Does a natural enemy limit the diet breadth of a generalist predator?

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Abstract

This dissertation explores the ways in which predators respond to novel prey, and in particular, how interactions among predators shape the habitat and prey use of predatory insects. Although predator habitat and prey use are commonly described in terms of responses to prey quality and density, interactions among predators may also be very important. In many systems, predators consume not only herbivorous prey, but also predators within their feeding guild. This interaction, termed intraguild predation, can alter the habitat use of predators that also act as prey, and thus limit access to some prey. Using a system in which a predator is simultaneously confronted with a novel prey and an aggressive intraguild predator, I consider how these forces work together to shape predator prey use.

Aphid-feeding lady beetles have been extensively studied due to their importance in suppression of agricultural pests. Moving from habitat to habitat over the course of a growing season, they prey upon diverse aphid (and other) species. Although many species seem to aggregate in the areas with the densest aphid populations, others deviate from this expectation, particularly in responding to a novel resource. For example, the predatory lady beetle *Coleogilla maculata*, native to the Americas, seldom feeds on the soybean aphid, established in the Midwestern United States in 2001, despite the fact that it is available at the same time as the commonly used corn leaf aphid and the fact that maize and soybean are typically planted very close to one another. One possibility for the failure of this native predator to incorporate the novel prey into its diet is its interaction with another predator, the exotic lady beetle *Harmonia axyridis*, which is the most common lady beetle in soybean habitats. This species is an aggressive intraguild predator, and has been blamed for the decline in several species of native lady beetles. To better understand the factors limiting the prey use of the native predator, I conducted a series of field experiments in maize and soybean, as well as laboratory predation trials.

In my first chapter, I describe how predator communities differ between maize and soybean, using visual counts of insects over two seasons of field experiments in maize and soybean, focusing on the two most common predator species, the native *C.*

maculata and the exotic *H. axyridis*. While the species are equally common in maize, the exotic is far more common in soybean. This observation fits with observations of lady beetles collected on sticky cards or in sweep nets in other studies across North America, but goes further in identifying immature stages and eggs. Comparing eggs by habitat, it is clear that either the native species does not use soybean for laying eggs, or eggs laid in soybean are quickly preyed upon.

The second and third chapters examine the quality of maize and soybean resources, as well as the likelihood of survival and predation in each habitat. In chapter two, I give an overview of the factors that affect selection of foraging habitats, considering the quality of the novel prey as a resource for the native beetle, as well as the potential effect of predation on survival on the novel prey. Comparing with a null model created by randomizing survey data across maize and soybean treatments, I examine differences in mortality by crop, species and stage and conclude that the native survives as well in soybean as in maize. In chapter 3, I examine predation on sentinel eggs in both habitats as well as the presence of other predators and resources available at the time of predation. With this study I conclude that maize is a more dangerous habitat than is soybean, and that this may be because of higher aphid numbers in soybean. In both chapters, I conclude that the native beetle is not excluded by the exotic.

The fourth and fifth chapters further explore the importance of intraguild predation among lady beetles in maize and soybean by examining how contact in the field might relate to coexistence of predators that engage in both intraguild predation and cannibalism. In chapter four, I explore the role of avoidance behavior in determining contact among potential intraguild predators by comparing observed contact at the plant level to expected contact given predator densities. Using both density and distribution data from my 2008 and 2009 surveys in maize and soybean, as well as data from a published study on avoidance among coccinellids in tansy, I conclude that except among pupae, avoidance does not prevent contact among potential intraguild predators. In chapter five I combine the field contact data described in chapter four with instantaneous attack rates on native and exotic lady beetle pupae determined in the laboratory. I then compare the likelihood of pupal mortality by cannibalism and. intraguild predation in

maize and soybean and use the products of intraspecific and interspecific interactions to predict coexistence or exclusion by habitat. I emphasize the potential importance of cannibalism in determining the outcome of interactions among species.

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Chapter 1. Comparison of lady beetle (Coleoptera: Coccinellidae) communities in soybean and maize

Introduction

The introduction of pests and pathogens from other continents can cause outbreaks in habitats that had previously enjoyed relative freedom from pests (Mack et al., 2000). For example, the soybean aphid (*Aphis glycines* Matsumura), native to Asia, was first detected in North America in soybean fields in the Midwestern USA in 2000 and rapidly became established in northern soybean growing regions (Ragsdale et al. 2011). Soybean aphids are currently present in over 20 states, as well as 3 provinces in Canada (Ragsdale et al. 2011). Predators and pathogens that attack soybean aphid in North America have been only moderately effective in suppressing aphid populations. In the Midwestern United States soybean aphids commonly reach levels that cause yield losses and necessitate the use of foliar insecticides (Ragsdale et al., 2007) which can harm natural enemies (Croft & Brown, 1975; Croft & Morse, 1979; Theiling & Croft, 1988; Desneux et al. 2007) and lead to aphid resistance to insecticides (Mota-Sanchez et al., 2008).

Coccinellids currently provide the most effective natural control of soybean aphids in North America (Costamagna & Landis, 2007; Fox et al., 2004), so it is important to understand the extent to which different species of aphid feeding coccinellids use soybean habitats. Although aphidophagous coccinellids are known to be fairly generalist when it comes to choosing among aphid prey, coccinellid communities differ among habitats, and this may be determined by differences in prey use among species (Comont et al., 2012). Foraging aphid predators respond both to prey density and prey identity (Hemptinne et al., 1993). Coccinellids aggregate to dense aphid colonies (Wright & Laing, 1980), but the strength (Elliott & Kieckhefer, 2000) and scale (Schellhorn & Andow, 2005) at which they respond differs by species. Further, differences among coccinellid species in their prey preferences and responses to novel prey are influenced by their unique evolutionary histories (Sloggett & Majerus, 2000). Coccinellids use volatile plant chemicals to detect aphid prey (Ninkovic et al., 2001) and

display differences in their responses to the feeding of different aphid prey (Ninkovic et al., 2001; Sarmento et al. 2007), further suggesting that prey identity could play an important role in determining habitat use.

Coccinellid species native to Asia might more easily add soybean aphid into their diets than those species that historically had no contact with the aphid. It can take generations of exposure to a prey for some predators to develop the ability to locate it (Hassel & Southwood, 1978) and develop on it (Rana et al., 2002). Because soybean in North America was free of colonizing aphids prior to the introduction of soybean aphid (Kogan & Turnipseed, 1987; Hartman & Hill, 2010), native coccinellids have had a short history of exposure both to the soybean aphid directly and to volatiles released by soybean plants when aphids feed. In contrast, *Harmonia axyridis*, which is one of the most common aphid predators observed feeding on soybean aphid in China (Wu et al. 2004) would have a shared evolutionary history with soybean aphid, and thus might respond to it more quickly. These differences, along with the known diversity of coccinellid responses to any resource, might make the composition of the coccinellid community in soybean distinct from that of other nearby habitats.

Characterizing the coccinellid community in soybean is important because although generalist predators can be very effective biological control agents (Murdoch et al., 1985), interactions among them may lead to non-additive effects that disrupt (Rosenheim et al., 1995) or enhance (Losey & Denno, 1998) suppression of prey. Further, coccinellid adults disperse readily (Kieckhefer & Olson, 1974), so coccinellid performance in soybean is also likely to affect coccinellid abundance and biological control in nearby crops.

Comparing the coccinellid community present in soybean to that of maize, where the corn leaf aphid, *Rhopalosiphum maidis* has historically been available (Foott, 1977), we sought to identify differences in the coccinellid species using the two habitats. We used visual counts of coccinellids and aphids from soybean and maize fields in Minnesota, USA, to document the composition of coccinellid communities in these habitats just less than 10 years after the establishment of soybean aphid. Both *R. maidis* (DiFonzo et al., 1997) and *A. glycines* (Ragsdale et al., 2004) appear in June and peak in

early August, giving coccinellids the opportunity to forage in maize or soybean. By carefully tracking aphid and coccinellid densities in these crops over two growing seasons, we sought to gain insights into how different coccinellid species responded to novel vs. traditional prey, both in terms of initial habitat selection as well as performance in each habitat over time.

Methods

Field Experiment

To characterize the composition of coccinellid communities in maize and soybean, we observed the abundance of predatory coccinellids and aphid prey in plots of maize and soybean at the University of Minnesota Agricultural experiment site in central Minnesota during the 2008 and 2009 growing seasons. We established 4 blocks, each of which contained two 10 by 10 meter plots that were randomly assigned be planted with maize (Green Giant Code 63) or soybean (Northrop King S19R5), at 5.18 and 24.70 plants/ m^2 respectively. Crops were planted in 30 inch rows on June 10 in 2008 and on June 4 in 2009.

Since previously published work with a similar maize variety in this region suggested that coccinellids would colonize in mid to late July (Schellhorn, 1998), we began scouting for coccinellids and aphids on a few plants per plot in the first week of July. We initiated sampling on July 9 in 2008 and July 12 in 2009 and continued until the no immature (larval or pupal) coccinellids were sampled.

At weekly intervals, we visually inspected plants at 15 randomly selected locations per plot. Each sample consisted of two adjacent maize plants or two adjacent 18 cm sections of soybean row. Weekly sampling for the duration of the coccinellid season gave us a total of 6 sample dates in 2008 (with one week missed due to rain) and 9 sample dates in 2009, when coccinellids persisted later in the season. When aphids were sparse, we counted individual aphids. However, in maize, aphids sometimes reached high numbers in tassels, and in these cases we chose a representative subsection of the densely populated part of the plant, counted aphids on it, and multiplied by the number of similar subsections that existed on the plant. A similar method was used to estimate

soybean aphid numbers. Since soybean aphids move to different parts of soybean plants over the season (McCornack et al., 2008) we chose a vertical subsection of all plant parts and counted aphids within it, multiplying by the number of similar subsections present in the sample.

Coccinellid numbers were reported by species and stage, with coccinellid egg masses collected for identification in the laboratory. Egg masses were checked daily and emerged coccinellid larvae were identified and returned to their natal plots within 48 hours. Although holding egg masses in the laboratory prevented them from being attacked by predators, their low numbers relative to the egg masses present in the plots would make the effect of this minimal. Insect densities from each pair of adjacent maize plants or adjacent sections of soybean row were averaged and then converted to density/ m^2 to make maize and soybean samples comparable. For maize samples, we multiplied each plant sampled by plants per square meter, while in soybean we multiplied each 18cm section of soybean row by 7.93 because there was 141 cm of row/ m^2.

Statistical Analysis

Data for each year were analyzed with a repeated measures, randomized complete block ANOVA. Whole plot treatments were habitat (maize or soybean) with sampling date nested within treatment. Aphid density/ m^2 +1 was log transformed prior to analysis to meet the assumption of homoscedasticity of error variance. Coccinellid densities by species were analyzed using a similar ANOVA, with coccinellid species added as a second factor at the whole plot level. For this analysis we included data on all life stages, eggs through adults. Species that made up more than 1 percent of total coccinellids observed were included, and these were *Harmonia axyridis*, *Coleomegilla maculata*, *Coccinella septempunctata* and *Cycloneda munda*. We also used a planned contrast to examine whether there was a numerically dominant coccinellid within each habitat, by testing whether the densities of the most abundant coccinellid were statistically different from those of all other coccinellids common in the habitat. Immature coccinellid density was analyzed with the same model, but was restricted to only the two most common species, *H. axyridis* and *C. maculata*. We analyzed egg densities separately from the densities of larvae and pupae.

Lastly, we compared species richness (number of species) and Shannon's index of diversity (Shannon & Weaver, 1963) across treatments using paired t-test with treatments of maize and soybean paired by block. Species richness and diversity were calculated using all coccinellids observed over the season, without considering differences by date.

Results

The most abundant aphids in maize and soybean were *R. maidis* and *A. glycines*, respectively. Both aphid species had colonized all plants sampled by the time their populations peaked, August 8 in 2008 and August 12 in 2009. In addition to *R. maidis*, we observed two other aphid species, *Rhopalosiphum padi* and *Sitobium avenae*, in maize plots. *R. padi* made up less than 5 percent of the total aphid population. Because *R. padi* and *R. maidis* are closely related (Papasotiropoulous et al., 2013), we included both species together in our aphid estimates. *S. avenae* was quite rare, appearing on only a few plants per season.

There were significant differences in aphid abundance by date, but these seasonal differences did not mask the difference in treatments (Table 1.1). *A. glycines* was significantly more abundant in soybean than *R. maidis* was in maize during both years (Figure 1.1, $p < .001$ for both years). This difference was most striking in 2008, when there were about three times as many *A. glycines*/ m^2 as there were *R. maidis.* In 2009 the difference was more subtle, with *A. glycines* only slightly more abundant than *R. maidis*. The biggest difference in aphid abundance between the two habitats occurred at the beginning and end of each growing season. Based on aphid abundance, both maize and soybean are suitable habitats for aphidophagous coccinellids, and the similar phenology of the two aphid species in these habitats would make them available to coccinellids at about the same time. The abundance of *A. glycines* in 2008 suggests that soybean might support higher coccinellid populations in this year than would maize.

Coccinellid densities peaked at the same time as aphid densities in both years and habitats, but the composition of the coccinellid community differed between the two habitats (Table, 1.2, Figure 1.2). While *H. axyridis* was dominant in soybean, where it made up 84% and 94% of all coccinellids sampled in 2008 and 2009, both *H. axyridis*

and the native *C. maculata* were common in maize in both years sampled. In 2008, when aphids were very abundant in both habitats, *H. axyridis* was more abundant in maize than was *C. maculata*, comprising 61% of the coccinellid community. The following year, however, when aphid densities were considerably lower, *C. maculata* made up 58% of the coccinellid community in maize (Figure 1.2). Together, these two species made up over 90% of the coccinellids sampled in maize during the two years.

The other coccinellids sampled, the exotic *Coccinella septempunctata* and the natives, *Cycloneda munda*, *Hippodamia tredecimpunctata* and *Hippodamia convergens* were all present in both maize and soybean in at least one sampling year. Although more species were observed in soybean than in maize, mean diversity (Shannon's index) was lower in soybean in both years, and this difference was statistically significant in 2009 ($p<.01$) (Table 1.3).

Examining adult densities of the two most abundant species, *H. axyridis* and *C. maculata,* revealed that adults had an early density peak that occurred with egg peaks, and then a second density peak later in the season (Figure 1.3). The 20 to 35 day gap between adult peaks suggests that the latter peak represents the emergence of a new generation of adults from eggs laid at the start of the season. Adults of both species were abundant in maize except in the latter portion of the 2008 season, when *C. maculata* decreased in abundance.

Each species maintained a distinct egg laying pattern over the two years, reflected in the significant species by habitat interaction present in both 2008 ($p=0.09$) and 2009 ($p=0.01$) (Table 1.4). *C. maculata* eggs were found almost exclusively in maize in both years, although there were more aphids available in soybean, particularly during the 2008 egg laying period. There were high densities of *H. axyridis* eggs in both habitats over both years (Figure 1.4). A comparison of egg densities within each habitat revealed that *H. axyridis* eggs were significantly more abundant than *C. maculata* eggs in soybean over 2008 and 2009 (Table 1.4) and eggs of the two species were equally abundant in maize in 2008 ($p=0.29$) while *C. maculata eggs* were more abundant in maize than *H. axyridis* eggs in 2009 ($p= 0.01$) (Table 1.4).

Not surprisingly, *C. maculata* larvae and pupae, here collectively grouped as immatures, were also rare in soybean, the habitat in which eggs were seldom observed (Figure 1.5). A within treatment comparison of species densities revealed that *H. axyridis* immatures were much more abundant than those of *C. maculata* in soybean (Table 4, p=<0.01 for both years). The patterns were more complicated in maize. In 2008, when aphids reached very high densities, *H. axyridis* immatures were more common in maize than were *C. maculata* immatures (Table 1.4, p=0.02), but in the following year, however, when aphids peaked at a lower level, *C. maculata* immatures were more abundant in maize than were those of *H. axyridis* (Table 1.4, p=0.02).

Discussion

Our observation that *H. axyridis* was the dominant coccinellid in Minnesota soybean was consistent with published field surveys in North American soybean after the establishment of the soybean aphid. In the eastern United States, more than 70 and 90% of the coccinellids captured in sweep net surveys in Maryland and Kentucky respectively, were *H. axyridis* (Seagraves et al., 2011). Moving west, sticky card surveys in Wisconsin yielded 62% *H. axyridis* (Gardiner et al., 2009) and sticky card and visual counts in Michigan reported 41 to 74% of adult coccinellids as *H. axyridis* (Fox et al., 2005;Costamagna & Landis, 2007;Gardiner et al., 2009) with the exception of 2002, when *H. axyridis* was absent from soybean, despite having comprised 70% of the coccinellid community the year before (Fox et al., 2005). Just west of the Mississippi, in Minnesota and Iowa, about 45% of adults captured on sticky cards were *H. axyridis*, with *H. convergens* and *C. septempunctata* also fairly common (Gardiner et al., 2009).

Our Minnesota soybean surveys showed relative coccinellid abundances similar to those found in Wisconsin and Michigan, with *H. axyridis* comprising more than 80% of the coccinellid community in both 2008 and 2009. Given that forested landscapes support higher populations of *H. axyridis* than do grassland or agricultural landscapes (Gardiner et al., 2009), this result is not surprising. Our survey was conducted in eastern Minnesota, just north of St. Paul, which is more forested than locations further south and

west, and thus likely more similar to Wisconsin and Michigan than Iowa and southern Minnesota.

Coccinellid communities in Canada and South Dakota soybean differed from those in Minnesota and the eastern United States in that *H. axyridis* was not the most abundant coccinellid. In Canada, the exotic *Propylea quatordecimpunctata* was the most abundant coccinellid, while *H. axyridis* made up about 22% of the coccinellid community in 2002 and 2003 (Lucas et al., 2007). South Dakota coccinellid communities were more even with three co-dominant species. Depending on the study and year, the native *H. convergens* (Hesler & Kieckhefer, 2008; Seagraves et al., 2011) and the exotic *C. septempunctata* (Hesler & Kieckhefer, 2008), were most abundant, with *H. axyridis* making up 18 and 33% of the coccinellid community in 2003 and 2002 based on visual counts (Hesler & Kieckhefer, 2008) and 32% of adults based on sweep net surveys (Seagraves et al., 2011).

The exotic *C. septempunctata*, observed in our Minnesota survey, was found in all of the soybean surveys we examined from North America, although its proportion varied from 1% in Kentucky (Seagraves et al., 2011) to 80% in one Michigan survey (Fox et al., 2005). No clear geographic pattern for this species was evident. Similar to our own results, these studies also reported adults of the native lady beetle *C. maculata* in soybean, but at very low levels. We observed a few *C. maculata* larvae in Minnesota soybean, but none were reported in visual counts in Michigan soybean (Costamagna & Landis, 2007) and thus *C. maculata* larvae are probably absent or rare in soybean. The natives *Hippodamia convergens*, *Hippodamia tredecimpunctata*, and *Cycloneda munda*, observed in our soybean surveys, comprised a small percentage of the coccinellid community in soybean fields across North America, with the exception South Dakota where *H. tredecimpunctata* was absent and *H. convergens* was much more common (Hesler & Kieckhefer, 2008; Seagraves et al., 2011). We did not encounter the native *H. parenthesis*, although it was reported making up 4% or less of the coccinellid community in Iowa, Michigan, Minnesota, and Wisconsin (Gardiner et al., 2009) and South Dakota (Hesler & Kieckhefer, 2008). We did not find *H. variegata* in any of our studies, although the species was observed in nearby maize and soybean fields starting in 2009

(Heidel & Morey, 2011) and was also reported in several Michigan surveys (Costamagna & Landis, 2007; Gardiner et al., 2009; Woltz & Landis, 2013).

Our observation that *H. axyridis* was less dominant in maize than it was in soybean was consistent with other studies that used visual counts to quantify coccinellids by species in maize in the years following 2000. In Canada (Lucas et al., 2007) and New York (Musser & Shelton, 2003) *H. axyridis* and the native *C. maculata* were similarly abundant, with each of the species slightly more abundant in some years and less in others. In Pennsylvania, *H. axyridis* was more abundant (Hoheisel & Fleischer, 2007) and in South Dakota, *C. maculata* was more abundant (Hesler & Kieckhefer, 2008) although both species were present in both of these locations in all sampling years. These observations further support the general pattern of *H. axyridis* being more abundant relative to other coccinellids in the eastern states.

The exotic *C. septempunctata* was observed in all maize studies, but was much less common than it had been in soybean. In some year/habitat combinations none were observed at all, and the highest percentage observed was in Canada sweet corn at 12% (Lucas et al., 2007). *H. tredecimpunctata* and *H. convergens*, which we observed in Minnesota maize, were also observed in South Dakota (Hesler & Kieckhefer, 2008). Although we observed *C. munda* in Minnesota maize, this species was not found in maize in the other surveys examined. Given that no maize study reported *H. variegata* and two other species, *C. munda* and *C. septempunctata*, were much less common in maize than in soybean, species richness tended to be lower maize, with a maximum of five coccinellid species (Hesler & Kieckhefer, 2008) present in the same location and year, compared to the maximum of 10 reported in soybean (Gardiner et al., 2009).

Differences in prey abundance and quality might explain some of the differences between coccinellid communities in maize and soybean. In our study there were higher aphid densities in soybean than in maize, thus coccinellids that respond strongly to high aphid densities, such as *H. axyridis* (Lundgren et al., 2004), would be expected to move into soybean habitats. Similar to *H. axyridis*, *C. septempunctata* and *C. munda* reached the highest densities in the habitats/years with the highest aphid densities. *Coleomegilla maculata*, in contast, did not seem to colonize where aphids were densest, a finding that

is consistent with previous observations of the species not responding to differences in aphid abundances in maize (Lundgren et al., 2004) and dominating coccinellid communities in maize only in years when aphids are least abundant (Schellhorn, 1998).

Prey identity might also be important in determining coccinellid distributions. Predator preferences influence the composition of predator communities (Straub & Snyder, 2006) and even those predatory insects deemed "generalist" focus on a subset of available and suitable prey (Finlay-Doney & Walter, 2012). Although aphid-feeding coccinellids are known to switch among prey species (Evans & Youssef, 1992; Sloggett & Majerus, 2000), they have distinct preferences that are influenced by their ecology and their evolutionary history (Sloggett & Majerus, 2000). Since *H. axyridis* feeds on *A. glycines* in its native China, it is not surprising that *H. axyridis* readily colonizes soybean in North America and quickly responds to the presence of soybean aphid.

Predator preferences for prey abundance and identity do not seem to fully explain the differences we observed between lady beetle communities in maize and soybean in North America, however. Although the species present in each habitat changed from study to study, the basic community structure was consistent, with soybean dominated by a single predator and maize dominated by two or more predators. It is unclear from our study why this may be. Janssen et al observed that intra-guild predators are more likely to coexist in complex habitats (Janssen et al., 2007) and maize, with its taller stature and combination of dense and sparse aphid regions, is arguably a more complex plant than is soybean. In such a habitat, less aggressive predators might be able to evade attack by more aggressive predators. Further, niche partitioning might be possible to a greater degree than it would be in a habitat with more uniform resources, such as soybean.

Aphidophagous coccinellids have proven more effective in suppressing soybean aphid populations than have other natural enemies (Costamagna & Landis, 2007). Given the composition of coccinellid communities in North America, it seems that this control is mostly provided by *H. axyridis* in the eastern United States and *P. quatourdecimpunctata* in Canada. Whether a more balanced coccinelllid community, such as that seen in South Dakota soybean or in maize would provide better aphid control is unclear. Direct studies correlating coccinellid community diversity and *A. glycines*

suppression are lacking. And while coccinellids are known to keep *R. maidis* populations below economically damaging levels (Wright & Laing, 1980), the role coccinellid diversity might play in this is unclear. Thus, whether increased predator diversity would improve biological control via niche complementarity (Losey & Denno, 1998; Aquilino et al. 2005) , or release prey from predation (Rosenheim et al., 1993; Finke & Denno, 2003) would depend on the foraging behavior of the predators involved (Chalcraft & Resetarits, 2003) as well as the interactions among them (Finke & Denno, 2005; Straub & Snyder, 2006).

If soybean habitats support fewer niches and fewer predators, then the lack of native predatory lady beetles in soybean may be due more to habitat structure than to adaptation to soybean aphid prey. Although it has been shown that diverse predators may interact to better suppress prey (Cardinale et al., 2003), in a more uniform habitat like soybean, one main predator, such as *H. axyridis* or *P. quattourdecimpunctata* may be just as effective. Our study suggests that although many lady beetle species are able to feed on soybean aphids, high species diversity is unlikely in soybean habitats.

Table 1.1. Analysis of variance for the effect of habitat treatment (Trt) and date (D) on aphid density per square meter. Densities were natural log transformed. For each year, all sampling dates were included.

2008	**Df**	**MS**	***F***	***P***
Trt	1	101.8	255.2	0.001
Block	3	1.5	3.7	0.155
Error 1	3	0.4		
D	5	30.8	53.2	<0.001
Trt x D	5	6.8	11.7	<0.001
Error2	30	0.6		
2009	**Df**	**MS**	***F***	***P***
Trt	1	38.0	426.7	<0.001
Block	3	0.2	1.8	0.317
Error 1	3	0.1		
D	8	2.6	26.0	<0.001
Trt x D	24	3.0	29.3	<0.001
Error2	48	0.1		

Table 1.2. Analysis of variance for the effect of habitat treatment (Trt), date (D) and species (Sp) on coccinellid density per square meter. Coccinellid densities were natural log transformed. For each year, all sampling dates were included.

All stages 2008	**Df**	**MS**	***F***	***P***
Blk	3	108	1.59	0.21
Trt	1	97	1.44	0.24
Sp	4	2942	43.54	<0.01
Trt x Sp	4	219	3.24	0.03
Error 1	27	68		
D	5	632	13.77	<0.01
Trt x D	5	368	8.01	<0.01
D x Sp	20	246	5.36	<0.01
Trt x D x Sp	20	215	4.69	<0.01
Error 2	150	46		
Contrast most abundant species vs all others				
maize	1	2522	37.33	<0.01
soybean	1	4113	60.88	<0.01
All stages 2009	**Df**	**MS**	***F***	***P***
Blk	3	19	1.06	0.38
Trt	1	11	0.60	0.44
Sp	4	943	53.22	<0.01
Trt x Sp	4	442	24.92	<0.01
Error 1	27	18		
D	8	184	12.81	<0.01
Trt x D	8	49	3.41	<0.01
D x Sp	32	70	4.87	<0.01
Trt x D x Sp	32	52	3.58	<0.01
Error 2	240	14		
Contrast most abundant species vs all others				
maize	1	747	42.16	<0.01
soybean	1	2180	122.98	<0.01

Table 1.3. Mean species richness and diversity (± SE) in maize and soybean treatments during 2008 and 2009. Significant differences based on a t-test with 3 degrees of freedom.

species richness (number species)

year	maize	soybean	t	*p*
2008	4.00 (0.00)	4.75 (0.25)	-3.00	0.06
2009	3.00 (0.41)	4.00 (0.41)	-1.41	0.25

species diversity (Shannon's Index)

year	maize	soybean	t	*p*
2008	0.78 (0.06)	0.57 (0.09)	1.96	0.15
2009	0.68 (0.03)	0.19 (0.04)	12.2	<0.01

Table 1.4. ANOVA for the effect of treatment (Trt) and date (D) on density per square meter of immature *H. axyridis* and *C. maculata*. Densities were natural log transformed. Contrasts conducted to determine differences in species density by treatment.

Eggs 2008	**Df**	**MS**	***F***	***p***
Blk	3	147	1.83	0.17
Trt	1	343	4.28	0.03
Sp	1	600	7.49	0.01
Trt x Sp	1	180	2.25	0.09
Error 1	9	80		
D	5	716	9.50	0.00
Trt x D	5	989	13.13	0.00
D x Sp	5	117	1.55	0.30
Trt x D x Sp	5	402	5.34	0.00
Error 2	60	75		
Contrast *C. maculata* vs *H. axyridis*				
maize	1	61	0.77	0.29
soybean	1	718	8.97	<0.01

Imm 2008	**Df**	**MS**	***F***	***p***
Blk	3	222	1.44	0.29
Trt	1	508	3.31	0.10
Sp	1	3387	22.04	<0.01
Trt x Sp	1	309	2.01	0.19
Error 1	9	154		
D	5	1298	12.19	<0.01
Trt x D	5	833	7.82	<0.01
D x Sp	5	263	2.47	0.04
Trt x D x Sp	5	431	4.05	<0.01
Error 2	60	106		
Contrast *C. maculata* vs *H. axyridis*				
maize	1	824	5.37	0.02
soybean	1	2872	18.69	<0.01

Eggs 2009	**Df**	**MS**	***F***	***p***
Blk	3	27	0.65	0.56
Trt	1	129	3.12	0.05
Sp	1	1	0.03	1.00
Trt x Sp	1	422	10.26	<0.01
Error 1	9	41		
D	8	420	14.21	<0.01
Trt x D	8	104	3.53	<0.01
D x Sp	8	5	0.16	0.10
Trt x D x Sp	8	121	4.11	<0.01
Error 2	96	30		
Contrast *C. maculata* vs *H. axyridis*				
maize	1	235	5.71	0.01
soybean	1	188	4.57	0.02

Imm 2009	**Df**	**MS**	***F***	***p***
Blk	3	36	0.23	0.87
Trt	1	46	0.30	0.60
Sp	1	240	1.56	0.24
Trt x Sp	1	1224	7.97	0.02
Error 1	9	154		
D	8	440	9.50	<0.01
Trt x D	8	107	13.13	<0.01
D x Sp	8	25	1.55	0.30
Trt x D x Sp	8	138	5.34	<0.01
Error 2	32	177		
Contrast *C. maculata* vs *H. axyridis*				
maize	1	190	4.61	0.02
soybean	1	1274	30.87	<0.01

Figure Legends

Fig. 1.1 Log mean aphid densities (± SE) in soybean (solid) and maize (dash).

Fig. 1.2 Log mean densities (± SE) of the most abundant coccinellids (egg to adult) by year and crop. Natives *Coleomegilla maculata* (filled circles) and *Cycloneda munda* (open circles) shown in solid lines, exotics *Harmonia axyridis* (filled circles) and *Coccinella septempunctata* (open circles) in dashed lines.

Fig. 1.3 Log mean densities (± SE) of *C. maculata* (solid) *and H. axyridis* (dash) adults in soybean (filled circles) and maize (open circles).

Fig. 1.4 Log mean densities (± SE) of *C. maculata* (solid) *and H. axyridis* (dash) eggs in soybean (filled circles) and maize (open circles).

Fig. 1.5 Log mean densities (± SE) of *C. maculata* (solid) *and H. axyridis* (dash) larvae and pupae in maize (open circles) and soybean (filled circles).

Figure 1.1.

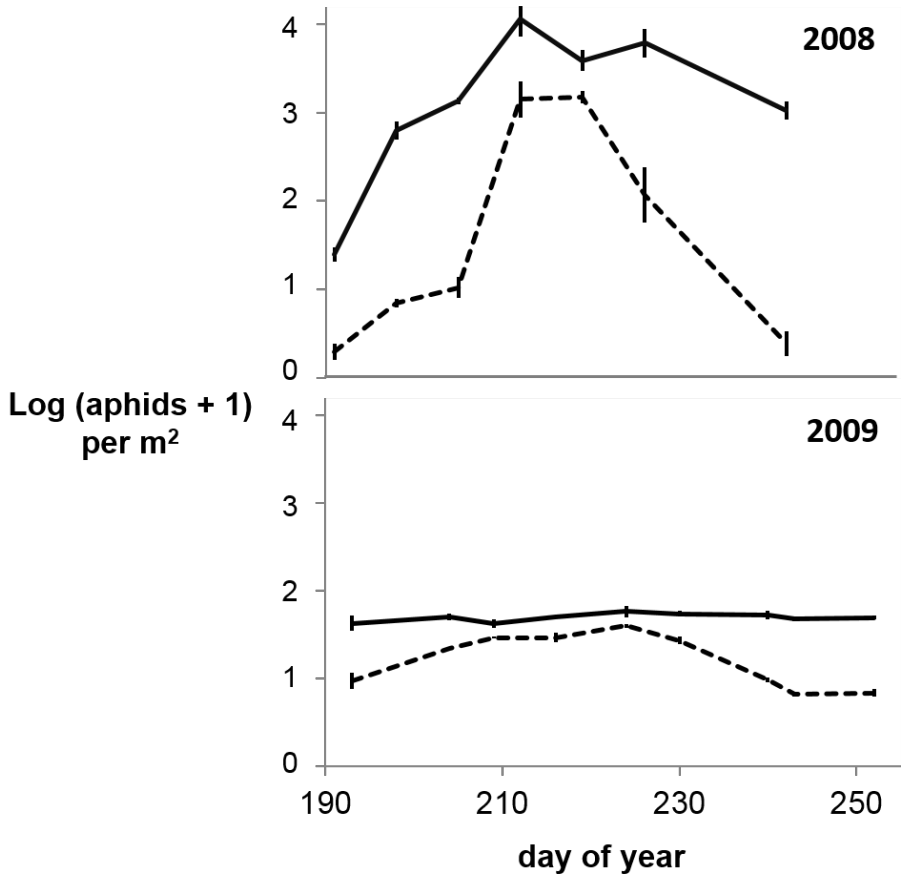


Figure 1.2.

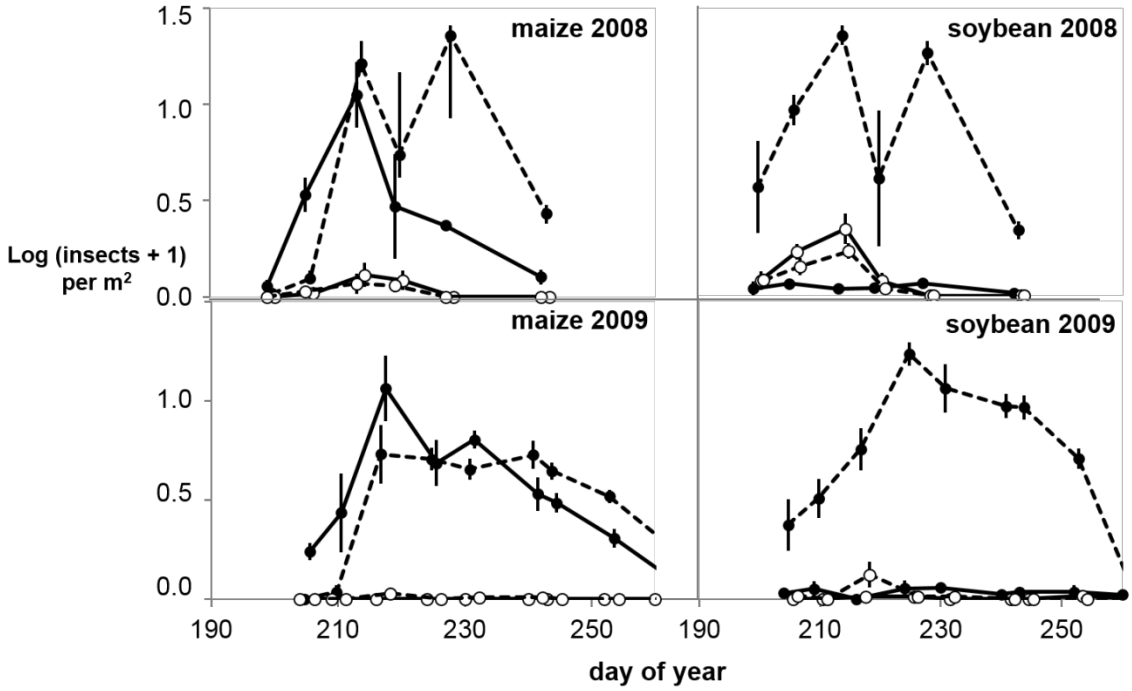

maize 2008
soybean 2008
maize 2009
soybean 2009
Log (insects + 1) per m²
1.5
1.0
0.5
0.0
190
210
230
250
day of year

Figure 1.3.

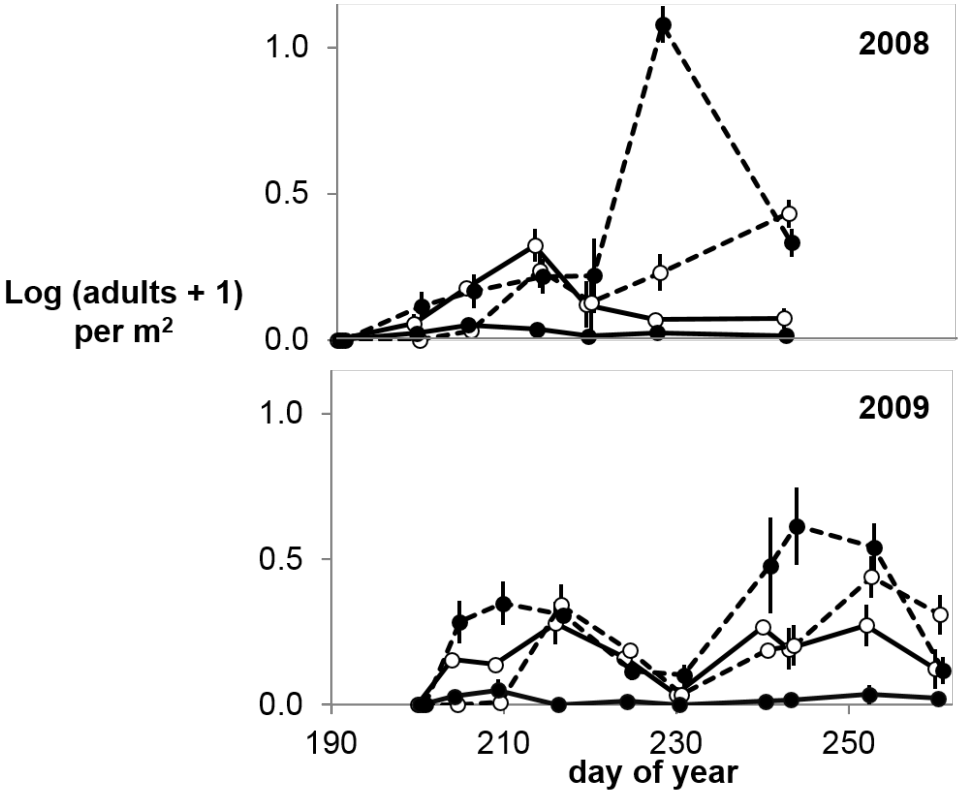

Log (adults + 1) per m²
1.0
0.5
0.0
2008
1.0
0.5
0.0
2009
190
210
230
250
day of year

Figure 1.4.

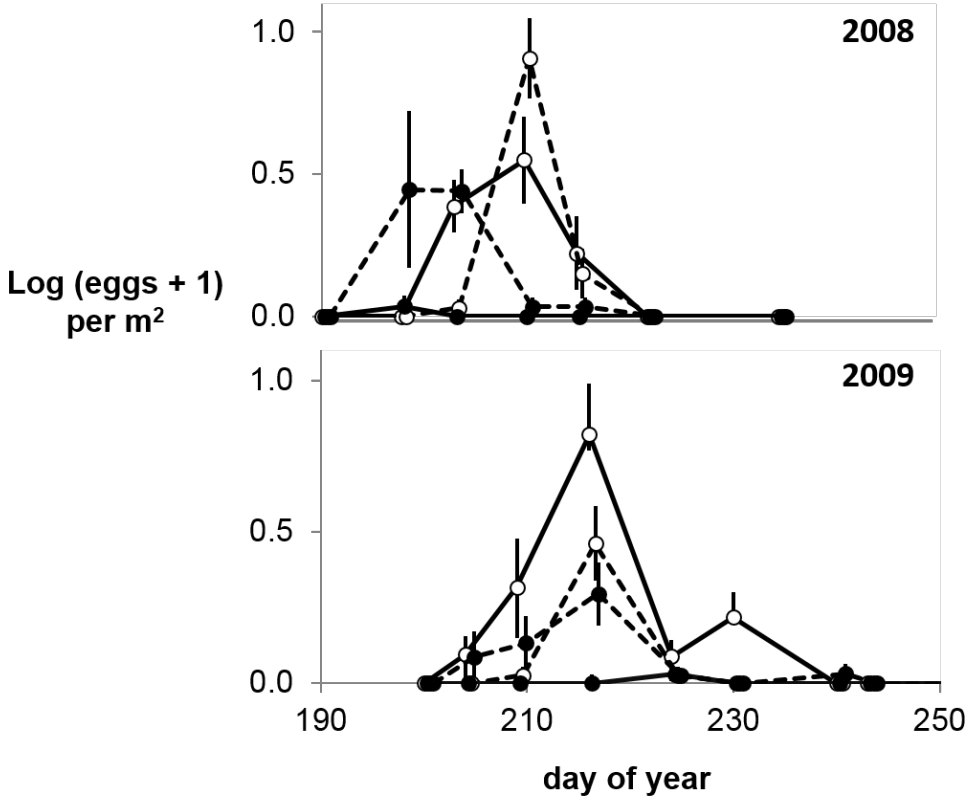
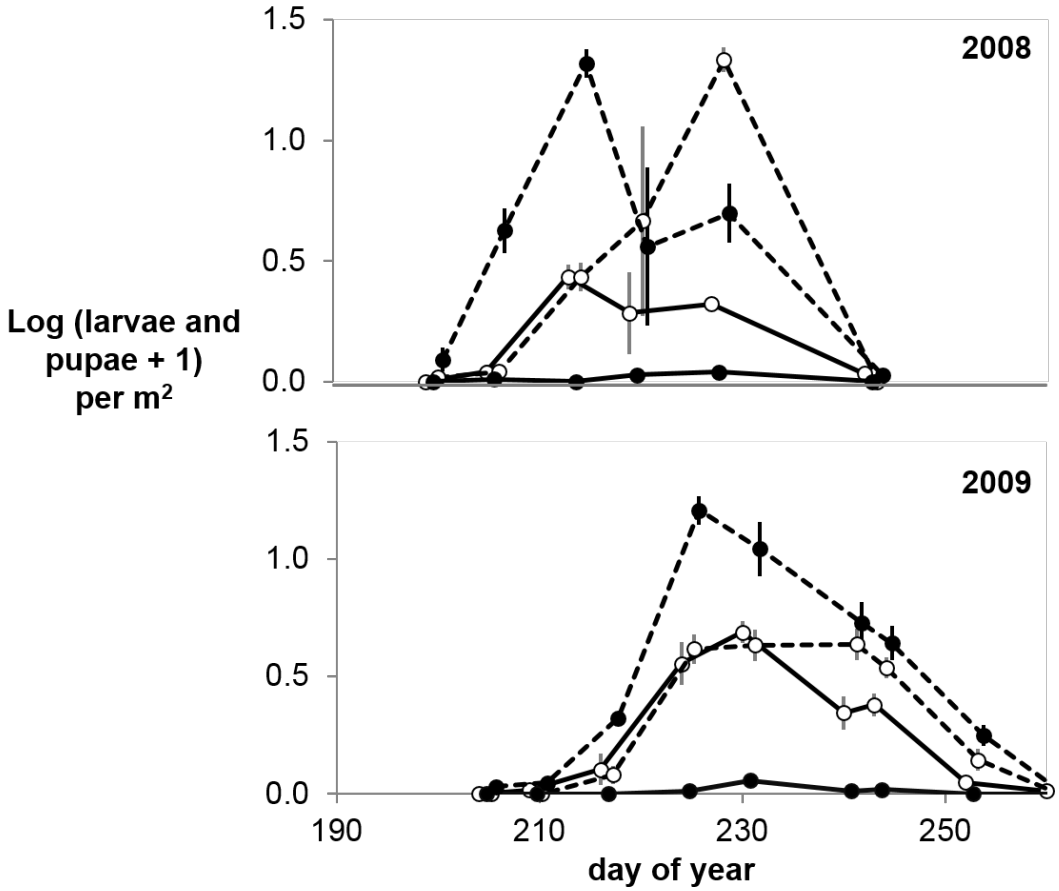

Log (eggs + 1) per m²
1.0
0.5
0.0
2008
1.0
0.5
0.0
2009
190
210
230
250
day of year

Figure 1.5.


Log (larvae and pupae + 1) per m²
1.5
1.0
0.5
0.0
2008
2009
190
210
230
250
day of year

Chapter 2. Does intra-guild predation limit a native predator's adaptation to a novel prey?

Introduction

Generalist predators should maximize their energy gain by selecting habitats in which they can obtain the most nutritional benefit from prey at the least energetic cost. This nutritional benefit relates both to prey quality and quantity; higher quality prey yield a higher per prey benefit from feeding and dense prey minimize the time a predator spends between encounters with prey (MacArthur & Pianka, 1966; Charnov, 1976; Pyke, 1984). An extensive body of research devoted to this type of decision making suggests that foraging behavior can often be predicted by considering how a forager can best maximize energy gain (Stephens & Krebs, 1986). A generalist predator, then, should expand its diet to include novel prey as long as they are as abundant and nutritionally suitable as prey already eaten (MacArthur & Pianka, 1966).

Predators that ignore nutritious and abundant novel prey challenge this simple model of maximizing energy gain. In addition to making a foraging decision based on how novel prey compare to those prey already eaten, a predator must evaluate other risks associated with consuming prey. Avoidance of predation can be more important than selecting the highest quality resources (Lima & Dill, 1990; Houston et al., 1993). Although predation risk is more often used to explain patterns of resource use among herbivores (Jeffries & Lawton, 1984), avoidance of enemies can also explain patterns of resource use among predators (Rosenheim, 2001), particularly at younger stages, when small size and lower mobility may leave them vulnerable to attack (Wissinger, 1992). Immobile eggs and pupae, as well as vulnerability during molting, put some predatory insects at great risk of predation. Thus for predators, refuge from become prey themselves could be as important as finding a good food source.

Sometimes a particular resource is associated with increased predation risk. In a review of foraging and predation risk, Verdolin (2006) found that trade-offs between foraging on a quality resource and being exposed to increased predation risk were common in terrestrial systems. This is particularly likely in systems in which generalist

predators share prey and also prey upon each other. Polis et al. (1989) argued that this intra-guild predation is common in nature and shapes predator communities. Systems with intra-guild predation would promote trade-offs between acquiring prey and risking predation because foraging on shared prey would expose a predator to the risk of being eaten by other predator species that share the prey. Habitats containing potential predators also vary in how dangerous they are. For example, complex habitats may provide more refuges and allow a forager to avoid predation, whereas habitats lacking such refuges could be very dangerous (Finke & Denno, 2002; Finke & Denno, 2006; Janssen et al., 2007; Amarasekare, 2008) and thus should be avoided.

Could predation risk limit the adaptation of a predatory insect to novel prey? Insects, including some predators, lay eggs in locations to minimize predation risk to their offspring (Ballabeni et al., 2001; Bond et al., 2005; Putra et al., 2009; Mishra et al., 2012). Further, predation on vulnerable predator stages could prevent young predators from encountering prey in some habitats. When a novel prey is a habitat specialist, a predator could therefore be prevented from feeding on it if the risk of predation in its habitat is high.

We tested the hypothesis that predation excludes a native predator from feeding on a novel, suitable prey. Such a finding would suggest that the diet breadth and habitat selection of the generalist predator might be shaped more by avoidance of risk than by resource availability.

To determine whether or not habitats containing the novel prey were associated with higher predation risk, we compared the mortality of a generalist predator in the open field habitats containing novel prey to mortality in a habitat containing a prey already commonly used in the diet. In a separate experiment we examined the impact of predation risk on the survival of native predator larvae foraging on novel and traditional prey under controlled predator levels.

If we find that mortality is higher in the habitat with the novel prey, then the failure of the native predator to feed on the novel prey might be explained by increased predation risk or poorer quality of the novel prey in the habitat. If inadequate prey quality limits the expansion of the native onto the novel prey, we would expect to see

lower survival of larvae on the novel prey than on the traditional prey even in the absence of predators. Further, we would expect high mortality among larval stages in the open field because larvae with inadequate nutrition will fail to properly molt or pupate. In contrast, if predators are driving the system, we'd expect that in the absence of predators native larvae will survive and develop on the novel prey and that in the open field high mortality will be seen on eggs and pupae because they are most vulnerable to predation. Lastly, if overall survival is higher in the novel habitat than the preferred habitat, the native predator may not have an adaptive reason for avoiding the novel habitat. This finding would warrant further research into constraints on the native predator's use of the novel prey.

Study System

We tested the hypothesis that predation limits predator diet expansion using a case in which a native, aphid-feeding lady beetle, *Coleomegilla maculata*, fails to include an exotic prey, the soybean aphid, *Aphis glycines*, in its diet. Prior to the establishment of *A. glycines*, soybean plants in the Americas were largely free of colonizing aphids (Kogan & Turnipseed, 1987). However, after its discovery in 2001 in Wisconsin, the aphid spread rapidly, becoming abundant across the Midwest (Ragsdale et al., 2011). Soybean aphid alternates between its primary overwintering host, buckthorn (*Rhamnus* spp) and its secondary host, soybean (Ragsdale et al., 2004). Lady beetles are the main predators of *A. glycines* and currently provide the most effective biological control against outbreaks of the aphid (Fox et al., 2004; Costamagna & Landis, 2007). Although a variety of lady beetle species have been observed feeding on *A. glycines* in soybean fields across the United States, most soybean fields are dominated by *Harmonia axyridis* (KKP, chapter 1), an exotic that was introduced from Asia prior to the establishment of soybean aphid (Koch, 2003). In a comparison of lady beetle species found on soybean and maize treatments in Minnesota, the abundance of *H. axyridis* was proportional to that of aphid prey across the habitats, suggesting that the beetle foraged wherever more prey were available. The native *C. maculata*, in contrast, rarely colonized soybean even when aphids were much more abundant in soybean than in maize (KKP, chapter 1).

That *C. maculata* so rarely feeds on *A. glycines* is surprising, because the species is considered to have one of the broadest diets among the aphid predators (Hodek, 1973), and it feeds on a variety of other aphids in the soybean growing region. Although aphids are best for its development and reproduction, the native predator also feeds on inferior, non-prey resources such as fungi and pollen (Lundgren & Weidenmann, 2004). In a study of the development of several lady beetle species foraging on soybean plants inoculated with *A. glycines*, Mignault et al. (2006) concluded that the soybean aphid was a high quality prey for *C. maculata*. Matos and Obrycki (2006) compared the development of *C. maculata* and *H. axyridis* on two other aphid species, *Acyrthosiphon pisum* and *Myzus lythri*, fed ad libitum in petri dishes. Both *C. maculata* and *H. axyridis* developed more quickly on *A. pisum* than on *M. lythri*, but within each prey category the development times of the two predators were very similar to each other, just as they were on soybean aphid (Figure 2.1). Since *H. axyridis* frequently feeds on *A. glycines* in the field, it is reasonable to expect *C. maculata* to also do so, given that the prey is of similar quality for both beetle species.

Since *A. glycines* is a habitat specialist, *C. maculata* might be excluded from feeding on it if foraging in soybean puts the native at risk of being preyed upon. Predation is an important source of mortality in the native beetle. In a study of egg predation by Schellhorn and Andow, between 20 and 50 percent of lady beetle eggs were eaten over 48 hours (Schellhorn & Andow, 1999a). Similarly, pupae are commonly eaten (Schellhorn & Andow, 1999b). Lady beetles sequester alkaloids that make them distasteful to many other predators (Pasteels et al., 1973; King & Meinwald, 1996) thus their most dangerous predators may be other lady beetles—either conspecifics (cannibalism) or heterospecifics (intra-guild predation).

H. axyridis, which is the most abundant predator in soybean fields where *A. glycines* is found (Fox et al., 2004), is known as an aggressive intra-guild predator (Pell et al., 2008). Intra-guild predation between the two species is probably asymmetrical, with *H. axyridis* acting much more frequently as the predator. Laboratory studies have shown that *H. axyridis* readily attacks eggs (Cottrell, 2007) and larvae (Cottrell & Yeargan, 1998) of *C. maculata*. In contrast, *C. maculata* cannibalizes its own eggs more often than

it attacks the eggs of *H. axyridis* (Cottrell, 2007) and seldom successfully attacks larvae of the exotic species. In addition to acting as an aggressive predator in laboratory studies, the guts of field collected *H. axyridis* have contained *C. maculata* DNA (Gagnon et al., 2011), suggesting that predation may be common in the field as well.

Predation by *H. axyridis* could prevent *C. maculata* from feeding on soybean aphid by causing the native to avoid soybean or by consuming those natives present in soybean. Since larvae cannot easily migrate outside their natal habitats, either of these possibilities would prevent most larvae from encountering soybean aphid. We would then expect survival of those *C. maculata* developing in soybean to be low in open field crops, even if the native could survive on *A. glycines* in the absence of predators.

Methods

Mortality in maize and soybean fields

Experimental design

To compare the mortality of *C. maculata* and *H. axyridis* in maize and soybean habitats, we estimated their abundance in plots of maize and soybean at the University of Minnesota Agricultural experiment site in central Minnesota during the 2008 and 2009 growing seasons. We established 4 blocks, each of which contained two 10 x 10 m plots that were randomly assigned to be planted with maize (Green Giant Code 63) or soybean (Northrop King S19R5), at 5.18 and 24.70 plants/ m^2 respectively. Crops were planted in 30 inch rows on June 10 in 2008 and on June 4 in 2009.

Since previous work with a similar maize variety in this region suggested that coccinellids would colonize in mid to late July (Schellhorn, 1998), we began scouting for coccinellids and aphids on a few plants per plot in the first week of July and initiated sampling after coccinellid adults had been observed, July 9 in 2008 and July 12 in 2009. No coccinellid eggs were observed on the first sampling date in 2008. On the first sampling date in 2009 2 egg masses were observed-- one in soybean and one in maize. Because of these very low numbers and the absence of coccinellids on plants prior to these dates, we assumed that one week prior to these dates there were no coccinellid eggs in either maize or soybean.

At weekly intervals, we visually inspected plants at 15 randomly selected locations per plot. Each sample consisted of two adjacent maize plants or two adjacent 18 cm sections of soybean row. Weekly sampling for the duration of the coccinellid season gave us a total of 6 sample dates in 2008 (with one week missed due to rain) and 9 sample dates in 2009, when coccinellids persisted later in the season. We concluded weekly sampling when no immature coccinellids were present on any of the plants sampled in either habitat.

Coccinellid numbers were recorded by species and stage, and all sampled coccinellid egg masses were collected for identification in the laboratory. Egg masses were checked daily in the lab, and emerged coccinellid larvae were identified to species and returned to their natal plots within 48 hours. Although holding egg masses in the laboratory prevented them from being attacked by predators, their low numbers relative to the egg masses present in the plots would make the effect of this minimal.

Statistical analysis

We calculated stage-specific mortality for both coccinellid species by habitat using the Kiritani-Nakasuji-Manly method (Manly, 1976). The mortality of each stage is calculated by dividing the area under the time-density curve for the stage by the summed area under the time-density curves for that and all subsequent stages. We estimated mortality in three stages-- eggs, young larvae (instars 1 and 2), and older larvae (instars 3 and 4). Since adults present could have emerged from resident pupae or have moved into the habitat from elsewhere, we did not attempt to estimate pupal mortality. In addition, we estimated total egg to pupae mortality.

To determine whether the dependent variable, mortality, differed by habitat, we used an RCB ANOVA model with habitat and block. We estimated p-values using a randomization test by comparing the observed F value to randomly generated F values. A randomization test was used because mortality data did not meet the assumptions of a parametric statistical model.

To establish the null distribution of F values, we randomized samples to habitat (while preserving assigned blocks and dates). We then calculated the mortality statistics and the habitat F value using the same RCB ANOVA as described above. We replicated this 500,000 times in R (script provided as an appendix to this chapter). Simulations that generated mortality values outside the biologically possible range (0 to 1) were counted and discarded. Values <0 and >1 were equally common and affected <10% of the simulations. This analysis was conducted for each year separately, and then results were combined across the two years using a Fisher exact test to calculate pooled p-values for the combined results.

Larval mortality on caged plants by habitat and predator treatments

Experimental Design

To determine the effect of habitat and aphid resource (maize with aphids vs. soybean with aphids) and predators (*H. axyridis* larvae) on the survival of *C. maculata* neonates, we conducted an experiment using exclusion cages with controlled resource and predator levels. In each of 32 trials, we divided *C. maculata* full-sib neonates among 4 treatment cages-- maize with predators, maize without predators, soybean with predators and soybean without predators and allowed them to forage for 48 hours.

Obtaining full sibling C. maculata larvae (experimental "prey")

To control for genetic variation among prey, we generated families of full sibling prey to spread across treatments. On June 2, 2009, adult *Coleomegilla maculata* were hand collected from stands of purple loosestrife in Roseville, Minnesota. In the laboratory, females (potential "grandmothers") were separated into individual petri dishes and held overnight to lay eggs. Females that did not lay eggs on their first night in captivity were housed individually at 23C, 16:8 L:D cycle and provisioned with a chicken liver based diet (Atallah & Newsom, 1966) and water. These "grandmothers" were checked daily for eggs. We used the same method to obtain additional eggs a week later, this time collecting adults from barley fields at the Minnesota Agricultural Experiment Station in Saint Paul, Minnesota (MAES).

After emerging, *C. maculata* larvae were reared in their sibling groups in petri dishes. As larvae grew they were separated into smaller groups, and ultimately held individually as adults. Females (potential "mothers") were mated with marked, field-collected males to obtain full sibling broods. Mothers were checked daily for eggs, which were removed and held at 16C. When a single mother had produced 40 full-sibling eggs, we moved the eggs to room temperature and to hatch the larvae for use in the experiment below. Only eggs from distinct, field-collected "grandmothers" were used.

Obtaining Harmonia axyridis larvae (experimental "predators")

We collected 3rd and 4th instar *H. axyridis* larvae from soybean fields at MAES just prior to the initiation of the experiment. Larvae were held in large containers with aphid infested soybean leaves for up to 24 hours prior to their use in the experiment.

Preparing treatment cages

Maize and soybean were planted on multiple dates, so that we were able to use similarly staged plants regardless of the trial start date. Maize plants used had at least 12 full leaves, but did not have exposed tassels. Soybean plants were at beginning to full flowering, but had not yet produced pods. Exclusion cages (1x1x1 m) were constructed using PVC pipe frames covered by no-see-um mesh. We placed cages over single maize plants and portions of soybean row (typically 3-5 plants) and buried 10 cm of the cage mesh around each cage to prevent the movement of insects into and out of the cage. We cleared each cage of all insects except for aphids. To avoid disturbing aphids and to make sure their densities reflected aphid densities that might naturally occur on plants, we did not manipulate aphid levels. Instead, we grouped cages with similar aphid densities for each experimental replicate. Although all plants had at least 20 aphids at the start of each replicate trail, initial aphid densities ranged up to 2000 aphids in maize and 4500 in soybean.

Each cage had 10 *C. maculata* larvae, all of which were full siblings. In the laboratory, we gently divided neonates from each full sibling family into four groups of 10 larvae, immediately transported the family groups in coolers to the field site at MAES (a five minute walk), and transferred 10 larvae to the lower leaves of the caged plant(s) in

each treatment. This was repeated for each family ready for placement (at least 40 walking individuals) on that day. Eight fourth instar *H. axyridis* larvae were then added to the tops of plants in each predator treatment cage.

After 48 hours, we returned to the caged plants to estimate the survival of *C. maculata* larvae by visually inspecting (without touching) the caged plant, the surrounding soil and the mesh cage itself for coccinellids. We then placed a white sheet around the base of the caged plant and thoroughly searched the plant. After counting and removing any visible larvae from the plant and sheet, we bagged and removed the plant for dissection and further inspection in the laboratory. All *C. maculata* and *H. axyridis* larvae were counted and missing larvae were presumed dead.

Statistical analysis

To demonstrate that we were able to create and maintain the desired experimental conditions across caged treatments, we conducted ANOVAs using habitat and predator treatments as predictors of initial aphid numbers (natural log of aphids per cage) and final predator numbers (number of *H. axyridis* larvae per cage). If there were no bias in assigning cages to receive *H. axyridis* predators, we should not see differences in initial aphid numbers within one habitat type. It was also necessary to confirm that even if some fourth instar *H. axyridis* in predator treatments were to pupate or escape over the course of the experiment, significant predator pressure remained at the end of the 48 hours in both predator treatments.

Our primary interests were in whether the survival of *C. maculata* larvae differed when feeding on *R. maidis* on maize or *A. glycines* on soybean, and how the presence of *H. axyridis* predators affected survival on each of these habitats. To assess this we ran a generalized linear model in the MASS package in R, specifying a logit link and binomial error. Binomial error best represented the nature of the response (each *C. maculata* larva either survived or did not) and the logistic model best matched our data distribution. We considered the two main factors relating to our treatment groups-- habitat (maize or soybean) and predator (*H. axyridis* larvae added or not) as predictors and number of *C. maculata* larvae recovered (or not) as the response. There was natural variation in initial aphids per cage, so we also added this as a factor in the model. A positive relationship

between initial aphids and survival would suggest that the *C. maculata* neonates were indeed using aphid resources in these habitats.

Since members of each of the 32 full-sibling families were tested simultaneously across the four treatment conditions, Family is a blocking factor for variation in experimental conditions (weather during trial, etc.), as well as genetic differences among families.

To examine the relationship between initial aphid levels and *C. maculata* survival, we also ran log linear regressions of *C. maculata* survival on the natural log of initial aphids in the MASS package in R. As previously, we used a logit link and binomial error, but this time we excluded the two treatments to which predators were added, to isolate the effect of predation from the effect of habitat on survival.

Results

Mortality in open field studies

Coccinellid density curves showed overlap among stages, but followed the expected pattern with eggs appearing first, followed by young larvae, older larvae and pupae (Figure 2.1). All species and stage combinations had clear start dates before which no individuals were observed. Clear end dates were observed for all stage/species/habitat categories except fourth instar larvae in 2008, when some individuals remained on the last sampling day—*C. maculata* in maize and *H. axyridis* in soybean. These fourth instar larvae were at very low densities, representing about a tenth and a hundredth of peak densities respectively. Given the low densities of these larvae and the fact that very few food resources remained in maize or soybean, it is very unlikely that these would have survived to pupation, and thus our estimates of survival based on these data are reliable.

Calculated stage-specific mortality varied greatly over species and stage, habitat and year (Table 2.1). For both *C. maculata* and *H. axyridis*, the highest estimated mortality was among fourth instars becoming pupae in soybean in 2008 (100 and 75% respectively). The 100% mortality of *C. maculata* fourth instars in soybean is not surprising, given the very low sample size of older larvae in soybean, and is reflected in the lack of statistical differences in the mortality of older larvae between habitats.

Although our focus was on differences in stage specific mortality by habitat, examining mortality by stage also revealed that, with the exception of *C. maculata* eggs in maize, mortality was high among older larvae and lower among younger larvae and eggs (Table 2.1).

In general, stage-specific mortality tended to be higher in maize than in soybean. Both species showed higher mortality in maize across stages in 2009, while in 2008 mortality was higher in maize for eggs of both species. Differences in mortality between maize and soybean were more evident in *C. maculata* than in *H. axyridis* (Figure 2.3). Although the patterns in mortality by habitat were fairly consistent, only 2009 *C. maculata* egg mortality was significantly higher in maize based on our randomization and F test (Figure 2.3, $p=0.03$). A separate comparison of total mortality revealed no differences in overall mortality (egg to pupa) by habitat for either species (Table 2.1). This makes sense given the high mortality among eggs in maize and fourth instar larvae in soybean in 2008.

Cage studies with controlled predator exposure

After 48 hours, less than one predator (*H. axyridis* larva) was recovered, on average, from each "no predator" treatment cage. In contrast, a mean of over 4 predators per cage were recovered from predator treatments (Figure 2.4). These differences were statistically significant based on an ANOVA testing the effect of habitat and predator treatments on predator recovery per cage (Table 2.2). There was no relationship between the number of predators recovered at 48 hours and the habitat type (Table 2.2), showing that there were no differences in predator numbers between maize and soybean habitats in predator treatment cages.

There were significant differences in initial aphid numbers among treatments (Table 2.2). Specifically, within one habitat type, starting aphid numbers were similar across predator treatments (Table 2.2), but across habitat types, soybean habitats tended to have more aphids per cage at the start of each trial than did maize habitats (Figure 2.4). Since surveys of maize and soybean habitats in 2008 and 2009 showed that soybeans consistently had a higher density of aphids than did maize (KKP Chapter 1), these differences likely reflect real differences between habitats.

The number of prey (*C. maculata* larvae) recovered after 48 hours on caged plants ranged from none to all 10, but mean larvae recovered was low for all of the predator and habitat combinations (Figure 2.5). Larvae caged on soybean plants with predators added had the lowest survival, while the highest survival was among larvae on maize plants from which predators had been removed. Log-linear regression of habitat treatment, predator treatment, and initial aphids on prey survival showed that all three of these factors significantly influenced the survival of *C. maculata* prey, with low aphid densities, the presence of predators and being placed in a soybean habitat all resulting in lower prey survival. The effect of predators was similarly negative on both maize and soybean. However, a significant interaction between initial aphid numbers and habitat type suggests that aphid numbers had different effects in maize and soybean (Table 2.3). We further examined this interaction by plotting prey survival by initial aphid numbers for each habitat separately (Figure 2.6). Although natural log of starting aphids showed a positive effect on larval survival within the soybean no predator treatment, there was no clear relationship between starting aphids and survival within the maize no predator treatment (Figure 2.6, Table 2.4).

Discussion

A predator might fail to feed on a nutritious novel prey because it cannot locate or recognize the novel prey. Predatory insects, for example, rely in part on innate responses to volatile chemicals released by plants to locate prey (Steidle & van Loon, 2003; Penaflor et al., 2011), and thus may not immediately identify novel prey by its chemical cues. Further, there might be inherent limits to the diversity of resources that can be used. Slower information processing has been used to explain limits on the resource use (Bernays, 1989; Janz & Nylin, 1997; Janz, 2003) and oviposition site selection (Courtney, 1983) of herbivorous insects. Similarly, predators feeding on diverse prey have been shown to be less efficient than more specialized predators (Dukas & Kamil, 2001).

Assuming a novel prey is as abundant and nutritious as those prey already eaten, nutritionally suitable prey might be ignored if it is more difficult to locate, capture or

digest than are alternative prey (Stephens & Krebs, 1986). Additionally, some researchers have found that when a forager incorporates a novel resource into its diet, it may fare worse on its traditional resources (Fry, 2003; Scheirs et al., 2005). Although these factors can be important, they are not likely sufficient to explain why a predator would fail to feed on novel prey that possess no special defenses and are nutritionally very similar to preferred prey.

C. maculata pupae were not recovered from soybean habitats, but this is unlikely to mean that *C. maculata* were unable to survive in the habitat. Instead, the very low initial numbers of *C. maculata* in soybean made survival difficult to estimate at all stages, thus failure to observe pupae may be a function of sample size. Given the variation around survival estimates by habitat, *C. maculata* were statistically just as likely to survive in soybean as they would have in maize, thus contrary to our prediction, the native predator experienced the same total mortality in soybean as in its preferred habitat over the two years studied. Similarly, *H. axyridis*, which was very abundant in both soybean and maize, had the same mortality across habitats. Since the mortality of both species across habitats was similar it remains surprising that the native is rare in soybean, while *H. axyridis* distributes itself across both habitats. Given the relative success of the native on *A. glycines* in soybean, failure to colonize soybean and feed on *A. glycines* may well be maladaptive.

Examining mortality in greater detail, older larvae of both species examined had very high mortality. Since larvae were limited to pupating on soybean or maize plants, it seems that these larvae were either too malnourished to pupate or pupated and were subsequently preyed upon. When larvae are too weak to pupate or must resort to preying on the pupae of other lady beetles, it is likely that prey resources are very scarce (Schellhorn & Andow 1999b). When aphid populations crash lady beetle larvae may be left without food at a time when they are particularly voracious. This is consistent with the decline in aphid numbers that we observed in both habitats late in the season as many larvae were pupating (KKP, chapter 1).

Young larvae experienced much lower mortality—only about half that of older larvae. This is not surprising since smaller larvae have lower nutrition requirements

(Lundgren & Weber, 2010) and also access to more abundant prey resources (Dixon, 2007). This also suggests that cannibalism and intraguild predation on young larvae, while often observed in the laboratory (Cottrell & Yeargan 1998; Pell et al. 2008), is not so common in the field. One notable effect of habitat, was that young larvae of both the native and the exotic had lower survival in maize than in soybean. This could have been because food resources were scarce in maize or because larvae were at greater risk of being preyed upon in maize, or both. Our data do not allow us to distinguish among these possibilities, however it is seems that the lower aphid densities observed in maize would both make it harder for young larvae to find prey and leave adults and other predators hungrier and more likely to attack.

Although past work suggested egg mortality would be high in maize (Schellhorn & Andow 1999a), we found that egg mortality of both species in soybean and of *H. axyridis* in maize was similar to that of young larvae and that older larvae were the least likely to survive. Mortality of older larvae could be the result of limited resources (older larvae fail to pupate) or predation (older larvae pupate but are eaten before we observe them). Although this information does not allow us to distinguish between resources driving mortality and predation driving mortality, it does suggest that the late larval stage is particularly important and warrants further investigation both from a resources and a predation standpoint.

A surprising exception to the overall trends of mortality by stage described above was mortality of *C. maculata* eggs in maize (73% in 2008, 81% in 2009) which was higher than the mortality of any other species/stage/habitat combination. Since egg mortality is primarily due to predation, we can assume that *C. maculata* eggs were significantly more likely to be preyed on in maize than in soybean, and within maize, were more likely to be preyed upon than were *H. axyridis* eggs. A possible explanation is that while both *C. maculata* and *H. axyridis* attacked *C. maculata* eggs in maize, only *H. axyridis* attacked *H. axyridis* eggs. Cottrell observed these preferences when examining egg predation between these species in small laboratory arenas (Cottrell, 2004). Although the native strongly preferred to feed on its own eggs, the exotic did not show this preference (Cottrell 2004). In our study, since the two species were similarly

abundant in maize, *C. maculata* eggs would be exposed to about twice as many potential predators as would *H. axyridis* eggs. In contrast, in soybean, where *C. maculata* were rare, the eggs of both species would have experienced similar predation rates by *H. axyridis*.

The survival of *C. maculata* neonate larvae on caged soybean and maize provide insights into the interaction of the native beetle with its resources. Since these larvae were placed on plants just after eclosion, they are most similar to the eggs becoming young larvae category from the open field study. However, since we allowed them to emerge before placing them, predation before eclosion was impossible, allowing us a unique opportunity to examine how young larvae cope with the dual pressures of finding resources and escaping predation.

Although in the absence of predators, *C. maculata* neonates were less likely to survive on caged soybean plants than on maize plants, the difference in survival was small. Further, the significant, positive relationship between starting aphid number and neonate survival suggests that the native used *A. glycines* as a food resource and supports the conclusion made by Mignault et al. (2006) that soybean aphid is a suitable resource for the native lady beetle. Further, the positive impact of initial aphids on survivorship within predator treatments supports the idea that increased extra guild prey (in this case, soybean aphid) reduces the impact of intra guild predation, as previously reported by Lucas et al. (1998).

It is possible that although the native feeds on *A. glycines*, something about the soybean habitat itself makes foraging on soybean difficult. Eisner et al. (1998) found that despite quality resources, *Hippodamia convergens* struggled to forage on plants with hooked trichomes. Similarly, movement on the surface of a hairy soybean plant might be challenging for young larvae foraging for aphids. In this case higher aphid densities would make it possible to forage successfully with less movement on the plant.

The absence of a positive relationship between aphid number and survival on maize is more difficult to explain. All other insects had been removed from the caged plants and they were not yet producing pollen. It may be that even at very low aphid levels, *C. maculata* neonates are able to forage effectively enough on maize to survive, as

found by Schellhorn and Andow (1998). Although neonate survival was lower in treatments with *H. axyridis* predators, the lack of interaction between predation and habitat type suggests that the effect of predation is equally strong in both habitats and independent of habitat type.

Our results were not consistent with either a resource-based or a predator-based explanation for why the native predator does not fully exploit resources in soybean. If resources were inadequate for the native in soybean we would have seen lower overall survival in soybean compared to maize, particularly of larvae. Further, we would have expected a stronger positive relationship between resource density and survival in maize compared to soybean in the absence of predators. We saw neither of those things and in fact saw a very strong positive relationship between densities of the novel aphid, *A. glycines* and *C. maculata* survival in soybean cages.

The ability of *C. maculata* to exploit resources in soybean is not likely limited by predation by *H. axyridis*. Despite the exotic lady beetle's reputation as an aggressive intra-guild predator, the patterns we observed suggest that the high levels of predation we saw on native eggs in maize were likely due to cannibalism by other *C. maculata*, rather than predation by *H. axyridis*. The effect of *H. axyridis* predation on caged *C. maculata* neonates was small, and did not differ between soybean and maize habitats, further suggesting that predation by the exotic was not a limiting factor for the native's use of soybean aphid, at least at the egg and early larval stage.

The mismatch we observed between *C. maculata* habitat preference and performance regarding maize and soybean warrants an examination of possible constraints on the ability of the native beetle to forage optimally across these habitats. At best, the native predator is unable to identify the good time periods during which to forage in soybean. At worst, the native consistently ignores a high quality resource and misses out on the opportunity to expand into suitable new habitats. If maize is frequently the better habitat, always laying eggs in maize might be a better long term strategy than would always laying eggs in both habitats. However, such a strategy still implies a behavioral constraint because it would be better to recognize good *A. glycines* time periods and expand into soybean accordingly.

It would be interesting to examine *C. maculata* preferences for ovipositing in soybean and maize under controlled conditions to discover if there is a predictable point at which conditions on soybean are good enough for *C. maculata* to lay eggs there. It may be that if soybean were not just similar to maize but actually better than maize, *C. maculata* would oviposit there. Similarly, by varying predator and prey levels, we could investigate how *C. maculata* perceives its surroundings, particularly with regard to soybean. Is *C. maculata* better able to evaluate conditions on maize compared to soybean for example?

Another possibility is that *A. glycines* is a consistently suitable prey but *C. maculata* doesn't recognize it as a resource. Coccinellids locate aphids using the volatile chemicals released by plants when the aphids feed (Ninkovic et al., 2001; Sarmento et al., 2007; Girling & Hassall, 2008) so the absence of soybean aphid prior to 2001 would mean that the unique volatile profile of aphid infested soybean would be relatively new to *C. maculata*. Since soybean lacked aphid prey prior to the arrival of soybean aphid, the offspring of any lady beetle laying eggs in it would have died, and any propensity for ovipositing in soybean might have been selectively eliminated. Futuyma et al. (1995) found that insects can lack genetic diversity in oviposition behavior such that they more quickly adapt to feeding on a new resource than to ovipositing on it (Futuyma et al., 1995). In contrast, *H. axyridis*, which originated in Asia, has a shared evolutionary history with both soybean and its aphids. This difference in the evolutionary history of the two predators might explain why *H. axyridis* readily accepts soybean for egg laying, and can use soybean according to aphid abundance.

Predatory coccinellids switch among aphid prey easily, and this has been attributed in part to the very broad diets of their ancestors (Sloggett & Majerus, 2000; Giorgi et al., 2009). Thus, although herbivores may take many years to begin colonizing novel plants (Andow & Imura, 1994; Braendle et al., 2008), similar lags in predatory coccinellids may be less common. Our study shows that feeding on *A. glycines* would be adaptive for *C. maculata* to use, thus this may be a case in which there is a lag in the response of a predator to a novel resource. Since females display a variety of egg laying behavior, it is likely that some females are more likely to oviposit on soybean than others.

By examining the heritability of preference for soybean, we could better predict whether we should expect *C. maculata* to begin ovipositing in soybean in the near future. If there is not much variation in egg laying behavior with respect to acceptance of soybean, we might expect this gap to persist for years to come.

Table 2.1. Mean mortality by species, stage, habitat and year. *F* values based on ANOVA comparing mortality by habitat within each category. P-values based on randomization test.

	2008				2009			
C. maculata	**Maize**	**Soybean**	***F***	**p**	**Maize**	**Soybean**	***F***	**p**
egg	0.73	0.31	4.6	0.07	0.81	0.22	6.8	**0.03**
larva 1,2,3	0.59	0.30	5.5	0.06	0.21	0.09	1.0	0.42
larva 4	0.93	1.00	1.7	0.28	0.43	0.29	1.3	0.35
total	0.98	1.00	2.3	0.21	0.96	1.00	0.7	0.48
H. axyridis	**Maize**	**Soybean**	***F***	**p**	**Maize**	**Soybean**	***F***	**p**
egg	0.45	0.43	0.0	0.90	0.11	0.00	10.1	0.26
larva 1,2,3	0.17	0.29	5.0	0.07	0.49	0.02	11.6	0.25
larva 4	0.62	0.75	6.5	0.04	0.73	0.65	0.2	0.75
total	0.83	0.90	1.0	0.43	0.78	0.89	3.3	0.12

Table 2.2. Analysis of variance of predator and aphid densities. ANOVA using habitat and predator treatments as predictors of the final densities of predators by cage and the densities of initial aphids by cage.

H. axyridis larvae remaining per cage after 48 hours

factor	**DF**	**Ms**	***F***	**p**
habitat	1	11.3	2.6	0.108
predator	1	450.0	104.5	<0.001
habitat x predator	1	1.1	0.3	0.610
residuals	124	4.3		

Natural log of aphids per cage at the start of each trial

factor	**DF**	**Ms**	***F***	**p**
habitat	1	12.4	12.3	0.001
predator	1	1.1	1.1	0.304
habitat x predator	1	0.1	0.1	0.766
residuals	124	1.0		

Table 2.3. Analysis of deviance table for *C. maculata* neonate larval survival by habitat and predator treatments in the cage experiment.

Factor	**Df**	**Deviance**	***p***
habitat	1	12.4	<0.001
predator	1	40.7	<0.001
log(aphid density)	1	15.3	<0.001
habitat*predator	1	0.4	0.531
habitat*log(aphid density)	1	16.8	<0.001
predator*log(aphid density)	1	0.6	0.421
habitat*predator*log(aphid density)	1	0.9	0.355

Table 2.4. Regression of *C. maculata* survival after 48 hours on natural log of initial aphids. Analyses conducted separately for soybean and maize habitats (predator treatments excluded), using a general linear model in R. Glm(survival~ log(initial aphids), family=binomial(logit)).

Soybean	**Estimate**	**Std. Error**	**z**	***p***
Intercept	-4.287	0.857	-5.004	<0.001
Log (aphids)	0.519	0.147	3.535	<0.001

Maize	**Estimate**	**Std. Error**	**z**	***p***
Intercept	-0.951	0.654	-1.454	0.146
Log (aphids)	0.009	0.133	0.067	0.946

Figure Legends

Figure 2.1. Mean preimaginal development (egg to adult) in days (± SE) of *C. maculata* (solid) and *H. axyridis* (stripes) on 3 aphid species at 24°C. *Myzus lythri* and *Acyrthosiphon pisum* development from Matos and Obrycki (2006), *Aphis glycines* from Mignault et al. (2006). Mean development times and standard error for *M. lythri* and *A. pisum* were taken directly from Matos and Obrycki (2006). We combined means and errors of the sexes reported by Mignault et al. (2006) to generate a single mean for each species to compare to the data of Matos and Obrycki (2006).

Figure 2.2. Mean density by date for *H. axyridis* in soybean in 2008. This illustrates data used to estimate stage specific mortality—eggs (light grey), young larvae (grey), older larvae (dark grey) and pupae (black).

Figure 2.3. Mean difference in stage specific mortality (± SE) of *C. maculata* (solid) and *H. axyridis* (dash) between maize and soybean habitats. Grey lines show values from 2008, black lines from 2009. Values above the zero line indicate that mortality was higher in maize, while those below indicate that mortality was higher in soybean.

Figure 2.4. Mean aphid and predator (*H. axyridis* larva) densities (± SE) per treatment cage. N = 32 replicates. Aphid density (shown in light grey) at the start of the experiment are $\log_{10}$ transformed. Number of *H. axyridis* larvae (shown in black) recovered per cage at the end of the experiment. Error bars are standard error of the mean. Aphid numbers differed significantly between habitats but not predator treatments, while predator numbers differed significantly between predator treatments but not habitats (Table 2).

Figure 2.5. Mean number of *C. maculata* larvae recovered by treatment (± SE). N = 32 replicates. Error bars are standard error of the mean. More *C. maculata* were recovered

from maize than from soybean ($p<0.001$) and more were recovered in the absence of predators ($p<0.001$). (Table 3).

Figure 2.6. *C. maculata* larvae recovered by initial aphid number in no predator treatments. Light circles represent maize, and dark circles represent soybean. Regression of survival on initial aphid number showed no relationship in maize, but a statistically significant positive relationship in soybean (Table 4).

Figure 2.1.

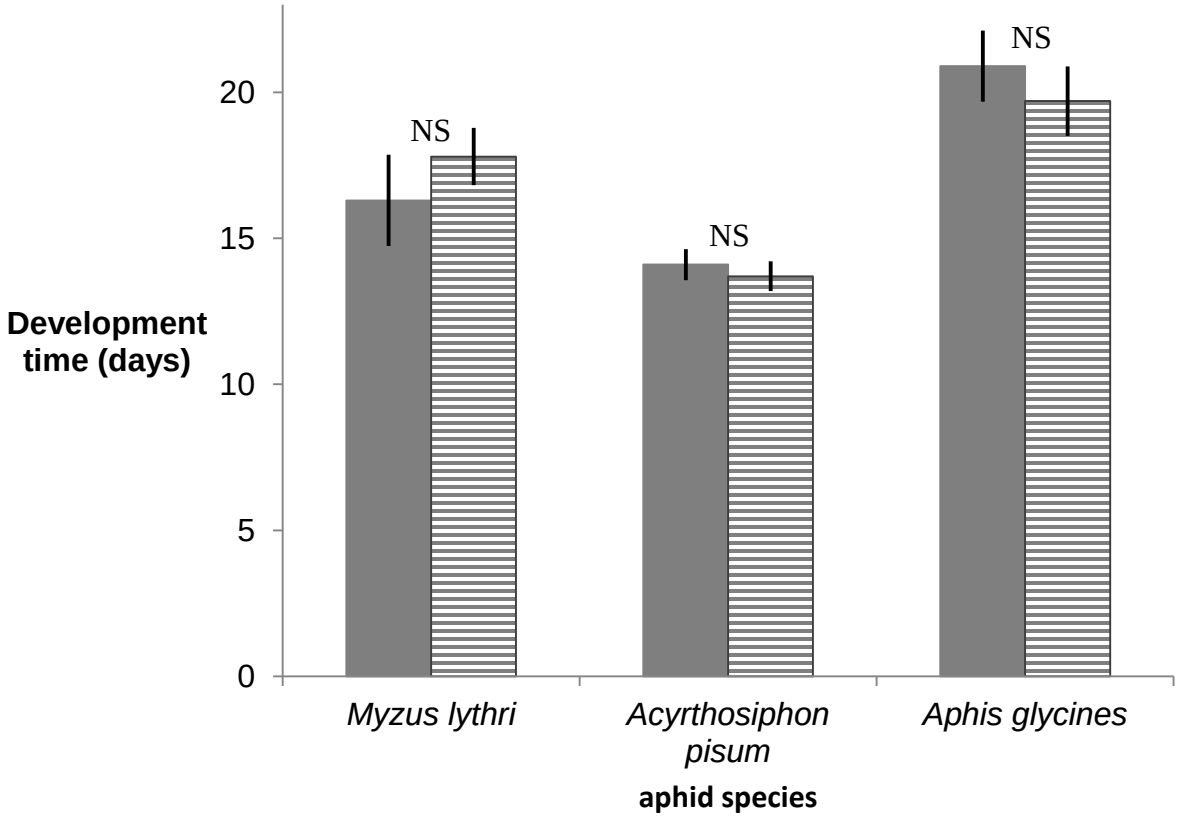

Development time (days)
0
5
10
15
20
NS
NS
NS
Myzus lythri
Acyrthosiphon pisum
Aphis glycines
aphid species

Figure 2.2.

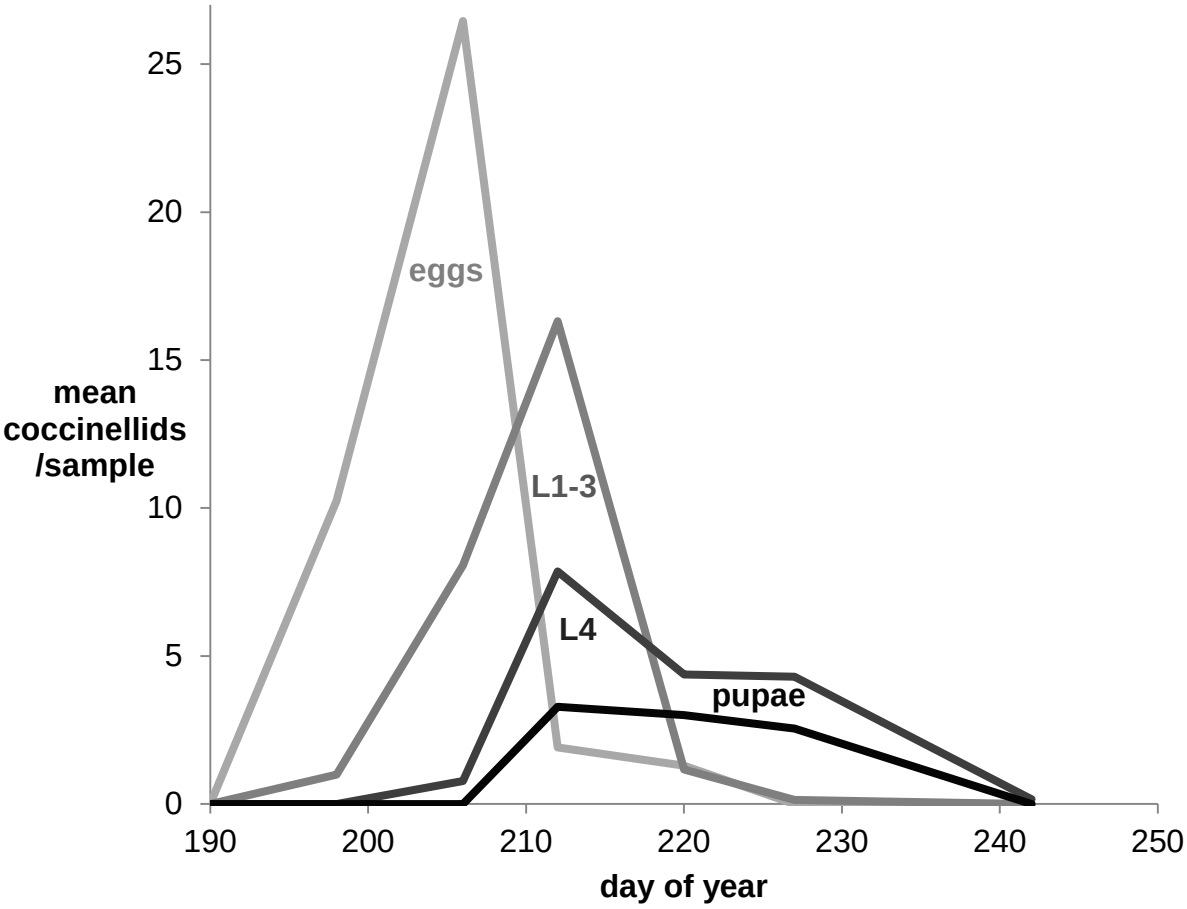

25
20
15
10
5
0
mean coccinellids /sample
eggs
L1-3
L4
pupae
190
200
210
220
230
240
250
day of year

Figure 2.3.

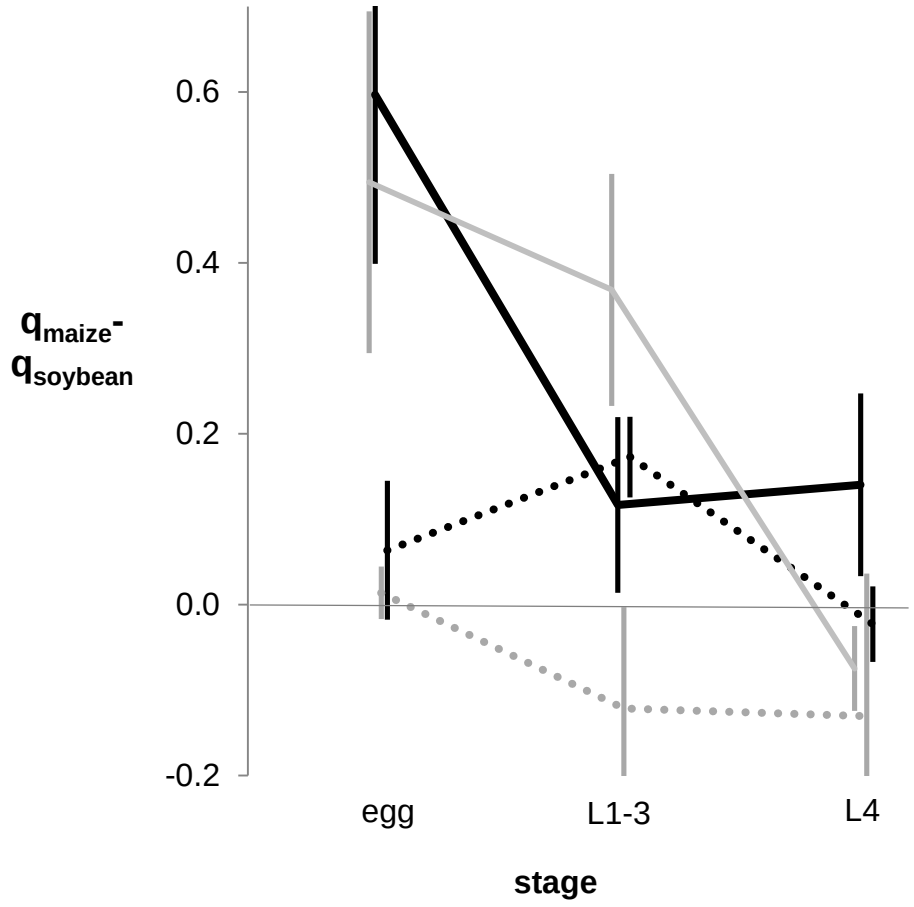


Figure 2.4.

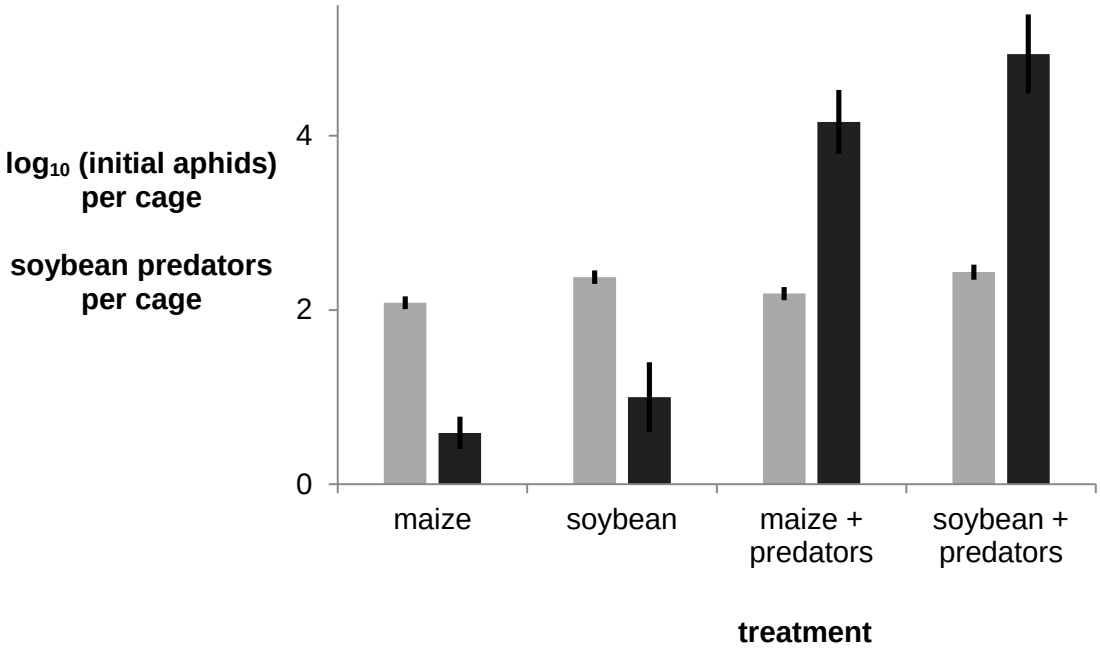

log10 (initial aphids) per cage
soybean predators per cage
4
2
0
maize
soybean
maize + predators
soybean + predators
treatment

Figure 2.5.

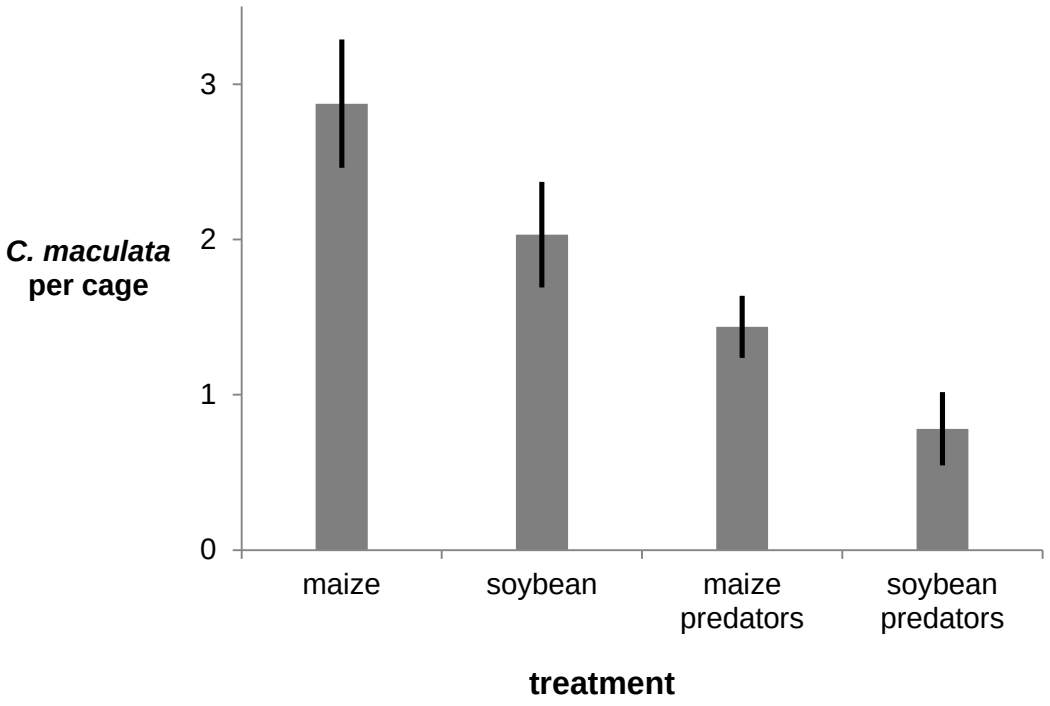
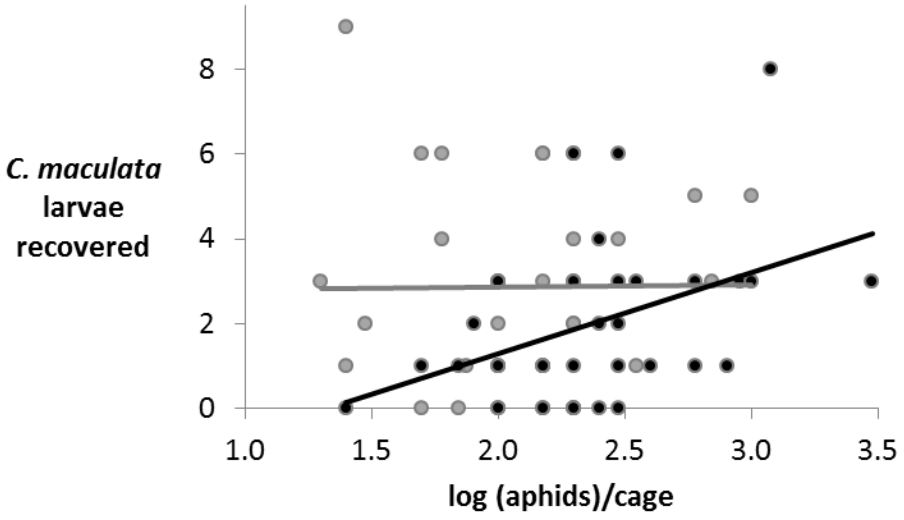

C. maculata per cage
3
2
1
0
maize
soybean
maize predators
soybean predators
treatment

Figure 2.6.


C. maculata larvae recovered
8
6
4
2
0
1.0
1.5
2.0
2.5
3.0
3.5
log (aphids)/cage

Appendix.

Program to randomize density data across habitats and compare Fstats from randomized data to those calculated using real data. Written for R version

Summary

- This program randomly assigns density data from samples to habitats (maize or soybean) to generate F statistics for the difference in mortality of a given species and age by habitat.
- The program adds zeroes to the 2 dates before the first observation and after the last observation of each species/age and substitutes any "NAN"s generated with dummy values that do not change the difference between habitats.
- The program compares F stats generated from the randomized data to those based on real data and reports the number of randomized Fs that are above the real Fs.

Inputs (all are .csv files)

1. The file "pairmeansyeartype" which contains observed insect densities for each sample (adjacent maize plants or 36 cm section of soybean row). This data frame must be called "pairmeans." The first 4 columns are the factors "Date", "P" (plot), "Cr" (habitat) and "Blk" (block) followed by aphid and then coccinellid density data.

#2. The file "fstatyeartype" which contains Fstat values calculated from ANOVAS using real data that will be compared to randomly generated data for mortality by stage/species. The columns are "Group" and "Fstat."

#3. The file "species and stagesyeartype" that contains a list of species included in mortality calculations.

Outputs (.csv files)

The main output is a data frame containing "group" (to identify the species and stage under consideration), "sum<f" (number of times the randomized F statistic was less than the real F statistic), "reps" (number of randomizations run) and "successes"(number of

randomizations run that generated mortality values within the biologically possible range 0 and 1). All fstats generated are also saved in data frame called “fstat rand.”

---

rm(list=ls())

ReP<-100000 #adjust for number randomizations that will be run

#1. LOADING DATA FRAMES AND PREPARING THEM FOR USE IN PROGRAM

pairmeans<-read.csv("C:/Users/Kristina/Documents /pairmeans2008c3.csv",header=T)

species <-read.csv("C:/Users/Kristina/Documents/species and stages2008c3.csv",header=T, na.strings="-999")

fstatreal<- read.csv("C:/Users/Kristina/Documents/fstat2008c3.csv",header=T, na.strings="-999")

dim(pairmeans)

col=dim(pairmeans)[2]

row=dim(pairmeans)[1]

by3<-pairmeans[,"P"]

by2<-pairmeans[,"Date"]

plotmeans<-aggregate(x=pairmeans, by=list(by3,by2),FUN="mean")

totsurvstages <- sum(species[,3]) #this gives me the number of columns I need in my fstat matrix in step 3a below.

fstatreal<-as.data.frame(fstatreal)

fstatreal$Fstat<-as.numeric(fstatreal$Fstat)

#2. SET UP FOR RANDOMIZATION (PAIR DATA SAMPLED ACROSS BLOCKS AND RANDOMLY ASSIGNED TO HABITAT).

#2A. USE COMMAND “LENGTH” TO SHOW # OF PLANTS BY CATEGORY SO THAT RANDOM SAMPLING CAN ACCURATELY REFLECT ORIGINAL DATA

```
by4<-pairmeans[,"Blk"]
by5<-pairmeans[,"Date"]
by6<-pairmeans[, "Cr"]
aggcrop<-aggregate(x=pairmeans,by=list(by4,by5,by6),FUN="length")
aggnocrop<-aggregate(x=pairmeans, by=list(by4,by5),FUN="length")
aggcrop<-aggcrop[with(aggcrop,order(Group.1,Group.2,Group.3)),]
aggnocrop<-aggnocrop[with(aggnocrop,order(Group.1,Group.2)),]
```

#2B. SET UP MATRIX WITH BLOCKS AS COLUMNS, DATES AS ROWS

```
aggnocrop1<-subset(aggnocrop,Group.1==1)
uniquedate<-dim(aggnocrop1)[1]
dimnocrop<-matrix(,uniquedate,0) #storage spot
block1<-aggnocrop[1:uniquedate,2]
block2<-aggnocrop[(uniquedate+1):(2*uniquedate),2]
block3<-aggnocrop[((2*uniquedate)+1):(3*uniquedate),2]
block4<-aggnocrop[((3*uniquedate)+1):(4*uniquedate),2]
dimnocrop<-cbind(dimnocrop,block1, block2,block3,block4)
```

#This gets the number of unique dates to be placed into the matrix “dimnocrop” which has blocks as its columns and dates as its rows.

#3. RUNNING THE RANDOMIZATION.

#3A. SETTING UP MATRICES TO STORE RANDOMIZED DATA AND FSTATS CALCULATED FROM IT.

```
dimp<-dim(pairmeans)
randpairmeans<-matrix(,0,dimp[2]) #destination of randomized data
numspecies <- dim(species)[1]
```

```
t1 <- sum(species[,3] <= 1) #The number of species for which survival will NOT be calculated.
numsurvspecies<-(numspecies-t1)
totstages <- sum(species[,2])
totsurvstages <- sum(species[,3])
survspecies <- species[,3] > 1 #logical vector TRUE for species with survival estimated
survcalcs<-totsurvstages-(sum(survspecies))

fstatsum<-matrix(0,survcalcs,2)
colnames(fstatsum)<-c("group","Fstat")
fstatsum<-as.data.frame(fstatsum)
fstatsum$Fstat<-as.numeric(fstatsum$Fstat)
test<- 0 #gives number of zloop runs attempted
success<-0 #gives number fstats calculated
failure<-0 #gives number rejected due to 2 or more NaNs in a column
fstatrand<-matrix(-999,survcalcs,1)
under<-0
over<-0
Nantotal<-matrix(0,1,(survcalcs+2))
Under<-matrix(0,1,(survcalcs))
Over<-matrix(0,1,(survcalcs))

#3B.  THIS LOOP TELLS PROGRAM HOW MANY TIMES TO RANDOMLY SAMPLE (IN THIS EXAMPLE I SAMPLE 1 TIMES)
        for (z in 1:ReP) {
fstatrand<-matrix(-999,survcalcs,1) #stores randomized f-values if we need to

#3C.  LOOP SAMPLES ACROSS BLOCKS AND DATES AND ASSIGNS HABITAT
                for (i in 1:4) {
                block1<-subset(pairmeans,Blk==i)
```

```
                for (j in 1:uniquedate) {
                blockdate<-subset(block1,Date==dimnocrop[j,i])
                randblockdate<-blockdate[sample(nrow(blockdate),(aggcrop[(((i-
                1)*(2*uniquedate))+((2*j)-1)),4]),replace=T),]
                randblockdate[1:aggcrop[(((i-1)*(2*uniquedate))+((2*j)-
                1)),4],3]<-1
                randpairmeans<-rbind(randpairmeans,randblockdate)
                randblockdate<-blockdate[sample(nrow(blockdate),(aggcrop[(((i-
                1)*(2*uniquedate))+(2*j)),4]),replace=T),]
                randblockdate[1:aggcrop[(((i-1)*(2*uniquedate))+((2*j))),4],3]<-2
                randpairmeans<-rbind(randpairmeans,randblockdate)
                } #closes j
        } #closes i

# 4 INITIALIZE SOME VALUES THAT WILL BE USED IN THE PROGRAM
data<-randpairmeans #rename data to make compatible with survivorship program
na.string <- -999
block <- 4
crop <- 2

#5.CALCULATE PLOTMEANS BY EA. OBSERVATIONAL UNIT (PLOT) & DATE.
by3<-data[,"Cr"]
by2<-data[,"Date"]
by1<-data[,"Blk"]
plotmeans <- aggregate(x=data, by=list(by1,by2,by3),FUN="mean")
plotmeans <- plotmeans[,-c(1,2,3)]

#6.  SET UP PLACEHOLDER MATRICES TO HOLD ESTIMATED AREAS (INSECT
LOAD) AND ESTIMATED SURVIVAL RATES FOR EA. OBSERVATIONAL UNIT
```

```
Allplots <- matrix(-999,block*crop,(2 + totsurvstages - numsurvspecies))  #Dimensions are NumPlots by Total stages for which survival will be estimated
InsLoad <- matrix(-999,block*crop,(2 + totstages))  #Dimensions are NumPlots by Total stages in 'DATA'
#The b loop sets up first column as block number, and the c loop sets up the second column as the crop number
for (b in 1:block){
    for (c in 1:crop){
        Allplots[(2*b)-2+c,2] <- c  #crop column
        Allplots[(2*b)-2+c,1] <- b  #block column
        InsLoad[(2*b)-2+c,2] <- c  #crop column
        InsLoad[(2*b)-2+c,1] <- b  #block column
        } #end for c
    } #end for b

#7. CALCULATE INSECT LOAD FOR ALL DATA AND SURVIVAL FOR SPECIFIED DATA ON EACH OBSERVATIONAL UNIT SEPARATELY, AND STORE RESULTS INTO ALLPLOTS AND INSLOAD

#7A. Isolate data from a single observational unit, using values for block and habitat to isolate the data into its own data frame, called datas.
for (b in 1:block){
    for (c in 1:crop){
        datas<-subset(plotmeans, Blk==b) #assigning block values
        datas<-subset(datas, Cr==c)

#7B. Ensure that the first and last records have zeroes for all values
        #Find the smallest and largest date
        mindate <- min(datas[,1])
        maxdate <- max(datas[,1])
```

```
#Loop to create a vector to pad the data
zerovec <- c(0,0,0)
for (i in 1:totstages){
    zerovec<- c(zerovec,c(0))
    } #end for i
#Pad beginning and end of data so that survival calculations will work
zeroa <- c(mindate-7,zerovec)
datas<-rbind(zeroa,datas)
zerob<- c(mindate-14,zerovec)
datas<-rbind(zerob,datas)
zeroc<- c(maxdate+7,zerovec)
datas<-rbind(datas,zeroc)
zerod<-c(maxdate+14,zerovec)
datas<-rbind(datas,zeroc)
```

#7C. Set up a matrix (AreaComp) to hold the values for the area components under the population density curves. The areas are called Ai by KNM.

```
numdates <- dim(datas)[1] #Find the number of unique dates in datas.
AreaComp<-matrix(-999, numdates-2, totstages)  #Dimensions are numdates-2,
    because the padded end dates are not counted, by the total number of stages in
    the data frame
```

#7D. Calculate the area components and put them into AreaComp

```
for (j in 1:totstages){
    for (i in 1:(numdates-2)){
        AreaComp[i,j]<-((datas[i,(j+4)] - datas[(i+2),(j+4)])/2*(datas[(i+1),1]))
        } #end for i
    } #end for j
```

#7E. Add up the components to calculate the total area and put these in Area

```
        Area<-colSums(AreaComp)

#7F. Store these areas into the proper location in 'InsLoad'
        for (i in 1:totstages){
            InsLoad[(2*b)-2+c,i+2] <- Area[i]
            }#end for i

#7G. For each species, calculate mortality by stage and place in Surv
        initscol <- c(1) #initial source column in Area
        initdcol <- c(1) #initial destination column in Surv
        Surv<-matrix(-999,1,(totsurvstages-numsurvspecies))
        for(sp in 1:numspecies){
            #Find the correct starting source column in Area
            if(sp!=1){initscol <- initscol + species[sp-1,2]}
            #Find the correct starting destination column in Surv

            if(sp!=1) {if(survspecies[sp-1]){initdcol <- initdcol + species[sp-1,3]-1}}
            #Update the destination pointer if sp != 1 and the previous species had
            survival calculated, survspecies[sp-1].  The two conditions need to be tested
            sequentially so that there is no error in referencing survspecies when sp = 1.

            #Calculate mortality only for the intended species
            if(survspecies[sp]){
                #Sum areas for all stages used to calculate survival for species sp
                allstage<-0
                for(j in 1:(species[sp,3])){
                    allstage <- allstage + Area[j + initscol -1]
                    } #end for j
                #Calculate survival by stage from the areas calculated above and a
                decremented sum.
```

```
                for (j in 1:(species[sp,3]-1)){
                    Surv[j + initdcol - 1] <- Area[j + initscol - 1]/allstage
                    allstage <- allstage - Area[j + initscol - 1]
                    } #end for j
                } #end if species[sp,3]
            } #end for sp

#7H. Store the survival rates in the proper location in Allplots
        for (i in 1:(totsurvstages-numsurvspecies)){
            Allplots[(2*b)-2+c,i+2]<-Surv[i]
            }#end for i
        } #end for c
    } #end for b

#8.  LABEL COLUMNS OF ALLPLOTS AND INSLOAD AND MAKE THEM INTO DATA FRAMES.
lab <- paste(c("Sp"), species[1,1]:numspecies, sep="")
#Column labels for InsLoad
labI <- c()
for(j in 1:numspecies){
    labI <- c(labI, paste(c(lab[j]), 0:(species[j,2]-1), sep=""))
    } #end for j
colnames(InsLoad)=c("Blk","Cr", labI)
InsLoad <- as.data.frame(InsLoad)

#Column labels for Allplots
labA <- c()
for(j in 1:numspecies){
    if(survspecies[j]){labA <- c(labA, paste(c(lab[j]), 0:(species[j,3]-2), sep=""))}
    } #end for j
```

```
colnames(Allplots)=c("Blk","Cr", labA)

#9.  GENERATE F STATISTIC FOR ALLPLOTS AND STORE IT IN FSTAT, THEN
CALCULATE NUMBER REPETITIONS FOR WHICH RANDOMLY GENERATED
FSTAT IS BELOW FSTAT GENERATED WITH REAL DATA.

Allplots<-as.data.frame(Allplots)
#Allplots$Blk<-as.factor(Allplots$Blk)
#Allplots$Cr<-as.factor(Allplots$Cr)
#Allplots[is.na(Allplots)]<-(-999)
Allplots2<-Allplots #Just so I know that Allplots below will have dummies
under<-0#to store dummies <0
over<-0 #to store dummies>1
under<-matrix(0,1,(length(Allplots2)-2))
over<- matrix(0,1,(length(Allplots2)-2))
nansums<-colSums(is.nan(as.matrix(Allplots2))) # vector with NaN sums by column

#9A.  PREPARE RANDOMIZED DATA FOR FSTAT CALCULATION.  DUMMIES
WILL BE SUBSTITUTED FOR NANS WHEN THERE IS 1 NAN OR FEWER PER
COLUMN.  THIS KEEPS THE DIFFERENCE BETWEEN HABITATS CONSTANT,
WITHIN THE BOUNDS OF POSSIBILITY (#S BETWEEN 0 AND 1).

{if((max(nansums))<2){
#CALCULATE AND STORE FSTATS FOR DFS GENERATED BY THE
RANDOMIZATION PROGRAM.  DISCARDS DFS WITH > 1NaN/COLUMN.

fstatif1<-1
    {if((sum(nansums))>0){
    Nantotal<-Nantotal+nansums
    # NAN DF IDENTIFICATION.  Selects dfs with NaNs
```

```
Elsecount<-0
replaceif1<-1
fortest<-0
{for(i in 1:(length(Allplots2)-2)){
#REPLACEMENT LOOP.  Identifies and replaces NaNs by column
replaceloop<-1
fortest<-fortest+1
dumrow<-which(Allplots2[,i+2]=="NaN",arr.ind=TRUE) # gives row of NaN
        {if((length(dumrow))!=0){
        #DUMMIES BY COLUMNS.   Inserts dummy values as needed.
        dummyif1<-1
        Blkdum<-as.numeric(Allplots2[dumrow,1])  #block associated with NaN
        Crdum<-as.numeric(Allplots2[dumrow,2])  #habitat associated with NaN
        Allplots3<-subset(Allplots2,Blk!=Blkdum)  #df with NaN block excluded
        by1<-Allplots3[,"Cr"]
        Crmeans<-aggregate(x=Allplots3, by=list(by1),FUN="mean") #means
        Crvar=Crmeans[1,4]-Crmeans[2,4] # difference bet/habitats w/o NaNblock
        notdummy<-subset(Allplots2, Blk==Blkdum)
        notdummy<- (subset(notdummy, Cr!=Crdum)) #value same block as NaN
        notdum<-as.numeric(notdummy[,i+2])
        orderdum<-(Crdum-1.5)*2
        #habitat associated w/dummy determines if variance will be added or
subtracted.
        dummy<-Crvar-(notdum*orderdum)#works no matter habitat of dummy
        Allplots2[,i+2]<-replace(Allplots2[,i+2],Allplots2[,i+2]=="NaN",dummy)
        dummyif2<-2
        }else{  #skips columns with no NaNs
        dummyelse<-3
        Elsecount<-Elsecount+1
```

```
            }
            #ends if/else
            }
    }

    #ends replacement loop over columns
    }

    replaceif2<-2
    underdum<-sum(Allplots2<0) #number dummies replaced with 0 for this run
    overdum<-sum(Allplots2[,-c(1,2)]>1) #number dummies replaced with 1 for this run
    under<-under+underdum #keeps total of dummies replaced with 0s
    over<-over+overdum #keeps total of dummies replaced with 1s
    Allplots2<-replace(Allplots2,Allplots2<0,0) #replaces negative values with 0
    Allplots2[,-c(1,2)]<-replace(Allplots2[,-c(1,2)],Allplots2[,-c(1,2)]>1,1) #replaces
values above 1 with 1
    replaceif3<-3
    }else{ #if no NaNs in df, above steps skipped
    replaceelse<-3
    } #ends if/else
    }
success<-success+1
Allplots2$Blk<-as.factor(Allplots$Blk)
Allplots2$Cr<-as.factor(Allplots$Cr)
Totsurvstages<-sum(species[,3])
Survspecies<-species[,3]>1
Survcalcs<-Totsurvstages-(sum(Survspecies))
Survcalcs
fstat<-matrix(-999,Survcalcs,2)
specieslabel<-colnames(Allplots2)
```

```
specieslabel<-specieslabel[-c(1,2)]
specieslabel<-noquote(specieslabel)
fstat[,1]<-(specieslabel)

        for (i in 1:(length(specieslabel))) {
        fvalue<-as.data.frame(matrix(summary(aov
        (Allplots2[,i+2]~Cr+Blk,data=Allplots2)), 2,6)[1,1]) [1,4]
        fstat[i,2]<-fvalue
        }

        fstatrand<-cbind(fstatrand,fstat[,2])

        for (i in 1:(length(specieslabel))){
        if (fstat[i,2]>fstatreal[i,2]){fstatsum[i,2]<- fstatsum[i,2]+1}

        }

}else{
#else should run if more than 2 NaNs in one column
fstatelse<-3
failure<-failure+1
}
}


} #this closes z

fstatsum[,1]<-(specieslabel)
Runs<-c(ReP)
```

```
for (i in 1:(length(specieslabel)-1)) {
Runs<-c(Runs,c(ReP))
}
Successes<-c(success)
for (i in 1:(length(specieslabel)-1)) {
Successes<-c(Successes,c(success))
}
fstatsum<-cbind(fstatsum,Successes)
fstatsum<-cbind(fstatsum,Runs)
fstatrand[,1]<-specieslabel
```

Chapter 3. Are lady beetle egg laying preferences explained by predation on lady beetle eggs?

Introduction

When an insect lays her eggs, she determines the habitat in which her offspring will develop. Females that prefer habitats in which their offspring develop the best are likely to leave more descendants than those who do not; thus optimal egg laying behavior should evolve (Jaenike, 1978; Mangel, 1987). A recent meta-analysis supported this positive relationship between preference and performance in herbivorous insects, showing that females oviposited on those plants that were best for the development of their offspring (Gripenberg et al., 2010). Still, ovipositing insects accept fewer plants for oviposition than their larvae can develop on (Wiklund 1975 and Smily 1978), suggesting that resource quality alone does not determine where a female lays her eggs.

The relationship between an insect and its food is clearly very important in egg laying decisions, but in many cases it is necessary to extend the decision making context to include consideration of interactions with natural enemies (Price et al., 1980; Dicke, 2000; Mooney et al., 2012). Often the objectives of providing quality food and protecting larvae from predators align, for example when slow development on a low quality resource increases the opportunity for predators to attack (Moran & Hamilton, 1980). However, these goals can come into conflict (Ohsaki & Sato, 1994; Bjorkman et al., 1997) , making the relative importance of resource quality and predation fundamentally important to understanding how insects use resources (Denno et al., 1990; Camara, 1997; Lill & Marquis, 2001). When predation risk is relatively high, selecting habitats to avoid natural enemies has been invoked to explain changes in resource use (Atsatt, 1981; Jeffries & Lawton, 1984; Gratton & Welter, 1999) and resulting speciation events (Jaenike & Grimaldi, 1983; Nylin & Wahlberg, 2008) among herbivorous insects.

Predatory insects face many of the same decisions as their herbivorous counterparts. In selecting an oviposition site, predators must balance the need to provide their offspring with quality resources with that of limiting the risk of harm from their own natural enemies. The latter has become the focus of more study as researchers recognize

the importance of interactions among predators in shaping community structure (Polis et al., 1989; Moran & Hurd, 1994; Polis & Strong, 1996; Rosenheim, 1998). However, resource use is often framed in the context of interactions between predators and their prey (Hairston,1959; Hassell, 1978) with interactions among predators typically limited to an intraguild predation framework (Rosenheim, 2001) that largely ignores avoidance of predation and the interaction between predator avoidance and resource use.

Consideration of natural enemies in selecting oviposition sites may be important, though, if higher order predators reduce the profitability of an otherwise high quality resource (Lima & Dill, 1990). In studies of oviposition location and predation, predators such as lady beetles (Schellhorn & Andow, 1999a) and hoverflies (Putra et al., 2009) have been shown to minimize predation by ovipositing away from predators, even if this reduces access to prey. Thus predation by natural enemies could limit predator access to prey. The exclusion of predators from habitats by other predators has been predicted theoretically (Holt & Polis, 1997) and shown experimentally (Barkai & McQuaid, 1988; Wissinger et al., 1996; Fincke, 1999; Rosenheim, 2001; further suggesting that predation risk likely shapes predator oviposition behavior (Blaustein et al., 2004).

Could intraguild predation exclude a generalist predator from using a novel prey? If intraguild predation creates sink habitats, predators may be restricted in their use of resources within those sink habitats. This could happen because ovipositing females avoid habitats associated with predation risk, or because those eggs laid in these habitats are eaten, preventing larvae from feeding there. Predator diets may be limited in this way when prey are habitat specialists and when predators face high mortality from attacks by other predators. We examined the role an aggressive intraguild predator might play in limiting the diet of another generalist predator, the native lady beetle *Coleomegilla maculata*, in a community of lady beetles feeding on traditional and novel aphid prey.

Predatory interactions are common within aphidophagous guilds and play an important role in community dynamics (Rosenheim et al., 1995; Lucas, 2005). Additionally, each aphid species tends to specialize on just a few plant species (Moran, 1992). Because they cannot fly, lady beetle larvae, particularly those that find themselves in agricultural monocultures, are much less likely than adults to move to new habitats.

This makes a lady beetle's selection of oviposition habitat a key determinant of the aphid species her larvae will encounter.

In the simplest model of lady beetle oviposition behavior, females lay eggs near prey because they spend more time foraging there than in areas with fewer prey (Evans & Dixon, 1986). However, in addition to the quantity and quality of resources present, predation risk also plays a role in selecting oviposition sites. Lady beetle larvae are vulnerable to intraguild predation by other lady beetles (Moser & Obrycki, 2009). Similarly, eggs (Schellhorn & Andow, 1999a; Gardiner et al., 2011) and pupae (Schellhorn & Andow, 1999b) face high mortality due to predation.

The importance of this predation is reflected in the strategies lady beetles have developed to limit it. Females respond to oviposition-deterring pheromones from conspecific (Merlin et al. 1996; Ruzicka, 1997) and heterospecific (Agarwala et al., 2003; Michaud & Jyoti, 2007) lady beetles and therefore lay fewer eggs in the vicinity of other lady beetles. It has also been shown that lady beetles respond more strongly to the odors of some lady beetle species than others (Magro et al., 2010); thus they may be able to avoid predators that are especially dangerous for them. Given these behaviors, it seems reasonable to expect lady beetles to balance predation risk with the need to provide adequate resources to offspring when selecting oviposition sites. Further, the higher the mortality by predation in a population, the more important predation should be in this balance.

It has been suggested that predation by aggressive introduced lady beetles may exclude natives from shared habitats (Snyder & Evans, 2006; Pell et al. 2008;Gardiner et al., 2011). The multicolored Asian lady beetle, *Harmonia axyridis*, for example, is known as an aggressive predator of other lady beetles. In small lab arenas, *H. axyridis* engages in asymmetric intraguild predation with native lady beetles, attacking eggs, larvae and pupae (Pell et al., 2008). In a study of egg predation between *H. axyridis* and the native lady beetle *Coleomegilla maculata, C. maculata* strongly preferred to cannibalize its own eggs. In contrast, *H. axyridis* showed no preference for its own eggs (Cottrell, 2007) and in fact could complete development on *C. maculata* eggs (Cottrell, 2004). Similarly, in an open field study, predation was higher on eggs of a native species than on *H. axyridis*

eggs (Gardiner et al., 2011), possibly because *H. axyridis* eggs are more toxic than those of most other lady beetles (Rieder et al., 2008). Gut content analysis of field collected *H. axyridis* has revealed *C. maculata* DNA, suggesting that predatory interactions in the field are common (Gagnon et al., 2011). Given the apparent asymmetry of these interactions, exclusion of the native from habitats containing the exotic might explain patterns of resource use by the native predator.

The case of the native lady beetle *C. maculata* only rarely eggs in soybean might be one in which exclusion by predation could explain an otherwise unexpected pattern of resource use. Although surveys in maize and soybean showed that the exotic *H. axyridis* distributes itself between maize and soybean proportionally to aphid densities (KKP, chapter 1), the native species remains rare in soybean even when resource densities are very high. This is surprising because *C. maculata* is considered to be the most generalist of the lady beetles (Hodek, 1973). Although aphids are its ancestral food source (Giorgi et al., 2009), the native also feeds on other prey and even fungi and pollen (Lundgren, 2009). Along with its broad diet, the native species lays eggs on diverse plants, including legumes such as alfalfa (Kiekheffer et al. 1992). Further, soybean aphid, *Aphis glycines*, has been shown to be a suitable and even high quality prey for *C. maculata* (Mignault et al., 2006), and this prey is present at the same time as another preferred prey of *C. maculata*, corn leaf aphid, *Rhopalosiphum maidis* (KKP, chapter 1).

To investigate the possible exclusion of *C. maculata* from soybean by predation, we compared predation on sentinel *C. maculata* eggs in soybean to predation in maize. Additionally, we surveyed predators and prey in the two habitats to see how predator and resource densities affected predation on *C. maculata* eggs. Higher predation on eggs in soybean might explain the native beetle's seemingly sub-optimal habitat use, and this could be explained by the presence of more coccinellid predators, fewer prey, or both in soybean habitats. Further, if higher predation in soybean is related to high densities of the exotic *H. axyridis*, this might lend credence to the idea that native lady beetle decline is related to the introduction of the exotic. Given the known aggressiveness of *H. axyridis*, we hypothesized that in soybean habitats, where *H. axyridis* is relatively more abundant, predation on *C. maculata* eggs would be greater than in maize.

Methods

To determine whether predation on coccinellid eggs differed by habitat, we observed the fate of sentinel coccinellid eggs in plots of maize and soybean at the University of Minnesota Agricultural experiment site (MAES) in central Minnesota during the 2010 growing season. We established 4 blocks, each of which contained two 10 by 10 m plots that were randomly assigned to maize (Green Giant Code 63) or soybean (Northrop King S19R5), at 5.18 and 24.70 plants/ m^2 respectively. Crops were planted in 30 inch rows in early June.

We conducted the experiment at the time of the season when lady beetles began to oviposit in maize and soybean (July 31st) and again 5 days later as coccinellid oviposition peaked (August 4). The timing was determined by observing when the first lady beetle eggs occurred in the experimental plots (late July), as described in Chapter 1 (KKP, chapter 1). We hand-collected *Coleomegilla maculata* adults in nearby maize and soybean fields, mated them in the laboratory and then transferred females to individual containers lined with waxed paper. The following day we removed the egg masses that had been laid, relined the containers, and added 15-30 live *Aphis glycines*. Egg masses were immediately transferred to a storage chamber where they were held at 10C. This was repeated over 3 days until 80 *C. maculata* egg masses had been obtained. Egg masses for the second trial were collected in the same manner.

To prevent the effect of egg mass size from being confounded with the effect of treatment, we sorted the egg masses from smallest to largest (4 to 23 eggs, mean 11.4), and assigned them in pairs to blocks 1-4, repeating this until all egg masses had been assigned to blocks. We then randomly assigned one egg mass from each pair to the maize or soybean treatment, giving 10 sentinel egg masses for each of 8 plots (4 blocks by 2 treatments).

Between 1 and 4 p.m. the same day, we attached each sentinel egg mass to the bottom of a leaf in the bottom third of a maize or soybean plant. This location was selected because it is the preferred oviposition location of the native *C. maculata* in maize (Schellhorn and Andow 1999a) and also the location in which we observed coccinellid egg masses in soybean (KKP, personal observation). We attached each egg

mass to a plant by pinning the wax paper to which it was attached so that the paper was flush with the bottom of the leaf. Each pin was secured using a small foam backing on the top of the leaf. We returned to check egg masses for predation at 24 and 48 hours.

While this experiment was in progress, we surveyed maize and soybean plants to establish the densities of predators and prey present. Between 9 and 11:00 AM the morning after the sentinel eggs were placed, we surveyed plants at each of 10 randomly selected locations per plot. At each location, we surveyed two adjacent maize plants or two adjacent 18 cm sections of soybean row. Coccinellid and aphid numbers were counted in the manner described in chapter 1, and naturally laid egg masses were collected from plants and identified to species in lab when larvae emerged (KKP, chapter 1).

Insect densities from each pair of adjacent maize plants or adjacent sections of soybean row were averaged and then converted to density/ m^2 to make maize and soybean samples comparable. For maize samples, we multiplied each plant sampled by plants per square meter, while in soybean we multiplied each 18cm section of soybean row by 7.93 because there was 141 cm of row/m^2.

Between 1 and 4 that afternoon, 24 hours after sentinel egg masses were placed, we returned to each egg mass and used a hand lens to determine how many eggs had been attacked. Attacked eggs were classified as having either chewing damage (eggs chewed down to bases) or sucking damage (eggs hollowed out). This was repeated again at 48 hours after placement of sentinel eggs. Because we were interested in the impact of intraguild predators, which chew on eggs, we separated incidence of chewing and sucking predation in our analyses.

Statistical Analyses

To determine whether there were significant differences in densities of predators (coccinellid adults and larvae) and prey (aphids) between habitats and dates, we conducted analysis of variance using habitat (maize or soybean), date (first or second trial) and block as factors affecting predator and prey densities. Because of differences in variance among treatments, prey densities were natural log transformed prior to the analysis. The effects of habitat and block were tested against the habitat by block

interaction (Error 1), and the effect of date was tested against the habitat by block by date and block by date interactions (Error 2). We did this separately with prey densities as the response and with predator (adult coccinellid) densities as the response.

We analyzed sucking and chewing predation on lady beetle eggs separately with generalized linear models using Poisson error. We used the MASS package in R to conduct Chi square tests for significance of model factors. Factors included were habitat (maize or soybean), Date, Block and Size (number of eggs in initial egg mass). All higher order interactions were estimated. In addition, to better understand how prey density affects sucking and chewing predation on lady beetle egg masses, we used models in which aphid density (natural log of aphids per square meter) was substituted for the habitat factor. Lastly, since chewing predation is most likely the result of attacks by coccinellids, we added a third model for chewing predation in which we substituted the density of adult coccinellids (adults/ m^2) for habitat. As the degrees of freedom used in these models was the same, the total deviance explained by biologically relevant factors in these models was compared to determine the best fitting model.

Results

Predator and prey by treatment

Maize and soybean were colonized primarily by the aphids *R. maidis* (over 95% of aphids observed in maize) and *A. glycines* (100% of aphids observed in soybean) and the coccinellid predators (in order of abundance) *H. axyridis*, *C. maculata*, *Cycloneda munda*, *Hippodamia convergens* and *Coccinella septempunctata.* Across habitats, 97% of the coccinellids observed were either *H. axyridis* or *C. maculata*. Based on our previous findings in 2008 and 2009, this community composition was typical for these habitats in central Minnesota (KKP, chapter 1).

Coccinellid eggs were observed in both maize and soybean by the last week in July, although we didn't find coccinellid eggs on surveyed plants until August 6, when they had become much more abundant (Figure 3.1). Densities of adult coccinellids (potential chewing predators) ranged from about 2.5 individuals/ m^2 on the first sampling date in maize to almost 4 individuals/ m^2 on the same date in soybean (Figure 3.1). This

pattern was reversed on the following sampling date, thus although there were no significant differences in adult predator densities either by habitat or by date, there was a significant interaction between the two ($p = 0.02$) (Table 3.1).

The differences in aphid abundances by habitat and date were more dramatic. Aphids were much more abundant in soybean than in maize on both sampling dates (Figure 3.1) and this was reflected in a significant effect of habitat on aphid density (Table 3.1, $p < 0.01$). Although aphid densities were higher in both habitat s on the second sampling date, neither this difference nor the interaction between habitat and date were statistically significant (Table 3.1).

Sentinel eggs by treatment

Of the 160 *C. maculata* egg masses we placed on plants, we recovered 159, representing 1834 eggs. Over a fourth of eggs placed were eaten during the first 24 hours. Attack rates were similar between 24 and 48 hours, suggesting constant attack rates during these short periods of time. However, by 48 hours some larvae had emerged from eggs, potentially affecting the likelihood of attack by predators. We therefore examined only 24 hour data in subsequent analyses.

Damage on coccinellid eggs from both sucking and chewing predators was found across trial dates and habitats, although there was consistently more chewing predation than sucking predation (Figure 3.2). There was less chewing damage, on average, in soybean, where incidence of sucking and chewing damage was more similar (Figure 3.2).

Separate analyses sucking and chewing damage on coccinellid eggs revealed distinct patterns in these predation types (Figure 3.2). Our analysis of the effect of habitat and date on sucking predation (eggs hollowed out) showed that sucking damage occurred more frequently in soybean than in maize ($p = 0.006$) and more frequently on the first sampling date than on the second ($p = 0.007$) (Table 3.2). The majority of sucking events happened in soybean during the first trial, while sucking predation in maize remained relatively constant over the two sampling dates (Figure 3.2). This difference is reflected in the significant effect of the habitat by date interaction (<0.001) on the occurrence of sucking predation (Table 3.2).

A second model in which we substituted aphid density for habitat showed that while aphid densities alone did not explain the patterns observed, interactions between aphid densities and date did have a significant effect on the incidence of sucking predation ($p < 0.001$), as did date alone ($p = 0.005$). A comparison of the total deviance explained by each of these models suggested that the model using aphid densities (deviance explained 83) might fit better than the habitat based model (deviance explained 60) (Table 3.2).

A comparison of chewing predation by habitat and date revealed that chewing predation was much more common in maize than in soybean (habitat effect, $p<0.001$) and on the first date compared to the second (date effect $p=0.012$) (Figure 3.2), with no significant interaction between these factors (Table 3.3). Substituting aphid density for habitat showed that aphid density was also a significant predictor of chewing predation ($p<0.001$), with fewer aphids predicting more attacks (Table 3.3). In this model date did not predict chewing predation on its own, but there was a significant effect of the interaction between aphid density and date ($p<0.001$) (Table 3.3). We also tried an additional model in which we substituted adult coccinellid density for habitat. In this model, we found that adult density ($p = 0.003$) and the interaction between adult density and date ($p<0.001$) both had a significant effect on chewing predation (Table 3.3).

Although habitat, aphid densities and predator densities all had some predictive power when combined with date and egg mass size, the total deviance explained by the biologically relevant factors considered differed greatly among models (Table 3.4). Habitat and aphid density explained more of the observed deviance (352 and 342 respectively) than did predator density (148). Thus, habitat and aphid densities might be more important predictors of chewing attacks than are predator densities. Because aphid densities were always higher in soybean (Figure 3.1), with no interaction with date, it is not possible to distinguish effects of aphid densities from effects of habitat identity, thus either habitat, aphid density, or both contribute to explaining the patterns in chewing attacks on eggs.

Discussion

Our hypothesis that predation on eggs by other lady beetles excludes the native lady beetle *Coleomegilla maculata* from soybean habitats was not supported by the data. Although the high rates of chewing damage on lady beetle eggs suggest that older lady beetles frequently prey on them, such damage on sentinel eggs was more common in maize. Sucking predation, which was likely due to attack by insects with sucking mouthparts, such as *Orius insidiosus* and *Chrysoperla carnea*, was slightly more common in soybean, but this type of predation was relatively rare compared to chewing predation, making maize the consistently more dangerous habitat from an egg predation standpoint. Thus it seems that the native beetle prefers to oviposit in maize despite the fact that eggs laid in maize are more likely to suffer attack by chewing predators.

Several factors might contribute to the relative predation risk in maize vs. soybean habitats—for example the presence of refuges in the habitat, the density of predators present in the habitat, and the density of alternative prey in the habitat. Structurally complex habitats are known to reduce predation risk by providing spatial refuges for prey (Janssen et al., 2007), but although Schellhorn and Andow found evidence of the existence of such refuges for lady beetle eggs on maize plants (Schellhorn & Andow, 1999a), we saw no difference in predation on eggs placed on the safest locations (bottom of a lower leaf) in maize plants compared to a similarly "safe" location (bottom leaf) on soybean plants. Therefore, in this case, the structural complexity and tall stature of maize did not reduce chewing or sucking predation sufficiently to make maize the safer habitat overall.

The presence of predators associated with habitats is arguably the most obvious causal factor in predation risk, but we saw only a very limited effect of coccinellid predator densities on the likelihood of chewing predation in our study. Although the effect of predators on attacks on eggs was significant, it explained little of the observed variation in predation compared to habitat (soybean vs. maize) or prey densities. This was not surprising because the predator densities that we observed were similar across habitats and dates, which made it very difficult to detect any effect of predators. Thus, while the density of coccinellid predators might be an important factor in determining

chewing damage by predation, it was not the main factor responsible for the differences we observed.

Because we did not observe sucking predators in maize and soybean, we cannot make a comparison between predator density and sucking predation. However, the sucking predator *Orius insidiosus* is common in both maize and soybean habitats (Isenhour & Yeargan, 1981) and has been identified as an important soybean aphid predator (Rutledge et al. 2004) suggesting it may have an important role in soybean habitats. Further, molecular analysis has shown that *O. insidiosus* also consumes lady beetle eggs in soybean (Harwood et al. 2007). Additionally, we occasionally observed the green lacewing, *Chrysoperla carnea*, in both maize and soybean. This predator has also been shown to interact with *C. maculata* via intraguild predation (Phoofolo & Obrycki, 1998).

The availability of shared extraguild prey also affects the likelihood of intraguild predation (Vance-Chalcraft et al. 2007). Coccinellid predators, *C. carnea* and *O. insidiosus* commonly attack aphid prey, thus aphid densities may be important in determining the likelihood of chewing or sucking predation. Aphid densities differed greatly by habitat, with more aphids available in soybean than in maize, and more aphids available in both habitats during the second trial than the first. Lower extraguild prey densities were associated with more chewing attacks on eggs, both when considering predation by habitat and predation within each habitat on dates with more vs. fewer prey. This makes sense because upon contact with an egg mass, a hungry predator is likely to eat more than is a satiated predator. This effect has been modeled (Daugherty et al., 2007) and demonstrated in laboratory arenas, where adding more aphid prey can lead to fewer attacks among predators (Ingels & De Clercq, 2011; Lucas, Coderre, & Brodeur, 1998). Similarly, in field studies on predation of eggs and pupae, Schellhorn and Andow found that as prey became scarce, mortality by chewing predation increased dramatically (Schellhorn & Andow, 1999b). This is in contrast to a previous result in which higher intraguild predation was observed on predators on plants with dense prey (Chacon & Heimpel, 2010)

The relationship between resources and sucking predation was more complex. There was a significant interaction between aphid densities and date that affected sucking predation, but this seems to be driven by very high predation in the trial one soybeans, and it is difficult to make general conclusions about it, except to say that on this day, higher aphid densities correlated with higher sucking predation in soybean. Because *O. insidiosus* is comparatively small relative to its prey, it may have low enough resources needs that the density of prey was less important in determining hunger level during this study. *C. carnea* was at very low levels on plants we observed, thus it was unlikely to have much influence over differences in sucking predation by habitat.

Although differences in prey densities explain the observed differences in egg predation between maize and soybean, a puzzling question remains. Why are predators choosing to oviposit in maize when it is clearly the more dangerous habitat for eggs? Given the higher densities of naturally laid eggs observed in maize compared to habitat, as well as the increased likelihood of predation on eggs in maize, it seems fair to say that maize was the preferred habitat for oviposition. This could be because there are benefits to maize habitats (or dangers in soybean habitats) that we did not observe in this study or because lady beetles are not laying eggs in soybean despite the fact that it is a superior habitat.

In considering the relative quality of maize and soybean habitats, it is important to remember that mortality at the egg stage is only one component of overall survival by habitat. Clearly any eggs that are eaten have zero survival. However, if maize is a much better habitat for developing offspring than is soybean, it might still be better for overall survival. Since we didn't examine mortality at other stages in this study, it is unclear how larvae fared in each habitat. These studies were conducted as eggs were just beginning to be laid in both habitats. Aphids reproduce very rapidly and experience boom and bust cycles as they exploit their resources (Moran, 1992). Thus the aphid densities we observed at the end of July would be very different than those we would expect even a few weeks later. Although there were fewer prey resources in maize on the days we conducted this study, we cannot assume that resources would continue to be inferior in maize. Dixon suggests that lady beetles should lay as aphid populations are beginning to

increase, because the offspring of those lady beetles laying when aphids are already very dense will still be developing when aphid populations crash later in the season (Dixon, 2007). According to this theory, while soybean appeared to be rich in resources in this study, it might already be too late for lady beetles to lay eggs there. This is further supported by research showing that *A. glycines* reproduction slows drastically at 30C and above (typical of mid and late summer temperatures in Minnesota) and the aphids emigrate rapidly from senescing soybean plants (McCornack et al., 2004).

In addition to changes in aphid densities, it is also possible that differences exist in the quality of resources in each habitat. Such differences are unlikely to relate to the relative quality *of R. maidis* and *A. glycines* because feeding studies have shown that *A. glycines* is a suitable and in fact high quality resource for *C. maculata*. Although it is smaller than *R. maidis*, it tends to reach higher densities, which would easily make up for this difference. The existence of alternative resources, however, might be very important. Pollen, for example, becomes a very important resource for lady beetles in maize late in the season as prey decline. Prey are a superior to pollen for *C. maculata* development (Hodek, Ruzicka, & Hodkova, 1978; Lundgren & Weidenmann, 2004), however, when prey become scarce, *C. maculata* can complete its development on maize pollen (Lundgren & Weidenmann, 2004). Soybean lacks the high volumes of pollen available in maize, leaving *A. glycines* as virtually the only resource available.

It is also worth considering the possibility that soybean is a superior oviposition location and lady beetles are ovipositing maladaptively. Sometimes, contrary to the theory of optimal oviposition, insects lay their eggs in locations in which their larvae fare worse (Thompson, 1988). Given that lady beetles are attracted to plants, and therefore oviposition sites, via volatile chemicals released by feeding aphids (Girling & Hassall, 2008; Ninkovic et al., 2001; Sarmento et al., 2007b) and that these chemicals differ by prey and plant (McCormick et al., 2012), it is possible that volatile chemicals released by aphids feeding on soybean are not recognized by *C. maculata*. Since *C. maculata* tends to respond more strongly to plant identity than to the presence of aphids (Griffin & Yeargan, 2002), it may be limited in its ability to recognize soybean aphid as a viable resource. In an overview of lady beetle oviposition behavior, Seagraves noted that

limitations in recognizing appropriate resources should be considered, along with the quality of resources and the presence of prey (Seagraves, 2009).

In contrast to the comparatively naïve native, the most common lady beetle species ovipositing in maize, *H. axyridis*, has a shared evolutionary history with *A. glycines* and soybean in its native Asia. We might expect this beetle to be more adept at detecting aphids in soybean and effectively using this habitat compared to *C. maculata*, whose first contact with *A. glycines* would have been after its establishment in North America in 2001. It is possible that even though soybean would be a good habitat for *C. maculata*, it is experiencing an evolutionary lag in the ability to recognize the aphid/plant complex. If this is the case, we would expect that those *C. maculata* that oviposit in soybean would leave more offspring than those that do not, and that the species would gradually begin to use soybean.

Whether or not *C. maculata* is ovipositing where it "should" to ensure the long term survival of its offspring, at the time of oviposition the native lays eggs in a way that neither minimizes predation risk, nor maximizes use of available resources. Further investigation of long term survival of lady beetles in maize and soybean habitats might shed some light on this unexpected behavior. If resources improve in maize over time, *C. maculata* survival might ultimately be better there, and thus, early in the season, choosing maize regardless of predation risk and prey densities might often lead to the greatest survival. In contrast, if survival does not improve in maize, this would suggest an evolutionary lag in using the novel resource, *A. glycines* on soybean. If this is the case, we should see native beetles fail to respond to the volatile chemicals released by aphids feeding in soybean. The native beetle seldom ovipositing in soybean when it is ultimately the superior habitat would be a very surprising finding, as the diversity of oviposition behavior within a species is thought to make evolutionary lags like this very brief. Such a finding would suggest that perhaps enough credence has not been given to the idea that it takes time for native predators to respond to a novel resource.

Table 3.1. Analysis of variance conducted with adult coccinellid densities and natural log of aphid densities in response to habitat and date.

Adult coccinellids per m^2 by habitat, block and date

Factor	Df	MS	*F*	*p*
habitat	1	0.4	0.4	0.58
block	3	0.3	0.3	0.62
Error 1	3	1.1		
date	1	0.0	0.0	0.84
habitat x date	1	5.9	11.2	0.02
Error 2	6	0.5		

Ln (aphids) per m^2 by crop, block and date

Factor	Df	MS	*F*	*p*
habitat	1	18.5	75.6	<0.01
block	3	0.8	3.1	0.19
Error 1	3	0.2		
date	1	0.1	0.9	0.37
habitat x date	1	0.4	2.9	0.14
Error 2	6	0.2		

Table 3.2. Analysis of deviance tables for sucking attacks on eggs. The first model considers factors of habitat, date, and egg mass size, while the second substitutes aphid densities for habitat.

Factor	**Df**	**Deviance**	***p***
habitat (maize vs soybean)	1	7.6	0.006
date	1	7.2	0.007
size	1	1.4	0.230
habitat x date	1	40.1	<0.001
habitat x size	1	1.9	0.165
date x size	1	1.2	0.270
habitat x date x size	1	0.8	0.374
total deviance			60
Factor	**Df**	**Deviance**	***p***
aphid density	1	0.0	0.879
date	1	7.8	0.005
size	1	1.6	0.204
aphids x date	1	65.0	<0.001
aphids x size	1	0.1	0.788
date x size	1	0.1	0.716
aphids x date x size	1	8.5	0.004
total deviance			83

Table 3.3. Analysis of deviance tables for chewing attacks on eggs. The first model considers factors of habitat, date, and egg mass size, while the second and third substitute aphid and adult coccinellid densities for habitat respectively.

Factor	**Df**	**Deviance**	***p***
habitat (maize vs soybean)	1	335.2	<0.001
date	1	6.2	0.012
size	1	3.6	0.058
habitat x date	1	2.3	0.126
habitat x size	1	2.2	0.141
date x size	1	1.7	0.191
habitat x date x size	1	0.9	0.333
total deviance			352
Factor	**Df**	**Deviance**	***p***
aphid density (ln aphids/m^2)	1	304.1	<0.001
date	1	1.1	0.289
size	1	3.5	0.062
aphids x date	1	27.2	<0.001
aphids x size	1	3.8	0.051
date x size	1	2.1	0.150
aphids x date x size	1	0.0	0.837
total deviance			342
Factor	**Df**	**Deviance**	***p***
adults (adult coccinellids/m^2)	1	9.0	0.003
date	1	3.2	0.074
size	1	3.7	0.056
adults x date	1	128.2	<0.001
adults x size	1	0.0	0.934
date x size	1	0.2	0.622
adults x date x size	1	3.4	0.066
total deviance			148

Figure Legends

Figure 3.1. Mean densities per m^2 (± SE) of aphids (black), coccinellid adults (grey) and coccinellid eggs (white) by habitat and trial: early (July 30) and as oviposition peaked (August 4). ANOVA revealed no significant differences among coccinellid densities by date or habitat, however, there were significant differences in aphid densities by habitat ($p<0.01$).

Figure 3.2. Mean eggs (± SE) per egg mass either unharmed (white), with sucking damage (grey) or with chewing damage (black) by habitat and trial: early (July 30) or peak (August 4).

Figure 3.1.

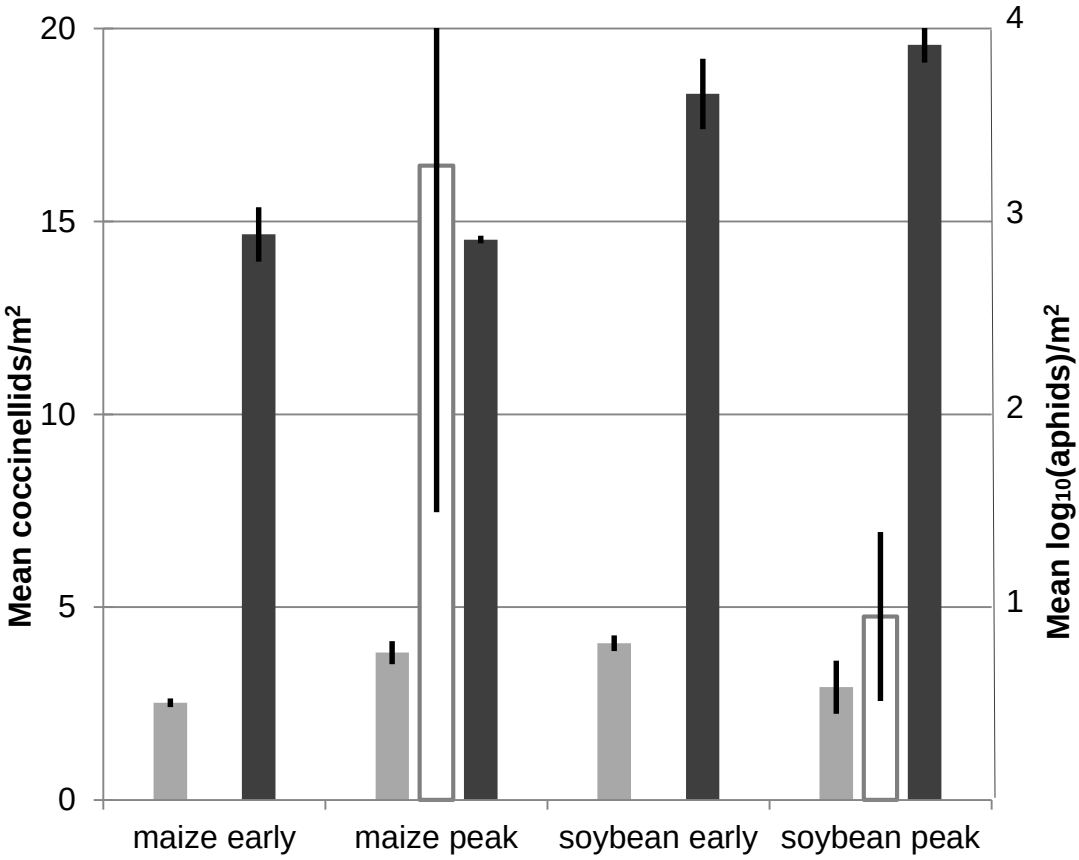
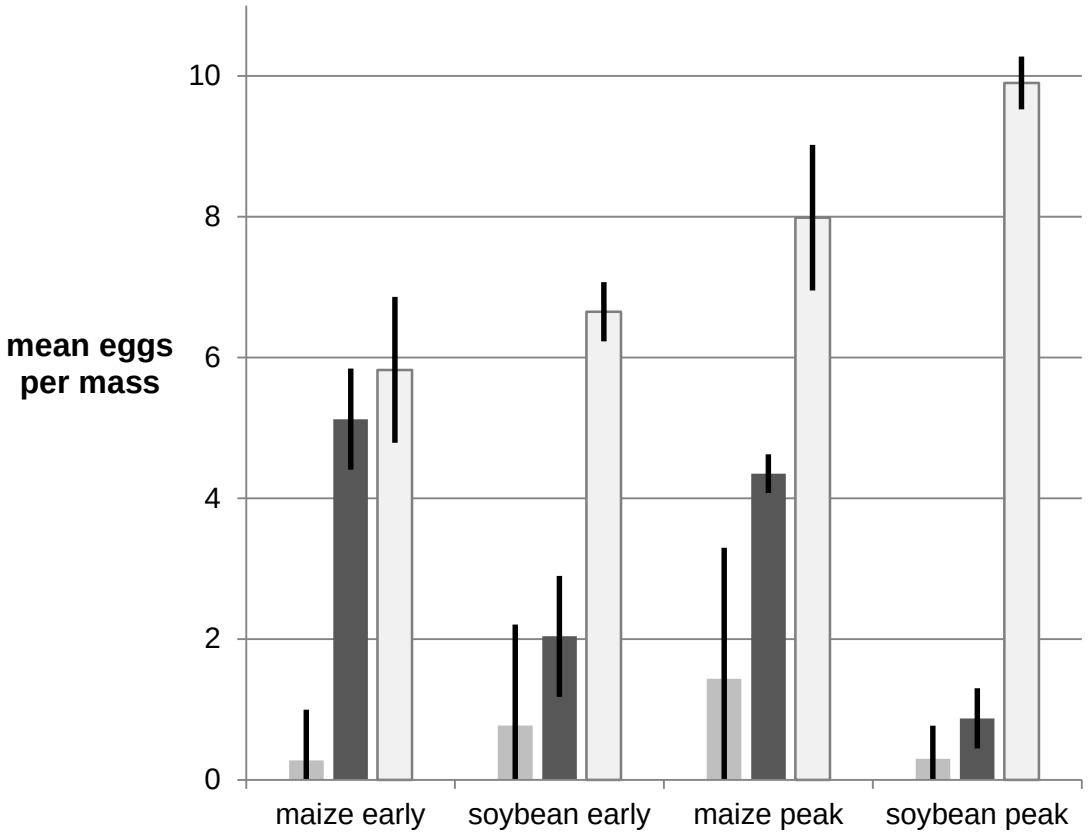

20
15
10
5
0
4
3
2
1
Mean coccinellids/m²
Mean log10(aphids)/m²
maize early
maize peak
soybean early
soybean peak

Figure 3.2.


mean eggs per mass
10
8
6
4
2
0
maize early
soybean early
maize peak
soybean peak

Chapter 4. Contact rates among intraguild predators: avoidance or aggregation?

Introduction

Coexistence of similar species should be rare in nature, because one species will always be "better" at exploiting a given niche and thus should exclude other species (Gause & Witt, 1935). However, aphid feeding predators often share habitats and prey species. These aphid predators, in addition to sharing prey, feed upon each other in an interaction termed intra-guild predation (IGP) (Phoofolo & Obrycki 1998, Amarasekare, 2008). In this interaction one predator, the intra guild (IG) predator, feeds upon a second predator, termed the IG prey, in addition to feeding on the resource that the two share. Intra guild predation theory predicts that such predators should coexist readily only when resources are limited and the IG prey is better at exploiting the common prey resource than is the IG predator (Polis et al., 1989). However, this mechanism is limited in its ability to explain cases commonly observed in the field, such as the coexistence of IG predators and prey in resource rich environments, where the exclusion of IG prey is predicted (Amarasekare, 2008). Further complicating matters, some predators can act as IG predators or IG prey, depending upon their age or size relative to that of other predators (Polis et al., 1989; Wissinger, 1992) .

Another mechanism that has been suggested for the maintenance of coexistence among IG predators and prey in the field is avoidance behavior among IG prey. Since prey are under strong selection to escape predation, they should detect and avoid predators whenever possible (Lima & Dill, 1990). If avoidance behaviors effectively limit contact between potential IG predators and their prey, incidents of IGP might be relatively rare, allowing coexistence of IG predators and prey. However, the extent to which these behaviors limit contact is unclear. In less complex habitats, avoidance may be difficult (Janssen et al., 2007). The distribution of prey within a habitat might also affect the extent to which predators are able to avoid each other while foraging. Foraging predators maximize their resource consumption by aggregating to areas of dense prey (Wright & Laing, 1980; Evans & Youssef, 1992; Ives et al., 1993; Elliott & Kieckhefer, 2000). However the close contact among predators makes IG predation

likely, particularly when resources begin to become depleted (Agarwala & Dixon, 1992; Schellhorn & Andow, 1999b). Predators must therefore balance the potentially conflicting objectives of avoiding predation and maximizing prey consumption (Lima & Dill, 1990).

Aphid feeding predators may be an ideal community in which to investigate the effect of avoidance of heterospecifics on IGP. Aphidophagous lady beetles aggregate to large aphid colonies (Dixon, 1959; Ives et al., 1993) making them effective at suppressing aphid pests (Obrycki et al. 2009). However, aggregation also leaves them vulnerable to predation by other lady beetles, which is a significant source of mortality in lady beetle populations (Wright & Laing, 1982; Osawa, 1989; Schellhorn & Andow, 1999b). In studies in the field (Hironori & Katsuhiro, 1997; Schellhorn & Andow, 1999a) and in small arenas (Agarwala et al., 1998; Cottrell & Yeargan, 1998), adult and larval lady beetles readily preyed upon lady beetle eggs, as well as molting larvae and pupae. In PCR based analysis of four species of field collected lady beetles, all species contained heterospecific DNA (Gagnon et al., 2011), suggesting that intra guild predation is somewhat common. Although the extent to which IG predation affects community structure is unclear, intra guild interactions are thought to be important in structuring communities of aphid predators in agricultural habitats (Lucas, 2005).

The extent of IGP among aphid feeding predators might be limited by avoidance behaviors. Lady beetles are known to use chemical cues to avoid laying eggs (Merlin et al., 1996; Ruzicka, 1997) and foraging (Agarwala et al., 2003) on plants that have been exposed to conspecific larvae. Heterospecific chemical cues also seem to reduce oviposition (Agarwala et al., 2003; Michaud & Jyoti, 2007) and foraging (Moser et al., 2010; Meisner et al., 2011) in some coccinellids. In addition to recognizing and avoiding signs that other lady beetles are nearby, some species lay eggs far from aphid aggregations, which reduces predation by other lady beetles (Schellhorn & Andow, 1999a). Similarly, although larvae molt where they forage, they frequently venture further from aphid aggregations to pupate, reducing their likelihood of falling victim to IGP (Lucas et al. 2000).

Using a model of predator behavior and an empirical study of aphid predators in tansy, Kindlmann and Houdkova (2006) argued that avoidance behaviors effectively limit opportunities for IGP in the field, rendering IGP unimportant. Reinforcing this idea, in a study of coccinellid predators on caged maize plants, Hoogendoorn and Heimpel found that the putatively less aggressive coccinellid, *Coleomegilla maculata*, limited its foraging area on maize plants in the presence of a more aggressive coccinellid, *Harmonia axyridis* (2004). Similarly, Musser and Shelton found a population level effect of avoidance among potential IG predators in an open field study of the same two lady beetle species (Musser & Shelton, 2003). While these studies suggest the possibility of a population level effect of avoidance behaviors, the many studies that document IGP among aphid predators suggest that further study on the extent to which these predators are able to limit predation by heterospecifics is warranted.

We measured heterospecific contact among lady beetles and other aphid feeding predators in maize and soybean and also analyzed the empirical data reported by Kindlmann and Houdkova (2006) in tansy. Using contact data from visual counts of aphid predators and their prey we used maximum likelihood to determine whether or not observed contact among potential intra guild predators deviated significantly from random contact. This analysis allowed us to examine the extent of avoidance in these three habitats and also to identify differences in avoidance and aggregation patterns among habitats and species. Further, we examined the changes in contact over the course of a growing season in maize. Based on IG predation reported among aphid predators in in the field and on predator aggregation to prey, we hypothesized that avoidance would be insufficient to limit contact among aphid feeding predators and prevent IG predation , particularly late in the season when predators aggregate around dwindling aphid resources.

Methods

Experimental Design

To determine whether potential IG predators effectively avoid each other in time and space, we observed contact among aphidophagous coccinellids over the course of

two growing seasons in maize and soybean, and also analyzed similar contact data reported by Kindlmann and Houdkova (2006) for aphid predators in tansy. We then compared this observed contact to the contact that would be expected if insects were distributed at random. Like Kindlmann and Houdkova, we considered heterospecifics to be in contact if they were present on the same plant at the same time. We compared all species combinations at all stages (egg, larva, pupa and adult) and distinguished those heterospecific pairings that would likely result in predation (a mobile stage from one species with an immobile stage of another species) from those for which IGP would be less likely (two mobile stages) or impossible (two immobile stages).

Coccinellid surveys in maize and soybean

In the 2008 and 2009 growing seasons we established 4 blocked treatments of maize and soybean at the University of Minnesota Agricultural experiment site in central Minnesota. Maize (Green Giant Code 63) and soybean (Northrop King S19R5) were planted in 10 x 10 m plots, at planting densities of 5.18 and 24.70 plants/ m^2 respectively.

Crops were planted on June 10 in 2008 and on June 4 in 2009. Since previously published work with a similar maize variety in this region suggested that coccinellids would colonize in mid to late July (Schellhorn, 1998), we began scouting for coccinellids and aphids on a few plants per plot in the first week of July. We initiated sampling on July 9 in 2008 and July 12 in 2009 and continued until the no immature (larval or pupal) coccinellids were sampled.

At weekly intervals, we visually inspected plants at 15 randomly selected locations per plot. Each sample consisted of two adjacent maize plants or two adjacent 18 cm sections of soybean row. Weekly sampling for the duration of the coccinellid season gave us a total of 6 sample dates in 2008 (with one week missed due to rain) and 9 sample dates in 2009, when coccinellids persisted later in the season. Coccinellid numbers were reported by species and stage, with coccinellid egg masses collected for identification in the laboratory. Egg masses were checked daily and emerged coccinellid larvae were identified and returned to their natal plots within 48 hours (KKP, chapter 1).

When aphids were sparse, we counted individual aphids. However, in maize, aphids sometimes reached high numbers in tassels, and in these cases we chose a

representative subsection of the densely populated part of the plant, counted aphids on it, and multiplied by the number of similar subsections that existed on the plant. A similar method was used to estimate soybean aphid numbers. Since soybean aphids move to different parts of soybean plants over the season (McCornack et al., 2008) we chose a vertical subsection of all plant parts and counted aphids within it, multiplying by the number of similar subsections present in the sample. Although we observed only *Aphis gylcines* in soybean, in maize we observed both *Rhopalosiphum padi* and *Sitobium avenae* in addition to the most common species, *Rhopalosiphum maidis*. *R. padi* made up less than 5 percent of the total aphid population, with the rest made up almost exclusively by *R. maidis*. Our aphid estimates include both of these closely related aphid species (Papasotiropoulous et al., 2013). *S. avenae* was quite rare, appearing on only a few plants per season.

Statistical analysis

We constructed 2 x 2 contingency tables for presence/absence data of all pairs of potential IG predator groups. Because IG predation generally occurs between a mobile predator and an immobile prey, we classified individuals by stage-- egg, larva, pupa or adult.

After performing a preliminary analysis to determine that patterns in observed and expected contact did not differ between 2008 and 2009, we pooled data across years and removed species/stage combinations observed on five or fewer plants. We Yates corrected the remaining contingency tables as needed and used maximum likelihood based on a log linear model to identify pairings in which observed contacts differed significantly from expected contacts. Significance was evaluated using an experimentwise $p<.05$ standard with Bonferoni correction.

To pinpoint when IGP might be most likely, and whether there are differences in avoidance over the course of the season, we also used the methods described above to compare observed contact to random contact among heterospecific predators before, at and after aphid densities peaked in maize. Grouping the data by aphid densities, we considered data from the beginning of sampling through peak aphids as “increasing aphids” and the remaining data (through week 6 and week 15 for 2008 and 2009) as

"declining aphids". In general, separating the data by weeks should decrease the effect of temporal avoidance, allowing us to more readily determine if predators present at the same time in the season were avoiding each other in space. Unfortunately, in soybean we had only one coccinellid species that was abundant. Since other species were relatively rare, dividing the data into subcategories yielded such low numbers that we did not have the statistical power to detect avoidance or aggregation by date in soybean.

In addition to analyzing contingency tables generated from our own survey data in maize and soybean, we constructed contingency tables in the same manner to statistically analyze the survey data collected by Kindlmann and Houdkova (2006) in tansy. This analysis goes beyond determining whether observed contact is greater or less than random contact for each predator pairing, as originally reported by Kindlmann and Houdkova, because by using maximum likelihood we could discern significant deviations from random contact. Before conducting this analysis, we eliminated those species/stage combinations for which individuals were observed on 5 or fewer plants, because these cannot be reliably analyzed.

Results

Insect densities

Aphids and lady beetles were sufficiently abundant to observe patterns in avoidance and aggregation among predators. Over the course of 2008 and 2009 growing seasons, we sampled 1553 soybean and 1557 maize plants for aphids and aphid feeding coccinellids. Both soybean and maize contained ample prey for coccinellids, with aphids present on 99% of soybean and 84% of maize plants sampled. When aphid densities peaked, all plants were colonized and there was an average of just over 2,000 aphids per plant in both crops. Kindlmann and Houdkova did not report aphid densities over the season, but they inoculated all plants with the aphid *M. tanacetaria*.

Overall we observed 3409 coccinellids in soybean and 6207 in maize, with at least one individual present on 63% of soybean and 57% of maize plants at the time of survey. At their peak in the first week in August, coccinellids were present on 94% of soybean and 96% of maize plants sampled. Examining coccinellids by species, *H.*

axyridis was the most abundant in soybean, where it made up 76 and 93% percent of the coccinellid community in 2008 and 2009 respectively. In maize *H. axyridis* and *C. maculata* were similarly abundant, together making up over 97 and 98% of the coccinellid community in 2008 and 2009. Other coccinellids observed included *Cycloneda munda*, *Coccinella septempunctata* and the *Hippodamia* species *H. tredecimpunctata and H. convergens*. (KKP, chapter 1)

The predator densities reported by Kindlmann and Houdkova in tansy were lower than those we observed in soybean and maize. The most abundant predators in tansy, syrphid larvae, were present on 15% of plants, compared with the most abundant predators in soybean and maize, larvae of the coccinellid *H. axyridis*, which were present on 36 and 23% of soybean and maize plants respectively.

Observed vs. random contact

In general, observed contact among heterospecific predators was not distinguishable from that expected assuming predators distributed themselves randomly with regard to other predators. Of 103 predator pairings examined, 90 showed the same contact as random, while only 10 showed more and 3 showed less contact than random (Table 4.1). Despite the fact that in most cases contact was the same as random, insights could be gained from careful examination of the characteristics of those pairings for which significantly more or less contact than random was observed. We considered this with regard to habitat, species involved, and stages involved.

Whether pairings were considered in soybean, maize or tansy seemed to matter. In both soybean and maize there were more predator pairings with greater than random contact than those with less than random contact. For example, of the 44 pairings examined in soybean, none showed evidence of avoidance while 2 showed evidence of aggregation (Table 4.2). In maize 7 pairings showed more contact than random, while only one pairing out of 41, *C. maculata* eggs and *H. axyridis* pupae, showed less (Table 4.2). Similar to soybean and maize, most pairings in tansy had contact that did not differ significantly from random. However, more pairings (2 out of 26) showed evidence of avoidance, while only one showed evidence of aggregation (Table 4.2). Since two of these pairings showing avoidance involved coccinellid eggs, which were not separated by

species, it was impossible to distinguish conspecific and heterospecific contact, leaving only one pairing, syrphid larvae and coccinellid eggs, that had significantly less heterospecific contact than random.

Interestingly, all pairings with aggregated contact involved the lady beetle *Harmonia axyridis* and most (7 of 10) involved at least one species of coccinellid larva. Since immobile stages might be more susceptible to predation than other stages, we also examined combinations that involved a mobile stage (larva or adult) in contact with an immobile stage (egg or pupa). We found that contact did not differ significantly from random for any such combinations, except for 2 pairings in maize, *H. axyridis* eggs/*C. maculata* adults and *H. axyridis* pupae/*C. maculata* larvae, for which more contact was observed than random (Table 4.2).

Contact over time in maize

Corn leaf aphid densities peaked in the 3rd week (August 1) of sampling in 2008 and in the 4th week (August 12) of sampling in 2009. We found that whether extraguild prey were increasing or decreasing had no effect on contact among coccinellids (Table 4.3). More contact than random was seen between the larvae of the two species examined, *C. maculata* and *H. axyridis*, but this was consistent regardless of whether aphids were increasing or decreasing. One other pairing, *C. maculata* adults and *H. axyridis* eggs, showed more contact than random when aphids were increasing, but this relationship was also present when the data were not divided.

Discussion

The hypothesis that contact among aphid predators would be significantly limited by avoidance was not supported by the data. Our soybean and maize surveys revealed contact among potential IG predators to be the same as or higher than one would predict assuming that predators were randomly distributed among plants. Further, although Kindlmann and Houdkova (2006) concluded that avoidance was common in tansy, our statistical analysis of contact among 26 predator pairs in tansy revealed only one case in which heterospecific predators were in less than random contact. Thus, although studies have shown predator behaviors such as adult avoidance of larval residues among

coccinellids to affect individual foraging and egg laying behavior (B. K. Agarwala et al., 2003; Meisner et al., 2011), at a population level we observe no trend of avoidance among potential IG predators and their prey.

Given the lack of significant differences between observed and random contact among IG predators, the likelihood that heterospecific IG predators share a plant would be determined by the proportion of plants occupied by each predator species, with a higher proportion of occupied plants leading to more contact. Thus in resource rich monocultures like the ones we studied, predator densities, rather than interspecific interactions such as avoidance, appear to determine IG contact. Since contact is not limited by avoidance in the field and lab studies have shown that IGP is likely when coccinellid predators come into contact with each other (Cottrell & Yeargan, 1998; Lucas et al., 1998;Felix & Soares, 2004; Yasuda et al. 2004; Lucas, 2005; Noia et al., 2008; Ware & Majerus, 2008) and with other aphid predators (Phoofolo & Obrycki, 1998; Noia et al., 2008; Noppe et al., 2012) IGP is likely to occur when densities of aphid predators are high, a situation common in very productive agricultural habitats.

Although most observed contact did not differ from random contact, there were cases in which predators were in more contact than expected, which would increase the likelihood of IGP. This was rare in tansy and soybean but much more common in maize, suggesting that habitat affects interspecific interactions among predators. This is consistent with the idea that habitats with clumped distributions of prey tend to increase predator interaction (Lucas, 2005). Although aphids in maize and soybean were equally clumped at the spatial scale of the whole plant, corn leaf aphid in maize tended to form huge colonies in plant tassels, which attracted groups of interacting coccinellids. In soybean, where aphids were distributed more evenly throughout each plant, coccinellids were more evenly distributed within the plant. Further, at the start of surveys in maize and soybean, only about half of maize plants were colonized by aphids, while all soybean plants were colonized. Thus in maize, prey were clumped initially among plants and later within plants, so coccinellids might have been compressed to interact in few places.

In addition to differences in the frequency of intraguild contact by habitat, the identity of the predators involved seemed to affect the likelihood of interaction. In

particular, the predatory coccinellid *H. axyridis* was involved in all interactions in maize and soybean for which more contact was observed than random. The high abundance of *H. axyridis* in maize and soybean probably made it easier to detect deviations from random contact involving *H. axyridis* than it might have for rarer species. However, the fact that *H. axyridis* was only very rarely involved in pairings with less contact than expected suggests that the high frequency of cases with more contact than expected was not solely due to high abundance, but a reflection of *H. axyridis* ecology. *H. axyridis* might be disproportionately involved in pairings with high levels of contact because the species is less vulnerable to IGP than are other species. For example, *H. axyridis* larvae (Cottrell & Yeargan, 1998) and eggs (Cottrell, 2007; Smith & Gardiner, 2013) are less susceptible to predation by other coccinellids than the reverse. This reduced vulnerability would leave *H. axyridis* with a smaller benefit from avoiding aggregation around food patches, in contrast to other species, such as *C. maculata*, that have been shown to adjust its position on plants in response to the presence of *H. axyridis* (Musser & Shelton, 2003). *H. axyridis* might also be relatively less sensitive than other coccinellids to heterospecific tracks. *H. axyridis* did not adjust oviposition or foraging behavior based on the presence *of C. septempunctata* (Meisner et al., 2011) or *Propylea japonica* (Agarwala et al., 2003) tracks, although both of these species avoided *H. axyridis*.

Another notable pattern was that most predator pairings with more contact than random involved larvae. Larvae can be expected to have more contact with each other and with other stages because of their residence time on the plant. While eggs and pupae are only present for a few days, and thus are likely to be temporally segregated from other species/stages, larvae of *C. maculata* and other coccinellids persist for weeks (Wright & Laing, 1978). In contrast to adults, larvae are less likely to move among habitats to seek prey, but instead must search for resources within their natal habitats. As larvae move among plants in search of prey, aggregation to patches of dense prey is likely. Larvae tend to molt near aphid aggregations (Lucas et al., 2000) where predators feed, making them periodically vulnerable to IGP. More often, larvae also take on the role of IG predators. Studies of captured coccinellid larvae have revealed high levels of

alkaloids from other coccinellid species (Hautier et al., 2011) and in lab studies coccinellid larvae attack other larvae (Yasuda et al., 2004), pupae (Ware & Majerus, 2008) and eggs (Cottrell, 2004), suggesting that interactions with larvae can lead to intraguild predation.

There were only two heterospecific pairings in which less contact was observed than random - syrphid larvae and coccinellid eggs in tansy and *C. maculata* eggs and *H. axyridis* pupae in maize. Syrphids and coccinellids have been observed to interact strongly via IGP, and although syrphids are more frequently the prey, young coccinellid larvae and eggs do suffer attacks by syrphids (Agarwala & Yasuda, 2001; Almohamad et al., 2010), thus there is benefit to coccinellids in avoiding oviposition near syrphids. Also, since syrphids tend to live in established aphid colonies (Coderre et al.,1987), and some lady beetle species lay eggs away from aphid colonies (Schellhorn & Andow, 1999a) or prior to the establishment of aphid colonies (Dixon, 2007), contact may be much less likely. *C. maculata* eggs and *H. axyridis* pupae would not be in contact due to temporal segregation—*C. maculata* eggs sometimes appear just as aphids begin colonizing, and *H. axyridis* pupae appear later, after several weeks of larval development. Thus, lack of contact between these stages is due to temporal segregation. However, IGP is not possible between these stages.

When contact data were separated into two time periods, as aphids were increasing and after aphid densities had peaked, patterns were similar to when all data were grouped together. Thus, although the incidence of IGP might increase (Chacon & Heimpel, 2010) or decrease (Lucas et al., 1998) with local prey abundance, our study suggests that in maize this would not be related to changes in avoidance behaviors. Further, the lack of obvious differences between the combined data and data divided by resource availability suggests that aggregation/avoidance observed among species/stages was due to the distribution of insects in space as well as time.

In summary, heterospecific contact among aphid predators was mostly determined by their densities. Contact among IG predators was more likely than random when resources were patchy, potentially causing predators to cluster around resources. Despite the apparent random distribution of predators, the fact that those stages and species least

likely to be vulnerable to intra guild predation (mobile stages, *H. axyridis*) were more likely than random to be in contact with heterospecific predators suggests that vulnerability to predation may play a role in the distribution of these species despite the lack of obvious avoidance. It is possible that most predators balance close temporal and spatial tracking of patchy prey resources with some level of avoiding heterospecifics, resulting in what appears to be a random distribution of predators. In contrast, those predators focused only on tracking patchy resources may appear to aggregate and have greater than random contact. Since the net effect is that contact was either the same as or more than expected than due to random chance, IGP can be expected in any habitat with high predator populations and is unlikely to be limited significantly by avoidance among predators.

Table 4.1. Number of predator pairings by contact category (less, more, or the same as random) and crop. Numbers in parentheses represent the subset of listed pairings that combine a mobile stage (adult or larva) with an immobile stage (egg or pupa). These are combinations in which contact is more likely to lead to intraguild predation or cannibalism.

	Pairings by crop and category			
crop	**less**	**more**	**same**	**total**
soybean	0 (0)	2 (0)	42 (6)	44 (6)
maize	1 (0)	7 (2)	25 (14)	33 (16)
tansy*	2 (1)	1 (0)	23 (5)	26 (6)

*Coccinellid eggs were not separated by species in tansy, thus some pairings may include conspecifics.

Table 4.2. Log odds observed vs. expected contact among predators. Negative numbers suggest less contact than expected, positive numbers suggest more contact than expected. Cells with contact that differed significantly from random contact ($p<.05$ level, Bonferoni corrected to $p<.002$) are shaded. For each species/stage combination, the number of individuals observed is given in bold type, followed by the number of plants sampled that contained at least one individual from this category. For tansy, the number of plants with 1 or more individuals from each category is reported.

Maize log odds observed vs. random contact (N=1577 plants observed)							
Species and stage	**individuals, plants**	**HaE**	**HaL**	**HaP**	**HaA**	**MuA**	**C7A**
Coleomegilla maculata eggs	**1923, 104**	1.20	0.06	-1.98	0.03	1.33	-inf
C. maculata larvae	**524, 267**	0.54	0.91	0.39	-0.04	1.31	0.80
C. maculata pupae	**80, 59**	-0.72	-0.20	0.98	0.33	-inf	-inf
C. maculata adults	**292, 226**	0.83	0.09	0.08	0.13	0.96	-inf
Harmonia axyridis eggs (HaE)	**1420, 55**					1.28	1.97
H. axyridis larvae (HaL)	**1065, 358**					1.20	1.20
H. axyridis pupae (HaP)	**434, 220**					-0.11	-0.11
H. axyridis adults (HaA)	**319, 251**					0.86	0.45
Cycloneda munda adults (MuA)	**9, 8**						3.20
Coccinella septempuncatata adults (C7A)	**9, 8**						

Soybean log odds observed vs. random contact (N=1553 plants observed)										
Species and stage	**individuals, plants**	**HaE**	**HaL**	**HaP**	**HaA**	**MuL**	**MuP**	**MuA**	**C7L**	**C7A**
C. maculata larvae	**16, 15**	-inf	-0.08	0.29	0.57	1.00	-inf	1.87	-inf	-inf
C. maculata adults	**31, 25**	-inf	-0.12	-1.32	-0.28	0.49	1.49	-inf	0.37	-inf
H. axyridis eggs (Ha0)	**1017, 43**					1.05	-inf	-inf	-inf	-inf
H. axyridis larvae (HaL)	**1191, 562**					0.64	0.68	0.73	0.51	0.32
H. axyridis pupae (HaP)	**293, 232**					0.34	-0.74	0.23	-0.47	0.11
H. axyridis adults (HaA)	**578, 411**					-1.62	-inf	-0.34	-1.05	0.23
C. munda larvae (MuL)	**41, 38**								1.05	1.23
C. munda pupae (MuP)	**14, 14**								0.95	2.22
C. munda adults (MuA)	**16, 16**								1.91	2.09
C. sepempunctata larvae (C7L)	**54, 43**									
C. septempuncatata adults (C7A)	**12, 12**									

Tansy log odds observed vs. random contact (N=3000 plants observed)									
Species and stage	**plants**	**eggs**	**LwE**	**C7L**	**AdL**	**LwL**	**SyL**	**C7A**	**P14A**
coccinellid eggs, sps (eggs)	**264**		-inf	1.49	-inf	-inf	-inf	-inf	-inf
Chrysopid eggs (LwE)	**85**			0.33	-inf		-0.27	-inf	0.87
C. septempuctata larvae (C7L)	**51**				-inf	-inf	-0.35		0.69
A.bipunctata larvae (AdL)	**9**					-inf	-inf	-inf	-inf
Chrysopid larvae (LwL)	**10**						-0.33	-inf	-inf
Syrphid larvae (SyL)	**417**							-0.29	-1.41
C. septempunctata adults (C7A)	**115**								1.13
Propylea quatourdecimpunctata (P14A) adults	**59**								

Table 4.3. Log odds observed vs. random contact as aphids increase (left) and decrease (right). Contact shown between the most common species, C. maculata (Cm) and H. axyridis (Ha). Stages for which individuals were observed on 5 or fewer plants have been removed from the data. Cases with significantly more contact than expected due to chance are in italics.

		Pre Peak					**Post Peak**		
		Ha egg	Ha larva	Ha adult			Ha larva	Ha pupa	Ha adult
		51	**128**	**80**			**230**	**214**	**171**
Cm egg	**92**	0.62	-0.06	0.55	Cm larva	**154**	***0.68***	0.23	-0.42
Cm larva	**113**	0.58	***1.22***	0.51	Cm pupa	**52**	-0.42	0.44	-0.23
Cm adult	**122**	***0.77***	0.28	0.38	Cm adult	**104**	-0.02	0.18	0.01

Chapter 5. Cannibalism and coexistence among intraguild predators

Introduction

When two consumers share a resource, theory predicts that the consumer best able to compete for the shared resource will exclude the inferior competitor (Gause & Witt, 1935; Hardin, 1960; Tilman, 1982). Such exclusion seems to be common in arthropod communities, particularly after the addition of a novel species (Reitz & Trumble, 2002). However, among predators that both share resources and prey upon one another, coexistence is also common (Vance-Chalcraft et al., 2007). Predators sharing a resource often engage in intraguild predation (IGP), in which one predator, termed the intraguild predator, preys upon the other predator, termed the intraguild prey (Polis et al., 1989). Models of intraguild interactions predict coexistence when the intraguild prey is also the better competitor for the shared, extraguild prey (Holt & Polis, 1997). In terrestrial systems, intraguild prey do tend to better suppress shared prey than do intraguild predators (Vance-Chalcraft et al., 2007), supporting this prediction. However, Holt and Polis (1997) also predicted that this mechanism of coexistence functions only at intermediate resource levels-- high or low resource levels would favor the stronger intraguild predator or the better competitor for extraguild resources, respectively.

In contrast to the narrow range of circumstances in which the models of Holt and Polis predict coexistence, intraguild predators seem to coexist in a wide variety of conditions even when habitat and resource overlap is extensive (Vance-Chalcraft et al., 2007). Although some consumers may successfully use temporal or spatial avoidance to limit contact with potential intraguild predators (Mylius et al., 2001; Hampton, 2004), they often face very high mortality from predation (Polis et al., 1989). This high mortality from predation along with a marked lack of evidence for avoidance among some intraguild predators (KKP, Chapter 4) suggest that in some systems, other factors must be important.

One such factor may be cannibalism. Often, in communities of predators that both feed on and compete with each other, larger or more mobile stages of predators attack younger, more vulnerable stages of both conspecifics and heterospecifics (Polis et

al., 1981). When cannibalism is size or stage specific, as it is in communities of insect predators, it generally has a stabilizing effect within populations (Kaewmanee & Tang, 2003; Claessen et al., 2004; Buonomo et al., 2010). In communities in which predators interact via both competition and predation, cannibalism may broaden the range of conditions in which coexistence is possible (Rudolf, 2007; Chakraborty & Chattopadhyay, 2011). If predators are under greater threat of cannibalism than of intraguild predation, cannibalism could tip the balance in favor of coexistence rather than exclusion by one species over the other, particularly where cannibalism is common within the stronger intraguild predator (Rudolf, 2007).

We investigated the relative likelihood of mortality by intraguild predation and cannibalism on a pupa in two generalist, intraguild insect predators feeding on shared prey— one species known as a strong intraguild predator and another known to act more often as the intraguild prey. In such a system, cannibalism, particularly within the stronger intraguild predator, could allow coexistence of the two species despite their shared resources. We used feeding experiments to estimate the likelihood that a vulnerable pre pupa of each species would be eaten by a cannibal or an intraguild predator given the two came into contact. We then used observed contact between the species to quantify the opportunities for intraguild predation and cannibalism in two habitats—one in which the intraguild predator and prey are equally common and another in which the intraguild prey are rare. We predicted that prey of both species would be more likely to experience cannibalism than IGP, and that cannibalism might be more likely in the shared habitat.

Methods

Study system

We examined the relative impact of cannibalism and intraguild predation using two species of lady beetles (Coleoptera: Coccinellidae), the native twelve spotted lady beetle, *Coleomegilla maculata* and the exotic multicolored Asian lady beetle *Harmonia axyridis*. These species prey primarily on aphids (Hodek, 1973; Koch, 2003), but also on conspecifc and heterospecific eggs, larvae and pupae. Several studies have shown that

intraguild interactions between *C. maculata* and *H. axyridis* tend to be asymmetrical, with *C. maculata* more likely to be attacked by heterospecifics than the reverse (Cottrell & Yeargan, 1998; Cottrell, 2007; Pell et al., 2008).

However, if lady beetles are much more likely to cannibalize than to prey on heterospecifics, asymmetrical interactions among heterospecifics might be relatively unimportant for determining coexistence. Agarwala and Dixon (1992) examined predation on eggs among four species of lady beetles and concluded that cannibalism was more common than intraguild predation, suggesting that species might be chemically protected from heterospecific attacks. The importance of cannibalism among lady beetles was further supported in a study by Schellhorn and Andow (1999a) that showed stronger intraspecific predatory interactions than interspecific predatory interactions among lady beetles foraging in maize. Similarly, in a laboratory study Burgio et al. (2002) found that *H. axyridis* larvae and adults were more likely to prey on conspecific eggs than those of another coccinellid, *Adalia bipunctata*. Cottrell (2005, 2007) also found that in small laboratory arenas *H. axyridis* and *C. maculata* were very likely to cannibalize eggs.

Because mortality among coccinellid pupae has been shown to be high in maize (Schellhorn & Andow, 1999b) and lady beetle mortality was the highest for older larvae becoming pupae in both maize and soybean (KKP, chapter 2), we focused on the role of cannibalism and intraguild predation on pupal mortality between these two species in maize where both species are common, and in soybean, where *C. maculata* is rare (KKP, chapter 1).

Few studies have examined intraguild predation and cannibalism on pupae, but a study by Ware and Majerus (2008) showed that *H. axyridis* larvae readily preyed on the pre-pupae of many other species, and that in 10 out of 12 species considered, this predation was unidirectional- with *H. axyridis* always acting as the predator. *C. maculata* was not included in the Ware and Majerus study, but given the generally asymmetric intraguild predation between *H. axyridis* and *C. maculata* observed at other stages (Pell et al., 2008) and how rarely other species attacked *H. axyridis* pre pupae in the Ware and Majerus study, it is reasonable to expect very asymmetric intraguild predation with *C.*

maculata acting as the prey when adults or larvae of these species come into contact with pre pupae.

Although asymmetric intraguild predation might lead to the exclusion of *C. maculata* from shared habitats, if cannibalism is more important than intraguild predation, coexistence might be possible. This could happen either because both species prefer to cannibalize pre-pupae of their own species, or because contact is more common among conspecifics than heterospecifics in an open field setting.

Attacks on pre pupae by conspecific vs. heterospecific predators

H. axyridis and *C. maculata* 3rd and 4th instar larvae were hand collected from small plots of maize and soybean at MAES in Minnesota between July 25 and August 22, 2012. Collected larvae were separated by species and held in small groups in a growth chamber at 25°C with night day cycle of 16:8. These larvae were provided with water and soybean aphids *ad libitum* until they neared pupation. Large fourth instar larvae were moved to individual petri dishes lined with wax paper and allowed to form pre-pupae, which were cut from wax paper and held at 10°C until they could be used (maximum of 48 hours). Those pre-pupae that completely shed their cuticle, thereby completing pupation during this time were discarded.

As the larvae in the laboratory neared pupation, adult *H. axyridis* and *C. maculata* were collected in the same plots at MAES, and females subsequently isolated in 5.5 cm petri dishes on a filter paper substrate, with water provided via cotton wicks. These females were starved for 24 hours in the 25°C growth chamber prior to the start of the experiment. Because both adults and larvae were field collected, the age and quality of adult predators and pre-pupal prey in this experiment can be expected to be typical of what would be seen in the field at this time.

Feeding trials were conducted in 8.5 cm petri dish arenas using each of the four possible predator (adult) and prey (pre-pupa) combinations. Each arena was lined with filter paper and provisioned with one pre-pupa and water accessible via cotton wick. One 24 hour starved female was then placed about 3 cm from the pre-pupa. After one hour, we observed pre-pupae for evidence of predation. Each feeding arena was then held in the 25°C growth chamber and observed at 6, 24, and 48 hours after the start of the trial.

We conducted 52 trials each for *C. maculata* pre-pupae paired with *C. maculata* and paired with *H. axyridis* adults, and 43 trials each for *H. axyridis* pre-pupae paired with *C. maculata* and paired with *H. axyridis* adults.

Beyond 24 hours predation dropped off sharply, thus we limited our analysis of attack data generated in the laboratory to observations through 6 hours. Within this time frame, the relationship between the natural log of survival and observation time was linear, yielding constant attack rates. Assuming predators are actively foraging in the field, contact with another insect on the same plant within 6 hours seems reasonable. We then used the Survival package in R to examine the effect of prey and predator species (and their interaction) on the likelihood of attack. We used a Cox proportional hazards model based on an exponential baseline hazard function with the response as time of attack (data right censored when no attacks occurred by 6 hours) to consider how these factors affected the instantaneous mortality rate. Chi squared test was used to determine if there were significant differences based on prey or predator species.

Intraguild predation and cannibalism by habitat

To estimate the probability of IGP and cannibalism on pupae in each habitat, we multiplied the probability that a pupa in the given habitat/year would encounter each predator type by the likelihood that contact between this predator and pre-pupal prey would result in a fatal attack on the prey (previously estimated as described above).

We estimated the probability of contact between *C. maculata* and *H. axyridis* pupae and potential predators using data from surveys of coccinellids in experimental plots of maize and soybean in 2008 and 2009 (KKP, chapter 4). For each year, block and habitat, we estimated the probability that a pupal prey would come into contact with a given predator by dividing the number of times the predator and prey were present in the same sample (2 adjacent plants in maize, or two adjacent 18 cm sections of soybean row) by the total number of samples in which the prey was present. Since *C. maculata* pupae were not observed in soybean samples, we estimated the likelihood a hypothetical *C. maculata* pupa in soybean would come into contact with a predator based on the probability of finding the predator in a sample. Because there was little evidence of aggregation or avoidance among the predators and prey we sampled (KKP, Chapter 4),

the likelihood that a sample contains a predator should be similar to the likelihood that a given prey would encounter that predator.

For each pupal prey (*C. maculata* or *H. axyridis*), we used a two sample t-test to compare the likelihood of being attacked in maize to the likelihood of being attacked in soybean. Because variances were unequal, we used Welch's t test for this comparison. Similarly, for each potential prey within a habitat, we compared the likelihood of the prey experiencing cannibalism or IGP using Welch's t test. For both of these tests 2008 and 2009 were considered separately and larval and adult predators were considered separately.

Intra vs. interspecific interaction strength by habitat

Finally, we used these data to compare the product of the intraspecific interactions (cannibalism) to the product of the interspecific interactions (IGP) by habitat and year. According to competition theory, if the product of the intraspecific interactions is greater than the product of the interspecific interactions, then coexistence is predicted. For intraspecific interactions we multiplied the probability of *C. maculata* predation on *C. maculata* pupae by the probability of *H. axyridis* predation on *H. axyridis* pupae. Similarly, for interspecific interactions we multiplied the probability of intraguild predation when *H. axyridis* was the prey by the probability of intraguild predation when *C. maculata* was the prey. We calculated this separately for interactions with adult predators and with larval predators.

Within each habitat and year, we compared the products of intraspecific and interspecific interactions, using a two sample t test to determine if these were different. To compare whether intraspecific interactions were stronger or weaker than interspecific interactions within each habitat and year, we used Welch's two sample t test assuming unequal variances.

Results

Attacks on pre-pupae by conspecific vs. heterospecific predators

The feeding trials conducted in laboratory revealed different hazard rates depending on treatment (Chi-square = 75.3, df = 3, $p < 0.0001$, Table 5.1). Prey species

significantly affected the likelihood of attack ($p < 0.0001$) with *C. maculata* pre-pupae attacked at a rate about four times that of *H. axyridis* pre-pupae (Table 5.1). There was also a significant effect of predator species ($p < 0.0001$), with attack occurring about 1.5 times faster when the predator was *H. axyridis* than when the predator was *C. maculata* (Table 5.1). The magnitude of the differences we observed in hazard rates suggest that even if field predators were much hungrier or more satiated than those we tested in the laboratory, these differences would persist.

The instantaneous hazard rate also depended on the predator/prey combination, and this is reflected in a significant interaction between these factors ($p < 0.0001$), as well as in the estimated hazard rates themselves (Figure 5.1). Both species of predators were more likely to attack conspecifics than heterospecifics, and this difference was particularly marked among *C. maculata* predators, which were more than 60 times as likely to attack a conspecific pre-pupa than a heterospecific pre-pupa (Figure 5.1). Although *H. axyridis* also cannibalized more often than they attacked heterospecifics, the difference was comparatively small, with attack on conspecifics 1.3 times as likely as attack on heterospecifics (Figure 5.1). Thus, given contact, *C. maculata* pre-pupae are at higher risk of being preyed upon than are *H. axyridis* pre-pupae, and both are more likely to experience cannibalism than intraguild predation.

Cannibalism and intraguild predation on pupae by habitat

Absolute predation estimates for each prey, predator and habitat combination were affected both by differences in hazard rate and contact. These ranged from nearly zero (*C. maculata* larvae or adults attacking *H. axyridis* pupae in 2009 soybean) to 0.45 (*H. axyridis* larvae cannibalizing *H. axyridis* pupae in 2008 soybean) (Table 5.2). In both habitats and years, *H. axyridis* pupae were more likely to be preyed upon by cannibals than by intraguild predators ($p < .001$ for all comparisons). In contrast, *C. maculata* pupae were more likely to be attacked by intraguild predators in soybean over both years ($p < .001$ for all comparisons) and in maize in 2008 for adult predators ($p<0.001$). Among larval predators in 2008 maize, no significant difference existed between likelihood of attack by cannibalism vs. IGP. In 2009, *C. maculata* faced more cannibalism than IGP by adults and by larvae ($p<0.001$). Statistical comparisons were conducted separately for

larval and adult predators, but both predator types showed similar patterns, with *H. axyridis* facing more cannibalism and *C. maculata* facing more IGP in soybean, and either IGP or cannibalism in maize depending on year and predator stage compared (Table 5.2).

An examination of predation by habitat revealed that the likelihood of cannibalism differed by habitat for both prey species. *C. maculata* pre-pupae were more likely to be cannibalized by both adults and larvae in maize than in soybean, a pattern that held true for 2008 and 2009 (Figure 5.2). *H. axyridis*, in contrast, tended to face greater risk of cannibalism in soybean, although this pattern was less consistent, with significant differences observed only among attacks by adult predators in 2008 and larval predators in 2009 (Figure 5.2). Intraguild predation did not differ significantly by habitat for either prey species, though there were some interesting trends. *C. maculata* showed higher IGP in maize for both predator stages and in both years, while *H. axyridis* faced higher IGP in maize in 2008 but in soybean in 2009 (Figure 5.2).

Aside from our focal species, few other predators were present in maize and soybean, thus examining the likelihood of attack by *C. maculata* and *H. axyridis* adults and larvae on each prey type yields a reasonable estimate of its likelihood of being attacked in each habitat. *C. maculata* prey were more than twice as likely to be attacked in maize (0.96 and 0.78 in 2008 and 2009) as in soybean (0.37 and 0.32 in 2008 and 2009), while the total likelihood of predation on *H. axyridis* was slightly lower in maize (0.64 and 0.31 in 2008 and 2009) than in soybean (0.78 and 0.62 in 2008 and 2009).

Intra and interspecific interaction strength by habitat

Although for *H. axyridis* cannibalism was clearly stronger than intraguild predation, a comparison of the product of intraspecific interactions (cannibalism) and interspecific interactions (IGP) in these habitats requires taking into account predation on *C. maculata* pupae as well. Despite the fact that intraguild predators did attack *C. maculata* pupae, sometimes more often than did conspecifics, the product of intraspecific interactions was greater than that of interspecific interactions in both habitats and both years and both predator stages (Table 5.3).

Discussion

Intraguild predation

Intraguild predation theory yields powerful predictions about when interacting predator species will coexist, and when one of them will be excluded. In simple models of two predator species and a shared extraguild prey, Polis and Holt (1997) show that coexistence of predator species is predicted only when the intraguild predator is a weaker competitor for the shared resource and when resources are at an intermediate level. At very high resource levels, competition for shared prey is less important than predatory interactions, excluding the intraguild prey, while at low resource levels, the superior competitor is able to survive at lower resource conditions, thereby excluding the intraguild predator (Polis and Holt, 1997). In their meta-analysis of IGP, Vance-Chalcraft et al. (2007) found that often, IG prey were better competitors for shared resources, generally supporting this idea.

Our system matches well to the basic intraguild predation scenario modeled by Polis and Holt. Maize and soybean are heavily fertilized and productive crops, each home to very high densities of one dominant aphid species (soybean aphid and corn leaf aphid respectively) that serves as the primary extraguild prey for coccinellids. Coccinellids are by far the most abundant aphid predators in the system (KKP, chapter 1) and although they suffer high mortality from attack by other coccinellids (KKP, chapters 2 and 3) they have few other predators, and parasites and diseases seldom greatly affect their population dynamics (Riddick et al., 2009). Given this relatively simple system, with a productive extraguild resource fed on by two species of omnivorous predators, we would predict exclusion of the intraguild prey, particularly if it is not a superior competitor for the shared resource. This seems to be an appropriate characterization of our system. Although each coccinellid species is capable of attacking vulnerable stages of the other, we found that IGP was highly asymmetrical. In small laboratory arenas, adult *H. axyridis* frequently attacked *C. maculata* pre-pupae, while cases of adult *C. maculata* attacking *H. axyridis* pre-pupae were extremely rare. Further, our estimated field predation rates show that *H. axyridis* would rarely be attacked by an IG predator in

the field, while *C. maculata* would face much higher rates of IG predation. Both of these results suggest that *H. axyridis* regularly acts as IG predator and *C. maculata* as IG prey.

Other researchers investigating intraguild interactions between *H. axyridis* and *C. maculata* reached a similar conclusion. Comparing the growth rate of the two species, Labrie et al. (2007) suggested that the fast growth of early instars of *H. axyridis* would protect them from IGP by *C. maculata*, while *C. maculata* would be vulnerable. In studies of direct interactions in small arenas, Cottrell (2005) found that *H. axyridis* preyed more readily on *C. maculata* eggs than the reverse, and Cottrell and Yeargan (1998) found *H. axyridis* to be the IG predator and *C. maculata* the IG prey when larvae came into contact.

The establishment of *H. axyridis* as the IG predator suggests that coexistence would depend on the ability of *C. maculata* to more efficiently exploit resources. In a laboratory study feeding the two species pea aphids, *H. axyridis* consumed aphids at a faster rate than did *C. maculata* (Labrie et al. 2007), suggesting that *H. axyridis* may be the superior forager. Although smaller coccinellids like *C. maculata* are typically able to persist at lower resource levels (Dixon, 2007), Grill et al. (1997) found great phenotypic plasticity among *H. axyridis* with regard to size. At low resource levels *H. axyridis* were able to complete development at a small size (Grill et al., 1997), potentially allowing it to survive low resource levels as well as *C. maculata*. Comparing the resource use and relative growth of the two beetles, Labrie et al. (2007) concluded that *H. axyridis* was likely to be both the superior competitor and stronger IG predator, potentially excluding *C. maculata* from habitats.

In summary, exclusion of the IG prey would be predicted in both maize and soybean based on IGP theory. Displacement by introduced coccinellids, particularly *H. axyridis*, is correlated with the decline of native coccinellids (Harmon et al., 2007) and IGP and competition has been used to explain the decline of native coccinellids and to predict their exclusion from shared habitats (Alyokhin & Sewell, 2004; Snyder & Evans, 2006; Evans et al., 2011). Despite this prediction, however, *C. maculata* has not been excluded from maize, despite the fact that *H. axyridis* has been present in maize in Minnesota since the late 1990s (Hesler et al., 2001), and *H. axyridis* is currently very

abundant in maize (KKP, chapter 1). This situation is not unusual. In 2007, Vance-Chalcraft et al. found that while intraguild prey often better compete for shared extraguild resources, there are also many cases in which IG prey are inferior competitors, yet they coexist with IG predators (Vance-Chalcraft et al., 1997).

Cannibalism

Although Holt et al. (1989) acknowledge that cannibalism is common among omnivores, IGP theory typically ignores the role of cannibalism in structuring communities. Cannibalism within the IG predator is widely thought to promote coexistence by limiting predator population growth (Amarasekare, 2003; Rudolf, 2007; Chakraborty & Chattopadhyay, 2011; Ohlberger et al., 2012). By integrating cannibalism into IG interactions modeled by Polis and Holt (1997), Rudolf (2007) confirmed that the addition of size structured cannibalism within an IG predator broadens the circumstances under which coexistence is predicted because at high population levels the IG predator population will limit itself and because at low resource levels the IG predator can subsist via cannibalism. The addition of cannibalism within the IG prey narrows the conditions under which coexistence is predicted very slightly, making the overall impact of cannibalism within both IG predator and IG prey an increased range of conditions under which coexistence is possible. Most importantly, the IG prey no longer need be the superior competitor for coexistence to be predicted. This finding suggests that the addition of cannibalism within predator, prey, or both, would have the general effect of broadening the conditions for coexistence in many systems, making empirical observations match predicted outcomes more closely.

In laboratory studies of adults interacting with pre-pupae, we found that both the IG predator, *H. axyridis*, and the IG prey, *C. maculata*, were more likely to cannibalize than to prey on a heterospecific, showing that cannibalism would be important in making coexistence predictions for these species. We did not examine cannibalism at other stages, but Cottrell (2005) found that adults of both species readily cannibalize eggs when coming into contact with them in small arenas. Although attacks on eggs, molting larvae and pupae are probably most common and thus most relevant, a study placing active

larvae in contact in the laboratory revealed that cannibalism was common within *H. axyridi*s but rare in *C. maculata* (Cottrell & Yeargan, 1998).

Our study, along with these laboratory studies on interactions among these species, suggests that attack by cannibalism is common for all immature stages of these lady beetle species. Thus, although lady beetles tend to move between more and less vulnerable stages throughout development, the fact that immatures tend to suffer cannibalism while adults do not, make this system similar to the one modeled by Rudolf (2007) in which cannibalism is possible up to a certain age. Varying the time of vulnerability to cannibalism and IGP did not affect conditions for coexistence greatly (Rudolf, 2007), suggesting that the conclusions of these models—that cannibalism will allow for more coexistence, even in cases in which the IG prey is not a superior competitor for the shared resource, likely apply to our system. While in the absence of cannibalism *C. maculata* should be excluded from the system, cannibalism, particularly within the IG predator *H. axyridi*s, makes coexistence possible in this system, even at fairly high resource levels.

Whether cannibalism makes coexistence possible for the species we studied likely depends on the relative importance of cannibalism and IGP in interactions. We considered this by modeling cannibalism and IGP as interference competition and comparing the product of cannibalistic interactions to the product of IG interactions for predators interacting with pupal prey. Since the product of cannibalistic interactions was consistently significantly higher than the product of IG interactions, we conclude that in our system, species interact more strongly with themselves than with each other, predicting coexistence. Thus incorporating cannibalism into our consideration of interactions between predators and pupae changes the prediction from exclusion of the native to coexistence of both species. Although we considered only pupae, the propensity of both species to cannibalize at other stages suggests that we would reach a similar conclusion if we had examined another life stage.

Estimating IGP and cannibalism in the field

Most studies of IGP and cannibalism are limited to small laboratory arenas. By incorporating the likelihood of contact in the field into our estimates, we were able to

evaluate our initial prediction that in addition to preferring to cannibalize, each species would be more likely to encounter a conspecific than to encounter a heterospecific, making cannibalism far more common in the field. Our estimates show that, as predicted, the likelihood of cannibalism was much greater than that of IGP for *H. axyridis*. However, for *C. maculata* prey, the importance of cannibalism vs. IGP was driven by differences in the relative density of each predator species because adults and larvae of both species might prey on *C. maculata* given contact. Since *C. maculata* were rare in soybean, they were far more likely to encounter heterospecifics, leading to IGP. In maize, cannibalism was more common when *C. maculata* was more abundant and IGP was more common when *H. axyridis* was more abundant. These results suggest that laboratory results alone not sufficient to predict the relative incidence of cannibalism vs. IGP in the field.

The total likelihood of attack on *C. maculata* prey was higher in maize than in soybean, and our results suggest that this is because *C. maculata* prey faced cannibalism and IGP in maize but only IGP in soybean. This generally higher level of predation in maize was consistent with our experimental results showing that sentinel *C. maculata* eggs were more likely to be eaten in maize than in soybean (KKP, chapter 2). In contrast, the probability of *H. axyridis* being preyed upon tended to be higher in soybean. Interestingly, despite the lower probability of being eaten in soybean (KKP, chapter 3) and the abundant and suitable prey available there (KKP, chapter 2) *C. maculata* seems to prefer maize habitats (KKP, chapter 1).

IGP, cannibalism and colonization of new habitats

Observed declines in native lady beetles in the Americas (Harmon et al. 2007; Hesler et al. 2008) have been blamed on displacement by introduced lady beetles (Snyder & Evans, 2006; Crowder &Snyder, 2007). Researchers have noted that the broad diets (Crowder & Snyder, 2007) and efficient prey use (Labrie et al., 2007) of introduced predators helps them successfully colonize novel habitats. Asymmetrical IGP may play a critical role in facilitating both the colonization of introduced species and their displacement of natives (Snyder et al. 2004; Evans et al., 2011). Introduced predators can

be prevented from successfully colonizing habitats when they are sufficiently preyed upon by natives (Ehler & Hall, 1982)

Prior to the invasion of *H. axyridis*, *C. maculata* was often the dominant species in maize in our area of study (Schellhorn, 1998). Given our finding that attacks by *C. maculata* on *H. axyridis* were very rare, but cannibalism of *H. axyridis* on immobile stages of conspecifics were common, those *H. axyridis* that initially colonized maize must have enjoyed a relative freedom from predation. Thus we would predict that populations of *H. axyridis* would rapidly increase in maize habitats until they reached a high enough density that *H. axyridis* prey would be likely to encounter conspecifics and be cannibalized. This seems to be what has happened with *H. axyridis*, which has achieved roughly even densities with the native *C. maculata* in maize. In contrast, we would predict that *C. maculata* might not successfully colonize a habitat already occupied by *H. axyridis* unless it were a better competitor for the shared resource because it would experience no similar freedom from predation. Instead, *C. maculata* prey would be readily attacked by conspecifics and heterospecifics. This seems to be what is seen in soybean habitats, where *C. maculata* and other native species have failed to colonize despite abundant extraguild prey (KKP, chapter 1).

Although most researchers cite mainly IGP when considering displacement of natives by introduced coccinellids, cannibalism is likely equally important. Rudolf (2007) suggests that low population densities will render cannibalism unimportant in the colonizing species, but that cannibalism within the inhabiting species would facilitate invasion. When predicting the colonization of Florida citrus by *H. axyridis*, Michaud (2002) noted that high levels of cannibalism within the native coccinellid, *Cycloneda sanguina*, would enable *H. axyridis* to invade. Similarly, high rates of cannibalism among *C. maculata* would have enabled *H. axyridis* to invade maize habitats, where *C. maculata* was historically the dominant species, while the lower rate of cannibalism that we observed within *H. axyridis* might help to explain why *C. maculata* and other natives are rare in soybean habitats.

Although IGP by *H. axyridis* and *C. septempunctata* has been blamed for the declines in native coccinellids observed in many habitats (Snyder et al., 2004), these

decreases in relative native abundance do not mean that native species will be excluded from these habitats. More extensive consideration of cannibalism, in addition to IGP, will greatly improve our ability to interpret interactions in these predator communities. In our consideration of IGP and cannibalism between a native and an exotic in maize and soybean, cannibalism is critical to coexistence. Although asymmetric IGP would lead to the expectation that *C. maculata* would be excluded from shared habitats (Holt & Polis, 1997), inclusion of cannibalism this system predicts coexistence. This prediction is borne out in maize where both species are common, as well as in soybean, where the native is rare, but present (KKP, chapter 1).

Table 5.1. Influence of prey and predator species and their interaction on instantaneous hazard. Parameter estimates from the Cox proportional hazards model with a baseline exponential mortality function. Chi square for model = 75.31, df=3, N = 190, p = <0.0001.

Prey, Predator	Cox PH parameter	value	z	p
Ha, Ha	(Intercept)	1.40	9.27	<0.0001
Cm, Ha	Prey 2	4.16	4.11	<0.0001
Ha, Cm	Predator 2	1.42	5.10	<0.0001
Cm, Cm	Prey2 – Predator 2 interaction	-4.47	-4.19	<0.0001

Table 5.2. Mean probability of predation (± sd) on pupae by prey species, predator type and habitat. *H. axyridis* (Ha) were more likely to face cannibalism. For *C. maculata* (Cm), IGP was more likely (Welch's t test, $p < .001$), with exceptions shown in italics. In 2008, *C. maculata* were equally likely to face cannibalism or IGP by larvae in maize, while in 2009, cannibalism was more likely in maize (Welch's t test, $p < .001$).

2008	prey	predator	stage	contact	N	attack	N	predation	df
maize	Cm	Cm	adult	0.13 (0.18)	2	0.88 (0.33)	52	0.11 (0.03)	52
			larva	***0.38 (0.18)***				***0.33 (0.21)***	
		Ha	adult	0.63 (0.16)	2	0.35 (0.48)	52	0.22 (0.31)	52
			larva	***0.88 (0.07)***				***0.30 (0.42)***	
	Ha	Cm	adult	0.14 (0.07)	4	0.04 (0.19)	43	0.01 (0.03)	45
			larva	0.53 (0.11)				0.02 (0.10)	
		Ha	adult	0.35 (0.16)	4	0.46 (0.51)	43	0.16 (0.21)	45
			larva	0.98 (0.03)				0.45 (0.50)	
soy	*Cm	Cm	adult	0.04 (0.01)	4	0.88 (0.33)	52	0.03 (0.02)	54
			larva	0.03 (0.01)				0.02 (0.01)	
		Ha	adult	0.41 (0.03)	4	0.35 (0.48)	52	0.14 (0.20)	54
			larva	0.52 (0.07)				0.18 (0.25)	
	Ha	Cm	adult	0.01 (0.03)	4	0.04 (0.19)	43	0.00 (0.01)	45
			larva	0.04 (0.03)				0.00 (0.01)	
		Ha	adult	0.71 (0.11)	4	0.46 (0.51)	43	0.33 (0.37)	45
2009			larva	0.82 (0.13)				0.38 (0.43)	
maize	Cm	Cm	adult	***0.41 (0.14)***	4	0.88 (0.33)	52	***0.36 (0.19)***	54
			larva	***0.22 (0.12)***				***0.20 (0.14)***	
		Ha	adult	***0.30 (0.05)***	4	0.35 (0.48)	52	***0.10 (0.15)***	54
			larva	***0.25 (0.12)***				***0.09 (0.14)***	
	Ha	Cm	adult	0.36 (0.21)	4	0.04 (0.19)	43	0.01 (0.08)	45
			larva	0.25 (0.08)				0.01 (0.05)	
		Ha	adult	0.35 (0.16)	4	0.46 (0.51)	43	0.16 (0.21)	45
			larva	0.29 (0.08)				0.13 (0.16)	
soy	*Cm	Cm	adult	0.03 (0.01)	4	0.88 (0.33)	52	0.02 (0.01)	54
			larva	0.02 (0.01)				0.01 (0.01)	
		Ha	adult	0.40 (0.10)	4	0.35 (0.48)	52	0.14 (0.20)	54
			larva	0.44 (0.08)				0.15 (0.21)	
	Ha	Cm	adult	0.00 (0.00)	4	0.04 (0.19)	43	0.00 (0.00)	45
			larva	0.02 (0.02)				0.00 (0.01)	
		Ha	adult	0.49 (0.19)	4	0.46 (0.51)	43	0.22 (0.28)	45
			larva	0.60 (0.18)				0.28 (0.33)	

**Estimate based on the likelihood a hypothetical prey would encounter predators.*

Table 5.3. Comparison of the products of intraspecific (cannibalism) and interspecific (IGP) interactions by habitat and predator stage. Standard deviation shown in parentheses. All interactions use pupae as the focal prey. Products of intraspecific interactions were significantly bigger than were those of interspecific interactions for all comparisons based on Welch's t test.

2008 product of interactions by habitat

	predator	intra vs inter			p value
maize	adult	0.018	>	0.001	<0.01
		(0.051)		(0.012)	
	larva	0.149	>	0.006	<0.01
		(0.217)		(0.056)	
soy	adult	0.010	>	0.000	<0.01
		(0.014)		(0.001)	
	larva	0.008	>	0.000	<0.01
		(0.012)		(0.003)	

2009 product of interactions by habitat

	predator	intra vs inter			p value
maize	adult	0.057	>	0.001	<0.01
		(0.089)		(0.014)	
	larva	0.026	>	0.001	<0.01
		(0.041)		(0.008)	
soy	adult	0.005	>	0.000	<0.01
		(0.008)		(0.000)	
	larva	0.004	>	0.000	<0.01
		(0.007)		(0.002)	

Figure Legends

5.1. Mean instantaneous mortality rates (± SD) by prey and predator pairings, determined with Cox proportional hazards model. Prey are *C. maculata* (Cm) or *H. axyridis* (Ha) pre-pupae, while predators are adults of the same two species. Both prey and predator species significantly affected the likelihood that the prey would be killed in the interaction (Table 1).

5.2. Difference in predation between maize and soybean habitats for each pre-pupae and predator combination. Bars above zero suggest predation is more likely in maize. Results for *C. maculata* prey shown in black, *H. axyridis* in stripes. Predators by species (*C. maculata* listed as Cm, *H. axyridis* as Ha) along the x axis. Significant differences in predation by habitat based on a Welch's t test are marked with asterisks ($p < .01$).

Figure 5.1.

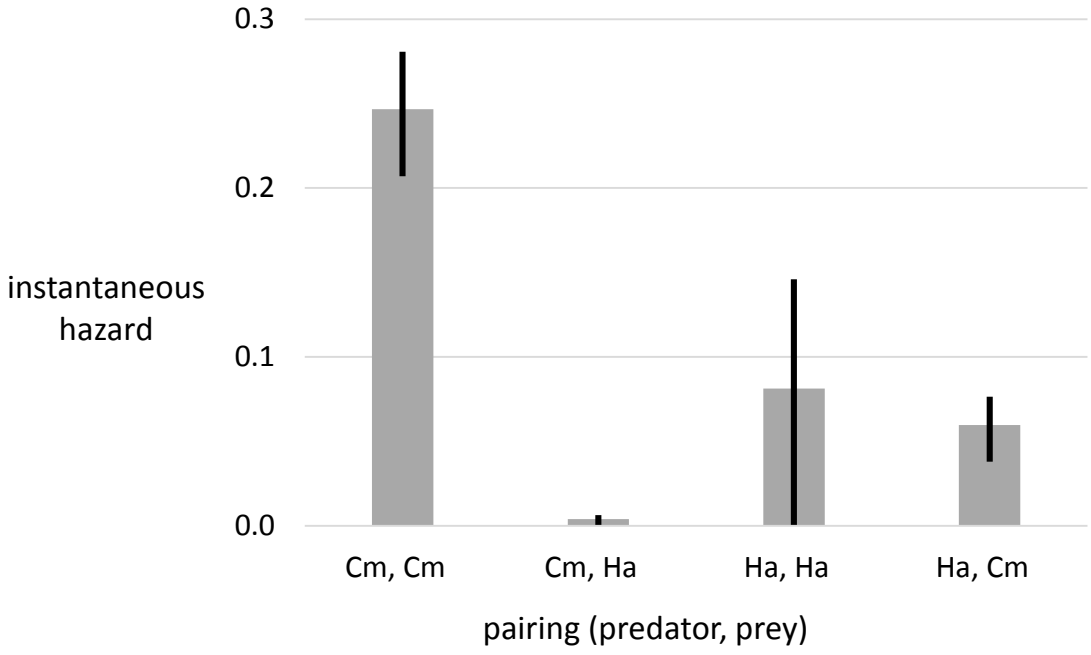
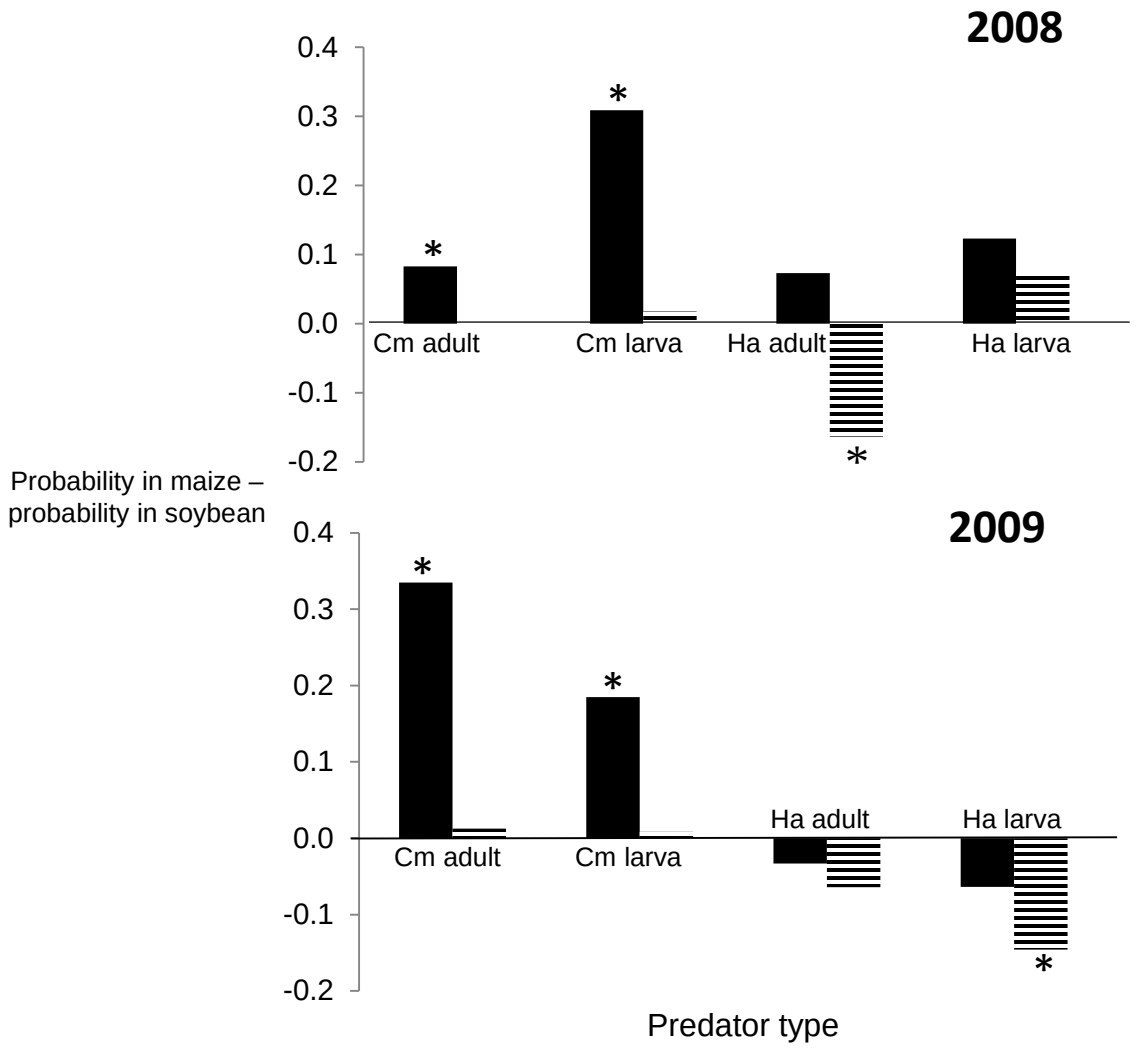

0.3
0.2
0.1
0.0
instantaneous hazard
Cm, Cm
Cm, Ha
Ha, Ha
Ha, Cm
pairing (predator, prey)

Figure 5.2.


2008
2009
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
*
Cm adult
Cm larva
Ha adult
Ha larva
Probability in maize – probability in soybean
Predator type

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