CALIFORNIA LEAFY GREENS RESEARCH BOARD
Annual Research Report
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Project title:
Integrating conservational biocontrol and chemical tactics for managing aphids and thrips in lettuce

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Abstract:
Alyssum flowers successfully promote the presence of hover flies in organic lettuce fields by providing pollen as a food source and alternative habitat. These flies are one of the most efficient aphid predators in the lettuce agroecosystem. On the other hand, the lack of plant diversity and flowers in conventionally-planted lettuce fields limits the presence of these hover flies. With upcoming regulations on the use of broad-spectrum pyrethroids and neonicotinoids, there is an opportunity for using insectary plants in conventional lettuce to promote conservational biocontrol. This project was an effort to showcase the feasibility of intercropping alyssum as an insectary plant with lettuce in conventional fields. During the establishment of these trials, we learned that the herbicide Kerb, normally effective at controlling weeds in the mustard family, did not drastically reduce the stand count of alyssum plants. We corroborated this in-field observation for Kerb by performing a greenhouse assay. Once alyssum was established and blooming, we recorded hover fly adults in our experimental plots. We also recovered very few hover fly maggots, only inside lettuce from plots with alyssum. Overall, aphid populations in lettuce were reduced when both 1) pyrethroids and neonicotinoids and 2) alternative insecticides to pyrethroids and neonicotinoids were sprayed. Meanwhile lower density trends were found in plots with alyssum flowers. Additionally, thrips densities were similar between plots with and without alyssum, and with and without insecticide applications. Future efforts will look into scaling up the intercropping of alyssum in conventional lettuce fields, without changing the selection of herbicides.

Objectives:

1. Compare aphid and thrips densities between leaf lettuces managed using different pesticide regimes (grower standard vs. alternative approach) with and without insectary and banker plants. Insectary plants provide alternative food and shelter for beneficials. A banker plant promotes the presence of non-pestiferous aphids as prey item for beneficials.

2. Compare and document number of insecticide applications, yield, and economic net return between leaf lettuces under different pesticide regimes (grower standard vs. alternative approach) with and without insectary and banker plants.

Procedures:

<u>Layout of experimental plots</u>

Trials were set up in two commercial lettuce fields during 2019 in Castroville, CA. The experimental design was a strip plot, where treatments were arranged as randomized complete blocks within each strip, with four replications (r = 4). Treatments were: 1) control with no insecticide applications, 2) current regime, including pyrethroid and neonicotinoid applications, 3) first alternative insecticide regime, and 4) second alternative insecticide regime. Table 1 shows details for our four experimental treatments. Strips (s = 2) were constituted by plots planted with insectary and banker plants, and plots without insectary and banker plants.

Table 1. Insecticides (brand name, active ingredient, rate) used for our experimental treatments

Treatment	Commercial name	IRAC group	Chemical class	Active ingredient	Rate / acre
Control	None	None	None	None	0 fl oz
Current	1. Admire Pro	4A	Neonicotinoid	Imidacloprid	1.3 fl oz
	& Silencer	3B	Pyrethroid	Lambda-cyhalothrin	3.84 fl oz
	2. Movento	23	Tetronic acid	Spirotetramat	5 fl oz
	& Perm-Up	3B	Pyrethroid	Permethrin	8 fl oz
	3. Sivanto	4D	Butenolides	Flupyradifurone	14 fl oz
	& Silencer	3B	Pyrethroid	Lambda-cyhalothrin	3.84 fl oz
First Alternative	1. Sivanto	4D	Butenolides	Flupyradifurone	14 fl oz
	2. Fulfill	9B	Pyridines	Pymetrozine	2.75 oz
	3. Sequoia	4C	Sulfoximines	Sulfoxaflor	2 fl oz
Second Alternative	1. Movento	23	Tetronic acid	Spirotetramat	5 fl oz
	2. Sequoia	4C	Sulfoximines	Sulfoxaflor	2 fl oz
	3. Beleaf	29	Flonicamid	Flonicamid	2.8 oz

The first experimental field, planted in late July, was Tropicana lettuce and direct seeded on 40-inch beds with two seedlines per beds. Plots were four beds wide by 35 ft long. The second field, planted in early August, was Romaine lettuce and also direct seeded on 80-inch beds with six seedlines per bed. Experimental plots were two beds wide by 35 ft long. Alyssum was direct seeded in both experimental fields using a commercial lettuce planter. Raw alyssum seed was coated similarly to the lettuce at 13.0 diameter. We used a mixture of 97% lettuce seed + 3% alyssum seed by weight to fill up the hoppers of the planter. After the lettuce + alyssum planting was concluded, we manually added 12 seeds of barley in each plot. Both experimental fields were sprayed with Kerb (active ingredient Pronamide) and Prefar (active ingredient Bensulide) herbicides following grower standard practices by the end of the day of planting. At thinning, the thinning crew tried to keep as many alyssum plants as possible. However, to avoid affecting the stand count per area, the crew favored the presence of lettuce plants and sometimes took out the alyssum plants.

<u>Evaluating the effect of Kerb on alyssum</u>

An experiment was conducted to assess the effect of the herbicide Kerb on the germination of pelletized alyssum seed. Two different types of soil (clear lake clay and loamy sand) were placed in 2-ft plastic germination trays. Twenty four pelletized alyssum seeds were sown equally distant in each tray. A solution of Kerb was prepared targeting 1.25 pts/acre. Our experimental treatments were 1) Control (no herbicide application), 2) 1.25 pts/acre (trays sprayed once), 3) 3.75 pts/acre (trays sprayed three times), and 4) 5.00 pts/acre (trays sprayed four times). Treatments were arranged as a completed randomized block design with three replications (r = 3). Sprayed trays with soil and alyssum seeds were housed under greenhouse conditions (~80°F and ~40% RH). Trays were watered once a day. Germination rates, expressed as percent emergence, were recorded 10 days after planting.

<u>Objective 1</u>

Two lettuce plants were collected from each experimental plot during three different dates, seven days after each insecticide application. Before the first collection of lettuce samples, we visually inspected the middle of each plot for one minute to record the presence of hover fly adults. Lettuce samples were bagged in the field and took them to the Entomology laboratory in Salinas, CA. Each sampled plant was cut into individual leaves, and every leaf was visually inspected to find and count aphids (adults and nymphs), thrips (adults and larvae), and hover fly maggots.

<u>Objective 2</u>

Different active ingredients were used depending on the experimental treatment (Table 1). All insecticide applications were deployed using a CO_2 backpack sprayer, with either four nozzles / bed for Romaine lettuce and two nozzles / bed for Tropicana lettuce; targeting 60 gallons of water / acre and using a silicone-based adjuvant. Applications were made early in the morning to avoid windy conditions. Table 1 also presents the order in which different insecticides were sprayed throughout this project.

<u>Statistical analysis</u>

To analyze alyssum seed percent emergence and aphid and thrips counts per lettuce head, we used generalized linear mixed models to test for differences among treatments. These treatments included Kerb application, insecticide regimes, and the presence of insectary plants.

Degrees of freedom were corrected using the Kenward and Roger procedure. Mean separations were performed using Tukey's test.

Results:

Layout of experimental plots

We were successful in establishing alyssum from seed and to intercrop this insectary plant with lettuce in commercial and conventional fields, even though these fields were sprayed with both herbicides Kerb and Prefar. When planted as seed, alyssum flowering period coincided with the thinning activities in lettuce. On the other hand, our banker plants failed to establish in our experimental plots. Therefore, we report treatment effects due to the planting of alyssum as insectary plants alone for these trials.

Evaluating the effect of Kerb on alyssum

In loamy sand soil, applications of the herbicide Kerb at different rates per acre did not significantly reduce the germination of coated alyssum seeds under greenhouse conditions (Fig. 1). However, there was ~37% reduction in the germination of alyssum when planted in clear lake clay soil and sprayed with the herbicide Kerb (Fig. 1).

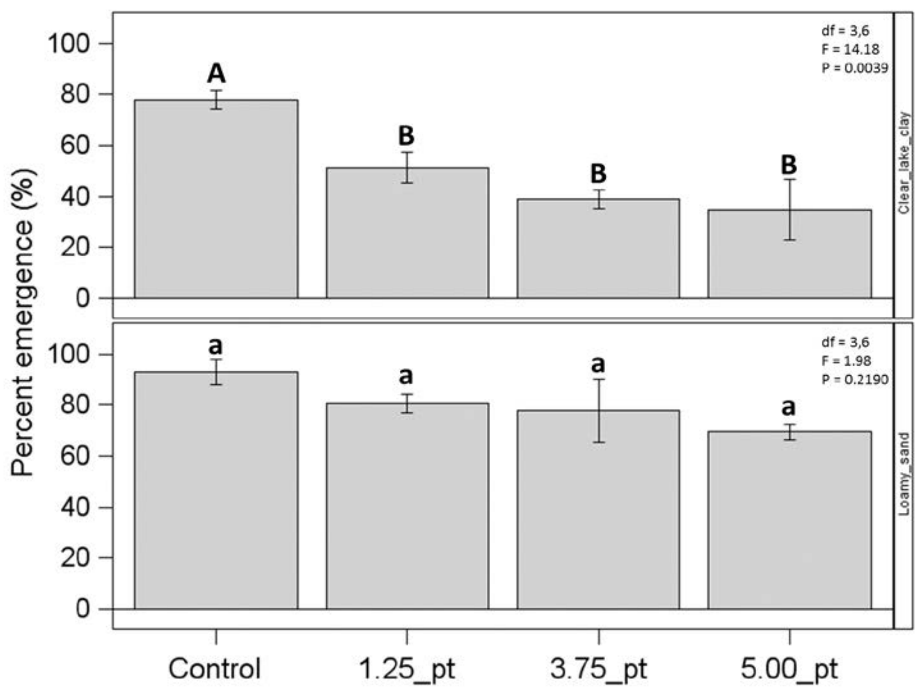

Fig. 1. Average germination rates ± standard errors, expressed as percent emergence, of pelletized alyssum seed planted in clear lake clay soil (top panel) and loamy sand soil (bottom panel). After planting seeds, the top layer of the soil was sprayed with the herbicide Kerb at 1.25, 3.75, and 5 pints per acre (X-axis). A control with no herbicide application was also included in this experiment. Gray bars sharing the same letters are not statistically different. Upper- and lower-case letters denote separate statistical analyses.

Objective 1

Visual scouting of hover fly adults

We recorded a total of 10 hover fly adults on alyssum flowers at only the experimental site planted with Tropicana (Fig. 2). No hover fly adults were found at any of the strips without insectary plants.

Fig. 2. Picture depicting a hover fly adult resting on an alyssum flower (specimen inside the black circle). Alyssum was direct seeded and interplanted with Tropicana lettuce in Castroville, CA during July 2019.

Insect densities

We counted a total of 451 aphids (adults and nymphs), 1,270 thrips (adults and larvae), and 6 hover fly maggots from our two experimental trials. All adult aphids were identified as the lettuce aphid (*Nasonovia ribisnigri*, Hemiptera: Aphididae) and adult thrips as the western flower thrips (*Frankliniella occidentalis*, Thysanoptera: Thripidae).

Aphid densities were influenced by the insecticide treatment used in both of our experimental fields (Fig. 3). Lettuce plants from control plots had the highest densities of aphids (Fig. 3). Similar aphid numbers were recorded from treatments mimicking the 'current insecticide regime' or any of the 'alternative regimes' (Fig. 3). Additionally, aphid densities per lettuce head did not vary statistically between plots with or without alyssum as an insectary plant in both experimental fields. However, the arithmetic mean of aphid densities was lower from plots with insectary plants (2.44 aphids / head) compared to plots with no insectary plants (3.27 aphids / head). The interaction between strip × treatment also did not influence aphid numbers in our lettuce fields.

Thrips densities were not influenced by any of our experimental treatments (insectary plants nor insecticide treatments). Thrips numbers were similar when comparing sampled lettuce plants between plots with and without insectary plants (Fig. 4). None of our insecticide treatments reduced the thrips density per lettuce head.

All of the hover fly maggots were only recovered from plots with insectary plants. The majority of those maggots were found inside lettuce collected from our control plots, with no insecticide applications. Only one maggot was recovered from one lettuce sample collected from a plot that received the 'Second Alternative' insecticide regime.

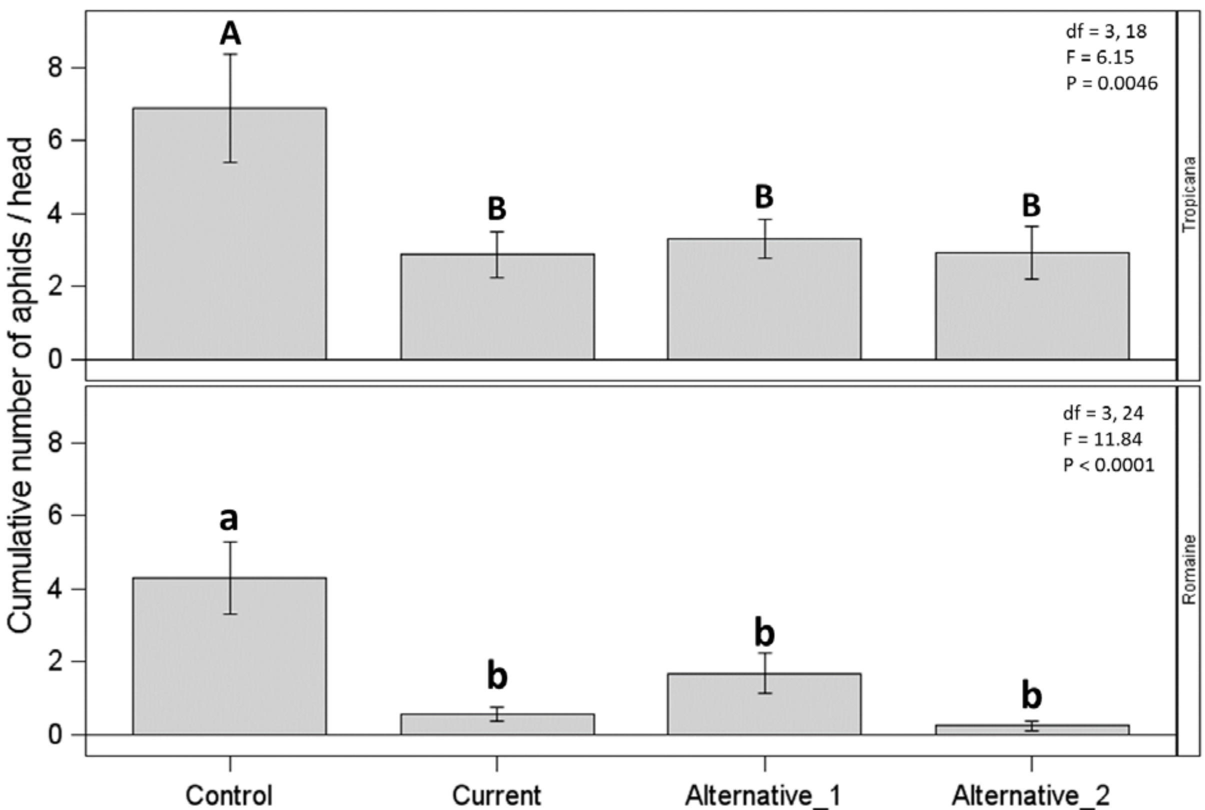

Fig. 3. Average ± standard errors of cumulative number of aphids per lettuce head documented from our insecticide experimental treatments (X-axis): a) Control with no insecticide applications, 2) Current, spraying pyrethroids and neonicotinoids, and 3) and 4) Alternative regimes, spraying alternative insecticides to pyrethroids and neonicotinoids. Gray bars sharing the same letters are not statistically different. Upper- and lower-case letters denote separate statistical analyses. Top panel presents means for the Tropicana lettuce, and the bottom panel is for the Romaine lettuce.

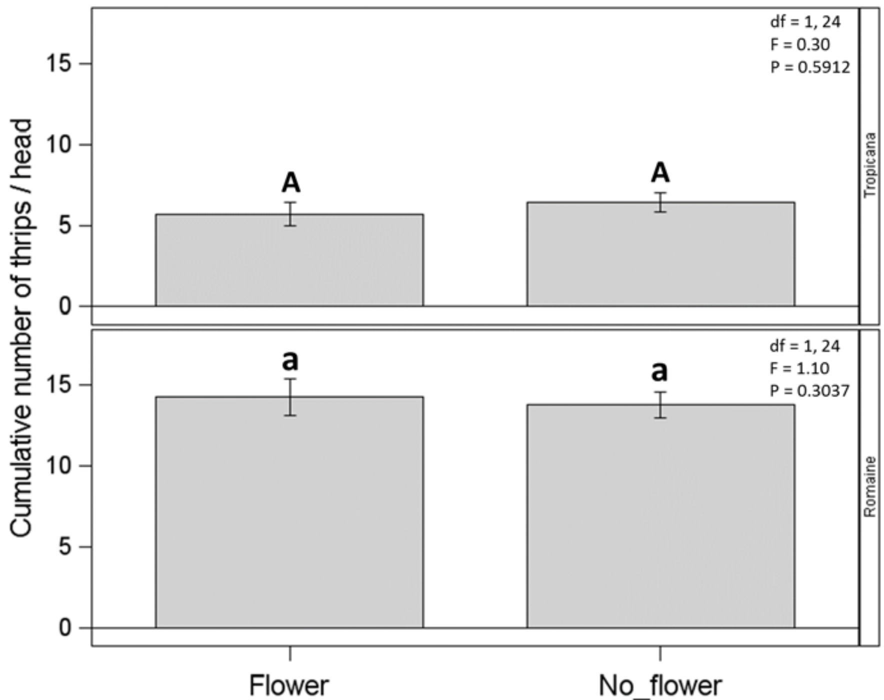

Fig. 4. Average ± standard errors of cumulative number of thrips per lettuce head documented from our insectary plant strip treatments (X-axis): a) Flower, plots planted with alyssum insectary plants and b) No flower, plots without the insectary plants. Gray bars sharing the same letters are not statistically different. Upper- and lower-case letters denote separate statistical analyses. Top panel presents means for the Tropicana lettuce, and the bottom panel is for the Romaine lettuce.

Objective 2

The presence or absence of alyssum as insectary plants did not statistically influence the number of aphids per lettuce plant. Therefore, the number of insecticide applications in our two experimental fields did not vary. All plots received a total of three insecticide applications, except for the control plots that had no insecticide applications. Information on the insecticides used and their sequence of application are presented in Table 1.

We did not collect yield data from any of our experimental plots, since the number of insecticide applications were identical among insecticide treatments. We did not calculate the economic net return for any of our treatments since lettuce head wet weight was not expected to differ between plots with and without insectary plants (Del-Pozo 2018 CLGRB final report).

Discussion:

Upcoming regulations on the use of pyrethroids and neonicotinoids seems to be inevitable, due to their nature as surface water contaminants. However, these two insecticide classes play an important role in managing pests, especially aphids, in conventionally-grown lettuce. Limiting the use of these two insecticide classes will incite growers and PCAs to experiment and find alternative solutions for managing pests. This project was intended to showcase interplanting alyssum as an insectary plant in conventional lettuce as part of the alternatives to using pyrethroids and neonicotinoids. We also proved that alternative insecticides to pyrethroids and neonicotinoids significantly reduced aphid densities in conventional lettuce.

This research project set the example that it is feasible to successfully interplant alyssum in conventionally-grown lettuce. We proposed the use of 97% lettuce seed + 3% alyssum seed by weight for mixing seeds in each planter's hopper. Additionally, it is not necessary to make any changes on the herbicide application regime. Spraying both Prefar and Kerb will not drastically influence alyssum germination in the field. It was thought that Kerb would have prevented alyssum from germinating, since Kerb target some crucifer weeds. However, our greenhouse assay showed that the herbicide application of Kerb in sandy soils, at different rates per acre, did not significantly reduce the germination of alyssum seeds, when compared to the control without an herbicide application. In clay soils, it is expected that an herbicide application of Kerb will reduce up to 37% of the germination rate for alyssum seeds.

Avoiding changes on the herbicide regime will make more attractive interplating alyssum in conventional lettuce for growers. However, it will limit the establishment of any annual grass banker plant, such barley. The original proposal was to test both insectary and banker plants. Banker plants would have been infested with a non-pestiferous aphid for lettuce, such as the grain aphid. Having other aphids as available prey items would have promoted the presence of hover flies in our plots with these banker plants. We could not test this assumption since our barley banker plants failed to establish in our experimental fields. It is likely that the herbicide application of Prefar and Kerb prevented the germination of the barley, leaving our experimental plots without banker plants.

Our working hypothesis for this research project was to expect lower aphid densities on lettuce collected from plots with alyssum insectary plants. Flowers would have attracted hover flies, these adults would have laid eggs in the lettuce, and then the maggots of these flies would have provided some predation services. We have data from this project that anecdotally support that hypothesis. There was a difference between the arithmetic means of aphid from plots with and without insectary plants; where lower aphid trends were found in lettuce from plots with insectary plants. Hover fly adults were only found in our plots with alyssum flowers. In addition, hover fly maggots were only recovered inside lettuce collected from plots with insectary plants. Our small plot set-up did not attract a significantly large population of hover flies. On the contrary, we only recorded a total of 10 hover fly adults and six maggots in the course of these two field trials.

Alyssum flowers not only attract beneficials, but also attract the western flower thrips. Thrips are the only vectors of the Impatiens necrotic spot virus (INSV). Having thrips flying around alyssum flowers in lettuce fields is also a concern due to potential INSV infections. Data from this research project showed that there is no significant difference in thrips densities per plant, when lettuce was collected from either plots with or without alyssum insectary plants. We recorded up to 5% of INSV infection rate in both experimental fields, regardless of the presence or absence of insectary plants. A future research project could look into using alyssum as a trap crop for thrips, where flowers could attract migrating adults and keep them away from the lettuce. If alyssum would be planted as a trap crop, additional management strategies should be considered to lower thrips densities in the alyssum and to avoid being a source of thrips for the lettuce crop.

In conclusion, it is feasible to successfully establish alyssum as insectary plants from seed, when coated at 13.0, mixed with lettuce seed, and planted under conventionally-grown conditions. It is also documented that alternative insecticides to pyrethroids and neonicotinoids were successful in lowering aphid densities in our experimental trials. The reduction in using broad-spectrum insecticides due to upcoming regulations may result in promoting conservational biocontrol in conventional lettuce by planting insectary plants. Alternative insecticides to pyrethroids and neonicotinoids are less harmful to beneficial insects; therefore, coupling selective chemicals and conservational biocontrol may be possible in conventional lettuce.

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