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DO VARYING NATURAL ENEMY ASSEMBLAGES IMPACT
APHIS GLYCINES POPULATION DYNAMICS?

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Ph.D. degree in Entomology

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DO VARYING NATURAL ENEMY ASSEMBLAGES IMPACT *APHIS GLYCINES* POPULATION DYNAMICS?

By

Alejandro Carlos Costamagna

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Entomology
and
Program in Ecology, Evolutionary Biology and Behavior

2006

ABSTRACT

DO VARYING NATURAL ENEMY ASSEMBLAGES IMPACT *APHIS GLYCINES* POPULATION DYNAMICS?

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The soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae), was detected for the first time in North America in Wisconsin during 2000, and it is currently present in 22 states. Since its detection, it has developed outbreak populations that resulted in up to 50% yield reduction during alternating years. I conducted field studies that demonstrated the crucial role of existing generalist natural enemies in suppressing soybean aphid population growth below economic threshold levels. By using predator exclusion cages, I demonstrated that ambient levels of predators exert strong suppression on soybean aphid (3 to 20-fold reduction) in comparison with open controls and sham cages. Through work at the NSF-Long Term Ecological Research site at the Kellogg Biological Station of Michigan State University, I found that this control occurs over a broad range of agricultural production systems, ranging from conventional practices with or without tillage, to organic soybeans. This suppression occurs regardless of initial aphid infestation, with aphid populations being reduced below threshold levels at high, medium and low initial aphid densities. The strength of this suppression results in a trophic cascade, with similar soybean biomass and yield in treatments where aphids were exposed to predation as in treatments completely protected from aphids by aphid exclusion cages. Direct field observations revealed that the coccinellids *Harmonia axyridis* and *Coccinella septempunctata* combined provided most of the mortality on soybean aphid, were very effective on a per capita basis, and responded to aphid density by increasing their abundance, time within the aphid patch, and consumption rate. Exposure to predation significantly shifted the aphid's within-plant distribution towards lower nodes on the plants, suggesting indirect effects of predation.

I also conducted studies using cages that differentially excluded large versus small predators and parasitoids, which showed that ambient levels of naturally occurring parasitoids provide only minor or no reduction of soybean aphid populations, even in treatments in which parasitoids were provided with a refuge from intraguild predation by coccinellids. Similar results were obtained in a separate study using artificial infestations with the parasitoid *Lysiphlebus testaceipes*. I observed direct evidence of intraguild predation on parasitoid mummies by generalist predators, in which up to 80% of mummies were killed by predators, but percentage parasitism did not differ from controls, suggesting that intraguild predation did not disrupt parasitism. Finally, I developed a mathematical model for soybean aphid population growth by fitting the data of field studies under predator exclusion conditions, using a novel approach that assumes a linear decrease in the intrinsic rate of increase of the aphids. Overall, these findings provide one of the first empirical tests of recent theoretical models in which the role of generalist predators is predicted to be an important source of insect population regulation. In addition, these results support theoretical predictions that intraguild predators with strong impacts on herbivores may not disrupt, or may even enhance biological control. From the pest management perspective, it suggests the incorporation of natural enemies as an important factor to take into account when determining soybean aphid thresholds.

To Marisol, Ailén and Chiara, for their continuos love and support

ACKNOWLEDGEMENTS

I would like to thank Dr. Doug Landis for being an outstanding advisor, providing support, enthusiasm, dedication and patience. His vast knowledge on insect ecology and biological control, high research standards, and encouragement to pursue interesting questions were fundamental parts of my Ph.D. experience. I would also like to thank the members of my Ph.D. Committee, Jim Miller, Mike Brewer, Chris DiFonzo and Phil Robertson, their expertise and support contributed significantly to improve the findings of this research.

I am very grateful to all the Landis lab people, Chris Sebolt, Anna Fiedler, Jeff Evans and Mary Gardiner, for sharing ideas, resources, and providing support throughout these studies, including hard days of work in the field. Special thanks to Chris, who was responsible for maintaining the campus study field site, and coordinated the field workers very effectively each field season. This study would have not been possible without the hard work of many undergraduate helpers: M. Wood, R. MacDonald, C. Stahlman, J. Steffen, T. Gibbons, A. Berro, N. Vuljaj, E. Knoblock, C. Richards, M. Wayo, R. Alderson, T. Lehman, D. Richards, K. Newhouse and S. Gottschalk.

I would also like to thank P. Robertson, J. Simmons, A. Corbin and all the KBS-LTER staff for their help in this study, including facilitating biomass and yield measurements from the study site. I am grateful to D. Prokrym and the staff of the USDA APHIS PPQ Laboratory at Niles, MI, for providing advice and support for rearing *A. glycines*. Very helpful statistical advice was given by S. Kravchenko and X. Huang (SCC-CANR). B. Graff generously helped process soybean samples (Agronomy Farm-

MSU). I am also indebted for the support provided by all the Faculty and staff in the Department of Entomology at MSU, especially Fred Stehr, Ed Grafius, Rich Merritt, and Jill Kolp. K. Pike, M. Brewer, T. Noma and G. Parsons provided their valuable expertise for insect identifications. Finally, I am very grateful to Wopke van der Werf and Felix Bianchi for all their support during the modeling part of this dissertation and for being excellent hosts during my visit to Wageningen University.

Funding for this research was provided through a teaching assistantship from the Department of Entomology, and by a USDA-CSREES grant 2004-35302-14811, the NSF Long-Term Ecological Research Program at the Kellogg Biological Station, the Michigan Agricultural Experiment Station, a MSU-Sustainable Agriculture grant, a summer fellowship from the Ecology, Evolutionary Biology and Behavior program at MSU, and a C. S. Mott Fellowship in Sustainable Agriculture.

Finally, many thanks to my dear wife Marisol and my daughters Ailén and Chiara, whose continuous support, encouragement, understanding and love made the completion of this degree possible.

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KEY TO SYMBOLS AND ABBREVIATIONS

ANOVA	analysis of variance
^{o}C	degrees Celsius
cm	centimeters
d	days
df	degrees of freedom
F	Fisher distribution
g	grams
h	hours
m	meters
mm	milimiters
n	number of observations
ns	not significant
P	probability under the assumption that the null hypothesis is true
r	Pearson's coefficient of correlation
r^2	coefficient of determination
RH	relative humidity
SD	standard Deviation of the Mean
SE	standard Error of the Mean
spp.	species
t	t-statistic

CHAPTER 1

APHIS GLYCINES IN NORTH AMERICA

Introduction

The soybean aphid, *Aphis glycines*, Matsumura (Hemiptera: Aphididae) is an important pest of soybean (*Glycine max* L.) in China and Southeast Asia, causing yield losses up to 70% (Wang et al. 1962, Hirano et al. 1996, Van den Berg et al. 1997, Wu et al. 2004). In the year 2000, *A. glycines* was discovered for the first time in North America and Australia (Fletcher and Desborough 2000, Wedburg 2000, Venette and Ragsdale 2004). Since 2000, it has expanded its distribution to 22 states in the USA and three provinces of Canada (Losey et al. 2002, Hunt et al. 2003, Landis et al. 2003, Venette and Ragsdale 2004, Mignault et al. 2006). In 2003, over 7 million acres of soybean were sprayed with insecticides against *A. glycines*, which infested over 50 million acres of soybean in the USA (Landis et al. 2003). Before the arrival of *A. glycines,* soybean in the North Central USA had few serious insect pests and was not usually treated with insecticides. Thus, soybean has played an important role as reservoir of natural enemies for other crops (Heimpel and Shelly 2004). Previous studies have shown significant impacts of natural enemies on *A. glycines* in Asia (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004). In the US, recent studies have also demonstrated a key role of natural enemies in suppressing *A. glycines* populations (Fox 2002, Fox and Landis 2003, Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Nielsen and Hajek 2005, Rutledge and O'Neil 2005). Generalist predators currently dominate the assemblage of *A. glycines* natural enemies, with parasitoids only rarely observed from 2000-2002. Interestingly, in 2003, surveys in Michigan, Minnesota and New York have revealed the presence of both native and introduced parasitoid species beginning to appear in natural enemy assemblages (Brewer and Noma 2003, Heimpel and Wu 2003, Nielsen and Hajek 2005). Observations indicate the presence of parasitoids, particularly *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) in 8 of 12 North Central States (Landis et al. 2003).

The invasion of *A. glycines* into North America provides a novel system to test ecological theory related to herbivore population dynamics. These include possible tests of the relative strength of bottom-up and top-down forces in the control of herbivorous species, and the interactions between generalist and specialist natural enemies, particularly in regard to the impact of intraguild predation on top-down control. The very limited levels of field parasitism to date provide a unique opportunity to explore the role of intraguild predation in influencing the integration of native parasitoids into natural enemy assemblages. This information could be used to anticipate the potential benefits and desired traits that introduced parasitoid species (classical biological control agents) should have to be successful in this system. In this dissertation, I first studied the relative role of top-down versus bottom-up control of *A. glycines* (Chapter 2). Then, after a prominent role of top-down regulation was established, I investigated if this resulted in a trophic cascade and whether it was affected by trophic interactions within the natural enemy guild, particularly intraguild predation (Chapters 3 and 4). I then investigated the dynamics of predation at the within field level assessing predation at different initial aphid densities and different plant parts (Chapter 5). Finally, I assessed the relative importance of each predator group attacking *A. glycines* through direct field observations (Chapter 6), and combining data from all my experiments I developed a model of *A. glycines* population growth under field conditions as a tool to investigate potential controls for this pest (Chapter 7).

Top-down and bottom-up forces in herbivore control

Hairston et al. (1960) proposed that top-down control of herbivore abundance by consumers was the dominant force shaping natural communities. This view proved to be controversial and it was subsequently proposed that in several communities bottom-up (plant resources) are the dominant forces (Murdoch 1966, Ehrlich and Birch 1967), a view supported by several empirical studies (Hunter et al. 1997, Denno et al. 2002, Gratton and Denno 2003b). Latter reviews suggest emerging consensus that both top-down and bottom-up forces are present in natural communities and that a more appropriate approach is to estimate their relative strength within ecosystems (Hunter and Price 1992, Power 1992, Walker and Jones 2001). Most empirical research in the role of these two forces has been conducted in aquatic ecosystems, but several recent studies have focused on terrestrial ecosystems (Dyer and Letourneau 1999, Walker and Jones 2001, Moran and Scheidler 2002, Gruner 2004). Several complex interactions and factors have been shown to significantly affect the relative strength of top-down versus bottom-up forces in terrestrial ecosystems. Among these are: environmental productivity (Power 1992, Fraser and Grime 1998, Dyer and Letourneau 1999, Fraser and Grime 1999, Moran and Scheidler 2002), consumer efficiency (Power 1992), temporal versus spatial variation of herbivore populations (Hunter et al. 1997), herbivore life stages

(Walker and Jones 2001), year-to-year (Walker and Jones 2001, Gratton and Denno 2003b) and seasonal changes in abiotic and biotic conditions (Boyer et al. 2003, Gratton and Denno 2003a), differential predation risk among herbivore species (Denno et al. 2003), and herbivore body size (Sinclair et al. 2003). No studies explicitly testing the relative role of top-down versus bottom-up forces has been conducted in agroecosystems, although comparisons of the effects of fertility regimes and host plant resistance (bottom-up) versus the effects of natural enemies (top-down) was utilized by Walker and Jones (2001) to infer the strength of this interaction from applied studies.

Trophic cascades

Higher order trophic levels can affect the abundance, biomass or productivity of populations across more than one link in a food web in a phenomenon termed a "trophic cascade" (Pace et al. 1999). There is still an open debate regarding the prevalence and importance of trophic cascades in different ecosystems, and particularly on what ecosystem characteristics lead to trophic cascades (Pace et al. 1999, Polis et al. 2000). Empirical evidence shows that trophic cascades frequently occur in more simple systems (i.e. less reticulated food webs) with many cases documented in aquatic environments, leading to the postulate that "all trophic cascades are wet" (Strong 1992, Pace et al. 1999). However, several studies have demonstrated trophic cascades in terrestrial ecosystems (Fraser and Grime 1998, Moran and Hurd 1998, Dyer and Letourneau 1999, Schmitz et al. 2000, Halaj and Wise 2001, Terborgh et al. 2001, Moran and Scheidler 2002, Finke and Denno 2004, 2005), and particularly in agroecosystems (Carter and Rypstra 1995, Snyder and Wise 1999, Colfer and Rosenheim 2001, Snyder and Wise

2001, Cardinale et al. 2003, Lang 2003). Several factors postulated as important characteristics of systems exhibiting trophic cascades are present in agroecosystems. They include: homogeneous habitat, prey turnover faster than predator turnover, prey uniformly edible, and simple trophic stratification of the system (Polis et al. 2000, Müller and Brodeur 2002). These factors make agroecosystems suitable subjects for study trophic cascades and is in fact form the theoretical foundation of biological control practices, i.e. a reduction of plant damage (with expected increased in crop yield) through deleterious effects of natural enemies (native or imported) on herbivore pests (Polis et al. 2000).

Generalist versus specialist natural enemies

There is increasing consensus supported by empirical evidence that top-down control is typically exerted by communities of multiple enemies rather than single species (Losey and Denno 1998a, Sih et al. 1998, Chang and Kareiva 1999, Symondson et al. 2002). This view contrasts with traditional biological control practices that have focused on the use of one or a few natural enemy species and that have generally favored specialist (or at least stenophagous species) rather than assemblages of generalist (polyphagous) species (Chang and Kareiva 1999, Symondson et al. 2002). The role of generalists as effective top-down regulators of herbivores in agroecosystems has received more support in recent theoretical and empirical reviews (Chang and Kareiva 1999, Symondson et al. 2002, Stiling and Cornelissen 2005). The traditional view suggest that specialists have increased capacity to control pests due to 1) a stronger numerical response (i.e. more offspring produced per prey than generalists) and 2) a greater functional response that is not limited by satiation in the case of parasitoids or diminished by the abundance of alternative prey (Symondson et al. 2002). In addition, Kindlmann and Dixon (2001) postulate that large relative generation time ratios between predators and prey prevent predators from exerting top-down control. They suggest herbivore prey are either regulated by bottom-up processes or self-regulated.

Utilization of generalists species in classical biological control programs (i.e. importation of exotic generalists) is not a viable practice due to potential non-target effects (Howarth 1983, 1991, Simberloff and Stiling 1996, Louda et al. 2003, Stiling and Cornelissen 2005). However, the use of native or previously established exotic generalists may have several advantages (Symondson et al. 2002). Generalist natural enemies have the potential to be present early in the season, before the pest arrives or builds up its population in the field (Chang and Kareiva 1999, Symondson et al. 2002). Using a simple theoretical model, Chang and Kareiva (1999) demonstrated that early predation by generalist predators has a similar effect as latter immigration of more specialized natural enemies. Landis and Van der Werf (1997) showed that the assemblage of generalist predators present in sugar beet fields early in the season significantly diminished the population of aphids and the impact of viruses transmitted by the aphids. Generalist predators are typically adapted to use several different habitats and can therefore re-invade agricultural fields faster than specialists after disturbances (Symondson et al. 2002). Finally, assemblages of generalists have the potential to contain natural enemies that attack all the stages of the pests throughout the season, avoiding stage specific or temporal refuges that may limit more specialized species (Symondson et al. 2002).

Top-down control by multiple enemy assemblages

Multiple enemy assemblages are typically composed of both generalist and specialist natural enemies (Symondson et al. 2002). Within consumer assemblages, interactions such as predator interference, cannibalism, predator avoidance behavior, and intraguild predation may modify the strength of top-down control of herbivores (Sih et al. 1998, Snyder and Wise 1999, Symondson et al. 2002), and multiple enemies within an assemblage may interact to have synergistic, additive, or non-additive effects (Polis et al. 1989, Sih et al. 1998, Dixon 2000). Synergist interactions among natural enemies result in greater impact on the prey population than that expected by adding individual predator impacts (Ferguson and Stiling 1996, Dixon 2000). Additive effects occur when the effect of natural enemies together is equivalent to adding the effects of each one separately (Ferguson and Stiling 1996, Dixon 2000). For this to occur, there has to be either an absence of direct (i.e. predation) or indirect (i.e. competition, interference) interactions among natural enemies or an overall compensation of interactions. Finally, non-additive effects can result when the control exerted by all the enemies together is less than the addition of the effect of each one separately, indicating negative interactions among consumers (Ferguson and Stiling 1996, Dixon 2000).

Intraguild predation

The presence of generalist natural enemies often leads to the existence of intraguild predation, i.e. trophic interactions among members of the same guild (Polis et al. 1989). Intraguild predation (IGP hereafter) is a particular case of omnivory in which consumer species interact eating each other (direct effect, +-), and/or competing for a shared resource (indirect effect, --) (Polis et al. 1989). IGP is common in many trophic webs including freshwater and marine aquatic ecosystems as well as terrestrial ecosystems (Polis et al. 1989, Rosenheim et al. 1995, Rosenheim 1998, Brodeur and Rosenheim 2000, Müller and Brodeur 2002). Two characteristics have been commonly associated with IG predators: larger size than other guild members, and a broad range diet (Polis et al. 1989).

Advantages and consequences of IGP

The widespread existence of IGP interactions in trophic webs could be attributed to several advantages received by the IG predator. IGP provides an alternative source of energy to the IG predator, reduces the number of competitors, and in the cases of mutual IGP also reduces predation risk (Brodeur and Rosenheim 2000). Mutual IGP refers to symmetrical interactions between natural enemies, i.e. both predators have similar capacities to prey on each other (Lucas et al. 1998), and thus reducing the number of the other predator species not only diminishes competition, but also reduces the chances of been eaten (Brodeur and Rosenheim 2000). For IG omnivores that primarily feed on plants, IGP could also represent an important source of nitrogen in the diet (Polis et al. 1989). It has been shown that IGP can increase survivorship during periods of low abundance of the primary prey, by both direct increase in food intake and indirect benefit in reducing competition (Polis et al. 1989). This may be especially important in ephemeral habitats, where IG predators that use both producer and consumer trophic levels (e. g. *Orius* spp.) can persist under low prey populations by using plant resources, and therefore be present in the field with very low prey densities (Coll and Guershon 2002).

In order to achieve strong IGP, natural enemies or at least those acting as IG predators should aggregate in response to prey density (Rosenheim 1998, Müller and Brodeur 2002). As an example, Tscharntke (1992) showed that parasitoids attacking hosts at high host densities were more affected by bird predation, whereas parasitoid species that prefer low host densities were relatively unaffected by avian IGP. Parasitoid species that showed a density-independent response to hosts were affected by IGP proportionally to their abundance (Tscharntke 1992). IGP has a direct impact on the predatory guild by removing more susceptible genotypes or by promoting niche shifting (Polis et al. 1989). Niche shifting may be achieved through behavioral modifications, morphological adaptations or development of toxins (Polis et al. 1989). Theoretical models predict that stability between guild members that engage in IGP can be achieved when the IG prey is a better exploiter of the shared resource than the IG predator, a finding also documented in the field (Polis et al. 1989, Holt and Polis 1997, Müller and Brodeur 2002). When both members of the guild have similar capacities to consume each other, priority effects (relative initial densities) are predicted to influence the outcome of the interaction, with the first species arriving in the habitat usually displacing the other (Polis et al. 1989, Müller and Brodeur 2002). Finally, equilibrium models showed that although IGP could result in stable equilibria, these may occur at a higher density of the shared resource (Briggs and Borer 2005). Therefore, IGP may result in a lower level of biological control, if the shared resource is an agricultural pest.

Factors affecting IGP

The intensity of IGP is also predicted to vary in habitats with different productivities (Müller and Brodeur 2002). In highly productive habitats theory predicts an increase in the presence of the IG predator, whereas the contrary is true for low productivity habitats (Polis et al. 1989, Müller and Brodeur 2002). This prediction was empirically demonstrated by the differential abundance of two parasitoids of the California red scale that engage in IGP on three citrus cultivars representing a gradient of productivity (Borer et al. 2003). Structural complexity is another factor that mediates the intensity of IGP. Finke and Denno (2002) showed significantly less IGP between the mirid *Tyttus vagus* and the wolf spider *Pardosa littoralis* in patches of vegetation with dense thatch (structurally complex) than in patches in which thatch was removed. The abundance of extraguild prey is also predicted as an important factor mediating IGP, and was shown to modify the outcome of the interactions between Cecidomyiidae, Coccinellidae and Chrysopidae species that prey on the potato aphid, *Macrosiphum euphorbiae* (Lucas et al. 1998).

Impacts of IGP on top-down control in agroecosystems

IGP can cause disruption, enhancement or have no effect on top-down control of herbivores (Polis et al. 1989, Rosenheim et al. 1995, Rosenheim 1998, Brodeur and Rosenheim 2000, Müller and Brodeur 2002, Symondson et al. 2002). Disruptions are caused by non-additive interactions that can cascade down producing an increase in the population of the herbivore (Rosenheim et al. 1995). In natural ecosystems top-down control is exerted more commonly by predators than parasitoids (Hawkins et al. 1999).

Finke and Denno (2003) demonstrated that together the the mirid *Tyttus vagus* and the wolf spider *Pardosa littoralis* exerted less control on *Prokelisia* planthoppers than the two predator species alone, indicating strong disruption of top-down control due to IGP . However, other studies shown no impact of IGP on top-down control of herbivores in natural ecosystems (Polis et al. 1989).

Although IGP is likely to occur in most natural enemy communities in agroecosystems, very few studies had been conducted to quantify its impact on herbivore regulation (Rosenheim et al. 1995). In agroecosystems, top-down control of herbivores has been shown to be predominantly the result of parasitism rather than predation (Hawkins et al. 1999). Empirical models predict both enhancement or disruption of biological control by IGP (Rosenheim et al. 1995). However, most models assume that attacks on each prey is based on its relative abundance, thus no preference between IG or extraguild prey, which at least in some systems seems not to be the case (Rosenheim et al. 1995). Where biological control has been disrupted by IGP, predator-predator IGP interactions dominate (Rosenheim et al. 1995). This system differs fundamentally from parasitoid-predator and pathogen-parasitoid IGP in that in these last two systems IGP occurs within a shared host, and therefore high levels of IGP also imply high host mortality (Rosenheim et al. 1995). By contrast, in predator-predator IGP systems, high levels of IGP may cause a relaxation of the top-down control on the herbivore, leading to pest increase (Rosenheim et al. 1995, Prasad and Snyder 2006).

Despite this general prediction, several field experiments have shown either no effect, or enhancement of top-down control by assemblages of predators that engage in IGP. Lang (2003) showed that despite strong IGP, winter wheat aphid populations were significantly more impacted by ground beetles (Carabidae) and spiders (Lycosidae, Lynphiidae) together than in plots were only one group of predators was present. In other systems, predator facilitation has been demonstrated to result in synergistic effects among predators. The impact of the coccinellid *Harmonia axyridis*, the damsel bug *Nabis* sp., and the parasitoid *Aphidius ervi* together on the aphid *Aphis craccivora* was significantly higher than the summed impact of the three enemies alone (Cardinale et al. 2003). In greenhouse conditions, the addition of the predator *Delphastus pusillus* to the enemy guild composed by the parasitoids *Encarsia formosa* and *E. pergandiella* also increased control of the whitefly *Bemisia argentifolii*, but in this case the predator was more effective than the parasitoids alone and also showed a significant non-preference for older parasitized hosts or parasitoid mummies (Heinz and Nelson 1996). A similar synergistic effect was found between introduced parasitoids of the winter moth *Operophtera brumata* (L.) and staphilinid predators that attack proportionally less parasitized than unparasitized winter moth pupae (Roland and Embree 1995). Finally, in parasitoid-predator systems where the predator showed strong IGP and prey have a refuge from predation but not from parasitism, IGP is predicted to significantly reduce biological control (Brodeur and Rosenheim 2000).

Intraguild predation in Aphid – Predator– Parasitoid Systems

Predator-parasitoid IGP is usually asymmetric, with all the stages of the parasitoid vulnerable to IGP, but with no attack of the predator by the parasitoid (Brodeur and Rosenheim 2000, Meyhöfer and Klug 2002). In this way, IGP has been suggested as a mechanism that may explain the failure of aphid parasitoids to regulate their hosts

(Ferguson and Stiling 1996, Rosenheim 1998, Brodeur and Rosenheim 2000, Colfer and Rosenheim 2001). Generalist predators commonly attack immature parasitoid stages, while the adult parasitoid is not typically consumed because of its mobility (Rosenheim et al. 1995, Brodeur and Rosenheim 2000). However, predation on adults has been reported and can achieve levels that limit the impact of parasitoids on herbivores (Rees and Onsager 1982, 1985, Völkl and Kraus 1996, Heimpel et al. 1997, Rosenheim 1998).

Usually, predators eat immature parasitoids inside their hosts without discerning their presence (Brodeur and Rosenheim 2000, Colfer and Rosenheim 2001), termed coincidental IGP by Polis et al. (1989). However, at least some predators have the ability to discriminate parasitized hosts, and this ability usually increases as the immature parasitoid develops inside its host, exhibiting less preference for parasitized than unparasitized hosts (Rosenheim et al. 1995, Rosenheim 1998). The ability to avoid parasitized hosts may maximize predator fitness, since there is evidence that parasitized hosts and mummies are of nutritional less quality than unparasitized hosts (Takizawa et al. 2000). Conversely, Meyhöfer and Klug (2002) showed that the coccinellid *Coccinella septempunctata* consume proportionally more parasitized aphids and mummies than unparasitized aphids, including consumption of empty mummies, indicating inability of this species to evaluate the suitability of food items. Predation on mummies is a case of direct predation and can also reach high levels in agroecosystems (Brodeur and Rosenheim 2000, Meyhöfer and Hindayana 2000, Colfer and Rosenheim 2001, Meyhöfer 2001, Meyhöfer and Klug 2002).

Types of predator-parasitoid IGP

If there is no discrimination between parasitized and unparasitized hosts the relative impact of a IG predator would be a function of encounter rates and attack rates with each type of host (Rosenheim 1998). IGP may occur simply due to a higher ratio between IGP prey versus non-guild prey (passive IGP), or can be due to a behavioral shift (active IGP) that results in the inclusion of more IG prey in the diet or an increase in the rate of encounter with IG prey due to increased predator mobility (Polis et al. 1989). Passive increase in IGP could additionally occur due to differential behaviours in parasitized hosts that makes them more susceptible to IGP (Brodeur and Rosenheim 2000). Among them are immobility that resulted in easy prey capture (Brodeur and Rosenheim 2000, Snyder and Ives 2001), increased dropping behavior that elevated the risk of predation by other predator species (McAllister and Roitberg 1987, Losey and Denno 1998a), and increase in honeydew production that could lead to aggregative responses of predators in patches with high parasitism (Brodeur and Rosenheim 2000). Conversely, certain immature parasitoids have the ability to manipulate host behavior to reduce the potential impact of predators and hyperparasitoids (Brodeur and McNeil 1992, Rosenheim 1998).

Forage behavior of natural enemies and IGP

The foraging patterns of different natural enemies could increase or decrease rates of IGP over what it may be expected by their relative abundance alone (Rosenheim 1998). Several parasitoids of gall insects have differential preference for gall sizes that in turn interacts with the gall size preference of predators to determine the intensity of IGP (Rosenheim 1998). Functional responses of parasitoids and predators also interact to determine different intensities of IGP, and most species of predators and parasitoids seem to respond positively to aphid density, increasing the chance for IGP interactions to occur (Rosenheim 1998, Brodeur and Rosenheim 2000). In a multi-year study of old field aphids in Great Britain, aphid parasitoids did not show a positive response to host density and therefore their effect as IG predators was reduced in comparison with predators that exhibited positive responses (Müller and Brodeur 2002). There is also evidence that adult parasitoids avoid patches where secondary parasitoids and predators are foraging (Höller et al. 1993, Taylor et al. 1998, Müller and Brodeur 2002). Other factors affecting the intensity of IGP against immature parasitoids include differential location of parasitized hosts in the plant canopy, immature parasitoid developmental time, parasitoid pupal morphology, aphid density, predator species and relative abundance, aphid-ant mutualisms and predator foraging behavior (Brodeur and McNeil 1992, Brodeur and Rosenheim 2000, Snyder and Ives 2001). IGP has been found to have non-additive effects on predator-parasitoid systems in both laboratory and field experiments, due to the direct effect of predation on immature parasitoids (Press et al. 1974, Ferguson and Stiling 1996). Field studies with the braconid *Lysiphlebus testaceipes* in combination with the coccinellid *Hipodamia convergens* showed that despite high predation on parasitoid mummies, suppression of *Aphis gossypii* populations was increased when both enemies were present (Colfer and Rosenheim 2001). Aphid-ant mutualism could also interfere with the intensity of IGP since ants can differentially interfere with the enemies in the guild (Völkl 1992, Rosenheim et al. 1995). Moreover, Meyhöfer and Klug (Meyhöfer and Klug 2002) conclude that *Lysiphlebus fabarum* (Marshall) (Hymenoptera:

Braconidae) lacks strategies to avoid IGP, probably due to its preference to utilize the "enemy free space" provided by ant-tended aphid colonies.

The *Aphis glycines*-natural enemy system

Aphis glycines life history

Aphis glycines is a heteroecious holocyclic aphid species that uses *Rhamnus* spp. as primary host and *Glycine* spp. as a secondary host (Wang et al. 1962, Van den Berg et al. 1997, Ragsdale et al. 2004, Wu et al. 2004). In China, *A. glycines* uses *R. davurica* Pall as it primary host. *Glycine max* and wild species of *Glycine* are used as secondary hosts (Wu et al. 2004). In North America, the most important primary host is the exotic invasive species *R. cathartica* L., however *A. glycines* can also successfully overwinter on native *R. alniflora* L' Héritier (Voegtlin et al. 2004, Yoo et al. 2005). Tests on another eight *Rhamnaceae* present in North America failed to produce succesfull overwintering (Voegtlin et al. 2004, Yoo et al. 2005). The *A. glycines* life cycle begins with egg hatch in spring on *Rhamnus* spp., where up to three generations develop in China (Wang et al. 1962, Wu et al. 2004), and an undetermined number in North America (Ragsdale et al. 2004) . Alate viviparous females from *Rhamnus* spp. migrate to soybeans on summer, where several (10 to 22 in Asia) parthenogenetic generations of apterous females are produced (Wu et al. 2004), On soybean, alates are also produced if densities are crowded or host quality declines (Ragsdale et al. 2004, Wu et al. 2004). In the fall, a generation of alate females called gynoparae is produced and migrates back to *Rhamnus* spp. They produce both apterous females called oviparae and alate males (Ragsdale et al. 2004, Wu et al. 2004). These last two morphs will mate in *Rhamnus* spp. and oviparae will lay the overwintering eggs on this host (Ragsdale et al. 2004, Wu et al. 2004).

Aphis glycines damage to soybeans

Aphis glycines cause direct damage to soybeans by sap removal as a consequence of very high densities of aphids feeding per plant. Yield reductions of between 50-70% have been reported in Asia (Wu et al. 2004) and up to 40% in USA (DiFonzo and Hines 2002). Plant symptoms from heavy infestation include "wrinkled and distorted foliage, early defoliation, underdeveloped roots, shortened stems and leaves, stunting, reduced branch number, lower pod and seed counts, reduced seed weight, and under severe infestations, plant death" (Wu et al. 2004, Mensah et al. 2005). However, a recent study has also shown that even relatively low aphid densities, feeding can significantly affect photosynthesis and potentially reduce plant yield (Macedo et al. 2003). High aphid densities also result in increased amounts of honeydew deposited on leaves which allows development of sooty molds which themselves reduce yield via reduced photosynthesis (Wu et al. 2004). Finally, *A. glycines* is vector of several important plant viruses that not only affect soybeans, but also other crops such as snap beans and seed potato (Venette and Ragsdale 2004). *Aphis glycines* has been shown to vector *Soybean mosaic virus* (SMV) in China and North America (Hill et al. 2001, Wang and Ghabrial 2002, Wu et al. 2004, Burrows et al. 2005), as well as *Alfalfa mosaic virus* (AMV), and *Tobacco ringspot virus* (TRSV) (Hill et al. 2001, Clark and Perry 2002). Other viruses that are known as transmissible by *A. glycines* in other parts of world and thus represent an important potential threat in North America, include *Bean pod mottle virus* (BPMV), *Bean yellow* *mosaic virus* (BYMV), *Peanut mottle virus, Soybean stunt virus, Abaca mosaic, Beet mosaic, Tobacco vein-banding mosaic virus, Mungean mosaic virus, Peanut stripe poty virus*, and *Peanut mosaic virus* (Clark and Perry 2002, Wang and Ghabrial 2002, Wu et al. 2004). Recently it was demonstrated that *Soybean dwarf virus* is not transmitted by *A. glycines* in Illinois, USA (Harrison et al. 2005).

Factors affecting A. glycines abundance

Environmental factors have been correlated with *A. glycines* outbreaks in Asia (Wu et al. 2004). Hirano et al.(1996) showed in laboratory conditions that temperatures from 22 to 27 °C are optimal for immature development, with survivorship declining sharply at 32 °C. The threshold temperature for development was estimated as 9.5 °C. Adults reproduce faster at 27 than at 22 °C, but their longevity is lower. However, the intrinsic rate of increase (r_m) is higher at 27 than at 22 °C. Other studies found the range of optimal temperatures for *A. glycines* between 22 and 25 °C (Wang et al. 1962, Ragsdale et al. 2004). Similar results were reported for *A. glycines* in North America, with a basal developmental threshold of 8.6 °C, an upper threshold of 34.9 °C, and an optimum of 27.8 °C (McCornack et al. 2004). The supercooling point for eggs was estimated as -34 °C, restricting the overwintering likelihood for *A. glycines* in northen United States and Canada (McCornack et al. 2005). Models utilizing average temperatures and precipitation produced significant correlations of these factors with aphid densities in some regions of China, but the conditions varied from region to region (Wu et al. 2004). The climate in the area of distribution of *A. glycines* in Asia and

Australia allowed Venette and Ragsdale (2004) to conclude that this pest has the potential to be distributed through most of the soybean production area in North America.

Bottom-up effects

Several sources of bottom-up effects have been reported that can potentially affect *A. glycines*. First, Van den Berg et al. (1997) showed in Indonesia that plant phenology significantly affected population growth of soybean aphid, diminishing its population growth 50% latter in the season. While, the results of Van den Berg et al. (1997) were not confirmed in trials performed in Indiana (Rutledge et al. 2004, Rutledge and O'Neil 2006), anecdotal evidence from Michigan suggests less *A. glycines* damage on early-planted soybeans (C. DiFonzo, pers. comm.). A second potential source of bottom-up effects is host plant resistance, and resistant varieties have been found in both Asia and North America, as well in lines of the ancestor of soybean *G. soja* Sieb. & Zucc. (Hill et al. 2004a, Li et al. 2004, Wu et al. 2004). Third, Myers et al. (2005b) showed that *A. glycines* fed on potassium deficient plants resulted in lower population growth in laboratory assays, although they could not replicate this finding in field studies. Finally, other factors that may affect *A. glycines* population include cultural practices, such as cropping, rotation (Wu et al. 2004).

Top-down effects

Top-down effects of natural enemies on *A. glycines* have been demonstrated in its native range and more recently in North America. In China, the most important natural enemies are the coccinellids *Propylaea japonica* (Thunberg) and *Harmonia axyridis* Pallas, and several species of syrphids and lacewings (Wu et al. 2004). Parasitism by *Lysiphlebia japonica* (Ashmead) (Hymenoptera: Braconidae) reached levels between

10.3 – 52.6%, and maintained *A. glycines* populations below threshold level at rates of parasitism of 34% (see Wu et. al., 2004, for a complete list of *A. glycines* natural enemies reported in China and South Korea). In China, using a combination of exclusion cages and sampling, Liu et al. (2004) showed a 12 fold reduction in *A. glycines* population growth when aphids were not protected from natural enemies, and 4 fold reduction when populations were exposed only to small size (< 2 mm) natural enemies. The most abundant natural enemies were the parasitoid *Lysiphlebus* sp., the coccinellid predators *P. japonica*, *Scymnus* (*Neopullus*) *babai* Sasajai, and the syrphid *Paragus tibialis* (Fallén). Of them, only *S. babai* showed a trend towards a numerical response, with its populations tracking aphid populations during the season. However, when they exposed (i.e. previously caged) high *A. glycines* populations, they obtained significant aggregation of *P. japonica*. Van den Berg et al. (1997) showed that the coccinellid *Harmonia arcuata* (Fabricius) was responsible for reducing *A. glycines* population latter in the season in Indonesia. In Japan, the absence of *A. glycines* outbreaks is mainly attributed to the action of natural enemies (Heimpel and Shelly 2004). Finally, the success in reducing *A. glycines* density by intercropping soybean with corn has been attributed to the concomitant increase in natural enemy abundance (Wu et al. 2004).

In North America, several studies have been conducted demonstrating that the assemblage of generalist predators naturally occurring in the field can provide effective top-down control of *A. glycines*. Using a combination of clip-cages that exclude predators but allowed *A. glycines* emigration, Fox et al. (2005) showed that generalist predators could significantly impact the initial number of adults that establish in the field at the beginning of the season in Michigan. Excluding predators during 5 weeks in early-mid season resulted in a 20-fold increase in *A. glycines* populations (Fox et al. 2004). Furthermore, exposure of formerly protected populations or conversely, protecting formerly exposed populations resulted in a reversal of population trends over 3 weeks (Fox et al. 2004). During these trials in Michigan, the most abundant foliar predators were the coccinellid *H. axyridis*, the anthocorid *Orius insidiosus* (Say), and the chamaemyiid *Leucopis* spp. Parasitoids were not observed in these studies in 2001-02. Similar patterns of species abundance were found in Indiana, although a much more relevant role is attributed to *O. insidiosus* (Rutledge et al. 2004, Rutledge and O'Neil 2005). Interestingly, predator populations that were effectively acting against *A. glycines* never exceeded 5 predators / m^2 (Fox et al. 2004), supporting the idea that predation early in the season can successfully maintain low pest levels (Chang and Kareiva 1999, Symondson et al. 2002).

The assemblage of natural enemies of A. glycines *in Michigan*

The most abundant and important natural enemy of *A. glycines* in Michigan is *H. axyridis* (Fox 2002, Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005). *Harmonia axyridis* is a very effective aphid predator in Asia (Osawa 1993, 2000), and it was introduced in the USA in 1988 (Chapin and Brou 1991). Since its introduction *H. axyridis* has become the most abundant coccinellid in several regions, displacing the previous dominant invasive species, *C. septempunctata* and other native coccinellid species (Brown and Miller 1998, Colunga-Garcia and Gage 1998, Michaud 2002, Snyder et al. 2004). The success of *H. axyridis* in invading new habitats is at least in part due to its behavior as IGP of other coccinellid species (Yasuda et al. 2001, Sato et al. 2003, Snyder et al. 2004). *Orius insidiosus* is an omnivorous predator commonly present in many crops, including soybeans where it was found in association with *A. glycines* (Rutledge et al. 2004, Rutledge and O'Neil 2005). As true omnivore, *O. insidiousus* can survive on plant resources in the absence of insect prey, allowing it to persist at very low levels of pest density (Coll and Guershon 2002). *Lysiphlebus testaceipes* is a native parasitoid widely distributed in North America in many different habitats (Pike et al. 2000). It has a wide host range and has been shown to effectively suppress aphid populations in several habitats (Pike et al. 2000). In 2003, *L. testaceipes* was observed attacking *A. glycines* in Michigan at very low levels (Brewer and Noma 2003). This species has been shown susceptible to IGP in cotton, where 98-100% of the mummies of this parasitoid can be attacked and killed by the coccinellid *Hippodamia convergens* Guerin (Coleoptera: Coccinellidae) (Colfer and Rosenheim 2001). However, the same study showed that suppression of the aphid *A. gossyppi* was enhanced in the presence of both natural enemies together. The parasitoid *Aphelinus albipodus* (Hymenoptera: Aphelinidae) was introduced in the USA two decades ago to control the Russian wheat aphid *Diuraphis noxia* (Brewer and Noma 2003). In Asia it was found parasitizing *A. glycines* at very low host densities and a 'strain' obtain from Wyoming populations has been released in Minnesota and Wisconsin in 2002 (Heimpel and Shelly 2004). *Aphelinus albipodus* has also been detected parasitizing *A. glycines* in Michigan in very low levels (Brewer and Noma 2003). Other natural enemies present in Michigan have been shown capable to attack *A. glycines* in pretri dish trials, including *Nabis* spp. (Heteroptera: Nabidae), *Chrysopa* spp. (Neuroptera: Chrysopidae), *Coccinella septempunctata* (L.), *Coleomegilla maculata* De Geer and *H. convergens*, *Forficula auricolaria* Linnaeus (Coleoptera: Forficulidae), *Philonthus thoracicus* (Grav.)

(Coleoptera: Staphylinidae), and eight species of Carabidae (Coleoptera) (Fox 2002). Finally, syrphid larvae and the larvae of the midge *Leucopis* sp., have also been shown attacking *A. glycines* in the field in Michigan (Fox 2002, Fox et al. 2004).

CHAPTER 2

PREDATORS EXERT TOP-DOWN CONTROL OF SOYBEAN APHID ACROSS A GRADIENT OF AGRICULTURAL MANAGEMENT SYSTEMS

Abstract.

The discovery of soybean aphid, *Aphis glycines* Matusumura, in North America in 2000 provides the opportunity to investigate the relative strength of top-down and bottom-up forces in regulating populations of this new invasive herbivore. At the Kellogg Biological Station Long Term Ecological Research site in agroecology, I contrasted *A. glycines* establishment and population growth under three agricultural production systems which differed markedly in disturbance and fertility regimes. Agricultural treatments consisted of a conventional tillage high input system, a no tillage high input system, and a zero chemical input system under conventional tillage. By selectively restricting or allowing predator access I simultaneously determined aphid response to top-down and bottom-up influences. Irrespective of predator exclusion, agricultural manipulations did not result in bottom-up control of *A. glycines* intrinsic rate of increase or realized population growth. In contrast, I observed strong evidence for top-down control of *A. glycines* establishment and overall population growth in all production systems. Abundant predators including *Harmonia axyridis*, *Coccinella septempunctata*, *Orius insidiosus* and various predaceous fly larvae significantly reduced *A. glycines* establishment and population increase in all trials. In contrast to other systems in which bottom-up forces control herbivore populations, I conclude that *A. glycines* is primarily

controlled via top-down influences of generalist predators under a wide range of agricultural management systems. Understanding the role of top-down and bottom-up forces in this context allows agricultural managers to focus on effective strategies for control of this invasive pest.

Introduction

Ecologists have long been interested in understanding the relative strength of top-down and bottom-up forces in shaping natural communities (Hairston et al. 1960, Hunter and Price 1992, Power 1992, Walker and Jones 2001). In terrestrial ecosystems both forces are widespread (Gruner 2004), and several factors have been shown to significantly affect the relative strength of their effects, including plant morphology (Kareiva and Sahakian 1990), productivity (Power 1992, Fraser and Grime 1998, 1999, Dyer and Letourneau 1999, Moran and Scheidler 2002), consumer efficiency (Power 1992), temporal versus spatial variation of herbivore populations (Hunter et al. 1997), herbivore life stages (Walker and Jones 2001), seasonal and year-to-year changes in abiotic and biotic conditions (Walker and Jones 2001, Boyer et al. 2003, Gratton and Denno 2003b, b), differential predation risk among herbivore species (Denno et al. 2003), and herbivore body size (Sinclair et al. 2003). Although not explicitly framed in a bottom-up versus top-down context, several studies in agroecosystems have tested the effects of fertility regimes and host plant resistance versus the effects of natural enemies on pest control (Walker and Jones 2001). Agroecosystems often consist of highly simplified food webs with strong reciprocal influences between adjacent trophic levels (Polis et al. 2000), resulting in ideal systems to manipulate bottom-up versus top-down forces. Despite this potential, no simultaneous manipulations of both forces have been conducted in agroecosystems (but see Dyer and Stireman 2003). Furthermore, understanding the relative strength of top-down versus bottom-up forces in agricultural systems has the potential to provide insights on fundamental ecological questions, as well as help guide agroecosystem management.

I tested the relative strength of top-down versus bottom-up influences on the population growth of soybean aphid, *Aphis glycines*, Matsumura (Homoptera: Aphididae). *Aphis glycines* is an important pest of soybean (*Glycine max* L.) in China and Southeast Asia, causing yield losses of up to 70% (Wang et al. 1962, Hirano et al. 1996, Van den Berg et al. 1997, Wu et al. 2004). In the year 2000, *A. glycines* was discovered for the first time in North America and as of 2004 was reported present in 21 states in the USA and three provinces of Canada (Hunt et al. 2003, Landis et al. 2003, Venette and Ragsdale 2004). In 2003, over 7 million acres of soybean were sprayed with insecticides against *A. glycines*, which infested over 50 million acres of soybean in the USA (Landis et al. 2003). Before the arrival of *A. glycines,* soybean in the North Central USA had few serious insect pests and was not usually treated with insecticides. Thus, soybean has played an important role as reservoir of natural enemies for other crops (Heimpel and Shelly 2004). Previous studies have detailed significant impacts of natural enemies on *A. glycines* in Asia (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004), suggesting potential for top-down control. Generalist predators currently dominate the assemblage of *A. glycines* natural enemies in the US, with parasitoids only rarely observed from 2000-2002 (Fox 2002, Fox and Landis 2003, Fox et al. 2004, Rutledge et al. 2004).

In the soybean aphid system, I manipulated different agricultural management systems as a potential source of bottom-up forces under realistic agronomic practices. At the same time, I manipulated the existing natural enemy assemblage as a source of top-down control by using exclusion cages. Specifically, I tested the relative strength of bottom-up versus top-down influences in determining *A. glycines* 1) early season establishment and 2) population increase. In addition, I tested the effects of agricultural management systems on 3) *A. glycines* survival and intrinsic rate of increase, and on 4) un-manipulated populations of aphids and their natural enemies during the entire season.

Materials and methods

Study site

Experiments were conducted at the Kellogg Biological Station Long Term Ecological Research in Row Crops Agriculture site (KBS-LTER) in 2003. The site contains seven different cropping systems, representing levels of disturbance from secondary succession to high input agriculture plots that have been managed under these practices since 1988 (see http://lter.kbs.msu.edu/ for detailed information). These differing production systems are known to significantly affect weed (Menalled et al. 2001) and natural enemy populations, including ground beetles (Clark et al. 1997) and ladybeetles (Maredia et al. 1992, Colunga-Garcia and Gage 1998), but their influence on herbivore populations has not previously been studied.

Three of the annual crop treatments, which were planted to soybean and represent a broad range of agricultural practices, were utilized. The high input treatment utilizes conventional tillage and standard chemical inputs to simulate typical grower inputs in the region (conventional). A second high input treatment is identical to the first except for the use of a no tillage regime (no–till). These were contrasted to a conventionally tilled treatment that received no chemical input of any kind (zero-chemical input) but which utilizes a winter leguminous cover crop to help maintain fertility. These treatments effectively bracket the range of practices used to produce soybean in the region and are likely to affect soybean plant quality to herbivores. Crop yields obtained in this site are representative of yields reported by the U. S. Department of Agriculture for the North Central region (Robertson et al. 2000). All treatments were planted between 26 and 29 of May 2003, at a rate of 72,845 seeds / ha, using the cultivar NK S20-F8 (Syngenta Seeds). Conventional and no-till treatments were planted on a 38 cm row spacing, and were fertilized at a rate of N= 1.0 kg/ha, P= 4.8 kg/ha, K= 11.0 kg/ha, on 04/26/2003. No insecticides were applied in any of the treatments during 2003. The zero-chemical input treatment was planted on 76 cm rows, with no previous fertilization and weed control by cultivation only. Treatments were replicated six times in 1 ha plots arranged in a randomized complete block design (see http://lter.kbs.msu.edu/ for plot layout and details of all agricultural operations). Using these plots I conducted five studies to assess the impact of agricultural management system and predation on *A. glycines*.

Soybean aboveground net primary production and yield

The effects of bottom-up manipulations on the soybean plants were assessed by comparing Aboveground Net Primary Production (ANPP) and yield among the three agricultural management systems. This information is collected systematically as part of the LTER-KBS sampling protocol and detailed procedures as well as raw data are available at http://lter.kbs.msu.edu. Sampling of ANPP was performed on 9/02/2003, during soybeans peak biomass, on five quadrates (1 m^2) per plot. On each quadrate soybeans were clipped at ground level and then dried at 60 °C for 48 h to obtain dry weight. Yield was determined by harvesting with conventional techniques on 10/06/03 and standardized to 13% moisture.

Aphid establishment

I first tested the effects of agricultural management system and predation on aphid survival during the establishment phase in the field. On June 26, *A. glycines* were placed within clip-cages in the three agronomic treatments described previously, at a rate of five apterous adults per clip-cage. Clip cages were cylinders of a 1.8 (diameter) x 1 (length) cm Cresline PVC pipe, covered on the top by a fine-mesh brass screen (33 threads/cm) that allow air exchange but prevent aphid movement (Fox et al. 2005). The clip-cage was secured to the abaxial surface of a leaflet of the first or second trifoliate by a metallic clip, which allowed cage removal with minimal disturbance of the aphids. Naturally occurring aphids are common on these plant parts at this crop stage (A. C. Costamagna, personal observation). After a 3 h acclimatization period, the groups of five aphids were indiscriminately assigned to one of three predator treatments: 1) *predator exclusion*, in which aphids were protected from predators by clip-cages, 2) *emigration control*, in which aphids were enclosed by clip-cages with a 3 mm opening (covered by a cork during the acclimatization period) that allowed aphid emigration but restricted predator access, and 3) *open*, in which clip-cages were removed, allowing both predation and aphid emigration. The 3 mm opening prevents entry by large predators (i.e. Coccinellidae), but small predators such as *Orius insidiosus* (Heteroptera: Anthocoridae) may theoretically enter cages, although this was never observed by us or in previous studies (Fox et al. 2005). Therefore, predation measured as the difference between the open and the emigration control treatments represents a conservative estimation (Fox et al. 2005). After 24 h the numbers of live *A. glycines* adults and nymphs were quantified separately. The experiment was conducted on the six replicates of agricultural treatments, with five clip-cages per agricultural and predator treatment combination, for a total of 270 clip-cages.

Aphid population increase

In a second experiment, I tested the effects of agricultural management system and predation on the population growth of *A. glycines* enclosed in 1 m^2 areas during the population increase phase. In each agricultural treatment plot 1 m^2 areas were established with 1) a field cage that excluded predators, 2) a sham field cage that allowed predator access, and 3) a 1 m^2 open area as a no cage control. The predator exclusion field cage consisted of a PVC frame (1 x 1 x 1 m) covered by fine-mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA) on the upper portion to exclude foliar predators, and a basal plastic barrier (10 cm buried in the soil, 20 cm above soil surface), connected to the netting by Velcro, to exclude ground-dwelling predators (after Fox et al. 2004). The sham treatment consisted of a similar field cage, but open from 0 to 15 cm above the soil, and with a second 20 cm wide opening at the canopy level, allowing access to both ground and foliar predators. The open treatment consisted of a 1 m^2 area delimited by wire flags. In each plot of the three agricultural treatments, three 1 m^2 areas were arranged as the vertices of a triangle, separated by 3 m, and were randomly assigned using a table of random numbers, to a predator manipulation treatment. In each 1 m^2 area resident predators and aphids were removed and then plots were re-infested at the average aphid density in the field at large (110 aphids/m^2). Aphids were released at a rate of 11 aphids per plant on 10 plants interspersed throughout the 1 m^2 area. Five blocks were infested using naturally occurring aphids from the same or nearby block, and one block was infested using artificially reared aphids from the USDA Niles PPQ lab at Niles, Michigan. No subsequent difference in the performance of natural vs. artificially reared aphids was detected and therefore all blocks were considered for data analysis. The experiment was initiated on July 14th with aphid and natural enemy populations assessed in all cages 6 and 14 days after infestation. Natural enemy sampling consisted of 3 min non-intrusive visual counting of larger predators (Fox et al. 2004), followed by a more detailed search on 6 to 10 plants within the cage (reducing sampling effort with aphid density increase over time) to detect small or cryptic natural enemies (i.e. predaceous fly larvae, syrphid larvae, lacewing larvae, *O. insidiosus*, and first and second instar coccinellid larvae; see results). Sample plants were selected without bias as to size, presence of natural enemies and aphids, or previous sampling history. The numbers of small-size predators and parasitoid mummies per plant were adjusted for the total number of plants per cage to compare with the number of large-size predators. Aphid sampling consisted of total counts of aphids on the same 6 to 10 plants.

Aphid intrinsic rate of increase

A third experiment was conducted to assess the effect of differing agricultural practices on survival, longevity, fecundity and intrinsic rate of increase (r) of *A. glycines*. *A. glycines* cohorts were followed using clip-cages in the three agricultural treatments utilized in the previous studies, during the same time period as the large cage study (July 16 to August 21). Clip-cages were the same as previously described but ventilated with the addition of a 3 mm cylindrical opening covered with the same fine mesh screen as above to reduce potential heat stress. Three to five apterous adult *A. glycines* were enclosed in a clip-cage to produce offspring. After 24 h all but three nymphs were removed. These nymphs were followed until the first adults were observed (indicated by the presence of newborn nymphs). At this point all but one adult aphid were removed in order to obtain estimates of per capita fecundity (van den Berg 1997). From the survivorship, longevity, and fecundity data I estimated the intrinsic rate of increase (r) for each block using the method developed for aphids by Wyatt and White (1977). The experiment was replicated in the six blocks, with 10 clip-cages per agricultural treatment plot.

Natural population sampling

I further assessed the effect of agronomic management system on *A. glycines* and natural enemy populations by conducting weekly sampling of un-manipulated populations in the six plots of each agricultural treatment from June 24 to August 18, 2003. There were other herbivores present, mostly thrips (Insecta: Thysanoptera) and leafhoppers (Homoptera: Cicadellidae), but due to their low abundance no attempt was made to quantify them. Sampling consisted of 3 samples of 1 linear m of soybean plants within a row (14.6 ± 2.7 plants / linear m, mean ± standard deviation), in each plot. New sampling points were interspersed in each field plot, for each sampling date. In each sample, foliar natural enemies were sampled by three-minute non-intrusive visual counts, followed by more detailed inspection of plant foliage, as described in the aphid population increase section. Whole plant counts of 4 to 10 plants were used to assess *A. glycines* populations (reducing sampling effort as aphid density increased over time).

Statistical analysis

Soybean ANPP and yield were analyzed using ANCOVA on a completely randomized block design with number of plants / m^2 and number of plants / ha as covariates, respectively. The number of surviving aphids in the aphid establishment experiment was analyzed using a split-plot design with agricultural treatment as the whole plot factor and predator manipulation as the sub-plot factor. Numbers of adults and nymphs were analyzed independently to separate adult mortality/emigration from reproduction. Number of aphids, predators and parasitoid mummies in the aphid population increase experiment were analyzed using a split-split-plot design, with agricultural treatment as whole-plot factor and predator manipulation and time as sub-plot factors. A Poisson regression using the GLIMMIX Macro link of SAS in PROC MIXED (SAS Institute 2001) was used to analyze these data due to the absence of a suitable transformation to normalize them, except in the aphid counts within large cages which were transformed (ln [x+1]) and analyzed by ANOVA. The effect of agricultural treatments on *A. glycines* longevity, fecundity and r was assessed by one-way ANOVA. The effect of agricultural treatments on un-manipulated populations was assessed by one-way ANOVA (aphids) and MANOVA (natural enemies) utilizing sampling date as a repeated measures factor. Due to the large number of samples with zeros, natural enemy data were condensed in three ways: 1) subsamples within each sample were combined (addition of all individuals per group), 2) the first two sampling dates were excluded from the analysis, and 3) groups representing less than 5% of the natural enemies collected were not used in the analyses. Standardized canonical coefficients were used to interpret the discriminant function of the MANOVA (Scheiner 2001).

Significant interactions in the ANOVA models were further explored via slicing by main effects (Quinn and Keough 2002), and means were separated using Least Squares Means Difference (LSMD , SAS Institute 2001). A significant agricultural treatment by date interaction obtained in the MANOVA was explored using pre-planned contrasts among agricultural practice treatments within each sampling date. When necessary to meet the assumptions of the ANOVA and MANOVA, variables were transformed (ln [x+1]) before analysis. Blocks and the interaction terms involving them were modeled as random effects in all models. ANOVAs were performed using PROC MIXED and MANOVA was performed using PROC GLM (SAS Institute 2001).

Results

Soybean aboveground net primary production and yield

Agricultural management systems significantly affected soybean ANPP and yield. ANPP was significantly lower in the zero chemical input treatment (322.2 ± 22.3 g / m^2) than in the conventional treatment (515.9 ± 9.5 g / m^2), with an intermediate response in the no-till treatment (462.3 ± 14.1 g / m^2) ($F_{2,9} = 5.10$, $P = 0.0331$; LSMD test: $P < 0.02$). The number of plants per m^2 was not a significant covariate ($F_{1,9} = 0.00$, $P = 0.9971$), and blocking did not affect the results ($F_{5,9} = 1.07$, $P = 0.4374$). Yield also differed among treatments ($F_{2,9} = 5.54$, $P = 0.0271$), with significantly higher levels in the no-till (1854. 7 ± 35.7 kg / ha) and in the conventional (1620.8 ± 78.5 kg / ha), than in the zero-chemical input treatment (1009.5 ± 78.7 kg / ha; LSMD tests, $P < 0.04$). The number of plants per hectare and block effects were not significant ($F_{1,9} = 0.04$, $P = 0.8406$; and $F_{5,9} = 1.39$, $P = 0.3139$, respectively).

Table 2.1. Poisson regression results of the effect of agricultural management system and predator manipulation treatments on the establishment of *A. glycines* at the KBS-LTER site, Michigan, 2003.

a) Fixed effects

		Adults		*Nymphs*	
	df	*F*	*P*	*F*	*P*
Agricultural treatment	2, 10	0.48	0.6314	0.35	0.7103
Predator treatment	2, 30	8.77	0.001	4.41	0.021
Agricultural treat. x pred. treat.	4, 30	0.39	0.8156	1.02	0.4125

b) Random effects

	Adults			*Nymphs*		
	Estimate	*Z*	*P*	Estimate	*Z*	*P*
Block	0.06 ± 0.05	1.15	0.1241	0.06 ± 0.09	0.73	0.233
Block x Agricultural treat.	0.02 ± 0.03	0.45	0.3265	0.12 ± 0.11	1.13	0.1284
Residual	0.35 ± 0.10			1.75 ± 0.49		

Aphid establishment

There were significant effects of predator manipulation on adult survivorship ($F_{2, 30} = 8.77$, P = 0.0010) and nymph production ($F_{2, 30} = 4.41$, P = 0.0210) after 24 h of establishment (Figure 2.1, Table 2.1). Exposure to predation significantly reduced adults and nymphs (ca. 32%) in the open versus the emigration control treatment (Figure 2.1). Adults and nymphs were 12 and 6% less abundant in the emigration control versus the predator exclusion treatment; however, these differences were not significant (Figure 2.1). There was no significant effect of agricultural management system on either adults

($F_{2,10} = 0.48$, $P = 0.6314$), or nymphs ($F_{2,10} = 0.35$, $P = 0.7103$), or the interaction agricultural management system x predator manipulation (adults: $F_{4,30} = 0.39$, $P = 0.8156$; nymphs: $F_{4,30} = 1.02$, $P = 0.4125$), indicating an absence of bottom-up effects in this test.

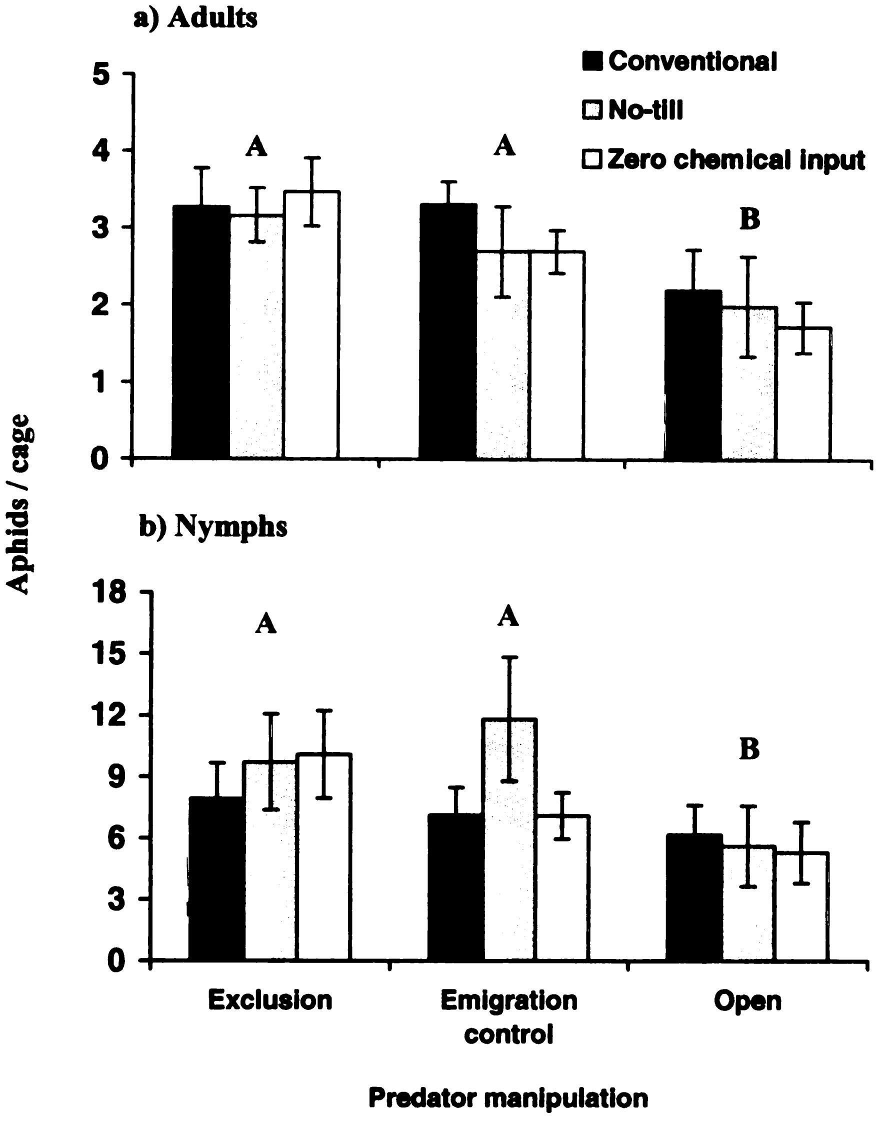


Figure 2.1. Effect of natural enemies and agricultural management system on the number of *A. glycines* (mean ± SE) alive after 24 h at the KBS-LTER site, Michigan, 2003. Different letters above bars indicate significant differences among predator manipulation treatments ($P < 0.05$, LSMD , SAS Institute 2001).

Aphid population increase

Top-down control significantly reduced aphid numbers in the large field cages during the two weeks of the experiment (predator manipulation effect: $F_{2, 27} = 94.63$, $P < 0.0001$, Figure 2.2, Table 2.2). Aphids increased significantly from the first to the second week ($F_{1, 42} = 644.46$, $P < 0.0001$), but differences among predator treatments ranging from 4 to 7 fold more aphids in the exclusion cages were persistent between weeks (Figure 2.2). The significant impact of predation on aphid population growth was consistent despite a marginally significant interaction between agricultural management system and predator manipulation ($F_{4, 27} = 2.50$, $P = 0.0661$), with higher number of aphids in predator exclusion cages under all agricultural management systems (all slicing tests with $P < 0.001$, Table 2.2). Agricultural treatments did not affect significantly the number of aphids in exclusion ($F_{2, 27} = 1.43$, $P = 0.2572$), and open ($F_{2, 27} = 2.03$, $P = 0.1508$; Figure 2.2) treatments, but significantly higher aphid suppression in sham cages in the no-till treatment in comparison with conventional and zero chemical input ($F_{2, 27} = 8.28$, $P = 0.0016$) was observed. Sham and open treatments differed in the no-till treatment (LSMD, $P = 0.0144$), suggesting a cage effect rather than an effect of the different agricultural management systems. No cage effects were observed in conventional or zero-chemical input treatments. Therefore, I found strong top-down control on *A. glycines* population growth, but no evidence of bottom-up effects due to differing crop management systems.

The natural enemy assemblage of *A. glycines* was dominated by generalist predators and a generalist parasitoid species. Large-size predators sampled within the field cages consisted of the lady beetles (Coleoptera: Coccinellidae) *Coccinella*

Table 2.2. ANOVA results for a) fixed, and b) random effects, and c) slicing tests results of the effect of agricultural management system and predator manipulation treatments on *A. glycines* within large field cages (ln [number of aphids / plant + 1]) at the KBS-LTER site, Michigan, 2003.

a) Full model with fixed effects

	df	*F*	*P*
Agricultural treatment	2, 10	5.56	0.0237
Predator treatment	2, 27	94.63	< 0.0001
Agricultural treat. x predator treat.	4, 27	2.50	0.0661
Date	1, 42	644.46	< 0.0001
Agricultural treat x date	2, 42	0.55	0.5827
Predator treat x date	2, 42	1.47	0.2424
Agricultural treat. x predator treat. x date	4, 42	0.65	0.6278

b) Random effects

	Estimate	*Z*	*P*
Block	0.090 ± 0.071	1.25	0.1053
Agricultural treat x block	0.006 ± 0.033	0.19	0.4248
Agricultural treat x predator treat x block	0.094 ± 0.048	1.97	0.0427*
Residual	0.143 ± 0.031		

* Graphical exploration of the significant 3-way interaction revealed consistently higher number of aphids on the exclusion cages in comparison with open and sham cages within each agronomic treatment and block. Therefore, predator manipulation effects were not affected by the 3-way interaction. However, the number of aphids obtained in the exclusion cages for each agronomic treatment varied in different blocks, showing an inconsistent effect of agronomic treatment in this experiment.

Table 2.2 (cont'd)

c) Slicing tests for significant interaction terms of fixed effects.

Agricultural treat. x predator treat.	df	*F*	*P*
Conventional	2, 27	49.07	< 0.0001
No-till	2, 27	36.20	< 0.0001
Zero-chemical input	2, 27	17.03	< 0.0001
Predator exclusion	2, 27	1.43	0.2572
Sham	2, 27	8.28	0.0016
Open	2, 27	2.03	0.1508

septempunctata (9.4 ± 4.4% of total predators sampled), and *Harmonia axyridis* (5.5 ± 2.8%), whereas small-size predators consisted of *O. insidiosus* (23.3 ± 6.3%), first and second instar coccinellid larvae not identified to species (18.4 ± 4.9%), syrphid larvae (Diptera: Syrphidae) (20.5 ± 6.1%), and predaceous fly larvae (Diptera: Cecidomyiidae and Chamaemyiidae) (7.9 ± 3.7%) (Table 2.3). Mummies of the native parasitoid *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) were also detected at low levels (Table 2.3). Exclusion cages significantly reduced the abundance of large-size predators ($F_{2,27} = 4.81$, $P = 0.0163$), but they had no significant effect on small-size predators ($F_{2,27} = 1.39$, $P = 0.2662$; Table 2.3). By contrast, parasitoid mummies showed the opposite trend, with significantly more mummies found in exclusion than in open cages ($F_{2,27} = 3.29$, $P = 0.0525$; Table 2.3), suggesting that exclusion cages may have protected parasitoids from intraguild predation. Although parasitoids were not able to enter the mesh of the exclusion cages, some parasitism may have occurred within exclusion cages if field collected aphids used to initially infest the cages had already been parasitized.

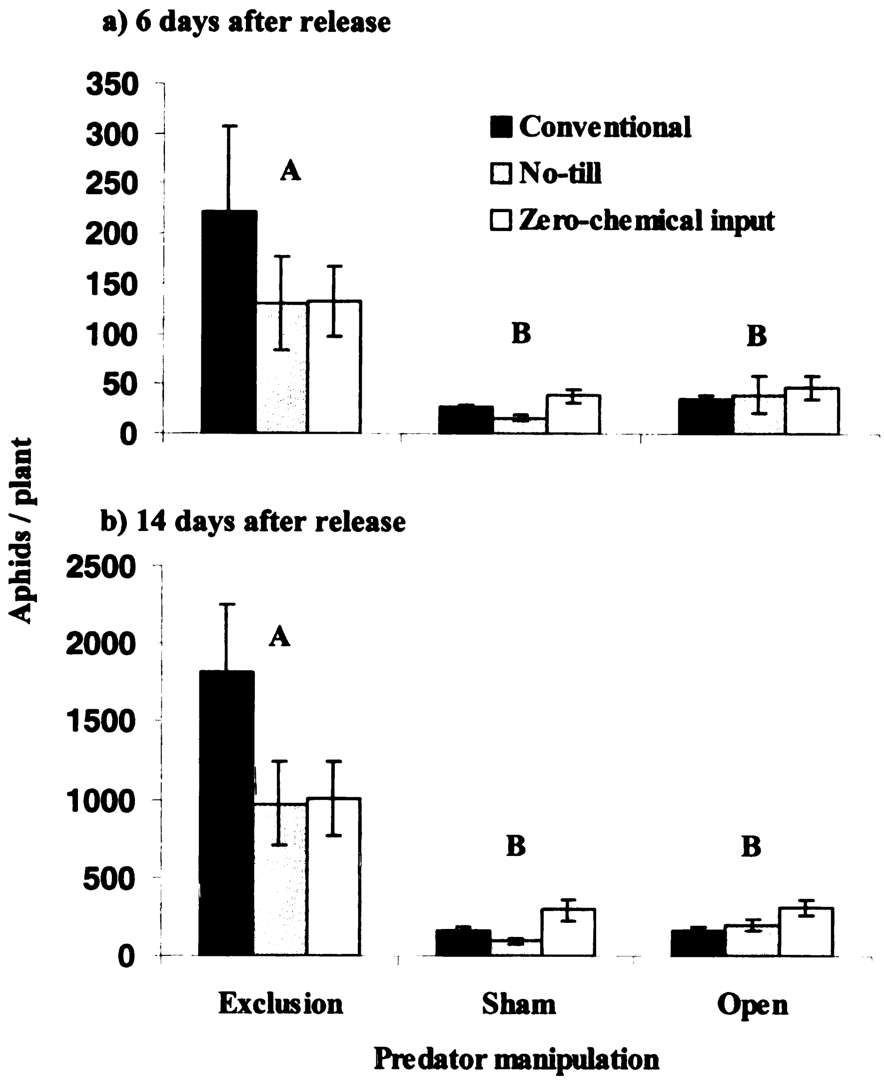


Figure 2.2. Effect of natural enemies and agricultural management systems on the number of *A. glycines* (mean ± SE) after a) 6 days, and b) 14 days of manipulation using large field cages at the KBS-LTER site, Michigan, 2003. Different letters above bars indicate significant differences among predator manipulation treatments ($P < 0.0001$, LSMD , SAS Institute 2001).

Agricultural treatments significantly affected the abundance of small-size predators ($F_{2, 10} = 6.21$, $P = 0.0177$), with higher number of predators in the conventional treatment (Table 2.3), but did not affect the abundance of large-size predators ($F_{2, 10} = 0.62$, $P = 0.5569$), or parasitoids ($F_{2, 10} = 0.61$, $P = 0.5649$; Table 2.3). These results suggest that the significant increase in aphid density obtained in predator exclusion cages was associated with reduction in the action of large-size predators (mainly Coccinellidae) rather than small-size predators and parasitoids.

Aphid intrinsic rate of increase

Rearing aphid cohorts with clip-cages confirmed the absence of effects of agricultural management system on *A. glycines* life history parameters. Agricultural management systems did not affect significantly *A. glycines* longevity (18.4 ± 0.6 d, n = 179; $F_{2, 10} = 1.93$, P = 0.1954), fecundity (28.9 ± 1.6 females x female^{-1}, n = 174; $F_{2, 10} = 0.68$, P = 0.5287), or intrinsic rate of increase (0.32 ± 0.01 females x female^{-1} x day^{-1}, n = 18; $F_{2, 10} = 2.38$, P = 0.1423; Table 2.4). My estimates (r = 0.30 – 0.33) are consistent with other field estimates of r = 0.310 reported by van den Berg (1997).

Natural population sampling

Un-manipulated field populations of *A. glycines* differed among agricultural management systems on three of the seven dates of sampling (agricultural management system by date interaction: $F_{12, 88} = 2.21$, P = 0.0175), but there was no main effect of agricultural treatment ($F_{2, 10} = 2.36$, P = 0.1446). Aphids increased exponentially from the end of July to mid August (sampling date: $F_{6, 88} = 638.95$, $P < 0.0001$), and slicing by the interaction between agricultural management system and date revealed significantly fewer aphids in the no-till treatment on July 16 ($F_{2, 88} = 4.29$, P = 0.0167). This trend later reversed, with significantly less aphids in the conventional treatment on August 5 ($F_{2, 88} = 6.85$, P = 0.0017) and 13 ($F_{2, 88} = 3.87$, P = 0.0246) (Figure 2.3a).

Table 2.3. Mean (± SE) of large- and small-size predators[1] (individuals / m^2) and parasitoids (*L. testaceipes* mummies / m^2) for different combinations of agricultural management system and predator manipulation treatments, within the large field cages in the aphid population increase experiment the KBS-LTER site during 2003.

Date	Predator treat.	Agricultural treatment	Big predators	Small predators	Parasitoids
7/21/03	Exclusion	Conventional	0.00 ± 0.00	1.34 ± 0.95	2.12 ± 1.62
		No-till	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		Zero-chemical input	0.00 ± 0.00	1.32 ± 1.32	1.95 ± 0.72
	Sham	Conventional	0.67 ± 0.49	0.71 ± 0.71	0.42 ± 0.42
		No-till	0.17 ± 0.17	0.00 ± 0.00	0.43 ± 0.43
		Zero-chemical input	0.33 ± 0.33	1.07 ± 0.50	0.91 ± 0.41
	Open	Conventional	0.33 ± 0.21	2.75 ± 1.23	0.00 ± 0.00
		No-till	0.17 ± 0.17	0.46 ± 0.46	0.46 ± 0.46
		Zero-chemical input	0.50 ± 0.34	0.28 ± 0.28	0.37 ± 0.37
7/28/03	Exclusion	Conventional	0.00 ± 0.00	0.79 ± 0.79	13.22 ± 8.66
		No-till	0.50 ± 0.50	2.02 ± 1.17	7.66 ± 7.66
		Zero-chemical input	0.00 ± 0.00	0.00 ± 0.00	21.73 ± 20.27
	Sham	Conventional	1.17 ± 0.48	14.28 ± 5.20	1.29 ± 1.29
		No-till	0.50 ± 0.34	0.00 ± 0.00	0.00 ± 0.00
		Zero-chemical input	0.50 ± 0.34	1.85 ± 0.85	0.00 ± 0.00
	Open	Conventional	0.17 ± 0.17	4.64 ± 2.45	3.07 ± 1.57
		No-till	0.00 ± 0.00	4.48 ± 4.48	3.21 ± 1.56
		Zero-chemical input	0.17 ± 0.17	1.68 ± 0.79	2.47 ± 1.35

[1] Large-size predators were counted through 3-minute non-intrusive visual sampling within the whole cage and included *H. axyiridis*, *C. septempunctata*, nabids (Heteroptera: Nabidae), and spiders (Aracnidae). Small-size predators and mummies were counted by a detailed, intrusive search on 10 (7-21) and 6 (7-28) plants per cage and were adjusted to m^2 by multiplying the average per plant by the total number of plants within the cage. Small-size predators included: *O. insidiosus*, first and second instar coccinellid larvae, syrphid larvae, predaceous fly larvae (Diptera: Cecidomyiidae and Chamaemyiidae), and lacewing larvae (Neuroptera: Chrysopidae).

Table 2.4. Mean longevity (days, ± SE), fecundity (total females / female) and intrinsic rate of increase (r, females/female/day*) of *A. glycines* reared on soybean produced under three different agricultural management systems in the KBS-LTER during 2003.

Agronomic practice	Longevity	Fecundity	*r*
Conventional	16.8 ± 1.0	26.0 ± 2.6	0.30 ± 0.01
No-till	18.7 ± 1.0	29.8 ± 2.7	0.33 ± 0.01
Zero chemical input	19.6 ± 1.0	30.8 ± 2.8	0.33 ± 0.01

*estimated per block using the method developed for aphids by Wyatt and White (1977).

Five groups of natural enemies were included in the analysis: *C. septempunctata* (29.4%), *H. axyridis* (23.1%), coccinellid larvae (16.0%), *O. insidiosus* (10.1%) and syrphid larvae (Diptera: Syrphidae) (5.5%), accounting for 84.1% of the total foliar natural enemies observed during the five sampling dates included in the analysis (Figure 2.3b-f). The rest of the natural enemies included *L. testaceipes* (3.2%), predaceous fly larvae (3.2%), nabids (Heteroptera: Nabidae) (2.8%), other coccinellid species (2.6%), spiders (Arachnida: Araneae) (1.7%), lacewing larvae (Neuroptera: Chrysopidae) (1.1%), opilionids (Arachnida: Opiliones) (0.9%), and carabids (Coleoptera: Carabidae) (0.3%). The results for the MANOVA showed significant agricultural management system ($F_{10, 148} = 2.25$, $P = 0.0176$), date ($F_{4, 75} = 20.73$, $P < 0.0001$), and management system x date interaction ($F_{8, 152} = 2.84$, $P = 0.0057$) effects on the assemblage of *A. glycines* natural enemies (Figure 2.3b-f, Table 2.5). Pre-planned contrasts showed that natural enemies assemblages in the no-till treatment differed significantly from the zero chemical input treatment (July 22 and July 30, $P = 0.05$) and from the conventional treatment (August 13, $P = 0.01$). The standardized canonical coefficients (SCC) of the first canonical

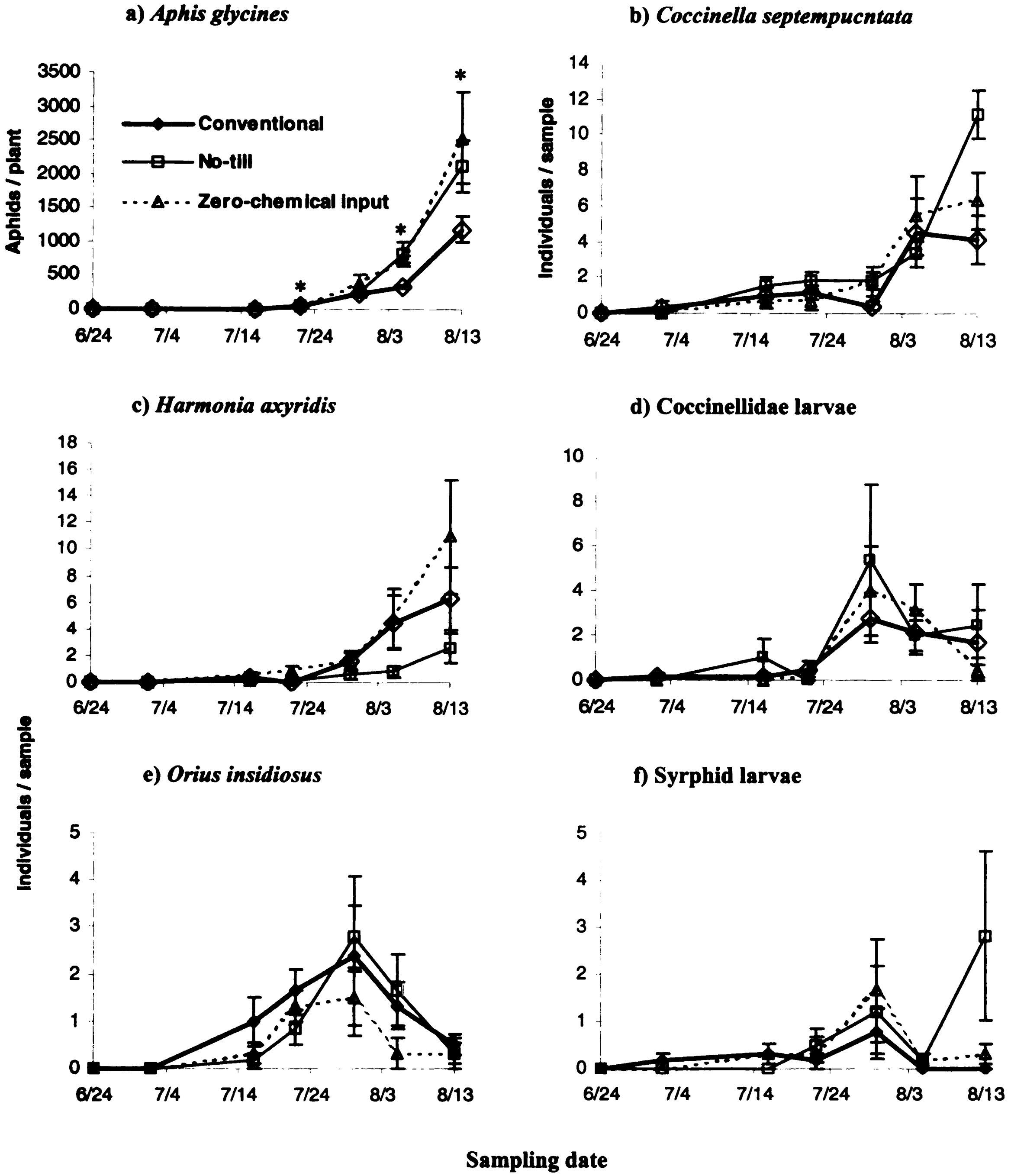


Figure 2.3. Un-manipulated *A. glycines* (a) and natural enemy (b-f) populations (mean ± SE) in soybeans under conventional, no-till and zero-chemical input treatments at the KBS-LTER site during 2003. Asterisks above lines indicate significant differences among agricultural management system treatments within sampling dates ($P < 0.05$) (LSMD , SAS Institute 2001).

variate showed that *C. septempunctata* (SCC = 0.5110) and syrphid larvae (SCC = 0.4004), which were more abundant in the no-till treatment, explained most of the

Table 2.5. MANOVA table for the effect of agricultural practices on the five more abundant *A. glycines* foliar predators at the KBS-LTER site during 2003.

Effect	Pillai's Trace	df	*F*	*P*
Agricultural practice	0.2613	10, 148	2.25	0.0176
Block	0.4157	25, 390	1.41	0.0912
Date	0.5251	4, 75	20.73	<0.0001
Agricultural practice x Date	0.2603	8, 152	2.84	0.0057
Block x Date	0.2733	20, 312	1.14	0.3034

variability and were negatively correlated with *H. axyridis* (SCC = -0.4876), which was less abundant in the no-till plots. There was an increase of all the abundant foliar predators late in the season, following the increase in aphid populations, with the exception of *O. insidiosus*, which declined in the last two samples (Figure 2.3e).

Discussion

These results provide strong evidence of top-down regulation of *A. glycines* due to predator assemblages, but only weak evidence of bottom-up effects under the range of conditions tested. Generalist predators dominated the natural enemy assemblages, with parasitoids only accounting for less than 4% of the natural enemies sampled in the un-manipulated study. However, parasitoids showed increased numbers within exclusion cages (46% of total natural enemies in the big cages study), suggesting that cages may have reduced intraguild predation (Colfer and Rosenheim 2001). Ground predators (i.e. spiders and carabids) were probably underestimated by mysampling methods, however the absence of dropping behavior in *A. glycines* (A. C. Costamagna, and D. A. Landis, personal observations), suggest a prevalent role of foliar predators in this system. The assemblage of generalist predators was dominated by the coccinellids *C. septempunctata* and *H. axyridis*, which were the dominant species previously reported attacking *A. glycines* in North America (Fox et al. 2004, Rutledge et al. 2004). Both species responded numerically to the increase in *A. glycines* natural populations later in the season; however, *C. septempunctata* was more abundant in the no-till system, whereas *H. axyridis* predominated in the conventional and zero-chemical input systems (Figure 2.3b and c). *H. axyridis* has been shown to be a strong intraguild predator of other coccinellids, and in particular of *C. septempunctata* larvae (Yasuda et al. 2001). Thus, the different agricultural system preferences found for these coccinellids may result in an attenuation of negative interactions between them.

Despite the significant differences in the assemblages of natural enemies in different agricultural production systems, overall suppression of *A. glycines* did not differ, suggesting a complementary role of the different predator species. Suppression of *A. glycines* occurred both during aphid establishment early in the season and during aphid population increase at mid season. In the aphid establishment experiment, only a third of the aphids survived when there was no protection against predation. The large impact on aphid survivorship obtained in the relatively short time frame of this experiment (24 h) suggests that this is a conservative measure of predation on aphid establishment. My results are in agreement with other studies that showed significant effects of predation on establishing pest populations. Using a simple theoretical model, Chang and Kareiva (1999) demonstrated that early predation by generalist predators has a similar effect as latter immigration of more specialized natural enemies. Landis and Van der Werf (1997) showed that the assemblage of generalist predators present in sugar beet fields early in the season significantly diminished the population of aphids and the impact of viruses transmitted by the aphids. In *A. glycines*, Fox et al. (2005) also found a significant reduction on aphid establishment due to generalist predators, independently of the presence of predator refuge strips within the field. Östman et al. (2001) found significant impacts of farming practices on bird cherry oat aphid (*Rhophalosiphum padi* [L.]) (Homoptera: Aphididae) establishment on barley, but there was no subsequent difference in aphid population growth between farming practices mediated by natural enemies.

Top-down control by generalist predators significantly reduced *A. glycines* population increase (4 to 7 fold) across all agricultural production systems. Top-down control of herbivores has been shown important in several terrestrial systems, leading to trophic cascades that release plants from herbivory pressure (Spiller and Schoener 1990, Carter and Rypstra 1995, Moran and Hurd 1998, Schmitz et al. 2000, Halaj and Wise 2001, Snyder and Wise 2001). In agroecosystems, both parasitoids and predators have been shown to be effective in suppressing several aphid species (Hopper et al. 1995, Obrycki and Kring 1998, Colfer and Rosenheim 2001, Symondson et al. 2002, Cardinale et al. 2003, Lang 2003, Schmidt et al. 2003, 2004). Natural enemies suppress *A. glycines* in its original area of distribution (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004) and in its invaded range in North America (Fox et al. 2004). My results expand these findings by showing that generalist predators suppressed *A. glycines* establishment and growth across a wide gradient of agricultural production systems.

Several factors have been shown to independently lead to bottom-up effects on aphid population growth in agroecosystems. Among them are significant impacts of fertilization (Cisneros and Godfrey 2001, Morales et al. 2001, Nevo and Coll 2001), tillage (Andersen 2003, Hesler and Berg 2003, Gencsoylu and Yalcin 2004), and cover crops (Tillman et al. 2004). On *A. glycines*, Van der Berg et al. (1997) observed a negative impact of plant age on population increase, whereas Myers et al. (2005b) found positive effects of soil potassium deficiency. In mystudy, differing input regimes (treatments) resulted in significant differences in ANPP and soybean yield, confirming successful manipulation of bottom-up resources; however, aphid population increase was not significantly affected. This suggests that manipulation of chemical inputs and tillage under realistic agricultural production systems does not change the quality of the soybean plant sufficiently to cause significant bottom-up effects on *A. glycines* establishment or population growth, even in the absence of top-down controls. Although other studies have shown significant bottom-up effects on aphid population growth within shorter time frames than myexperiments (Van den Berg et al. 1997, Nevo and Coll 2001, Myers et al. 2005b), I can not rule out that bottom-up effects may become more evident over longer periods of time.

In my study, field populations of *A. glycines* eventually exceeded the economic threshold (250 aphids per plant, University of Wisconsin 2003). However, my experiment demonstrates that if predators were not continually suppressing aphids, damaging aphid populations would have occurred much earlier in the season (Figure 2.2). Other studies have documented that season-long predator suppression of *A. glycines* can occur as well (Fox et al. 2004). Understanding the mechanism of aphid escape in some but not all years is the focus of ongoing studies.

Top-down controls dominated the dynamics of the invasive *A. glycines* populations during the period of this study. These findings differ from results obtained in natural systems in which bottom-up forces has been shown to exert the primary control on herbivore populations (Stiling and Rossi 1997, Denno et al. 2002), but supports the prevalence of top-down control on determining herbivore populations found in other studies (Dyer and Letourneau 1999, Walker and Jones 2001). The two high input production systems utilized in my study represent the most common strategies to grow soybean in North America while the zero chemical input system represents an extreme of low inputs. Given this wide range, these results suggest that there is little scope to manage *A. glycines* impacts via such bottom-up influences. However, other sources of bottom-up control on *A. glycines* such as manipulation of plant phenology (Van den Berg et al. 1997), and plant resistance (Hill et al. 2004a, b, Li et al. 2004) should be investigated.

CHAPTER 3

SUPPRESSION OF SOYBEAN APHID BY GENERALIST PREDATORS RESULTS IN A TROPHIC CASCADE IN SOYBEANS

Abstract

Top-down regulation of herbivores in terrestrial ecosystems is pervasive and can lead to trophic cascades that release plants from herbivory. Due to their relatively simplified food webs, agroecosystems may be particularly prone to trophic cascades, a rationale that underlies biological control. However, theoretical and empirical studies show that within multiple enemy assemblages, intraguild predation (IGP) may lead to a disruption of top-down control by predators. A factorial field study was conducted to test the separate and combined effects of predators and parasitoids in a system with asymmetric IGP. Specifically I combined ambient levels of generalist predators (mainly Coccinellidae) of the soybean aphid, *Aphis glycines* Matsumura, with controlled releases of the native parasitoid *Lysiphlebus testaceipes* (Cresson) and measured their impact on aphid population growth and soybean biomass and yield. Generalist predators provided strong, season-long aphid suppression, which resulted in a trophic cascade that doubled soybean biomass and yield. However, contrary to expectations, *L. testaceipes* provided minor aphid suppression and only when predators were excluded, which resulted in non-additive effects when both groups were combined. I found direct and indirect evidence of IGP, but since percentage parasitism did not differ between predator exclusion and ambient predator treatments, I concluded that IGP did not disrupt parasitism during this

study. These results support theoretical predictions that intraguild predators which also provide strong herbivore suppression do not disrupt top-down control of herbivores.

Introduction

Top-down regulation of herbivores has been strongly debated since it was postulated as a major force organizing terrestrial ecosystems by Hairston et al. (1960). This view of three defined trophic levels that interact directly and indirectly with lower trophic levels has lead to the concept of trophic cascades, in which organisms in higher-order trophic levels affect the abundance, biomass or productivity of other species across more than one link in a food web (Pace et al. 1999). Empirical evidence shows that trophic cascades frequently occur in simple systems (i.e. less reticulated food webs) with many cases having been documented in aquatic environments (Strong 1992, Pace et al. 1999). Other studies have demonstrated trophic cascades in terrestrial ecosystems (Schmitz et al. 2000, Halaj and Wise 2001), and particularly in agroecosystems (Carter and Rypstra 1995, Snyder and Wise 1999, Colfer and Rosenheim 2001, Snyder and Wise 2001, Cardinale et al. 2003, Lang 2003, Matsumoto et al. 2003, Rypstra and Marshall 2005). Several characteristics of agroecosystems contribute to the development of trophic cascades, including homogeneous habitat, faster prey than predator turnover, uniformly edible prey, and simple trophic stratification of the system (Polis et al. 2000, Müller and Brodeur 2002). Moreover, trophic cascades in agroecosystems form the theoretical foundation of biological control, i.e. a reduction of plant damage (with expected increased in crop yield) through deleterious effects of natural enemies (native or imported) on herbivore pests (Polis et al. 2000, Rosenheim et al. 2004a).

However, despite the relative trophic simplicity suggested for agroecosystems, the consumer assemblages present in them typically have multiple natural enemies, and thus interactions such as predator interference, cannibalism, predator avoidance behavior, and intraguild predation may modify the strength of top-down control of herbivores (Sih et al. 1998, Snyder and Wise 1999, Halaj and Wise 2001, Symondson et al. 2002, Rosenheim and Corbett 2003, Rosenheim et al. 2004a). Multiple consumers within an assemblage may interact with synergistic, additive, or non-additive effects on herbivore populations (Polis et al. 1989, Sih et al. 1998, Dixon 2000). Intraguild predation (IGP hereafter), i.e. trophic interactions among members of the same guild within multiple natural enemy assemblages, may be particularly important in determining the overall impact of predators on herbivores (Polis et al. 1989). Within agroecosystems, empirical studies show that the presence of multiple natural enemies that engage in IGP can enhance (Cardinale et al. 2003), have no effect on (Colfer and Rosenheim 2001, Lang 2003, Snyder and Ives 2003, Rosenheim et al. 2004b), or disrupt (Rosenheim et al. 1993, Snyder and Ives 2001, Snyder and Wise 2001, Yasuda and Kimura 2001, Persad and Hoy 2004, Philpott et al. 2004, Rosenheim et al. 2004a) biological control. Disruptions are caused by negative non-additive interactions that cascade down, producing an increase in the population of the herbivore (Rosenheim et al. 1995). In this case, the IG predator exerts stronger pressure on an IG prey that in turn is effectively suppressing an herbivore, releasing the herbivore from control, thus acting functionally as a fourth trophic level (Polis and Holt 1992, Rosenheim and Corbett 2003).

In natural ecosystems top-down control is commonly exerted by predators, whereas parasitoids are postulated as more important control agents in agroecosystems

(Hawkins et al. 1999). Interactions between parasitoids and predators often result in asymmetric IGP, with all the stages of the parasitoid vulnerable to the predator, but with no attack of the predator by the parasitoid (Brodeur and Rosenheim 2000). As a result, IGP has been suggested as a possible mechanism to explain the failure of aphid parasitoids to regulate their hosts (Ferguson and Stiling 1996, Rosenheim 1998, Brodeur and Rosenheim 2000, Colfer and Rosenheim 2001). Although IGP is likely to occur within most of the natural enemy communities present in agroecosystems, relatively few studies have been conducted to quantify IGP's impact on herbivore regulation (Rosenheim et al. 1995). Colfer and Rosenheim (2001) and Snyder and Ives (2003) found no impact of IGP on herbivore suppression, while Snyder and Ives (2001) documented a disruptive effect on biological control.

Even in relatively simplified agroecosystems many factors may influence the extent of herbivore suppression by multiple natural enemies. However, there is a lack of studies manipulating natural enemies in experiments that allow distinction among their separate and combined direct effect on herbivores and indirect effect on plants (Lang 2003). A notable exception is Cardinale et al. (2003), in which synergistic interactions between predators and parasitoids resulted in a reduction of herbivore abundance and an increase of plant biomass, although they did not directly test for IGP interactions.

The recent invasion in North America by the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) provides an ideal system to test the impact of assemblages of multiple natural enemies. Several studies demonstrate that assemblages of naturally occurring generalist predators can provide effective top-down control of *A. glycines* in North America (Fox et al. 2004, Fox et al. 2005, Costamagna and Landis

2006). Parasitoids were observed at very low levels in these studies and the most common species collected was *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) (Brewer and Noma 2003, Costamagna and Landis 2006). *L. testaceipes* is a generalist parasitoid shown to be the target of intense IGP by coccinellids in cotton agroecosystems, although remains as an important biocontrol agent of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) (Colfer and Rosenheim 2001). Moreover, in a previous study I found significantly greater numbers of *L. testaceipes* mummies in cages that excluded *A. glycines* predators than in sham cages and open treatments. I postulated that exclusion cages provided refuge from IGP (Costamagna and Landis 2006). These results suggest that in the *A. glycines* system, IGP may be limiting the impact of parasitoids, and may impair future attempts to establish additional parasitoid species by importation biological control.

I conducted a field study to test the impact of ambient levels of generalist predators and controlled releases of the native parasitoid *L. testaceipes* on *A. glycines* suppression and soybean biomass and yield. Using exclusion cages allow separation of the effects of large predators versus *L. testaceipes*, and testing whether their combined effect resulted in additive control. In addition, intraguild predation on parasitoid mummies was assessed to determine to what extent IGP disrupts biological control by *L. testaceipes*. Specifically, I asked 1) can ambient levels of generalist predators provide season-long suppression of *A. glycines*, 2) can parasitoids alone suppress *A. glycines*, 3) does the combined impact of predators and parasitoids result in synergistic, additive or non-additive control of *A. glycines*, 4) does IGP disrupt biological control by parasitoids, and finally, 5) do predators, parasitoids or their combined impacts on *A. glycines* populations indirectly increase soybean biomass and yield?

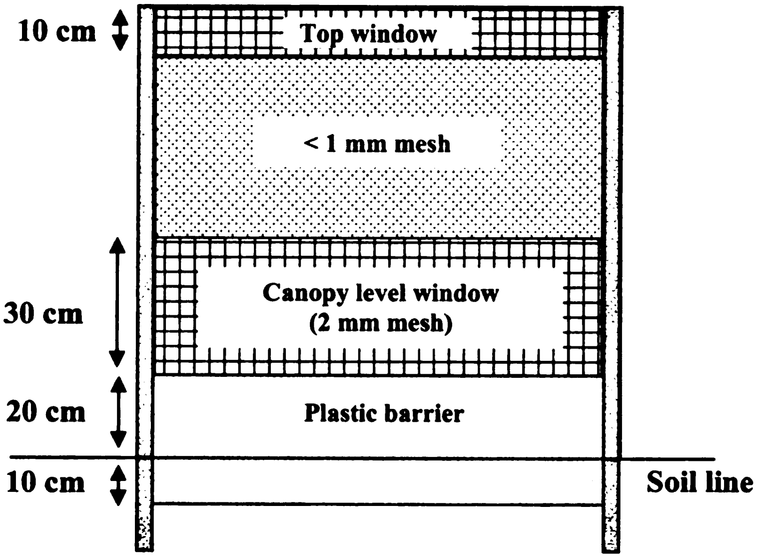
Materials and methods

The experiment was conducted in a 99 x 94 m soybean field at the Michigan State University Entomological Research Farm, Ingham County, Michigan, USA. Soybean (Pioneer 92B13) was planted on May 7, 2004, using a Great Plains Seed Drill planter on 38 cm wide rows, at a population of 494,209 seeds / ha, and weeds were controlled using Roundup WeatherMax (Monsanto Co.) tank-mixed with ammonium sulfate at standard rates. The experiment was designed as a 2 x 2 factorial in 6 completely randomized blocks, with predator and parasitoid manipulations as main factors. The four treatments were: 1) aphid control, with added aphids only; 2) parasitoid, in which parasitoids were released and large predators were excluded; 3) predator, in which ambient levels of predators were allowed access to the aphids; and 4) predator + parasitoid, similar to the predator, but with parasitoids released as in the parasitoid treatment. The rationale for the approach of augment ambient parasitoid populations was that parasitoid immigration into the aphid and predator treatments would be minimal due to the very low background levels of field parasitism found in the study area (Costamagna et al. unpublished results). To further ensure low levels of parasitism in the aphid and predator treatments, mummies observed during the weekly samplings were recorded and then removed. Thus, treatments contrasted ambient levels of generalist predators versus increased levels of parasitoids.

The predator exclusion cage consisted of a PVC frame (1 x 1 x 1 m) covered by fine-mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA) on the upper portion to exclude foliar predators, and a basal plastic barrier (10 cm buried in the soil, 20 cm above soil surface), connected to the netting by Velcro, to exclude ground-dwelling predators (Figure 3.1a) (after Fox et al. 2004, Costamagna and Landis 2006). To allow alate aphid and parasitoid emigration from cages, the upper 10 cm of the lateral walls consisted of a band of mesh with coarse openings (2 mm). A second band of the same coarse mesh (30 cm height) was placed at the canopy level after the 3rd sampling to mimic the openings on the sham cages (see below). This design prevented large predators from entering cages and was used for the aphid and parasitoid treatments. Sham cages were utilized to allow access of predators in the predator and predator + parasitoid treatments. The sham cage had the same design as the exclusion cage, but with the upper and canopy level windows completely uncovered (Figure 3.1b). To ensure parasitoid establishment, all cage windows were covered during the five initial days of the experiment by an external band of the fine mesh secured by Velcro, and this was performed to all treatments to avoid bias.

To this design I added three control treatments. First, one open reference area of 1 m^2 (open treatment) was flagged in each block as a control for potential cage effects. Second, to estimate effects on plant biomass and yield I included an exclusion cage treatment completely closed (i.e. without lateral bands of coarse mesh) that was maintained free of aphids by weekly inspections and removal of any aphids present (no-aphid treatment). The four treatments and the two controls were located in blocks separated by at least 10 m. Within blocks, all treatments were separated by at least 3 m

a) Exclusion cage



b) Sham cage

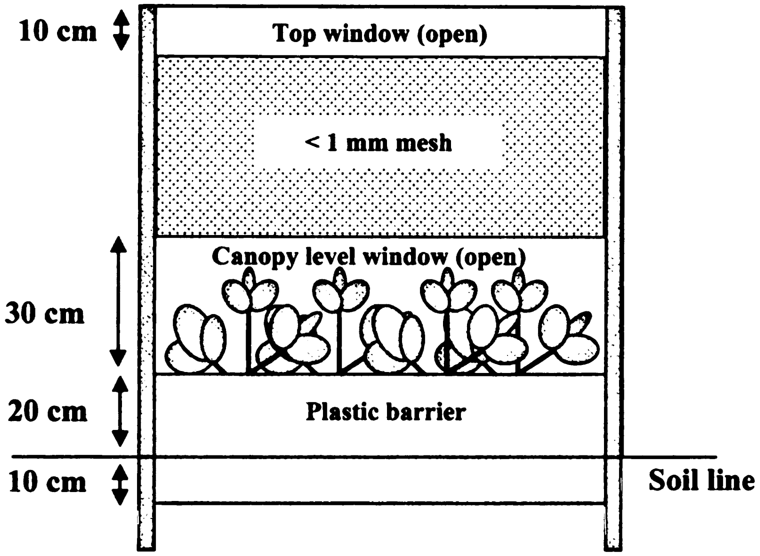


Figure 3.1. Design of predator exclusion cages (A) used in aphid and parasitoid treatments, and sham cages (B) used in predator and predator + parasitoid treatments. The fine mesh (< 1 mm) prevents aphid, parasitoid and predator movement, whereas the coarse mesh (2 mm openings) prevents only large predator access (i. e. mainly Coccinellidae). All cages were covered on top by fine mesh. Ground-dwelling predator movement was reduced by means of a transparent plastic barrier buried in the soil (see details in the methods).

from each other. In all treatments I included Raid® Ant Baits II (S. C. Johnson & Son, Inc., Racine, WI) to prevent ants from establishing and tending aphids. Finally, I established a third control consisting of totally un-manipulated 1 m^2 areas located 30 m distant from the main experiment to avoid contamination by insect manipulations (field reference).

In all but the field reference, all resident predators and aphids were initially removed by hand and then plots were re-infested with aphids at similar densities to those found at the beginning of the season during outbreak years (100 aphids / m^2, Fox et al. 2004, Costamagna and Landis 2006). In the no-aphid treatment the same manipulation was performed, but no aphids were added. Aphids reared on soybeans in the laboratory were released on June 16th, 2004, at a rate of 10 aphids per plant on 10 random plants per cage. The soybean plants were at the V3 stage and cages enclosed (mean ± SD) 29.1 ± 2.0 plants / m^2. Parasitoids were established in the parasitoid and predator + parasitoid treatments using mummies obtained from a colony of *L. testaceipes* maintained by the USDA APHIS PPQ Laboratory at Niles, Michigan. Mummies were placed in 2 oz plastic cups (Sweetheart Cup Company Inc., Owings Mills, MD) covered by a coarse mesh (2 mm openings) at the top that allowed parasitoid emigration from the cup but protected them from large predators. Cups were held 20 cm above ground level by a wooden stake and were protected from rainfall by an inverted white plastic cup (7.5 x 8 cm). Three consecutive releases were performed on June 16, 23, and 25, at a rate of 1 cup per cage. For each release, 20 mummies were placed in each cup, which generated a 1:5 (parasitoid to aphid) ratio for the first release. This ratio was successfully used in preliminary tests in the same field establishing *L. testaceipes* in small mesh cages. During each release, I deployed control release cups completely closed by a fine mesh (n = 15 randomly selected cages), that were checked daily for 4 days to assess parasitoid emergence.

Aphid and natural enemy populations were assessed weekly in all cages for six weeks following aphid and parasitoid establishment. By week 3 it was evident that, contrary to expectations, the sham cages were interfering with predator activity, resulting in higher aphid densities than in the open control (see results). Thus, on July 9 windows at the canopy level for both exclusion and sham cages (previously described) were opened, which resulted in a significant reduction in aphid abundance. Natural enemies were sampled by conducting visual counts in cages for 3 minutes (Fox et al. 2004), followed by aphid and parasitoid sampling which consisted of total counts of aphids and mummies on 4 to 10 plants within the cage (reducing sampling effort with aphid density increase, Costamagna and Landis 2006). Mummies were classified as intact (fully closed mummy), emerged (with openings made by emerging adult parasitoids), or damaged by predation (mummies opened in irregular patterns, consistent with damage by coccinellid feeding observed in preliminary laboratory trials). P parasitism was assessed based on the number of intact mummies, and therefore the measure of parasitism is conservative, since some aphids could have been parasitized but not yet mummified. Soybean plants were harvested for total above ground biomass in 3 randomly selected blocks on August 16th, and the remaining 3 blocks were harvested on October 13 to estimate yield. Plants were dried at 60 °C until no change in weight was detected and then dry weights for whole plants and seeds were recorded.

Statistical Analysis

The effects of predator and parasitoid manipulations on aphid, predator, and parasitoid populations were analyzed as a 2 x 2 factorial ANOVA using a complete randomized block design (CRBD) with time as a repeated factor (Proc Mixed, SAS Institute 2001). Significant interactions were further explored by slicing main effects (Quinn and Keough 2002), and individual means were compared by the Least Square Means Difference adjusted by the Tukey-Kramer method for multiple comparisons (LSMD-TK, SAS Institute 2001). I also assessed 1) effects of caging by comparing the predator treatment with the open reference plots, and 2) effects of predator and parasitoid manipulations on soybean yield and biomass, using 1-way ANOVAs on a CRBD with time as a repeated factor. Predator, mummy, and aphid counts, and plant biomass and yield data, were log-transformed, whereas percentage parasitism and proportion of damaged mummies (mummies damaged/ [mummies damaged + new mummies]) were arcsine-transformed, to meet assumptions of normality and homoscedasticity. Additionally, using log-transformed data in the analysis allowed testing interactions using a multiplicative risk model, which avoids bias when predation levels are high (Sih et al. 1998). I did not record predators and mummies during the first two weeks of sampling in most samples, and therefore these weeks were not included in statistical analyses for those variables.

Results

Effectiveness of predator manipulations

The assemblage of *A. glycines* predators was dominated by coccinellids (Coleoptera: Coccinellidae; 53.7% of total number of predators sampled), with the most abundant species being *Harmonia axyridis* (Pallas) (69.9%), followed by *Coccinella septempunctata* L. (4.2%), *Coleomegilla maculata* (De Geer) (2.2%), and *Hippodamia variegata* (Goeze) (2.2%). First and second instar coccinellid larvae (21.5% of total coccinellid counts) were not identified to species, but were most likely also dominated by *H. axyridis*. Predator and predator + parasitoid treatments had significantly higher abundance of coccinellids than did aphid and parasitoid treatments after the opening of the canopy-level windows (significant predator x date interaction in Table 3.1, Figure 3.2a), indicating successful manipulation of this group of predators. During the last week of sampling, this trend reversed, with coccinellids occurring in higher abundances on the aphid and parasitoid treatments (Figure 3.2a) where higher numbers of aphids occurred (Figure 3.3). Small predators including midge larvae (Diptera: Cecidomyiidae and Chamaemyiidae; 58.1%) and *Orius insidiosus* (Say) (41.9%) accounted for 34.3% of total predators. In contrast to coccinellids, small predators were not affected by predator and parasitoid manipulations (Table 3.1, Figure 3.2b). Although the visual sampling method utilized may have underestimated small predators hidden in the canopy of the plants, the detection of small predators at low densities in all treatments indicates an absence of bias among treatments.

Both coccinellids and small predators increased significantly throughout the season, following the increase in *A. glycines* densities (Table 3.1, Figure 3.2). Other

Table 3.1. ANOVA results of predator and parasitoid manipulations on the abundance of predators, parasitoids and aphids using field cages in Michigan during 2004.

		Coccinellids		Small Predators		*L. testaceipes*		*A. glycines*		
	df	*F*	*P*	*F*	*P*	*F*	*P*	df	*F*	*P*
Block	5, 15	0.49	0.7764	2.99	0.0455	1.82	0.1695	5, 15	1.16	0.3744
Predator	1, 15	11.55	0.004	1.16	0.2985	16.47	0.001	1, 15	181.39	<.0001
Parasitoid	1, 15	1.32	0.2689	0.16	0.693	32.72	<.0001	1, 15	2.4	0.1419
Predator x Parasitoid	1, 15	1.88	0.1901	1.05	0.3221	4.82	0.0444	1, 15	9.54	0.0075
Date	3, 60	16.24	<.0001	7.7	0.0002	4.7	0.0052	5, 100	203.08	<.0001
Date x Predator	3, 60	27.52	<.0001	1.88	0.1432	9.65	<.0001	5, 100	76.74	<.0001
Date x Parasitoid	3, 60	0.02	0.9972	0.12	0.9451	2.14	0.105	5, 100	0.58	0.7122
Date x Predator x Parasitoid	3, 60	0.37	0.7761	0.01	0.997	5.94	0.0013	5, 100	1.21	0.3113

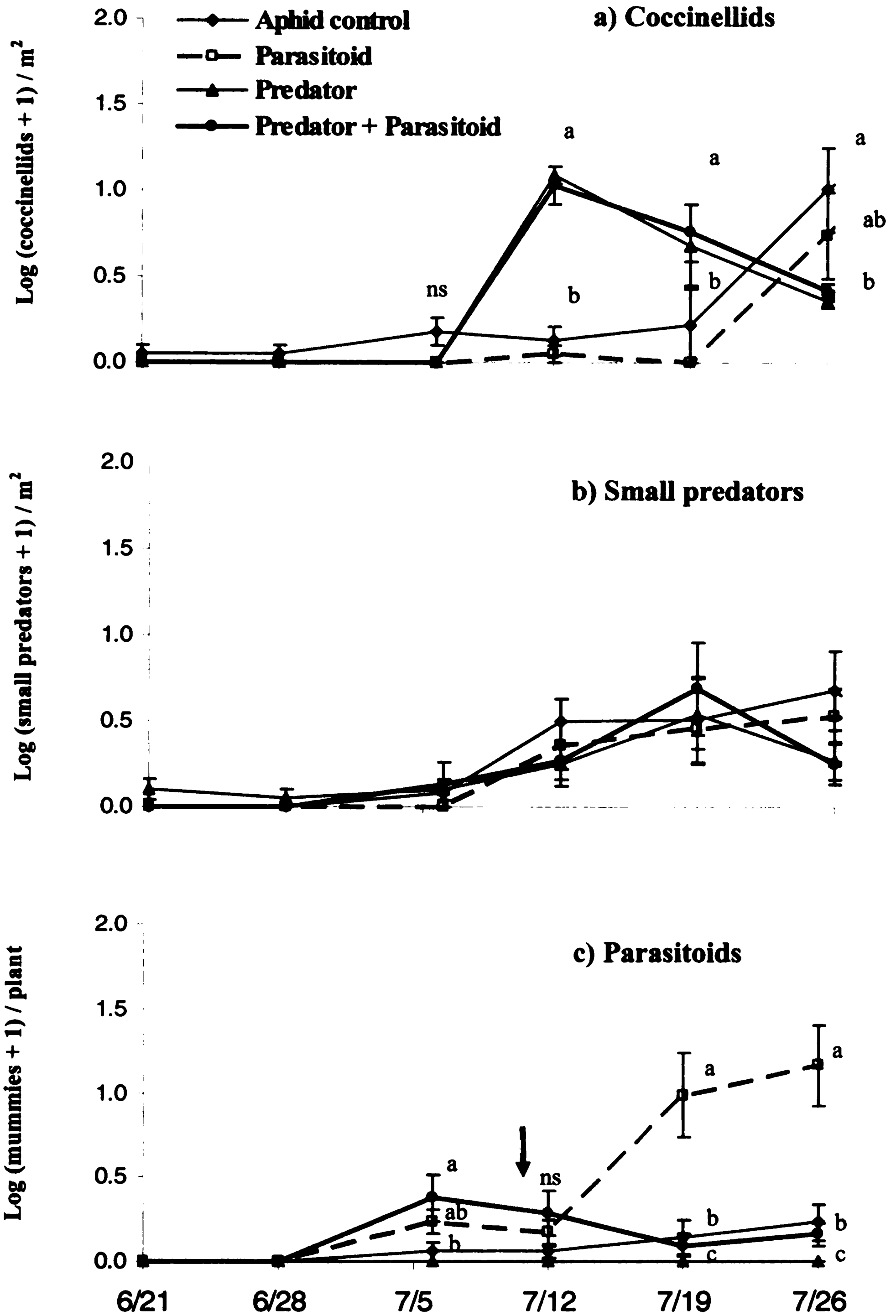


Figure 3.2 Mean (± SE) abundance of a) coccinellids, b) small predators (midge larvae and *O. insidiosus* combined), and c) parasitoid mummies in field cages with *A. glycines* that combine predator and parasitoid manipulations. The arrow indicates the opening of a canopy-level window (see methods). Different letters within each sampling date represent significant differences ($p < 0.05$) using slicing tests for the 2 and 3-way significant interactions (Table 3.1); ns = not significant.

predator groups were also sampled, but low abundance and irregular distribution among samples prevented statistical analysis. However, none of these groups showed trends of association with any of the treatments, suggesting that they probably played only a minor role in aphid suppression. Those predators included (in order of abundance): syrphid larvae (Diptera: Syrphidae, 7.4%), lacewing larvae (Neuroptera: Chrysopidae, 2.6%), and nabids (Hemiptera: Nabidae, 0.9%), followed by carabids (Coleoptera: Carabidae), spiders (Arachnidae: Aranae), and staphylinids (Coleoptera: Staphylinidae) (1.1% combined). Since samplings were conducted during the day, predators with nocturnal habits such as carabids and spiders were undoubtedly underestimated.

Parasitoid effectiveness and intraguild predation

Data from the control release cups indicated that 46.7 ± 12.0% (mean ± SD) of the *L. testaceipes* mummies emerged successfully within 4 days. Releases resulted in parasitoid establishment in the cages and a significant increase of *L. testaceipes* mummies (Table 3.1, Fig 3.2c). This effect was consistent through time, as indicated by the non-significant parasitoid x date interaction (Table 3.1). The presence of predators significantly reduced mummy abundance, but only during the last two sampling dates, after opening the canopy-level window (Table 3.1, Fig 3.2c). There was a significant predator x parasitoid interaction, indicating non-additive effects of predator and parasitoid manipulations. The significant interaction term resulted from the significant reduction in the number of mummies when predators and parasitoids were combined (Figure 3.2c). Parasitoid abundance increased significantly over time in the parasitoid treatment (Table 3.1, Fig 3.2c).

To test whether predators disrupted aphid control by parasitoids, I compared the percentage parasitism between the parasitoid and the predator + parasitoid treatments. Despite the significantly lower number of parasitoid mummies found in the treatments with predators, there were no significant differences in percent parasitism between treatments with parasitoids exposed versus protected from large predators ($F_{1,5} = 0.01$, $P = 0.9455$), indicating that predators did not reduce parasitism by *L. testaceipes*. Overall, parasitism was very low (parasitoid: 0.46 ± 0.16%; predator + parasitoid: 0.59 ± 0.21%; mean ± SE averaged across weeks 3-6, n = 24) and did not vary among blocks ($F_{5,5} = 1.34$, $P = 0.3774$). In addition, parasitism decreased significantly over time ($F_{3,30} = 4.67$, $P = 0.0085$), indicating a lack of response of *L. testaceipes* to aphid increase. Very low levels of damaged mummies were observed throughout the season, and despite a trend for higher total number of mummies damaged per plant in the predator + parasitoid treatment (1.8 ± 1.4, mean ± SE) than in the parasitoid treatment (1.1 ± 0.6), differences were not significant for total ($F_{1,5} = 0.01$, $P = 0.9104$) or proportion of mummies damaged ($F_{1,5} = 0.86$, $P = 0.3950$). Thus, these results indicate that parasitism by *L. testaceipes* was not disrupted by predators through IGP.

Aphis glycines *control*

Natural enemy manipulations resulted in large and significant differences in *A. glycines* populations after 6 weeks (Figure 3.3). Large predators (mainly Coccinellidae) significantly reduced aphid populations (Table 3.1), resulting in an average 176-fold aphid reduction compared with the aphid control and parasitoid addition treatments. Predator impacts on aphid abundance were underestimated during the initial part of the experiment due to the restrictive size of the sham cage openings, which resulted in

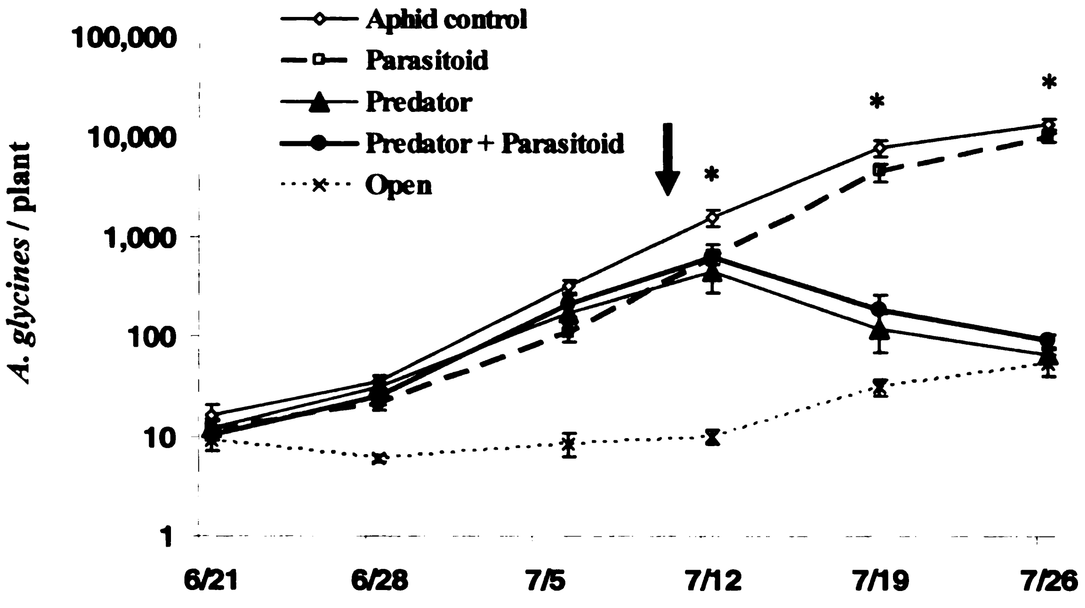


Figure 3.3 Mean (± SE) number of *A. glycines* in field cages with different combinations of predators and parasitoids. The arrow indicates the opening of a canopy-level window (see methods). Asterisks indicate significant differences between treatments with ambient levels of predators versus predator exclusion treatments (predator x date slicing tests, P < 0.0005).

significant differences between predator and open treatments ($F_{1,5} = 52.18$, $P = 0.0008$; Figure 3.3). However, predator effectiveness increased in both treatments after opening the canopy-level windows, which resulted in a significant predator x date interaction (Table 3.1, Figure 3.3). Ultimately, the predator treatment did not differ significantly from the open treatment during the last sampling date (slicing by date, $F_{1,50} = 0.61$, $P = 0.4379$; for the last sampling date; Figure 3.3). In contrast, parasitoid addition resulted in a weak reduction of *A. glycines* populations and only when predators were excluded (Figure 3.3). Aphid abundance was not affected by the opening of the canopy-level windows in the parasitoid treatment (Figure 3.3). Natural *A. glycines* abundance was very low in the field references, reaching a maximum of 2.3 ± 0.6 aphids / plant at the end of July, and was significantly lower in the field references than in the open treatment (Figure 3.3, $F_{1,14} = 211.70$, $P < 0.0001$).

There was a significant predator x parasitoid interaction, indicating non-additive effects, and those were consistent among sampling dates (non-significant predator x parasitoid x date interaction, Table 3.1). Slicing the interaction by main effects indicates that predators reduced the number of aphids per plant, with or without added parasitoids ($F_{1,15} = 53.86$, $P < 0.0001$, and $F_{1,15} = 137.07$, $P < 0.0001$, respectively), whereas parasitoids reduced aphids when predators were excluded ($F_{1,15} = 10.76$, $P = 0.0051$) but not when predators were present ($F_{1,15} = 1.18$, $P = 0.2937$). These results show that generalist predators (mainly coccinellids) provided strong suppression of *A. glycines* and that their presence eliminated the small impact of *L. testaceipes*, resulting in significant non-additive effects of the two groups of natural enemies.

Trophic cascade

Predator manipulations resulted in a trophic cascade that restored plant biomass and yield ($F_{4,8} = 7.70$, $P = 0.0075$; and $F_{4,8} = 12.08$, $P = 0.0018$, respectively) to the same levels as the no-aphid control (Figure 3.4). Exposure to ambient levels of predators resulted in a 2-fold biomass and a 1.6-fold yield increase over the aphid control. Adding parasitoids alone resulted in an intermediate increase in biomass, but did not improve plant yield over the aphid control (Figure 3.4). Block was not significant in either analysis. These results indicate that the strong suppression of *A. glycines* by generalist predators cascades down to result in levels of plant biomass and yield similar to plants where aphids were excluded.

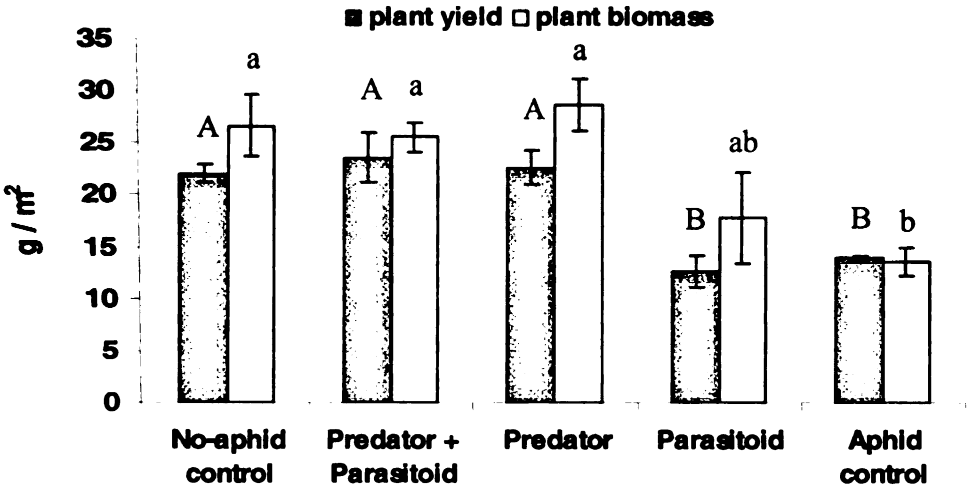


Figure 3.4. Mean (± SE) soybean yield and above ground dry biomass in field cages without *A. glycines* (no-aphid control), and with *A. glycines* and different combinations of predators and parasitoids. Different letters among columns represent significant differences ($P < 0.05$, LSMD-TK).

Discussion

The established assemblage of generalist predators significantly impacted *A. glycines* populations throughout the entire season. By contrast, controlled releases of the native parasitoid *L. testaceipes* had only minor effects in *A. glycines* suppression when large predators were excluded and did not add control when combined with them. Significant impacts of generalist predators on *A. glycines* was previously shown in Asia (Van den Berg et al. 1997, Liu et al. 2004), and North America (Fox et al. 2004, Fox et al. 2005, Costamagna and Landis 2006), but my results extend these findings by spanning an entire field season, and by demonstrating for the first time that this control cascades down to increase plant biomass and yield.

The use of exclusion cages to test for the impact on natural enemies has been extensively used for aphids in general (e.g. Chambers et al. 1983, Hopper et al. 1995, Ferguson and Stiling 1996, Jervis and Kidd 1996, Brown 2004) and also recently for *A. glycines* (Fox et al. 2004, Costamagna and Landis 2006, Costamagna et al., unpublished results). However, others have criticized cages as potentially preventing aphids from emigrating and thus artificially inflating apparent predation impacts (e. g. Kindlmann et al. 2005). Several lines of evidence support the role of predator exclusion rather than aphid enclosure in explaining higher aphid abundances in predator exclusion cages. Brown (2004) demonstrated similar levels of increased spirea aphid populations by excluding predators with cages or with insecticide treatments in open plots. Chambers et al. (1983) showed that removal of alates from exclusion cages did not result in lower aphid populations in comparison with cages in which alates were confined. In my study I did not observe significant accumulation of alates in exclusion cages even at high aphid populations, suggesting that alates were able to successfully emigrate from the cages. Finally, a significant reduction in aphid abundance has been shown for treatments with both aphids and predators enclosed together in comparison to controls in which only aphids were enclosed, demonstrating the ability of predators to control aphid populations (e.g. Cardinale et al. 2003, Snyder and Ives 2003). Thus, I feel confident that the reduction in *A. glycines* in my study was due to predation rather than to artificial confinement of aphids.

The different mesh sizes used in the cages allowed us to separate the effects of large versus small predators on *A. glycines*. Small predators, such as midge larvae and *O. insidiosus*, did not differ in abundance between exclusion and sham cages. Since aphids increased exponentially in the exclusion cages in the presence of small predators, these results suggest a minor role for small predators in *A. glycines* control at this location. This result contrasts with other studies that suggest that *O. insidiosus* is an important predator of *A. glycines* (Rutledge et al. 2004, Rutledge and O'Neil 2005).

Although extensive data supports the importance of coccinellids as aphid predators (Frazer 1988, Hodek and Honěk 1996, Obrycki and Kring 1998), there is still debate concerning their ability to suppress aphid populations (Dixon 2000). My results demonstrate strong aphid suppression by un-manipulated ambient levels of coccinellids. Both predator treatments resulted in significant *A. glycines* control during the entire 2004 field season, which mimicked un-manipulated field populations. By contrast, in treatments that excluded predation by large predators, *A. glycines* increased exponentially, indicating that abiotic factors were suitable and did not limit aphid growth. The similarity in abiotic conditions in exclusion versus sham cages and open controls was previously demonstrated (e.g. Hopper et al. 1995, Schmidt et al. 2003, Fox et al. 2004). Thus, the absence of outbreak aphid populations in commercial fields during 2004 was not directly attributable to unfavorable abiotic conditions. As in previous reports (Fox et al. 2004, Fox et al. 2005, Costamagna and Landis 2006), these results suggest that the coccinellids *H. axyridis* and *C. septempunctata*, are the main predators responsible for *A. glycines* suppression in the study area.

Controlled releases of the native parasitoid *L. testaceipes* failed to suppress *A. glycines* population growth, even when large predators were reduced. There was only a small impact of *L. testaceipes* on *A. glycines*, and this effect disappeared when ambient levels of large predators were combined with parasitoids, resulting in non-additive interactions. Although I obtained evidence of predator interference on parasitism, the very low impact of *L. testaceipes* on *A. glycines* in predator exclusion cages indicates that this disruption was trivial in comparison with the strong impact of large predators in suppressing *A. glycines*. However, one important caveat of my experiment is that the low efficiency of *L. testaceipes* prevents us from making a generalized conclusion regarding the disruptive effect of IGP on more effective parasitoids. Nevertheless, the strong top-down control exerted by coccinellids suggests that even more efficient parasitoids will add little control and most likely suffer high IGP (Colfer and Rosenheim 2001) under similar conditions as in my experiment. Therefore, my results confirm the theoretical prediction that IG predators that have strong impacts on the primary prey (i.e. the herbivore) will not disrupt top-down control, regardless of how much suppression they exert upon the IG prey (Polis et al. 1989, Holt and Polis 1997).

The failure of *L. testaceipes* to suppress *A. glycines* was unexpected. Other studies report show that *L. testaceipes* significantly suppressed aphid populations in cotton (Colfer and Rosenheim 2001), sorghum (Fernandes et al. 1998), and cereals in general (Brewer and Elliott 2004), and is the most abundant parasitoid attacking *A. glycines* at the study area (Kaiser, Noma and Brewer, personal communication; Costamagna and Landis 2006). Moreover, laboratory colonies of *L. testaceipes* developed successfully on *A. glycines* for several generations (ACC personal observation) indicating that *A. glycines* is a suitable host for this parasitoid. Field observations suggest that the lack of control of *A. glycines* by *L. testaceipes* cannot be explained by unsuitable abiotic factors. Visual inspections of releases performed in completely closed sleeve cages in the same field two weeks prior to the main experiment revealed continued high levels of parasitism throughout the season, with a cumulative number of 512.2 ± 152.1 mummies per plant (mean ± SE, n = 4). In contrast, the cumulative number of mummies observed in the parasitoid treatment was significantly lower (73.4 ± 32.5 mummies per plant, $t = -3.61$, $df = 8$, $P = 0.0068$). Thus, it is likely that the lower parasitism levels obtained in field cages were due to parasitoid emigration rather than from potential unfavorable weather conditions. Why *L. testaceipes* would leave a patch with abundant suitable hosts is unclear, but may include a preference to search in other habitats where it is adapted to find hosts (Vinson 1976), imperfect host discrimination (Rosenheim and Mangel 1994), variability in host suitability across scales (Ives et al. 1999) and different responses to cues from different host species (Grasswitz and Paine 1992).

There were a higher number of mummies in the parasitoid treatment, but since percent parasitism was not affected by predation, mummy increase may be a response to increased host density rather than the effect of a refuge from predation. I did not estimate predation on parasitized aphids before mummy formation or on adult parasitoids, both of which has been shown as potential sources of IGP (Heimpel et al. 1997, Meyhöfer and Klug 2002). In addition, I can not rule out a more general, indirect effect of intraguild interference (sensu Lang 2003) as another potential factor reducing mummy numbers in the treatment combining parasitoids and predators. Recent studies showed that female parasitoids spend less time, attack fewer aphids, and avoid leaves on which coccinellids or their semiochemicals are present (Taylor et al. 1998, Nakashima et al. 2004).

This study adds to the growing body of evidence that generalist predators have strong effects on prey, and is novel in demonstrating that these effects cascade down to increase plant biomass and yield. Trophic cascades are pervasive in terrestrial ecosystems (Schmitz et al. 2000, Halaj and Wise 2001, but see Polis et al. 2000), and have been demonstrated in agricultural systems, including vegetable (Snyder and Wise 1999, 2000, 2001), perennial (Cardinale et al. 2003), and annual crops (Carter and Rypstra 1995, Colfer and Rosenheim 2001, Rypstra and Marshall 2005), although some studies fail to show significant impacts (e. g. Vichitbandha and Wise 2002). My findings showed that strong top-down control by ladybeetles can completely release soybean plants from aphid pressure and restore plant biomass and yield. This study differs from previous experiments investigating top-down control and trophic cascades in agroecosystems in two aspects. Previously it has been argued that strong trophic cascades in agroecosystems may be at least partially an artifact of increasing the levels of natural enemies above ambient levels, and by measuring plant damage, which may not be an adequate surrogate of plant fitness (Polis et al. 2000, Halaj and Wise 2001). These concerns were addressed by conducting factorial natural enemy manipulations that allowed ambient levels of predation to respond to aphid density and avoided forcing interactions by allowing free emigration of aphids and parasitoids from all treatments. This also makes me confident that aphid pressure on plants was not artificially inflated. Finally, plant biomass and yield were measure directly, this last being a good surrogate for plant fitness.

In summary, I observed that strong suppression of soybean aphid by generalist predators led to a trophic cascade that restored soybean biomass and yield. On the other hand, the most abundant native parasitoid attacking soybean aphid, *L. testaceipes*, was ineffective in reducing aphid populations. Although I obtained direct evidence of IGP in field conditions, the low suppression exerted by *L. testaceipes* is not currently limited by IGP. However, there is still a very poor knowledge of factors leading to *A. glycines* outbreaks, and thus it is still an open question whether despite potential IGP more effective parasitoids may add control during seasons in which aphids escape predation.

CHAPTER 4

ARE EXISTING SOYBEAN APHID PARASITOIDS LIMITED BY INTRAGUILD PREDATION?

Abstract

Generalist predators are increasingly recognized as important sources of mortality for agricultural pests. However, their role as intraguild predators has also been demonstrated, and disruption by intraguild predation (IGP) has been proposed to explain the failure of some parasitoids to suppress pest populations. The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), present in North America since 2000, is widely attacked by predators, but only sustains very low levels of parasitism by endemic species. During 2004 and 2005, I conducted field experiments in Michigan, USA, to test whether parasitism is increased by providing parasitoids with refuges from IGP. I used field cages that differentially excluded large predators (primarily coccinellids) and parasitoids, and tested their impact on controlled releases of *A. glycines*. Three species of resident parasitoids were observed attacking *A. glycines*. However, even under levels of IGP as high as 94%, I found no evidence for disruption in the level of parasitism, which was uniformly low (< 1%). In contrast, coccinellids provided strong suppression of *A. glycines* (86 – 36 fold reduction), which resulted in a trophic cascade that restored soybean biomass and yield to similar levels as controls without aphids. Parasitoids and small predators (primarily *Orius insidiosus*) combined did not prevent exponential growth of *A. glycines,* and soybean biomass and yield suffered similar reduction as in the aphid control treatment. These results indicate that large predators exert strong

suppression of *A. glycines*, but do not disrupt the currently low levels of parasitism. However, the potential for IGP in this system should be considered when introducing exotic parasitoids as biological control agents for *A. glycines*.

Introduction

Exotic species that invade new habitats face an established assemblage of natural enemies, including generalists which may attack them as a novel resource. The Enemy Release Hypothesis proposes that invasive species become pests due to the inability of the existing generalist natural enemies to control them (Shea and Chesson 2002). The success of classical biological control programs involving importation of natural enemies from the original area of distribution of a pest provides support to this theory (Shea and Chesson 2002). Parasitoids are commonly selected for classical biological control of arthropods, due to their host specificity and life histories which are frequently tightly coupled with the pest (Hawkins et al. 1999, van Lenteren et al. 2003, Stiling and Cornelissen 2005). However, many attempts to introduce parasitoids as biological control agents have failed to achieve adequate control and negative interactions with existing natural enemies have been suggested as one possible reason for lack of success (Rosenheim et al. 1995, Brodeur and Rosenheim 2000, Müller and Brodeur 2002).

There is increasing recognition that established generalist predators can provide pest suppression, due to their voracity and earlier presence in the field (Chang and Kareiva 1999, Symondson et al. 2002, Stiling and Cornelissen 2005). However, trophic interactions are likely within multiple natural enemy assemblages that involve generalists, a phenomenon termed intraguild predation (IGP hereafter, Polis et al. 1989, Polis and Holt 1992). In addition to direct nutritional gains, intraguild predators obtain an indirect benefit from eliminating potential competitors for the resource they share (Polis et al. 1989). IGP has been shown to be widespread among food webs, where it is postulated to confer stability in trophic relationships (Polis et al. 1989, Rosenheim et al. 1995, Rosenheim 1998, Brodeur and Rosenheim 2000, Polis et al. 2000, Finke and Denno 2002, Müller and Brodeur 2002, Finke and Denno 2003).

Generalist predators usually engage in IGP and their body size is the main determinant of whether they act as intraguild predators or prey (Polis and Holt 1992). Thus, generalists have the potential to provide pest suppression, but also to disrupt control by other natural enemies, resulting in what has been called the 'the indeterminacy of predator function' (Rosenheim and Corbett 2003). By contrast, parasitoids that are specialized on herbivores typically do not have the potential to attack other members of the guild, with the exception of other parasitoids species sharing the same host (Brodeur and Rosenheim 2000, Borer 2002, Borer et al. 2003). Instead, parasitoids are themselves frequently susceptible to asymmetric IGP by predators in both immature and adult stages (Rosenheim et al. 1995, Heimpel et al. 1997, Brodeur and Rosenheim 2000, Meyhöfer and Klug 2002, Müller and Brodeur 2002). Moreover, IGP has been suggested as a potential explanation for the failure of parasitoids to suppress their hosts (Ferguson and Stiling 1996, Rosenheim 1998, Brodeur and Rosenheim 2000, Colfer and Rosenheim 2001).

Empirical evidence shows that IGP can either disrupt (Snyder and Ives 2001, Finke and Denno 2004) or have no effect (Colfer and Rosenheim 2001, Snyder and Ives 2003) on herbivore suppression by natural enemies. Moreover, additional factors mediate the strength of the effect of IGP on herbivore control. Cardinale et al. (2003) showed that the presence of alternative prey for the predator resulted in enhancement of aphid suppression by parasitoids in alfalfa. Finke and Denno (2002) showed that increased structural complexity of vegetation resulted in lower levels of IGP and increased herbivore suppression in a salt marsh community. Thus, the potential effect of IGP on herbivore suppression is difficult to predict (Rosenheim et al. 1995, Brodeur and Rosenheim 2000).

The soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae) is a pest of soybeans that is originally from Asia and has been found in North America since 2000 (Ragsdale et al. 2004, Venette and Ragsdale 2004). Several studies have shown significant impacts of natural enemies on *A. glycines* in Asia (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004) and North America (Fox and Landis 2003, Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Costamagna and Landis 2006). In Asia, the complex of natural enemies attacking *A. glycines* includes the predators *Propylaea japonica* (Thunberg), *Harmonia axyridis* (Pallas), and *H. arcuata* (Fabricius) (Coleoptera: Coccinellidae), and several species of syrphids and lacewings (Van den Berg et al. 1997, Wu et al. 2004). In addition, 10 – 53% parasitism by the parasitoid *Lysiphlebia japonica* (Ashmead) has been reported in China (Wu et al. 2004). In North America, the assemblage of *A. glycines* natural enemies is dominated by generalist predators, mainly the coccinellids *H. axyridis* and *Coccinella septempunctata* L. (Fox et al. 2004, Fox et al. 2005, Costamagna and Landis 2006, Mignault et al. 2006). There are only anecdotal references of field parasitism (Landis et al. 2003, Costamagna and Landis

2006). Due to the lack of effective parasitoids in North America, efforts are currently underway to research and possibly introduce parasitoids from Asia (Heimpel et al. 2004).

One potential explanation for the lack of parasitism of *A. glycines* in North America is the occurrence of asymmetric IGP on parasitoids. In a recent survey, Kaiser et al. (personal communication) detected six parasitoids species from sentinel *A. glycines* in Michigan, suggesting that North American parasitoid assemblages have the potential to attack soybean aphid. In addition, results of a 2003 field cage study revealed higher number of the native parasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) in cages in which predators were excluded, supporting the hypothesis of IGP limitation of parasitism (Costamagna and Landis 2006). Here I report the results of field experiments manipulating natural enemies to test the potential role of IGP in limiting extant North American parasitoids of *A. glycines*. Specifically, I tested whether refuge from IGP by large predators increased parasitoid attack of soybean aphid. The level of IGP was determined directly by quantifying the number of mummies damaged by predation. Finally, I also measured the impact of the different natural enemy groups on aphid suppression, plant biomass and yield.

Material and methods

Field site

Field experiments were conducted in the biodiversity study of the Kellogg Biological Station - Long Term Ecological Research site (Hickory Corners, Michigan), during 2004 and 2005. The biodiversity study has a series of 21 different agronomic treatments that vary in plant species diversity in time and space, ranging from 0 to >15 species in 3-year rotation cycles. Treatments are replicated in four randomized blocks in 9.1 x 27.4 m plots. Within this site, I utilized the three most similar treatments (all corn-soybean-wheat rotations) that varied only in the presence of cover crops in the non-soybean portions of the rotations. These included system "b" with a legume and a small grain cover crop in corn and wheat, system "c" with only a legume cover crop in corn, and system "d" with no cover crop. My rationale in selecting these systems was primarily to increase replication, rather than explore the effects of cover crop systems *per se*, since previous work had shown little impact of cover crops legacy in *A. glycines* population growth (Costamagna and Landis 2006). A detailed description of the layout of the treatments and protocols of agricultural management is available at http://lter.kbs.msu.edu/.

Natural enemy manipulations

In each plot aphid and natural enemies were manipulated using three types of cages. An exclusion cage consisted of a cylindrical framework of wire of 0.4 x 1.0 m, covered by a fine-mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA), buried 25 cm in the soil and tied to the top, enclosing three soybean plants. The fine mesh used prevented most insects (even small aphids) from moving in or out. The parasitoid refuge cage was similar, but was covered with a coarse mesh that had 2-mm openings. This cage allowed free movement of parasitoids and other small insects (primarily *Orius insidiosus* (Say) [Heteroptera: Anthocoridae]), but prevented large predators (i.e. coccinellids) from entering cages. Finally, I used a sham cage identical to the previous one, except the mesh was held 5 cm above the ground surface, attached with metal clips to four wooden stakes, and with eight rectangular openings (2 x 20 cm) arranged in 3 circular patterns alternating in a manner such that there were openings in all directions. The rings of openings were located from 10 to 30 cm above the soil, encompassing the height range of the plant canopy. Thus, the sham cage was designed to allow movement of insects of all sizes, while minimizing the differences with the parasitoid refuge cage.

Using these cages, I established five natural enemy treatments in each plot: 1) a no-aphid treatment, in which soybean plants free of insects were enclosed within exclusion cages, 2) an aphid treatment, in which a controlled number of aphids at the beginning of the season were enclosed within exclusion cages, 3) a parasitoid refuge treatment, in which aphids were enclosed in the same manner as in the previous treatment, but within parasitoid refuge cages, and finally two treatments that allowed all natural enemies: 4) a sham treatment, in which aphids were enclosed within sham cages, and finally 5) an open treatment, in which soybean plants were infested with aphids but without enclosing them in a cage. In all treatments groups of 3 soybean plants were visually inspected and all resident arthropods were removed at the beginning of the experiment (7/1/04 and 6/24/05). Then, I randomly assigned to each group of plants one of the five natural enemy treatments and infested them (except in the no-aphid control) at a rate of 15 aphids per plant on the central plant. This infestation rate was equivalent to approximately 165 aphids / m^2, which is slightly higher than the number used in previous studies (i. e. 100 - 110 aphids / m^2, Costamagna and Landis 2006, Costamagna et al., unpublished results), to ensure aphid establishment in all treatments. Aphids were from a colony maintained on soybeans at Michigan State University, in growth chambers at 24 °C, 16:8 h photoperiod, and 60 – 80% RH.

Each cage was sampled once a week after initial infestation until aphid populations clearly started to decline in the aphid and the parasitoid refuge treatments (mid August to early September). On each sampling date all the aphids and natural enemies present in each cage were quantified by carefully inspecting each plant. Parasitoid mummies were identified as Braconidae or Aphelinidae, and I also determined whether they were intact, emerged or damaged by predation. On each sampling date few individual mummies (1 – 5) were collected for rearing to determine parasitoid species. After the third week of sampling, all but one plant in each cage were removed (typically the central one unless damaged), to allow plants to grow uncrowded by the cage. Before that date, the number of aphids was quantified on the three plants and calculated the average per plant in each cage, in order to compare these data with subsequent dates. During 2004 beginning two weeks after the start of the experiment, I detected ants tending the aphids in the parasitoid refuge, sham and open treatments in seven of the five experimental plots. To prevent this in other plots, I added plastic rings (60 cm diameter) around the cages, buried 15 cm in the soil and rising 20 cm above the soil, coated with Tanglefoot (The Tanglefoot Company, Grand Rapids, MI) on the outside. Ant-exclusion rings were placed around the ant-free plots with no attempt to prevent ant tending in the seven plots where ants were already present. This allowed us to compare the effects of ant tending on aphid abundance. In 2005, I included ant-exclusion rings to all treatments from the outset. I did not observe ants tending aphids in cages with ant-exclusion rings during either year of the experiment.

Natural population sampling

In the surrounding field at large, natural populations of aphids and natural enemies were sampled at the same time cages were sampled. During 2004, sampling consisted of total counts of aphids and natural enemies on one specific plant per plot throughout the season. During 2005 a more comprehensive sampling program consisted of direct counts of predators in 3 quadrats of 1 m^2 in the same plots with natural enemy treatments, changing their location on each sampling date. In each quadrat the number of large predators was recorded during 3 minute (weeks 1 – 4) to 1 minute (weeks 5-8) periods. In order to sample smaller predators (i.e. *O. insidiosus*, midge larvae, and syrphid larvae), a more detailed search was conducted in one quadrat per plot after the initial non-intrusive observation. All values of natural enemies are expressed as number of insects per m^2. I also determined plant phenology and counted the number of *A. glycines* in the plant located in the Northeast corner of each quadrat.

Statistical analysis

Aphid abundance was analyzed using ANOVA (PROC MIXED, SAS Institute 2001). Treatment structure was a 3 x 4 factorial in a split-plot design, with agronomic treatment as a whole plot factor with 3 levels and natural enemy treatment as the subplot factor with 4 levels. The experiment was conducted over two years. The statistical model included year, agronomic treatment, natural enemy treatment and sampling dates, and all the interactions between them as fixed effects. Blocks and the interactions between them and other factors were treated as random effects. The error term used for year was the year x block interaction. Sampling dates were nested within year. I analyzed the first 6 weeks after infestation, because after that aphid populations started to decline. In all analyses, sampling dates within each year were treated as repeated measures obtained from each subplot with autoregressive covariance structure selected as the most appropriate covariance structure based on AIC (Akaike Information Criteria) values (Littell et al. 1996). I performed pre-planned contrasts using the ANOVA model above to assess whether the artificial aphid releases were representative of natural populations (open treatment versus field sampling), whether field populations differed between years (2004 versus 2005), and whether the no-aphid treatment resulted in significantly reduced aphids (no-aphid treatment versus field sampling). To rule out potential effects of ants tending the aphids during 2004, I compared treatments with and without ants using a similar ANOVA model, but including ant control treatment at the whole-plot level. The effect of agronomic treatments on the abundance of coccinellids and *O. insidiosus* was assessed with ANOVA for 2005 data, whereas the reduced sampling performed in 2004 prevented statistical comparisons. The cumulative number of mummies (i.e. added across sampling dates) was analyzed using a Split-Plot ANOVA, with agronomic treatment in whole-plot in a CRBD, and the natural enemy treatments as the subplot factor. Individual means were compared using the Least Square Mean Difference, adjusted for multiple comparisons by the sequential Bonferroni method (Rice 1989). Data were log- or arcsine-transformed before analyses to meet the assumptions of ANOVA, when necessary. Data that did not fit the assumptions of ANOVA after transformation were analyzed using non-parametric statistical tests. Natural enemy abundance within cages was analyzed using the Kruskal-Wallis tests to compare the effect of natural enemy manipulations independently within years and dates, and the effect of year on the total number of natural enemies sampled in each date (SYSTAT

Software Inc. 2004). Similarly, the same test was used to assess the effect of agronomic treatments on un-manipulated populations of large and small predators averaged across all sampling dates for 2005. Finally, mummy abundance, percentage parasitism and proportion of mummies damaged by IGP were compared between the parasitoid refuge and sham treatments using separate Wilcoxon tests for each sampling date (SYSTAT Software Inc. 2004).

Results

Aphis glycines *field versus caged populations*

Un-manipulated field populations of *A. glycines* differed significantly between years ($F = 262.44$; df = 1, 36; $P < 0.001$). Aphid populations were low throughout 2004, but exceeded the action threshold of 250 aphids / plant during 2005 (Figure 4.1). Un-manipulated field populations were significantly lower than those of the open treatment during both years (Figure 4.1 and 2, $F = 145.16$; df = 1, 36; $P < 0.001$), suggesting that the initial infestation levels resulted in higher aphid establishment than the natural aphid immigration in the field. Caging plants successfully reduced aphids in the no-aphid treatment in comparison with the open treatment and aphid field populations (mean aphids / plant ± SE for the no-aphid treatment, 2004 = 0.4 ± 0.2; 2005 = 31.6 ± 12.4; contrasts with field, $F = 152.93$; df = 1, 36; $P < 0.001$; with open, $F = 596.07$; df = 1, 36; $P < 0.001$). I did not detect significant effects of agronomic treatments on aphids ($F = 1.92$; df = 2, 6; $P = 0.2623$).

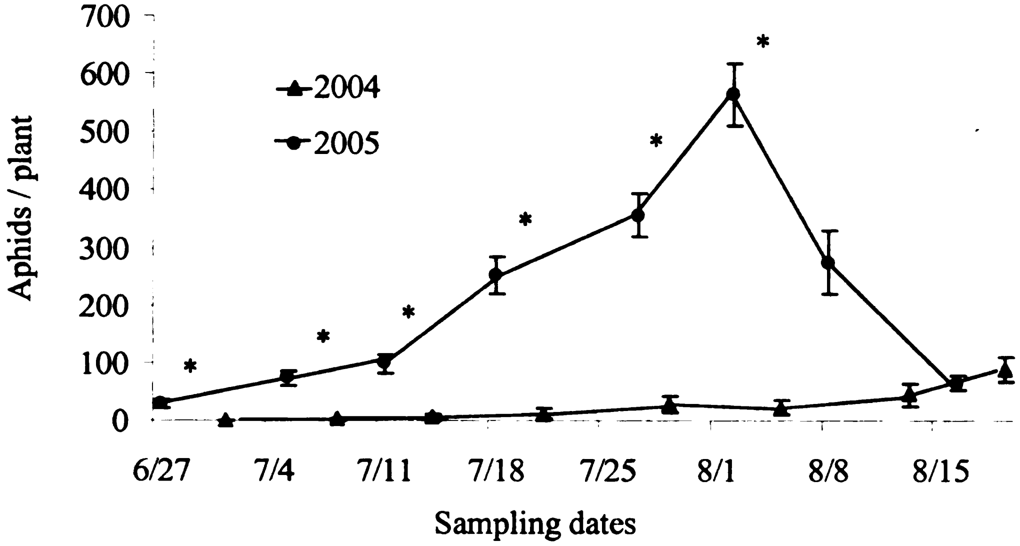


Figure 4.1. Open field populations of *A. glycines* at the biodiversity plots of the Kellogg Biological Station – Long Term Ecological Research site in Michigan during 2004 and 2005. Asterisks above the line denote significant differences between years in equivalent sampling weeks (pre-planned contrasts, $P < 0.001$, performed on initial six weeks of sampling).

Aphid suppression

The presence of ants in 2004 did not significantly reduce aphid abundance either as a main effect (F = 3.49; df = 1, 2, $P = 0.2027$) or as an interaction with agronomic or natural enemy treatments (all P > 0.20), therefore this factor was removed from subsequent analysis. Although I did not detect significant effects of agronomic treatments (Table 4.1, P = 0.1116), there was a significant agronomic treatment by natural enemy by date (year) interaction (Table 4.1, P = 0.0365). Slicing by main effects revealed that aphid abundance was significantly affected by agronomic treatment in only 5 out of the 48 potential contrasts, indicating the absence of a consistent effect of agronomic treatments on natural enemy treatments and sampling dates. Therefore, I present the results averaged across agronomic treatments (Figure 4.2). Natural enemy

Table 4.1. Results of the ANOVA for effects of year, agronomic treatment, and natural enemy treatment on *A. glycines* abundance.

Source	df	*F*	*P*
Year	1, 3	40.86	0.0078
Agronomic Treatment	2, 6	3.23	0.1116
Agronomic Treatment x Year	2, 6	0.26	0.7804
Natural enemies	3, 54	65.67	<.0001
Agronomic Treatment x Natural Enemies	6, 54	1.38	0.241
Natural Enemies x Year	3, 54	7.96	0.0002
Agron. Treat x Natural Enemies x Year	6, 54	1.12	0.3624
Date (Year)	10, 360	155.02	<.0001
Date (Year) x Agronomic Treatment	20, 360	1.62	0.0457
Date (Year) x Natural Enemies	30, 360	7.85	<.0001
Date (Year) x Agron. Treat. x Natural Enemies	60, 360	1.39	0.0365
Block	3, 1.2	6.66	0.2429
Block x Year	3, 6	0.57	0.6567
Block x Agronomic Treatment	6, 6	1.2	0.4142
Block x Year x Agronomic Treatment	6, 54	1.32	0.2659
Block x Year x Agron. Treat. x Natural Enemies	54, 360	4.45	<.0001

manipulations had significant effects on aphid abundance that changed in intensity with year and sampling date (significant natural enemy by date [year] interaction, Table 4.1). Large predators dramatically reduced aphid populations during 2004 (207-fold reduction comparing aphid and open treatments, and 86-fold reduction comparing parasitoid refuge and open treatments at peak aphid populations) and although this effect was lower in

2005 (63- and 36-fold reduction, respectively) in both cases it was highly significant (Table 4.1, Figure 4.2).

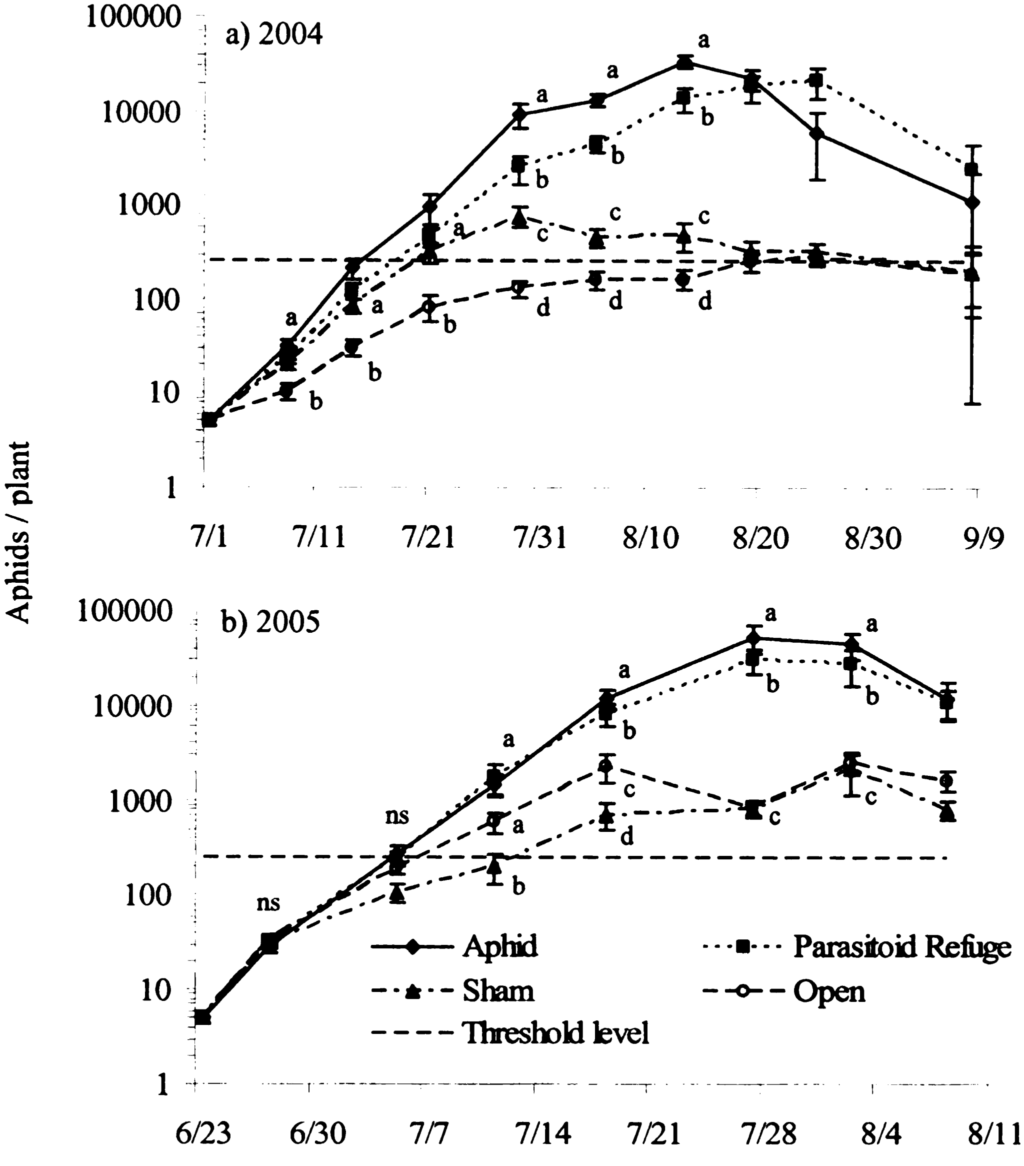


Figure 4.2. Effect of natural enemy treatments on the number of *A. glycines* (mean ± SE) during a) 2004, and b) 2005. Different letters on each sampling date indicate significant differences ($P < 0.05$, Least Mean Square Differences adjusted by the sequential Bonferroni method for multiple comparisons). Statistical comparisons were restricted to the six initial sampling dates, when aphids reach peak populations.

Aphid populations increased rapidly in the aphid and parasitoid refuge treatments and exceeded threshold levels three (2004) and two (2005) weeks after initial infestation with aphids. By contrast, large predators maintained aphids below threshold for the entire season in 2004 in the open treatment, whereas in the sham treatment a maximum of approximately 800 aphids / plant were observed 4 weeks after infestation and then declined to the same level as in the open treatment (Figure 4.2a). In 2005, an outbreak of *A. glycines* occurred throughout the US Midwest, and aphids reached threshold populations four weeks after infestation, with a maximum level of 2,500 aphids / plant at week 6 in the sham and open treatments. However, these population levels were reached 3 weeks earlier in the aphid and parasitoid refuge treatments, in which large predators were excluded (Figure 4.2b).

During 2004, I observed a significant delay (approximately two weeks) in the timing of peak aphid density in the parasitoid refuge versus the aphid treatment (Figure 4.2a). A shorter delay was observed in 2005, although in general aphid populations were slightly lower in the parasitoid refuge (Figure 4.2b). Since there was much more parasitism in 2004 than in 2005 (see 3.5), I tested whether the delay in aphid peak populations may have been a result of parasitism. To test this, aphid abundance was contrasted for the first 6 weeks of the experiment in the parasitoid refuge treatment between plots in which I found parasitoids (n= 5) versus plots in which I did not find parasitoids (n = 7), irrespective of block and agronomic treatment. There were no significant differences related to the presence of parasitoids (F = 2.70, df = 1, 10; P = 0.1311) and a trend opposite to the expected, with more aphids in plots with parasitoids.

Thus, the lower numbers of aphids in the parasitoid refuge treatment in comparison with the aphid treatment were not attributable to parasitism.

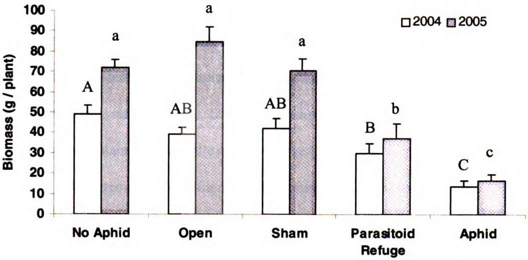
Soybean biomass and yield

Agronomic treatment did not affect soybean biomass (F = 0.71; df = 2, 15, P = 0.5081) or yield (F = 0.53; df = 2, 15, P = 0.5965), and therefore results were averaged across the three treatments. I observed significant differences in biomass (F = 42.69; df = 4, 80, $P < 0.0001$) and yield (F = 70.11; df = 4, 80; $P < 0.0001$) due to natural enemy manipulation treatments (Figure 4.3). The presence of soybean aphids decreased biomass and yield between 72 to 90% in comparison with the controls with no aphids, but exposure of aphids to predators in the sham and open treatments resulted in a trophic cascade that restored both plant yield and biomass (Figure 4.3). Biomass was significantly lower in 2004 than in 2005 (F = 10.77; df = 1, 15; P = 0.0050). I observed a non-significant trend of lower yield in 2005 in all except the open treatment (year by treatment interaction: F = 5.65; df = 4, 80; P = 0.0005). These results confirm independent findings of the potential for extant predator assemblages to reduce *A. glycines* populations to levels that prevent significant plant damage (Costamagna et al., unpublished results).

Predator abundance

In 2004, the single plant sampling yielded only 17 natural enemies, and thus these data were excluded from statistical comparisons. By contrast, in 2005 a total of 1,197 natural enemies were observed in the quadrats, which were dominated by Coccinellidae (76.8% of all predators), including *H. axyridis* (36.4%), *Hippodamia variegata* (Goeze) (4.4%), *Coccinella septempunctata* L. (3.6%), and *Coleomegilla maculata* (De Geer),

a) Soybean biomass



b) Soybean yield

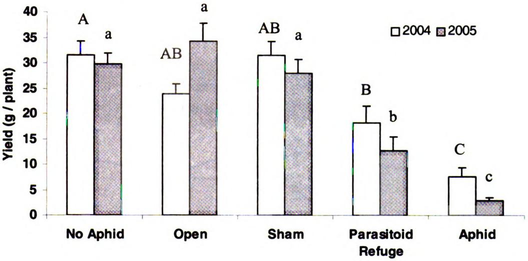


Figure 4.3. Effects of natural enemy manipulations on soybean a) above ground biomass and b) yield (g of seed / plant), in the biodiversity plots of the KBS-LTER site, during 2004 and 2005. Different letters indicate significant differences ($P < 0.05$, LSMD, adjusted by the sequential Bonferroni method) among treatments within the same year (capital letters for 2004, lower case letters for 2005).

Cycloneda munda (Say), and *Hippodamia convergens* Guérin-Menéville, accounting for the rest (2.5%). In addition, I sampled first and second instar coccinellid larvae (19.6%)

and pupae (10.2%), that were not possible to identify to species in the field, but most likely were *H. axyridis*. Other large-size predators included nabids (3.3%), spiders (2.6%), mirids (2.5%), and lacewing larvae (1.7%), and their abundance was compared among agronomic treatments grouping them together as "other large predators" (Figure 4.4). Among small-size predators, *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) was the most abundant (9.8%), followed by midge (2.3%) and syrphid (1.2%) larvae (these last two groups were combined as "other small predators," Figure 4.4). Overall, predator abundance was low (Figure 4.4), and was not affected by agronomic treatment (coccinellids: $F = 1.28$; df = 2, 6; $P = 0.3445$; *O. insidiosus*: $F = 1.45$; df = 2, 6; $P = 0.3055$; and, averaged across sampling weeks, other large predators: $\chi^2 = 0.546$; df = 2; $P = 0.761$; and other small predators: $\chi^2 = 3.512$; df = 2; $P = 0.173$).

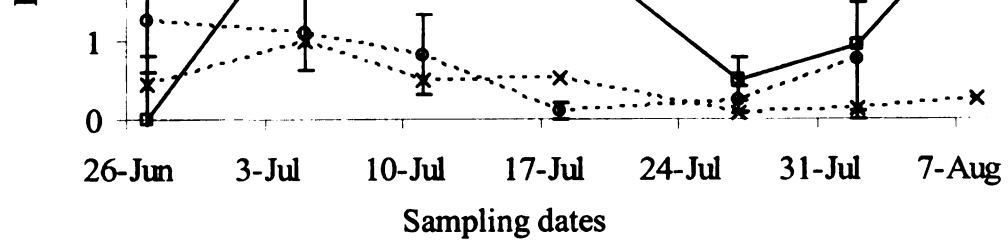


Figure 4.4. Open field predator populations sampled in the biodiversity plots of the KBS-LTER site during 2005. Large-size predators includes nabids, spiders, small carabids, mirids and lacewing larvae; small-size predators includes midge and syrphid larvae.

Cage manipulations resulted in significant differences in the number of predators present in each treatment (Figure 4.5). During both years coccinellids were successfully excluded from the aphid and parasitoid refuge treatments, although in weeks 5 and 6 of 2005, coccinellid larvae were found in parasitoid refuge treatments (Figure 4.5b). The abundance of *O. insidiosus* was initially reduced only in the aphid treatment, and showed a trend of higher abundance in the parasitoid refuge treatment (Figure 4.5c and d). Large predators other than coccinellids were significantly more abundant in sham cages only during the fourth week of 2004, although at very low levels (Figure 4.5e and f). Small predators (i.e. midge and syrphid larvae) were initially higher in the sham and open treatments at low levels, and then reached high but variable abundances in the parasitoid refuge and aphid treatments, probably as a response to the increase densities of aphids on those treatments (Figure 4.5g and h). Thus, cage manipulations successfully excluded coccinellids, but did not prevent small predators and *O. insidiosus* from occurring in parasitoid refuge cages. To test for a potential difference in the abundance of natural enemies between 2004 and 2005, I compared the total number of natural enemies observed, grouped for all four natural enemy treatments, for each sampling date. Overall, predators were more abundant in 2005, probably responding to increased aphid densities in the field (Figure 4.1). In 2005, significantly more *O. insidiosus* and coccinellids were observed in weeks 2, 5 and 6 after the beginning of the experiment, and more large predators in weeks 2 and 5 (Figure 4.5a - f). The abundance of small predators did not differ between years (Figure 4.5g and h).

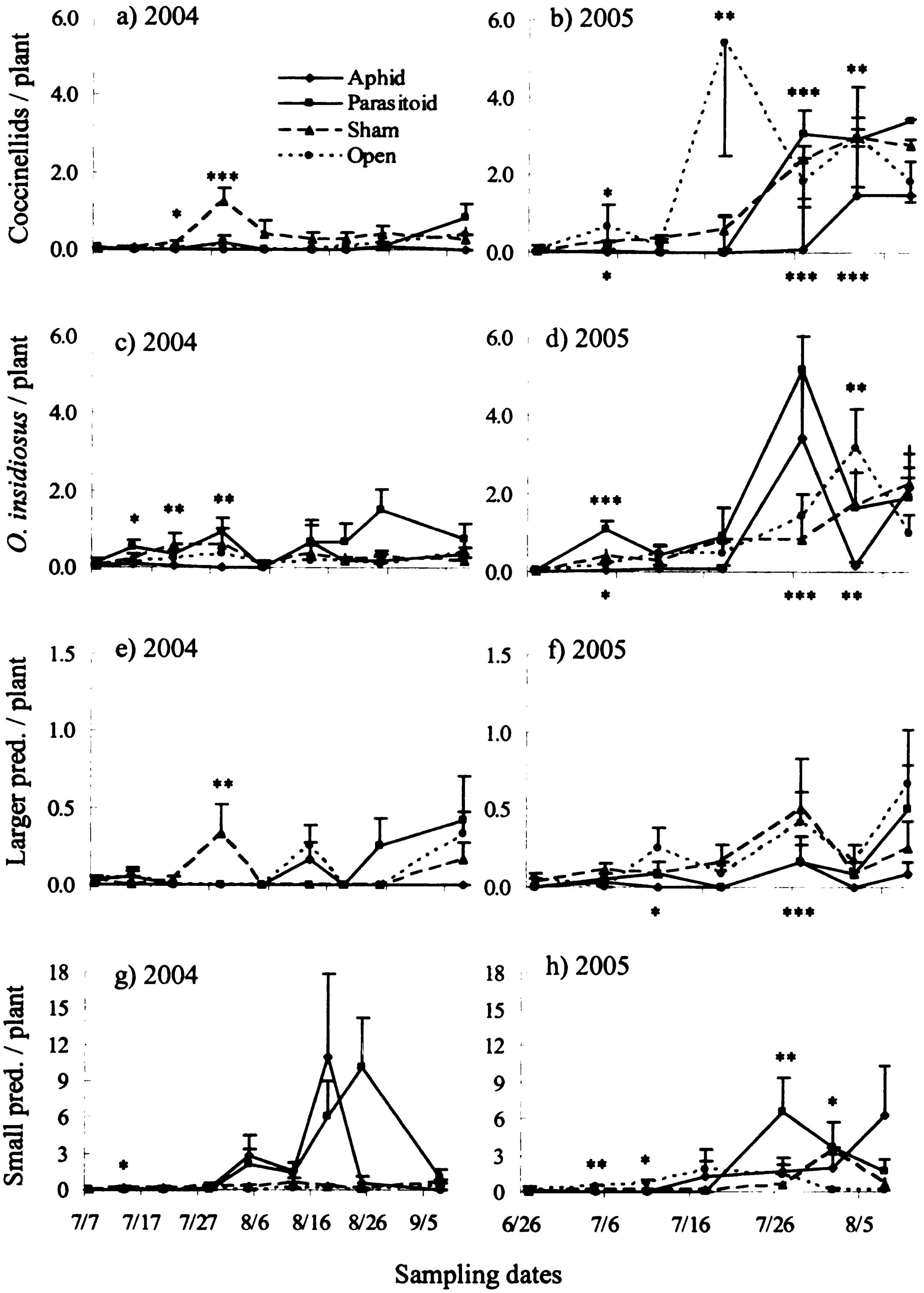


Figure 4.5. Effect of natural enemy manipulations on the abundance (mean + SE) of the four major groups of predators sampled in the biodiversity plots of the KBS-LTER during 2004 and 2005. Asterisks above treatment lines indicate significant differences among natural enemy treatments, asterisks below sampling dates indicate significantly

Figure 4.5 (cont'd)
higher abundance for all treatments combined in that year (Kruskal-Wallis tests, * = $P < 0.10$, ** = $P < 0.05$, *** = $P < 0.01$). Statistical comparisons were performed for the six initial weeks.

Parasitoid abundance and intraguild predation

Sampling of natural populations did not yield any parasitoids outside cage manipulations during 2004. However, 1,934 parasitoids were observed in cage manipulations, most of which were Braconids (85%) and the rest were Aphelinidae (15%). Despite the aphid outbreak and the more intensive sampling performed in 2005, only 5 braconid mummies were detected outside of cages, and 42 mummies in cage manipulations (95% Braconidae). Therefore, only data from 2004 were considered in the following analyses of parasitism. I collected three parasitoid species: the braconids *Lysiphlebus testaceipes* (Cresson) and *Binodoxys kelloggensis* Pike et Stary, and the aphelinid *Aphelinus asychis* (Walker). There were no parasitoids in the aphid treatment and only one mummy in the open treatment, and therefore these treatments were excluded from statistical comparisons.

I observed significantly higher cumulative number of mummies in the parasitoid refuge than in the sham treatment ($F = 8.30$; df = 1, 9; $P = 0.0182$). Mummy counts were not affected by agronomic treatment ($F = 0.24$; df = 2, 6; $P = 0.7953$), and therefore this factor was excluded for the following analyses with parasitoids. Non-parametric comparisons per date showed that the trend of higher mummy abundance observed in the parasitoid refuge treatment was significantly different on only two dates (Figure 4.6a). To test whether this difference was due to intraguild predation or to a response to the higher aphid densities of the parasitoid refuge treatment, I compared the percentage parasitism

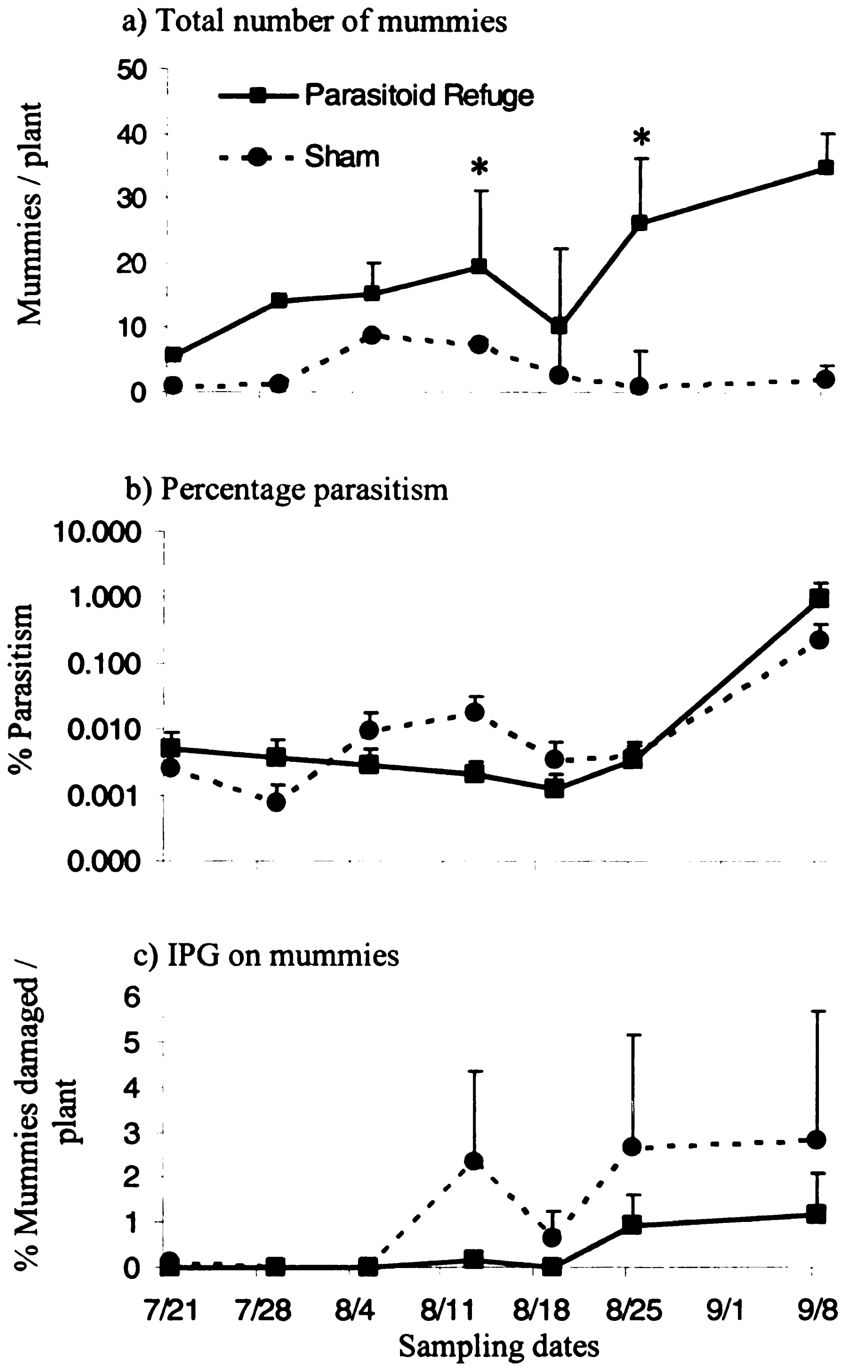


Figure 4.6. Effect of predator manipulations on a) number of mummies / plant, b) percentage parasitism (proportion of mummies to aphids x 100), and c), number of mummies damaged by predators; averaged across agronomic treatments. Only one mummy was obtained in the open treatment and none in the aphid treatment, and therefore these treatments were excluded from statistical comparisons. Treatments were compared independently for each date using Wilcoxon tests (* = $P < 0.10$).

among treatments, and found no significant difference between parasitoid refuge and sham treatments (Wilcoxon tests, all $P > 0.176$, Figure 4.6b). Finally, IGP was directly assessed by comparing the number and percentage of mummies damaged by predation between the parasitoid refuge and the sham treatment. Although I observed a trend of more damaged mummies in the sham treatment, the highly variable incidence of IGP in this treatment resulted in an absence of significant differences (Wilcoxon tests, all $P > 0.180$, Figure 4.6c). Contrasting IGP on all the possible pairs in which parasitism was obtained in both treatments within the same plot, revealed that proportionally significantly more mummies were damaged in the sham (18.1 ± 7.6%, range 0 – 94.4%) than in the parasitoid refuge treatment (3.7 ± 2.4%, range 0 – 36.4%, Wilcoxon test $z = 2.1$, $P = 0.036$). This result shows that IGP is occurring at the very low levels of parasitism observed in this study.

Discussion

Despite an expected positive role of ant tending on aphids (e.g. Völkl 1992, Stadler et al. 2002), I did not find significant effects of ants on aphid abundance. However, this interaction can not be ruled out from the *A. glycines* system, since my study was not explicitly designed to test this factor and I did not carry on observations on this interaction during 2005. As anticipated, cover crops did not affect significantly aphid abundance in my experiment, confirming previous results of experiments using similar agronomic treatments (Costamagna and Landis 2006).

During the two years of study, I observed strong suppression of *A. glycines* due to generalist predators, but I did not find significant effects of extant parasitoids. These findings thus support an increasing number of studies showing an important role of generalist predators controlling *A. glycines* in North America (Brown et al. 2003, Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Nielsen and Hajek 2005, Rutledge and O'Neil 2005, Costamagna and Landis 2006). Moreover, my results showed that even during outbreak years such as 2005, predators were able to delay aphid increase above threshold levels in comparison with controls in which they were excluded, confirming earlier studies (Fox et al. 2004, Costamagna and Landis 2006). In addition, I showed that soybean aphid has the potential to cause a 3.5 to 10-fold reduction on soybean yield and biomass, but that both were restored to normal levels by exposure to predation, during both years of study. Similar results were obtained in a different experiment using 1 m^2 field cages by Costamagna et al. (unpublished results) during 2004. These findings suggest that predators are a key factor in soybean aphid suppression and their input should be taken into account in determining action thresholds.

The different cages used for the natural enemy treatments allowed separation of the effect of *O. insidiosus* and small predators from Coccinellids and other large predators. Previous studies have suggested that *O. insidiosus*, due to its abundance on soybeans early in the season, and potential for aphid consumption in laboratory conditions, may play an important role reducing *A. glycines* population growth (Rutledge et al. 2004, Rutledge and O'Neil 2005). I observed similar or even higher numbers of *O. insidiosus* and midge and syrphid larvae in the parasitoid refuge cages than in sham and open cages, confirming that these predators had similar access to aphids in those treatments. However, aphids increased exponentially to very high numbers in the parasitoid refuge treatment during both years, despite being exposed to predation by small predators. These results indicate that *O. insidiosus* and other small predators were unable to prevent *A. glycines* exponential growth. However, field experiments were started at levels of 5 aphids / plant, and thus I can not rule out a potentially greater impact of *O. insidiosus* at lower aphid densities, earlier in the season. In conclusion, I observed strong reduction in aphid numbers only in the treatments that had high numbers of coccinellids and large predators (sham and open treatments), indicating that these were the predators that had the greater impact on aphid populations in the field.

On the other hand, my study suggests that *O. insidiosus* and other small predators may have had a low to moderate impact on aphids. There was a delay in peak aphid populations in the parasitoid refuge treatment with respect to the aphid treatment in 2004, and slightly less overall aphid densities during 2005 (Figure 4.2). However, there were very few parasitoids during 2005, and there was no significant association between parasitism occurrence and aphid abundance in 2004. Therefore, I concluded that the lower aphid densities observed were not a consequence of parasitism. An alternative explanation is that alate aphids were confined in the aphid treatment due to the fine mesh of the cage, and inflated aphid density with respect to the parasitoid refuge treatment. To test that, I compared the proportion of alates (number of alates / total number of aphids) between these two treatments and found that the proportion of alates was significantly higher in the aphid treatment than in the parasitoid refuge only during the fourth week of the experiment, during both years (natural enemy by date [year] interaction: $F = 3.03$; df $= 10, 80$; $P = 0.0014$, LSMD tests on week four, P values < 0.001). In general alates were at low densities (at week 4: 0.3 versus 2.7% in 2004, 1.8 versus 6.4% in 2005, for the parasitoid refuge and aphid treatment, respectively), indicating that probably a

combination of alate emigration and the impact of small predators is likely to explain the difference on aphid densities observed between the parasitoid refuge and aphid treatments. In addition, these results suggest that to conservatively assess the impacts of large predators, aphid densities on the sham and open treatments should be compared to the parasitoid refuge treatment.

Finally, three species of parasitoids were observed attacking *A. glycines*, but there was no significant effects of parasitism in reducing aphid populations, even when IGP was minimized with predator exclusion cages. I did observe direct evidence of IGP, by quantifying damaged mummies, and this measure is likely to be conservative, since predators may completely dislodge some of the parasitoid mummies during consumption (Brodeur and Rosenheim 2000). The maximum levels of IGP detected were around 94% of mummies damaged by predation, although on average, IGP was less than 20%. Colfer and Rosenheim (2001) studied IGP of *L. testaceipes* by the coccinellid *Hippodamia convergens* in cotton and found that despite levels of IGP of up to 98 – 100%, the addition of the coccinellid added suppression of cotton aphid over the presence of the parasitoid alone. By contrast, in the *A. glycines* system I obtained very low parasitism levels, typically around 1%, and no evidence of reduction in aphid growth due to parasitism.

In conclusion, generalist parasitoids are attacking *A. glycines* in Michigan soybean production systems and they experience significant IGP by generalist predators. However, IGP did not affect the percent parasitism, suggesting the absence of any disruptive effect of predation at the levels of parasitism detected. The relatively few studies on the effect of IGP on herbivore control by parasitoids involves systems where parasitism levels are high, and therefore the potential for disruption is greater (Colfer and Rosenheim 2001, Snyder and Ives 2001, 2003). In the *A. glycines* system, the recent arrival of this exotic pest to North America results in a lack of adaptation of the extant parasitoid species to use it as a host, as seen with other aphid species (Brewer et al. 2005). My results showed that resident parasitoids of *A. glycines* are not currently disrupted through IGP. Generalist predators act as major sources of *A. glycines* mortality, although during some seasons aphids escape their control. However, I demonstrate a potential for IGP that may reach high levels in some cases and may contribute to delay the adaptation process of resident parasitoid species to *A. glycines*. Moreover, the potential disruptive effect of generalist predators may be important if more effective parasitoids species are added to the system and this should be considered in any classical biological control effort involving parasitoids.

CHAPTER 5

DOES *APHIS GLYCINES* HAVE REFUGES FROM TOP-DOWN CONTROL BY GENERALIST NATURAL ENEMIES?

Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) recently invaded North America, causing significant economic losses. Previous studies demonstrated strong suppression of *A. glycines* by natural enemies, however, in some years *A. glycines* reach outbreak populations. I investigated the existence of refuges from predation as one of the potential factors leading to *A. glycines* escape from predation. I tested the presence of 'numeric refuges', in which colonies with higher aphid densities were released from predator control, and spatial refuges at the within-plant level. I followed naturally occurring *A. glycines* populations at high, medium, and low initial densities and compared them with populations of the same initial level protected by predator exclusion cages. In addition, I compared the vertical distribution of the aphids at the within-plant level for the same colonies. Predators provide strong and significant reductions of *A. glycines* populations regardless of initial aphid density. Interestingly, there were lower per capita rates of increase at higher than at lower initial aphid populations. Exposure to predators significantly altered *A. glycines* distribution within the plant, with proportionally more aphids at the lower plant nodes in colonies exposed to predation. These results suggest that predator suppression is effective under the naturally

occurring range of aphid densities and that the lower portion of the plant may represent a partial refuge from predation for *A. glycines*.

Introduction

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) is a new invasive pest in North America (Losey et al. 2002, Ragsdale et al. 2004). The potential of natural enemies to suppress *A. glycines* populations has been shown both in its original area of distribution in Asia (Liu et al. 2004, Wu et al. 2004), and recently in North America (Fox 2002, Fox and Landis 2003, Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Rutledge and O'Neil 2005). However, outbreak populations of *A. glycines* have led to significant yield reductions during alternating years since its first detection in 2000 (DiFonzo and Hines 2002, Venette and Ragsdale 2004, Myers et al. 2005a). The causes of the escape of *A. glycines* from natural enemy control during those outbreak years remain poorly understood.

One potential explanation of the lack of control of *A. glycines* is the existence of refuges from predation. Here the term refuge is used broadly for any spatial, temporal or numerical scenario that release prey populations from suppression by predators. Thus, aphid colonies of high density may constitute a refuge from predation through a dilution effect on the mortality risk (Turchin and Kareiva 1989). If that is the case I predict that field patches with higher aphid densities will increase aphid density more rapidly than field patches with lower aphid densities, constituting a 'numerical refuge' for the aphids. In addition, differential foraging by natural enemies has been shown to result in refuges at the within-plant scale (Hacker and Bertness 1995, Gonzales et al. 2001, Magalhães et al. 2002). I conducted field studies to determine the existence of numerical and spatial refuges from predation in the *A. glycines* system. Specifically I tested for the presence of refuges in field patches with different densities of *A. glycines* and within the vertical distribution of the aphids on soybean plants.

Materials and methods

Experiments were conducted in a conventional soybean field (50 x 100 m) located on the Entomological Farm of Michigan State University, Michigan, USA during 2003. Soybeans (Pionner 92B38) were planted on 05/22/2003, and maintained with conventional agricultural techniques. In this field, naturally occurring colonies of *A. glycines* were caged at different patch densities to test whether initial aphid density influenced suppression by predators. On 7/3/2003, the field was divided in 50,10 x 10 m patches that were sampled to determine aphid density. Sampling consisted of counting the total number of aphids (nymphs, apterous adults, alates) on 10 randomly selected plants per patch. *A. glycines* densities obtained in the grid sampling were used to classify field patches in three categories according to their initial aphid density: low (< 5 aphids / plant), medium (5 – 10 aphids / plant) and high (> 10 aphids / plant). In each category, 6 patches were randomly chosen and received three predator manipulation treatments: predator exclusion, a sham cage or no-cage. The exclusion cage consisted in a cylindrical framework of wire of 0.4 x 1.0 m, covered by a fine mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA), buried 25 cm in the soil and tied to the top. The sham cage was identical except the mesh was held 5 cm above ground surface attached by metal clips to four wooden stakes, and had eight rectangular openings (2 x 20 cm) arranged in 3 circular patterns alternating in a manner such that there were openings in all directions. These rings of openings were located from 10 to 30 cm above the soil, encompassing the height range of the canopy. Finally, the no cage control ("open") consisted of three contiguous uncovered plants marked by flags nearby.

Each cage enclosed the focal plant containing a naturally occurring aphid colony within the range desired and, for the first two weeks, two additional soybean plants initially free of aphids. After two weeks, the plants were reduced to one per cage, leaving typically the central one (unless it was damaged), to allow plants to growth uncrowded by the cage. The experiment started on 7/03/2003, when plants were at the V3 - V4 stage (Ritchie et al. 1994). Once a week the total number of aphids and their location within the plant was assessed by direct counts. After two weeks I calculated the per capita rate of increase as $PCRI = N_{t0} / N_{t14}$, where N_{t0} is initial aphid density, and N_{t14} is the density at day 14 after the beginning of the experiment. To rule out potential local effects of the soybean plants on aphid growth, exclusion and sham cages were switched in a subset of the cages after two weeks. On 7/17/2003 I matched cages in pairs according to the density of aphids in the exclusion cages, and in one of the plots the sham and exclusion cages were switched, and in the other they were left un-switched as controls, for a total of 6 plots switched and 6 control plots. The experiment was conducted for three additional weeks, but sampling of the control plots was stopped at week 4 due to the extraordinary number of aphids produced and the fact that alates could not emigrate, artificially inflating numbers in exclusion versus sham and open treatments.

Statistical analysis

The effects of initial aphid density and natural enemy control on *A. glycines* population growth and per capita rate of increase were assessed by ANOVA using a split-plot design, with aphid density as the whole-plot factor and natural enemy as the sub-plot factor (Proc MIXED, SAS Institute 2001). For the aphid population increase analysis, sampling date was analyzed as a repeated measures factor. Significant interactions were explored slicing by main effects (Quinn and Keough 2002). Means were compared using Least Square Mean Difference tests adjusted for multiple comparisons by the sequential Bonferroni test (Rice 1989). Within-plant aphid distribution was analyzed by MANOVA on a split-plot design with initial aphid density as the whole-plot factor and natural enemy as the subplot factor using Proc GLM (SAS Institute 2001). The proportions of aphids present in each node were analyzed as the dependent variable of the MANOVA and separate analysis were conducted for each date due to the variable number of nodes. Roy's greatest root was reported as the statistic for the MANOVA due to its power to detect differences among treatments (Scheiner 2001). Data were log or arcsine-transformed previous to statistical analysis to meet the normality and homocedasticity assumptions.

Results

Initial A. glycines density

After two weeks of manipulation, predator exclusion resulted in significantly higher aphid densities, ranging 9 to 28 times above the sham and open controls (Figure 5.1, Table 5.1). Initial aphid density also significantly affected aphid abundance, with higher

Table 5.1. ANOVA results for the effect of initial aphid density and natural enemy control on the population growth of *A. glycines* natural populations in Michigan.

Source	df	*F*	*P*
Aphid density	2, 15	12.36	0.0007
Natural enemies	2, 118	153.29	<.0001
Aphid density x natural enemies	4, 118	2.67	0.0355
Date	2, 118	303.32	<.0001
Aphid density x date	4, 118	0.91	0.4621
Natural enemies x date	4, 118	46.77	<.0001
Aphid density x natural enemies x date	8, 118	0.47	0.8756

aphid abundances at the high than at the medium and low initial aphid densities (*P*s < 0.05, LSMD tests). Slicing the significant aphid density by natural enemy interaction revealed that natural enemy treatments differed significantly under all initial aphid densities (*P*s < 0.005), and initial aphid density affected aphid abundance in all natural enemy treatments (*P*s < 0.005). The significant interaction is therefore due to the lower aphid abundance observed in the sham and open treatments of the lower aphid density in comparison with medium and high (Figure 5.1). The significant natural enemy by date interaction is due to the expected lack of differences between natural enemy treatments at the start of the experiment (*P* = 0.9734, Figure 5.1a). I did not observe significant cage effects at high and low initial aphid densities, and only small differences between sham cages and open plots at the medium density, suggesting that potential cage effects were minimal (Figure 5.1). In summary, although there was a significant effect of initial aphid density, this effect was more important in exclusion cages, where treatments exposed to

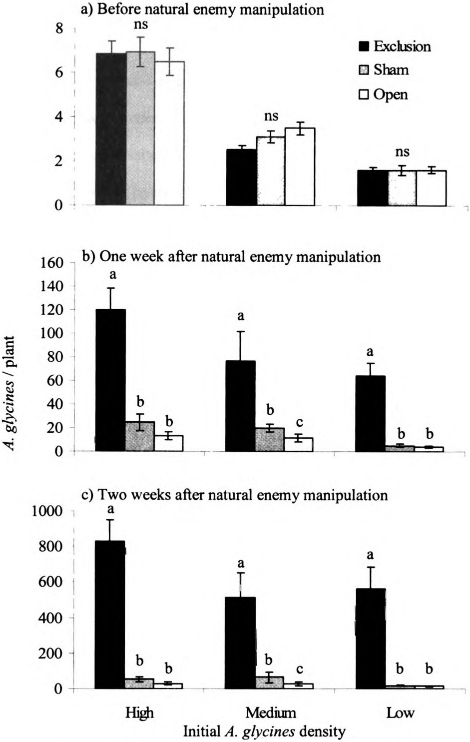


Figure 5.1. *A. glycines* population growth from three initial densities under three predator manipulation treatments. Mean (± SE) aphid abundance is shown at a) before natural enemy manipulation, and b) one week, and c) two weeks after manipulation. Different letters represent significant differences among natural enemy treatments within patch density treatments ($P < 0.05$, Least Square Mean Differences).

predation maintained were well below the 250 aphids per plant threshold level in all cases (Figure 5.1).

Comparing the per capita rate of increase (PCRI) showed similar results, with strong effects of natural enemies ($F = 129.75$; df = 2, 29; $P < 0.0001$), marginally significant effects of initial aphid density ($F = 3.48$; df = 2, 15; $P = 0.0573$) and no significant aphid density by natural enemy interaction ($F = 1.05$; df = 4, 29; $P = 0.3934$; Figure 5.2). Comparing the means of the natural enemy treatments revealed a 16-fold decrease in PCRI on sham and open treatments in comparison with the predator exclusion treatment (LSMD tests, Ps < 0.0001), and no significant differences between the sham and open treatments (LSMD test, $P > 0.05$, Figure 5.2). Interestingly, I observed up to 3-fold lower PCRI plots that had high versus low initial aphid density, and that difference was marginally significant (LSMD test, $P = 0.0638$, Figure 5.2). This result suggests a potential self-limitation on *A. glycines* population growth.

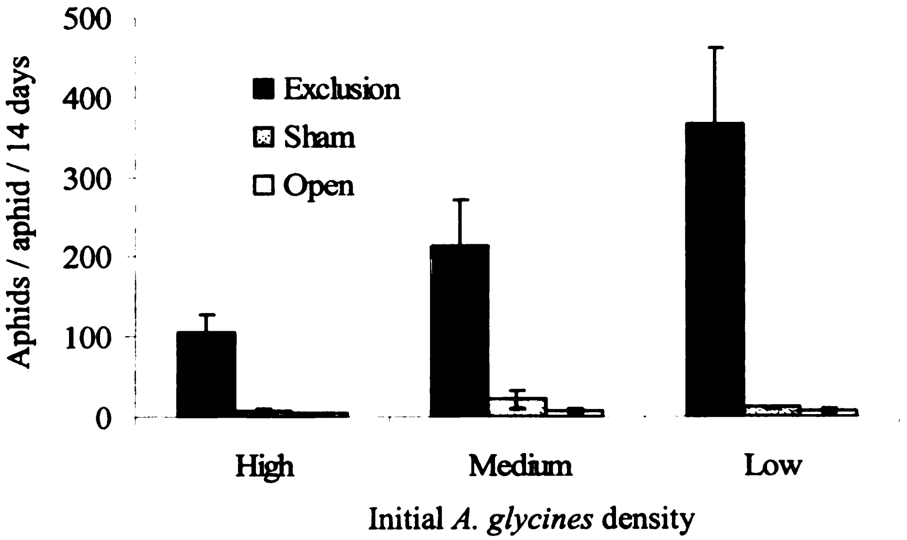


Figure 5.2. Mean (± SE) per capita rate of increase of *A. glycines* at three initial densities under three natural enemy manipulation treatments after 14 days. Significant main effects are described in the text.

Switching of the cages confirmed high impacts of predation; aphid populations differed significantly among predator manipulation treatments ($F = 13.75$; df = 5, 25; $P < 0.0001$), and sampling dates ($F = 35.11$; df = 1, 30; $P < 0.0001$). Reversing of the trajectories of switched versus control cages is indicated by a significant cage by date interaction ($F = 4.21$; df = 5, 30; $P = 0.0051$, Figure 5.3a and b). Due to the extraordinarily high aphid populations observed the control cages were interrupted on week 4. Therefore, *A. glycines* densities for the fifth week were compared separately, showing a complete reversal in the trajectories of the aphid populations, with significantly higher densities in the exclusion than the open treatment ($F = 3.98$; df = 2, 10; $P = 0.0534$; Figure 5.3c).

Within-plant distribution

Aphid distributions within plants were not affected by initial aphid density (Table 5.2). Most of the plants were at the V4 stage (few were V3 or V5), and most of the aphids were located within the fifth node, which represented the youngest tissue available in most cases (Figure 5.4). One week after natural enemy manipulation I observed significant effects of aphid initial density on the within-plant distribution (Table 5.2), with aphids at the low density treatment concentrated more on the youngest tissue (nodes 5 to 7, Figure 5.5c), whereas at the low and medium initial density treatments proportionally more aphids were at lower nodes (Figure 5.5a and b). Plants were at the V5, V6 or R1 developmental stage, and in some cases had secondary trifoliates emerging from the first and second node. Two weeks after manipulation, exposure to natural enemies resulted in highly significant differences on aphid distribution, whereas initial aphid density was only marginally significant (Table 5.2). I observed that the within-plant distribution of *A.*

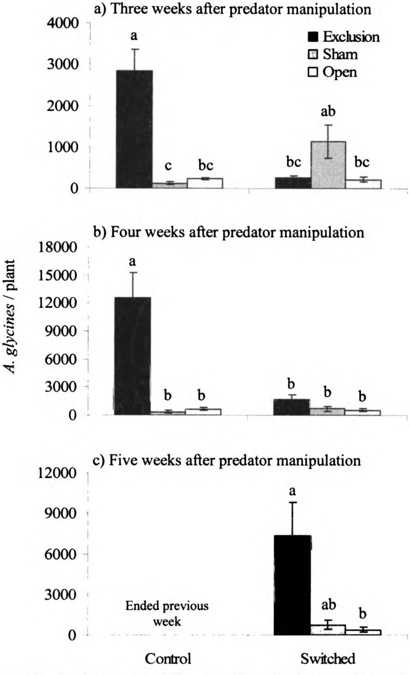


Figure 5.3. *A. glycines* population growth under three predator manipulation treatments. Mean (± SE) aphid abundance is shown at a) three, c) four, and c) five weeks after predator manipulation. Plots were paired according to aphid density in the exclusion treatment at week 2 and randomly chosen to switch exclusion and sham cages (switched) or remained un-switched (control). Control treatments were interrupted at week four due

to the extraordinary high aphid densities achieved, and therefore week five was analyzed separately (see methods). Different letters represent significant differences among natural enemy treatments ($P < 0.05$, LSMD adjusted by the sequential Bonferroni method for multiple comparisons).

Table 5.2. MANOVA results for the effects of different initial aphid densities and natural enemy manipulations on the within-plant distribution of *A. glycines*.

	df	F^1	P
Before manipulation			
Aphid density	6, 26	1.37	0.2619
Natural enemies	6, 26	2.12	0.0847
Aphid density x natural enemies	6, 28	2.67	0.0357
One week after manipulation			
Aphid density	9, 20	3.44	0.0103
Natural enemies	9, 20	2.24	0.0644
Aphid density x natural enemies	9, 22	2.05	0.0814
Two weeks after manipulation			
Aphid density	12, 18	2.23	0.0607
Natural enemies	12, 18	6.04	0.0004
Aphid density x natural enemies	12, 20	1.71	0.1391

[1] F values of Roy's greatest root statistic (Scheiner 2001).

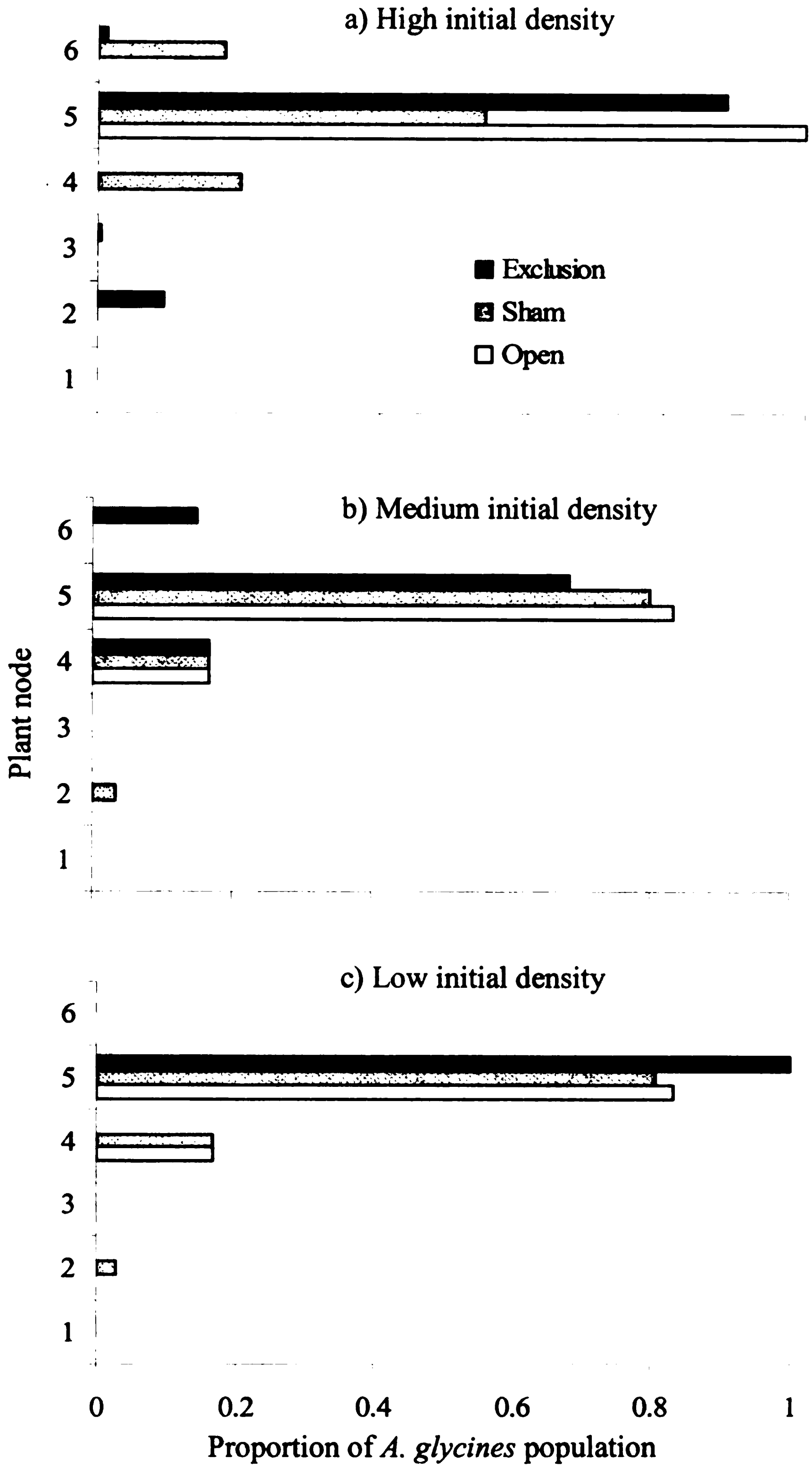


Figure 5.4. Within-plant distribution of naturally occurring *A. glycines* at a) high, b) medium, and c) low densities before assigning the predator manipulation treatments. Cages were placed immediately after sampling aphid abundance. The statistics for this figure are presented in Table 5.2.

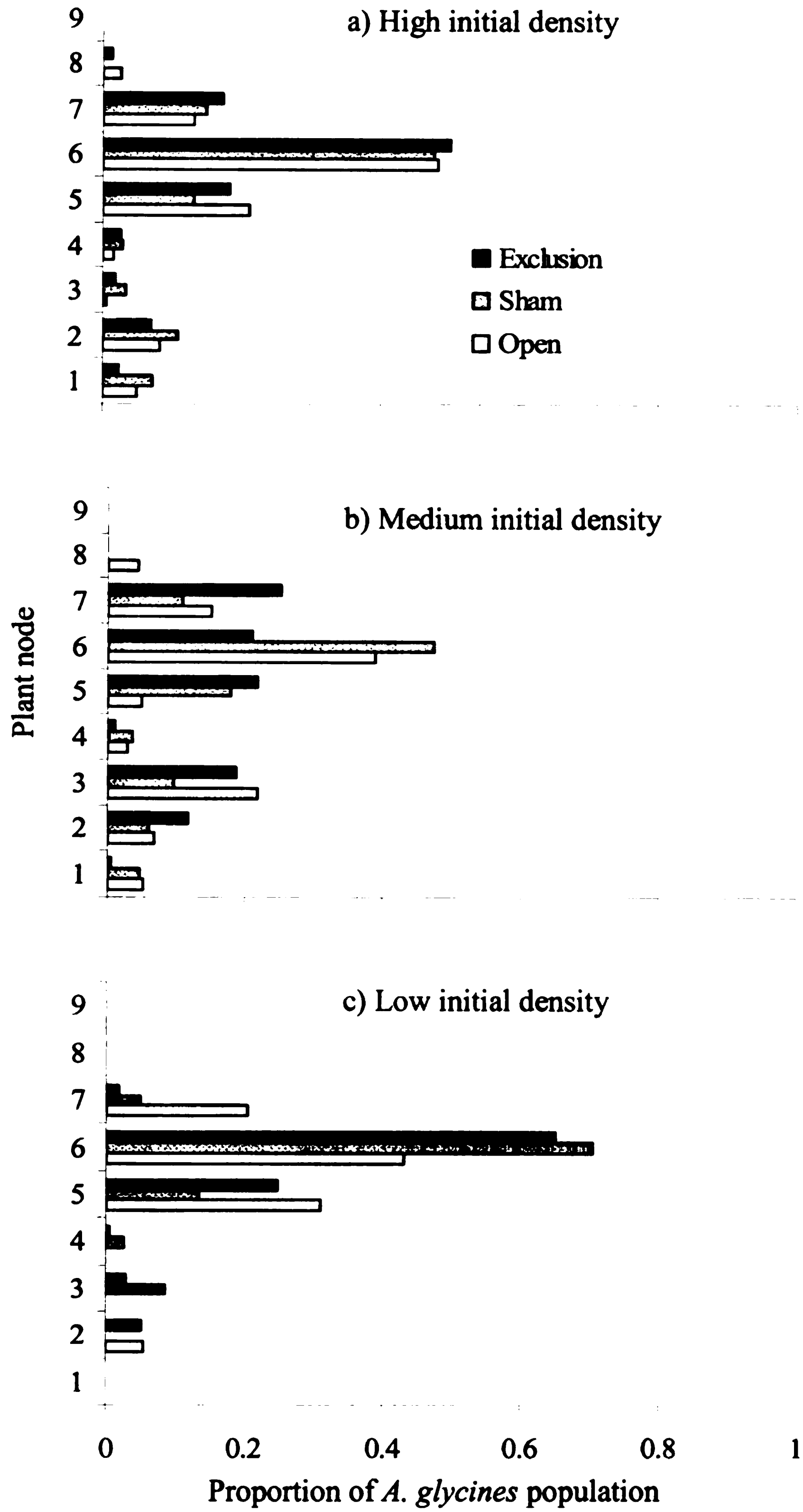


Figure 5.5. Within-plant distribution of naturally occurring *A. glycines* at a) high, b) medium, and c) low densities one week after predator manipulation treatments. The statistics for this figure are presented in Table 5.2.

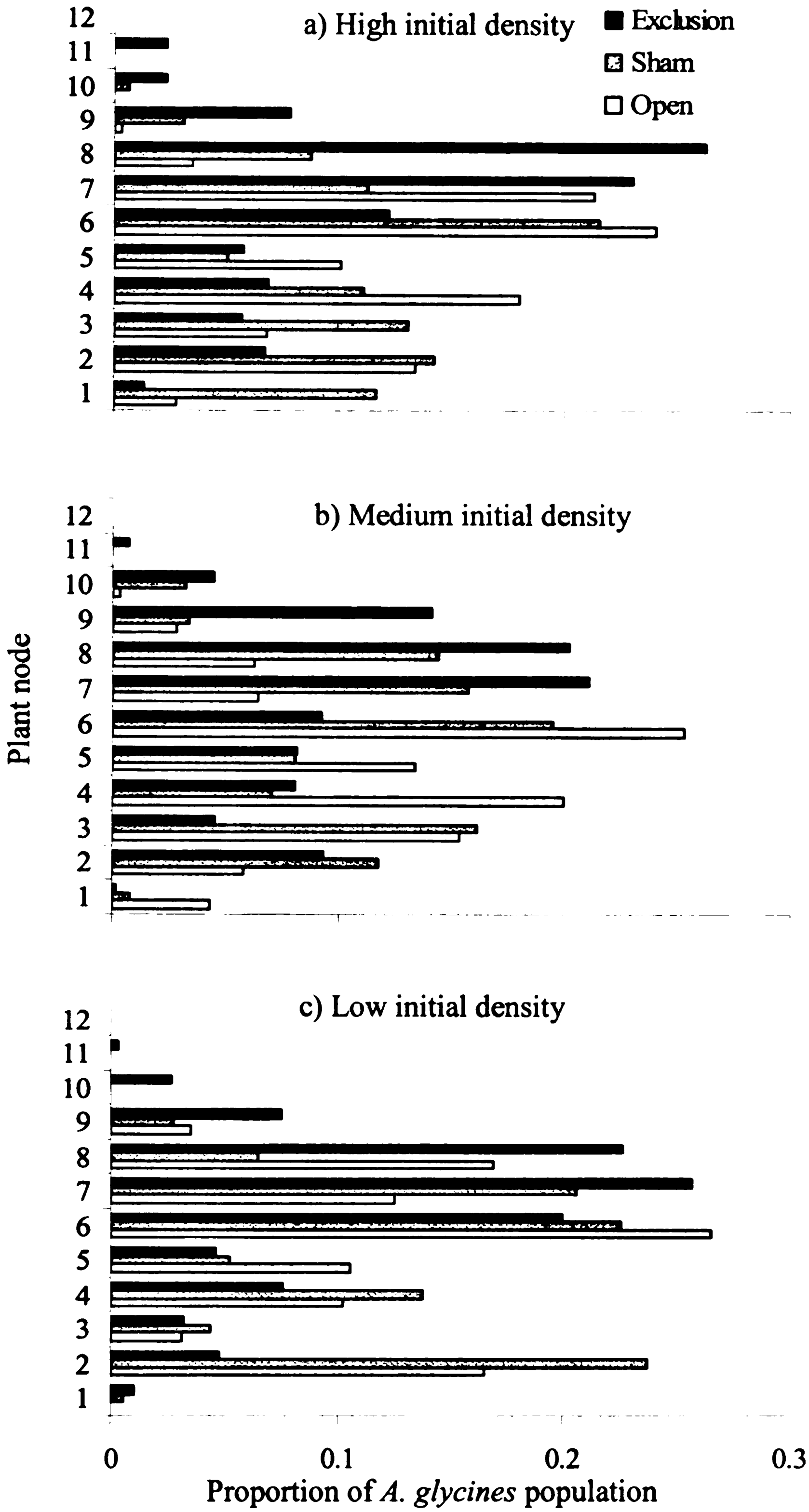


Figure 5.6. Within-plant distribution of naturally occurring *A. glycines* at a) high, b) medium, and c) low densities two weeks after predator manipulation treatments. The statistics for this figure are presented in Table 5.2.

glycines in the predator exclusion treatment tended to be shifted towards the upper portion of the plant, whereas in the sham and open treatments a relatively high proportion of the population was concentrated in lower parts of the plant (Figure 5.6). Plants were mostly at the R1 stage and many had secondary trifoliates up to the fourth and fifth node.

After switching exclusion and sham cages, a similar pattern was obtained (Figure 5.7). After three and four weeks of manipulation, aphid within-plant distribution continue to differed significantly among natural enemy treatments in unswitched controls ($F = 23.26$; df = 12, 5; $P = 0.0014$, and $F = 14.60$; df = 12, 5; $P = 0.0041$, respectively). Aphids were more evenly distributed in exclusion cages, probably due to the extraordinary densities achieved (average around 12,000 aphids / plant, Figure 5.3b) forced them to move to less prefered portions of the plant. In switched cages, aphid densities were much lower and with a good proportion of the aphids remaining at lower nodes of the plant, despite protection from predation, which resulted in only marginally differences in week 3 and significant but small differences in week 4 ($F = 4.63$; df = 12, 5; $P = 0.0511$, and $F = 5.22$; df = 12, 5; $P = 0.0399$, respectively, Figure 5.8a and b).

Discussion

In addition to a remarkable reproductive potential, the existence of refuges from predation, in combination with other factors may provide an explanation for the development and persistence of outbreak populations in aphids (Dixon 1987, 1998). Turchin and Kareiva (1989) demonstrated that *Aphis varians* (Hemiptera: Aphididae) at high density colonies benefited from a 'dilution effect' of lower predation risk in comparison with colonies with lower aphid densities. I postulated that if such a

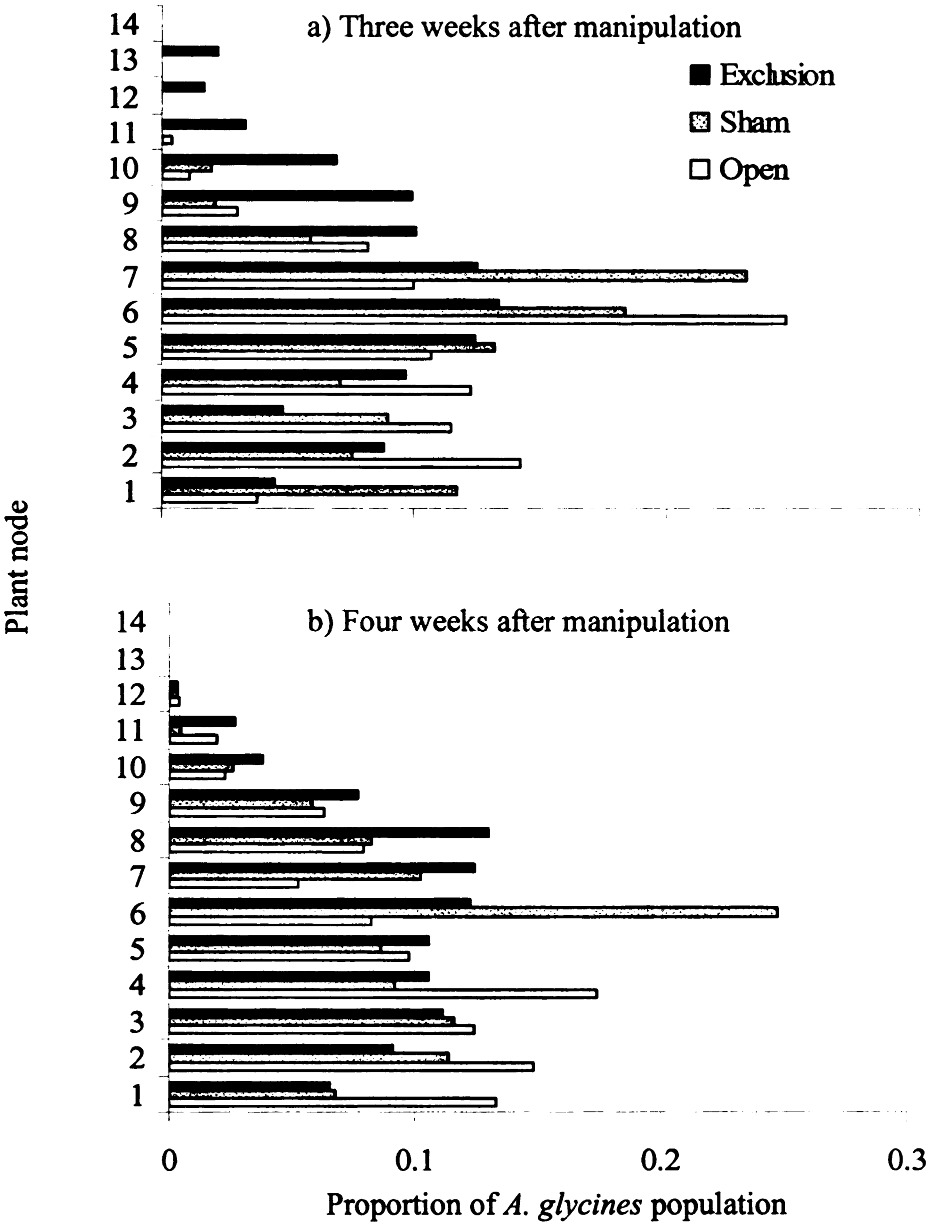


Figure 5.7. Within-plant distribution of naturally occurring *A. glycines* at a) three, and b) four weeks after predator manipulation treatments. Exclusion and sham cages were not switched, serving as controls for the switching treatment (see Figure 5.8).

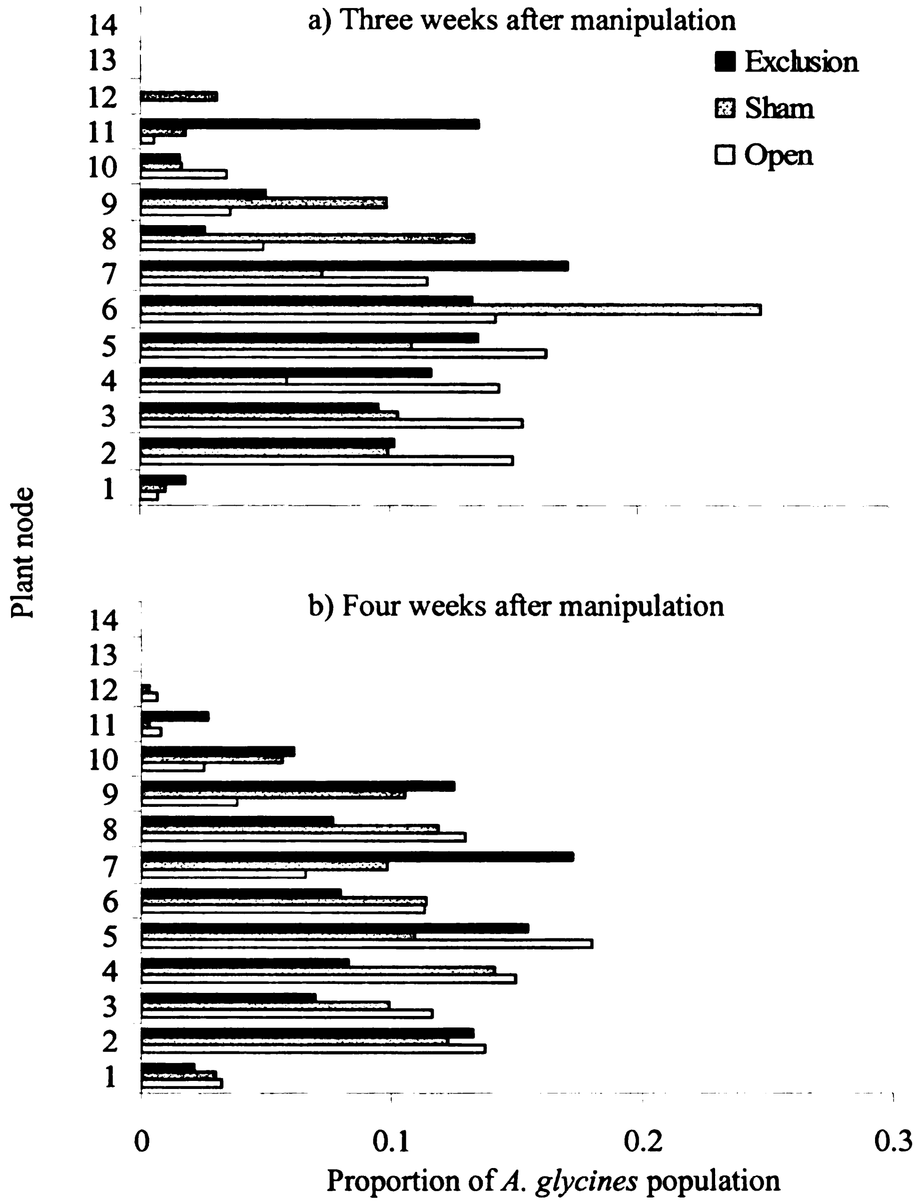


Figure 5.8. Within-plant distribution of naturally occurring *A. glycines* at a) three, and b) four weeks after predator manipulation treatments. Exclusion and sham cages were switched after the second week.

'numerical refuge' exists for *A. glycines*, I would detect lower impacts of predation on high density aphid patches that may lead to an eventual escape from predation. My results showed that predators exerted strong suppression at all the aphid densities within the range detected in naturally occurring *A. glycines* populations during 2003. Moreover, this pattern was even more convincingly demonstrated when aphid population trajectories were reversed as a result of switching exclusion and sham cages. Interestingly, I observed that *A. glycines* populations showed lower per capita rate of increase at higher aphid densities, suggesting a potential self-limitation in population growth. Thus, the potential benefits of lower predation risks at high density patches may be offset by a reduction in reproduction. Since I did not determine rates of predation in this study, it is not possible to separate the effect of these two factors determining *A. glycines* abundance. This pattern of reduce reproduction at higher aphid densities provides support to the model developed in Chapter 7, which assumes a linear decrease in the intrinsic rate of increase.

There was a significant shift in the within-plant distribution of aphids as a result of exposure to predation. Similar changes in spatial distribution in response to natural enemies have been shown for different mites and aphid species. Magalhães et al. (2002) showed that the green cassava mite, *Mononychellus tanajoa* Bondar, migrated vertically in specific patterns in response to odors from different predatory mites. Hacker and Bertness (1995) showed higher *Uroleucon ambrosiae* (Thomas) densities on shorter than taller plants as a response to the forage behaviour of its coccinellid predator. Hopkins and Dixon (1997) showed lower predation levels on the nymphs of the aphid *Monaphis antennata* (Kaltenbach) adapted to live on the upper surfaces of leaves and petioles, an unusual place for other aphids and even the adults of this species. Gonzáles et al. (2001) showed that *Rhopalosiphum maidis* (Fitch) located in the whorl of corn were less susceptible to attacks from the parasitoid *L. testaceipes*. An extreme adaptation is the dropping behavior exhibited by the pea aphid, *Acyrthosiphon pisum* (Losey and Denno

1998b), although this behavior could result in increased mortality by ground based predators (Losey and Denno 1998a, 1999).

I observed that *A. glycines* exposed to predation shifted its distribution towards lower nodes in the plant. This is consistent with avoiding predation by coccinellids that are observed more frequently foraging at the upper portion of plants (Hacker and Bertness 1995, Nakashima and Akashi 2005). In the *A. glycines* system, there were significantly more predation events on the upper portion of the plants (Chapter 6), supporting the existence of 'enemy free space' at lower portions of the plant. The development of predator avoidance behavior usually involves a tradeoff of reduced reproduction or other fitness components (Hacker and Bertness 1995, Lima 1998, Magalhães et al. 2002). The quality of plants for aphid feeding differs with leaf age, with younger leaves more suitable due to the concentration of metabolites (Dixon 1998). Thus, it is likely that shifts in *A. glycines* within-plant distribution as a consequence of predation result in indirect negative effects on aphid population growth. Further studies should be conducted to address the importance of this potential indirect effect of predation and its consequences in *A. glycines* suppression.

CHAPTER 6

QUANTIFYING PREDATION ON SOYBEAN APHID THROUGH DIRECT FIELD OBSERVATIONS

Abstract

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), is a new pest attacking soybeans in North America since 2000. Several studies have documented strong impacts of generalist natural enemies on *A. glycines* populations using predator exclusion cages and correlating predator and aphid abundances. However, to date no studies were conducted to directly determine the natural enemy groups that attack *A. glycines* in North America under field conditions. In 2005, 72 h of direct observations of predation on natural populations of *A. glycines* were conducted in Michigan. Observations were conducted during three consecutive weeks during morning, afternoon and evening, in replicated 1 m^2 areas. A total of 643 predators within 11 groups and 211 predation events on *A. glycines* were observed. Transient predators such as *Harmonia axyridis* and *Coccinella septempunctata* (Coleoptera: Coccinellidae) accounted for most of the mortality observed and were very effective on a per capita basis, despite relatively short residence time in the observed patches. Transient predators responded positively to increased *A. glycines* field densities. By contrast, resident predators, particularly *Orius insidiosus* (Hemiptera: Anthocoridae), were abundant and accounted for many predation events, but they were not as effective on a per capita basis and did not respond to changes in aphid density. I observed lower predation on *A. glycines* located at the bottom portion

of the plants and on alate aphids. I concluded that coccinellids exerted most of the mortality observed and have the potential to rapidly respond to changes in aphid density with high per capita rates of predation.

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae) is a new pest of soybeans in North America and has rapidly extended its distribution since its detection in 2000 to over 22 States in the USA and 3 Canadian provinces (Ragsdale et al. 2004, Venette and Ragsdale 2004, Mignault et al. 2006). Several studies have demonstrated strong impacts of natural enemies on soybean aphids in Asia (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004). In North America, field experiments suggest suppression of *A. glycines* by generalist predators (Fox et al. 2004, Rutledge et al. 2004, Fox et al. 2005, Nielsen and Hajek 2005, Rutledge and O'Neil 2005, Costamagna and Landis 2006, Costamagna et al., unpublished results). However, most studies to date relied on indirect methods to establish predator effects, including comparison of predator exclusion versus sham cages (Fox et al. 2004, Liu et al. 2004, Fox et al. 2005) and correlations between aphid and predator abundances (Rutledge et al. 2004, Nielsen and Hajek 2005, Rutledge and O'Neil 2005). These techniques have distinguished the major predator groups of *A. glycines* according to their size, based on different types of exclusion cages (Costamagna et al, unpublished data), or suggested the most important predator species based on their co-occurrence with the aphids in the field. However, direct evidence of which are the most important predator species attacking *A. glycines* in the field is very limited.

The only study assessing the relative importance of different predator species attacking *A. glycines* in North America was conducted in laboratory conditions by Rutledge et al. (2004). This study suggests that within the North American guild of predators, the coccinellids *Harmonia axyridis* Pallas, *Coccinella septempunctata* L., and *Hippodamia convergens* G.-Mén., the earwig *Forficula auricularia* L., the nabid *Nabis* sp. and lacewing larvae of the genera *Chrysopa* sp. have the highest potential to consume *A. glycines*. Predators with nocturnal habits, such as carabids and staphylinids showed lower impacts. However, these were 24 h trials carried out using Petri dishes and realized predation rates under field conditions may differ.

A more realistic, although time consuming method to assess predation consist of conducting direct observations of predation events on the field. Direct observation is the most useful technique to establish trophic relationships and determine predation rates under un-manipulated field conditions and has been used for a variety of organisms, including leafhoppers and aphids (Luck et al. 1988, Powel et al. 1996). Van den Berg et al. (1997) conducted direct observations of predation events on *A. glycines* by three predator groups in Indonesia: *Harmonia arquata* (Fabricius) adults, *Harmonia* larvae, and the staphylinid *Paederus fuscipes* Curtis. In that study, the authors followed individual predators for 10 minutes, obtaining information on the per capita consumption for each predator, but they did not assess the impact of the entire predator guild on *A. glycines*.

In this study, I present observations assessing the relative importance of different diurnal predator species attacking *A. glycines* under un-manipulated field conditions. The specific objectives of the study were to determine: a) which species attack *A. glycines* in field conditions and their relative importance, b) their response to naturally occurring aphid densities, and c) the potential existence of refuges from predation. I considered refuges in a broad sense as locations on the soybean plant and sizes of the aphids that experienced lower predation rates.

Material and methods

The interaction of *A. glycines* and its natural enemies was observed in a soybean field located at the Entomology Research Farm of Michigan State University, East Lansing, MI. The field was planted with variety Pionner 92B38, in 37.5 cm rows, on 5/5/05, and was maintained using standard agronomic techniques. Observations were conducted on 7/8, 7/12, 7/14, 7/19 and 7/21/2005, on plants ranging from V4 to R2 stage (Ritchie et al. 1994). On each observation date, three 1 m^2 areas (hereafter patches) were established. Patches were delimited by placing quadrates made by PVC tubes on the soil, each of which encompassed two soybean rows. The three patches were observed simultaneously during 2 h periods with one observer per patch during three time slots per day: 10 – 12 AM (total = 30 h), 2 – 4 PM (30 h), and 7 – 9 PM (12 h). In each patch, all predation events on *A. glycines* and information on predator species and behavior were recorded.

Based on preliminary observations, I divided *A. glycines* predators in two groups. Transient predators (i.e. coccinellid adults, mirids, nabids), only resided in an observation patch for a short time and consumed aphids very rapidly, requiring continuous attention. By contrast, resident predators (i.e. *O. insidiosus*, spiders, and lacewing, midge and coccinellid larvae) usually stayed in the observed patch during the entire observation period and consumed aphids more slowly. By noting where these individuals were foraging, I was able to periodically check on their activity. In order to balance observations of predation events by transient and resident predators, I set three rules for observations. First, priority was assigned to follow the behavior of the predators that consumed aphids more rapidly and were the most mobile. This included, in order of priority for observation, coccinellid adults > third and fourth instars coccinellid larvae > mirids > lacewing larvae > first and second instar coccinellid larvae > *O. insidiosus* > syrphid larvae > midge larvae. Second, individual resident predators were relocated and observed at least every 20 minutes (typically much more fragmentally), interrupting observation of transient predators if necessary to ensure observation of predation events by these predators. Finally, c) observation of small predators (i.e. *O. insidiosus*, first and second instar coccinellid larvae, syrphid larvae, and midge larvae) was focused on the proximal soybean row, whereas transient predators were followed in both rows. To facilitate observation of resident predators, at the beginning of each observation period the location of each resident predator was noted on a chart containing the plants of the proximal row. Total number of small predators per m^2 (NSP / m^2) was estimated as: NSP / m^2 = NSP in focal row / number of plants in focal row x total number of plants / m^2, and the number of predation events due to small predators was similarly adjusted. Due to logistical reasons, observations during the evening were shorter (84.0 ± 17.1 minutes, mean ± SD) and thus observations were adjusted for comparison with the morning and afternoon observations.

The distribution of predation events within plants and among aphid size-classes was also characterized. Plants were divided in three parts: the upper-third ("top"), the middle-third ("middle"), and the lower-third ("bottom") and predation events were classified according to where they occurred. The size of the aphids eaten was recorded as large, medium, and small-size apterous, and alate aphids. In addition to predation events, the residence time (RT), i.e. the time spent by transient predators in the observed patch, and the consumption time (CT), i.e. the time needed by predator to consume each *A. glycines* after they located an aphid, were recorded. Not all predation events and RT were timed, and thus I restricted statistical comparisons to groups with sufficient data collected ($n \geq 9$). Predation by coccinellid adults was typically very fast, with individual aphids consumed in a few seconds. There was a trade off between recording and timing of predation events by coccinellids, and therefore to facilitate observation of predation events, I recorded CT of coccinellids only to the half minute. Consequently, CT by coccinellid adults is undoubtedly overestimated and comparisons with other groups should be interpreted with caution. Immediately after the two hour periods of observation, the number of plants per patch and *A. glycines* present on 4 – 6 random plants, were recorded. With this information I estimated the total number of aphids per m^2 for each patch.

Statistical analysis

Predator abundance (predators / m^2 / 2 h), number of aphids eaten (aphids eaten / m^2 / 2 h) and per capita consumption of aphids (aphids eaten / predator / 2h, PCCR) were analyzed with two-way ANOVAs using a factorial design (time of the day x predator group), and date of observation as a random blocking factor (PROC GLM, SAS Institute 2001). Residence time and consumption time were compared among predator groups using one-way ANOVAs considering each predation event an independent observation.

Similarly, the distribution of predation events among plant portions and on different aphid size-classes was analyzed with one-way ANOVAs. Data was log-transformed to meet the assumptions of ANOVA before analysis when necessary. Means were compared using the Least Square Mean Difference, adjusted by the Tukey method for multiple comparisons (SAS Institute 2001). The responses of predator abundance, residence time and predation on aphids to aphid density were investigated by calculating Pearson's correlation coefficient using SYSTAT 11 for Windows (SYSTAT Software Inc. 2004). The distribution of predation events by different predator groups on different plant parts and on different aphid size-classes were compared calculating the Pearson's χ^2 statistics for two-way tables (SYSTAT Software Inc. 2004).

Results

A total of 643 predators in 12 groups that attacked *A. glycines* were observed during the 72 h of observations (Table 6.1). In addition, I found two unidentified 3rd instar coccinellid larvae eating 1 aphid each, and adults of *Hippodamia convergens* Guérin-Méneville (n = 1) and *Cycloneda munda* (Say) (n = 1), syrphid larvae (n = 33), small carabid beetles (n = 11) and small staphyllinids (n = 3), but I did not observe predation by these taxa and therefore they were excluded from statistical analyses. Two predator species not previously reported feeding on *A. glycines* were observed: *Plagiognathus* spp. Fieber, and *Chlamydatus associatus* (Uhler) (Hemiptera: Miridae). Very little about the biology of these two species is known. The first genera is broadly consider phytophagous, with no previous references of predation, whereas the second

Table 6.1. Predator abundance, total aphids consumed (TC), consumption time and residence time, observed per m^2 during 2 h periods in a soybean field in Michigan.

Predator[1]		Mean ± SE Abundance (predators / m^2)								
		Morning	Afternoon	Evening	Total	TC[2]	%	CT[3]	n	Residence Time[4]
O. insidiosus	Adults	8.37 ± 1.34	8.66 ± 1.28	6.89 ± 2.13	318	63 [31]	20.7	9.7 ± 1.4 **a**	23	---
	Nymphs	5.78 ± 1.62	5.51 ± 1.60	5.47 ± 2.00	219	46 [19]	15.1	9.6 ± 1.7 **a**	13	---
H. axyridis	Adults	4.53 ± 0.89	2.00 ± 0.38	1.44 ± 0.65	111	27 [25]	8.8	0.8 ± 0.1 **d**	23	11.0 ± 1.6 **a**
	Larvae 3 and 4	1.93 ± 0.54	1.27 ± 0.36	0.33 ± 0.17	51	45 [43]	14.8	1.7 ± 0.5 **cd**	42	---
Coccinellid larvae 1 and 2		2.28 ± 0.80	2.67 ± 1.36	2.95 ± 1.37	101	40 [19]	13.2	4.2 ± 0.8 **b**	15	---
C. septempunctata	A.	0.27 ± 0.12	0.40 ± 0.19	0.22 ± 0.15	12	25 [24]	8.1	0.6 ± 0.1 **d**	24	16.9 ± 8.0 **ab**
	Larvae 3 and 4	0.40 ± 0.13	0.53 ± 0.24	0.78 ± 0.28	21	15 [13]	5.0	2.7 ± 1.1 **bc**	12	---
Midge larvae		1.04 ± 0.64	0.16 ± 0.16	0.28 ± 0.28	21	15 [8]	4.9	13.8 ± 4.0	4	---
Lacewing larvae		0.52 ± 0.28	0.57 ± 0.35	0.00 ± 0.00	16	10 [10]	3.3	3.4 ± 2.0 **b**	9	---
H. variegata	Adults	1.20 ± 0.44	1.07 ± 0.38	0.11 ± 0.11	35	9 [9]	3.0	0.5 ± 0.0	3	17.9 ± 5.8 **ab**
C. maculata	Adults	2.80 ± 0.31	1.07 ± 0.41	1.89 ± 0.77	75	3 [3]	1.0	0.5	1	5.1 ± 1.0 **b**
Spiders		2.11 ± 0.62	2.00 ± 0.67	1.78 ± 0.81	78	2 [2]	0.7	17.0	1	---
Plagiognathus spp.		0.27 ± 0.15	0.47 ± 0.19	0.00 ± 0.00	11	2 [2]	0.7	11.0 ± 5.0	1	31.0 ± 8.6 **a**
C. associatus		0.27 ± 0.12	0.07 ± 0.07	0.00 ± 0.00	5	2 [2]	0.7	---		---
Nabis spp.		0.40 ± 0.13	0.33 ± 0.21	0.50 ± 0.34	14	1 [1]	0.3	10.0	1	17.6 ± 5.6 **a**

Table 6.1 (cont'd)

[1] A. = adults, coccinellid larvae refers to all 1st and 2nd instars observed, which were not identified to species.

[2] TC = total aphids eaten / m^2 / 2h. Numbers within brackets are actual numbers observed before scaling (see methods for details).

[3] CT= consumption time; mean ± SE minutes / aphid / predator; different letters indicate significant differences ($P < 0.05$, LSMD-TK tests); n = sample sizes.

[4] Minutes; different letters indicate significant differences ($P < 0.05$, LSMD-TK tests)

species is also reported as phytophagous, but have been documented feeding on pollen and eggs as well (Schuh 2001, Schuh and Schwartz 2005).

Time of the day did not affect significantly the number of predators observed (F = 1.85; df = 2, 66; P = 0.1648), aphids eaten (F = 1.20; df = 2, 66; P = 0.3089) and per capita consumption rate (PCCR) (F = 1.18; df = 2, 39; P = 0.3191), although in general there were lower values for these parameters during the evening observations (data not shown). The number of *A. glycines* per plant tended to increase with sampling date (Figure 6.1), but there were no significant effects of date on predator abundance (F = 0.70; df = 2, 66; P = 0.5923), number of aphids eaten (F = 0.25; df = 2, 66; P = 0.9111), and PCCR (F = 1.54; df = 2, 39; P = 0.3098). Therefore, I present results averaged across observation dates and observation times (Figure 6.2).

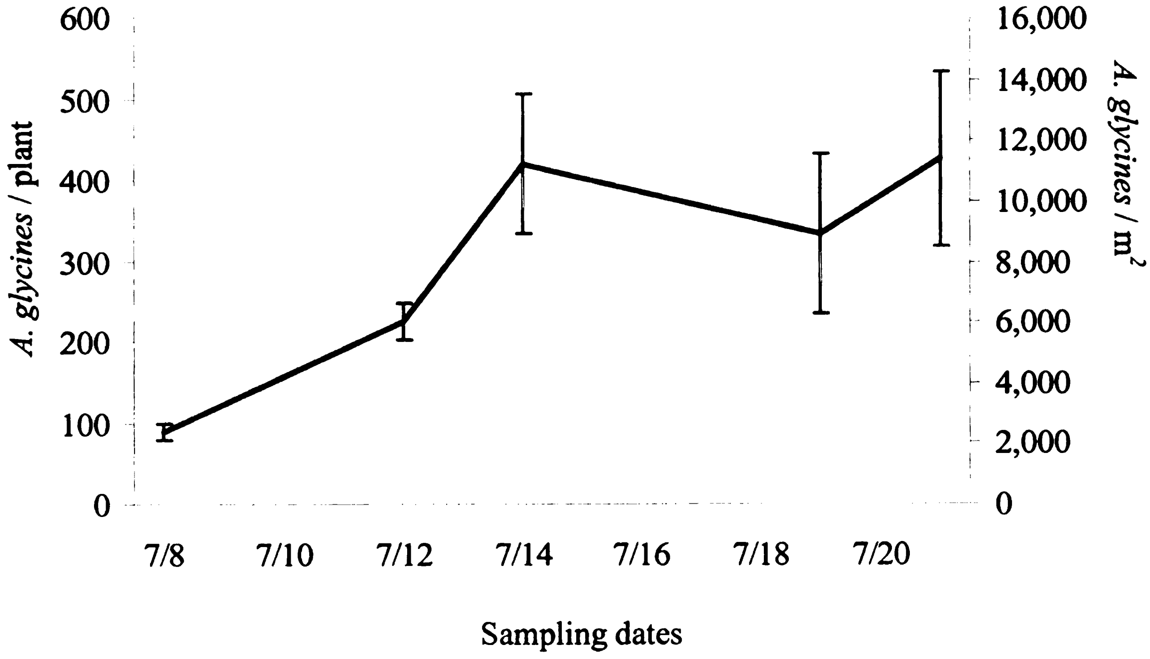


Figure 6.1. *A. glycines* abundance in the soybean field observed for predation, Entomology Farm, Michigan State University, during 2005.

The most abundant predator observed was the minute pirate bug, *O. insidiosus* (49.4% of total predators), which was present during all observation periods and was approximately three-fold more abundant than the next more abundant species (*H. axyridis*, 14.9%) ($F = 38.95$; df = 6, 66; $P < 0.0001$; Fig 6.2a). Accordingly, significantly more predation events were observed by *O. insidiosus* (35.9% of total predation events) than other predators with the exception of *H. axyridis* (23.6%) ($F = 7.67$; df = 6, 66; $P < 0.0001$; Figure 6.2b). On a per capita basis, however, the most effective predators were the coccinellids *C. septempunctata* and *H. axyridis*, followed by first and second instar coccinellids ($F = 3.96$; df = 4, 39; $P = 0.0086$; Figure 6.2c). Other coccinellids and *O. insidiosus* were the least effective, eating between half to a fifth of the number of aphids than the most voracious coccinellids (Figure 6.2c). The low frequency of predation events observed for midge and lacewing larvae (n = 6, for each one) prevented statistical analysis.

The time required to consume *A. glycines* varied significantly among predators ($F = 39.16$; df = 7, 153; $P < 0.0001$; Table 6.1). Both adult and nymphal *O. insidiosus* required on average almost 10 minutes to consume an individual *A. glycines*, and a similar result was obtained for other hemipterans and midge larvae. For coccinellids, adults of *H. axyridis*, *C. septempunctata*, and *Hippodamia variegata* (Goeze) consumed aphids in less than 1 min (mean between 0.5 – 0.8 min), while large larvae (third and fourth instars) required 1.7 to 2.7 min, and small larvae (first to second instars) 4.2 min (Table 6.1). Lacewing larvae required similar consumption times as coccinellid larvae (Table 6.1). This result suggests there was a higher chance of missing predation events from coccinellids (particularly adults), than by *O. insidiosus* and other resident predators.

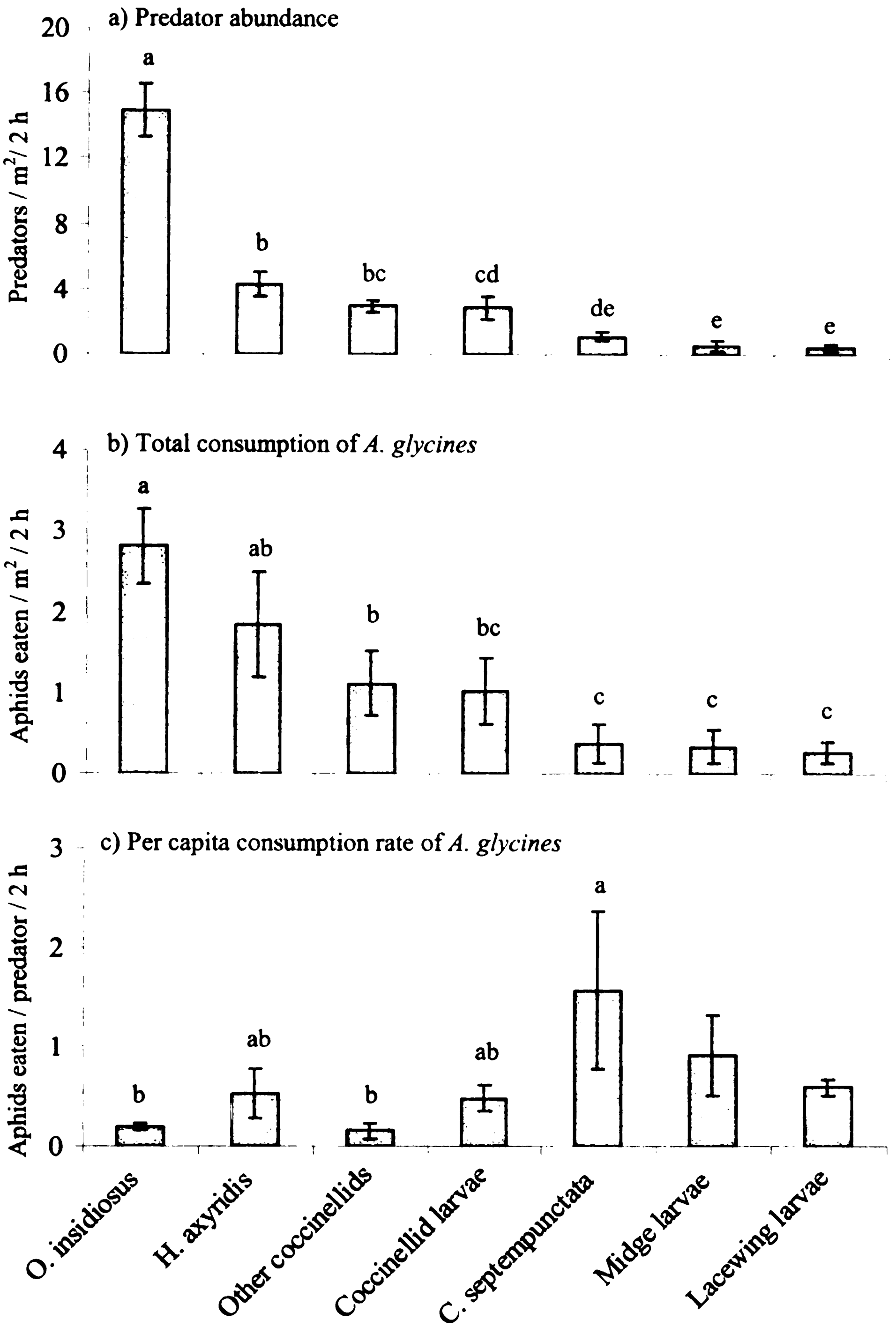


Figure 6.2. Mean ± SE, a) predator abundance, and b) total, and c) per capita rate of consumption of *A. glycines* by the most abundant predator groups observed during 2 h periods in a soybean field in Michigan, USA. *H. axyridis* and *C. septempunctata* combine adults and 3rd and 4th instar larvae, Coccinellid larvae includes all the 1st and 2nd instar larvae, since they were not identify to species. Different letters indicate significant difference between predator groups (LSMD-TK, $P < 0.05$). Per capita rates of consumption of midge and lacewing larvae were not included in the statistical comparisons due to their low frequency (15% of cases).

However, since the sampling scheme was accommodated to follow more closely coccinellids, and high density clusters of aphids were not observed, I feel this potential bias was minimized. The residence time of transient predators in the patch also differed significantly ($F = 6.35$; df = 5, 213; $P < 0.0001$; Table 6.1). While abundant, *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) spent significantly less time in each plot (mean = 5.1 min) compared to other coccinellids, nabids and *Plagiognathus* spp., and had a very low consumption of *A. glycines* (Table 6.1).

Observations were conducted over a range of naturally occurring aphid densities from 1,290 to 24,678 aphids / m^2 (48 – 784 aphids / plant; Figure 6.1), and thus I explored the response of predators to aphid density within that range. I did not observe a significant correlation between the abundance of all predators combined and aphid density (r = 0.001, P = 0.996). However, when predators were classified according to their vagility, there was a significant correlation between transient predator abundance and aphid density (r = 0.479, $P = 0.002$), whereas a marginally significant but opposite trend in the response of resident predators to aphid density was observed (r = -0.281, $P = 0.083$; Figure 6.3a). When predator groups were considered separately there were significant positive associations between aphid density and the abundance of *H. axyiridis* adults, and negative associations of immature *C. septempunctata* and *O. insidiosus* (Figure 6.4).

By contrast, there was a significant positive correlation of aphid density and predation by all predators combined (r = 0.338, P = 0.036), although this response was largely due to predation by transient predators (r = 0.514, P = 0.001), since resident predators did not show a significant correlation with aphid abundance (r = - 0.144, P =

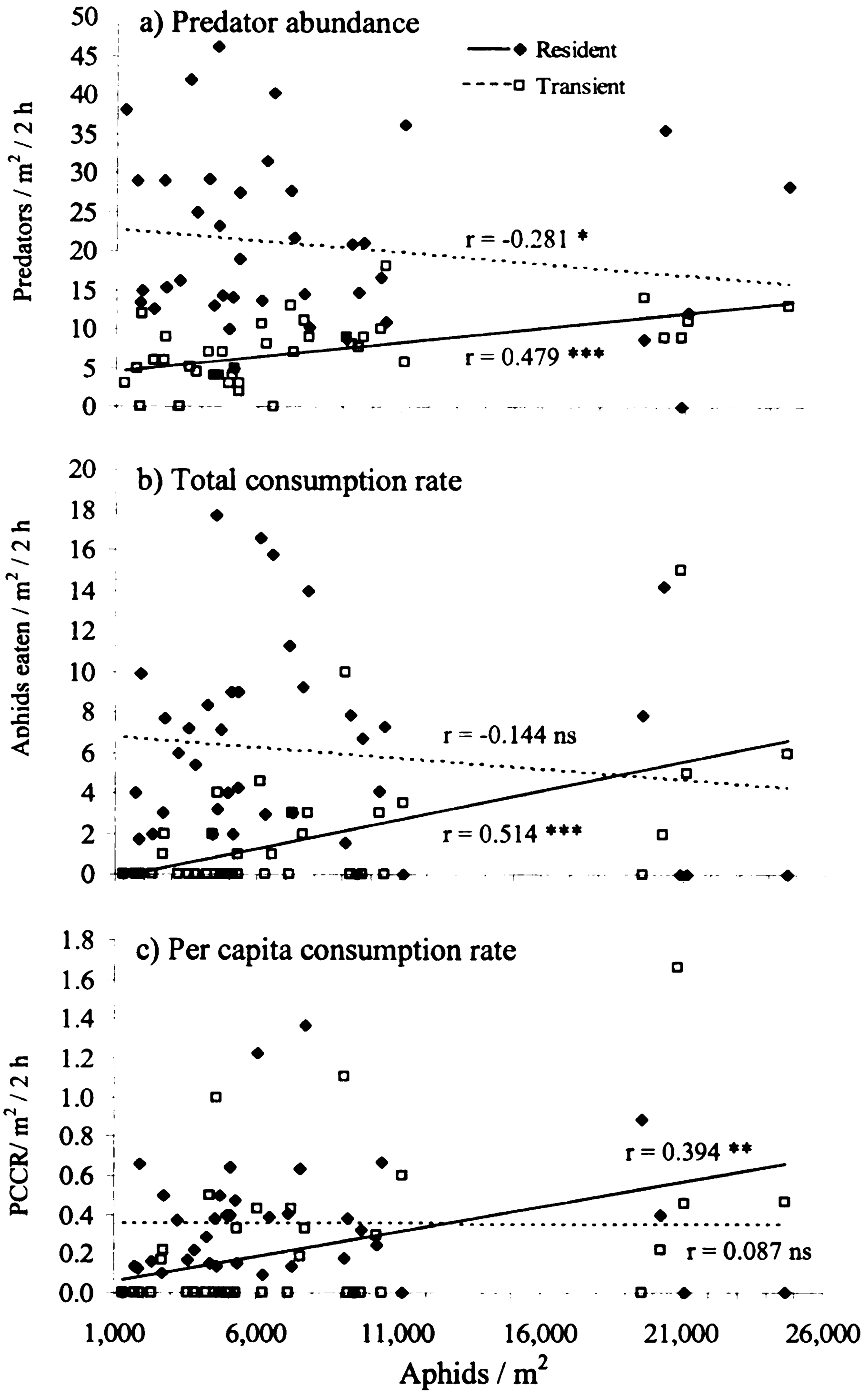


Figure 6.3. Responses of resident (closed symbols) and transient predators (open symbols) to aphid density: a) predator abundance, b) total consumption rate, and c) per capita consumption rate. Resident predators included *O. insidiosus*, midge, lacewing, and coccinellid larvae. Transient predators included coccinellids adults, *Nabis* spp., *Plagiognathus* spp., and *C. associatus*. Lines indicate linear trends, r is Pearson's correlation coefficient for the closest line, * indicates $P < 0.10$, ** indicates $P < 0.05$, *** indicates $P < 0.01$, ns = not significant.

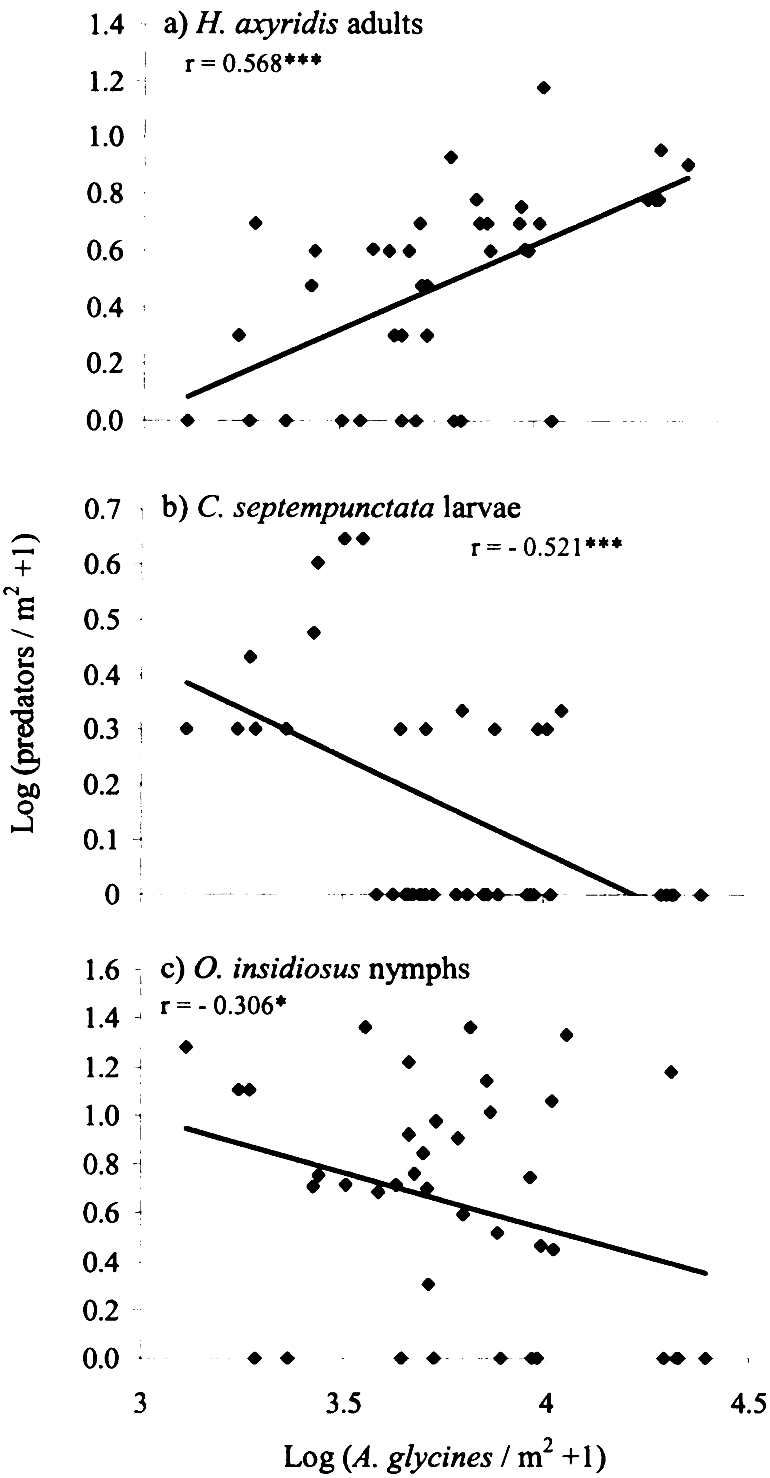


Figure 6.4. Correlations between aphid abundance and the abundance of a) *H. axyridis* adults, b) *C. septempunctata* larvae, and c) *O. insidiosus* nymphs. Other predator groups did not show significant associations. Lines indicate linear trends, r is Pearson's correlation coefficient, * indicates P < 0.10, ** indicates P < 0.05, *** indicates P < 0.01, ns = not significant.

0.383; Figure 6.3b). Separate analysis for each predator group revealed significant positive correlations with aphid density for adults of *H. axyridis* and *C. septempunctata*, and negative correlations for *C. septempunctata* larvae, with the rest of the predator groups not showing significant responses (Figure 6.5). There was a significant correlation between aphid abundance and PCCR ($r = 0.310$; $P = 0.055$), but again this positive correlation was due to the response of transient predators ($r = 0.394$ $P = 0.017$), since resident predators did not respond significantly to aphid increase ($r = 0.087$; $P = 0.602$; Figure 6.3c). At the species level, only *C. septempunctata* adults showed a marginally significantly positive correlation between PCCR and aphid abundance ($r = 0.625$, $P = 0.054$; Figure 6.6). Lastly, I assessed the potential relationship between residence time in the patch and aphid density for the adults of five transient predator groups in which there was at least 10 observations (*C. septempunctata* [n =12], *H. axyirids* [n = 95], *Plagiognathus* spp. [n =10], *C. maculata* [n =72], and *H. variegata* [n =23]). Only *C. septempunctata* adults exhibited a positive response to aphid abundance (Figure 6.7). None of the other predator groups showed a change in their residence time in the patch associated with aphid density.

Finally, I compared predation impacts on different parts of the plant and on different aphid stages. Significantly more aphids were killed in the upper-third of the plants than in the bottom-third when predation by all predators was combined ($F = 8.38$, df = 2, 114; $P = 0.0004$; Table 6.2). When considered separately, different predator groups varied in their impact on aphids at different parts of the plant (Pearson's correlation $\chi^2 = 54.9$, df = 18, $P < 0.001$), with *H. axyridis* larvae and *O. insidiosus* nymphs having proportionally higher predation rates in both the upper and lower thirds of

Table 6.2. Percent predation of *A. glycines* by different predators on different plant parts and aphid sizes, recorded by direct observation of 211 predation events in a soybean field in Michigan during 2005.

		Plant Location (%)[1]			Aphid size (%)[2]				
		Bottom	Middle	Top	Alate	Large	Medium	Small	Total
O. insidiosus	Adults	1.1	3.7	**11.6**	0.0	**5.9**	**7.5**	3.2	31
	Nymphs	3.7	1.1	**5.3**	0.5	3.2	3.2	3.2	19
H. axyridis	Adults	1.6	2.1	**8.9**	3.2	2.1	4.8	2.7	24
	Larvae 3 and 4	**5.8**	**10.5**	**6.8**	1.6	2.7	**9.6**	**9.6**	44
Coccinellid larvae 1 and 2		1.1	3.2	4.2	0.0	2.1	1.6	4.8	16
C. septempunctata	Adults	0.0	1.1	**10.0**	0.5	1.6	**8.6**	2.1	21
	Larvae 3 and 4	1.6	1.1	4.2	1.1	2.1	2.7	1.1	13
Midge larvae		0.0	2.6	0.5	0.0	0.5	0.5	2.1	6
Lacewing larvae		0.0	3.2	2.1	0.5	1.6	1.6	1.6	10
H. variegata	Adults	0.0	0.5	2.6	---	---	---	---	6
mean[3]		0.74 **B**	1.59 **AB**	2.92 A	0.46 **b**	1.21 **ab**	2.18 **a**	1.49 **ab**	
SE		0.22	0.32	0.54	0.12	0.24	0.44	0.38	

[1] Percent of predation events observed on the different plant portions. Percentages higher than 5% are indicated in bold.

[2] Percent of predation events observed for different aphid body sizes. Percentages higher than 5% are indicated in bold.

[3] Means of number of aphids eaten / m^2 / 2 h by all predators combined. Different capital letters indicate significant differences in the number of predation events among plant parts, whereas different lower-case letters indicate differences among aphid sizes ($P < 0.05$, LSMD-TK tests).

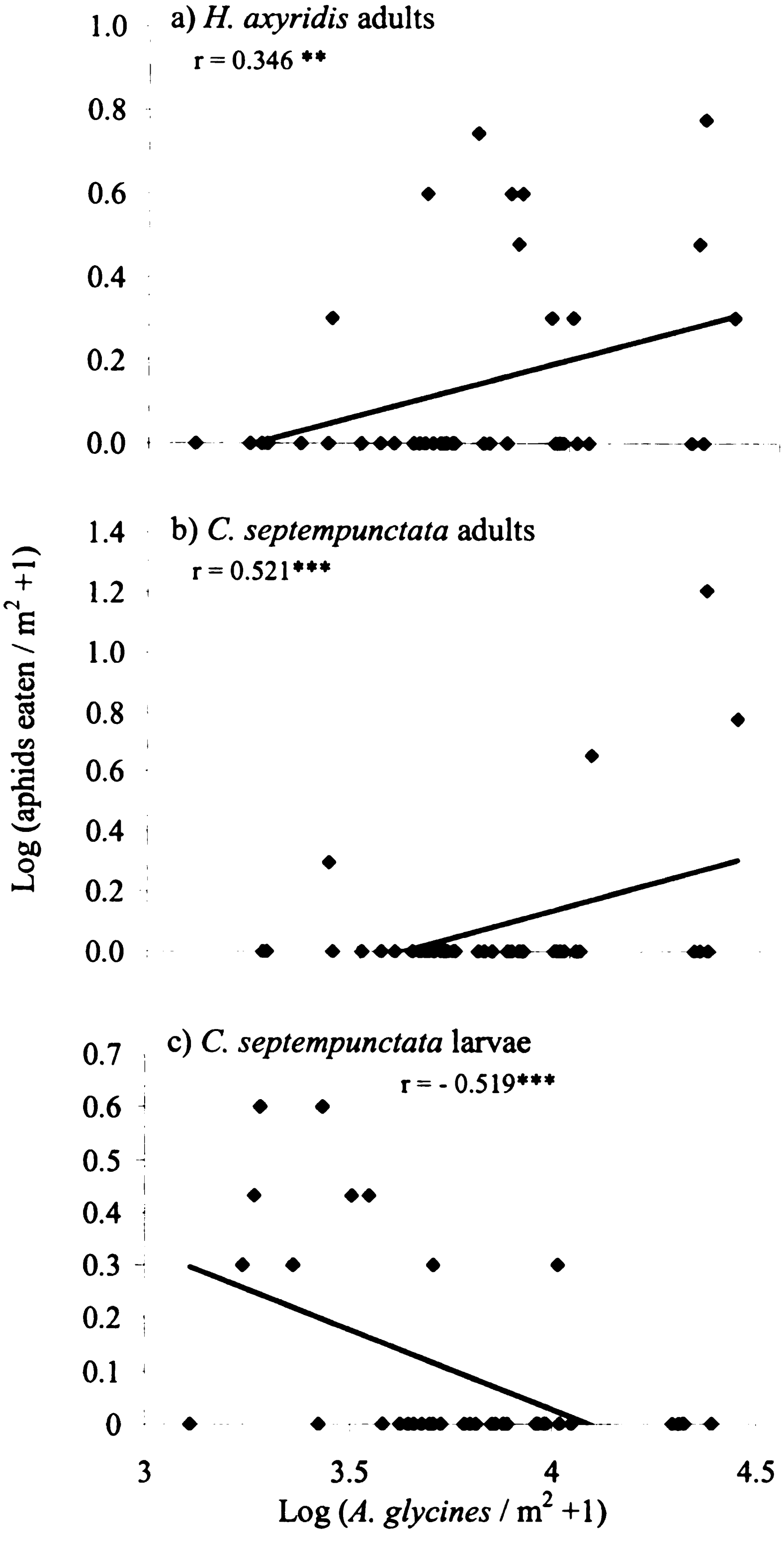


Figure 6.5. Correlations between aphid abundance and total aphids eaten by a) *H. axyridis* adults, b) *C. septempunctata* adults, and c) *C. septempunctata* larvae. Other predator groups did not show significant associations. Lines indicate linear trends, r is Pearson's correlation coefficient, * indicates $P < 0.10$, ** indicates $P < 0.05$, *** indicates $P < 0.01$.

the plants, whereas the rest of the predators concentrate their attacks on the upper and middle portions of the plants (Table 6.2). Predation also differed significantly among different aphid sizes ($F = 6.68$, $df = 3, 152$; $P = 0.0003$; Table 6.2), with alates being preyed less on average than apterous adults and nymphs. The response of the different predator groups to the different aphid size-classes also differed significantly (Pearson's correlation $\chi^2 = 43.1$, $df = 24$, $P = 0.01$), with *H. axyridis* adults consuming proportionally more alates and their larvae proportionally more small aphids than the rest of the predators, that concentrate their attacks on medium and large size aphids (Table 6.2)

Discussion

Coccinellids were the most important predators of *A. glycines* in this study. Altogether, *H. axyridis*, *C. septempunctata*, first and second unidentified coccinellid larvae, and adult coccinellids of other species, accounted for 37.4% of all predators, and 53.7% of predation events on *A. glycines*. In absolute terms, *H. axyridis* eat proportionally more aphids (23.6%), than *C. septempunctata* (13.0%), but the former was more abundant, and thus on a *per capita* basis there was a trend of more aphids eaten by *C. septempunctata* (Figure 6.2c). Therefore, these results confirm previous reports of field (Fox et al. 2004, Fox et al. 2005, Costamagna and Landis 2006, Costamagna et al. unpublished results) and laboratory experiments (Rutledge et al. 2004), that suggested these two species as the most important predators of *A. glycines* in North America. The second most abundant coccinellid, *C. maculata*, was very mobile, spending between a half to a third of the time per patch in comparison with other coccinellids, and its impact

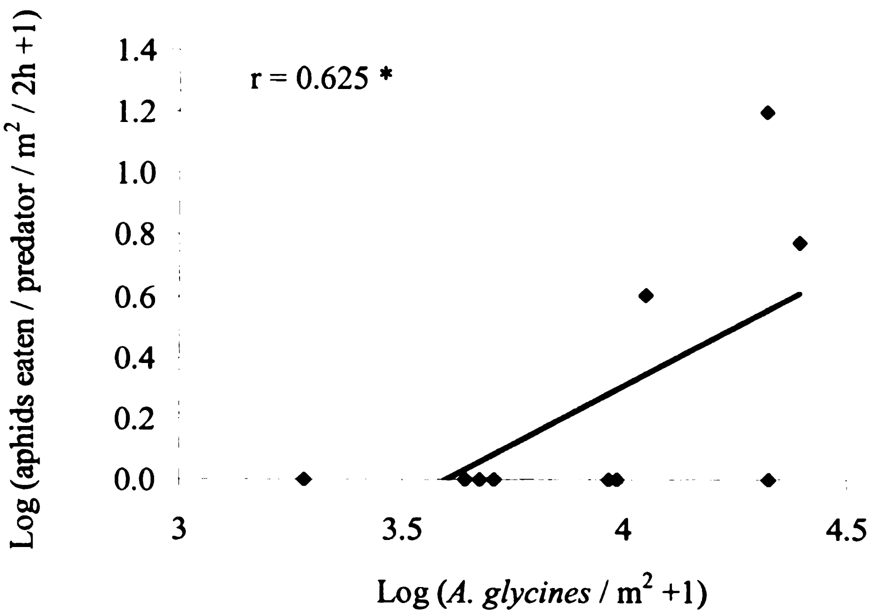


Figure 6.6. Correlation between aphid abundance and per capita consumption of *C. septempunctata* adults. Other predator groups did not show significant associations. Line indicates the linear trend, r is Pearson's correlation coefficient, * indicates $P < 0.10$.

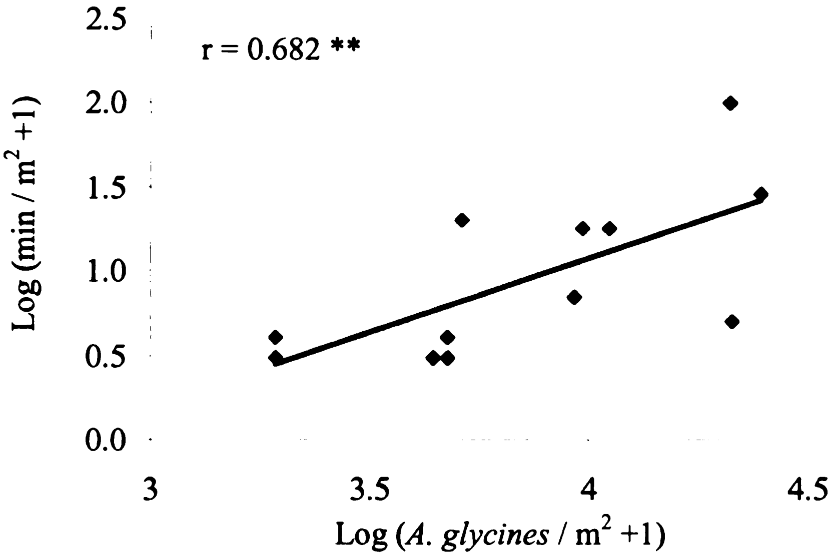


Figure 6.7. Significant correlations between aphid abundance and residence time of *C. septempunctata* adults. Other predator groups did not show significant associations. Line indicates the linear trend, r is Pearson's correlation coefficient, ** indicates $P < 0.05$.

on *A. glycines* was minor in absolute terms (1.4% of predation events) and on a *per capita* basis (Figure 6.2c, combined with other coccinellids). This result is in agreement with previous findings of low predation by *C. maculata* on *A. glycines* obtained confining adults in Petri dishes (Rutledge et al. 2004).

The role of *O. insidiosus* in *A. glycines* suppression is less clear. Manipulative laboratory experiments and field studies correlating the abundance of *O. insidiosus* and *A. glycines* suggest a potentially important role of this predator suppressing aphid increase (Rutledge et al. 2004, Rutledge and O'Neil 2005). However, field manipulations using cages that differentially excluded coccinellids but not *O. insidiosus* resulted in exponential increase of *A. glycines* to similar levels as populations in which all predators were excluded, indicating that aphid suppression observed in open plots and sham cages were mainly due to predation by coccinellids (Costamagna et al., unpublished results). The difficulty of completely excluding *O. insidiosus* in field experiments makes difficult to establish the separate contribution of this species to *A. glycines* suppression, and whether it has additive effects with coccinellids (Costamagna et al., unpublished results). In the present study there was only a marginally significant correlation between the abundance of *A. glycines* and *O. insidiosus*, and no correlation between *A. glycines* abundance and total and per capita aphid consumption by *O. insidiosus*, suggesting a limited role for this species in controlling *A. glycines*. However, my studies were conducted at a relatively high density of *A. glycines* per plant, and I can not rule out a more important role of *O. insidiosus* at lower aphid densities and on younger and smaller plants (Rutledge and O'Neil 2005).

I did not detect differences in the mortality of aphids relative to the size of apterous adults and nymphs, although lower predation was observed on alate aphids. This could be due to the proportionally lower number of alates observed (mean ± SD, 1.9

± 2.2% of total aphids). Selective mortality on larger-size *A. glycines* was demonstrated for the parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) and resulted in stronger aphid suppression than mortality factors concentrated on smaller aphids (Lin and Ives 2003). In my study there were no significant differences in the distribution of predation events according to the size of nymphs and apterous *A. glycines* adults. However, I did not establish the proportion of each aphid size in the population, so I could not assess the relative impact of predation on each size category in apterous individuals.

Predation was more intense on the upper third of the plant, and diminished in intensity towards the bottom. Since the within-plant distribution of aphids was not surveyed, it is not possible to assess whether this was due to higher densities of aphids at the upper third of the plant or more intense searching of predators in that portion of the plant. However, in a separate experiment (Chapter 5), I observed proportionally more aphids on the lower part of plants when predators were present versus when they were excluded. Together these results suggest that the lower portion of the soybean plant may constitute a partial refuge from predation for *A. glycines*.

Resident and transient predators showed different patterns of interactions with *A. glycines*, which resulted in different predator impacts. When combined, resident predators did not change their abundance and total and per capita consumption rates, in response to increased aphid abundance. However, individual responses of *C. septempunctata* larvae and *O. insidiosus* nymphs showed a significant negative correlation with aphid density. Since patch immigration/emigration by these resident predators was negligible, these results indicate that lower aphid populations could be the result of consumption by these two predators or predator avoidance by the aphids. By

contrast, transient predators such as *H. axyridis* and *C. septempunctata* adults significantly aggregated, stayed longer within the patch, and increased total and per capita consumption of aphids in function of aphid abundance. Similar responses of increased abundance and residence time were obtained for adult *C. septempunctata* and *H. variegata* to varying densities of fireweed aphis at different scales (Ives et al. 1993). Several studies have demonstrated the response of different coccinellid species to aphid cues that result in the orientation towards aphid clusters (Dixon 2000 and references therein). In addition, *C. septempunctata* is attracted to *A. glycines*-induced volatiles produced by soybeans (Zhu and Park 2005), supporting the pattern that I observed in the field. These observations suggest that resident predators provide a basal mortality level that does not react rapidly to changes in aphid abundance, at least in the scales measured in this study. To the contrary, transient predators mainly consisting of adult coccinellids, spent relatively little amount of time per patch (on average, less than 18 minutes of the 120 observed), but provide strong aphid suppression (Chapters 1 - 5) coupled with positive responses to aphid increase.

In summary, my observations indicate that the coccinellids *H. axyridis* and *C. septempunctata* are the major consumers of *A. glycines*. Moreover, transient predators in general and adult coccinellids in particular, have the ability to respond to changes in aphid density by increasing their abundance, and total and per capita feeding. By contrast, resident predators in general and *O. insidiosus* in particular, contribute to *A. glycines* mortality due to their abundance, but they do not respond significantly to changes in aphid abundance. Predation is not equally distributed among plant portions and aphid size classes. This study is the first one to provide a direct assessment of the importance of different predator species attacking *A. glycines* in North America, using un-manipulated field populations. More studies focusing on the interaction of *A. glycines* and its predators under field conditions, including different times of the season and observation of nocturnal predators, are needed to completely assess the role of the different predator species in the regulation of this pest.

CHAPTER 7

PREDICTIVE MODEL FOR THE GROWTH OF FIELD POPULATIONS OF *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) IN SOYBEAN

Abstract

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) is an invasive pest that was detected in North America in 2000, and rapidly became the most important insect pest of this crop in the American Midwest. Strong suppression by natural enemies occurs in some years, but *A. glycines* populations escape natural suppression in other years. The potential causes of this escape are poorly understood. Here I present a mathematical model parameterized using data from four field experiments in which *A. glycines* populations grew in predator exclusion cages in two locations in Michigan, USA, between 2003 and 2005. These data were fit an exponential growth model in which the intrinsic rate of increase (r) decreases linearly with time. This model, in contrast to the traditional exponential and logistic growth models, accurately predicts *A. glycines* population dynamics in soybeans, including the population decline towards the end of the season. The model is robust against variability within and between experiments and a generalized model combined the data of the four experiments. This generalized model has better explanatory power ($R^2 = 0.95$) when is fitted on an *A. glycines* degree-days scale, suggesting the important role of temperature in aphid population dynamics. Potential causes of r declining with time are discussed. This model provides a simple and accurate tool to model *A. glycines* populations and explore the effects of natural enemies and different control measures to develop management strategies for this pest.

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is an invasive pest originally from Asia that was observed for the first time in North America in 2000 (Ragsdale et al. 2004, Venette and Ragsdale 2004). Since its detection, *A. glycines* has expanded its distribution throughout the soybean producing region in North America, including both the United States and Canada, and became the most important insect pest of this crop (Venette and Ragsdale 2004, Mignault et al. 2006). Yield losses due to *A. glycines* ranging from 50 – 70% have been reported in Asia (Wu et al. 2004) and up to 40% in USA (DiFonzo and Hines 2002). Yield reduction is mostly caused by direct feeding of outbreak populations, but even small populations may cause yield losses due to reducing the photosynthetic rates of plants (Macedo et al. 2003). Indirect damage due to *A. glycines* includes facilitating the development of sooty mold and virus transmission, although severe viral outbreaks have not yet been reported in North America (Hill et al. 2001, Clark and Perry 2002, Wang and Ghabrial 2002, Wu et al. 2004). Strong impacts of natural enemies on *A. glycines* have been demonstrated in Asia (Van den Berg et al. 1997, Liu et al. 2004, Wu et al. 2004) and in North America (Fox and Landis 2003, Fox et al. 2004, Fox et al. 2005, Nielsen and Hajek 2005, Costamagna and Landis 2006). However, despite substantial suppression by natural enemies, during some years *A. glycines* develops outbreak populations, and the causes of this escape remain unclear (Costamagna and Landis 2006). Thus, accurate mathematical models to describe *A. glycines* population dynamics may provide an important tool to manage this pest and to help elucidate potential causes for population outbreaks.

Besides predation, aphid population dynamics are influenced by several other factors under field conditions, including temperature, and host plant quality (Dixon 1987, 1998). Temperature effects on aphids are well documented affecting growth, developmental and respiration rates, and honeydew production (Dixon 1998). At the population level, these effects result in changes in the intrinsic rate of increase that is expressed (Dixon 1998). In general, aphids increase their individual growth rates from a basal temperature until an optimum and then growth rates decline until a maximum tolerable temperature (Dixon 1998). In the case of *A. glycines*, temperature effects have been characterized under the range of summer temperatures, with a basal temperature of 8.6 °C, optimum around 20 - 25 °C, and a maximum threshold of 34.9 °C (McCornack et al. 2004).

Several bottom-up factors influencing host plant quality have been shown to affect aphids populations, including fertilization (Cisneros and Godfrey 2001, Morales et al. 2001, Nevo and Coll 2001), tillage regime (Andersen 2003, Hesler and Berg 2003, Gencsoylu and Yalcin 2004), and cover crops (Tillman et al. 2004). Manipulating potential bottom-up factors resulting from different regimes of tillage, fertilization and weed control, did not affect population growth of *A. glycines* in field experiments conducted in Michigan (Costamagna and Landis 2006), however, deficiencies in soil potassium have been shown to increase *A. glycines* populations in Wisconsin (Myers et al. 2005b). Finally, plant phenology has been shown as an important factor affecting aphid population growth (Rossing et al. 1994, Williams et al. 1999). In the *A. glycines* system, Van den Berg et al. (1997) demonstrated a linear decrease of the intrinsic rate of increase on aphids reared at three different soybean phenological stages under field conditions in Indonesia.

The objective of this work was to develop an analytical model to accurately describe *A. glycines* population growth under field conditions in the absence of predation. The ultimate goal of this study is to develop a mathematical model that may serve as a tool to explore the consequences of different levels of predation, control measures, use of resistant varieties and abiotic factors in *A. glycines* management. I utilized data from four field experiments in which *A. glycines* was reared in predator exclusion cages to determine parameters of population growth. Previous evidence suggest that the intrinsic rate of increase (r) declines with time due to host plant quality on other aphid species (Rossing et al. 1994, Williams et al. 1999) as well as on *A. glycines* (Van den Berg et al. 1997). Thus, I incorporated a linearly decreasing r into an exponential growth model (further referred to as the "decreasing r model") and compared this model with the traditional exponential and logistic growth models, to test which one best describes the field data. Then data from all experiments were combined to create a more general predictive model and its accuracy was further improved by fitting two additional models accounting for temperature effects (aphid degree-days since initial infestation) and plant phenology effects (soybean degree-days since sowing).

Materials and methods

Study sites and field experiments

I utilized data obtained in four field experiments in which 48 *A. glycines* populations were reared in predator exclusion cages. All field experiments were conducted at one of two locations in Michigan (USA): the Entomology Farm or the biodiversity study site of the Kellogg Biological Station Long Term Ecological Research site in row crop agriculture (KBS-LTER) of Michigan State University, between 2003 and 2005. The details of the experimental designs are summarized in Table 7.1, and are fully described in the previous Chapters. The experiments were conducted by caging aphids naturally infesting plants, or by placing controlled numbers of aphids reared in the laboratory on soybean plants using a camel-hair brush (Table 7.1). Planting date varied among experiments, resulting in different plant phenology at equivalent dates. All aphid inoculations were performed between the V2 – V4 stage of plant phenology (Ritchie et al. 1994).

Two cage designs were utilized, sleeve cages that enclosed aphids on one to three plants, and larger cages that enclosed a 1 m^2 area (29.1 ± 2.0 plants / m^2, mean ± SD). Sleeve cages consisted of a cylindrical frame of wire (0.4 m of diameter by 1.0 m of height), that was completely covered by fine-mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA) and that was buried 15 cm in the soil. The larger field cages consisted of a PVC frame (1 x 1 x 1 m) covered by the same material on the upper portion and with a basal plastic barrier (10 cm buried in the soil, 20 cm above soil surface), connected to the netting by Velcro. Two bands of mesh with coarse openings (2 mm) were included around the sides of the larger cages, one located at the top (10 cm wide) and the other at the canopy level (30 cm wide). These bands of coarse mesh were required to allow alate aphids to escape, but also allowed some small predators to enter (primarily *Orius insidiosus* (Say) [Heteroptera: Anthocoridae]). My previous experiments have shown a very limited impact of *O. insidiosus* on *A. glycines* population

Table 7.1. Characteristics of four experiments studying *A. glycines* population dynamics under field conditions in predator exclusion cages. For a detailed description of each experiment see the methods section and Costamagna 2006).

						A. glycines inoculation				
Experiment	Year	Cage	n[1]	Location[2]	Planting Date	Source	Date	Rate[3]	Plant Phenology	Mean Temp.[4]
1	2003	Sleeve	18	Entomol. Farm	May 22	Field	July 3	4.6	V3 - V4	22.4 ± 0.3 a
2	2004	$1\ m^2$	6	Entomol. Farm	May 7	Colony	June 16	3.4	V3	18.8 ± 0.6 c
3	2004	Sleeve	12	KBS-LTER	June 7	Colony	July 1	5	V2	20.6 ± 0.5 b
4	2005	Sleeve	12	KBS-LTER	May 25	Colony	June 23	5	V3 - V4	23.1 ± 0.6 a

[1] Number of cages per experiment, in exp. 1 reduced to 6 cages for the last two sampling dates.

[2] Entomol. Farm: Entomology Farm, and KBS-LTER: Kellogg Biological Station – Long Term Ecological Research site in row crop agriculture, at Michigan State University, MI, USA.

[3] Number of *A. glycines* / plant.

[4] Mean ± SE temperature for the first 28 days after infestation, different letters indicate significant differences among experiments ($P < 0.05$, Least Square Means Difference adjusted by the Tukey-Kramer method for multiple comparisons)

growth in these cages, however, my results should be consider a conservative measure of aphid increase. Sampling was performed weekly by counting the total number of aphids on all the enclosed plants in the sleeve cages, and on 4 to 10 random plants, diminishing sampling size as the population increased, on the larger cages (Costamagna and Landis 2006). All counts are expressed as number of *A. glycines* per plant.

Models of A. glycines growth

The *A. glycines* population growth curves obtained in the four experiments were fitted separately to exponential, logistic and a decreasing r models of population growth. Exponential growth was modeled using the equation:

$$N_t = N_0 e^{r \cdot t} \qquad \text{(Eq. 1)}$$

where t is time (days), N_t is the aphid population size at time t (aphids / plant), N_0 is the initial aphid population (aphids / plant), and r is the intrinsic rate of increase (aphids / aphid / day).

Logistic growth was modeled using:

$$N_t = \frac{K}{1 + \frac{K - N_0}{N_0} e^{-rt}} \qquad \text{(Eq. 2)}$$

where K is the carrying capacity (aphids / plant). Finally, *A. glycines* growth was modeled assuming a linear decrease of r using the equation presented in Williams et al.(1999):

$$N_t = N_0 e^{\int r dt} \qquad \text{(Eq. 3)}$$

with

$$\int r dt = r_{\max} t \left(1 - \frac{1}{2} at \right) \qquad \text{(Eq. 4)}$$

By substitution of Eq. (4) in Eq. (3) we obtain:

$$N_t = N_0 \cdot e^{r_{\max} t \left(1 - \frac{1}{2} a t\right)} \quad \text{(Eq. 5)}$$

where r_{max} is the maximum intrinsic rate of increase (aphids / aphid /day), and a is the reciprocal of the time to peak population (1 / days) . Thus, this model has an r that decreases in time, starting at a maximum r_{max} when $t = 0$ and decreasing linearly until $r = 0$, when $t = 1 / a$ and the population is at its peak, and after that the population declines (Williams et al. 1999). Finally, I calculated the intrinsic rate of increase r between sampling intervals using:

$$r = \frac{\ln(N_{t+\Delta t}) - \ln(N_{t-\Delta t})}{2\Delta t} \quad \text{(Eq. 6)}$$

Statistical analysis

Fitting of the three models was performed by nonlinear regression on log-transformed data and parameter values ± SE and R^2 are reported for each model (Proc NLIN, SAS Institute 2001). Potential within-experiment variability that may result in discrepancies with the linear decreasing r model, was assessed by fitting models for the individual aphid populations followed in each experiment (i.e. considering each cage a separate aphid population). Differences in mean temperature among experiments were assessed for the first 28 days after infestation, which was the duration of the shortest experiment, using ANOVA with day as a blocking factor (Proc MIXED, SAS Institute 2001). I fit the decreasing r model to the combined data of all experiments, and tested whether inclusion of aphid physiological or plant phenological scales resulted in a higher R^2. I expect that when population dynamics of *A. glycines* are sensitive to changes in temperature, a better fit will be obtained using a degree-day scale based on aphid growth (basal temperature = 8.6 °C, McCornack et al. 2004) rather than time since inoculation (calendar time). Similarly, if plant phenology were the most important factor driving the population dynamics of *A. glycines*, the model will provide the best fit when scaled on a scale of degree-days since sowing, based on soybean phenology (basal temperature = 10 °C, Zhang et al. 2001). In addition, linear regressions of the intrinsic rate of increase *r* with calendar time, aphid degree-days and soybean degree-days were performed to check which scale provides the best fit (Proc REG, SAS Institute 2001).

Results

The decreasing r model was the only model that accurately represented population dynamics of *A. glycines* in all four experiments ($R^2 > 0.94$; Figure 7.1, Table 7.2). Exponential and logistic models produced very good fits for experiments 1 and 2 ($R^2 > 0.98$, Fig 7.1a and b, Table 7.2), which were conducted before aphids reached peak populations, but there was a clear lack of fit for experiments 3 and 4 when the number of aphids decreased after having reached their peak ($R^2 = 0.42 – 0.90$, Figure 7.1a and b, Table 7.2). In experiments 3 and 4, exponential and logistic models underestimated *r* to account for the latter decrease in aphid population, resulting in low to intermediate *r* values in comparison with values of r_{max} estimated by the decreasing *r* model (Table 7.2). Thus, the decreasing *r* model provides a complete description of *A. glycines* population dynamics on soybeans during the whole season.

To test the robustness of the decreasing r model against variability within experiments, I fitted separate models for each aphid population within each of the four experiments. All the models (n = 36 aphid populations) were significant ($P < 0.01$) and

Table 7.2. Parameters (mean ± SE) obtained by fitting three population growth models to mean population abundances of *A. glycines* in four field experiments in Michigan, USA (for details on the parameters see the methods section)

	Experiments			
	1	2	3	4
Exponential				
r	0.273 ± 0.204	0.215 ± 0.012	0.094 ± 0.033	0.113 ± 0.050
log (N_0)	0.939 ± 0.424	0.614 ± 0.123	1.832 ± 0.546	1.806 ± 0.687
R^2	0.981	0.985	0.505	0.418
P	0.001	< 0.001	0.021	0.060
Logistic				
r	0.315 ± 0.188	0.236 ± 0.016	0.294 ± 0.069	0.341 ± 0.138
log (N_0)	0.828 ± 0.233	0.531 ± 0.114	0.650 ± 0.375	0.737 ± 0.620
log (K)	4.189 ± 3.122	4.368 ± 0.284	3.990 ± 0.203	3.992 ± 0.379
R^2	0.993	0.991	0.902	0.774
P	0.002	< 0.001	< 0.001	< 0.001
Decreasing r				
r_{max}	0.395 ± 0.282	0.252 ± 0.045	0.398 ± 0.023	0.560 ± 0.063
log (N_0)	0.753 ± 0.464	0.532 ± 0.159	0.518 ± 0.148	0.421 ± 0.298
a	0.395 ± 0.282	0.252 ± 0.045	0.398 ± 0.023	0.560 ± 0.063
R^2	0.999	0.987	0.982	0.943
P	0.002	< 0.001	< 0.001	< 0.001

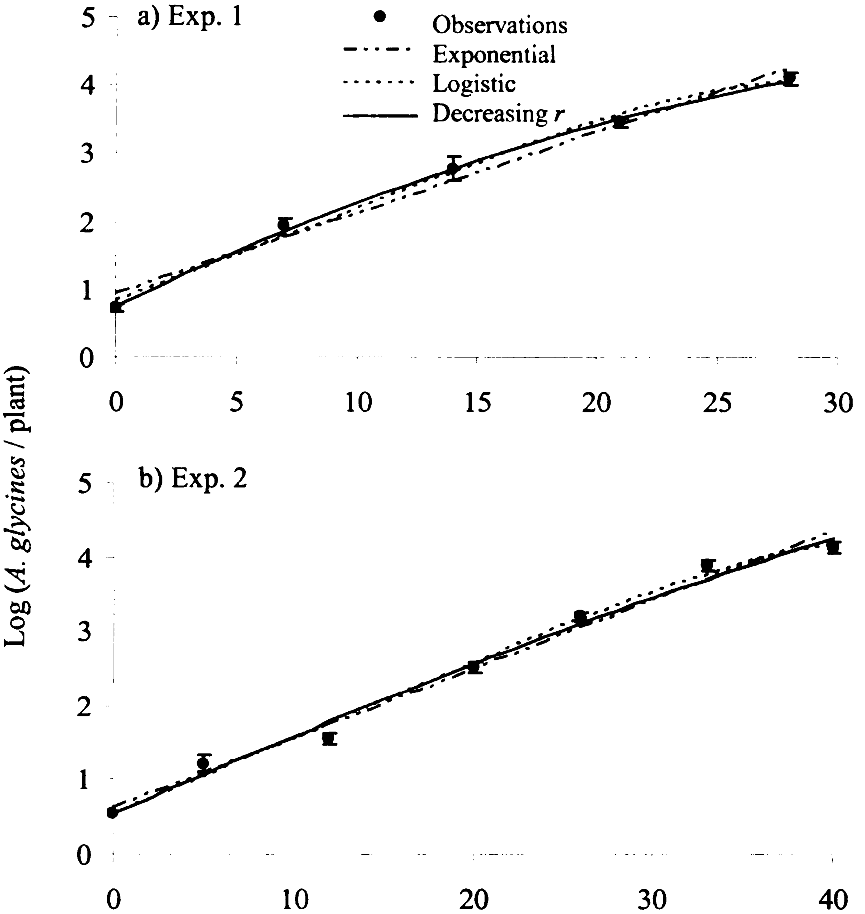


Figure 7.1. Observed population dynamics of *A. glycines* in four field experiments using predator exclusion cages and the fit of three competing models, for experiments 1 (a), 2 (b), 3 (c) and 4 (d). Observations (mean ± SE) are indicated by points and the exponential, logistic, and decreasing r models by dashed, dotter and solid lines, respectively. Parameter values and statistics for these models are given in Table 7.2.

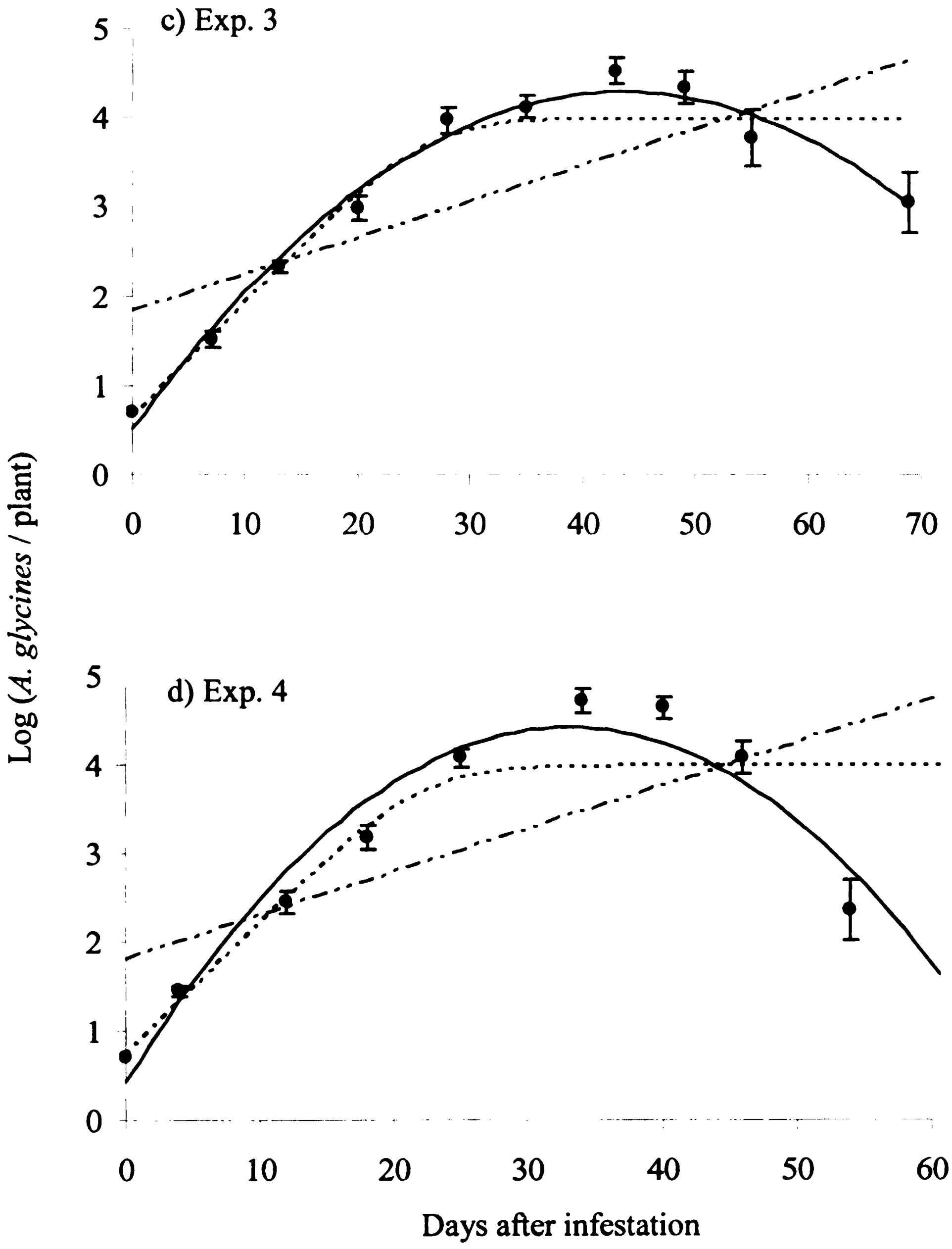


Figure 7.1. (cont'd)

all the values for the parameters r_{max}, $\ln(N_0)$, and a, had overlapping 95% confidence intervals within each experiment. The R^2 values indicate a very good fit of individual aphid populations to the model (exp. 1: all $R^2 > 0.99$ [n = 6]; exp. 2: all $R^2 > 0.97$ [n = 6]; exp. 3: $R^2 > 0.79$ [n = 7] and $R^2 > 0.90$ [n = 5]; and exp. 4: $R^2 > 0.69$ [n = 1], $R^2 > 0.79$ [n = 5], and $R^2 > 0.90$ [n = 6]). These results show that the decreasing *r* model accurately describes the growth of individual *A. glycines* populations and therefore supports the use of the means obtained in each experiment to investigate differences among experiments.

A more generalized model of *A. glycines* population growth was developed by fitting the model to the data of all four experiments combined. The fit of the model to the observed data expressed at the scale of days after infestation resulted in very good fit ($R^2 = 0.90$, Figure 7.2a, Table 7.3). Mean temperatures registered during the first 28 days after infestation differed significantly among experiments, with higher mean temperatures recorded in 2003 and 2005 ($F = 17.82$; df = 3, 84; $P < 0.0001$, Table 7.1). Thus, to incorporate the effect of temperature on the population dynamics of *A. glycines*, the model was fit using *A. glycines* degree-days instead of days after infestation, obtaining substantially higher explanatory power on this scale ($R^2 = 0.95$, Figure 7.2b, Table 7.3). Similarly, using soybean degree-days improved the model explanatory power over using calendar days, however it was slightly less predictive than the model using *A. glycines* degree-days ($R^2 = 0.943$, Figure 7.2c, Table 7.3). In all cases, the assumption of a linear decrease of r in time was met, with all three linear regressions being highly significant (Table 7.3).

Table 7.3. Parameters (mean ± SE) obtained by fitting the decreasing *r* model to observations scaled to days after infestation, *A. glycines* degree-days and soybean degree-days as time scales. At the bottom, results of the statistical analysis testing the assumption of linear decrease of *r* in time are presented for each model.

	Days after infestation	*A. glycines* degree-days	Soybeans degree-days
Decreasing r model			
r_{max}	0.3978 ± 0.0306	0.03550 ± 0.00179	0.06310 ± 0.00385
log (N_0)	0.5825 ± 0.0306	0.24480 ± 0.12410	-5.69220 ± 0.46370
a	0.0240 ± 0.0010	0.00193 ± 0.00005	0.00138 ± 0.00002
R^2	0.897	0.953	0.943
P	< 0.001	< 0.001	< 0.001
Linear regression r			
β_0	0.4284 ± 0.0412	0.43576 ± 0.04110	0.70554 ± 0.06913
β_1	-0.0113 ± 0.0014	-0.00090 ± 0.00011	-0.00102 ± 0.00012
R^2	0.725	0.737	0.744
P	< 0.001	< 0.001	< 0.001

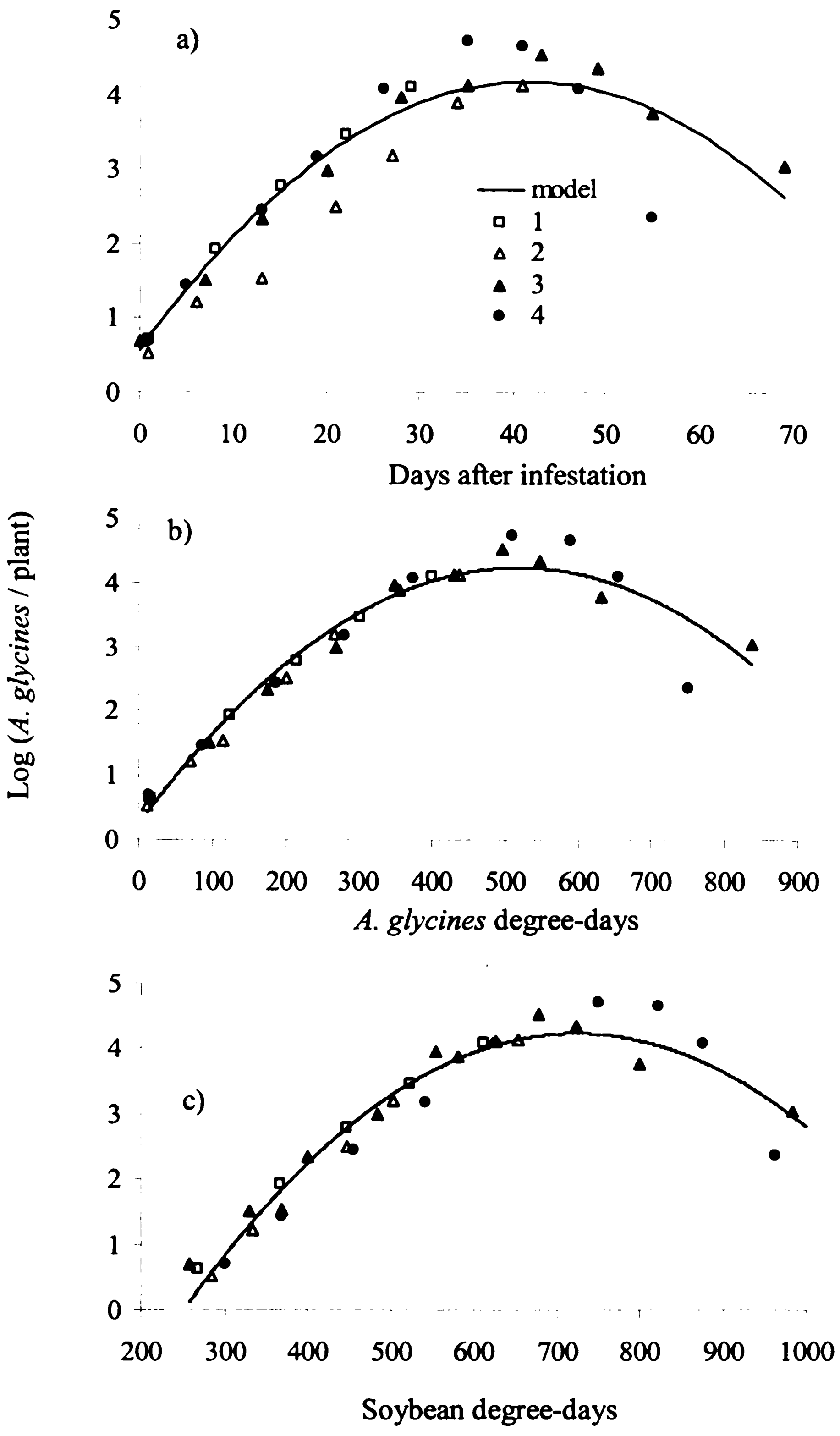


Figure 7.2. Fit of the decreasing *r* model (solid line) when fitted to observations of the four field experiments combined, scaled to days after infestation (a), *A. glycines* degree-days (b), and soybean degree-days (c). Different symbols represent the means of the four experiments. Parameter values for these models are given in Table 7.3.

Discussion

The population dynamics of *A. glycines* on soybean in the absence of predation can be well described using a model for exponential growth with the intrinsic rate of increase *r* that decreases linearly in time. The model was robust against variability among four experiments that were conducted in two separate locations during three different years. In addition, the model provided high explanatory power when fitted separately to data of the 36 individual aphid populations followed in the four experiments. Similar models assuming a decrease of *r* with time were previously used to simulate aphid population dynamics of *Sitobion avenae* (F.) (Hemiptera: Aphididae) in wheat (Rossing et al. 1994, Bianchi and van der Werf 2004) and three aphid species colonizing sugar beets in England (Williams et al. 1999). In addition, Van den Berg et al. (1997) used a similar approach to model *A. glycines* population growth under field conditions in Indonesia, decreasing the value of *r* according to estimations obtained rearing cohorts of aphids on plants at three different phenological stages. These studies measured the effects of plant quality either on individual aphids (Van den Berg et al. 1997, Williams et al. 1999) or on aphid field populations but without excluding predation (Rossing et al. 1994). My study presents a similar decreasing *r* model predicting *A. glycines* population dynamics based on data of field populations excluding mortality due to predation. Therefore, these studies show that plant quality affects population dynamics of a range of aphid species on a variety of crops and thus, can be considered a general phenomenon.

The generalized model developed for *A. glycines* population growth is highly predictive under the range of environmental conditions of the four experiments conducted. The model provided a very good fit when fitted to data expressed at the scale of days after infestation. This is most likely caused by the relatively narrow range of phenological stages of the plants when infested with aphids ($V_2 - V_4$) and temperatures that generally fell within the optimum range for *A. glycines* development in all experiments (McCornack et al. 2004). Notably, *A. glycines* populations in the experiments where lower mean temperatures occurred (2 and 3), were below the curve predicted by the model, whereas in experiments 1 and 4 populations were above the predicted values (Figure 7.2a). However, when the model was fitted using physiological scales for aphids and soybeans, these differences disappeared, indicating an effect of temperature on *A. glycines* population growth (Figs. 7.2b and c). The very similar results obtained using aphid and soybean degree-days may be due to two factors. First the basal temperatures for *A. glycines* and soybeans are very close, resulting in a relatively small difference in scale (8.6 versus 10 °C, respectively, Zhang et al. 2001, McCornack et al. 2004). Second, and most importantly, my experiments were conducted under a narrow range of plant phenology; greater effects could be expected on populations reared on more separated soybean phenological stages (Van den Berg et al. 1997).

These results supports the use of population growth models with decreasing r for systems in which the quality of the resources vary significantly with time and this change is directly translated into reduced reproduction. The observed decrease in the intrinsic rate of increase of *A. glycines* in time could be due to several factors. First, plant phenology may have a deleterious effect on the quality of resources for aphids, as was previously mentioned (Van den Berg et al. 1997). Second, rapidly increasing aphid populations may deteriorate host plant quality, as documented for several aphid species

(Dixon 1998, Williams et al. 1999). Finally, programmed reductions in aphid fecundity in response to seasonal changes in habitat quality have also been demonstrated (Wellings et al. 1980). Since my experiments were not designed to distinguish among these possible mechanisms, I can not rule out any of them affecting *A. glycines*. In summary, I developed a mathematical model for the population dynamics of *A. glycines* in the absence of predators, which provides season-long accurate and robust predictions. This model can be used as a simple tool to explore effects of different control strategies and predation levels on field populations of *A. glycines*.

CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

Aphis glycines invasion in North America

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) was detected in North America for the first time in Wisconsin in 2000 (Ragsdale et al. 2004, Venette and Ragsdale 2004). Since then, it has rapidly expanded to 22 States and three Canadian provinces, causing yield losses up to 40% in the USA (DiFonzo and Hines 2002, Losey et al. 2002, Hunt et al. 2003, Landis et al. 2003, Venette and Ragsdale 2004, Myers et al. 2005a, Mignault et al. 2006). During 2003, *A. glycines* was detected in over 50 million acres in the USA, of which 7 million acres were sprayed with insecticide to control this aphid (Landis et al. 2003). Thus, *A. glycines* arrival dramatically changed the dynamics of the insect communities associated with soybeans, a crop that was very seldom treated with insecticides before soybean aphid arrival (Heimpel and Shelly 2004).

Several studies were conducted in response to *A. glycines* invasion, including field studies for development of sampling protocols and threshold levels (Hodgson et al. 2004, Myers et al. 2005a, Onstad et al. 2005a, Onstad et al. 2005b), experiments determining optimal abiotic conditions for *A. glycines* development (McCornack et al. 2004, McCornack et al. 2005), and trips to its original area of distribution exploring for natural enemies (Heimpel and Shelly 2004). In addition, Fox and Landis (2003), Fox et al. (2004, 2005), Rutledge et al. (2004), and Rutledge and O'Neil (2005), assessed the impact of resident natural enemies on *A. glycines* suppression. In this dissertation, I investigated several aspects of the interactions between *A. glycines* and its resident natural enemies in Michigan.

Top-down versus bottom-up forces in *A. glycines* population control

The source of herbivore population control has been intensively debated since the proposal by Hairston et al. (1960) of a dominant top-down control on natural communities (Hunter and Price 1992, Power 1992). At present, both forces have been shown determining herbivore abundances on several ecosystems and their relative importance within each system is the focus of current research (Hunter et al. 1997, Fraser and Grime 1998, Dyer and Letourneau 1999, Fraser and Grime 1999, Walker and Jones 2001, Denno et al. 2002, Gratton and Denno 2003a). In the soybean aphid system, I observed strong suppression of *A. glycines* due to generalist predators both at aphid establishment and population growth phases, but only a weak, non-significant and variable effect of bottom-up forces (Chapter 1). I explored bottom-up factors by conducting experiments over a range of agricultural treatments, from conventional to organic agriculture, present at the Long Term Ecological Research Site at the Kellogg Biological Station of Michigan State University. These agricultural treatments altered plant quality as measured by above ground net productivity and yield, but did not significantly affect aphid populations even in the absence of top down controls, suggesting little scope for the management of *A. glycines* by manipulating agricultural practices. Similar results were obtained in experiments conducted on soybeans under different rotation systems (Chapter 4). However, other sources of bottom-up factors have been shown to affect *A. glycines*, including potassium soil deficiencies (Myers et al.

2005b), soybean resistant varieties (Hill et al. 2004a, b, Li et al. 2004, Wu et al. 2004, Mensah et al. 2005), plant phenology (Van den Berg et al. 1997), and alternative cropping systems (Wu et al. 2004). These should also be investigated to explore the possibility of combining them with existing sources of top-down control.

Strong top-down control may result in a trophic cascade. Trophic cascades occur when higher trophic levels significantly affect the abundance of lower trophic levels across more than one link in a food web (Pace et al. 1999, Polis et al. 2000). In agroecosystems in which pest suppression is exerted by natural enemies, a trophic cascade of increased plant yield represents a measure of biological control efficacy (Polis et al. 2000). In three separate experiments over two years I observed trophic cascades, as evidenced by increased soybean biomass and yield, due to the reduction of *A. glycines* populations by ambient levels of generalist predators (Chapters 3 and 4). These findings suggest that natural enemies play a prominent role in suppressing aphids during non-outbreak years and during much of the season in outbreak years.

Aphis glycines natural enemy assemblages and intraguild predation

In a multiple natural enemy guild, trophic interactions among its members may result in a cumulative effect on the prey, leading to increased (synergistic interactions) or reduced prey suppression (non-additive interactions) (Polis et al. 1989, Ferguson and Stiling 1996, Sih et al. 1998). Thus, in addition to attacking the focal prey, generalist predators may attack other members of the natural enemy guild, thus acting as intraguild predators (Polis et al. 1989, Polis and Holt 1992). In particular, aphid parasitoids have been shown to be susceptible to high levels of intraguild predation, since their immature stages develop within the aphids and later mummify (pupate) in close association with them (Rosenheim et al. 1995, Brodeur and Rosenheim 2000, Müller and Brodeur 2002). The assemblage of *A. glycines* natural enemies in North America is currently dominated by generalist predators, and parasitoids have only occasionally been detected in field infestations (Landis et al. 2003, Rutledge et al. 2004). By contrast, parasitism levels up to 53% have been reported in China, potentially contributing substantially to *A. glycines* suppression (Wu et al. 2004).

Thus, *A. glycines* provides an ideal system to test the potential limitation of parasitoid effectiveness by generalist predators through intraguild predation. I obtained evidence of intraguild predation on ambient and augmented levels of North American parasitoids of *A. glycines* in two separate field experiments (Chapters 3 and 4). Intraguild predation did not disrupt parasitism in these experiments, since the percentage parasitism was very low (< 1% in ambient levels of extant parasitoids). However, intraguild predation may slow down the process of adaptation of local parasitoids to this new host. Moreover, my results show that intraguild predation may become more important under higher levels of field parasitism. Thus, efforts directed at introducing more effective parasitoids of *A. glycines* in North America should consider detrimental effects of intraguild predation and try to assess whether new natural enemies will provide additive or synergistic aphid suppression or, in contrast, their effect will be diluted by non-additive interactions with the resident assemblage of natural enemies.

Aphis glycines – predator interactions

Despite strong predator suppression, *A. glycines* still increase to outbreak levels that result in significant yield reductions and economic losses. The causes of these outbreak populations are not well understood but may include a combination of abiotic and biotic factors. I developed a mathematical model of *A. glycines* population growth under field conditions as a first step in understanding the population dynamics of this pest (Chapter 7). The model is an exponential growth model in which the intrinsic rate of increase of *A. glycines* decreases linearly with time, and provides very good fit to the data obtained from four field experiments where predators were excluded (Chapters 1 to 5). A decreasing intrinsic rate of increase could be the result of decreasing resource quality due to plant phenology (Van den Berg et al. 1997), aphid feeding (Dixon 1998, Williams et al. 1999) or programmed responses to seasonal changes (Wellings et al. 1980). This model could be used to determine the effects of different control strategies and mortality due to natural enemies to successfully manage *A. glycines*.

Aphis glycines shows a highly aggregated spatial distribution (Hodgson et al. 2004), suggesting that certain areas in the field will develop higher aphid populations and may constitute a relative refuge from predation due to a dilution effect (Turchin and Kareiva 1989). By comparing predator protected versus unprotected aphid populations I determined that predators suppressed aphids below the threshold level irrespective of the initial aphid density (Chapter 5). Interestingly, I found a trend of lower per capita increase rates in high density aphid colonies, suggesting self limitation for *A. glycines*. In addition, exposure to predation significantly affected aphid distribution within the plant, with proportionally lower aphid densities on the upper nodes of the plant when they were exposed to predators (Chapter 5). This could have significant consequences for *A. glycines* population growth if the suitability of the soybean plant varies at different nodes and should be the focus of future research.

A significant role of the entire assemblage of predators suppressing *A. glycines* in North America was demonstrated in previous studies (Fox 2002, Fox and Landis 2003, Fox et al. 2004, Fox et al. 2005) and Chapters 1 to 5. However, these studies involved excluding partial or entire assemblages of predators, thus not allowing determination of the individual contribution of each predator group. During 2005, I conducted direct field observations and recorded 211 predation events on un-manipulated *A. glycines* populations. These results showed that the coccinellids *H. axyridis* and *C. septempunctata* were the most effective predators of *A. glycines*, consuming more aphids per capita and responding to changes in aphid density with increased aggregation in space and time and per capita consumption rate (Chapter 6). Thus, investigating the population dynamics of these two species across the landscape may provide insights on the causes of *A. glycines* outbreak populations.

In summary, these studies provide strong support for the key role of natural enemies, in particular generalist predators, in suppressing *A. glycines* populations across a range of conditions in Michigan. However, during outbreak years *A. glycines* is able to escape control from natural enemies and the causes of this escape remain unclear. In addition, several aspects of the interaction between *A. glycines* and its natural enemies are still poorly understood. The current major gap in our knowledge is regarding the population dynamics of the main predators of the system, the coccinellids *H. axyridis* and *C. septempunctata*. Since these are vagile predators that develop early season and populations overwinter in areas outside soybean crops, a study of their populations at a landscape level should provide insights on their interaction with *A. glycines*. In addition, their foraging behavior and factors influencing it should also be investigated. A more complete understanding of the interactions between *A. glycines* and its natural enemies will allow the implementation of better management practices and may represent the key to control of this pest.

APPENDIX

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2006 - 03

Title of thesis or dissertation (or other research projects):

DO VARYING NATURAL ENEMY ASSEMBLAGES IMPACT *APHIS GLYCINES* POPULATION DYNAMICS?

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Alejandro Carlos Costamagna

Date 05/01/2006

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:
Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 1.1

Voucher Specimen Data

Page 1 of 2 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Aphis glycines Matsumura	USA MI, Niles, USDA APHIS PPQ Laboratory - Lab Culture, VI 2003			20		20			
Harmonia axyridis (Pallas)	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 25 2005		2			10			
Coccinella septempunctata L.	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 25 2005					6			
Hippodamia variegata (Goeze)	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 25 2005					10			
Coleomegilla maculata De Geer	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 21 2005					6			
Orius insidiosus (Say)	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 25 2005					10			

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Alejandro Carlos Costamagna

Date 5/1/2006

Voucher No. 2006-03

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator 2 MAY 2006

Date

Appendix 1.1

Voucher Specimen Data

Page 2 of 2 Pages

		Number of:							
Species or other taxon	Label data for specimens collected or used and deposited	Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Nabis spp.	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 25 2005					10			
Chlamydatus associatus (Uhler)	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 1 2005					1			
Plagiognathus spp.	USA MI Ingham Co., East Lansing MSU - Entomology Farm, VII 1 2005					1			
Lysiphlebus testaceipes (Cresson)	USA MI Kalamazoo Co., Kalamazoo LTER-KBS biodiversity study, VII 11 200					9			
Aphelinus asychis (Walker)	USA MI Kalamazoo Co., Kalamazoo LTER-KBS biodiversity study, VIII 13 200					3			
Bynodoxis kelloggensis Pike et Stary	USA MI Kalamazoo Co., Kalamazoo LTER-KBS biodiversity study, VII 29 200					5			

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Alejandro Carlos Costamagna

Date 5/1/2006

Voucher No. 2006-03

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

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