BOTTOM-UP AND TOP-DOWN MANAGEMENT OF SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA) IN APHID-RESISTANT SOYBEAN (*GLYCINE MAX* (L.) MERRILL)

By

T. Michael Kates

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ABSTRACT

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Since the discovery of soybean aphid (*Aphis glycines* Matsumura) (Hemiptera: Aphididae) in North America in 2000, researchers have identified the top-down suppression of aphid populations by natural enemies, and the bottom-up effects of soil nutrition, specifically soil potassium on aphid populations. In addition to these factors, genes that convey aphid resistance have also been identified. We studied how several of these genes interacted with the ecosystem services of natural enemies, and how they preformed in potassium deficient soil. The overall number of natural enemies was no different between resistant and susceptible plots. There were significant differences in the density of certain taxa, corresponding with different aphid density in resistant and susceptible plots. There was no significant difference in the ecosystem services provided by natural enemies in resistant and susceptible plots. No significant difference in aphid populations was detected between plants grown in potassium deficient and potassium amended soil. We also used molecular markers to explore the relationships of aphid populations on resistant and susceptible soybean. We found significant impacts of geography on aphid populations and that populations of aphids from each resistant line were significantly different from the other resistant line.

To my grandfathers-
The men to whom I owe my love of nature, science, and the creation.

ACKNOWLEDGEMENTS

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ECOSYSTEM SERVICES PROVIDED BY APHID PREDATORS IN APHID-RESISTANT AND APHID SUSCEPTIBLE SOYBEAN

Abstract

Natural enemies are an important factor in managing soybean aphid (*Aphis glycines* Matsumura), but are sometimes unable to maintain aphid numbers below economically damaging levels. Several genes that convey resistance to soybean aphid have been discovered. In this study, we survey natural enemy populations in aphid-resistant and susceptible soybean and measure the ecosystem services they provide using a biological control services index (BSI). The overall number of natural enemies was no different between resistant and susceptible plots, while there were significant differences in the density of certain taxa- specifically coccinellid lady beetles. There were significantly more coccinellid beetles in susceptible soybean than in resistant, corresponding with different aphid density. There was no significant difference in the BSI between resistant and susceptible plots.

Introduction

Ecosystems provide goods and services that are beneficial to human beings (Daily *et al.* 2000). These ecosystem services can be tangible things, such as timber and wild fisheries, or intangible processes, such as pollination of crops by insects, carbon sequestration by forests, and filtration of water by wetlands. By expansion, ecosystem services can be monetized through ecotourism or carbon offsets. Although ecosystem services are vital to human well being, it is difficult to quantify them and include their value in everyday decision-making (Barbier and Heal 2006, Daily *et al.* 2009, Nelson *et al.* 2009).

Entomophagous (insect-eating) arthropods offer significant ecosystem services by regulating pest populations in a wide array of environments (Losey and Vaughan 2006). Beneficial insects provide efficient, resilient, and environmentally friendly reduction and suppression of populations of pest species in many agricultural systems. The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an example of a pest strongly impacted by biological control. Soybean aphid is a native of Asia, where it is a pest of soybean (*Glycine max* (L.) Merrill). The soybean aphid was first discovered in North America in 2000. By 2003, soybean aphid moved across the northern United States and eastern Canada, causing billions of dollars in crop loss (NCSRP 2004, Ragsdale *et al.* 2004), and a dramatic increase in the application of insecticides to soybean in outbreak years (DiFonzo 2009). In infested soybean fields, natural enemies significantly reduce aphid numbers (Costamagna and Landis 2006, Miao *et al.* 2007, Landis *et al.* 2004, Gardiner *et al.* 2009, Costamagna *et al.* 2007). Minute pirate bugs (Hemiptera: Anthocoridae), coccinellid beetles (Coleoptera: Coccinellidae), syrphid flies (Diptera: Syrphidae), and parasitoid wasps consume aphids and

often keep aphid populations below established thresholds (Rutledge *et al.* 2004, Costamagna *et al.* 2007, Kaiser *et al.* 2007, Ragsdale *et al.* 2007, Schmidt *et al.* 2008, Gardiner *et al.* 2009, Ragsdale *et al.* 2010). But natural enemies do not always keep soybean aphid populations below economically damaging levels. When outbreaks occur, insecticide applications effectively manage soybean aphid populations and mitigate yield loss (Myers *et al.* 2005, Johnson *et al.* 2009). However, insecticides have both lethal and sublethal effects on natural enemies (Elzen 2001, Bosik 2006, Desneaux *et al.* 2006, Desneaux *et al.* 2007, Ohnesorg *et al.* 2009), add significant cost to soybean production (Song *et al.* 2006), and have negative environment impact.

To control soybean aphid while reducing the application of insecticides, soybean breeders identified several sources of host plant resistance, with Rag (Resistance *Aphis glycines*) genes. The first sources had single, dominant genes- *Rag*, *Rag1* (Hill *et al.* 2004, Hill *et al.* 2006, Li *et al.* 2007), or *Rag2* (Mian *et al.* 2008, Kim *et al.* 2010). Mensah *et al.* (2008) screened over 2,000 plant introductions (PIs) from China and reported two additional sources of aphid resistance. These two sources, PI 567541B and PI 567598B, each contained two recessive genes (*rag1c* and *rag4*, *rag1b* and *rag3*, respectively) that conveyed epistatic (synergistic) aphid resistance (Chiozza *et al.* 2008). *Rag1* was available in commercial varieties beginning 2010 and other sources of resistance were listened for use by seed companies.

Soybean aphid biotypes that colonize *Rag1* (Kim *et al.* 2008) and *Rag2* (Hill *et al.* 2010) were reported relatively quickly after the genes were discovered. The ability of aphids to overcome host-plant resistance is well documented (Dreyer and Campbell 1987, Dixon 1998). Aphid life cycles are typified by sexual and parthenogenic reproduction, telescoping

generations, and host alternation. Many aphid species also have symbiotic bacteria that allow aphids to live on poor food sources. These characteristics also apply to soybean aphid (Michel *et al.* 2011), increasing the risk of soybean aphids overcoming all known resistance genes. To lengthen the time that aphid resistant genes are effective, it is important to consider multiple integrated tactics in including the impacts of biological control.

The coupling of host plant resistance and biological control can have additive or synergistic effects on the suppression of aphids. Natural enemies take longer to reproduce than aphids. Host plant resistance can slow the growth of an aphid population, allowing natural enemies to keep pace, and have a greater impact than on a susceptible plot (Dreyer and Campbell 1987). Conversely, host plant resistance may decrease an aphid populations and eliminate the prey base that attracts and sustains natural enemies, making biological control less than in a susceptible crop. The effects of antibiosis resistance have been seen to negatively impact two trophic levels of predators feeding on insects feeding on resistant soybean (Orr and Boethel 1986). How the reduced number of soybean aphids and the antibiotic effects of resistant plants will effect natural enemy populations is not well understood.

How predator populations and the ecosystem services they provide are effected by prey density can be difficult to categorize. In spite of this difficulty, certain aphid predators are density-dependent in a multitude of systems. Coccinellid beetles are strongly affected by aphid numbers (Evans and Nadeer 1992, Ives *et al.* 1993, Schellhorn and Andow 2005). These patterns also hold true in soybean (Donaldson *et al.* 2007) By dramatically reducing aphid numbers, resistant soybean fields may recruit and support fewer natural enemies,

allowing low density aphid populations (for example isolated colonies of a biotype) to persist.

The objective of this study was to compare the numbers, diversity and ecosystem services (as measured by aphid population suppression) of natural enemies in susceptible and aphid-resistant soybean.

We expected to see lower natural enemy density in plots with fewer aphids, leading to lower numbers and diversity of natural enemies as well as lower ecosystem services in resistant plots when compared to susceptible plots.

Materials and Methods

The study was conducted at the Michigan State University (MSU) Entomology Field Research Station in East Lansing, Michigan (42°41'26.12"N, 84°29'38.92"W), and the MSU Saginaw Valley Research and Extension Center in Frankenmuth, Michigan (43°23'54.81"N, 83°41'44.76"W) in 2010 and 2011.

At both locations in 2010, four replicates of three soybean lines were planted. These lines represented three different levels of resistance to soybean aphid: susceptible (SD01-76R), *Rag1* resistant (LD16060), and *rag1b* and *rag3* resistant (E06902). Soybeans were planted in East Lansing on 19 May and in Frankenmuth on 5 May in 0.1ha plots in 76 cm rows. Within a day of planting, 16 seeds in each plot were removed by hand and replaced with 16 SD01-76R seeds. This created single susceptible plants, or 'aphid islands,' in a background of susceptible or resistant soybeans. Aphid islands were arranged in a

rectangular, four-by-four grid in the center of each plot, separated by 2 m within a row and 2.3 m across two rows (Figure 1.1).

After emergence, each aphid island was covered with a predator-exclusion cage. Cages consisted of 35 cm diameter tomato-ring covered with a mesh bag made of No-See-Um nylon netting (24 holes/cm^2, Outdoor Wilderness Fabrics Inc., Caldwell, ID). The bottom of each mesh bag was buried around the base of the plant and the top was tied to exclude insects. On 6 July (East Lansing), 22 July (Frankenmuth) and eight aphid islands in each plot were infested with five aphids collected from a commercial field of susceptible soybean (Asgrow AG2108) near East Lansing. Aphids were placed on the upper trifoliate of each island with a camelhair brush. This created point source infestations that simulated isolated colonies of a virulent soybean aphid biotype. Four islands were randomly selected to remain covered to exclude natural enemies, while the remaining mesh bags were removed from the other four islands. The infested islands were left for 14 days.

The natural aphid population in each plot was monitored by counting aphids on ten randomly selected plants 7 and 14 days after infestation. Yellow sticky cards (Multigard, Scentry Biologicals, Billings, MT) were hung in each plot on the day of infestation and changed after 7 and 14 days, the numbers and taxa of natural enemies on each card were identified (Table 1.1). On day 14, aphid islands were destructively sampled to count the number of soybean aphids per plant. The suppression of aphid populations between caged and open aphid islands showed the impact of predation. The study was repeated using the remaining eight aphid islands in each plot beginning 8 August (Frankenmuth) and 27 July (East Lansing) for a later timing at each location.

In 2011, four replicates of two soybean lines were planted on 11 May in Frankenmuth in the same structure as 2010: susceptible (Pioneer 92M33) and *rag1b* and *rag3* resistant (E06902 and E06905). Plots were 0.1 ha, planted in 76 cm rows. On 18 May, aphid islands (aphid-susceptible Pioneer 93M33) were planted in each plot and caged in the same arrangement as in 2010 (Figure 1.1). On 30 June, eight aphid islands in each plot were infested with five aphids. Four islands were randomly selected to remain covered to exclude natural enemies, while the remaining mesh bags were removed from the other four islands. The infested islands were left for 14 days while the natural aphid population in each plot was monitored by counting aphids on ten randomly selected plants. Natural enemies were collected from these same plants. Four yellow sticky cards were placed in each plot (Figure 1.1) and changed 7 and 14 days after infestation sticky cards were changed. At the same time, 100 sweeps were taken from the center of each plot, and samples were frozen. On day 14, aphid islands were destructively sampled to count soybean aphids per plant. Insects collected on plants, sticky cards, and sweeps were identified to family. Coccinellids, were identified to species.

For both years, the suppression of aphids by natural enemies in each plot was determined by comparing the number of aphids on caged and open aphid islands using a biological control services index (BSI) after Gardiner *et al.* (2009).

The total number of natural enemies collected was compared between resistant and susceptible plots. In addition, we separated numbers of coccinellids and anthocorids and compared the mean numbers of both taxa between resistant and susceptible plots. In addition to the quantitative analysis of natural enemies in each plot, a qualitative analysis was

also conducted using: Shannon's index of diversity (a measure of species evenness), and Simpson's diversity index (a measure of species richness). To conduct these analyses, the relative density ($P_i=n_i/N$) of each taxa in each plot was calculated, then used to calculate Shannon's ($D=\sum p_i^2$) and Simpson's ($H'=-\sum(p_i \ln p_i)$) to determine the diversity of the taxa represented in each plot.

All statistical analyses were conducted using SAS 9.1 (SAS Institute, Inc. 2009. Cary, North Carolina) with an alpha of 0.05 to determine significance. For data from 2010, aphid numbers, BSI, and natural enemy numbers was compared among lines using proc mixed. In 2011, aphid numbers, natural enemy numbers, and BSI were compared between lines using Student's t-test (proc ttest).

Results

In 2010 and 2011 there were low numbers of soybean aphid in Michigan. In 2010, the greatest number of aphids detected per susceptible plant was 25 in Frankenmuth on 10 August. In 2011, the greatest number of aphids detected was 6 aphids per susceptible plant in Frankenmuth on 11 August. This was below the economic threshold of 250 aphids per plant (Ragsdale *et al.* 2007). Although overall numbers were low, there were detectable and significant differences in the number of aphids in resistant and susceptible plots (Table 1.1, 2010: $F=11.9$, $p<0.0001$; 2011: $t=4.84$, $p<0.0001$).

In all instances, aphid islands left open to natural enemies, regardless of surrounding plants, had significantly lower numbers of aphids than caged aphid islands (Figure 1.2). In 2010, open aphid islands had an average of 15 and 11 aphids per plant and closed islands

had an average of 262.2 and 47.4 aphids per plant in East Lansing (t=2.96, p=0.006) and Frankenmuth (t=3.97, p=0.006), respectively. In 2011, open aphid islands averaged of 3 aphids per plant, and closed islands averaged of 336 aphids per plant (t=3.64, p=0.0024). This demonstrated the effect of natural enemies in reducing aphid numbers. When natural enemies were excluded, aphid numbers rose above the economic threshold of 250 aphids per plant (Ragsdale *et al.* 2007).

In both years however, there was no difference in the suppression of aphid populations between resistant and susceptible plots (2010: F=0.04, p=0.957, 2011:t=1.11, p=0.29). Natural enemies reduced aphid numbers just as efficiently in resistant plots as susceptible plots (Table 1.2).

In 2010, we collected 310 natural enemies on yellow sticky cards, consisting primarily of coccinellid beetles (N=136, 44% of total) and syrphid flies (N=117, 38% of total). This is unsurprising as both taxa are mobile and likely to be caught by a sticky trap. There was no significant difference in the overall numbers of natural enemies collected between the three lines (F=0.0168, p=0.98) or by location (t=0.44, p=0.66). The diversity of natural enemies did not differ significantly between resistant and susceptible plots (Shannon: F=1.61, p=0.21, Simpsons: F=2.76, p=0.079), by date (Shannon: F=0.18, p=0.68, Simpsons: F=0.84, p=0.55), or by location (Shannon: t=-1.07, p=0.91, Simpsons: t=0.11, p=0.91).

In 2011, we collected 2,248 natural enemies on whole plants (N=73, 3% of total), sticky cards (N=1359, 56% of total), and sweeps (N=996, 41% of total), consisting primarily of anthocorids (N=1,747, 72%), syrphid flies (N=333, 14% of total), and coccinellid beetles (N=245, 10% of total). The larger proportion of anthocorids caught in 2011 when compared

to 2010 is due to the inclusion of sweep netting and of natural enemies on plants. There were no significant differences in the number of natural enemies collected in resistant and susceptible plots overall (t=-0.75, p=0.46), and for each individual date (Table 1.3). The diversity of natural enemies did not differ significantly between resistant and susceptible plots (Shannon: F=1.84, p=0.21, Simpsons: F=1.97, p=0.19) or by date (Shannon: F=0.18, p=0.68, Simpsons: F=0.01, p=0.93)

In 2011, the number of anthocorids collected in resistant and susceptible plots did not differ significantly (Table 1.4). We collected 91 anthocorids per plot in *rag1b* and *rag3* plots and 96 per plot in susceptible plots (t=2.23, p=0.81). In contrast to anthocorids, there were significant differences in the number of coccinellid beetles collected in resistant and susceptible plots (Table 1.4). We collected 7 coccinellids per plot in *rag1b* and *rag3* plots and 18 per plot in susceptible plots (t=-3.33, p=0.0082). Coccinellids are primarily aphidophagous, so their attraction to plots with more aphids is unsurprising, although given the low numbers of aphids, the strength of the differences between resistant and susceptible plots is high.

Discussion

We saw significant differences in the number of soybean aphid on caged and open plants. In East Lansing in 2010 and Frankenmuth in 2011, aphid numbers rose above economic thresholds (Ragsdale *et al.* 2007). In these cases the impact of predation by natural enemies is shown. The ability of natural enemies to maintain aphid numbers below economically damaging levels has been well documented (Costamagna and Landis 2006,

Miao *et al.* 2007, Landis *et al.* 2004, Gardiner *et al.* 2009, Costamagna *et al.* 2007). Without the ecosystem services provided by natural enemies, susceptible plots may have gone above threshold. In this case, the positive economic benefits of aphid population suppression by natural enemies are apparent.

The three primary groups of insects that likely provided this suppression of aphid populations were the anthocorid bugs, syrphid flies, and coccinellid beetles. These three families of insects were a large majority of the natural enemies collected and are known for their importance as biological control agents. There was no difference in the numbers of anthocorids collected in resistant and susceptible plots, which would be expected due to their ability to eat a wider diet than just aphids. The number of coccinellid beetles collected in susceptible plots was greater than those collected in resistant plots. Ladybird beetles feed primarily on aphids and are known to be drawn to high density aphid populations (Evans and Youssef 1992, Elliott and Kieckhefer 2000, Koch 2003, Noma *et al.* 2010. An increase of their numbers in plots with greater density of aphids is not surprising, but the size of the increase based on a small difference in the numbers of aphids demonstrates the efficacy of ladybird beetles in detecting elevated prey populations. Although there more coccinellid beetles collected in susceptible plots, there was not a significant overall difference in the number of natural enemies between resistant and susceptible soybean.

There was no difference in the suppression of aphid populations measured by BSI between in resistant plots and susceptible plots. There was a significant difference in the density of aphids on naturally colonized plants surrounding the aphid islands. Although significantly different, the number of aphids on resistant plants did not rise above 25 aphids

per plant in aphid susceptible plots, in comparison to 8 and 9 aphids per plant on *rag1b* and *rag3* and *Rag1* plants respectively, on 10 August 2010 in East Lansing. This difference is statistically significant, but when compared to the economic threshold of 250 aphids per plant it is not a great enough difference to merit any differences in aphid management and is not biologically significant.

Although some natural enemies were found associated with higher aphid numbers, the number and diversity of natural enemies did not differ significantly between resistant and susceptible soybean lines.

Under low soybean aphid pressure we could not detect any impact on ecosystem services, as measured by BSI, in aphid resistant soybean. The results may be different in years with higher aphid numbers.

APPENDIX

Figure 1.1: Diagram of soybean plots and aphid islands used in 2010 and 2011to sample ecosystem services and natural enemy numbers in East Lansing and Frankenmuth, MI.

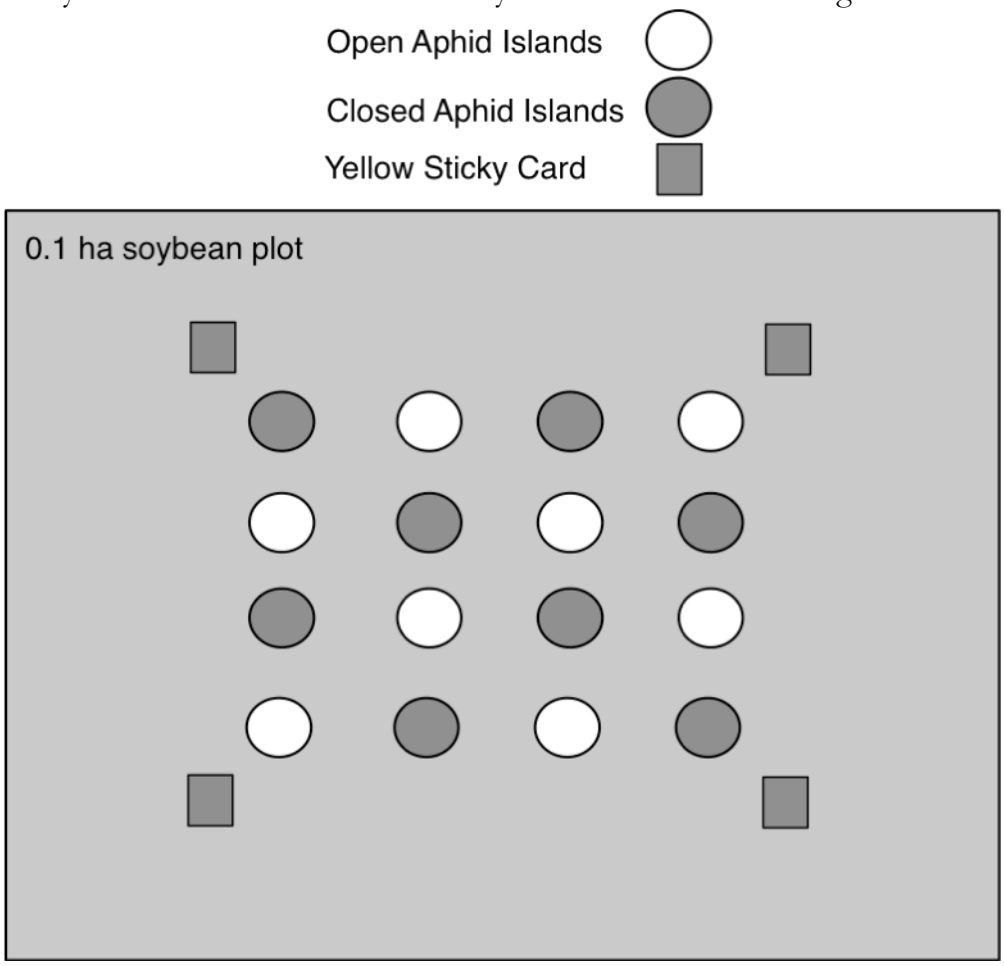

Table 1.1: Mean aphid population size per plant (± Standard Error) per plant aphid-resistant and susceptible soybean plots during 2010 and 2011 in East Lansing and Frankenmuth, MI. Different letters signify significantly different means.

Location (Year)	Line (Gene)	Date			
		7/13	**7/20**	**8/3**	**8/10**
East Lansing (2010)	SD01-76R (Susceptible)	0.7±0.3a	0.8±0.3a	21.1±3.1a	24.7±4.3a
	LD160160 (*Rag1)*	0±0b	0.1±0.08b	8.6±3b	8.2±3a
	E06902 (*rag1b* and *rag3)*	0±0b	0.1±.07b	3.5±0.8c	8.5±2.4a
		7/29	**8/5**	**8/19**	**8/26**
Frankenmuth (2010)	SD01-76R (Susceptible)	11.9±4.5a	17±1.3a	5.5±2.3a	3.1±0.7a
	LD160160 (*Rag1)*	5.1±2.4b	5±0.9b	3.7±1.4a	2.3±0.9a
	E06902 (*rag1b* and *rag3)*	1.2±0.7c	0±0c	1.1±0.5b	0.5±0.4b
		7/7	**7/14**	**8/4**	**8/11**
Frankenmuth (2011)	Pioneer 92M33 (Susceptible)	3.9±2.6a	2.7±1.4a	2.2±1.3a	6.3±3.9a
	E06902 and E06905 (*rag1b* and *rag3)*	0.05±0.1b	0.03±0.07b	0.03±0.08b	0.1±0.2b

Figure 1.2: Mean population size of aphids per plant (± Standard Error) on open and closed aphid islands during 2010 and 2011.

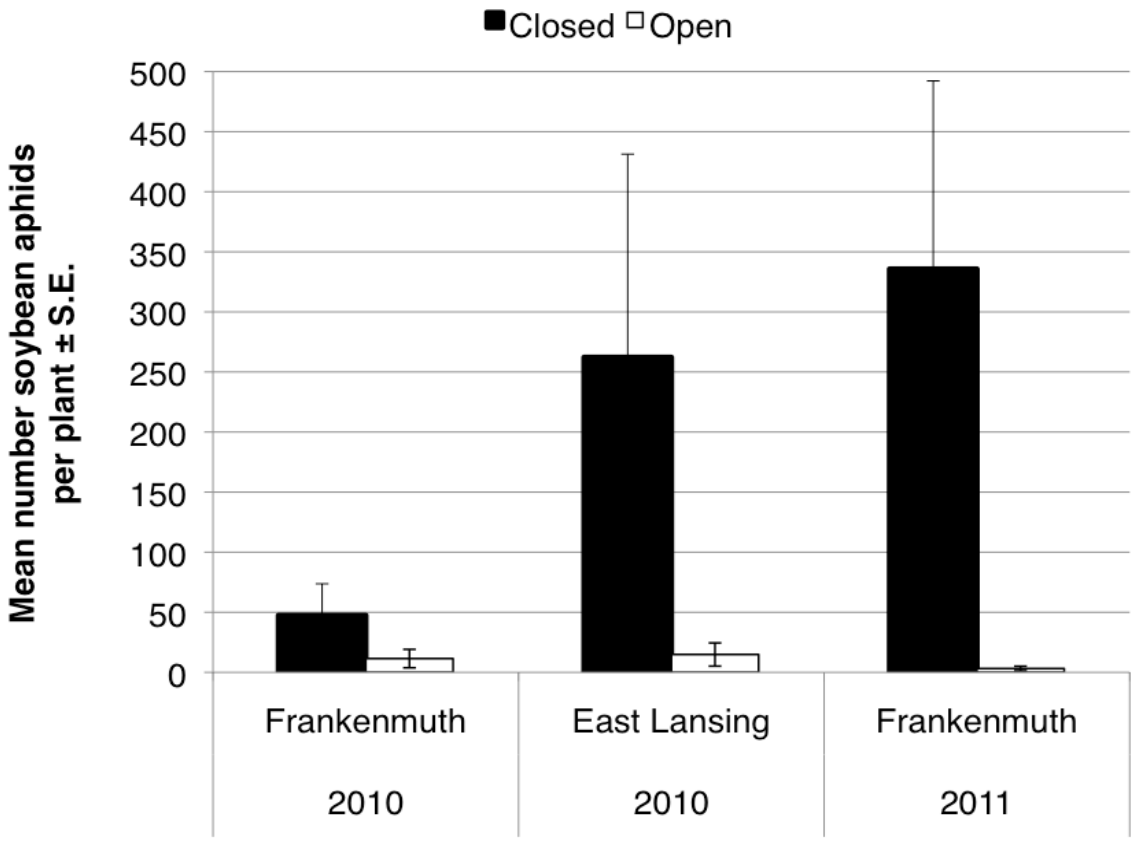

Table 1.2- Biological control service index (BSI) values (± Standard Error) in aphid suppression on aphid islands in aphid-resistant and susceptible soybean (*Glycine max*). Different letters signify significantly different means.

Location (year)	Line (Gene)	Date	
		7/20	**8/10**
East Lansing (2010)	SD01-76R (Susceptible)	0.99±0.0a	0.54±0.2a
	LD160160 (*Rag1)*	0.96±0.02a	0.64±0.1a
	E06902 (*rag1b* and *rag3)*	0.95±0.03a	0.37±0.2a
		8/5	**8/26**
Frankenmuth (2010)	SD01-76R (Susceptible)	0.74±0.1a	0.75±0.2a
	LD160160 (*Rag1)*	0.64±0.1a	0.89±0.05a
	E06902 (*rag1b* and *rag3)*	0.76±0.2a	0.96±0.0a
		7/14	**8/11**
Frankenmuth (2011)	Pioneer 92M33 (Susceptible)	0.95±0.01a	0.85±0.2a
	E06902 and E06905 (*rag1b* and *rag3)*	0.98±0.1a	0.68±0.1a

Table 1.3: Mean quantity (± Standard Error) and diversity of natural enemies collected in aphid-resistant and susceptible soybean (*Glycine max*) in East Lansing and Frankenmuth.

Location (Year)	Line/Variety (Gene)	Date				Diversity	
		7/13	**7/20**	**8/3**	**8/10**	*Simpsons (D)*	*Shannon (H')*
East Lansing (2010)	SD01-76R (Susceptible)	4±1.7	5.8±3.1	5.3±1.2	8.5±2.9	0.90±0.13	0.60±0.12
	LD160160 (*Rag1)*	0.7±0.6	1.7±0.3	4±2.8	1.5±0.4	0.54±0.10	0.76±0.11
	E06902 (*rag1b* and *rag3)*	0.8±0.5	3±1.4	1±0.5	1±0	1.0±0.22	0.58±0.07
		7/29	**8/5**	**8/19**	**8/26**		
Frankenmuth (2010)	SD01-76R (Susceptible)	5.3±1.3	1.7±0.3	1±0	3.5±1.5	0.64±0.12	0.59±0.07
	LD160160 (*Rag1)*	2.3±0.9	1±0	2±0	2.3±1.5	0.56±0.10	0.63±0.06
	E06902 (*rag1b* and *rag3)*	2±0	2±0.7	1±0	1±0	0.62±0.11	0.38±0.08
		7/7	**7/14**	**8/4**	**8/11**		
Frankenmuth (2011)	Pioneer 92M33 (Susceptible)	49±7.0	51±7.6	117±15.2	63.8±18.9	0.28±0.05	1.9±0.08
	E06902 and E06905 (*rag1b* and *rag3)*	47.8±6.5	52.3±12.8	74.5±15.3	66.3±20.1	0.20±0.02	1.7±0.18

Table 1.4: Mean (± Standard Error) coccinellid beetles and anthocorid bugs collected in aphid resistant aphid susceptible soybean (*Glycine max*) and Frankenmuth, MI during 2011. Different letters signify significantly different means.

Line/Variety (Gene)	Taxa	7/1-7/14	7/28-8/11
Pioneer 92M33 (Susceptible)	Anthocoridae	32±9a	160±35d
	Coccinellidae	25±3c	16±2e
E06902 and E06905 (*rag1b* and *rag3)*	Anthocoridae	54±8a	128±27d
	Coccinellidae	10±1b	8±1f

Record of deposition of voucher specimens

The Specimens listed on the following page have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2011-08

Title of thesis:

BOTTOM-UP AND TOP-DOWN MANAGEMENT OF SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA) IN APHID-RESISTANT SOYBEAN (*GLYCINE MAX* (L.) MERRILL)

Museum where deposited:
The Michigan State University (MSU) Albert J. Cook Arthropod Research Collection

Specimens

Family		**Life Stage**	**Quanitity**	**Preservation**
Aphididae	*Aphis glycines*	Adult	1 Vial	Ethanol
Anthocoridae	*Oruis spp.*	Nymph	10	Pinned
	Oruis spp.	Adult	10	Pinned
Chrysopidae		Adult	2	Pinned
Coccinellidae	*Coccinella septempunctata*	Adult	10	Pinned
	Coleomegilla maculata	Adult	3	Pinned
	Cycloneda munda	Adult	2	Pinned
	Hippodamia variegata	Adult	10	Pinned
	Hippodamia parenthesis	Adult	1	Pinned
	Harmonia axyridis	Adult	10	Pinned
	Propylea quatuordecimpunctata	Adult	1	Pinned
Hemerobiidae		Adult	1	Pinned
Lampyridae		Adult	3	Pinned
Nabidae		Adults and Nymphs	10	Pinned
Syrphidae		Adult	2	Pinned

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CHAPTER 2:
HOST PLANT RESISTANCE TO SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA) IN POTASSIUM DEFICIENT SOILS

Abstract

When soybean aphids were first discovered in the United States in 2000, researchers and growers noticed the damage associated with large aphid numbers and soils that were low in potassium. Researchers observed that aphids on potassium deficient plants had higher fecundity, survivorship, and population density than aphids on plants with sufficient potassium. Plant breeders quickly identified several aphid resistance genes that significantly suppress the number of aphids. How effective these aphid-resistance genes were in plants grown in potassium deficient soils was unknown. Soybean plants with single gene resistance (*Rag1*), double gene resistance (*rag1b* and *rag3*, and *rag1c* and *rag4*), as well as susceptible soybean were planted in aphid and predator-proof cages in a potassium deficient field. Half of the cages were amended with potash while the other half remained potassium deficient. Plants were infested with aphids and monitored through the growing season. No significant difference in aphid populations was detected between plants grown in potassium deficient and potassium amended soil. Resistant plants had significantly lower aphid populations than susceptible plants in both potassium deficient and potassium-amended cages.

Introduction

The soybean aphid (*Aphis glycines* Matsumura) is an important pest of soybean (*Glycine max* (L.) Merrill) in North America and East Asia. The soybean aphid was first identified in the United States in 2000 (Venette and Ragsdale 2004). By 2003, it was established in 21 states and 3 Canadian provinces (NCSRP 2004). Its introduction resulted in an estimated $1.7 billion in annual soybean crop loss (NCSRP 2004) and a 130-fold increase in the amount of insecticides applied to soybean between 2000 and 2006 (Ragsdale *et al.* 2010). Aphids feed by tapping into the plant phloem and suck sap from soybean plants. This inhibits photosynthesis (Macedo *et al.* 2003, Diaz-Montano *et al.* 2007) and during outbreaks, soybean aphids can reduce seed size, yield, and oil content (Ragsdale *et al.* 2007, Beckendorf *et al.* 2008). Soybean aphid vectors viruses of soybean (Pederson *et al.* 2007, Wang *et al.* 2006, Burrows *et al.* 2005, Wang and Ghabrial 2002) and other important crops, including dry beans (DiFonzo and Agle 2008), snap beans (Larsen *et al.* 2008) and potato (Davis *et al.* 2005, Wang *et al.* 2006, Davis and Radcliffe 2008).

In 2000, when soybean aphid was discovered in the US, growers in southwest Michigan reported yellowing of soybean leaves in aphid-infested fields. Foliar tissue analysis revealed that these plants were potassium deficient and field observations suggested that aphid numbers were elevated in these fields (DiFonzo and Hines 2001). In laboratory studies Myers *et al.* (2005) determined that aphids on leaves from potassium deficient plants had a significantly greater intrinsic and finite rate of increase as well as in increased net reproductive rate. Myers and Gratton (2006) demonstrated that aphids on plants growing in low potassium soil in a laboratory had a greater rate of population increase when compared

to aphids on plants growing in soil with higher levels of potassium. Walter and DiFonzo (2007) showed that aphids on potassium deficient plants had s shorter time to first reproduction and greater production of nymphs per day. They also recorded that aphid density was greater on plants in potassium-deficient soybean fields.

Plant phloem is rich in sugar but is an otherwise poor source of nutrients. Nitrogen is a limiting factor in aphid population growth (Dixon 1998). The most efficient source of nitrogen for aphids is free amino acids in plant sap, which are either essential or can be converted to essential amino acids by symbiotic bacteria within the aphid (Dixon 1998). Walter and DiFonzo (2007) found that the sap of potassium deficient plants had an elevated percentage of asparagine, one such free amino acid. The increased concentration of free amino acids, such as asparagine, could account for the increased fecundity of soybean aphids on potassium deficient plants. In a region-wide survey of ecological factors Noma *et al.* (2010) found negative correlation between soil potassium level and soybean aphid populations across the midwest. The link between soybean aphid population growth and soil potassium level is now generally accepted.

Shortly after the discovery of soybean aphid in North America, soybean breeders began to screen for host plant resistance. Hill *et al.* (2006) reported the first aphid resistance gene, *Rag1* (Resistance *Aphis g*lycines *1*); a second gene, *Rag2* was reported by Mian et al (2012). In Michigan, Mensah *et al.* (2005) screened over 2,000 plant introductions (PIs) for aphid resistance, and found two expressing recessive two-gene epistatic (synergistic) resistance. These genes were named *rag1b* and *rag3*, and *rag1c* and *rag4* (Mensah *et al.* 2005). Commercial varieties of soybean with *Rag1* genes have been available since 2010 and the

introduction of additional aphid resistance genes is expected. The ultimate goal of aphid-resistant soybean is to reduce environmental and economic cost of insecticide use, making soybean production more profitable and sustainable.

Currently identified aphid-resistance genes negatively impact soybean aphid populations through antibiosis, although the exact mechanism is not yet fully understood. Chiozza *et al.* (2010) determined that aphid-resistant *Rag1* soybean had significantly lower concentrations of several free amino acids, including asparagine, than aphid susceptible plants and that the concentration of free amino acids strongly correlated with aphid density on plants. The antibiotic effects of some or all soybean aphid resistance genes could be a product of the nutritional quality of resistant plants. If this is the case, the efficacy of aphid resistant soybean in potassium deficient soils is questionable.

The use of host plant resistance to manage soybean aphid is still new and relatively untested. How aphid-resistant soybean lines perform under varying conditions is not known. The objective of this study was to determine if soybean aphid resistance is maintained under potassium deficient conditions.

Materials and Methods

This study was conducted in 2009, 2010, and 2011 in a potassium deficient field in Montcalm County, Michigan. The specific location of the study within the field shifted each year to avoid working on the same place. Preliminary soil tests of the field averaged between 44 and 62 parts per million potassium, approximately 50-70 parts per million below optimum levels (Staton and Wylie 2009). In all years, square (1m x 1m x 1m) or rectangular

(1m x 1m x 2m) predator-proof cages (Lumite Inc., Gainsville GA. 32 x 32 amber mesh) were and assigned one of two treatments, not-fertilized (K-) or fertilized (K+), by adding 0-0-62 potash (Northern Star Minerals, Okemos, MI). The amount of potash applied was based on soil test results and the recommendations from the Michigan State University (MSU) Soil Testing Lab, East Lansing, MI. The potash was spread by hand evenly across the soil surface in the cage and incorporated using a hand cultivator. After fertilization, soybean seeds were planted in each cage. Soil and tissue samples from each cage were taken and analyzed at the MSU Soil Testing Lab (Table 2.1). After emergence plants were thinned to predetermined numbers and infested with aphids collected from susceptible soybean at the MSU Entomology Research Farm, East Lansing, MI. To sample soybean aphid numbers, ten plants per line in each cage were randomly selected and counted until the average number of aphids per plant was above 1,000 on the line with the most aphids. Between 1,000 and 5,000 aphids per plant, five plants per line were counted, above 5,000 aphids per plant, three plants per line were counted.

In 2009, eight square cages were erected. Four cages were fertilized with 0.07 kg of potash on 17 June and the remaining four cages were not treated. On 24 June, 120 seeds of an aphid-resistant line, E06902 (*rag1b* and *rag3*), were hand planted in each cage. On 15 July, plants were thinned to 80 plants per cage. On 22 July, each plant in every cage was infested with 5 aphids. Beginning seven days after infestation, plants were destructively sampled every seven days for 35 days. Soil samples were taken from each cage 29 July. Tissue samples were taken on 5 Aug, dried, and submitted to the MSU Soil Testing Laboratory for foliar potassium levels.

In 2010, the study was repeated with more aphid resistant lines. On 7 June, ten rectangular cages were erected. On 9 June, five cages were fertilized with 0.1kg of potash while the remaining five cages remained untreated. Fertilized cages received an additional 0.05 Kg of potash 9 July. On 17 July soil was re-tested to determine if the fertilization successfully increased soil K levels to optimum levels. On 17 June, 60 seeds from three soybean aphid-resistant lines, LD16060 (*Rag1*), E06902 (*rag1b* and *rag3*), and E07906-2 (*rag1c* and *rag4*) were planted in randomly assigned blocks within each cage. On 15 July each plant in every cage received was infested with 5 aphids. On 26 July, plant samples were collected from each cage, dried and submitted for analysis. Beginning seven days after the infestation of replications, plants were destructively sampled every seven days for 42 days.

In 2011, the study was repeated with the inclusion of an aphid-susceptible line to compare the numbers of aphids on aphid-resistant soybean to susceptible soybean. Twenty-four square cages were erected on 24 and 25 May. Twelve cages were fertilized 31 May with 0.07 kg of potash. Each cage was planted to one of three soybean lines, SD01-76R (aphid-susceptible), LD016060 (*Rag1*), or E06902 (*rag1b* and *rag3*). On 31 May, 30 soybean seeds were planted in each cage. On 29 June, every plant in each cage was infested with a single aphid. Beginning three days after infestation, whole-plant nondestructive counts were taken every 3-4 days for 42 days. Soil samples from each cage were taken 1 July. Plant tissue samples were taken 19 July.

In 2010, several E06902 plants had high aphid populations. To determine if a *rag1c* and *rag3* virulent biotype was present, samples of these aphids were collected and placed in an incubator (16:8 light: dark, 25°C day, 20°C night) and maintained on susceptible soybeans

(Pioneer 92M33). Aphids collected from this colony as well as aphids from a colony maintained in the MSU Field Crop Entomology greenhouses were placed on susceptible soybean leaves in Petri dishes (100mm x 20mm, Corning Inc., Corning, NY) with wet filter paper substrate. Soybean leaf stems were wrapped in damp gauze to prevent desiccation. Petri dishes were placed in an incubator for 24 hours. Single, newly deposited nymphs were removed with a damp camelhair brush and placed on the upper trifoliate of V1 susceptible (Pioneer 93M33) and aphid resistant (E06902) soybeans. Ten susceptible plants received aphids from the field colony and another ten received single aphids from the lab colony. This was repeated with resistant plants making four treatments; field colony on susceptible, field colony on resistant, lab colony on susceptible, and lab colony on resistant. Each plant was covered with a clear plastic dome made by cutting off the bottom of a clear, 20 oz soda bottle. Aphids were counted every two to three days for fourteen days.

Data from each year were analyzed separately using SAS 9.1 and JMP 8.0.2 (SAS Institute, Inc. Cary, North Carolina). For all analyses, a 95% confidence interval was used. Differences in foliar and soil potassium levels were compared using Students t-Test (proc ttest). Repeated measures analysis (proc glm) was conducted on aphid numbers. This including analysis of covariance (mancova) and allowed us to directly compare the difference between aphid populations on fertilized and unfertilized plants, as well as the interaction between fertilization time to determine if the rate of change of aphid populations (slope) was significantly different between fertilized and unfertilized soybean plots.

Results and Discussion

In all three years, potash fertilization resulted in a significant increase in both on soil and foliar potassium levels (Table 2.1) within fertilized cages.

In 2009, the E06902 was very effective in reducing soybean aphid populations (Figure 2.1). The average number of aphids per plant did not rise above 9 and there were no significant differences between treatments ($F= 3.09$, $p=0.09$). There was no significant difference between the slopes of the two treatments ($F=0.8$, $p=0.53$).

In 2010, when lines were planted together, there were much larger numbers of aphids in the cages than in 2009 (Figure 2.2). Despite these large numbers, there were no statistically significant differences between fertilized and unfertilized plants of each line (LD16060- $F=2.92$, $p=0.12$; E06902- $F=1.67$, $p=0.23$; E07906-2- $F=0.33$, $p=0.58$). In each case slope of aphid population growth was not significantly different between fertilized and unfertilized cages.

In 2011, we again saw large numbers of aphids (Figure 2.3). Resistant lines had significantly fewer aphids than susceptible soybeans in both unfertilized ($F=13.32$, $p<0.0001$) and fertilized ($F=67.92$, $p<0.0001$) cages. There were no significant differences between treatments (SD01-76R, $F=0.96$, $p=0.36$, *Rag1*: $F=0.03$, $p= 0.87$; *rag1b* and *rag3*- $F=1.89$, $p=0.22$). In each case, slope of aphid population growth on resistant plants was not significantly different between fertilized and unfertilized cages (SD01-76R, $F=1.06$, $p=0.41$, *Rag1*-$F=0.03$, $p=1$; *rag1b* and *rag3*-$F=0.98$, $p=0.47$).

Aphids removed from E06902 plants in 2010 did not have significantly greater survival on E06902 leaflets than those from the laboratory colony ($F=4.02$, $P=0.0524$).

When comparing the slope of aphid population growth there is no significance difference between aphids collected from E06902 plants and those from the laboratory colony ($F=2.77$, $P=0.10$).

Host plant resistance is an important tool in the integrated pest management of soybean aphid. As aphid resistance gene becomes more ubiquitous in commercial seed supply it is important to understand the mechanism of resistance and any conditions in which it fails. We were unable to detect any difference in aphid populations between fertilized and potassium deficient soils after Walter and DiFonzo 2007. The role of amino acids and aphid resistance has been well documented, but the connection between soil potassium and amino acid levels is still not well understood.

APPENDIX

Table 2.1: Mean potassium levels (± Standard Error) of soybean plants and soil in potassium amended (K+) and potassium deficient (K-) cages in a potassium-deficient field in Montcalm County, MI. Different letters signify significantly

Year	Line	Aphid-resistance gene	Potassium Treatment	Soil Potassium (PPM)	Foliar Potassium (%)
2009	E06902	*rag1b* and *rag3*	K+	296±35a	1.5±0.25a
			K-	48±3b	1.1±0.11b
2010	E06902	*rag1b* and *rag3*	K+	155±12a	2.5±0.11a
	E07906-2	*rag1c* and *rag4*			
	LD16060	*Rag1*	K-	53±7b	1.5±0.02b
2011	E06902	*rag1b* and *rag3*	K+	105±6a	2.1±0.16a
			K-	54±2b	1.2±0.25b
	LD16060	*Rag1*	K+	144±16a	2.4±0.19a
			K-	63±10b	1.6±0.23b
	SD01-76R	Susceptible	K+	150±15a	2.0±0.07a
			K-	62±17b	1.5±0.41b

Figure 2.1: Mean population size (± Standard Error) of soybean aphid on aphid-resistant soybean on potassium deficient and potassium amended soil during 2009.

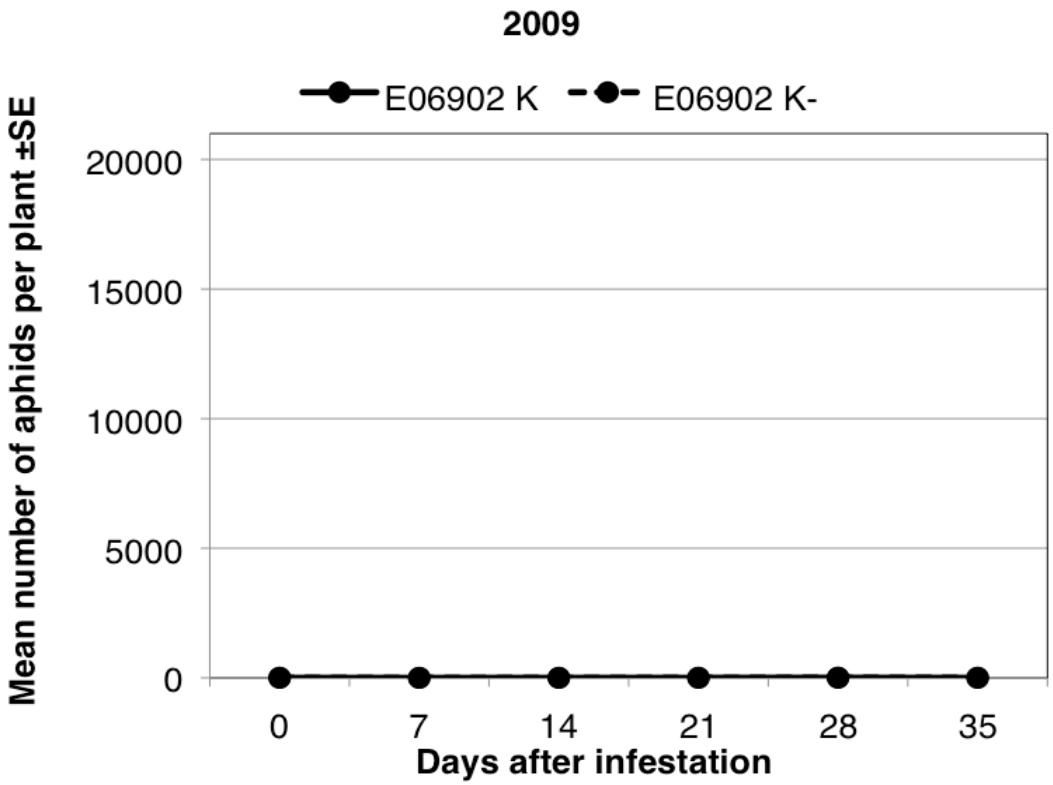


Figure 2.2: Mean population size (± Standard Error) of soybean aphid on aphid-resistant soybean lines on potassium deficient and potassium amended soil during 2010.

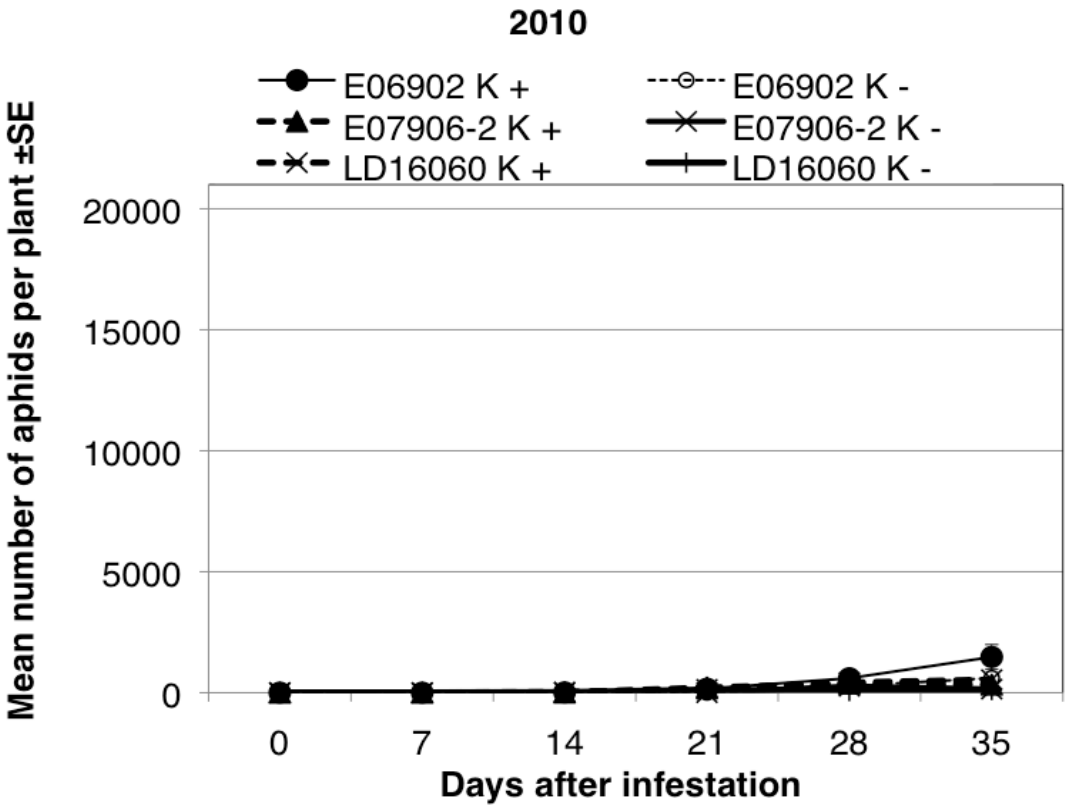
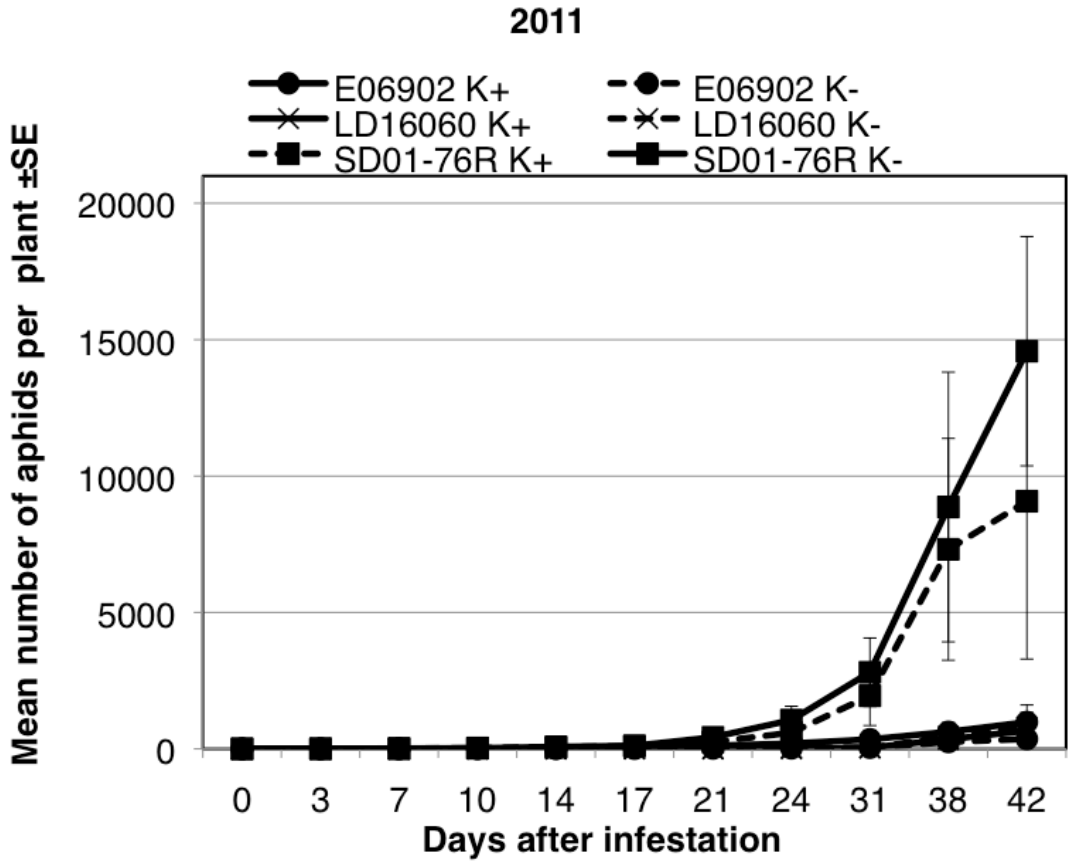


Figure 2.3: Mean population size (± Standard Error) of soybean aphid on resistant and susceptible soybean lines on potassium deficient and potassium amended soil during 2011.



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CHAPTER 3:
MOLECULAR MARKER PROFILES OF SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA) ON APHID-RESISTANT AND SUSCEPTIBLE SOYBEAN

Abstract

Soon after soybean aphids were discovered in North America in 2000, molecular techniques were adapted for investigating population dynamics. Around the same time, aphid biotypes were observed that colonized aphid-resistant plants. We used 7 molecular markers to compare populations of aphids from one susceptible and two aphid-resistant soybean lines from two locations in Michigan. We found that aphids collected from both locations were significantly different each other. We also found that the populations of aphids from each resistant line were significantly different from the other resistant line. Aphids collected from *Rag1* plants in Frankenmuth were significantly different from susceptible plants.

Introduction

The soybean aphid (*Aphis glycines* Matsumura) is an important pest of soybean (*Glycine max* (L.) Merrill) in North America and East Asia. The soybean aphid was first identified in the United States in 2000 (Venette and Ragsdale 2004). By 2003 it was established in 21 states and 3 Canadian provinces (NCSRP 2004), resulting in an estimated $1.7 billion in annual soybean crop loss (NCSRP 2004) and a 130-fold increase in insecticides applied to soybean between 2000 and 2006 (Ragsdale *et al.* 2010). Soybean aphids suck sap from soybean plants and inhibit photosynthesis (Macedo *et al.* 2003, Diaz-Montano *et al.* 2007). During outbreaks, large numbers of soybean aphids significantly reduce seed size, yield, and oil content (Ragsdale *et al.* 2007, Beckendorf *et al.* 2008). Soybean aphids vector viruses of soybean (Pederson *et al.* 2007, Wang *et al.* 2006, Burrows *et al.* 2005, Wang and Ghabrial 2002) and other important crops, including dry beans (DiFonzo and Agle 2008), snap beans (Larsen *et al.* 2008) and potato (Davis *et al.* 2005, Wang *et al.* 2006, Davis and Radcliffe 2008).

Shortly after the discovery of soybean aphid in North America, breeders began screening soybean germplasm for host-plant resistance. Several dominant *Rag* genes (Resistance to *Aphis g*lycines) were identified by Hill *et al.* (2004, 2006 - *Rag* and *Rag1*), Mian *et al.* (2008 - *Rag2*) and Zhang *et al.* (2010 - *Rag3*). Mensah *et al.* (2005) described two plant introductions (PIs) from China, PI 567541B and PI 567598B, each with two recessive aphid-resistance genes (Zhang *et al.* 2009), *rag1b* and *rag3*, *rag1c* and *rag4,* respectively. In a multi-state field screen, Chiozza *et al.* (2008) reported that soybean lines with these genes were extremely effective in limiting aphid populations. *Rag1* was the first gene licensed for incorporation in commercial soybean lines, with other genes to follow.

The ability of aphids to overcome host-plant resistance is well documented (Dixon 1998, Dreyer and Campbell 1987). Aphids are relatively unique in their ability to reproduce asexually, through parthenogenesis, or sexually, alternate hosts, and rely on gut symbionts to augment poor diets. These aspects of aphid biology enhance the ability of aphids to adapt quickly to new selection pressures; soybean aphids are no exception (Michel *et al.* 2011). At least three biotypes of soybean aphid have been identified. Biotype-1 populations do not colonize known aphid-resistant sources. Biotype-2 populations are virulent on *Rag1* soybean (Hill *et al.* 2009, Kim *et al.* 2008), and biotype-3 populations are virulent on *Rag2* soybean (Hill *et al.* 2010). These biotypes were found in research and breeding trials before the widespread commercial planting of aphid-resistant soybean. There is no known biotype that is virulent on *rag1b* and *rag3* or *rag1c* and *rag4* soybean. However, due to the suspected variability of virulence within aphid populations, the potential exists for a biotype that is virulent on *rag1b* and *rag3* or *rag1c* and *rag4* soybean to be discovered.

Because soybean aphid multiplies during the summer by parthenogenesis and no sexual reproduction and genetic recombination occurs until the fall, the genetic makeup of soybean aphid populations is fixed until the fall. The maximum overall genetic diversity of a soybean aphid population is highest in the spring when fundatricies hatch from eggs on buckthorn, and decreases until mating occurs in the fall. Within this system soybean aphid evolution occurs in discreet periods of genetic reproduction (mating in the fall) and selection (the summer months).

Molecular markers provide a tool to understand population dynamics of soybean aphid in North America and the relationships of aphid populations on a landscape scale. By

comparing genetic markers in soybean aphid populations, the effects of geography and selection can be determined. Michel *et al.* (2009), using molecular markers, compared the diversity of Asian and North American soybean aphid populations and how aphid populations interact on a landscape scale (Michel *et al.* 2009, Michel *et al.* 2009 Michel *et al.* 2009). The use of these same molecular techniques can be used to discover how aphid populations are impacted by aphid resistant soybean. Hill *et al.* (2010) suggested that a wide range of virulence existed within soybean aphid populations in North America based on the fact that biotypes were quickly identified on *Rag1* and *Rag2* soybean.

The objective of this study was to use single nucleotide polymorphisms (SNPs) to examine the diversity and relationships of soybean aphid populations on resistant and susceptible soybean. We expected to find distinctly different populations of soybean aphids on resistant plants than on susceptible plants.

Materials and Methods

Soybean Lines

Three soybean lines representing three levels of resistance to soybean aphid were planted in blocks at the Michigan State University (MSU) Entomology Field Station in East Lansing, Ingham County, MI (42°41'26.12"N, 84°29'38.92"W) and at the MSU Saginaw Valley Research and Extension Center in Frankenmuth, Saginaw County, MI (43°23'54.81"N, 83°41'44.76"W). These lines were aphid-susceptible SD01-76R, single gene resistant LD16060 (*Rag1*), and multi-gene resistant E06902 (*rag1b* and *rag3*). Plots were 0.1 ha

and replicated four times in a randomized complete block design. Plots were maintained using conventional weed control and agronomic practices.

Aphid Populations

Soybean aphids were initially detected on 15 June in East Lansing and 1 July in Frankenmuth. Aphids were sampled weekly beginning early July. Infested trifoliates were collected 10 August (East Lansing) and 12 and 19 August (Frankenmuth). Trifoliates were returned to the laboratory, where one aphid was removed from each infested trifoliate with a camelhair brush, and placed individually in a 2ml microfuge tube. Tubes were stored in a −20ºC freezer until analysis. A minimum of 32 aphids were collected from each plot with the exception of two E06902 plots at Frankenmuth. Due to the extremely low aphid numbers, only 19 aphids were collected from these plots.

Aphid Genotyping

Genotyping of aphids was conducted at The Ohio State University Ohio Agriculture Research and Development Center (OARDC) in Wooster, OH. Individual aphids were placed in PCR plates with 100 µl of QuickExtract Seed DNA Extraction Solution (Epicentre Biotechnologies, Madison WI) for DNA extraction. Each aphid was crushed with a micropipette tip to ensure that DNA was suspended in the extraction solution. Separate tips were used for each aphid to avoid cross contamination. PCR plates were vortexed and placed in an PCR cycler (Eppendorf Mastercycler, Eppendorf, Hamburg, Germany) for a single cycle of 65º C for 6 minutes followed by 98º C for 2 minutes.

After extraction, one µl of DNA suspension from each sample was transferred to a 96 well PCR plate. Twenty-five µl of Qiagen Multiplex PCR solution (Qiagen, Valencia CA),

five μl of primer mix (See Table 1), and 18 μl of RNAase-free water (Qiagen, Valencia CA) were added to the DNA to bring the total volume in each well to 50 μl. Plates were vortexed then centrifuged for one minute at 1,000 rpm then returned to the PCR cycler. Samples were held at 95º C for 15 minutes for enzyme activation and cycled for 30 seconds at 94º C, 55-58.5º C for 30 seconds, and 72ºC for 30 seconds. This cycle was repeated 35 times, then samples were held at 4ºC until processing. Samples were again vortexed and centrifuged for one minute at 1,000 RPM. Three μl of the PCR products to 96 well PCR plates and ExoSAP-it PCR product cleaner (1.2 μl, Affymetrix, Santa Clara, CA) was added to each well. Each plate was vortexed then centrifuged for one minute at 1,000 rpm. The samples were incubated at 37ºC for 30 minutes and inactivated by heating to 80ºC for 15 minutes.

An allele-specific primer extension (ASPE) assay was then conducted using ASPE primer mix (Orantes 2011). dNTP-dCTP (Invitrogen Corporation, Carlsbad, CA) was added to the products of the Multiplex PCR reaction, bringing each sample to 10 μl of solution. Samples were vortexed and centrifuged for 1 minute at 1,000 rpm and placed in a PCR cycler. Samples were held at 96ºC for two minutes and followed by 30 30-second cycles at 94ºC, 58ºC-60ºC for one minute, and 74ºC for 2 minutes, then held at 4ºC.

Each ASPE reaction mixture was then hybridized with 40 μl of FlexMAP microsphere mixture (Millipore Corporation, Billerice, MA) to create a total volume of 50 μl. Plates were mixed at 700 rpm for 1 minute. Samples were heated to 96ºC for 90 seconds to denature DNA, then samples were held at 37ºC for 60 minutes to allow for hybridization. Samples were placed in a centrifuge to pellet the beads and hybridized DNA. Supernatant was removed by turning the plate upside down, allowing the liquid to be absorbed by a

KimWipe (Kimberly-Clark, Dallas, TX). Each sample was washed with 75 µl of TX Hybridization buffer, centrifuged, and inverted on a KimWipe. This was repeated twice. The pelleted beads were resuspended in 40 µl of TM Hybridization buffer containing 2 µl/ml Streptavidin-R-phycoerythrin and incubated for 30 minutes at 37ºC. Plates were vortexed for 1 minute at 700 rpm. Each sample was analyzed using a Luminex 100 analyzer (Millipore Corporation, Billerice, MA).

Genetic distance among the six populations was calculated using GenAlEx 6.4 (Peakall and Smouse 2006). Genetic distances were used to conduct principal component analysis. The relationship between the six populations was calculated using Fst values generated by FSTAT 2.9.3 (Goudet 2002).

Results

Aphid numbers at both locations were low, never reaching the economic threshold of 250 aphids per plant (Ragsdale *et al.* 2007). Despite the low aphid population, there were significantly fewer aphids per plant on resistant soybean lines than on susceptible lines (Data not shown). In each location, the resistant lines had fewer aphids than susceptible soybean lines (Frankenmuth, $F=36.4$, $p<0.0001$; MSU, $F=17.7$, $p<0.0001$).

Principal component analysis (Figure 3.1) showed that the populations separated along both principal component one and two. The populations separated by location (East Lansing and Frankenmuth) along component one (47.78% of variation). Populations separated by line along component two (36.48% of variation).

According to Fst values (Table 3.1), there was a significant difference among populations by location. Populations from Frankenmuth and East Lansing were not similar. Within locations, there were significant differences in populations collected from different lines. Aphids collected from *Rag1* and *rag1b* and *rag3* lines were significantly different from each other at both locations. However, aphids collected from *rag1b* and *rag3* plants were similar to aphids from susceptible plants. In East Lansing, aphids from *Rag1* plants were not significantly different from susceptible plants. In Frankenmuth, aphids from *Rag1* plants were significantly different than aphids from both susceptible and *rag1b* and *rag3* aphid resistant plants.

Discussion

Using molecular markers, we are able to examine how aphid-resistance genes effect aphid populations. The aphids that are able to persist on these resistant lines represent a small but highly virulent subset of the entire aphid population. Our results indicate two distinctly different populations in the two locations. The aphids collected at East Lansing and those collected from Frankenmuth were significantly different. This goes against current thought about soybean aphid biology. The difference between populations collected at both locations is not consistent with past studies that found no correlation between genetic distance and geographical distance (Michel *et al.* 2009). We expected soybean line to be the primary factor in differentiating populations with no significant difference between aphids collected from both locations on the same soybean line. Not only did this not occur, but populations graphed to the opposite quadrants of the principal component analysis. This

signifies a large impact of geography on genetics of soybean aphid in this study. A possible explanation for this result is the low numbers of soybean aphid in Michigan soybean fields. In a year with heavy aphid pressure there is an increased production of winged aphids when aphid populations reach high densities (Ragsdale *et al.* 2004, Hodgson *et al.* 2005, Ragsdale *et al.* 2010). The movement of these alates from field to field would contribute to homogeneity of aphid populations. The low density of aphids in soybean fields leading to a smaller number of highly mobile alates could contribute to the differences in populations between the two locations.

The overlap between aphids collected on resistant and susceptible plants was expected; aphid biotypes that are virulent on resistant plants will also be virulent on susceptible plants. The significantly different populations of aphids on the two resistant lines suggest that selection is occurring. Because different resistant genes are present in LD16060 and E06902, the probability of an aphid being virulent to both sources of resistance is low.

This study has significant implications for the management of soybean aphid. One of the goals of implementing these genes is to prolong the efficacy of the resistance. Our findings indicate that there could be a spatial component to developing aphid biotypes. These results may only be valid in years when aphid numbers are low and aphid flights are reduced. In years with higher soybean aphid pressure and substantial flights, the effect of location may be lessened.

APPENDIX

Figure 3.1: Principal component analysis of soybean aphid populations collected from aphid-resistant and susceptible soybean lines in East Lansing and Frankenmuth MI, during August, 2010 using 7 SNP markers.

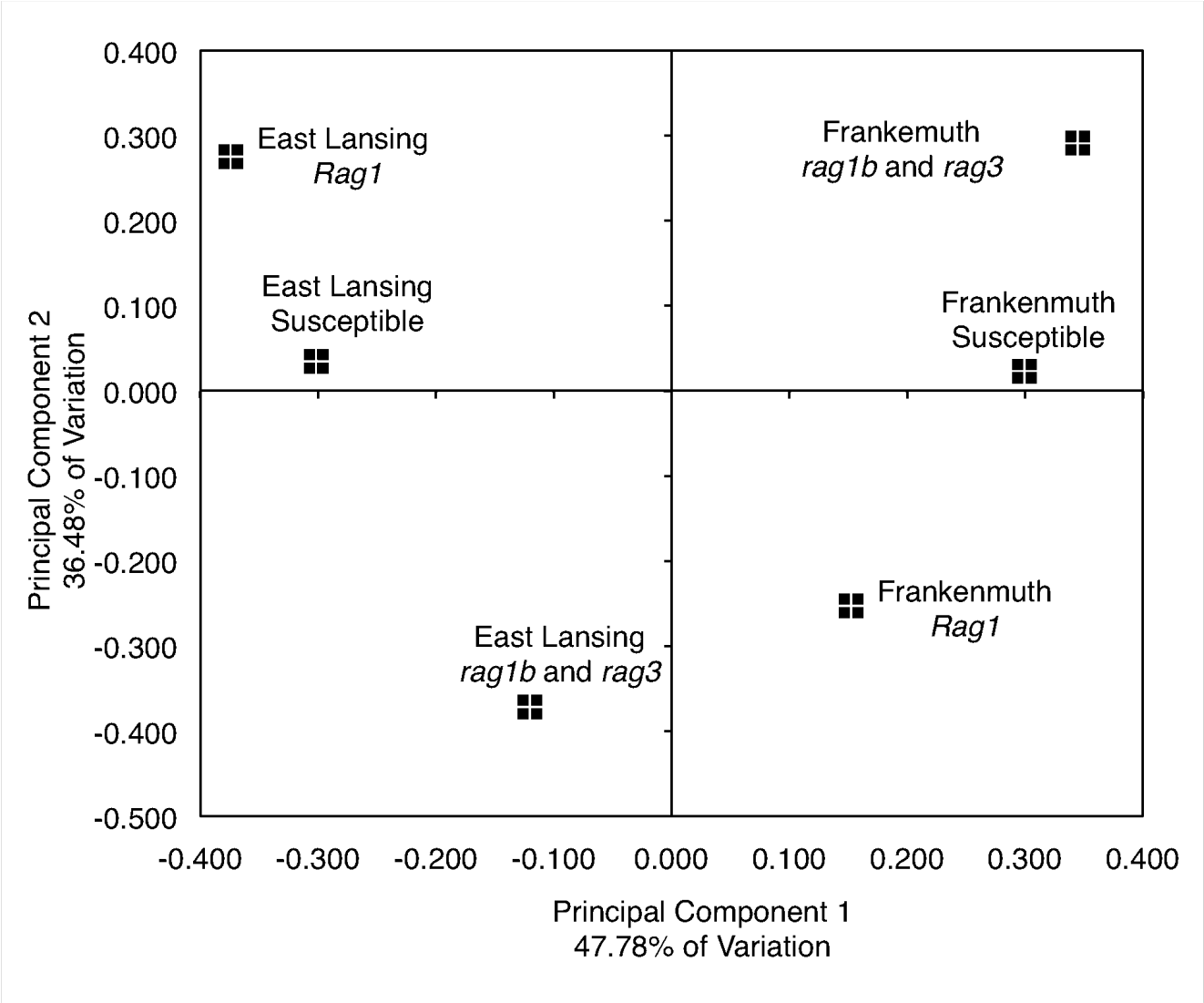

Table 3.1: Comparison of aphid populations collected from aphid-resistant and susceptible soybean lines in East Lansing and Frankenmuth MI in August 2010. Genetic distance (Fst values) between populations by FSTAT 2.9.3 (Goudet 2002) X's signify significant differences between populations using 7 SNP markers.

	Frankenmuth *Rag1*	Frankenmuth *rag1b* and *rag3*	East Lansing *rag1b* and *rag3*	East Lansing *Rag1*	East Lansing Susceptible
Frankenmuth *rag1b* and *rag3*	**X**	**NS**	**X**	**X**	**X**
Frankenmuth *Rag1*		**X**	**X**	**X**	**X**
Frankenmuth Susceptible			**X**	**X**	**X**
East Lansing *rag1b* and *rag3*				**X**	**NS**
East Lansing *Rag1*					**NS**

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