ABSTRACT

Title of Document: THE INFLUENCE OF PREDATOR SPECIES RICHNESS ON PREY MORTALITY: IMPLICATIONS TO CONSERVATION BIOLOGICAL CONTROL.

Scott Asher Lewins, Master of Science, 2006

Directed By: Professor, Pedro Barbosa, Department of Entomology

Understanding how changes in biodiversity affect the function of agroecosystems is paramount to conservation biological control. The Species Assemblage Control Hypothesis predicts increasing species richness of predator assemblages can increase the assemblages' ability to suppress pests. I hypothesized that an increase in species richness of a predator assemblage leads to an increase in prey mortality and predator species identity can alter the relationship. An assemblage of predators identified from an assessment of a collard agroecosystem was evaluated to find that only some predators fed on larval *Pieris rapae*, they did not differ in their per capita consumption, and some intraguild predation occurred. In testing the hypotheses I found a significant relationship between predator species richness and prey mortality; however, predator species identity altered the relationship. These findings highlight the importance in understanding predator assemblages before conservation decisions that effectively suppress pests can be made.

THE INFLUENCE OF PREDATOR SPECIES RICHNESS ON PREY MORTALITY: IMPLICATIONS TO CONSERVATION BIOLOGICAL CONTROL.

By

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Thesis submitted to the Faculty of the Graduate School of the
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Dr. Pedro Barbosa, Chair
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Acknowledgements

I have a great many people to thank. My research committee members Drs. Paula Shrewsbury, Galen Dively and my major advisor, Dr. Pedro Barbosa for their feedback and support throughout this project. Carlo Moreno, my collaborator, with whom I shared much of the pain and enjoyment of this project. Members of the Barbosa lab, Dr. John Kemper, Dr. Astrid Caldas, Dr. Raul Medina, Eric Lind, Megan Paustian, and Gwen Shlicta, research assistants Kelsey Phelps, Rebeka Friedrich and Nat Lichten, and UMD farm managers Kevin Conover, Mike Newell and Mark Spicknall were all integral in the completion of this project. Identifications were made with the help of Dr. Michael Gates, Dr. Thomas Henry, and Dr. Charles Staines. A special thanks to Grace Pazdan for her endless support and undying love during this undertaking.

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For hundreds of years citrus growers in ancient China would place predaceous ant colonies, *Oecophylla smaragdina subnitida* Emery, between trees to protect their harvest (DeBach 1964). The use of predators as biological control agents in conventional U.S. agriculture began with the introduction of the Vedalia Beetle, *Rodolia cardinalis* Mulsant, in 1889 to combat cottony cushion scale, *Icerya purchasi* Maskell, an introduced species that was threatening the citrus industry in California at that time (Doutt 1967, Caltagirone 1981). The latter is an example of classical biological control. In this approach to biological control, an exotic natural enemy, i.e. a parasitoid, pathogen or predator, is intentionally introduced from the region of origin of the pest to the new region of the pest for the purpose of establishing long-term suppression usually of a coevolved pest (Eilenberg et al. 2001). Augmentative biological control is the repeated release of native or non-native natural enemies by way of mass rearing programs in order to increase their abundance in habitats where their populations are low or non-existent. Both classical and augmentative biological control have historically been where much of the success in biological control has been achieved (Caltagirone 1981, Denoth et al. 2002). However, in addition to the successes there have been numerous examples in which the release of an introduced species for the purpose of biological control of a pest has resulted in negative consequences to non-target species (Howarth 1991, Pearson and

Callaway 2003, Stiling 2004). It is perhaps for this reason that a third approach, conservation biological control, has recently received increasing interest among researchers. Conservation biological control employs tactics that enhance the survival and/or performance of native natural enemies in order to enhance their effectiveness (Barbosa 1998). This can be achieved through the manipulation of the habitat in ways that benefit natural enemies such as providing alternative food like pollen or nectar, providing a microclimate for natural enemies to seek refuge from environmental extremes or pesticides, and providing habitat for alternative prey (Landis et al. 2000, Barbosa et al. 2005).

In contemporary agriculture, generalist predators have typically been viewed as ineffective at natural suppression of pests and have been neglected as biological control agents in comparison to their specialists counterparts (predators and parasitoids) (Chang and Kareiva 1999). Despite this disparity, there is little evidence that suggests indigenous generalist predators cannot effectively suppress pests. A 2002 review of generalist predators found that in about 75% of manipulative field studies, generalist predators significantly reduced pest numbers (Symondson et al. 2002). Further, they found that assemblages (sensu Claridge 1987) of generalist predators were equally as effective at lowering pest numbers as single predators, and in most cases, their predation led to an increase in yield or decrease in plant damage. More recently, the increased interest in the effect of biodiversity on ecosystem function (Chapin et al. 2000, Loreau 2000, Tilman 2000) has resulted in a corresponding increase in research on how the biodiversity of predator assemblages affect their ability to control pests (Ives et al. 2005, Snyder et al. 2005, Wilby et al.

2005, Straub and Snyder 2006). The evidence suggests that increased biodiversity can enhance biological control. But is all of the biodiversity of predator assemblages necessary for effective biological control? If the entire assemblage is not necessary for effective pest suppression, then conservation efforts could focus on tactics that benefit the subset of the assemblage actually responsible for pest suppression. Current conservation biological control tactics may not benefit all predators similarly (Barbosa et al. 2005). Therefore, it may be more appropriate to evaluate the effect of potential tactics on the assemblage subset responsible for suppression. In order to identify the subset of a predator assemblage most likely to impose mortality on the target pest, it is necessary to determine (a) what predators, potentially interacting with the target pest actually feed on the target pest, (b) the relative effectiveness and differences in effectiveness among the predators that feed on the target pest, and (c) whether the predators in the group that do consume the target pest engage in any antagonistic interactions like intraguild predation, which can potentially reduce the impact of the assemblage.

The increased interest in the influence of biodiversity on ecosystem function has highlighted the importance of understanding how the species in a predator assemblage interact and ultimately influence the assemblage's ability to suppress pests. Research has shown that increasing natural enemy species richness (a measure of biodiversity) can lead to decreased biocontrol because of negative interactions like interference, cannibalism and intraguild predation (Rosenheim et al. 1993, Hochberg 1996, Denoth et al. 2002). Debach (1974) went as far as suggesting that biological control would be most effective if relatively few specialized natural enemies were

employed as biological control agents. That is, with agents that are able to survive within the same range of the pest, capable of high reproductive capacity relative to the pest and highly efficient at searching for the pest. The Species Assemblage Control Hypothesis (SACH) epitomizes the opposing view. Central to SACH is the belief that an assemblage of generalist predators, if conserved properly, can suppression associated prey populations more effectively than a single predator species (Riechert and Lockley 1984, Provencher and Riechert 1994, Riechert and Lawrence 1997). Riechert and Lockley (1984) argue that certain characteristics of generalist predator assemblages (spiders in this case) make the assemblage as a whole more effective then any single agent of natural suppression. They contend that the assemblage is self-dampening because of factors like cannibalism and intraguild predation, lending stability in periods of low prey availability. In times of high prey availability, the assemblage can exhibit a strong numerical response through aggregation and reproduction. They go on to suggest that as predator richness in an assemblage increases, a more diverse set of foraging behaviors and sizes should be present, which should enhance the probability that prey of various sizes and species will be killed. This anticipated advantage of greater species diversity is what has been term sampling effect (Loreau et al. 2001). On the other hand, biological control theory predicts that increasing predator richness should lead to an increase in potentially negative predator-predator interactions, and limit effective suppression of pests (DeBach 1974, Rosenheim et al. 1995). However, there have been examples of predator assemblages suppressing pests despite the occurrence of negative predator-predator interactions (Lang 2003, Snyder and Ives 2003, Snyder et al. 2004).

The relationship between biodiversity of predator assemblages and pest suppression is of great importance due to recent trends in agriculture that have led to a loss of predator biodiversity (Snyder et al. 2005). Increased biodiversity has been shown to increase pest suppression (Cardinale et al. 2003, Snyder et al. 2006). The goal of this study was to elucidate the relationship between biodiversity and pest suppression, specifically the relationship between species richness of a predator assemblage and pest suppression.

Larvae of the cabbage white butterfly, *Pieris rapae* Linnaeus, are significant economic pests of crucifers worldwide (Schmaedick and Shelton 2000) and in Maryland (G. Dively personal communication). Larvae are preyed on by a myriad of generalist predators (Dempster 1967, Ashby 1974, Schmaedick and Shelton 2000). Life tables constructed by Dempster (1967) indicated that 1st instars are the most vulnerable life stage. Assemblages of generalist predators, which could potentially affect 1st instar *P. rapae,* have been identified in crucifers across a wide range of geographic regions such as Japan (Suenaga and Hamamura 2001), Hawaii (Hooks et al. 2003), and the Northwestern (Snyder et al. 2006) and Midwestern continental United States (Schellhorn and Sork 1997). Therefore this study system, consisting of collards, *P. rapae* and the associated assemblage of generalist predators found on collards in Maryland, is an appropriate focus for research on the relationship between predator species richness and pest suppression.

Mesocosm studies, using protocols that are scaled-down versions of field experiments have been used by researchers as an intermediate approach between field studies and laboratory Petri dish studies (Dinter 2002, Finke and Denno 2004,

Madsen et al. 2004). Laboratory studies done in the sterile environment of a small arena (such as a Petri dish) may be over simplified. The circumstances created in these simplified arenas may foster interactions that are unlikely to occur in the field. Conversely field studies, although most relevant in an applied context, can be costly, time intensive, and logistically impractical. Mesocosms provide a useful alternative. By conducting this study in mesocosms I was able to simulate, to a certain extent, the environment present in a collard field, while at the same time maintaining certain experimental parameters constant. The results can then be used to infer what might take place under field conditions.

Given that some assemblages of predators have been shown to be more effective at controlling pests than a single predator species, the number and identity of predator species in an assemblage influences the assemblages effectiveness, and that interactions between predator species may impact the effectiveness of the assemblage, I chose to explore two questions. Does the level of pest suppression provided by the assemblage increase as the number of predator species in an assemblage increases? And, how does species identity of predators in the assemblage affect the relationship between predator species richness and pest suppression? Thus, I hypothesized that an increase in the species richness of an assemblage of predators leads to an increase in prey mortality imposed by the assemblage. Further, I hypothesized that predator species identity can alter the relationship between species richness of a predator assemblage and prey mortality.

In order to test these hypotheses it was necessary to accomplish the following objectives: (1) I evaluated the assemblage of generalist arthropod predators found in collard agroecosystems in Maryland to assess the relative abundance of species in the assemblage and their identity, (2) I then further evaluated the predators identified through this assessment to confirm that they fed on larval *P. rapae*, their relative effectiveness and differences in consumption of *P. rapae* larvae, and determine if they engaged in intraguild predation in the absence of *P. rapae,* (3) I then tested the hypotheses that increasing predator species richness increases the mortality imposed on larval *P. rapae* and that predator species identity can alter the relationship between species richness of an predator assemblage and prey mortality using the cohort of predator species selected as a result of the evaluations.

Objective (1) provided information on the assemblage of generalist arthropod predators found in collard agroecosystems in Maryland. The assessment of the predator assemblages within each microhabitat (epigeal, aerial, and foliar) was conducted with three common sampling methods: pitfall trapping, sweep, and visual sampling. From this assessment, I generated species abundance distributions (SADs) for each of the microhabitats in order to identify the numerically dominant and subdominant predators. It is from the SADs that I identified a subset of predators in their presumed order of importance (assuming the most abundant species present is the most important in the suppression of the focal pest species P. rapae). Predators most likely to interact with P. rapae larvae were identified to the species level. For objective (2) I performed a series of feeding trials in small mesocosms wherein I first determined which of the predators present in the collard agroecosystem actually fed

on P. rapae larvae by comparing P. rapae mortality in the presence of each predator species to that of the no predator control. Then, I estimated the per capita consumption for the predators that were determined to feed on P. rapae using a second more rigorous series of feeding trials where a no predator control was used to adjust for background mortality. In order to evaluate the potential for intraguild predation to occur between those predators, I undertook a third set of trials in which combinations of two individuals of a different species were combined in the absence of prey. The results of the feeding trials (objective 2) enabled me to choose the appropriate predators for the experimental assemblage needed to test my hypotheses. In objective (3), I tested my hypotheses by manipulating the number of predator species in assemblages within larger mesocosms to determine if there were significant changes in larval P. rapae mortality as species richness increased, and explored the affect species identity had on the hypothesized relationship.

Chapter 2: Evaluation of Predator Community Affecting *Pieris rapae* on Collards in Maryland.

Introduction

In contemporary agriculture, generalist predators have typically been viewed as ineffective at natural suppression of pests and have been neglected as biological control agents in comparison to their specialists counterparts (predators and parasitoids) (Chang and Kareiva 1999). Despite this disparity, there is little evidence that suggests indigenous predators cannot effectively suppress pests. A 2002 review of generalist predators in biological control found that in about 75% of manipulative field studies, generalist predators significantly reduced pest numbers (Symondson et al.). Further, they found that assemblages (sensu Claridge 1987) of generalist predators were equally as effective at lowering pest numbers as single predators, and in most cases, their predation led to an increase in yield or decrease in plant damage. More recently, the increased interest in the effect of biodiversity on ecosystem function (Chapin et al. 2000, Loreau 2000, Tilman 2000) has resulted in a corresponding increase in research on how the biodiversity of predator assemblages affect their ability to suppress pests (Ives et al. 2005, Snyder et al. 2005).

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Methods

Study Sites

Study sites included the Wye Research and Education Center (WREC) on the eastern shore of Maryland and the Central Maryland Research and Education Center (CMREC) - Upper Marlboro (UP) facility. Two plots of collard greens (Vates variety) were planted on May 2 and June 1, 2004 at WREC and on May 8, 2004 at

CMREC. The collards were grown using standard agricultural practices. The two plots at WREC were conventionally tilled, and the two at UP were not tilled. The plots were all 23 x 33m and the rows in a plot were approximately 1 meter apart from each other. There were 25 rows per plot at WREC and 26 per plot at UP.

Community Assessment

The community of arthropods on collards, including *P. rapae* and the assemblages of generalist predators in three microhabitats (epigeal, foliar and aerial), were assessed once a week for six weeks starting five weeks after the collards were planted (from early June to late July 2004). The assessment of the predator assemblages within each microhabitat was conducted with the following common sampling methods: pitfall traps (for epigeal), sweeping (aerial), and collections by hand (foliar).

The pitfall trap sampling within the epigeal microhabitat was conducted using two 473ml clear plastic cups (with a 9.7 cm diameter opening; Solo Cup Co.®, Urbana, Illinois), one inside the other. A plastic plate (Solo Cup Co. ®, Urbana, Illinois) serving as a roof and fastened 2 cm above the trap with three 7.62cm bolts was used to prevent the cups filling with rain water. Approximately 60ml of automobile antifreeze was added to each trap, as a killing agent and preservative. Once weekly, pitfalls were left in the field for 24 hours, after which their contents emptied. Nine traps were placed in 1m X 1m grids within each plot. This was accomplished by replacing a plant, every eight meters of every eighth row, thus each pitfall was approximately 8m apart from adjacent traps (Figure 2.1). The contents of each trap were emptied into a clean plastic cup, labeled according to site, date, time, plot No., row No., location within row, and identified as a pitfall collection. Samples

were brought back to the lab, washed, and stored in 95% EtOH until they were identified.

The community inhabiting the aerial microhabitat was sampled with sweep nets. A standard 30cm diameter sweep-net composed of linen net was used for sweep samples. The samples consisted of ten replicate sweep transects; each comprised of ten double swings taken while walking ten paces. Each sweep was initiated at a randomly selected row and starting point using a random numbers table and sweeps sampled the air immediately above the plants. The first and last five rows, as well as the first and last eight meters inward from plot edges (Figure 2.1) were excluded from sampling to minimize edge effect. The sweeping took place once a week at the approximate same time of day, for six weeks. The contents of each replicate sweep were individually bagged, labeled according to site, date, time, plot No., row No., location within row, identified as a sweep sample and kept on ice until brought back to the lab where they were placed into 95% EtOH for later identification.

The foliar assemblage of arthropods actually represented the assemblage of arthropods found on foliage as well as stems but was described as "foliar assemblage." It was sampled by visually inspecting individual collard plants and hand collecting all arthropods. The visual inspection of all the above ground parts of ten randomly selected collard plants was conducted by carefully searching the individual plant for five minutes. All of the arthropods found on the observed plant were collected into glass vials containing 95% EtOH with the aid of feather forceps (BioQuip®, Rancho Dominguez, CA). The first eight meters from the edge along the entire perimeter of the plots (Figure 2.1) were excluded in the visual sampling to

minimize edge effect. The samples were all taken at the approximate same time every week. The vials were labeled according to site, date, time, plot No., row No., location within row, identified as a visual sample, and brought back to the lab for identification.

Constructing Species Abundance Distributions

Species abundance distributions (SADs) for each of the microhabitats were generated in order to compare the structures of the predator communities in each microhabitat, with the aim of identifying which species were numerically dominant and subdominant predators. The abundances of all predator morphospecies collected from the four plots were pooled to construct SADs for each microhabitat separately. All life stages were included in the SADs. It is from the SADs that I generated a list of predators in rank order to provide insight into their potential importance as predators of *P. rapae* (assuming the most abundant species present is the most important).

Results/Discussion

Due to the large number of individuals collected, and the difficulty in identifying to the species level, specimens were identified to family and sorted by species and morphospecies. In the foliar microhabitat, 239 individuals representing 8 species and 44 morphospecies, were collected (Figure 2.2). Sweeping of the aerial microhabitat yielded 567 individuals representing 14 species and 31 morphospecies (Figure 2.3). From within the epigeal microhabitat, 3060 individuals representing 19 species and 151 morphospecies were collected (Figure 2.4). Abundance distributions

for the microhabitats were informative because they show the relative abundances for each morphospecies. Barbosa et al. (2005) developed a method of comparing assemblages using "Robin's Curves," however statistical comparisons of SADs between assemblages found in the different microhabitats would not be appropriate because not all of the individuals collected have been identified to species.

Although there were numerous predator species in all three microhabitats, not all are predators of *P. rapae*. Certain foraging behaviors and life-history traits of predators, particularly in relation to the traits and behavior of *P. rapae* make it unlikely that they would find, attack and consume larval *P. rapae*. For example, given that larval *P. rapae* rarely, if ever, leave their natal plant except to pupate (Harcourt 1961, Jones 1977) the predator species most likely to be important mortality factors would be in the foliar microhabitat. Within the foliar microhabitat, only the adults of each species were considered, because testing both adults and larvae/nymphs of each predator species would have created far too many combinations to evaluate. Further, identification of immatures was not always possible (especially spiders). Web-building spiders were excluded from consideration as *P. rapae* predators because they would be unlikely to interact with relatively sessile larval *P. rapae*. Social predators, such as ants, also were excluded because their social behavior makes testing individual predators difficult and unlikely to produce accurate data since the use of nests in mesocosm trials would be unfeasible. The remaining predators in the foliar predator assemblage were *Nabis roseipennis, Coleomegilla maculata, Lygus lineolaris, Coccinella septempunctata, Podisus maculiventris, Geocoris punctipes,* and *Chauliognathus marginatus* (Figure 2.5).

Lycosids and carabids feed on *P. rapae* larvae (Dempster 1967, Ashby 1974, Schmaedick and Shelton 2000), so *Pardosa sp* (Lycosidae) and *Pterostichus lucublanduds* (Carabidae), two numerically dominant predators found in the epigeal microhabitat (216 and 116 individuals collected, respectively), also were evaluated. After confirming the status of these predators as *P. rapae* predators, I was then able to use them to test the relationship between increases in predator species richness and increases in larval *P. rapae* mortality and how the relationship is affected by predator species identity.

The subsets of generalist predator assemblages responsible for suppression of *P. rapae* in collard agroecosystems in other regions are very similar to those in collards in Maryland. The common predators of 1st instar *P. rapae* on collards in New York state include *C. maculata, Nabis americoferus, Lygus lineolaris, Orius insidiosus,* and *Pardosa milvina* (Schmaedick and Shelton (2000). Predators of aphids on collards, and potentially *P. rapae,* in Washington state include *C. maculata, C. septempunctata, Geocoris pallens,* and a combination of *N. americoferus* and *Nabis alternatus* (Snyder et al. (2006). The only two predator species selected to be apart of the assemblage tested in mesocosms that haven't been reported in crucifers was *P. maculiventris*, which is commonly known to feed on lepidopteran larvae, and *C. marginatus*, which was found along the collard field margins in great numbers during the 2005 season.

From this evaluation of the assemblage of generalist arthropod predators found in collard agroecosystems in Maryland, I was able to ascertain those predators most likely to interact with, and inevitably influence *P. rapae* populations. The

predators were then tested in mesocosm trials to confirm that they fed on larval *P. rapae*, and determine if they would feed on each other in the absence of *P. rapae* (see chapter 3). The assemblage was ultimately tested in mesocosms to examine the relationship between species richness of a predator assemblage and mortality of larval *P. rapae* imposed by the assemblage and the influence of predator identity on that relationship (see chapter 4).

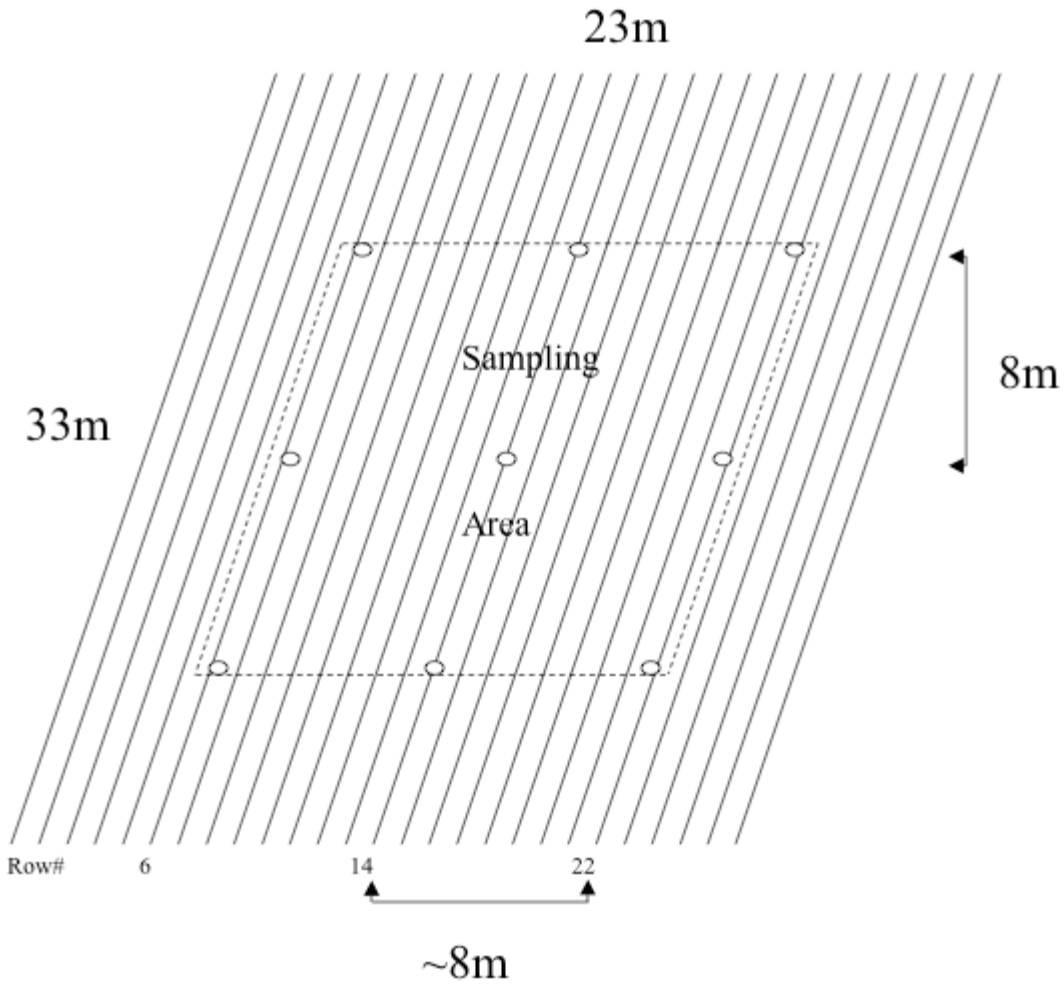

Figure 2.1. Sampling design

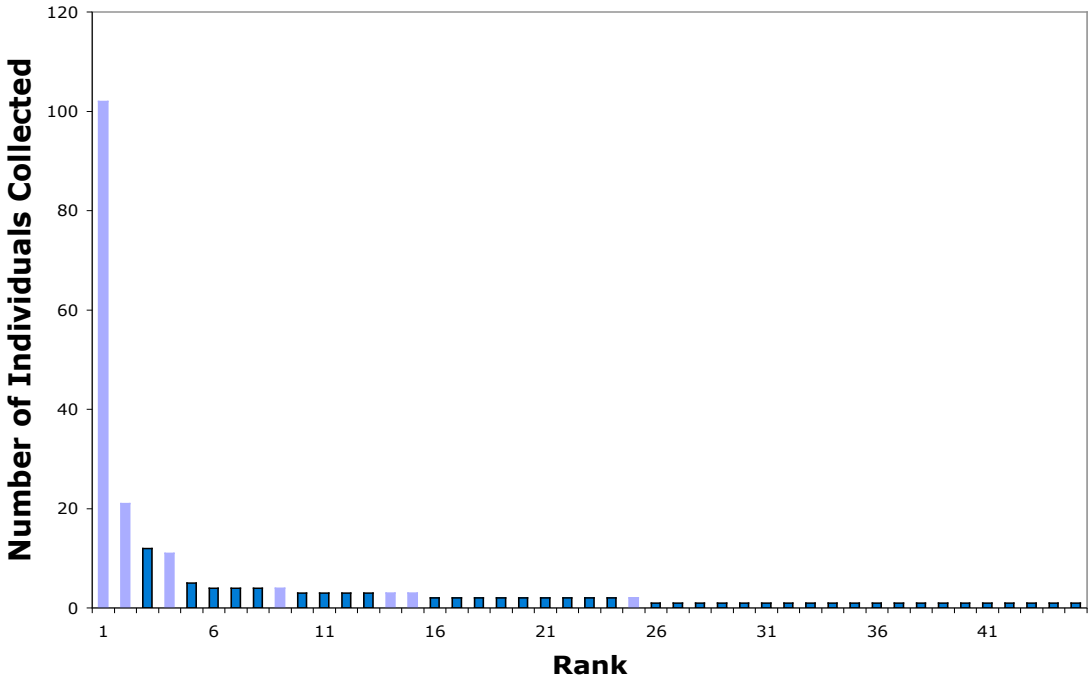

Figure 2.2. Foliar abundance distribution. Abundance distribution of all foliar predator morpho-species collected during sampling period from June through August 2004. Darkened bars represent web-building, social arthropods.

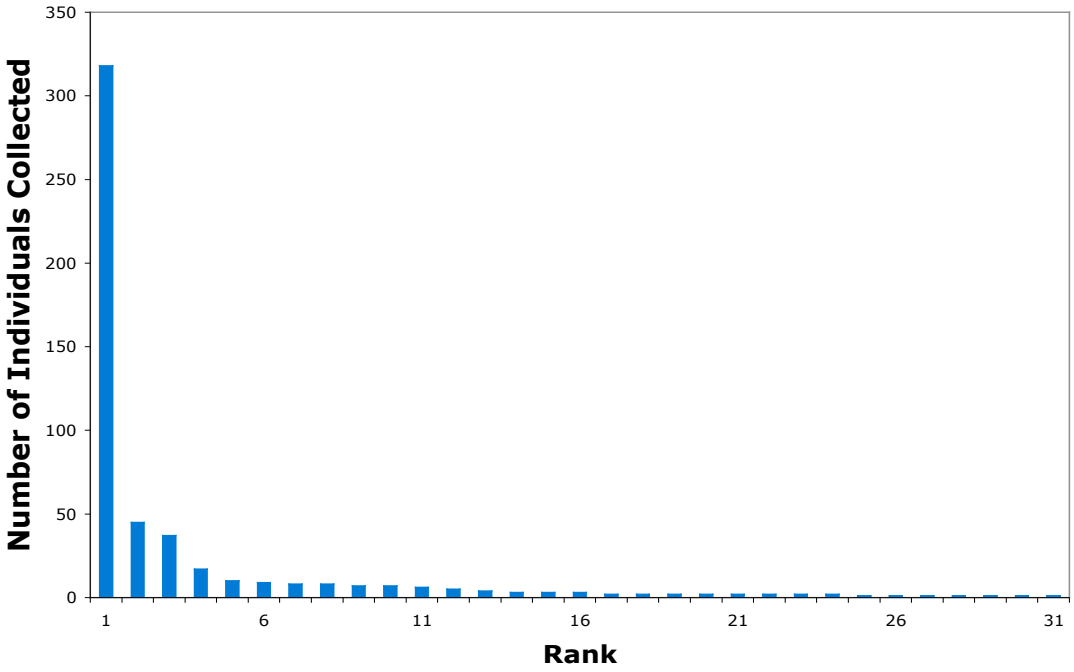

Figure 2.3. Aerial abundance distribution. Abundance distribution of aerial predator morpho-species species collected during sampling period from June through August 2004.

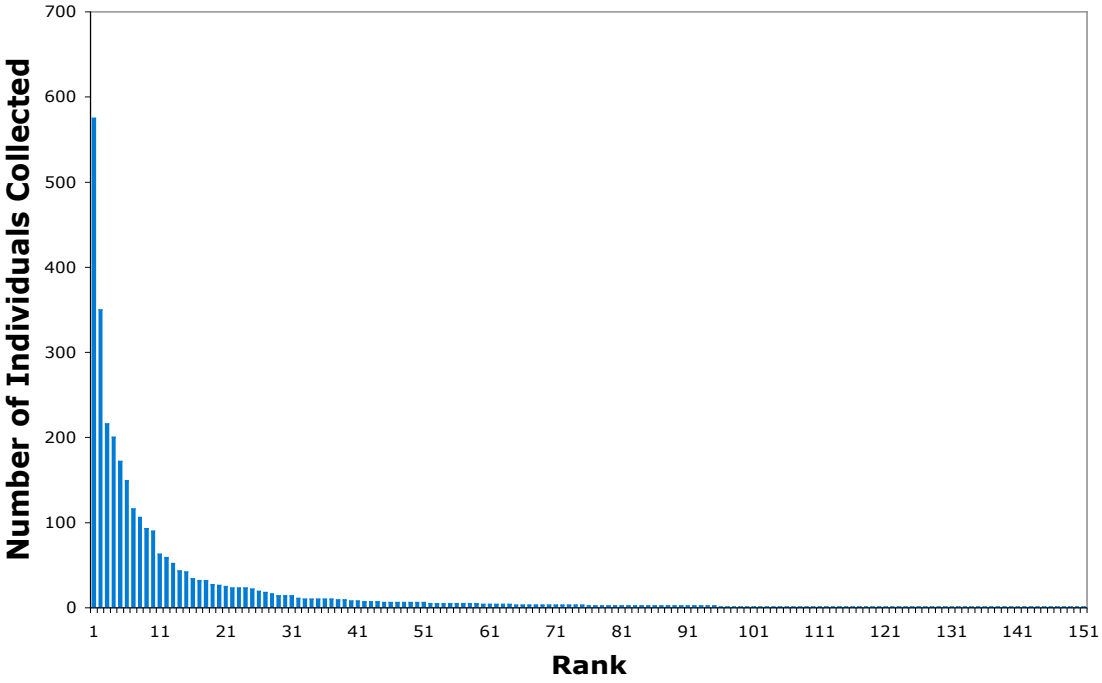

Figure 2.4. Epigeal abundance distribution. Abundance distribution of epigeal predator morpho-species species collected during sampling period from June through August 2004.

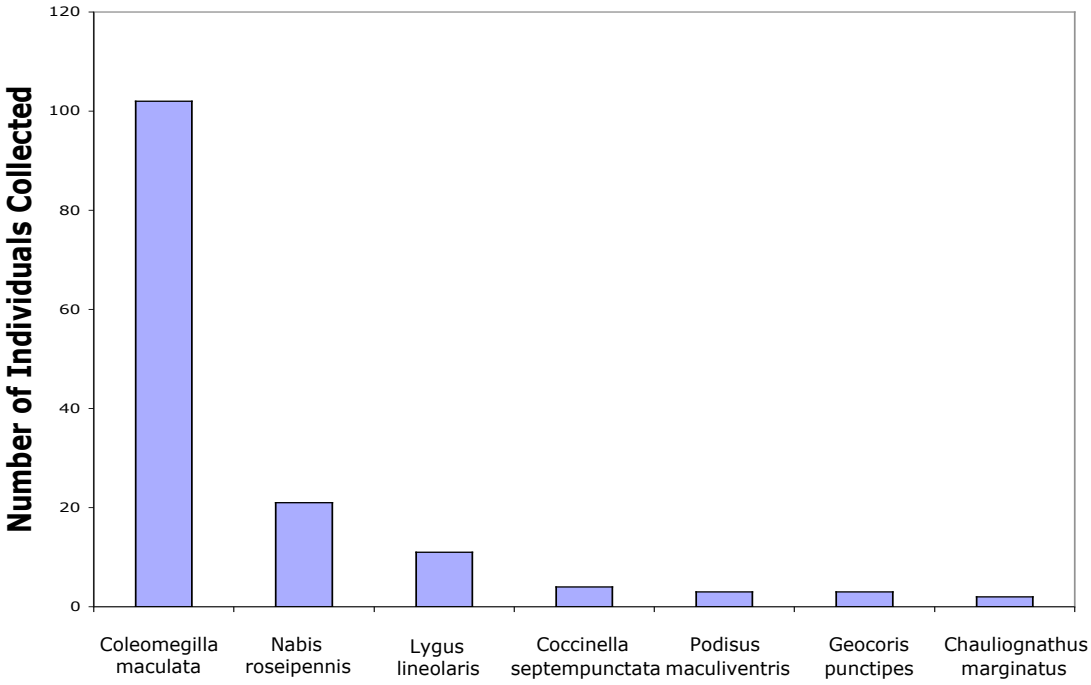

Figure 2.5. Assemblage species abundance distribution. Species abundance distribution of non-web-building and non-social predators collected in the foliar microhabitat. This represents the assemblage of potential predators of 1st instar *P. rapae* that were evaluated in chapter 3, with the addition of *Pardosa sp* and *Pterostichus lucublanduds*, two numerically dominant predators found in the epigeal microhabitat.

Chapter 3: Testing Predator-Prey and Predator-Predator Relationships.

Introduction

The use of predators as biological control agents in conventional U.S. agriculture began with the introduction of the Vedalia Beetle, *Rodolia cardinalis* Mulsant, in 1889 to combat cottony cushion scale, *Icerya purchasi* Maskell, an introduced species that was threatening the citrus industry in California at that time (Doutt 1967, Caltagirone 1981). The latter is an example of classical biological control. In this approach to biological control, an exotic natural enemy, i.e. a parasitoid, pathogen or predator, is intentionally introduced from the region of origin of the pest to the new region of the pest for the purpose of establishing long-term suppression usually of a coevolved pest (Eilenberg et al. 2001). Augmentative biological control is the repeated release of native or non-native natural enemies by way of mass rearing programs in order to increase their abundance in habitats where their populations are low or non-existent. Both classical and augmentative biological control have historically been where much of the success in biological control has been achieved (Caltagirone 1981, Denoth et al. 2002). However, in addition to the successes there have been numerous examples in which the release of an introduced species for the purpose of biological control of a pest has resulted in negative consequences to non-target species (Howarth 1991, Pearson and Callaway 2003,

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2005, Straub and Snyder 2006). The evidence suggests that increased biodiversity can enhance biological control. But is all of the biodiversity of predator assemblages necessary for effective biological control? If the entire assemblage is not necessary for effective pest suppression, then conservation efforts could focus on tactics that benefit the subset of the assemblage actually responsible for pest suppression. Current conservation biological control tactics may not benefit all predators similarly (Barbosa et al. 2005). Therefore, it may be more appropriate to evaluate the effect of potential tactics on the assemblage subset responsible for suppression. In order to identify the subset of a predator assemblage most likely to impose mortality on the target pest, it is necessary to determine (a) what predators, potentially interacting with the target pest actually feed on the target pest, (b) the relative effectiveness and differences in effectiveness among the predators that feed on the target pest, and (c) whether the predators in the group that do consume the target pest engage in any antagonistic interactions like intraguild predation (IGP), which can potentially reduce the impact of the assemblage.

Larvae of the cabbage white butterfly, *Pieris rapae* Linnaeus, are significant economic pests of crucifers worldwide (Schmaedick and Shelton 2000) and in Maryland (G. Dively personal communication). Larvae are preyed on by a myriad of generalist predators (Dempster 1967, Ashby 1974, Schmaedick and Shelton 2000). Life tables constructed by Dempster (1967) indicated that 1st instars are the most vulnerable life stage. Assemblages of generalist predators, which could potentially affect 1st instar *P. rapae,* have been identified in crucifers across a wide range of geographic regions such as Japan (Suenaga and Hamamura 2001), Hawaii (Hooks et

al. 2003), and the Northwestern (Snyder et al. 2006) and Midwestern continental United States (Schellhorn and Sork 1997). In this study, the predators identified through the field assessment of the predator community (chapter 2) were further evaluated to confirm that they feed on larval *P. rapae*, and determine if they engaged in intraguild predation, in the absence of *P. rapae*. The cohort of predator species selected as a result of these evaluations will then be used to test the hypotheses that increasing predator species richness increases the mortality imposed on larval *P. rapae* and that identity of the predators in assemblages influences the relationship between species richness and levels of prey mortality (see chapter 4).

Methods

Objectives: (1) To determine if there is a significant difference in mean *P. rapae* larval mortality imposed by each of the selected predator species compared to that in a no-predator treatment. (2) To determine the per capita consumption by predators which were found to consume 1st instar *P. rapae*. (3) To determine whether the predators of 1st instar *P. rapae* engage in intraguild predation in the absence of prey.

Study System

Experiments were conducted from May to September in 2005 and 2006. Collards (Vates variety) were grown from seed in a controlled environment in the University of Maryland research greenhouse. Plants used for feeding trials and colony maintenance were grown in 10cm square pots (multiple seeds were planted per pot to ensure germination). Seeds were planted in MM510 soil (The Scotts Co.®,

Marysville, OH) treated with Multicote®, a controlled release fertilizer. Four flats of 15 pots (for a total of 60 pots) were planted every week.

Adult *P. rapae* were collected in the field, placed in glassine envelopes, kept cool, and brought back to the lab to initiate a lab colony. *P. rapae* were kept in 60 X 60 X 60cm screen cages (BioQuip®, Rancho Dominguez, CA). Cages were placed adjacent to a lab window, and provided with supplemental lighting, at ambient room conditions (16:8 L:D, ~24°C and ~50% rH). Cages contained yellow sponges soaked with honey water for nutrition and two collard plants on which adults could oviposit. Collards were replaced daily, the eggs on them were allowed to hatch, some of the larvae were used in experiments and others were reared to adults to maintain the colony. Once the collard leaves had been completely skeletonized, the larvae were removed with feather forceps and reared in Petri dishes with filter paper in groups of five to ten (depending on their size) and fed fresh collard leaves, *ad libitum*. Upon pupation, they were removed from the Petri dish and placed in 473ml plastic deli cups (Solo Cup Co. ®, Highland Park, IL) to allow space for the adults after emergence. When the adults emerged they were used to maintain a lab colony in a 60 X 60 X 60cm cage.

An assessment of composition of the predator community in collards was undertaken in 2004 (described in chapter 2). In 2005, adult predators known to exist within the foliar microhabitat were evaluated in laboratory experiments to determine if in fact they feed on 1st instar *P. rapae* (Objective 1). The foliar predator assemblage tested included *Nabis roseipennis* (Reuter), *Coleomegilla maculata* (DeGeer), *Lygus lineolaris* (Palisot de Beauvois), *Coccinella septempunctata* (Linnaeus), *Podisus*

maculiventris (Say), *Geocoris punctipes* (Say), and *Chauliognathus marginatus* (Fabricus). These predators, along with two numerically dominant predators found in the epigeal microhabitat, *Pardosa sp.* and *Pterostichus lucublandus* (Say), were collected in order to determine if they would feed on 1st instar *P. rapae.* Predators were field collected, placed in 29.6ml plastic cups (Solo Cup Co.®, Highland Park, IL), kept cool and brought back to the lab to be used in experiments. Even though the predators were originally collected in collards, they are generalists that are commonly found in many cropping systems as well as non-managed systems. Thus, in order to maximize collection an emphasis was placed on searching alfalfa, however some individuals were collected in collards, sweet corn, and other vegetable and forage crops. Sites in which predators were collected were located throughout central and eastern Maryland. Collected predators were maintained individually in 473ml plastic deli cups (Solo Cup Co. ®, Highland Park IL) in the lab at ambient conditions (~24°C and ~50%rH) until used in experiments. At the conclusion of each experiment, all predators were placed back in 473ml plastic deli cups, reared with moist cotton and fed *P. rapae*.

In 2006, those species that were confirmed to be predators of 1st instar *P. rapae* from objective 1, and were collected in sufficient numbers, were used in experiments evaluating per capita consumption (objective 2) and intraguild predation (objective 3). Per capita consumption was determined for *C. maculata* (a numerically dominant predator), and *C. septempunctata* and *P. maculiventris* (two numerically sub-dominant predators) (see chapter 2). Although *N. roseipennis* was collected in moderate numbers in prior years, in 2006 it was not able to be collected in sufficient

numbers, so *N. roseipennis* was not included in the rest of the study. A *P. maculiventris* colony was established with individuals collected through the use of pheromone traps set out at Patuxent National Wildlife Refuge (Laurel, MD) and Beltsville Agricultural Research Center (Beltsville, MD). Traps and pheromone were obtained from Aldrich, J. R. (USDA-ARS, Beltsville, MD). *P. maculiventris* eggs were also purchased through Biocontrol Network® (Brentwood, TN) and reared to adults. Once in the lab, adult predators were reared in cages with moist cotton and fed Colorado potato beetle larvae obtained from a lab colony (maintained by G. Dively, Entomology Department, UMD) as well as on black cutworms. Coccinellid colonies were kept in 25 X 25 X 25cm Plexiglas® cages in the lab at ambient conditions (~24°C and ~50%rH) until they were used in mesocosm experiments. All stages of *P. maculiventris* were reared in 473ml plastic deli cups, at a density of 3-5 individuals per cup to cut down on cannibalism, with moist cotton and a food source. *C. septempunctata* were collected in alfalfa and other forage crops, small grains and various vegetables Species known to be cannibalistic, such as larval coccinellids were kept individually in 473ml plastic deli cups (Solo Cup Co. ®, Highland Park, Il). A *C. maculata* colony was established with individuals from USDA-ARS Biocontrol Laboratory (Beltsville, MD) and supplemented with individuals collected in collards, sweet corn, alfalfa, and other forage and vegetable crops. *C. maculata* were kept in 25 cm^3 screen cages and fed artificial bee pollen obtained from the USDA-ARS Biocontrol Laboratory (Beltsville, MD). *C. septempunctata* were kept in 25 cm^3 screen cages and fed *P. rapae* larvae, black cutworm larvae (*Agrotis ipsilon* Hufnagel) obtained from Dow AgriSciences® (Indianapolis, IN), or aphids collected

from alfalfa. At the conclusion of each experiment all living predators were returned to their respective colonies.

Mesocosm Design

All experiments were conducted in laboratory mesocosms. Each mesocosm consisted of a 10 cm^2 pot containing a single collard plant, covered with a 1-gallon nylon paint strainer bag (National/Ruskin, Inc. ®, Hatfield, PA). The bags had an elastic band, which secured them to the pots, with the aid of 15mm binder clips. Each mesocosm contained a three-week-old collard plant. Ten *P. rapae* larvae from the lab colony were haphazardly placed on the plant. In 2005, leaf segments on which larvae were feeding were cut off and transferred to experimental plants. The larvae were allowed 12 hours to move from the leaf segment to the experimental plant. If, after 12 hours, larvae remained on the leaf segments, they were moved onto the plant with a small brush. In 2006, larvae were transferred with feather forceps (BioQuip®, Rancho Dominguez, CA). Mortality of larvae was approximately the same using the two methods, 12.5% in 2005 and 10.6% in 2006. Haphazardly selected adult predators were removed from the colony, placed in 29.6ml plastic cups, and starved for 24 hours prior to running an experiment. When adding them to the mesocosms, predators were placed on the soil.

In order to avoid the loss of degrees of freedom when using a repeated measures analysis, novel plants, and *P. rapae* larvae were used for each experiment. Novel predators (whenever possible) were randomly assigned for each experiment. If a predator individual was used in multiple experiments the experimental treatment to which it was assigned was re-randomized. The location within the environmental

chamber in which each mesocosm was placed was re-randomized for each experiment.

Experimental Protocol

Objective (1): To determine if there is a significant difference in mean *P. rapae* larval mortality imposed by each of the selected predator species compared to that in a no-predator treatment.

Experiments in 2005 were designed to determine if mean larval *P. rapae* mortality in the presence of each predator differed significantly from that in control treatments in which no predator was added to the mesocosm. The design was unbalanced because sample sizes were dependent on the number of each predator species collected (Table 3.1). Mesocosms were randomly placed in an environmental chamber (16:8 L:D, 28°/18°C, ~70% rH) for 24 hours. At the end of that time period predators were removed and the number of living *P. rapae* larvae noted. 28 control replicates, and at least four replicates of each predator treatment were run at a single time (Table 3.1), when sufficient numbers of predators were collected. The response variable in the experiment was the number of dead *P. rapae* larvae at the end of a 24-hour time period (i.e., larval *P. rapae* mortality). A one-tailed one-way analysis of variance (PROC MIXED, SAS® 1999) was used to compare mean larval *P. rapae* mortality between treatments (predator species). Contrasts were used to determine if mean larval *P. rapae* mortality for each predator species was greater than that of no-predator control. The data satisfied the assumption of normality, however, the variances were heterogeneous. In order to run the ANOVA it was necessary to group

the variances. A Bonferroni adjustment was made to control for experiment-wise error rate.

Objective (2): To determine the per capita consumption by predators of 1st instar *P. rapae*.

Experiments conducted in 2006 determined per capita larval *P. rapae* consumption of each predator species. Control treatments (in which no predator was added to the mesocosm) were established to assess background mortality of *P. rapae* larvae. Per capita consumption of *P. rapae* differs from *P. rapae* mortality in that per capita consumption of *P. rapae* represents the mean mortality of *P. rapae* in the presence of each predator species minus background mortality. Mesocosms were randomly placed in an environmental chamber (16:8 L:D, 28°/18°C, ~70% rH) for 24 hours. At the end of that time period predators were removed and the number of living *P. rapae* larvae noted. Up to 13 mesocosms (i.e., replicates) of each predator treatment were run at a single time, when sufficient numbers of predators were collected. A total of 15 replicates of each predator treatment and 16 controls were tested. The response variable in the experiment, was the number of dead *P. rapae* larvae at the end of a 24-hour time period, adjusted for the mortality observed in controls (i.e. the no predator treatment). Per capita consumption represented the difference between number of *P. rapae* larvae alive at the beginning (10) and at the end of the time period, minus the mean number of larvae that died in the no-predator treatment (an average of 1.06). The adjusted values represented the number of larvae consumed by predators. A one-way analysis of variance (PROC MIXED, SAS®

1999) was used to compare mean per capita consumption of predator species. The data satisfied all of the assumptions of ANOVA and a Bonferroni adjustment was made to control for experiment-wise error rate.

Objective (3): To determine whether the predators of 1st instar *P. rapae* engage in intraguild predation (IGP) in the absence of prey.

To test for IGP, the mesocosm protocol was used, but no *P. rapae* larvae were introduced into each mesocosm. Instead, two predator individuals of differing species were placed in the same mesocosm. The IGP mesocosms were subjected to the same abiotic conditions noted above. After 24 and 48 hours the survival of the each predator was noted. If both predators were still living after 48 hours it was determined that no IGP occurred. However, if that was not the case, then I concluded that IGP did occur. Control treatments, in which a single predator individual was placed in a mesocosm, were used to determine background mortality, and were necessary for the purpose of making comparisons. Fifteen replicates of each predator combination, as well as each control, were tested. Predator survival in each of the predator combinations was compared to that of the corresponding control using a Chi-squared test (PROC FREQ, SAS® 1999). For tests in which 50% of the cells had expected counts less than 5, the Chi-squared test may not be valid, so a Fisher's exact test was used to generate p-values.

Results

There was a significant difference in the mean *P. rapae* larval mortality imposed by the collard agroecosystem predators tested (Figure 3.1; $F = 10.5$, $p < 0.001$). Mean 1st instar *P. rapae* mortality was significantly greater in the presence of *C. maculata* (mean mortality = 40%), *C. septempunctata* (mean mortality = 58%), *N. roseipennis* (mean mortality = 34.4%), and *P. maculiventris* (mean mortality = 35%) than in their absence (mean control mortality of 12.5% and p-value of $p = 0.001$, $p < 0.001$, $p = 0.045$, $p = 0.001$, respectively; Figure 3.1). Mean mortality of 1st instar *P. rapae* imposed by *Chauliognathus marginatus* (mean mortality = 20%; $p = 1.00$), *Geocoris punctipes* (mean mortality = 20%; $p = 1.00$), *Lygus lineolaris* (mean mortality = 7.1%; $p = 1.00$), *Pardosa spp.* (mean mortality = 6%; $p = 1.00$) and *Pterostichus lucublandus* (mean mortality = 16%; $p = 1.00$) was not significantly different from controls.

The mean *P. rapae* mortality observed in the control treatment (1.06 out of 10) represented background mortality and this value was used to obtain an accurate value for per capita consumption of each predator (Table 3.2). The per capita consumption of 1st instar *P. rapae* by *P. maculiventris* (5.1 out of 10) appeared to be higher than that of *C. maculata* and *C. septempunctata* (2.9 and 3.1 out of 10, respectively). However, these differences were not statistically significant (Figure 3.2; $F = 2.83$, $p = 0.070$).

There was evidence of intraguild predation in only one of the predators. In the absence of another predator, all 15 *C. maculata* individuals remained alive through the end of the 48-hour period (Figure 3.3). In the presence of *C. septempunctata* all

15 *C. maculata* survived. When paired with *P. maculiventris*, 12 of the 15 *C. maculata* individuals survived, although this level of survival was not significantly different from the control ($\chi^2 = 3.33$, $p = 0.224$). In the absence of another predator, 13 of the 15 *C. septempunctata* individuals survived at the end of the 48-hour period (Figure 3.3). In the presence of *C. maculata*, 13 *C. septempunctata* remained living, although not a significant difference ($\chi^2 = 0.370$, $p = 1.00$). When paired with *P. maculiventris* there was a significant difference in survival, i.e., only 4 of the 15 *C. septempunctata* individuals survived ($\chi^2 = 11.0$, $p < 0.001$). In the absence of any other predator, all 15 *P. maculiventris* individuals remained alive through the end of the 48-hour period (Figure 3.3). There was no significant difference in *P. maculiventris* in the presence of *C. maculata,* i.e., 13 of the 15 *P. maculiventris* survived ($\chi^2 = 2.14$, $p = 0.483$). When paired with *C. septempunctata*, all of the 15 *P. maculiventris* individuals survived.

Discussion

The purpose of this study was to determine which of the predators found in the foliar microhabitat during 2004 were in fact predators of larval *P. rapae*, and their rate of consumption of larval *P. rapae*. Although large numbers of predators were collected in the collard agroecosystem, only a small proportion of those collected are predators of larval *P. rapae. P. maculiventris*, *C. septempunctata, C. maculata*, and *N. roseipennis* are foliar predators that feed on larval *P. rapae*. Additionally, in this system, *L. lineolaris, G. punctipes, C. marginatus. Pardosa spp.* and *P. lucublanduds* were not predators of 1st instars. Previous studies on predators found in crucifers have

also concluded that not all predators collected are predators of 1st instar *P. rapae.* Ashby (1974), conducted feeding trials using potted cabbage plants and Schmaedick and Shelton (2000) using preciptin tests (using many of the same species or congeners of those I used), and found complimentary results, with only one exception. Schmaedick and Shelton (2000) found that *Pardosa milvina* (Hentz) consumed 0.90±0.38 (mean±SE) 1st instar *P. rapae* in 24 hours. However, no statistical analyses were performed so it is impossible to make comparisons to what I found. Nevertheless, these results enabled me to determine which of the predators found in the collard agroecosystem actually feed on *P. rapae* and to identify the subset of a predator assemblage most likely to affect the biological control of *P. rapae* in the field.

When the consumption rates of three confirmed predators of *P. rapae*, *P. maculiventris*, *C. septempunctata, C. maculata*, were tested in mesocosms on potted collard plants, *P. maculiventris* tended to consume more than the two species of individuals of coccinellids (Table 3.2), although the difference turned out to not be signifcant. The lack of signifcance is consistent with the results from the 2005 experiments where per capita consumption was highest in *C. septempunctata* (Table 3.1). In fact in a previous study, *C. maculata* was found to consume 5.7±1.07 (mean±SE) 1st instar *P. rapae* in 24 hours (Schmaedick and Shelton 2000), which is greater than that found for both *C. septempunctata* in 2005 and *P. maculiventris* in 2006. However, had the trials run for a longer period of time (e.g., 48 Hours), the results may have been different in that *P. maculiventris* consumption may have decrease because it's searching behavior limits the number of prey it attacks

(Wiedenmann and O'Neil 1991). In order to identify the subset of a predator assemblage most likely to affect *P. rapae*, it was necessary to determine the relative effectiveness and differences in effectiveness among the predators that feed on *P. rapae*. The numerically dominant predator (*C. maculata*) did not seem to vary in it's consumption of the target pest than that of the two numerical subdominant predators (*C. septempunctata* and *P. maculiventris*). These results suggest that the assumption, the most abundant predator is the most effective, may not always be true. Therefore other factors, such as predator species richness or predator identity, may be contributing to an assemblage's effectiveness more than the relative abundances of the predators in the assemblage.

Intraguild predation was found to occur in this system, however it was asymetrical. When I measured intraguild predation between the three predators, the only intraguild predator observed was *P. maculiventris* on *C. septempunctata*, its intraguild prey. Intraguild predation has been shown to occur by *C. maculata* (Schellhorn and Andow 1999), *C. septempunctata* (Agarwala and Dixon 1992) and *P. maculiventris* (Mallampalli et al. 2002). In all of these studies the intraguild predation was asymetrical, however, unlike in my study none of the intraguild prey were adults. In using these three predator species, and by only using adults, I was able to limit the potential for intraguild predation to occur when assembled in the mesocosms I used to test the relationship between predator species richness and prey mortality (in chapter 4). Intraguild predation has been shown to effect predator's ability to suppress pests (Rosenheim et al. 1993). With this assemblage it was important to consider that only *P. maculiventris* engages in intraguild predation and that *C septempunctata* is their

intraguild prey. *P. maculiventris*, being a numerically subdominant predator in the assemblage, highlights the fact that tactics that are aimed at conserving more of the assemblage may be more likely to increase negative interactions between predators in the assemblage. These findings reinforce my speculation that conservation biological control might be best accomplished by using targeted tactics. Those tactics should focus on predators that don't engage in detrimental interaction with other predators in order to get the greatest impact on target pests.

In summary, I found that not all predators present in the agroecosystem actually feed on the target pest, the relative effectiveness among the predators that feed on the target pest appear to be equivalent and some, but not all of the predators in the group that do consume the target pest engage intraguild predation. This evidence supports the idea that in fact a subset of the generalist predators found in an agroecosystem is responsible for suppression of the target pest. In making biological control decisions to properly conserve the entire subset of predators responsible for suppressing target pest populations, it is necessary to not only take in to account the interactions occurring between the predators in the assemblage and the pest, but one must also consider the negative interactions occurring between predators species in the subset assemblage.

Table 3.1. Determination of predators of 1st instar *P. rapae*. In 2005, comparisons were made between mean *P. rapae* larval mortality imposed by each predator and that in a no-predator treatment.

Treatment	n	Mean Mortality (%)	SEM
Control	28	12.5	0.324
Podisus maculiventris	6	35.0	0.500
Coccinella septempunctata	15	58.0	0.776
Coleomegilla maculata	14	40.0	0.620
Nabis roseipennis	9	34.4	0.603
Geocoris punctipes	5	20.0	0.548
Lygus lineolaris	7	7.10	0.286
Pardosa spp.	5	6.00	0.400
Chauliognathus marginatus	4	22.5	0.478
Pterostichus lucublandus	5	16.0	0.748

Table 3.2. Per capita consumption of 1st instar *P. rapae*. In 2006 the per capita consumption of the three predators species collected in sufficient numbers, and that were found to consume 1st instar *P. rapae,* was determined. Per capita consumption represents the mean mortality in the presence of each predator species minus background mortality. Therefore, per capita consumptions reflect adjusted mortalities. The design was balanced, in that sample size was 15 for all treatments, unlike in 2005.

Treatment	Mean Mortality (%)	Per Capita Consumption	SEM
Coleomegilla maculata	39.3	2.87	0.480
Coccinella septempunctata	42.0	3.14	0.825
Podisus maculiventris	61.3	5.07	0.786

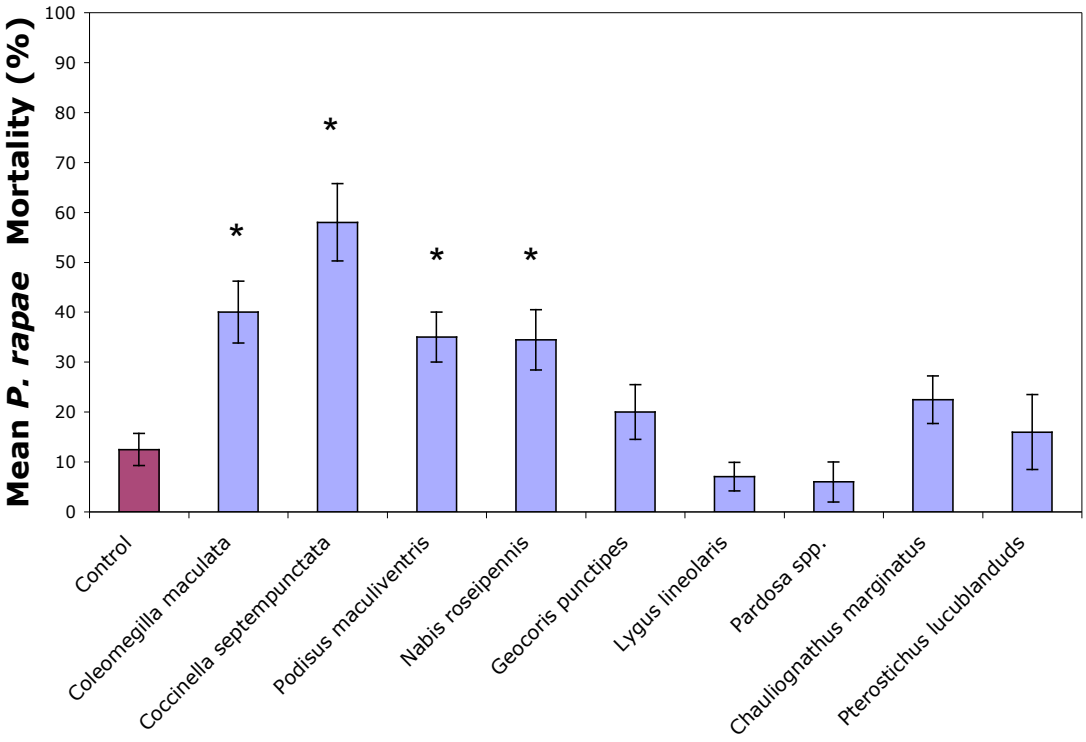

Figure 3.1. Feeding trials to determine which predators feed on larval *P. rapae.* Each predator treatment was compared to the no predator control. Significant differences were found when *C. maculata, C. septempunctata, P. maculiventris* and *N. roseipennis* were compared to the no predator control.

* Denotes p-values less than 0.05, and error bars represent standard error of the mean

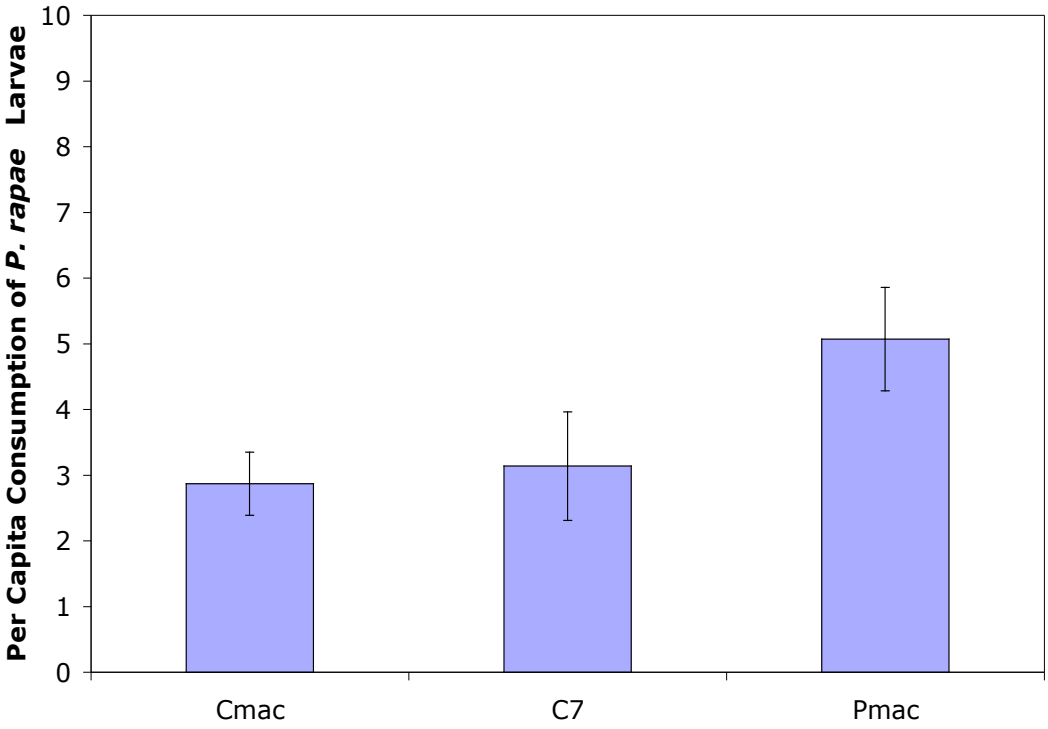

Figure 3.2. Per capita consumption of 1st instar *P. rapae* by each predator species. There was no significant difference in consumption between any of the predators. Error bars represent standard error of the mean.

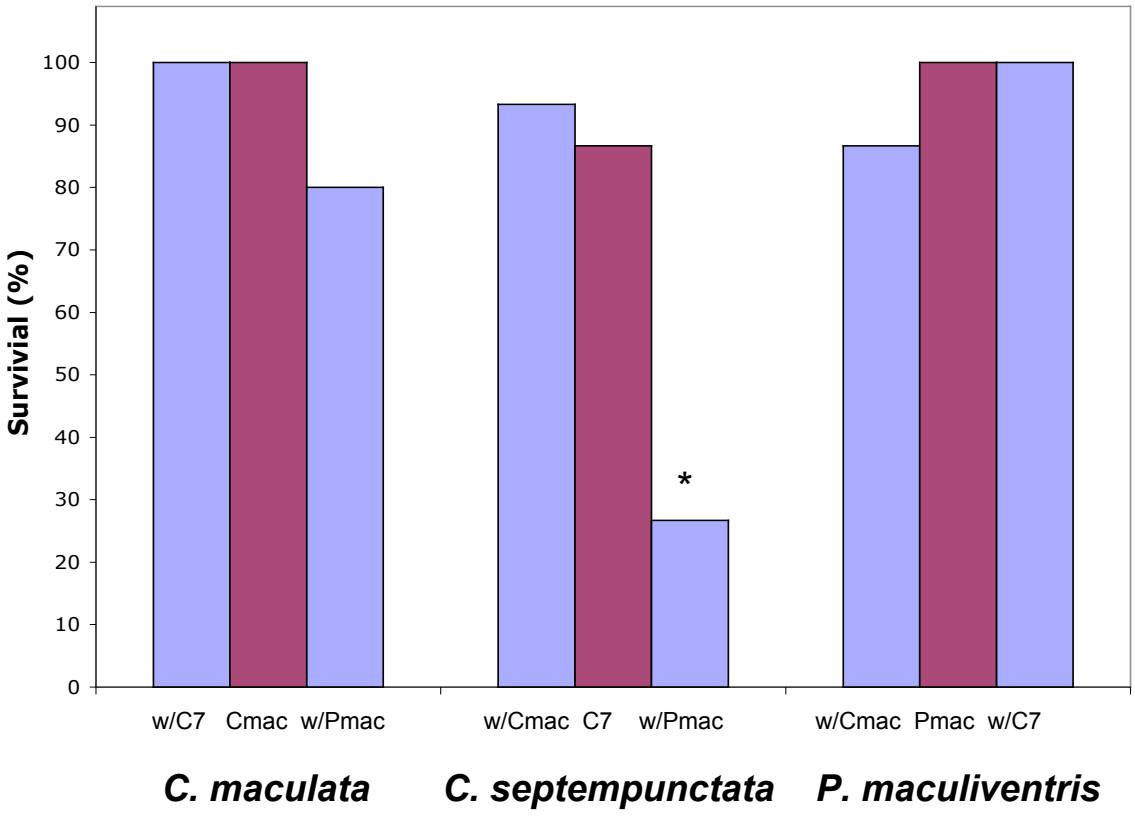

Figure 3.3. Test for Intraguild Predation. The first set of bars represents *C. maculata* in the presence of *C. septempunctata* (left) control (center) and in the presence of *P. maculiventris* (right). The second set of bars represents *C. septempunctata* in the presence of *C. maculata* (left), control (center) and in the presence of *P. maculiventris* (right). The third set of bars represents *P. maculiventris* in the presence of *C. maculata* (left), control (center) and in the presence of *C. septempunctata* (right).

* Denotes a p-value less than 0.05

Chapter 4: The Relationship Between Predator Species Richness and Prey Mortality.

Introduction

Recent research on biodiversity suggest that increasing loss of biodiversity can lead to a corresponding loss of ecosystem function (Chapin et al. 2000, Loreau 2000, Tilman 2000). More recently, the interest in the effect of biodiversity on ecosystem function has resulted in more research on how the biodiversity of predator assemblages (sensu Claridge 1987) affects their ability to suppress pests (Ives et al. 2005, Snyder et al. 2005, Wilby et al. 2005, Straub and Snyder 2006). The increased interest in the functioning of predator assemblages has highlighted the importance of understanding how the species in a predator assemblage interact and ultimately influence the assemblage's ability to suppress pests. Research has shown that increasing natural enemy species richness (a measure of biodiversity) can lead to decreased biological control because of negative factors like interference, cannibalism and intraguild predation (Rosenheim et al. 1993, Hochberg 1996, Denoth et al. 2002). Debach (1974) went as far as to suggested that biological control would be most effective if relatively few specialized natural enemies were employed as biological control agents. That is, with agents that are able to survive within the same range of the pest, capable of high reproductive capacity relative to the pest and highly efficient at searching for the pest. The Species Assemblage Control Hypothesis (SACH) epitomizes the opposing view. Central to SACH is the belief that an assemblage of

generalist predators, if conserved properly, can suppress associated prey populations more effectively than a single predator species (Riechert and Lockley 1984, Provencher and Riechert 1994, Riechert and Lawrence 1997). Riechert and Lockley (1984) argue that certain characteristics of generalist predator assemblages (spiders in this case) make the assemblage as a whole a more effective agent of natural control than any single species. They contend that a predator assemblage is self-regulating because of factors like cannibalism and intraguild predation, lending stability in periods of low prey availability. In times of high prey availability, the assemblage can exhibit a strong numerical response through aggregation and reproduction. They go on to suggest that as predator richness in an assemblage increases, a more diverse set of foraging behaviors and sizes should be present, which should, in turn, enhance the probability that prey of various sizes and species will be killed. This anticipated advantage of greater species diversity is what has been termed a sampling effect (Loreau et al. 2001). On the other hand, biological control theory predicts that increasing predator richness should lead to an increase in potentially negative predator-predator interactions, and limit effective suppression of pests (DeBach 1974, Rosenheim et al. 1995). However there have been examples of predator assemblages suppressing pests despite the occurrence of negative predator-predator interactions (Lang 2003, Snyder and Ives 2003, Snyder et al. 2004).

The relationship between biodiversity of predator assemblages and pest suppression is of great importance due to recent trends in agriculture that have led to an increasing loss of predator biodiversity (Snyder et al. 2005). Increased biodiversity has been shown to increase pest suppression (Cardinale et al. 2003, Snyder et al.

2006). In this study, I examined the relationship between biodiversity of a predator assemblage, specifically species richness, and pest suppression (henceforth prey mortality). In addition, I explored how predator species identity can alter the relationship between species richness of a predator assemblage and prey mortality.

In general, if there are three levels of predator richness for an experimental design, there are nine possible results (Figure 4.1). (A) There is no relationship between predator richness and prey mortality (Figure 4.1a). (B) There is a direct positive correlation between predator richness and prey mortality (Figure 4.1b). (C) There is a direct correlation between predator richness and prey mortality up to a point, after which there is a leveling off of mortality as richness increases (Figure 4.1c). (D) Predator richness is directly correlated with prey mortality up to a point, after which they are negatively correlated (Figure 4.1d). (E) There is a direct negative correlation between predator richness and prey mortality (Figure 4.1e). (F) Increasing predator richness does not change prey mortality up to a point, after which increasing richness leads to decreased mortality (Figure 4.1f). (G) Increasing predator richness does not change prey mortality up to a point, after which increasing richness leads to increased prey mortality (Figure 4.1g). (H) Predator richness is directly negatively correlated with prey mortality up to a point, after which further increases in richness does not change prey mortality (Figure 4.1h). (I) Predator richness is directly negatively correlated with prey mortality up to a point, after which further increases in richness leads to increased prey mortality (Figure 4.1i).

Testing of my hypotheses would help determine which of the potential outcomes outlined may occur in collard agroecosystems in Maryland. Thus, I tested

the hypothesis that an increase in the species richness of an assemblage of predators leads to an increase in larval *Pieris rapae* (Linnaeus) mortality imposed by the assemblage. Further, I tested the hypothesis that predator species identity can alter the relationship between species richness of a predator assemblage and *P. rapae* mortality.

Methods

Study System

Experiments pertaining to this section took place from May to September 2006. Collard greens, *Brassica oleracea* var. acephala (Vates variety), were grown from seed in a controlled environment at the University of Maryland research greenhouse. Plants used for the assemblage manipulations and colony maintenance were planted every week. For the assemblage manipulations, three collard plants were planted 10 cm apart from one another in 30cm diameter plastic pots. More than one seed was put in each pot to ensure germination. Sixteen pots were planted each week to insure that there would be sufficient plants for the experiment. Collards for the maintenance of *P. rapae* colonies were grown in $10cm^2$ pots. Four flats of 15 pots (for a total of 60 pots) were planted every week. Seeds were planted in MM510 soil treated with Multicote®, a controlled release fertilizer.

Adults were collected in the field, placed in glassine envelopes, kept cool, and brought back to the lab to initiate a lab colony of *P. rapae.* They were kept in 60 X 60 X 60cm screen cages (BioQuip®, Rancho Dominguez, CA). Cages were placed adjacent to a lab window, but with supplemental lighting, at ambient room conditions (16:8 L:D, ~24°C and ~50%rH). Cages contained yellow sponges soaked with honey

water for nutrition and two collard plants on which adults could oviposit. Collards were replaced daily, the eggs on them were allowed to hatch, and some of the resulting larvae used in experiments. Once the collard leaves had been completely skeletonized, the larvae were removed with feather forceps. Remaining larvae were reared in Petri dishes with filter paper in groups of five to ten (depending on their size) and fed fresh collard leaves, *ad libitum*. Upon pupation, they were removed from the Petri dish and placed in 473ml plastic deli cups (Solo Cup Co.®, Highland Park, IL) to allow space for the adults after eclosion. When the adults emerged they were used to maintain a lab colony in a 60 X 60 X 60cm cage.

Predator collections focused on the numerically dominant *Coleomegilla maculata* (DeGeer), and two sub-dominant predators *Coccinella septempunctata* (Linnaeus) and *Podisus maculiventris* (Say). These species were predators that were members of the foliar predator assemblage on collards during the summer 2004 (see chapter 2) and known to be predators of *P. rapae* (see chapter 3). Predators were collected in the field, placed in 29.6ml plastic cups (Solo Cup Co.®, Highland Park, IL), kept cool and brought back to the lab to be used in experiments. Even though the predators were originally collected in collards, they are commonly found in many cropping systems as well as non-managed systems. In order to maximize collection, efforts targeted different crops for each predator. Sites in which predators were collected were located primarily in Maryland, although some early season collection took place in the North Carolina piedmont area. A *C. maculata* colony was first established with individuals from USDA-ARS Biocontrol Laboratory (Beltsville, MD) and supplemented with individuals collected in collards, sweet corn and alfalfa,

and other forage and vegetable crops. *C. maculata* were fed artificial bee pollen obtained from the USDA-ARS Biocontrol Laboratory (Beltsville, MD). *C. septempunctata* were collected in alfalfa, other forage crops, small grains, and various vegetables. They were fed *P. rapae* larvae, black cutworm larvae (*Agrotis ipsilon* Hufnagel) obtained from Dow AgriSciences® (Indianapolis, IN), or aphids collected from alfalfa. The *P. maculiventris* colony was established with individuals collected through the use of pheromone traps set out at Patuxent National Wildlife Refuge (Laurel, MD) and Beltsville Agricultural Research Center (Beltsville, MD). Traps and pheromone were obtained from Aldrich, J. R. (USDA-ARS, Beltsville, MD). *P. maculiventris* eggs were also purchased through Biocontrol Network® (Brentwood, TN) and raised to adulthood. Once in the lab, adult coccinellids were reared with moist cotton and their respective food source in 25 X 25 X 25cm Plexiglas® cages in the lab at ambient conditions at (~24°C and ~50%rH) until they were used in mesocosm experiments. Species known to be cannibalistic, such as larval coccinellids were kept individually in 473ml plastic deli cups (Solo Cup Co.®, Highland Park, Il). All stages of *P. maculiventris* were reared in 473ml plastic deli cups, at a density of 3-5 individuals per cup to cut down on cannibalism, with moist cotton. *P. maculiventris* were fed Colorado potato beetle larvae obtained from a lab colony (maintained by G. Dively, Entomology Department, UMD) as well as black cutworms. At the conclusion of each experiment all living predators were returned to their respective colonies.

Mesocosm Design

All experiments were conducted in laboratory mesocosms. Mesocosms for assemblage manipulations consisted of 30 cm diameter pots containing collard plants covered with 5-gallon nylon paint strainer bags (National/Ruskin® Inc. Hatfield, PA) supported by a tomato trellis. The bags had an elastic band, which secured them to the pots.

Experimental Protocol

The experimental protocol for this study consisted of determining whether the number of first instar *P. rapae* consumed increased significantly when in the presence of assemblages with the same abundance of predator individuals but comprised of different number of predator species. Thus, the design of this experiment was a substitutive experimental design (Snyder et al. 2006), in that the absolute abundance of predator individuals remained constant throughout all experimental treatments, while the number of species was varied. This design allows for the isolation of the effect that predator species richness on prey mortality while eliminating the potentially confounding effects of total predator density and species composition (see below for further details).

Each mesocosm contained three five-week-old plants (about 30cm tall). Ten first instar *P. rapae* from the lab colony were placed on each of the three plants (30 per mesocosm). This density was higher than that found in the field (personal observation) in order to emulate the average number of larvae consumed by the most voracious predator species (5.8 *P. rapae* larvae/individual predator, see chapter 3). In

order to infest the plants in the mesocosms *P. rapae* larvae were gently transferred using feather forceps (BioQuip®, Rancho Dominguez, CA). A treatment was then randomly assigned to each mesocosm following the substitutive experimental design (Snyder et al. 2006). In this design, the absolute number of predator individuals in each treatment remained constant (six predator individuals) while species richness was varied. Such a design, with three predator species yielded the following treatments 1) six *C. maculata* individuals, 2) six *C. septempunctata* individuals, 3) six *P. maculiventris* individuals, 4) three *C. maculata* individuals and three *C. septempunctata*, 5) three *C. maculata* individuals and three *P. maculiventris* 6) three *C. septempunctata* individuals and three *P. maculiventris*, and 7) two individuals of each of the three predator species. A no predator control was included to measure background larval *P. rapae* mortality. Predators were taken from the lab colony and randomly assigned to each appropriate treatment. Each individual predator was starved for 24 hours prior to being placed into the mesocosm. The mesocosms were randomly assigned a location within a growth chamber in the lab (set at 16L:8D, 24°C, ~70% rH). At the end of a 48-hour time period the predators were removed from the mesocosms, and the number of living *P. rapae* larvae counted. All eight treatments were replicated ten times over time for a total of 80 mesocosms. The number of replicates of each treatment that were ran at a particular time depended on the availability of each predator species, but no more than eight were run at a given time. In order to avoid the loss of degrees of freedom when using a repeated measures analysis, novel plants and *P. rapae* larvae were used for each replicate, and the

treatment to which predators were assigned was randomized. The location within the chamber that each mesocosm was placed was re-randomized each time.

The response variable in the experiment, was the number of dead *P. rapae* larvae at the end of a 48-hour time period, adjusted for the mortality observed in controls (i.e., the no predator treatment). Adjusted mortality represented the difference between number of *P. rapae* larvae alive at the beginning (30) and at the end of the time period, minus the mean number of larvae that died in the no-predator treatment (6.08). These data represented the number of larvae consumed by predators. The mean *P. rapae* larval mortality observed among treatments with the same species richness were pooled, and the means for each richness level were compared. Pooling treatments was an appropriate approach because the variances were homogeneous. A one-tailed one-way analysis of variance (PROC MIXED, SAS® 1999) was used to determine the effect of predator species richness on larval *P. rapae* mortality, in which the pooled mean *P. rapae* mortality for the three one-predator treatments combined, the pooled mean *P. rapae* mortality for the three two-predator treatments combined, and the mean larval *P. rapae* mortality for the three-predator treatment were compared. The data satisfied all of the assumptions of ANOVA and a Bonferroni adjustment was made to control for experiment-wise error rate. The following six sets of pair-wise comparisons were also made to determine the effect of predator identity on the relationship between predator richness and prey mortality: (1) predator treatments 1, 4 and 7 (from above) and (2) predator treatments 1, 5 and 7, which represent the *C. maculata* containing treatments; (3) predator treatments 2, 4 and 7 and (4) predator treatments 2, 6 and 7, which represent the *C. septempunctata*

containing treatments; (5) predator treatments 3, 5 and 7, and (6) predator treatments 3, 6 and 7, which represent the *P. maculiventris* containing treatments. Bonferroni adjustments were made for each set of pair-wise comparisons to control for experiment-wise error rate.

Results

There was a significant relationship between species richness of an assemblage of predators and prey mortality; however, the relationship was non-linear (Figure 4.2). Increased predator species richness from one species to two species led to a significant increase in mean larval *P. rapae* mortality ($F = 14.7$, $p = 0.001$) however, there was no significant increase in mean larval *P. rapae* mortality when the richness was increased from two species to three ($F = 4.03$, $p = 0.727$). In fact, although the mean larval *P. rapae* mortality in the three species treatment was greater than that of the one species treatments, that difference was not significant ($F = 0.49$, $p = 0.074$) and thus the change in prey mortality from two to three species may indeed represent a decline in prey mortality.

Predator species identity did affect the relationship between species richness of a predator assemblage and prey mortality. In the first two set of comparisons, where I focused on *C. maculata*-containing assemblages, as predator species richness increased prey mortality also increased. However, the increase in prey mortality appeared to level off in the three species assemblage (Figure 4.3). In comparison 1 (Figure 4.3a), there was no significant difference in mean larval *P. rapae* mortality imposed by *C. maculata* (the single species "assemblage") and the two species

assemblage consisting of *C. maculata* and *C. septempunctata* (t = -2.16, p = 0.051). However, there was a significant increase the mortality imposed by the three predator species assemblages (t = -2.46, p = 0.025) compared to *C. maculata* alone, although there was no significant differences imposed by the three species assemblage and the assemblage comprised of *C. maculata* and *C. septempunctata* (t = -0.29, p = 1.00). In comparison 2 (Figure 4.3b), mean larval *P. rapae* mortality imposed by the two species (*C. maculata* and *P. maculiventris*) assemblage as well as the three species (*C. maculata*, *P. maculiventris* and *C. septempunctata*) assemblage were significantly greater than *C. maculata* alone (t = -3.83, p = 0.001 and t = -2.46, p = 0.025, respectively). There was no significant difference imposed by the two and three species assemblages (t = 1.38, p = 0.260).

The next two sets of comparisons focused on *C. septempunctata*-containing assemblages. For this set of comparisons, the relationship between predator richness and prey mortality differed from those noted above (Figure 4.4). In the first comparison (Figure 4.4a), there was no relationship between predator richness and prey mortality. That is, there was no significant difference in mean larval *P. rapae* mortality imposed by *C. septempunctata* alone and the two species (*C. septempunctata* and *C. maculata*) assemblages (t = -1.72, p = 0.135), or *C. septempunctata* alone and the three species (*C. septempunctata*, *C. maculata* and *P. maculiventris*) assemblages (t = -2.02, p = 0.072). However, the second comparison (Figure 4.4b) differs in that there is a relationship between species richness and prey mortality when one compares *C. septempunctata* alone and the two species (*C. septempunctata* and *P. maculiventris*) assemblage (t = -2.65, p = 0.015). There was

no significant difference between the three species assemblage and *C. septempunctata* alone (t = -2.02, p = 0.072) or when the two and three species assemblages were compared. (t = 0.06, p = 1.00).

The final two sets of comparisons focused on *P. maculiventris*-containing assemblages. There was no relationship between predator richness and prey mortality (Figure 4.5). In the first comparison (Figure 4.5a), there was no significant difference in the mean larval *P. rapae* mortality imposed by *P. maculiventris* alone, a two species (*P. maculiventris* and *C. maculata*) assemblage, or a three species (*P. maculiventris*, *C. maculata* and *C. septempunctata*) (t = -1.82, p = 0.110 and t = -0.44, p = 1.00, respectively). There was also no significant difference in mortality imposed by the two and three species assemblages (t = 1.38, p = 0.260). The second comparison (Figure 4.5b) yielded the same results. That is, there was no significant difference in the mean larval *P. rapae* mortality imposed by *P. maculiventris* alone, a two species (*P. maculiventris* and *C. septempunctata*) assemblage (t = -1.08, p = 0.425), or a three species (*P. maculiventris*, *C. maculata* and *C. septempunctata*) assemblage (t = -0.44, p = 1.00). There was also no significant difference in mortality imposed by the two and three species assemblages (t = 0.64, p = 1.00).

Discussion

The results of the analyses supported the hypothesis that increased species richness of an assemblage of predators can lead to an increase in prey mortality imposed by the assemblage. However, this relationship was not found to be linear, and dependent on the identity of the species in the assemblage. When all one species assemblages were compared to all two and three species assemblages (Figure 4.2),

larval *P. rapae* mortality increased initially, but then leveled off at the highest level of species richness. When put in the context of the nine possible outcomes (discussed in the introduction) the relationship was best described by the lines in Figures 4.1c and 4.1d. That is, an increase in mortality imposed when a single species is compared to a two species assemblage followed by either a leveling off of mortality (as in Figure 4.1c), or a decline in mortality imposed (as in Figure 4.1d). The results of statistical analysis do not allow a clear distinction of these two interpretations of the changes between two and three species assemblages.

Predator identity, however, does have an impact on the relationship between species richness and prey mortality, and thus results vary across assemblages. I explored how species identity affected the results through six sets of comparisons. In some cases the results were ambiguous, as in the pooled results, such that the relationship between richness and mortality was not clear. For example, in comparisons in which *C. septempunctata* was the focal predator, the addition of the third predator (i.e., *C. maculata*) did not lead to a significant increase in *P. rapae* *m*ortality (Figure 4.4b). In fact, mortality imposed by the three-predator assemblage was not significantly different from that imposed by *C. septempunctata* alone. Thus the relationship was that which is depicted by either Figure 4.1c or 4.1d. In contrast, in the comparison in which *C. maculata* was the focal predator, the addition of *C. septempunctata* to create a two species assemblage did not lead to a significant change in *P. rapae* mortality, but when all three predators were present there was a significant difference in mortality compared to *C. maculata* alone (Figure 4.3a). This relationship between predator richness and prey mortality was best represented by the

relationship depicted in Figures 4.1c and 4.1g. For the other sets of comparisons, the interpretation was more straightforward. In comparison 2 (Figure 4.3b), there was a direct correlation between predator richness and prey mortality up to a point, after which there was a leveling off of mortality as richness increased. For three of the comparisons, 3, 5 and 6 (Figures 4.4a, 4.5a and 4.5b respectively), no relationship existed between predator richness and prey mortality. The fact that such a variety of conclusions can be made from these data suggests that the relationship between predator richness and prey mortality can be mitigated by the identity of predator species in the assemblage.

The idea, that species identity may dictate an assemblage's effectiveness, has been recently supported by Wilby (2005) and Straub and Snyder (2006). These findings suggest there is an idiosyncratic nature to how effective assemblages of predators can be depending upon the identity of the predators. This idea along with the findings of my study, suggest that a thorough understanding of the predator species in the assemblage being conserved, as well as an understanding of the interactions between those species, are necessary for conservation biological control to be effective.

The leveling off of, and in some cases the decrease in, prey mortality as predator richness increased may be explained by considering the interactions occurring between predator species. When multiple species occur in an assemblage, interactions between the species are inevitable. The outcome of those interactions can affect the functioning of the ecosystem in which the community resides. In terms of predator assemblages, interactions can yield beneficial ecosystem functions [so-called

positive multiple predator effects (MPEs), sensu (Sih et al. 1998)] as well as outcomes that are detrimental to the functioning of ecosystems (i.e., negative MPEs). Mechanisms by which positive MPEs arise include synergism (Losey and Denno 1998), facilitation (Bruno et al. 2003) and resource partitioning (Townsend and Hildrew 1979). Facilitation and synergism differ in that, while facilitation benefits one participant in the interaction, in synergistic predator-predator interactions both predators are benefited. There exist far greater evidence of mechanisms for how negative MPEs such as intraguild predation (Polis et al. 1989, Rosenheim et al. 1993) and interference (Snyder and Wise 1999) occur. In order for an assemblage to effectively suppress pests, the positive MPEs must outweigh the negative MPEs. In fact, assemblages have been shown be effective at influencing pest numbers despite the potentially negative effects of intraguild predation (Snyder and Ives 2003) and interference (Lang 2003). In my study, I asked if increasing the number of predators in an assemblage, despite the increased likelihood of negative MPEs occurring, can still lead to a situation in which there is an increase in natural suppression of pests. What I found was that increasing the species richness of a predator assemblage can lead to a corresponding increase in prey mortality, but that relationship is not linear. In fact, it may have been the case that at higher species richness prey mortality decreased. In order to explain this pattern, further investigation into negative MPEs other than intraguild predation (i.e. cannibalism and interference) would be necessary.

A recent meta-analysis looked at the effects of species richness of various trophic groups including predators and their depletion of a resource, prey in the case of predators (Cardinale et al. 2006). Although diverse “polycultures” of predators on

average consumed more prey than "monocultures," the highest performing 'monoculture' was not statistically distinguishable from the "polyculture." This suggests that a single predator could be more effective at suppressing pests than an assemblage of predators. To the contrary, the Species Assemblage Control Hypothesis suggests that an assemblage of predators can be more effective at suppressing pest than a single natural enemy (Riechert and Lockley 1984, Provencher and Riechert 1994, Riechert and Lawrence 1997). And, the results of my study show that it's not the entire assemblage of predators present in the agroecosystem that is responsible for pest suppression, as suggested by SACH. Rather, it's a subset of the predator assemblage that is actually responsible for pest suppression, and that the identity of predators in the assemblage influences how the assemblage functions. So how do we reconcile the contradictory evidence emerging about the relationship between biodiversity and biological control? In the future, experiments that look at assemblages with greater numbers of predators in field conditions are needed to gain further insight into the relationship biodiversity and biological control, and determine how this relationship can be altered by predator species identity. It is clear that a better understanding of the predator assemblage influencing a target pest, specifically an understanding of predator-predator interactions as well as predator-prey interaction, is needed before conservation decisions can be made that provide effective pest suppression.

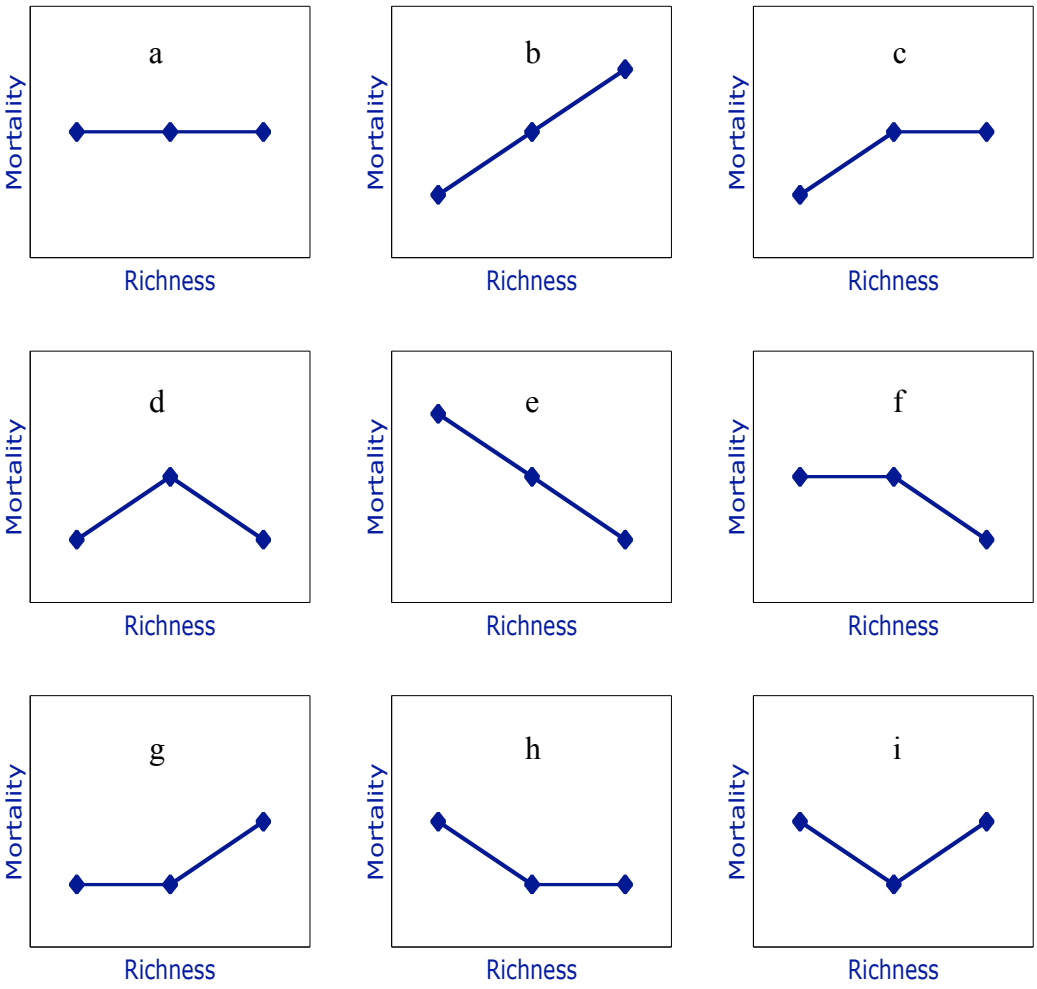

Figure 4.1. Generalized results graphs. All possible results for an experimental design with three levels of species richness.

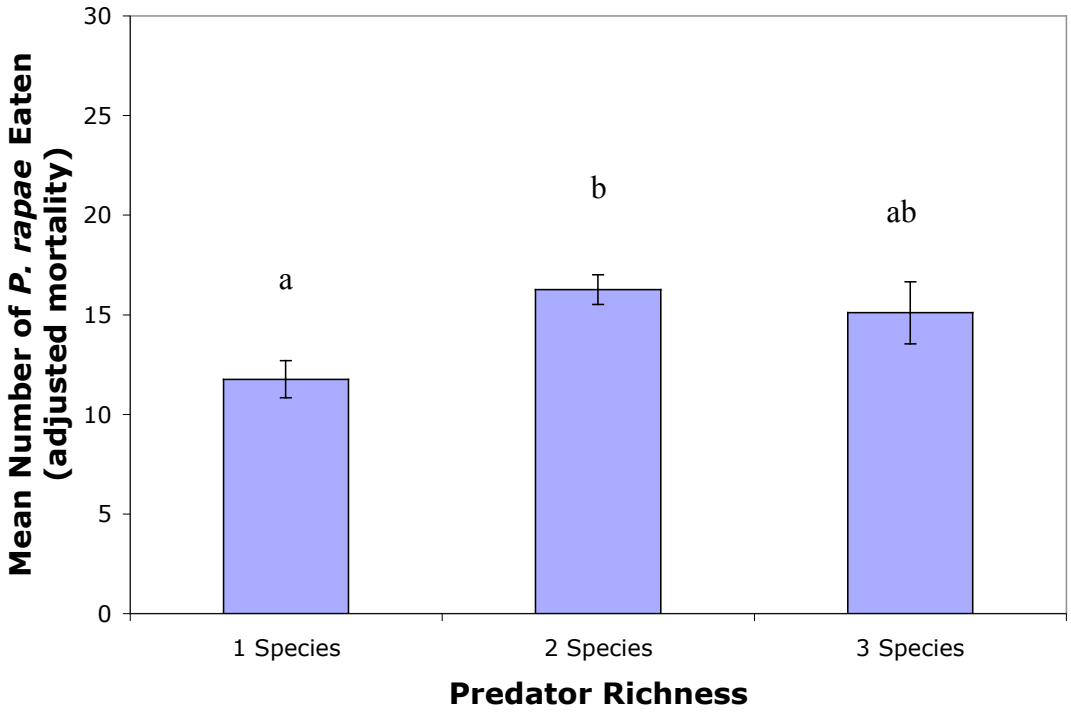

Figure 4.2. Relationship between predator richness and prey mortality. Adjusted *P. rapae* mortality was pooled across all treatments with the same richness and then compared between treatments with 1 species, 2 species and 3 species.

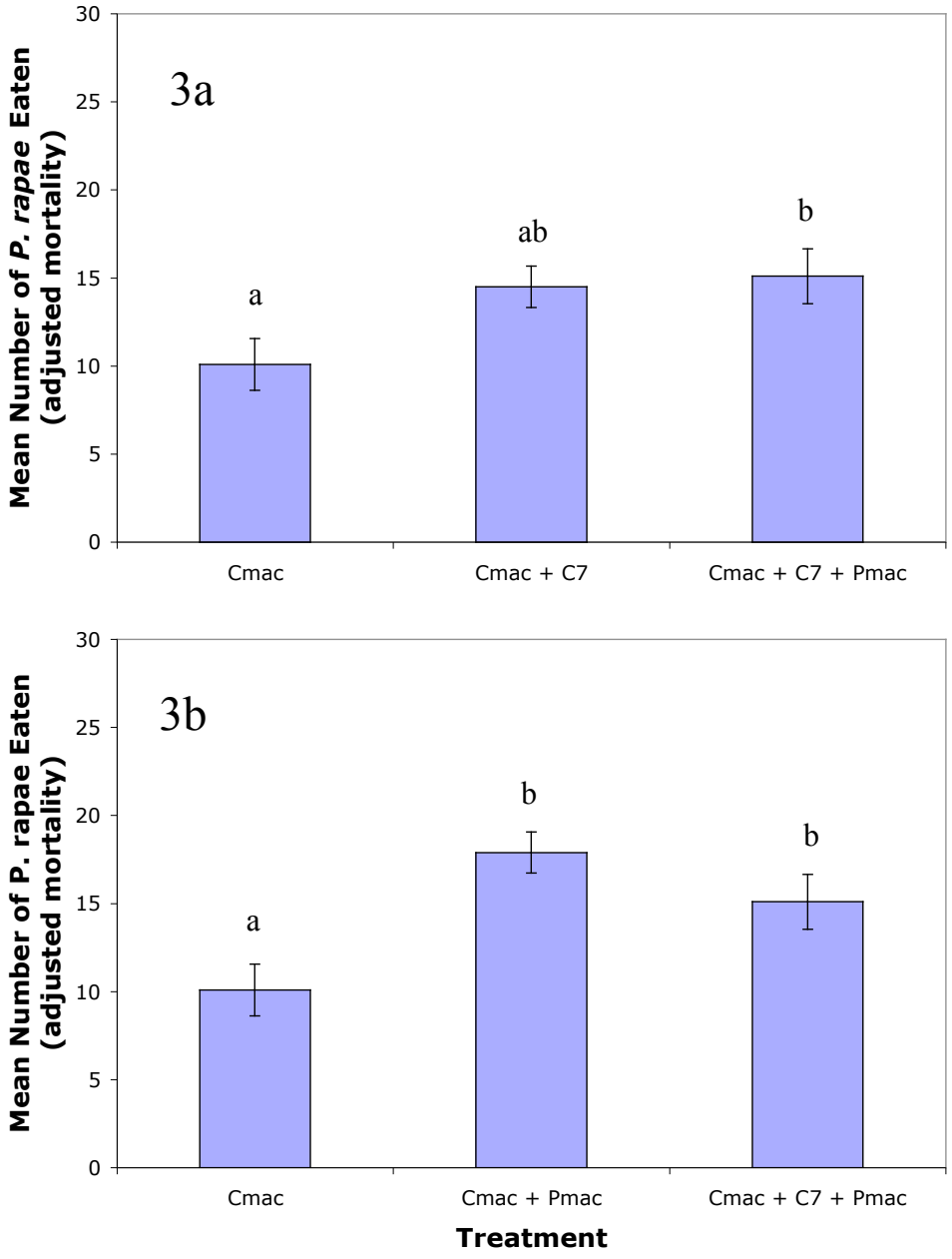

Figure 4.3. *C. maculata* (Cmac) in one, two and three species assemblages. In figure 4.3a, treatments with *C. maculata* alone, treatments with *C. maculata* and *C. septempunctata* (C7), and treatments with all three predators were compared. Figure 4.3b differs in that the two-predator treatments contained *C. maculata* and *P. maculiventris* (Pmac), however the same comparisons were made.

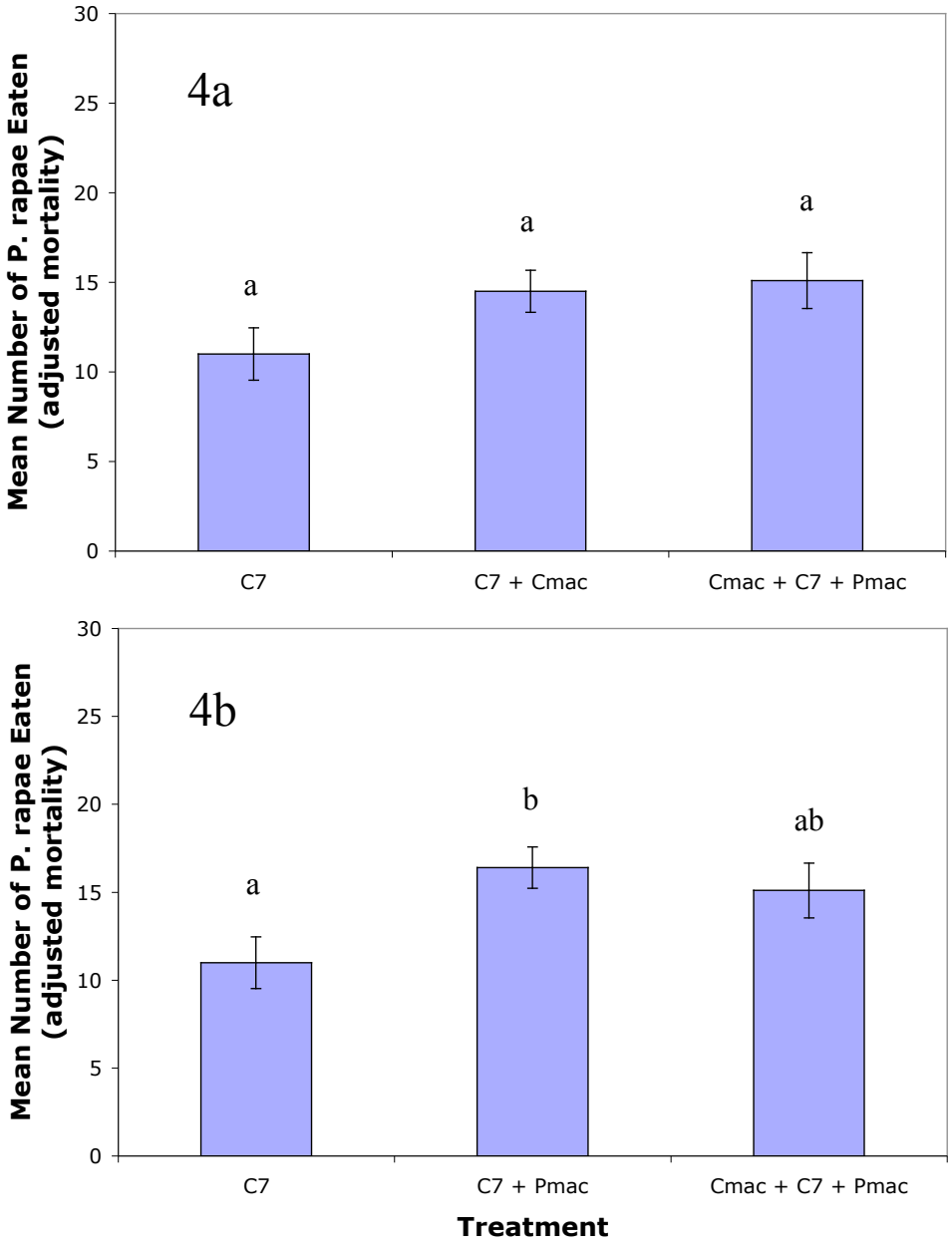

Figure 4.4. C. septempunctata (C7) in one, two and three species assemblages. In figure 4.4a, treatments with C. septempunctata alone, treatments with C. septempunctata and C. maculata (Cmac), and treatments with all three predators were compared. Figure 4.4b differs in that two-predator treatments contained C. septempunctata and P. maculiventris (Pmac), but same comparisons were made.

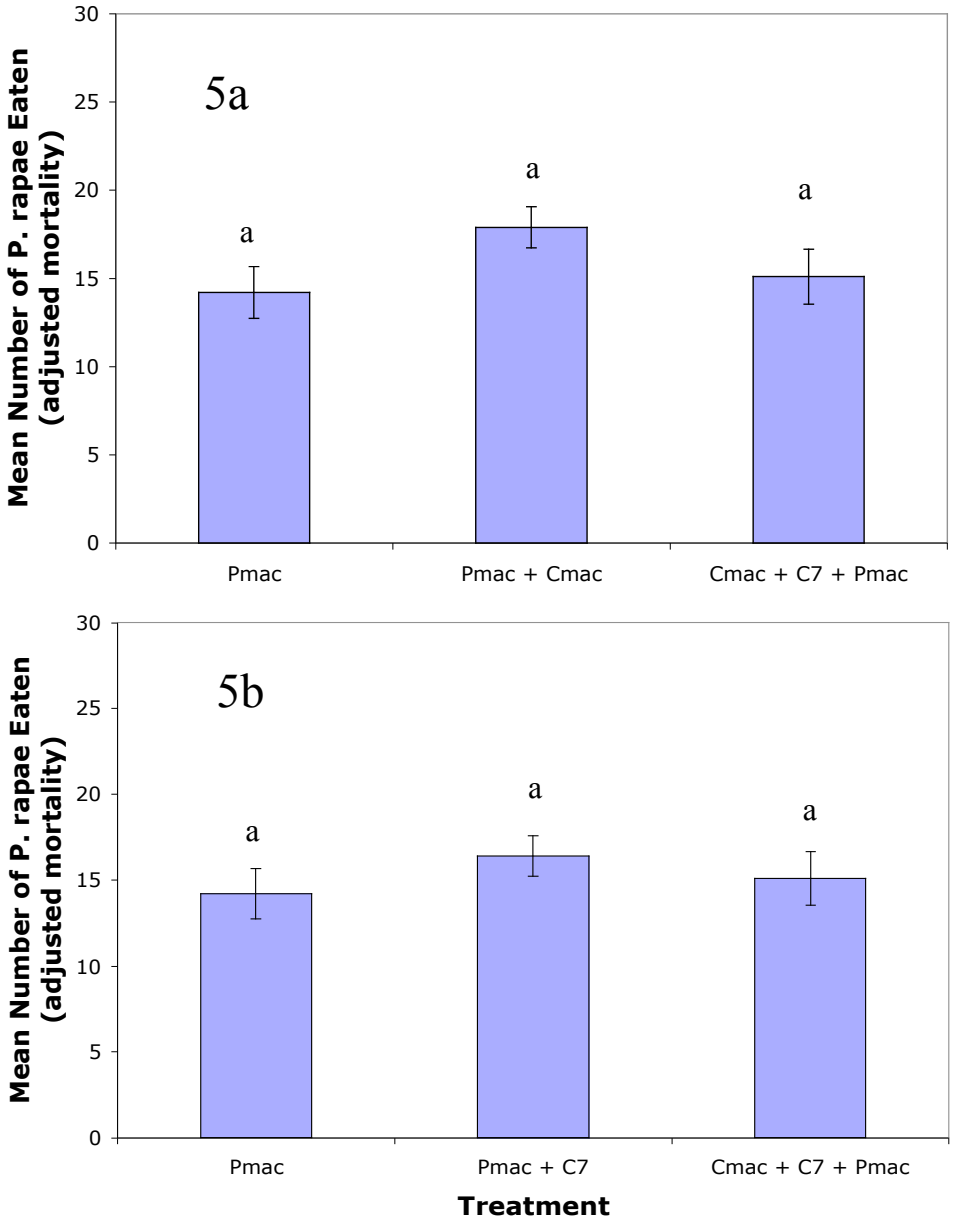

Figure 4.5. P. maculiventris (Pmac) in one, two and three species assemblages. In figure 4.5a, treatments with P. maculiventris alone, treatments with P. maculiventris and C. maculata (Cmac), and treatments with all three predators were compared. Figure 4.5b differs in that the two-predator treatments contained P. maculiventris and C. septempunctata, however same comparisons were made.

Appendix: Data from the assessment of the predator assemblages found in the epigeal, foliar and aerial microhabitats of the collard agroecosystem

Pitfall Samples

Heteroptera

Rank	Abundance	ID	Species	Family
117	1	2.1.04	*Orius insidiosus*	Anthocoridae
82	2	2.1.02	*Nabis roseipennis*	Nabidae
118	1	2.1.05	*Barce fraterna*	Reduviidae
119	1	2.1.06	*Melanolestes picipes*	Reduviidae
81	2	2.1.01	*Micracanthia humilis*	Saldidae
120	1	2.1.08		Unknown

Hymenoptera

Rank	Abundance	ID	Species	Family
2	350	4.1.01	*Lasius alienus*	Formicidae
5	172	4.1.03, 4.1.12, 4.1.13	*Pheidole bicarinata vinelandica*	Formicidae
8	106	4.1.04		Formicidae
13	52	4.1.02	*Tetramorium caespitum*	Formicidae
16	34	4.1.05	*Aphaenogaster sp.*	Formicidae
58	5	4.1.07	*Monomorium minimum*	Formicidae
59	5	4.1.09	*Aphaenogaster sp.*	Formicidae
94	2	4.1.10	*Tetramorium sp.*	Formicidae
95	2	4.1.15		Formicidae
145	1	4.1.11	*Tetramorium sp.*	Formicidae
147	1	4.1.16	*Formica (fusca) sp.*	Formicidae
148	1	4.1.17	*Hypoponera sp.*	Formicidae
149	1	4.1.18		Formicidae
146	1	4.1.14		Mutilidae

Coleoptera

Rank	Abundance	ID	Species	Family
7	116	3.1.03	*Pterostichus lucublandus*	Carabidae
14	43	3.1.15	*Bembidion semistrictum*	Carabidae
17	32	3.1.14		Carabidae

20	26	3.1.18	*Stenolophus ochropezus*	Carabidae
23	23	3.1.06		Carabidae
26	19	3.1.09		Carabidae
35	10	3.1.07		Carabidae
34	10	3.1.12		Carabidae
49	6	3.1.02		Carabidae
50	6	3.1.04		Carabidae
48	6	3.1.16		Carabidae
54	5	3.1.05		Carabidae
64	4	3.1.20		Carabidae
85	2	3.1.08		Carabidae
83	2	3.1.11		Carabidae
84	2	3.1.13		Carabidae
121	1	3.1.01		Carabidae
122	1	3.1.10		Carabidae
123	1	3.1.17		Carabidae
124	1	3.1.19		Carabidae
125	1	3.1.21		Carabidae
31	14	3.3.2, 3.3.1, 3.3.3	*Coleomegilla maculata*	Coccinellidae
1	575	3.2.05	*Amisch sp.*	Staphylinidae
15	42	3.2.04	*Amisch sp.*	Staphylinidae
18	32	3.2.06		Staphylinidae
24	23	3.2.01		Staphylinidae
28	16	3.2.03		Staphylinidae
30	14	3.2.19		Staphylinidae
32	11	3.2.15		Staphylinidae
36	10	3.2.02		Staphylinidae
44	7	3.2.08		Staphylinidae
43	7	3.2.25		Staphylinidae
55	5	3.2.14		Staphylinidae
56	5	3.2.18		Staphylinidae
57	5	3.2.26		Staphylinidae
71	3	3.2.07		Staphylinidae
72	3	3.2.09		Staphylinidae
70	3	3.2.23		Staphylinidae
86	2	3.2.16		Staphylinidae
87	2	3.2.17		Staphylinidae
88	2	3.2.22		Staphylinidae
126	1	3.2.10		Staphylinidae
127	1	3.2.11		Staphylinidae

128	1	3.2.12		Staphylinidae
129	1	3.2.13		Staphylinidae
130	1	3.2.20		Staphylinidae
131	1	3.2.21		Staphylinidae
132	1	3.2.24		Staphylinidae
133	1	3.2.27		Staphylinidae
37	10	3.4.07		Unknown
41	8	3.4.16		Unknown
51	6	3.4.06		Unknown
73	3	3.4.01		Unknown
74	3	3.4.13		Unknown
91	2	3.4.03		Unknown
92	2	3.4.04		Unknown
93	2	3.4.05		Unknown
89	2	3.4.10		Unknown
90	2	3.4.20		Unknown
141	1	3.4.02		Unknown
143	1	3.4.08		Unknown
144	1	3.4.09		Unknown
134	1	3.4.11		Unknown
135	1	3.4.12		Unknown
136	1	3.4.14		Unknown
137	1	3.4.15		Unknown
138	1	3.4.17		Unknown
139	1	3.4.18		Unknown
140	1	3.4.19		Unknown
142	1	3.4.21		Unknown

Araneae

Rank	Abundance	ID	Species	Family
11	63	1.2.02		Linyphiidae
21	25	1.2.09		Linyphiidae
22	23	1.2.06		Linyphiidae
25	22	1.2.03		Linyphiidae
29	14	1.2.07		Linyphiidae
33	10	1.2.13		Linyphiidae
42	7	1.2.01		Linyphiidae
46	6	1.2.17		Linyphiidae
47	6	1.2.08		Linyphiidae
53	5	1.2.31		Linyphiidae
61	4	1.2.14		Linyphiidae
62	4	1.2.30		Linyphiidae

63	4	1.2.32		Linyphiidae
67	3	1.2.20		Linyphiidae
68	3	1.2.24		Linyphiidae
69	3	1.2.27		Linyphiidae
76	2	1.2.18		Linyphiidae
77	2	1.2.22		Linyphiidae
78	2	1.2.23		Linyphiidae
79	2	1.2.26		Linyphiidae
97	1	1.2.10		Linyphiidae
98	1	1.2.11		Linyphiidae
100	1	1.2.19		Linyphiidae
101	1	1.2.21		Linyphiidae
102	1	1.2.25		Linyphiidae
103	1	1.2.28		Linyphiidae
104	1	1.2.29		Linyphiidae
116	1	1.2.05		Linyphiidae
3	216	1.1.08	*Pardosa sp.*	Lycosidae
4	200	1.1.02	*Pardosa sp.*	Lycosidae
6	149	1.1.11	*Pardosa sp.*	Lycosidae
9	93	1.1.07	*Pardosa sp.*	Lycosidae
10	90	1.1.01	*Pardosa sp.*	Lycosidae
12	59	1.1.12	*Pardosa sp.*	Lycosidae
27	18	1.1.05	*Pardosa sp.*	Lycosidae
38	9	1.1.14	*Pardosa sp.*	Lycosidae
39	9	1.2.15		Lycosidae
40	8	1.2.04		Lycosidae
45	6	1.1.04	*Pardosa sp.*	Lycosidae
52	5	1.1.09		Lycosidae
60	4	1.1.06	*Pardosa sp.*	Lycosidae
65	3	1.1.10		Lycosidae
66	3	1.1.13	*Pardosa sp.*	Lycosidae
96	1	1.1.03	*Pardosa sp.*	Lycosidae
99	1	1.2.16		Thomisidae
80	2	1.2.36		Unknown
105	1	1.2.33		Unknown
106	1	1.2.34		Unknown
107	1	1.2.35		Unknown
108	1	1.2.37		Unknown
109	1	1.2.38		Unknown
110	1	1.2.39		Unknown
111	1	1.2.40		Unknown
112	1	1.2.41		Unknown

113	1	1.2.42		Unknown
114	1	1.2.43		Unknown
115	1	1.2.44		Unknown

Opiliones

Rank	Abundance	ID	Species	Family
151	1	6.1.1		Unknown

Chilopoda

Rank	Abundance	ID	Species	Family
19	27	5.1.1		Unknown
75	3	5.1.2		Unknown
150	1	5.1.3		Unknown

Visual Samples

Heteroptera

Rank	Abundance	ID	Species	Family
4	11	2.1.01, 2.1.06, 2.1.08	*Lygus lineolaris*	Miridae
2	21	2.1.02	*Nabis roseipennis*	Nabidae
15	3	2.1.03	*Podisus maculiventris*	Pentatomidae
40	1	2.1.11	*Euschistus servus*	Pentatomidae

Hymenoptera

Rank	Abundance	ID	Species	Family
42	1	4.1.1	*Tetramorium sp.*	Formicidae

Diptera

Rank	Abundance	ID	Species	Family
43	1	5.1.1		Syrphidae

Coleoptera

Rank	Abundance	ID	Species	Family
25	2	3.4.1	*Chauliognathus marginatus*	Cantharidae
1	102	3.3.1	*Coleomegilla maculata*	Coccinellidae
9	4	3.3.5	*Coccinella septempunctata*	Coccinellidae
41	1	3.4.2		Lampyridae

Araneae

Rank	Abundance	ID	Species	Family
8	4	1.4.1		Araneidae

11	3	1.2.02		Araneidae
5	5	1.1.02		Lycosidae
10	3	1.1.03		Lycosidae
26	1	1.1.01		Lycosidae
13	3	1.2.15		Salticidae
21	2	1.2.18		Salticidae
45	1	1.2.24		Salticidae
3	12	1.3.01		Tetragnathidae
39	1	1.5.1		Thomisidae
6	4	1.2.07		Unknown
7	4	1.2.17		Unknown
12	3	1.2.10		Unknown
14	3	1.3.02		Unknown
16	2	1.2.01		Unknown
17	2	1.2.03		Unknown
18	2	1.2.04		Unknown
19	2	1.2.12		Unknown
20	2	1.2.13		Unknown
22	2	1.2.21		Unknown
23	2	1.2.22		Unknown
24	2	1.2.26		Unknown
27	1	1.2.05		Unknown
28	1	1.2.06		Unknown
29	1	1.2.08		Unknown
30	1	1.2.09		Unknown
31	1	1.2.11		Unknown
32	1	1.2.14		Unknown
33	1	1.2.16		Unknown
34	1	1.2.19		Unknown
35	1	1.2.20		Unknown
44	1	1.2.23		Unknown
36	1	1.2.25		Unknown
37	1	1.2.27		Unknown
38	1	1.2.28		Unknown

Sweep Samples

Heteroptera

Rank	Abundance	ID	Species	Family
2	45	2.1.03	*Orius insidiosus*	Anthocoridae
14	3	2.1.17	*Jalysus wickhami*	Berytidae
17	2	2.1.10	*Geocoris punctipes*	Geocoridae
1	318	2.1.02,	*Lygus lineolaris*	Miridae

		2.1.11, 2.1.04, 2.1.07		
4	17	2.1.12	*Polymerus basalis*	Miridae
18	2	2.1.09	*Trigonotylus caelestialium*	Miridae
25	1	2.1.01	*Nabis roseipennis*	Nabidae
26	1	2.1.19	*Sinea sp.*	Reduviidae
13	4	2.1.06, 2.1.13	*Micracanthia humilis*	Saldidae

Hymenoptera

Rank	Abundance	ID	Species	Family
11	6	4.1.1, 4.1.2	*Monomorium minimum*	Formicidae
19	2	4.1.3	*Lasius alienus*	Formicidae

Coleoptera

Rank	Abundance	ID	Species	Family
3	37	3.1.3, 3.1.2	*Coleomegilla maculata*	Coccinellidae
20	2	3.1.5, 3.1.7	*Harmonia axyridis*	Coccinellidae
29	1	3.1.6		Carabidae
15	3	2.1.05	*Chauliognathus marginatus*	Cantharidae
21	2	3.1.4		Staphylinidae
7	8	5.1.2		Unknown
30	1	3.1.01		Staphylinidae

Araneae

Rank	Abundance	ID	Species	Family
5	10	1.1.05		Unknown
6	9	1.1.08		Unknown
9	7	1.1.03		Unknown
10	7	1.1.04		Unknown
12	5	1.1.07		Unknown
23	2	1.1.02		Unknown
24	2	1.1.06		Unknown
27	1	2.1.16		Unknown
28	1	2.1.18		Unknown
31	1	1.1.10		Unknown
8	8	1.1.01		Tetragnathidae
22	2	1.1.09		Salticidae

Chilopoda

Rank	Abundance	ID	Species	Family
16	3	5.1.1		Unknown

References

Agarwala, B. K., and A. F. G. Dixon. 1992. Laboratory Study of Cannibalism and Interspecific Predation in Ladybirds. Ecological Entomology **17**:303-309.

Ashby, J. W. 1974. A Study of Arthropod Predation of Pieris rapae L. Using Serological and Exclusion Techniques. The Journal of Applied Ecology **11**:419-425.

Barbosa, P., editor. 1998. Conservation Biological Control Academic Press, San Diego, CA.

Barbosa, P., A. Caldas, and S. E. Reichert. 2005. Species abundance distribution and predator-prey interactions: theoretical and applied consequences. *in* P. Barbosa and I. Castellanos, editors. Ecology of Predator-Prey Interactions. Oxford University Press, Oxford.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution **18**:119-125.

Caltagirone, L. E. 1981. Landmark Examples in Classical Biological-Control. Annual Review of Entomology **26**:213-232.

Cardinale, B. J., C. T. Harvey, K. Gross, and A. R. Ives. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. Ecology Letters **6**:857-865.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran, and C. Jouseau. 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature **443**:989-992.

Chang, G. C., and P. Kareiva. 1999. The case for indigenous generalists in biological control. Pages 103-115 *in* B. A. Hawkins and H. V. Cornell, editors. Theoretical Approaches to Biological Control. Cambridge University Press, Cambridge, UK.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Diaz. 2000. Consequences of changing biodiversity. Nature **405**:234-242.

Claridge, M. F. 1987. Insect assemblages - diversity, organization, and evolution. *in* J. H. R. Gee and P. S. Giller, editors. Organization of communities. Past and present. 27th Symposium of the British Ecological Society. Blackwell Science, London.

DeBach, P., editor. 1964. Biological control of insect pests and weeds. Chapman & Hall, London.

DeBach, P. 1974. Biological control by natural enemies. Cambridge University Press, London ; New York, N.Y.

Dempster, J. P. 1967. The Control of Pieris rapae with DDT. I. The Natural Mortality of the Young Stages of Pieris. The Journal of Applied Ecology **4**:485-500.

Denoth, M., L. Frid, and J. H. Myers. 2002. Multiple agents in biological control: improving the odds? Biological Control **24**:20-30.

Dinter, A. 2002. Microcosm studies on intraguild predation between female erigonid spiders and lacewing larvae and influence of single versus multiple predators on cereal aphids. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie **126**:249-257.

Doutt, R. L. 1967. Biological control. . Pages 4-29 *in* W. W. Kilgore and R. L. Doutt, editors. Pest Control: Biological, Physical and Selected Chemical Methods. . Academic Press, New York, NY.

Eilenberg, J., A. Hajek, and C. Lomer. 2001. Suggestions for unifying the terminology in biological control. Biocontrol **46**:387-400.

Finke, D. L., and R. F. Denno. 2004. Predator diversity dampens trophic cascades. Nature **429**:407-410.

Harcourt, D. G. 1961. Spatial pattern of the imported cabbageworm, Pieris rapae (L.)(Lepidoptera: Pieridae), on cultivated cruciferae. The Canadian Entomologist **93**:945-952.

Hochberg, M. E. 1996. Consequences for host population levels of increasing natural enemy species richness in classical biological control. American Naturalist **147**:307-318.

Hooks, C. R. R., R. R. Pandey, and M. W. Johnson. 2003. Impact of avian and arthropod predation on lepidopteran caterpillar densities and plant productivity in an ephemeral agroecosystem. Ecological Entomology **28**:522-532.

Howarth, F. G. 1991. Environmental Impacts of Classical Biological-Control. Annual Review of Entomology **36**:485-509.

Ives, A. R., B. J. Cardinale, and W. E. Snyder. 2005. A synthesis of subdisciplines: predator-prey interactions, and biodiversity and ecosystem functioning (vol 8, pg 102, 2005). Ecology Letters **8**:782-782.

Jones, R. E. 1977. Search behaviour: a study of three caterpillar species. Behaviour **60**:237-257.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology **45**:175-201.

Lang, A. 2003. Intraguild interference and biocontrol effects of generalist predators in a winter wheat field. Oecologia **134**:144-153.

Loreau, M. 2000. Biodiversity and ecosystem functioning: recent theoretical advances. Oikos **91**:3-17.

Loreau, M., S. Naeem, P. Inchausti, J. Bengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman, and D. A. Wardle. 2001. Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science **294**:804-808.

Losey, J. E., and R. F. Denno. 1998. Positive predator-predator interactions: Enhanced predation rates and synergistic suppression of aphid populations. Ecology **79**:2143-2152.

Madsen, M., S. Terkildsen, and S. Toft. 2004. Microcosm studies on control of aphids by generalist arthropod predators: Effects of alternative prey. Biocontrol **49**:483-504.

Mallampalli, N., I. Castellanos, and P. Barbosa. 2002. Evidence for intraguild predation by *Podisus maculiventris* on a ladybeetle, *Coleomegilla maculata*: Implications for biological control of Colorado potato beetle, *Leptinotarsa decemlineata*. Biocontrol **V47**:387-398.

Pearson, D. E., and R. M. Callaway. 2003. Indirect effects of host-specific biological control agents. Trends in Ecology & Evolution **18**:456-461.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The Ecology and Evolution of Intraguild Predation - Potential Competitors That Eat Each Other. Annual Review of Ecology and Systematics **20**:297-330.

Provencher, L., and S. E. Riechert. 1994. Model and Field-Test of Prey Control Effects by Spider Assemblages. Environmental Entomology **23**:1-17.

Riechert, S. E., and K. Lawrence. 1997. Test for predation effects of single versus multiple species of generalist predators: spiders and their insect prey. Entomologia Experimentalis Et Applicata **84**:147-155.

Riechert, S. E., and T. Lockley. 1984. Spiders as Biological-Control Agents. Annual Review of Entomology **29**:299-320.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild Predation Among Biological-Control Agents: Theory and Evidence. Biological Control **5**:303-335.

Rosenheim, J. A., L. R. Wilhoit, and C. A. Armer. 1993. Influence of Intraguild Predation among Generalist Insect Predators on the Suppression of an Herbivore Population. Oecologia **96**:439-449.

Schellhorn, N. A., and D. A. Andow. 1999. Mortality of coccinellid (Coleoptera : Coccinellidae) larvae and pupae when prey become scarce. Environmental Entomology **28**:1092-1100.

Schellhorn, N. A., and V. L. Sork. 1997. The impact of weed diversity on insect population dynamics and crop yield in collards, Brassica oleraceae (Brassicaceae). Oecologia **111**:233-240.

Schmaedick, M. A., and A. M. Shelton. 2000. Arthropod predators in cabbage (Cruciferae) and their potential as naturally occurring biological control agents for Pieris rapae (Lepidoptera : Pieridae). Canadian Entomologist **132**:655-675.

Sih, A., G. Englund, and D. Wooster. 1998. Emergent impacts of multiple predators on prey. Trends in Ecology & Evolution **13**:350-355.

Snyder, W. E., S. N. Ballard, S. Yang, G. M. Clevenger, T. D. Miller, J. J. Ahn, T. D. Hatten, and A. A. Berryman. 2004. Complementary biocontrol of aphids by the ladybird beetle Harmonia axyridis and the parasitoid Aphelinus asychis on greenhouse roses. Biological Control **30**:229-235.

Snyder, W. E., G. C. Chang, and R. P. Prasad. 2005. Conservation Biological Control: Biodiversity Influences the Effectiveness of Predators. *in* P. Barbosa and I. Castellanos, editors. Ecology of Predator-Prey Interactions. Oxford University Press, Oxford.

Snyder, W. E., and A. R. Ives. 2003. Interactions between specialist and generalist natural enemies: Parasitoids, predators, and pea aphid biocontrol. Ecology **84**:91-107.

Snyder, W. E., G. B. Snyder, D. L. Finke, and C. S. Straub. 2006. Predator biodiversity strengthens herbivore suppression. Ecology Letters **9**:789-796.

Snyder, W. E., and D. H. Wise. 1999. Predator Interference and the Establishment of Generalist Predator Populations for Biocontrol. Biological Control **15**:283-292.

Stiling, P. 2004. Biological control not on target. Biological Invasions **6**:151-159.

Straub, C. S., and W. E. Snyder. 2006. Species identity dominates the relationship between predator biodiversity and herbivore suppression. Ecology **87**:277-282.

Suenaga, H., and T. Hamamura. 2001. Occurrence of carabid beetles (Coleoptera : Carabidae) in cabbage fields and their possible impact on lepidopteran pests. Applied Entomology and Zoology **36**:151-160.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology **47**:561-594.

Tilman, D. 2000. Causes, consequences and ethics of biodiversity. Nature **405**:208-211.

Townsend, C. R., and A. G. Hildrew. 1979. Resource Partitioning by Two Freshwater Invertebrate Predators with Contrasting Foraging Strategies. The Journal of Animal Ecology **48**:909-920.

Wiedenmann, R. N., and R. J. O'Neil. 1991. Searching behavior and time budgets of the predator *Podisus maculiventris*. Entomologia Experimentalis Et Applicata **60**:83-93.

Wilby, A., S. C. Villareal, L. P. Lan, K. L. Heong, and M. B. Thomas. 2005. Functional benefits of predator species diversity depend on prey identity. Ecological Entomology **30**:497-501.