DENSITY AND MOVEMENT OF SOYBEAN APHID, APHIS GLYCINES (HEMIPTERA: APHIDIDAE) IN RESPONSE TO TEMPERATURE AND RESISTANT SOYBEAN PLANTS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Movement is one way herbivores respond to their host plant, yet the movement of relatively immobile insects has received little attention. We studied how the movement and density of apterous soybean aphids responds to a resistant soybean variety and different temperatures. In Chapter One, we examined aphid movement both within and between soybean plants that varied in their resistance to aphids. Aphids on resistant plants had a wider dispersal, apparently due to greater aphid movement. Consequently, aphids on resistant plants could move to neighboring susceptible plants, thereby increasing their density. In Chapter Two, we measured aphid density and dispersal on resistant and susceptible plants when insects and plants were exposed to two different temperatures. Here, movement behavior was affected by both plant resistance and temperature. Moreover, temperature and plant resistance interacted to influence aphid density. Our results indicate the important role that movement can play in an herbivore's response to plant resistance.

ACKNOWLEDGMENTS

I'd like to thank my advisor Jason Harmon for his patience and tireless support throughout my time at NDSU. I'm grateful to Deb and Bob Whalen, Robert Whalen, Weston Berg and Derek Krewedl. Each played a part in ensuring my success in Fargo and the field of entomology.

Thank you to Don Carey, for constructing any contraption I demanded, and the entire faculty and staff of the Department of Entomology.

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Abstract

Herbivorous insects can move and distribute according to the quality of the plant they are on, and this behavior may help determine the effectiveness of resistant plants in agricultural systems. However, when an insect is normally considered sedentary, there is much less known about whether any movement behavior is important. We performed experiments to determine if a resistant soybean variety alters the movement and distribution, both within and between plants, of the soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). We did this by counting apterous (wingless) aphid populations on plant leaves of resistant and susceptible soybean plants for several days. In individual plant tests, aphid distribution was different between susceptible and resistant soybeans, most notably aphids were quickly found off the original leaf they were placed on when on resistant plants. Aphids were widely distributed throughout resistant soybeans after a week, while aphids on susceptible plants stayed primarily on their initial leaf of placement. Follow up experiments indicate that this difference was primarily based on the movement of individuals and not differential demography on various plant parts. In experiments where aphids were able to walk from their initial plant to an adjacent plant there was a net movement of aphids off resistant plants and on to adjacent susceptible plants. Consequently, aphid populations on susceptible plants were higher when the plant was adjacent to a resistant plant than when adjacent to another susceptible plant. The effect of resistant plants on aphid movement and distribution could lead to unintended side-effects such as greater spread of plant viruses, ineffective scouting practices, or altered effectiveness by biological control agents.

Introduction

Herbivorous insects have a range of responses to a poor quality host plant. Common responses to less desirable plants are to move to another part of the plant (Hodar et al. 2002) or to move off the plant entirely (Honek et al. 1998). However, insects that are relatively immobile may be limited in their response options. Most research on insect movement has involved relatively mobile insects that either have wings, such as harlequin bugs (Englishloeb and Collier 1987) or the capacity to frequent a number of host plants in a lifetime, such as the polyphageous European tarnished plant bug (Hannunen and Ekbom 2002). However, relatively sedentary herbivores likely also respond to host plant quality in some way, but studies investigating how poor quality food affects their movement behavior are relatively scarce.

Aphids are an example of an herbivore where one species can be extremely mobile or quite immobile, depending on wing dimorphism. Both winged (alate) and non-winged (apterous) aphids must spend a great deal of time actively feeding in order to attain sufficient nitrogen from their host plant (Wilkinson and Douglas 1995, Dixon 1998). Movement not only uses stored energy but can also be extremely detrimental in terms of lost feeding time (Schultz 1983, Nelson 2007). Moving can also place aphids at greater risk to predators (Schultz 1983, Losey and Denno 1998, Day et al. 2006) or to the environment if, for instance, high ground temperature increases the risk of death by desiccation (Roitberg and Myers 1979). Various situations prompt aphid movement and dispersal. For instance, movement behaviors have been linked to predator presence (Dixon 1958), plant age (Hodgson 1978, Fernandes et al. 2012), aphid age (Honek et al. 1998) and low levels of nutrients in the phloem ingested by aphids (Harrewijn 1978).

Soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae), make good subjects for the study of movement behavior of a small, relatively immobile insect. The choice to move

off its host plant may carry extreme consequences for soybean aphids, in part because of the amount of time necessary for beneficial feeding to begin. Soybean aphids have an extended period of intracellular and intercellular probing with its stylet (Diaz-Montano et al 2007). One study showed that, on average, it takes more than three hours for a soybean aphid to reach phloem of susceptible soybean (Diaz-Montano et al. 2007). Therefore, movement to another plant could mean hours of lost feeding time. While the precise effect of lost feeding time on soybean aphids is unknown, even one hour of lost feeding time in pea aphids, a relatively larger and more mobile aphid, has been linked to decreased weight and lower fecundity (Nelson 2007); for pea aphids, reproduction was reduced by 9 percent for each day it was starved for one hour.

Soybean aphids may also be considered less mobile because of limited options for defensive behavior if predators are encountered. Soybean aphids may release a sticky substance secreted by its cornicles when attacked, a response common to many aphids (Butler and O'Neil 2006). Another study recorded body raising, kicking and body rotation when confronted by a parasitoid (Wyckhuys et al. 2008), but the overall effectiveness of these behaviors is unknown. In comparison, pea aphids have a range of defensive behaviors, which include dropping from the plant, kicking, and attacking with frontal horns (Roitberg et al. 1979, Villagra et al. 2002).

It is evident that the behavioral response of an aphid to actively search for another feeding site carries a number of biological consequences, although these could be of less overall harm to the aphid's longevity or fecundity than eating a poor quality plant.

Soybean plants with resistance to soybean aphids were available for purchase by farmers in the United States starting in 2010 (Ragsdale et al. 2011). In this first widely developed resistant soybean variety, resistance is due to a dominant gene, *Rag1* (Hill et al. 2006a, b, Kim et al. 2010). Soybean aphids restricted to *Rag1* resistant plants have a shorter lifespan, lower

fecundity and longer development time (Li et al. 2004) which leads to much smaller aphid populations than those kept on susceptible plants (e.g. Ghising 2011, Ghising et al. 2012).

Soybean aphid behavioral response to resistant plants is interesting since the mechanisms behind soybean resistance are not clear. Generally speaking, both antixenosis and antibiosis have been reported as categories of resistance in soybeans with the *Rag1* gene (Diaz-Montano et al. 2007), however, these categories are not easily distinguishable (Smith 2005). Antibiosis indicates that the plant in some way affects the biology of the aphid, including changes in fecundity, longevity and development time. Antixenosis, which indicates that the plant is of poor quality to the aphid, can result in a behavioral reaction, including movement on or off the plant. Tantalizing research suggests that in a choice environment with multiple varieties the distribution of soybean aphids between and within plants may be influenced by the presence of resistant varieties (Hesler and Dashiell 2007, 2008). However, it is unclear whether resistant soybeans are causing such differences, and, if so, what behavioral or demographic mechanisms might generate such patterns.

Here, we quantify the distribution of aphids on resistant and susceptible plants in choice and no-choice situations while also examining the mechanisms underlying these patterns. To investigate how plant quality affects the distribution of this herbivore, we studied soybean aphids on high and low quality soybean plants represented by two near-isogenic varieties, one susceptible and one resistant to soybean aphids. We first compared the within plant distribution on susceptible and resistant soybeans. We then quantified fecundity on different areas of each plant variety. This allowed us to determine whether distribution patterns were due to different reproduction and survival on various plant parts on susceptible and resistant plants. Next, we compared movement of adult aphids on susceptible and resistant plants over 48 hours. This let us

determine if the overall distributions could be caused by differential movement and whether any difference in movement was exhibited by the majority of the population or just a few individuals. We then examined the movement of aphids between plants of different quality and recorded the resulting size of aphid populations per plant. This allowed us to answer the question of how soybean aphid's movement response may affect adjoining plants of different quality. We discuss what our patterns of movement and distribution may tell us about the mechanism of resistance in soybeans and how aphid movement might impact population growth, biological control efforts and virus transmission.

Materials and Methods

Study system

The soybean aphid was first detected in North America in 2000 (Ragsdale et al. 2004) and is now considered the most important pest to soybean in North America (Ragsdale et al. 2007). The U.S. population originated in Asia and as of 2009 it had spread to 30 states and 3 Canadian provinces (Ragsdale et al. 2011). The soybean aphid can cause yield losses up to 40% in the U.S. (Ragsdale et al. 2007). North Dakota is one of the top producers of soybeans in the United States, with 4.2 million acres planted in 2012 (USDA-National Agricultural Statistics Service (NASS) 2012). Scouting for aphids in soybean fields is recommended in North Dakota as far west as the Missouri River. In 2002 and 2003, fields with significant infestations were treated for soybean aphids (NDSU Extension Service 2004).

A soybean variety resistant to soybean aphids in the U.S. was first reported in 2004 (Hill et al. 2004b), and a number of soybean genotypes are now known to show some level of resistance to soybean aphid (Hill et al. 2004a, Diaz-Montano et al. 2006). The soybean aphid resistant varieties available for purchase have a single dominant gene, *Rag1* (Resistance to *Aphis*

glycines gene 1) that is known to impart resistance to soybean aphid (Hill et al. 2006a, b). Field studies suggest that biological control by soybean aphid parasitoids and natural enemies could be compromised when used in conjunction with *Rag1* soybeans (Chacon et al. 2008, Ghising 2011, Ghising et al. 2012). There is some evidence that resistance confers less nutritional quality for soybean aphid (Chiozza et al. 2010). However, the mechanism of resistance to the soybean aphid is still unclear, and there are few studies examining the influence of resistant plants on soybean aphid behavior. A better understanding of resistance mechanisms could help determine compatibility with natural enemies and predict differential spatial distribution, which is crucial for controlling virus or pathogen spread.

Aphid colony

Soybean aphids were reared in a laboratory colony in the Department of Entomology, North Dakota State University (Fargo, ND). The soybean aphids were originally collected from soybean fields located at Prosper Agricultural Experimental Station (Prosper, ND), and had been kept in culture for approximately 24 months before experiments began. The aphids were maintained on a susceptible soybean line RG607RR in a wood and wire mesh cage 26±4ºC, 60-80% RH under a L16:D8 photoperiod. To maintain aphid colonies, older soybean plants (vegetative stage V3-V4: three or four fully expanded first trifoliates; soybean growth stages are described according to Fehr and Caviness 1977) were replaced with young soybean plants (vegetative stage V1: fully expanded first trifoliate) every 4-5 d depending on plant quality.

Plants

The susceptible cultivar RG607RR and the resistant plant used in experiments are near isogenic lines. For the resistant plant, soybean line Dwight was crossed with a soybean line consisting of Loda crossed with Dowling (Brian Diers, University of Illinois, Urbana-

Champaign, IL); this resulted in the source for the *Rag1 gene*, the soybean line LDXG04018-3. Our susceptible plant, which is a common breeding line used in North Dakota, RG607RR, was then crossed with LDXG04018-3, the line which contained the *Rag1* gene. Three backcrossings with the susceptible plant followed. This resulted in the resistant plant that we used. This soybean line was tested in the field, in lab experiments using SSR markers, and in greenhouse screenings for resistance to soybean aphids (Hochalter 2009) and yield (T. Helms, unpub. data). The resistant plants were confirmed to be resistant using aphid growth rate experiments (Hochalter 2009) and confirmed to have the *Rag1* gene using PCR (Ghising 2011).

All soybeans for experiments were grown in a greenhouse at the USDA-Agricultural Research Service, Northern Crop Science Laboratory, Fargo, ND. Soybean lines were grown in the greenhouse at 26C±4ºC, 60-80% RH under a L16:D8 photoperiod, under high pressure sodium lights, from May 2010 through September 2011. Plants were grown by planting two seeds of each soybean line in 10.2 × 10.2 cm plastic pots and new plants were planted weekly. All plants were thinned to one plant per pot before starting experiments. Plants were potted in commercial horticultural mix (Sunshine Mix LC1, Sun Gro Horticulture, Vancouver, BC).

Effect of resistant plants on intraplant aphid distribution

We conducted a no-choice experiment to investigate the effect of soybean resistance on the intraplant distribution of soybean aphid. We evaluated the distribution of aphids on each part of a single plant and calculated how the distribution changed off the original leaf of placement through time and the distribution throughout the plant at the end of the experiment. Susceptible soybean plants were used as a control.

At the beginning of the experiment soybean plants were at vegetative stage V1 (fully expanded unifoliate leaves and partially expanded first trifoliate leaves). Ten aphids were placed

on a marked unifoliate leaf of each susceptible and resistant plant using a fine paint brush. A random mix of late-stage nymphs and adults were used. Aphids were chosen based on size rather than age because differentiating between late-instar and adult apterous aphids would require every aphid in all experiments to be examined under a microscope to confirm an extended cauda on adults (Hodgson et al. 2005). Extensive handling can harm or even kill aphids, so we made as little contact as possible. The pot was placed in a larger 21.6 cm diameter round pot. Each pot was covered with a plastic and mesh cage (31 cm long X 19 cm length) with a nylon mesh top (19 cm diameter) to prevent aphids from moving between plants.

The experiment began when aphids were placed on plants (day 0). The aphid population, per leaf and on the stem, was recorded on day 1, 2, 5 and 7. Offspring and adults were counted separately on days 1 and 2 and were never removed. We stopped counting juveniles after day 2 since it was impossible at this point to tell whether the older juvenile aphids were still the original juvenile aphids that had not developed or whether they were progeny of the original aphids. The experiment was conducted in three blocks between May and October 2010. For each block 8 to 20 replicates of each treatment, susceptible and resistant, were used. A total of 38 plants per treatment were used over all blocks (76 total). The experiment was performed on a bench top in the greenhouse at 26C±4ºC under a L16:D8 cycle.

A repeated measure analysis of variance (ANOVA) was used to determine the effect of plant quality on aphid population density through time (PROC MIXED, SAS Institute, 2009). The main effect was treatment and day was the repeated measure factor. The random effect was block. Means were separated using Fisher's LSD.

To quantify aphid distribution we used two different measures. The first measure focused on the proportion of the aphids on the plant that were on the original leaf aphids were placed on.

To see how this distribution changed through time on the two plant varieties we used a repeated measure analysis of variance (ANOVA) (PROC MIXED, SAS Institute 2009). The main effect was treatment and day was a repeated measure factor. The random effect was block. Means were separated using Fisher's LSD.

We used a second, complementary measure of distribution by looking at the proportion of aphids on different plant parts at the end of the experiment using a MANOVA (Wilks Lambda criteria, PROC GLM, SAS Institute, 2009). There were four different response variables that corresponded to the proportion of aphids on a plant that were found on the unifoliate, first trifoliate, second trifoliate, and "rest", which was primarily the stem and new growth that would become the third trifoliate. The proportion of aphids in all of these categories would equal one for any given plant, therefore to ensure each response variable is an independent measure only three independent responses can be analyzed within the model (Cisneros and Rosenheim 1998). Therefore we analyzed the arc-sin square root proportion of aphids on the unifoliate leaves, first trifoliate leaves, and second trifoliate leaves as response variables, plant type as the independent variable, and block as a random effect.

Aphids per plant part on resistant vs. susceptible plants

To determine whether the distribution patterns on resistant and susceptible plants observed in the first experiment (Figures 2-3) could have been caused by differential demography, with different rates of survival and fecundity on certain plant parts depending on plant variety, we counted aphids confined to individual plant parts of either a resistant or a susceptible plant. If differential demography is responsible for the distribution patterns observed earlier, aphids on unifoliate leaves of susceptible plants must do better than the aphids on other parts of susceptible plants. Moreover, aphids on unifoliate leaves on resistant plants should do

relatively worse than the other plant parts, but all other plant parts should be relatively equally beneficial for aphids. If we see these patterns the observed distribution could be due primarily to aphids doing relatively better or worse on certain plant parts and not by large differences in aphid movement on resistant compared to susceptible plants.

Individual adult aphids were placed in clip cages on one of three locations on a resistant or susceptible plant on day 0. Plants were at V1-stage with one plant per pot. Each plant was randomly assigned to receive one adult aphid on one of three places: stem, unifoliate leaf, or the first trifoliate leaf. These three locations correspond to the three general areas that were available to the aphids at the start of the distribution experiment. The aphid was placed on the assigned location with a fine paint brush and confined within a 2.5 cm diameter X 1.9 cm height clip cage. Each of these 3 placements was repeated for an equal number of resistant and susceptible plants. Clip cages were made from two pieces of cut celluloid tube of 1.6 mm wall thickness and 1.6 mm closed cell foam to form a tight seal between the top and bottom of the cage. The top and bottom of the clip cage was covered with nylon organdy screen mesh and the two pieces of tube were held together by a 10.2 cm stainless steel hair clip. A 15.2 cm wooden stake, with a 17.8 cm copper wire at the tip, was placed in the soil, and an alligator clip was attached to the hair clip to keep the clip cage in place.

Aphids and cages were left undisturbed for three days then the clip cages were removed and adults and nymphs counted. There were three blocks of the experiment for a total of 18 replicates for each location on each plant type (108 total). The experiment was performed on a bench top in the greenhouse at 26±5ºC under a L16:D8 cycle in June and July of 2011.

Mean difference in the overall number of aphids (adults+nymphs) on each plant part was examined via analysis of variance (ANOVA) (PROC MIXED, SAS Institute, 2009). Population

data was log(aphids + 0.05) transformed. The fixed effects were treatment, plant part, and the treatment x plant part interaction, and the random effect was block. Planned contrasts between the clip cage location and plant type were also performed to determine overall effect of plant location on aphid population per clip cage. We also performed a nominal logistic model (SAS Institute 2000) to determine if plant type and clip cage location influenced whether the original adult aphid was alive or dead at the end of the experiment.

Effect of resistance on movement of adult soybean aphid

An alternative explanation to the distribution patterns observed in Experiment 1 is that soybean aphid movement is affected by resistant plants. To determine whether resistant plants encourage the majority of an aphid population to move off the initial leaf of placement, we performed a no-choice test in which the movement of aphids on susceptible plants was compared with movement on resistant plants. A secondary aim of the experiment was to gauge whether the distribution patterns seen previously on resistant plants were due to a few aphids or many aphids moving off the initial leaf of placement. By taking more frequent counts, as well as removing nymphs, we were able to track aphids more closely and separate apparent movement from the distribution of adult and juvenile aphids. The previous experiment showed approximately half of the aphids on resistant plants were found away from their initial leaf of placement after 24 hours. By examining this apparent movement several times in the first 12 hours, we could figure out if a majority of the aphids quickly left the resistant leaf, or if a few quickly left the leaf and the ones that stayed behind were the first to die.

Soybean plants were at vegetative stage V1 with fully expanded unifoliate leaves and partially expanded first trifoliate leaves. Plant location was randomized and there were 13 replicates of each line (26 total). The plant pot was placed in a larger 21.6 cm diameter round

pot. The round pot was covered with a plastic and mesh cage, as described in Experiment 1. Each plant and cage was then placed in a tray. The experiment was conducted over 48h in July 2011. We placed 10 aphids onto a marked unifoliate leaf of each resistant and susceptible plant. Aphids were transferred to the unifoliate leaf using a fine paint brush. A random mix of late-stage nymphs and adults were used. Counts of aphids and their location on each plant was recorded over the next 48 h for a total of 18 counts. Seven counts were done in the first 12h. At each count, nymphs were removed. The experiment was performed on a bench top in the greenhouse, at 26±5ºC under a L16:D8 cycle, over the course of two days in July 2011.

We used a repeated measure ANOVA to determine the effect of resistance on aphid movement throughout the plant (PROC MIXED, SAS Institute, 2009). The fixed effects were treatment, location and time. A first-order autoregressive was used for covariance structure and least-squares means (LSMEANS) was used for mean comparisons among treatment and time effects.

Mean difference in aphid density between the two plant treatments at the final count was examined via analysis of variance (ANOVA) (PROC GLM, SAS Institute, 2009). The fixed effect was treatment.

Effect of resistance on interplant aphid density

This experiment was done to determine if differences in movement on resistant and susceptible plants may influence the population size and distribution of adjacent soybean plants of different types. We evaluated the distribution, per leaf and per plant, of aphids on whole soybean plants with a choice experiment consisting of two adjacent soybean plants. The adjacent soybeans were either both susceptible, both resistant, or one of each.

Two VC (stage immediately preceding V1 and consisting of unifoliate leaves expanded and no expanded first trifoliate) soybeans were transplanted to 21.6 cm diameter round pots. Pots were assigned to receive one of three plant combinations: two susceptible plants, two resistant plants, or one resistant and one susceptible plant.

The experiment was started when plants were at V1 stage. In each pot, a bridge was created between the two plants by clipping together one unifoliate leaf from each plant. On day 0, five aphids were placed on the clipped unifoliate leaf of each plant with a fine paint brush, for a total of 10 aphids per pot. A mix of late-stage nymphs and adults were used. Pots were covered with a large cage, as described above. There were 10 plants per treatment in each of two temporal blocks for 20 plants per treatment (60 total). Interplant movement experiments were performed in May and June 2011. The experiments were performed on a bench top in the greenhouse at 26±5ºC under a L16:D8 cycle.

The experiment was first analyzed as a 2x2 factorial with the type of plant (resistant or susceptible) crossed by the adjacent type of plant (is it the same as the focal plant or different). ANOVA (PROC MIXED SAS Institute 2009). This was used to determine any effect of adjacent soybean variety on aphid population per plant. Initial plant quality, neighboring plant quality and their interaction were included in the model. ANOVA was used to determine any effect of adjacent soybean variety on aphid population per pot (both adjacent plants together) (PROC MIXED, SAS Institute 2009). The main effect was pot combination and block was included as a random effect. An analysis of variance ANOVA was used to determine any effect of adjacent soybean on aphid population of susceptible plants (PROC MIXED, SAS institute, 2009). The fixed effect was treatment, defined as two susceptible plants or one susceptible and one resistant plant. Block was included as a random effect.

Results

Effect of resistant plants on intraplant aphid distribution

Aphid density was higher on susceptible plants compared to resistant plants, and this difference became larger over time (Figure 1). Even though aphid densities were significantly different after just one day ($F_{1,72}$=48.1, p<.0001), the difference between treatments continued to increase through time, leading to a significant time x treatment interaction ($F_{3,70}$=23.8, p<.0001). This is the expected result given previous experiments with these varieties (e.g. Hochalter 2009, Ghising 2011).

The aphids were originally distributed on just a single leaf on the plant. However, this distribution quickly changed for aphids on resistant plants; after 24 hours, almost half of the aphids on resistant plants were away from their original leaf (Figure 2). Seven days after placement, less than 30% of the aphid population on resistant plants was on the original leaf, while 80% of aphids on susceptible plants were still on the original leaf. As with aphid density, this difference between treatments increased with time leading to a significant time x treatment interaction (time x treatment: $F_{3,72}$ = 4.30, p = 0.008).

To get a better sense of where aphids were throughout the plant, we looked at a finer break down of aphid location on resistant and susceptible plants at the end of the experiment. Aphids on resistant plants were more evenly distributed throughout the plant compared with aphids on susceptible plants, which were predominately found on the unifoliate leaves that included the one unifoliate leaf they were originally placed on (Figure 3; $F_{3,70}$ =28.15, p<.0001). About 40 percent of aphids on resistant plants were on the unifoliate leaves, while about 84 percent of aphids on susceptible plants were on the unifoliate leaves. The aphids on resistant plants were found to be more spread out (21 percent on first trifoliate, 16 percent on second

trifoliate, and 23 percent on the rest of the plant). The 16 percent of aphids on susceptible plants that weren't on the unifoliate were on the second trifoliate (8 percent) or the rest of the plant (7 percent).

Figure 1. Number of aphids per susceptible or resistant plant over time (mean ± 1 SE).

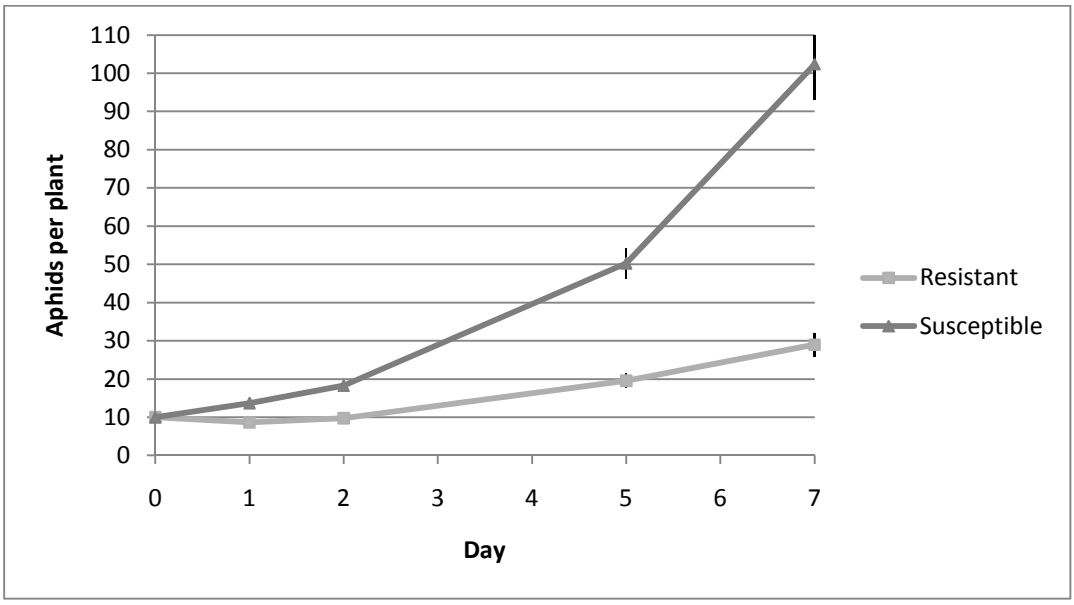


Figure 2. Proportion of aphids on the original leaf of a susceptible or a resistant plant over time (mean ± 1 SE).

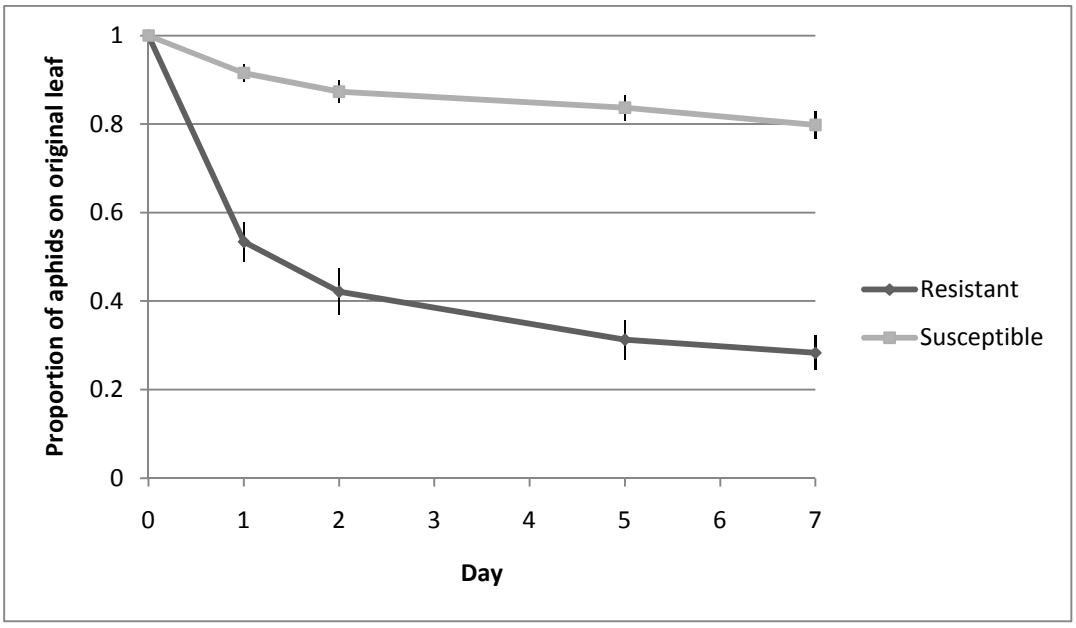
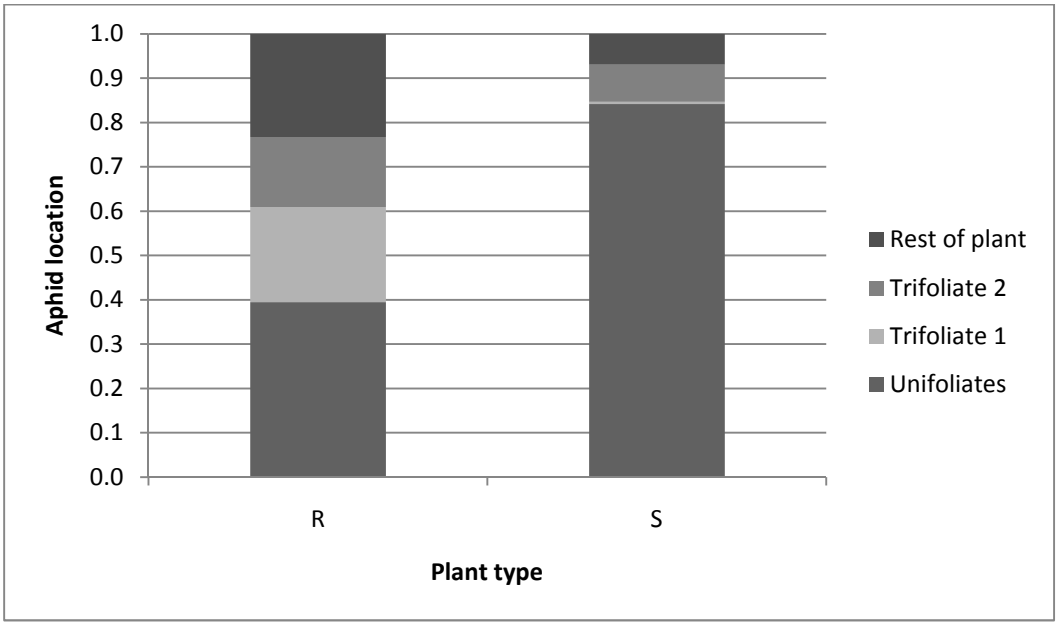


Figure 3. Proportion of the aphid population located on different plant parts of a resistant or a susceptible plant at the end of the experiment.



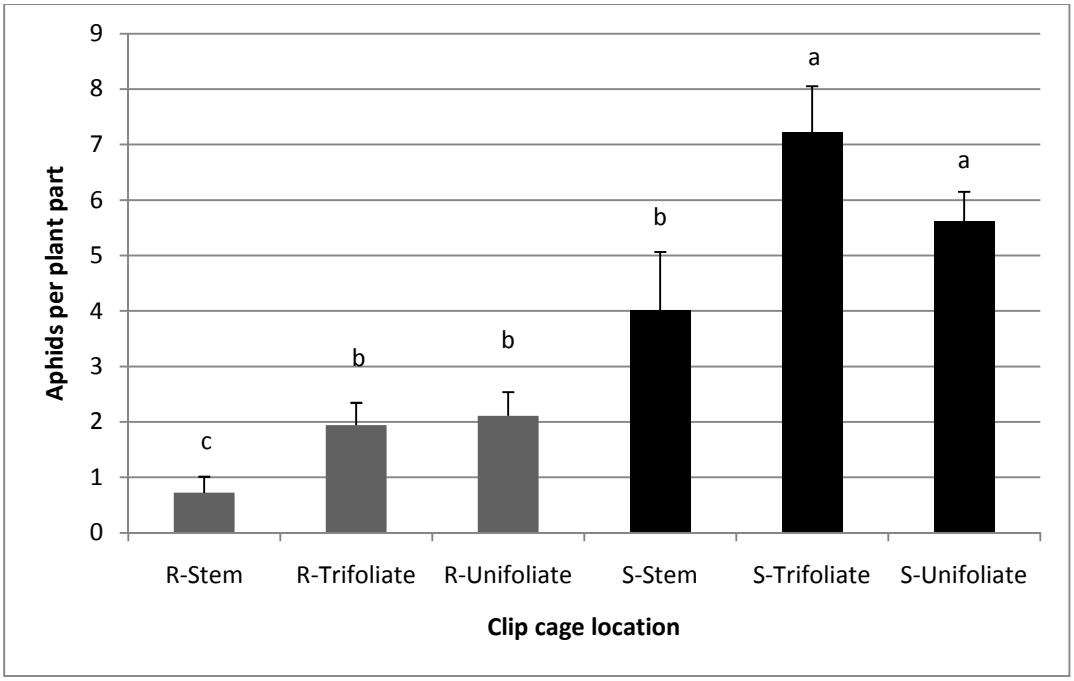
Aphids per plant part on resistant vs. susceptible plants

In the clip-cage experiment comparing numbers of aphids when restricted to different parts of resistant and susceptible soybeans, we found that the type of plant did not interact with plant part to influence the number of aphids (treatment x part $F_{2,100} = 0.20$, $p = 0.82$; Figure 4). However, both plant type and plant part each independently influenced aphid density. As expected, there were more aphids on susceptible plants across all plant parts (treatment $F_{1,100} = 74.69$, $P < 0.0001$). Across both plant treatments there was also a significant difference in aphid numbers by location ($F_{2,100}=14.76$, $p=0.0007$). Contrasts across treatments showed fewer aphids on the stem compared with other locations ($F_{1, 100}=29.30$, $p<0.0001$) but no significant difference in aphids on trifoliates vs. unifoliates ($F_{1,100}=0.26$, $p=0.61$).

To better understand the results of this experiment we looked at whether the plant type and location influenced the likelihood of survival for the original adult. We found that there

were some differences in how many of the original adults were alive after four days (R unifoliate: 13/18; R trifoliate: 10/18; R stem: 6/18; S unifoliate: 18/18, S trifoliate: 18/18, S stem: 14/18). However, like the measure of overall aphid numbers there was not a significant interaction (treatment x location $F_{2, 102}$=3.52, p=0.17), but instead significant effects of both the plant treatment main effect ($F_{1, 102}$=27.5, p<0.0001) and location main effect ($F_{2, 102}$=14.0, p=0.001). The overall patterns for these main effects matched those seen with the measure of total aphid numbers per plant part with survivorship higher on susceptible plants compared to resistant plants and survivorship lower on the stem compared to the other plant parts.

Figure 4. Number of aphids per plant part on susceptible or resistant plant on day 3 (mean + 1 SE).

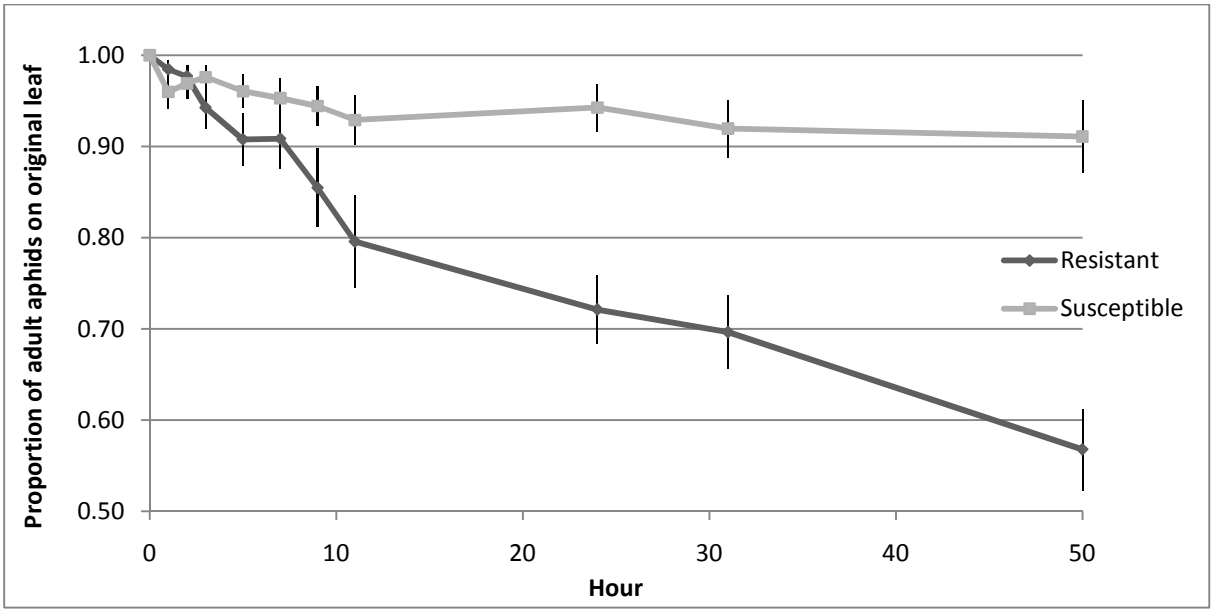


Effect of resistance on rate of movement of adult soybean aphid

We saw a significant time X treatment interaction in the counts of adult aphids on resistant and susceptible plants ($F_{9,216}$=5.16, p<.0001). This significance is due to similar aphid

counts on resistant and susceptible plants for the beginning of the experiment with the difference in aphid counts between the two treatments becoming bigger over time (Treatment $F_{1,24}$=19.3, p=.0002). A significant difference in aphid movement between treatments was apparent 10 hours after aphids were placed (t_{24}=2.09, p=.038) (Figure 5). Aphid populations on resistant plants moved off their original leaf faster than aphids on susceptible plants: after 10 hours, 20 percent of the original aphids had left the original leaf, while only 7 percent of original aphids on susceptible plants had left the leaf. After 31 hours, 30 percent of the original aphids on resistant plants had left the original leaf, compared with 8 percent of aphids on susceptible plants leaving the original leaf (t_{24}=4.35, p<0.0001). This pattern continued until the final count (t_{24}=5.73, p<0.0001). The aphid population after 49 hours was significantly lower on the resistant plants ($F_{1,24}$=31.6, p<.0001). After 49 hours, the susceptible plants had an average of 8.77 original aphids alive, and resistant plants had an average of 5.54 original aphids alive.

Figure 5. Proportion of adult aphids on original leaf of susceptible or resistant plant over time (mean ± 1 SE).



Effect of resistance on interplant aphid density

The aphid density per plant depended on both the variety of the focal plant being measured and whether the neighboring plant was the same type of plant or different (treatment X neighbor: $F_{1,73}$=5.22, p=0.025; Figure 6). After seven days, the aphid density of a susceptible plant, when in a pot with another susceptible plant, was significantly lower than the aphid density of a susceptible plant when in a pot with a resistant plant (t_{73}=3.07, p=.0030). This wasn't the case with aphid densities on resistant plants. After seven days, the aphid density of a resistant plant, when in a pot with another resistant plant, was low and not distinguishable from the aphid density of a resistant plant when in a pot with a susceptible plant (t_{73}=0.16, p=0.87).

The overall aphid density per pot was significantly different depending on what plants were present ($F_{2,55}$=26.6, p<.0001) (Figure 7). It was not surprising that two resistant plants next to each other had the lowest total aphid density. However, what was surprising was that when a susceptible plant neighbored a resistant plant, the aphid density per pot (both the resistant and susceptible plants together) was not significantly different than a pot with two susceptible plants (t_{55}=1.15, p=0.25).

Figure 6. Number of aphids per resistant or susceptible plant in each pot combination on day 7 (mean ± 1 SE).

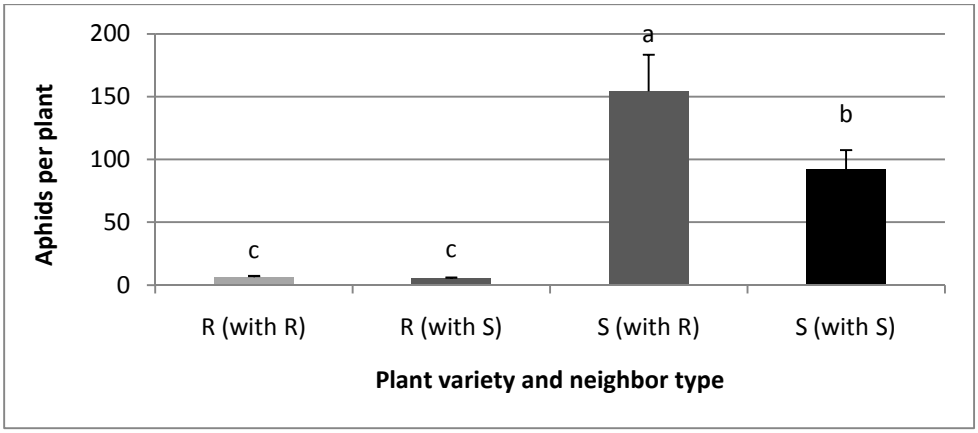
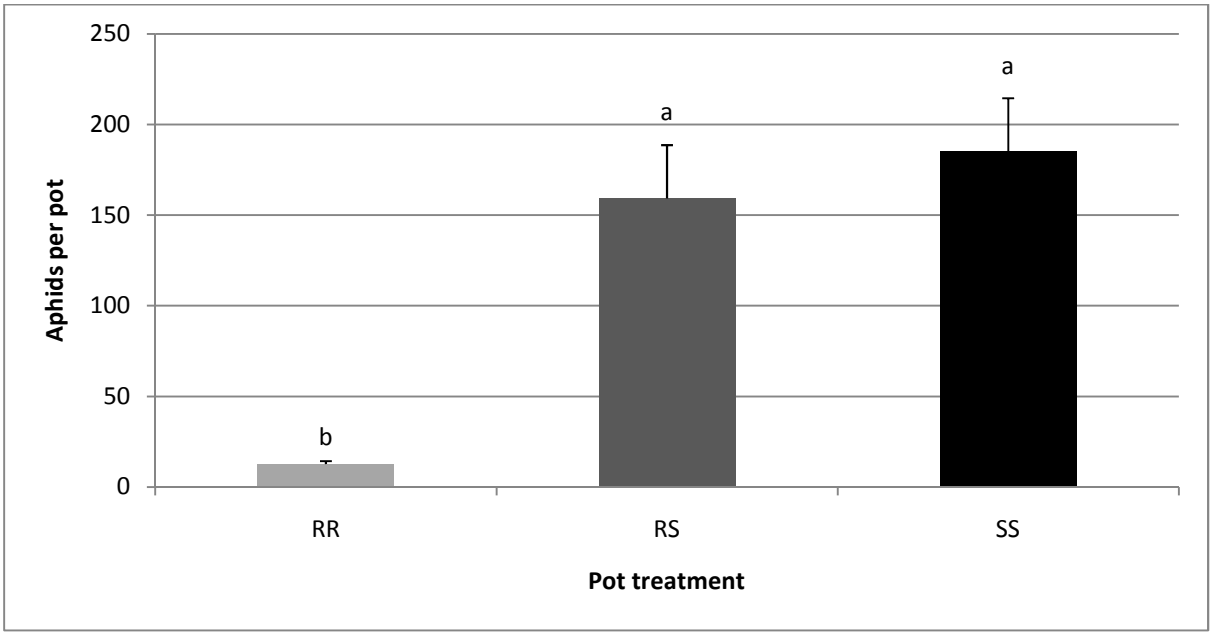


Figure 7. Number of aphids per pot (both plants added together) in each pot combination on day 7 (mean ± 1 SE).



Discussion

We found that soybean aphids on resistant plants had a different distribution where they quickly were found away from their original leaf and became more evenly dispersed than aphids on susceptible plants (Figure 2). We hypothesized that this difference in distribution could have been caused by either differential demography, with aphids surviving and producing more progeny on certain plant parts differently on the two plant types, or by differential movement of aphids when on resistant compared to susceptible plants, or by a combination of both.

The results showed that soybean aphids respond to resistant soybeans by quickly moving to another location within the plant or off the resistant plant, and this differential movement likely resulted in the observed differences in aphid distribution on resistant compared with susceptible plants. We draw this conclusion from several lines of evidence. First, when individual adult aphids were confined to different parts of a plant, we saw that aphid numbers on

the unifoliate, stem and trifoliates of resistant plants were proportionally similar to those on susceptible plants (Figure 4). This suggests that differential fecundity was unlikely to be the driving mechanism behind the distribution patterns we observed. In order for differential fecundity rates to produce the distribution patterns, we would have needed to see, for instance, low rates of fecundity on the unifoliate compared to other parts of the plant when on resistant plants. This could produce the observed distribution patterns we observed on our intraplant experiments, but for reasons of demography, not movement. Our second line of evidence for the importance of movement comes from tracking adult aphids. This data reinforced that the majority of the population, not just a few individuals, were moving soon after being placed on resistant plants (Figure 5), and this large scale movement away from the unifoliate leaves on resistant plants was likely responsible for the increased movement patterns.

A potential consequence of differential movement on resistant and susceptible plants can be seen when the two plant types are placed next to each other. We found that the soybean aphid population of susceptible plants was higher when the plant was adjacent to a resistant plant than when it was neighboring another susceptible plant (Figure 6). This means that not only are aphids more likely to move when on a resistant plant, but if next to a neighboring plant of higher quality they will readily move to and thrive on that plant. Since previous experiments showed that aphids on resistant plants tend to move more, this strongly suggests that there was a net migration off of resistant plants and on to susceptible plants when these plants were neighbors. We didn't see a big difference in aphid numbers per plant on resistant plants in either treatment (R different vs. R same). In other words, even though it seems that some aphids left resistant plants and walked to adjacent susceptible plants, it didn't significantly diminish the end population density of aphids remaining on resistant plants.

Our interplant movement experiment demonstrates the possible benefit to the aphid of increased movement when faced with an inferior host plant. Aphids on resistant plants, while not necessarily orienting themselves toward susceptible plants, appeared to move to a much higher quality plant. In our intraplant experiment, we saw that up to half the aphids on resistant plants left their original leaf after 24 hours. If there was not a tendency toward a high rate of movement on resistant plants, aphids might die before finding a higher quality plant.

A recent study on the intraplant distribution of apterous and alate cotton aphids on transgenic and non-transgenic Bt cotton plants also showed a marked increase in movement by aphids when on plants that are correlated with lower fecundity and survival (Fernandes et al. 2012). This result is in line with our findings of apparent increased movement on lower quality host plants.

Despite a clear genetic link, the mechanisms of resistance to soybean aphids aren't clear for resistant plants. It was obvious that aphids on resistant plants performed poorly, as expected (Li et al. 2004). We found that one behavioral effect of resistance is an increase in aphid movement within and between plants. Whether due to poor food quality, inability to sustain feeding, or some other reason, almost half of aphids on resistant plants moved to other parts of the plants after 24 hours. However, we did not find evidence that aphids went to a specific part of the plant. This suggests that resistance per se may not be apparent to the aphid until a feeding site is reached.

The differential movement and distribution of aphids on resistant plants could be in response to many proximate factors. We didn't find evidence that soybean aphids on resistant plants detected neighboring susceptible plants and we can't say they moved deliberately to the susceptible plant. Few studies have investigated the relationship of host plant volatiles and

soybean aphid behavior, though one olfactometer test showed no preference by the soybean aphid when resistant and susceptible plants were close to each other (Lamont 2010). In the vegetative stages, *Rag1* soybeans have a lower concentration of amino acids associated with high nutritional quality for aphids than susceptible soybeans (Chiozza et al. 2010). It is possible that aphids may be able to detect this type of nutritional difference while feeding which could explain why aphids aren't prone to staying and feeding at the initial site on resistant plants like they are on susceptible plants. We do know that aphids on resistant plants have different probing patterns and make at least some contact with phloem upon initial investigation (Diaz-Montano et al. 2007), which indicates that movement probably isn't due to a waxy surface or trichomes that make it impossible for stylet penetration. Dense trichomes on soybeans have been shown to prevent feeding by other herbivores (Bernays and Chapman 1994). Since we have no evidence of aphids on resistant plants moving in any particular direction, plant architecture, in terms of trichome density or leaf wax, doesn't appear to be influencing their movement. We often found aphids on the stem, which was entirely covered in trichomes. There are multiple factors that are known to prompt aphid within-plant movement including stress by drought (Dickson and Laird 1962) plant pathogens (Fereres et al. 1999) plant injury (Delaney and Macedo 2001) physical disturbances (Ferrar 1969, Schotzko and Knudsen 1992) and predator presence (Dixon and McKay 1970). It is not clear how any of the factors operating in these instances would apply to resistant plants in particular. However, it does suggest that soybean aphids may have some sort of a general response to environments that are relatively detrimental.

Our study focused on the effects of resistance on soybean aphid movement behavior. Our experiments were designed to get a clear picture of the plant-insect relationship with little variation due to outside influence, such as predators or plant disease. This placed restrictions on

the length of time experiments could be run and where they could be performed. For instance, our research focused on aphid movement during the early to mid-vegetative stages of soybean growth, although soybean aphids will occupy almost any stage of growth. Since resistance is thought to stay constant as soybeans in vegetative stages grow older (Hill et al. 2004b) our results should hold with older plants, however we did not test this. Experiments were performed with small groups of aphids, so the movement response of a single aphid was not tested. Since soybean aphids on susceptible plants generally didn't leave their original leaf, there's no evidence that a group of 10 aphids would have lead to more movement on resistant plants. We performed all experiments in a greenhouse, so our results could vary in different settings. In the field, the combination of factors including other herbivores, predators, parasitoids would make it difficult for us to target the aphid's movement specifically in response to the host plant. We focused on aphid movement in 1-2 week periods. It would be interesting to see how movement patterns change over the course of a season, and in periods of drought, frequent rain or high wind.

A poor quality plant can have greater impact than simply decreasing the number of a given insect on the plant. Even a small, relatively immobile insect such as an aphid will react differently depending on its host plant. This reaction, whether it be within-plant redistribution (Kennedy et al. 1950) or movement onto other plants (Knudsen and Schotzko 1999, Underwood et al. 2011), interacts with a range of other ecological functions, including predator interactions (Turchin and Kareiva 1989) and effectiveness of parasitoids (Honek et al. 1998). One pest management tactic involves the intercropping of resistant and susceptible plants (e.g. wheat, Wang et al. 2009). Moreover, the interplant movement could have implications for the effectiveness of seed mixes of resistant and susceptible plants used together. If susceptible and

resistant varieties are equally susceptible to a plant virus, transmission could also be higher across resistant plants due to increased aphid movement (Kennedy 1976). Soybean aphids on resistant plants have been shown to probe the plant before deciding its unsuitability (Diaz-Montano et al. 2007), so there is the possibility of virus transmission, when the aphid decides the plant is unsuitable, moves, and the virus in the resistant plant now continues to be spread to other plants (Kennedy 1976). There is also the possibility of the virus affecting aphid resistance, such as the loss of aphid resistance in sugar beet lines infected with certain viruses (Baker 1960). An increase in interplant movement by a given population in a resistant field could be even more important to virus spread than in a susceptible field (Irwin et al. 2007, Van Emden and Harrington 2007). As is shown in our interplant movement experiment, aphids initially on resistant plants are able to locate, move to, and thrive on a susceptible plant. Whatever the *Rag1* mechanism is, we didn't find evidence that it immediately kills aphids that initially fed on it. If a disease can be transmitted to the aphid in a few probes, which can happen with nonpersistent viruses (Kennedy 1970) there's a possibility the aphid could then transmit the disease.

Host plants can directly affect insect movement behavior (Schotzko and Smith 1991). Our experiments indicate an example of lower plant quality being responsible for an increase in aphid movement, and one consequence of this movement is the possibility of emigration to neighboring plants. Aphid distribution on agricultural plants has been linked to many variables, including parasitoid presence (Gonzales et al. 2001) age of plant (Hodgson 1978), presence of other aphids (Turchin and Kareiva 1989) and cultivar in the case of cotton (Fernandes et al. 2012). We've shown that initial movement and distribution of soybean aphid depends on resistance, and the trend toward higher aphid populations on susceptible soybeans when near resistant soybeans. Future research could attempt to link intraplant walking behavior with finding

a higher quality neighboring plant, hence the rapid and relatively random distribution of aphids to all parts of a poor-quality plant.

References

Baker, P. F. 1960. Aphid behavior on healthy and on yellow-virus infected sugar beet. Annals of Applied Biology **48**:384-391.

Bernays, E. A. and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman & Hall, New York.

Butler, C. D. and R. J. O'Neil. 2006. Defensive response of soybean aphid (Hemiptera : Aphididae) to predation by insidious flower bug (Hemiptera : Anthocoridae). Annals of the Entomological Society of America **99**:317-320.

Chacon, J. M., D. A. Landis, and G. E. Heimpel. 2008. Potential for biotic interference of a classical biological control agent of the soybean aphid. Biological Control **46**:216-225.

Chiozza, M. V., M. E. O'Neal, and G. C. MacIntosh. 2010. Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant soybean plants in response to the soybean aphid (Hemiptera: Aphididae). Environmental Entomology **39**:856-864.

Cisneros, J. J. and J. A. Rosenheim. 1998. Changes in the foraging behavior, within-plant vertical distribution, and microhabitat selection of a generalist insect predator: an age analysis. Environmental Entomology **27:**949-957.

Day, K. R., M. Docherty, S. R. Leather, and N. A. C. Kidd. 2006. The role of generalist insect predators and pathogens in suppressing green spruce aphid populations through direct mortality and mediation of aphid dropping behavior. Biological Control **38**:233-246.

Delaney, K. J. and T. B. Macedo. 2001. The impact of herbivory on plants: yield, fitness, and population dynamics. Pages 135-158 *in* R. K. D. Peterson and L. G. Highly, editors. Biotic Stress and Yield Loss. CRC Press, Boca Raton, FL.

Diaz-Montano, J., J. C. Reese, J. Louis, L. R. Campbell, and W. T. Schapaugh. 2007. Feeding behavior by the soybean aphid (Hemiptera : Aphididae) on resistant and susceptible soybean genotypes. Journal of Economic Entomology **100**:984-989.

Diaz-Montano, J., J. C. Reese, W. T. Schapaugh, and L. R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera : Aphididae) in several soybean genotypes. Journal of Economic Entomology **99**:1884-1889.

Dickson, R. C. and E. F. Laird. 1962. Green peach aphid populations on desert sugar beets. Journal of Economic Entomology **55**:501-&.

Dixon, A. F. G. 1958. Escape responses shown by certain aphids to the presence of *Adalia decempunctata.* . Transactions of the Royal Entomological Society of London **110**:319-334.

Dixon, A. F. G. 1998. Aphid Ecology : An Optimization Approach. 2nd edition. Chapman & Hall, London.

Dixon, A. F. G. and S. McKay. 1970. Aggregation in sycamore aphid *Drepanosiphum platanoides* (Schr) (Hemiptera: Aphididae) and its relevance to regulation of population growth. Journal of Animal Ecology **39**:439-&.

Englishloeb, G. M. and B. D. Collier. 1987. Nonmigratory Movement of Adult Harlequin Bugs Murgantia-Histrionica (Hemiptera, Pentatomidae) as Affected by Sex, Age and Host Plant-Quality. American Midland Naturalist **118**:189-197.

Fehr, W. R. and C. E. Caviness. 1977. Stages of soybean development. Agriculture and Home Economics Experiment Station Iowa State University of Science and Technology, Ames, Iowa.

Fereres, A., G. E. Kampmeier, and M. E. Irwin. 1999. Aphid attraction and preference for soybean and pepper plants infected with potyviridae. Annals of the Entomological Society of America **92**:542-548.

Fernandes, F. S., F. S. Ramalho, J. Nascimento, J. B. Malaquias, A. R. B. Nascimento, C. A. D. Silva, and J. C. Zanuncio. 2012. Within-plant distribution of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), in Bt and non-Bt cotton fields. Bulletin of Entomological Research **102**:79-87.

Ferrar, P. 1969. Interplant movement of apterous aphids with special reference to *Myzus persicae* (Sulz.) (Hemptera: Aphididae). Bulletin of Entomological Research **58**:653-660.

Ghising, K. 2011. Impact of *Rag1* aphid resistant soybeans on *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), a parasitoid of soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). Thesis (M S). North Dakota State University 2011.

Ghising, K., J.P. harmon, P.B. Beauzay, D.A. Prischmann-Voldseth, T.C. Helms, P.J. Ode, and J.J. Knodel. 2012. Impact of Rag1 aphid resistant soybeans on Binodoxys communis (Hymenoptera: Braconidae), a parasitoid of soybean aphid (Hemiptera: Aphididae). Environmental Entomology **41:**282-288.

Gonzales, W. L., E. Gianoli, and H. M. Niemeyer. 2001. Plant quality vs. risk of parasitism: within-plant distribution and performance of the corn leaf aphid, *Rhopalosiphum maidis*. Agricultural and Forest Entomology **3**:29-33.

Hannunen, S. and B. Ekbom. 2002. Within species variation in host plant quality and movement behavior of *Lygus rugulipennis* nymphs. Entomologia Experimentalis Et Applicata **104**:95-101.

Harrewijn, P. 1978. Role of plant-substances in polymorphism of the aphid *Myzus persicae*. Entomologia Experimentalis Et Applicata **24**:398-414.

Hesler, L. S. and K. E. Dashiell. 2007. Resistance to *Aphis glycines* (Hemiptera : Aphididae) in various soybean lines under controlled laboratory conditions. Journal of Economic Entomology **100**:1464-1469.

Hesler, L. S. and K. E. Dashiell. 2008. Identification and characterization of new sources of resistance to *Aphis glycines* Matsumura (Hemiptera : Aphididae) in soybean lines. Applied Entomology and Zoology **43**:197-206.

Hill, C. B., Y. Li, and G. L. Hartman. 2004a. Resistance of Glycine species and various cultivated legumes to the soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology **97**:1071-1077.

Hill, C. B., Y. Li, and G. L. Hartman. 2004b. Resistance to the soybean aphid in soybean germplasm. Crop Science **44**:98-106.

Hill, C. B., Y. Li, and G. L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Science **46**:1601-1605.

Hill, C. B., Y. Li, and G. L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Science **46**:1606-1608.

Hodgson, E.W., R.C. Venette, M. Abrahamson, and D.W. Ragsdale. 2005. Alate production of soybean aphid (Homoptera: Aphididae) in Minnesota. Environmental Entomology **34:**1456-1463.

Hodar, J. A., R. Zamora, and J. Castro. 2002. Host utilisation by moth and larval survival of pine processionary caterpillar Thaumetopoea pityocampa in relation to food quality in three Pinus species. Ecological Entomology **27**:292-301.

Ghising, K. 2011. Impact of *Rag1* aphid resistant soybeans on *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), a parasitoid of soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). Thesis (M S). North Dakota State University 2011.

Hochhalter, J. 2009. Integrated pest management of soybean aphid (*Aphis glycines*) in North Dakota. Thesis (M.S), North Dakota State University.

Hodgson, C. J. 1978. Distribution and movement of apterous *Myzus persicae* on rapidly growing turnip plants. Ecological Entomology **3**:289-298.

Honek, A., V. Jarosik, L. Lapchin, and J. M. Rabasse. 1998. The effect of parasitism by *Aphelinus abdominalis* and drought on the walking movement of aphids. Entomologia Experimentalis Et Applicata **87**:191-200.

Irwin, M. E., G. E. Kampmeier, and W. W. Weisser. 2007. Aphid movement: process and consequences. Pages 153-186 *in* H. F. Van Emden and R. Harrington, editors. Aphids as Crop Pests. CABI, Wallingford, UK.

Kennedy, G. G. 1976. Host plant resistance and spread of plant viruses. Environmental Entomology **5**:827-832.

Kennedy, J. S., A. Ibbotson, and C. O. Booth. 1950. The distribution of aphid infestation in relation to leaf age .1. *Myzus persicae* (Sulz) and *Aphis fabae* Scop on spindle trees and sugar-beet plants. Annals of Applied Biology **37**:651-679.

Kim, K. S., S. Bellendir, K. A. Hudson, C. B. Hill, G. L. Hartman, D. L. Hyten, M. E. Hudson, and B. W. Diers. 2010. Fine mapping the soybean aphid resistance gene *Rag1* in soybean. Theoretical and Applied Genetics **120**:1063-1071.

Knudsen, G. R. and D. J. Schotzko. 1999. Spatial simulation of epizootics caused by *Beauveria bassiana* in Russian wheat aphid populations. Biological Control **16**:318-326.

Lamont, N. 2010. Physiological and behavioral response of the soybean aphid (*Aphis glycines* Matsumura) to susceptible and resistant soybean. University of Windsor, Windsor, ON.

Li, Y., C. B. Hill, and G. L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology **97**:1106-1111.

Losey, J. E. and R. F. Denno. 1998. Interspecific variation in the escape responses of aphids: effect on risk of predation from foliar-foraging and ground-foraging predators. Oecologia **115**:245-252.

Nelson, E. H. 2007. Predator avoidance behavior in the pea aphid: costs, frequency, and population consequences. Oecologia **151**:22-32.

Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annual Review of Entomology **56**:375-399.

Ragsdale, D. W., B. P. McCornack, R. C. Venette, B. D. Potter, I. V. Macrae, E. W. Hodgson, M. E. O'Neal, K. D. Johnson, R. J. O'Neil, C. D. Difonzo, T. E. Hunt, P. A. Glogoza, and E. M. Cullen. 2007. Economic threshold for soybean aphid (Hemiptera : Aphididae). Journal of Economic Entomology **100**:1258-1267.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Annals of the Entomological Society of America **97**:204-208.

Roitberg, B. D. and J. H. Myers. 1979. Behavioral and physiological adaptations of pea aphids (Homoptera Aphididae) to high ground temperatures and predator disturbance. Canadian Entomologist **111**:515-519.

Roitberg, B. D., J. H. Myers, and B. D. Frazer. 1979. Influence of predators on the movement of apterous pea aphids between plants. Journal of Animal Ecology **48**:111-122.

SAS Institute Inc. 2000. JMP Statistics and Graphics Guide JMP Version 4. SAS Institute, Cary, NC, USA.

SAS Institute Inc. 2009. SAS 9.2 Reference, Second Edition. SAS Institute, Cary, NC, USA.

Schotzko, D. J. and G. R. Knudsen. 1992. Use of geostatistics to evaluate a spatial simulation of Russian wheat aphid (Homoptera, Aphididae) movement behavior on preferred and nonpreferred hosts. Environmental Entomology **21**:1271-1282.

Schotzko, D. J. and C. M. Smith. 1991. Effects of host plant on the between-plant spatial-distribution of the Russian wheat aphid (Homoptera, Aphididae). Journal of Economic Entomology **84**:1725-1734.

Schultz, J. C. 1983. Habitat selection and foraging tactics of caterpillars in heterogeneous trees.*in* R. F. D. a. M. S. McClure, editor. Variable plants and herbivores in natural and managed systems. Academic Press, New York.

Smith, C. M. 2005. Plant resistance to arthropods molecular and conventional approaches. Page 423 p. Springer, Dordrecht, The Netherlands.

Turchin, P. and P. Kareiva. 1989. Aggregation in *Aphis varians* - an effective strategy for reducing predation risk. Ecology **70**:1008-1016.

Underwood, N., S. Halpern, and C. Klein. 2011. Effect of host-plant genotype and neighboring plants on strawberry aphid movement in the greenhouse and field. American Midland Naturalist **165**:38-49.

USDA-National Agricultural Statistics Service (NASS). 2012. From Washington State University Site: Soybean Acreage by Year, US [Online] Available at http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/index.asp. USDA-NASS, Washington, D.C.

Van Emden, H. F. and R. Harrington. 2007. Aphids as crop pests / edited by Helmut F. van Emden and Richard Harrington. CABI, Wallingford, UK ; Cambridge, MA.

Villagra, C. A., C. C. Ramirez, and H. M. Niemeyer. 2002. Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. Animal Behaviour **64**:677-683.

Wang, W., Y. Liu, J. Chen, X. Ji, H. Zhou, and G. Wang. Impact of intercropping aphid-resistant wheat cultivars with oilseed rape on wheat aphid (Sitobion avenae) and its natural enemies. Acta Ecologica Sinica **29:**186-191.

Wilkinson, T. L. and A. E. Douglas. 1995. Aphid feeding, as influenced by disruption of the symbiotic bacteria - an analysis of the pea aphid (Acyrthosiphon-Pisum). Journal of Insect Physiology **41**:635-640.

Wyckhuys, K. A. G., L. Stone, N. Desneux, K. A. Hoelmer, K. R. Hopper, and G. E. Heimpel. 2008. Parasitism of the soybean aphid, *Aphis glycines* by *Binodoxys communis*: the role of aphid defensive behaviour and parasitoid reproductive performance. Bulletin of Entomological Research **98**:361-370.

TEMPERATURE ALTERS THE INTERACTION BETWEEN AN HERBIVORE AND A RESISTANT PLANT

Introduction

Environmental factors are well known to affect the behavior, demography, and overall fitness of individual organisms, especially poikliotherms such as insects (Wellington 1957, Gullan and Cranston 1994). More recently, increased attention has been given to understanding how these environmental factors can also influence the ecological interactions between two species (Dixon 2003, Tylianakis et al. 2008). One such environmentally-sensitive interaction is between plants and herbivores. Previous research has shown that plant-herbivore interaction may change depending on temperature (Gingery et al. 2004, Post and Pedersen 2008) precipitation (Suttle et al. 2007), and CO_2 (Hunter 2001). The quality of a host plant, specifically how resistant it is to an herbivore, is also critical in shaping plant-herbivore interactions (Bennett and Wallsgrove 1994, Bernays and Chapman 1994). Despite the known importance of each factor independently, there has been little work to understand if changing an environmental factor such as temperature may interact with plant resistance to influence the outcome of a plant-herbivore interaction.

Temperature is known to influence such life aspects as fecundity, longevity and development rate in herbivorous insects (Uvarov 1931, Schowalter 2006). Temperature also affects other rate-based processes like movement in aphids (Harrington et al. 1995). Extreme temperature has been linked to differential distribution of aphids on plants (Wiktelius 1987) as well as major possible shifts in aphid ecology and evolution (Harmon et al. 2009).

The effects of temperature on host plant and herbivorous insect interactions haven't been as widely studied, however there are some examples. Temperature was found to expand the

number of host species and the parts of each host exploited by an arctic psyllid in Greenland (Hodkinson 1997). Fossil evidence shows an increase in herbivore diversity and herbivory intensity with rising temperature in a past global warming interval (Wilf and Labandeira 1999). A recent study showed that locusts experiencing food shortage will move to lower temperatures to maximize nutrient use (Coggan et al. 2011). The locust chose to prioritize the efficiency of the limited ingested food at the expense of a slower growth rate, which in many ectotherms is associated with lower temperatures. This suggests a differential effect of host plant quality on grasshopper movement depending on temperature. The study also points to the idea that temperature change can influence insect movement decisions and how movement can be used to offset an environmental change. These studies demonstrate the complexity of plant-herbivore interactions that need to be investigated when determining how an insect will respond to abiotic factors.

Plant resistance can have many of the same effects on insect herbivore demography as temperature. For instance, green peach aphids have decreased fecundity on resistant peaches (Sauge et al. 1998). Lettuce root aphids have much lower population on many resistant lettuce cultivars (Ellis et al. 2002) and spotted alfalfa aphid has a significantly lower intrinsic rate of increase on resistant alfalfa cultivars (Ruggle and Gutierrez 1995). Resistance, like temperature, is also known to affect insect movement (Underwood et al. 2011, Fernandes et al. 2012) (Chapter 1). The study of resistance and its affect on plant-insect interactions is one way to help us better understand how insects respond to poor quality resources.

Since temperature and resistance are each known to affect movement, the study of both factors together may give important insights in to how temperature can change an insect's reaction to plant resistance. The reaction in terms of movement of insects to plant resistance can

help us with predicting when and where a population will thrive. For instance, some insects respond to plant resistance by moving to a different part of the plant (Paschold et al. 2007), while some insects respond to plant resistance by moving to another plant entirely (Hoy et al. 1998). The movement behavior of an insect, thus, has implications for the resistant plant-herbivore interaction and its response to temperature. Likewise, both temperature and plant resistance may affect many of the same aspects of aphid demography, and therefore may provide interesting effects on plant-herbivore interactions when the two factors are allowed to interact.

The goal of our study was to examine how temperature alters a plant-insect interaction, specifically whether temperature interacts with plant resistance to ultimately affect an herbivorous insect. For our first experiment, we compare soybean aphid performance, in terms of total population density, adult survival, nymph density, and per capita fecundity on different-quality host plants while exposed to either a warm or a cool temperature. We then compare aphid distribution on resistant plants vs. susceptible plants in each of those two temperatures. In a second experiment we quantify aphid longevity and fecundity when aphids are exposed to very nutrient-poor conditions in different temperatures. This allows us to better understand an unexpected result from our first experiment. Together these experiments allow us to look at how plant resistance and temperature may interact to ultimately affect the demography and movement of an important herbivorous insect.

Materials and Methods

Study system

Soybean aphid is the principle pest of soybeans in the US, and was first detected in North America in 2000 (Ragsdale et al. 2004). The phloem-feeders were introduced from Asia, and, in part because of insufficient biological control from predators and parasitoids, can reach outbreak

proportions. Soybean aphid is known to be influenced by both temperature and resistant host plants. For example, soybean aphid reproduction on susceptible plants increases as temperature increases until 25°C and aphid longevity decreases when above 20°C (McCornack et al. 2004). These results on soybean aphid demography and population dynamics are similar to what has been seen in other plant-aphid systems (e.g Morgan et al. 2001). Soybeans bred to include resistance to soybean aphid were available to farmers in 2010 (Ragsdale et al. 2011). Direct effects of *Rag1* resistant soybeans on soybean aphid fitness include reduced survival, longevity and fecundity (Li et al. 2004). Behavioral effects include changes in the distribution of aphids and aphid movement (Chapter 1). Indirectly, *Rag1* soybeans can have negative effects on soybean aphid parasitoids in terms of fitness (Ghising 2011), and later-season density-dependence (Chacon et al. 2012). Because of the recent commercial availability of resistant soybeans, the effectiveness of resistant soybeans at different temperatures is not well studied.

Aphid colony

Soybean aphids were reared in a laboratory colony in the Department of Entomology, North Dakota State University (Fargo, ND) and were of the same origin as those described in Chapter 1. The aphids were maintained on susceptible soybeans in a wire and mesh cage, 26±4°C, 60-80% RH under a L16:D8 photoperiod. Aphid colonies were maintained by replacing older plants (vegetative stage V3-V4) with younger plants every 4-5 days.

Plants

The resistant and susceptible soybean plants used in this experiment were the same as those described in Chapter 1. Susceptible and resistant cultivars were near-isogenic lines. Plants were grown in the Department of Entomology, North Dakota State University (Fargo, N.D.) from August 2011 through November 2011. Soybeans were grown at 25±2°C, 60-80% RH under

a L16:D8 photoperiod, under fluorescent lights (t5 bulbs).The soybean growth stages are described according to Fehr and Caviness (Fehr and Caviness 1977). Plants were potted in commercial horticultural mix (Sunshine Mix LC1) and were planted on a weekly basis. Pots contained two seeds of either resistant or susceptible soybeans and were thinned to one plant per pot before beginning experiments.

Effects of temperature and plant resistance on aphid dynamics and distribution

We wanted to test whether aphid dynamics and behavior were temperature dependent on resistant and susceptible plants. We also evaluated the aphids' distribution on resistant plants at different temperatures. We looked at an aphid-plant-temperature interaction by analyzing groups of treatments as 2X2 factorials. The four treatments are as follows: 1) aphids on susceptible plants in warm chambers, 2) aphids on susceptible plants in cool chambers, 3) aphids on resistant plants in warm chambers, 4) aphids on resistant plants in cool chambers. For each of these treatments, we looked at the effects of plant quality and temperature on aphid population size, adult survival, per capita fecundity, and within-plant distribution.

Soybean plants at the start of the experiments were at late VC stage/early V1 stage, with fully formed unifoliates and partially expanded first trifoliate leaves. A random mix of 10 aphids that were either adults or late instar nymphs were placed on a marked unifoliate leaf of each susceptible and resistant plant using a fine paint brush. Each pot was covered with a plastic and mesh cage (31 cm long X 19 cm length) with a nylon mesh top (19 cm diameter). Cages were used to ensure aphids didn't migrate to neighboring plants.

The experiments were conducted in climate-controlled growth chambers. A chamber assigned to a cool treatment held an average temperature of 18.6°C and a warm chamber held an average temperature of 26.8°C, both with a photoperiod of L16:D8. These temperatures

correspond with the extreme average temperatures for a cool or warm week in Fargo during July and August. These temperatures are well within the upper and lower developmental threshold for soybean aphid (McCornack et al. 2004). Three growth chambers were used for the experiments and the assignment of warm or cool temperature was switched for each block to ensure that any observed differences in temperature treatments was due to temperature and not to any differences between chambers. Plants were randomly assigned temperature treatments and all chambers held a mix of resistant and susceptible soybean plants. The plants were approximately 91 cm from the lights. Cages were placed on a wire rack approximately 46 cm from the bottom of the growth chamber to promote airflow within the chamber.

Aphid placement was done on day 0 and the experiment ran for 7 days. Aphid populations and location on each plant were counted on days 1, 2, 4, and 7. Plants were removed to do counts, then replaced in their original location and chamber. Nymphs were recorded separately for the first two days. By day 4, it was impossible to discern which aphids were originally placed on the plant and which were born on the plant. Therefore, the combined total of adult and immature aphids was counted on day 4 and day 7. Nymphs were not removed during the experiment.

The experiment was conducted in three temporal blocks between October and November 2011. There were 13 resistant and 11 susceptible plants in the warm treatment, and 14 resistant and 13 susceptible in the cool treatment (51 plants total).

Differences in aphid population size over the 7-day experiment between the four treatments were examined via a repeated measure analysis of variance (ANOVA). The fixed effects were plant treatment, temperature, and their interaction. The random effect was block and the repeated measures factor was day (PROC MIXED, SAS Institute, 2009). Many covariance

structures are available for performing a repeated measures analysis in using PROC MIXED. We chose a first-order autoregressive for the within-subject covariance structure. We used this type of analysis because our correlation matrix showed relatively common variance and correlations getting smaller as time progressed. Means were compared using the least-squares mean difference test adjusted by Fisher's LSD.

We also compared differences in adult survival between the four treatments on day 2 using an analysis of variance (ANOVA) (PROC MIXED, SAS Institute, 2009) which included plant treatment, temperature, and their interaction as fixed effects and block as a random effect. We also compared differences in the number of juvenile aphids on day 2 using an analysis of variance (ANOVA) (PROC MIXED, SAS Institute, 2009) which included plant treatment, temperature, and their interaction as fixed effects and block as a random effect. In addition, we compared a measure of per capita fecundity across the four treatments. We calculated "per-adult fecundity" by dividing the nymph count on a plant on day 2 by the sum of the number of adults alive on that plant each day over the course of two days. We chose to use this measure of adults to account for not only how many adults were on each plant but also how long they lived. We did this because previous research indicated that adult survival on resistant and susceptible plants can differentiate very quickly (Chapter 1). We compared mean differences in per-adult fecundity between four treatments on day two using an analysis of variance (ANOVA) (PROC MIXED, SAS Institute, 2009) which included plant treatment, temperature, and their interaction as fixed effects and block as a random effect.

A repeated measures analysis of variance (ANOVA) (PROC MIXED, SAS Institute, 2009) was used to analyze the effects of plant resistance and temperature on aphid distribution, specifically the proportion of aphids on the original leaf of placement over the course of seven

days. Proportion data was arcsine transformed. Means were separated using Fisher's LSD. The fixed effects were plant treatment, temperature, and their interaction. The random effect was block and day was the repeated measures factor. A first-order ante-dependence was used for the within-subject covariance structure. We used this type of analysis because our correlation matrix showed no pattern in variance and correlations getting smaller as time progressed.

Effect of temperature on aphid survival

To investigate the effect of temperature on aphid survival we conducted a complementary experiment using aphids in Petri dishes placed in one of two growth chambers. We specifically wanted to test for effects of temperature on aphid longevity under an extremely bad nutritional situation where a water-only diet was the only available food source. This experiment tested whether temperature can change survival under poor nutrient conditions.

One diet (water only) was used for both temperature treatments. One-half of a 50 mm Petri dish was covered with parafilm. Onto each parafilm-covered dish was placed 2 ml distilled water. The water was then covered with another layer of parafilm. Five aphids were then transferred to the dish using a fine paint brush. We used a random mix of late-stage nymph and adult aphids for the experiment. The Petri dish was then covered with an identical ½ of a Petri dish and bound with parafilm (method developed by Wille and Hartman 2008).

Dishes were placed in one of two chambers. Each chamber had a wire shelf onto which the dishes were placed. The shelf was located approximately 46 cm from the bottom of the chamber to ensure air flow and minimal heating from the overhead lights. A chamber assigned to a cool treatment held an average temperature of 18.6°C and a warm chamber held an average temperature of 26.8°C, both with a photoperiod of 16L:8D. These settings were the same in temperature experiments that used whole-plant treatments.

Aphids were counted daily, with day of placement in the chamber considered day 0. Nymphs were removed daily. The experiment ended when all aphids were dead. There were two blocks of the experiment for a total of 40 replicates in the warm chamber and 38 replicates in the cool chamber (78 total). The experiments were performed in January 2012.

To determine whether aphid survival differed between the two treatments, we calculated the mean time until death for the 5 aphids in each Petri dish in warm and cool chambers. We used an analysis of variance to analyze the effects of temperature on mean days until aphid death (PROC MIXED, SAS Institute, 2009). The fixed effect was temperature and the random effect was block.

Results

Effects of temperature and plant resistance on aphid dynamics and distribution

Overall density – When we looked at aphid density over the course of the seven day whole-plant experiment, there was a significant plant treatment X temperature X day interaction ($F_{3,186}$=5.22, p=0.0017). This interaction with time was primarily due to the differences between treatments becoming larger throughout the course of the experiment (Figure 1). Focusing on the end of the experiment when treatment differences are most pronounced, we see a significant interaction between plant treatment and temperature on d7 (treatment X temperature $F_{1,45}$ =14.3, p=0.0005). The interaction at the end of the experiment is due to a difference in how temperature affected aphids on the two types of plants. Aphid populations on susceptible plants were higher in warm temperature than in cool temperature (t_{45}=5.56 p<.0001) (Figure 1). Conversely, aphid populations on resistant plants were higher in cooler temperature than in warm temperatures (t_{45}=2.18, p=0.031). This means that for some reason, warmer temperature was more conducive to higher aphid populations on susceptible plants, but cooler temperature was more conducive to

higher aphid populations on resistant plants. Given that aphid populations on resistant plants showed very limited growth, it can also be said that the significant interaction between plant treatment and temperature came from cooler temperatures slowing the aphid density increase that happens on susceptible plants, and simultaneously slowing the aphid density decrease that happens on resistant plants.

Adult survivorship, nymph density, and realized fecundity – Since we delineated nymphs from adults during counts on days 1 and 2, we decided to look at adult and nymph densities to try to better understand the overall density results. Just as occurred at the end of the experiment, there was a significant plant treatment by temperature interaction for total aphid density on day 2 of the experiment ($F_{1,45}$ =15.5, p=0.0003), with all of the treatments in the same relative order as there was at the end of the experiment (Figure 1).

To better understand this overall pattern in total aphid density, we first looked at the number of adults that were alive at day two of the experiment (Figure 2). As with total aphid density, there were more adult aphids in the susceptible treatments than in the resistant treatments ($F_{1,44}$=10.5, p=0.0023). There was a trend toward more adults in the cool than in the warm ($F_{1,44}$ =2.71, p=0.11), and there was no interaction between plant treatment and temperature ($F_{1,44}$ = 0.21, p=0.65).

We also looked at the number of nymphs in each treatment after 2 days (Figure 3). We saw at least double the number of nymphs on susceptible plants in the warm chamber than in any other treatment. This led to a significant plant treatment by temperature interaction ($F_{1,44}$ = 16.8, p=0.0002) for nymph density.

A difference in the number of nymphs can be caused by either differences in the number of adults producing those nymphs or by a difference in per capita fecundity. Therefore, we also

calculated a measure of per-adult fecundity on day two (Figure 4). Although there were only an average of 6 adult aphids per susceptible plant in the warm chamber, the per capita fecundity after two days (1.1) was more than twice that of any other treatment (resistant warm: 0.50; resistant cool: 0.48; susceptible cool: 0.49) (Figure 4). Accordingly, an ANOVA shows a highly significant treatment X temperature interaction ($F_{1,44}$ =13.82, p=0.0006) on per capita fecundity for day 2.

The extreme fecundity advantage for susceptible plants in warm chambers made up for slightly fewer adults in terms of total aphid population compared with susceptible in cool. Since the other three treatments showed very similar per capita fecundity, the relatively minor differences in total nymphs between these other three treatments was likely due to small differences in numbers of adults alive in warm vs. cool chambers.

Distribution – As in previous experiments (Chapter 1), the within plant distribution of aphids quickly differed between aphid populations on different plants and then changed to some extent over the course of the experiment (Figure 5). Therefore, we first look at the repeated measures analysis over seven days to get a sense of how the proportion of aphids on the original leaf of placement changed across the different treatments through time. We found that there was no plant treatment X temperature X day interaction ($F_{1,60}$=0.88, p= 0.45) on the proportion of aphids on the original leaf. There was, however, a significant plant treatment X day interaction ($F_{1,60}$ =4.15, p=0.0097) and a main effect of temperature ($F_{1,44}$ =4.94, p = 0.03). As in previous experiments (Chapter 1), on susceptible plants, most of the aphids on a plant were still on the original leaf in which they were placed, whereas on resistant plants, the aphids were quickly found throughout the plant, and this difference tended to magnify across time.

To better understand the role of temperature we did a separate ANOVA for the final day 7 when treatments had the longest amount of time to differentiate. We saw no interaction of treatment and temperature ($F_{1,45}$= 0.57, p=0. 45), a marginally significant effect of temperature ($F_{1,45}$= 3.50, p=0.068), and a significant effect of treatment ($F_{1,45}$ =21.1, d.f.=1,45, p<0.0001) on the proportion of aphids on original leaf. Taken together, these results seem to indicate that the effect of temperature was fairly constant and relatively weak throughout the experiment, whereas the effect of plant treatment changed through time. Over the course of the entire experiment, we found that in the cool treatments, aphids tended to be found on the original leaf more whereas in the warm treatment they were more likely to be found in other places. Since there was no interaction of plant treatment and temperature, either across time or on the last day, it is likely that plant treatment and temperature worked in an additive fashion such that aphid populations in the cool, susceptible plants were the most likely to be found on the original leaf and aphid populations on the warm, resistant plants were most likely to be found elsewhere on the plant.

Figure 8. Number of aphids on resistant and susceptible plants in warm and cool temperature over time (mean ± 1 SE).

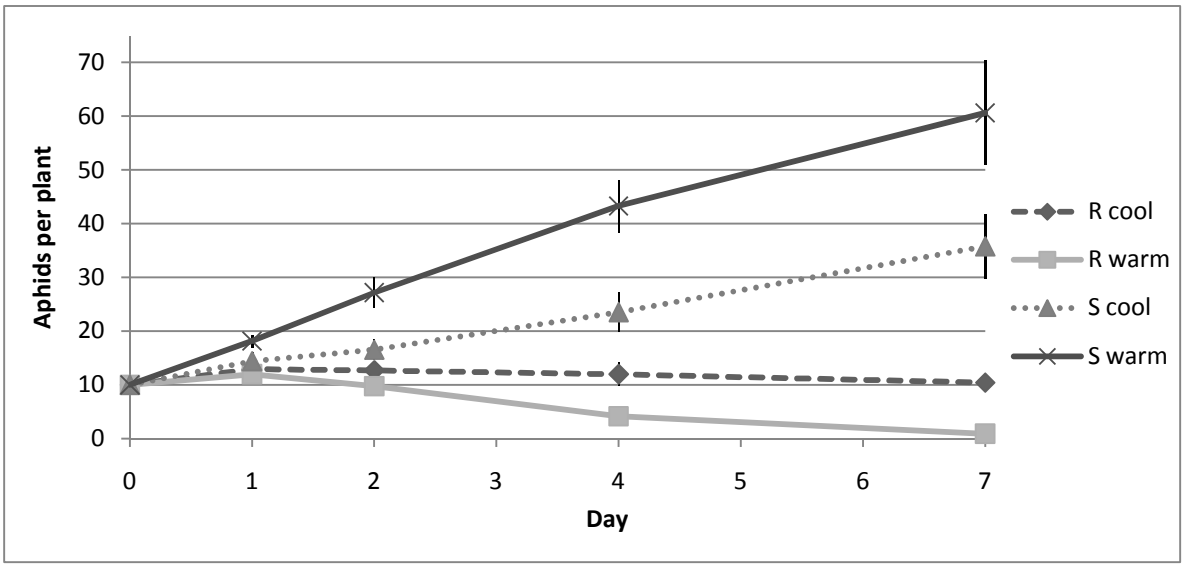


Figure 9. Number of adult aphids on resistant and susceptible plants in warm and cool temperature on day 2 (mean + 1 SE).

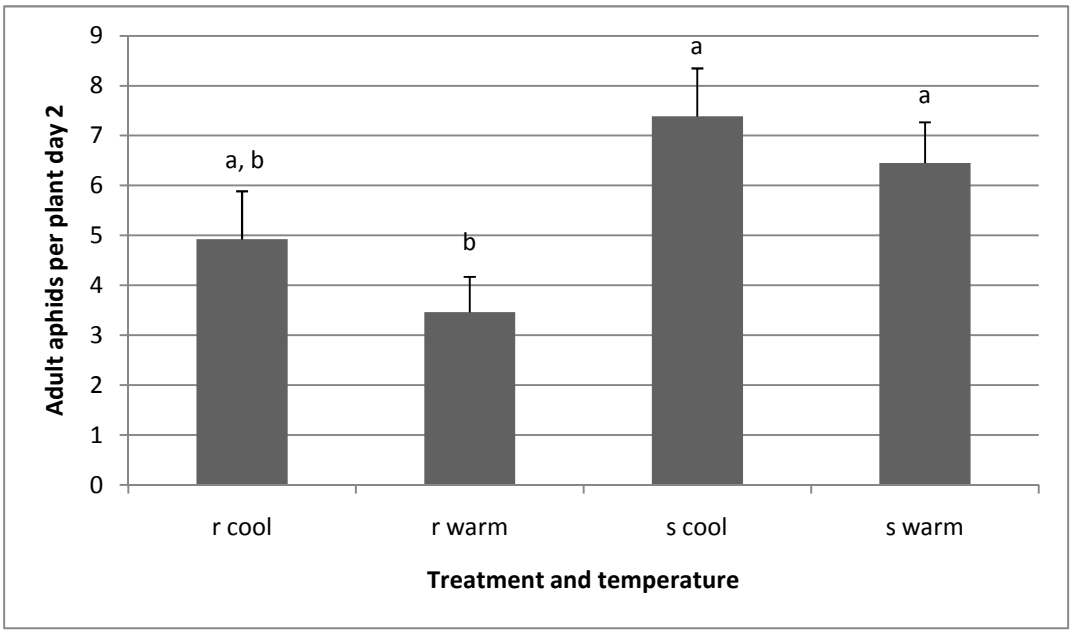


Figure 10. Number of nymphs per plant on resistant and susceptible plants in warm and cool temperature on day 2 (mean + 1 SE).

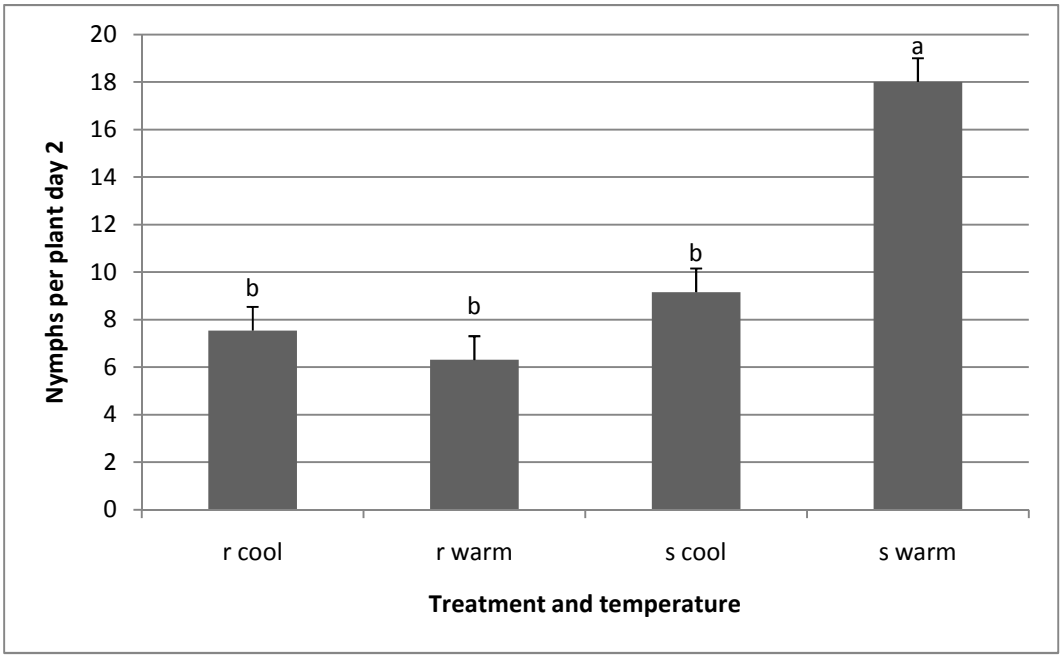


Figure 11. Per adult rate of fecundity on resistant and susceptible plants in warm and cool temperature on day 2 (mean + 1 SE).

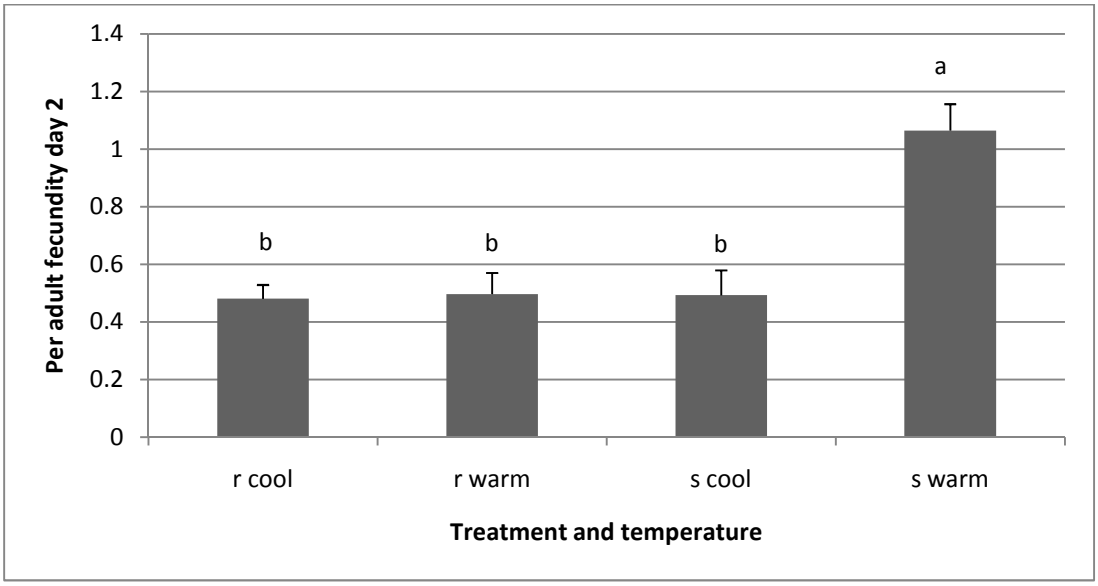
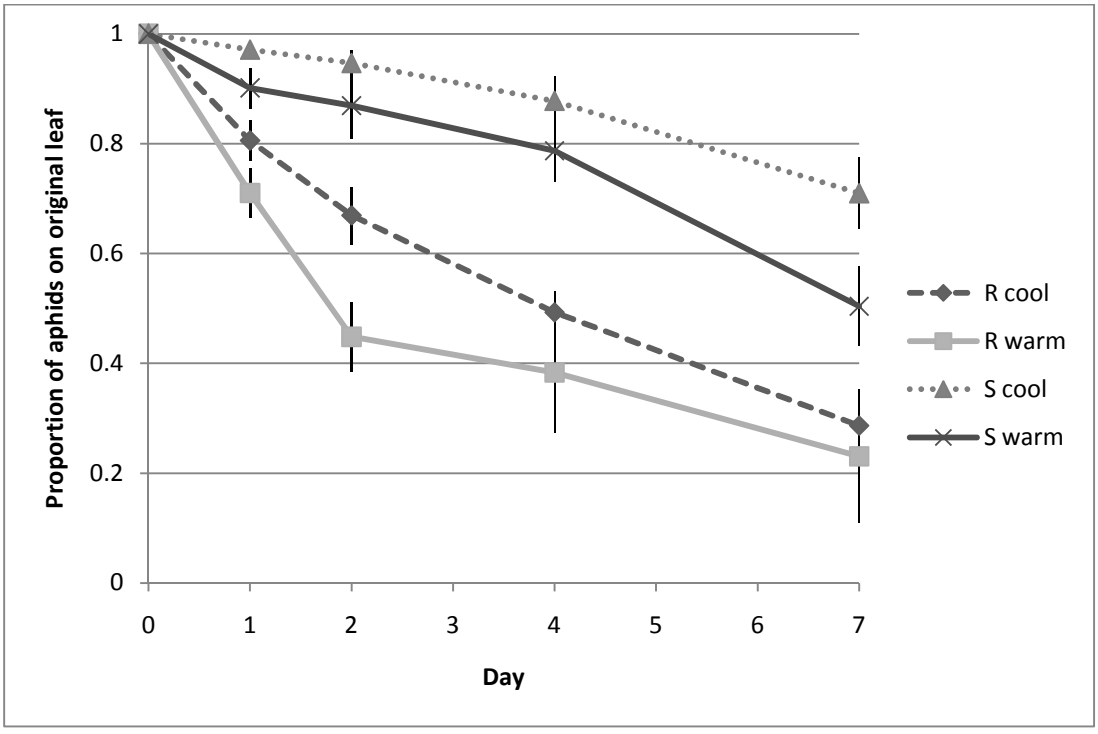


Figure 12. Proportion of aphids on original leaf of susceptible and resistant plants in warm and cool temperature over time (mean ± 1 SE).



Effect of temperature on aphid demography

Aphids in cool chambers lived a mean of 4.5 days, significantly longer than aphids in the warm chamber, which lived a mean of 2.9 days ($F_{1,76} = 122.7$, $p<0.0001$) (Fig. 13). Two days after aphids were placed in either the cool or warm chamber, there was a significant difference in the number of aphids alive per dish (Cool = 4.34, Warm = 3.05, $t_{76} = 5.23$, $p<.0001$) (Figure 14). From whole-plant temperature experiments, we found that aphids in cool chambers on resistant plants had, after 7 days, a higher population than resistant plants in warm chambers. This is in line with the Petri dish experiments, which showed that under stressful situation, aphids in a cooler environment tended to have survived longer. However, total aphid populations on susceptible plants in the warm chamber were consistently the highest of all the treatments. Since we saw in Petri dish experiment was the opposite, in terms of warm vs. cold, it indicates that higher populations on susceptible plants in warm chambers was likely primarily due to increased fecundity, not differences in the longevity of adults.

Figure 13. Days until aphid death (dish average) in warm and cool temperature (mean ± 1 SE).

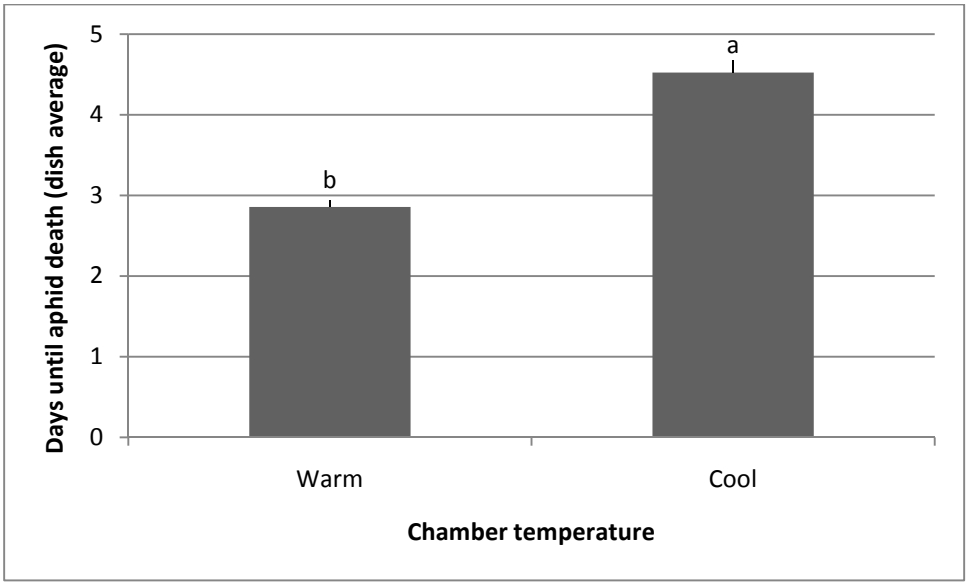
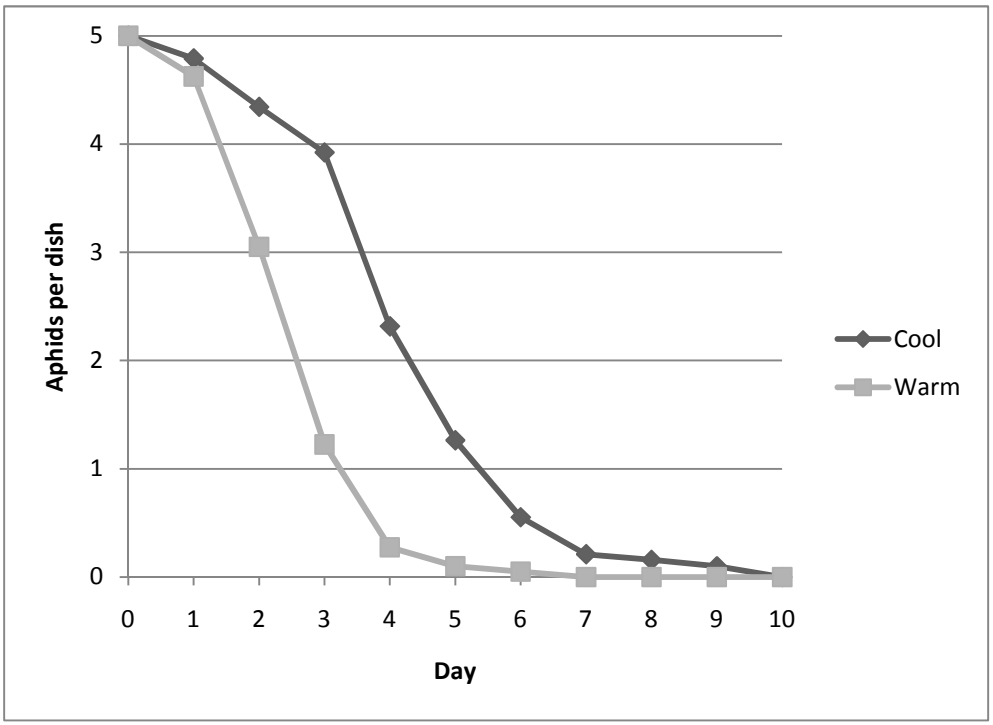


Figure 14. Number of aphids per dish in warm and cool temperature over time (mean ± 1 SE).



Discussion

Our study was performed to investigate whether plant resistance to herbivores interacts with temperature to influence herbivore populations and behavior. We found that aphid density is influenced by the interaction of plant resistance and temperature (Figure 1), and it did so in an unexpected way. Within a range of acceptable conditions, aphid populations are normally expected to be greater when on susceptible plants compared to resistant plants and when in warmer temperatures compared to cooler temperatures (e.g. Morgan et al. 2001, McCornack et al. 2004, Chiu et al. 2012). We did find more aphids in susceptible plants than on resistant plants, and when the aphids were on susceptible plants in a warmer temperature they did do better than the aphids on susceptible plants in a cooler temperature. However, we saw the opposite effect of

temperature on aphid density for resistant plants. Aphids on resistant plants in the warmer temperature actually did worse and were almost completely gone within 7 days compared to the aphids on resistant plants in the cooler temperature where we saw on average a fairly constant population size throughout the experiment.

A closer inspection of adults and nymphs two days into the experiment provided additional demographic information that helped produce this overall pattern. Overall, adult survival was higher on susceptible plants than resistant plants and slightly higher in the cooler temperature compared to the warmer (Figure 2). While adult survival may help explain the effect of temperature on resistant plants, it doesn't explain the pattern on susceptible plants. To better understand this pattern, we can look at the number of nymphs after two days (Figure 3) and our measure of juvenile production (Figure 4). We saw twice the number of nymphs on susceptible plants in the warmer temperature than in the cooler temperature. So despite there being fewer adults in the warm susceptible treatment, each adult in that treatment was producing far more nymphs than any of the adults in other treatments. Therefore, it appears that our overall pattern of aphid density was produced by both differential effects on survival, particularly of the original aphids as well as differential nymph production, especially for aphids on susceptible plants in the warmer temperature.

Previous experiments have shown that soybean aphids (McCornack 2004) and other aphid species (Morgan et al. 2001, Chiu et al. 2012) tend not to live as long in warm temperatures compared to cooler temperatures. Yet those experiments were performed on good quality, susceptible plant hosts. To get a better sense of what may happen on an extremely poor host we looked at survival when aphids had no access to food. We found that for aphids under extreme diet conditions, adult aphids had a longer lifespan when in a cool environment than in a

warm chamber (Figure 6). This corroborates the idea that aphids on resistant plants may be able to live longer and help better maintain the aphid population than those aphids in warmer conditions, leading to overall higher aphid populations on resistant plants in the cool compared to resistant plants in the warm temperature.

We did not find synergistic effects of plant resistance and temperature on aphid movement. Instead, we saw an additive effect of temperature on aphid distribution (Figure 5). As expected from previous results (Chapter 1), aphids on resistant plants distributed themselves more widely than on susceptible plants. Here we found that this differential distribution seemed to be amplified by temperature. Aphids in warm temperatures on resistant plants were slightly more likely to be found off of the original leaf than the other treatments and aphids in cool temperatures on susceptible plants were least likely to be found off the original leaf. Based on previous results (Chapter 1), it is likely that the observed distribution patterns were primarily caused by differential rates of movement.

Differential insect movement, even by a relatively sedentary aphid, could be a concern in agricultural crops. Aphids were slower to spread out on the plant when in cooler temperature, which could aid in decreasing the spread of virus (Harrington 1994). Another potential consequence is in the interaction between aphids and biological control agents. A wide distribution of prey can make predators less effective in biological control. If in a certain region the soybean aphid's dominant predator has trouble locating aphid between patches of prey (Casas and Djemai 2002) or is only attracted to a certain density of prey (Wiedenmann and Oneil 1992), there could be trouble using resistant plants in combination with predators, especially early in the season. For example, randomly distributed soybean aphids do not significantly

decline as numbers of their primary predator *O. insidiosus* increased, while clumped aphids did decline, in summer field experiments (Desneux et al. 2006).

We found that temperature does play a role in determining the effectiveness of resistant plants in terms of aphid density. On resistant plants, cooler temperatures showed longer lifespan for adults, and we saw no difference in fecundity rates in cool or warm temperatures. Although we looked at aphid demography over a short period of time, this could mean a relatively high initial aphid populations on resistant plants might persist through the season. However, there's a chance this may be balanced by a potential increase in predators, or an abiotic factors not considered. We used soybeans in the early vegetative stages, which are the stages aphids have been known to infest the plant (Ragsdale 2004). Learning about herbivores on young plants is important since soybean aphid populations may double in size in less than two days under the right temperature conditions (McCornack 2004). A few days can make a big difference in management decisions. Soybean aphids even at low density can impair photosynthetic processes in soybeans (Macedo et al. 2003), and knowing the rate of population growth in different temperatures on resistant plants will be valuable for pest management.

The effect of temperature on the effectiveness of the *Rag1* gene is not clear, in part because the exact mechanism behind its effect on soybean aphid populations is unknown. Exposure to warm or cool temperature could affect resistance to soybean aphid, but there are no generalizations as to how temperature affects plant defense, especially in terms of changes to plant secondary chemistry (Bidart-Bouzat and Imeh-Nathaniel 2008). If temperature was directly influencing how resistant the plants were, the aphids could be responding negatively to direct effects of warmer temperature, direct effect of poor plant quality, and indirect effect of temperature's affect on plant resistance. If warmer temperature increases the concentration of

certain secondary compounds in *Rag1* soybeans, for instance, it could give us results similar to what we found when comparing aphid populations on resistant plants in warm and cool temperatures. Certain secondary chemicals, such as tannin in some tree species, can increase or decrease in concentration with temperature (Dury et al. 1998) but this effect of temperature seems plant species and chemical specific, making it difficult to predict whether there are any temperature affects on soybean resistance. Further research of aphid development time, a known physiological process affected by temperature, on resistant and susceptible plants could give insight to impacts of temperature on *Rag1* operation.

Our study demonstrates that host plant resistance and temperature can interact in influencing herbivorous insects, but the nature of the interaction depended on the demographic characteristic being measured. This work helps demonstrate that the effects of climate change and other altered abiotic factors might not only have the potential to influence individual species and interactions between species, but these abiotic changes can also interact with various factors to substantially alter the species, sometimes in unexpected ways. Thus, this is another piece of evidence for the idea that we will need fairly detailed information about particular systems, their species, and their interactions in order to make meaningful predictions about the effects of future climate changes.

References

Bennett, R. N. and R. M. Wallsgrove. 1994. Secondary metabolites in plant defense-mechanisms. New Phytologist **127**:617-633.

Bernays, E. A. and R. F. Chapman. 1994. Host-Plant Selection by Phytophagous Insects. Chapman & Hall, New York.

Casas, J. and I. Djemai. 2002. Plant canopy architecture and multitrophic interactions. Pages 174-196 *in* T. Tscharntke and B. A. Hawkins, editors. Multitrophic Interactions. Cambridge University Press, Cambridge.

Chacon, J. M., M. K. Asplen, and G. E. Heimpel. 2012. Combined effects of host-plant resistance and intraguild predation on the soybean aphid parasitoid *Binodoxys communis* in the field. Biological Control **60**:16-25.

Chiu, M. C., Y. H. Chen, and M. H. Kuo. 2012. The effect of experimental warming on a low-latitude aphid, *Myzus varians*. Entomologia Experimentalis Et Applicata **142**:216-222.

Coggan, N., F. J. Clissold, and S. J. Simpson. 2011. Locusts use dynamic thermoregulatory behaviour to optimize nutritional outcomes. Proceedings of the Royal Society B-Biological Sciences **278**:2745-2752.

Desneux, N., R. J. O'Neil, and H. J. S. Yoo. 2006. Suppression of population growth of the soybean aphid, *Aphis glycines* Matsumura, by predators: The identification of a key predator and the effects of prey dispersion, predator abundance, and temperature. Environmental Entomology **35**:1342-1349.

Dixon, A. F. G. 2003. Climate change and phenological asynchrony. Ecological Entomology **28**:380-381.

Ellis, P. R., S. J. McClement, P. L. Saw, K. Phelps, W. E. Vice, N. B. Kift, D. Astley, and D. A. C. Pink. 2002. Identification of sources of resistance in lettuce to the lettuce root aphid, *Pemphigus bursarius* - Resistance to lettuce root aphid. Euphytica **125**:305-315.

Fehr, W. R. and C. E. Caviness. 1977. Stages of soybean development. Agriculture and Home Economics Experiment Station Iowa State University of Science and Technology, Ames, Iowa.

Fernandes, F. S., F. S. Ramalho, J. Nascimento, J. B. Malaquias, A. R. B. Nascimento, C. A. D. Silva, and J. C. Zanuncio. 2012. Within-plant distribution of cotton aphids, *Aphis gossypii* Glover (Hemiptera: Aphididae), in Bt and non-Bt cotton fields. Bulletin of Entomological Research **102**:79-87.

Ghising, K. 2011. Impact of *Rag1* aphid resistant soybeans on *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), a parasitoid of soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae). Thesis (M S). North Dakota State University 2011.

Gingery, R. E., R. J. Anderson, and M. G. Redinbaugh. 2004. Effect of environmental conditions and leafhopper gender on Maize chlorotic dwarf virus transmission by *Graminella nigrifrons* (Homoptera : Cicadellidae). Journal of Economic Entomology **97**:768-773.

Gullan, P. J. and P. S. Cranston. 1994. The Insects : An Outline of Entomology. 1st edition. Chapman & Hall, London ; New York.

Harmon, J. P., N. A. Moran, and A. R. Ives. 2009. Species response to environmental change: Impacts of food web interactions and evolution. Science **323**:1347-1350.

Harrington, R., N. E. Stork, and Royal Entomological Society of London. 1995. Insects in a Changing Environment. Academic Press, London ; San Diego.

Harrington, R. and L. R. Taylor. 1990. Migration for survival - fine-scale population redistribution in an aphid, *Myzus persicae*. Journal of Animal Ecology **59**:1177-1193.

Harrington, R. M., J.A., Plumb, R.T., Smith, A.J., Taylor, M.S., Foster, G.N., Holmes, S.J., Masterman, A.J., Tones, S.J., Knight, J.D. 1994. Monitoring and forecasting for BYDV control--the way forward? Aspects of Applied Biology **37**:197-206.

Hodkinson, I. D. 1997. Progressive restriction of host plant exploitation along a climatic gradient: The willow psyllid *Cacopsylla groenlandica* in Greenland. Ecological Entomology **22**:47-54.

Hoy, C. W., G. P. Head, and F. R. Hall. 1998. Spatial heterogeneity and insect adaptation to toxins. Annual Review of Entomology **43**:571-594.

Hunter, M. D. 2001. Effects of elevated atmospheric carbon dioxide on insect-plant interactions. Agricultural and Forest Entomology **3**:153-159.

Li, Y., C. B. Hill, and G. L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera : Aphididae). Journal of Economic Entomology **97**:1106-1111.

Macedo, T. B., C. S. Bastos, L. G. Higley, K. R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera : Aphididae) injury. Journal of Economic Entomology **96**:188-193.

McCornack, B. P., D. W. Ragsdale, and R. C. Venette. 2004. Demography of soybean aphid (Homoptera : Aphididae) at summer temperatures. Journal of Economic Entomology **97**:854-861.

Morgan, D., K. F. A. Walters, and J. N. Aegerter. 2001. Effect of temperature and cultivar on pea aphid, *Acyrthosiphon pisum* (Hemiptera : Aphididae) life history. Bulletin of Entomological Research **91**:47-52.

Paschold, A., R. Halitschke, and I. T. Baldwin. 2007. Co-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. Plant Journal **51**:79-91.

Post, E. and C. Pedersen. 2008. Opposing plant community responses to warming with and without herbivores. Proceedings of the National Academy of Science **105**:12353-12358.

Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annual Review of Entomology **56**:375-399.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Annals of the Entomological Society of America **97**:204-208.

Ruggle, P. and A. P. Gutierrez. 1995. Use of life-tables to assess host-plant resistance in alfalfa to *Therioaphis trifolii F maculata* (Homoptera, Aphididae) - Hypothesis for maintenance of resistance. Environmental Entomology **24**:313-325.

SAS Institute Inc. 2009. SAS 9.2 Reference, Second Edition. SAS Institute, Cary, NC, USA.

Sauge, M. H., J. Kervella, and T. Pascal. 1998. Settling behaviour and reproductive potential of the green peach aphid *Myzus persicae* on peach varieties and a related wild *Prunus*. Entomologia Experimentalis Et Applicata **89**:233-242.

Schowalter, T. D. 2006. Insect Ecology : An Ecosystem Approach. 2nd edition. Academic Press, Burlington, MA.

Suttle, K. B., M. A. Thomsen, and M. E. Power. 2007. Species interactions reverse grassland responses to changing climate. Science **315**:640-642.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters **11**:1351-1363.

Underwood, N., S. Halpern, and C. Klein. 2011. Effect of host-plant genotype and neighboring plants on strawberry aphid movement in the greenhouse and field. American Midland Naturalist **165**:38-49.

Uvarov, B. P. 1931. Insects and Climate. The Entomological Society of London, London.

Wellington, W. G. 1957. The synoptic approach to studies of insects and climate. Annual Review of Entomology **2**:143-162.

Wiedenmann, R. N. and R. J. Oneil. 1992. Searching strategy of the predator *Podisus maculiventris* (Say) (Heteroptera, Pentatomidae). Environmental Entomology **21**:1-9.

Wiktelius, S. 1987. Distribution of *Rhopalosiphum padi* (Homoptera, Aphididae) on spring barley plants. Annals of Applied Biology **110**:1-7.

Wilf, P. and C. C. Labandeira. 1999. Response of plant-insect associations to Paleocene-Eocene warming. Science **284**:2153-2156.

Wille, B.D. and G.L. Hartman. 2008. Evalution of artificial diets for rearing *Aphis glycines* (Hemiptera: Aphididae). J. Econ. Entomol. **101:**1228-1232.