

DOES FLORAL FARMSCAPING ENHANCE BIOLOGICAL CONTROL?

by

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(Under the Direction of John R. Ruberson)

ABSTRACT

Floral farmscaping is the planting of flowering plants in proximity of target crops in order to attract and enhance the populations, fitness, and biological control efficacy of natural enemies. Flowering plants provide food resources such as pollen, floral, and extrafloral nectar for natural enemies. These food resources can be critical for survival and reproduction of natural enemies, and have therefore provided a means of manipulating natural enemies to enhance their biological control efficacy for pest management, in cropping systems.

Flowering plants differ in their capacity to supply these food resources; therefore, it is important in designing a farmscaping system to screen potential flower plants to identify and work with those that attract and support desired natural enemies, while excluding those that might compromise the intended goal of pest suppression.

We investigated the effects of two flowering plants (buckwheat, *Fagopyrum esculentum* (Moench) and Indian blanket, *Gaillardia pulchella* Foug.) on adults of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) parasitoid, *Aridelus rufotestaceus* (Tobias) (Hymenoptera: Braconidae). We also assessed the suitability of three flower treatments (buckwheat; a combination of fennel, *Foeniculum*

vulgare (Mill.) and dill, *Anethum graveolens* (L.); and a combination of sunflower, *Helianthus annuus* (L.) and yarrow, *Achillea millefolium* (L.)) for enhancing parasitism of lepidopteran pests in an organic broccoli production system, and predation of sentinel eggs of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), in organic broccoli and cucumber systems.

Aridelus rufotestaceus lived longer on flowers and 5% honey solution than on water alone. Feeding on Indian blanket and 5% honey solution increased production of mature ova. Apart from few inconsistent significant differences among treatments in the response variables, the flower treatments did not enhance parasitism of lepidopteran pests, as well as predation of *S. exigua* eggs. The results imply that *F. esculentum* and *G. pulchella* can benefit *A. rufotestaceus* for managing *N. viridula*. Our results on parasitism of lepidopteran pests and predation of *S. exigua* eggs might have been confounded by the size of the plots, interactions among predators and available prey, and history of the land.

INDEX WORDS: Predation; parasitism; fecundity; longevity; *Fagopyrum esculentum*; *Foeniculum vulgare*; *Anethum graveolens*; *Helianthus annuus*, *Achillea millefolium*; *Gaillardia pulchella*

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2013

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DEDICATION

I would like to dedicate this to the memory of my late grandmother, Mrs. Mmaka Aduba and my living grandmother, Mrs. Chipaoke Eze

ACKNOWLEDGEMENTS

I would like to thank my major professor Dr. John Ruberson for his advice and guidance through this work and for going out of his way to make sure that things are going well with me.

I am thankful to Dr. Peter Hartel for accepting my application and giving me a chance, and also for guiding me through this work.

I am grateful to my committee members, Drs. Paul Guillebeau, Carl Jordan, and Michael Toews for reviewing my work and providing useful corrections and suggestions.

My thanks go to my family members; my father, Mr. Godwin Aduba; my mother, Mrs. Edith Aduba; my sister, Chioma Udeagbara; my brothers, Chidiebere Aduba and Kenechukwu Aduba; my uncles, Mr. Christian Onyema (for doing all the dirty work and heavy lifting), Mr. Benjamin Aduba (for pulling the strings when it mattered), Mr. David Aduba, and Ozo (Chief) Ike Iloputaife; and my aunties, Mrs. Fidelia Obasi, Chief (Mrs.) Nkechi Iroku, Mrs. Ijeoma Nzegwu, Mrs. Ulari Iloputaife, Mrs. Eucharika Aduba, and Mrs. Alberta Orjiekwe.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Beneficial organisms provide critical ecosystem services, among which natural pest suppression is of considerable importance to mankind (Campbell et al., 2012). Natural pest control is important for global food security and has been valued in global cropping systems at \$14 billion/year (Costanza et al., 1997), \$4.5 billion/year in the United States alone (Losey and Vaughan, 2006), and up to \$100/ hectare/ year in Canterbury, New Zealand (Sandhu et al., 2008). It is even more critical in food production in developing countries, where many farmers depend almost entirely on natural pest control for pest management (Wyckhuys et al., 2013).

Natural pest control is provided by natural enemies such as predators and parasitoids, which can be introduced into native ecosystems from exotic origins to provide permanent pest control of typically exotic pests (classical biological control), periodically released to establish control (augmentative biological control), or whose environment can be manipulated to enhance their populations or efficacy for pest control (conservation biological control) (DeBach, 1964; Hajek, 2004; Perdikis et al., 2011). The last approach (conservation biological control) is the most sustainable because it focuses on the resident natural enemies, integrates with production practices, and reduces the problems that beset other pest management approaches, such as environmental degradation, pest resistance to chemical pesticides, and

ecosystem risks associated with introduction of exotic species. One way to implement conservation biological control is through farmscaping.

Farmscaping is a pest management strategy that involves the use of insectary plants, hedgerows, cover crops, beetle banks, and water reservoirs to provide valuable resources that attract and enhance populations of beneficial organisms, such as insects, birds, and bats, and enhance their populations for pest control (Dufour, 2000). It is an ecological and sustainable approach to pest management that must be able to integrate economically, environmentally, and socially with production practices.

When flowering plants are used for farmscaping, it is termed floral farmscaping. Therefore, floral farmscaping is the planting of flowering plants in proximity to target crops in order to attract and enhance the populations, fitness, and biological control efficacy of natural enemies of pests, for enhanced natural pest control. Floral farmscaping is a form of conservation biological control (Ehler, 1998; Landis et al., 2000a). It also can be viewed as a form of ecological engineering, which involves habitat manipulation with cultural techniques to enhance biological pest control (Gurr et al., 2004).

1.2 Historical background of floral farmscaping

One of the earliest mentions of natural enemies visiting flowers was by Froggatt (1902), who reported *Scolia formosa* (Guérin-Méneville), a parasitoid of the grey cane beetle, *Lepidoderma albohirtum* (Waterhouse), visiting a flower. Subsequent records include Allen (1929); King and Holloway (1930); Nishida (1958).

However, not until the early 1930's was the connection between flower visit and biological control first made by Clausen et al. (1933), who reported that adult food (honeydew from aphids and nectar from flowers of umbelliferous and polygonaceous plants) was a major factor for *Tiphia matura* (Allen and Jaynes) (Hymenoptera: Tiphidae), a parasitoid of *Popillia cupricollis* (Hope) (Coleoptera: Scarabaeidae), limiting distribution and biological control effectiveness. Later, Wolcott (1942) reported that *Larra americana* (Saussure), a parasitoid of mole cricket, *Scapteriscus vicinus* (Scudder), was successfully introduced from Brazil into Puerto Rico because two local weeds: *Borreria verticillata* (L.) and *Hyptis atrorubens* (Poit.) provided nectar for the wasp.

One of the earliest records of targeted planting of insectary plants to provide food source and shelter for parasitoids was in New Zealand apple orchards in the 1960's, in which the Australian shrubs *Acacia* spp., *Eucalyptus* spp., *Grevillea rosmarinifolia* (A.Cunn.), *Hakea laurina* (R.Br.), and *Citrus* sp. were planted to support populations of the parasitoids *Trichogramma* spp. and *Apanteles* spp. for control of the light brown apple moth, *Epiphyas postvittana* (Walker) (Collyer and van Geldermalsen, 1975). Subsequently, Yan and Duan (1988) reported that planting white sweet clover, *Melilotus albus* (Desr.) between rows of apple trees had positive effects on predator community in the trees. And from 1990, the literature on the use of insectary plants as food sources to enhance population of natural enemies for pest control has grown considerably, for example, Haley and Hogue (1990); Bugg et al. (1991); Wyss (1995); Stephens et al. (1998); Bostanian et al. (2004); Blaauw and Isaacs (2012); Díaz et al. (2012a); Gontijo et al. (2013)

1.3 Benefits of floral farmscaping

Floral farmscaping can confer the following benefits when adequately implemented in the field. It can lead to reductions in the amount of pesticides that a farmer may need to use as part of an integrated pest management (IPM) program. This can reduce operation costs for the farmer, health risks associated with chemical residues from the pesticides application to humans, and deleterious effects on natural enemies, thereby conserving them. For example, (Yan et al., 1997) reported that a section of apple orchard managed with alfalfa, *Medicago sativa* (L.)/*Lagopsis supina* (Steph.), cover crop experienced mite (*Tetranychus ulmi* (Koch) and *T. vevennensis* (Zacher)) infestations below economic threshold and did not require any pesticide application unlike the section without the cover crop. It also can provide an opportunity to conserve native flora, when native plants are integrated into the system. Further, it can be an additional source of income to farmers, especially when high-value flowering plants are used (Dufour, 2000; Landis et al., 2000a).

1.4 Plant food resources for natural enemies

Many natural enemies (parasitoids and predators) are omnivores and, therefore, utilize non-host food (plant-based diets) in addition to host or prey (animal-based diets) in order to optimize their life histories (Coll and Guershon, 2002; Eubanks and Styrsky, 2005). Although natural enemies feed on a combination of these diets, the extent and timing at which they utilize these food resources vary. Many of them feed on a combination of these two diets throughout their feeding life stages (predators such as

coccinellids and heteropteran predators), and therefore are referred to as lifelong omnivores (Eubanks and Styrsky, 2005). Others feed on one of these diets at only certain life stages, (e.g., syrphid flies, which feed solely on prey in larval stages and only on a plant-based diet as adults), and are referred to as life-history omnivores (Wäckers and van Rijn, 2005). Yet others feed on a combination of these diets only in the adult stage (e.g., parasitoids that feed on hosts during their larval stages and on a combination of host and plant-based diets in the adult stage).

Omnivory by these natural enemies is particularly well-suited for biological control of pests in ephemeral agroecosystems because feeding on multiple trophic levels allows these natural enemies to survive and remain in the area when hosts or prey are scarce, reducing their risk of starvation or emigration from the target crop area (Eubanks and Denno, 1999; Beckman and Hurd, 2003; Welch et al., 2012). And as a result, they may continue to feed on pests at low densities, driving them to local extinction, thereby benefiting pest management. This is in contrast to what might happen to obligate, and especially specialist predators at low prey densities (Eubanks and Styrsky, 2005).

Plant-based diets include nectar (floral and extrafloral), phloem sap, and pollen (Olson et al., 2005; Wäckers, 2005a; Wäckers and van Rijn, 2005; Lundgren, 2009a).

Floral nectar is among the rewards that plants use to recruit pollinators and is derived from both phloem and xylem sap or phloem sap alone, and is secreted by nectaries. It is composed primarily of carbohydrates, but may contain some amino acids, vitamins, lipids and secondary plant metabolites (Fahn, 1988; Wäckers, 2005a).

Extrafloral nectar is produced from glands located in various plant parts external to the flower, such as stems, leaves, fruits, and bracts. Extrafloral nectar is usually not involved in plant pollination, but is used mainly to recruit parasitoids and predators for plant defense (Koptur, 1992). Similar to floral nectar, extrafloral nectar is composed primarily of carbohydrates, with some amino acids, lipids, and vitamins (Fahn, 1988; Wäckers, 2005a).

Pollen is the means of transferring male genetic information of plants from anthers to the stigma, and so is important for plant reproduction. It is also an important reward offered by insect-pollinated flowers to pollinators. Pollen is composed primarily of free amino acids and proteins with variable amounts of carbohydrates, lipids, and sterol (Roulston and Cane, 2000; Wäckers, 2005a).

These plant food resources have been reported to improve natural enemy development rates, survival, fecundity, dispersal, and distribution (Addison et al., 2000; Eubanks and Styrsky, 2005; Witting-Bissinger et al., 2008b; Díaz et al., 2012a; Géneau et al., 2012; Portillo et al., 2012). Improvement in these life history traits can be important in biological control. For example, decreased development time can provide opportunity for natural enemies to produce more generations in a season. Increased longevity can increase the length of time natural enemies may have access to prey and hosts (pests), while increased fecundity increases number of offspring natural enemies may have, and thereby enhances the numerical response of the enemies to pest populations, and increasing natural enemy populations with a concomitant increase in pest consumption.

Because natural enemies are associated with these food resources and because these food resources can enhance their life history traits, floral farmscaping provides an opportunity for biological control practitioners to deploy these resources to enhance biological control in cropping systems (Lundgren, 2009b). Planting of insectary plants in association with target crops for the purpose of pest management in cropping systems is based on this association.

1.5 Floral plants as sources plant-derived food

Pollen, floral nectar, and extrafloral nectar are produced by flowering plants. These flowering plants can be annual, in which they complete their lifecycle in one growing season (e.g., dill, *Anethum graveolens* (L.)), or perennial, in which they complete their lifecycle in more than two years (e.g., deergrass, *Muhlenbergia rigens* (Benth.) Hitchc). Perennial flowering plants tend to be better suited for insectary plants because they provide a more persistent resource for natural enemies, unlike the annuals that die at the end of the season, and may have to be replanted (Long et al., 1998; Landis et al., 2000a; Sokhangoy et al., 2012).

Flowering plants vary in their capacity to supply these food resources, in quantity, quality, accessibility, and length of supply. With respect to nutrient contents, some nectar is 'sucrose-dominant', for example buckwheat, *Fagopyrum esculentum* (Moench) nectar, while some are 'hexose-dominant,' for example coriander, *Coriandrum sativum* (L.). Sugar consumption may increase osmotic pressure in insects, with physiological consequences such as destabilization of water balance. This increase is more rapid with consumption of nectars dominated by monosaccharides such as glucose and

fructose than disaccharide-dominant nectars (Baker and Baker, 1983; Vattala et al., 2006). The quantity of nectar that a flower produces can determine the number of visitors it receives as insects are able to discriminate flowers based on nectar volume (Goulson, 1999). The size and shape of nectaries can significantly affect the community of natural enemies that benefits from a particular plant. Nectaries of some plants are highly accessible, typically characterized by shallow and wide corollae apertures, for example buckwheat (0.54 mm deep and 6.59 mm wide;(Baggen et al., 1999a)), while others limit access to certain natural enemy species by being too deep or narrow for many species to utilize. Some plants, such as Indian blanket, *Gaillardia pulchella* (Foug.) (observed to flower for 7-8 months in the field in southern Georgia), flower for prolonged periods of time, while some flower for a shorter period, for example buckwheat (observed to flower for 3-4 weeks in the field in southern Georgia), limiting their utility for longer growing seasons without staggered plantings.

Because of these variations, flowering plants differ in the types of natural enemies that they attract and their value to those natural enemies, and it is important to evaluate them in order to identify those that maximize these food resources for natural enemy utilization before they can be deployed as insectary plants.

1.6 Deployment of insectary plants

Insectary plants can be deployed in the field as 'flower strips', alternated with target crops, or they can be planted at borders surrounding the target crops. They can also be planted at the center field to create a "halo effect", so that when natural enemies are attracted they can move into the crop section to control the pests.

Natural enemies vary in the distances that they can travel; for example, *Hippodamia convergens* (Guérin-Ménéville) can travel more than 1 km (Sivakoff et al., 2012), while Tachinids are known to have long flight ranges of up to 200 m (Romina et al., 2011; Pfannenstiel et al., 2012). Therefore, it is important to locate insectary plants appropriately in space within or around crop fields, in order to maximize natural enemy area of influence.

Because flowers differ in when they initiate flowering and how long they subsequently flower, it is important in choosing flowering species mixes to select those that can complement each other temporally and extend the availability of appropriate floral resources for natural enemies.

1.7 Does floral farmscaping enhance biological control?

Since the importance of floral resources for fitness of natural enemies became evident, numerous studies have been carried out to determine how they can be used to enhance biological control and manage agricultural pests. A look at those studies shows that results have been equivocal. Some of those studies show positive effects of planting insectary plants in association with target crops with respect to pest control, some show negative effect of insectary plants, with increased risk of exacerbating pest problems, while others show negligible or neutral effects of insectary plants on pest control (Table 1.1).

1.8 Reasons for the mixed results

Potential reasons for the conflicting results obtained with floral farmscaping include:

- 1) *Natural enemy feeding habit*: many predators and parasitoids in agroecosystems are polyphagous, and feed on multiple taxa of prey (Coll and Guershon, 2002; Welch et al., 2012). These polyphagous natural enemies may switch prey based on preference [preferred (high-quality) vs. alternative (low-quality) prey] or nutritional demands and, therefore, the presence of preferred prey may influence how these polyphagous natural enemies respond to alternative prey. This presents problems in biological control, especially when the target prey is not the preferred prey for natural enemies, with the predators not responding as desired to pests of interest (Welch et al., 2012). Natural enemies can also feed on plant food and become satiated and not respond adequately to pests.
- 2) *Competition for resources*: natural enemies may compete among themselves for floral resources, when they are limited, thereby resulting in competitive exclusion of some. In some cases some beneficial insects may interfere with the ability of others to utilize floral resources. For example, Campbell et al. (2012) showed that parasitoid visitation to a flower of short corolla length was reduced by 50% when the flowers were mixed with other flowers of long corollae length, possibly due to competitive interference from bumble bees, which prefer flowers with long corolla length.

- 3) *Wrong insectary plants*: flowers differ in their capacity to supply plant food resources and, therefore, using wrong plants may end up not attracting the desired natural enemies. Floral resources may be more nutritious than prey and may draw the natural enemies away from the prey. On the contrary, floral resources may be nutritionally poor may be inadequate to sustain natural enemies in time of prey scarcity. Insectary plants may differentially favor recruitment of pests relative natural enemies.
- 4) *Relative size of insectary plant land*: the area of land devoted to insectary plants relative to target crop land can be too small to produce effects. Pfiffner and Wyss (2004) recommended that at least 10% of intensively cultivated area be set aside as wildflower strips for natural enemy conservation. In the lettuce-alyssum system in California, ~4% of cultivated land is devoted to natural enemy conservation, and this system has been successful in managing the currant-lettuce aphid (*Nasonovia ribisnigri* Mosley) with attracted hoverflies (Gillespie et al., 2011). The work by Gillespie et al. (2011) on the lettuce-alyssum system suggests that the area set aside for natural enemies can be reduced to 2% of the crop area without any effect on hoverfly abundance in the crop section. There are likely system-specific variations in the extent of area that must be devoted to insectary plants relative to target crop to achieve positive biological control results.
- 5) *Natural enemy mobility*: natural enemies vary in the distance and speed at which they travel in the field and therefore using insectary plants that attract mostly slow- moving natural enemies may not be as effective as those that

attract considerable number of fast-moving natural enemies. Fast-moving natural enemies with long dispersal range, depending on their foraging strategies, may likely encounter more pests and consequently improve biological control.

1.9 Present study

In view of these mixed results and potential system differences in how insectary plants can be used to enhance biological control, it is apparent that more work is needed to answer the question: does floral farmscaping enhance biological control? Additional studies are necessary to develop location- and system-specific farmscape systems for pest management.

Objective:

The main objective of the present study was to address the question “does floral farmscaping enhance biological control?”

Hypotheses tested:

- 1) Flowering plants will enhance longevity and fecundity of an important model parasitoid, *Aridelus rufotestaceus* (Tobias) (Hymenoptera: Braconidae), more than water alone.
- 2) Buckwheat will enhance parasitoid longevity and fecundity (of *A. rufotestaceus*) more than other flower treatments because of buckwheat’s copious nectar production and ready accessibility.

- 3) The availability of flowers will lower pest density and enhance their parasitism in an organic broccoli cropping system.
- 4) Buckwheat will lower pest density and enhance parasitism more than other flowers tested in an organic broccoli system because of its copious nectar production.
- 5) The presence of flowers will enhance predator abundance and predation of *Spodoptera exigua* (Hübner) eggs in organic broccoli and cucumber systems.
- 6) Buckwheat, in particular, will enhance predator abundance and predation of *S. exigua* eggs more than other flowers tested in organic broccoli and cucumber cropping systems because of copious nectar production and ease of its access in buckwheat.

This dissertation examined the questions above. This chapter (Chapter 1) presents a definition of floral farmscaping and its history, types of plant food resources, mixed results of floral farmscaping on biological control, and possible explanations for the mixed results.

Chapter 2 presents the results of a laboratory study investigating the effect of two flowering plants (buckwheat and Indian blanket) on adults of the stink bug parasitoid *A. rufotestaceus*. *Aridelus rufotestaceus* lived longer on flowers and 5% honey solution than on water alone. Feeding on Indian blanket and 5% honey solution increased production of mature ova relative to water. Body sugars of the wasps were similar after feeding on the treatments for 24 h, despite differences in sugar contents among the

treatments. The lack of significant differences in sugar contents of the wasps might be a result of a short feeding time (24 h) and/or the timing of the feeding assessment (very shortly after adult emergence). These results imply that buckwheat and Indian blanket can benefit *A. rufotestaceus* for managing the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae).

Chapter 3 presents results on the suitability of three flower treatments (buckwheat; a combination of fennel, *Foeniculum vulgare* (Mill.) and dill, *Anethum graveolens* (L.); and a combination of sunflower, *Helianthus annuus* (L.) and yarrow, *Achillea millefolium* (L.)) for enhancing biological control by parasitoids of lepidopteran pests in an organic broccoli production system. There were inconsistent significant differences among treatments, such as in percent parasitism of all lepidopteran pupae/plant in Athens in 2011; *Pieris rapae* (L) larval density in Tifton in 2011; percent parasitism of *P. rapae* pupae/plant in Athens in 2011; and *Plutella xylostella* (L.) larva density in Athens in 2011. These significant differences likely reflected random events rather than the effect of treatments, since they were unusual and inconsistent across locations and years. The results might have been confounded by the size of the plots and history of the land.

Chapter 4 presents results on suitability of three flower treatments (buckwheat; a combination of fennel and dill; and a combination of sunflower and yarrow) for enhancing predator abundance and predation of sentinel eggs of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), in organic broccoli and cucumber systems. There were few and inconsistent significant differences among treatments in number of sucking predators/plant in cucumber (only Athens in 2011) and

total numbers of predators/plant in cucumber (only Athens in 2010). Predation of beet armyworm eggs did not differ among treatments within locations and years. The few significant differences likely reflected random events rather than the effect of treatments, since they were unusual and inconsistent across locations and years. Plot size and interactions among predators and available prey in the systems may have masked any significant treatment effects.

Chapter 5 presents conclusions.

1.10 Conclusion

There is overwhelming evidence that floral resources can benefit natural enemies, with respect to enhancing their fitness (supported by the present study). But translating this into biological control and pest management has proven to be more difficult. This is mainly due to complex interactions among natural enemies, pests, and flowering plants in the field. The answer may lie in developing a farmscaping system that is unique to a cropping system and location rather than a system with broad applications. Despite the negligible impact of floral farmscaping in the present study, a future study that takes into account the area occupied by the insectary plants relative to the target crop and increases the distance between the plots, to eliminate any neutralizing effects, their proximity might yield results that separate treatment effects.

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Table 1.1 Effects of farmscape plants on biological control. + = positive effect, - = negative effect, and 0 – neutral effect.

| Citation | Flower treatment | Study | Findings | Effect |
|-----------------------------|--|---|---|--------|
| Gontijo et al. (2013) | Sweet alyssum, <i>Lobularia maritima</i> (L.) Desv. | Effect of sweet alyssum on woolly apple aphids, <i>Eriosoma lanigerum</i> (Hausmann), in apple orchard | Sweet alyssum planted close to apple trees infested with aphids attracted more syrphid flies and other predators that significantly suppressed aphid densities more than control (apple trees not close to sweet alyssum). | + |
| Thomson and Hoffmann (2013) | Woody vegetation comprising <i>Allocasuarina</i> spp., <i>Eucalyptus</i> spp., <i>Acacia</i> spp., grasses, flowering shrubs, and heath tea tree, bordering vineyard | Effect of woody vegetation on light brown apple moth, <i>Epiphyas postvittana</i> (Walker), in Australian vineyard | Predation and parasitism of sentinel <i>E. postvittana</i> eggs were significantly higher on grapevines nearer the wood vegetation than those farthest away. Predation and parasitism correlated with abundance of Coccinellidae and <i>Trichogramma</i> spp. | + |
| Woltz et al. (2012) | Buckwheat | Effect of buckwheat on soybean aphid, <i>Aphis glycines</i> (Matsumura), suppression in soybean fields. | Fields with buckwheat strips had a suppression of <i>A. glycines</i> comparable to those without buckwheat strips. | 0 |
| Wong and Frank (2012) | Black pearl pepper, <i>Capsicum annuum</i> (L.) 'Black Pearl', as a banker plant | Effect of <i>C. annuum</i> on augmented release of <i>Orius insidiosus</i> (Say) on predation of western flower thrips, <i>Frankliniella occidentalis</i> (Pergande) in nursery | Predation of western flower thrips was the same from augmentative release of <i>O. insidiosus</i> irrespective of the presence of banker plant as a pollen and nectar source. | 0 |
| Zumoffen et al. (2012) | Natural borders comprising flowering forbs | Effect of natural border vegetation on parasitism of aphids in alfalfa fields in Argentina. | There was significant parasitism of aphids in alfalfa fields with high proportions of natural borders compared to those with low proportions of natural borders | + |

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|------------------------|---|--|--|---|
| | | | (i.e., alfalfa field surrounded by another alfalfa field). | |
| Masetti et al. (2010) | Mixture of <i>Phacelia tanacetifolia</i> (Bentham), <i>Sinapis arvensis</i> (L.), <i>Borago officinalis</i> (L.), <i>Trifolium incarnatum</i> (L.), <i>T. alexandrinum</i> (L.), <i>T. pratense</i> (L.), <i>Vicia faba</i> (L.), and <i>V. sativa</i> (L.) | Effect of flowering plant mixture on parasitism of lettuce leafminers, <i>Liriomyza huidobrensis</i> (Blanchard) | Lettuce fields surrounded by flowering plants did not show significant reduction in <i>L. huidobrensis</i> compared to those surrounded by bare soil, overall. However, parasitism of <i>L. huidobrensis</i> in lettuce with flower treatment was higher in the first year of study. | 0 |
| Winkler et al. (2010) | Brown knapweed, <i>Centaurea jacea</i> (L.) | Effect of <i>C. jacea</i> on population of <i>Pieris rapae</i> (L.) in Brussels sprouts, <i>Brassica oleracea</i> (L.) plots | Brussels sprouts plots bordered by <i>C. jacea</i> had higher infestations of <i>P. rapae</i> than control. | - |
| Pfiffner et al. (2009) | Mixture of 24 wildflower species | Effect of wildflower strips on biological control of <i>Mamestra brassicae</i> (L.) and <i>P. rapae</i> (L.) in cabbage fields | Cabbage plots with wildflower strips did not show higher biological control (overall) compared to control (i.e., plots without wild flower strips). However, parasitism of <i>P. rapae</i> larvae and predation of <i>M. brassicae</i> eggs in one location were significantly enhanced in plots with wildflower strips. | 0 |
| Berndt et al. (2006) | Buckwheat | Effect of buckwheat on biological control of leafrollers, <i>Epiphyas postvittana</i> (Walker), <i>Ctenopseustis</i> spp., and <i>Planotortrix</i> spp. in vineyards in Marlborough, New Zealand | Presence of buckwheat significantly enhanced parasitism of leafrollers larvae in one vineyard but did not reduce the abundance of leafroller larvae significantly (overall). | 0 |
| Rebek et al. (2006) | <i>Trifolium repens</i> (L.); mixture of <i>Euphorbia epithymoides</i> (L.), <i>Coreopsis verticillata</i> (L.) var. 'Moonbeam,' and <i>Solidago</i> | Effect of the flower mix on biological control of euonymus scale, <i>Unaspis euonymi</i> (Comstock), a pest of <i>Euonymus fortunei</i> (Turcz.) var. | Flower plants surrounding <i>E. fortunei</i> plants did not lead to significantly higher parasitism of <i>U. euonymi</i> compared to control (i.e., <i>E. fortunei</i> not surrounded | 0 |

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| | <i>canadensis</i> (L.) var. 'Golden Baby.' | 'Coloratus' | by flower plants. | |
| Ellis et al. (2005) | Four flowering forbs; treasure-flower, <i>Gazania rigens</i> (L.); Shasta daisy <i>Leucanthemum</i> × <i>superbum</i> 'Alaska'; a compact aster, <i>Aster novi-belgii</i> 'Niobi,' 'Professor Kippenburg'; and a compact cultivar of goldenrod <i>Solidago canadensis</i> (L.) 'Golden Baby' | Effect of flowering forbs on parasitism of bagworm, <i>Thyridopteryx ephemeraeformis</i> (Haworth) on <i>Thuja occidentalis</i> (L.) by <i>Pimpla</i> (= <i>Coccygominus</i>) <i>disparis</i> (Vierick), <i>Itoplectis conquisitor</i> (Say), and <i>Gambrus ultimus</i> (Cresson), in urban landscapes | Parasitism of <i>T. Ephemeraeformis</i> was significantly enhanced on <i>T. occidentalis</i> surrounded by the forbs, compared to the control (i.e. <i>T. occidentalis</i> without forbs). | + |
| Lee and Heimpel (2005) | Buckwheat | Effect of buckwheat on parasitism of lepidopteran cabbage pests, <i>Trichoplusia ni</i> (Hübner), <i>P. rapae</i> , <i>P. xylostella</i> | Presence of buckwheat did not significantly reduce the egg, larval, and pupal densities of <i>T. ni</i> , <i>P. rapae</i> , and <i>P. xylostella</i> . However, buckwheat increased parasitism rates of <i>T. ni</i> larvae by <i>Voria ruralis</i> (Fallen) and <i>P. rapae</i> larvae by <i>Cotesia rubecula</i> (Marshall) over four years of study. | 0 |
| English-Loeb et al. (2003) | Cover crops, sod, <i>Dactylis glomerata</i> (L.), buckwheat, and ladino clover, <i>Trifolium repens</i> (L.) | Effect of cover crops on biological control of grape leafhoppers, <i>Erythroneura</i> spp. by <i>Anagrus</i> spp. in vineyards | Although buckwheat treatment significantly enhanced parasitism of <i>Erythroneura</i> spp. more than control, cover crops did not lead to significant reduction in <i>Erythroneura</i> spp. | 0 |
| Sengonca et al. (2002) | Weed species; wormwood, <i>Artemisia vulgaris</i> (L.), stinging nettle, <i>Urtica dioica</i> (L.), and tansy, <i>Tanacetum vulgare</i> (L.) | Effect of weed species on predators, <i>Coccinella septempunctata</i> (L.), <i>Adalia bipunctata</i> (L.), <i>Propylea quatuordecimpunctata</i> (L.), and | Presence of weeds significantly increased predator larva and adult densities and reduced aphids infestation rates in comparison to control (i.e. lettuce | + |

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|------------------------|--|--|--|---|
| | | <i>Chrysoperla carnea</i> (Steph.) and aphids on lettuce plants, <i>Lactuca sativa</i> (L.) | without weed species). | |
| Baggen and Gurr (1998) | Coriander and faba bean, <i>Vicia faba</i> (L.) | Effect of floral resources on the egg-larval parasitoid, <i>Copidosoma koehleri</i> (Blanchard) and potato moth, <i>Phthorimaea operculella</i> (Zeller) | Potato closer to floral resources were more heavily attacked by <i>P. operculella</i> . | - |
| Yan et al. (1997) | Mixture of cover crops alfalfa and <i>Lagopsis supina</i> (Steph.) | Effect of alfalfa/ <i>L. supina</i> cover crops on the control of two mites <i>Tetranychus ulmi</i> (Koch) and <i>T. vevnensis</i> (Zacher) in an apple orchards | The section of apple orchard managed with cover crops experienced mite below economic threshold and did not require any pesticide application unlike the section without the cover crop, which required pesticide application. | + |

CHAPTER 2
FLOWERING PLANT EFFECTS ON ADULTS OF THE STINK BUG PARASITOID
***ARIDELUS RUFOTESTACEUS* (HYMENOPTERA: BRACONIDAE) ¹**

¹ Aduba, O.L., D.M. Olson, J.R. Ruberson, P.G. Hartel and T.L. Potter. Submitted to *Biological Control*, 06/07/2013.

Abstract

Many parasitoids require food resources, such as nectar and pollen, besides hosts in order to optimize their life histories. This requirement has led to an interest in using these food resources in pest management. Here we assess the potential effects of two flowering plants, buckwheat (*Fagopyrum esculentum* Moench) and Indian blanket (*Gaillardia pulchella* Foug.), a 5% honey solution, and water (control) on the longevity and fecundity of *Aridelus rufotestaceus* (Tobias), an important parasitoid of the southern green stink bug, *Nezara viridula* (L.). *Gaillardia pulchella* and 5% honey solution significantly increased *A. rufotestaceus* fecundity compared to water ($P = 0.02$), with *G. pulchella* exhibiting the highest fecundity (138.0 ± 3.0 eggs), followed by 5% honey solution (134.0 ± 6.0 eggs), *F. esculentum* (123.0 ± 5.0 eggs), and water (109.0 ± 3.0 eggs). *Gaillardia pulchella*, *F. esculentum*, and 5% honey solution significantly increased longevity of *A. rufotestaceus* relative to water ($P < 0.00$), with *G. pulchella* yielding the highest longevity (11.0 ± 1.0 d), followed by 5% honey solution (10.0 ± 1.0 d), *F. esculentum* (9.0 ± 1.0 d), and water (4.0 ± 0.0 d). Body sugars (fructose, glucose, sucrose and maltose) of *Aridelus rufotestaceus* did not vary significantly among treatments after 24 h of parasitoid exposure to the treatments immediately after adult emergence. These results imply that *F. esculentum* and *G. pulchella* can benefit *A. rufotestaceus* for managing *N. viridula*.

Keywords

Non-host food; fecundity; longevity; nectar

2.1 Introduction

Many parasitoids require non-host food resources, in addition to hosts, in order to optimize their life histories. These non-host food sources include pollen, floral and extrafloral nectars, and honeydew (Lewis et al., 1998; Lavandero et al., 2005; Hogg et al., 2011b). These food resources serve as energy sources and may improve parasitoid fecundity, longevity, and efficacy in biological control (Lee and Heimpel, 2008). Because biological pest control has been associated with these plant-derived foods, there have been growing efforts to increase abundance and access to parasitoid food resources in modern agroecosystems, which are generally food-limited monocultures. Considering that food plants differ in traits such as nectar quality, quantity, and accessibility to parasitoids, it is important to screen plants to identify and utilize those that maximize these traits for the benefit of important natural enemies (Patt, 1997; Lundgren, 2009c), while minimizing benefits for intraguild enemies and pests.

The southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is a destructive pest of many agricultural crops, such as wheat, rice, cotton and soybean (Todd, 1989; McPherson and McPherson, 2000; Huang and Toews, 2012; Musolin, 2012). An important component of sustainable management of this pest is the action of its natural enemies. One natural enemy is the parasitoid *Aridelus rufotestaceus* (Tobias) (Hymenoptera: Braconidae: Euphorinae). *Aridelus rufotestaceus* is a thelytokous solitary endoparasitoid that parasitizes both nymphal and adult stages of *N. viridula*, but shows special preference for younger host stages (2nd and 3rd instars) (Shaw et al., 2001). The parasitoid was originally described from specimens collected near the Black

Sea in Georgia, but has been found in Italy (Shaw et al., 2001), the United States (Ruberson et al. 2010), and New Zealand (MAF Biosecurity New Zealand, 2010).

Although *A. rufotestaceus* parasitizes *N. viridula* in the field, the parasitism rate is low; Shaw et al. (2001) observed up to 21.7% parasitism, whereas Ruberson et al. (2010) found less than 5% parasitism of *N. viridula* collected in cotton and soybeans in Georgia, USA. The factors limiting parasitism are unknown, but availability of food resources in the cropping system may play a role.

One way to potentially enhance efficacy of *A. rufotestaceus* is by providing plant-derived food resources, such as nectar and pollen, through floral farmscaping in proximity to target crop systems. In order to design a good farmscape system to improve management of *N. viridula* with *A. rufotestaceus*, it is important to screen potential food plants and identify those that are accessible and have suitable nectar and/or pollen resources that enhance the parasitoid's life history.

In this study, we assessed the suitability of two floral plants, buckwheat (*Fagopyrum esculentum* Moench) and Indian blanket (*Gaillardia pulchella* Foug.), for enhancing the survivorship and fecundity of *A. rufotestaceus* relative to a 5% honey solution and water (control). We tested the following hypotheses:

- a) Flowering plants will enhance longevity and fecundity of *A. rufotestaceus* more than water alone, and
- b) Buckwheat will enhance longevity and fecundity of *A. rufotestaceus* more than other treatments because of its copious nectar production and relatively high accessibility.

2.2 Materials and methods

2.2.1 *Floral plants*

Buckwheat and Indian blanket were assessed for their suitability as non-host food sources for *A. rufotestaceus*. Buckwheat was chosen because of its abundant nectar production and its flower architecture that favors nectar accessibility, as well as its history of use in similar studies and in commercial production. Buckwheat has shallow corollae with wide apertures, which make its nectar easily accessible to many insects (Sim and Choi, 1999; Vattala et al., 2006). Although native to Asia, it is widely cultivated in many regions of the world, including the United States (Ohnishi, 1990). It starts flowering about one month after sowing and continues flowering for about 6 weeks (Li and Zhang, 2001; Quinet et al., 2004). Its seeds are inexpensive and can be purchased readily from most flower seed companies.

Indian blanket was selected because it is native to North America and has an extended flowering period. It is an annual flowering plant, although some can persist beyond one growing season, and it has the capacity to bloom all year round, depending on the climate (Hammond et al., 2007). It produces flowers with narrow and elongated corollae that can interfere with nectar access for foragers with short mouthparts (Mani and Saravanan, 1999). As a member of Compositae, its nectar production is relatively limited but it can be sustained for a long time (Mani and Saravanan, 1999; Hammond et al., 2007).

Organic buckwheat seed was procured from Johnny's Selected Seeds (<http://www.johnnyseeds.com>) with product ID: 966G.36. Organic Indian blanket seed

(variety SWF230) was obtained from Peaceful Valley Farm Supply (<http://www.groworganic.com>).

The two plant species were planted in organic germination mix, Fafard 20 (obtained from GROSouth; <http://www.grosouth.com/>), in a greenhouse located at the University of Georgia Entomology Department, Tifton Campus, under these conditions: $26 \pm 2^{\circ}\text{C}$, 14:10 (L:D) photoperiod, and $60 \pm 10\%$ RH. Buckwheat was sown every three weeks for the duration of the study to maintain a constant supply of flowers. Indian blanket was planted once and produced flowers throughout the experimental period. All plants were watered as needed, starting with once every two days and changing to once a day as the plants grew larger.

2.2.2 *Parasitoids*

Aridelus rufotestaceus were obtained from a culture maintained at the Entomology Department of the University of Georgia, Tifton Campus. The parasitoids had been in colony for two years (ca. 20 generations) and were reared on *N. viridula* nymphs maintained on shelled sunflower seeds and snap beans at $25 \pm 1^{\circ}\text{C}$ and L:D 14:10 after exposure to parasitoids. Newly emerged parasitoids were sexed and only females were used for the experiment (males were rare, less than 5% of emerging parasitoids).

2.2.3 *Longevity*

Survivorship of adult female *A. rufotestaceus* was determined with the following food treatments: (1) flowering buckwheat plant, (2) flowering Indian blanket plant, (3)

5% honey solution, and (4) water (control). The number of wasps used for the treatments were 15, 12, 16, and 16 for buckwheat, Indian blanket, 5% honey solution, and water respectively. Newly emerged female wasps were individually placed in transparent plastic cages (15.5 x 10.5 x 5.5 cm) with a hole cut in one side and sealed with a cloth screen to ensure ventilation and permit water and 5% honey solution replacement. Circular holes were also cut in the bottom of each cage to permit introduction of the flowering plants, with the gap around the stems of the flowering plants plugged with cotton batting to prevent the wasps from escaping. One flower head of Indian blanket was used per cage and a cluster of flowers of buckwheat was used per cage to ensure an abundant nectar supply. Water and a 5% honey solution were offered in microcentrifuge tubes with holes punctured in the lids and a cotton wick was introduced through the hole to ensure a constant supply of the fluids through capillary action. Water was offered in all the treatments in addition to the main treatments. The cages were held at 25 ± 1°C and 14:10 L:D, and wasps were observed twice daily until they died.

2.2.4 Fecundity

Fecundity of female *A. rufotestaceus* was assessed at emergence and five days after receiving the aforementioned four food treatments by counting the mature ova in dissected females. A total of 10 newly emerged females (≤ 24 h post-emergence) and

12, 15, 16, and 9 wasps fed on buckwheat, Indian blanket, 5% honey solution, and water treatments, respectively, for five days, under the same conditions as in the longevity experiment, were collected and immobilized on ice. The females were dissected in PBS (Phosphate Buffered Saline) solution to extract the ovaries. The extracted ovaries were slide mounted and all mature eggs in the ovaries counted at 40x magnification.

Before each female was dissected for the fecundity study, head width, right hind tibia length, and right forewing were measured with an ocular micrometer. These metrics were used as covariates to ensure that any observed differences were attributable to treatments rather than possible size differences in the parasitoids across treatments.

2.2.5 Sugar Analyses

To analyze the sugar contents of the wasps and treatments, the wasps were allowed to feed on the treatments for 24 h after emergence, whereas nectar from the flower treatments was obtained by rupturing the nectary gland and soaking up the nectar with a small section (4 cm²) of Kimwipe® tissue. Water and 5% honey solution samples were also obtained using Kimwipe® tissues. The fed wasps and Kimwipe® tissue containing the treatments were held in microcentrifuge tubes and placed in a freezer at -80°C until sample preparation.

Individual wasps (4 for each treatment) were dissected by cutting them just behind the prothorax so that only the abdomen with propodeum remained. The abdomen with propodeum was placed in 100µl of HPLC-grade water and ground with a plastic pestle. All fluid was removed with a pipette and placed in a vial.

The Kimwipe® tissues used to obtain the nectar from the flower, honey solution and the water as control were separately placed in 200µl of HPLC-grade water and left for 15 min. to dissolve the sugars. Subsequently, 100µl of the extracted fluid was removed with a pipette and placed into a vial.

Extracts from insects and food treatments were transferred to 2-mL glass autosampler vials and taken to dryness under a stream of N₂ gas. After addition of 40 µL of anhydrous pyridine and 200 µL of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % trimethylsilyl chloride (TMCS), vials were sealed with screw-caps fitted with Teflon faced septa, and heated at 70°C for 2 h. After cooling to room temperature, 300 µL of n-hexane and 2 µL of a 0.5 ug uL⁻¹ solution of phenanthrene-d₁₀ (P-d10) in hexane were added to each vial. The phenanthrene-d₁₀ was used as an internal standard. GC-MS analyses were performed on a ThermoQuest-Finnigan DSQII system (ThermoFisher Scientific, San Jose, CA). The GC column was a 30 m DB5MS® (Agilent, Santa Clara, CA, USA) with inner diameter, 0.25 mm, and film thickness, 0.25 µm. Helium carrier gas flow was fixed at 1.5 mL min⁻¹. Injections were in the splitless mode at 220°C with pressure surged to 250 kPa for 1 min after injection. Column over temperature at injection, 60°C, was held for 1 minute and then increased to 250°C at 10°C min⁻¹ and held for 10 minutes. Data acquisitions were in the selected ion monitoring mode. Ions monitored were m/z = 147, **204**, 217, 437 (fructose); 147, 91,

204, 217, (glucose); 217, **361**, 437 (sucrose); 191, **204**, 217, 361 (maltose), and 188 (phenanthrene-d₁₀). Ions in bold italics were used for quantitation. Confirmation criteria included retention time within ± 0.05 min, detection of all target ions, and the relative response ratio between the quantitation ion and the next most abundant ion within $\pm 20\%$ of analytical standards prepared in the same way as samples (Becker et al., 2013). All chemicals and standards were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2.6 Statistical Analyses

Fecundity and longevity of female *A. rufotestaceus* fed on buckwheat, Indian blanket, 5% honey solution, and water (control) were analyzed with generalized linear models (one-way ANOVA) (SAS, 2010). Fecundity and longevity data were square root-transformed to normalize the distribution and eliminate significance of replication. Fecundity data were regressed against head width, right hind tibia, and right forewing, and were analyzed for significant differences between treatments using generalized linear models (one-way ANOVA).

Sugars were not normally distributed and thus were analyzed using Kruskal–Wallis non parametric one-way analysis of variance (SAS, 2010).

3.0 Results

3.1 Longevity

At least one female *A. rufotestaceus* was observed feeding on each treatment used in this study. There was a significant food treatment effect on the longevity of female *A. rufotestaceus* ($F = 11.10$, $df = 3, 40$, $P < 0.00$). The wasps that fed on Indian

blanket, buckwheat, and 5% honey solution survived significantly longer than those that fed on water alone. Indian blanket yielded the numerically highest longevity (11.0 ± 1.0 d, $n = 12$), followed by 5% honey solution, buckwheat, and water (10.0 ± 1.0 , $n = 16$; 9.0 ± 1.0 , $n = 15$; and 4.0 ± 0.0 d, $n = 16$ respectively) (Fig. 2.1). Therefore, wasps that fed on Indian blanket, buckwheat, and 5% honey solution lived at least twice as long as those that fed on water alone.

3.2 Fecundity

After 5 d of feeding, the number of mature eggs (egg load) in the female wasps increased significantly from 80.0 ± 1.0 ($n = 10$) eggs at emergence to 109.0 ± 3.0 eggs ($n = 9$) (water), 123.0 ± 5.0 eggs ($n = 12$) (buckwheat), 134.0 ± 6.0 eggs ($n = 16$) (5% honey solution), and 138.0 ± 3.0 eggs ($n = 9$) (Indian blanket). Indian blanket and 5% honey solution significantly ($F = 3.91$, $df = 3, 33$, $P = 0.02$) increased the female wasp fecundity in comparison to those fed on water alone. Buckwheat resulted in an intermediate egg load that did not differ significantly from any of the other treatments after five days of feeding (Fig. 2.2).

3.3 Correlation of traits

Wing length ($F = 2.52$, $df = 3, 33$, $P = 0.076$), tibia length ($F = 0.74$, $df = 3, 33$, $P = 0.537$), and head width ($F = 0.51$, $df = 3, 33$, $P = 0.677$) did not differ significantly among treatments. However, there was a significant correlation between egg load and wing length for parasitoids in the buckwheat ($\alpha = 0.05$) and 5% honey solution ($\alpha = 0.01$) treatments. Hence, wing length explained 55% and 52% of the variability in the

number of mature eggs of the wasps fed on buckwheat and 5% honey solution, respectively, as well 51% of the variability in the number of mature eggs in newly emerged female wasps (Table 2.1).

3.4 Sugar Analyses

There were no significant differences among the treatments with respect to sugar content of the wasps after 24 h of exposure to the treatments (Table 2.2, fructose $\chi^2 = 2.60$, $df = 3$, $P = 0.46$; glucose $\chi^2 = 0.68$, $df = 3$, $P = 0.88$; sucrose $\chi^2 = 3.39$, $df = 3$, $P = 0.34$; and maltose $\chi^2 = 1.05$, $df = 3$, $P = 0.79$).

The five percent honey solution had significantly higher fructose ($\chi^2 = 7.53$, $df = 2$, $P = 0.02$), glucose ($\chi^2 = 7.57$, $df = 2$, $P = 0.02$), and maltose levels ($\chi^2 = 9.37$, $df = 2$, $P = 0.01$) than Indian blanket and buckwheat nectar, but similar levels of sucrose as buckwheat ($\chi^2 = 0.13$, $df = 1$, $P = 0.72$). Buckwheat nectar had significantly higher sucrose levels ($\chi^2 = 5.40$, $df = 1$, $P = 0.02$) than Indian blanket nectar. The glucose/fructose ratio did not differ significantly ($\chi^2 = 3.50$, $df = 2$, $P = 0.17$) among the treatments, whereas sucrose/hexose ratio differed significantly ($\chi^2 = 8.00$, $df = 2$, $P = 0.02$) among the treatments. Buckwheat nectar had a significantly higher sucrose/hexose ratio (2.10 ± 0.36) than the 5% honey solution (0.04 ± 0.00) and Indian blanket nectar (0.34 ± 0.21) and buckwheat nectar had comparable sucrose/hexose ratios.

4.0 Discussion

Female *A. rufotestaceus* provisioned with nectar from flowering plants (buckwheat and Indian blanket) or with 5% honey solution lived significantly longer than those with access to water only. This increased longevity with buckwheat and honey is consistent with findings in other wasps, such as *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) by Hopkinson et al. (2013), and *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) by Nafziger and Fadamiro (2011). The significant increase in longevity of the wasps with access to Indian blanket is interesting because this is the first time this plant has been shown to enhance survivorship of a natural enemy, although it has been used as part of commercial flower mixes to attract natural enemies (Braman et al., 2002). Despite production of considerable amounts of nectar by buckwheat (Sim and Choi, 1999), the Indian blanket treatment yielded numerically greater wasp longevity than buckwheat, although the difference was not statistically significant. This result implies that the sugar contents of the two flower species were qualitatively comparable for the wasps, and that the two plant species produced enough nectar to sustain the wasps equally.

Buckwheat nectar is “sucrose-dominant” (Vattala et al., 2006), while Indian blanket nectar is composed primarily of glucose. Sugar consumption may increase osmotic pressure in insects, with physiological consequences such as destabilization of water balance. This increase is more rapid with consumption of nectars dominated by monosaccharides such as glucose and fructose than disaccharide-dominant nectars (Baker and Baker, 1983; Vattala et al., 2006). However, the differing nectar sugar compositions of buckwheat and Indian blanket did not affect the wasps’ longevity in our

study, in agreement with the result obtained by Chen and Fadamiro (2006), in which the longevity of *Pseudacteon tricuspis* Borgmeier was similarly influenced by sucrose, fructose, and glucose intake. These results are the first time sugar contents of Indian blanket nectar have been reported and they appear to be similar to those of buckwheat, except in sucrose, where buckwheat was significantly higher.

Nectar quantity and accessibility did not matter for *A. rufotestaceus* with the flowers tested, as the wasps had comparable life spans despite buckwheat's considerable and easily accessible nectar (Sim and Choi, 1999; Vattala et al., 2006) compared to Indian blanket's flowers, which produce limited nectar and with more restricted access (Mani and Saravanan, 1999). However, *A. rufotestaceus* is a relatively large parasitoid, and may have experienced no difficulty in accessing nectar in Indian blanket flowers with its mouthparts.

The significant increase in the number of mature eggs by *A. rufotestaceus* from 80.0 ± 1.0 to at least 123.0 ± 5.0 with access to non-host food for five days indicates that the wasp is synovigenic, although females emerge with a large number of mature ova. Despite access to buckwheat nectar and pollen for five days by *A. rufotestaceus*, their egg load did not significantly differ from those that had access to water only. This result is inconsistent with results obtained for other parasitic wasps by Witting-Bissinger et al. (2008a) in which buckwheat significantly enhanced wasp fecundity relative to the water, although they evaluated realized fecundity over the lifetime of the wasps. Indian blanket, on the other hand, significantly increased *A. rufotestaceus* egg load in all of the wasps in comparison to the water, again highlighting its potential for use as a farmscaping plant.

Although wasp size metrics, such as wing length, tibia length, and head width, often correlate with longevity and fecundity, as was observed in the significant positive correlations between number of mature eggs and wing length in buckwheat and 5% honey treatments, the lack of significant differences in these metrics among the treatments indicate that the differences observed in longevity and fecundity are independent of parasitoid size.

Even though access to sugar significantly enhanced longevity and fecundity (Indian blanket and 5% honey solution only) of the wasps relative to water, the results did not correspond with observed differences in the sugar contents of the wasps, in which there were no significant differences in the treatments in any of the sugars (Table 2.2). The lack of significant differences in sugar contents of the wasps might be a result of a short feeding time (24 h) and/or the timing of the feeding assessment (very shortly after adult emergence). Wasps emerge with sugar reserves, as can be seen from the sugar contents of the wasps fed with water alone; therefore, there may not have been sufficient time post-emergence for the parasitoids to expend their pre-adult reserves, and to switch to reliance on adult foods. Therefore, we anticipate that allowing the wasps longer time to feed would yield significant differences in their body sugar.

The increase in longevity and fecundity of *A. rufotestaceus* when provisioned with carbohydrate-rich food sources can have important biological control implications in agroecosystems. The longer lifespan and higher number of eggs recorded with these food sources indicates that providing the wasps access to these resources can afford them longer time to access pests and more eggs with which to parasitize them, possibly resulting in greater pest suppression. Further, ready availability of carbohydrate

resources may retain the parasitoids more effectively in the area of targeted pest populations.

Indian blanket may be a potentially effective farmscaping plant for North American agricultural systems for several reasons. First, it is native to the region and exhibits a broad geographic range. Second, it exhibits prolonged flowering periods (we have observed flowering for 7-8 months in the field in southern Georgia). Third, in warmer climates, it can persist for two or more growing seasons. Fourth, its nectar quality was comparable to buckwheat for survival and fecundity of the parasitoid tested in the present study. However, the relatively deep corollae may present problems for smaller parasitoids to access the nectar, and additional studies of its relative effects on pest and other beneficial species are needed.

Although positive results were obtained with these plants in the enclosed system used in our study, where the wasps had no choice but to feed on what was provided to them, it is important to extend this study to the field where the effects of the plant species on the wasps can be evaluated under natural conditions before they are deployed as farmscaping plants for management of *N. viridula* or other pests. Under field conditions, the net benefit of these plants species for pest species can also be evaluated to ensure that the plants do not enhance pest risk.

Acknowledgments

We appreciate the identification of the parasitoid by Dr. Scott Shaw (University of Wyoming), and the support of the project by a USDA Southern Sustainable Agricultural Research and Education (SARE) award to J.R. Ruberson and P.G. Hartel. We thank Dr.

Michael Strand (University of Georgia) for helping with dissection of the wasps and Drs. Paul Guillebeau, Carl Jordan, and Michael Toews (University of Georgia) for their helpful comments on the manuscript. We appreciate Dr. Henry Fadamiro (Auburn University) for his suggestions on the experimental design.

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Table 2.1. Linear regression of egg numbers (dissected 5 d after emergence or upon emergence) against various traits in the food treatments. Traits measured in mm. For treatments, BW = buckwheat, IB = Indian blanket, HS = honey solution, and EM = emergence. For traits, Head = head width, Tibia = right metathoracic tibia length, Wing = wing length, Eggs = total eggs at dissection. R^2 = coefficient of determination, and SE = standard error. For P-values, * = significant at $\alpha = 0.05$ and ** = significant at $\alpha = 0.01$. Emergence = metrics of parasitoids within 24 h of adult emergence.

| Treatment | Trait | Mean \pm SE | R^2 | P | Equation |
|----------------|-------|-------------------|-------|--------|---------------------------------|
| Buckwheat | Head | 1.14 \pm 0.01 | 0.15 | 0.22 | Egg = -194.20 + (277.33 x Head) |
| | Tibia | 1.12 \pm 0.01 | 0.07 | 0.41 | Egg = -48.22 + (153.04 x Tibia) |
| | Wing | 3.24 \pm 0.04 | 0.55 | 0.05* | Egg = -234.00 + (110.16 x Wing) |
| | Eggs | 123.00 \pm 5.25 | | | |
| Indian Blanket | Head | 1.14 \pm 0.01 | 0.00 | 0.94 | Egg = 147.32 – (8.04 x Head) |
| | Tibia | 1.14 \pm 0.01 | 0.07 | 0.35 | Egg = 226.50 – (77.68 x Tibia) |
| | Wing | 3.20 \pm 0.03 | 0.07 | 0.36 | Egg = 229.04 – (28.39 x Wing) |
| | Eggs | 138.13 \pm 3.02 | | | |
| Honey | Head | 1.16 \pm 0.01 | 0.08 | 0.30 | Egg = -69.51 + (175.66 x Head) |
| | Tibia | 1.13 \pm 0.01 | 0.16 | 0.12 | Egg = -95.32 + (203.31 x Tibia) |
| | Wing | 3.11 \pm 0.04 | 0.52 | 0.00** | Egg = -200.82 + (107.45 x Wing) |
| | Eggs | 133.88 \pm 6.10 | | | |
| Water | Head | 1.15 \pm 0.01 | 0.22 | 0.21 | Egg = -43.41 + (131.77 x Head) |
| | Tibia | 1.13 \pm 0.01 | 0.00 | 0.98 | Egg = 106.00 + (2.35 x Tibia) |
| | Wing | 3.15 \pm 0.00 | 0.01 | 0.80 | Egg = 79.43 + (9.27 x Wing) |
| | Eggs | 108.67 \pm 3.45 | | | |
| Emergence | Head | 1.10 \pm 0.04 | 0.59 | 0.01** | Egg = 47.94 + (28.82 x Head) |
| | Tibia | 1.14 \pm 0.02 | 0.01 | 0.84 | Egg = 86.16 – (5.75 x Tibia) |
| | Wing | 3.13 \pm 0.06 | 0.51 | 0.02* | Egg = 27.44 + (16.68 x Wing) |
| | Eggs | 79.60 \pm 1.39 | | | |

Table 2.2. Mean \pm SEM sugar content ($\mu\text{g}/\text{insect}$) of wasps after 24 h of exposure to the treatments following emergence and mean \pm SEM sugar content of buckwheat and Indian blanket nectar and the 5% honey solution ($\mu\text{g}/100\mu\text{L}$). G/F = glucose:fructose ratio and S/H = sucrose: hexose (glucose+fructose) ratio. Differing letters across treatments indicate significant differences (Mann-Whitney U test, $P \leq 0.05$).

| Treatment | Sugar | | | | Sugar ratios | |
|-----------------------|---------------------------|---------------------------|--------------------------|-------------------------|-----------------|--------------------------|
| | Fructose | Glucose | Sucrose | Maltose | G/F | S/H |
| Parasitoid content | | | | | | |
| Buckwheat | 0.19 ± 0.11 | 8.46 ± 1.37 | 0.02 ± 0.01 | 0.05 ± 0.03 | NA | NA |
| Ind. Blanket | 0.23 ± 0.11 | 6.24 ± 2.42 | 0.14 ± 0.09 | 0.13 ± 0.08 | NA | NA |
| Honey | 0.29 ± 0.22 | 7.12 ± 3.45 | 0.06 ± 0.02 | 0.20 ± 0.15 | NA | NA |
| Water | 0.07 ± 0.03 | 5.84 ± 2.01 | 0.04 ± 0.01 | 0.08 ± 0.05 | NA | NA |
| Food resource content | | | | | | |
| Buckwheat | $3.11 \pm 1.30\text{b}$ | $3.76 \pm 0.83\text{b}$ | $13.14 \pm 4.31\text{a}$ | $0.01 \pm 0.00\text{b}$ | 2.23 ± 0.83 | $2.10 \pm 0.36\text{a}$ |
| Ind. Blanket | $2.96 \pm 2.33\text{b}$ | $5.03 \pm 2.78\text{b}$ | $0.83 \pm 0.23\text{b}$ | $0.03 \pm 0.01\text{b}$ | 8.34 ± 6.26 | $0.34 \pm 0.21\text{ab}$ |
| Honey | $163.8 \pm 57.86\text{a}$ | $158.8 \pm 40.18\text{a}$ | $13.17 \pm 2.95\text{a}$ | $4.59 \pm 1.27\text{a}$ | 1.19 ± 0.30 | $0.04 \pm 0.00\text{b}$ |
| Water | NA | NA | NA | NA | NA | NA |

Fig. 2.1. Mean \pm SEM longevity of *A. rufotestaceus* fed on different food treatments. Bars with different letters are significantly different (Ryan-Einot-Gabriel-Welsch Multiple Range, $P < 0.05$). Numbers above bars are numbers of individuals used for respective treatments.

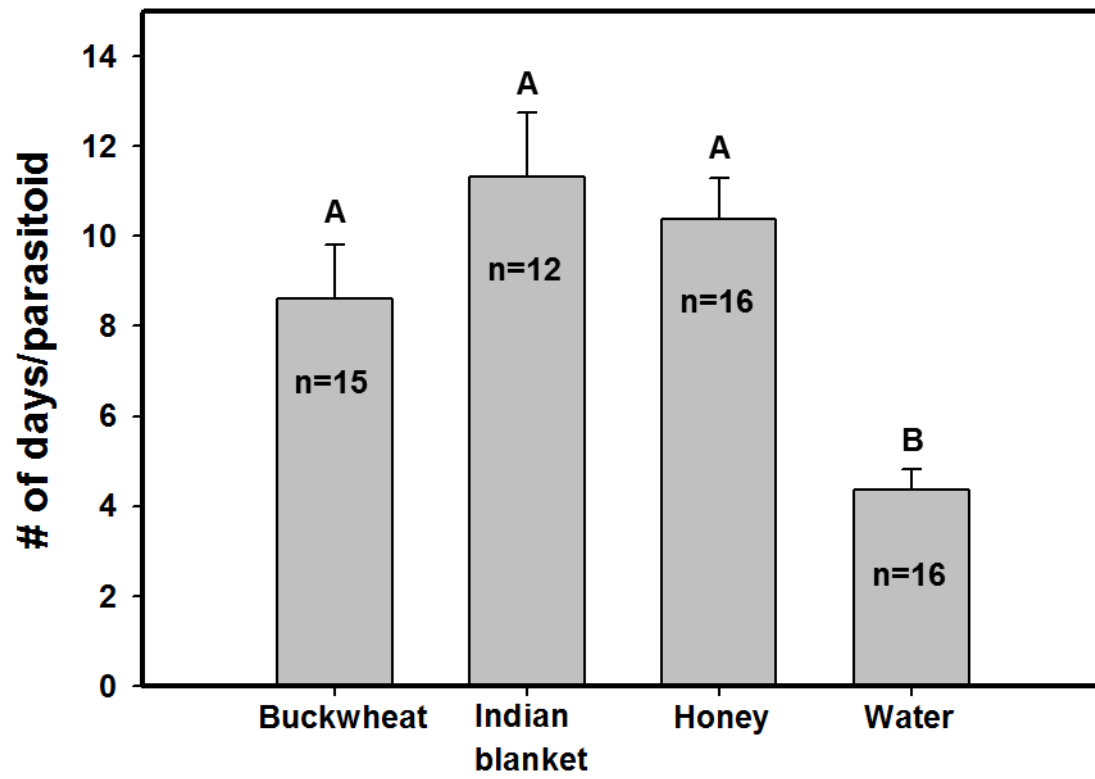
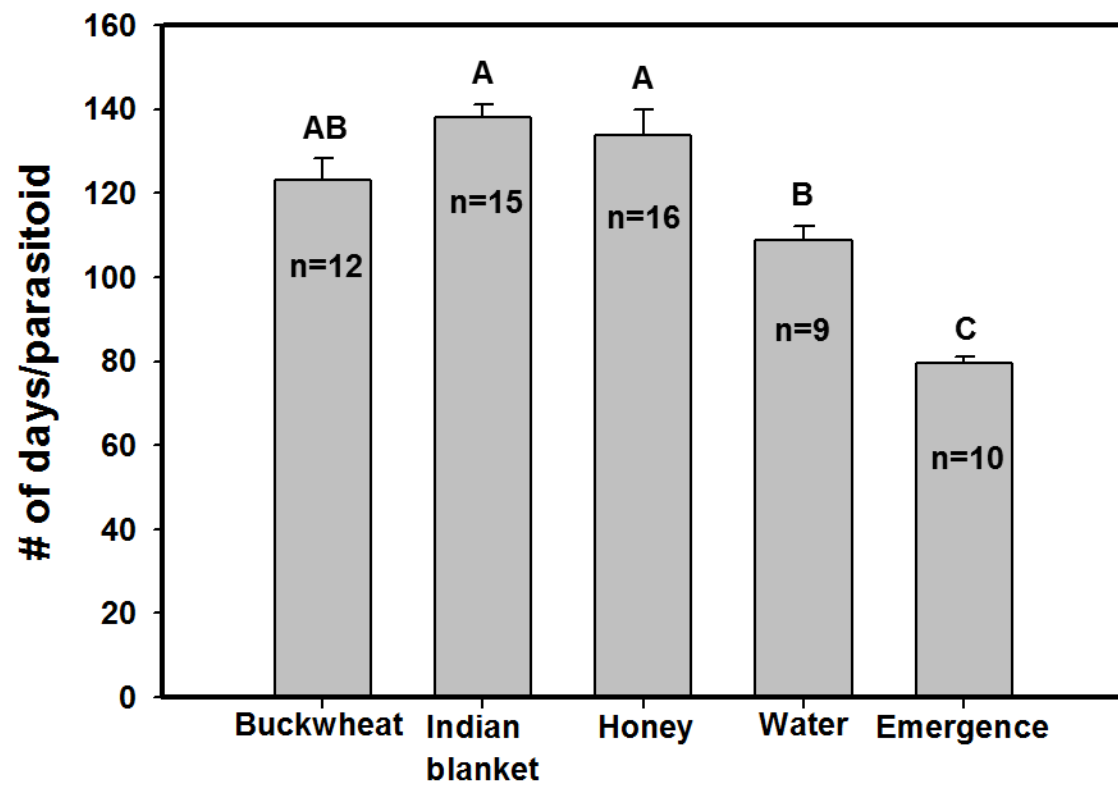


Fig. 2.2. Mean \pm SEM number of eggs per *A. rufotestaceus* at emergence and after five days of feeding on different food treatments. Bars with different letters are significantly different (Ryan-Einot-Gabriel-Welsch Multiple Range, $P < 0.05$). Numbers above bars are numbers of individuals dissected for the respective treatments.



CHAPTER 3
DOES FLORAL FARMSCAPING ENHANCE PARASITOID EFFICACY IN
BROCCOLI? ²

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Abstract

Non-host food resources, such as nectar and pollen, can be important for parasitoid life histories and may be provided in agroecosystems through floral farmscaping. These food resources provide energy and nutrients critical for parasitoid survival and reproduction. Therefore, flower resources can be used to enhance biological control efficacy of parasitoids in cropping systems. In the present study, we assessed the suitability of three flower treatments (buckwheat, *Fagopyrum esculentum* (Moench); a combination of fennel, *Foeniculum vulgare* (Mill.) and dill, *Anethum graveolens* (L.); and a combination of sunflower, *Helianthus annuus* (L.) and yarrow, *Achillea millefolium* (L.)) for enhancing biological control of lepidopteran pests in an organic broccoli production system over three years at two locations. Lepidopteran pest composition varied across years and locations with *Plutella xylostella* (L.), being the dominant pest in Athens in 2010 and Tifton in 2010 and 2011, while *Pieris rapae* (L.) was dominant in Athens in 2011 and Tifton in 2012. *Diadegma insulare* (Cresson) was the dominant parasitoid of *P. xylostella* in both locations and across the years of the study, while parasitism of *P. rapae* in Athens in 2011 and in Tifton in 2011 and 2012 was dominated by Tachinids, and by *Pteromalus puparum* (L.) in Tifton in 2010. There were inconsistent significant differences among treatments, such as in % parasitism of all lepidopteran pupae/plant in Athens in 2011; *P. rapae* larval density in Tifton in 2011; % parasitism of *P. rapae* pupae/plant in Athens in 2011; and *P. xylostella* larva density in Athens in 2011. These significant differences likely reflected random events rather than the effect of treatments, since they were unusual and inconsistent across locations

and years. Our results might have been confounded by the size of the plots and history of the land.

Keywords

Non-host food; biological control; parasitism.

3.1 Introduction

Non-host food resources can be very important for parasitoid life histories (Lundgren, 2009c; G  neau et al., 2012). Most parasitoids are synovigenic and must feed on host and/or non-host materials as adults to achieve maximal survival and egg production (Jervis et al., 2001; Olson et al., 2005). Therefore, for parasitoids requiring carbohydrates, availability of non-host food resources is critical for survival and optimal reproduction. These non-host foods include plant-derived foods such as floral and extra-floral nectar, and pollen, as well as honeydew (Baggen et al., 1999b; Landis et al., 2000b; W  ckers, 2005b; Pontin et al., 2006). Plant-derived foods have been reported to improve parasitoids' longevity, fecundity, and biological control efficacy, and scarcity of these food resources in modern monoculture agroecosystems has been implicated in the reduced natural pest control observed in these systems (Lee and Heimpel, 2005; Crowder et al., 2010; D  az et al., 2012b; G  neau et al., 2012). As a result, there are growing efforts to increase the availability of these food resources to natural enemies for pest management. One way to increase the availability of these food resources is through floral farmscaping, which is the planting of target crops in association with floral plants to provide resources that enhance the populations and function of natural enemies for pest control.

Although the concept of floral farmscaping, supported by the enemies hypothesis expounded by Root (1973), theoretically presumes pest suppression is increased by natural enemies in the presence of flowering plants, efforts to demonstrate this in the field have so far yielded conflicting results. For example, while parasitism of pests was enhanced by providing parasitoids access to flowering plants in some studies (Masetti

et al., 2010; Zhu et al., 2013), in other studies availability of floral resources to parasitoids did not translate into increased parasitism (Berndt et al., 2002; Brown et al., 2010). This discrepancy in results highlights the current unpredictability of the outcomes of the multi-trophic interactions among plants, pests, and parasitoids, and the gaps in our knowledge of how the concept can be utilized for pest management. Importantly, in implementing floral farmscaping for pest management, Gurr et al. (2005) pointed out the need for a “directed” rather than “shotgun” approach, since flowering plants differ in characteristics, such as food quality, quantity, and accessibility to natural enemies, as well as in their attraction for various pest species (Lundgren, 2009c; Wäckers and van Rijn, 2012). Therefore, this makes it imperative to screen potential flower plants to identify and work with those that attract and support desired natural enemies while excluding those that might compromise the intended goal of pest suppression (Carrié et al., 2012).

Broccoli, *Brassica oleracea* (L.) (Brassicales: Brassicaceae), is cultivated and eaten in many parts of the world, mainly because of its health benefits and ease of cooking and preparation (Jeffery and Araya, 2009; Walley et al., 2012). It is attacked by a variety of pests, including the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and the imported cabbageworm, *Pieris rapae* (L.) (Lepidoptera: Pieridae), both of which are indirect pests that can cause significant yield reductions. Developing a sustainable management system for these pests is important, especially for the diamondback moth, which has a strong tendency to quickly develop resistance to pesticides (Talekar and Shelton, 1993) and can be a devastating pest of many

Brassicas. This will particularly benefit organic broccoli producers, who may experience high pest pressure and have few options for pest management.

The objective of this work was to investigate the suitability of three floral farmscape treatments for enhancing biological control to manage lepidopteran pests in an organic broccoli production system. The study tested two hypotheses: 1) the presence of flowers will lower pest density and enhance their parasitism, and 2) buckwheat will lower pest density and enhance their parasitism more than other flowers tested because of its copious nectar production.

3.2 Materials and Methods

3.2.1 Floral plants

The treatments used were: 1) broccoli (control), 2) buckwheat, *Fagopyrum esculentum* (Moench) (Caryophyllales: Polygonaceae), 3) a combination of fennel, *Foeniculum vulgare* (Mill.) (Apiales: Apiaceae) and dill, *Anethum graveolens* (L.) (Apiales: Apiaceae), and 4) a combination of sunflower, *Helianthus annuus* (L.) (Asterales: Asteraceae) and yarrow, *Achillea millefolium* (L.) (Asterales: Asteraceae). All of these flowering plants were selected because they are either native to North America (in the case of sunflower) or cultivated for food and industrial uses, as well as attractants for natural enemies in cropping systems.

Sunflower is grown mainly for its seeds, which are a source of oil for food, feed for animals, and other industrial raw materials (Lu and Hoefft, 2009; Fernández-Martínez et al., 2010) and is known to attract a diverse array of insects, including natural enemies (Jones and Gillett, 2005; Adedipe and Park, 2010). It is an annual plant that produces

capitulae of 2 to 30 cm in diameter, depending on the variety (Cronn et al., 1997; Fambrini et al., 2007). The capitulum bears two kinds of flowers: ray and disc florets. Ray florets sometimes have nectaries that are usually smaller than those of disc florets and, therefore, produce less nectar than disc florets. Average disc floret corolla length ranges from 7.23 to 10.22 mm, while the mean nectar production per floret per day ranges from 0.24 to 0.38 μ L (Hadisoesilo and Furgala, 1986; Atlagić et al., 2003; Wist and Davis, 2006).

Yarrow is native to Western Asia and Europe, but grows in most temperate regions, including the United States. It is mainly cultivated for medicinal uses and it is becoming popular as an attractant for natural enemies (Applequist and Moerman, 2011; Dib et al., 2012). It is perennial and as a member of Asteraceae produces a flower head about 2 to 4 mm wide comprising about five to six ray florets and 10 to 30 disk florets, with corollae 2.2 to 3 mm long (Warwick and Black, 1982; Zhang et al., 1996; Sulborska and Weryszko-Chmielewska, 2006).

Dill is an annual flowering plant that is native to the Mediterranean and used mainly as a spice and medicine (Carrubba et al., 2008; Sokhangoy et al., 2012; Tian et al., 2012). It is also being used as a companion plant in cropping systems (Winkler et al., 2010). It produces flowers with no corolla depth, with average aperture size of 2.63 mm and easily accessible nectar (Winkler et al., 2009).

Fennel is a perennial flowering herb that is native to the Mediterranean region and cultivated for spice and medicinal use (Gross et al., 2008). It produces flowers with no corolla depth and easily accessible nectar (Winkler et al., 2009).

Buckwheat is native to Asia and is widely cultivated in many regions of the world, including the United States, for food and habitat management (Ohnishi, 1990; Li and Zhang, 2001; Wijngaard and Arendt, 2006; Lee and Heimpel, 2008). It has shallow corollae with wide apertures, which make its nectar easily accessible to many insects (Sim and Choi, 1999; Vattala et al., 2006).

3.2.2 *Experimental design*

The experiment was conducted in two locations: Athens (The *University of Georgia's Athens Horticulture Research Farm*) and Tifton (The *University of Georgia's Tifton Horticulture Research Farm*), Georgia, from 2010 to 2012. The study was conducted in spring 2010 and 2011 in Athens and spring 2010, 2011, and 2012 in Tifton. The land used in Athens was in transition to organic certification, while that used in Tifton was organically certified and had been in organic production for two years before the start of the experiment. The broccoli (var. Windsor F1), dill (var. Bouquet), fennel (var. Bronze), and buckwheat seeds used in the study were organic and were purchased from Johnny's Selected Seeds (<http://www.johnnyseeds.com/default.aspx>), while sunflower (var. Sunbright F1) was not organic and was obtained from the same vendor. Yarrow (White) was not organic and was obtained from Peaceful Valley Farm & Garden Supply (<http://www.groworganic.com>)

The year prior to spring planting, the land in Athens and Tifton was planted with cover crops [Athens: Austrian winter peas, *Pisum sativum* (L.) (Fabales: Fabaceae) and oat, *Avena sativa* (L.) (Poales: Poaceae) planted in late fall; Tifton: sunn hemp, *Crotalaria juncea* (L.) (Fabales: Fabaceae), planted in early summer]. The flower plants

were started in the greenhouse between January and February of each planting season to make sure that the flowers were at the flowering stage at the time of transplanting. Flowers were also replanted every three to four weeks to ensure that there were flowering plants in the field during sampling. Broccoli for both locations was started in the greenhouse between January and February by planting in Fafard germination mix in multi-celled trays with circular cells of 7.62 cm diameter. In Athens, broccoli (15-20 cm tall) and flower seedlings were transplanted to the field between 1 and 30 April in 2010 and between 4 and 20 April in 2011. In Tifton, broccoli (15-20 cm tall) and flower seedlings were transplanted on 22 and 23 March in 2010, 14 and 15 March in 2011, and 14 and 15 March in 2012.

3.2.3 *Field Layout*

In each location and each year, broccoli was transplanted into 16 blocks (4 replicates of 4 treatments) measuring 12x12 meters (144 m²) and separated from one another by 3m border of bare soil on all sides of the plot. Each block contained 6 twin-row beds, each measuring 12x1.2 meters. Broccoli was planted in Athens and Tifton at a spacing of 0.46 m between rows and 0.46 m between plants within rows. A treatment plot measuring 2x2 meters was established in the center of each block in which the respective treatments were placed, with the flower plants planted on the adjacent halves of the third and fourth beds. The treatments with two flowers - dill/fennel and yarrow/sunflower - were planted in such a way that one flower species was planted on one half of one bed and the other flower on the other half of the other bed, and alternated across the blocks to avoid bias. Forty flowering plants were planted in each

treatment plot, 20 plants on each half of the bed, in Athens. Forty eight flowering plants were planted in each treatment plot, 24 plants on each half of the bed, in Tifton. The flowers were close to flowering when transplanted and were transplanted immediately after broccoli.

The treatment plots were laid out in a randomized complete block design in Athens (because of land constraint) and Latin square design in Tifton. The plots were fertilized in Athens with feathermeal (11-14% N) at the rate of 3 g/ planting hole and in Tifton pelletized poultry litter was applied at a rate of 1467 kg/ha (3% N for 44 kg/ha of N) to the field in mid-February of each year. Water was supplied with drip irrigation as needed at both locations. Weed control was done by tillage and hand-pulling in 2010, and with tillage and black plastic mulch (0.25 ml) covering the beds in 2011 and 2012 at both locations.

3.2.4 Data collection and analyses

After transplanting the plants (broccoli and flowers), they were allowed about one to two weeks to establish before sampling began. In each location, broccoli plants were sampled for lepidopteran larvae and pupae at the center of the block next to the flowering plants, and 4 meters away from the center in the four cardinal directions, resulting in five sampling positions (east, west, north, south, and center) per block; i.e., five plants per block and 80 plants in each location. The plants were sampled once a week between 8:00 am and 12:00 pm in Athens 2010 (May 22 to June 8), Athens 2011 (April 26 to June 23), Tifton 2010 (April 20 to May 25), Tifton 2011 (April 13 to June 7), and Tifton 2012 (March 29 to May 24). Each plant was sampled by examining the

leaves, stem, and broccoli head and collecting all the lepidopteran larvae and pupae on it. Larvae and pupae collected from each plant were individually put in a diet cup (35 cm²) and feeding stages were provided fresh broccoli leaves until pupation. All specimens were held in the laboratory at $26 \pm 2^{\circ}\text{C}$ and RH of $60 \pm 10\%$ until either the lepidopteran adult or parasitoids emerged. Dominant parasitoids were identified to species level and the remainder to family.

Data on hosts/plant, parasitized hosts/plant, and % parasitism of hosts/plant were collected for total lepidopteran hosts (larvae and pupae), *Pieris rapae* (larvae and pupae), and *Plutella xylostella* (larvae and pupae) for each location and each year, and analyzed using generalized linear mixed models (repeated measures two-way ANOVA), with block as a random effect (SAS, 2010). Percentage parasitism was transformed using $\arcsin\sqrt{\%}$ before analysis with generalized linear mixed models (repeated measures two-way ANOVA).

3.3 Results

Lepidopteran hosts (larvae and pupae) obtained in our samples included *Plutella xylostella*, *Pieris rapae*, *Trichoplusia ni* (Hübner), *Spodoptera exigua* (Hübner), and *Spodoptera eridania* (Cramer). Table 3.1 presents all observed lepidopterans (larvae and pupae), while Tables 3.2 and 3.3 focus on *P. rapae* and *P. xylostella*, respectively, because they were the most abundant hosts obtained. In Tifton, *P. rapae* constituted approximately 3, 12, and 84% of all the lepidopteran hosts obtained in 2010, 2011 and 2012, respectively, while *P. xylostella* constituted 93, 80, and 5% in 2010, 2011, and 2012, respectively (Fig. 3.1). In Athens, *P. rapae* constituted approximately 31 and 53%

of all lepidopteran hosts in 2010 and 2011, respectively, while *P. xylostella* constituted 62 and 33% in 2010 and 2011, respectively (Fig. 3.1). Therefore, the remainder of this work will focus on *P. xylostella* and *P. rapae*.

The parasitoids obtained in our samples included the ichneumonid *Diadegma insulare* (Cresson) and the braconid *Microplitis plutellae* (Muesbeck), both of which are parasitoids of *P. xylostella*; and the Pteromalidae *Pteromalus puparum* (L.) and Tachinidae flies, both of which were collected only from *P. rapae*. Additionally, some specimens of the family Chalcididae were reared from both lepidopteran species, with apparently different parasitoid species attacking the two lepidopterans. In Athens, *D. insulare*, *M. plutellae*, and Chalcididae contributed 71, 22, and 7% respectively to parasitism of *P. xylostella* in 2010, while in 2011 they contributed 60, 20 and 20% respectively (Fig 3.2a). In Tifton, *D. insulare* and *M. plutellae* accounted for 70 and 30%, respectively, of parasitism of *P. xylostella* in 2010, and in 2011, *D. insulare*, *M. plutellae*, and Chalcididae accounted for 92, 5, and 3%, respectively, of parasitism of *P. xylostella*. In contrast, in 2012, *D. insulare* accounted for 100% of *P. xylostella* parasitism (Fig. 3.2a).

No parasitoids were recovered from *P. rapae* in Athens in 2010, while in 2011, Tachinidae, Chalcididae, and *P. puparum* accounted for 68, 13, and 19% of the parasitism (Fig. 3.2b). In Tifton, *P. puparum* and Tachinidae were respectively responsible for 100% of *P. rapae* parasitism in 2010 and 2011, while in 2012, Tachinidae, Chalcididae, and *P. puparum* accounted for 77, 12, and 10%, respectively, of *P. rapae* parasitism (Fig. 3.2b).

There were no significant differences among the treatments in any of the variables for total lepidopteran hosts (pupae and larvae) (Table 3.4), except in % parasitism of pupae/plant in Athens in 2011 ($F = 4.46$, $df = 3, 9$, $P = 0.04$), in which the dill/fennel combination treatment yielded the highest % parasitism of pupae/plant (52.10 ± 16.50 %, $n = 8$) and differed significantly from the other treatments: sunflower and yarrow combination (17.50 ± 8.10 %, $n = 14$), buckwheat (15.60 ± 11.40 %, $n = 9$), and broccoli (2.50 ± 2.50 %, $n = 10$) (Table 3.1).

For *Pieris rapae* (Table 3.5) there were significant differences among treatments in larvae/plant in Tifton 2011 ($F = 4.15$, $df = 3, 9$, $P = 0.04$) and % parasitism of pupae/plant in Athens 2011 ($F = 4.43$, $df = 3, 9$, $P = 0.04$). Broccoli treatment yielded significantly more larvae/plant (0.40 ± 0.10 , $n = 12$) than buckwheat (0.30 ± 0.00 , $n = 12$), dill/fennel (0.20 ± 0.00 , $n = 10$), and yarrow/sunflower combinations (0.30 ± 0.00 , $n = 9$) in Tifton in 2011 (Table 3.2). The dill/fennel treatment yielded significantly higher % parasitism of pupae/plant (52.10 ± 16.50 , $n = 8$) than broccoli (2.50 ± 2.50 , $n = 10$), buckwheat (15.60 ± 11.40 , $n = 9$), and sunflower/yarrow combination (18.90 ± 8.60 , $n = 13$) in Athens in 2011 (Table 3.2).

For *Plutella xylostella* (Table 3.6), there were significant differences among the treatments only in larval density in Athens in 2011 ($F = 5.54$, $df = 3, 9$, $P = 0.02$), with the sunflower/yarrow treatment (0.50 ± 0.10 , $n = 19$) having significantly greater larval density than broccoli (0.30 ± 0.00 , $n = 14$), buckwheat (0.40 ± 0.10 , $n = 16$), and dill/ fennel combination (0.40 ± 0.10 , $n = 19$) (Table 3.3).

3.4 Discussion

There were very few significant differences among treatments for any of the assessed variables. Given that the experiment was replicated five times in space and time, this finding suggests that, with this experimental design, the flowering plots had little or no impact on pest populations, or on parasitoid activity.

The lepidopteran pest composition in the studied system varied considerably across years and locations, highlighting the considerable challenges of spatiotemporal variability in the pest complexes that are presented to pest managers, and which must be considered in developing effective farmscaping systems. For example, in 2010 in Athens and Tifton, and in 2011 in Tifton, *P. xylostella* was the most abundant lepidopteran pest, while in Athens in 2011 and Tifton in 2012, *P. rapae* was the most abundant lepidopteran pest (Fig. 3.1). This variation also underscores the need for spatiotemporal diversity in assessing farmscaping systems to ensure the technology is appropriate for local conditions.

Diadegma insulare was the dominant parasitoid of *P. xylostella* in both locations and across the years of the study (Fig. 3.2a). Correspondingly, *D. insulare* is reported to be the main parasitoid of *P. xylostella* in the United States (Mitchell et al., 1997; Xu et al., 2001; Shelton et al., 2002). *Microplitis plutellae* was also relatively important at both locations, but occurred later in the season than *D. insulare*.

Parasitism of *P. rapae* in Athens in 2011 and in Tifton in 2011 and 2012 was dominated by Tachinidae; however, *P. puparum* was also important in Athens in 2011 and in Tifton in 2012 (Fig. 3.2b), when *P. rapae* was the most numerous herbivore in the system. Wold-Burkness et al. (2005) reported *P. puparum* as the most dominant

parasitoid of *P. rapae* in cabbage, in Minnesota, but the generalist *Compsilura concinnata* (Meigen) (Diptera: Tachinidae) was also important.

Significant differences occurred sporadically among treatments: for percent parasitism of all lepidopteran pupae/plant in Athens in 2011; *P. rapae* larval density in Tifton in 2011; percent parasitism of *P. rapae* pupa/plant (Table 3.2) in Athens 2011; and *P. xylostella* larva density (Table 3.3) in Athens 2011. Despite the occasional significant differences, the lack of consistency or pattern in the differences strongly suggests that they were random and did not reflect effects of the experimental treatments. Lee and Heimpel (2005) reported no significant differences in larval and pupal densities of *P. rapae* and *P. xylostella* in cabbage with buckwheat as a food source for natural enemies. They also found that overall parasitism of *P. rapae* by Tachinids and *P. puparum*, and of *P. xylostella* by *D. insulare* was not significantly influenced by buckwheat, similar to the present observations.

Various factors could have contributed to the general lack of significant differences in the variables and inconsistencies in those variables that showed significant differences, with respect to the treatments:

- (1) The land used in the study in Athens was in transition to organic production and that of Tifton had been in organic production for about two years before it was used for the study, and thus, the residual effects of conventional management practices that were used in the land could have had significant influence on the parasitoids' performance. This history should not exert differential effects across treatments within locations, but there may have

been some variation in plot history that may have contributed to the observed differences.

- (2) The size of the plots (12X12 m) and distance between the plots (3 m) was possibly too small to yield differences. Unfortunately, larger suitable parcels of land for the project were not available. Given the scale, it seems likely that the pests and at least some of the parasitoids were able to move readily among plots, thereby neutralizing any significant effects that the various treatments might have had. Tachinids are known to have long flight ranges, up to 200 m (Romina et al., 2011; Pfannenstiel et al., 2012). Another important factor may have been the size of the floral plot relative to the total area planted. In the present study the treatment plot occupied 2.8% of the total area and could have been too small to be effective. But increasing the size of the treatment plot risks consuming area that could be devoted to the crop and can add difficulties to management practices (such as tillage), and reduces the acceptability of such an integrated flower system for growers.
- (3) The flowers that we used might have not been appropriate for the system and, therefore, not as effective at attracting parasitoids of the pests in the broccoli system. Flowers are known to vary in their attraction to parasitoids (Sivinski et al., 2011; Zhu et al., 2013) . It is also possible that the flowers attracted other insects, such as bees and hoverflies that could have competitively diminished the resources that were available to the parasitoids and thereby supporting fewer parasitoid populations. Even *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), a pest was observed

feeding on floral resources of buckwheat, in present study. Possible competition for floral resources among flower visitors has been documented (Ambrosino et al., 2006; Hogg et al., 2011a; Campbell et al., 2012)

Considering these factors we cannot conclude with certainty that these flower treatments were not effective in the system. But for future studies on this system, we would recommend using a larger area for the crop and the treatment plot as well as increasing the distance between blocks to prevent the treatments from neutralizing the effects of one another. Further detailed analyses of the effects of the flowers used on the life histories of key parasitoids and pests in the system would be valuable. Understanding the key parasitoids in the system for respective pest species and the benefits of particular flower species for those parasitoids creates opportunities for developing appropriate flower blends to match the physical, nutritional, and phenological demands of the natural enemies.

Acknowledgments

We appreciate the support of the project by a USDA Southern Sustainable Agricultural Research and Education (SARE) award to J.R. Ruberson and P.G. Hartel. We thank Drs. Paul Guillebeau, Carl Jordan, and Michael Toews (University of Georgia) for their helpful comments on the manuscript. We appreciate Mr. Jerry Davis (University of Georgia) for helping with statistical analyses.

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Table 3.1. Total lepidopteran larval and pupal density (all species pooled), parasitized host density, and % parasitism of hosts/plant (seasonal means) by host, year, location, and treatment. For treatment, BR = broccoli, BW = buckwheat, D/F = dill and fennel combination, S/Y = sunflower and yarrow combination, and SE = standard error. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Host | Year | Location | Treatment | Host/plant (mean±SE) | Parasitized host/plant (mean±SE) | % parasitism of host/plant (mean±SE) |
|--------------|------|----------|-----------|-------------------------|-------------------------------------|---|
| <i>Larva</i> | 2010 | Athens | BR | 1.20 ± 0.10 | 0.10 ± 0.00 | 10.90 ± 2.40 |
| | | | BW | 1.20 ± 0.20 | 0.30 ± 0.10 | 31.70 ± 10.80 |
| | | | D/F | 1.60 ± 0.30 | 0.20 ± 0.10 | 10.80 ± 5.40 |
| | | | S/Y | 1.50 ± 0.20 | 0.30 ± 0.10 | 18.70 ± 4.20 |
| | | Tifton | BR | 2.60 ± 0.40 | 1.50 ± 0.2 | 52.20 ± 3.80 |
| | | | BW | 2.50 ± 0.20 | 1.50 ± 0.20 | 58.80 ± 3.80 |
| | | | D/F | 2.30 ± 0.20 | 1.30 ± 0.20 | 56.50 ± 4.30 |
| | | | S/Y | 1.80 ± 0.20 | 1.10 ± 0.20 | 56.70 ± 4.00 |
| | 2011 | Athens | BR | 1.40 ± 0.30 | 0.10 ± 0.00 | 14.00 ± 5.70 |
| | | | BW | 1.10 ± 0.20 | 0.10 ± 0.00 | 16.10 ± 5.60 |
| | | | D/F | 1.20 ± 0.20 | 0.10 ± 0.00 | 10.60 ± 4.50 |
| | | | S/Y | 1.10 ± 0.20 | 0.10 ± 0.00 | 11.00 ± 3.20 |
| | | Tifton | BR | 1.30 ± 0.200 | 0.70 ± 0.10 | 48.10 ± 6.40 |
| | | | BW | 1.10 ± 0.20 | 0.60 ± 0.10 | 43.00 ± 5.90 |
| | | | D/F | 1.00 ± 0.20 | 0.50 ± 0.10 | 51.90 ± 6.20 |
| | | | S/Y | 0.70 ± 0.10 | 0.40 ± 0.10 | 44.40 ± 7.00 |
| | 2012 | Tifton | BR | 5.50 ± 0.80 | 1.20 ± 0.30 | 17.40 ± 3.10 |
| | | | BW | 5.80 ± 0.80 | 1.30 ± 0.30 | 18.20 ± 2.60 |
| | | | D/F | 5.70 ± 0.90 | 1.30 ± 0.30 | 14.70 ± 2.10 |
| | | | S/Y | 6.10 ± 0.80 | 1.30 ± 0.30 | 14.60 ± 2.20 |

Table 3.1. (cont.)

| Host | Year | Location | Treatment | Host/plant (mean±SE) | Parasitized host/plant (mean±SE) | % parasitism of host/plant (mean±SE) |
|-------------|------|----------|-----------|-------------------------|-------------------------------------|---|
| <i>Pupa</i> | 2010 | Athens | BR | NA | NA | NA |
| | | | BW | NA | NA | NA |
| | | | D/F | NA | NA | NA |
| | | | S/Y | NA | NA | NA |
| | | Tifton | BR | 0.70 ± 0.10 | 0.50 ± 0.10 | 72.60 ± 5.90 |
| | | | BW | 0.70 ± 0.10 | 0.60 ± 0.10 | 79.90 ± 4.80 |
| | | | D/F | 0.70 ± 0.10 | 0.60 ± 0.10 | 83.30 ± 5.40 |
| | | | S/Y | 0.80 ± 0.10 | 0.50 ± 0.10 | 62.90 ± 6.40 |
| | 2011 | Athens | BR | 0.50 ± 0.10 | 0.00 ± 0.00 | 2.50 ± 2.50b |
| | | | BW | 0.60 ± 0.10 | 0.10 ± 0.00 | 15.60 ± 11.40b |
| | | | D/F | 0.50 ± 0.10 | 0.20 ± 0.10 | 52.10 ± 16.50a |
| | | | S/Y | 0.50 ± 0.10 | 0.10 ± 0.10 | 17.50 ± 8.10b |
| | | Tifton | BR | 0.40 ± 0.10 | 0.20 ± 0.00 | 38.10 ± 10.30 |
| | | | BW | 0.30 ± 0.10 | 0.10 ± 0.00 | 30.20 ± 10.40 |
| | | | D/F | 0.30 ± 0.10 | 0.10 ± 0.00 | 34.60 ± 12.00 |
| | | | S/Y | 0.50 ± 0.10 | 0.20 ± 0.10 | 25.60 ± 9.60 |
| | 2012 | Tifton | BR | 3.00 ± 0.40 | 2.00 ± 0.40 | 53.40 ± 6.20 |
| | | | BW | 2.80 ± 0.40 | 1.70 ± 0.30 | 51.30 ± 5.90 |
| | | | D/F | 3.10 ± 0.40 | 1.80 ± 0.40 | 43.10 ± 6.50 |
| | | | S/Y | 2.80 ± 0.40 | 2.00 ± 0.30 | 54.50 ± 6.20 |

Table 3.2. Total *Pieris rapae* (larva and pupa) density, parasitized *P. rapae* density, and % parasitism of *P. rapae* /plant (seasonal means) by host, year, location, and treatment. For treatment, BR = broccoli, BW = buckwheat, D/F = dill and fennel combination, S/Y = sunflower and yarrow combination, and SE = standard error. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Host | Year | Location | Treatment | <i>P. rapae</i> /plant (mean±SE) | Parasitized <i>P. rapae</i> /plant (mean±SE) | % parasitism of <i>P. rapae</i> /plant (mean±SE) |
|-------|------|----------|-----------|-------------------------------------|--|--|
| Larva | 2010 | Athens | BR | 0.40 ± 0.10 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | BW | 0.70 ± 0.20 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | D/F | 0.80 ± 0.10 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | S/Y | 0.70 ± 0.30 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | Tifton | BR | 0.30 ± 0.10 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | BW | 0.30 ± 0.10 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | D/F | 0.30 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | S/Y | 0.30 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | 2011 | Athens | BR | 1.50 ± 0.30 | 0.10 ± 0.00 | 4.60 ± 2.90 |
| | | | BW | 1.30 ± 0.30 | 0.10 ± 0.00 | 7.20 ± 5.20 |
| | | | D/F | 1.50 ± 0.20 | 0.10 ± 0.00 | 5.20 ± 3.10 |
| | | | S/Y | 1.10 ± 0.20 | 0.10 ± 0.00 | 6.70 ± 3.20 |
| | | Tifton | BR | 0.40 ± 0.10a | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | BW | 0.30 ± 0.00b | 0.10 ± 0.00 | 4.20 ± 4.20 |
| | | | D/F | 0.20 ± 0.00b | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | S/Y | 0.30 ± 0.00b | 0.00 ± 0.00 | 0.00 ± 0.00 |

Table 3.2 (cont.)

| Host | Year | Location | Treatment | <i>P. rapae</i> /plant (mean±SE) | Parasitized <i>P. rapae</i> /plant (mean±SE) | % parasitism of <i>P. rapae</i> /plant (mean±SE) |
|------------------|------|----------|-----------|-------------------------------------|--|--|
| Larva (cont.) | 2012 | Tifton | BR | 4.80 ± 0.70 | 1.10 ± 0.30 | 14.00 ± 2.50 |
| | | | BW | 4.90 ± 0.70 | 1.10 ± 0.20 | 15.90 ± 2.70 |
| | | | D/F | 4.80 ± 0.80 | 1.10 ± 0.30 | 14.80 ± 2.50 |
| | | | S/Y | 5.20 ± 0.70 | 1.20 ± 0.30 | 15.20 ± 2.50 |
| Pupa | 2010 | Athens | BR | NA | NA | NA |
| | | | BW | NA | NA | NA |
| | | | D/F | NA | NA | NA |
| | | | S/Y | NA | NA | NA |
| | | Tifton | BR | 0.20 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | BW | 0.20 ± 0.00 | 0.20 ± 0.00 | 100.00 ± 0.00 |
| | | | D/F | NA | NA | NA |
| | | | S/Y | NA | NA | NA |
| | 2011 | Athens | BR | 0.50 ± 0.10 | 0.00 ± 0.00 | 2.50 ± 2.50b |
| | | | BW | 0.60 ± 0.10 | 0.10 ± 0.00 | 15.60 ± 11.40b |
| | | | D/F | 0.50 ± 0.10 | 0.20 ± 0.10 | 52.10 ± 16.50a |
| | | | S/Y | 0.50 ± 0.10 | 0.10 ± 0.10 | 18.90 ± 8.60b |

Table 3.2 (cont.)

| Host | Year | Location | Treatment | <i>P. rapae</i> /plant (mean±SE) | Parasitized <i>P. rapae</i> /plant (mean±SE) | % parasitism of <i>P. rapae</i> /plant (mean±SE) |
|-----------------|------|----------|-----------|-------------------------------------|--|--|
| Pupa (cont.) | | Tifton | BR | 0.40 ± 0.20 | 0.10 ± 0.10 | 25.00 ± 25.00 |
| | | | BW | 0.20 ± 0.00 | 0.00 ± 0.00 | 16.70 ± 16.70 |
| | | | D/F | 0.20 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | S/Y | 0.20 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | 2012 | Tifton | BR | 2.90 ± 0.40 | 1.90 ± 0.40 | 49.60 ± 6.10 |
| | | | BW | 2.60 ± 0.30 | 1.60 ± 0.30 | 46.70 ± 6.40 |
| | | | D/F | 2.70 ± 0.40 | 1.60 ± 0.40 | 42.10 ± 6.70 |
| | | | S/Y | 2.60 ± 0.40 | 1.80 ± 0.30 | 50.90 ± 6.60 |

Table 3.3. Total *Plutella xylostella* (larva and pupa) density, parasitized *P. rapae* density, and % parasitism of *P. rapae*/plant (the whole seasonal mean) by host, year, location and treatment. For treatment, BR = broccoli, BW = buckwheat, D/F = dill and fennel combination, S/Y = sunflower and yarrow combination, and SE = standard error. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Host | Year | Location | Treatment | <i>P. xylostella</i> /plant (mean±SE) | Parasitized <i>P. xylostella</i> /plant (mean±SE) | % parasitism of <i>P. xylostella</i> /plant (mean±SE) |
|-------|------|----------|-----------|--|---|---|
| Larva | 2010 | Athens | BR | 1.10 ± 0.10 | 0.10 ± 0.00 | 12.60 ± 2.80 |
| | | | BW | 0.90 ± 0.20 | 0.30 ± 0.10 | 42.20 ± 11.10 |
| | | | D/F | 1.20 ± 0.20 | 0.20 ± 0.10 | 13.30 ± 5.70 |
| | | | S/Y | 1.10 ± 0.20 | 0.30 ± 0.10 | 27.40 ± 6.90 |
| | | Tifton | BR | 2.40 ± 0.40 | 1.50 ± 0.20 | 59.40 ± 4.20 |
| | | | BW | 2.30 ± 0.20 | 1.50 ± 0.20 | 64.90 ± 3.70 |
| | | | D/F | 2.10 ± 0.20 | 1.30 ± 0.20 | 61.90 ± 4.30 |
| | | | S/Y | 1.70 ± 0.20 | 1.10 ± 0.20 | 64.70 ± 5.10 |
| | 2011 | Athens | BR | 0.30 ± 0.00b | 0.10 ± 0.10 | 31.00 ± 11.20 |
| | | | BW | 0.40 ± 0.10b | 0.10 ± 0.00 | 39.10 ± 11.20 |
| | | | D/F | 0.40 ± 0.10b | 0.10 ± 0.00 | 19.80 ± 8.00 |
| | | | S/Y | 0.50 ± 0.10a | 0.10 ± 0.00 | 14.20 ± 4.90 |
| | | Tifton | BR | 1.10 ± 0.20 | 0.70 ± 0.10 | 62.40 ± 7.20 |
| | | | BW | 1.10 ± 0.20 | 0.70 ± 0.10 | 62.00 ± 6.80 |
| | | | D/F | 1.00 ± 0.20 | 0.60 ± 0.10 | 66.80 ± 6.20 |
| | | | S/Y | 0.70 ± 0.10 | 0.40 ± 0.10 | 63.60 ± 8.10 |
| | 2012 | Tifton | BR | 0.30 ± 0.00 | 0.20 ± 0.00 | 65.20 ± 9.60 |
| | | | BW | 0.30 ± 0.00 | 0.30 ± 0.00 | 85.50 ± 5.20 |
| | | | D/F | 0.40 ± 0.10 | 0.30 ± 0.00 | 71.20 ± 8.70 |
| | | | S/Y | 0.70 ± 0.10 | 0.40 ± 0.10 | 58.60 ± 8.20 |

Table 3.3 (cont.)

| Host | Year | Location | Treatment | <i>P. xylostella</i> /plant (mean±SE) | Parasitized <i>P. xylostella</i> /plant (mean±SE) | % parasitism of <i>P. xylostella</i> /plant (mean±SE) |
|------|------|----------|-----------|--|---|---|
| Pupa | 2010 | Athens | BR | NA | NA | NA |
| | | | BW | NA | NA | NA |
| | | | D/F | NA | NA | NA |
| | | | S/Y | NA | NA | NA |
| | | Tifton | BR | 0.70 ± 0.10 | 0.50 ± 0.10 | 73.60 ± 5.80 |
| | | | BW | 0.70 ± 0.10 | 0.60 ± 0.10 | 79.90 ± 4.80 |
| | | | D/F | 0.70 ± 0.10 | 0.60 ± 0.10 | 83.30 ± 5.40 |
| | | | S/Y | 0.80 ± 0.10 | 0.50 ± 0.10 | 62.90 ± 6.40 |
| | 2011 | Athens | BR | NA | NA | NA |
| | | | BW | NA | NA | NA |
| | | | D/F | NA | NA | NA |
| | | | S/Y | NA | NA | NA |
| | | Tifton | BR | 0.30 ± 0.00 | 0.20 ± 0.00 | 41.00 ± 11.90 |
| | | | BW | 0.30 ± 0.10 | 0.10 ± 0.00 | 40.60 ± 13.80 |
| | | | D/F | 0.30 ± 0.00 | 0.10 ± 0.00 | 42.40 ± 13.40 |
| | | | S/Y | 0.40 ± 0.10 | 0.20 ± 0.10 | 21.40 ± 8.80 |
| | 2012 | Tifton | BR | 0.40 ± 0.00 | 0.30 ± 0.10 | 69.80 ± 11.00 |
| | | | BW | 0.30 ± 0.10 | 0.30 ± 0.10 | 71.90 ± 10.20 |
| | | | D/F | 0.40 ± 0.10 | 0.30 ± 0.10 | 77.30 ± 8.10 |
| | | | S/Y | 0.30 ± 0.10 | 0.30 ± 0.00 | 91.80 ± 5.40 |

Table 3.4. Treatment ANOVA table for total lepidopteran larval and pupal (all species pooled), by host, year, and location. P value followed by * is significant at $\alpha = 0.05$.

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|------------------------|-------|------|----------|-----|------|-------------|---------|--------|
| Total host/plant | Larva | 2010 | Athens | 3,9 | 1.08 | 0.36 | 0.60 | 0.63 |
| | | | Tifton | 3,9 | 8.43 | 2.81 | 0.88 | 0.49 |
| | | 2011 | Athens | 3,9 | 0.22 | 0.07 | 0.07 | 0.98 |
| | | | Tifton | 3,9 | 0.70 | 0.23 | 0.24 | 0.87 |
| | | 2012 | Tifton | 3,9 | 2.55 | 0.85 | 0.12 | 0.95 |
| | | | | | | | | |
| | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 3,9 | 0.10 | 0.03 | 0.31 | 0.82 |
| | | 2011 | Athens | 3,9 | 0.06 | 0.02 | 0.25 | 0.86 |
| | | | Tifton | 3,9 | 0.01 | 0.00 | 0.05 | 0.99 |
| | | 2012 | Tifton | 3,9 | 0.56 | 0.19 | 0.13 | 0.94 |
| | | | | | | | | |
| Parasitized host/plant | Larva | 2010 | Athens | 3,9 | 0.20 | 0.07 | 1.63 | 0.25 |
| | | | Tifton | 3,9 | 2.35 | 0.78 | 0.64 | 0.61 |
| | | 2011 | Athens | 3,9 | 0.00 | 0.00 | 0.01 | 1.00 |
| | | | Tifton | 3,9 | 0.06 | 0.02 | 0.07 | 0.97 |
| | | 2012 | Tifton | 3,9 | 0.13 | 0.04 | 0.03 | 0.99 |
| | | | | | | | | |

Table 3.4 (cont.)

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|--------------------------------|-------|------|----------|-----|------|-------------|---------|--------|
| Parasitized host/plant (cont.) | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 3,9 | 0.03 | 0.01 | 0.26 | 0.86 |
| | | 2011 | Athens | 3,9 | 0.10 | 0.03 | 1.46 | 0.29 |
| | | | Tifton | 3,9 | 0.03 | 0.01 | 0.40 | 0.76 |
| | | 2012 | Tifton | 3,9 | 0.89 | 0.30 | 0.41 | 0.75 |
| | | | | | | | | |
| % parasitism of host/plant | Larva | 2010 | Athens | 3,9 | 0.52 | 0.17 | 2.05 | 0.18 |
| | | | Tifton | 3,9 | 0.12 | 0.04 | 0.54 | 0.66 |
| | | 2011 | Athens | 3,9 | 0.15 | 0.05 | 0.33 | 0.80 |
| | | | Tifton | 3,9 | 0.59 | 0.20 | 1.75 | 0.23 |
| | | 2012 | Tifton | 3,9 | 0.11 | 0.04 | 0.44 | 0.73 |
| | | | | | | | | |
| | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 3,9 | 0.61 | 0.20 | 1.22 | 0.36 |
| | | 2011 | Athens | 3,9 | 1.91 | 0.64 | 4.46 | 0.04* |
| | | | Tifton | 3,9 | 0.39 | 0.13 | 1.14 | 0.39 |
| | | 2012 | Tifton | 3,9 | 1.27 | 0.42 | 1.30 | 0.28 |

Table 3.5. Treatment ANOVA table for total *Pieris rapae* (larvae and pupae), by host, year, and location. P value followed by * is significant at $\alpha = 0.05$.

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|-------------------------|-------|------|----------|-----|------|-------------|---------|--------|
| Total hosts/plant | Larva | 2010 | Athens | 3,7 | 1.21 | 0.40 | 3.12 | 0.10 |
| | | | Tifton | 3,6 | 0.03 | 0.01 | 1.10 | 0.42 |
| | | 2011 | Athens | 3,9 | 0.78 | 0.26 | 0.21 | 0.89 |
| | | | Tifton | 3,9 | 0.04 | 0.01 | 4.15 | 0.04* |
| | | 2012 | Tifton | 3,9 | 1.41 | 0.47 | 0.10 | 0.96 |
| | | | | | | | | |
| | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 1,0 | 0.00 | 0.00 | NA | NA |
| | | 2011 | Athens | 3,9 | 0.06 | 0.02 | 0.24 | 0.86 |
| | | | Tifton | 3,4 | 0.03 | 0.01 | 0.19 | 0.90 |
| Parasitized hosts/plant | Larva | 2010 | Athens | 3,7 | 0.00 | 0.00 | NA | NA |
| | | | Tifton | 3,6 | 0.00 | 0.00 | NA | NA |
| | | 2011 | Athens | 3,9 | 0.00 | 0.00 | 0.02 | 1.00 |
| | | | Tifton | 3,9 | 0.00 | 0.00 | 0.19 | 0.90 |
| | | 2012 | Tifton | 3,9 | 0.11 | 0.04 | 0.02 | 0.99 |
| | | | | | | | | |

Table 3.5 (cont.)

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|---------------------------------|-------|------|----------|-----|------|-------------|---------|--------|
| Parasitized hosts/plant (cont.) | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 1,0 | 0.02 | 0.02 | NA | NA |
| | | 2011 | Athens | 3,9 | 0.13 | 0.04 | 1.79 | 0.22 |
| | | | Tifton | 3,4 | 0.01 | 0.00 | 0.68 | 0.61 |
| | | 2012 | Tifton | 3,9 | 0.84 | 0.28 | 0.39 | 0.77 |
| | | | | | | | | |
| % parasitism of host/plant | Larva | 2010 | Athens | 3,7 | 0.00 | 0.00 | NA | NA |
| | | | Tifton | 3,6 | 0.00 | 0.00 | NA | NA |
| | | 2011 | Athens | 3,9 | 0.01 | 0.00 | 0.03 | 0.99 |
| | | | Tifton | 3,9 | 0.01 | 0.00 | 0.19 | 0.90 |
| | | 2012 | Tifton | 3,9 | 0.03 | 0.01 | 0.38 | 0.77 |
| | | | | | | | | |
| | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 1,0 | 1.23 | 1.23 | NA | NA |
| | | 2011 | Athens | 3,9 | 1.90 | 0.63 | 4.43 | 0.04* |
| | | | Tifton | 3,4 | 0.78 | 0.26 | 0.68 | 0.61 |
| | | 2012 | Tifton | 3,9 | 0.22 | 0.07 | 2.22 | 0.16 |

Table 3.6. Treatment ANOVA table for total *Plutella xylostella* (larvae and pupae) density, by host, year, and location. P value followed by * is significant at $\alpha = 0.05$

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|-------------------------|-------|------|----------|-----|------|-------------|---------|--------|
| Total host/plant | Larva | 2010 | Athens | 3,9 | 0.20 | 0.07 | 0.19 | 0.90 |
| | | | Tifton | 3,9 | 7.39 | 2.46 | 0.74 | 0.55 |
| | | 2011 | Athens | 3,9 | 0.45 | 0.15 | 5.54 | 0.02* |
| | | | Tifton | 3,9 | 0.22 | 0.07 | 0.07 | 0.97 |
| | | 2012 | Tifton | 3,9 | 0.15 | 0.05 | 0.47 | 0.71 |
| | | | | | | | | |
| | Pupa | 2010 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 3,9 | 0.12 | 0.04 | 0.38 | 0.77 |
| | | 2011 | Athens | NA | NA | NA | NA | NA |
| | | | Tifton | 3,9 | 0.07 | 0.02 | 0.34 | 0.79 |
| Parasitized hosts/plant | Larva | 2010 | Athens | 3,9 | 0.30 | 0.10 | 2.81 | 0.10 |
| | | | Tifton | 3,9 | 2.35 | 0.78 | 0.64 | 0.61 |
| | | 2011 | Athens | 3,9 | 0.02 | 0.01 | 0.24 | 0.87 |
| | | | Tifton | 3,9 | 0.06 | 0.02 | 0.07 | 0.98 |
| | | 2012 | Tifton | 3,9 | 0.04 | 0.01 | 1.21 | 0.36 |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

Table 3.6 (cont.)

| Variable | Host | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|------------------------------------|-------|------|------------------|-----------|------------|-------------|------------|------------|
| Parasitized hosts/plant (cont.) | Pupa | 2010 | Athens Tifton | NA 3,9 | NA 0.04 | NA 0.01 | NA 0.32 | NA 0.81 |
| | | 2011 | Athens Tifton | NA 3,9 | NA 0.06 | NA 0.02 | NA 0.77 | NA 0.54 |
| | | 2012 | Tifton | 3,9 | 0.14 | 0.05 | 0.38 | 0.77 |
| % parasitism of host/plant | Larva | 2010 | Athens | 3,9 | 1.19 | 0.40 | 2.44 | 0.13 |
| | | | Tifton | 3,9 | 0.15 | 0.05 | 0.67 | 0.59 |
| | | 2011 | Athens | 3,9 | 1.09 | 0.36 | 0.59 | 0.63 |
| | | | Tifton | 3,9 | 0.53 | 0.18 | 0.76 | 0.54 |
| | | 2012 | Tifton | 3,9 | 0.70 | 0.23 | 0.65 | 0.60 |
| | | | | | | | | |
| | Pupa | 2010 | Athens Tifton | NA 3,9 | NA 0.62 | NA 0.21 | NA 1.29 | NA 0.34 |
| | | 2011 | Athens Tifton | NA 3,9 | NA 1.32 | NA 0.44 | NA 1.70 | NA 0.24 |
| | | 2012 | Tifton | 3,9 | 0.57 | 0.19 | 0.43 | 0.74 |

Fig. 3.1. Incidence of lepidopteran host species in broccoli trials as percentage of overall numbers collected, by year and location. *Trichoplusia ni* (Hübner), *Spodoptera exigua* (Hübner), and *Spodoptera eridania* (Cramer).

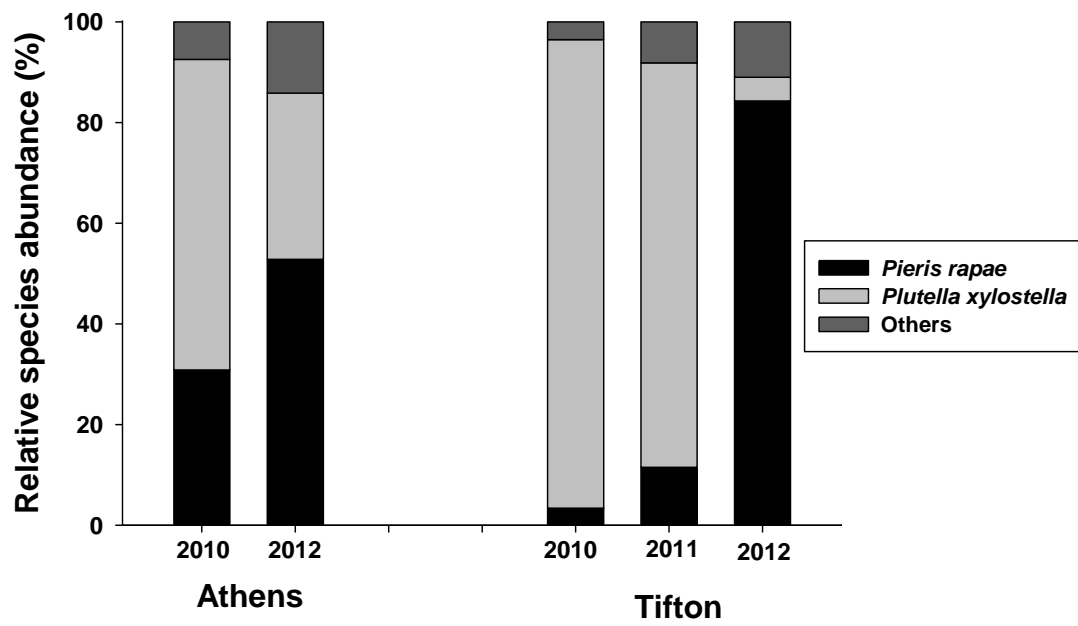


Fig. 3.2a. Relative abundance of parasitoid taxa reared from *P. xylostella* larvae and pupae as percentage of overall parasitoid numbers reared from this species, by year and location.

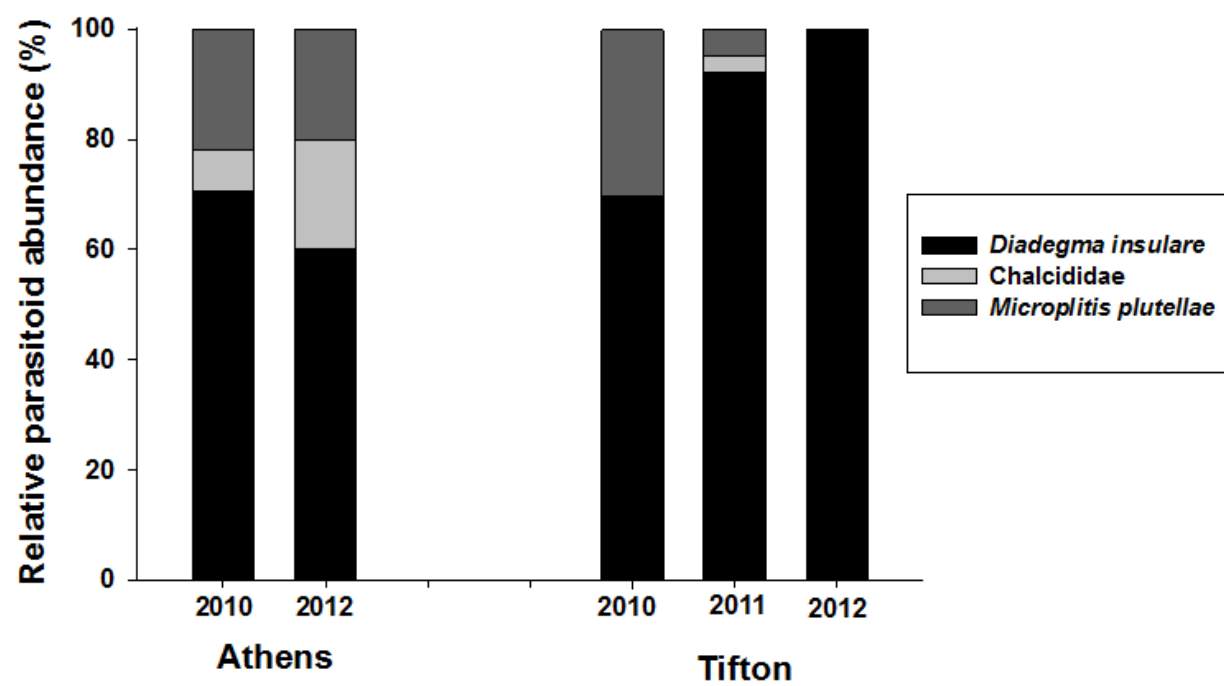
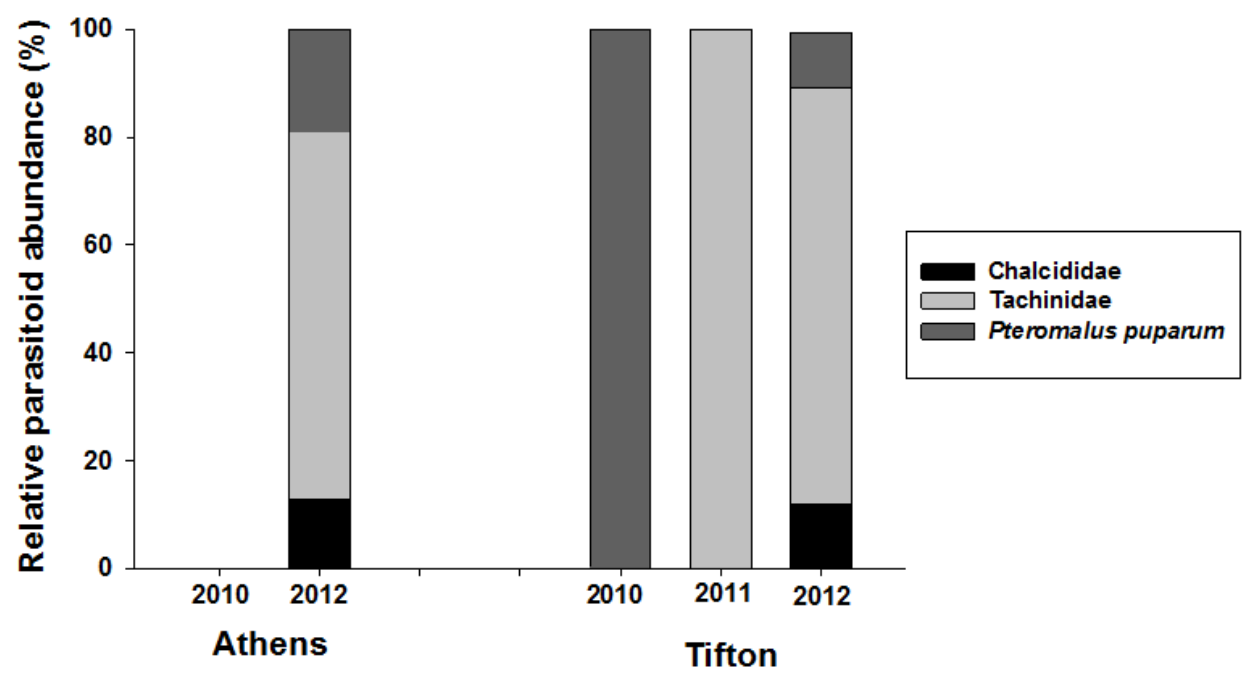


Fig. 3.2b. Relative abundance of parasitoid taxa reared from *P. rapae* larvae and pupae as percentage of overall parasitoid numbers reared from this species, by year and location.



CHAPTER 4
PREDATOR ABUNDANCE AND PREDATION OF SENTINEL PREY EGGS IN
RELATION TO FLORAL FARMSCAPING IN BROCCOLI ³

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Abstract

Predatory arthropods play important roles in natural pest control in agroecosystems. Simplification of modern agroecosystems through monoculture cropping practices has led to decreased abundance, diversity, and impact of these predatory insects. However, due to side effects of chemical insecticides on the environment and human health, natural pest control has grown in emphasis. Abundance, fitness, and biological control efficacy of predatory arthropods can be enhanced by providing them access to non-prey food sources. In the present study, we assessed the suitability of three flower treatments (buckwheat, *Fagopyrum esculentum* (Moench); a combination of fennel, *Foeniculum vulgare* (Mill.), and dill, *Anethum graveolens* (L.); and a combination of sunflower, *Helianthus annuus* (L.), and yarrow, *Achillea millefolium* (L.)) for enhancing predator abundance and predation of sentinel eggs of the beet armyworm, *Spodoptera exigua* (Hübner), in organic broccoli and cucumber systems in Athens and Tifton, Georgia, from 2010 to 2012. There was no evidence for effects of the flower treatments on predator abundance or efficacy. There were few and inconsistent significant differences among treatments in number of sucking predators/plant in cucumber (only Athens in 2011) and total numbers of predators/plant in cucumber (only Athens in 2010). Predation of beet armyworm eggs did not differ among treatments within locations and years. The few significant differences likely reflected random events rather than the effect of treatments, since they were unusual and inconsistent across locations and years. Plot size and interactions among predators and available prey in the systems may have masked any significant treatment effects.

Keywords

Non-prey food; *Spodoptera exigua*; broccoli; cucumber.

4.1 Introduction

In agroecosystems, natural enemies provide important ecosystem services, chief among which is natural pest suppression (Campbell et al., 2012). The effects of natural enemies in agroecosystems have been diminished by simplification through monoculture cropping practices (Stoate et al., 2009; Rusch et al., 2013). However, due to detrimental environmental and health side effects of synthetic insecticides and development of insecticide resistance, the importance of natural enemies has been an increasing emphasis in recent years, and efforts to enhance natural biological control have been growing, especially among organic farmers and Integrated Pest Management (IPM) practitioners (Hole et al., 2005; Gibson et al., 2007).

Many insect predators are omnivores (Lundgren, 2009), and thus require a combination of prey and non-prey foods to optimize their fitness. These non-prey foods may include pollen, floral nectar, extrafloral nectar and honeydew. Because plant diversity is often associated with arthropod species diversity and richness, it is often assumed that increasing plant diversity in agroecosystems will lead to pest suppression. However, this is not always the case as the interactions among the plants, herbivores, and predators are complex, with outcomes that can be difficult to predict (Risch et al., 1983; Sheehan, 1986; Bianchi et al., 2006).

Increased plant diversity can have positive, negative, or neutral effects on biological control of herbivores (Bianchi et al., 2006; Jacometti et al., 2010). On one hand, plants can provide food resources to predators that complement their nutrition from prey and enhance their abundance (via immigration or in situ reproduction), fitness, and biological control activity. On the other hand providing non-crop plants as a

source of alternate food can enhance herbivore pest populations, satiate predators with non-prey foods and distract them from feeding on prey, or enhance the effect of the fourth trophic level and interfere with biological pest control (Stephens et al., 1998; Lavandero et al., 2006; Spellman et al., 2006; Jonsson et al., 2009).

Given that providing food resources to predators may interfere with biological control, it is important to identify the most appropriate combinations of plants to maximize the biological control efficacy of resident natural enemies.

In this study, we assessed the effects of three floral farmscape treatments on predator abundance and pest predation in vegetable crops. We used egg masses of beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), as sentinel prey to monitor activity of the predator complex in organic broccoli, *Brassica oleracea* (L.) (Brassicales: Brassicaceae), and cucumber, *Cucumis sativus* (L.) (Cucurbitales: Cucurbitaceae), cropping systems. The study tested two hypotheses: 1) the presence of flowers will enhance predator abundance and predation of *S. exigua* eggs, and 2) buckwheat will enhance predator abundance and predation of *S. exigua* eggs more than other flowers tested because of the copious nectar production and ease of its access in buckwheat.

4.2 Materials and Methods

4.2.1 Floral plants

The treatments used were: 1) broccoli or cucumber (control), 2) buckwheat, *Fagopyrum esculentum* (Moench) (Caryophyllales: Polygonaceae), 3) a combination of fennel, *Foeniculum vulgare* (Mill.) (Apiales: Apiaceae) and dill, *Anethum graveolens* (L.)

(Apiales: Apiaceae), and 4) a combination of sunflower, *Helianthus annuus* (L.) (Asterales: Asteraceae) and yarrow, *Achillea millefolium* (L.) (Asterales: Asteraceae). We selected these flowers because they are either native to North America (in case of sunflower) or grown as commercial crops or as attractants for natural enemies in agricultural systems.

Dill is native to the Mediterranean and cultivated for spice and medicine (Carrubba et al., 2008; Sokhangoy et al., 2012; Tian et al., 2012). It is used as a companion plant in some cropping systems (Winkler et al., 2010). It is an annual flowering plant and produces flowers with no corolla depth, with average aperture size of 2.63 mm and easily accessible nectar (Carrubba et al., 2008; Winkler et al., 2009).

Fennel is native to the Mediterranean region and cultivated for spice and medicinal use (Gross et al., 2008). It is a perennial flowering herb and produces flowers with no corolla depth and easily accessible nectar (Gross et al., 2008; Winkler et al., 2009).

Yarrow is native to Western Asia and Europe, but grows in most temperate regions, including the United States. It is mainly cultivated for medicinal uses and it is becoming popular as an attractant for natural enemies (Applequist and Moerman, 2011; Dib et al., 2012). It is perennial and as a member of Asteraceae produces a flower head about 2 to 4 mm wide comprising about five to six ray florets and 10 to 30 disk florets, with corolla depth measuring 2.2 to 3 mm (Warwick and Black, 1982; Zhang et al., 1996; Sulborska and Weryszko-Chmielewska, 2006).

Sunflower is cultivated for oil, feed, and industrial raw materials (Lu and Hoeft, 2009; Fernández-Martínez et al., 2010). It attracts a suite of beneficial insects, including

predators and parasitoids (Jones and Gillett, 2005; Adedipe and Park, 2010). It is an annual plant and produces capitulae of 2 to 30 cm in diameter, depending on the variety (Cronn et al., 1997; Fambrini et al., 2007). The capitulum bears two kinds of flowers: ray and disc florets. Ray florets do not usually have nectaries and, when present, they are very small and do not secrete nectar. Disc florets have nectaries that secrete nectar and average disc floret corolla depth ranges from 7.23 to 10.22 mm, while the mean daily nectar production per floret ranges between 0.24 to 0.38 μL (Hadisoesilo and Furgala, 1986; Mani and Saravanan, 1999; Atlagić et al., 2003; Wist and Davis, 2006).

Buckwheat is native to Asia and is widely cultivated in many regions of the world, including the United States for food and habitat management (Ohnishi, 1990; Li and Zhang, 2001; Wijngaard and Arendt, 2006; Lee and Heimpel, 2008). It has shallow corollae with wide apertures (0.54 mm deep and 6.59 mm wide) (Baggen et al., 1999), which make its nectar easily accessible to many insects (Sim and Choi, 1999; Vattala et al., 2006).

4.2.2 *Experimental design*

The experiment was carried out at experimental plots in two locations, Athens and Tifton, Georgia, from 2010 to 2012. The study was conducted in spring and summer 2010 and 2011 in Athens, and spring 2010, 2011, and 2012, in Tifton. Broccoli was grown in spring in both locations while cucumber was grown only in Athens. The land used in Athens was in transition to organic certification, while that used in Tifton was organically certified and had been in organic production for two years before the start of the experiment. The broccoli (var. 'Windsor F1'), cucumber (var. 'Marketmore

76') dill (var. 'Bouquet'), fennel (var. 'Bronze'), and buckwheat seeds used in the study were organic and were purchased from Johnny's Selected Seeds (<http://www.johnnyseeds.com/default.aspx>), while sunflower (var. 'Sunbright F1') was not organic and was obtained from the same vendor. Yarrow (White) was not organic and was obtained from Peaceful Valley Farm & Garden Supply (<http://www.groworganic.com>)

The year prior to spring and summer planting, the land in Athens [(Austrian winter peas, *Pisum sativum* (L.) (Fabales: Fabaceae), 22.4 kg/ha and oat, *Avena sativa* (L.) (Poales: Poaceae), 11.2 kg/ha, planted in late fall)] and Tifton [(sunn hemp, *Crotalaria juncea* (L.) (Fabales: Fabaceae), 33.6 kg/ha planted in early summer)] was planted with cover crops. The flower treatment plants were started in the greenhouse between January and February of each year to make sure that the plants were at the flowering stage at the time of transplanting. Flowers were also replanted every three to four weeks as needed to ensure that there were flowering plants in the field throughout the sampling period. Broccoli for both locations was started in the greenhouse between January and February, while cucumber in Athens was started in May. All transplant starters were planted in Fafard germination mix in 7.62 cm diameter trays, under $26 \pm 2^{\circ}\text{C}$ and 14:10 (L:D) photoperiod.

Broccoli (15-20 cm tall) and flowering plants for the broccoli trials in Athens were transplanted to the field from 1 to 30 April in 2010 and from 4 to 20 April in 2011, while cucumber (15-20 cm tall) and flowering plants for cucumber trials were transplanted from 23 June to 7 July in 2010 and from June 15 to 21 in 2011. Broccoli (15-20 cm tall)

and flowering plants in Tifton were transplanted on 22 and 23 March in 2010, 14 and 15 March in 2011, and 14 and 15 March in 2012.

4.2.3 *Field Layout*

Broccoli and cucumber were transplanted into 16 blocks (4 replicates of 4 treatments) measuring 12x12 meters, i.e. 144 m², and separated from one another by a 3m border of bare soil, with each block containing 6 twin-row beds, each measuring 12x1.2 meters, in each location and each year. Broccoli and cucumber were planted in Athens and broccoli in Tifton at a spacing of 0.46 m between rows and 0.46 m between plants within rows. A treatment plot measuring 2x2 meters was established in the center of each block in which the respective flowering treatments were placed. The treatment plots were laid out in a randomized block design in Athens (because of land constraint) for both broccoli and cucumber and in a Latin square design for broccoli in Tifton.

The flowering plants were planted on the adjacent halves of the third and fourth beds of each plot in the 2x2 m subplot. The treatments with two flower species (dill/fennel and yarrow/sunflower) were planted in such a way that one flower species was planted on one half of one bed and the other flower on the other half of the other bed, and sides were alternated across the blocks to avoid bias. In Athens, 40 flowering plants were planted in each treatment plot, 20 plants on each half of the bed, and 20 plants for each of the flower species in treatments with two flower species. In Tifton, 48 flowering plants were planted in each treatment plot, 24 plants on each half of the bed, and 24 plants for each of the flower species in treatments with two flower species. The

flowers were close to flowering when transplanted and were transplanted immediately after broccoli transplanting.

The plots were fertilized at planting in Athens with feathermeal (11-14% N) at the rate of 3 g/ planting hole, and prior to planting in Tifton with pelletized poultry litter applied at a rate of 1467 kg/ha (3% N for 44 kg/ha of N) to the field in mid-February of each year. Water was supplied with drip irrigation as needed. Weed control was done by tillage and hand-pulling in 2010 and with tillage and black plastic mulch (0.25 ml) covering the beds in 2011 and 2012 at both locations.

4.2.4 *Beet armyworm eggs*

Beet armyworm egg masses used in the experiment were obtained from a beet armyworm culture maintained at the Entomology Department of the University of Georgia, Tifton Campus. The beet armyworm colony was maintained on artificial diet prepared with pinto bean meal, wheat germ, agar, yeast, ascorbic acid, sorbic acid, and vitamin mixture (Burton, 1969). The beet armyworm colony was held at $25 \pm 1^{\circ}\text{C}$ and 14:10 L:D.

4.2.5 *Data collection and analyses*

After transplanting the plants (broccoli, cucumber, and flowers), they were allowed about 1-2 weeks to establish before sampling. Individual broccoli and cucumber plants were sampled for predators at the center of the block next to the flowering plants (1 plant), and 1 plant each 4 meters away from the center in the four cardinal directions, resulting in five sampling positions (east, west, north, south, and center) per block; i.e.,

five plants per plot and 80 plants in each location; except in broccoli in Tifton in 2011, where we had three sampling positions (north, central, and south) per block; i.e three plants per plot and a total of 48 plants. The plants were sampled once a week between 8:00 am and 12:00 pm in Athens 2010 (May 22 to June 8 for broccoli and July 20 to September 5 for cucumber), Athens 2011 (April 26 to June 23 for broccoli and July 6 to July 26 for cucumber), Tifton 2010 (April 20 to May 25), Tifton 2011 (April 13 to June 7), and Tifton 2012 (March 29 to May 24).

Beet armyworm moths of the lab colony were provisioned with a white paper towel oviposition substrate (28x18.5 cm). Egg masses of 20 to 60 eggs on the paper towel were counted under the microscope and cut out to be used as sentinel prey. One egg mass was stapled on the underside of one leaf of the sampled plants (i.e., five egg masses per plot and 80 egg masses per location, except in broccoli in Tifton in 2011, where we had three egg masses per plot and a total of 48 egg masses) and photographed 24, 48, and 72 h [using Canon camera (Canon EOS Digital Rebel XT), image size of ~ 3500x2300 squared pixels, and at distance of about 5 cm from egg masses] after putting out the eggs. The photographs were uploaded to the computer and predated eggs at each photo interval were determined by subtracting the eggs remaining at each time from the total eggs that were put out initially.

Sucked eggs were recognized by their collapsed pyramidal shape. Chewed eggs were characterized by extensive damage to the chorions, with yolk sometimes spilled on the paper containing the eggs, and sometimes a part of the paper also was chewed. Some eggs hatched prior to the 72-hour period late in the season when field temperatures were high (hatched eggs were readily recognized by transparent, dry, and

empty chorions left after the larvae emerged) and they were excluded from the analyses. Missing eggs were considered incidental loss and were excluded from the analyses. This omission undoubtedly led to some underestimates of predation, but incidental dislodgement of eggs occurs (from rain, heavy dews, wind vibration on leaves, etc.), and in the absence of clear data on the proportion lost due to abiotic factors, we were unable to adequately assign lost eggs to a definitive fate. Further, the number of missing eggs was unaffected by treatment (see below), so outcomes were not affected by their omission.

Data on predator density (number of chewing predators/plant, number of sucking predators/plant, and total number of predators/plant), percent chewed beet armyworm eggs/plant by 72 h, percent sucked beet armyworm eggs/plant by 72 h and total percent predation of beet armyworm eggs/plant by 72 h for each location and each year were collected and analyzed using generalized linear mixed models (repeated measures two-way ANOVA), with block as a random effect (SAS Institute, 2010). Percentage predation was transformed using $\arcsin\sqrt{\%}$ before analysis with generalized linear mixed models (repeated measures two-way ANOVA).

4.3 Results

There were no significant differences among the treatments in number of chewing predators/plant for either cucumber or broccoli in any of the years and locations. Number of sucking predators/plant differed significantly among treatments ($F = 6.53$, $df = 3, 9$, $P = 0.01$) in cucumber in one year and site (Athens in 2011), with the cucumber control treatment (1.06 ± 0.14) having the highest sucking predator density

and differing significantly from the dill/fennel treatment (0.56 ± 0.11), while having comparable sucking predator density to the buckwheat (0.99 ± 0.13) and yarrow/sunflower (0.84 ± 0.12) treatments. Total numbers of predators/plant differed significantly among treatments ($F = 4.07$, $df = 3, 9$, $P = 0.04$) only in cucumber in Athens 2010, with the cucumber control treatment having the highest predator density (0.44 ± 0.07) and differing significantly from the yarrow/sunflower (0.29 ± 0.09) and dill/fennel (0.24 ± 0.05) treatments, but not from buckwheat (0.40 ± 0.09). Buckwheat total predator density also differed significantly from both yarrow/sunflower and dill/fennel in Athens cucumbers in 2010, while yarrow/sunflower and dill/fennel did not differ significantly from one another (Table 4.1). Total predator density did not differ among treatments in cucumber for Athens 2011 ($F = 3.30$, $df = 3, 9$, $P = 0.07$) or for broccoli in Athens 2010 ($F = 1.70$, $df = 3, 9$, $P = 0.24$), Athens 2011 ($F = 0.50$, $df = 3, 9$, $P = 0.69$), Tifton 2010 ($F = 1.41$, $df = 3, 9$, $P = 0.30$), Tifton 2011 ($F = 0.40$, $df = 3, 9$, $P = 0.76$), and Tifton 2012 ($F = 0.41$, $df = 3, 9$, $P = 0.75$).

Predators obtained in the on-plant samples included the sucking predators *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), *Orius insidiosus* (Say) (Hemiptera: Anthrenidae), *Geocoris* spp. (Hemiptera: Geocoridae), and *Nabis* sp. (Hemiptera: Nabidae). Chewing predators consisted of *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae), *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae), and *Hippodamia convergens* (Guérin-Ménéville) (Coleoptera: Coccinellidae). For individual predator densities (Table 4.2), there were significant differences among treatments only in *H. convergens* densities in broccoli in

Athens in 2010 ($F = 1.96$, $df = 3, 9$, $P = 0.02$) and in cucumber in Athens in 2011 ($F = 4.98$, $df = 3, 9$, $P = 0.03$), and *Geocoris* spp. density in Athens in 2011 ($F = 3.14$, $df = 3, 9$, $P = 0.05$). The broccoli treatment in broccoli in Athens in 2010 had the highest *H. convergens* density (0.31 ± 0.07 per plant) and significantly differed from the buckwheat (0.08 ± 0.03) treatment but had comparable density to yarrow/sunflower (0.21 ± 0.06) and dill/fennel treatment (0.19 ± 0.06) treatments. Dill/fennel treatment for cucumber in Athens in 2011 had the highest *H. convergens* density (0.15 ± 0.04), and differed significantly from yarrow/sunflower (0.05 ± 0.02) and cucumber (0.00 ± 0.00) treatments, but had comparable density to the buckwheat (0.09 ± 0.03) treatment. The control treatment had the highest *Geocoris* spp. density in the cucumber trial in Athens in 2011 (1.01 ± 0.14), differing significantly from the dill/fennel treatment (0.55 ± 0.11) but exhibiting similar density as in the buckwheat (0.85 ± 0.13) and yarrow/sunflower (0.76 ± 0.11) treatments.

The most abundant predators were *H. convergens* for broccoli in Tifton in 2011 (67.9% of all predators) and in 2012 (63.4%) and for cucumber in Athens in 2010 (30.7%); *O. insidiosus* for broccoli in Athens in 2010 (52.1%); *L. lineolaris* for broccoli in Athens in 2011 (39.8%); and *Geocoris* spp. for cucumber in Athens 2011 (62.6%). *Hippodamia convergens*, despite not being the most abundant predator, had a high relative abundance in broccoli in Athens in 2010 and 2011 (Table 4.3).

Table 4.4 presents total percent predation of beet armyworm eggs/plant by 72 h, separated by whether the eggs were chewed or sucked for both broccoli and cucumber studies in both locations, from 2010 to 2012. There were no significant differences among the treatments in any of the variables (percent chewed beet armyworm

eggs/plant by 72 h, percent sucked beet armyworm eggs/plant by 72 h and percent predation of beet armyworm eggs/plant by 72 h) in either broccoli or cucumber trials for both locations.

Comparison of percentage predation of beet armyworm eggs/plant in broccoli and cucumber studies shows that predation was consistently higher in cucumber than in broccoli in each year and each location. More of the eggs were chewed than sucked in cucumbers in each year of study, while the results in broccoli were mixed (Table 4.4). Percentage predation of beet armyworm eggs/plant increased with time in both crops. The increase was typically more rapid and achieved higher predation levels across the observation period in cucumber than in broccoli, with most of the predation in cucumber occurring within 48 h (Figs. 4.1 and 4.2).

Predators captured in the photographs in association with the beet armyworm eggs included the sucking predators/omnivores *L. lineolaris* [broccoli, Athens 2010 (n = 6) and 2011 (n = 3) and broccoli, Tifton 2010 (n = 2), 2011 (n=2) and 2012 (n = 6)] , *O. insidiosus* [broccoli, Athens 2011 (n = 1), Tifton 2011 (n=1)] , and *Geocoris uliginosus* (Say) [broccoli, Athens 2011 (n = 1)]. The chewing predators included *S. invicta* [broccoli, Tifton 2010 (n = 3)], *C. maculata* [broccoli, Athens 2010 (n = 1) and 2011 (n = 1)], and *H. convergens* [broccoli, Athens 2011 (n = 1) and broccoli, Tifton 2010 (n = 1), 2011 (n = 1), and 2012 (n = 1)].

Predator density in broccoli (0.45 ± 0.05 , n = 320) did not differ significantly ($t = 1.64$, $df = 1$, $P = 0.10$) from that in cucumber (0.34 ± 0.04 , n = 400) in Athens 2010, while in Athens 2011 broccoli predator density (0.25 ± 0.02 , n = 720) was significantly ($t = 11.18$, $df = 1$, $P < 0.00$) less than that of cucumber (1.27 ± 0.09 , n = 320).

4.4 Discussion

Results from our experiments show no significant differences among the treatments in any of the variables [chewing predator density, sucking predator density, and total predator density (Table 4.1); individual predator species density (Table 4.2); and percent chewed beet armyworm eggs/plant by 72 h, percent sucked beet armyworm eggs/plant by 72 h, and percent predation of beet armyworm eggs/plant by 72 h (Table 4)] evaluated in either the broccoli or cucumber studies for both locations. This is with the exception of sucking predator density in cucumbers in Athens 2011 and in total predator density in Athens 2010 (Table 4.1), and *Geocoris* spp. density in cucumber in Athens in 2011 and *H. convergens* density in broccoli in Athens in 2010 and cucumber in Athens in 2011 (Table 4.2).

Considering that the experiment was replicated five times with broccoli and three times with cucumbers in space and time, this suggests that the flower treatments did not impact predator density and predation of beet armyworm eggs, with this experimental design. Therefore, the sporadic significant differences recorded in some of the variables were likely random and did not reflect effects of the experimental treatments.

The consistently higher predation of beet armyworm eggs in cucumber than broccoli, as can be seen in Fig. 4.1, may be attributable to higher predator density in cucumber than broccoli in Athens 2011. However, differential predator density cannot explain the higher predation of beet armyworm eggs in cucumber than broccoli in Athens 2010 as predator densities for both crops were comparable. Nor do the predator numbers adequately explain the very low predation rates in Tifton 2012 broccoli relative to 2010 and 2011, since the predator numbers were highest in 2012.

Predation was expected to be higher in cucumber than broccoli for several reasons. First, cucumber was planted in summer when the temperature was higher and insects more active than in spring when broccoli was planted. Further, predator (and herbivore) species abundance is higher later in the season, which may contribute to higher predation rates. Finally, cucumber plant and growth architecture generates a very different micro-habitat than that presented by the more-vertical and open structure of broccoli. Cucumbers expand and cover the ground, providing shade and a presumably more favorable micro-environment for insects relative to broccoli.

The change from tillage and hand pulling of weeds in 2010 to plastic mulch and tillage for weed management in 2011 also may have affected the outcomes of the study. Predation rates in both broccoli and cucumbers declined from 2010 to 2011, and failed to rebound in 2012 in Tifton although predator numbers on plants were comparable with those observed in 2010.

The plastic mulch may have significantly affected the microhabitat for foraging predators, and changed their activity against the sentinel eggs. Plastic mulch have shown to affect insect communities in the field (Tuovinen et al., 2006; Žanec et al., 2009)

Besides microhabitat variability, the predation results may also have been affected by the diverse feeding habits of the predators present. All of the predators observed are polyphagous, and many are omnivorous. The abundance and species composition of available herbivores in the broccoli crop varied across locations and years (Chapter 3), and these variations in herbivorous prey may have resulted in variation across locations and years. Predators are known to switch prey based on preference (preferred vs. alternative prey) and, therefore, their responses to *S. exigua*

eggs could have been influenced by temporal and spatial variations in the abundance of other prey species around them (Welch et al., 2012). However, the possibilities for intraguild predation may have varied within locations and across years, as well, adding another element of variation into the predation outcomes with sentinel prey. Further, the relative demands of carnivory and herbivory in the omnivores may also have added significant variation to the observed predation rates on the sentinel egg masses (Welch et al., 2012).

The lack of differences in predation observed here (and parasitism reported in Chapter 3) suggests no benefit obtained from the flower plantings. However, plot sizes may have significantly influenced the lack of treatment effect. The size of the plots (12x12 m) and distance between the plots (3 m) was possibly too small for detecting effects of treatments. Unfortunately, larger suitable parcels of land for the project were not available. Given the scale, it seems likely that many of the predators were able to move readily among plots, possibly negating any significant effects that the various treatments might have had. Predators, such as *Geocoris* sp., *H. convergens*, *Lygus* sp., and *Nabis* spp., are highly mobile and have been recorded to disperse more than 1 km (Sivakoff et al., 2012).

Another important factor may have been the size of the treatment plot relative to the total area planted. In the present study the flowering treatment area occupied ~3% of the total area and could have been too small to be effective. Pfiffner and Wyss (2004) recommended that at least 10% of intensively cultivated area be set aside as wildflower strips for natural enemy conservation. In the lettuce-allysum system in California, ~4% of cultivated land is devoted to natural enemy conservation, and this system has been

successful in managing the currant-lettuce aphid, *Nasonovia ribisnigri* (Mosley) with attracted hoverflies (Gillespie et al., 2011). The work by Gillespie et al. (2011) on the lettuce-alyssum system suggests that the area set aside for natural enemies can be reduced to 2% without any effect on hoverfly abundance in the crop section. There are likely system-specific variations in the extent of area to be devoted to insectary plants, relative to target crop. However, significantly increasing the size of the treatment plot relative to the crop area brings costs in terms of lost yield, and land and management expenses (e.g., labor, fuel, and time to manage weeds in the floral planting) that would need to be compensated for by pest suppression from natural enemies or other valued services to justify producer adoption. For example, increasing the area set aside for insectary plant in the lettuce-alyssum system from 2% to 8% reduced the yield of lettuce by ~7% (Brennan, 2013). Future studies with significantly larger plot sizes would be of value.

Acknowledgments

We appreciate the support of the project by a USDA Southern Sustainable Agricultural Research and Education (SARE) award to J.R. Ruberson and P.G. Hartel. We thank Drs. Paul Guillebeau, Carl Jordan, and Michael Toews (University of Georgia) for their helpful comments on the manuscript. We appreciate Mr. Jerry Davis (University of Georgia) for helping with statistical analyses.

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Table 4.1. Number of chewing predators/plant, # of sucking predators/plant, and total # of chewing predators/plant for broccoli and cucumber (seasonal means) by year, location, and treatment. For treatment, BR = broccoli, CU = cucumber, BW = buckwheat, D/F = dill and fennel combination, Y/S = sunflower and yarrow combination, and SE = standard error. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Crop | Year | Location | Treatment | # of chewing predators/plant (mean±SE) | # of sucking predators/plant (mean±SE) | Total predators/plant (mean±SE) |
|----------|------|----------|-----------|--|--|---------------------------------|
| Broccoli | 2010 | Athens | BR | 0.31 ± 0.07 | 0.26 ± 0.07 | 0.58 ± 0.12 |
| | | | BW | 0.11 ± 0.05 | 0.24 ± 0.07 | 0.35 ± 0.10 |
| | | | D/F | 0.23 ± 0.06 | 0.15 ± 0.06 | 0.38 ± 0.10 |
| | | | Y/S | 0.23 ± 0.06 | 0.26 ± 0.07 | 0.49 ± 0.10 |
| | | Tifton | BR | 0.48 ± 0.08 | 0.05 ± 0.02 | 0.53 ± 0.11 |
| | | | BW | 0.63 ± 0.10 | 0.08 ± 0.03 | 0.72 ± 0.11 |
| | | | D/F | 0.40 ± 0.07 | 0.03 ± 0.01 | 0.43 ± 0.08 |
| | | | Y/S | 0.37 ± 0.07 | 0.08 ± 0.03 | 0.44 ± 0.08 |
| | 2011 | Athens | BR | 0.09 ± 0.03 | 0.12 ± 0.03 | 0.21 ± 0.04 |
| | | | BW | 0.13 ± 0.03 | 0.17 ± 0.04 | 0.30 ± 0.06 |
| | | | D/F | 0.11 ± 0.03 | 0.10 ± 0.03 | 0.21 ± 0.04 |
| | | | Y/S | 0.17 ± 0.04 | 0.11 ± 0.03 | 0.28 ± 0.05 |
| | | Tifton | BR | 0.29 ± 0.05 | 0.02 ± 0.01 | 0.31 ± 0.05 |
| | | | BW | 0.24 ± 0.05 | 0.01 ± 0.01 | 0.25 ± 0.05 |
| | | | D/F | 0.26 ± 0.04 | 0.01 ± 0.01 | 0.27 ± 0.04 |
| | | | Y/S | 0.33 ± 0.08 | 0.03 ± 0.01 | 0.36 ± 0.08 |
| | 2012 | Tifton | BR | 0.45 ± 0.06 | 0.10 ± 0.02 | 0.55 ± 0.07 |
| | | | BW | 0.57 ± 0.09 | 0.05 ± 0.00 | 0.62 ± 0.09 |
| | | | D/F | 0.45 ± 0.06 | 0.13 ± 0.03 | 0.58 ± 0.07 |
| | | | Y/S | 0.46 ± 0.06 | 0.10 ± 0.03 | 0.56 ± 0.07 |

Table 4.1 (cont.)

| Crop | Year | Location | Treatment | # of chewing predators/plant (mean±SE) | # of sucking predators/plant (mean±SE) | Total predators/plant (mean±SE) |
|----------|------|----------|-----------|--|--|------------------------------------|
| Cucumber | 2010 | Athens | CU | 0.29 ± 0.06 | 0.15 ± 0.04 | 0.44 ± 0.07a |
| | | | BW | 0.27 ± 0.08 | 0.13 ± 0.04 | 0.40 ± 0.09a |
| | | | D/F | 0.12 ± 0.04 | 0.12 ± 0.03 | 0.24 ± 0.05b |
| | | | Y/S | 0.24 ± 0.09 | 0.05 ± 0.02 | 0.29 ± 0.09b |
| | 2011 | Athens | CU | 0.40 ± 0.11 | 1.06 ± 0.14a | 1.46 ± 0.18 |
| | | | BW | 0.43 ± 0.10 | 0.99 ± 0.13a | 1.41 ± 0.17 |
| | | | D/F | 0.34 ± 0.08 | 0.56 ± 0.11b | 0.90 ± 0.15 |
| | | | Y/S | 0.46 ± 0.15 | 0.84 ± 0.12ab | 1.30 ± 0.19 |

Table 4.2. Number of predator species/plant for broccoli and cucumber (seasonal means) by year, location, and treatment. For treatment, BR = broccoli, CU = cucumber, BW = buckwheat, D/F = dill and fennel combination, Y/S = yarrow and sunflower combination, and SE = standard error. For species, *L. lin* = *Lygus lineolaris*, *O. ins* = *Orius insidiosus*, *S. inv* = *Solenopsis invicta*, *C. mac* = *Coleomegilla maculata*, *H. axy* = *Harmonia axyridis*, *C. sep* = *Coccinella septempunctata*, and *H. con* = *Hippodamia convergens*. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Crop | Year | Location | Treatment | <i>L. lin</i> /plant (mean±SE) | <i>O. ins</i> /plant (mean±SE) | <i>Geocoris</i> spp./plant (mean±SE) |
|----------|------|----------|-----------|-----------------------------------|-----------------------------------|---|
| Broccoli | 2010 | Athens | BR | NA | 0.26 ± 0.07 | NA |
| | | | BW | NA | 0.24 ± 0.07 | NA |
| | | | D/F | NA | 0.15 ± 0.06 | NA |
| | | | Y/S | NA | 0.26 ± 0.07 | NA |
| | | Tifton | BR | 0.04 ± 0.02 | 0.01 ± 0.01 | NA |
| | | | BW | 0.07 ± 0.03 | 0.00 ± 0.00 | NA |
| | | | D/F | 0.03 ± 0.01 | 0.00 ± 0.00 | NA |
| | | | Y/S | 0.04 ± 0.03 | 0.01 ± 0.01 | NA |
| | 2011 | Athens | BR | 0.08 ± 0.02 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| | | | BW | 0.13 ± 0.04 | 0.02 ± 0.01 | 0.02 ± 0.01 |
| | | | D/F | 0.08 ± 0.03 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| | | | Y/S | 0.08 ± 0.03 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| | | Tifton | BR | NA | 0.01 ± 0.01 | 0.01 ± 0.01 |
| | | | BW | NA | 0.00 ± 0.00 | 0.01 ± 0.01 |
| | | | D/F | NA | 0.01 ± 0.01 | 0.01 ± 0.01 |
| | | | Y/S | NA | 0.01 ± 0.01 | 0.02 ± 0.01 |

Table 4.2 (cont.)

| Crop | Year | Location | Treatment | <i>L. lin</i> /plant (mean±SE) | <i>O. ins</i> /plant (mean±SE) | <i>Geocoris</i> spp./plant (mean±SE) |
|---------------------|------|----------|-----------|-----------------------------------|-----------------------------------|---|
| Broccoli (cont.) | 2012 | Tifton | BR | 0.06 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.01 |
| | | | BW | 0.04 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | D/F | 0.09 ± 0.02 | 0.00 ± 0.00 | 0.01 ± 0.01 |
| | | | Y/S | 0.05 ± 0.02 | 0.01 ± 0.01 | 0.02 ± 0.02 |
| Cucumber | 2010 | Athens | CU | NA | 0.00 ± 0.00 | 0.08 ± 0.03 |
| | | | BW | NA | 0.00 ± 0.00 | 0.07 ± 0.03 |
| | | | D/F | NA | 0.00 ± 0.00 | 0.03 ± 0.02 |
| | | | Y/S | NA | 0.00 ± 0.00 | 0.01 ± 0.01 |
| | 2011 | Athens | CU | 0.01 ± 0.01 | 0.04 ± 0.02 | 1.01 ± 0.14a |
| | | | BW | 0.03 ± 0.02 | 0.11 ± 0.05 | 0.85 ± 0.13ab |
| | | | D/F | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.55 ± 0.11b |
| | | | Y/S | 0.00 ± 0.00 | 0.08 ± 0.04 | 0.76 ± 0.11ab |
| Broccoli | 2010 | Athens | BR | NA | NA | NA |
| | | | BW | NA | NA | NA |
| | | | D/F | NA | NA | NA |
| | | | Y/S | NA | NA | NA |
| | | Tifton | BR | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | | | BW | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.02 |
| | | | D/F | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.01 ± 0.01 |
| | | | Y/S | 0.02 ± 0.02 | 0.01 ± 0.01 | 0.00 ± 0.00 |

Table 4.2. (cont.)

| Crop | Year | Location | Treatment | Reduviidae/plant (mean±SE) | <i>Nabis</i> sp./plant (mean±SE) | <i>S. inv</i> /plant (mean±SE) |
|---------------------|------|----------|-----------|-------------------------------|-------------------------------------|-----------------------------------|
| Broccoli (cont.) | 2011 | Athens | BR | NA | NA | 0.00 ± 0.00 |
| | | | BW | NA | NA | 0.02 ± 0.01 |
| | | | D/F | NA | NA | 0.00 ± 0.00 |
| | | | Y/S | NA | NA | 0.04 ± 0.03 |
| | | Tifton | BR | NA | NA | 0.02 ± 0.01 |
| | | | BW | NA | NA | 0.01 ± 0.01 |
| | | | D/F | NA | NA | 0.03 ± 0.01 |
| | | | Y/S | NA | NA | 0.09 ± 0.07 |
| | 2012 | Tifton | BR | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.03 ± 0.02 |
| | | | BW | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.08 ± 0.04 |
| | | | D/F | 0.00 ± 0.00 | 0.03 ± 0.01 | 0.01 ± 0.01 |
| | | | Y/S | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Cucumber | 2010 | Athens | CU | 0.07 ± 0.03 | NA | 0.00 ± 0.00 |
| | | | BW | 0.06 ± 0.02 | NA | 0.00 ± 0.00 |
| | | | D/F | 0.09 ± 0.03 | NA | 0.00 ± 0.00 |
| | | | Y/S | 0.04 ± 0.02 | NA | 0.08 ± 0.07 |
| | 2011 | Athens | CU | NA | NA | 0.38 ± 0.11 |
| | | | BW | NA | NA | 0.34 ± 0.10 |
| | | | D/F | NA | NA | 0.18 ± 0.07 |
| | | | Y/S | NA | NA | 0.39 ± 0.15 |

Table 4.2. (cont.)

| Crop | Year | Location | Treatment | <i>C. mac</i> /plant (mean±SE) | <i>H. axy</i> /plant (mean±SE) | <i>C. sep</i> /plant (mean±SE) | <i>H. con</i> /plant (mean±SE) |
|----------|------|----------|-----------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Broccoli | 2010 | Athens | BR | NA | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.31 ± 0.07a |
| | | | BW | NA | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.08 ± 0.03b |
| | | | D/F | NA | 0.01 ± 0.01 | 0.03 ± 0.02 | 0.19 ± 0.06ab |
| | | | Y/S | NA | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.21 ± 0.06ab |
| | | Tifton | BR | NA | NA | 0.03 ± 0.03 | 0.40 ± 0.07 |
| | | | BW | NA | NA | 0.02 ± 0.01 | 0.53 ± 0.09 |
| | | | D/F | NA | NA | 0.01 ± 0.01 | 0.36 ± 0.07 |
| | | | Y/S | NA | NA | 0.00 ± 0.00 | 0.37 ± 0.06 |
| | 2011 | Athens | BR | NA | NA | 0.01 ± 0.01 | 0.07 ± 0.02 |
| | | | BW | NA | NA | 0.00 ± 0.00 | 0.09 ± 0.02 |
| | | | D/F | NA | NA | 0.00 ± 0.00 | 0.10 ± 0.03 |
| | | | Y/S | NA | NA | 0.00 ± 0.00 | 0.10 ± 0.03 |
| | | Tifton | BR | NA | 0.01 ± 0.01 | 0.04 ± 0.02 | 0.23 ± 0.04 |
| | | | BW | NA | 0.02 ± 0.01 | 0.05 ± 0.01 | 0.017 ± 0.04 |
| | | | D/F | NA | 0.00 ± 0.00 | 0.06 ± 0.02 | 0.18 ± 0.03 |
| | | | Y/S | NA | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.23 ± 0.05 |
| | 2012 | Tifton | BR | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.26 ± 0.04 |
| | | | BW | 0.04 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.30 ± 0.04 |
| | | | D/F | 0.02 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.30 ± 0.04 |
| | | | Y/S | 0.04 ± 0.02 | 0.04 ± 0.01 | 0.01 ± 0.01 | 0.32 ± 0.05 |

Table 4.2. (cont.)

| Crop | Year | Location | Treatment | <i>C. mac</i> /plant (mean±SE) | <i>H. axy</i> /plant (mean±SE) | <i>C. sep</i> /plant (mean±SE) | <i>H. con</i> /plant (mean±SE) |
|----------|------|----------|-----------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Cucumber | 2010 | Athens | CU | NA | 0.10 ± 0.04 | 0.08 ± 0.03 | 0.11 ± 0.03 |
| | | | BW | NA | 0.06 ± 0.03 | 0.05 ± 0.02 | 0.16 ± 0.05 |
| | | | D/F | NA | 0.03 ± 0.02 | 0.04 ± 0.02 | 0.05 ± 0.02 |
| | | | Y/S | NA | 0.02 ± 0.01 | 0.04 ± 0.02 | 0.10 ± 0.04 |
| | 2011 | Athens | CU | NA | 0.03 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00b |
| | | | BW | NA | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.09 ± 0.03ab |
| | | | D/F | NA | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.15 ± 0.04a |
| | | | Y/S | NA | 0.00 ± 0.00 | 0.03 ± 0.02 | 0.05 ± 0.02b |

Table 4.3. Relative abundance of each species (%) for broccoli and cucumber by year and location. For species, *L. lin* = *Lygus lineolaris*, *O. ins* = *Orius insidiosus*, *S. inv* = *Solenopsis invicta*, *C. mac* = *Coleogomegilla maculata*, *H. axy* = *Harmonia axyridis*, *C. sep* = *Coccinella septempunctata*, and *H. con* = *Hippodamia convergens*.

| Crop | Year | Location | Relative abundance (%) | | | | |
|----------|------|----------|------------------------|---------------|---------------------|------------|------------------|
| | | | <i>L. lin</i> | <i>O. ins</i> | <i>Geocoris</i> sp. | Reduviidae | <i>Nabis</i> sp. |
| Broccoli | 2010 | Athens | NA | 52.10 | 0.00 | NA | NA |
| | | Tifton | 8.80 | 0.80 | NA | 1.30 | 0.80 |
| | 2011 | Athens | 39.80 | 5.40 | 9.00 | NA | NA |
| | | Tifton | NA | 2.10 | 3.00 | NA | NA |
| | 2012 | Tifton | 13.00 | 0.80 | 2.20 | 0.30 | 3.00 |
| | | | | | | | |
| Cucumber | 2010 | Athens | NA | 0.00 | 13.90 | 19.00 | NA |
| | 2011 | Athens | 1.00 | 4.40 | 62.60 | NA | NA |

Table 4.3 (cont.)

| Crop | Year | Location | Relative abundance (%) | | | | |
|----------|------|----------|------------------------|---------------|---------------|---------------|---------------|
| | | | <i>S. inv</i> | <i>C. mac</i> | <i>H. axy</i> | <i>C. sep</i> | <i>H. con</i> |
| Broccoli | 2010 | Athens | NA | NA | 0.70 | 2.10 | 45.00 |
| | | Tifton | 1.70 | NA | NA | 2.90 | 83.60 |
| | 2011 | Athens | 6.60 | NA | NA | 0.60 | 38.60 |
| | | Tifton | 12.40 | NA | 1.70 | 12.80 | 67.90 |
| | 2012 | Tifton | 6.50 | 5.70 | 4.10 | 1.10 | 63.40 |
| | | | | | | | |
| Cucumber | 2010 | Athens | 5.80 | NA | 15.30 | 15.30 | 30.70 |
| | 2011 | Athens | 25.10 | NA | 0.50 | 0.70 | 5.70 |

Table 4.4. Total % predation of beet armyworm eggs/plant by 72 h after egg placement, % sucked beet armyworm eggs/plant by 72 h, and % chewed beet armyworm eggs/plant by 72 h (seasonal means) by crop, year, location, and treatment. For treatment, BR = broccoli, CU = cucumber, BW = buckwheat, D/F = dill and fennel combination, Y/S = sunflower and yarrow combination, and SE = standard error. Means within year by location followed by letters that are the same are not significantly different. ($P > 0.05$, LSD).

| Crop | Year | Location | Treatment | % chewed/plant (mean \pm SE) | % sucked/plant (mean \pm SE) | % predation/plant (mean \pm SE) |
|----------|------|----------|-----------|-----------------------------------|-----------------------------------|--------------------------------------|
| Broccoli | 2010 | Athens | BR | 56.40 \pm 6.30 | 28.40 \pm 4.90 | 97.50 \pm 1.70 |
| | | | BW | 64.80 \pm 6.50 | 18.70 \pm 4.70 | 94.60 \pm 3.00 |
| | | | D/F | 73.50 \pm 5.80 | 20.70 \pm 4.40 | 94.20 \pm 2.80 |
| | | | Y/S | 62.20 \pm 6.30 | 23.60 \pm 4.60 | 91.10 \pm 3.40 |
| | | Tifton | BR | 24.40 \pm 3.70 | 38.40 \pm 3.90 | 62.90 \pm 3.90 |
| | | | BW | 26.60 \pm 3.90 | 42.10 \pm 3.80 | 68.30 \pm 3.40 |
| | | | D/F | 28.50 \pm 4.10 | 39.20 \pm 4.10 | 67.70 \pm 4.00 |
| | | | Y/S | 24.80 \pm 3.90 | 40.70 \pm 3.80 | 65.20 \pm 3.40 |
| | 2011 | Athens | BR | 17.50 \pm 3.00 | 25.60 \pm 2.80 | 43.10 \pm 3.40 |
| | | | BW | 21.90 \pm 3.30 | 26.30 \pm 2.60 | 48.20 \pm 3.70 |
| | | | D/F | 17.40 \pm 3.10 | 21.50 \pm 2.10 | 38.90 \pm 3.20 |
| | | | Y/S | 17.00 \pm 2.90 | 30.20 \pm 2.80 | 47.10 \pm 3.30 |
| | | Tifton | BR | 16.90 \pm 2.90 | 17.10 \pm 3.20 | 34.00 \pm 3.70 |
| | | | BW | 11.60 \pm 2.30 | 12.50 \pm 2.40 | 24.00 \pm 3.00 |
| | | | D/F | 17.10 \pm 2.40 | 18.50 \pm 3.40 | 35.60 \pm 3.80 |
| | | | Y/S | 16.20 \pm 2.30 | 16.50 \pm 2.60 | 32.80 \pm 2.90 |

Table 4.4 (cont.)

| Crop | Year | Location | Treatment | % chewed/plant (mean±SE) | % sucked/plant (mean±SE) | % predation/plant (mean±SE) |
|----------|------|----------|-----------|-----------------------------|-----------------------------|--------------------------------|
| Cucumber | 2012 | Tifton | BR | 23.30 ± 2.50 | 15.50 ± 1.80 | 41.40 ± 2.90 |
| | | | BW | 20.90 ± 2.40 | 14.40 ± 1.70 | 38.80 ± 2.90 |
| | | | D/F | 24.10 ± 2.60 | 12.30 ± 1.40 | 39.90 ± 2.90 |
| | | | Y/S | 24.60 ± 2.60 | 11.00 ± 1.50 | 39.70 ± 3.00 |
| | 2010 | Athens | CU | 74.40 ± 4.70 | 25.40 ± 4.60 | 99.80 ± 0.10 |
| | | | BW | 69.10 ± 5.10 | 30.70 ± 5.10 | 99.70 ± 0.20 |
| | | | D/F | 69.80 ± 5.10 | 26.80 ± 5.00 | 96.60 ± 1.90 |
| | | | Y/S | 74.70 ± 4.80 | 25.00 ± 4.80 | 99.70 ± 0.20 |
| | 2011 | Athens | CU | 61.90 ± 5.00 | 9.70 ± 2.60 | 95.50 ± 1.90 |
| | | | BW | 64.10 ± 5.10 | 7.50 ± 2.50 | 94.30 ± 2.60 |
| | | | D/F | 62.20 ± 5.00 | 10.10 ± 2.70 | 96.40 ± 1.40 |
| | | | Y/S | 54.30 ± 5.20 | 9.50 ± 2.70 | 85.00 ± 4.20 |

Table 4.5. Treatment ANOVA table for total % predation of beet armyworm eggs/plant by 72 h following egg placement, % sucked beet armyworm eggs/plant by 72 h, and % chewed of beet armyworm eggs/plant by 72 h, by crop, year, and location. P value followed by * is significant at $\alpha = 0.05$.

| Crop/variable | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|--------------------|------|----------|-----|------|-------------|---------|--------|
| Broccoli (chewed) | 2010 | Athens | 3,9 | 0.48 | 0.16 | 0.44 | 0.73 |
| | | Tifton | 3,9 | 0.11 | 0.04 | 0.21 | 0.89 |
| | 2011 | Athens | 3,9 | 0.28 | 0.09 | 0.61 | 0.63 |
| | | Tifton | 3,9 | 0.36 | 0.12 | 1.40 | 0.31 |
| | 2012 | Tifton | 3,9 | 0.28 | 0.09 | 0.59 | 0.64 |
| | | | | | | | |
| Cucumber (chewed) | 2010 | Athens | 3,9 | 0.34 | 0.11 | 0.19 | 0.90 |
| | 2011 | Athens | 3,9 | 0.94 | 0.31 | 1.51 | 0.28 |
| Broccoli (sucked) | 2010 | Athens | 3,9 | 0.62 | 0.21 | 0.81 | 0.52 |
| | | Tifton | 3,9 | 0.09 | 0.03 | 0.10 | 0.96 |
| | 2011 | Athens | 3,9 | 0.65 | 0.22 | 1.87 | 0.20 |
| | | Tifton | 3,9 | 0.31 | 0.10 | 0.67 | 0.59 |
| | 2012 | Tifton | 3,9 | 0.59 | 0.20 | 1.90 | 0.20 |
| | | | | | | | |
| Cucumber (sucked) | 2010 | Athens | 3,9 | 0.17 | 0.06 | 0.08 | 0.97 |
| | 2011 | Athens | 3,9 | 0.13 | 0.04 | 0.45 | 0.72 |

Table 4.5 (cont.)

| Crop/variable | Year | Location | DF | SS | Mean Square | F Value | Pr > F |
|--------------------------|------|----------|-----|------|-------------|---------|--------|
| Broccoli (all predation) | 2010 | Athens | 3,9 | 0.27 | 0.09 | 1.24 | 0.35 |
| | | Tifton | 3,9 | 0.34 | 0.11 | 1.92 | 0.20 |
| | 2011 | Athens | 3,9 | 0.99 | 0.33 | 1.23 | 0.35 |
| | | Tifton | 3,9 | 1.12 | 0.37 | 2.05 | 0.18 |
| | 2012 | Tifton | 3,9 | 0.38 | 0.13 | 0.58 | 0.64 |
| | | | | | | | |
| Cucumber (all predation) | 2010 | Athens | 3,9 | 0.19 | 0.06 | 2.44 | 0.13 |
| | 2011 | Athens | 3,9 | 1.26 | 0.42 | 1.62 | 0.25 |

Fig. 4.1. Percent predation of beet armyworm eggs/plant by 24, 48, and 72 hrs after placement in broccoli for each treatment, location and year. The bars on the points are SEM bars.

Figure 4.1

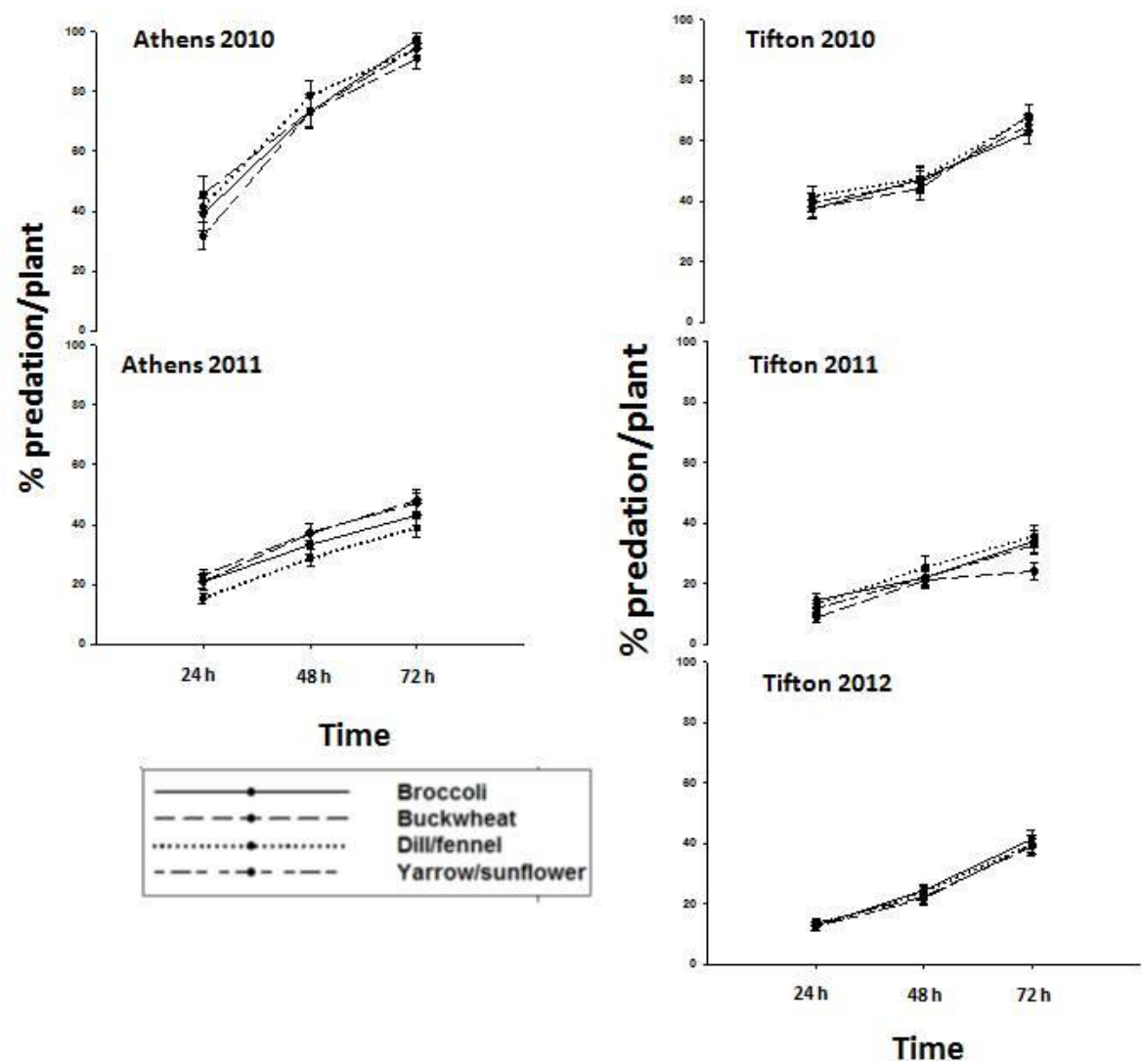
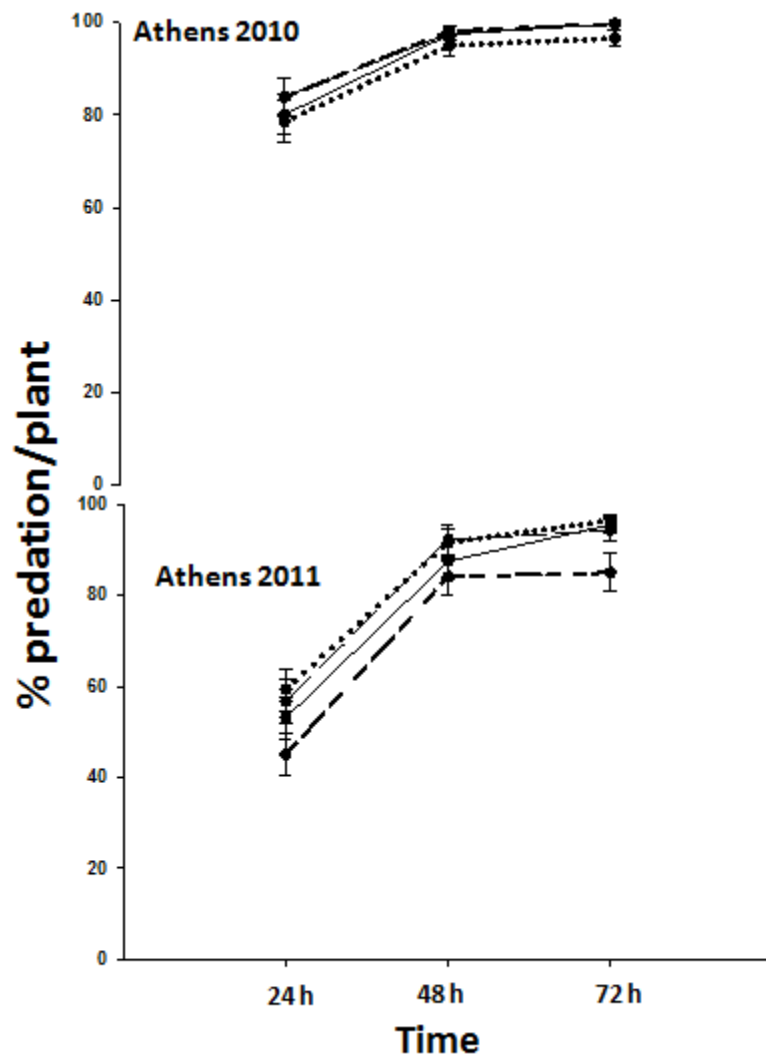


Fig. 4.2. Percent predation of beet armyworm eggs/plant by 24, 48, and 72 hrs after placement in cucumber for each treatment and year in Athens. The bars on the points are SEM bars.



CHAPTER 5

CONCLUSION

There is overwhelming evidence that floral resources can benefit natural enemies, with respect to enhancing their fitness (supported by the present study). But translating this into biological control and pest management has proven to be more difficult. This is mainly due complex interactions among natural enemies, pests, and flowering plant in the field. The answer may lie in developing a farmscaping system that is unique to a cropping system and location rather than system with broad applications. Despite the negligible impact of floral farmscaping in the present study, a future study that takes into account the area occupied by the insectary plants relative the target crop and increases the distance between the plots, to eliminate any neutralizing effects their proximity might yield results that separate treatment effects.