APHID INTERACTIONS WITH ENVIRONMENTAL VARIATION IN THE FIELD AND LAB

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By
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Title

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MASTER OF SCIENCE

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ABSTRACT

A basic tenet of ecology is that organisms are affected by both abiotic factors and other organisms; therefore, there is value in understanding interactions in our changing world. Aphids are model organisms for questions regarding many interactions. I explored three aphid-centered studies. (1) Rearing aphids in a certain temperature changed their response to exposure to different temperatures. Short term exposure to warmer temperatures increased fecundity, but being raised in higher temperatures lowered fecundity across treatments. (2) Feeding on aphids by lady beetles in the lab was measured after exposure to varying temperatures with or without prey. Warming without prey was detrimental, but warming while continually fed invoked more predation than the cooler temperature. (3) Soybean aphids, natural enemies, and other pests vary in space and time. We surveyed soybean to explore the effect of field locations, management, and year on arthropod community structure and found a predominant year effect.
ACKNOWLEDGEMENTS

I would like to thank the many people who supported me as I worked on my degree. Nearly every day was made better by getting so say hello to Danelle, Diane, and Judy; I feel lucky to have been a part of the Department of Entomology at NDSU. My advisor Dr. Jason Harmon supported me throughout the process and always encouraged and guided me to find my way and continue to get better, I cannot thank him enough. I’m grateful to my other committee members Dr. Devan McGranahan and Dr. Marion Harris for their input and role in making my experience more meaningful. I appreciate the many people that helped with my survey, but a special thank you to Sarah and Mary for taking on roles of responsibility and being people I am proud to have befriended. Michael was the best I could have hoped for to help with my lab experiments; his positivity and curiosity are contagious and he is yet another person I am now better for knowing. Thank you to my family, especially Greg and Mellissa for their love and reassurance. And last, but in no way least, a thank you to my daughter Cheltzie, my foundation that helped me keep perspective through everything.
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CHAPTER 1. THERMAL ACCLIMATION MODIFIES THE ABSOLUTE VALUES BUT NOT THE SHAPE OF AN APHID’S THERMAL PERFORMANCE CURVE

Abstract

Temperature is vitally important to insects, yet short-term responses to temperature can vary widely between individuals of the same species. The temperature an individual has previously been exposed to is a proposed explanation of this variability. We exposed genetically identical pea aphids (Acyrthosiphon pisum) to one of three different acclimation temperatures (17°C, 21°C, and 25°C) for their entire lives. Individual fecundity was examined from each acclimation temperature at each of three experimental temperatures. This let us determine how previous acclimation temperatures modified aggregate thermal performance curves—a measure of how aphids respond to temperature—for each acclimation group. All aphids showed increased fecundity as temperature increased, however, the absolute value of the curves varied with previous acclimation temperature: individuals raised at higher temperatures produced fewer nymphs than those raised at lower temperatures. We hypothesized that adult size may have been connected to our results and measured hind tibia length. Although adult size strongly influenced fecundity and was itself influenced by acclimation temperature, it did not explain how the three performance curves changed with acclimation temperatures. These results suggest a potential negative feedback between temperature and pea aphid performance: in the short term, higher temperatures are associated with increased fecundity, but when higher temperatures are maintained for longer periods, overall fecundity may decrease in those aphids.

Introduction

Temperature makes fundamental contributions to the ecology and performance of insects (Bale et al. 2002, Battisti 2004). As such, it has been extensively studied, including a relatively
recent upswing of interest in environmental variation and the consequences of climate change (Dury et al. 1998, Walther et al. 2002, Deutsch et al. 2008). While there are numerous methods for studying the effects of temperature on organisms (Berrigan 1997, Davis et al. 2006, Harmon et al. 2009, Bubliy et al. 2012), a foundational approach uses manipulative experiments to measure short-term performance of individuals across a range of temperatures. Such thermal performance curves can be valuable when integrated with larger experiments and theoretical models (e.g. Schulte et al. 2011). However, results can vary across and even within populations of an organism (Kingsolver and Gomulkiewicz 2003, Kingsolver et al. 2004). Understanding this variation is crucial to correctly interpreting and utilizing information provided by thermal performance curves.

Thermal acclimation—also referred to as the beneficial acclimation hypothesis (BAH)—is one potential explanation for some of this variation within populations. The BAH states that the performance of an organism at a given temperature depends on what temperature the organism was raised in and acclimated to (Leroi et al. 1994, Lagerspetz 2006). This means that insects will perform best at temperatures they have been acclimated to (e.g. Geister and Fischer 2007, Huey et al. 1999, Sorensen et al. 2013), however, the BAH has been rejected as a universal rule across all organisms (Huey and Berrigan 1996, Marais and Chown 2008). Regardless of the ability for exact predictions, experiments examining thermal acclimation suggest the potential importance of developmental plasticity as it relates to performance across temperatures (Wilson and Franklin 2002). Moreover, thermal acclimation has the potential to explain some of the variation found in thermal performance curves. A thermal performance curve shows how performance generally increases, between a low temperature threshold and upper threshold, as
temperature increases. The trend of increased performance with increased temperature typically holds true, but individual performance can vary along that curve.

The pea aphid, *Acyrthosiphon pisum* H. (Hemiptera: Aphididae), has become a model organism for numerous inquiries, including studies on temperature (Morgan et al. 2001). Its asexual reproduction allows researchers to control for difference in genetics (e.g. Caillaud et al. 2002) and the presence/absence of potentially important facultative, bacterial symbionts (Montllor et al. 2002, Russell et al. 2013). Studies of temperature effects on pea aphids suggest short-term fecundity is a useful temperature-sensitive metric to see temperature effects on a function (e.g., Meisner et al. 2014), and fecundity is often closely related, though not exactly equivalent, to other variables that contribute to overall population response (Richardson et al. 2011).

To test for effects of thermal acclimation on pea aphids we established three genetically identical colonies at different acclimation temperatures 17°C, 21°C, and 25°C, and used aphids from each colony to assess fecundity in experimental temperatures and produce thermal performance curves for each acclimation temperature. We also determined the effect of adult body size on thermal performance, as adult size has been shown to correlate with fecundity (Nicol and Mackauer 1999). Our results indicate that thermal acclimation can be important to aphid fecundity, but not in the way we expected.

**Materials and Methods**

**Colonies and Rearing**

We used a colony of genetically uniform pea aphids that originated from a single aphid clone collected on alfalfa in Pennsylvania, USA, and which contained no known facultative symbionts (*K. M. Oliver unpubl. data*). Aphids were reared on fava, *Vicia faba*, and maintained
using standard methods (Meisner et al. 2014, Chapter 2) until separate colonies were established
for experimentation. Three colonies used for experimentation were kept in separate growth
chambers (three Vemco BOD Low Temp Incubators model number 2015 and one Conviron
model number 125L) set at constant temperatures of 17°C, 21°C, or 25°C with L:D 16:8 h. To
ensure that all individuals had been exposed to the same temperature since birth, all colonies
were kept at the acclimation temperatures for at least 15 days before experimentation. To prevent
confounding acclimation temperature with potential differences in growth chambers, we
randomly reassigned each chamber to a new temperature approximately weekly, maintaining
colony target acclimation temperatures while rotating among chambers.

**Experimental Procedure**

To determine pea aphid performance, the fecundity of individual adult pea aphids was
examined over a 48-hour period. Our experiment had a 3x3 factorial design (Figure 1.1), with the
three levels of acclimation temperatures crossed by three levels of experimental temperatures
(17°C, 21°C, and 25°C). Our temperatures were chosen based on previous investigations that
showed pea aphids can grow and reproduce throughout this range of temperatures (Morgan et al.

For each replicate, a single fava leaf was placed in a 5 cm diameter plastic petri dish, and
a single, apterous, adult aphid was placed on the leaf. The stem of the leaf was wrapped in moist
cotton to prevent dehydration. Aphids from each acclimation temperature were then randomly
assigned to experimental temperatures. After 24h and 48h nymphs laid were counted and
removed to examine 48h individual fecundity while avoiding potential overcrowding from
nymphs. We then placed individual adults into a freezer until hind tibia could be measured,
which can be a relative measure of size and potential fecundity in some aphids (Nicol and Mackauer 1999).

**Figure 1.1.** Experimental set up showing how individuals from the three acclimation temperatures were placed in each of the three experimental temperatures to examine fecundity.

Three temporal blocks of the experiment were performed with each of the nine treatments replicated 8-24 times within each block and n=39-49 for each treatment, with N=382 aphids overall (9 treatments acclimation→experimental; 17°C→17°C=39, 17°C→21°C=41, 17°C→25°C=43, 21°C→17°C=39, 21°C→21°C=39, 21°C→25°C=48, 25°C→17°C=41, 25°C→21°C=43, 25°C→25°C=49). Each block used aphids from the same colonies, so that with successive blocks the colonies had been kept at the acclimation temperature for more generations. The first block’s aphids were acclimated for 1-2 generations, the second block’s for
2-3 generations, and the third block’s for 3-4 generations. To avoid confounding effects with temperature, chambers were reassigned to different experimental temperatures between each block.

Statistical analyses were performed in JMP 9.0 (SAS Institute Inc. 2010) and we used an alpha of 0.05. The effect of acclimation temperature and experimental temperature on aphid fecundity was examined with both independent variables considered categorical. Because of the potential for the length of colony acclimation to influence the results, we first ran an ANOVA that specifically tested for interactions amongst a continuous blocking factor, the acclimation temperature, the experimental temperature, and the three way interaction. The ANOVA uses a Type III sum of squares, the default for JMP. There were no interactions between blocks and the other main effects (Table 1.1, p>0.05). We therefore reran the analysis the way it was originally intended looking at acclimation temperature, experimental temperature, their interaction, and a simple continuous block. We also investigated how aphid size may be influenced by acclimation and experimental temperatures using the same ANOVA structure. Finally, we looked at the correlation between aphid size and fecundity.

**Table 1.1.** Results of ANOVA with continuous blocking factor and dependent variable of number of nymphs born.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>SS</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>65.66</td>
<td>3.9</td>
<td>0.049</td>
</tr>
<tr>
<td>Acclimation temperature</td>
<td>2</td>
<td>92.96</td>
<td>2.76</td>
<td>0.064</td>
</tr>
<tr>
<td>Experimental temperature</td>
<td>2</td>
<td>1453.59</td>
<td>43.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acclimation temperature * experimental temperature</td>
<td>4</td>
<td>71.5</td>
<td>1.06</td>
<td>0.37</td>
</tr>
<tr>
<td>Block * Acclimation temperature</td>
<td>2</td>
<td>26.64</td>
<td>0.79</td>
<td>0.45</td>
</tr>
<tr>
<td>Block * Experimental temperature</td>
<td>2</td>
<td>39.22</td>
<td>1.17</td>
<td>0.31</td>
</tr>
<tr>
<td>Block * Acclimation temperature * Experimental temperature</td>
<td>4</td>
<td>73.66</td>
<td>1.09</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Results and Discussion

Aphid fecundity was influenced by both the acclimation temperature an aphid developed in, and the short-term experimental temperature (Figure 1.2, Table 1.2). However, the two temperature treatments did not interact and had opposite effects on reproductive performance. Looking at these two factors as categorical variables allow us to look for results expected regarding the BAH e.g. those raised at 21°C performed best at 21°C. We found that fecundity was influenced by acclimation temperature ($F_{2,372}=3.21$, $p=0.042$) and experimental temperature ($F_{2,372}=43.36$, $p<0.0001$), but not their interaction ($F_{4,372}=0.97$, $p=0.32$). For acclimation temperature, the highest fecundity was observed at 17°C, and was greater than fecundity at 25°C ($p<0.05$), with 21°C being intermediate and not significantly different than the other two (means ± 1 S.E.: 11.9 ± 0.4 17°C, 11.7 ± 0.5 21°C, 10.7 ± 0.3 25°C). For experimental temperature, all three treatments were different from each other with the greatest fecundity at 25°C and decreased fecundity with lower temperatures (means ± 1 S.E.: 8.8 ± 0.4 17°C, 11.3 ± 0.4 21°C, 13.7 ± 0.4 25°C).

Table 1.2. Results of ANOVA for acclimation and experimental temperature and dependent variable of number of nymphs born.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable: number of nymphs born</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>1</td>
<td>57.78</td>
<td>3.43</td>
<td>0.065</td>
</tr>
<tr>
<td>Acclimation temperature</td>
<td>2</td>
<td>108.16</td>
<td>3.21</td>
<td>0.042</td>
</tr>
<tr>
<td>Experimental temperature</td>
<td>2</td>
<td>1461.95</td>
<td>43.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Acclimation temperature * experimental temperature</td>
<td>4</td>
<td>65.35</td>
<td>0.97</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Figure 1.2. The effect of the short-term, experimental temperature on aphid fecundity for three groups of aphids that were raised at one of three acclimation temperatures. Individual points are averages (± 1 S.E.).

We hypothesized that acclimation temperature may have been related to a change in the size of adults, which could be correlated with fecundity. Adult size and fecundity were correlated (across all treatments $t_{379}=4.94$, $p<0.0001$), and acclimation temperature did influence size (Table 1.3, $F_{2,371}=10.65$, $p<0.0001$). However, adult size per se did not seem to explain our results. To produce the overall pattern (Figure 1.2), we expected that aphids grown at 17°C would be the largest and 25°C would be the smallest. However, in this study aphids grown at 21°C were larger than 17°C and 25°C ($p<0.05$), and the latter two were not distinguishable (means ± 1 S.E.: 2.5 ± 0.02 mm 17°C, 2.6 ± 0.02 mm 21°C, 2.5 ± 0.02 mm 25°C). Thus, size does not provide an overall explanation for our acclimation effect, as acclimation and
experimental temperature did not affect number of nymphs born and hind tibia length in the same way.

**Table 1.3.** Results of ANOVA for acclimation and experimental temperature and dependent variable of hind tibia length (aphid size).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>1.87</td>
<td>10.78</td>
<td>0.0011</td>
</tr>
<tr>
<td>Acclimation temperature</td>
<td>2</td>
<td>3.7</td>
<td>10.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Experimental temperature</td>
<td>2</td>
<td>0.28</td>
<td>0.81</td>
<td>0.44</td>
</tr>
<tr>
<td>Acclimation temperature * exp temperature</td>
<td>4</td>
<td>0.67</td>
<td>0.81</td>
<td>0.43</td>
</tr>
</tbody>
</table>

We designed this experiment to test for a clear effect of both acclimation temperature and experimental temperature. Thus, there are a number of important factors to consider when extrapolating or generalizing our findings. For example, we specifically chose temperatures at which we expected a positive, linear relationship between temperature and fecundity. Higher temperatures can cause complicating effects, including those from short-term heat shocks (Harmon et al. 2009). We also chose to keep aphids at the acclimation temperature for their entire life in hopes it would make it easier to see the strongest signal, although from previous studies it is not clear how long acclimation might take. Finally, we also chose to use a single aphid clone with no known secondary bacterial symbionts. The ecological properties of pea aphids can vary with both aphid genetics and symbiont association (Oliver et al. 2010), thus further testing would need to explore the relative role of these in relation to temperature acclimation and thermal performance curves.

Our study demonstrates the potential importance of acclimation or development temperature in determining the response of insects to temperature sensitive experiments. While the results did not necessarily match the proposed effects of thermal acclimation or changes in body size, they represent a clear source of variation for thermal performance curves and similar
experimental temperature-dependent tests of insect performance. In addition, these results suggest the potential for interesting interactions between short-term and long-term effects of increasing or variable temperature on aphid dynamics. In the short-term, higher temperature could increase the population growth rate of aphids because of higher fecundity. However, when maintained at higher temperatures, the insects’ potential fecundity may be reduced, thereby negating some of the effect. Understanding the full consequences of temperature on species’ population dynamics may therefore require knowledge of temperature effects and how those effects change with temporal variation.

References


CHAPTER 2. LADY BEETLE PREDATION AFTER VARIABLE TEMPERATURE EXPOSURE AND PREY AVAILABILITY

Introduction

Altered abiotic conditions, including rising temperatures and other factors associated with climate change, can alter biotic interactions among organisms (Tylianakis et al. 2008). Insight on these factors responses to rising temperatures comes from comparing interacting organisms in two different abiotic settings. Those comparisons look at differences between conditions now and predicted average conditions in the future. Yet this approach does not necessarily capture the consequences of more discrete events of climate change. For example, precipitation events are expected to be less frequent but more intense (Trenberth et al. 2003) and heat shocks—warm temperatures for a short time period—are expected to be more common (Diffenbaugh et al. 2005). While it is clear that species interactions can change during these short-duration events (e.g., Harmon et al. 2009), it remains to be determined if species interactions are modified following an abiotic change once the event is complete. The potential change after exposure could be particularly important for predation events, which can be crucial in determining how climate change might influence species (Harmon and Barton 2013).

Although often studied as long term averages, temperature is not constant in environments. Particularly warm afternoons can invoke heat shocks and harm organisms unable to move or produce heat shock proteins to help mitigate the stress (Feder and Hofman 1999). Climate change also affects temperatures differently over the course of a 24-hour period. During the last hundred years, nights have been warming at twice the rate of days (IPCC 2007), one possible explanation being that increasing cloud cover leads to slower cooling from day to night. As nights stay warmer, activity patterns could change and potentially alter organism interactions.
This differential warming across time periods needs to be examined, as opposed to looking at temperature change as a constant average difference.

Predation rates for insects are influenced by temperature (Abbott et al. 2014), but predator activity is not constant. When and how effectively a predator forages can be affected by fluctuating factors including temperature (Simonsen et al. 2009), light (Harmon et al. 1998), and diel patterns. Diel patterns differ between predators, which are reflected in foraging patterns of predators in various ecological settings (Lundgren et al. 2009, Pfannestiel and Yeargan 2002). This variability in feeding creates scenarios in which discrete changes in temperature within a given day might alter discrete predation events; the two may offset each other and create the possibility of temperature influencing predation rates in the near future.

Sevenspotted lady beetles, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), are a dominant aphid predator originally introduced to North America from Europe and now present in many crop systems (Hodek and Michaud 2008). *Coccinella septempunctata* preys upon pea aphids, *Acyrthosiphon pisum* H. (Hemiptera: Aphididae), which are soft-bodied insects that feed on many legumes and are commonly used for feeding studies (Ugine and Losey 2014). Lady beetles such as *C. septempunctata* are important natural enemies in many systems, and their interactions can affect both their prey and other lady beetles (Harmon et al. 2007). Thus direct effects of abiotic changes to lady beetles such as predation rate at different temperatures (Sentis et al. 2012) can have knock-on effects to other species, as shown through the effect of wind on ladybeetle predation and resulting aphid abundance (Barton 2014).

Our study on *C. septempunctata* was motivated by the current differential warming rates being observed over day versus night. Previous work has found that *C. septempunctata* adults may feed at night, but feeding rates are lower in the dark as it likely suppresses their foraging
(Nakamuta 1987). To verify this observation, we performed a preliminary investigation of larval activity by measuring feeding rates under a temperature treatment of 20°C from 6am to 10pm and 17°C from 10pm to 6am with a 16L:8D photoperiod. Feeding was recorded every eight hours over two days. As Nakamuta (1987) found with adults, we observed lower larval feeding during the night than the day, although some feeding occurred in each period. Thus there is the potential for feeding throughout the day and night, but there is reason to expect lower activity at night.

We examined the consequence of different temperature exposure on subsequent predation by exposing adult and larval *C. septempunctata* to 17°C or 25°C for a period and quantified their feeding rates after exposure, when placed together at a common, moderate temperature of 21°C. Two experiments were performed: one in which predators were denied access to prey during the exposure period, and another in which half were provided food during the exposure period. Typically, insect feeding will increase as temperature increases, but little to no work reports on feeding after temperature exposure. Simulating different prey availability during temperature exposure can give further insight to any effects seen from exposure temperature on subsequent feeding. Our results indicate that for some life stages temperature can alter feeding rates after the exposure period to the temperature, even once temperatures have returned to a moderate level.

**Materials and Methods**

**Lady Beetle Colony**

On May 28th 2014 approximately 40 adult individuals of *C. septempunctata* were collected in alfalfa near the campus of North Dakota State University in Fargo, ND and brought to the lab. These lady beetles and their offspring were maintained through the following experiments. The lady beetles were housed in two mesh cages containing fava plants, *Vicia faba*. 
In colony cages, the lady beetles fed upon pea aphids from another laboratory colony; aphids were replenished as needed. Pea aphids for feeding came from lab and greenhouse colonies created using aphids collected from alfalfa fields near NDSU’s campus in the summer of 2012 and replenished each year to maintain genetic variation. Cages were cleaned and plants replaced as needed, approximately every two weeks.

To obtain larvae for the experiments, adults were collected from the two colony cages, secluded, and then combined in an empty cage to form mating pairs. Each pair was then placed in its own 9 cm petri dish with aphids and a water supply, allowing for the collection of eggs. Once eggs hatched, larvae were provided aphids and dishes were cleaned as needed. We combined broods by date the eggs were laid as they grew and moved them to larger 14 cm petri dishes. Larvae from petri dishes not used in the experiments were returned to the colony cages or allowed to pupate in the dishes and were returned to the colony as adults.

**Lady Beetle Feeding After Periods of Exposure Temperatures While Starved**

*Lady Beetle Adults*

We examined feeding rates of adult lady beetles after an exposure period of being starved while under one of two temperature treatments. Starving represents an extreme condition where either no prey is available or the predator chooses not to forage. By only allowing predation after being exposed to the temperatures, we can isolate subsequent effects of temperature exposure without having to account for potential differential predation during the exposure period (see night starved vs. fed ladybeetles).

We removed adult lady beetles from the lab colony, combined them in large petri dishes, and allowed them to feed until satiation prior to the experiment for three hours to standardize hunger level. Individual lady beetles were randomly placed in a 5cm petri dish for the duration of
the experiment and experienced 16L:8D photoperiods. The dish included moist cotton and was randomly assigned to either the 17°C or 25°C exposure treatment. Respective dishes were then placed into the designated growth chamber from 4:30 pm until 9:30am (all steps of experiment listed in Table 2.1). We randomly rotated temperature treatments between four climate controlled growth chambers between blocks to avoid a chamber effect (three Vemco BOD Low Temp Incubators model number 2015 and one Conviron model number 125L).

Table 2.1. Experimental timing for lady beetle feeding after periods of exposure temperatures 17°C or 25°C and while starved during exposure.

<table>
<thead>
<tr>
<th>Set up day</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed aphids and set at common temp 9:30am</td>
<td>Fed aphids and set at common temp 9:30am</td>
<td>Fed aphids and set at common temp 9:30am</td>
</tr>
<tr>
<td></td>
<td>Checked at 11:30am</td>
<td>Checked at 11:30am</td>
<td>Checked at 11:30am</td>
</tr>
<tr>
<td></td>
<td>Checked at 1:30pm</td>
<td>Checked at 1:30pm</td>
<td>Checked at 1:30pm</td>
</tr>
<tr>
<td>Fed to satiation starting at 1pm</td>
<td>Checked and aphids removed at 4:00pm</td>
<td>Checked and aphids removed at 4:00pm</td>
<td>Checked and aphids removed at 4:00pm</td>
</tr>
<tr>
<td>Placed into exposure treatments 4:30pm</td>
<td>Placed into exposure treatments 4:30pm</td>
<td>Placed into exposure treatments 4:30pm</td>
<td></td>
</tr>
</tbody>
</table>

In the morning 20 mid to late instar pea aphids were counted out for feeding each individual. To increase certainty that lady beetles were the cause of aphid mortality, we tested a total of 20 dishes with 10 aphids each as aphid-only controls in 5cm petri dishes containing moist cotton; our experimental set-up lacking the lady beetle. The average death rate per dish was 0.9 aphids (±0.2). Thus, we are confident counting aphids remaining alive from those supplied would accurately represent lady beetle feeding.

At 9:30am, after the exposure period, we removed adults from growth chambers and provided individuals 20 aphids. Then we placed all lady beetles in an open laboratory space,
averaging around 21°C, for the duration of the common period. Two feeding checks were done during the common period so we could add more aphids if needed. Sets of 10 aphids for supplemental feeding were counted prior to each check at 11:30am and 1:30pm. We added 10 more aphids if there were 5 or fewer remaining alive. The final check was done at 4:00pm; we recorded the remaining number of live aphids and cleared petri dishes of aphids and any aphid remains for the night. At 4:30pm we placed lady beetles back in their exposure treatments. Feeding and checking was repeated for 3 days. Three blocks of the experiment were performed with 10 adults used per treatment in each block. Seven adults did not provide data over all three days and were removed, for a grand total of 53 adults in this experiment.

*Lady Beetle Larvae*

The experimental set-up was the same as for adults, but here we used larval lady beetles to examine the effect of an exposure period, at two temperature treatments with no food, on subsequent feeding rates. Larvae used in the experiments were fourth instars that had developed from broods of eggs laid the same day. Three hours prior to the experiment, broods were combined in large petri dishes and fed until satiation to standardize hunger levels. As before, 5cm petri dishes were set up with moist cotton for the experiment and individual larvae were randomly assigned a dish and an exposure temperature. We used the same schedule as for adults and exposed larvae to a temperature during which they were starved over the evening and night and fed during the day with predation rates recorded (Table 2.1). The experiment was replicated four times (temporal blocks) and a total of 79 larvae had predation data for all three days.

Statistical analyses for adults and larvae used the same procedures but were performed separately in JMP 9.0 statistical software (SAS Institute Inc. 2010). Two different analyses were performed for each life stage. The first was an ANOVA using the total number of aphids a
predator consumed over all three days as dependent/response variable. Temperature was the independent variable and the temporal block was included as a random variable. A second analysis was performed separately for each life stage in the form of a repeated measure. This used the predation of each day separately and allowed us to test for difference in predation as the experiment progressed over time. Treatment was again the primary independent variable and the temporal block was included. For all three analyses the dependent variable was log transformed to meet the assumption of normality.

**Lady Beetle Larvae After Periods of Exposure Temperatures While Starved versus Fed**

The previous experiment represented an extreme condition where predators were unable or unwilling to consume prey during the exposure period. This experiment relaxes that assumption and directly compares predation rates when predators are allowed to feed during the exposure period compared to those that are starved.

The experiment was set up as a 2x2 factorial with two exposure period temperatures and being either starved or fed during the exposure period (Table 2.2). Fourth instar larvae were used and the methodology closely follows to the larval experiment above (Table 2.1). The primary difference in this experiment being that the mass of aphids consumed was used as the response variable. There were two blocks of the experiment. For the first block 0.02 g (±0.001g) of aphids were supplied at the beginning of the common period for all lady beetles and again at the beginning of the exposure period for those in the fed treatments; aphids were weighed out with a Mettler Toledo AG285 balance. In block two, 0.04 g (±0.001g) was supplied to the correct groups at the two times. After exposure and common periods the mass of aphids remaining was determined using the balance and the mass of aphids consumed was calculated based on the mass originally given. The other difference being that the experiment was performed for 48h total. For
each of the 2 blocks there were 10 individual larvae in each of the 4 treatments. In the first block 9 larvae molted during the experiment and were excluded for a total of 71 larvae.

**Table 2.2.** Experimental treatments for the lady beetles, exposure periods at one of two temperatures with fed or starved treatments during exposure.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Common</th>
</tr>
</thead>
<tbody>
<tr>
<td>17°C/Fed</td>
<td>17°C /prey</td>
</tr>
<tr>
<td>17°C/Starved</td>
<td>17°C /no prey</td>
</tr>
<tr>
<td>25°C/Fed</td>
<td>25 °C /prey</td>
</tr>
<tr>
<td>25°C/Starved</td>
<td>25 °C /no prey</td>
</tr>
</tbody>
</table>

Three statistical analyses were performed in JMP 9.0 statistical software (SAS Institute Inc. 2010) using three different response variables: total predation, predation after exposure (during common period), and predation during exposure. ANOVA was used in all three cases including a blocking factor for the temporal block. For the first two analyses the independent variables were exposure temperature, feeding treatment (fed during exposure period or starved), and their interaction. Since there was no feeding in the starved treatment during exposure, that analysis only included exposure temperature and the blocking factor. Tukey HSD was used to make comparisons across treatments. Repeated measures analyses were also performed to look for patterns across the two days of the experiment. The only significant effect (p<0.05) of time was found for the overall time effect when just considering predation during the day (Mean ± 1 SE: Day 1 14.5 ± 0.05 mg vs. Day 2 16.8 ± 0.06 mg; F1,66=11.4, p=0.036). All other time and
time by factor interactions for each of the three response variables were non-significant and thus not included in the results.

Results

Lady Beetle Feeding After Periods of Exposure Temperatures While Starved

Lady Beetle Adults

Lady beetle adult feeding was not affected by exposure temperature treatments (Figure 2.1). Total predation over all three days did not vary with treatment \( (F_{1,50}=0.38, p=0.54) \). When broken into individual days there was no time*treatment interaction \( (F_{2,48}=0.32, p=0.73) \). Across both treatments there was a weak, but non-significant effect of time itself across the three days \( (F_{2,48}=2.46, p=0.10) \) with a trend towards more predation in the third day compared to the first two.

![Figure 2.1](image)

Figure 2.1. Adult lady beetle feeding over three days after experiencing periods of exposure temperatures; lady beetles were not given food over the exposure period. No difference in predation between treatments \( (F_{1,50}=0.38, p=0.54) \). Average aphids eaten per lady beetle \( (± 1SE) \).

Lady Beetle Larvae

Lady beetle larvae consumed slightly more aphids after being exposed to 17°C compared to 25°C during the exposure period (Figure 2.2). Across all three days this was about a 15%
difference and resulted in a significant effect of exposure temperature (F\(_{1,74}=15.85\), p=0.01).

When broken down by day there was a non-significant time*treatment interaction (F\(_{2,73}=2.39\), p=0.10) with the two treatments being very similar on the first day but differing more the longer the experiment was performed.

![Graph showing average aphids eaten per lady beetle (± 1SE)](image)

**Figure 2.2.** Lady beetle larval feeding between exposure temperature treatments over the three days of the experiment. Predation differed between treatments (F\(_{1,74}=15.85\), p=0.01). Average aphids eaten per lady beetle (± 1SE).

**Lady Beetle Larvae After Periods of Exposure Temperatures While Starved versus Fed**

Throughout the experiment total predation was again affected by exposure period temperatures, but the effect reversed depending on whether the lady beetles were given food throughout the entire day or were starved during the exposure time. Combined predation (predation during the exposure period plus during the common period) showed a significant interaction between exposure temperature and feeding treatment (F\(_{1,66}=47.6\), p<0.01; Figure 2.3). When larvae were starved during the exposure period, predation was again higher in 17 °C compared to the 25 °C. However, when larvae were allowed access to food throughout the day, total predation was lower when the exposure temperature was 17 °C than 25 °C.
We can better understand patterns over the entire day by separating out what happened during the exposure period and what happened over the common period. Just during the exposure period, predation varied with temperature treatment: at 25 °C predation was 57% greater than in 17 °C (F\(_{1,32}=59.7, \ p<0.01; \) Figure 2.4). The starved treatment had no prey available during the exposure period so there was no predation to compare. Predation in the common period after exposure followed the same pattern with a significant interaction between exposure temperature and feeding treatment (F\(_{1,66}=26.7 \ p<0.01; \) Figure 2.5). Just considering predation during the common period, when all treatments were held at the same temperature, being starved during the exposure period results in more predation when coming from 17 °C compared to 25 °C, and when fed throughout the day, there is more predation when exposed to 25 °C compared to 17 °C.

![Figure 2.3](image)

**Figure 2.3.** Combined, exposure period and common period, predation of lady beetle larvae in the four treatments, two exposure temperatures in which starved or fed. Exposure temperature by feeding treatment interaction (F\(_{1,66}=47.6, \ p<0.01\)). Average aphids eaten per lady beetle (± 1SE).
Figure 2.4. Larval feeding during the exposure period at two temperatures for those in the fed treatment. Predation varied with temperature treatment ($F_{1,32}=59.7$, $p<0.01$). Average aphids eaten per lady beetle ($\pm$ 1SE).

Figure 2.5. Larval feeding during the common period, all treatments under same temperature conditions, but having previously experienced different exposure temperatures. Exposure temperature by feeding treatment interaction ($F_{1,66}=26.7$ $p<0.01$). Average aphids eaten per lady beetle ($\pm$ 1SE).
Discussion

Many studies have examined how predator-prey interactions can change while insects are exposed to different temperatures, but this study focuses on how interactions can change after exposure to different temperatures. Adult feeding rates did not differ between temperature treatments, whereas larvae starved and exposed to 25 °C consumed fewer aphids following exposure compared to those starved at 17 °C.

The exposure temperature effect on larval predation depended on the opportunity for larvae to feed. Our second experiment confirmed that larvae starved over the exposure period had lower predation after being exposed to 25 °C compared to 17 °C. However, when those larvae were allowed access to prey during the exposure period, 25 °C resulted in more predation than 17 °C. Larvae in 25 °C ate more prey during the exposure period than those in 17 °C. Moreover, this effect also carried over to the next day as larvae from 25 °C also ate more prey when in the same environment as their counterparts exposed to 17 °C.

Previous experiments reported that lady beetle functions—reproduction, development, foraging—change while exposed to various temperatures. For example, short-term effects such as foraging rates show prey consumption tends to increase to a threshold, beyond which it becomes too warm and feeding is suppressed (Sentis et al. 2012). Our results corroborate these findings: when larvae in our study were given prey during the exposure period, those from the warmer temperature had higher predation. Over a longer period of time temperature influences physiological processes of lady beetles, such as larval growth rates (Miller 1992) and adult fecundity (Stathas et al. 2001). In natural environments beyond the lab, temperature alters predator-prey interactions and overall populations (Estay et al. 2014). Given that these real-world conditions would likely experience differential periods of warm and cool, it may be that these
situations influence predators both during warmer periods and after warmer periods, as we found here.

Though not as abundant as temperature related studies, effects of starvation on lady beetle physiology have also been studied. Depriving lady beetle larvae for various times has differential effects on development rates for different species (Phoofolo et al. 2008). Varying timing of feeding so that predators were fed daily rather than less frequently also altered larval size, development time, and survival (Santos-Cividanes et al. 2011). Thus, feeding, or lack thereof, was shown to affect lady beetle traits that temperature can also affect.

Our findings indicate that temperature and prey availability affect lady beetle predation, but the mechanisms underlying our results are less well-defined. Both temperatures used in our experiments (17°C and 25°C) fall within the range of temperature that still support overall lady beetle development and survival (Bakr et al. 2009). Since we tested within hospitable temperatures, reduced predation in 25°C when starved is not likely an effect due to deleterious heat. If the temperature range had been harmful, the same negative effect would likely be seen even with larvae fed at 25°C. Alternatively, the combination of a warmer temperature and lack of food could have resulted in deleterious effects to the predator. In an aphid species, warmer temperatures resulted in quicker mortality when the aphid was only given water (Whalen 2012). Changes in predation rates we observed after exposure in both experiments could potentially be behavioral if the inclination to forage is related to how much effort went into foraging and the relative success previously experienced.

Our observed effects might influence longer term patterns of predation and larval development. Temperature differences themselves can influence development rates, and food availability would confound that (Gyenge et al. 1998). If differential predation continues through
time, the speed of larval development and subsequent size of the predators would likely be altered. Since lady beetle larvae eat more prey as they grow larger, differences in development time would subsequently influence how much prey is eaten. In this way, it may be that the differences between treatments observed here could accumulate and become larger if larvae were continually exposed to these treatments.

We used very specific parameters for temperature and food availability in our experiment. We purposefully chose a temperature difference approaching typical upper and lower limits for insect success. A broader temperature range could be tested to examine boundaries for these effects. Along with a broader temperature range, different periods of starving and temperature exposure could be tested. Our length of time was chosen for logistical reasons, but more testing of different timing, including what time of the day temperature changed, could show differences of potentially important effects related to circadian rhythms and day/night temperatures.

Temperature and prey availability naturally vary throughout time and space, but it is often difficult to know what the ecosystem consequences are, if any. For example, assuming temperature ranges do not become too extreme, some studies have proposed that the average temperature will have the same effect as a varying temperature (e.g., Liu et al. 1995). Our results suggest that might not be the case if temperature influences predation both during and after increases in temperature. Moreover, our results suggest the potential for an interaction between varying temperature and prey availability, which complicates efforts to find and justify average temperatures over variable ones. More pieces of the puzzle, such as more insight for both predator and prey activity under different night temperatures, could help further understanding of what interactions might occur and what population level effects might result.
References


CHAPTER 3. ECOLOGICAL FACTORS SHAPING SOYBEAN ARTHROPOD COMMUNITIES

Introduction

Arthropod communities are susceptible to and experience variation in member composition, especially those communities occupying annual crops. As crops grow and establish each season, arthropods must colonize, thus creating the opportunity for different arthropod communities to form. In contrast, habitats having perennial vegetation can support arthropods until they colonize annual crops (Letourneau et al. 2012). At broad spatial scales, arthropod community composition in a given agroecosystem varies with climate and species ranges. At finer spatial scales, arthropod communities also vary between fields of the same crop and across seasons. The mechanisms behind arthropod community composition variations are difficult to determine. Observable correlations give some insight; prey density per location can affect predator density per locations even within a field (Desneux et al. 2006). Ecological factors may also influence arthropod community composition from field to field.

Soybean is an economically important crop grown widely in the eastern and midwestern parts of the United States and worldwide. In 2014, more acres of soybean were planted and produced in the United States than ever before (NASS 2014). Soybean fields host crop-damaging arthropod pests as well as natural enemies capable of controlling pests. Soybean arthropod communities have shown variation in the diversity and density of arthropods present. For example, populations of the soybean aphid (*Aphis glycines*) have varied considerably from season to season since their 2000 discovery in the United States, the dynamics of which have been explored from many angles without a definite answer (Ragsdale et al. 2004, Noma et al. 2010, Bahlai et al. 2010). Soybean aphid density might be related to variability in the broader
arthropod community of soybean fields, but it’s likely not the only factor given the associations shown between arthropod communities and ecological factors such as field management, location, and year.

Organic versus conventional field management can influence arthropod communities, even for the same primary crop plant. The primary difference between these two management strategies is pesticide use. When applied to control crop-damaging pests, insecticides affect the entire arthropod community. In soybeans, for example, foliar predator populations decline with insecticide use in response to high soybean aphid populations (Lundgren et al. 2013). Field studies show organic farming supports greater biodiversity than conventional crop production, but it is unclear how that biodiversity relates to ecological pest control (Letourneau and Bothwell 2008). Weedy soybean habitats support greater species diversity, including beneficial predators, than weed-free soybeans (Shelton and Edwards 1983). Weed communities tend to be more abundant in organic fields than conventional fields due to the lack of herbicide usage, which in turn may support more biodiversity.

Field location encompasses a wide variety of potentially important spatially-explicit factors, including composition of surrounding landscape and field-specific characteristics such as soil type, many of which influence arthropod community composition. For example, the complexity of agricultural landscapes will alter production-related arthropod biodiversity (Tscharntke et al. 2005). Whether the field is adjacent to similar or different cropland, residential areas, or unmanaged lands can change potential arthropod immigration and emigration. Arthropods vary in their ability and necessity to disperse, so some arthropods may be more affected by location than others. In soybean fields, landscape diversity measures, assessed with the Simpson’s index, and biological control services index values have increased together.
(Gardiner et al. 2009). Soil type also varies with field location and can affect plant parameters, which subsequently can alter insect preference and/or performance. Soybean plants deficient in potassium, due to low potassium concentrations in the soil, are preferable to soybean aphids as they experienced increased population growth (Myers and Gratton 2006).

Arthropod community composition can demonstrate inter-annual variability even when crop composition remains constant, as in many crop systems, including soybeans. Soybean plant nutrients and landscape type affect soybean aphid densities year to year (Noma et al. 2010). Non-crop plant communities surrounding fields, which affect arthropod community composition, is also temporally dynamic (Mayse and Price 1978).

Weather, particularly temperature and precipitation, will affect arthropod communities as it can differ considerably across years in a given field and beyond. This can change the timing of colonization in terms of incoming arthropods compared to resident plants. Timing is important: early soybean production is implemented to avoid certain pests because early planted soybean supports a different arthropod community than a comparable field planted later (Baur et al. 2000). Moreover, soybean aphid population growth rates differ depending upon plant stage at infestation (Meihls et al. 2010) and air temperature (McCornack et al. 2004). Thus, the environmental conditions that soybean aphids and other insects experience could influence population growth rates and, potentially, interactions with other species.

To explore differences in arthropod community composition in relation to management type, location, and between seasons, a three-year survey was conducted in soybean fields in Eastern North Dakota and Western Minnesota. Knowledge of variation in arthropod communities, and the nature of those differences, could be used to support practical and efficient management of soybean agroecosystems.
Materials and Methods

Study Sites

For three consecutive growing seasons, we surveyed six soybean fields at three locations along the Red River Valley of North Dakota and Minnesota. The fields were all managed by the land owners; we only sampled at the fields and did not alter any production decisions. At each location, we sampled two fields within 1-2 miles of each other, one managed organically and the other managed conventionally. Between years the organic or conventionally managed soybean fields were either planted in the exact spot or were planted within a mile from the previous location per the crop rotation schedule. Conventional fields received herbicides, which kept them weed free. Organic fields were weeded manually, if at all, and field weed cover and composition fluctuated in and between fields and seasons. Field soil type was silty clay for all fields, aside from one silty clay loam.

Data Collection and Preparation

Sampling

We sampled arthropods bimonthly in 2011 and weekly in 2012 and 2013. On each sampling date, ten random sets of sweep samples per field were taken with a 38 cm diameter sweep net to collect arthropods (except soybean aphid). Sweep sampling is an effective method to collect a breadth of life stages and species of aphidophagous predators in soybean fields (Schmidt et al. 2008). Each sweep sample was conducted for a count of 60 sweeps at one of 10 random locations per field, while being mindful to avoid overlapping areas sampled. Sweep samples were frozen until processed. To determine soybean aphid densities, we counted aphids on ten randomly chosen plants per field per sampling date.
Taxonomic Classification

Collected arthropods were identified to various taxonomic levels (Table 3.1). Classification was to a coarse taxonomic level for many of the arthropods. In 2011 soybean aphids and four natural enemies were recorded. This lowered the level of sample scrutiny by limiting data collection to arthropods known as relevant to the soybean system. In 2012 and 2013, a total of 13 taxa were accounted for; we only recorded arthropods we deemed potential natural enemies of soybean aphids or potential herbivores of soybean. Those 13 taxa were further divided into trophic groups. While omnivory is possible, we simplified classifications as primarily predatory or herbivorous.

Table 3.1. Arthropods of the survey identified and grouped into taxa, and divided into four community scenarios as used for analysis.

<table>
<thead>
<tr>
<th></th>
<th>5 taxa Community 3 years</th>
<th>13 taxa Community 2 years</th>
<th>Herbivore Community 2 years</th>
<th>Predator Community 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean aphid</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Lady Beetles</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lacewings</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Orius</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Arachnida</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Nabidae</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Predatory Flies</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Miridae</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthoptera</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicadomorpha (hoppers)</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flea Beetles</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentatomidae</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern Corn Rootworm</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) is a pest of concern to soybean. Native to Asia, it is now fairly prevalent in most areas of soybean production and can be an influential pest (Ragsdale at al. 2011).
Stinkbugs (Hemiptera: Pentatomidae) are typically herbivorous. Twelve of fourteen species of Minnesota stinkbugs in soybean fields were documented as herbivorous, and as soybean plants mature stinkbug populations tend to increase more quickly (Koch and Pahs 2014).

Flea beetles (Coleoptera: Chrysomelidae) are a species-rich group of defoliators found in soybean fields, which we grouped flea beetles as Tribe Alticini for analysis. There were several morphotypes including two species: the red-headed flea beetle, *Systena frontalis*, and the striped flea beetle, *Phyllotreta striolata*. Striped flea beetle populations have been found to vary between years, crops, and management types (Tonhasca 1994).

The infraorder Cicadomorpha includes many types of leafhoppers (Hemiptera). Leafhoppers were identified to family Cicadellidae, but spittle bugs were included and widened the taxa group. As a group, leaf hoppers can have economic influence on soybean crops; *Empoasca terminalis* is a pest capable of 70% yield loss in Indonesia and has been spreading (Nasruddin et al. 2014), while damage from the potato leafhopper, *Empoasca fabae*, called for a need to set economic injury levels in soybean (Hunt et al. 2000).

Miridae (Hemiptera: Miridae) are a group known to be plant pests. We found several types of mirids in the fields. The tarnished plant bug, *Lygus lineolaris*, is most likely to cause damage in soybean at the time of flowering. It’s found to reproduce on soybean even though it’s a marginal host, but early season it offers a previously unavailable crop option (Snodgrass et al. 2010).

We identified the taxa containing grasshoppers (Orthoptera) to order. Grasshoppers were grouped to suborder Caelifera, though we included others such as katydids, suborder Ensifera. Grasshoppers often become pests of row crops, including soybean, and sampling techniques can
give an accurate picture of densities and potential Orthopteran threat levels in soybean (Browde et al. 1992).

Southern corn rootworms, *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae) are a known pest of soybeans in the south, but occur in low numbers in this study’s area. The potential exists for their range to spread as temperatures change, and their feeding preferences on soybean varieties have been tested (Bruner et al. 2013).

We classified spiders to Order Araneae, though harvestmen (Order Opiliones) were also included in this taxa group, opening it to class Arachnida. Crab spiders, family Thomisidae, and long-jawed orb weavers, family Tetragnathidae, were notably found. Spiders are generalist predators known to prey upon many pests in soybean, but their success as biocontrol agents depends on the presence of other predators and pests (Vichitbanhada and Wise 2002).

Damsel bugs (Hemiptera: Nabidae) are another generalist predator found in soybean. Nabids primarily reside on the leaves as opposed to the pods and flowers, which differs from some other predators such as Orius (Clements and Yeargan 1997) and makes them a forager of a different niche in the system.

Insidious flower bugs, *Orius insidiosus* (Hemiptera: Anthocoridae) are small predators. They are common in soybean and can be capable of suppressing soybean aphid populations (Rutledge and O’Neil 2005).

Lady beetles (Coleoptera: Coccinellidae) were identified to family Coccinellidae. We mostly found the multicolored Asian lady beetle, *Harmonia axyridis*, the sevenspotted lady beetle, *Coccinella septempunctata*, and the convergent lady beetle, *Hippodamia convergens*. Lady beetles are well-established, important natural enemies for pests of many seasonal crops. In
Lacewings (Neuroptera: Chrysopidae and Hermerobiidae) are to suborder Hermerobiiformia, to include both green and brown lacewings. Lacewings are well-known natural enemies of soybean aphids, and their densities can be very high. Lacewing eggs tend to be present in the field throughout the growing season, which may show their capacity for steady pest suppression (Hesler 2014).

We grouped predatory flies (Diptera) to suborder Brachycera, and the families included Dolichopodidae and Syrphidae. Within the family Syrphidae, two genera *Toxomerus* and *Eupeodes* dominated. Predatory flies, especially Syrphidae, are known to be predators in the soybean system though with fairly sporadic populations from season to season (Noma and Brewer 2008).

**Data Analysis**

We used different combinations of samples and response variables in different analyses. To calculate the inverse Simpson’s diversity index, we used the sum of individuals for each arthropod taxon per field for each year. For community multivariate analysis, we used the sum of individuals of each taxon each week per each field; in other words, we combined the ten sweeps or aphid plant counts per field per sampling date. We removed weeks with zeroes for all samples, resulting in 1,680 total samples analyzed.

For the diversity indices and community multivariate analyses we established and evaluated four different community scenarios (Table 3.1). The communities include a 5-taxa community comprised of all three years of the survey (but we did not record all taxa the first year so it is most simplified in terms of potential arthropod presence). A 13-taxa community includes...
the latter two years and all the potential considered taxa of the survey. The herbivore and predator communities are the division of 13-taxon community into two trophic groups. After statistical analysis of community composition, we plotted total abundance of each taxonomic group over time by combining fields per week for each year.

We compared the inverse Simpson’s diversity index across both management type and year. Inverse Simpson’s index yields values up to the number of species (taxa) considered and more weight is given to more abundant species than rare species for the index value, which was desirable as rarity was not a concern of ours. We calculated inverse Simpson’s diversity index values at field level each year with our four community scenarios using the diversity function in the vegan package for the R statistical environment (Oksanen 2009, R Development Core Team 2014). We used the multcomp package to test Tukey contrasts among group means by year and management type (Hothorn et al. 2008).

We used non-metric multidimensional scaling (NMDS), to describe patterns in community composition in relation to management, location, and year. We used the metaMDS function in vegan to project arthropod community ordinations based on Bray-Curtis dissimilarity matrices in K=4 dimensions. NMDS stress levels were 0.05 for 5-taxon community, 0.11 for the 13-taxon community, 0.07 for herbivores, and 0.09 for predators. In ordination space, being closer together indicates similarity among samples. We used the envfit function envfit to test associations between each community ordination and the ecological factors management, location, and year.

We plotted the sum of a taxon’s weekly abundance across all fields for each year. The community ordination combined all time and all taxa for analyses; plotting abundance in this way allowed for visual comparison between years for individual taxon.
Results

Diversity

Inverse Simpson’s diversity index differed through years and management types (Figure 3.1). In post-hoc Tukey comparisons within the 5-taxa community, arthropod diversity in fields between years was significantly different: 2012 was greater than 2013 and both were greater than 2011 ($F_{2,14}=57.88$, $p<0.01$ and $p < 0.01$, respectively). Diversity did not vary between years as tested for the 13-taxa community. Diversity did not differ between organic and conventional management for any community scenario.

**Figure 3.1.** Inverse Simpson’s diversity values plotted for each field. Post-hoc Tukey comparisons were done to compare diversity values between years and between management types. Different letters indicate significant differences between years.

Ordinations

The ordinations show our arthropod community composition varying as the taxa relate to our ecological factors in question, in particular year. For an example of taxa relations, in the 5 taxa community ordination lady beetles and orius are more dissimilar than lacewing and soybean aphids because they are placed further away from each other. The 5 taxa community ordination (Figure 3.2) showed the taxa to vary throughout site scores, and year ($p<0.01$) significantly
contributed to the community variation. The 13 taxa community ordination (Figure 3.3) showed taxa differences and all three factors —management (p=0.02), location (p<0.01), and year (p<0.01)—were significant to arthropod variation. Management type (p<0.01), location (p<0.01) and year (p<0.01) were significant for the herbivore community, while for the predator community, year was the only factor associated with patterns of variation in community composition (p<0.01) (Figure 3.4).
Figure 3.2. The 5 taxa community ordination, (A) species scores plotted as text among the site scores, (B) points for species scores and spider plots group site scores by year.
Figure 3.3. The 13 taxa community ordination, (A) species scores plotted as text among the site scores, (B) points for species scores and spider plots group site scores by year.
Figure 3.4. The herbivore and predator community ordinations. Species scores plotted as points and spider plots group samples by each of the three factors—management, location, and year.
**Abundance**

As we plotted total abundance it shows year contributing to variation at the individual taxon level (Figure 3.5). The 5 taxa community ordination shows lady beetles as variable and year tested as contributing to variation; lady beetle total abundance over time shows the difference between years, though you can see they peaked week 10 in both 2011 and 2013. Taxa variation in the communities was most consistently attributed to year, out of the three factors considered. We can see potential relationships, for example, in 2011 when soybean aphids were high, then the predators orius, lady beetles, and lacewings peaked with or just after them, while arachnida dropped off as others peaked. Predatory flies were more present in 2013. Herbivore groups rotated between two years; in 2012 pentatomids and southern corn rootworms were more abundant, while in 2013 flea beetles and mirids were more abundant. Cicadomorpha varied in which week(s) they peaked between years, as did Orthoptera and nabids, but abundance didn’t vary between years as much as for other taxa.
Figure 3.5. Total abundance, total number of individuals per taxon found per sampling date over all fields per each week, over the three years. Weeks spanning late June to mid-September.
Figure 3.5. Total abundance, total number of individuals per taxon found per sampling date over all fields per each week, over the three years (continued). Weeks spanning late June to mid-September.

Discussion

Soybean field arthropod community diversity and composition varied among sampling years more than among locations and management types. But other studies of arthropods in soybean fields conducted over multiple years generally do not consider inter-annual variation in community composition. Multi-year arthropod data are often combined to test other factors, such
as sample techniques (Schmidt et al. 2008) or soybean aphid to natural enemy ratios (Noma et al. 2010). Alternatively, studies may look at the arthropods across years to compare how they react to variables such as soybean variety/management, but not directly compare arthropod diversity or density differences between years (Yu et al. 2014).

Differences in community composition among years is a difficult factor from the standpoint of crop production because control over seasonal variation in abiotic conditions is almost nonexistent, unlike location and management type with which choices can be made (Swift et al. 2004). This inter-annual effect influences arthropod communities and needs to be considered. To help mitigate potential effects from year to year fluctuations we need to be aware of climate effects and recognize arthropod populations of concern versus a manageable arthropod community.

Variation in community composition across management groups suggested herbivores were more affected by management than predators were. Herbivores could be affected because of weed management choices for a field in that weeds are more prevalent in organic fields. Arthropod populations will react differently to plant diversity whether or not they are a generalist or specialist (Andow 1991). Beyond the soybean aphid, herbivores in the soybean fields studied here were generalists, and as such might have been more successful under organic management with more available food sources from weeds. It is difficult to separate the interactions from our community analysis. Research has shown that while plant diversity increases arthropod diversity, small spatial scale herbivore and natural enemy diversity are largely maintained by one another (Siemann et al. 1998). Natural history and interactions would be specific to each arthropod type, distinguished to taxon in our study. We observed a relationship between management type and
community composition associated more with herbivores, but we are unable to explore the specifics as to why with our data.

Location was associated with affecting herbivores, in a similar way to management type. One explanation could be that our study locations were relatively close to one another, at no farther than 35 miles apart, and that there was little variation between location landscape types, all surroundings were heavily agricultural. Locations may have been too similar to result in being a significant factor in shaping arthropod community variation. In the future, landscape structure analysis for locations would be useful in explaining possible location effects, or lack thereof. Many studies have found relationships between landscape structure in and around agricultural areas and the arthropods present (Woltz et al. 2012, Mitchell et al. 2014, Gardiner et al. 2009).

The survey data show potential for more questions and next steps. Accounting for all arthropods regardless of potential or known ecological roles could have provided community differences we didn’t see with limited taxa consideration. Detailed climate data could contribute to models and lead to further understanding of seasonal differences and build upon the arthropod data. Week level data of field climate (Ma et al. 2015), plant development (Waltz and Whitham 1997), and arthropods collected would create a more complete year to year picture for the community and why year was predominantly associated with community variation. Further exploration of taxon within a community may give insight in regards to interactions, some taxon may support each other’s populations while others may oppose. Identifying arthropods to finer taxonomic levels would be more meaningful for analysis and findings. Individual species functions can differ from one another more than is represented in the taxon we established, especially considering omnivory (Gillespie et al. 2012, Coll and Izraylevich 1997).
Our data contrasts with conventional wisdom of arthropod community composition in crop systems, which believes that management type has a large impact on arthropods (Hole et al. 2005). A lack of spatial variation has been used to explain management type and location being less significant than expected to arthropods (Boutine et al. 2009). The inter-annual effect exhibiting the greatest effect on arthropod communities in our study and suggests climate might play a role in shaping arthropod composition and undoubtedly climate and agriculture are deeply connected (Selvaraju et al. 2011). Climate dependent events for an agroecosystem such as the timing of planting, plant development, temperature ranges, and precipitation events can shape seasonal success of the crop and the arthropods that will be present.

Conclusion

We examined soybean field arthropod communities on a relatively small spatial scale, which showed year to year seasonal variation as the predominate factor of arthropod variation. Exploring specific arthropods and their relationships in more detail could provide insight on the variation we found. In the changing world, the potential exists for a pest to threaten a crop as its populations or range shift. Changes can be monitored for, with respect to what is typical for crops and the arthropods they host each year, making management more effective.

References


