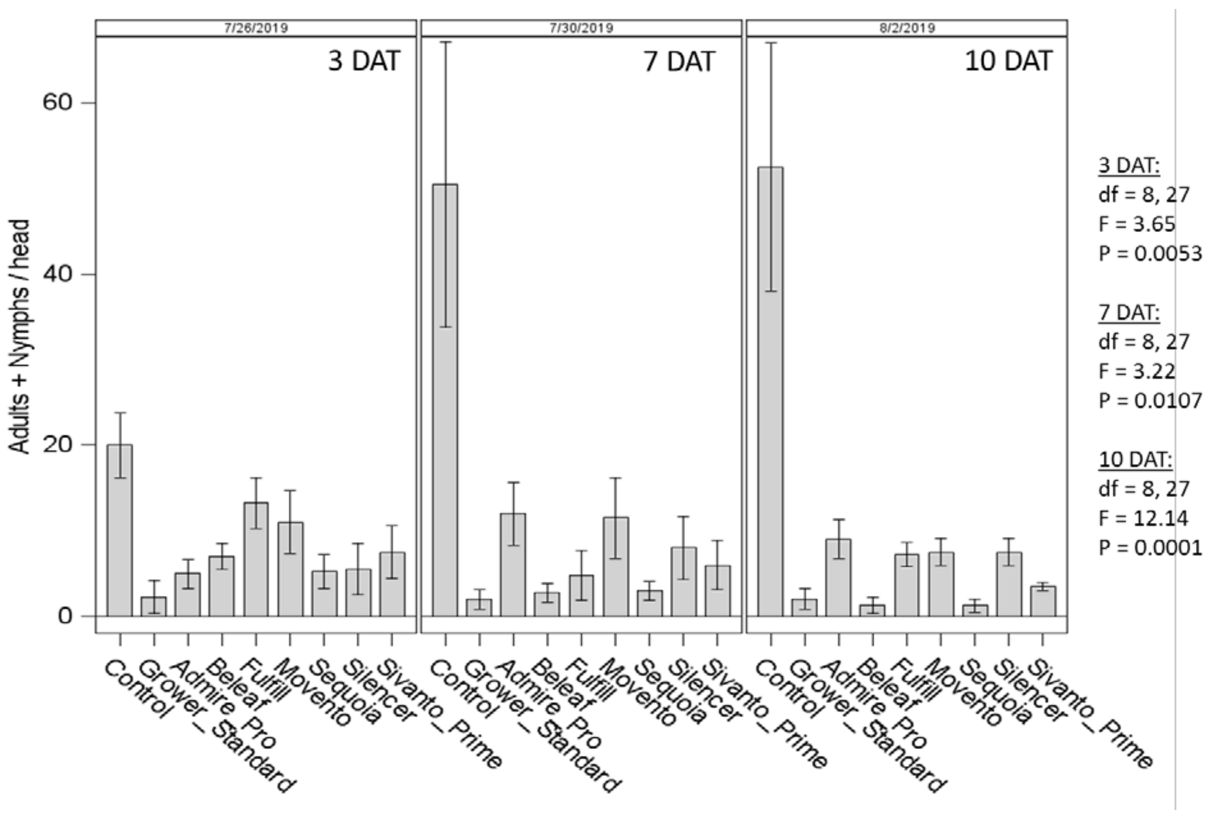


Fig. 1. Average (± standard error) aphid densities (adults + nymphs) per lettuce head from an insecticide efficacy field trial located in Castroville, CA. This trial was planted on June 25, 2019 with Romaine lettuce on 80-inch beds with 5 seedlines. All insecticide treatments (X-axis) were

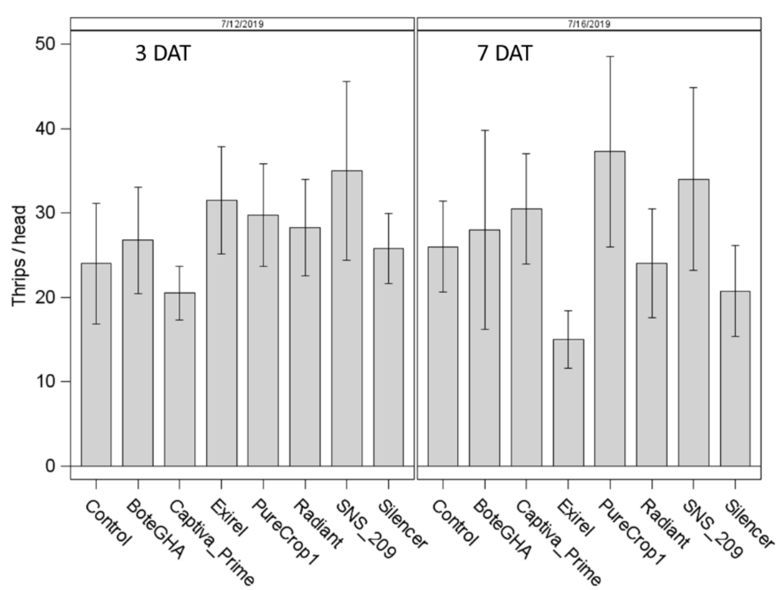
sprayed on July 23, 2019. Aphid densities significantly varied among experimental treatment at 3, 7, and 10 days after treatment (DAT).

Table 2. Overall ranking for insecticide performance controlling aphids in lettuce across eight field trial locations. A lower ranking number represents a higher insecticide performance relative to the untreated control. N/A = not applicable.

	Locations / Rankings								
Treatment	Spence_1	Spence_2	Spence_3	Castro_1	Castro_2	Gonz_1_A	Gonz_1_B	Gonz_2	Avg. Performance
Control	4	5	4	5	7	7	5	5	100% low
Grower_Standard	N/A	N/A	N/A	1	1	1	1	1	100% high
Admire_Pro	1	4	2	4	6	2	3	2	63% high; 37% low
Beleaf	N/A	N/A	N/A	2	5	5	3	2	60% high; 40% medium
Fulfill	3	3	3	4	3	6	4	4	50% medium; 50% low
Movento	2	2	1	4	5	3	1	3	63% high; 37% medium
Sequoia	3	1	2	2	2	3	2	2	75% high; 25% medium
Silencer	N/A	N/A	N/A	4	4	4	1	3	40% high; 60% low
Sivanto_Prime	2	4	1	3	5	3	2	2	50% high; 50% medium

<u>Objective 1 – Insecticide field efficacy for thrips</u>

Thrips densities per plant did not vary among control and insecticide-treated plots from our two field locations (df = 7, 24; $P > 0.05$ for both trials). However, there was a numerical difference from plots treated with Exirel (chlorantraniloprole) when compared to untreated plots at 7 days after treatment in the trial located at the USDA Spence Farm (Fig. 2).



3 DAT:
df = 7, 24
F = 0.40
P = 0.8959

7 DAT:
df = 7, 21
F = 0.87
P = 0.5458

Fig. 2. Average (± standard error) thrips densities (adults + nymphs) per lettuce head from an insecticide efficacy field trial located at the USDA Spence Farm in Salinas, CA. This trial was planted on May 6, 2019 with Romaine lettuce on 40-inch beds with 2 seedlines. All insecticide treatments (X-axis) were sprayed on July 9, 2019. Thrips densities did not significantly vary among experimental treatment 3 or 7 DAT.

<u>Objective 2 – Monitoring 'practical' insecticide resistance in aphids</u>

All assayed aphid adults, regardless of the location, and species, died after 120 hours of being exposed to all leaf-disks treated with our insecticide treatments (Table 3). Adult mortality rates in the control experimental units ranged from 38 to 55% (Table 3). While mortality rates in the control were high, mortality for our treatments reached 100% indicating specimens were susceptible to all our treatments. For instance, adults of the potato aphid, *Macrosiphum euphorbiae* – the red morph, collected from Soledad, CA died at different rates, depending on the insecticide treatment (Fig. 3). Potato aphid adults died quickly and within the first 48 hours after treatment when exposed to leaf-disk treated with Silencer (lambda-cyhalothrin) and Sequoia (sulfoxaflor) (Fig.3). A slower mortality rate was observed when adult aphids were exposed to Movento (spirotetramat) and Fulfill (pymetrozine), where more than 80% of aphids died 96 hours after treatment (Fig. 3). Mortality rates in control experimental units never exceeded 41% of the total specimens per container 120 hours after treatment (Fig. 3).

Table 3. Adult mortality rates at 120 hours after treatment (HAT) for specimens from three aphid species, under laboratory assay conditions. Both color morphs (red and green) for the potato aphid, *Macrosiphum euphorbiae*, were collected in Soledad, CA in April 2019. The green peach aphid, *Myzus persicae*, was collected in Castroville, CA, in September 2019 and in Salinas, CA in October 2019. The lettuce aphid, *Nasonovia ribisnigri*, was collected in Gonzales, CA in August 2019. Mortality rates under the column labeled 'Treatments' summarizes percent adult mortality for all the experimental treatments across three assay repetitions, which were 100% for all species-location-treatment combinations.

		(%) Mortality (120 HAT)	
Aphid type	Location	Treatments	Control
Potato aphid (red morph)	Soledad	100	41
Potato aphid (green morph)	Soledad	100	38
Green peach	Castroville	100	45
Green peach	Salinas	100	42
Lettuce aphid	Gonzales	100	55

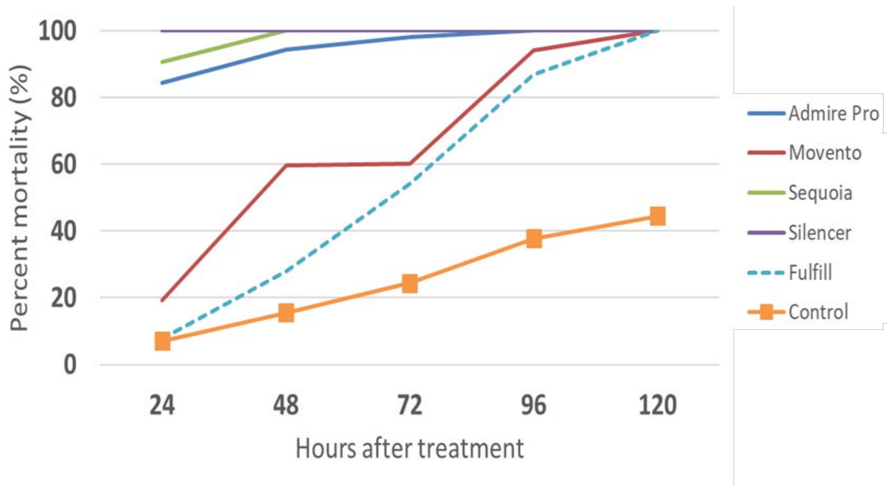


Fig. 3. Average percent of the adult mortality recorded every 24 h for five consecutive days (120 hours, on the X-axis) for the potato aphid, *Macrosiphum euphorbiae* – red color morph, exposed to leaf-disk treated with five different insecticides. Another set of aphids were included as a control where leaf-disks were only treated with distilled water.

<u>Objective 2 – Monitoring 'practical' insecticide resistance in thrips</u>

Adults of western flower thrips, *Frankiniella occidentalis*, collected from Chualar, CA in February 2020 died at different rates, depending on the insecticide treatment (Fig. 4). By the end of this assay (168 hours after treatment), the highest mortality rate (100%) was documented from experimental units with leaf-disk treated with Radiant (spinoteram) and Sivanto (flupyradifurone). Radiant acted very quickly, with 100% mortality after 24 h. The second highest mortality rates (85 – 90%) were documented from cups receiving disks treated with Exirel (chlorontraniliprole), Sequoia (sulfoxaflor). Lower thrips mortality rates (65 to 80%) were documented from cups with disks treated with Pounce WP (permethrin) and Mustang (zeta-cypermethrin). Mortality rates recorded in cups with leaf-disks treated Warrior (lambda-cyhalothrin) were similar to the mortality rates from the control experimental units (Fig. 4).

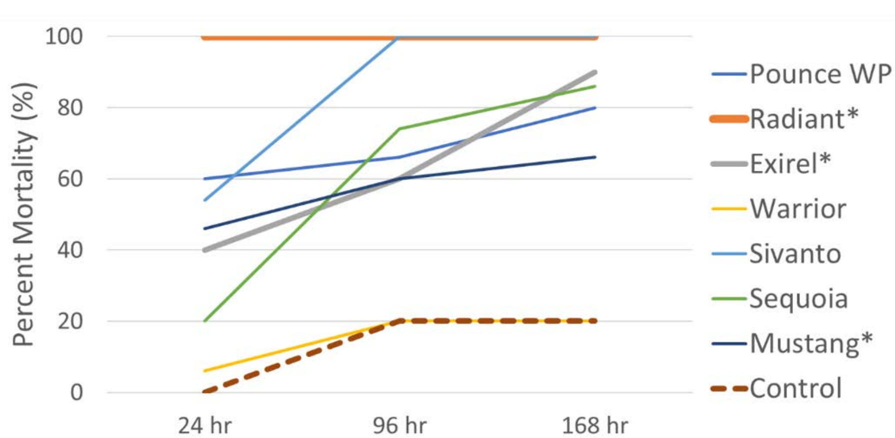


Fig. 4. Average percent of the adult mortality recorded at 24 h, 96 h and 168 h after treatment for the western flower thrips, *Frankiniella occidentalis*, exposed to leaf-disk treated with five

different insecticides. Another set of thrips were included as control (dashed line) where leaf-disks were only treated with distilled water. Asterisks by the product names (legend) denote insecticides that have control of thrips on their labels. The rest of the selected insecticides claimed only suppression of thrips from their labels.

Objective 3

Data originated from this project was presented at four grower meetings, including one Continuing Education meeting, one Grower Shipper Association Pest Management meeting, during the mid-year report meeting for the CLGRB, and during the 2019 Salinas Valley Entomology meeting.

Discussion:

Several insecticides, both organic-certified and conventional, are used to lower population densities of aphids and thrips in lettuce. These active ingredients range from broad-spectrum and contact insecticides, such as carbamates or pyrethroids, to selective and systemic insecticides such as flonicamid, diamides, or spynosyns. Information on the field efficacies of those insecticides influences how growers and PCAs select and use them in their chemical rotations. Therefore, there has been a need to generate replicated and multi-site field insecticide efficacy data for labeled active ingredients to control aphids and thrips in lettuce.

The seven insecticides tested during this project successfully reduced aphid densities in lettuce, when compared to our untreated control plots. The performance of these insecticides varied in the field. The location of these trials, as well as planting dates of experimental plots might have influenced the population dynamics of aphids infesting our experimental lettuce plots. However, across these locations and dates, the ranking for performance controlling aphids could be divided into three distinct groups. High insecticide performance came with the use of Admire Pro, Beleaf, Movento and Sequoia. Intermediate performance was recorded using Sivanto Prime. Fullfill and Silencer had the lowest insecticide performance.

To build upon field efficacy data, we were also interested in testing the susceptibility of field-collected aphids and thrips to selected insecticides under laboratory conditions. Both pests are managed consistently with insecticides due to their role as vectors of plant viruses and their low tolerance as contaminants in the final product. There is a possibility that a portion of their populations may have diminished susceptibility to certain active ingredients. Our laboratory bioassays did not indicate any substantial issues with insecticide resistance with the tested insecticides. Sequoia and Silencer killed almost 100% of the assayed aphid adults within the first 48 hours of our bioassay. On the other hand, aphids died at a slower rate over time when exposed to Admire, Movento, and Fulfill. The nature of these compounds, some of them acting as contact poison (i.e Sequoia) and some others being absorbed by the plant tissue (i.e., Movento), plays a role in understanding how quickly they are expected to work.

Our data on field efficacy of insecticides to control thrips is limited. The lack of differences between our control plots and the treated plots was heavily influenced by the timing of the insecticide applications. The biggest constraint for these experiments was timing, and we initiated our trials late within each crop growing cycle. Therefore, it was difficult to control an already established thrips population inside the lettuce heads. Future field trials addressing the efficacy of insecticides to control thrips will be initiated at thinning. However, thrips mortality varied depending on the insecticide treatment for our laboratory leaf-disk bioassays. By the end of these assays, higher thrips mortality rates were documented from Radiant, Sivanto Prime, Exirel, and Sequoia. Lower thrips mortality under leaf-disk bioassay conditions were recorded with Pounce WP and Mustang. Additionally, mortality rates were the lowest when thrips collected from Chualar, CA. were exposed to leaf-disks treated with lambda-cyhalothrin. It is important to clarify that Sivanto Prime and Sequoia labels indicate these products might suppress thrips populations in the field; therefore, they were included in these assays. Higher mortality rates from these two insecticides during these assays might reflect a best case scenario for control because they are a non-choice setup and thrips being confined and directly exposed to these insecticide-treated leaf tissue.

In conclusion, the application of our selected insecticides for this project successfully reduced aphid densities in lettuce under both field and laboratory conditions. There is no indication from our assays that aphid populations in the Salinas Valley are developing insecticide resistance to the selected insecticides. However, we remain concerned that insecticide resistance could threaten the continued efficacy of some materials. In contrast, thrips might be losing susceptibility to the pyrethroid lambda-cyhalothrin, based on our laboratory bioassays. Additional field collections of thrips will be needed to expand on this potential susceptibility issue. And finally, further validation in the field will be needed to corroborate the effect of both Sequoia and Sivanto Prime on thrips. We will continue to look for alternative insecticides to control both aphids and thrips under field conditions. It is our goal to widen the toolbox for these two pests in lettuce and help maintain the tools we currently have.

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