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**THE EFFECTS OF COVER CROPS AS INSECTARY PLANTS ON INSECT NATURAL ENEMIES AND
THEIR POTENTIAL FOR CONSERVATION BIOLOGICAL CONTROL**

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Entomology

by

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ABSTRACT

Agronomic cropping systems are often highly disturbed, lacking alternative resources for natural enemies critical for suppressing pests. Under these conditions, natural enemy survival and biological control potential may be reduced. As a conservation biological control approach, insectary plants may be introduced to provide supplemental nectar, pollen, and habitat to improve the performance and survival of natural enemies. However, natural enemies exhibit preferences toward specific flowering plants. Furthermore, different plant species may provide different resources. Therefore diverse insectary mixtures may be deliberately designed to more effectively support targeted natural enemies. I established buckwheat, *Fagopyrum esculentum*, and cowpea, *Vigna unguiculata*, in monocultures and mixtures adjacent to corn, *Zea mays*, to test the effects of insectary provisioning on the natural enemy community, predator dispersal between cover and cash crops, and potential for pest suppression. To measure these effects, I used sweep net sampling, protein-based mark-recapture and sentinel prey. Results suggest that predator abundance increases with increasing density of inflorescences and extrafloral nectaries. *Coleomegilla maculata* and *Orius insidiosus*, two key generalist predators, as well as crab spiders (Thomisidae) were more abundant in buckwheat monoculture and buckwheat-cowpea mixture treatments than in the cowpea monoculture. Recovered protein-marked *C. maculata* and *O. insidiosus* indicated migration between the insectary border and corn, as well as predation on sentinel prey. Despite higher predator abundance and confirmed predator dispersal, sentinel egg predation, while high, did not differ between treatments. Landscape factors may play a greater role than field-scale management in influencing predator dynamics at this site.

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Chapter 1

Impacts of habitat modification and plant diversification on the potential to attract natural enemies and suppress pest arthropods

In the chapters presented in this dissertation, I contribute to the growing body of research aimed at evaluating strategies to promote natural enemies in agricultural systems through the implementation and timely management of flowering non-crop, or insectary, plant species. I begin by discussing the importance of natural enemies as a source of biological control in agricultural systems and continue by reviewing habitat management strategies through which natural enemies have been previously supported. I continue with a brief review of recent literature pertaining to the use of habitat management strategies, specifically the addition of floral resources, while highlighting knowledge gaps and limitations surrounding natural enemy response to flowers. I conclude this introductory chapter with broad objectives of dissertation my research to understand strategies through which natural enemies may be supported in organic agricultural systems.

Challenges Natural Enemies Face

Natural enemies play a critical role in suppressing insect pests below economically damaging levels. In the United States, the value of pest suppression provided by natural enemies is estimated to be as high as \$4.5 billion annually (Losey and Vaughan 2006). Although pest suppression by natural enemies is a valuable ecosystem service, frequent disturbance, habitat fragmentation, and loss of diversity due to increased agricultural intensification and

simplification may create conditions unsuitable for many natural enemy species and may reduce their potential to suppress insect pests (Ehler 1998, Letourneau et al. 2011, Jonsson et al. 2012). Even though they are primarily predatory or parasitic, many important natural enemy species depend on resources provided by non-crop plants, such as food (nectar, pollen, alternate prey) and shelter (favorable microclimate, oviposition sites) to successfully complete development, especially during periods of limited prey availability (Landis et al. 2000, Lee and Heimpel 2008, Amaral et al. 2013, Gurr et al. 2017a). Loss of essential non-crop resources due to disturbances such as tillage or applications of broad-spectrum pesticides may challenge natural enemy survival and reduce their potential to suppress pests (Thorbek and Bilde 2004).

Conservation Biological Control: An Alternative Approach

Conservation biological control, the strategic use of pest management practices and features of the local environment to enhance locally-occurring natural enemy species, has been described as one approach to mitigate the detrimental effects of disturbance and enhance the survival and effectiveness of natural enemies (Ehler 1998). Currently, routine or preventive applications of broad-spectrum insecticides are typically used to manage pest insects in agricultural settings. However, these insecticides, and often herbicides, may disturb important natural enemies by directly killing them, their prey, and floral resources which they are dependant for their survival (Moser and Obrycki 2009, Bommarco et al. 2011). Two important approaches to conservation biological control though which natural enemies may be protected from the adverse effects of pesticides are reducing the frequency of disturbing pesticide

applications and choosing more selective insecticides specific to targeted pests (Lu et al. 2012). Natural enemies spared from the consequences of pesticides may be maintained at levels high enough to respond to and suppress insect pests (Varenhorst and O'Neal 2012). Other sources of disturbance such as tillage and landscape simplification may directly result in loss of overwintering sites, oviposition sites, and preferred host plants from which natural enemies receive food resources and shelter (Cronin 2004). Conservation biological control theory posits that minimizing the effects of disturbance via ecologically based approaches to pest management with special emphasis on manipulating the local landscape may conserve natural enemies and improve control of pest insects (Letourneau 1998b, Lundgren 2009b, Gurr et al. 2017a).

Natural Enemy Response to Habitat Diversification

Natural enemy populations may benefit from conservation biological control through habitat modifications where undisturbed non-crop patches are deliberately maintained and provide food resources in or along field edges to provide refuge and resources to beneficial insects (Letourneau et al. 2011). In contrast to classical and augmentative approaches to biological control where natural enemies are imported or purchased and released repeatedly, conservation biological control relies on enhancement of native or locally available natural enemies. Providing resident natural enemies with undisturbed habitat and food resources may increase their longevity, fecundity, and predation and parasitism rates (Thomas et al. 1991,

Begum et al. 2006, Berndt 2006, Lee and Heimpel 2008, Araj et al. 2009, Geneau et al. 2011, Bickerton and Hamilton 2012).

Because the conservation approach to biological control relies on the enhancement of native natural enemies for pest suppression rather than the introduction of exotic natural enemies, it may be an approach that poses lower environmental risk than classical biological control (Ehler 1998). For example, the multicolored Asian lady beetle (*Harmonia axyridis*) has been successfully released throughout the United States to suppress several species of aphid pests (Koch 2003). However, evidence suggests *H. axyridis* may be contributing to the displacement and decline of native lady beetles (Michaud 2002). There has been increasing awareness of the ecological risks associated with the introduction of non-native species and the non-target effects of pesticides, which has resulted in growing interest in ecologically sound and environmentally safe approaches to pest management (Andow 1991, Howarth 1991, Altieri 1999, Landis et al. 2000, Zehnder et al. 2006). Correspondingly, more research has been focused on investigating conservation biological control approaches to promote natural enemies and suppress pests (Berndt 2006, Vattala et al. 2006, Araj et al. 2011). While conservation biological control may pose lower environmental risk, there are associated disadvantages. Successful implementation of conservation biological control often requires intimate knowledge of the target pests and their natural enemies, including life history traits, phenology, habitat and prey preferences (Gurr et al. 2017a). From a practical standpoint, habitat manipulation practices may be initially more labor and cost intensive to implement. Growers may be unwilling to shoulder the costs of taking land out of production and fuel, machinery, and labor costs needed to prepare and establish non crop or insectary plants that

may not be feasible for harvest. In addition to these costs, habitat management strategies also require specialized knowledge of the plants, pests, and targeted natural enemies locally available. Growers must carefully consider how to manage an insectary plant used for natural enemy attraction to avoid competition with the cash crop for natural enemies or weeds. Though it may seem counterproductive due to additional labor and equipment costs, habitat conservation and enhancement of natural enemies can be relatively self-sustaining and may be more likely to have long-term effects in contrast to augmentative releases (Gurr et al. 2017a). Implementation of conservation biological control can be achieved through a variety of management strategies including altering tillage practices and insecticide use, as well as implementing crop diversification schemes (Landis et al. 2000).

Increasing the spatial and/or temporal diversity of plant species is a strategy by which pest insect colonization and establishment in cash crops can be reduced and natural enemy enhanced (Landis et al. 2000, Lundgren 2009b). Crop diversification schemes include practices such as polycultures, crop rotation, hedgerow establishment, and incorporation of cover crops or insectary plants into cash crop rotations (Altieri 1999). The concept of crop diversification to reduce pest and enhance natural enemy populations relies on several ecological principles. Charles Elton (1958) predicted that habitats with taxonomically distinct plant communities would be less likely to be colonized by pest insects, while taxonomically similar habitats would be more likely to be colonized. He suggested that non-host plants would confer an *associational resistance* to host plants through a dilution of host plant density. Tahvanainen and Root (1972) and Root (1973) furthered this hypothesis by proposing the *resource concentration* hypothesis, which suggests that insects are more likely to locate, colonize and establish in large, dense,

uninterrupted host patches such as monocultures common in agricultural settings. Therefore, by reducing patch size and density, pest populations may be reduced. The *enemies* hypothesis suggests that diverse habitats are more likely to have higher abundances of natural enemies due to the increased likelihood of species present that can provide insects with food and shelter resources. Crop diversification can influence pest-natural enemy dynamics through two general mechanisms: bottom-up and top-down regulation of insect pests. Through bottom-up regulation, additional plant species may reduce pest insect colonization by disrupting their ability to locate host plants or creating unfavorable habitat. Crop diversification may also promote top-down regulation of pests by providing plant species that are attractive to predators and parasites through the provision of resources such as food (nectar, pollen, alternate prey) and shelter.

Cover Crops: Insectary Resources

Cover cropping represents one crop diversification approach that may promote natural enemies and accordingly reduce pest-associated species. Currently in agricultural systems, cover crops are primarily used for soil conservation (Snapp et al. 2005). Cover crops are extensively used for maintaining and improving soil organic matter content, nutrient retention and cycling, and weed suppression (Delgado et al., 2007). However, in addition to these soil-associated benefits, cover cropping can have a significant influence on the insect community (Gurr et al., 2004). For example, in organic cropping systems where genetically modified crop varieties and synthetic pesticides are prohibited, pest management options are largely preventive, focusing on reducing the likelihood of colonization and establishment of insect

pests (Zehnder et al. 2006). Cover crops can function as barrier plants, non-crop/non-host plants, trap crops, and/or insectary plants which inhibit pest insect colonization and establishment by reducing their ability to locate host cash crops and creating less suitable habitats for pests (Bach 1980, Risch 1980, Letourneau 1986, 1987, Manandhar et al. 2009), or by attracting and supporting natural enemies (Letourneau 1987, Lee and Heimpel 2005, Lee and Heimpel 2008, Gardiner et al. 2009).

Insectary plants, that bear nectar-producing flowers and/or extrafloral nectaries, are highly attractive food sources to predators, parasitoids, and pollinators and can function to improve their longevity, fecundity and biological control potential (Geneau et al. 2011, Pumariño and Alomar 2012). Insectary plant species may additionally benefit natural enemies such as ground and web-building spiders by providing ground cover and habitat complexity (Rypstra et al. 1999). There have been considerable research efforts aimed at examining and reviewing the effects of crop diversification and the implementation of insectary plant species to promote natural enemies (Altieri and Letourneau 1982, Andow 1991, Landis et al. 2000, Poveda et al. 2008, Letourneau et al. 2011, Woltz et al. 2012, Gurr et al. 2017a). Several studies have reported significantly higher abundances of natural enemies when a cash crop is bordered or interplanted with flowering cover crops (Berndt et al. 2002, Irvin et al. 2006, Walton and Isaacs 2011, Morandin et al. 2014). For example, *Dolichogenidea tasmanica* (Hymenoptera: Braconidae), was significantly more abundant when buckwheat was used as a cover crop in vineyards compared to vineyards without cover crops (Berndt 2006). Parasitism of leafrollers did not consistently increase despite higher parasitoid abundance in this case. Higher abundances of beneficial insects in response to insectary plantings often result in increased

predation and parasitism of pests and subsequent decline in their populations (Baggen and Gurr 1998, Diaz et al. 2012). The encyrtid wasp, *Copidosoma koehleri* parasitized the potato tuber moth (*Phthorimaea operculella*) more frequently in the presence of flowering coriander and faba bean compared to treatments lacking these plants (Baggen and Gurr 1998). Similarly, the predatory syrphid *Eupeodes fumipennis* was enhanced when provisioned with sweet alyssum (*Lobularia maritima*) to control *Ostrinia nubilalis* in a lettuce cropping system (Hogg et al. 2011b). Predatory syrphid abundance, adult survivorship, fecundity increased when provisioned with sweet alyssum. Correspondingly, aphid abundance in lettuce heads significantly decreased when *E. fumipennis* was provisioned with sweet alyssum. There has been considerable research towards investigating the use of supplemental nectar and floral resources to support both generalist and specialist natural enemies (Landis et al. 2000, Berndt 2006, Letourneau et al. 2011, Géneau et al. 2012, Pumariño et al. 2012, Gurr et al. 2017a).

Using insectary plants to promote natural enemies is a potentially effective tactic. However, while many studies find positive responses of natural enemies to non-crop diversification, studies often suggest negative or non-significant impacts on natural enemies in response to the presence of non-crop resources (Poveda et al. 2008). *Coleomegilla maculata* experienced lower abundance and lower predation rate in a corn-bean-squash intercropped system compared to a corn monoculture. Differences in lady beetle abundance and predation rates were attributed to different alternate prey densities between the polyculture and monoculture plots (Andow 1991). Buckwheat enhanced parasitism of *Acyrtosiphon pisum* by the parasitoid, *Aphidius ervi* in an alfalfa system (Araj et al. 2009). However, buckwheat provisioning also enhanced hyperparasitism on *A. ervi* by its hyperparasitoid, *Dendrocerus*

aphidum and negatively impacted the natural enemy of the targeted pest. Another study found that despite increased abundances of coccinellid predators in flowering buckwheat strips, coccinellid predators did not increase correspondingly in an adjacent soybean cash crop. The authors suggested that lack of effects may be due to landscape patterns and individual predator biology (Woltz et al. 2012). Finally, reduction in predation by thrips predator, *Orius laevigatus*, was found when provided with supplemental food. Despite being a suitable insectary plant, *Ricinus communis* served to also distract *O. laevigatus*. Copious pollen resulted in lower rates of predator dispersal and corresponding predation rates.

While the benefits of floral diversification and supplementation for natural enemy populations may seem self evident, non-crop plant species and their impacts on targeted pests and natural enemies should carefully be considered and tested to maximize natural enemy enhancement. Haphazardly providing floral resources may result in unintentional consequences such as nectar exploitation by the targeted pest and lack of natural enemy dispersal from the non-crop resource (Winkler et al. 2009, Quinn et al. 2017).

Designing the Ideal Insectary

Throughout the scientific literature, inconsistent responses of natural enemies to resource diversification and floral provisioning strategies are due to non-target effects and unintended interactions between flowering plants and arthropods (Skirvin et al. 2007, Araj et al. 2009, Winkler et al. 2009, Quinn et al. 2017). This suggests that the role of non-crop vegetation in supporting natural enemies to improve biological control in crops is not well understood.

Therefore, more research must be undertaken at local and landscape scales to understand more thoroughly how natural enemies respond to and use resources provided through conservation biological control practices such as floral provisioning. Several considerations must be made before implementing insectary plants in an agroecosystem as natural enemies may respond differently depending factors such as landscape and local plant characteristics may influence how natural enemies respond to insectary provisioning. Natural enemies are often highly mobile and capable of dispersing between crop and non-crop areas (Tscharrntke et al. 2007). Therefore the surrounding landscape may influence the impacts of resource provisioning for natural enemies. Evidence suggests that local provisioning floral resources may have a more pronounced effect on natural enemy abundance and biological control in simple landscapes compared to increasingly heterogeneous landscapes more abundant in non-crop resources (Tscharrntke et al. 2007, Rusch et al. 2016). Woltz et al. (2012) found that lady beetle abundance and pest suppression was not enhanced, even when supplemented with buckwheat borders, in study plots surrounded by higher proportions of semi-natural habitat.

Ideally, insectary species should be well-suited to the needs of targeted natural enemies (Landis et al. 2000). For example, characteristics of potential insectary species such as vegetative biomass production, vegetative structure, floral structure and phenology, and nutritional content are features that must be considered when selecting a cover crop to conserve natural enemies.

Biomass production and structure affect habitat complexity provided by canopy closure and ground coverage, which can serve to attract generalists such as ground spiders and ground beetles (Thorbeck and Bilde 2004). This type of habitat may function as refuge or shelter for

natural enemies and may harbor alternate prey items that may support natural enemy feeding (Gurr et al. 2017a). Floral structure may affect the different types of insect taxa attracted.

Nectar-producing structures should be accessible to the natural enemies desired for biological control. Plants that produce multiple, shallow, open flowers are generally more accessible and likely to host a wide range of natural enemies compared to flowers with fewer flowers with longer corolla whose nectar is harder to access. (Vattala et al. 2006, Géneau et al. 2012).

Flowers characterized by a long, narrow corolla are likely to be inaccessible to short tongued bees, parasitoids, and other generalists that lack the ability to access nectar hidden deep in flowers. Floral phenology for insectary cover crops should not only coincide with the cash crop species and targeted pests, but should also synchronize with natural enemy emergence and activity. Longer blooming insectary cover crops, if selectively exploited by natural enemies and not pests, are more likely to serve as sources of natural enemies throughout key parts of cash crop growing season. Similarly, considerations must be made to ensure that chosen insectary plants do not support pest species and antagonists of natural enemies that can exploit floral resources intended for natural enemies and exacerbate pest problems (Baggen et al. 1999, Araj et al. 2009).

Consideration should also be given to the nutritional composition and availability of the nectar and pollen source. Nectar primarily consists of glucose, sucrose and fructose, but also contains small amounts of complex sugars, amino acids, lipids and defensive compounds (Lundgren 2009a). Nectar availability varies depending on plant species as well as nectar source. Floral nectar is often abundant, but short-lived and contains defensive compounds to deter nectar theft whereas extrafloral nectar is available for longer periods of time and serves

to attract natural enemies to protect plants from herbivory (Cawoy et al. 2008, Lundgren 2009a, Lundgren 2009b). Pollen is a significant food source for natural enemies and consists of sugars (glucose, fructose, sucrose), starches, protein, lipids, vitamins and minerals (Lundgren 2009b). Pollen, similar to nectar, may have morphological and chemical defenses. Variation in nectar and pollen composition and availability may optimally support particular natural enemy species, while being deficient for others.

In addition to morphological traits suited for natural enemies, insectary species should be compatible with grower identified needs such as crop window and other ecosystem services such as nitrogen supply and weed suppression. Determining the usefulness of a potential insectary plant species to targeted natural enemies would benefit more from a structured approach that demonstrate natural enemy utilization of the resource, improved fitness in the presence of the resource, natural enemy attraction to the resource, and dispersal between the provided resource and main crop (Heimpel and Jervis 2005). Several studies have attempted to determine optimal insectary species for targeted natural enemies by screening several insectary species and examining how they are used by natural enemies (Vattala et al. 2006, Kehrlı and Bacher 2008, Géneau et al. 2012, Araj and Wratten 2015).

While many species are screened and identified as good candidates to provide resources for natural enemies, relatively few studies examine the effect of insectary diversification on the natural enemy community (Pontin et al. 2006, Pumariño et al. 2012). The dominant approach to understanding the presence of natural enemies and enhancement of their biological control potential is to target the single most effective natural enemy species and provision it with a single insectary species that is optimal for that particular natural enemy. This

strategy focuses on a single natural enemy species, but ignores the biological control potential for other natural enemies present in the system. Natural enemies often display preferences or host fidelity to particular insectary species based a variety of characteristics such as plant morphology (Colley and Luna 2000). Therefore, an insectary plant species suitable for one natural enemy may not be suitable or even accessible by others (Vattala et al. 2006). An alternative approach would be to instead include multiple insectary species in mixtures to provide complementary resources usable by multiple species of natural enemies. Such an approach could further improve pest insect suppression and contribute to more resilient cropping systems (Snyder et al. 2006). In the event that the target natural enemy has unusually low abundances in one particular year, the alternate natural enemy species supported by resource subsidies may compensate and maintain levels of pest suppression. Alternatively, increased predator biodiversity may result in reduced levels of pest suppression due to negative predator-predator interaction such as intra-guild predation (Finke and Denno 2004). These types of outcomes further emphasize the importance of closely examining how natural enemy communities use and respond to resource subsidies.

Research Overview

In this dissertation I aim to contribute to the growing body of research that seeks to understand natural enemy use of non-crop resources, as well as management strategies to promote the presence of natural enemies in agro-ecosystems. I aim to contribute to some of the aforementioned knowledge gaps by examining insectary diversification and natural enemy dispersal associated with non-crop insectary plants. When considering the use of habitat

management strategies and insectary plants, it is critical to understand potential natural enemy responses. Will resource plants necessarily support my targeted natural enemies? Can providing complementary floral resources in increasingly diverse mixtures provide greater support for natural enemies compared to less diverse mixtures of single species? Will natural enemies necessarily move between resource rich resources patches and the main crop?

I conducted laboratory and field experiments to examine the impacts of non-crop resource plant on native generalist predator abundance and performance in an organic corn agroecosystem. I further examined the impacts of diversifying the insectary plant resources to determine if diverse insectary plant mixtures result in a greater diversity of natural enemies compared with monocultures of insectary plants. Finally, I conducted an experiment to determine if natural enemies disperse between insectary plant treatments and an adjacent crop. Here, I will briefly summarize the research presented in the following chapters.

In Chapter 2, I conducted an experiment to examine the influences of cover crop diversification on the abundance and diversity of natural enemies in a field corn agroecosystem. Monocultures and mixtures of commonly used cover crops such as cereal rye (*Secale cereale*), red clover (*Trifolium pratense*) and canola (*Brassica napus*) were planted and succeeded by a corn crop. I monitored early spring natural enemy abundance and predation within a main crop that had been preceded by different cover crop treatments to determine how natural enemies respond to early season cover crop resources. From this study I learned the importance of the timing of cover crop management may have on natural enemies and the implications are discussed.

In Chapter 3, following lessons learned from the cover crop field study, I tested two species, buckwheat (*Fagopyrum esculentum*) and cowpea (*Vigna unguiculata*), for their potential to support *Orius insidiosus*, an economically important natural enemy. Buckwheat and cowpea were provided singly and in mixtures to *O. insidiosus* in laboratory bioassays. In this setting, I determined the potential for these insectary plant species to support *O. insidiosus*. To measure overall performance, I measured *O. insidiosus* survival, fecundity and rates of predation on sentinel prey in the presence of resource plants. I discuss the implications of this experiment for resource plant screening.

In Chapter 4, I conducted a field experiment to determine if buckwheat and cowpea, established as insectary borders can serve natural enemies in an adjacent corn agroecosystem. I also determined the influence of these two insectary plant species grown in a mixture on natural enemies when the insectaries are established adjacent to the main crop to allow spatial and temporal overlap between the insectary, natural enemies, and crop. I monitored natural enemy abundance and predation of sentinel prey in both the insectary borders and corn crop. I discuss the potential for these two insectary species to support natural enemies as well as considerations that must be made prior to using insectary plants in the field.

In Chapter 5, I used a protein-based immunomarking technique to monitor the potential for *Coleomegilla maculata* and *O. insidiosus* to disperse between an insectary border and the main crop in the field. My goal was to determine the proportion of sentinel prey attacked by natural enemies previously inhabiting the insectary borders by using a secondary protein marker. I discuss the importance of understanding natural enemy dispersal in habitat management contexts.

Finally in Chapter 6, I summarize my overall results and discuss the implications for use of cover crops as insectary plants. Additionally, I suggest directions for future research aimed at natural enemy enhancement via non-crop resource plants.

Chapter 2

Cover Crop Cocktails: Impacts of cover crop diversification on natural enemy abundance and pest suppression

Introduction

The conservation biological control approach to pest regulation aims to enhance the abundance of arthropod natural enemies through the preservation of a relatively stable non-crop, resource rich environment in which natural enemy arthropod populations can be maintained during periods of low pest activity (Ehler 1998). These environments may further function as a source of natural enemies that can colonize crop fields early in the season when crop plants are most vulnerable (Thomas et al. 1991). Within a non-crop habitat, populations of insect natural enemies may build up to levels that can more quickly respond to increasing pest populations that colonize crop fields throughout the growing season; thereby keeping pests at economically tolerable levels and reducing the likelihood of pest outbreaks.

Cover crops are non-crop plants established in fields that are not currently in cash crop production. Cover crops are most commonly used to address crop and soil management concerns such as nutrient supply, nutrient retention, organic matter quantity and quality, erosion control, weed suppression and improvement in crop yield. There is a wide array of cover crops from a wide range of plant families that can be used to address specific functions in an agricultural landscape. For example, legumes fix nitrogen and when incorporated into the soil can supplement subsequent crops with nitrogen (Sullivan et al. 1991). Similarly, cereal rye (*Secale cereale*) can effectively suppress weeds (Akemo et al. 2000). Cover crops have traditionally been planted as monocultures intended to address on-farm ecosystem functions singly. For example, nitrogen-fixing legumes such as field peas (*Pisum sativum*) or red clover

(*Trifolium pratense*) are often used to supply nitrogen (Sullivan et al. 1991, Dabney et al. 2001). However, recently there has been growing interest in using multiple cover crop species in mixtures to enhance multiple ecosystem services such as nitrogen supply, retention, and erosion control (Schipanski et al. 2014, Finney and Kaye 2016).

While cover crops are most often used for soil conservation and weed suppression, planting cover crops, especially flowering cover crops, can be considered a form of habitat management that contribute to conservation biological control as they may impact the insect community. Insects are highly dependent on resources provided by the existing plant community, which may include cover crops, the main crop and surrounding vegetation in fencerows, woodlots, and weedy borders (Landis et al. 2000). Flowering cover crops can function as an insectary, or a source of food such as nectar, pollen, and alternate prey. Cover crops may additionally provide favorable habitat in terms of microclimatic conditions, preferred oviposition sites, and habitats to avoid disturbance and predators (Parajulee and Slosser 1999). From these plant-based resources, natural enemy insect populations may achieve higher abundances, increased longevity, and increased fecundity (Thomas et al. 1991, Begum et al. 2006, Berndt 2006, Lee and Heimpel 2008, Araj et al. 2009, Geneau et al. 2011, Bickerton and Hamilton 2012).

Similar to how cover crops differently affect ecosystems services on a farm, floral resources may differentially influence the arthropod taxa that inhabit them. Natural enemies benefit from different types of cover crops differently; therefore, certain cover crops may be more beneficial than others for natural enemies depending on several cover crop characteristics (Géneau et al. 2012). Characteristics such as plant architecture, presence of

floral or extrafloral nectaries, pollen production, the appearance and presence of floral and extrafloral structures, accessibility and nutrition can influence the types of insects that can benefit from a cover crop species (Vattala et al. 2006). Cover crops have been traditionally used in monocultures to help target specific natural enemies (Spellman et al. 2006, Venzon et al. 2006, Woltz et al. 2012, Tavares et al. 2015); however, studies examining the impact of diversification of cover crops as an insectary have been less common (Lundgren et al. 2009, Walton and Isaacs 2011, Pumariño et al. 2012). As natural enemies respond differently to different cover crops, it may be feasible to diversify plantings of cover crop to positively impact the visiting insect community. For example, I can ask, “What is the potential for increasingly diverse mixtures of flowering cover crops to serve as a resource for higher abundances or wider range of natural enemies?”

To investigate the impacts of cover crop diversification on early season recruitment of natural enemies in an organic cropping system in central Pennsylvania, I surveyed cover crop monocultures and mixtures designed to address particular functions. My objectives were to identify natural enemy arthropods associated with particular cover crop monocultures as well their representation in more diverse mixtures, assess the time of arrival beneficial insects to particular cover crop treatments, and assess levels of predation in the succeeding cash crop. I hypothesized that more diverse cover crop mixtures would attract a higher abundance and diversity of natural enemies, as well as a subsequently increase predation in following cash crop due to greater numbers of natural enemies in the area.

Materials and Methods

This study was conducted as a part of a larger field study in Centre County, Pennsylvania, to examine the benefits and costs associated with the use of increasingly diverse cover crop mixtures to address several grower identified priority functions, or ecosystem services. These services included nutrient supply, nutrient retention, erosion control, insect regulation, yield, and profitability. While the larger study aimed to observe effects on multiple ecosystem services, I was specifically interested in determining the effect of cover crop diversification on arthropod natural enemies.

Treatments

Cover crop mixture treatments were specifically designed to improve multiple ecosystem services simultaneously. The six plant species included in the cover crop mixtures, each with different traits, included red clover (*Trifolium pratense*), Austrian winter pea (*Pisum sativum*), forage radish (*Raphanus sativus*), winter canola (*Brassica napus*), cereal rye (*Secale cereale*), and oats (*Avena sativa*). Red clover is a legume that can produce rapid biomass, increase nitrogen supply, and can overwinter for cover and floral bloom in the spring. Field pea is similarly a legume that can provide nitrogen, however spring biomass may be limited because pea does not overwinter consistently in Centre County, PA. Forage radish winter-kills and was included for rapid fall biomass, nitrogen retention, water infiltration and weed suppression. Winter canola grows rapidly in the fall for nitrogen retention and weed suppression. Winter canola consistently overwinters and blooms in early spring. Cereal rye produces high spring biomass that can help retain nitrogen and has been reported to attract natural enemies at

pollen-shed in the spring (Tillman et al. 2004, Clark 2007). Oats provide rapid fall biomass for nitrogen retention and weed suppression, but winter kills. Several of these species have been suggested to provide beneficial resources to natural enemies. These resource-provisioning “insectary” species were red clover, Austrian winter pea, winter canola and cereal rye.

Field Experiment Establishment

This study was a part of a larger ~12 hectare field experiment, based on a three-year rotation of organic wheat, corn and soy with two cover crop windows after wheat and after corn. The study was implemented at the Russell E. Larson Agricultural Research Center in Centre County, Pennsylvania. This experiment was managed in transition to organic certification and as such, all crop management followed USDA organic regulations (NASS 2015). To focus on the effects of cover crops on insect pest regulation, I chose to focus on corn and a subset of the cover crop treatments preceding corn, and to examine potential carryover effects in the following corn cash crop. Each block of each cash crop was divided into 12 cover crop treatment plots, including a bare fallow treatment, replicated across four blocks. Cover crop treatment plots measured 24m x 29m and were seeded in August of the previous year following the harvest of wheat. I collected samples from 6 of the cover crop treatments chosen for their potential to serve as insectaries. These treatments included monocultures of red clover (seeded at a rate of 600 plants/m²), Austrian winter pea (60 plants/m²), cereal rye (500 plants/m²), and winter canola monoculture (400 plants/m²); a 4-species mix (4SPP: cereal rye (100 plants/m²), winter canola (200 plants/m²), red clover (300 plants/m²), Austrian winter pea (30 plants/m²), and a 6-species mix (6SPP: cereal rye (100 plants/m²), winter canola (100

plants/m²), red clover (150 plants/m²), Austrian winter pea (15 plants/m²), oats (75 plants/m²), and forage radish(20 plants/m²) (Murrell et al. 2017).

I conducted the study in two consecutive growing seasons, 2014 and 2015, in corn that followed wheat followed by fall-planted cover crops in the preceding years. Corn was established after termination of the cover crop treatments by mowing and soil incorporation in the spring. In 2013, cover crops to precede corn were seeded on 8 August, and terminated and incorporated by 19 May in following spring in 2014. Untreated, non-transgenic corn (Master's Choice 4050) was planted on the 2 June, 2014. In 2014, cover crops were seeded on 15 August and terminated and incorporated by 14 May in spring of 2015. Corn was planted on 28 May 2015. In 2015, to observe the potential for extended presence of cover crops on natural enemies, I preserved cover crop strips measuring 1.5m x 29m on the edge of the treatment plots for 2 weeks after the rest of the plot had been terminated. The study was terminated when corn was harvested on September 15 and 14 in 2014 and 2015, respectively.

Sweep Net Sampling

I collected sweep net samples from the cover cropped plots to determine the arthropod identity and abundance early in the spring. Sweep net sampling began on 2 May in 2014 and 2015, during cover crop bloom, which occurred in late April to mid May and continued until cover crop termination. Sweep net samples were performed weekly at midday, when insects are most active, until all of the cover crop treatments were terminated on 13 May in 2014 and 20 May 2015. I avoided sampling on days that were excessively windy or cloudy. A 25.4 cm diameter sweep net was used to collect arthropods from three random locations from each

cover crop treatment plot. I transferred collected insects into labeled freezer-safe bags that were frozen until identification. I identified all arthropods to various levels the lowest taxonomic level possible and recorded their numbers (Goulet et al. 1993, Triplehorn et al. 2005).

Sentinel Predation Assays

To determine if natural enemies in the preceding cover crops could influence pest suppression in the following corn crop, I deployed European corn borer, *Ostrinia nubilalis*, eggs as a sentinel prey. Eggs were obtained commercially (Benzon Research, Carlisle, PA) and prepared by gluing single egg masses (<48 hours old) to card stock and recording the number of eggs per card. Only egg masses containing at least 12 individual eggs were used in the field. Predation assays were conducted biweekly throughout the duration of the study in corn after cover crop termination during corn establishment. I began deploying sentinel prey on 1 July in 2014 and 15 July in 2015, 5 to 6 weeks after corn was planted. I conducted sentinel prey assays biweekly until 29 August, 2014 and 26 August, 2015. In each crop plot, six sentinel prey cards were placed randomly on corn plants in each plot by stapling prepared prey cards to the underside of corn leaves where *O. nubilalis* usually lays eggs and where generalist predators often forage for prey (Wang et al. 1997). Sentinel eggs were left in the field for 48 hours, when they were collected and returned to the laboratory and examined under the microscope for signs of predation. Eggs were categorized as "chewing predator" (mangled or missing eggs), "sucking predator" (flat, punctured eggs), "parasitized" (blackened eggs), "hatched" (undamaged eggs), "dead" (no larval emergence) or "missing" (egg card missing) (Andow 1990).

Characterization was confirmed by keeping eggs until larvae emerged from all egg masses which were stored under laboratory conditions in sealed plastic bags. All larvae typically emerged within 48 hours after being collected from the field. Mean proportions of dead eggs, successfully hatched eggs, and eggs killed by chewing and sucking predators and parasitism were calculated and recorded.

Statistical Analysis

Data includes arthropod counts collected from sweep net samples in 2014 and 2015 and predation rates obtained from sentinel corn borer prey. As predation rate is presented as proportion of eggs attacked, data was arcsine square root transformed to meet assumptions of homogeneity of variances. Data were analyzed using SAS statistical analysis software (SAS Institute 2002). A repeated measured ANOVA (Proc MIXED) was constructed to determine the effects of main plot factors of cover crop treatment effects over the course of the growing season. When a significant treatment by date interaction was detected, simple effect means were compared using LS means. Regression analyses were conducted to test the effects of cover crop species diversity on the abundance of key natural enemy taxa. Effects were considered significant when $P < 0.05$.

Results

Arthropod Survey

In sweep net samples initiated at cover crop bloom, the most consistently occurring natural enemy taxa during both years included spiders (Araneae), lady beetles (Coleoptera: Coccinellidae), and parasitic wasps (Hymenoptera: Parasitica)(Tables 1 and 2).

In 2014, spider abundance increased over time, peaking on the third sampling event on 5 May before decreasing by 26 May. There was a significant time by treatment interaction ($F_{12, 244} = 2.38, P < 0.0001$) for spiders; therefore, a separate analysis was conducted to determine treatment effects by time. Spiders were significantly more abundant in red clover compared to canola ($F_{4, 39} = 2.72, P = 0.0097$), cereal rye ($F_{4, 39} = 3.74, P = 0.0006$), 4SPP ($F_{4, 39} = 3.91, P = 0.0004$) and 6SPP ($F_{4, 39} = 3.23, P = 0.0025$) on 20 May (Figure 2.1a). In 2014, the influence of cover crop species on spider abundance was marginally significant ($F_{1, 279} = 3.83, P = 0.0051$). Spider abundance decreased with increasing cover crop diversity (Figure 2.1b).

Lady beetle abundance was generally low and therefore their abundances were pooled. The only lady beetle species collected were *C. maculata* and *Harmonia axyridis*. However, their abundances increased over the course of the study, peaking by 20 May. There was a significant treatment by time interaction for lady beetles ($F_{12, 244} = 4.56, P < 0.0001$). Because there was a significant interaction, a separate analysis was conducted to determine the simple effects on collected lady beetles. Lady beetles were significantly more abundant in the red clover cover monoculture compared to canola ($F_{4, 39} = 2.68, P = 0.0108$), cereal rye ($F_{4, 39} = 3.40, P = 0.0016$), 4SPP ($F_{4, 39} = 3.40, P = 0.0016$) and 6SPP ($F_{4, 39} = 3.40, P = 0.0016$) on 20 May (Figure 2.2a). Regression analysis suggest that cover crop diversity significantly influence coccinellid

abundance in 2014 ($F_{1,279} = 6.04$, $P=0.0146$). Similar to spider abundance, coccinellid abundance decreased with increasing cover crop diversity in 2015 (Figure 2.2b).

Parasitic wasps were combined into the super group total parasitoids for analysis. Total parasitoid abundance was relatively low at the beginning of the sampling period, but increased over the course of the study, peaking on 20 and 26 May. There was a significant date by treatment interaction for total parasitoids ($F_{12,244} = 1.83$, $P=0.0448$). Cover crop treatment effects were significant on all sampling dates (2 May, 15 May, 20 May, and 26 May) (Figure 2.3a). On 2 May, total parasitoids were more abundant in red clover than in canola ($F_{4,79} = 4.43$, $P<0.0001$), cereal rye ($F_{4,79}=4.14$, $P<0.0001$), 4SPP ($F_{4,79} = 3.30$, $P=0.0014$), and 6SPP ($F_{4,79} = 3.44$, $P=0.0009$) treatments. On 15 May, total parasitoid numbers were greater in red clover than in canola ($F_{4,39} = 4.93$, $P<0.0001$), cereal rye ($F_{4,39}=4.56$, $P<0.0001$), 4SPP ($F_{4,39} = 4.18$, $P=0.0002$), and 6SPP ($F_{4,39} = 4.93$, $P<0.0001$) treatments. Total parasitoids were greater on 20 May in red clover compared to canola ($F_{4,39} = 3.58$, $P=0.009$), 4SPP ($F_{4,39} = 3.35$, $P=0.0018$), and 6SPP ($F_{4,39} = 2.34$, $P=0.0244$) treatments. Total parasitoids collected in sweep net sampling on 26 May were more abundant in red clover compared to canola ($F_{4,39} = 3.53$, $P=0.0011$), cereal rye ($F_{4,39}=3.28$, $P=0.0022$), 4SPP ($F_{4,39} = 3.03$, $P=0.0043$), and 6SPP ($F_{4,39} = 2.90$, $P=0.0060$) treatments.

Parasitoids abundance was significantly impacted by cover crop species diversity ($F_{1,279} = 5.46$, $P=0.0202$). Parasitoid abundance decreased with increasing cover crop diversity (Figure 2.3b).

In 2015, spider abundance in sweep net samples decreased over the course of the study in all treatments ($F_{3,217} = 3.63$, $P=0.0137$), with a significant treatment by time interaction ($F_{23,205} = 2.11$, $P=0.0177$)(Figure 2.1c). There were no significant effects of treatments on any date

sampled. Sampling date, however, was a significant factor in the model. Cover crop diversity did not influence spider abundance during the 2015 study year ($P>0.05$) (Figure 2.1d).

Lady beetles were less abundant in 2015 than in 2014. Additionally, in 2015 lady beetle abundance decreased at each sampling event and there was a significant time by cover crop treatment ($F_{12,205} = 1.94$, $P=0.317$) (Figure 2.2c). Lady beetles were found in greater abundances on 2 May in red clover compared to canola ($F_{4,40} = 3.71$, $P=0.0006$), cereal rye ($F_{4,40} = 3.71$, $P=0.0006$), 4SPP ($F_{4,40} = 3.71$, $P=0.0006$), and 6SPP ($F_{4,40} = 3.71$, $P=0.0006$) treatments. On 7 May, lady beetle abundances were higher in red clover compared to canola ($F_{4,40} = 3.87$, $P=0.0004$), cereal rye ($F_{4,40} = 2.76$, $P=0.0086$), 4SPP ($F_{4,40} = 3.32$, $P=0.00019$), and 6SPP ($F_{4,40} = 3.87$, $P=0.0004$) treatments. Coccinellid abundance was significantly impacted by the number of species in cover crop mixtures ($F_{1,239} = 6.18$, $P=0.0136$). Similar to the previous year, coccinellid abundance decreased with increasing cover crop diversification (Figure 2.2d)

Unlike in 2014, total parasitoid abundance was initially high, but decreased at each sampling event. There was a significant treatment by time effect on total parasitoid wasps in 2015 ($F_{12,205} = 11.82$, $P<0.0001$). Cover crop treatment effects were significant on all sampling dates (2 May, 7 May, 14 May, and 19 May) (Figure 2.3c). On 2 May, total parasitoids were more abundant in red clover than in canola ($F_{4,40} = 5.83$, $P<0.0001$), cereal rye ($F_{4,40} = 5.81$, $P<0.0001$), 4SPP ($F_{4,40} = 5.20$, $P<0.0001$), and 6SPP ($F_{4,40} = 5.45$, $P<0.0001$) treatments. On 7 May, total parasitoid numbers were greater in red clover than in canola ($F_{4,40} = 7.92$, $P<0.0001$), cereal rye ($F_{4,40} = 7.30$, $P<0.0001$), 4SPP ($F_{4,40} = 6.98$, $P<0.0001$), and 6SPP ($F_{4,40} = 7.71$, $P<0.0001$) treatments. Total parasitoids were significantly greater on 14 May in red clover compared to canola ($F_{4,40} = 9.77$, $P<0.0001$), cereal rye ($F_{4,40} = 9.77$, $P<0.0001$), 4SPP ($F_{4,40} = 10.46$, $P<0.0001$),

and 6SPP ($F_{4,40} = 10.46$, $P < 0.0001$) treatments. Parasitoids abundance was again significantly influenced by cover crop species diversification ($F_{1,239} = 4.86$, $P = 0.0285$). Abundance decreased with increasing cover crop diversity (Figure 2.3d).

Sentinel Prey Predation

In 2014, predation by chewing predators was initially low, but increased over the course of the season and peaked at approximately 80% predation of eggs. Cover crop treatment did not significantly influence rates of sentinel prey predation ($P = 0.70$). However, there was a significant effect of time on rates of predation ($F_{4,829} = 65.66$, $P < 0.0001$) (Figure 2.4a). Predation by sucking predators was initially relatively low at approximately 10% before declining later in the season. Similar to predation by chewing predators, treatment did not significantly affect rates of predation by sucking predators. Time, however did significantly affect predation by sucking predators as predation decreased over the course of the season ($F_{4,830} = 16.66$, $P < 0.0001$) (Figure 2.5a).

In 2015, there was a significant cover crop treatment by time interaction ($F_{18,637} = 1.81$, $P = 0.0208$). On 15 July, in corn that followed the cover crop treatments, chewing predation was greatest in the 6SPP mixture and was greater than in the 4SPP ($F_{6,157} = 2.36$, $P = 0.0216$), canola ($F_{6,157} = 2.41$, $P = 0.0172$), and cereal rye ($F_{6,157} = 3.45$, $P = 0.0007$) treatments. Chewing predation in red clover was significantly greater than in cereal rye ($F_{6,157} = 2.61$, $P = 0.0100$). Chewing predation in winter pea was greater than in cereal rye ($F_{6,157} = 2.06$, $P = 0.0407$). On 29 July, chewing predation was significantly greater in red clover than in 6SPP ($F_{6,157} = 3.19$, $P = 0.0017$) and 4SPP ($F_{6,157} = 2.52$, $P = 0.0127$) treatments. On this sampling date predation in 6SPP was the

lowest compared to winter pea ($F_{6,157}=2.26$, $P=0.0250$), canola ($F_{6,157}=2.14$, $P=0.0343$), and cereal rye ($F_{6,157}=2.17$, $P=0.0317$) treatments (Figure 2.4b). There were no significant differences across cover crop treatment for chewing predation on any subsequent sampling date. With regard to predation by sucking predators, predation rates were similar to 2014 when predation was low at approximately 10%. There was a significant treatment by time interaction for sucking predation ($F_{18,657}=2.66$, $P=0.0002$). On 1 July, predation was the highest in red clover compared to winter pea ($F_{6,157}=3.96$, $P<0.0001$), fallow ($F_{6,157}=2.79$, $P=0.0059$), canola ($F_{6,157}=3.96$, $P=0.0001$), cereal rye ($F_{6,157}=2.34$, $P=0.0203$), 4SPP ($F_{6,157}=3.73$, $P=0.0003$), and 6SPP ($F_{6,157}=3.35$, $P=0.0010$) treatments.

Discussion

This study aimed to determine the impact of cover crop diversification on natural enemy recruitment and potential pest suppression in the succeeding corn cash crop. My hypothesis that more diverse cover crop mixtures would support greater abundances of natural enemies was not consistent with my results and observations.

Our current understanding of natural enemy responses to habitat management suggests that natural enemy numbers would be expected to be enhanced when provisioned with relatively undisturbed resource-rich patches (Letourneau 1998a, Lundgren 2009b, Gurr et al. 2017a). Increasingly diverse mixtures would be expected to provide a greater diversity and abundance of nectar, pollen, alternative prey resources that can support a wide range of natural enemy preferences (Vattala et al. 2006, Géneau et al. 2013). Our regression analyses suggest this was not the case. For each key natural enemy taxa, increasingly diverse cover crop

mixtures appeared to correspond to reduced abundances of these natural enemy groups. In this study, this may have been due to the red clover being the most consistently attractive species. Reduced clover representation in the four and six species cover crop mixtures may be the cause of lower arthropod abundances.

Due to management and timing constraints, cover crops may not have been able to bloom sufficiently and overlap with the timing of natural enemy emergence and activity in my experiment. Although, the cover crops used in the experiment are reported to have insectary traits, insects did not appear to be supported by any species, excluding red clover, in monocultures or mixtures.

The red clover monoculture was attractive to the most common natural enemy taxa, including spiders, lady beetles and parasitoids. In 2014 spiders, lady beetles and total parasitoids were significantly more abundant in red clover compared to other cover crop monoculture and mixture treatments. In 2015, this trend only held for lady beetles and total parasitoids. During the cover crop bloom window, red clover establishment was patchy and did not reliably bloom in monoculture and mixture treatments (Murrell et al. 2017). In mixtures, red clover was poorly represented and the more aggressive cover crop species such as canola and cereal rye often dominated (Murrell et al. 2017). Despite this uneven representation of red clover, natural enemies were still most abundant in the red clover monoculture compared to other treatments. Red clover may be attractive to natural enemies due to the provisioning of resources such as nectar, pollen, alternative prey and shelter (Clark 2007). Diaz et al (2012), found that the parasitoid, *Trichogramma atopovirilia*, survived longer, was more fecund and parasitized more prey when provided with red clover blooms compared to borage (*Borago*

officinalis), coriander (*Coriandrum sativum*), moricandia (*Moricandia* sp.) and sweet alyssum (*Lobularia maritima*) under laboratory, greenhouse and field conditions.

The pattern of predation by *Coleomegilla maculata* and *Orius insidiosus*, which were both frequently observed directly feeding on sentinel European corn borer eggs were consistent with that described by Conrad (1959). In my experiment, predation by chewing insects was initially low in spring, but increased over the course of the study, peaking by the end of the study in August. This may have been related to the fact that under warmer temperatures, insect growth and develop faster (Obrycki and Tauber 2012). During the spring and summer months as mean temperature rise, insects increasingly emerge from overwintering sites in search of prey and mates. As pest populations establish within the cash crop, natural enemies may respond by migrating into plots in response to greater prey availability. Additionally pollen shed by corn in early August may have attracted higher numbers of chewing predators that are also pollenivorous, such as the pink spotted lady beetle, *C. maculata*, seeking to supplement their diet. Chewing predators, like *C. maculata*, appeared responsible for the high rates of predation recorded by chewing predators. I frequently observed *C. maculata* actively feeding on sentinel prey in the field and under laboratory conditions. *C. maculata* is reported to be an important natural enemy of *O. nubilalis* (Conrad 1959). The main sucking predator likely responsible for sentinel predation was the insidiosus flower bug, *O. insidiosus*. This insect is reported to be an important natural enemy of the European corn border and is often abundant in corn agroecosystems (Conrad 1959, Dicke and Jarvis 1962). Sucking predation was initially low and decreased in each subsequent sampling event before becoming negligible by early August. Rates of sucking predation may have been underestimated due to

the feeding habit of *O. insidiosus*. As a sucking predator, *O. insidiosus* feeds on the yolk of prey eggs, leaving the intact egg chorion behind. Lady beetles were often observed in the field and confirmed in the laboratory to feed on prey egg chorions left behind by sucking predators. In these cases, it appeared that chewing predators were responsible for predation. In addition, the apparent egg predation by *O. insidiosus* may have actually decreased due to the tendency of this insect to move into developing corn ears and tassels to feed on plant pollen and tissues during pollen shed (Dicke and Jarvis 1962).

While I did observe some significant differences in particular treatments on certain sampling days, there did not appear to be any clear and consistent carry over impacts of cover crop diversification on the proportion of sentinel prey attacked by chewing or sucking predators. For example, despite higher natural enemy abundances in red clover, there were no corresponding increases in predation due to this cover crop treatment on sentinel prey in the succeeding cash crop. This raises important habitat management implications. Each year, for agronomic reasons, cover crops were terminated in early May, before many of the cover crop species could reach peak bloom. Although canola achieved peak bloom in late May, red clover and Austrian winter pea did not overwinter or bloom reliably, thereby limiting their potential to provide resources to natural enemies in spring. In the second year of the experiment, I left a border strip of cover crops for an additional 2 weeks to prolong the occurrence of the cover crop bloom window. However, this resource conservation measure did not result in any increases to natural enemy taxa. In spring, many natural enemy groups, coccinellids, for example, emerge from overwintering sites starved and in search of prey and plant-based resources before mating. Arthropods often rely on early season wildflowers and weeds in

bloom and alternative prey when primary prey is scarce to provide energy for migration and mate-finding (Lundgren 2009a). Terminating plant-based resources before peak bloom may negatively impact natural enemies as it destroys a potentially valuable resource that can help them thrive under the relatively harsh early season conditions. Therefore, it is critical to consider bloom phenology of cover crops intended as insectaries, as well as compatibility with the needs of crop management, e.g., planting dates.

Cover crop termination by incorporation of moldboard plow may represent a significant disturbance and source of mortality to arthropods inhabiting cover crops. This type of large scale disturbance disrupts arthropod habitat, forces local emigration and may directly kill arthropods. Holland and Reynolds (2003) found reduced numbers of six carabid beetle species in deep plowed plots compared to undisturbed fields. Another study found consistent increases in mortality and population decline in several arthropod taxa after a range of crop management strategies including superficial soil disturbance, non-inversion deep soil disturbance, weed harrowing, grass cutting and plowing (Thorbeck and Bilde 2004). During my study, the soil remained largely barren for several weeks after cover crop termination and before corn planting prior to corn establishment and growth. This disconnect in resource availability between the cover crop and cash crop may have resulted in direct mortality or emigration of natural enemies from cover crop into nearby fields or other areas where resources were available. This disturbance due to management, which would be common in this region, may reduce the likelihood that any beneficial arthropod which may have been supported by the cover crops early in the season, would remain in the vicinity to migrate into the succeeding cash crop to suppress pests.

Conclusion

In this study, increasing the diversity of the cover crop mixtures did not necessarily support higher numbers or diversity of natural enemies compared to monocultures. However, the abundances of spiders, lady beetles, and parasitoids were higher in the red clover monoculture compared with other cover crop monocultures and mixtures, despite the low abundance of red clover in both monocultures and mixtures. Several management aspects were incompatible with our goal of supporting natural enemies in this particular cropping system. For example, cover crop and natural enemy phenology, cover crop representation within mixtures, lack of spatio-temporal overlap between cover crop and cash crop, and cover crop management by inversion tillage may affect potential of this system to support early season beneficial insects that can carry over to provide biological control in the following cash crop.

In central Pennsylvania, cover crops in organic cropping systems need to be terminated early in May before corn can be planted in mid-May to early June. At this time, most commonly used flowering cover crops will not have reached peak bloom that could support spring emerging natural enemies and other beneficial insects, such as pollinators (Ellis and Barbercheck 2015). To more effectively support beneficial insects, cover crop bloom should be available when beneficial insects emerge and throughout the spring so that they may be supported until prey establishes in the main crop. Ideally, peak bloom should coincide with natural enemy emergence to meet the early-season resource requirements of natural enemies. In this study, natural enemy abundance remained low throughout the early spring when cover crops were available as a resource, i.e., cover crop blooms were available too early in the

season for key natural enemies, such as *C. maculata* and *O. insidiosus*, to be able to benefit from these resources.

In this cropping system, alternative cover crop planting schemes and management strategies should be considered if the goal is to support natural enemies and other beneficial insects. For example increasing the spatio-temporal overlap of the cover crop, main crop, and natural enemy populations could increase the benefits conferred to natural enemies. Insectary border planting, in which undisturbed flowering borders are maintained adjacent to the developing main crop may help to enhance natural enemy activity. This leaves an undisturbed habitat that may attract and support natural enemies by providing nectar, pollen, alternative prey, shelter and oviposition sites where their populations can develop alongside the main crop. Future studies aimed at enhancing natural enemies and biological control through provisioning of plant-based resources could involve screening additional cover crop species for their potential to attract and sustain natural enemies, and to establish in mixtures. In addition, the further development of planting schemes to maintain uninterrupted resource may facilitate natural enemy dispersal into the main crop is needed.

Chapter 2 Tables and Figures

Table 2.1. Accumulated arthropods collected via sweep net sampling in cover crop monocultures and mixtures in 2014.

Trophic Group	Taxa Found	Canola	Red Clover	Rye	4-Species	6-Species	Grand Total
Predatory/Parasitic	Araneae	42	68	25	31	30	196
	Carabidae	0	6	0	0	0	6
	Coccinellidae	13	31	3	6	3	56
	Hymenoptera-Braconidae	2	17	5	6	6	36
	Hymenoptera-Parasitica	4	48	11	10	9	82
	Ichneumonidae	3	7	14	8	10	42
	Nabidae	0	4	1	3	0	8
	Platygastridae	2	47	2	3	9	63
	Staphylinidae	0	2	2	1	0	5
	Syrphidae	2	2	0	1	0	5
	Total Parasitoids	11	119	32	27	34	223
Total Natural Enemies	79	351	95	96	101	722	
Heribvorous	Aphidididae	1	19	3	3	1	27
	Chrysomelidae	2068	24	10	41	18	2161
	Curculionidae	2368	200	13	206	89	2876
	Hemiptera	4	5	0	2	3	14
	Homoptera	3	89	13	9	9	123
	Lepidoptera	2	9	2	3	3	19
	Miridae	55	111	13	10	20	209
	Pentatomidae	0	5	1	0	0	6
	Thysanoptera	1	31	379	249	234	894
	Total Herbivores	4502	493	434	523	377	6329
Neutral	Apoidea	55	3	9	16	5	88
	Coleoptera	7	37	6	5	4	59
	Collembola	75	488	21	11	46	641
	Diptera-Brachycera	105	178	54	53	52	442
	Diptera-Nematocera	56	93	75	69	51	344
	Elateridae	2	3	0	0	1	6
	Formicidae	0	4	0	0	0	4
	Total Neutral	300	806	165	154	159	1584
	Total Arthropods						8635

Table 2.2. Accumulated arthropods collected via sweep net sampling in cover crop monocultures and mixtures in 2015.

Trophic Group	Taxa Found	Canola	Red Clover	Rye	4-Species	6-Species	Grand Total
Predatory/Parasitic	Anthocoridae	1	1	0	0	0	2
	Araneae	25	40	25	39	44	173
	Carabidae	1	2	0	1	0	4
	Coccinellidae	1	14	2	1	1	19
	Hymenoptera-Braconidae	9	80	11	24	13	137
	Hymenoptera-Parasitica	31	133	36	57	83	340
	Ichneumonidae	5	11	3	11	4	34
	Nabidae	0	9	2	2	2	15
	Platygastridae	12	362	15	35	35	459
	Opilionidae	0	1	0	0	0	1
	Staphylinidae	1	7	0	2	1	11
	Syrphidae	3	4	1	4	5	17
	Total Parasitoids	57	586	65	127	135	970
	Total Natural Enemies	146	1250	160	303	323	2182
Heribvorous	Aphidididae	2	24	7	6	13	52
	Chrysomelidae	69	15	5	2	0	91
	Curculionidae	566	101	40	152	61	920
	Hemiptera	0	2	3	0	1	6
	Homoptera	1	130	2	3	44	180
	Lepidoptera	11	12	3	12	11	49
	Miridae	132	89	34	140	62	457
	Pentatomidae	2	0	0	0	1	3
	Thysanoptera	44	465	942	537	663	2651
	Total Herbivores	827	838	1036	852	856	4409
Neutral	Apoidea	106	4	8	31	8	157
	Coleoptera	46	27	7	7	10	97
	Collembola	2	642	6	1	6	657
	Diptera-Brachycera	263	484	148	327	193	1415
	Diptera-Nematocera	212	665	249	340	232	1698
	Elateridae	2	0	0	1	0	3
	Formicidae	0	0	0	3	0	3
	Symphyta	0	3	1	2	2	8
	Total Neutral	631	1825	419	712	451	4038
Grand Total						10629	

Figures

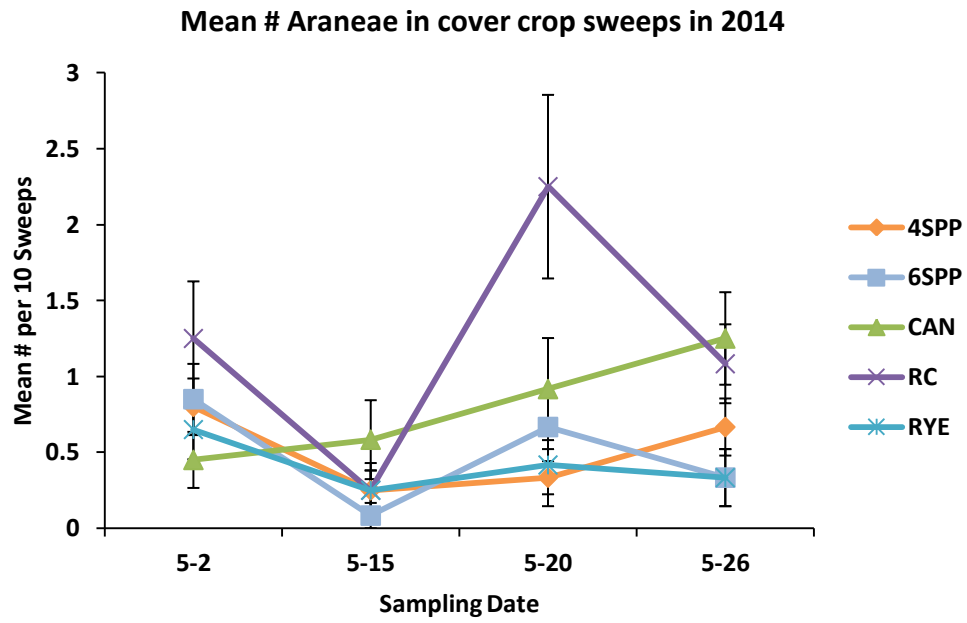


Figure 2.1a Mean number of Araneae collected per 10 sweeps in sweep net samples in cover crop treatments during the 2014 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

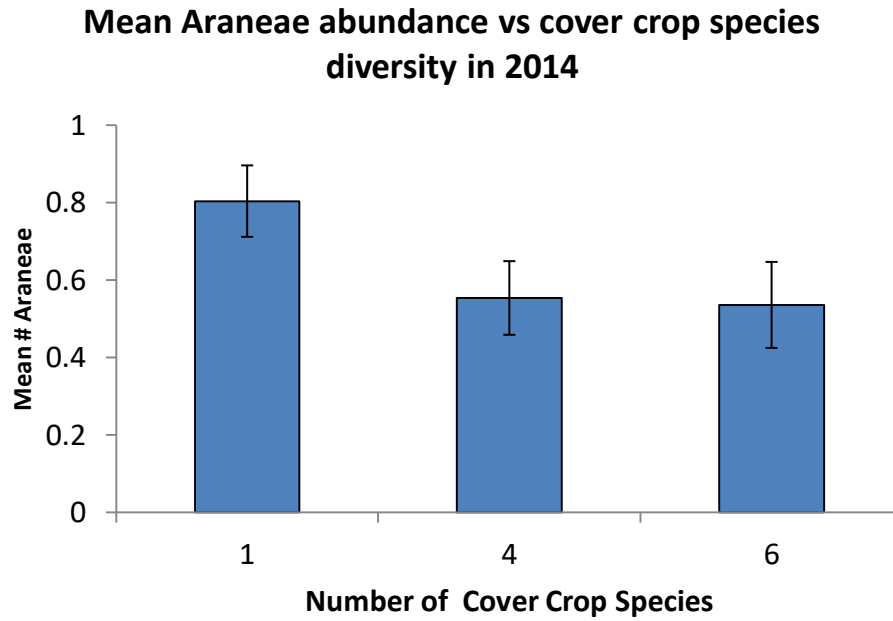


Figure 2.1b Mean number of Araneae collected per 10 sweeps in sweep net samples in cover crop treatments containing 1, 4, or 6 plant species during the 2014 study year.

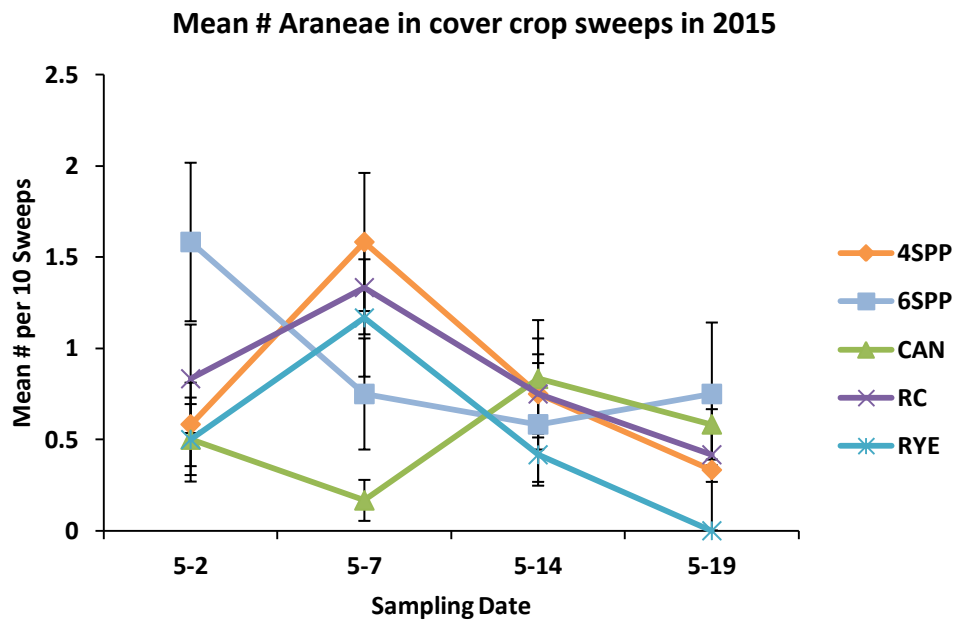


Figure 2.1c Mean number of Araneae collected per 10 sweeps in sweep net samples in cover crop treatments during the 2015 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

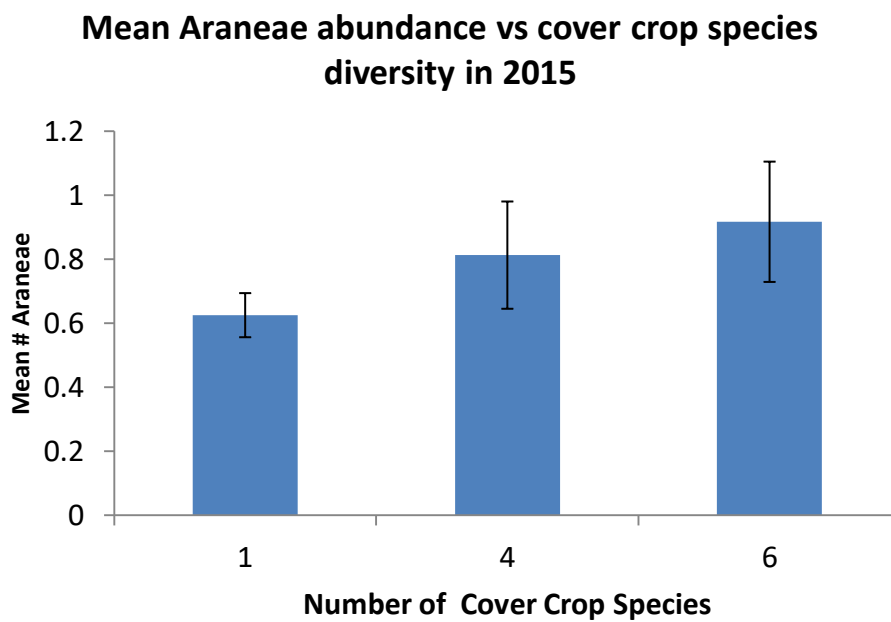


Figure 2.1d Mean number of Araneae collected per 10 sweeps in sweep net samples in cover crop treatments containing 1, 4, or 6 plant species during the 2015 study year.

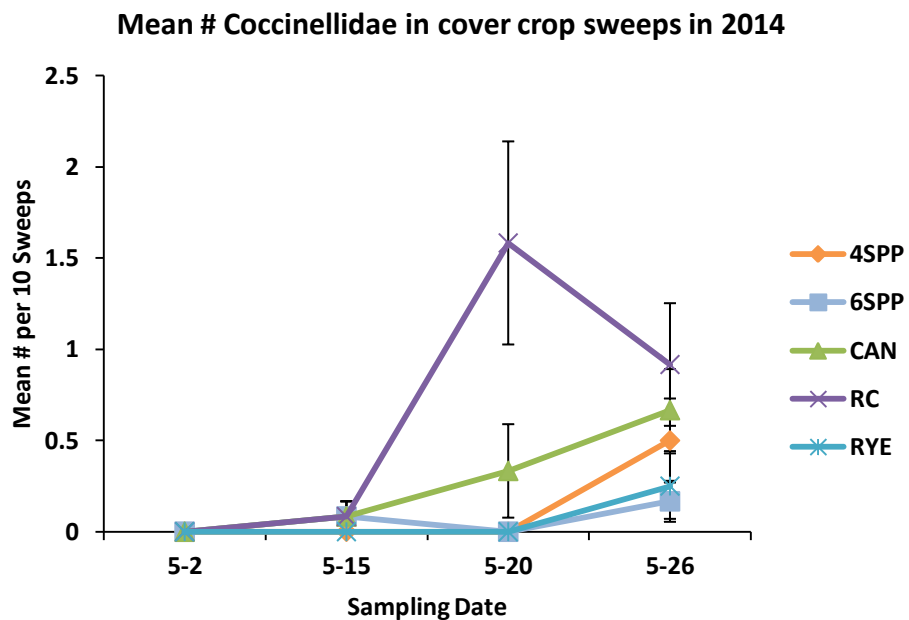


Figure 2.2a Mean number of Coccinellidae collected per 10 sweeps in sweep net samples in cover crop treatments during the 2014 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

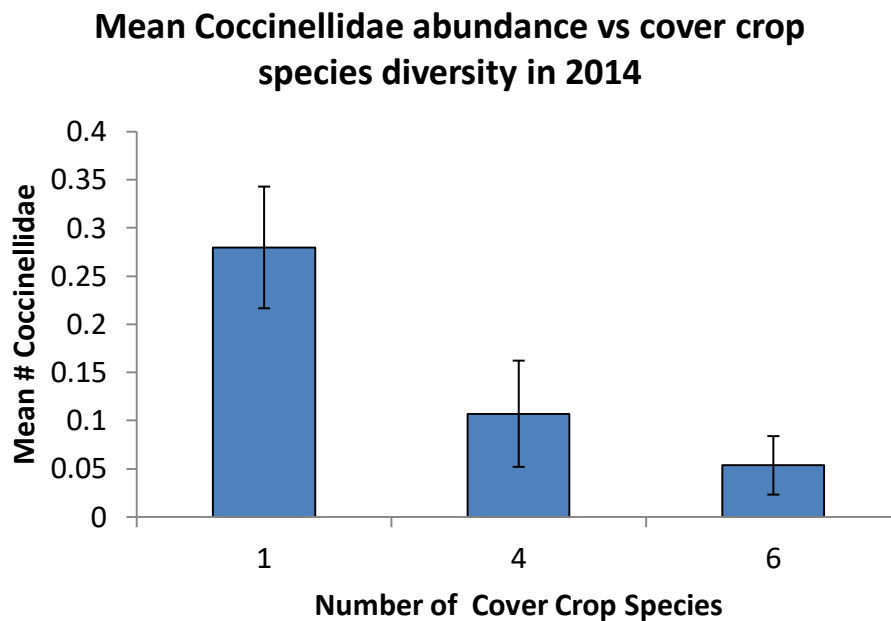


Figure 2.2b Mean number of Coccinellidae collected per 10 sweeps in sweep net samples in cover crop treatments containing 1, 4, or 6 plant species during the 2014 study year.

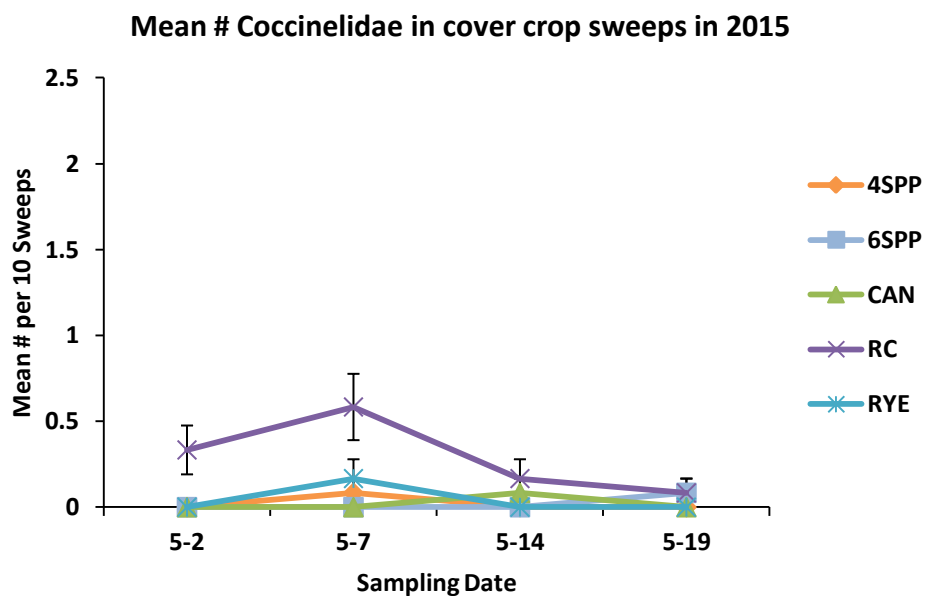


Figure 2.2c Mean number of Coccinelidae collected per 10 sweeps in sweep net samples in cover crop treatments during the 2015 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

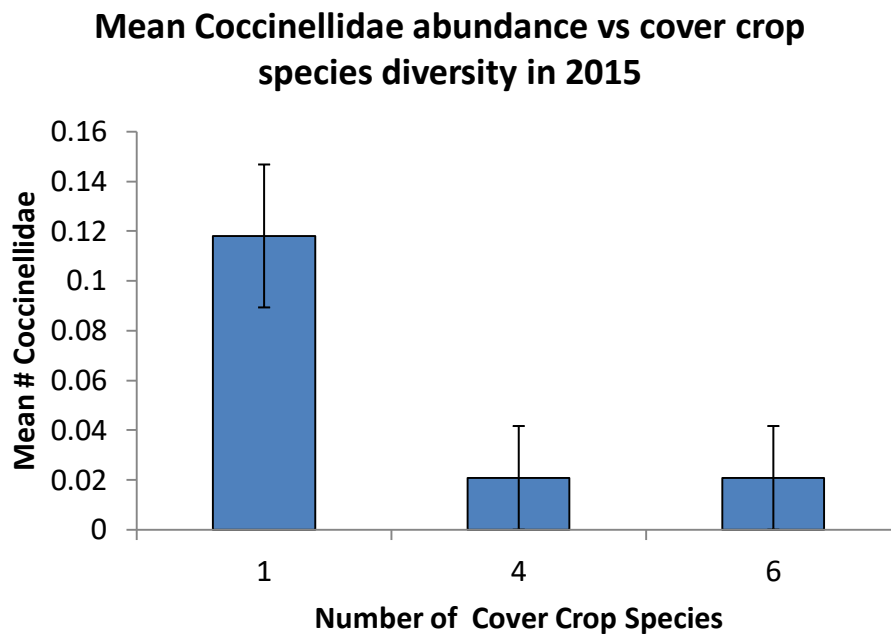


Figure 2.2d Mean number of Coccinellidae collected per 10 sweeps in sweep net samples in cover crop treatments containing 1, 4, or 6 plant species during the 2015 study year.

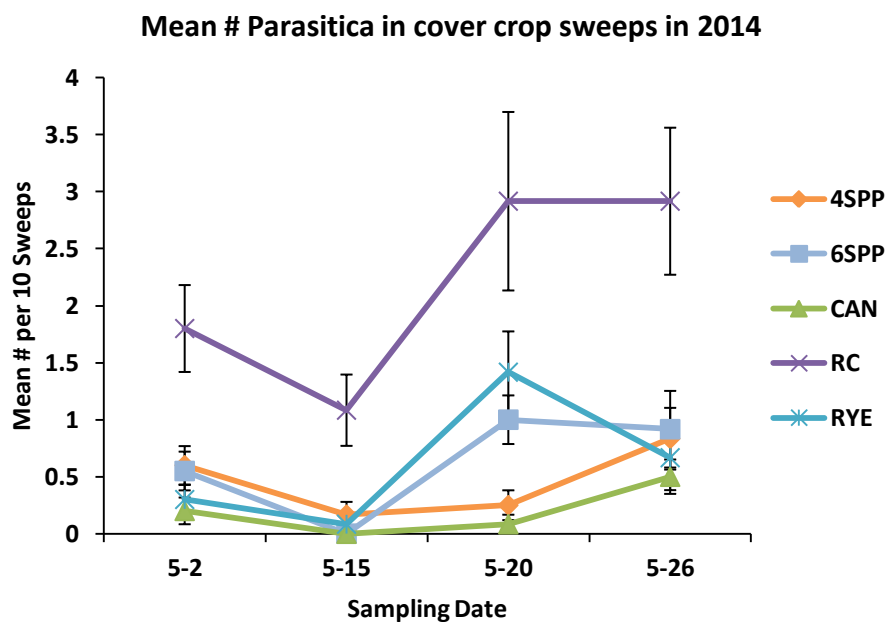


Figure 2.3a Mean number of hymenopteran parasitoids collected per 10 sweeps in sweep net samples in cover crop treatments during the 2014 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

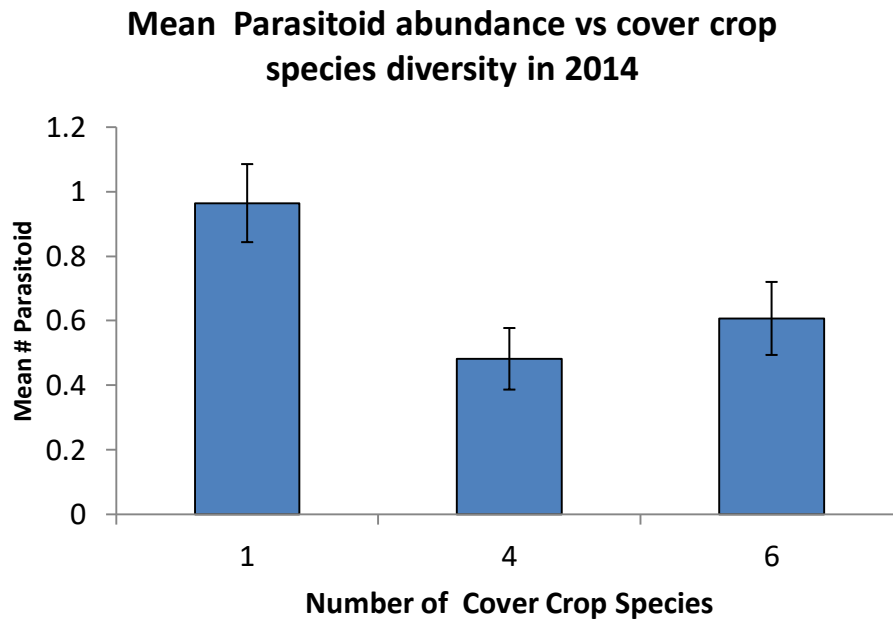


Figure 2.3b Mean number of Parasitica collected per 10 sweeps in sweep net samples in cover crop treatments containing containing 1, 4, or 6 plant species during the 2014 study year.

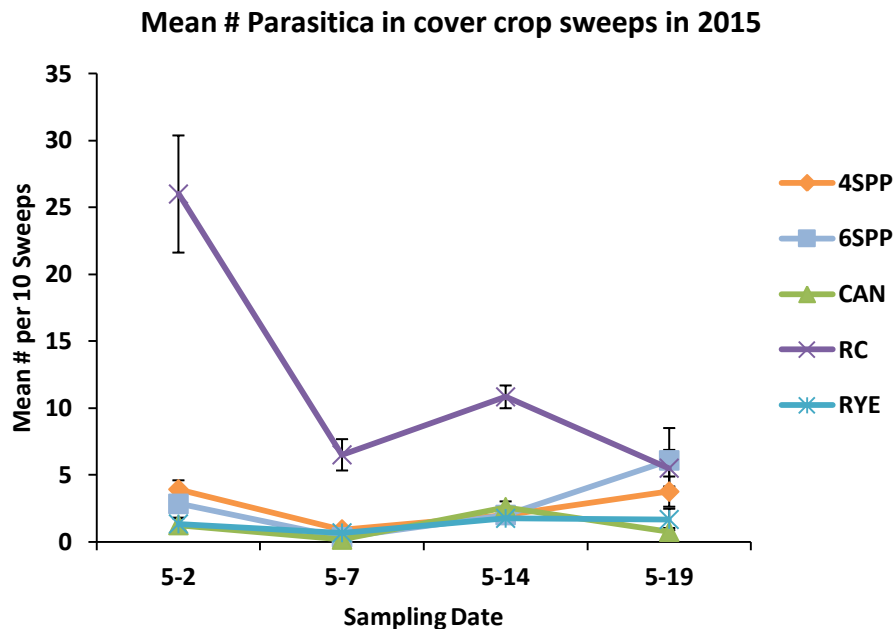


Figure 2.3c Mean number of hymenopteran parasitoids collected per 10 sweeps in sweep net samples in cover crop treatments during the 2015 study year (4SPP: 4 species mix, 6SPP: 6 species mix, CAN: canola, RC: red clover and rye).

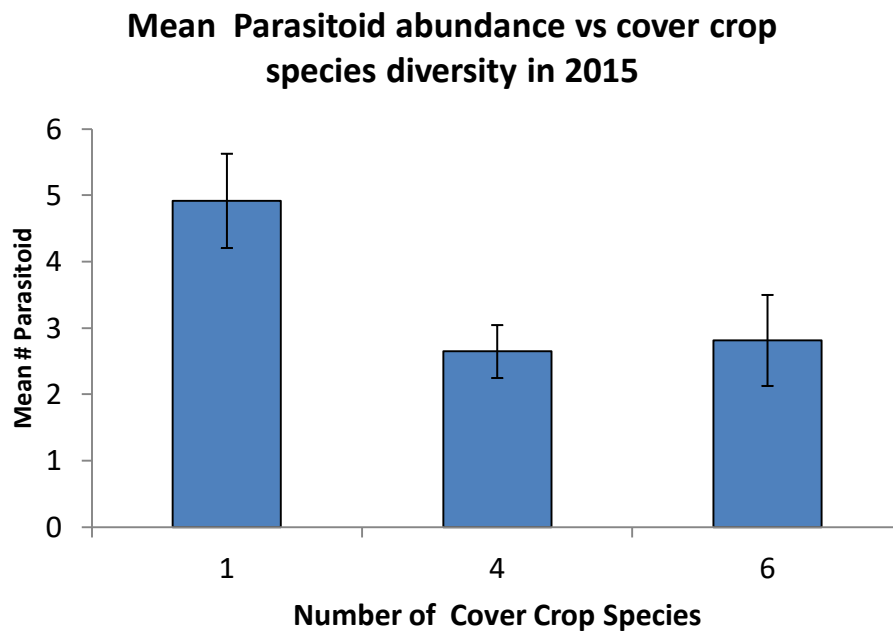


Figure 2.3d Mean number of Parasitica collected per 10 sweeps in sweep net samples in cover crop treatments containing containing 1, 4, or 6 plant species during the 2015 study year.

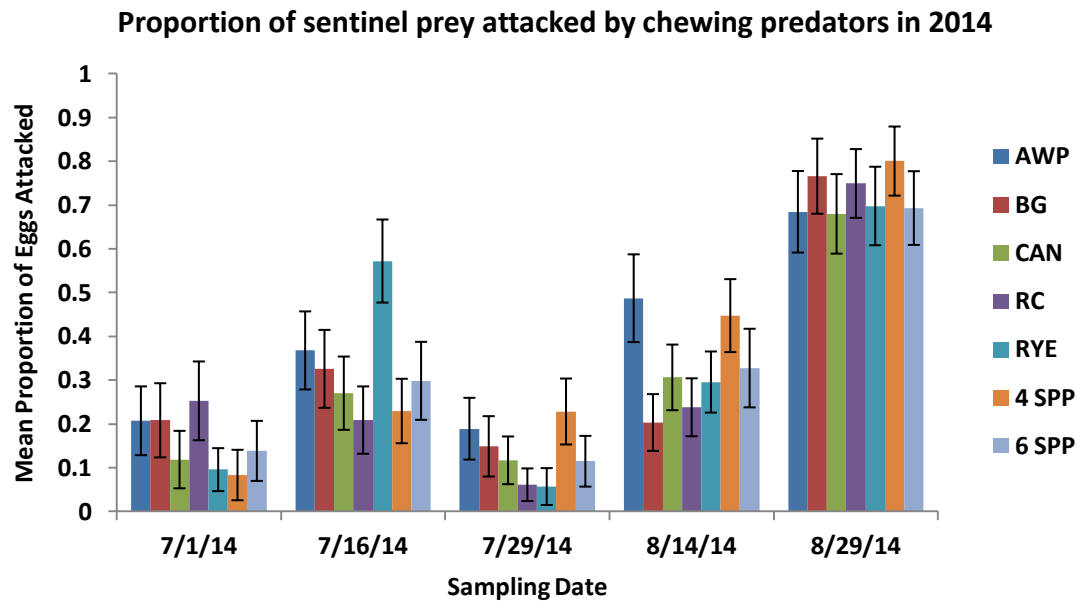


Figure 2.4a Mean proportion of European corn borer sentinel prey attacked by chewing predators during the 2014 study year .

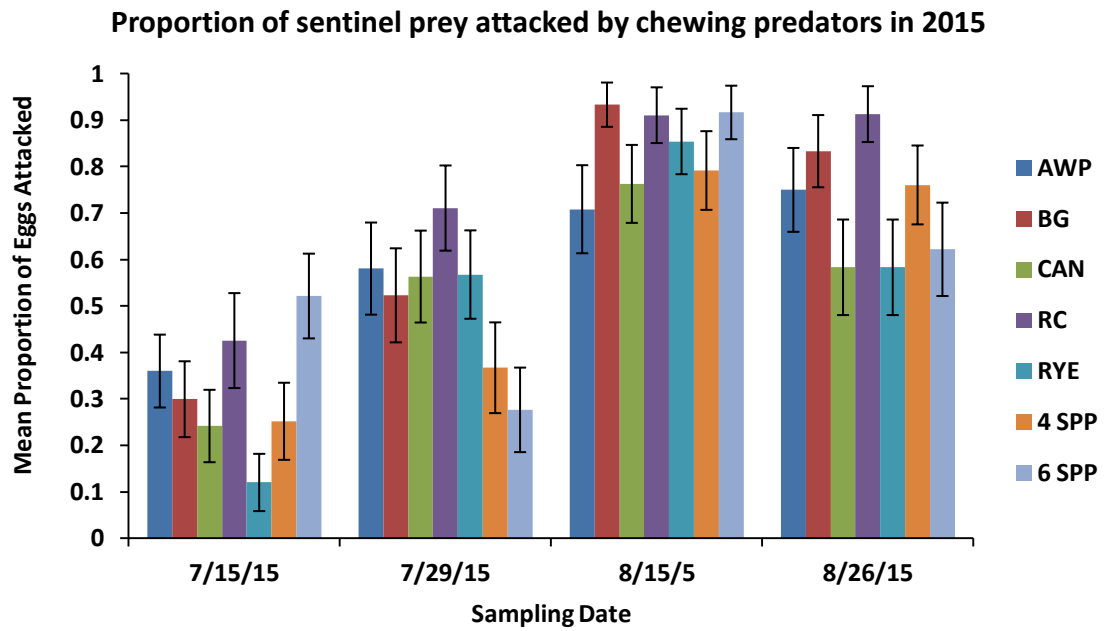


Figure 2.4b Mean proportion of European corn boreer sentinel prey attacked by chewing predators during the 2015 study year.

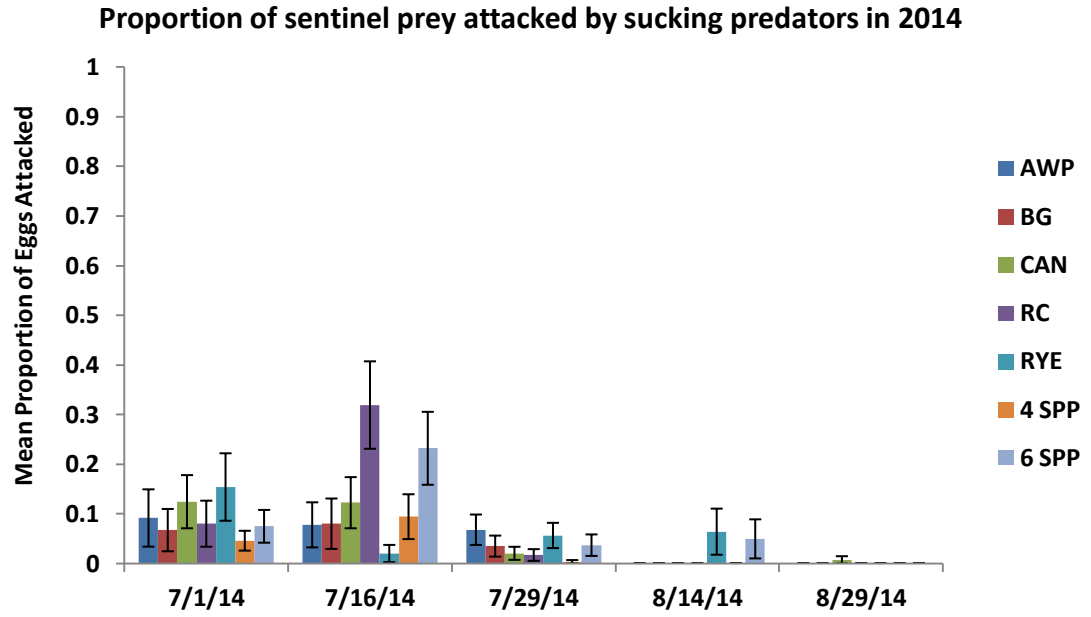


Figure 2.5a Mean proportion of European corn boreer sentinel prey attacked by sucking predators during the 2014 study year.

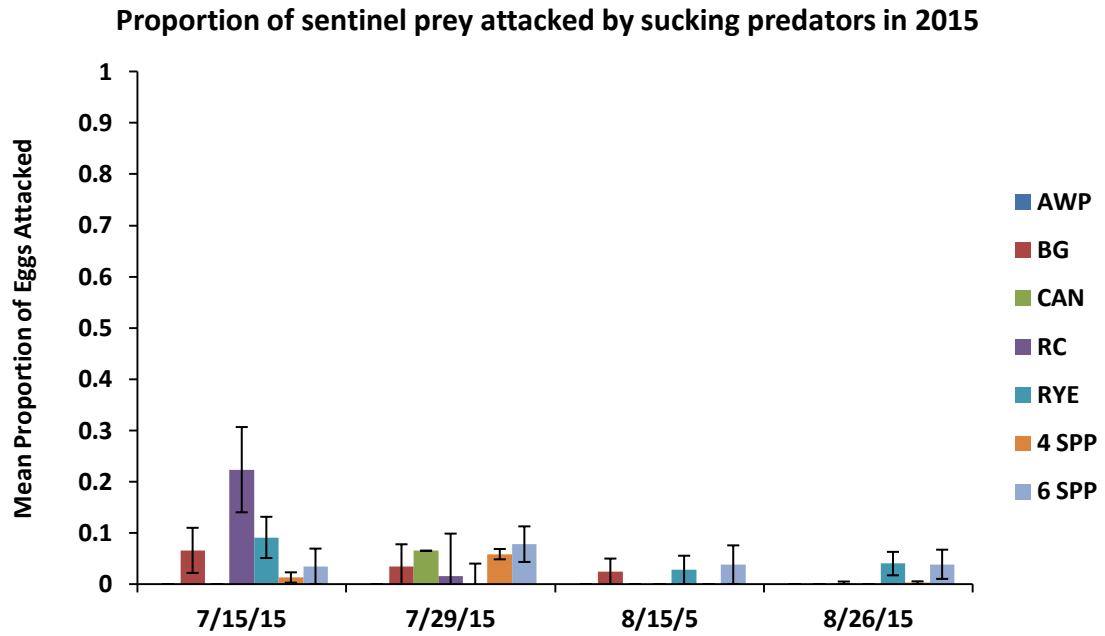


Figure 2.5b Mean proportion of European corn boreer sentinel prey attacked by sucking predators during the 2015 study year.

Chapter 3

Impacts of nectar and pollen provisioning by *Fagopyrum esculentum* and *Vigna unguiculata* on performance of *Orius insidiosus*

Introduction

There is an increasing interest in the role of non-crop, plant-based, resources to support natural enemies for biological control of economically important agricultural pests. Habitat management, a conservation biological approach to pest management often focuses on incorporation of insectary, or resource-producing, plants into agricultural landscapes. Insectary plants provide nectar, pollen, alternative prey and shelter that natural enemies can exploit to increase their likelihood of survival in harsh, resource-poor, and fragmented landscapes and agroecosystems (Ehler 1998, Landis et al. 2000, Gurr et al. 2017a).

When provided with supplemental food resources, natural enemies survive longer and produce more offspring than in absence of nectar and pollen (Baggen and Gurr 1998, Vattala et al. 2006, Nafziger and Fadamiro 2011, Géneau et al. 2012, Pumariño et al. 2012, Géneau et al. 2013). While nectar and pollen can support natural enemies, studies suggest natural enemies may respond differently depending on functional traits of particular plant species. Insects prefer flowering species based on a suite of morphological characteristics. These can include flower color, anatomy and nectary structure, plant growth habit and nutritional content (Vattala et al. 2006, Géneau et al. 2013). When considering non-crop plants to support natural enemies, special consideration must be giving to plants that are not attractive or accessible for exploitation by pest species (Araj et al. 2009, Winkler et al. 2009). These findings suggest that multiple plant species must be screened for suitability for targeted natural enemies and impacts on potential pest and antagonistic species. Because natural enemies may respond to

supplemental food differently, it is critical to assess the potential of each insectary plant species to influence natural enemy performance.

In addition to screening insectary plants singly, the potential for increasingly diverse mixtures containing complementary resources should be considered. A mixed diet of prey and nectar may improve natural enemy longevity and fecundity beyond levels previously observed for either prey or nectar resource alone (Lundgren 2009a, Lundgren et al. 2011, Pumariño and Alomar 2012, Choate and Lundgren 2013). Studies often focus on comparing single species insectary borders to a control or compare wildflower mixtures to a control lacking plant-based resources (Gareau et al. 2013). Few studies, however, deliberately assess the impacts of insectary plant mixtures, containing multiple species, on natural enemy performance compared to monoculture borders (Pumariño et al. 2012). This approach may elucidate each insectary species' contribution to supporting natural enemies in mixtures. When provided in mixtures, multiple insectary species may provide complementary resources that natural enemies can choose from for optimal growth, development, and fecundity.

The goal of this research was to examine the role of two potential insectary plant species on the performance of *Orius insidiosus* in controlled laboratory assays. This important generalist natural enemy feeds on a variety of economically important pests such as thrips (Thysanoptera: Thripidae), European corn borer (*Ostrinia nubilalis*) and other soft-bodied arthropods in many agroecosystems and greenhouse environments (Van den Meiracker and Ramakers 1991). I chose buckwheat (*Fagopyrum esculentum*) and cowpea (*Vigna unguiculata*) as resource plants as they provide abundant nectar and pollen that can support natural enemies (Lee and Heimpel 2005, Clark 2007, Quinn et al. 2017). Buckwheat produces nectar

through its numerous, open, white flowers while cowpea secretes nectar through extrafloral nectaries at the base of its leaves (Kuo and Pate 1985). An additional goal of this research was to investigate the impact of insectary plants on *O. insidiosus* performance when provided a biculture compared to either species singly. A biculture of cowpea and buckwheat may provide complementary resources that can potentially enhance *O. insidiosus* performance beyond the effects of either insectary species alone.

The specific objectives of this study were to test the impacts of insectary plant species provided singly and in a more complex biculture on *O. insidiosus* longevity, fecundity and predation rates on sentinel European corn borer. I hypothesized that when compared to a control treatment lacking a nectar resource, *O. insidiosus* will survive longer and lay more eggs in the presence of nectar (Heimpel and Jervis 2005, Géneau et al. 2013). For a biculture, I hypothesized that survival and fecundity of *O. insidiosus* would be greater in plant biculture than monoculture as a greater diversity of plant-based resources would be available for *O. insidiosus*' growth and development. I also hypothesized that predation would similarly be greater in the presence of supplemental nectar compared to no nectar treatments as *O. insidiosus* would be more vigorous and able to forage and attack prey.

Materials and Methods

Insect rearing and Insectary plant establishment

A colony of *O. insidiosus* was reared from purchased individuals (Beneficial Insectary, Redding, CA). Insects were reared under controlled conditions at 27°C, 70% relative humidity, and L14:D10 photoperiod. Rearing containers consisted of 236.5 mL paper food containers

topped with matching meshed lids to provide adequate ventilation. Each container housed approximately 30-50 individuals. Shredded paper was added to each rearing cage to provide refuge and reduce cannibalism. Insects were reared on a diet of purchased *Ephestia kuehniella* eggs (Beneficial Insectary, Redding, CA) provided on card stock. Insects were provided with sections of fresh green beans (*Phaseolus vulgaris*) to provide a water source and an oviposition substrate. Rearing containers were checked and maintained every 2 days, when new food and green bean sections were added. During colony maintenance, dead individuals were aspirated from rearing containers and, if necessary, soiled containers were replaced. Nymphs were reared separately by cohort from green bean sections taken from adult cages. Under these rearing conditions *O. insidiosus* typically completed development in 10 days after oviposition and were sexually mature 12 days evidenced by the first appearance of eggs in rearing cages. Voucher specimens were deposited in the Frost Entomological Museum.

Buckwheat (*F. esculentum*) (Lakeview Organic Grain, Penn Yan, NY) and cowpea (*V. unguiculata* variety: Iron and Clay, Hancock Seeds, Dade City, FL) were planted weekly in the greenhouse and grown under ambient conditions. Under greenhouse conditions buckwheat typically bloomed after 21 days. Cowpea extrafloral nectaries were observed secreting nectar during this similar time frame. For laboratory assays, cut buckwheat and cowpea stem and leaf sections similar in size were used as floral and extrafloral nectar sources.

Experimental Arenas

Experimental arenas consisted of 14cm-diameter Petri dishes lined with filter paper and fitted with a mesh lid and weather sealant tape, and sealed with masking tape to prevent predators from escaping. Before each experimental trial, newly emerged (<24 hrs) adults were

collected from rearing cages and separated by sex. Insects were then starved for 24 hours prior to initiation of the experiment. Experimental cages were prepared by cutting assay plants, wrapping their stems in cotton and placing their stems in a water wick. One *E. kuehniella* food card and one green bean with cut ends sealed with paraffin wax were added to each cage to provide a protein source and an oviposition substrate, respectively. Ten *O. insidiosus* individuals consisting of 4 males and 6 females, representative of the colony's sex ratio, were added to each Petri dish. Experimental cages were maintained under similar rearing conditions to the laboratory colony. Fresh food, plants and green bean sections were added to refresh the rearing cages every two days.

Longevity and Fecundity: *Epehstia kuehniella* Trials

To test the effect of insectary plant provisioning and diversification on the longevity and fecundity of *O. insidiosus*, three treatments and a control were used. Experimental treatments included buckwheat or cowpea offered singly, and a biculture of buckwheat and cowpea. The control consisted of a cotton topped water wick to provide water. In each trial, all *O. insidiosus* individuals belonged to the same age cohort. Initially treatments were replicated 8 times during the first trial, but increased to 10 in the second and third *E. kuehniella* trials.

I monitored *O. insidiosus* survival by checking experimental arenas daily and recording the number of living males, females and total number insects per experimental arena. Dead individuals were removed via aspiration and their sex confirmed before discarding them. Survival and fecundity data collection continued for 20 days or until all insects were dead.

Fecundity was recorded every 48 hours. Green bean and plant sections taken from experimental arenas were examined under a stereomicroscope and number of *O. insidiosus* eggs found in green bean, buckwheat and cowpea recorded. All plant material including adaxial and abaxial leaf surfaces, stems and flowers were dissected and carefully examined for the presence of eggs.

Longevity, Fecundity and Predation: *O. nubilalis* Trials

To determine the effects of floral resource provisioning and diversification on predation, similar experiments were conducted as those described above using similar treatments. I conducted a second set of laboratory trials using European corn borer eggs as a food source representative of field prey. Predation assays were conducted across four trials. Longevity and fecundity were again recorded due the change in prey species. Treatments were replicated times 9 during the first two trials but increased to 10 for the second two trials.

Similar to the previous experiments, survival was recorded daily, while fecundity and predation was recorded every 48 hours when new food plants were added. Experimental cages were prepared as described above. However, European corn borer eggs were prepared by gluing egg masses to card stock and added to arenas. Initially predators were provided with ~15 eggs, but tripled in subsequent treatments to ensure ample prey was available. Prey cards were examined under the microscope and predation recorded as the proportion of *O. insidiosus* eggs attacked. Food cards were kept and double checked after 48 hours to confirm viable *O. insidiosus* hatched. Data collection continued for 8 days.

Statistical Analysis

Analyzed data included mean proportion of *O. insidiosus* surviving, mean number of eggs laid per 48 hours and cumulative mean number of eggs by plant substrate for the *E. kuehniella* trials. Data from all *E. kuehniella* trials were pooled prior to analysis. Proportion data was arcsine square root transformed to meet assumptions of homogeneity of variances. Untransformed data is reported. Data for the *O. nubilalis* predation trials was similarly handled, however, predation data was included and analyzed as mean proportion of sentinel *O. nubilalis* attacked. Predation data was arcsine square root transformed prior to analysis. For each test, a model was constructed to determine the effects of main treatment effects over the course of the experiment. Longevity and fecundity treatment means were compared using repeated measures ANOVA (SAS Institute, 2002). Cumulative mean number of eggs produced and predation rate was analyzed as a one-way ANOVA. Treatment effects were considered significant when $P < 0.05$.

Results

Longevity and fecundity: *E. kuehniella* Trials

O. insidiosus survived in experimental cages for approximately 20 days. Insectary plant significantly affected the proportion of insects surviving. During the *E. kuehniella* trials, insectary plant significantly impacted *O. insidiosus* survival ($F_{3,2339} = 19.21$, $P < 0.0001$) (Figure 3.1). On each day sampled, a greater proportion of insects survived in biculture compared to cowpea ($P = 0.003$) and buckwheat ($P = 0.034$), which were statistically similar ($P = 0.406$). There were significantly fewer insects surviving in the water only control compared to any treatment containing insectary plants ($P < 0.0001$).

O. insidiosus successfully oviposited and completed development on all experimental plant species. Insectary plant had a significant effect on the mean number of eggs laid by *O. insidiosus* ($F_{3,1107} = 22.55$, $P < 0.0001$). *O. insidiosus* oviposited significantly greater numbers of eggs in the biculture compared to control ($P < 0.0001$), buckwheat ($P = 0.0004$) and cowpea ($P = 0.041$) (Figure 3.2). Statistically similar numbers of eggs were observed in cowpea compared to buckwheat ($P = 0.135$). Predators in the control treatment consistently oviposited the fewest number of eggs (Figure 3.2).

O. insidiosus displayed a strong preference to oviposit into insectary plants compared to green bean (Figure 3.3). Whenever cowpea or buckwheat was available, *O. insidiosus* tended to exclusively lay eggs into insectary host plants compared to the recommended green bean host (Figure 3.3). In biculture, there appeared to be a clear preference for cowpea over buckwheat as majority of eggs were laid in cowpea plant hosts compared to buckwheat. Eggs were observed most frequently in close proximity to cowpea extrafloral nectaries and at the base of the undersides of leaves near stems. Few eggs were laid in buckwheat flowers or on stems of either plant.

Longevity and fecundity: *O. nubilalis* Predation Trials

Insectary plant significantly affected *O. insidiosus* survival in predation trials where predators were fed European corn borer eggs ($F_{3,1327} = 34.67$, $P < 0.0001$). There were significantly more *O. insidiosus* surviving each day in biculture compared to buckwheat ($P = 0.002$) and cowpea ($P < 0.0001$), which were statistically similar ($P = 0.061$) (Figure 3.4). Fewer predators survived in water control compared to all other treatments ($P < 0.0001$) (Figure 3.4).

Insectary plant host significantly affected the mean number of eggs oviposited by *O. insidiosus* ($F_{3,595} = 33.41$, $P < 0.0001$). *O. insidiosus* laid significantly more eggs in biculture compared to buckwheat, cowpea and least in the water control ($P < 0.001$) (Figure 3.5). Statistically similar number of eggs were found in buckwheat and cowpea ($P = 0.395$). There were significantly greater numbers of eggs in the insectary plant treatments compared to the water control (Figure 3.5a).

O. insidiosus oviposition was greater in insectary plant hosts compared to green bean as predators appeared to prefer to oviposit in cowpea and buckwheat instead of green bean whenever available (Figures 3.6). In biculture, a greater number of eggs were oviposited in cowpea compared to buckwheat and fewest in green bean (Figures 3.6).

Effects on Insectary Plant Provisioning on Predation

O. insidiosus readily attacked sentinel prey of *O. nubilalis* eggs in laboratory assays. Results were omitted from the first predation trial where predation was overestimated by the low number of prey eggs provided. This was corrected by providing more prey in subsequent trials. Signs of *O. insidiosus* predation on European corn borer eggs were consistent with those previously described by Conrad (1959). Predation ranged from 40-50% eggs attacked. Insectary plant provisioning significantly influenced predation by *O. insidiosus* ($F_{3,451} = 2.75$, $P = 0.042$) (Figure 3.7). Predation rate was significantly reduced in biculture compared to cowpea ($P = 0.0053$), but only marginally significant compared to buckwheat ($P = 0.052$). Biculture was not significantly different compared to the water only control ($P = 0.123$).

Discussion

This study was aimed at determining the potential of two insectary plants, buckwheat and cowpea, to enhance the performance of *O. insidiosus*, an important natural enemy of economically important pest species. Resource provisioning through the use of insectary plants has been well documented in other studies (Skirvin et al. 2007, Straub et al. 2013, Gurr et al. 2017a). Insects, however, may prefer particular plants, and insectary plants may differentially attract and impact insect performance. Therefore, screening insectary plants may provide insights into the potential of a particular plant species to support natural enemies in a controlled environment before choosing to implement a plant species or mixture in the field to support particular natural enemies.

This study is the first to provide empirical evidence that cowpea can function as an insectary plant species that can support *O. insidiosus*. My hypothesis that *O. insidiosus* would perform better in a biculture treatment compared to a monoculture was supported. *O. insidiosus* survived longer when provided with buckwheat and cowpea compared to the water-only control. *O. insidiosus* maintained in the biculture tended to survive longer overall and have higher daily mean survival rate. Similar studies have found improved survival rates of a natural enemy when prey and supplemental nectar resources are provided (Nafziger and Fadamiro 2011, Pumariño and Alomar 2012, Pumariño et al. 2012, Géneau et al. 2013). A related species, *Orius majusculus*, survived longer when reared with supplemental alyssum, *Lobularia maritima*) compared with a water-only control. In comparison to a diet of alyssum with and without supplemental prey, *O. majusculus* survived longer on alyssum without prey compared to a water-only control. However, when provided with both supplemental prey and nectar, *O.*

majusculus survived longer than in the alyssum-only treatment or in the control. None of these studies investigated the use of multiple plant species on natural enemy longevity, and few empirical studies are available. A study by Pumariño et al. (2012) was one of few that compared longevity of *O. insidiosus* reared in the presence of supplemental resource plants in monocultures and a mixture. In that study, supplemental resources included alyssum (*L. maritima*), buckwheat (*F. esculentum*), phacelia (*Phacelia tanacetifolia*), fava bean (*Vicia faba*) and chamomile (*Matricaria chamomilla*), and a mixture containing all species. The mixture, although providing a diversity of resources, did not improve *O. insidiosus* survival beyond any plant species alone (Pumariño et al. 2012). My results contrast with those observed by (Pumariño et al. 2012). In my experiments, *O. insidiosus* survived significantly longer on a mixture of cowpea and buckwheat compared to a water control or either plant alone. It is possible that the mixture may provide complementary nutrients that can better support *O. insidiosus* development compared to either plant species offered separately. Fecundity was similarly affected by insectary plant species. *O. insidiosus* laid the greatest numbers of eggs when provided with a mixture of cowpea and buckwheat compared to each plant grown in monoculture. It is possible that the nutrient combination provided by these plants may be more optimal for *O. insidiosus* growth and development, as more eggs were laid in the mixture treatment compared to in either plant alone. In similar studies, natural enemy fecundity was greater when provided with supplemental nectar (Pumariño and Alomar 2012, Pumariño et al. 2012, Wong and Frank 2013). *O. majusculus* was more fecund when reared with supplemental alyssum and prey compared to either prey separately or alyssum added alone (Pumariño and Alomar 2012). In a study that compared *O. insidiosus* fecundity reared on insectary plants and

mixture, more eggs were laid in the presence of supplemental nectar compared to a water control (Pumariño et al. 2012). Similar numbers of eggs were laid in all resource treatments, but were significantly greater than in the control. The results of their study were consistent with the effects on fecundity I observed. This may be also one of the first studies to document the impact of cowpea extrafloral nectaries on *O. insidiosus* fecundity. My observations of oviposition choice revealed that *O. insidiosus* prefers cowpea as the majority of eggs laid were near the extrafloral nectaries of cowpea and on the undersides of cowpea leaves (Pumariño et al. 2012) similarly found changes in oviposition preferences in *O. insidiosus* when provided with a diverse mix of resource plants. In this study, fava bean, which was the least preferred by *O. insidiosus* when provided alone, became most attractive when offered in a mixture. They suggested that *O. insidiosus* may prefer less favorable host plants in the presence of ample resources to support egg development. Ample resources would more likely be provided by a mixture of species.

Cowpea has been anecdotally cited to be beneficial to natural enemies (Clark 2007). It is possible that nectar secreted by cowpea may be more nutritionally optimal for *O. insidiosus* development (Kuo and Pate 1985, Pate et al. 1985). Alternatively, the leaf structure of cowpea may influence its suitability as an oviposition substrate (Lundgren and Fergen 2006). Lundgren et al. (2008) found that relative trichome density and epidermis thickness may drive reproductive host suitability. *O. insidiosus* laid the most eggs into plant species with thinnest layers of epidermal cells. This often correlated with higher mean survival of offspring compared to those oviposited into thicker leaves. Additionally, these results revealed cowpea leaf morphology may be more desirable to females than green bean and buckwheat.

Results from predation assays suggest that insectary plant provisioning can influence the predation rates. My hypothesis that predation would increase in the presence of supplemental nectar compared to a water only control was not supported. Predation was consistently lower when provided with the biculture containing both buckwheat and cowpea. Initially, I expected higher predation rates in the water control compared to insectary treatments. However predation in the water only control was similar to buckwheat and cowpea alone. This may have been due to increased predator mortality in water control arenas. Fewer surviving predators were available to attack available to prey, resulting in lower overall predation in the water control. Predation was relatively high in cowpea and buckwheat despite the presence of floral and extrafloral nectaries. Higher predator survival and fecundity, may have contributed to higher proportions of prey attacked. This suggests that cowpea and buckwheat, when provided singly may provide sufficient nectar to support survival and egg development, but not sufficient to distract it from prey items (Lundgren 2009b). Predators reared in biculture arenas consistently attacked fewer *O. nubilalis* eggs. In these cases excess nectar and pollen may have satiated predators making prey eggs less appealing to *O. insidiosus*. Although nectar and pollen may often benefit target natural enemies and enhance pest suppression, predation may be reduced when resources provided in excess (Cottrell and Yeargan 1998, Spellman et al. 2006). Skirvin et al. (2007) found that predation on thrips by *Orius laevigatus* was reduced when supplemental floral pollen was provided. This raises important considerations for designing insectary borders in field for habitat management purposes. Management of insectary plants should be carefully considered to avoid cases when excess resources provide may compete with or distract from pest suppression.

Conclusion

My experiments suggest that nectary provisioning improves *O. insidiosus* survival and fecundity compared to a lack of plant-based resources. Additionally, when the insectary resource was diversified by adding a second plant species, longevity and fecundity increased further. Studies such as these demonstrate the ability to assess the potential insectary plants to support natural enemies prior to establishment in the field. In this case, I provided experimental evidence that cowpea can support development of *O. insidiosus*. These effects may persist in the field environment. Future studies can further screen and identify potential insectary species as different insects are attracted to and benefit differently from different plants (Vattala et al. 2006). Promising species can then be tested under more complex field conditions to determine viability and compatibility with grower management goals and constraints. Eventually, insectary mixtures can be customized around the predator, prey and plant community in different agroecosystems and utilized to more effectively suppress pests. In addition to insectary composition, the abundance of nectar and pollen provided by each species in the mixture should be considered as excess may result in detrimental effects on pest suppression.

Chapter 3 Figures

Figures

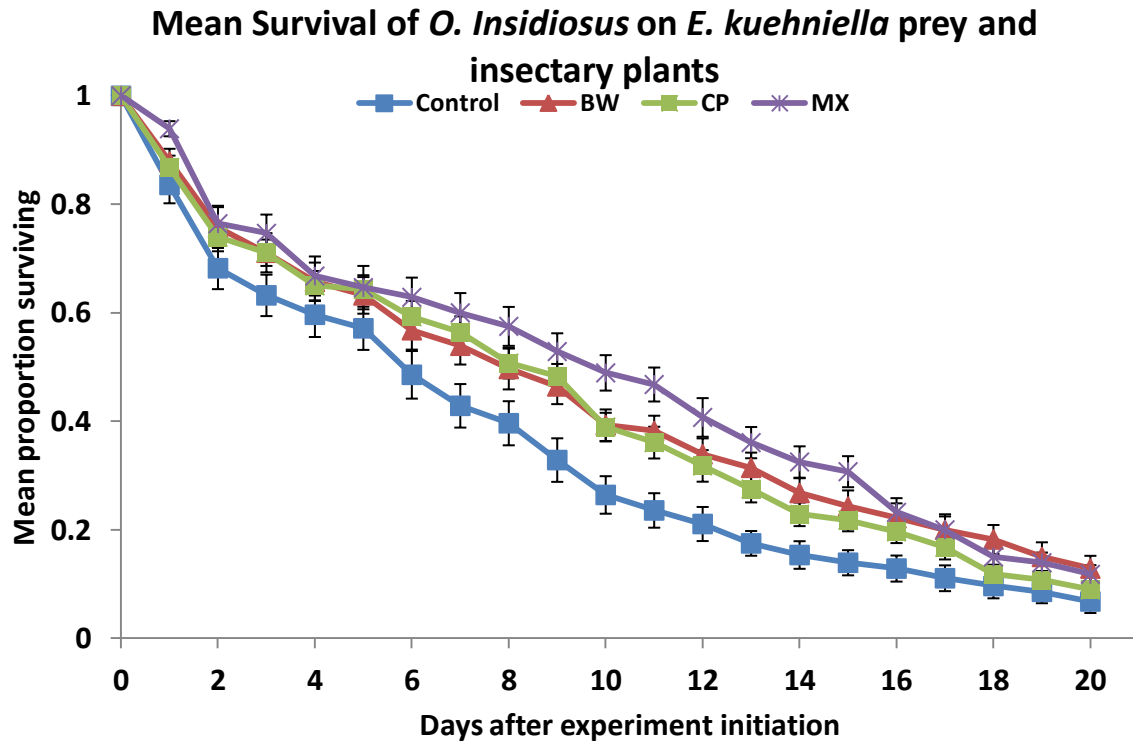


Figure 3.1. Mean survival of *O. insidiosus* individuals reared in cages fed with *E. kuehniella* prey and nectar resource. Treatments were the water control, buckwheat (BW), cowpea (CP) and biculture (MX) treatments.

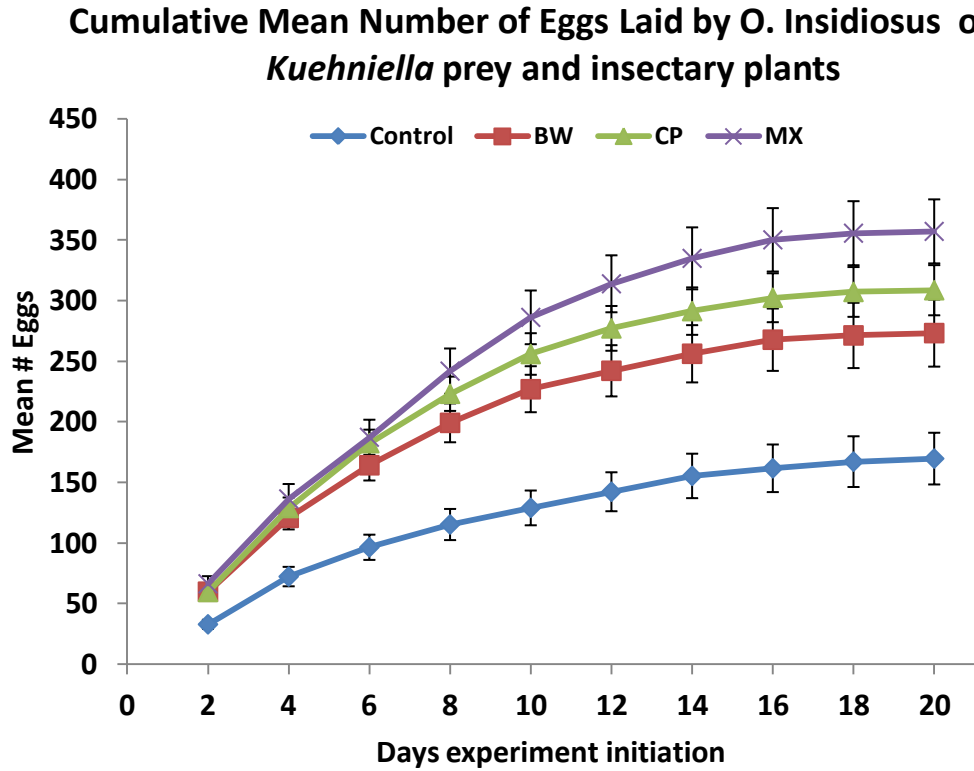


Figure 3.2. Mean number *O. insidiosus* eggs oviposited into plant sections in the water control, buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *E. kuehniella* trials. Data is presented as accumulated mean number of eggs.

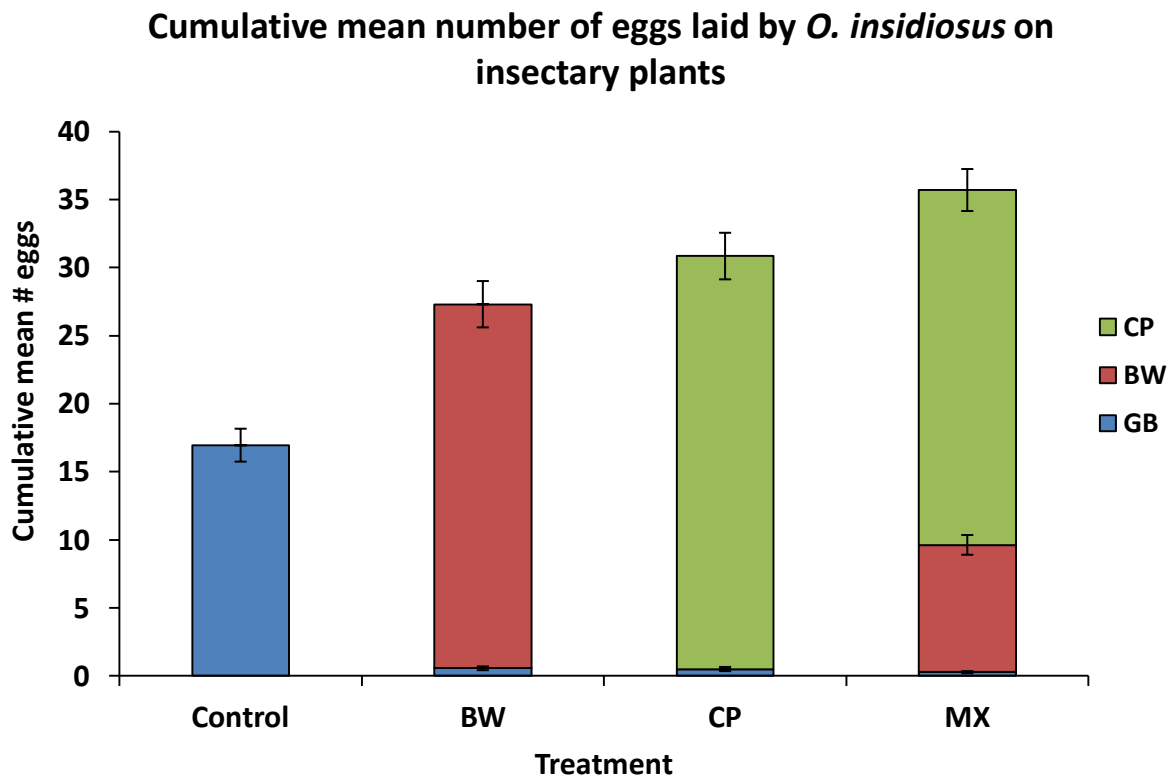


Figure 3.3. Cumulative mean number of *O. insidiosus* eggs oviposited into green bean (GB) buckwheat (BW) and cowpea (CP) plant sections in the water control (C), buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *E. kuehniella* trials.

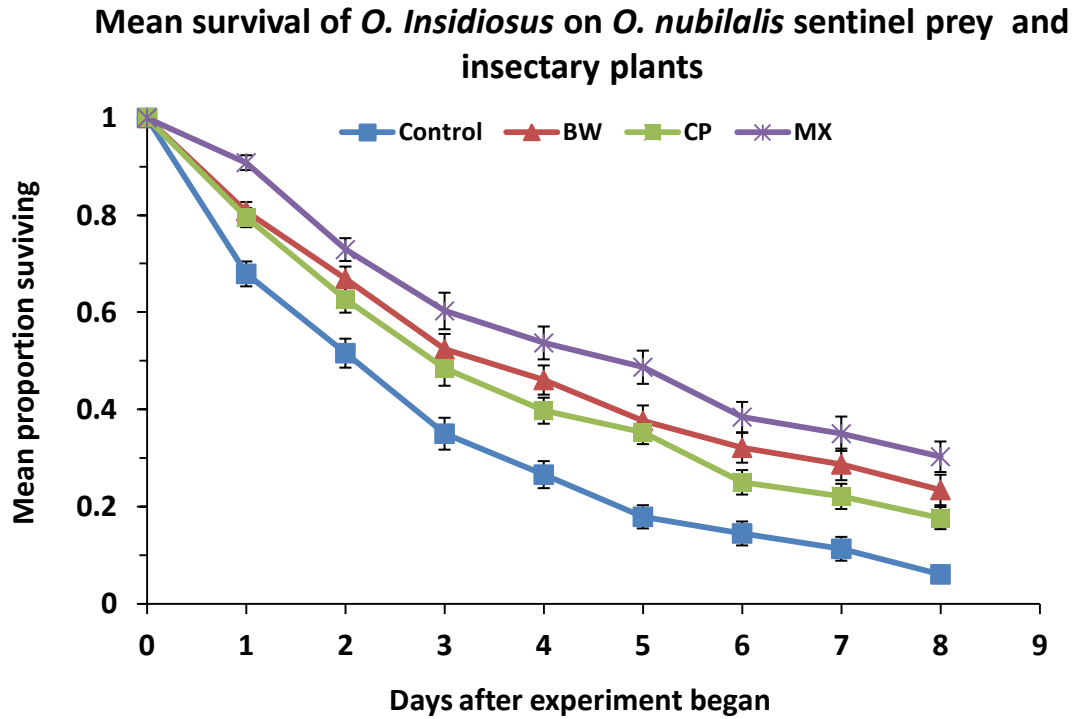


Figure 3.4. Mean survival of *O. insidiosus* individuals reared in cages on *O. nubilalis* prey and insectary plant. Treatments were the water control , buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *O. nubilalis* predation trials.

Cumulative Mean Number of Eggs Laid by *O. insidiosus*

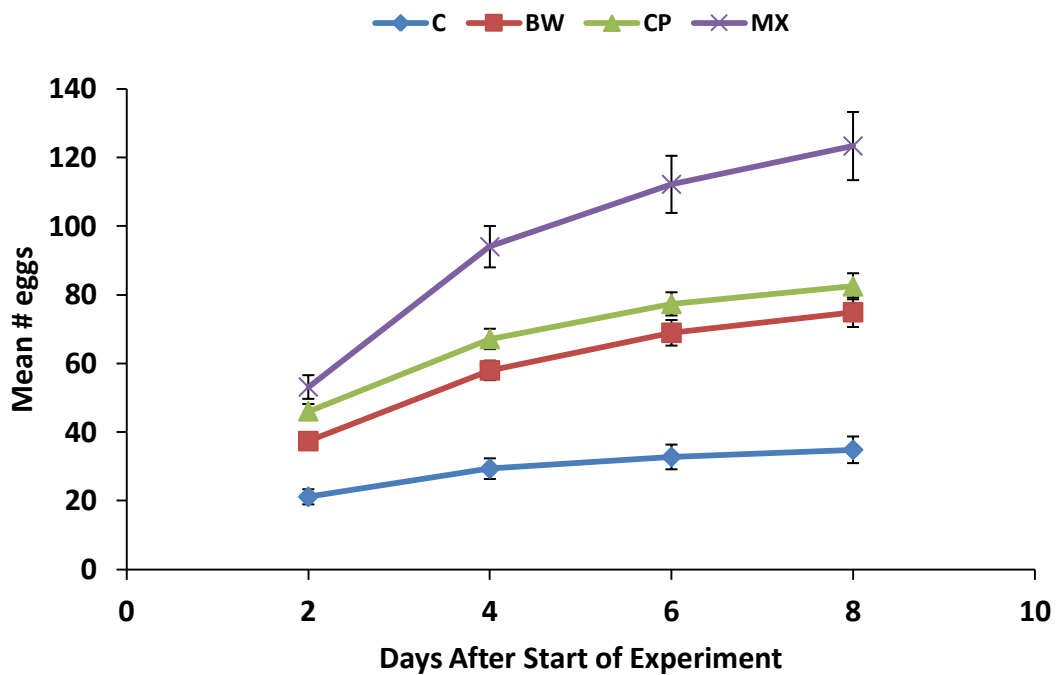


Figure 3.5. Mean number *O. insidiosus* eggs oviposited into plant sections in the water control , buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *O. nubilalis* predation trials. Data is presented as accumulated mean number of eggs.

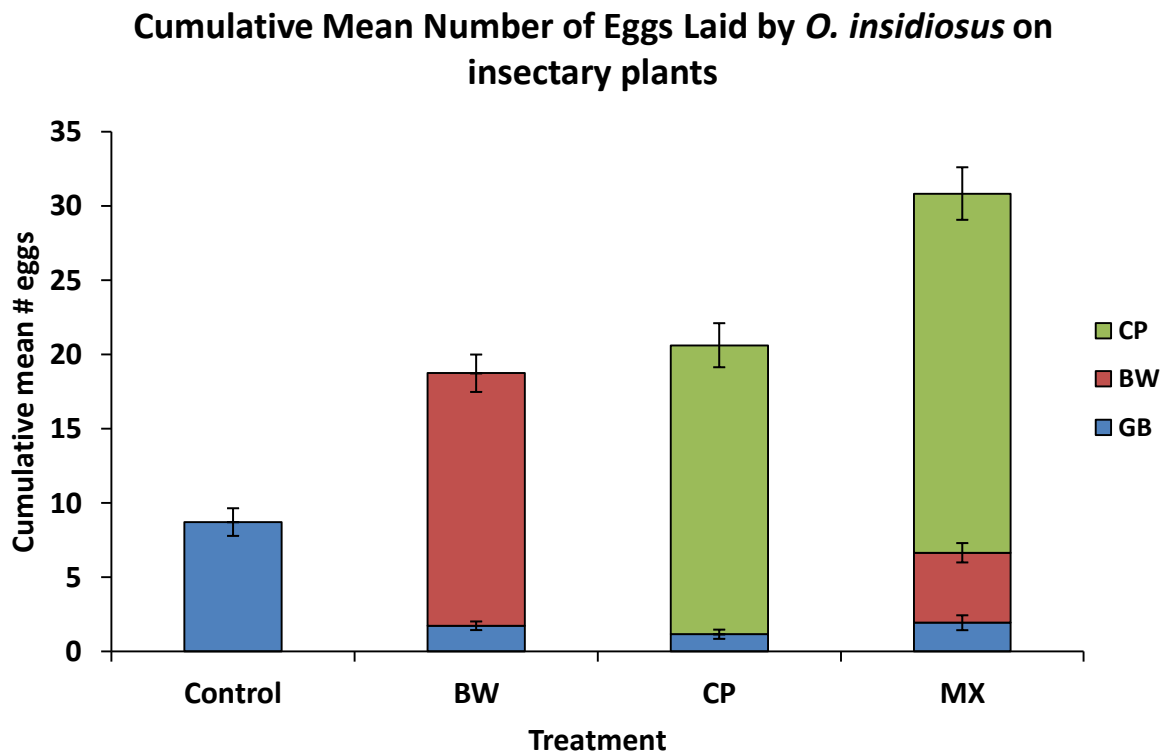


Figure 3.6. Cumulative mean number of *O. insidiosus* eggs oviposited into green bean (GB) buckwheat (BW) and cowpea (CP) plant sections in the water control, buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *O. nubilalis* trials.

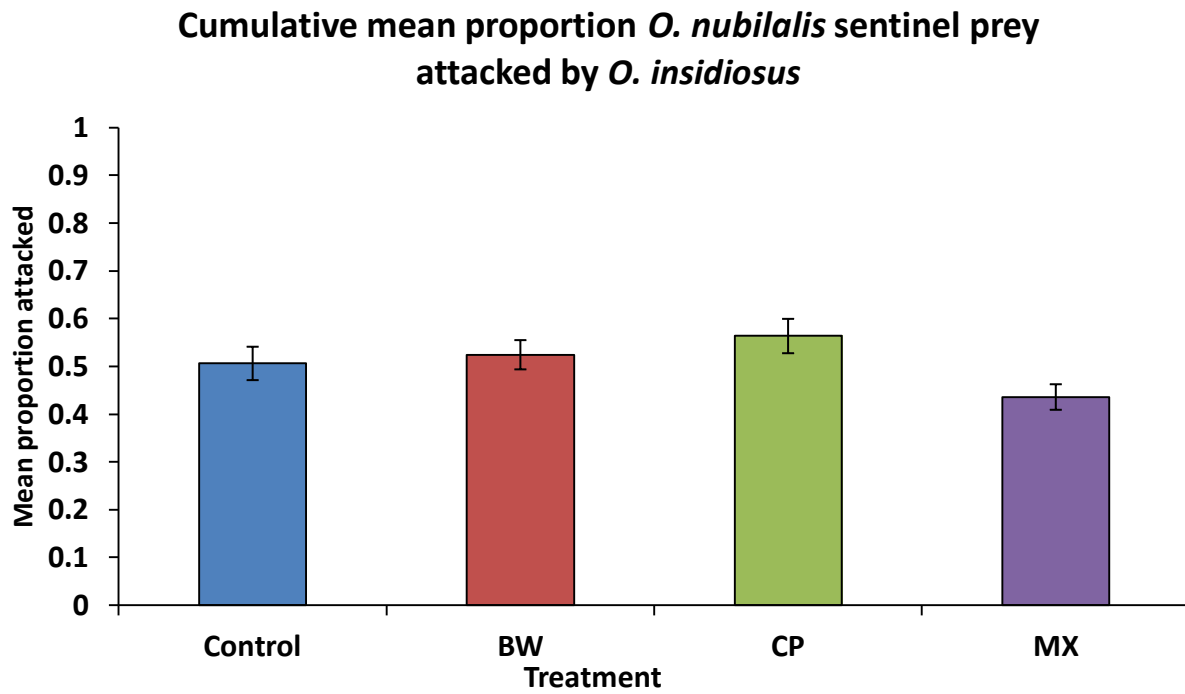


Figure 3.7. Mean proportion of *O. nubilalis* sentinel prey attacked in the water control, buckwheat (BW), cowpea (CP) and biculture (MX) treatments during *O. nubilalis* predation trials

Chapter 4

The role of insectary plant diversification on natural enemies and pest suppression in an insectary border

Introduction

Natural enemies play a critical role in suppressing insect pests below economically damaging levels. In the United States, the value of pest suppression provided by natural enemies is estimated to be as high as \$4.5 billion annually (Losey and Vaughan 2006). Although pest suppression by natural enemies is a valuable ecosystem service, frequent disturbance, habitat fragmentation, and loss of diversity due to increased agricultural intensification and simplification may create conditions unsuitable for many natural enemy species and may reduce their potential to suppress insect pests (Letourneau et al. 2011). Although primarily predatory or parasitic, many important natural enemy species depend on resources provided by non-crop plants, such as food (nectar, pollen, alternative prey) and shelter (favorable microclimate, oviposition sites) to successfully complete development, especially during periods of limited prey availability (Lee and Heimpel 2008, Amaral et al. 2013). Loss of such non-crop resources due to frequent disturbances such as tillage or applications of broad-spectrum insecticides and seed treatments challenge natural enemy survival and reduce their potential to suppress pests (Barbosa, 1998; Seagraves and Lundgren, 2012; Douglas and Tooker, 2016).

Conservation biological control, the modification of pest management practices and features of the local environment to enhance locally-occurring natural enemy species, is one approach to mitigate the detrimental effects of disturbance and enhance the survival and effectiveness of natural enemies (Ehler 1998). Currently, routine and preventive applications of

broad-spectrum insecticides are typically used to manage pest insects in agricultural settings. However, these insecticides, and often herbicides, may negatively impact the natural enemies of the targeted pest species as well as host plants important to their survival (Moser and Obrycki 2009, Bommarco et al. 2011, Douglas and Tooker 2015, 2016). By reducing disturbances and application frequency or alternatively choosing selective insecticides, natural enemy populations may be protected from the adverse effects of insecticides and may be maintained at abundances high enough to respond to and suppress insect pest populations (Varenhorst and O'Neal 2012). Similarly, large scale disturbances such as conventional plowing and tillage and further landscape simplification directly result in loss of overwintering sites, oviposition sites, and loss of preferred host plants from which natural enemies receive food resources and shelter.

Natural enemy populations directly benefit from habitat modifications where tillage and soil disturbance is reduced or relatively undisturbed non-crop patches are deliberately maintained in or along field edges to provide refuge and resources to beneficial insects (Letourneau et al. 2011, Schellhorn et al. 2015, Gurr et al. 2017b). Deliberately providing resident natural enemies with undisturbed habitat and food resources, such as insectary plants, can increase natural enemy longevity, fecundity, predation and parasitism rates (Thomas et al. 1991, Begum et al. 2006, Berndt 2006, Lee and Heimpel 2008, Araj et al. 2009, Geneau et al. 2011, Bickerton and Hamilton 2012). Deliberate re-introduction of insectary plant species, plants that bear nectar-producing flowers and/or extrafloral nectaries, into the farmscape or agroecosystem can potentially attract and sustain natural enemy species that would be otherwise deterred by simpler, resource-poor agroecosystems (Landis et al. 2000, Gurr et al.

2017b). Floral resources provided by insectary plant species not only support foliar natural enemies, but may also benefit other natural enemies such as ground-dwelling arthropods and web-building spiders, which are attracted by the ground cover and habitat complexity provided by insectary plant species (Rypstra et al. 1999, Langellotto and Denno 2004, Balmer et al. 2013).

Using insectary plants is a potentially effective tactic to promote natural enemies. However, while natural enemies may be supported by insectary plants, they may differentially respond to and benefit from particular plant species (Vattala et al. 2006, Géneau et al. 2013). Variable responses of natural enemies to resource diversification suggest that the role of non-crop vegetation, such as insectary plants, is not fully understood. Therefore, considerable research must be undertaken to further understand how natural enemies respond to and use resources provisioned through conservation biological control practices.

Several key considerations must be made prior to implementing insectary plants in an agroecosystem. Ideal insectary species should be well-suited to the needs of targeted natural enemies and should avoid benefitting pest species (Landis et al. 2000). Characteristics such as biomass production, vegetative structure, floral structure, and bloom time are features that must be considered when selecting an insectary crop to conserve natural enemies (Wackers 2004, Begum et al. 2006, Vattala et al. 2006, Fiedler and Landis 2007, Hogg et al. 2011a). These factors directly and indirectly influence how arthropod natural enemies access and use resources provided by individual insectary plant species. For example, flowers that have long, narrow corolla are unlikely to be accessed and used by generalist predators and smaller parasitoids that lack the ability to access nectar and pollen hidden deep within (Vattala et al., 2006). In addition to insectary plant morphological traits, cover crops that are used as insectary

species should be compatible with grower-identified needs such as the available crop growth window and other desired ecosystem services such as nitrogen supply and retention and weed suppression. Longer blooming cover crops are more likely to serve as resources for natural enemies throughout key parts of cash crop growing season.

Currently, in the northeastern United States, corn (*Zea mays*) is an economically important cash crop grown annually and primarily used as feed for livestock and biofuels. Historically, this crop has been subject to attack by many insect pests such as fall armyworm (*Spodoptera frugiperda*), black cutworm (*Agrotis ipsilon*), aphids (Aphididae), and most notably European corn borer (*Ostrinia nubilalis*), one of its most destructive pests (Penn-State 2015). Corn pests are commonly managed through the use of foliar insecticide applications, genetic modification for insect resistance, and seed treatments (Douglas and Tooker 2015). However, these practices are not available to organic producers, who must rely on the naturally occurring established complex of predators and parasitoids in the corn agro-ecosystem (USDA 2017). Predatory and parasitic arthropods such as damsel bugs (Nabidae), ground and tiger beetles (Carabidae), rove beetles (Staphylinidae), parasitic wasps and flies (Braconidae, Ichneumonidae, Tachinidae), predatory hoverflies (Syrphidae), arachnids (Thomisidae, Tetragnathidae, Salticidae), lacewings (Chrysopidae), insidious flower bugs (Anthocoridae) and lady beetles (Coccinellidae) inhabit corn cropping systems and can contribute to the suppression of corn pests (Barber 1936, Andow and Risch 1985, Coderre et al. 1987, Losey et al. 1992, Clark et al. 1997). Populations of predators and parasitoids may be enhanced by providing resource plants, since many of these natural enemies benefit from plant-based resources including nectar,

pollen, habitat complexity, and oviposition sites (Landis et al. 2000, Sedlacek et al. 2012, Gurr et al. 2017b).

Cover crop species such as buckwheat and cowpea have been used to attract and support beneficial insects that suppress insect pests; however, reports are often anecdotal or inconsistent (Kyamanywa et al. 1993, Clark 2007). Buckwheat (*Fagopyrum esculentum*) is a warm season, summer annual that produces many small, open, white flowers, attractive to a range of beneficial insects such as lady beetles, hoverflies, lacewings, minute pirate bugs, parasitic flies and wasps. Buckwheat typically blooms three weeks after sowing and continues until frost-killed. Because buckwheat flowers throughout the summer, it is a good candidate insectary species for use in or alongside a variety of summer annual crops. Many studies have demonstrated increased abundance of natural enemies in a variety of cropping systems in the presence of buckwheat (Baggen and Gurr 1998, Berndt et al. 2002, Lee and Heimpel 2005, Irvin et al. 2006, Sedlacek et al. 2012, Araj and Wratten 2015). Cowpea (*Vigna unguiculata*) is another warm season, summer annual characterized by quick establishment and high biomass production. The habitat complexity and ground coverage provided by cowpea foliage may be conducive to harboring beneficial ground dwelling predators that are attracted to these microhabitats. Cowpea produces flowers and extrafloral nectaries that can support predatory insects such as minute pirate bugs, assassin bugs, big-eyed bugs, parasitic wasps, and hoverflies. Cowpea typically flowers 60-90 days after planting, which is typically outside of the timing window afforded in the corn cropping systems. However, extrafloral nectaries, which develop sooner, can be a valuable nutritional resource for natural enemies (Clark 2007).

Many studies focus on provisioning a single, targeted natural enemy using a single optimal insectary species (Spellman et al. 2006, Kehrli and Bacher 2008, Balmer et al. 2013, Quinn et al. 2017). This approach fails to address the role of alternate natural enemies and their biological control potential. Therefore, I tested a biculture of cowpea and buckwheat to determine if it could provide complementary, morphologically distinct, resources that can synergistically work to attract and sustain greater numbers and a wider diversity of natural enemy species. Buckwheat produces nectar and pollen through its numerous open flowers, while cowpea provides extrafloral nectar. Because natural enemies exhibit preferences toward different resources, these two commonly used cover crop species can potentially attract different species of generalist predators, as has been observed for parasitoids on flowering plants featuring different morphologies (Vattala et al., 2005).

The goal of this research was to examine the impact of non-crop resource provisioning plants on generalist predator abundance and biological control in a corn agroecosystem. This study placed special emphasis on *C. maculata*, the pink spotted lady beetle, and *O. insidiosus*, the insidious flower bug as they are both reported to be economically important natural enemies in the corn agroecosystem (Dicke and Jarvis 1962, Cottrell and Yeargan 1998). I hypothesized that the abundance of *C. maculata* and *O. insidiosus* would be enhanced in the presence of buckwheat nectar and pollen. I further hypothesized that a mixture of buckwheat and cowpea would support higher abundances of natural enemies and a corresponding increase in predation on a sentinel pest. Specific objectives were to: 1.) Document insectary border growth and development measured as floral and extrafloral nectar densities for *C. maculata* and *O. insidiosus*; 2.) Assess the foliar arthropod community associated with

buckwheat and cowpea monocultures, and a cowpea-buckwheat mixture compared to a fallow control; and 3.) Monitor predation on prey insects within the insectary border and adjacent corn crop. For each of the corresponding objectives, I hypothesized that 1.) A biculture containing buckwheat and cowpea, two resource rich plant species, would contain a greater cumulative abundance of resources compared to either species alone; 2.) There will be greater abundances of natural enemies, specifically *C. maculata* and *O. insidiosus* in the biculture compared to either monoculture; and 3.) A greater proportion of sentinel prey would be attacked in biculture compared to monocultures.

Materials and Methods

Field Establishment

To assess the potential of cover crops to support natural enemies and enhance pest suppression in adjacent corn, *Zea mays*, I established field experiments at the Russell E. Larson Agricultural Research Center, Rock Springs, PA in 2014 and 2015. The experimental site consisted of 16 experimental treatment plots, each measuring 18.2 m x 13.7m, arranged in a randomized complete block design. Each block was replicated 4 times. In 2014, treatment plots consisted of two areas, an insectary border region (13.7m x 4.5m) and an adjacent corn crop (13.7m x 13.7m). In 2015, the experimental layout was similar; however treatment plot sizes were larger, where the insectary border and cash crop portion of each individual plot was 18.2m x 4.5m and 18.2m x 27.4m, respectively (Figures 4.1 and 4.2).

In early June, cover crop species were established as insectary borders containing buckwheat (*Fagopyrum esculentum*) (BW), cowpea (*Vigna unguiculata*) (CP), a

buckwheat-cowpea biculture (MX), or a weedy fallow treatment (WF) along the edges of corn cash crop plantings. In monoculture plots, buckwheat (Lakeview Organic Grain, Penn Yan, NY) was planted at the recommended seeding rate of 61.6 kg/ha. Cowpea (variety: Iron and Clay, Hancock Seeds, Dade City, FL) was planted at rate of 78 kg/ha, a rate higher than recommended to compensate for poor germination observed during a preliminary study. The seeding rate of the biculture consisted of an adjusted 16.8 kg/ha for buckwheat and 48 kg/ha for cowpea to reduce the likelihood of buckwheat dominating the mixture as observed in a preliminary study. The weedy fallow treatment consisted of naturally occurring weed species as a control. Untreated, non-transgenic corn (Master's Choice 4050) was planted immediately after the insectary border at the recommended rate of 81,500 plants per hectare. I began data collection 3 weeks after planting, when insectary borders and corn had germinated, and continued through mid-September, when I ended of the study. The crop was managed according to USDA National Organic Standards, and received no pesticides or synthetic fertilizers during the period of the experiment (USDA 2017).

Insectary Border Establishment

To monitor the establishment of the insectary border treatments, I measured several characteristics of the established cover crops in each treatment plot, including: plant density, plant height, and number of true leaves, inflorescences, and extrafloral nectaries per plant. Beginning three weeks after planting, cover crops and just before bloom, I used a 0.25m² quadrat to randomly sample the density of germinated plants within each insectary border. At each sample location, I recorded the density of germinated buckwheat and/or cowpea by

counting each plant within the quadrat. For weedy fallow treatments, I identified and recorded the density of germinated weedy plant species. Immediately after the density measurement, I randomly chose one plant within the quadrat, and recorded plant height, leaf number and the number of inflorescences and extrafloral nectaries. Plant parameters were summarized as mean resource density per plot. This was recorded as the mean number of inflorescences or extrafloral nectaries present on plants within the 0.25m^2 quadrat and calculated as the mean number of inflorescences or extrafloral nectaries (EFN) multiplied by mean plant density. Weather data such as weather conditions, and accumulated growing degree days since date of planting were also recorded.

Sweep Net Sampling

To characterize the insects inhabiting the insectary borders, I initiated sweep net sampling three weeks after planting and prior to insectary border bloom. Sampling occurred weekly and close to midday when insects are most active. I avoided sampling on rainy and excessively windy days. To minimize the effect of time of day on arthropods collected via sweep net sampling, I used a random number generator to determine the sampling order of plots. Sweep net sampling consisted of 10 sweeps collected at 3 random locations within each insectary border treatment. Collected insects were immediately transferred to labeled freezer safe bags and then frozen until samples were processed. During processing, all insects were identified to the lowest taxonomic level possible and their numbers recorded.

Timed Counts of Predators

As developing corn cannot be effectively sampled using a sweep net, I conducted timed counts of insects on plants weekly. Three-minute timed counts were conducted along predetermined transects within the corn area of the plots 1.5m, 4.5m, 7.6m and 13.7m from the adjacent insectary borders in 2014. These distances were adjusted to 6m, 12m, 18m, and 24m in 2015 to account for the larger sized insectary borders (Figure 4.1). During each timed count, corn plants were inspected for dominant natural enemy species such as *C. maculata*, *O. insidiosus* and *H. axyridis*. On each plant, the upper and lower sides of leaves, stems, ears and silk were inspected for arthropods. Sampling continued weekly for the duration of the study.

Sentinel Predation

I examined predation rates using sentinel prey eggs of *Ostrinia nubilalis*, the European corn borer. Predation assays were conducted biweekly throughout the duration of the study within the insectary border and in the adjacent corn area of the plot. European corn borer eggs were purchased (Benzon Research, Carlisle, PA) and prepared by recording the number of eggs per mass and gluing individual egg masses to index card backing. I initiated sentinel predation assays each year approximately 5 weeks after planting when corn and insectary border treatments were established. To determine predation rates at increasing distances from insectary borders, I placed 20 sentinel egg masses in each treatment, four per transect, stapled to the underside of a healthy corn leaf on each sampling date. I collected the sentinel egg masses after 48 hours of field exposure and examined them under microscope for signs of predation. Eggs were characterized as "chewing predator" (mangled or missing eggs), "sucking predator" (flat, punctured eggs), "parasitized" (blackened eggs), "hatched" (undamaged eggs),

"dead" (no larval emergence) or "missing" (egg card missing) (Andow 1990). Characterization was confirmed by keeping eggs until larvae emerged from all egg masses which were stored under laboratory conditions in sealed plastic bags. All larvae typically emerged within 48 hours after being collected from the field. Mean proportions of dead eggs, successfully hatched eggs, and eggs killed by chewing and sucking predators and parasitism were calculated and recorded.

Statistical Analysis

Analyzed data includes floral density of the insectary border, arthropod counts from sweep net sampling, timed predator counts in standing corn, and proportion of sentinel eggs attacked by predators in insectary border and corn areas. Whenever necessary, data was transformed to meet assumptions of homogeneity of variances. Proportion data, for example, were arcsine square root transformed. Data were analyzed using SAS statistical analysis software (SAS Institute 2002). A model was constructed to determine the effects of main plot factors, or treatment effects, over the course of the growing season. In each case, treatment means were compared using repeated measures ANOVA (SAS Institute 2002). When a significant treatment by date interaction was detected, simple effect means were compared using LS means. Significance values for means were compared using a Bonferroni adjustment.

Results

Insectary Border Establishment

In each year, insectary borders were planted in early June. Data collection began two weeks after planting and continued until the borders were terminated in late July.

Buckwheat monocultures germinated 7 days after planting, bloomed approximately 3 weeks after planting and continued blooming until termination. In each year, cowpea germination rates were low, despite adjusting seeding rates upward in 2015. Cowpea germinated 7 days after planting and extrafloral nectaries were present as early as 2 weeks after planting. In both study years, the cowpea-buckwheat biculture treatment was dominated by buckwheat.

Cowpea was represented at approximately half the stand density of buckwheat, despite the adjusted seeding rate. Weeds in fallow plots consisted primarily of redroot pigweed (*Amaranthus retroflexus*), common lambsquarters (*Chenopodium album*), velvetleaf (*Abutilon theophrasti*) and black medic (*Medicago lupulina*). These weed species did not flower during the insectary border flowering period.

In 2014, buckwheat resource density was significantly higher in buckwheat monocultures compared to the cowpea monoculture, biculture, and weedy fallow plots 5 ($F_{3, 121} = 72.28, P < 0.0001$), 6 ($F_{3, 121} = 96.94, P < 0.0001$) and 7 ($F_{3, 121} = 59.79, P < 0.0001$) weeks after planting (Figure 4.3a). Cowpea monocultures contained significantly more cowpea extrafloral nectaries per 0.25m^2 than in mixture plots on all dates sampled ($F_{3, 761} = 945.28, P < 0.0001$) (Figure 4.3b.). In bicultures, buckwheat inflorescences were represented at half their monoculture mean densities. Cowpea was poorly represented in bicultures, occurring at one-fifth the monoculture density by the end of the season. In the cowpea-buckwheat biculture, cowpea plant density and EFN number were lower compared to the monoculture. Results from 2015 were similar to 2014 in that mean density of buckwheat inflorescences were significantly higher in buckwheat monoculture compared to cowpea, biculture and weedy fallow plots ($F_{3, 633} = 125.09, P > 0.0001$) (Figure 4.4a). Cowpea extrafloral nectaries were similarly more abundant in

cowpea monoculture plots compared to the biculture treatment on all dates sampled, despite increasing the seeding rate of cowpea in bicultures to reduce the likelihood of buckwheat dominating the biculture treatments in 2015 ($F_{3,633} = 582.71$, $P > 0.0001$) (Figure 4.4b).

Sweep Net Sampling

In 2014, arthropod collections were conducted weekly via sweep net sampling initiating on July 2 and continuing until insectary border termination on 25 July. Common natural enemies collected from border treatments included crab spiders (Araneae: Thomisidae), the pink spotted lady beetle (*Coleomegilla maculata*) and the insidious flower bug (*Orius insidiosus*). There was a significant effect of treatment on spider abundance ($F_{3,173} = 6.23$, $P = 0.0005$). Crab spiders were significantly more abundant 7 weeks after planting in buckwheat than in cowpea ($P = 0.0002$) and the weedy fallow treatments ($P < 0.0001$) (Figure 4.5a). Similarly, crab spiders were more abundant in biculture treatments than in cowpea ($P = 0.0042$) or weedy fallow ($P = 0.0017$) (Figure 4.5a). Spider abundance was similar between the buckwheat and biculture treatments. Although lady beetles appeared to be more abundant in the buckwheat and biculture treatments, by 7 weeks after planting, treatment effects were not statistically significant for any week sampled (Figure 4.5b). Insidious flower bugs were influenced by treatment ($F_{3,173} = 80.69$; $P < 0.0001$) (Figure 4.5c), and were more abundant in buckwheat compared to cowpea and weedy fallow 6 ($P < 0.0001$; $P < 0.0001$) and 7 ($P < 0.0001$; $P < 0.0001$) weeks after planting, respectively (Figure 4.5c). Insidious flower bugs were more abundant in biculture treatments compared to cowpea 6 ($P < 0.0001$; $P < 0.0001$) and 7 ($P < 0.0001$; $P < 0.0001$)

weeks after planting (Figure 4.5c). *O. insidiosus* numbers were low in cowpea treatments on all dates.

In 2014, several potentially important herbivorous insect species were captured during sweep net sampling. These included pests such as aphids (Hemiptera: Aphididae), the tarnished plant bug (*Lygus lineolaris*) and the potato leafhopper (*Empoasca fabae*). However, there were no significant treatment effects on aphids (Figure 4.5d). Insectary treatment significantly affected tarnished plant bug numbers ($F_{3, 173} = 48.11$, $P < 0.0001$). Tarnished plant bugs were more abundant in buckwheat compared to cowpea 5 ($P < 0.0001$), 6 ($P = 0.0012$) and 7 ($P < 0.0001$) weeks after planting (Figure 4.5e). However, tarnished plant bugs were only significantly more abundant in buckwheat compared to weedy fallow 5 weeks after planting ($P < 0.0001$). Tarnished plant bugs were also more abundant in biculture treatments compared to cowpea and weedy fallow treatments 5 ($P = 0.0004$; $P = 0.0001$), 6 ($P < 0.0001$; $P = 0.0156$) and 7 ($P < 0.05$; $P < 0.0001$) weeks after planting, respectively (Figure 4.5e). Potato leafhoppers were significantly impacted by insectary border treatments ($F_{3, 173} = 188.57$, $P < 0.0001$). Leafhopper abundance was significantly higher in cowpea treatments compared to buckwheat, biculture, and weedy fallow treatments 4 ($P < 0.0001$), 6 ($P < 0.0001$) and 7 ($P < 0.0001$) weeks after planting, but not 5 weeks after planting (Figure 4.5f).

In the 2015 study year, sweep net sampling began on June 29 and continued through July 22, shortly before insectary borders were terminated. Similar to 2014, the most commonly collected predatory arthropods included crab spiders, pink spotted lady beetles and minute pirate bugs. There was a significant effect of treatment on crab spider abundance ($F_{3, 172} = 5.33$, $P = 0.0015$). Crab spiders were significantly more abundant in the buckwheat monoculture

compared to weedy fallow 7 weeks after planting ($P = 0.048$) (Figure 4.6a). Abundances were similar across treatments on all other days sampled. In 2015, there were no significant effects of insectary treatment on pink spotted lady beetle abundance although they appeared to be more abundant in buckwheat and biculture by 7 weeks after planting ($P = 0.0756$) (Figure 4.6b). There was a significant effect of insectary treatment on *O. insidiosus* abundance in 2015 ($F_{3,172} = 97.06, P < 0.0001$). Minute pirate bugs were significantly more abundant in buckwheat monoculture compared to weedy fallow ($P < 0.0001$) and cowpea monoculture ($P < 0.0001$), but not significantly different from biculture treatments on all dates sampled (Figure 4.6c). Similarly, *O. insidiosus* was more abundant in biculture treatments than in cowpea monoculture ($P < 0.0001$) and weedy fallow treatments ($P < 0.0001$) on all dates sampled.

Patterns of pest species abundance were similar to those observed in 2014. The most commonly captured herbivorous insects included aphids, tarnished plant bugs and potato leafhoppers. In 2015, insectary border treatment were significant for aphid abundance ($F_{3, 172} = 4.62, P = 0.0039$). Aphids were more abundant in the weedy fallow compared buckwheat ($P = 0.0141$), cowpea (marginally significant at $P = 0.0731$) and the biculture treatments (marginally significant at $P = 0.074$) throughout the duration of the study (Figure 4.6d). Tarnished plant bugs were generally more abundant in buckwheat and the biculture treatments, which both contained buckwheat inflorescences ($F_{3, 172} = 43.95, P < 0.0001$). There were no significant differences among treatments in abundance of tarnished plant bugs until 7 weeks after planting, when they were more abundant in buckwheat compared to weedy fallow ($P < 0.0001$) and cowpea treatments ($P < 0.0001$) (Figure 4.6e). Similarly, they were more abundant in the biculture compared to weedy fallow ($P < 0.0001$) and cowpea ($P < 0.0001$) treatments 7 weeks

after planting. Potato leafhoppers were captured only in the cowpea monoculture treatment and, as such, were significantly more abundant in the cowpea insectary treatment compared to other treatments on all days sampled, with abundance peaking at week 7 ($F_{3,172} = 72.16$, $P < 0.0001$) (Figure 4.6e).

Timed Counts in Corn

In 2014, arthropods in corn were not significantly affected by transect distance from insectary borders; therefore, this factor was removed from further analyses. *C. maculata* was initially found on 10 July and peaked by 30 July before declining toward the end of the study (Figure 4.7a). Insectary border treatment had a significant impact on *C. maculata* abundance in corn ($F_{3,477} = 2.74$, $P = 0.0430$). *C. maculata* abundance was significantly higher adjacent to buckwheat plots compared to cowpea plots throughout the study ($P = 0.0060$) (Figure 4.7a). Pink spotted lady beetle abundance in corn did not differ significantly among the biculture, cowpea or weedy fallow treatments. *O. insidiosus* was initially observed on 18 July and consistently for two sampling events before declining by 30 July (Figure 4.7b). However, insectary treatment did not appear to significantly influence the abundance of insidious flower bug in corn on any date sampled (Figure 4.7b) ($P = 0.1200$). The multicolored Asian lady beetle (*Harmonia axyridis*) in corn was similarly not influenced by insectary border treatment in 2014 ($P = 0.1700$).

In 2015, arthropods in corn were not significantly affected by transect distance, therefore distance was removed as a factor from further analyses. *C. maculata*, unlike in 2014, was not significantly influenced by insectary border treatment ($P = 0.1400$). Neither *O.*

insidiosus nor *H. axyridis* abundance in corn were influenced by insectary border treatment ($P = 0.4200$ and $P = 0.0700$, respectively) in 2015.

Predation on Sentinel ECB Eggs

In 2014, I monitored predation on European corn borer sentinel prey throughout the season. There were no significant effects of transect distance on the proportion of eggs consumed in 2014 for chewing or sucking predators ($P = 0.5800$ and $P = 0.1200$, respectively). Therefore, transect distance was removed from further analyses. However, both treatment and week significantly influenced proportion of sentinel eggs attacked by chewing predators (Treatment: $F_{3, 1185} = 2.8900$, $P = 0.0350$; Week: $F_{4, 1185} = 58.4900$, $P < 0.0001$). In general, chewing predation increased over time, but declined 9 weeks after planting before peaking at 13 weeks after planting at the end of the study. Analysis of sentinel prey suggests that within the corn crop, chewing predation was significantly higher in plots adjacent to weedy fallow treatment plots compared to those adjacent to buckwheat treatment plots ($F_{3, 1245} = 2.0100$, $P = 0.0442$) (Figure 4.8a). Cowpea treatments also exhibited significantly higher mean predation by chewing predators compared to buckwheat and marginally more significant than biculture ($F_{3, 1245} = 2.78$, $P = 0.0056$ and $F_{3, 1245} = 1.72$, $P = 0.0850$, respectively) (Figure 4.8a). Buckwheat and biculture treatments had the lowest mean predation due to sucking predators and were statistically similar ($P > 0.0500$). Sentinel prey attacked by sucking predators were not significantly impacted by treatment, however week significantly influenced mean predation ($F_{3, 1245} = 14.2000$, $P < 0.0001$) (Figure 4.8b). Predation decreased by sucking predators until being imperceptible by 11 weeks after planting.

Results from 2015 were similar to 2014 in that transect did not significantly impact mean predation and was therefore removed from analysis in 2015 ($P > 0.05$). Time after planting and treatment significantly impacted mean proportion of eggs attacked by chewing predators (Time: $F_{3,1242} = 134.5$, $P < 0.0001$ and Treatment: $F_{3,1242} = 4.51$, $P = 0.0037$, respectively). Similar to the previous year, mean proportion of sentinel prey attacked by chewing predators increased before crashing 8 weeks after planting and peaking by 11 weeks after planting. While results were similar to the preceding year, treatments performed differently in 2015. Chewing predation in cowpea was significantly greater compared to biculture ($F_{3,1242} = 3.03$, $P = 0.0025$), but similar to buckwheat and weedy fallow (Figure 4.9a). Buckwheat was significantly greater than biculture ($F_{3,1242} = 3.23$, $P = 0.0013$), but similar to cowpea and weedy fallow (Figure 4.5a). Sucking predation was similar to the previous year in that treatment was not significant ($P > 0.5$). Sucking predation was significantly impacted by week, decreasing over time before becoming imperceptible later in the season ($F_{3,1245} = 14.20$, $P < 0.0001$).

Discussion

The objective of this study was to investigate whether insectary border diversification can attract, bolster, and sustain natural enemies in an organic corn agroecosystem. To do this, insectary border treatments were established adjacent to corn. The effects of the treatments were assessed through measuring border insectary plant characteristics, arthropod populations, and sentinel predation in border and adjacent crop. Initially, I hypothesized that the biculture containing both buckwheat and cowpea would have a greater abundance of plant-based resources than either of the monocultures alone. Biculture treatments would contain

both buckwheat floral nectaries and cowpea extrafloral nectaries whereas the monoculture plants only contained either type of plant-based resource. Tillman (1997) predicts that higher biodiversity typically leads to overall greater productivity. In other words, I can expect higher floral resource abundance in a biculture plot compared to a monoculture of either floral species. Multiple studies have reported higher overall biomass production in experimental treatments containing a higher diversity of plant species (Troumbis and Memtsas 2000, Tilman et al. 2001, Balzan et al. 2016). With regard to natural enemy abundance, I hypothesized that there would be greater abundances of *C. maculata* and *O. insidiosus* in the biculture compared to either monoculture. Root's (1973) *enemies* hypothesis suggests that higher diversity of plant species is predicted to support greater abundances and a wider range of natural enemies than would be supported by a monoculture. Multiple plant species may provide natural enemies with complementary sources of nectar, pollen, alternative prey, oviposition and refuge sites (Andow 1991, Straub et al. 2013). Finally, I hypothesized that a greater proportion of sentinel prey would be attacked in the biculture treatment compared to the monoculture and weedy fallow treatments. Because the biculture is expected to have higher plant-based resource density and natural enemy abundance, I expected that this would lead to a greater degree of pest suppression by increased natural enemy abundance. Meta-analyses from Poveda (2008) and Letourneau (2011) found that increased vegetational diversity strongly corresponded to increased natural enemy abundance and reduced pest densities and insect associated damage.

Insectary resource provisioning

Floral resources and other characteristics of the insectary border tended to vary by experimental treatment. Floral density in 2014 and 2015 suggest that buckwheat is resource rich, but its rapid development may dominate other insectary species used in border mixtures. During both study years, buckwheat monocultures produced significantly greater densities of inflorescences per 0.25m² compared to the buckwheat and cowpea biculture. This could be attributed to the seeding rate of each species in the mixture as well as the growth habit of buckwheat. Buckwheat planted in the biculture produced significantly fewer inflorescences compared to the buckwheat monoculture. This was likely due to the lower seeding rate for buckwheat in the biculture to accommodate for cowpea seeds. Buckwheat is reported to compensate for lower seeding density by producing significantly more branches per individual plant and therefore more inflorescences per plant (Ali-Khan 1973). However, while I did observe this change in buckwheat growth habit with decreased seeding density, in general, the bicultures contained significantly lower densities of buckwheat inflorescences per unit area than the monoculture. During both years of the study, cowpea generally exhibited poor germination rates in both monoculture and biculture, despite germination testing to confirm viable seeds, inoculation, and a higher seeding rate in 2015 to account for poorer germination. Poor germination and performance in cowpea could have been due to suboptimal growing conditions as well as competition from buckwheat. Cowpeas typically thrive in moist, hot zones (Clark 2007). However, when planted in central Pennsylvania (plant hardiness zone 6b) in early June, growing conditions were cooler with an average minimum and maximum temperatures of 15°C and 23°C, respectively and moister with an average June monthly precipitation of 1mm per day (NOAA 2017). Current recommendations suggest that cowpea achieves maximum

productivity at 27°C, which was uncommon during the cowpea growth window. Therefore, cowpea may not have had sufficient accumulated growing degree days to contribute to adequate cowpea growth and development at the site of the experiment (Clark 2007, NOAA 2017).

Competition with weeds is another factor that can explain poor cowpea establishment during study years (Remison 2009). Cool moist weather, combined with cowpea's poor establishment was likely conducive to the establishment of weedy species in the open canopy of cowpea treatments. Similarly, in biculture border treatments, cowpea appeared to be dominated by the rapidly growing buckwheat that additionally compensates for poor cowpea establishment by adopting its branched growth habit (Ali-Khan 1973). In biculture treatments, cowpea was represented at about half the density as in cowpea monocultures. While the insectary plant species appear to establish well as monocultures, establishing these species in a biculture appears to result in the underperformance of one or both species as suggested by significantly fewer buckwheat inflorescences and cowpea extrafloral nectaries in bicultures compared to monoculture treatments. This effect is not uncommon as a similar underperformance in increasingly diverse cover crop mixtures was reported by Creamer and Bennet (1997) while evaluating cover crop mixtures for use in vegetable production systems. They found that certain species in their mixtures, tall fescue (*Festuca arundinacea* L.), perennial ryegrass (*Lolium perenne* L.) and orchardgrass (*Dactylis glomerata* L.) did not compete as well and had poor representation in diverse cover crop mixtures with taller, more vigorous species. Similarly, another study evaluating the effects planting date and seeding rates has on achieving diverse cover crop mixtures observed that in mixtures, brassica and legume cover crops may

underperform compared to monocultures of each as indicated by biomass measurements (Murrell et al. 2017).

Arthropod abundance/ Distribution within treatments

The most consistently abundant natural enemy species found in the insectary border treatments included economically important natural enemy taxa such as crab spiders, the pink spotted lady beetle and the insidious flower bug. Each of these species has been reported to benefit from floral and extrafloral nectar and pollen. Additionally, each species feeds on economically important pests of corn such as thrips, aphids, European corn borer and corn rootworm (Barber 1936, Van den Meiracker and Ramakers 1991, Cottrell and Yeargan 1998).

My hypothesis that there would be a greater abundance of natural enemies within the biculture treatment was partially consistent with my observations. Throughout both study years each taxa exhibited a progressive increase in abundance over the course of the experimental season, peaking by its end at 7 weeks after planting. Additionally, all three natural enemy taxa were significantly more abundant in the buckwheat and biculture treatments compared to cowpea and weedy fallow by the end of the insectary border growing window 7 weeks after planting. Natural enemy abundance in buckwheat and biculture did not consistently differ. These observed increases in natural enemy abundances corresponded with higher resource densities in buckwheat and biculture treatments recorded from floral establishment data and which similarly peaked 7 weeks after planting (Figs 2.1 and 2.2).

Increased natural enemy abundance in the presence of increasing floral resource abundance and diversity is predicted by the enemies hypothesis, which attributes this to

supplemental resources provided by non-crop plant resources, especially when prey is scarce. Several similar studies have found increased natural enemy abundance and performance in the presence of additional floral resources (Berndt et al. 2002, Spellman et al. 2006, Letourneau et al. 2011, Géneau et al. 2012, Géneau et al. 2013, Quinn et al. 2017). For instance, in a recent study comparing the insectary potential of flowering weeds, shepherd's purse (*Capsella bursa-pastoris*) and white rocket (*Diplotaxis eruroides*), to more commonly used insectary border plants, buckwheat (*Fagopyrum esculentum*) and sweet alyssum (*Lobularia maritima*), Araj and Wratten (2015) found that the aphid parasitoid, *Diaeretiella rapae*, lived significantly longer and was significantly more fecund compared to a control treatment consisting of water only. The experimental plants exhibited differential effects on parasitoid longevity and fecundity. Buckwheat in particular, resulted in the greatest increases in parasitoid longevity and fecundity followed by sweet alyssum and the two flowering weed species. Another study investigating the impacts of floral strips on beneficial insect abundance and cucumber yield in a cucurbit agroecosystem found increased abundance of honeybees and predatory syrphid flies in sweet alyssum, buckwheat and mustard strips compared to a control of cucumber crop only (Quinn et al. 2017). Further, meta-analyses of studies examining the impacts of increasing vegetational diversity found that natural enemy abundance increases with increasing vegetational diversity (Letourneau et al. 2011, Gurr et al. 2017a).

While it is fairly well documented that insectary plants may increase natural enemy abundance and diversity, the potential for insect pests to benefit from insectary plant resources must also be considered. Observations from this study suggest that economically important pest species may have also benefited by non-crop resources provided by insectary borders.

Aphids (Hemiptera: Aphididae), tarnished plant bugs *Lygus lineolaris* (Hemiptera: Miridae), and potato leafhoppers, *Empoasca fabae* (Hemiptera: Cicadellidae), were the most commonly encountered pest species during the study and were found to be significantly more abundant in particular treatments. Tarnished plant bugs were consistently more abundant in buckwheat and biculture treatments compared to cowpea monoculture and weedy fallow treatments, while potato leafhoppers were significantly more abundant in cowpeas compared to any other treatment. Tarnished plant bugs are well documented to be generalists, feeding on a wide range of plants as well as their nectar producing flowers (Young 1986). It is unclear whether, I observed higher abundances of potato leafhopper on cowpea due to the presence of extrafloral nectar or due to the fact that potato leafhoppers specialize on plants in the legume family such as alfalfa and cowpea (Lamp et al. 1994). Aphid abundances were sporadic, but appeared most abundantly in weedy fallow treatments.

Nectar exploitation by herbivorous insects has been reported in several studies in which targeted pests experience increased longevity and fecundity in the presence of floral and extrafloral nectar (Baggen et al. 1999, Araj et al. 2009, Winkler et al. 2009, Géneau et al. 2012). In many cases, this negative effect of insectary resource is offset by the relatively greater benefits to the targeted natural enemy by the presence of insectary plants that can counter effects of the pest insects despite benefits to them gained from nectar exploitation. When screening insectary plants compatible for a particular crop, targeted natural enemies and pests, it is critical to consider the potential for insectary resources to exacerbate pest problems, especially if the species exploiting nectar is a specialist on surrounding crops. For example, the higher abundance of potato leaf hopper in the cowpea insectary border can serve as a source of

pests that can colonize nearby soybean or alfalfa fields. In this case, cowpea may not be desired as a potential insectary crop.

Sentinel Predation

My observations on predation rate in the presence of insectary plants were not fully consistent with my original hypothesis that there would be higher levels of predation in main crops adjacent to biculture compared to weedy fallow or monoculture treatment. The characteristics of predation for chewing and sucking predators were consistent with findings by (Andow 1990) and personal observations where both *C. maculata* and *O. insidiosus* readily feed on *O. nubilalis* eggs. Predation observed in my sentinel trials may be attributed to these two predators as they were the most abundant in the field at the time of sampling.

In general, during both study years, predation by chewing and sucking predators increased over time, peaking by the end of the season. Chewing predation accounted for 20-30% predation early in the season to 85-95% by the end of the season. This observation is consistent with established theory that insect development and activity increases over time with increasing degree day accumulation (Damos and Savopoulou-Soultani 2012). In addition to growing degree days, precipitation, daylight availability, floral bloom and prey availability increase during the summer season and may influence insect development, abundance and activity (Danilevskii 1965, McCall and Primack 1992).

During both study years, I observed low egg mortality by sucking predators like *O. insidiosus*. These observations may have been due to behaviors such as migration of *O. insidiosus* into the developing corn whorls, ears and tassels to feed on plant tissues and pollen

(Dicke and Jarvis 1962). Additionally, low egg predation by *O. insidiosus* may be due to their feeding habit. *O. insidiosus* use their mouthparts to pierce prey eggs and feed on their yolk contents, but leave the intact egg chorion behind. In the field and laboratory, I observed *C. maculata* feeding on egg chorion left behind after predation by *O. insidiosus*. This could have resulted in underestimation of predation by sucking predators.

Insectary border treatment did not appear to consistently influence predation in chewing or sucking predators across years. Despite high natural enemy abundance in buckwheat and biculture recorded during similar time frames, there was no corresponding increase in predation in corn plots adjacent to buckwheat and biculture plots. Conversely, despite low natural enemy numbers in cowpea borders, predation in corn adjacent to cowpea was significantly higher than or similar other treatments. These observations suggest that these natural enemies may not have migrated into the adjacent corn crops to attack prey. In many cases, it is not known whether natural enemies regularly disperse from supplemental resource plots into adjacent plots to suppress pests (Lavandero et al. 2004).

There was a drastic reduction in predation 8 and 9 weeks after planting in 2014 and 2015, respectively. On these sampling dates, predation on sentinel prey in corn dropped from approximately 30-40% to 10-20% in both years. This could have been due to the beginning of corn anthesis which occurred around the same time as these recorded sampling events. High corn pollen density could have served as a more easily accessible food source and may have competed for the attention of *C. maculata* and *O. insidiosus*, both of which have been recorded to readily feed on corn pollen (Corey et al. 1998, Duan et al. 2002). Previously, studies have recorded reductions in predation due to increases in supplemental foods such as nectar and

pollen. Skirvin et al (2007) reported a 40% reduction in predation of thrips by the *Orius laevigatus* when provided with supplemental *Ricinus communis* pollen. Similarly, Cottrell and Lundgren (1998) found a reduction in predation and cannibalism by *C. maculata* in corn plots during anthesis compared to plots where corn was detasseled and pollen absent. They suggested that high pollen abundance may cause natural enemies to become satiated and distracted from insect prey. Two weeks later, after corn pollen shed expired, predation returned to previous levels and peaked by the end of the season.

Conclusion

This study was aimed at investigating the impacts that insectary provisioning has on two key natural enemies in a corn agroecosystem. Used as an insectary plant in central Pennsylvania, buckwheat established quickly to provide a high density floral resource to attract and support higher abundances of economically important natural enemies compared to cowpea and weedy fallow treatments. Cowpea did not perform as well as buckwheat when planted in June in central Pennsylvania. This was likely due to slow degree day accumulation and high precipitation, which is detrimental to cowpea since it thrives in heat and is drought tolerant. When established in mixtures, buckwheat outcompeted cowpea, minimizing its representation in the biculture. These observations suggests that extreme care must be taken when designing insectary mixtures aimed at supporting natural enemies and suppressing targeted pests. Some of these considerations include plant growth habit, floral morphology, establishment time, bloom window, nectar and pollen resource abundance, targeted natural enemies and target pests among others. Further studies to screen plants for insectary

properties will serve to identify more plants and mixtures that could more effectively support natural enemies in agroecosystems.

Although higher abundances of non-crop resources and natural enemies were recorded in buckwheat and biculture, this did not translate into corresponding increases in natural enemy abundance or higher rates of predation within the adjacent corn plots. This may be due to a variety of reasons, such as pollen abundance and lack of natural enemy dispersal from insectary into the main crop. Abundant pollen and nectar provided by insectary plants or even within the main crop may satiate natural enemies, causing them to reduce their reliance on prey for nutrition. Pollen abundance has been suggested to negatively impact predation in certain cases, especially when pollen is highly abundant (Cottrell and Yeorgan 1998, Skirvin et al. 2007). Absence of a corresponding increase in predation rates adjacent to insectary treatments may also be due to the lack of natural enemy dispersal between resource and crop patches. It is not clear whether sentinel eggs were killed by natural enemies that resided in flowering insectary treatment plots, extrafloral nectar insectary plots or the weedy fallow as I did not measure insect dispersal patterns between the insectary treatment plots and the adjacent corn plots in this experiment.

Future studies should assess natural enemy dispersal between insectary and cash crop plots to further evaluate insectary or natural enemy potential. This can be done by incorporating mark recapture techniques into field studies (Hagler et al. 2014). Marking an insectary border and recapturing targeted natural enemies in different areas of the field may further inform approaches to conservation biological control.

Chapter 4 Figures

Figures

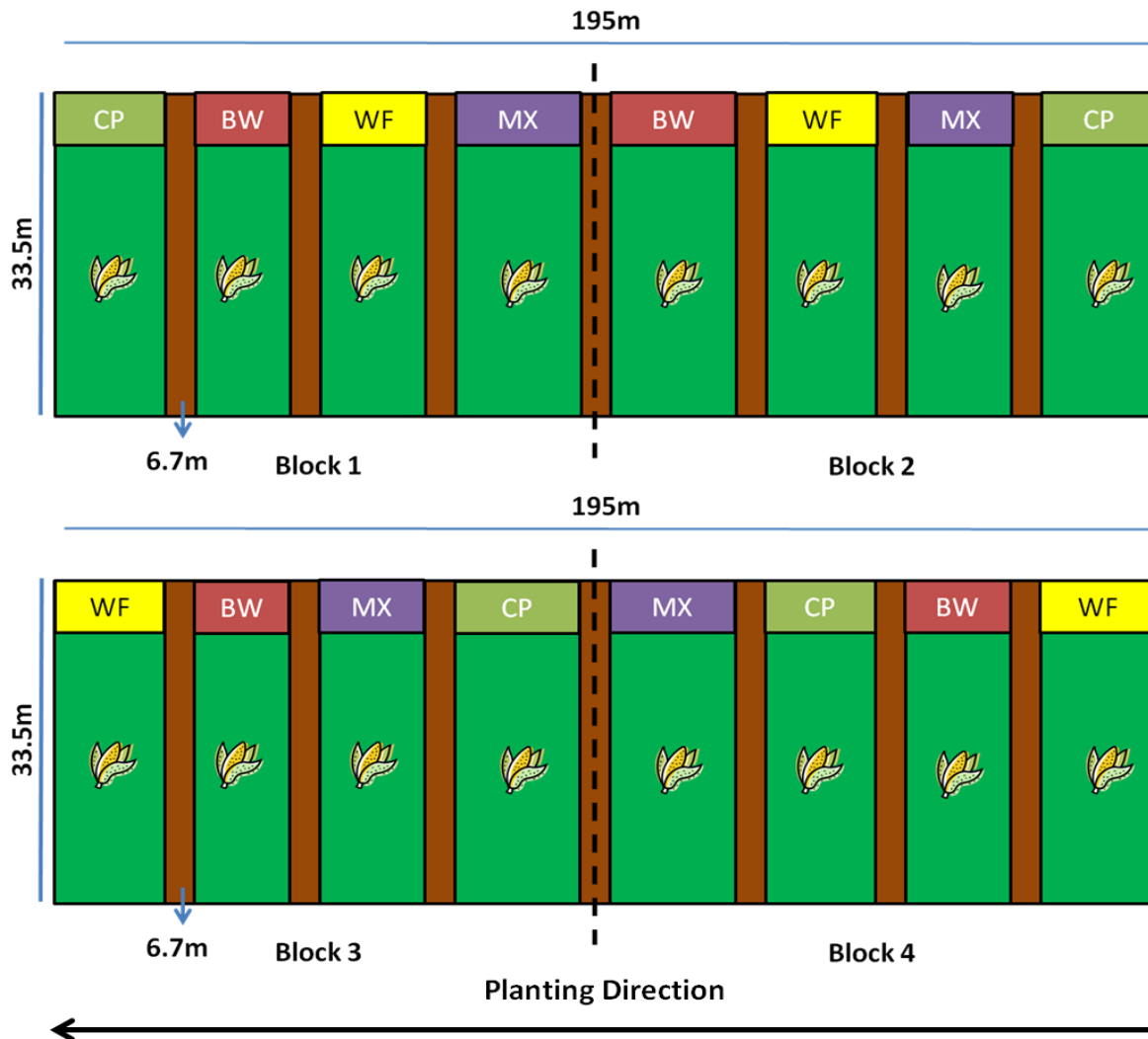


Figure 4.1. Diagram of the experimental site in 2015 study year. Treatment plots each measured 18m x 33.5m and consisted of an insectary border plot (18m x 6m) directly adjacent to a cash crop plot (18m x 27m). Individual plots were separated by a 6.7m mowed buffer strip.

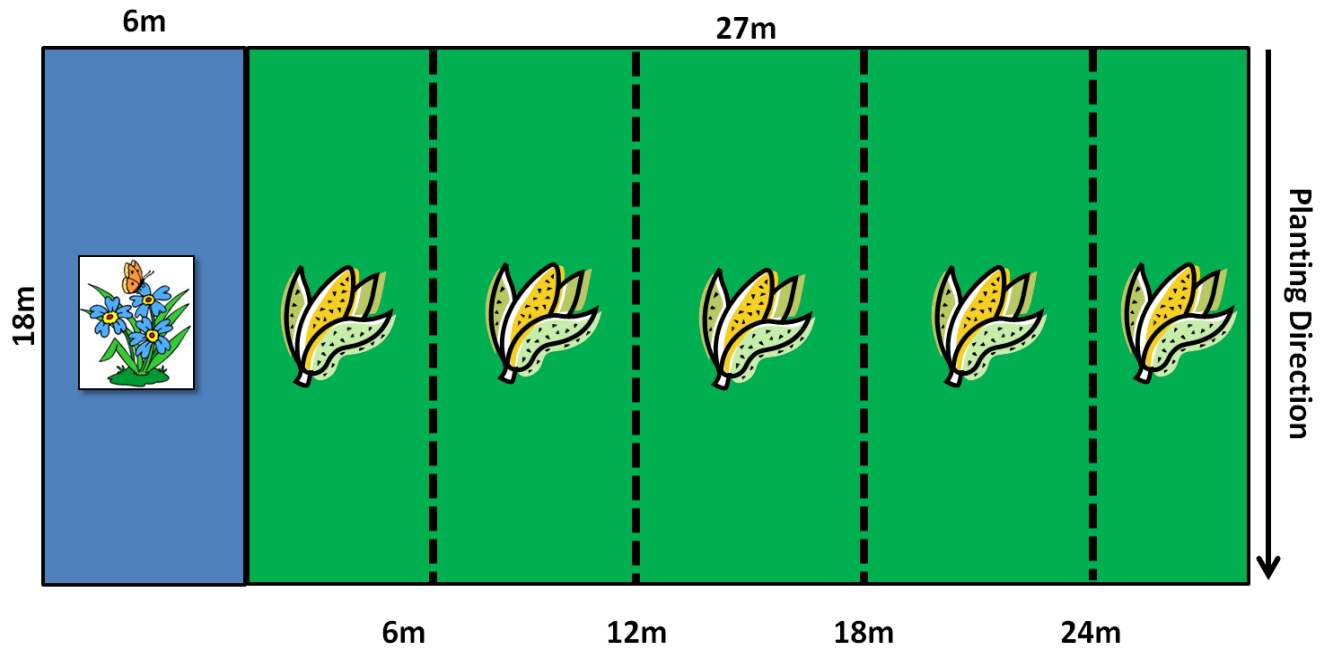


Figure 4.2. Diagram of an individual treatment plot in 2015. Individual plots each measured 18m x 33.5m and consisted of an insectary border (18m x 6m) directly adjacent to a cash crop plot (18m x 27m). Transects were established at 0m, 6m, 12m, 18m, and 24m distances from the insectary border for sampling of predation rates.

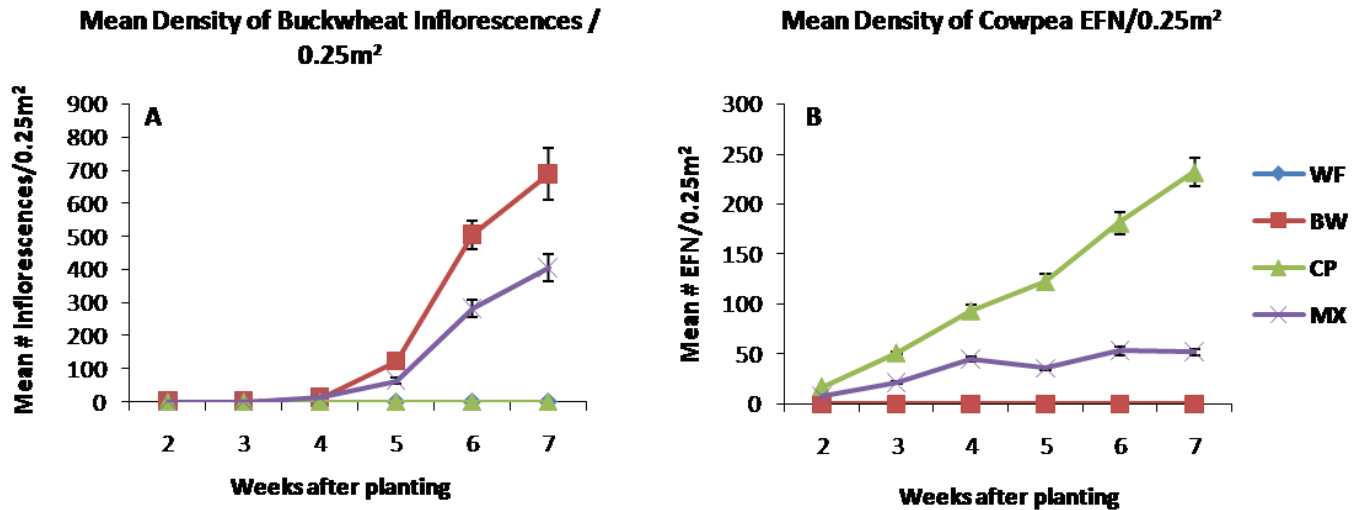


Figure 4.3 Resource density measured as mean number of buckwheat inflorescences (A) and cowpea extrafloral nectaries (B) per 0.25m² in 2014. . Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

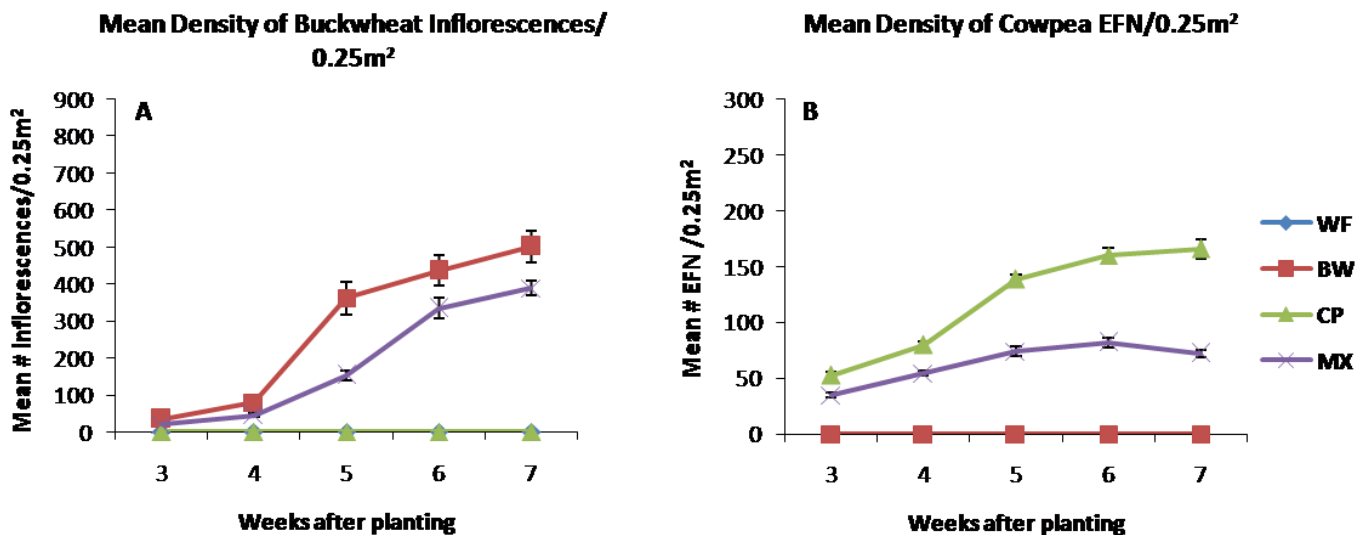


Figure 4.4 Resource density measured as mean number of buckwheat inflorescences (A) and cowpea extrafloral nectaries (B) per 0.25m² in 2015. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

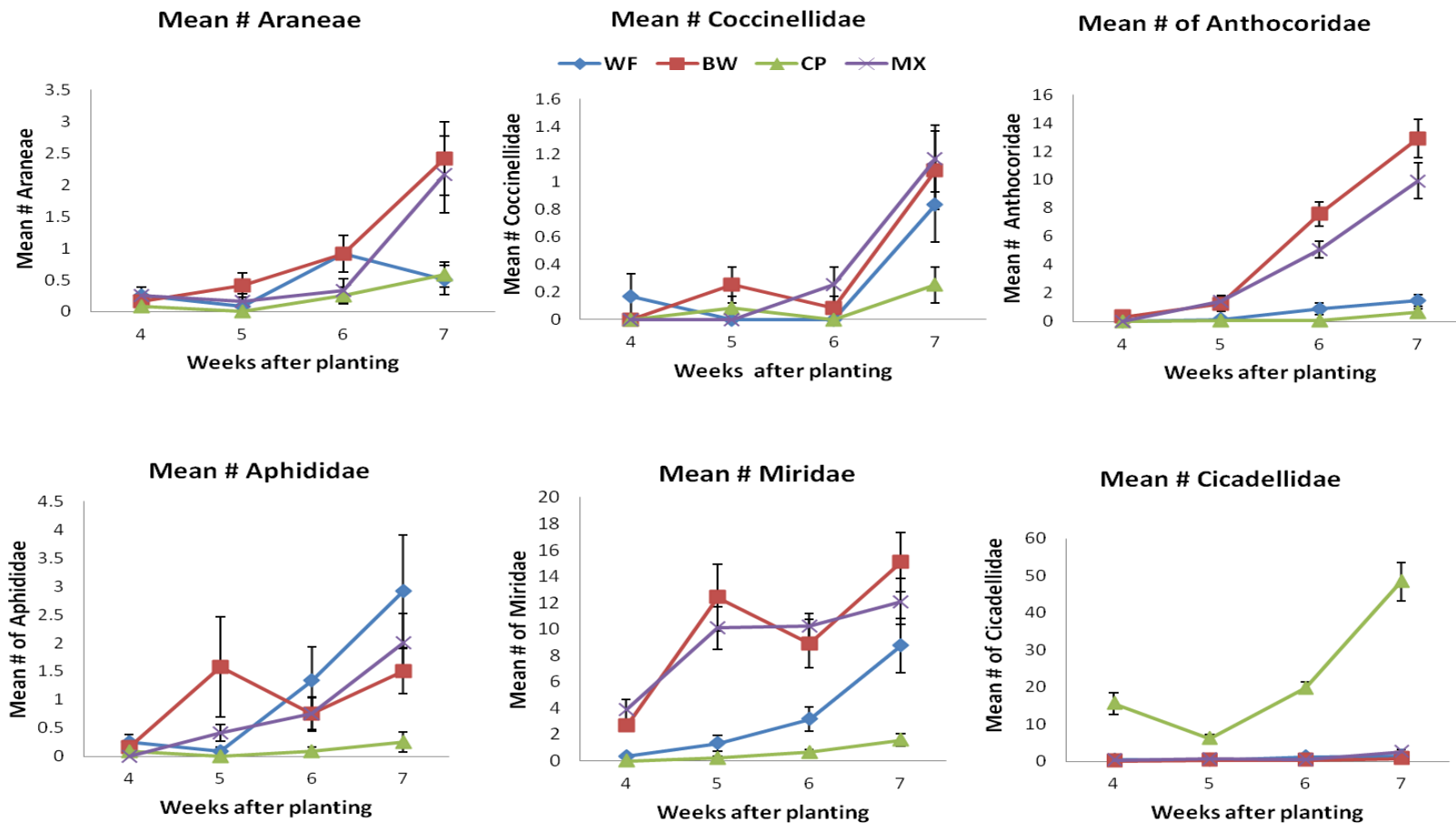


Figure 4.5 Mean number of (A.) Araneae, (B.) Coccinellidae, (C.) Anthocoridae, (D.) Aphididae, (E.) Miridae, (F.) Cicadellidae collected from sweep net samples collected from flowering insectary strips in 2014. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

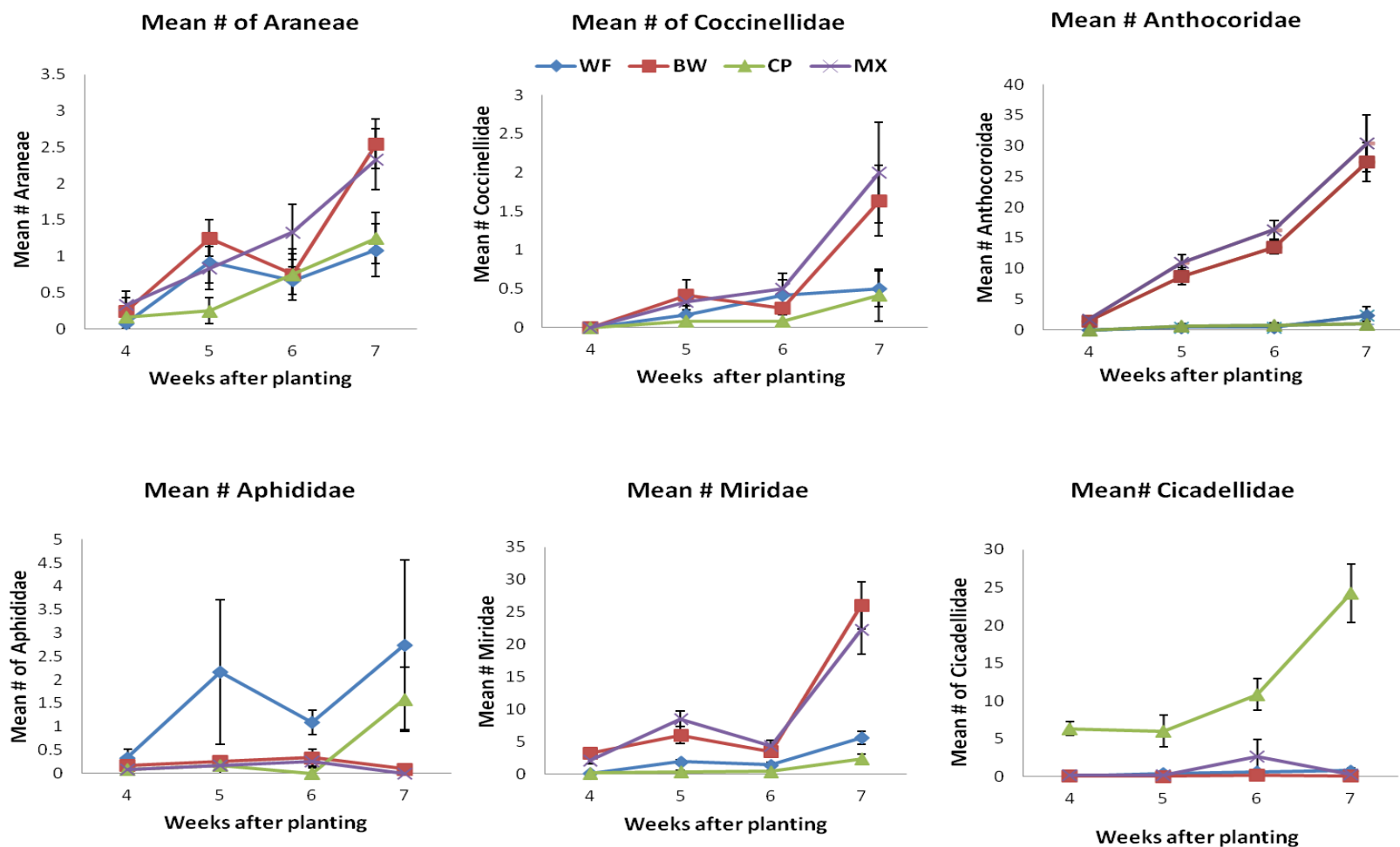


Figure 4.6 Mean number of (A.) Araneae, (B.) Coccinellidae, (C.) Anthocoridae, (D.) Aphididae, (E.) Miridae, (F.) Cicadellidae collected from sweep net samples collected from flowering insectary strips in 2015. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

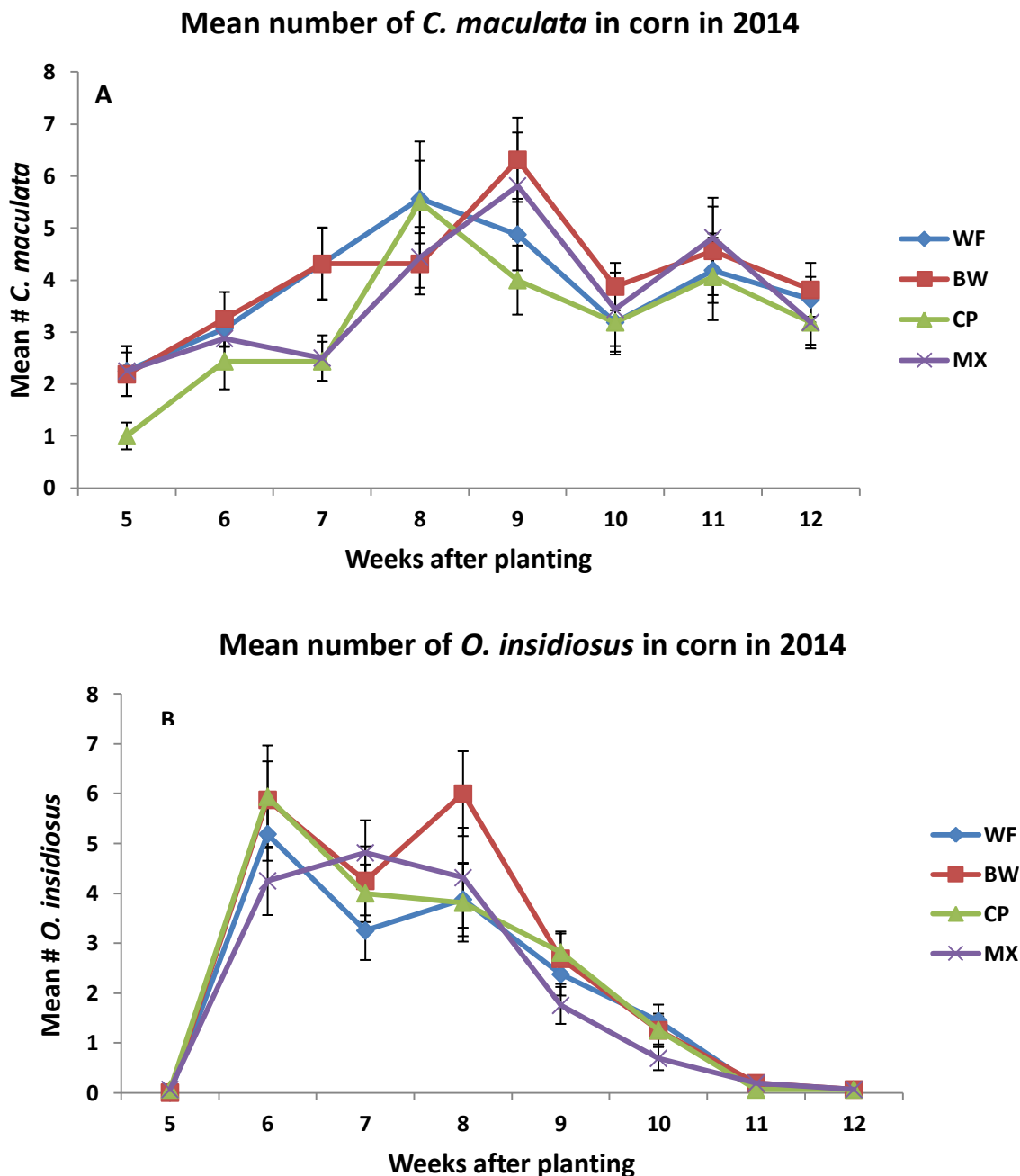


Figure 4.7 Mean number of A.) *C. maculata* and B.) *O. insidiosus* observed during timed counts conducted in corn adjacent to insectary border treatments in 2014. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

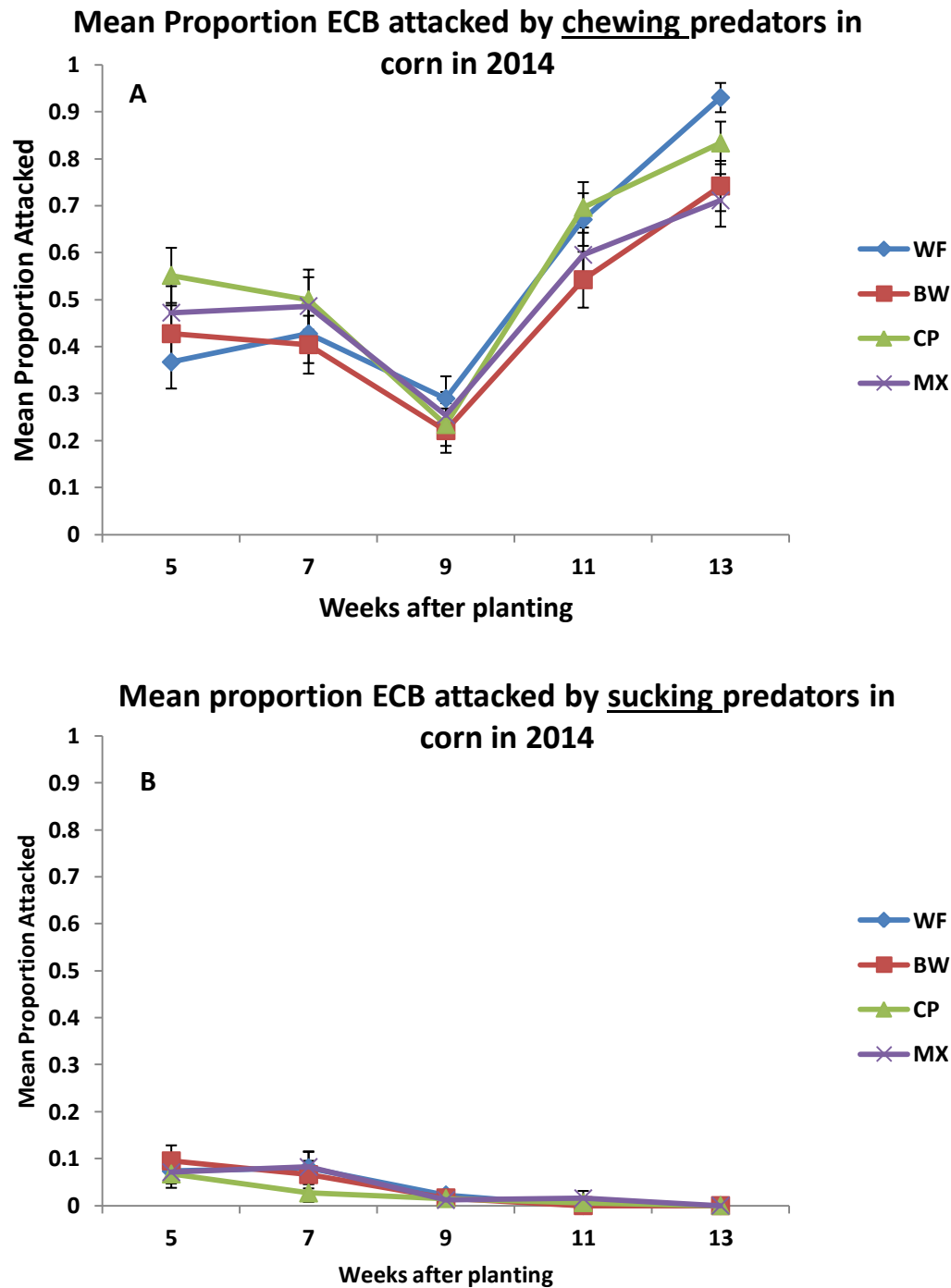


Figure 4.8 Mean proportion of European corn borer sentinel prey attacked by A.) chewing and B.) sucking predators in corn adjacent to insectary border treatments in 2014. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

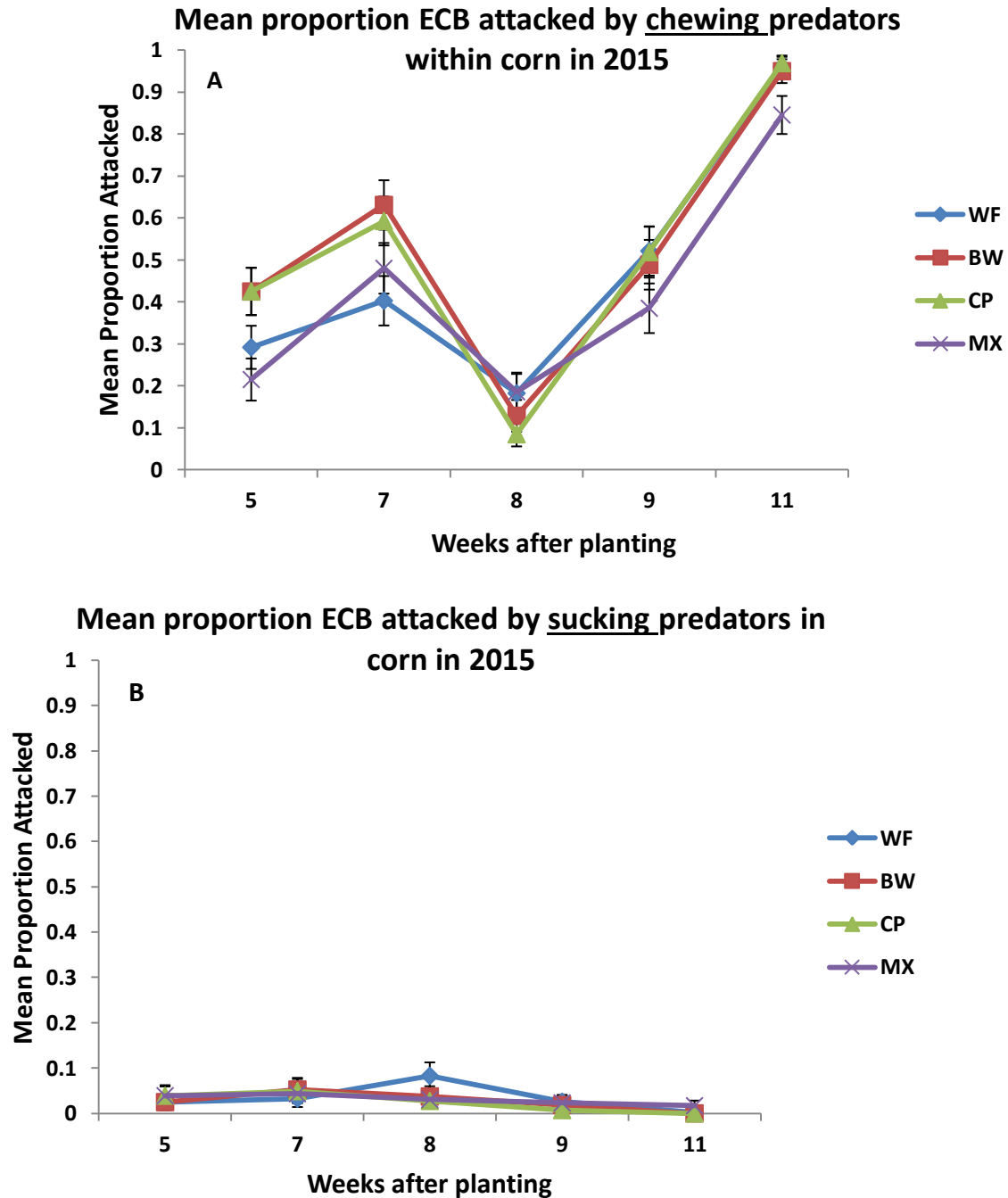


Figure 4.9 Mean proportion of European corn borer sentinel prey attacked by A.) chewing and B.) sucking predators in corn adjacent to insectary border treatments in 2015. Buckwheat (BW), cowpea (CP), biculture (MX) and weedy fallow (WF) treatments are denoted using squares, triangles, Xs and diamonds, respectively.

Chapter 5

Use of a protein-based immunomarking mark capture technique to track generalist predator dispersal between insectary borders and corn crop

Introduction

Habitat management to provide supplemental resources to enhance conservation biological control services in agricultural landscapes is receiving increasing interest (Landis et al. 2000, Gurr et al. 2017a). Providing supplemental resources through habitat management, such as the use of insectary plant borders has, in many cases, improved natural enemy performance as measured by survival, fecundity and predation rates (Pumariño and Alomar 2012, Wong and Frank 2013, Morandin et al. 2014). This practice provides natural enemies with supplemental nectar, pollen and alternative prey items during times of prey scarcity (Ehler 1998). Additionally, relatively undisturbed habitat can serve as a refuge in which arthropods can hide or reproduce (Ehler 1998). Studies have increasingly aimed to further understand the underlying mechanisms that drive natural enemy enhancement in agricultural landscapes. Results from these studies can serve to improve the effectiveness of ecologically-based pest management.

To better understand the mechanisms of enhanced biological control associated with supplemental provisioning, studies have investigated how insectary plant resources, plant diversification, nectar and pollen resources, and a natural enemy's ability to access flowers influence performance and ability to suppress pests in field and greenhouse settings (Vattala et al. 2006, Díaz et al. 2012, Géneau et al. 2013). Studies have further investigated how spatial factors, such landscape versus local-scale diversification, can influence local natural enemy

diversity and abundance (Gardiner et al. 2009, Woltz et al. 2012). Studies have even considered the potential for supplemental resources to support targeted pest species and exacerbate pest problems (Baggen and Gurr 1998, Baggen et al. 1999). Increasingly diverse mixtures of insectary plants that provide a functionally diverse array of resources such as floral and extrafloral nectar, pollen, alternative prey, favorable oviposition sites and shelter may further enhance natural enemy and corresponding pest suppression (Gurr et al. 2017a). While each of these factors are critical to developing a better understanding of mechanisms that influence natural enemy performance, the potential for natural enemies to disperse between a resource patch and a target crop and pest should be investigated.

Studies often report increased pest suppression by natural enemies when provided with supplemental food in more diverse habitats (Letourneau et al. 2011). However, there is evidence that the effects of supplemental resources on natural enemy performance and pest suppression may be neutral or negative. Tscharrntke et al. (2016) presented five hypotheses explaining why habitat management may fail to enhance biological pest control. Of these, the authors discussed the hypothesis that natural habitats provide more suitable habitat than does the crop and the natural enemy may not disperse from natural habitat in search of prey. Natural enemy dispersal depends on a variety of factors such as natural enemy species, insectary species, floral resource abundance, prey availability, and surrounding landscape composition. For example, buckwheat (*Fagopyrum esculentum*), yellow mustard (*Brassica hirta*) and sweet alyssum (*Lobularia maritima*) attracted more beneficial insects than did a cucumber control crop (Quinn et al. 2017). In that study, insects did not frequently disperse from insectary plants into the main cucumber crop. Therefore, there was no corresponding

increase in marketable yield of cucumber despite an increase in natural enemy abundance. Studies such as these emphasize the importance of considering natural enemy dispersal between crop and insectary plants. Because predator dispersal may be context-dependent, it must be studied in a variety of agroecosystems (Lavandero et al. 2004). Recent developments in mark-capture techniques have allowed the use of highly sensitive, but inexpensive and commercially available food proteins for broad-scale field marking and detection of insects (Jones et al. 2006, Hagler et al. 2014). These techniques may be used in the field on natural enemies inhabiting insectary patches to monitor natural enemy dispersal (Hagler and Jackson 2001).

I conducted an experiment to monitor the potential for two key generalist predators, *Coleomegilla maculata* and *Orius insidiosus*, to disperse from insectary borders into an adjacent corn (*Zea mays*) crop to attack sentinel prey. Buckwheat (*F. esculentum*) and cowpea (*Vigna unguiculata*) are summer annuals that establish rapidly and have insectary characteristics (Clark 2007). Buckwheat produces numerous, open flowers laden with nectar and pollen. Cowpea has extrafloral nectaries that may support natural enemies. These plants may serve as insectary borders capable of attracting and sustaining natural enemies while corn pests are establishing. It is, however, important to determine whether these key natural enemies will not only disperse into the main crop, but also attack pest insects. I used egg and milk proteins to mark insects and sentinel prey to determine the frequency of dispersal from an insectary border into a neighboring cash crop to understand the impact of the insectary border on the dispersal of *C. maculata* and *O. insidiosus* and their subsequent predation on marked prey. I hypothesized that 1.) marked insects will most frequently be found in the main crop adjacent to insectary borders;

and 2.) a higher proportion of dispersing insects will attack sentinel prey in insectary border treatments adjacent to corn compared to weedy fallow control. Insects inhabiting insectary borders would have been more likely to have fed on carbohydrate-rich nectar, and moved into the main crop to forage on marked prey (Panizzi and Parra 2012).

Materials and Methods

Field Establishment

The experimental site consisted of 16 experimental treatment plots, each measuring 18.2 m x 13.7m, arranged in a randomized complete block design. Each block was replicated 4 times. In 2014, treatment plots consisted of two areas, an insectary border region (13.7m x 4.5m) and an adjacent corn crop (13.7m x 13.7m). In 2015, the experimental layout was similar; however treatment plot sizes were larger, where the insectary border and cash crop portion of each individual plot was 18.2m x 4.5m and 18.2m x 27.4m, respectively (Figures 5.1, 5.2 and 5.3).

In early June, cover crop species were established as insectary borders containing buckwheat (*Fagopyrum esculentum*) (BW), cowpea (*Vigna unguiculata*) (CP), a buckwheat-cowpea biculture (MX), or a weedy fallow treatment (WF) along the edges of corn cash crop plantings. In monoculture plots, buckwheat (Lakeview Organic Grain, Penn Yan, NY) was planted at the recommended seeding rate of 61.6 kg/ha. Cowpea (variety: Iron and Clay, Hancock Seeds, Dade City, FL) was planted at rate of 78 kg/ha, a rate higher than recommended to compensate for poor germination observed during a preliminary study. The seeding rate of the biculture consisted of an adjusted 16.8 kg/ha for buckwheat and 48 kg/ha for cowpea to

reduce the likelihood of buckwheat dominating the mixture as observed in a preliminary study. The weedy fallow treatment consisted of naturally occurring weed species as a control. Untreated, non-transgenic corn (Master's Choice 4050) was planted immediately after the insectary border at the recommended rate of 81,500 plants per hectare. Insectary borders grew for 6-7 weeks before protein marking and subsequent termination 24 hours later.

Protein Marking - Dispersal

Six weeks after planting and prior to termination, I sprayed the insectary borders with a 15% solution of commercially-available egg whites (Great Value Liquid Egg Whites) using a piston backpack sprayer and boom attachment. I made several passes, using all 15 liters of the backpack sprayer's capacity to ensure the insectary borders were thoroughly coated. One day later, Insectary borders were terminated by flail mowing; thus, insects had a 24-hr window in which to acquire the protein mark. Termination of the insectary border was intended to encourage arthropods to disperse into the adjoining crop once there are no longer any buckwheat flowers or cowpea nectaries (Khan et al. 2000, Thorbek and Bilde 2004). Recovered insects were tested for the presence of chicken egg albumin protein using an ELISA assay, as described below (Hagler et al. 2014).

Protein Marking – Predation

To examine the potential for predators to migrate from resource patches into the main crop and attack pests I used a spray bottle to apply a 10% milk solution to European corn borer (*Ostrinia nubilalis*) eggs as sentinel prey. Protein marking has been previously used to internally

mark arthropods by marking their prey with egg albumin or bovine casein protein (Sivakoff et al. 2012). European corn borer eggs were obtained from a local insectary (Benzon Research, Carlisle PA) and prepared by gluing to card stock and recording the number of eggs per egg mass. I then applied a 10% solution of whole milk to the prepared eggs to thoroughly coat them. After air-drying, sentinel prey were deployed in the field, 20 per experimental plot located randomly in the insectary border and along 4 transects (6m, 12m, 18m, 24m) intervals from the insectary border (Figure 5.3). Insects feeding upon milk-marked prey were initially recovered 48 hours after insectary border termination and weekly afterwards. Recovered insects were tested for bovine casein, a protein present in milk, using ELISA, as described below (Hagler et al. 2014).

Arthropod Collection

I used both active and passive collection methods to recover protein-marked insects. Active collection of the key generalist predators, *C. maculata* and *O. insidiosus*, consisted of timed aspirator collections. I used a stopwatch to conduct three minute timed aspirator collections for each *C. maculata* and *O. insidiosus*. A team of two recorders simultaneously scanned corn plants along 4 transects parallel to the insectary in each experimental plot. Transects were 1.5m, 4.5m, 7.5m and 12m from the insectary border in 2014 and 6m, 12m, 18m, and 24m from the insectary border in 2015 (Figure 5.3). I searched along corn stems, adaxial and abaxial leaf surfaces and in corn silks and aspirated *C. maculata* and *O. insidiosus* into separate aspirator vials. Insects were stored in individual bags to avoid cross-contamination. Insects were frozen until used in ELISA assays.

I used post-mounted 7.62cm x 12.7cm yellow sticky cards placed along transects throughout corn plots to passively recover marked insects. Three yellow sticky cards were placed along transects 4.5m, 7.5m and 12m in 2014 and 7.5m, 15m and 24m in 2015 (Figure 5.4). Sticky cards were collected 7 days after placement, stored in individual bags and frozen until assayed. Additional posts were placed around the perimeter of the field to capture insects dispersing from the field. In 2014, 6 sticky traps were placed along the northern and southern field borders while 2 sticky traps were placed along the eastern and western borders. In 2015, with the larger field plots, 16 and 6 sticky traps were placed along the northern/southern borders and eastern/western borders, respectively (Figure 5.5). To document dispersal on a larger scale, 3 additional sticky cards per site were placed at 9 sites surrounding the experimental plots during the 2015 study year (Figure 5.6).

ELISA Assay

ELISA assays were conducted at the USDA Arid Land Agricultural Research Center in Maricopa, AZ as described in Hagler et al (2014). Indirect ELISA assays were used to test arthropods for egg albumin and bovine casein (Hagler and Machtley 2016). Insects collected from the mark-recapture study were removed from their individual bags and sticky cards and sorted into individual 1.5ml microcentrifuge tubes and labeled. Insects were first assayed for the presence chicken egg albumin. Insect specimens were then individually ground up using disposable pestle tips and re-assayed for the presence bovine casein, which internally marked insects that had fed on protein marked sentinel prey in the field. Microplates were read and optical density (OD) using a SpectraMax 250 microplate reader set at 650nm. Insects were

considered positively marked if the observed OD for that well was at least two standard deviations higher than the plate mean.

Statistical Analysis

Data was analyzed as the proportion of insects positively marked using a Kruskal-Wallis nonparametric test. A model was constructed to determine the effects of insectary border type on the mean proportion of insects positively marked. Due to relatively low recapture rates, plot data was pooled and transect removed from analysis. Proportion data was arcsine transformed to ensure homogeneity of variances. Treatment effects were considered significant when ($P < 0.05$)

Results

C. maculata and *O. insidiosus* Dispersal in 2014

During the 2014 study year, of 670 lady beetles captured during aspirator collection, a total of 19 insects (2.84%) were marked with chicken egg albumin, indicating dispersal from a previously marked plot (Figure 5.1a). Treatment did not significantly influence the proportion of insects marked ($P = 0.7611$). Passive collection via sticky cards captured 380 lady beetles. Of these, only 3 (0.79%) were marked with chicken egg albumin (Figure 5.1b). Treatment did not impact the proportion of insects marked on sticky traps in 2014 ($P = 0.7611$). Perimeter straps captured three lady beetles, however, none were marked (Figure 5.1c)

Active collection of *O. insidiosus* resulted in 7 of 453 insects (1.55%) marked with egg albumin (Figure 5.2a). Treatment did not influence the proportion of marked insects captured

via aspirator ($P = 0.6993$). Passive collection of *O. insidiosus* via sticky cards captured 844 insects, of which 8 were marked with the egg white protein (Figure 5.2b). Treatment did not influence the proportion of *O. insidiosus* captured by sticky traps in 2015 ($P = 0.5675$). Perimeter sticky traps captured 32 *O. insidiosus*. Of these, only 1 insect collected on the northern perimeter was marked (Figure 5.2c).

C. maculata and *O. insidiosus* Predation in 2014

With regard to lady beetles, of the 670 captured via aspiration, 15 lady beetles tested positive for bovine casein (Figure 5.3a). Treatment did not influence the proportion of marked lady beetles collected via aspiration in 2014 ($P = 0.0521$). I did not recover any marked lady beetles from sticky cards located within plots nor in perimeter traps in 2014 (Figure 5.3b and 5.3c).

I collected 453 *O. insidiosus* individuals via aspiration. Of these, 29 were marked with bovine casein (Figure 5.4a). Treatment did not influence the proportion of *O. insidiosus* marked ($P = 0.3323$). I captured 8 *O. insidiosus* individuals of 844 marked with the casein protein (Figure 5.4b). Treatment, however, did not influence the proportion of *O. insidiosus* marked in 2014 ($P = 0.3855$). I collected 21 *O. insidiosus* on perimeter sticky traps; however none were marked (Figure 5.4c).

C. maculata and *O. insidiosus* Dispersal in 2015

In 2015, I collected 474 lady beetles and 34 were marked positive for the presence of egg albumin (Figure 5.5a). Treatment did not influence proportion of lady beetles marked when

captured via aspiration in 2015 ($P = 0.5126$). Sticky traps recovered 399 lady beetles with 60 marked positive for egg albumin (Figure 5.5b). Treatment did not influence proportion of lady beetles marked ($P = 0.9532$). Perimeter traps collected 49 lady beetles with 7 marked positive for the egg protein (Figure 5.5c). Landscape sticky traps captured 1 lady beetle; however, none were marked (5.5d).

In 2015, I captured 263 *O. insidiosus* via aspirator collection. This year, an abnormally high (82.13%) proportion of insects marked with egg albumin protein (Figure 5.6a). Treatment did not significantly influence the proportion of insects marked with egg protein caught by aspiration in 2015 ($P = 0.7750$). Sticky traps captured 1,141 *O. insidiosus* individuals. Of these, 40 were marked (Figure 5.6b). Treatment did not influence the proportion of marked *O. insidiosus* recovered in 2015 ($P = 0.3539$). Perimeter traps captured 721 *O. insidiosus*, 15 were marked (Figure 5.6c). Location did not influence the proportion marked ($P = 0.2222$). The landscape sticky traps captured 87 *O. insidiosus* of which 6 were marked (Figure 5.6d). Location did not influence proportion marked with egg protein ($P = 0.4608$).

C. maculata and *O. insidiosus* Predation in 2015

In 2015, of the 474 lady beetles captured via aspiration, 6 were marked positive for bovine casein protein (Figure 5.7a). Treatment did not influence the proportion of casein-marked lady beetles captured via aspiration in 2015 ($P = 0.1070$). Sticky traps recovered 5 casein-marked lady beetles in 2015 (Figure 5.7b). Treatment did not influence the proportion of casein-marked lady beetles on sticky traps in 2015 ($P = .2584$). A single milk-marked lady beetle

was recovered on perimeter traps in 2015 along the western perimeter (Figure 5.7c). Landscape sticky traps did not recover any milk-marked lady beetles in 2015 (Figure 5.7d).

In 2015, 12 casein-marked *O. insidiosus* were recovered (Figure 5.8a). Treatment did not influence the proportion of casein-marked *O. insidiosus* ($P = 0.5723$). Sticky traps placed within treatment plots recovered 16 milk-marked *O. insidiosus* (Figure 5.8b). Treatment did not significantly impact the proportion of marked *O. insidiosus* ($P = 0.1566$). In 2015, perimeter traps captured 15 casein-marked *O. insidiosus* of 721 captured (Figure 5.8c). Effects of sticky trap location on proportion of *O. insidiosus* marked was not significant ($P = 0.2872$). Landscape sticky traps recovered 10 milk-marked *O. insidiosus* (5.8d). Location did not influence the proportion of milk-marked insects recovered on landscape sticky traps ($P = 0.5852$).

Discussion

This study aimed to determine the influence of insectary border crops on the dispersal of *C. maculata* and *O. insidiosus* between insectary borders and a main crop. I used bovine casein as a secondary mark to determine proportion of dispersing insects attacking marked prey. I hypothesized that marked insects would most frequently be found in the main crop adjacent to insectary borders, and with regard to predation, there would be a higher proportion of dispersing milk-marked insects in the main crop adjacent to insectary border compared to the weedy fallow control attacking sentinel prey. My data did not support either of my hypotheses. Insectary species did not influence the proportion of marked *C. maculata* or *O. insidiosus* collected by aspiration or sticky traps in either study year. Similarly, insectary border species did not influence the proportion of marked *C. maculata* and *O. insidiosus* attacking sentinel prey.

My analyses may not have detected any influence of insectary border treatment due to the low recapture rates of both *C. maculata* and *O. insidiosus* for both capture methods throughout the study. *O. insidiosus* dispersal data from 2015 was the exception to this when 82.13% of captured individuals tested positive the egg white dispersal protein. It was unlikely that there was such a high degree of positively marked insects. Instead, the corresponding micro plates exhibited signs of contamination and may be an outlier. In contrast to this study where recapture rates were low, similar studies examining field-scale dispersal on different arthropods have reported higher recapture rates for other arthropods in other agroecosystems (Horton et al. 2009, Sivakoff et al. 2012, Swezey et al. 2014, Blaauw et al. 2016). For example, in a study monitoring field dispersal of the brown marmorated stink bug, *Halyomorpha halys*, the proportion of insects marked ranged from 20-90%. In mark capture studies recapture rates may depend on a variety of factors ranging from arthropod-related to ambient aspects to study design.

Recapture rates may, in some cases, depend on the target arthropod's mobility or ability to disperse. It is possible that arthropods that are sensitive to disturbance may flee from marked vegetation when the mark is applied. In this study, I focused on *C. maculata* and *O. insidiosus*, which are both highly mobile and capable of dispersing several kilometers in search of food or new reproductive sites (Hodek et al. 1996, Saulich and Musolin 2009). In field experiments, researchers may be limited with their options to prevent arthropod dispersal from fields without the risk of imposing artificial conditions on the insects.

Recapture rates may also depend on experimental design considerations such as the frequency that the protein marks are applied and trap efficiency. In this field study, the

dispersal mark, the egg white protein, was applied once just prior to termination of the insectary. The choice to apply the mark once may have resulted in fewer insects acquiring the protein mark. Similar studies often apply protein mark on a weekly basis (Swezey et al. 2014, Blaauw et al. 2016). This may result in an accumulating number of insects being repeatedly marked over time. Both chicken egg albumin and bovine casein were found to persist in the field for up to 21 days after application through rain events and ambient UV radiation (Jones et al. 2006). Higher recapture rates were found in a similar study where fields were marked up to four times to monitor dispersal of *H. halys* in corn (Blaauw et al. 2016). While still variable, the authors reported higher mean recapture rates ranging from 50-80% marked insects recovered. Recapture rates may also be influenced by the trap effectiveness as traps that can capture more insects are more likely to recover a marked individual.

While insectary treatment did not influence the proportion of marked individuals recovered, this data suggests that visual collection of lady beetles using aspirators were more effective at capturing lady beetles while yellow sticky traps were more effective at capturing *O. insidiosus*. This is consistent with previous studies that successfully used aspiration and yellow sticky traps to sample *C. maculata* and *O. insidiosus* (Elliott et al. 2002, Musser et al. 2004). Musser et al. (2004) found that after pollen shed, *C. maculata* were less attracted to yellow sticky cards. Therefore yellow sticky cards may be unreliable for sampling for the pink spotted lady beetle.

The strategy to utilize two protein marks, egg albumin to monitor dispersal, and bovine casein to test for predation on marked prey, resulted in only a single double marked insect being recovered. A single *O. insidiosus* was found ~3km northeast of the main experimental

plots. Perimeter and landscape sticky traps placed around the experimental site and the surrounding landscape, in rare cases, recovered protein-marked insects. Although I did not observe any statistically significant dispersal patterns from these traps, these instances confirm that both *C. maculata* and *O. insidiosus* may disperse not only out of the field, but up to 3km away after acquiring the protein mark. Similarly, there were a few instances where milk marked insects that previously fed on marked sentinel prey were found on both perimeter and landscape sticky traps.

Conclusion

The goal of the current study was to document dispersal of *C. maculata* and *O. insidiosus* in the presence of different insectary border treatments. I further attempted to determine the proportion of dispersing insects subsequently feeding on protein marked prey. Insectary treatment did not influence the proportion of dispersing *C. maculata* or *O. insidiosus* captured by either visual aspirator collection or yellow sticky traps. Our few positive captures do indicate that there is some degree of dispersal between insectary borders and the main crop. Although rare, perimeter and landscape sticky traps indicate that both *C. maculata* and *O. insidiosus* may disperse from the field after marking and up to 3km away in the surrounding landscape. I also recovered milked marked insects, suggesting both *C. maculata* and *O. insidiosus* fed on milk marked sentinel prey. However, only a single *O. insidiosus* individual tested positive for both the egg albumin and bovine casein mark, which indicated dispersal from marked insectary borders into the main crop and subsequent feeding on marked prey.

Due to low recapture rates, I am unable to determine the impact on insectary border on dispersal ability of targeted natural enemies. As a variety of factors can influence recapture rates, extra care should be taken to ensure maximizing the amount of insects captured during sampling. Frequency of application may expose higher numbers of arthropods to the protein mark. The type of sampling method as well as sampling interval must be appropriate for the targeted insect to maximize the amount of insects captured. Future studies aimed at maximizing recapture rates are needed to obtain more robust data sets necessary to draw inferences and better document and understand arthropod movement through the landscape in response to different management strategies.

Development of these relatively inexpensive protein-based immunomarking strategies has resulted in increased interest in arthropod dispersal in a variety of contexts. Studies are increasingly aimed at understanding movement patterns of natural enemies or pests to maximize effectiveness of habitat management and pest management strategies (Horton et al. 2009, Sivakoff et al. 2012, Choate and Lundgren 2014, Hagler et al. 2014, Swezey et al. 2014, Blaauw et al. 2016, Blaauw et al. 2017). With regards to conservation biological control, it is critical to understand how natural enemies are affected by habitat management strategies and how they move through the environment in response. Dispersal studies may allow us to further evaluate the effectiveness and feasibility of particular insectary species established to support natural enemies beyond the immediate benefits to fecundity and longevity. It is critical to confirm dispersal and enhanced predation by natural enemies in the presence of different insectary species. Many natural enemies may be supported by established insectary. However, they may not reliably disperse from resource patches to main crops and

suppress targeted prey. Insectary species that arrest natural enemy foraging may result in reduced predation and therefore reduced effectiveness of the habitat management strategy and natural enemy.

Chapter 5 Figures and Tables

Figures



Figure 5.1. Aerial view of experimental plots in 2015. Experimental plots were divided across two field strips, 2 blocks per strip. Within each block, the experimental treatments were buckwheat, cowpea, biculture and weedy fallow.

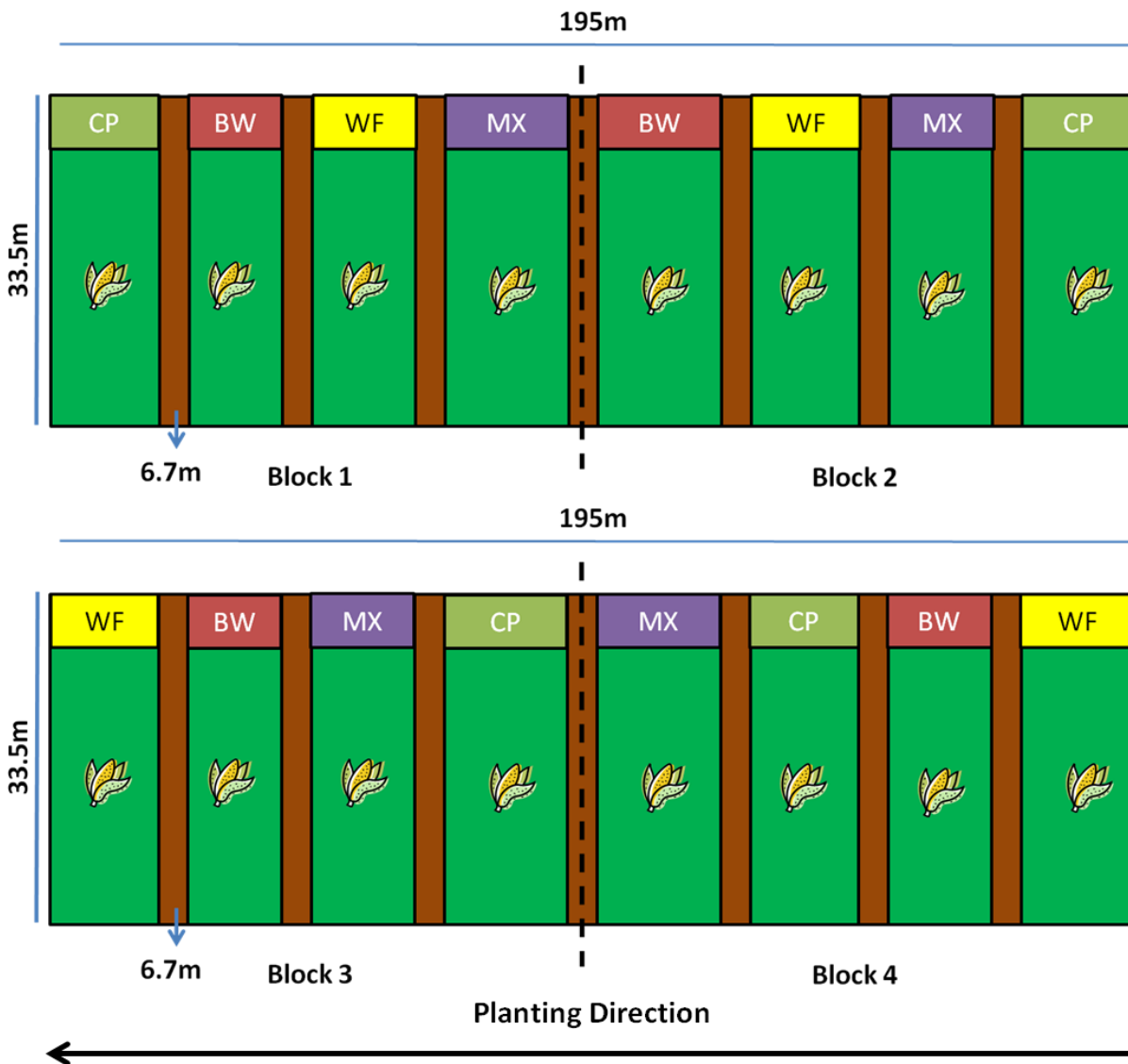


Figure 5.2. Diagram of field layout in 2015. The experiment consisted of 16 experimental plots across four blocks distributed across two 195m x 33.5m parcels of land.

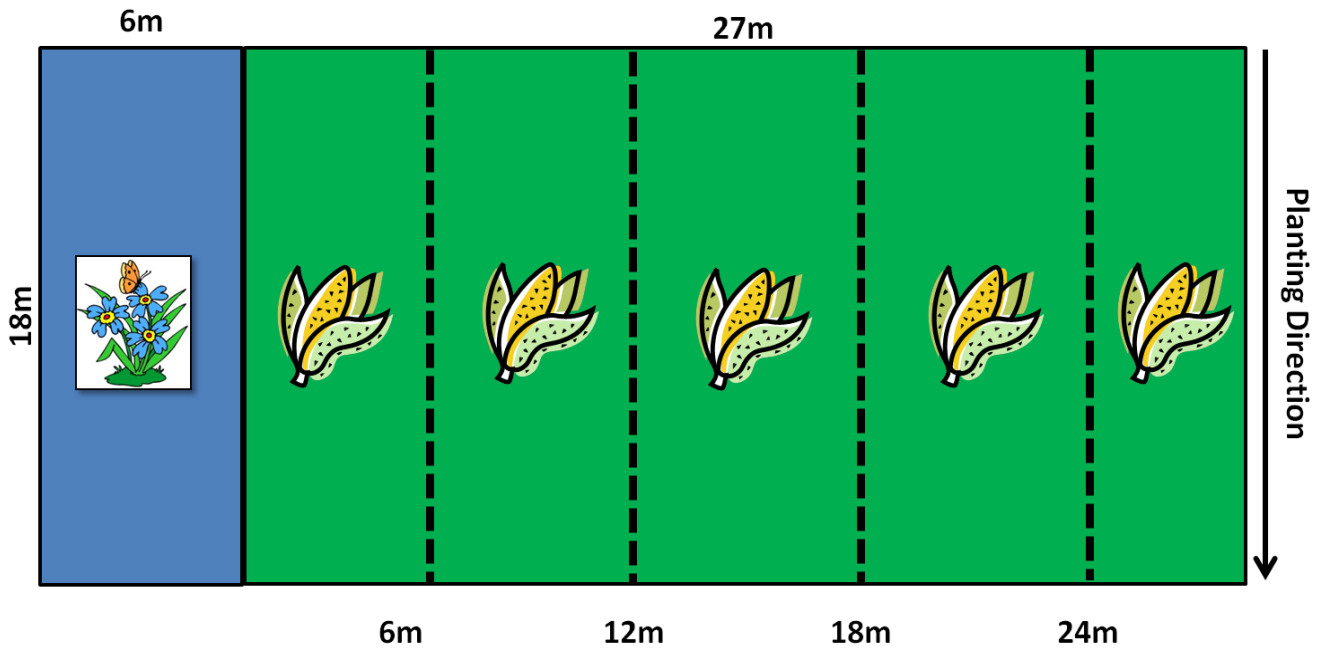


Figure 5.3. Diagram of an individual experimental plot. Each plot measured a total of 33m x 18m and consisted of a insectary border area (18m x 6m) and main crop area (27m x 18m). Predetermined transects at 6m, 12m, 18m, and 24m were used during insect recapture.

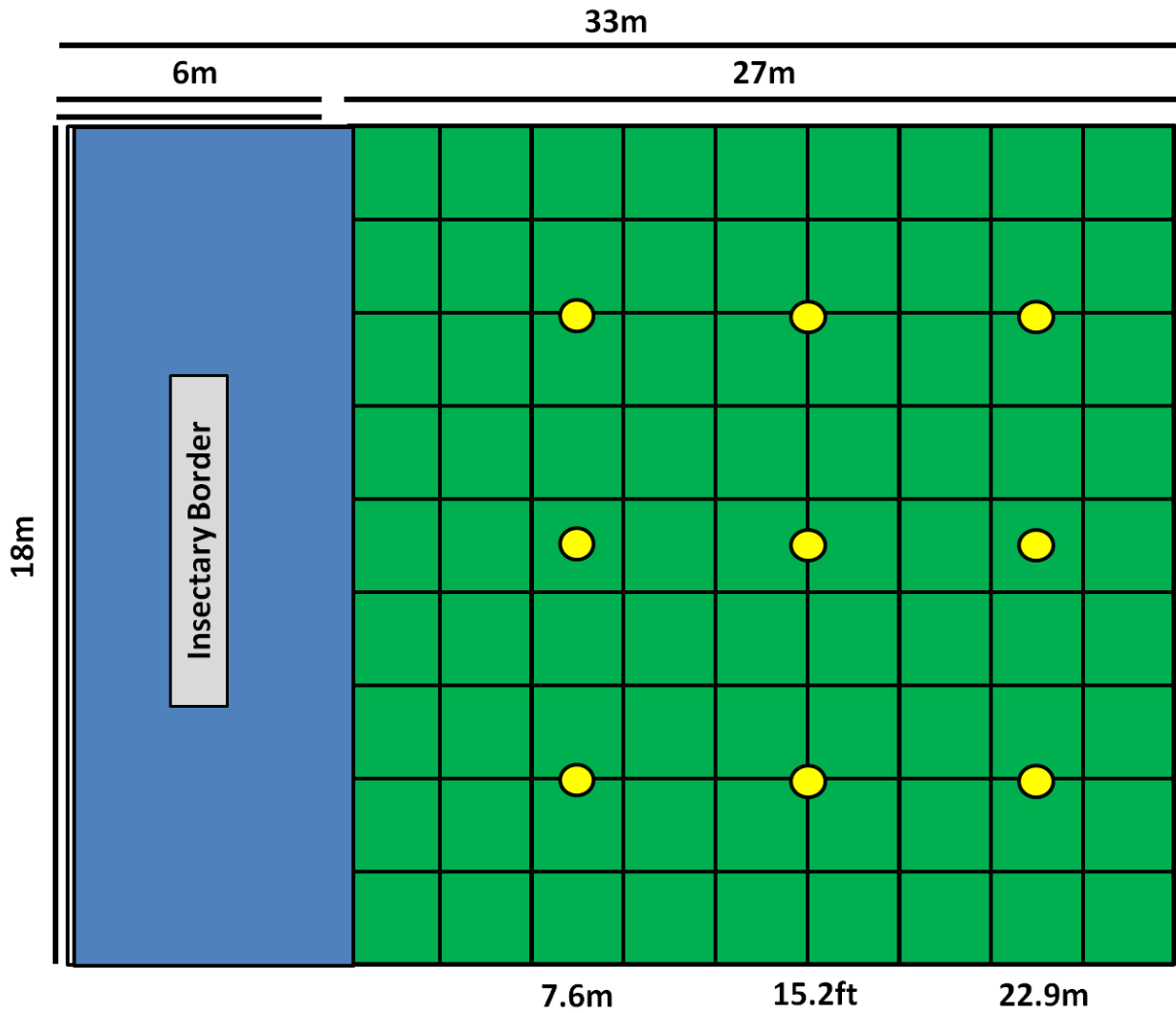


Figure 5.4. Diagram of plot sticky trap layout. Nine sticky traps were placed along three transects 7.6m, 15.2m, and 22.9m from insectary borders. Three sticky traps were placed in each transect.

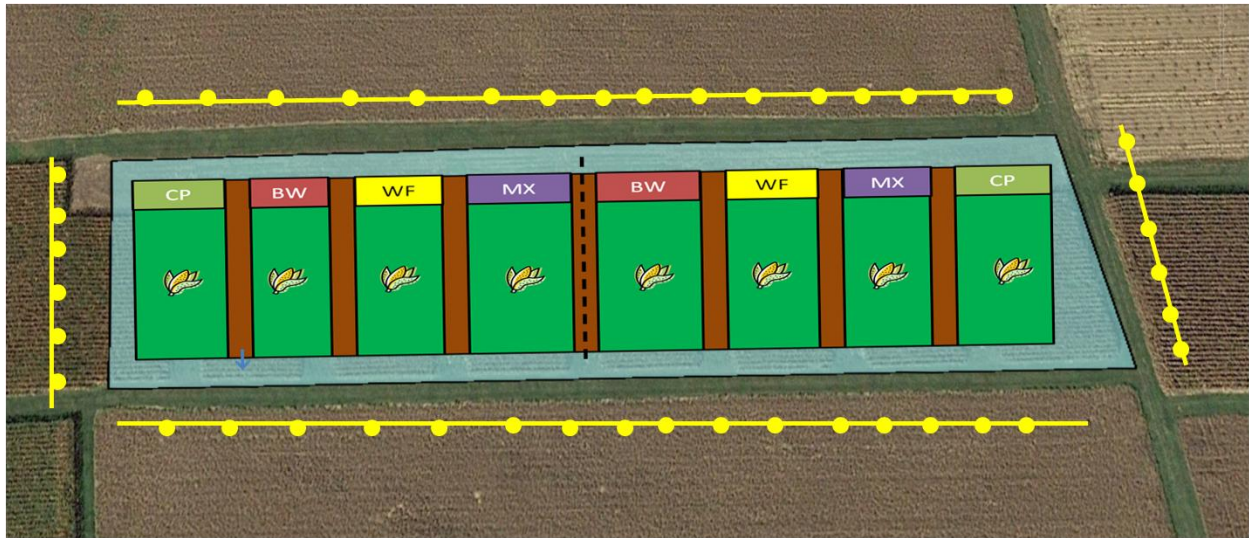


Figure 5.5. Diagram of perimeter sticky trap layout. Sticky cards were placed in adjacent fields surrounding the experimental strips to monitor potential dispersal from the field.

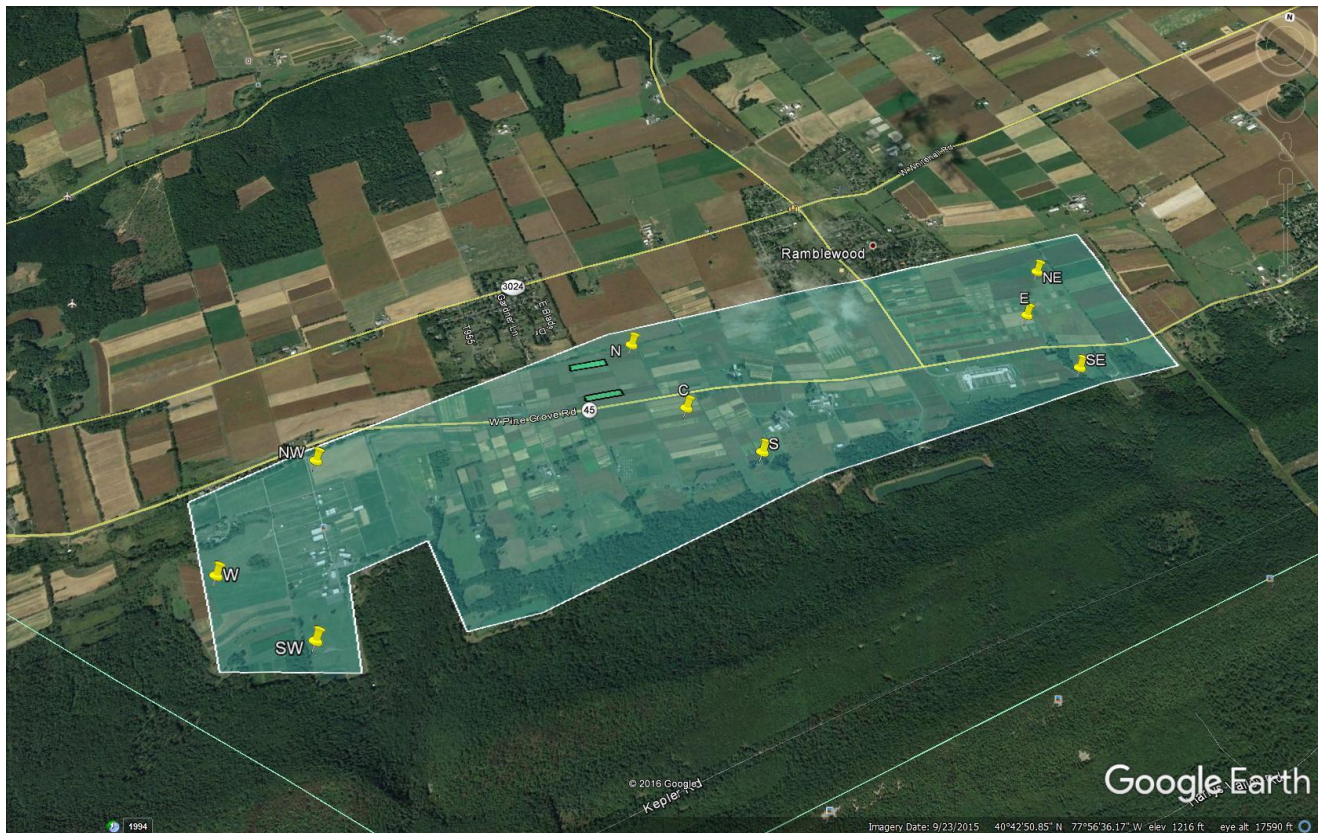


Figure 5.6. Aerial diagram of landscape scale perimeter sticky trap locations. Cards were placed around the landscape corresponding to Northwest, North, Northeast, West, Southwest, South, Southeast, East and Central.

Tables

Table 5.1a. Number of and percentage of total *C. maculata* collected during timed aspiration sampling that testing positive for egg albumin protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	6	187	3.21%
BW	4	151	2.65%
CP	4	171	2.34%
MX	5	161	3.11%
Total	19	670	2.84%

Table 5.1b. Number of and percentage of total of *C. maculata* collected on sticky traps that tested positive for egg albumin protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	1	85	1.18%
BW	2	112	1.79%
CP	0	96	0.00%
MX	0	87	0.00%
Total	3	380	0.79%

Table 5.1c. Number and percentage of total of *C. maculata* collected on perimeter sticky traps that tested positive for egg albumin in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	0	1	0.00%
South	0	2	0.00%
East	0	0	0.00%
West	0	0	0.00%
Total	0	3	0.00%

Table 5.2a. Number and percentage of total *O. insidiosus* collected during timed aspiration testing positive for egg albumin protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	3	132	2.27%
BW	1	111	0.90%
CP	2	111	1.80%
MX	1	99	1.01%
Total	7	453	1.55%

Table 5.2b. Number and percentage of total *O. insidiosus* collected on sticky traps testing positive for egg albumin protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	1	188	0.53%
BW	3	232	1.29%
CP	3	213	1.41%
MX	1	211	0.47%
Total	8	844	0.95%

Table 5.2c. Number and percentage of total of *O. insidiosus* collected on perimeter sticky traps that tested positive for egg albumin in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	1	4	25.00%
South	0	20	0.00%
East	0	0	0.00%
West	0	8	0.00%
Total	1	32	3.13%

Table 5.3a. Number and percentage of total *C. maculata* collected during timed aspiration testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	0	187	0.00%
BW	4	151	2.65%
CP	7	171	4.09%
MX	4	161	2.48%
Total	15	670	2.24%

Table 5.3b. Number and percentage of total of *C. maculata* collected on sticky traps testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	0	85	0.00%
BW	0	112	0.00%
CP	0	96	0.00%
MX	0	87	0.00%
Total	0	380	0.00%

Table 5.3c. Number and percentage of total of *C. maculata* collected on perimeter sticky traps that tested positive for bovine casein in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	0	5	0.00%
South	0	4	0.00%
East	0	0	0.00%
West	0	2	0.00%
Total	0	11	0.00%

Table 5.4a. Number of and percentage of total of *O. insidiosus* collected during timed aspiration testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	11	132	8.33%
BW	7	111	6.31%
CP	8	111	7.21%
MX	3	99	3.03%
Total	29	453	6.40%

Table 5.4b. Number of and percentage of total of *O. insidiosus* collected on sticky traps testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	1	188	0.53%
BW	4	234	1.71%
CP	1	213	0.47%
MX	2	209	0.96%
Total	8	844	0.95%

Table 5.4c. Number and percentage of total of *O. insidiosus* collected on perimeter sticky traps that tested positive for bovine casein in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	0	2	0.00%
South	0	12	0.00%
East	0	0	0.00%
West	0	7	0.00%
Total	0	21	0.00%

Table 5.5a. Number of and percentage of total *C. maculata* collected during timed aspiration sampling that testing positive for egg albumin protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	5	110	4.55%
BW	10	120	8.33%
CP	11	137	8.03%
MX	8	107	7.48%
Total	34	474	7.17%

Table 5.5b. Number of and percentage of total of *C. maculata* collected on sticky traps that tested positive for egg albumin protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	14	82	17.07%
BW	15	89	16.85%
CP	16	115	13.91%
MX	15	113	13.27%
Total	60	399	15.04%

Table 5.5c. Number and percentage of total of *C. maculata* collected on perimeter sticky traps that tested positive for egg albumin in 2015.

Direction	No. Insects Marked	Total Collected	Percent Marked
North	1	1	100.00%
South	0	1	0.00%
East	3	10	30.00%
West	3	37	8.11%
Total	7	49	14.29%

Table 5.5d. Number and percentage of total of *C. maculata* collected on landscape sticky traps that tested positive for egg albumin in 2015.

Direction	No. Insects Marked	Total Collected	Percent Marked
Northwest	0	1	0.00%
North	0	0	0.00%
Northeast	0	1	0.00%
West	0	0	0.00%
Central	0	1	0.00%
East	0	1	0.00%
Southwest	0	0	0.00%
South	0	0	0.00%
Southeast	0	0	0.00%
Total	0	1	0.00%

Table 5.6a. Number and percentage of total *O. insidiosus* collected during timed aspiration testing positive for egg albumin protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	46	55	83.64%
BW	55	71	77.46%
CP	44	58	75.86%
MX	71	79	89.87%
Total	216	263	82.13%

Table 5.6b. Number and percentage of total *O. insidiosus* collected on sticky traps testing positive for egg albumin protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	13	361	3.60%
BW	9	335	2.69%
CP	8	336	2.38%
MX	10	379	2.64%
Total	40	1411	2.83%

Table 5.6c. Number and percentage of total of *O. insidiosus* collected on perimeter sticky traps that tested positive for egg albumin in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	2	300	0.67%
South	7	230	3.04%
East	2	67	2.99%
West	4	124	3.23%
Total	15	721	2.08%

Table 5.6d. Number and percentage of total of *O. insidiosus* collected on landscape sticky traps that tested positive for egg albumin in 2015.

Direction	No. Insects Marked	Total Collected	Percent Marked
Northwest	1	7	14.29%
North	1	7	14.29%
Northeast	3	43	6.98%
West	0	7	0.00%
Central	0	11	0.00%
East	1	10	10.00%
Southwest	0	2	0.00%
South	0	0	0.00%
Southeast	0	0	0.00%
Total	6	87	6.90%

Table 5.7a. Number and percentage of total *C. maculata* collected during timed aspiration testing positive for bovine casein protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	0	110	0.00%
BW	1	120	0.83%
CP	4	137	2.92%
MX	1	107	0.93%
Total	6	474	1.27%

Table 5.7b. Number and percentage of total of *C. maculata* collected on sticky traps testing positive for bovine casein protein in corn adjacent to insectary borders in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	0	82	0.00%
BW	2	89	2.25%
CP	3	115	2.61%
MX	0	113	0.00%
Total	5	399	1.25%

Table 5.7c. Number and percentage of total of *C. maculata* collected on perimeter sticky traps that tested positive for bovine casein in 2015.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	0	1	0.00%
South	0	1	0.00%
East	0	1	0.00%
West	1	37	2.70%
Total	1	40	2.50%

Table 5.7d. Number and percentage of total of *C. maculata* collected on landscape sticky traps that tested positive for bovine casein in 2015.

Direction	No. Insects Marked	Total Collected	Percent Marked
Northwest	0	1	0.00%
North	0	0	0.00%
Northeast	0	1	0.00%
West	0	0	0.00%
Central	0	3	0.00%
East	0	1	0.00%
Southwest	0	0	0.00%
South	0	0	0.00%
Southeast	0	0	0.00%
Total	0	1	0.00%

Table 5.8a. Number of and percentage of total of *O. insidiosus* collected during timed aspiration testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	2	55	3.64%
BW	5	71	7.04%
CP	3	58	5.17%
MX	2	79	2.53%
Total	12	263	4.56%

Table 5.8b. Number of and percentage of total of *O. insidiosus* collected on sticky traps testing positive for bovine casein protein in corn adjacent to insectary borders in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
BG	8	361	2.22%
BW	1	335	0.30%
CP	3	336	0.89%
MX	4	379	1.06%
Total	16	1411	1.13%

Table 5.8c. Number and percentage of total of *O. insidiosus* collected on perimeter sticky traps that tested positive for bovine casein in 2014.

Treatment	No. Insects Marked	Total Collected	Percent Marked
North	6	300	2.00%
South	5	230	2.17%
East	0	67	0.00%
West	4	124	3.23%
Total	15	721	2.08%

Table 5.8d. Number and percentage of total of *O. insidiosus* collected on landscape sticky traps that tested positive for bovine casein in 2015.

Direction	No. Insects Marked	Total Collected	Percent Marked
Northwest	1	7	14.29%
North	2	7	28.57%
Northeast	2	43	4.65%
West	2	7	28.57%
Central	1	11	9.09%
East	2	10	20.00%
Southwest	0	2	0.00%
South	0	0	0.00%
Southeast	0	0	0.00%
Total	10	87	11.49%

Chapter 6

Conclusions and Future Directions

Pest suppression by natural enemies represents an important ecosystem service that result in suppression of economically important pest insects (Losey and Vaughan 2006). Currently, many agricultural landscapes in the United States are characterized by frequent disturbance, fragmented, resource-poor and low biodiversity (Thorbek and Bilde 2004, Jonsson et al. 2012). These environmental conditions represent significant challenges to natural enemies that depend on plant-based resources and alternative prey especially during periods of prey scarcity (Gurr et al. 2017a). Conservation biological control strategies emphasize deliberately managing the agricultural landscape with the intention of supporting natural enemies and enhancing the biological control of pests by natural enemies (Ehler 1998). Insectary plant establishment is one approach to habitat management where flowering plants are maintained in the landscape to provide shelter, nectar, alternative prey and pollen. Studies are increasingly investigating the impacts of insectary plants on natural enemy performance (Pfiffner et al. 2009, Silveira et al. 2009, Walton and Isaacs 2011).

The work presented in this dissertation aimed to contribute to this growing body of work by conducting experiments evaluating the potential for commonly used cover crops to function as insectary species that could attract and sustain generalist natural enemies. Many of the studies that have investigated insectary plant enhancement of natural enemies often focus on a single insectary plant species. I decided to additionally investigate the effect of presenting insectary plants in mixtures to determine if multiple plant-based resources would have a synergistic effect in enhancing natural enemy performance. I conducted protein based

immunomarking to determine natural enemy dispersal rates between insectary and main crop as well as predation by dispersing insects.

Chapter 1 provided an overview of the importance of natural enemies and highlighted the challenges they face under habitat intensification and simplification. I followed with a discussion of some of the developing solutions aimed at promoting natural enemies through the use of habitat management strategies such as insectary planting and diversification. I followed with a brief discussion highlighting some of the limitations of current studies and considerations important when deciding to implement cover crops as insectaries.

The study reported in Chapter 2 was conducted to determine the impact of cover crop diversification on early season natural enemy recruitment and pest suppression in a succeeding corn cash crop. Spiders, lady beetles and parasitoids were more abundant in a red clover monoculture compared to other monocultures and mixtures containing cereal rye, winter pea, and canola. These differences however did not correspond to increased predation based on cover crop mixture. Lessons learned from this study emphasize the importance of considering the compatibility of resource plant use with the crop, targeted natural enemies, and farm management operations. I experienced low abundances of natural enemies throughout the study, which may have been due to blooms being available before *C. maculata* and *O. insidiosus* had emerged each spring. In central PA, corn is often preceded by fall cover crops that must be managed before corn is planted in early May. Because of this, cover crops were often terminated prior to peak bloom and before many late emerging natural enemies could benefit from the cover crop habitat. Subsequent destructive termination of cover crops may have created a resource-poor, fragmented habitat that forced natural enemies out of fields. Since

this study was done as a part of a larger experiment, cover crop and management options were limited. In future studies, I would test the potential for several other cover crops implemented in monocultures and mixtures to support targeted natural enemies. Care would be taken to align peak bloom dates with spring emergence and activity of targeted natural enemies. Future studies would be designed to allow spatial and temporal overlap between the cover crop and succeeding cash crop as well as minimizing disturbance caused by insectary termination. In this study, cover crops could have been partially terminated, leaving a border as refuge while the main crop establishes. This could potentially reduce the emigration of natural enemies for terminated cover crops.

The lessons learned in this study were used to design the research conducted in Chapters 3 and 4. I focused on buckwheat (*F. esculentum*) and cowpea (*V. unguiculata*) as candidate insectary species because they have been reported to be resource rich and their phenologies are appropriate for a corn agroecosystem (Clark 2007, Bickerton and Hamilton 2012).

In Chapter 3, I conducted assays to determine the potential for buckwheat and cowpea to promote *O. insidiosus* performance. Nectar provisioning improved *O. insidiosus* longevity and fecundity compared to a water control. When provided as a biculture, longevity and fecundity was often enhanced beyond when either species was provided alone. Cowpea was found to be a favorable host for *O. insidiosus* as their performance on cowpea was similar to buckwheat. Cowpea may function as a preferred reproductive host to *O. insidiosus* as indicated by more eggs oviposited in cowpea compared buckwheat to buckwheat when presented as a biculture. There was no evidence that insectary provisioning enhanced predation of sentinel prey. I

instead found evidence that insectary provisioning may, in some cases, reduce predation.

Results from this study emphasize the importance of screening natural enemies on insectary plant species in smaller scale microcosm studies in absence of complex field conditions. Unlike the results in Chapter 4 where cowpea did not support any natural enemy sampled, here, *O. insidiosus* thrived on cowpea, experiencing higher longevity and fecundity. In a future study, I would screen additional flowering species singly and in complex mixtures for their potential to support different economically important natural enemy species. I would continue to pair microcosm studies with field studies to determine the true potential for particular plant species to function in an insectary. In cases such as this, issues such as poor establishment may cause certain plant species to appear ineffective. However, in other contexts, seemingly ineffective species may prove effective in enhancing performance of a different natural enemy species.

In Chapter 4, I conducted a field experiment to determine the potential for cowpea and buckwheat, established as monocultures and mixtures in insectary borders, to support and enhance natural enemies in a corn agroecosystem. When established as an insectary border in central PA in June, I found that buckwheat was a fast growing, resource that attracted several natural enemy taxa. Cowpea, however, did not establish well and was often distributed patchily throughout the border area. As such, cowpea was not effective in supporting any sampled arthropod taxa. Results from this study contrasted with those found from the microcosm studies conducted in Chapter 3. Here, cowpea did not support high abundances of *O. insidiosus*; while they performed similarly well on cowpea and buckwheat in microcosm studies. Under better growing conditions, cowpea may prove to be a valuable insectary plant capable of supporting *O. insidiosus* in the field. In future studies, I would test additional insectary species

for their potential to successfully establish in the field as well as supporting natural enemies. This study did not attempt to quantify pest injury or marketable yield of the crop. Since the goal of many conservation biological control programs is crop protection, a desirable insectary plant species should not only enhance natural enemy performance, but should also result in less damaged crops. In future studies I would monitor the frequency of targeted pest injury in the presence of insectary resource patches.

In Chapter 5, I used protein marks to document *C. maculata* and *O. insidiosus* dispersal between insectary border and main crop as well as subsequent predation of dispersing insects. Recapture rates were low throughout the study. Therefore, I did not find any evidence that insectary border treatment influenced the proportion of insects dispersing. Positive marks do, however, indicate some degree of dispersal between insectary and crop. I additionally found marked insects several kilometers from the marked insectary borders. Both *C. maculata* and *O. insidiosus* fed on marked sentinel prey, however, only a single *O. insidiosus* tested positive for both marks. In future dispersal studies I would try to maximize recapture rates by incorporating more frequent marking applications as well as increasing recapture duration and frequency. Capturing a higher proportion of marked insects would allow for me to more confidently determine natural enemy dispersal in response to the presence of insectary borders

Interest in implementing conservation biological control strategies (e.g. habitat management) has rapidly increased and resulted in a dramatic expansion of the field (Gurr et al. 2017a). Numerous works have contributed to developing our understanding of natural enemy responses to habitat management strategies and have found that many different behavioral and ecological processes such as species compatibility, dispersal ability, and source-sink

dynamics can heavily influence how natural enemies respond to habitat management strategies (Khan et al. 2000, Vattala et al. 2006, Sivakoff et al. 2012, Veres et al. 2013, Hagler and Machtley 2016, Gurr et al. 2017a). As agroecosystems are complex environments, arthropod and plant species often respond unpredictably to changes in the environment if not thoroughly considered. The contributions of these types of studies will serve to further our understanding of natural enemy response to habitat management as well as informing pest management strategies. Further development of emerging technologies such as protein-based immunomarking and advanced modeling software may be used in novel ways by future researchers to expand upon the contributions of previous works (Kean et al. 2003, Hagler and Machtley 2016, Gurr et al. 2017a).

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B.S. General Biology, 2009

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Professional Experience

Graduate Assistant, 2012-2017

Barbercheck Lab, Penn State University, University Park

Graduate Assistant, 2009-2012

Hooks Lab, University of Maryland, College Park

Undergraduate Assistant, 2008

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Selected Publications

Hinds, J., K.-H. Wang, C.R.R. Hooks. 2015. Growth and yield of zucchini squash (*Cucurbita pepo* L.) as influenced by a living mulch. *Biol. Agric. Hort.*

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Hinds, J., K.-H. Wang, S.P. Marahatta, S.L.F Meyer, C.R.R. Hooks. 2013. Sunn hemp cover cropping and organic fertilizer effects on the nematode community under temperate growing conditions. *J. Nematol.* 45:265-2671

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Selected Awards and Honors

- **Northeast SARE Graduate Student Grant**, 2014
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