Organic Farming Research Foundation Final Grant Report (2022-2023)

Project title: Companion plantings for organic management of a new invasive Brassica pest

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Project Summary

This project leveraged agricultural diversity to mitigate the recent attack of the invasive yellow-margined leaf beetle (*Microtheca ochroloma*) on leafy brassica greens across the Southeastern United States. This new pest is reviled among farmers in Alabama, Florida, Georgia and South Carolina, is rapidly expanding its range northward as winters become milder. Unfortunately, the biology of *M. ochroloma* is not well known, and organic farmers have not found a viable tool for protecting their brassica crops. However, strong host plant preferences revealed by *M. ochroloma* suggest that it might be successfully manipulated with strategic combinations of attractive trap crops and repellant intercrops. Our project focused on identifying companion plants that reduce destruction by *M. ochroloma* in the absence of other viable tools. We tested three methods to control *Microtheca ochroloma,* including intercropping, trap cropping, and biological control via predator gut content analysis. Our purpose was to identify accessible strategies that limit damage from this pest while minimizing pesticide use on organic farms. First, we found that lemongrass and tomato companion plants may reduce herbivore populations on turnip greens while commonly employed companion plants like basil and marigold may actually backfire to attract herbivores, relative to crop monocultures. Predatory insects were highly correlated with herbivore populations; suggesting that they are more influenced prey availability than companion plants. Next, we found that mizuna is a strongly preferred host plant by *M. ochroloma,* relative to other *Brassica napa* hosts, but may only serve as an appropriate trap if pest spillover is managed. Last, we identified several generalist predators that commonly consume *M. ochroloma* that were unknown previously by screening predator guts for *M. ochroloma* DNA.

Introduction to Topic

The yellowmargined leaf beetle (*Microtheca ochroloma*) is a new invasive pest that is prominent throughout the Southeastern United States and spreading north and westward. These beetles consume leafy greens in the Brassicaceae family including mustards, turnips, and cruciferous vegetables which are of high economic and cultural value in the Southeastern United States. In the past few years, many of our farmer collaborators throughout Georgia, South Carolina, and North Carolina have identified *M. ochroloma* on their farms as a new ravenous consumer of their *Brassica* greens and are searching for solutions. Some of these farmers have reluctantly used heavy and frequent applications of registered-organic insecticides to protect their crops. For example, in Fall of 2020, farmers at the UGArden (the student organic teaching farm at UGA) were spraying PyGanic insecticide on their turnip greens at least once every two weeks. Still, much of their turnip crop faced severe damage from *M. ochroloma* making them unsellable. Many small organic farmers hesitate to use insecticides since they can lead to insecticide resistance and kill beneficial insects like pollinators and predators. Unfortunately, little is known about *M. ochroloma's* biology along with non-pesticide management strategies. The lack of viable solutions for organic pest control prompted us to identify companion plants that can be used in a push-pull system where a deterrent intercrop pushes *M. ochroloma* away from turnip plants while a more preferred boarder plant pulls them away. We also worked to identify insects that consume *M. ochroloma* so that that uniquely tailored strategies to promote biological control can be implemented. With these discoveries, we hope to aid farmers in managing this destructive pest while minimizing insecticide use.

Objectives :

Our original objectives were to:

1. Identify a repellant companion plant that can be planted alongside turnip plants that limits *M. ochroloma* from establishing in turnip fields and increases marketable turnip yield.
2. "Herd" *M. ochroloma* away from turnip greens by combining an attractive trap crop (identified in previous field experiment as mizuna) and a repellant intercrop (identified in Objective 1).
3. Demonstrate successful polycultures on 10 collaborating farms.

Unfortunately, *M. ochroloma* was not present at our site during the experiment performed to address Objective 1, meaning Objective 3 could not be performed. Instead, we combined Objective 2 and 3 and created a new Objective 4 that yielded and additional useful tool for farmers.

2/3. "Herd" *M. ochroloma* away from turnip greens by planting a trap crop (mizuna) adjacent to the turnips. Demonstrate this on 5 collaborating farms.

4. Identify insects that predate on *M. ochroloma* by analyzing the gut contents of the most common predatory insects.

Materials and Methods:

<u>Objective 1: Identify repellant companion plants</u>

Experimental Setup

Our intercropping experiment took place on an organically certified plot at the UGA Durham Horticulture Farm in Watkinsville, Georgia from May 13, 2022 - July 12, 2022. Seeds were planted on May 13, 2022, and fertilized with 5 kg Nature Safe Organic Nitrogen Fertilizer (13-0-0) on June 3, 2022. Plants were watered with drip irrigation as needed.

Our six companion plant treatments included:

- French marigold (*Tagetes patula*)
- Lemongrass (*Cymbopogon flexuosus*)
- Thai basil (*Ocimum basilicum*)
- Tomato (*Solanum lycopersicum*)
- Fallow/weedy
- Sole turnip control (*Brassica rapa* var. *rapa*)

These plants were chosen for either their ability to repel pest insects or to attract predatory insects, based on previously published studies as well as folk knowledge. Plots were 2.7 m x 3.6 m and organized in a randomized complete block design shown below (Fig. 1). Each plot contained two beds with four rows of plants each: two rows of turnip on the inside, and two rows of companion plant on the outside. The turnip monoculture control only had the two inner turnip plantings. Turnips were planted 5 cm apart and companion plants were planted 30 cm apart. Companion plants were seeded in a greenhouse and transplanted after two weeks when turnip seeds were directly sown.

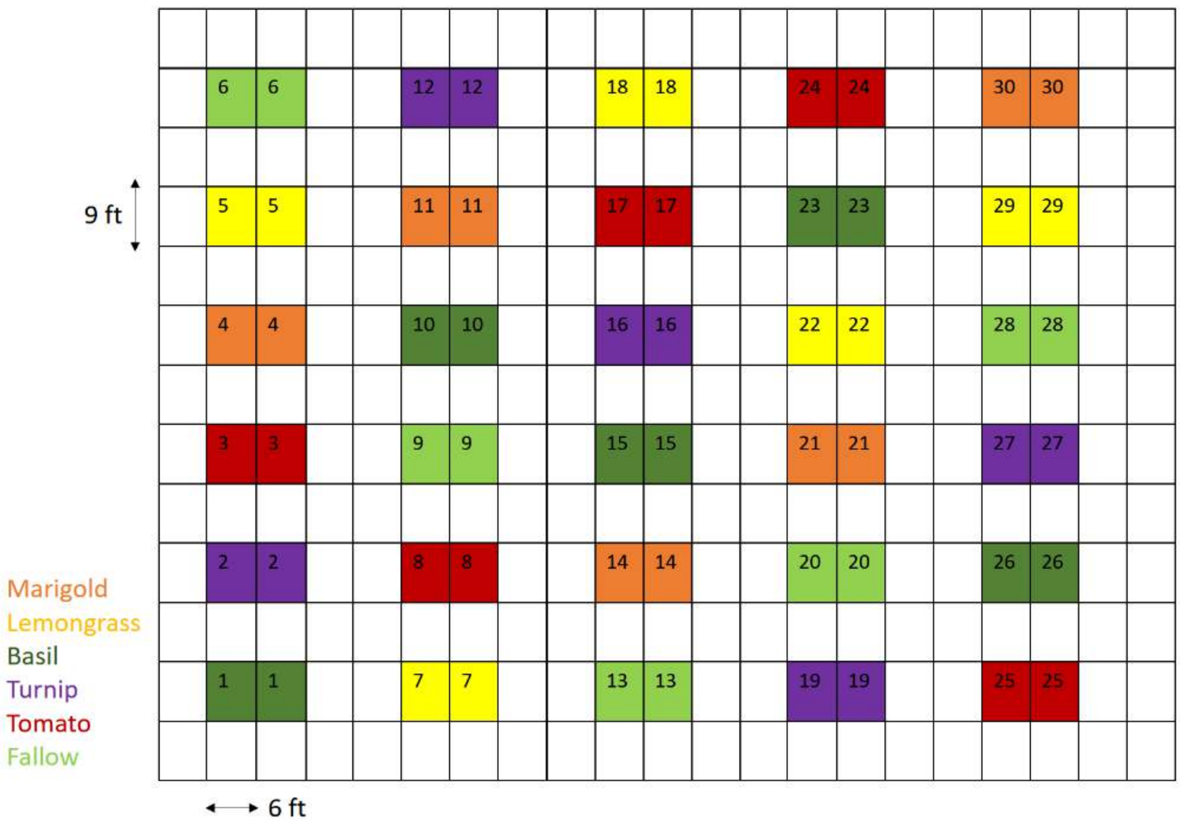

Figure 1: Field design for intercropping experiment

Data collection

Six randomly selected turnip plants, six random companion plants, and six 45 cm x 45 cm ground quadrats were visually surveyed for insects once a week for four weeks. Plant health and damage scores were recorded for the turnip plants according to an ordinal scale from 0-5. To quantify plant biomass, three randomly selected turnips and three companion plants were removed, dried in a 60°C oven, and weighed. Turnip bulbs were separated from leaves and weighed before drying.

<u>Objective 2/3: Demonstrate trap crops on-farm</u>

Experimental setup

Our trap crop experiment took place between October 20th, 2022 and November 11th, 2022 on five organic farms located in Northern Georgia and South Carolina (Fig. 2). Each farm contained two plots each, one with a mizuna trap crop, and one without (control). Plots were 6 m x 1 m and spaced > 24 m apart. Since *M. ochroloma* is known to lay eggs and pupate on dead plant material, plots were divided in half with one raking/ dead plant removal treatment (to eliminate preferred habitat where *M. ochroloma* lays eggs) and one non-raking control. Trap crop treatments contained one row of mizuna and three rows of turnips. The control plots contained three rows of turnips.

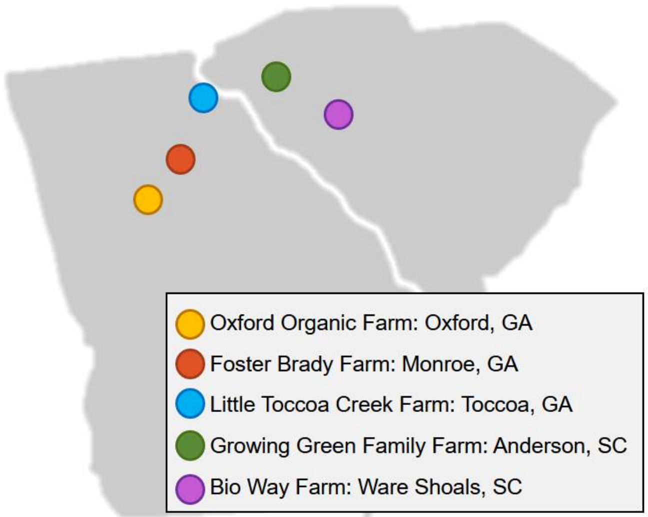

Figure 2: Map indicating the location of our five farms used for the trap crop experiment.

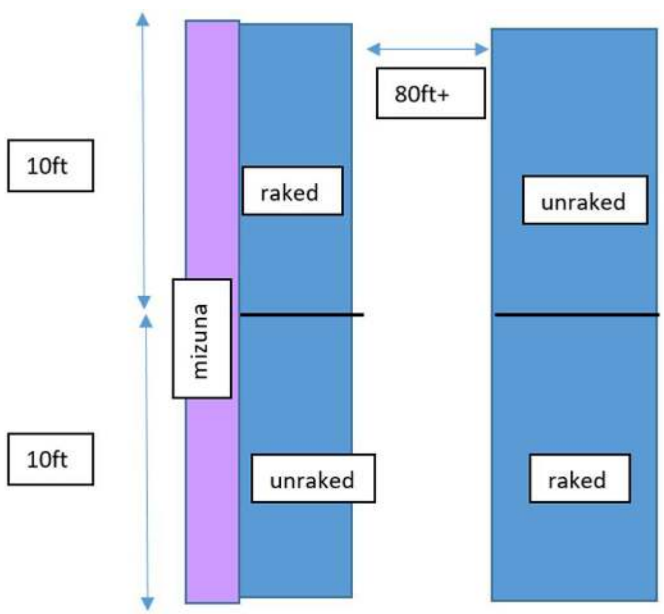

Figure 3: Diagram showing the experimental design for the trap crop experiment.

Data collection

We surveyed each farm four times. We chose three random plants in each subplot and recorded all insects on the leaves as well as in three 45 cm x 45 cm quadrats. Each week, we removed all dead plant material from the ground within the "raked" treatments. Health and damage scores were recorded for the according to an ordinal scale from 0-5. To quantify plant biomass, three randomly selected turnips and three companion plants were removed, dried in a 60°C oven, and weighed.

Objective 3: Identify native predators of yellowmargined leaf beetles

Experimental design

364 predatory insects were manually collected from six *Brassica spp*. plants (mizuna, turnip, mustard, choi sum, Chinese cabbage, and broccoli raab) from a previous host preference experiment in 2021 (Golan et al., in revision). Each predator was placed in cooled 100% ethanol and stored in a –20 until DNA extraction could occur. Whole body DNA extractions were performed on each predator using QIAGEN DNEasy185 Blood & Tissue Kits based on the manufacturer's animal tissue protocol. We performed PCR and gel electrophoresis to determine if *M. ochroloma* DNA occurred in the predator samples. If an insect tested positive for *M. ochroloma* DNA, we concluded that the insect likely consumed *M. ochroloma* recently.

Project Results:

Objective 1: Intercropping experiment

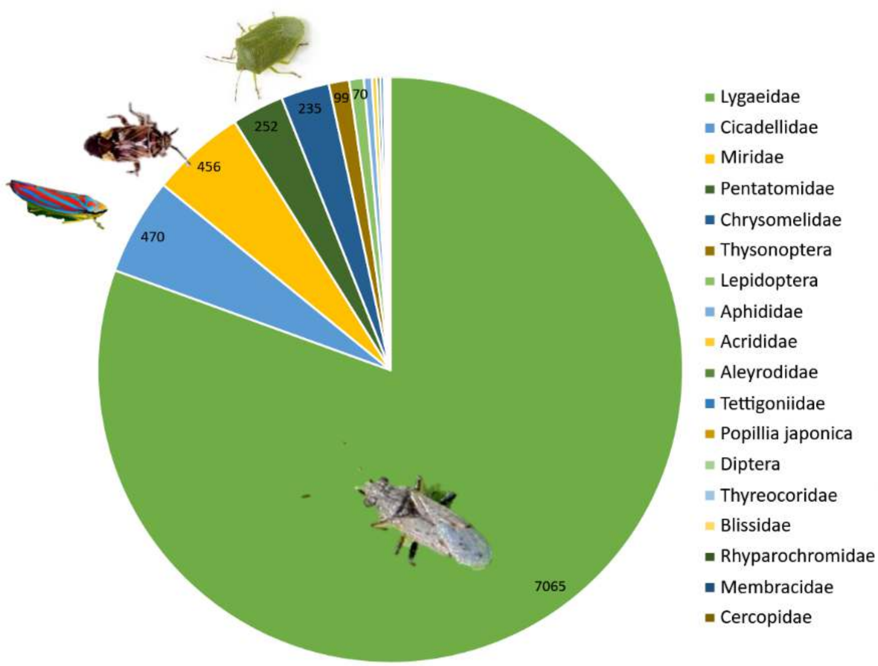

Figure 4: Pie chart showing the total season herbivore counts on turnips in the intercropping experiment.

The herbivore community on our turnips consisted mostly of Lygaidae, particularly the false chinch bug (*Nysius raphanus*). Unfortunately, only two *M. ochroloma* were identified throughout the season. Insects most similar to *M. ochroloma* in the family Chrysomelidae included striped flea beetles (*Phyllotreta striolata*), palestriped flea beetles (*Systena blanda*), and various other flea beetles.

We found that turnip treatments interplanted with tomato or lemon grass harbored fewer herbivores than turnip monocultures (control). Fallow, marigold, and basil treatments supported more herbivores than turnip monocultures with basil being the most numerous. The number of herbivores on the associated companion plants followed a similar pattern to that of the turnip plants.

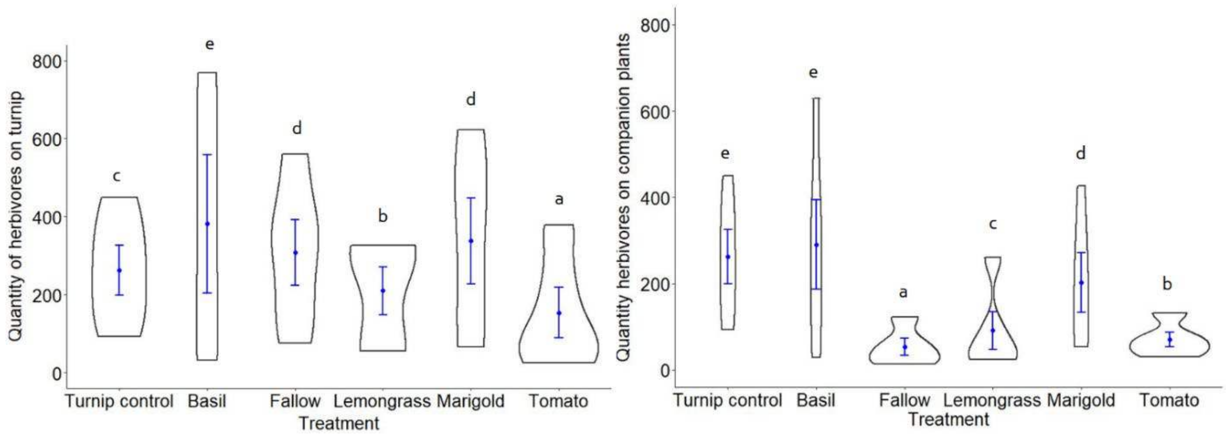

Figure 5: Mean annual counts (±SE) of herbivorous insects on turnips and their associated companion plants according to foliar count data. The top and bottom of the violin plot are the minimum and maximum values, and the thickness of the shapes represents the probability density of the data. Letters represent significant differences between treatments.

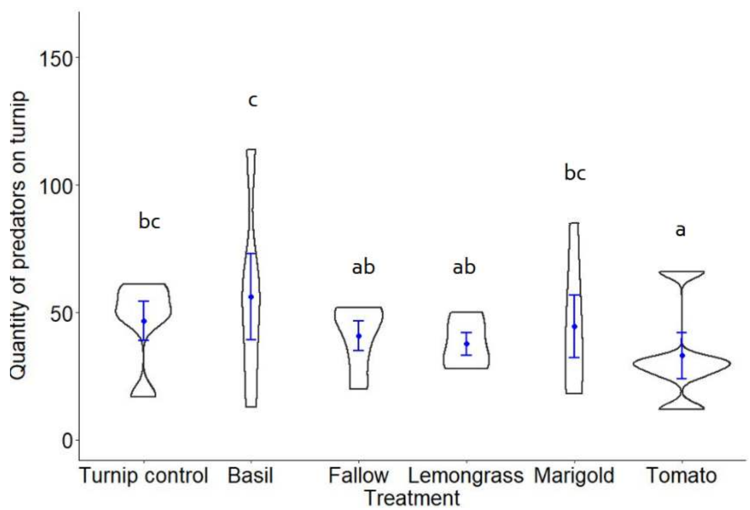

Fig 6: Mean annual counts (±SE) of predatory insects on turnips according to foliar count data. The top and bottom of the violin plot are the minimum and maximum values, and the thickness of the shapes represents the probability density of the data. Letters represent the significant differences between treatments.

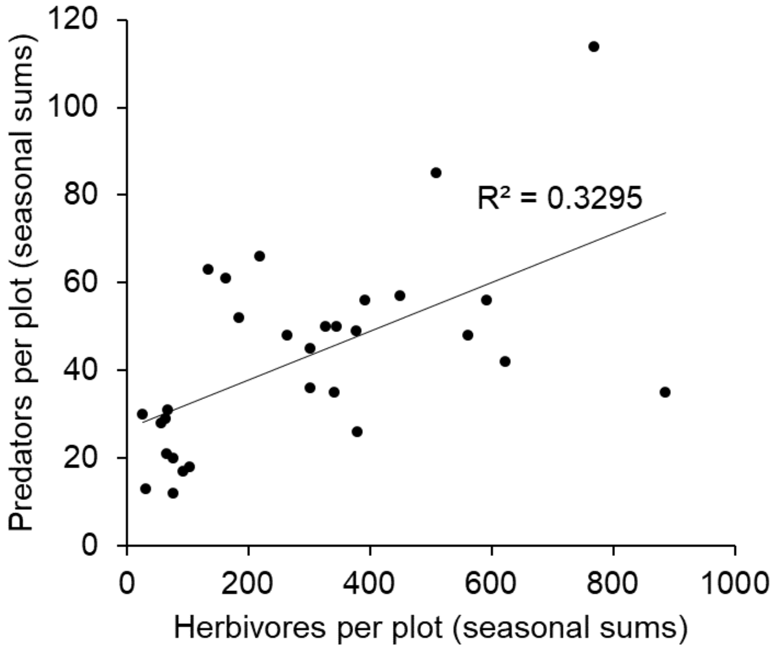

Fig. 7: Scatter plot indicating the relationship between herbivore and predator counts on turnip plants. Each dot represents a different plot. Includes a line of best fit.

We found that turnips adjacent to tomato plants harbored fewer predators than the turnip control while basil and marigold supported the most. We also found that predator abundance correlated closely with herbivore abundance (Estimate = 0.055, SE = 0.014, T = 3.709, P = 0.001. R^2 = 0.3295).

Objective 2/3: On-farm trap crop experiment

We found that mizuna was successful in attracting more *M. ochroloma* than the turnip plants (Estimate = -1.718, SE = 0.092, Z= -18.611, P = <0.001, Fig). However, the mizuna trap crop treatments had increased *M. ochroloma* infesting the turnip greens compared to the turnip monoculture controls (Estimate = 1.13, SE = 0.086, Z = 13.093, P = <0.001). Raking/sanitation treatments had no effect on *M. ochroloma* densities.

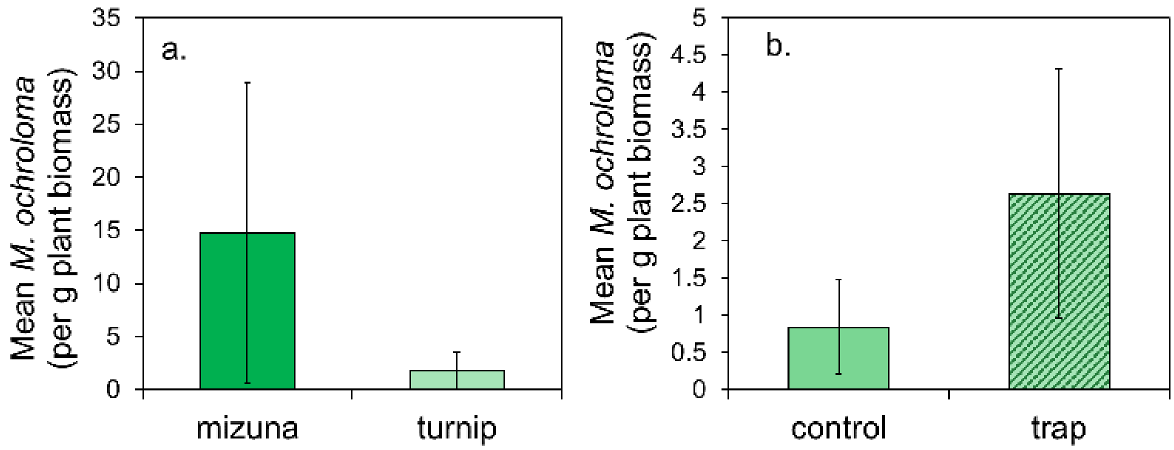

Fig. 8: Mean number of *M. ochroloma* per gram dried plant biomass and standard error on mizuna and turnip plants in trap crop treatmants(a). Mean number of *M. ochroloma* per gram dried plant biomass and standard error on turnip plants across the two trap crop treatments (b).

Objective 4: Gut content analysis

We found that 26.1% of our tested insects had *M. ochroloma* DNA in their guts. The most numerous predators tested were various Coccinelids (149), particularly the pink spotted lady beetle (93) followed by big-eyed-bugs (49), spiders (42) and rove beetles (33). Spined soldier bugs (91.7%), big-eyed-bugs (59.2%), and damsel bugs (33.3%) all contained *M. ochroloma* DNA at percentages higher than the average 26.1%.

Table 1: Results of the gut content analysis showing the predators that tested positively or negatively for *M. ochroloma* DNA.

Predator taxa	Count	Percent predators positive for YMLB DNA	Average concentration of YMLB DNA among positives (ng/µl)
Spined-soldier bug	12	91.70%	4.119
Big-eyed bugs	49	59.20%	2.803
Damsel bugs	9	33.30%	2.083
Ground beetles	**46**	**23.90%**	**0.701**
Spiders	42	21.40%	0.896
Total Coccinellids	149	20.10%	2.053
Larval Coccinellids	28	25.00%	2.031
Coleomegilla maculata	**93**	**23.70%**	**2.053**
Harmonia axyridis	5	20.00%	2.21
Coccinella septempunctata	12	0.00%	
Hippodamia convergens	4	0.00%	
Scymnus loewii	7	0.00%	
Misc. other predators [1]	18	5.60%	1.29
Rove beetle	33	3.00%	0.26
Orius	6	0.00%	
Grand Total	364	26.10%	

[1] Other predators included 3 Berytidae, 3 Miridae, 2 Scarabidae, 1 Symphyta, 1 unknown Chilopod (positive), 4 unknown Coleoptera, 1 unknown Diptera, 2 unknown Hemiptera, and 1 unknown Hymenoptera

Conclusions and Discussion

From our companion plant experiment, we discovered that turnip plants paired with tomato and lemongrass had fewer herbivores than the turnip monocultures, suggesting these crops may work to repel Brassica pests. Indeed, lemongrass has been shown to reduce the presence of flea beetles (*Chaetocnema confinis*) from cabbage (*Brassica oleracia*) (Lamba & Yaubi, 2021), as well as eggplant fruit and shoot borers (*Leucinodes orbonalis*) on eggplant (*Solanum melongena*) (Calumpang et al., 2013). In both cases, marigolds were tested and attracted more herbivores than their host counterparts, aligning with our results as well. This was particularly curious to us because marigolds are so frequently recommended as effective companion plants for pest management in extension literature and in the popular press (Riotte 1994), despite their attractiveness to herbivores (Lopez and Liburd 2022). Tomatoes may have helped in repelling insects because they were the bushiest of all the companion plants and may have physically blocked the turnips. Unfortunately, tomatoes are a summer crop while turnips are more suited for spring and fall. However, planting tomato seedlings next to turnips in early fall or late spring may stop early infestation. Additionally, shade from the growing tomatoes may protect the turnips from the summer heat. Herbivores were attracted to flowering plants like basil and marigold, and their populations spilled over onto turnip plants. Since predator and herbivore insect populations were highly correlated in our experiment, we believe that the predatory insects were attracted to the increased prey source on highly herbivore-infested plants instead of being attracted to the companion plant directly. These results were driven by the false chinch bug population, rather than *M. ochroloma,* meaning further experiments will need to be conducted to identify companion plants that specifically repel *M. ochroloma*.

Although we did not find that mizuna protected turnips from *M. ochroloma* infestations when they were planted adjacent to each other on our collaborators farms, we still believe that it could potentially succeed as a highly attractive trap crop. Most of the turnip damage occurred on plants directly next to the mizuna rows indicating a spillover from the mizuna to the turnips. We may have had more success with our trap crop if it was spaced further from the turnips. Future studies could test the success of using an organic herbicide on the mizuna, early removal of the mizuna, or covering the mizuna with insect-proof cloth before the insects have the chance to spill over onto the turnip greens.

To our surprise, many generalist predator insects including big-eyed bugs, lady beetles, spiders, and ground beetles consumed *M. ochroloma* in a highly infested field site. Previously, only spined soldier bugs, lady beetles, and lacewings were known to eat this pest. The high quantity and diversity of insects that consume *M. ochroloma* stresses the importance of limiting pesticide use that could harm these beneficial populations. Companion plants that attract these beneficial insects, particularly spined soldier bugs, should be identified in future research. Additionally, many of these insects can be reared and released in the field to increase the likelihood of biological control of this pest.

Overall, our studies identified tomato and lemongrass as possible companion plants that repel *Brassica* pests. We also found many potential biological control agents for *M. ochroloma*. This information can be used as a baseline for future *Brassica* companion plant research. Farmers should consider these intercrop options when experimenting with *Brassica* pest control.

Outreach:

The predator gut content analysis results are in revision at the journal Biological Control. We plan to publish the results of the intercropping experiment and on-farm experiments in winter 2023. Results have already been shared with farmers in our network in a webinar in winter 2022, and will be presented in several extension workshops in the coming year. We are also developing an article for eOrganic summarizing our results and will work with extension specialists throughout the Southeast to ensure that our new tools are delivered to farmers battling *M. ochroloma.*

Financial accounting:

Because objectives had to be adapted after the late freeze that led to sparse *M. ochroloma* populations in summer 2022, our on-farm experiments were more limited in number than originally planned. (i.e. We decided not to ask 10 farmers to test a combination of trap crops and repellent companion plants when we weren't able to evaluate repellent plants with an abundant population of *M. ochroloma* the previous year). Instead, we completed five smaller on-farm experiments focused on mizuna trap crops, and therefore saved half the budget associated with on-farm experiments.

	Budget	Cummulative Actuals	Balance
Salaries	$9,499.00	$9,738.53	$(239.53)
Total Personnel Services	**$9,500.00**	**$9,738.53**	**$(238.53)**
Travel	$3,393.00	$1,762.94	$1,630.06
Operating (materials)	$2,084.00	$1,883.45	$200.55
Farmer stipends	$5,000.00	$2,500.00	$2,500.00
Total Non-personnel services	**$10,477.00**	**$6,146.39**	**$4,330.61**
Total Direct Costs	$19,977.00	$15,884.92	$4,092.08
TOTAL PROJECT	**$19,977.00**	**$15,884.92**	**$4,092.08**

References:

Calumpang, S. M. F., Bayot, R. G., Vargas, D. G., Ebuenga, M. D., & Gonzales, P. G. (2013). Impact of intercropping lemon grass (Cymbopogon citratus Stapf.) on infestation of eggplant fruit and shoot borer (Leucinodes orbonalis Guenee) in eggplant (Solanum melongena L.). *Silliman Journal*, *54*(1).

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Riotte, L. (1998). *Carrots love tomatoes: secrets of companion planting for successful gardening*. Storey Publishing.

Photos and other addenda:

Early and late season photos of companion plant experiment. Marigold treatment is forefront in both photos.

Photos of the trap crop experiment. The first shows an uninfested plot with mizuna on the left and turnip on the right. The second photo shows a highly infested plot. Skeletonized mizuna can be seen on the left.