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EXAMINING THE EFFICACY OF COVER CROPS AS AN INTEGRATED PEST MANAGEMENT TOOL IN ORGANIC FARMS IN THE LOWER RIO GRANDE VALLEY

A Thesis

by

LILI M. MARTINEZ

Submitted to the Graduate College The University of Texas Rio Grande Valley In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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Major Subject: Biology

EXAMINING THE EFFICACY OF COVER CROPS AS AN INTEGRATED

PEST MANAGEMENT TOOL IN ORGANIC FARMS IN

THE LOWER RIO GRANDE VALLEY

A Thesis by LILI M. MARTINEZ

COMMITTEE MEMBERS

Dr. Rupesh R. Kariyat Chair of Committee

Dr. Alexis Racelis Committee Member

Dr. Pushpa Soti Committee Member

August 2020

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ABSTRACT

Martinez, Lili M., <u>Examining the Efficacy of Cover Crops as an Integrated Pest Management</u> <u>tool in Organic Farms of the Lower Rio Grande Valley.</u> Master of Science (MS), August 2020, 49 pp., 4 tables, 9 figures, 83 references.

In Chapter I, we review the current status of pest management in organic farming. We also review the components of plant defenses against insect herbivores, current management practices and how chemical ecology can be used as a tool to enhance pest management, especially in organic farming.

Chapter II discusses a two-year, four-season field experiment on the potential of various cover crops as a pest management tool for cash crops conducted at an organic farm in Edinburg, Texas. Manipulating cover crops as an integrated pest management strategy has recently gained popularity in both traditional and organic agroecosystems. However, little information is available for producers to make informed decisions about cover crop selection, management, and their potential use as a pest management tool. Our results indicate that cover crops support beneficial insects during the early summer season, while the time of growing season does not affect herbivore abundance. Crop-specific effects were found for herbivore abundance with possible cascading effects on insect community as well as damage levels on the subsequent cash crop, but without any impact on the growth traits of the cash crop. Together, our data suggests that cover crops, when carefully selected, can be an integral part of a pest management strategy for sustainable agriculture.

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CHAPTER I

INTRODUCTION

Organic farming

In 1949, the land area used for agricultural purposes made up 63% of the United States, but as of 2007 this has decreased to 51% (Nickerson and Borchers, 2012). However, this reduction of agricultural land has coincided with the increasing popularity of organic farming (USDA, 2016; Nickerson and Borchers, 2012). Both large- and small-scale producers have started to deviate from the use of synthetic fertilizers, herbicides, and pesticides due to the increased risk of chemical pollution affecting many animal species like fish, birds, and humans (Mahmood et al., 2016). For example, the well-documented pesticide DDT, Dichlorodiphenyltrichloroethane, used as an insecticide to control mosquito populations, has since been found to have negative effects on bird populations throughout the United States. DDT affects fish-eating bird eggs by thinning the shells, causing them to crack during brooding (Carson, 1962; Turusov et al., 2002). In general, synthetic pesticides have been found to cause side effects in the form of cancer or endocrine disruptors (Turusov et al., 2002). Organic farming excludes synthetic pesticides all together and instead aims at utilizing natural plant-based products such as neem oil, pepper sprays, or garlic sprays, and other commercially available products to combat herbivorous pests (Schmutterer, 1990; Zehnder et al., 2007). Although the detrimental effects of using synthetic chemicals and pesticides are eliminated in organic farming,

an increase in pest population is predicted, which cause significant damage to the crop/s and risk the introduction and establishment of more plant diseases (Andret-Link and Fuchs, 2005; Carter, 1962; Perilla-Henao and Cateel, 2016). This leads to both reduced yield and quality, thereby causing severe economic losses and discouraging farmers, making it difficult for them to refrain from using chemical pesticides (Oerke, 2006). Taken together, unless efficient non-synthetic pesticides are developed which incorporate sustainable, consistent, and continued resistance against pests and diseases, organic farming will continue to be a niche farming method, and not a norm.

Plant volatiles

Plant volatiles are high vapor pressure and low molecular weight organic compounds that are produced within plants through enzymatic reactions, and are categorized into several classes such as terpenoids, phenylpropanoids/benzenoids, fatty acid, and amino acid derivatives (Kariyat et al., 2012a; War et al., 2011). They are emitted from leaves, fruits, flowers, and roots and are produced by plants mainly as by-products of primary metabolism (Pare and Tumlinson, 1997). These volatiles are constitutively present and emitted from plants as part of their metabolic activities; however, both biotic and abiotic stresses can alter the amount and type of volatiles produced (qualitative and quantitative change; Kariyat et al., 2012a; Pare and Tumlinson, 1999). Insect herbivores, a major group of these biotic stressors, can induce plant volatiles which have been found to differ from mechanical wounding such as leaf tearing due to wind or artificial wounding methods (Howe and Jander 2008; Pare and Tumlinson, 1997; Reymond et al., 2000). The difference in these two types of damage responses are primarily due to the elicitors present in the saliva of insects that triggers the plants' defense mechanisms against the herbivores and the production of herbivore induce plant volatiles (HIPVs) and

underlying plant defense hormones- like jasmonic acid (JA) and salicylic acid (SA) (Kariyat et al., 2012b; Lopez-Larrea, 2012). The JA pathway is initiated by elicitors, found in the saliva of chewing insects, after mechanical damage is made through chewing or biting the plant (Mueller et al., 1993; Kariyat et al., 2012b). Meanwhile, the SA pathway is initiated by sucking damage from insects such as aphids or true bugs. As soon as the damage is done, elicitors will begin a reaction within the plant to produce or increase the quantity of specific volatiles. These constitutive and herbivore-feeding induced volatiles are made up of terpenoids, amino acids, fatty acid catabolites, and other compounds which vary depending on the type of plant (Pickett, 2014). These semiochemicals are then released by leaf tissues into the atmosphere to be used as signals by parasitoids, the natural enemies of herbivorous insects, to locate prey by attracting the attention of third trophic level insects (Kaur et al., 2020; Pare and Tumlinson, 1999). On the other hand, floral volatiles are produced as messages to pollinators to attract pollinators, and/or to encourage dispersal of pollen or seeds (War et al., 2011; Wright and Schiestl, 2009).

Plant volatiles mediated by direct (affecting host recognition; Kariyat et al., 2014) and indirect (attract predators and beneficial insects; Kariyat et al., 2012a; Kaur et al., 2020) defenses can be manipulated by organic farmers as a pest management tool. This can be achieved by implementing crops that are favorable (producing volatiles) to parasitoids and natural predators and offer shelter and hosts such as agricultural pest insects (Altierr and Schmidt, 1986; Kaur et al., 2020). The addition of cover crops on otherwise fallow land increases the presence of insects including beneficials that can potentially reduce the incidence of pests, therefore reducing crop damage (Hawkes, 2016; War et al., 2011). Taken together, these methods can collectively enhance benefits of organic farming in a sustainable way.

Integrated pest management

Integrated pest management (IPM) approaches consist of a systematic plan that requires frequent insect population evaluations of crops throughout the growing season to assess pest presence and aims at reducing pest numbers (Barzman et al., 2015; Chandler et al., 2011; IPM Institute of North America, 2018; United States Environmental Protection Agency, 2019). Procedures followed during IPM assessments include identifying pests, monitoring pest populations and activity, setting threshold limits, and implementing a plan for action in the case that pest populations become problematic (Barzman et al., 2015; IPM Institute of North America, 2018; United States Environmental Protection Agency, 2019). There are multiple methods of controls such as biological, cultural, mechanical, and chemical. Biological controls require the implementation of additional crops to attract the natural predators of pests (Chandler et al., 2011; Cook et al., 2007; IPM Institute of North America, 2018). Cultural controls can be implemented by manipulating the crop layout or changing irrigation practices in an effort to mitigate pests and diseases (Barzman et al., 2015; IPM Institute of North America, 2018). These two control methods are implemented in an organic farm by incorporating multiple cover crops and assessing their efficacy in reducing pest presence and damage to the subsequent cash crops (Chandler et al., 2011). Mechanical controls utilize physical barriers, unfavorable conditions, or traps to kill or keep pests away from crops (Chandler et al., 2011; IPM Institute of North America, 2018). Chemical control methods utilize pesticides, synthetic and/or natural, as sprays or bait stations to kill pests (Chandler et al., 2011; Cook et al., 2007; IPM Institute of North America, 2018; Kogan, 1998). Control methods can also be used in combination to amplify their effectiveness in reducing the presence of pests (Chandler et al., 2011; IPM Institute of North America, 2018; United States Environmental Protection Agency, 2019).

Cover Crops

Cover crops have become implemented in sustainable cropping systems and they can be leguminous plants, cereals, broadleaf plants, forage grasses, or mixes that provide benefits and improve the soil for future cash crops (Gfeller et al., 2018; Hartwig and Ammon, 2002; Snapp et al., 2005). Their benefits include nutrient enrichment, improved water retention, preventing erosion, eliminating weeds, helping control pests and diseases, and increasing biodiversity (Delgado et al., 2007; Fageria et al., 2005; Gfeller et al., 2018; Sarrantonio and Gallandt, 2008; Schipanski et al., 2014; Snapp et al., 2005). The crops are terminated by being plowed into the soil before flowering (Hartwig and Ammon, 2002). This improves the cash crop fitness in the first year and the benefits continue accumulating in future years, especially when implemented consistently (Delgado et al., 2007). The selection process depends on the preceding cash crop, time, and location (Schipanski et al., 2014). Leguminous cover crops, grown mostly in summer, offer increased nitrogen availability for cash crops, while non-leguminous cover cops are typically useful in removing excess nitrogen (Snapp et al., 2005). Mixes of cover crops can also be implemented as they provide multiple benefits at once. Another indirect benefit of cover cropping is an increase in pollinator diversity, as well as herbivore suppression (Kaur et al., 2020; Long et al., 1998; Muneret et al., 2018; Wang et al., 2008). Cover crop varieties include sorghum sudangrass, cowpea, and sunn hemp which are grown in the summer due to their hardy drought tolerant characteristics (Adler and Chase, 2007; Kaur et al., 2020; Mansoer et al., 1997; Soti and Racelis, 2020).

Although plant volatiles, cover cropping, and integrated pest management practices have been individually tested in filed and lab conditions, we still lack a complete understanding on how they can act in tandem. In this study we examined the efficacy of cover crops as a pest

management tool using organic farms in Lower Rio Grande Valley as our experimental plots. Our results will provide a direct benefit to the farming community in making better informed decisions when it comes to cover cropping.

Note: <u>Chapter II is published</u> as "Martinez, L., Pushpa, S., Kaur, J., Racelis, A., and Kariyat, R. (2020). Impact of cover crops on insect community dynamics is both cover and cash crop species dependent. *Agriculture, 10*(6), 209." doi: 10.3390/agriculture10060209

CHAPTER II

IMPACT OF COVER CROPS ON INSECT COMMUNITY DYNAMICS IS BOTH COVER AND CASH CROP SPECIES DEPENDENT

Abstract

Manipulating cover crops as an integrated pest management strategy has recently gained popularity in both traditional and organic agroecosystems. However, little information is available for producers to make informed decisions about cover crop selection, management, and their potential use as a pest management tool. To address this, we conducted a two-year, fourseason field experiment on the potential of various cover crops during the summer seasons of both years, followed by monocultures of cash crops during winters. We hypothesized that the cover crop treatments would attract beneficial insects and repel damaging herbivores in a species-specific manner, and the insect community dynamics would be bridged to the cash crops in the subsequent season. In addition, we hypothesized that cash crops would suffer lower herbivory damage following specific cover crop treatments. Our design comprised of three cover crops. Our results indicate that cover crops support beneficial insects during the early summer season, while the time of growing season does not affect herbivore abundance. Crop-specific effects were found for herbivore abundance with possible cascading effects on insect community as well as damage levels on the subsequent cash crop, but without any impact on the growth

traits of the cash crop. Together, our data suggest that cover crops, when carefully selected, can be an integral part of a pest management strategy for sustainable agriculture.

Keywords: cover crops; organic farming; biocontrol; beneficial insects; herbivores

Introduction

Harnessing natural predator–prey interactions to limit pest populations is one of the major goals of insect biological control, and farmers have employed both augmentative and classical strategies to increase beneficial insects in their cropping systems. This includes the use of beneficial organisms such as predatory insects and mites, parasitoids, beneficial nematodes, and other microbial agents (Bale et al., 2007; Rodriguez-Saona et al., 2012). In addition, increasing plant diversity in cropping systems can also play an important role in protecting and managing crops from herbivore damage, by increasing both natural enemy abundance and diversity, which in turn can suppress pest populations (Rodriguez-Saona et al., 2012). These community ecologybased pest management strategies can be mediated through plant volatiles, and their induction through herbivore damage (herbivore induced plant volatiles; HIPV) as a cue for predators and parasitoids through tritrophic interactions (Kariyat et al., 2012a), and also as a repellant for some herbivore species (Unsicker et al., 2009). However, these interactions are often species specific, and also depend on other contributing factors such as age, location, and health of the plants, just to name a few (Reicosky et al., 1998; Fageria et al., 2005).

Cover crops, or the intentional planting of non-cash crops for agroecosystem services such as weed suppression and water management (Lu et al., 2000), is a common method that has been found to increase above- and below-ground species diversity in agricultural farms. In addition to these commonly perceived benefits, cover cropping has also been found to serve as

an integrated pest management tool, attracting beneficial insects, thereby acting as an indirect plant defense (Bugg et al., 1991; Bugg and Waddington, 1994; Creamer and Baldwin, 2000; Tilman, 2004; Lundgren and Fergen, 2010). For example, Creamer and Baldwin (2000) found that Sesame (*Sesamum indicum*), in addition to outcompeting weeds, also attracted beneficial insects, clearly demonstrating the multiple benefits of cover crops. More interestingly, in a California organic vineyard system, the planting of summer cover crops buckwheat (*Fagopyrum esculentum* Moench) and sunflower (*Helianthus annus* Linnaeus) resulted in the reduced density of leafhoppers and thrips and increased the numbers of predators, including spiders, and *Anagrus epos* Girault (Hymenoptera: Mymaridae), a major leafhopper parasitoid (Nicholls et al., 2001). Proper incorporation of cover crops into the cropping system can have an added benefit of pest management along with soil fertility management and weed suppression, although this warrants additional empirical testing in a wide range of cover crop species across various cropping systems (Lu et al., 2000; Vukicevich et al., 2016).

Ideally, a cover crop should include species that provide nectar, pollen, refuge for insects and maintain relatively high populations of predators and parasitoids (Long et al., 1998), in addition to building healthy soils and providing weed control through their allelopathic properties (Hoffman et al., 1996). Selecting the right species of cover crop has been reported to reduce the dependency on synthetic chemical treatments (herbicides and insecticides), especially in organic systems where these options are inherently limited. However, these cover crops may also potentially serve as a refuge and alternative host for herbivorous insects and can exacerbate pest populations, consequently nullifying any on-farm benefits received from cover cropping (Bugg et al., 1991; Long et al., 1998). Furthermore, habitat complexity (mono vs. polycultures) (Asmah et al., 2016) can lead to reduced predator abundance and foraging efficiency. For

example, studies have found that both predators and parasitoid populations can be affected by habitat complexity, such as that of polycultures (Stamps and Linit, 1997; E Crews et al., 1997) when compared to monocultures (Costello, 1995; Bryant et al., 2013; Legrand and Barbosa, 2003). Among all these expected benefits, the cover crops should also provide these traits to the cash crop, including any additional yield gain through better pest management or improved nutrient status (Kaye and Quemada, 2017). However, it is quite clear that cover cropping systems are heavily dependent on species specificity, and any given benefits in one system are not necessarily reproducible in another. In addition, due to a dearth of studies showing the susceptibility of cover crops in harboring insect pests of subsequent cash crops (Bugg et al., 1991; Long et al., 1998; Costello, 1995; Bryant et al., 2013), special attention is needed in selecting appropriate species that can potentially serve as insectaries supporting beneficial insects while staying neutral or repelling herbivores (Bryant et al., 2013). Since herbivores, predators, and pollinators use plant-derived volatiles as olfactory cues (in addition to other cues), expecting a cover crop to selectively attract predators, pollinators, and natural herbivore enemies while repelling the damaging herbivores is tricky to put in practice (Costello, 1995; Bryant et al., 2013). More importantly, we lack enough field studies that have tested these effects with covercash crops and their specificity in attracting and repelling natural enemies and herbivores, respectively. Results from these studies can assist in the selection, management, and evaluation of cover crops and cash crops, and cover crop-mediated and integrated pest management for cropping systems.

To address this, we conducted a two-year, four-season field experiment on the potential of a cover crop and cash crop duo on insect community dynamics (beneficial insects including predators, parasitoids, pollinators, and herbivorous insects) in a certified organic cropping system in the Lower Rio Grande Valley (LRGV). Due to the subtropical climate with hot summers and mild winters, and high relative humidity, LRGV has always been a sweet spot for insects. This makes pest management a primary concern for the two million acres or more of agricultural land in the area, but also provides a unique opportunity to understand the mechanisms underlying a wide range of plant–herbivore–pollinator interactions, and devise management practices that have broad appeal for tropics and sub-tropic agroecosystems elsewhere. The cover crop species were selected based on their ability to successfully grow and develop in the warm and subtropical conditions pertinent to the study location. Specifically, we asked the following questions: i) Can cover crops enhance the biological control of insect pests?; ii) Are there species-specific effects of cover crops on the insect community dynamics?; and iii) Do these effects, if any, translate into enhanced pest management or yield increase for the subsequent cash crops?

Materials and Methods

Study Site

This study was conducted on a certified organic vegetable farm located in Edinburg, Texas (26°15′59.3″ N 98°05′28.1″ W) in the LRGV. The farm had been managed under organic farming specifications for the past nine years under a diverse rotation of organically grown crucifers (beets, cabbage, cauliflower, kohlrabi, kale), cucurbits (squash, zucchini, pumpkin, cucumber, watermelons), tomatoes, okra, and eggplants. Farm soil was sandy loam with 0.6% soil organic matter, 0.10% total N, 55.33 ppm total P, and soil pH 8.30. Average temperature during the summer and fall of the 2017 study period was 28.7 °C and 22.2 °C, respectively. Average temperatures during the summer and fall of the 2018 study period were 30.7 °C and 21.1 °C, respectively.

Cover Crop Treatments and Experimental Design

The cover crop treatments included in this study were selected for their relevance to summer conditions (extremely hot, dry) of the region, and included (1) sunn hemp (SH) (*Crotalaria juncea*), a legume extensively used as a green manure crop in the tropical and subtropical regions; (2) sudangrass (SG) (*Sorghum drummondii*), a high-biomass grass used for increasing soil organic matter with weed-suppression potential; (3) Chinese red cowpea (CP) (*Vigna unguiculata*); (4) mix of the three (Mix). Certified organic seeds of sudangrass and sunn hemp were purchased from Johnny's Seed Company (Fairfield, Maine) and cowpea seeds were purchased from Petcher Seeds (Fruitdale, AL; for seeding rates and cover crop details, see Table 1). In June 2017 and 2018, after the spring vegetable growing season, an area approximately 0.85 ha was disked and divided into 20–35 m × 7 m with a 1-m buffer space between, delineated with a rototiller. Each cover crop treatment (SH, SG, CP, Mix) and the control fallow were completely randomized during both the years. This experimental design (including four treatments and control) was replicated four times (see Table 2 and Figure 1).

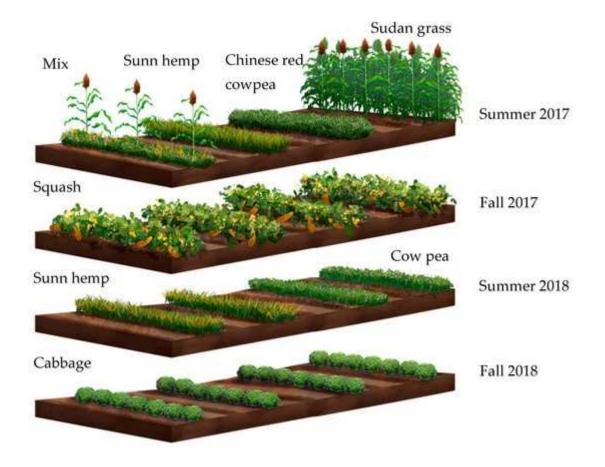


Figure 1. Schematic of planting summer and fall of 2017 and 2018. Summer of 2017 consisted of a mix of cover crops (sudangrass, sunn hemp, and cowpea), sunn hemp, Chinese red cowpea, and sudangrass plots. Fall of 2017 shows the zucchini cash crop. Summer of 2018 shows the cover crops sunn hemp and cowpea. Lastly, fall of 2018 shows the cabbage cash crop. Cartoon by Annette Diaz.

Table 1. Cover crop	o treatments,	species, cro	o types, a	and seeding 1	ate used in the study.
					·····

Cover crop treatment	Crop type	Seeding rate (kg/ha) 45	
Sudangrass (SG)	Grass		
Cowpea (CP)	Legume	28	
Sunn hemp (SH)	Legume	45	
Mix (Sudangrass + Cowpea + Sunn hemp)	-	16+ 10+ 16	
Control (C)	-	-	

Table 2. Summary of different crop treatments, traits observed for data collection and time of data collection with respect to the season and year are outlined. CP, SH and SG are abbreviations used for Chinese red cowpea, Sunn hemp and Sudangrass respectively. Years 1 and 2 refer to years 2017 and 2018, respectively.

Year	Season	Crop	Frequency	Data collected	
1	C	Cover crops: CP,	Early/late in	Herbivores and Beneficial	
1 Sum	Summer	SH, SG, Mix	season	insects	
	E-11	Cash aron: Sayash	Early/late in	Harbiyara damaga	
	Fall	Cash crop: Squash	season	Herbivore damage	
2 Sumn	Summer Cover crops: CP and SH	Cover crops: CP	Early/late in	Plant height, Herbivore	
		and SH	season	damage, Biomass	
	Fall	Cash crop: Cabbage	Early/late in	Herbivores and Beneficial	
			season	insects	

Planting and Termination

Cover crop seeds were planted using a hand-held seeder (Scotts Handy Green II®; Marysville, OH, USA) at recommended rates (see Table 1). All blocks and all treatments (including control) were flood-irrigated twice during the study (immediately after planting and three weeks after planting when the plants showed signs of water stress). All treatments were terminated using a flail mower at the end of 8 weeks after planting in both the years.

Insect Community Collection

Insect community data were collected from all the treatment blocks in 2017 and only from the leguminous cover crops (cowpea and sunn hemp) in 2018. Insect community data were sampled using three different techniques: sweep net (11" diameter), pit fall traps (8 fluid ounce plastic cups with 3/4ths of water and a drop of odorless detergent to break surface tension; [Kariyat et al., 2012]), and unbaited sticky traps (Pherocon® AM Yellow; Trece, Inc., Adair, OK, USA). Pit fall traps and sticky traps were installed randomly in each treatment plots on 15 August (7 weeks after planting). This method allowed us to collect both flying insects and soil dwellers to get a better understanding of the insect community associated with the system, rather than selectively collecting one or the other. All the collected insects were retrieved after 72 h (a total of 20 pitfall and 20 sticky traps). For sweep net collection, each of the cover crop treatments was swept (20 sweeps/plot) just before cover crop termination. The rationale behind using three different methods of sampling was to obtain comprehensive coverage of the community including insects and other arthropods at canopy and soil levels, including fliers and crawlers (for details, see Kariyat et al., 2012; 2018; Kaur et al., 2020). After the experiment, all sampled

insects were identified and grouped into guilds based on their functional roles: herbivores, predators, parasitoids, and pollinators. The summer of 2018 consisted of the same cover crops as the previous year but only sunn hemp and cowpea were observed since other cover crops had delayed germination and were patchy in growth. Data collection of summer 2018 consisted of two insect damage assessments and a sweep net method before termination. Damage was measured for 50 randomly chosen plants in each of the 3 sunn hemp and 3 cowpea plots for a total of >300 plants. Damage was measured visually on a scale of 0 to 4, 0 indicating no visible damage to the plant while a 4 indicated physical damage was present on almost all leaves of the plant (see Kaur et al., 2020 for methodological details). Insect collection using the sweep net method consisted of sweeping an insect net (11' diameter) three times over the cover crop plot of each of the six chosen fields plus three control plots and placing the contents into a mason jar containing 70% isopropyl alcohol for further storage and identification.

Cash Crop Planting & Pest Damage

In both 2017 and 2018, after two weeks of cover crops termination, all the experimental plots were disked to incorporate the cover crop biomass residue and then bedded into rows to prepare for fall planting. In 2017, each cover crop plot was divided into two equal plots for the cash crops: zucchini (*Cucurbita pepo*) and bush beans (*Phaseolus vulgaris*). Seeds of organic zucchini and bush beans were planted in rows as traditionally done by growers (row hills were 1.2 m apart and about 0.5 m between plants). The cash crop plants were drip irrigated and the edges around the treatment plots were hand-weeded as necessary. Since the bush bean plants did not suffer heavy insect damage, pest damage estimation was only done for zucchini. A visual pest damage estimate was conducted three times (as detailed above), twice for damage on plants and once for damage on fruit (zucchini). In 2018, the cash crops consisted of cabbage (*Brassica*)

oleracea var. *capitata*), cilantro (*Coriandrum sativum*), and kale (*Brassica oleracea* var. *sabellica*). Rows were distanced by 1 m and plants were sown 0.5 m apart. Drip irrigation was implemented after sowing. Two rows consisting of cilantro and cabbage were sown on opposite widths of the plot while seven rows of kale were sown in the center. Data collection consisted of pit fall cup setups and visual damage assessments. Pit fall cups were placed throughout the field at two months after sowing and three months after sowing with a total of 45 cups per replication.

Statistical Analysis

The statistical analysis was performed using generalized linear models (GLM). All analyses were carried out using Minitab 19® (by Minitab LLC) and plots were built using Graphpad Prism (GraphPad Software, CA, USA). For year one, we examined the total insect abundance as well as their separation according to functional roles across different cover crop treatments during the cropping season using GLM and Tukey's post-hoc tests. For this, we included time, cover crop species, and their interaction as factors, while the mean number of total insects, beneficial insects and herbivores were the response variable. For the damage levels recorded on cash crops (year 1), time and previous year cover crop plots were included as factors, while the damage levels were the response variables.

Since cowpea and sunn hemp were the main cover crops that showed promise, in the second year we focused on these two by examining their growth and damage levels. The data were analyzed with time and cover crop species, and their interactions were factors, plant height and damage levels as response variables, followed by Tukey's post-hoc tests. Cover crop biomass data at the end of the growing season was analyzed using a two-tailed t-test to test for significance between the two species. In the fall of year two among the cash crops planted, only cabbage survived, and we used similar analyses (GLM and Tukey's post-hoc tests) to examine

whether the cover crops from the previous season had any effect on both biomass and insect community dynamics on the cash crop.

Results

For the summer of year 1, when cover crops were planted, we found no significant difference among the three cover crop species and their mix for the total number of insects collected in the traps when pooled for each treatment (P=0.286; Table 3). Interestingly, a similar trend was also found for both the early season as well as the late season collections (P=0.598, F=0.053; Table 3). Additionally, we also separated the insects found in the traps into different groups based on the functional roles: herbivores such as white fly (Bemisia sp.; Hemiptera) and armyworm (*Pseudaletia unipuncta*; Lepidoptera), and beneficial insects (predators, parasitoids, and pollinators) such as big-eyed bug (Geocoris sp.) and parasitoid wasps (Cotesia sp.) (See appendix Table A1 for details). Our results suggest that beneficial insects significantly varied during the different growth periods (P=0.025, F=4.98; Table 3), as they were found significantly more during the early season of summer 2017 as compared to mid-season. However, this effect was not observed during other seasons or with respect to different crops (P= 0.387, F= 1.13; Table 3). On the contrary, we did not find any significant difference for the prevalence of the damaging herbivores with respect to the growth period during Summer 2017 (P=0.199, F=1.83; Table 3). However, it is interesting to note that damaging herbivore numbers were found to significantly vary for the different cover crops (P=0.026, F=3.93; Figure 2; Table 3). We found that herbivores were significantly lower on the treatment with a mix of sorghumsudangrass, sunn hemp, and cowpea as compared to control without any cover crops. However, none of the cover crops showed any significant difference among themselves for herbivore attraction (Tukey's post-hoc tests; Figure 2; Table 3). Taken together, cover crop insect

community dynamics showed little effect due to both growth season variation and cover crop species, except for beneficial insects which were more prevalent in the early season of cover crop growth.

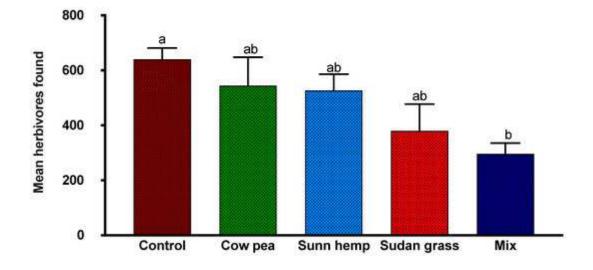


Figure 2. Results of mean herbivores abundance by different cover crop treatments during Summer 2017. Data was analyzed using Generalized Linear model and means were separated using post hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

Time	Trait	Test statistic	P value
2017 Cover crop	Total insects (Time)	F=0.53, df=2	0.598
	Total insects (Crop)	F= 1.41, df= 4	0.286
	Beneficial insects (Time)	F= 4.98, df= 2	0.025
	Beneficial insects (Crop)	F=1.13, $df=4$	0.387
	Herbivores (Time)	F= 1.83, df= 2	0.199
	Herbivores (Crop)	F=3.93, df= 4	0.026
2017 Cash crop	Damage (Early season)	F=4.36, df=4	0.015
	Damage (Mid season)	F= 1.58, df= 4	0.23
	Damage (Late season)	F=0.26, df=4	0.896
2018 Cover crop	Height (Time)	F=117.51, df=1	0.00
	Height (Crop)	F=617.74, df=1	0.00
	Height (Time* Crop)	F=53.73, df=1	0.00
	Damage (Crop)	F= 0.09, df= 1	0.762
	Damage (Time*Crop)	F= 225.83, df= 1	0.00
	Biomass	<i>t</i> = 3.96, df= 31	0.00
2018 Cash crop	Biomass	F=4.6, df=2	0.013
	Total insects (Time)	F= 0.37, df= 1	0.548
	Total insects (Cover crop)	F= 8.59, df= 2	0.002
	Cash crop	F=1.14, df=2	0.337
	Beneficial's (Time)	F= 0.74, df= 1	0.397
	Cover crop	F= 2.6, df= 2	0.095
	Cash crop	F=0.31, df=2	0.735
	Herbivores (Time)	F= 0.92, df= 1	0.348
	Herbivores (Cover crops)	F= 9.17, df= 2	0.001
	Herbivores (Cash crop)	F= 1.98, df= 2	0.16
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Table 3. Results of statistical analyses to examine the effects of cover crops, time of season and their interaction on the insect diversity, damage levels and growth traits in cover crops and cash crops over two years and four seasons.

Asterisk (*) denotes the interaction between two treatments. Bold values represent statistically significant effects at P < 0.05.

Following this, we performed the damage assessment on the cash crops (zucchini/squash) planted in fall 2017, after the termination of the cover crops to examine any remnant effects of the previously sown cover crops. We found that the herbivory incurred on the cash crops significantly varied by the growing season of cash crop. Specifically, observing the foliar damage levels at three time points during the growing season, we found significant variation in the damage levels during the early season (P= 0.015, F= 4.36; Figure 3; Table 3); however no difference was observed during the mid and late season (P= 0.23, F= 1.58; Figure 3; Table 3; P= 0.896, F= 0.26; Figure 3; Table 3). More precisely, during the early season of fall 2017, the squash crop grown in cowpea plots suffered significantly higher damage compared to squash grown in sudangrass and sunn hemp plots. While, damage levels did not vary significantly for the squash plants in the mix and control plots and their comparison to the squash plants grown in sorghum sudangrass, sunn hemp and cowpea plots (Tukey's post-hoc tests; Figure 3; Table 3).

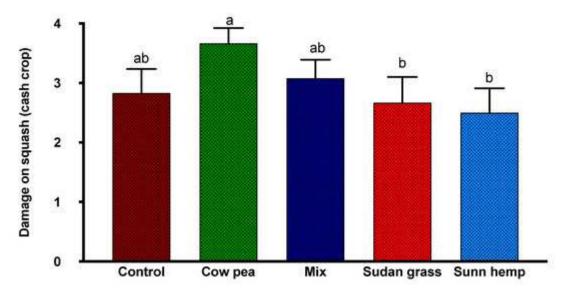


Figure 3. Results of mean damage levels on cash crop (squash) during Fall 2017 based on previous cover crop. Data were analyzed using generalized linear model and means were separated using post-hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

During the second year (summer 2018), we further recorded damage levels in the cover crops to understand the effect of cash crops on the subsequent cover crops in the next season. Our analysis showed a significant interaction between the cover crop and the time of the season or growth stage for the damage levels incurred on the plants following the cash crop (P=0.000, F= 225.83; Figure 4; Table 3). Furthermore, post hoc pairwise comparisons showed that early season cowpea and late season sunn hemp suffered significantly higher damage levels than early season sunn hemp and late season cowpea (Tukey's post-hoc tests) but the pairs did not differ among each other. We also found that early season cowpea suffered higher damage than late season cowpea while early season sunn hemp has lower damage than the late season sunn hemp (P= 0.000; Figure 4; Table 3). In addition, as expected, sunn hemp clearly displayed significantly higher growth traits than cowpea. Sunn hemp grew taller and gained higher biomass than cowpea during the summer 2018 (Two tailed t-tests; P= 0.000; Figure 5; Table 3; Two tailed t tests; P= 0.000; Figure 6; Table 3). In contrast, we did not find any significant difference in biomass of the cash crop cabbage grown in both sunn hemp and cowpea plots. However, the cabbages in the sunn hemp plots had significantly higher biomass than the control plot (P=0.013, F=4.6; Figure 7; Table 3).

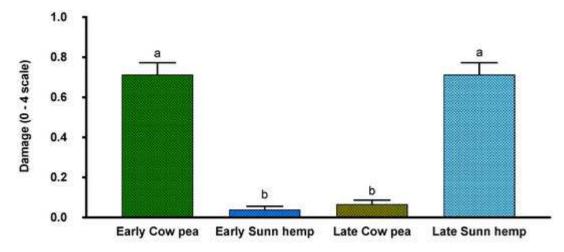


Figure 4. Results of damage levels on the cover crops during early and late season during summer 2017. Data were analyzed using generalized linear model and means were separated using post-hoc tests at a P value of 0.05. Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

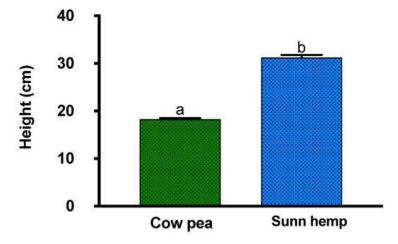


Figure 5. Results comparing mean height of cover crops during the summer 2018. Data were analyzed using generalized linear model and means were separated using post-hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

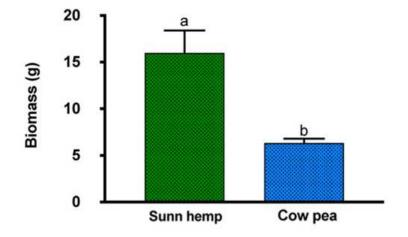


Figure 6. Results comparing mean biomass of cover crops during the summer 2018. Data were analyzed using generalized linear model and means were separated using post-hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

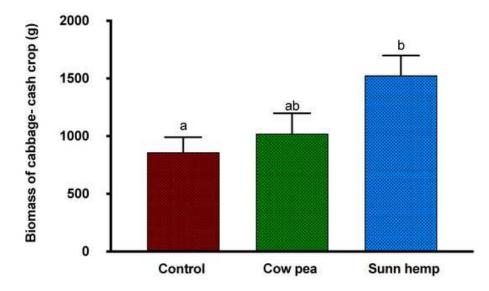


Figure 7. Results comparing mean damage levels on cash crop (squash) during Fall 2018 based on previous cover crop. Data were analyzed using generalized linear model and means were separated using post-hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05. Details are included in Table 3.

Finally, we also assessed the effects from previous season cover crops on the insect community associated with the only surviving cabbage cash crop. These analyses revealed no significant difference between the early and late season cash crops for total number of all the insects, beneficial insects and damaging insects (P= 0.548, 0.397, 0.348, F= 0.37, 0.74, 0.92; Figure 8a–c, respectively; Table 3). However, cabbage grown following cowpea planting attracted significantly more number of total insects, followed by cabbage grown after sunn hemp and the least number of insects were found on cabbage grown on control plots (P= 0.002, F= 8.59; Figure 9a; Table 3). Additionally, we also found that the damaging herbivores had a significantly higher affinity for both cowpea and sunn hemp, compared to the control plants (weedy plots mainly with pigweed, *Solanum elaeagnifolium*, cowpen daisy, and sunflower) (P= 0.001, F= 9.17; Figure 9b; Table 3; Tukey's post-hoc tests). Also, the mean number of beneficial insects did not significantly vary for any of the three treatments (P= 0.095, F= 2.6; Figure 9c; Table 3).

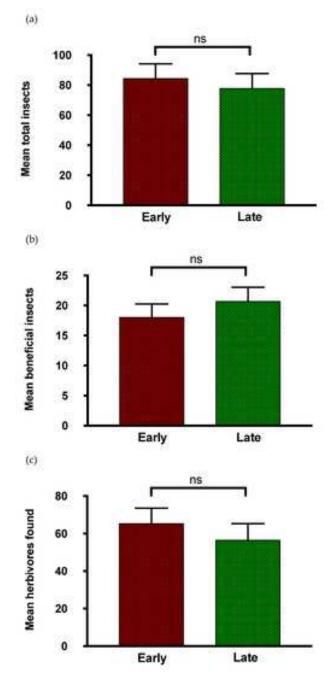


Figure 8. Results of the insect abundance (based on their functional role) at the early and late season during the year 2 (2018). (A–C) represent mean abundance of total insects comprising of (a) all the insects irrespective of their taxonomic orders, (b) beneficial insects- including predators, parasitoids and pollinators and (c) herbivorous insects collected. No significant difference was found for total, beneficial, or herbivorous insects when compared early and late during the season. Data were analyzed using a generalized linear model and treatment means were separated using post-hoc tests at a *P* value of 0.05. Different treatments are represented in difference of the graph. Bars with different lowercase letters denote statistically significant differences at *P* value of 0.05, while ns denotes non-significant results. Details are included in Table 3.

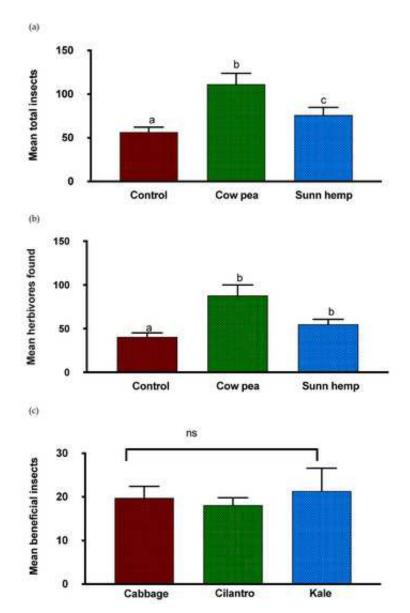


Figure 9. Year 2 insects by crop: Results comparing the insect abundance (based on their functional role) by cover crop during the year 2 (2018). (A- C) represents mean abundance of total insects, beneficial insects and herbivores, respectively by cover crop in year 2. Data was analyzed using Generalized Linear model and means were separated using post hoc tests at a P value of 0.05 Different treatments are represented in different colors in the graph. Bars with different lowercase letters denote statistically significant differences at P value of 0.05, while ns denotes non-significant results. Details are included in Table 3.

Discussion

This study was conducted to examine the effect of sorghum sudangrass, cowpea, sunn hemp, and their mix on the insect community dynamics in the field and whether their effects cascaded to the cash crop grown during the following season upon their termination. Overall, we found that crop specificity played a significant role in regulating the herbivore abundance in this cropping system, while the natural enemies' (predators and parasitoids) abundance was largely influenced by temporal variation during the same season.

Insect abundance during summer 2017 remained unaffected by the presence of different cover crop treatments and their growing period (time) during the season. Amongst these, beneficial insects comprising of predators, parasitoids, and pollinators were found to be more abundant earlier rather than later in the season, while no crop-specific effect was observed on their abundance. As plants emit a unique blend of volatile compounds induced upon herbivory, also known as herbivore-induced plant volatiles (HIPVs) (Kariyat et al. 2012; Kaur et al., 2020; Pare, 1999; Kariyat et al., 2014; Dicke and Baldwin, 2010), studies suggest that HIPVs can selectively attract the natural enemies like predators and parasitoids of the insect pests feeding on the host plant. For instance, a United States Department of Agriculture study suggested that sorghum sudangrass, upon herbivory by aphids, attracts their predators while cowpea, upon damage by stink bugs, leaf-footed bugs, and aphids, can attract parasitoid wasps and other beneficial insects (29). Interestingly, in our experimental traps, we found Cotesia sp. (Hymenoptera), an important braconid wasp that parasitises two major cabbage herbivores, *Trichoplusia ni* and *Pseudaletia unipuncta* (Lepidoptera) (Grasela et al., 2008; Capinera, 2012). Besides cabbage, P. unipuncta can damage cereals (Gramineae) like Sorghum sp. (Capinera, 2012). Our traps also collected various predatory bugs (Hemiptera) and spiders (Arachnae)

which can forage on *P. unipuncta* (Capinera, 2012). Egg parasitoid green lacewing (*Chrysoperla* carnea; Neuroptera), also known to predate on the whitefies (Bemisia argentifolii), was found in the traps (Legaspi et al., 1996; Inbar and Gerling, 2008). Therefore, the early season can be the most favorable for biocontrol of prevalent herbivores in this particular cropping system. In addition, intercropping is known to affect the host location of a suitable plant by the insect pest. For instance, Mansion-Vaquie (2019) reported the increased time taken by cereal aphids (Sitobion avenae) to locate wheat plants (Triticum aestivum) in a diversified cropping system than in a monoculture. So, it is possible that herbivore abundance remained constant during the early and the late season in the course of locating and feeding on the appropriate host plant. These results can have major implications in devising integrated pest management strategies for growing cash crops in the next season. However, the temporal influence on the insect diversity during the same season warrants additional examination of herbivore-induced plant chemistry, which can certainly play a major role in influencing the insect community dynamics in the field (Kariyat et al., 2012; Kariyat et al., 2018; Kaur et al., 2020; Pare, 1999; Kariyat et al., 2014). Our results suggest that herbivores had a more-or-less uniform affinity to the cover crops throughout the summer season, while the beneficial insects were mostly found in higher numbers during the early season.

In addition, significant variation in the selective herbivore affinity to the cover crops can be related to the collective effect of intercropping along with HIPVs. Past studies suggest that HIPVs released in a specific plant-insect system can also repel the herbivores. For instance, HIPVs induced in *Spodoptera frugiperda* (Lepidoptera) fed maize, a gramineae crop like sorghum sudangrass, have been found to repel the adult moths of the pest (War et al., 2011). Although statistically insignificant, we still found the lowest herbivores in the mix treatment

followed by the individual crops, and highest in the control. This reinforces the difficulty in host selection faced by the insects in a mixed system, possibly due to volatiles overload and mixing from multiple species and concentrations (Himanen et al., 2015; Leslie et al. 2020). Similarly, a study recorded lower aphid movement to the potatoes in onion intercropped fields (Ninkovic et al., 2013). However, these effects were not bridged to the next cash crop; no significant difference was recorded for damage levels on squash grown in control and mix plots. Therefore, our data do not support using mix treatment in cover cropping. Additionally, it involves an increased workforce, difficulty in carrying out pest control, and intercultural farm operations using mechanized implements and farm machinery, making it possibly uneconomical.

During year 2, our data indicated that squash plants grown in cowpea plots suffered the highest damage levels when compared to both sunn hemp and sorghum sudangrass plots. In fact, cowpea grown after the termination of squash again displayed the highest damage levels. Therefore, a possible plant morphological effect on the insect herbivory can be speculated in this case. As cowpea is a short cover crop when compared to tall growing sunn hemp and sorghum sudangrass (Mansoer et al., 1997; Taiwo and Akinjogunla, 2006), it is plausible that the insect pests harbored by cowpea's short canopy were bridged to the squash plants, another short-statured crop, in the following season and back to cowpeas grown in the next season. This reinforces our speculation that a unique microclimate due to a shorter canopy of the crop plants (both cowpea and squash) is a possible explanation for these results. This is known as the green bridge effect (Hickman, 2019), where the cover crops are expected to influence the insect diversity of the cash crop in the subsequent season. However, contrary to the expectations, the green bridge effect also has the risk of spreading insect pests to the cash crop (Hickman, 2019), where cover crops serve as a refuge in an otherwise bare fallow period.

Our results indicate that cowpea can potentially serve as a trap crop to attract and kill the prevalent insect pests in the field, similar to push-pull-based biocontrol [Leslie et al. 2020;,Khan et al., 2010; Ezzat et al., 2020), but this area warrants further examination. Furthermore, significantly high damage levels recorded on the early season cowpea during the second year reinforces the speculation of temporal effects on the insects prevalent in the area. With the progress of the summer season accompanied by a rise in ambient temperature, there was a significant decrease in the damage levels on the crop. However, significantly higher damage levels were observed on the sunn hemp later in the season, suggesting species-specific effects, as observed throughout the experiment. Additionally, we observed a positive effect of both cowpea and sunn hemp on the growth traits of our cash crop cabbage grown in the following season. Cabbage grown on the cover crop treatment plots had significantly higher biomass than the control cabbage. This is consistent with the benefits of cover crops reported in the earlier studies (Kaur et al., 2020; Soti and Racelis, 2020; Murrell et al., 2019). It is also important to note that there was a significant difference between the height of the two cover crops, with sunn hemp being taller than cowpea (Mansoer et al., 1997; Taiwo and Akinjogunla, 2006). This growth pattern might be beneficial and less of a concern for resource partitioning by growing sunn hemp in a cover cropping system alongside others. For instance, certain cover crops are known to have allelopathic effects which can hamper the growth of neighboring plants or voluntary plants (e.g., weed species) in the vicinity of the allelopathic plant (Bewick et al., 1994; Saha et al., 2018).

To conclude, our results are in congruence with other studies which collectively demonstrate that intercropping different cover crops can influence the insect diversity during the cropping season (SARE, 2020; Mansion-Vaquie et al., 2019; Himanen et al., 2015; Leslie et al. 2020; Ninkovic et al., 2013; Ezzat et al., 2020; Soti and Racelis, 2020) and bridge a few, if not

all, insects and their benefits, if any, to the subsequent cash crop (Hickman, 20199). However, it is important to plan and select appropriate cover crops for maximum benefits that are both species- and location-specific. Instances have been reported where plant volatiles have served as cues for herbivores and thus lead to additional herbivory (War et al., 2011). The results of this study also demonstrate how the effects of different cover crops on the insect community dynamics and growth benefits vary when transferred to the cash crops grown in the same field in the next season. Most importantly, our results have highlighted the need for a detailed examination of the effect of microclimates modified by a particular crop species in relation to the macroclimate of the entire field, having a significant impact on the insect community dynamics and thereby the major cash crops.

Author Contributions

Conceptualization, R.R.K., P.S., and A.R.; methodology, L.M., R.R.K. and P.S; formal analysis, R.R.K. and J.K., investigation, L.M., R.R.K. and P.S.; resources, R.R.K., P.S., and A.R.; writing—original draft preparation, L.M., R.R.K., P.S., writing—review and editing, L.M., R.R.K., P.S. and A.R.; visualization, supervision, R.R.K. and P.S.; project administration.; funding acquisition, R.R.K., P.S. and A.R. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

CHAPTER III

CONCLUSIONS AND FUTURE DIRECTIONS

The objective of this thesis was to offer field-based experimental results for organic farmers of the LRGV with relevant and locally-sourced information to assist in their cover crop selection process. Additionally, these experiments revealed possible mechanisms underlying the effects of cover cropping, beyond the well-documented soil benefits. For example, cover crops allow for the manipulation of plant-insect interactions through volatile signaling, which occurs when pests like chewing or sucking insects create tissue damage on plants, attracting natural predators and parasitoids, and reducing the number of herbivores. The benefits of utilizing this as a method of biocontrol also help reduce the need for synthetic pesticides. With little to no available information on the topics of cover crop selection, planting season impact, sustainable agroecosystem methodologies for various crops, or integrated pest management for LRGV, this thesis will hopefully initiate a movement for additional experimentation in this field.

This experiment took place in an organic farm located in Edinburg, Texas. The cover crops used in this experiment are very commonly used in the area, therefore further analysis of their effectiveness as a method of biocontrol was performed. The results showed crop specificity affected herbivore presence. Additionally, insect community dynamics were affected temporally

in the same season. In the first year of the experiment, the cover crops consisted of sunn hemp, cowpea, sudangrass, and a mix of the three. The proceeding squash cash crops sustained the most herbivore damage in the cowpea plots while sunn hemp and sudangrass had the least damage. In year 2, sunn hemp and cowpea were selected for further assessment. Sunn hemp plots consisted of significantly fewer total insects, fewer herbivores, and grew larger cash crop cabbage heads. Insect presence was significantly higher in cabbage plots that had previously grown cowpea. Cowpea plots growing cabbage also had a greater herbivore presence. Overall, parasitic wasps and predatory bugs were documented in higher numbers during the early growing season, but herbivorous insects remained constant throughout. This may have been due to the complexity of the cropping system negatively impacting the effectiveness of tracking down prey. *Ultimately, this problem offers opportunity to further examine the plant-insect interactions of agroecosystems with focus on herbivore-induced plant volatiles.*

To further extend the scope of this study, examining and quantifying species-specific volatiles (cover and cash crops), herbivore choice assays, and herbivore feeding assays should be conducted. This would allow for further understanding of pest-host preference and the assessment of volatile composition that entails feeding as well as predation. Future studies should also test the use of push-pull integrated pest management method. This is comprised of a pull crop that will attract herbivores and a push crop that will deter pest-feeding planted alternatively with a cash crop. This strategic cropping system facilitates the movement of herbivore pests off the cash crop and onto a trap/pull crop that the target pest has a greater preference for. Ultimately, this manipulation of tri-trophic interactions may be an additional method of biocontrol that local organic farmers can implement in their fields, thereby managing pests below damage threshold, to continue and prosper with sustainable agriculture.

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APPENDIX A

APPENDIX A

Table A1. Details of most common pests, their natural predators, and parasitoids found in the traps employed in different cover and cash crop treatments over two years of field study. Details of their common names, scientific names, taxonomic orders and families are included.

Herbivores	Order	Family		
White fly (Bemisia argentifolii [Bellows & Perring])	Hemiptera	Aleyrodidae		
Leaf hopper (<i>Empoasca fabae</i> [Harris])	Hemiptera	Cicadellidae		
Lygus bugs (Lygus lineolaris)	Hemiptera	Miridae		
Flea beetle (Chaetocnema hortensis)	Coleoptera	Chrysomelidae		
Armyworm (Pseudaletia unipuncta [Haworth])	Lepidoptera	Noctuidae		
Looper (Trichoplusia ni [Hübner])	Lepidoptera	Noctuidae		
Predators				
Ladybug beetle (Hippodamia convergens [Guérin-	Coleoptera	Coccinellidae		
Méneville])	Colcoptera	Coccilicitidae		
Minute pirate bug (Orius sp.)	Hemiptera	Anthocoridae		
Big eyed bug (Geocoris sp.)	Hemiptera	Geocoridae		
Spiders (multiple species)	Araneae			
Green lacewing (Chrysoperla sp.)	Neuroptera	Chrysopidae		
Damsel bug (<i>Nabis</i> sp.)	Hemiptera	Nabidae		
Assassin bug (Zelus sp.)	Hemiptera	Reduviidae		
Parasitoids				
Parasitoid Wasps (Cotesia sp.)	Hymenoptera	Braconidae		

BIOGRAPHICAL SKETCH

Lili M. Martinez studied in Weslaco, Texas for her Elementary, Middle, and High schooling. She began attending the University of Texas Rio Grande Valley in 2014 where she completed her Bachelor of Science with a Major in Biology and a Minor in Psychology (2018) and continued to complete her Master of Science, Major in Biology (2020). One of her many passions is to learn about the world and she hopes to attend UTRGV again one day to pursue a PhD in Biology and further her understanding of insect-plant ecology and sustainable agriculture. As of February 2020, she has accepted a position with the City of Pharr as their sole Urban Ecologist.

Email: lili.martinez01@utrgv.edu; martinez96lili@gmail.com