

EFFECTS OF SOYBEAN APHID RESISTANCE ON THE LADY BEETLE *HARMONIA*  
*AXYRIDIS* MEDIATED BY THE SOYBEAN APHID *APHIS GLYCINES*

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Title

EFFECTS OF SOYBEAN APHID RESISTANCE ON THE LADY BEETLE

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By

ANNE CHRISTINE THURN

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## ABSTRACT

Thurn, Anne Christine, MS, Department of Entomology, College of Agriculture, Food Systems, and Natural Resources, North Dakota State University, April 2010. Effects of Soybean Aphid Resistance on the Lady Beetle *Harmonia axyridis* Mediated by the Soybean Aphid *Aphis glycines*. Major Professors: Dr. Jason Harmon and Dr. Paul Ode.

Plants can directly and indirectly influence the natural enemies of their herbivores. Such trophic level effects apply to plants in natural and agronomic settings as well as to plants bred for pest resistance and biological control of herbivores. The effects of host plant resistance on herbivore natural enemies are highly variable, depending on the system. Currently, there is great interest in breeding soybean for resistance to the soybean aphid (*Aphis glycines* Matsumura). However, little is known about the effects of soybean aphid resistance traits on soybean aphid natural enemies. This study looks at the indirect effects of plant resistance to soybean aphid on the lady beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), an important natural enemy of the soybean aphid, using seven soybean varieties that exhibited variable resistance to the soybean aphid. Aphids were collected from greenhouse-reared soybean plants and fed to beetles in the laboratory. *Harmonia axyridis* larval development time, adult mass, and fecundity over a month were measured. Results indicated moderate and inconsistent indirect effects with highly resistant plant varieties while some moderately-resistant plant varieties reduced adult weight and egg production. My conclusion is that soybean aphid resistance is potentially compatible with *H. axyridis* biological control, but care must be taken when selecting varieties if there is more than one source of resistance or if plant resistance differentially affects soybean aphids and their natural enemies.

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# CHAPTER 1

## LITERATURE REVIEW

### Introduction

Price *et al.* (1980) stressed the important role of plants in plant-herbivore-natural enemy interactions. Plant effects on herbivores and natural enemies can be influenced by plant volatile cues, structure, epidermal characteristics (waxiness, trichome structure/density, etc.), plant interspecific interactions, and chemical composition (i.e., nutritional value to herbivores) (Bergman and Tingey 1979, Price *et al.* 1980). For example, an increase in the complexity of pea leaf structure reduced the foraging efficiency of the lady beetle *Coccinella septempunctata* (Linnaeus) searching for aphid prey (*Acyrtosiphon pisum* Harris) (Legrand and Barbosa 2003). The beetles spent more time searching the most complex plant leaves (highly branched; many small leaflets) before moving to a search new area in comparison to plants with the simpler four leaflets with tendril and seven leaflets without tendril pea lines (Legrand and Barbosa 2003). In this case, a change in the plant structure affected interactions with the natural enemies.

Herbivore quality, which is in large part influenced by host plant traits, often affects natural enemy fitness (Bottrell *et al.* 1998, Hare 2002). An example of this is found in Ode *et al.* (2004). The presence of high furanocoumarins (specifically xanthotoxin), a known plant chemical defense, in three host plants (wild parsnip *Pastinaca sativa* Linnaeus, hogweed *Heracleum sphondylium* Linnaeus, and giant hogweed *Heracleum mantegazzianum* Sommier & Levier) of parsnip webworm (*Depressaria pastinacella* Duponchel) reduced survivorship of the polyembryonic parasitoid wasp *Copidosoma*

*sosares* (Walker) (Ode *et al.* 2004). Furanocoumarins were able to pass unmetabolized into the hemolymph of the herbivores to affect the natural enemies. There are many more examples concerning multi-trophic interactions (see reviews Price *et al.* 1980, Kagata and Ohgushi 2006, Ode 2006); however, there are some interactions that are yet poorly understood. One example is the interaction of a predator, the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an herbivore, the soybean aphid *Aphis glycines* (Matsumura) (Hemiptera: Aphididae), and soybean aphid-resistant soybean plants (*Glycine max* Linnaeus).

Biological control, chemical control, and host plant resistance have all been used to control the soybean aphid, *A. glycines* (Heimpel *et al.* 2004, Hill *et al.* 2004a,b, McCornack *et al.* 2004, Ragsdale *et al.* 2004, Fox *et al.* 2005, Hesler and Dashiell 2007, Ragsdale *et al.* 2007). As is true in many integrated pest management systems, combinations of control approaches show the greatest promise in terms of effective long-term pest control. There are many promising soybean cultivars showing high resistance to *A. glycines*, such as Dowling, Jackson, and PI 567543C, although not all are appropriate in every growing region (Hill *et al.* 2004b, Mensah *et al.* 2005, Hesler and Dashiell 2007, USDA ARS 2009). A large multi-state study established economic threshold levels for soybean aphid management and guidelines for chemical treatment (Ragsdale *et al.* 2007). Scouting for biological control is important because natural enemies can reduce the aphid population to below-threshold levels (Ragsdale *et al.* 2007).

The multicolored Asian lady beetle, *H. axyridis*, is one of the more prominent biological control agents in soybean. This aphidophagous predator has been seen in soybean fields in sufficient numbers to cause or contribute to control of soybean aphid

populations both in the United States and in its native Asia (Han 1997, Fox and Landis 2001, Koch 2003, Landis *et al.* 2004, Fox *et al.* 2005, Nielsen and Hajek 2005). If soybean resistant plants were used along with biological control, some aphid populations could potentially be kept below threshold levels without growers needing to use insecticides.

However, the compatibility of soybean aphid resistance in soybean with biological control by *H. axyridis* is not well understood. The mechanism conferring soybean aphid resistance is still being studied (see Diaz-Montano 2007) and, depending on how host plant resistance traits interact with the herbivore, a natural enemy may or may not be harmed.

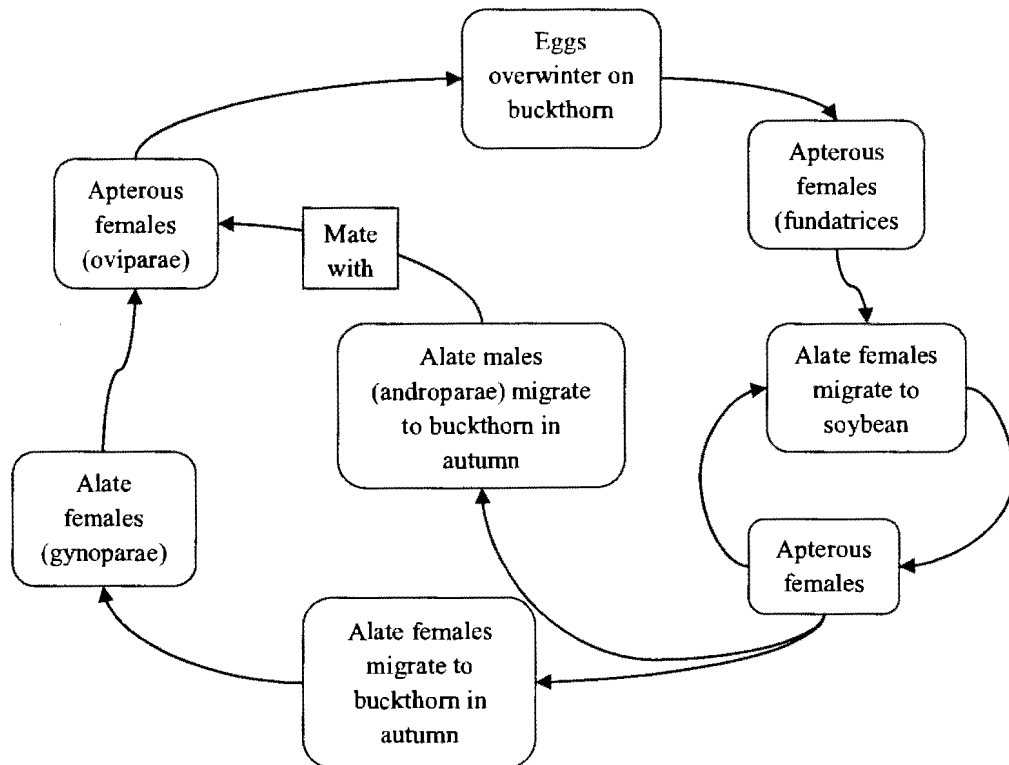
### Soybean

Soybean is an important agricultural plant (Hill *et al.* 2004a, Wu *et al.* 2004). It has been cultivated in Asia for thousands of years and is grown worldwide for its oil and protein, which are used in industry, medicine, and animal and human consumption (Chinese Ministry of Agriculture 2001, Wu *et al.* 2004). In 2007, the U.S. produced approximately 2.59 billion bushels of soybean valued at \$26.8 billion (USDA 2007). However, yields can be severely reduced if stresses (e.g., water, drought, insects, plant pathogens) occur during early reproductive stages (Fehr and Caviness 1977). In 2000, the soybean aphid arrived in the U.S. and severely affected soybean production and yields (Ragsdale *et al.* 2004, Voegtlin *et al.* 2004).

### Soybean Aphid

The soybean aphid is a heteroecious holocyclic species that is a major pest of the cultivated soybean (Wang *et al.* 1962, Wang *et al.* 1991, Wu *et al.* 2004). During its life cycle (Figure 1) it alternates host plants and between parthenogenesis and sexual

Figure 1. The heteroecious holocyclic life cycle of soybean aphid.



reproduction (Dixon 1998). In autumn, male and female aphids mate and lay eggs that will overwinter on leaf buds of buckthorn (*Rhamnus* spp.), which are the primary host plants (Wang *et al.* 1962, Wang *et al.* 1998, Ragsdale *et al.* 2004, Voegtlin *et al.* 2004, Wu *et al.* 2004). In the following spring, the eggs hatch into apterous females (fundatrices), which then give birth via parthenogenesis to alate females (Wang *et al.* 1962, Wang *et al.* 1998,

Ragsdale *et al.* 2004, Wu *et al.* 2004). These winged females migrate to soybean (i.e., *G. max* and also wild soybean, *Glycines soja* in Asia), the secondary host, to produce apterous females by parthenogenesis (Wang *et al.* 1962, Wang *et al.* 1998, Ragsdale *et al.* 2004, Venette and Ragsdale 2004, Wu *et al.* 2004). The aphids are attracted to soybean via volatile chemicals (Du *et al.* 1994) and reproduce rapidly on new leaves, stems, apexes, flower buds, and pods of the soybean plant, although older parts of the plant may be fed on as the population density increases (Wang *et al.* 1962, Ragsdale *et al.* 2004, Wu *et al.* 2004). Throughout the summer, alate females are produced as a result of overcrowding and may disperse to establish new colonies of apterous females on uninfested plants (Wang *et al.* 1962, Lu and Chen 1993, Wang *et al.* 1998, Ragsdale *et al.* 2004, Wu *et al.* 2004). As autumn approaches and temperatures decline, alate females (i.e., gynoparae) leave soybeans to find buckthorn hosts. Here they give birth to apterous females (i.e., oviparae) (Wang *et al.* 1962, Wang *et al.* 1998, Ragsdale *et al.* 2004, Voegtlin *et al.* 2004, Wu *et al.* 2004). These new females will mate with alate males (i.e., androparae; produced by apterous females) arriving from soybean hosts and lay eggs (Wang *et al.* 1962, Wang *et al.* 1998, Glogoza 2004, Ragsdale *et al.* 2004, Voegtlin *et al.* 2004, Wu *et al.* 2004).

Soybean aphids have flexible temperature-dependant reproductive rates. Successful aphid development and reproduction can occur when ambient temperatures are between 20 and 30°C with optimal growth and reproduction in the laboratory having been observed to occur at 27°C [McCornack *et al.* 2004]). In Minnesota, late summer temperatures range from 19-35°C is also when the densest aphid populations can be found in soybean fields (McCornack *et al.* 2004).

The first observation of soybean aphid in North America occurred in Wisconsin in 2000 (Ragsdale *et al.* 2004, Voegtlin *et al.* 2004), and by 2004 the insect had spread throughout the north central United States (Ragsdale *et al.* 2004). Facilitating the persistence and spread of the soybean aphid is the widespread distribution of invasive buckthorn (i.e., *Rhamnus cathartica* Linnaeus) and native buckthorn (e.g., *R. alnifolia* L'Héritier) species throughout North America (Wang *et al.* 1962, Takahashi *et al.* 1993, Ragsdale *et al.* 2004, Voegtlin *et al.* 2004). *Rhamnus* is the primary host plant genus in the soybean aphid's native range in Asia. Also, because of its small size, the soybean aphid is easily carried by wind. It is thought that this is the primary means of long-range dispersal for soybean aphid (Venette and Ragsdale 2004). Soybean aphid has the potential to spread wherever soybean is grown in North America because of the climatic similarity between its native Asia and where soybean is grown in North America (Venette and Ragsdale 2004).

Soybean aphid damages soybean in several ways. Their piercing-sucking, phloem feeding habit results in curling and wilting of leaves, stunted plant growth, virus (e.g., alfalfa mosaic virus) transmission, and even plant death (Hill *et al.* 2004, Wu *et al.* 2004, Wang *et al.* 1998). Aphids also secrete honeydew, which even in low aphid densities, creates an ideal environment for sooty mold growth that inhibits photosynthesis (Wu *et al.* 2004). Aphids pierce the undersides of leaves, apices of buds, pods, and even stems as plants mature (Wu *et al.* 2004). The feeding location is often determined by age of the plant (Wang *et al.* 1962, Hirano 1996, Wu *et al.* 2004). The results of this damage can include reduced pod numbers and lower seed quality. Dai and Fan (1991) reported up to 30% yield loss in field experiments in China. Sun *et al.* (2000) reported 30% average yield losses for Wangkui County, Heilongjiang Province during a severe outbreak of soybean

aphid in China; 6,000 ha out of 40,000 ha soybean fields experienced densities in excess of 3,000 aphids per plant and 100% yield loss. Projections of up to 50% loss in yield have been predicted if no measures are taken to control aphids during an outbreak year (Wang *et al.* 1962). In Minnesota, field experiments showed an average 45% yield loss in untreated plots versus sprayed plots (Ostlie 2002).

### Soybean Aphid Control

Methods for controlling soybean aphid include chemical, biological, and cultural control as well as breeding for resistant plants (e.g., Wang *et al.* 1962, Chung *et al.* 1980, Wang and Ba 1998, Li *et al.* 2000, Sun *et al.* 2000, Hill *et al.* 2004a,b, Ragsdale *et al.* 2004, Wu *et al.* 2004, Fox *et al.* 2005, Ragsdale *et al.* 2007). Cultural control tactics such as alternative planting dates and plant or row spacing are commonly suggested (e.g., Chung *et al.* 1980). Ragsdale *et al.* (2004) suggested that late planted soybeans (and therefore late plant senescence) are the source of gynoparae and andoparae for overwintering soybean aphid. Thus, planting earlier might reduce the aphid populations for the next year (Ragsdale *et al.* 2004). Wang and Ba (1998) determined that planting soybean with maize decreased aphid populations and increased natural enemy populations. Planting soybean with alfalfa also increased natural enemy populations and delayed aphid establishment (Schmidt *et al.* 2007). This kept soybean aphid populations under economic threshold levels though most of the growing season (Schmidt *et al.* 2007). By decreasing the aphid populations through cultural practices, natural enemies were often better able to control the remaining population.

Another important factor in the control of *A. glycines* is the presence of natural enemies. Thirty species of predators, 15 parasitoids, and one pathogen of *A. glycines* have been documented in China and South Korea (Wu *et al.* 2004). Since this exotic aphid has very few of its native natural enemies in its introduced environment in North America – *C. septempunctata* and *H. axyridis* being two exceptions – it can often reproduce unchecked, resulting in considerable damage (Wu *et al.* 2004). However, in the U.S., generalist predators, including carabid beetles (*Elaphorpus ancep* Le Conte, *Clavina impressifrons* Le Conte, *Bembidion quadrimaculatum* Say), spiders, coccinellids (*H. axyridis* and *C. septempunctata*), and minute pirate bug (*Orius insidiosus* Say), can be significant factors in controlling soybean aphid in soybean fields (Fox *et al.* 2005). Carabids appeared to control aphids more effectively early in the season, whereas coccinellids tended to provide more effective mid-season control.

Chemical pesticides are the predominant means of controlling soybean aphid (Wang *et al.* 1962, Li *et al.* 2000, Sun *et al.* 2000, Ostlie 2002) and can be very effective at reducing population levels (Wang *et al.* 1962, Ostlie 2002, Ragsdale *et al.* 2007). With proper monitoring of soybean aphid populations, chemical control only needs to be used on a population that is likely to exceed a density of 250 aphids per plant, the current economic threshold level, to protect crop yields (Ragsdale *et al.* 2007). Growers have a variety of chemicals from which to choose, including pyrethroids (e.g., Warrior, Asana XL, Mustang, and Baythroid), organophosphates (e.g., Dimethoate, Larsban 4E, PennCap M), and carbamates (e.g., Furadan 4F) (Ostlie 2002, Glogoza 2004, Easley and Hammond 2007).

Unfortunately, most insecticides are toxic to natural enemies (Lou 1983, Wang and Ba 1998, Wu *et al.* 1999, Sun *et al.* 2000). Careful monitoring of pest and natural enemy



populations are required because spraying too early or too often can decrease natural enemy populations, essentially releasing any surviving pests from natural biological control (Sun *et al.* 2000, Heimpel *et al.* 2004, Ragsdale *et al.* 2007). James (2004) demonstrated this with *H. axyridis* larvae and buprofezin, a chitin synthesis inhibitor used in grape IPM programs in Washington. Larvae of all stages were collected, sprayed with the recommended dosage of buprofezin for grape pests (specifically leafhoppers), and then monitored for development (James 2004). Most *H. axyridis* larvae were unable to complete development and died because ecdysis was either incomplete or inhibited (James 2004). Natural enemies of soybean aphid (e.g., coccinellids, parasitoids) are also likely affected by broad spectrum insecticides, which is the reason for careful monitoring of soybean fields (Sun *et al.* 2000, Heimpel *et al.* 2004, Ragsdale *et al.* 2007).

### Soybean Resistance to Aphids

The use of aphid-resistant soybean cultivars is another tactic to control soybean aphid. Breeding for plant resistance is becoming increasingly popular as the cost of chemical control increases and there is increased risk that soybean aphid may become resistant to chemical control as have other aphids (ffrench-Constant *et al.* 2004). Unfortunately, very little is known concerning the compatibility of soybean resistance and biological control. If they are compatible, together they would help keep aphid populations even lower, further reducing the need for chemical control, and reducing the likelihood of aphids becoming resistant to resistant soybean cultivars.

Differential resistance to soybean aphids has been detected among cultivars of soybean and lines of the wild soybean *G. soja* (Sun *et al.* 1991, Yue *et al.* 1989, He *et al.*

1995, Fan 1988, Wu *et al.* 2004, Hill *et al.* 2004a, Hill *et al.* 2004b, Mensah *et al.* 2005, Mian *et al.* 2008a). Resistance-associated genes from cultivars have been identified and are being introduced into commercial soybean varieties (Wu *et al.* 2004, Hill *et al.* 2004a, Hill *et al.* 2004b, Mensah *et al.* 2005, Mian *et al.* 2008a, University of Illinois Extension 2009, Zhang *et al.* 2010). Fan (1988) studied several soybean varieties over three years and found that some plants had resistance to soybean aphid. However, the degree of resistance was inconsistent in years with severe infestations (Fan 1988). In more recent surveys, Hill *et al.* (2004a) surveyed 798 (in 2001) and 644 (in 2002) different commercial soybean cultivars available in the United States for aphid resistance. They also surveyed 87 varieties that were ancestors or first progeny of North American cultivars. Of the cultivars surveyed, only five varieties of soybean were highly resistant to *A. glycines*: Palmetto, ‘CNS’, PI 71506, Dowling, and Jackson. These varieties all had aphid indices of <3 (Table 1). Other varieties in the same survey of 87 varieties were also found to exhibit

Table 1: Aphid index calculation for determining level of soybean aphid resistance (Hill *et al.* 2004a,b)

aphid index (0-9)	=	aphid density (0-3)	x	aphid damage (0-3)
0-3 = high resistance		0 = no aphids		0 = no damage
4-6 = moderate resistance		1 = low aphid population		1 = mild leaf damage
7-9 = no resistance		2 = moderate aphid population		2 = moderate leaf damage
		3 = high aphid population		3 = severe damage (including plant death)

reduced resistance (aphid indices of <5) consisting of light damage with moderate aphid populations: Tracy, Vansoy, Wye, Emerald, Verde, Curtis, Mejiro, Peking, and Bansei.

Several genes have been identified in resistant soybean as a source of soybean aphid resistance. Dowling has a single gene, *Rag1* (Hill *et al.* 2006a). Jackson also has a single gene at the same loci, named *Rag* (Hill *et al.* 2006b, Kim *et al.* 2008). *Rag2* is the source of resistance in PI 243540 (Mian *et al.* 2008b), and *Rag3* is thought to cause resistance in PI 567543C (Zhang *et al.* 2010). The interaction of a pair of genes (*rag1*\_provisional and *rag4*) confers soybean aphid resistance in PI 567541B (Zhang *et al.* 2009). All these genes cause different types of resistance interactions between soybean plant and soybean aphid (Kim *et al.* 2008, Zhang *et al.* 2010). The mechanism of soybean aphid resistance is not well understood. Further testing by Hill *et al.* 2004a indicated that the varieties ‘CNS’ and PI 71506 were repellent to soybean aphid and that varieties Dowling, Jackson, and Palmetto had negative effects on fecundity and survival of soybean aphids. Because their aphids did not show signs of complete starvation, Li *et al.* (2004) suggested that either inadequate nutrition or a toxic compound in the resistant leaves interferes with aphid metabolism. Aphids raised on Dowling, PI200538, and Jackson showed reduced fecundity and longevity, along with the highest mortality in the nymphal stage, compared with a susceptible variety Pana (Li *et al.* 2004). Chemical interference was also found in the interaction between soybean and the velvet bean caterpillar *Anticarsia gemmatilis* Hübner. Although *A. gemmatilis* has a different feeding pattern than aphids (chewing vs. piercing/sucking), soybean resistance to *A. gemmatilis* was caused by flavonoids adversely affecting the caterpillar’s weight, development time, and survivorship (Piubelli *et al.* 2005).

Plants also directly influence aphid populations through their nutritional value to the aphid. Soybean plants with a potassium deficiency had higher populations of soybean aphid than plants with higher potassium levels (Myers *et al.* 2005, Myers and Gratton 2006,

Walter and DiFonzo 2007). Potassium is involved in amino acid production, and a reduction in potassium would cause the amino acids, particularly a nitrogen storage molecule (asparagine), to accumulate in phloem. The more nitrogen available to aphids in the phloem, the faster they grow and reproduce (Walter and DiFonzo 2007). Conversely, inadequate nutrition was seen in aphids reared on soybean with high soybean aphid resistance (Hu *et al.* 1992, 1993). This was a result of higher lignin levels and lower nitrogen content in young leaves from high resistant plants than susceptible plants (Hu *et al.* 1992, 1993).

Insight into soybean aphid resistance comes from other plant-aphid systems. Expression of a gene in tomatoes caused interference with ingestion of phloem during probing of sieve elements by the potato aphid (*Macrosiphum euphorbiae* Thomas) (Kaloshian *et al.* 2000). Aphids were able to find and penetrate sieve elements in resistant and susceptible tomatoes in a similar amount of time, but aphids probing resistant plants were quickly forced to withdraw from sieve elements (Kaloshian *et al.* 2000). The authors suggested that the mechanism of resistance is not due to plant chemistry or physical barriers, but rather a prevention of ingestion or digestion of phloem. Resistance in melon (*Cucumis melo* Linnaeus) to the cotton aphid (*Aphis gossypii* Glover) appears to exhibit a similar mechanism (Klinger *et al.* 1998). A comparison of the life history and life table traits as well as the electrical penetration graph (EPG) waveforms of *A. gossypii* feeding on resistant (AR 5) and susceptible (PMR 5) melon (*C. melo*) plants showed that all aphids could puncture the plant's sieve elements (Klingler *et al.* 1998). However, aphids feeding on resistant melons took longer to salivate and spent a shorter time ingesting phloem than those feeding on susceptible plants (Klingler *et al.* 1998) thus suggesting that aphids are

rejecting phloem or are reacting to the plant's defensive mechanism of sealing off the penetrated sieve elements.

It is possible that soybean resistance to soybean aphid could be caused by a single complex mechanism or the interaction of several mechanisms. Diaz-Montano *et al.* (2007) tested four resistant soybean varieties (i.e., K1639, Pioneer 95B97, Dowling, and Jackson) and one susceptible (KS4202) for *A. glycines* feeding behavior using EPG. They found that aphids on resistant plants took twice as long to reach sieve elements than the aphids reared on susceptible plants and they spent only a few minutes digesting phloem compared to an hour or more for aphids on susceptible plants. Klingler *et al.* (1998 and 2000) produced similar results. However, Diaz-Montano *et al.* (2007) also suggested that xylem in resistant soybean may contain substances that affect *A. glycines* by either preventing sieve element penetration or by being toxic. The time aphids spent ingesting xylem was similar on both resistant and susceptible varieties (Diaz-Montano *et al.* 2007). It is likely many factors contribute to soybean aphid resistance in soybean, and the level of resistance may depend on soybean lines of ancestry or genes (Diaz-Montano *et al.* 2006, Hesler and Dashiell 2007, Kim *et al.* 2008) as well as plant composition (Hu *et al.* 1992, 1993, Walter and Difonzo 2007).

### Tritrophic Interactions with Resistant Soybean

Attempts at combining soybean pest resistance and biological control have produced mixed results. Emergence rate and fecundity of the parasitoid *Telenomus chloropus* Thomson were adversely affected when developing in stink bug eggs (*Nezara viridula* Linnaeus) laid by adults reared on stink-bug resistant (PI 171444) verses

susceptible soybean ('Davis') (Orr *et al.* 1985). The authors suggested a decrease in nutritional quality of the soybean pods fed to *N. viridula* caused the decrease in successful *T. chloropus* emergence even though its development time was not different between the varieties. However, development time was significantly greater for a parasitoid, *Copidosoma truncatellum* Dalman, of soybean looper (*Pseudoplusia includens* Walker) reared on resistant soybean (PI 227687) when compared to soybean looper reared on susceptible ('Davis') soybean (Orr and Boethel 1985). This is important since soybean looper resistance causes the greatest mortality in soybean looper larvae (Orr and Boethel 1985). Interrupted host development may adversely affect (*C. truncatellum*) populations that develop throughout the host larval period (Orr and Boethel 1985). *Pediobius foveolatus* Crawford, a parasitoid of Mexican bean beetle, showed decreased reproduction and survival and increased development time when reared in Mexican Bean Beetles (*Epilachna varivestis* Mulsant) fed a resistant soybean cultivar (Culter 71) than on soybeans 'Bonus' and 'Williams' and Lima Bean 'Henderson Bush' (susceptible varieties)(Kauffman and Flanders 1985). Even though *P. foveolatus* was adversely affected, the authors considered this biological control agent and soybean resistance to be compatible. Together they caused the greatest decrease in Mexican bean beetle populations, and *P. foveolatus* needed to be imported every year anyway (Kauffman and Flanders 1985). While all of these examples indicated negative effects of plant traits on the natural enemy, compatibility between the two control tactics was often dependant on how much more the pest population decreased and how many natural enemies were able to survive to consume more pests.

## Multicolored Asian Lady Beetle

Typical of predaceous coccinellids, *H. axyridis* can utilize a wide range of prey (Hodek and Honěk 1996). A generalist, but mostly aphidophagous predator it has been observed attacking a variety of soft-bodied insect species including aphids, scales, lepidopteran eggs and even other lady beetle species (Koch 2003, Koch *et al.* 2003, Snyder *et al.* 2004, Kajita *et al.* 2000, Sato *et al.* 2003, LaMana and Miller 1996, Coderre *et al.* 1995). In autumn, it also feeds on grapes, apples and other ripe or bruised fruit (Kovach 2004).

The multicolored Asian lady beetle plays an important role in suppressing a variety of crops pests (Landis *et al.* 2004, Colunga-Garcia and Gage 1998), and it is an important predator of *A. glycines* in Asia and North America (Koch 2003, Fox and Landis 2001, Landis *et al.* 2004, Fox *et al.* 2005). On apple trees, *H. axyridis* was the most important predator in biological control of spirea aphid, *Aphis spiraecola* Patch (Brown 2004), and in greenhouse roses, it complemented biological control by the parasitoid *Aphelinus asychis* Walker of the aphid species *M. euphorbiae* (Snyder *et al.* 2004). In Asia, it is listed as a common predator of *A. glycines* (Wang *et al.* 1998, Sun *et al.* 2000, Han 1997). Other natural enemies of *A. glycines* in Asia include approximately 43 other insect predators and parasitoids and one pathogen (Wu *et al.* 2004). *Harmonia axyridis* accounted for 29.6% (third-most dominant) of all natural enemies present during soybean vegetative and blooming stages in Henan province in China (Han 1997). In New York, coccinellids, specifically *H. axyridis*, were considered to be the most abundant predators of soybean aphid (Nielsen and Hajek 2005).

*Harmonia axyridis* is native to eastern Asia (Siberia, Manchuria, China, Formosa, Korea, Japan, Ryuku Islands, and Bonin Islands) (Chapin and Brou 1991). It has been released multiple times in the United States over the past 70 years as a biological control agent (Gordon 1985) for black pecan aphid (*Melanocallis caryaefoliae* Davis), blackmargined aphid (*Monellia caryella* Fitch), and yellow pecan aphid (*Monelliopsis pecanis* Bissell) on pecans (Teddars and Schaefer 1994, Dreistadt *et al.* 1995, Kidd *et al.* 1995), and is believed to have become established from a combination of accidental introductions at ports (Day *et al.* 1994) and intentional releases (Coderre *et al.* 1995, Colunga-Garcia and Gage 1998, Hesler *et al.* 2001, LaMana and Miller 1996, Tedders and Schaefer 1994). Releases occurred in California (1916, 1964, 1965, 1993), Washington (1978-1982), Nova Scotia (1981), and during 1978-1981 in Connecticut, Georgia, Louisiana, Maryland, Washington DC, Delaware, Maine, Mississippi, Ohio, and Pennsylvania (Gordon 1985, Dreistadt *et al.* 1995).

There is considerable evidence suggesting that *H. axyridis* has spread successfully from several initial points of introduction. Chapin and Brou (1991) reported that the first post-introduction detection of *H. axyridis* was in Louisiana in 1988 and then in Mississippi in 1990. Light traps, which had been continuously monitored since 1982, did not capture any specimens of *H. axyridis* prior to 1988. Day *et al.* (1994) suggested that because of the length of time and long distances between release and capture, the presence of this lady beetle in Louisiana and Mississippi is due to accidental introduction at the port of New Orleans in 1988. Whatever the method of introduction, *H. axyridis* has since spread throughout most of the lower 48 U.S. states (Alabama, Georgia, Florida, South Carolina [Teddars and Schaefer 1994], North Carolina, Virginia [Kidd *et al.* 1995], Washington,



California [Dreistadt *et al.* 1995], Oregon [Dreistadt *et al.* 1995, LaMana and Miller 1996], Michigan [Colunga-Garcia and Gage 1998], North Dakota [Fauske *et al.* 2003], South Dakota [Hesler *et al.* 2001], and Minnesota [Fauske *et al.* 2003, Hesler *et al.* 2001]), into Canada (Coderre *et al.* 1995), with the potential of invading much of South America as well (Koch *et al.* 2006). Montana and Wyoming are two states that do not have known populations of *H. axyridis*, in addition to some portions of the southwestern U.S. (Koch 2003). In many of the areas with established *H. axyridis* populations, the beetles were found on apple, poplar, alfalfa, soybean, corn, winter wheat, and a variety of other plants (Coderre *et al.* 1995, Colunga-Garcia and Gage 1998, Hesler *et al.* 2001, LaMana and Miller 1996) that are susceptible to aphids and other soft-bodied insect pests.

In its native east Asia, *H. axyridis* has a wide range of color variations, ranging from an orange or red elytra background with zero to many black spots (non-melanic) to a black elytra background with no to many red spots (melanic) (Chapin and Brou 1991). In North America, *H. axyridis* consists of the non-melanic morphs (Teddars and Schaefer 1994) with an exception in Oregon where melanic morphs are found very rarely (LaMana and Miller 1996). All individuals have black spots on the pronotum arranged in an M or W pattern, depending on orientation, against a white background (Chapin and Brou 1991). Chapin and Brou (1991) give a full description of the species with a key to amend Gordon's Coccinellidae of America North of Mexico (1985).

When temperatures rise in spring, *H. axyridis* emerge from overwintering sites, typically cracks and crevices of large rocks and buildings, and fly in search of prey (Hodek *et al.* 1993, Koch 2003, Nalepa *et al.* 2004). Females mate repeatedly with males and will lay clusters of approximately 20-30 oval-shaped eggs (Hodek and Honěk 1996, Koch

2003). Eggs are initially yellow and turn darker with age, becoming grey approximately one day prior to hatching (Hodek and Honěk 1996, Koch 2003). First-instar larvae spend their first day resting on their egg shells, often eating any nonviable eggs near them (Hodek and Honěk 1996, Koch 2003). Before fourth instars become pupae, they undergo a period of rest, called the pre-pupal period, where they do not eat and will not move unless disturbed (Hodek and Honěk 1996, personal observation). At the end of the pre-pupal period, the larva attaches to a substrate, curls, and sheds its larval exoskeleton to reveal an exposed pupa (Hodek and Honěk 1996, Koch 2003). Upon adult eclosion, adults emerge with a pale yellowish color, hang onto the pupal exuviae, and expand their elytra and hind wings until they harden (Hodek and Honěk 1996). Elytra color slowly develops with spots appearing within hours and background color continuously darkening over several weeks (Hodek and Honěk 1996).

The ability of predators to easily locate and consume large amounts of aphid prey is an important cause of aphid population crashes (Landis *et al.* 2004, Fox *et al.* 2005). Adult *H. axyridis* are able to consume higher numbers of aphids than adults of other lady beetles (Rongcai *et al.* 1994). The larval stages of *H. axyridis* can consume approximately 90 to 370 total aphids, depending on species (Koch 2003). It has been reported to consume 100-200 aphids per day in the field (Han 1997), however, lady beetle adult and larval stages were combined and other soybean aphid predators were present. In their study, Harmon *et al.* (1998) suggest that visual and chemical cues play a role in adult predation behavior at very small spatial scales, whereas larvae tend to use random movements, positive phototaxis and negative geotaxis to search for prey (Koch 2003). Both stages tend to outperform native species in prey location (Pervez and Omkar 2006).

## Influence of Prey on the Asian Lady Beetle

Duration of each stadium depends greatly on temperature and diet quality and quantity (Hodek and Honěk 1996, Koch 2003). Temperature affects respiration rate, which in turn regulates development rate (Acar *et al.* 2004). They develop in a temperature range of 18 – 30°C, with optimal temperature at 22°C (LaMana and Miller 1998). Higher temperatures decrease the length of each instar and thus, decrease adult weight, whereas, low temperatures increase instar duration and increase adult weight (LaMana and Miller 1998, Rongcai *et al.* 1994, Koch 2003). When reared at 24.6°C (average) and fed the green peach aphid (*Myzus persicae* Sulzer) reared on Chinese cabbage [*Brassica rapa* Linnaeus]), larval development time from 1<sup>st</sup> to 4<sup>th</sup> instar was 12 – 14 days (Teddars and Schaefer 1994). The same larval instars developed in  $10.2 \pm 1.0$  days at 26°C on pea aphid *A. pisum* reared on fava bean (*Vicia fabae* Linnaeus) (LaMana and Miller 1998). Ueno (2003) showed a larval development time of  $13.8 \pm 0.5$  days for males and  $13.1 \pm 0.3$  for females when raised at 25 °C on the same aphid, *A. pisum*, reared on bean plant (plant species not specified). While LaMana and Miller (1998) showed *H. axyridis* with the highest temperature and shortest development time, and Tedders and Schaefer (1994) showed *H. axyridis* with potentially the lowest temperature and longest development time, the differences between the results of Tedders and Schaefer (1994) and Ueno (2003) could be explained by differences in aphid species for prey.

The fact that generalist predators readily eat a wide range of prey does not indicate all prey species are equally beneficial to the predator. Prey species differ in their development time (Hodek and Honěk 1996, Koch 2003, Pervez and Omkar 2006). Suboptimal (i.e., marginal) prey are consumed as a maintenance (survival) tactic that

prolongs adult life in the short term but it can have long-term consequences (Michaud 2005). When reared on the aphid *A. pisum*, a favorable prey species, *H. axyridis* had a shorter development time and larger pupal mass than when reared on the comparatively poor-resource species *Aphis craccivora* Koch or artificial diet of honeybee larvae (Ueno 2003). Also, when given a choice, *H. axyridis* readily consumed both *Paraprociophilus tessellatus* Fitch and the hemlock wooly adelgid *Adelges tsugae* Annand even though it was unable to complete its life cycle on *A. tsugae* (Butin *et al.* 2004). Michaud (2000) studied seven common coccinellid species that control green citrus aphid (*Aphis spiraecola* Patch) and the newly introduced brown citrus aphid (*Toxoptera citricida* Kirkaldy), which vector citrus tristeza virus. *H. axyridis* developed faster and weighed more when raised on *T. citricida* (Michaud 2000).

Presence and consumption of prey species also had consequences on reproductive performance. *Harmonia axyridis* will eat suboptimal prey for survival but tend to limit egg laying when optimal prey are scarce. In alfalfa fields, *H. axyridis* laid the most eggs when they consumed pea aphids, smaller numbers of eggs on a combination of alfalfa weevil larvae and sugar water, and no eggs with weevil larvae or sugar water alone (Evans and Gunther 2005). There was no ovipositing *H. axyridis* when in the presence of *A. spiraecola* and  $15 \pm 10$  eggs per day with *T. citricida* (Michaud 2000). Similarly, the ladybeetle *Adalia bipunctata* Linnaeus readily laid eggs in the presence of suitable and moderately suitable aphids (*A. pisum* and *Aphis fabae*, respectively) but deposited fewer eggs in the presence of aphids known to be toxic (*Megoura viciae*) to *A. bipunctata* (Fréchette *et al.* 2006). Since *A. bipunctata* still oviposited in the presence of toxic aphids, Fréchette *et al.* (2006) reasoned that there was local population adaptation to these species

of aphids. Overall, they concluded that their results were due to scarcity of suitable prey or the presence of aphid or aphid honeydew as an oviposition cue (Fréchette *et al.* 2006). Chemicals and presence of honeydew apparently provides oviposition cues for the lady beetle *C. septempunctata* (Evans and Dixon 1986). The beetles laid eggs in the presence of the pea aphid *A. pisum* and in vials previously containing *A. pisum*, but few or no eggs were laid in clean vials (Evans and Dixon 1986). The beetles' normal oviposition rates resumed a few hours later when aphids were replaced (Evans and Dixon 1986). Ladybeetles might be able to detect suitable prey through chemical cues given by prey, possibly through honeydew, and if so, females could be choosy about where they lay eggs.

Like other lady beetles, *H. axyridis* can fly to plants with heavy aphid infestation, lay eggs there if aphid levels are high enough, and then move on to lay more eggs leaving larvae to feed on the aphid population (Osawa 2000, Fox and Landis 2001, Landis *et al.* 2004, Koch 2003, Pervez and Omkar 2006). Female lady beetles can determine the age of aphid populations through semiochemicals and therefore assess if the aphid population will be able to support the development of her offspring (Koch 2003). Since larvae are restricted to their substrate surface (i.e., they cannot fly to other prey), the female has an impact on the development of her offspring by choosing to lay her eggs in the presence of a particular prey species (Hodek and Honěk 1996).

### Tritrophic Interactions Involving Lady Beetles

Lady beetles encounter a wide range of prey (Hodek and Honěk 1996) but not all prey are equal in terms of beetle development (Hodek and Honěk 1996, Koch 2003, Michaud 2005) due to the influence of plant traits. The same principle can be applied to

the plants on which herbivores (i.e., aphids) feed. In a study by Francis *et al.* (2001), peach-potato aphid, *M. persicae*, was stimulated to feed when in the presence of the brassicaceous plants containing glucosinolates. The lady beetle *A. bipunctata*, when fed aphids that fed on plants containing high levels of glucosinolates, had a shorter development time, larger body size, lower fecundity, and continuously decreasing egg viability thus suggesting a delayed effect of the chemicals (Francis *et al.* 2001). Similarly, the lady beetle *Hippodamia convergens* Guerin also showed a change in life history when fed greenbugs (*Schizaphis graminum* Rondani) which had been raised on resistant sorghum (*Sorghum bicolor* Linnaeus) in comparison to susceptible sorghum-raised greenbug. The lady beetle showed increased larval development time, decreased female weight, increased male weight, and a decrease in survival (Rice and Wilde 1989). However, aphid-resistant plant cultivars may result in reduced feeding by aphids on the 'toxic' plants and therefore reduced sequestration of the toxin (van Emden 2002). Lady beetles that fed on these aphids were not affected because of the small levels of the toxin in their prey (van Emden 2002). Conversely, aphids feeding on resistant cotton cultivars accumulate higher amounts of fatty acid; lady beetles (*Propylaea japonica* Thunberg) feeding on these aphids had a shorter development time and increased weight than when fed aphids raised on susceptible cotton cultivars (Du *et al.* 2004). Clearly, plant-herbivore interactions can have a wide range of effects on the predator.

### Summary

The soybean aphid is a widespread pest of soybean. It can reproduce rapidly on soybean via parthenogenesis, and it is capable of causing considerable damage. Control of

damaging aphid populations is often achieved by chemical spraying, but biological control can help keep aphid populations under economic threshold levels. Another method of control involves breeding soybean for resistance. Several genes have been identified to confer soybean aphid resistance, but it is not known how the genes are expressed in the plant. Resistance may be caused by a potassium deficiency or by levels of lignin present in the plant. Another mechanism is interruption of phloem feeding through sieve elements. Neither how rejection of sieve elements is accomplished nor whether xylem feeding contributes to aphid resistance is known.

The multicolored Asian lady beetle was introduced to the U.S. as control for pecan aphid several decades ago, but has a wide host range. It is a major predator of soybean aphid and can contribute significantly to population control. Typical of most predacious coccinellids, larval development and fecundity are dependent on temperature and diet. Prey species and plant composition are factors in determining prey quality since not all prey are equal. Consuming less preferred prey is by coccinellids is a survival tactic but can be detrimental to larval development and egg laying.

Plant traits can influence natural enemies through their herbivore associates. Resistance in soybean to other herbivores (i.e., soybean looper) can affect the development of the herbivores' parasitoids. Coccinellid larvae, while not confined to a single individual for development, are confined to the area in which they were laid. Larval development is greatly affected by diet, and suboptimal prey can prolong or interrupt development. Soybean aphid showed rejection of sieve elements (less phloem feeding), reduced fecundity, and greater mortality on resistant varieties of soybean. It is not known if

soybean aphid resistance in soybean affects the quality of soybean aphids as prey for *H. axyridis*.

In the interest of maintaining a healthy biological control population to reduce soybean aphid, soybean aphid resistance and *H. axyridis* biological control should be tested for compatibility. In this study, I examined the effects of soybean varieties that varied in their resistance to the soybean aphid on the development and adult fitness of a widespread generalist aphid predator, *Harmonia axyridis*.

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## CHAPTER 2

# EFFECTS OF SOYBEAN APHID RESISTANCE ON THE LADY BEETLE *HARMONIA AXYRIDIS* MEDIATED BY THE SOYBEAN APHID *APHIS GLYCINES*

### Introduction

Successful biological control of a pest depends on a number of factors, including how the host plant directly and indirectly influences the natural enemies of the plant's herbivores. Direct effects include plant traits that influence an adult natural enemy's ability to locate and attack the herbivore. Plants can directly influence the natural enemies of its herbivores through structural and chemical means (Bergman and Tingey 1979, Price *et al.* 1980, Bottrell *et al.* 1998, Hare 2002). For example, plants can release chemicals that attract natural enemies (Kessler and Baldwin 2002) or alter natural enemy foraging behavior due to variation of trichome density (Bergman and Tingey 1979, Price *et al.* 1980, Bottrell *et al.* 1998, Obrycki and Kring 1998, Hare 2002, van Emden 2002).

Plants can also affect natural enemies indirectly by altering characteristics of the herbivore that serves as a prey or host (Bergman and Tingey 1979, Price *et al.* 1980, Hare 2002, Kagata and Ohgushi 2006, Ode 2006). For example, herbivores that sequester plant chemicals may become toxic to their natural enemies (Price *et al.* 1980, Obrycki and Kring 1998, Hare 2002, van Emden 2002). More subtly, plant species or varieties can differentially affect herbivore nutritional suitability for natural enemies (Price *et al.* 1980, Bottrell *et al.* 1998, van Emden 2002). When plant resistance to insect pests is artificially selected, plant resistance is likely to also indirectly affect the natural enemies of those herbivores (Price *et al.* 1980, Bottrell *et al.* 1998, Obrycki and Kring 1998, Hare 2002, van

Emden 2002). Understanding these indirect effects caused by changes in the host plant are therefore important for understanding the likelihood of success from using insects as agents of biological control.

Indirect effects of plant resistance on natural enemies have been studied for some herbivore of the soybean, *Glycine max* Linnaeus. Different resistant soybean lines have had variable effects on the natural enemies of target herbivores. For instance, the egg parasitoid *Telenomus chloropus* Thomson experienced lower emergence rates and fecundity when its stinkbug (*Nezara viridula* Linnaeus) host was reared on soybean with resistance to the stinkbug compared to susceptible plants (Orr et al. 1985). The parasitoid *Microplitis demolitor* Wilkinson had reduced survival when it developed in soybean looper, *Pseudoplusia includens* Walker, fed resistant soybeans compared to when it developed in loopers reared on susceptible soybeans (Yanes and Boethel 1983). In addition, the parasitoid *Copidosoma truncatellum* had a longer development time in *P. includens* fed resistant soybeans compared to susceptible soybeans (Orr and Boethel 1985). Despite the reduced performance of the biological control agents in these studies, soybean resistance and biological control were considered to be compatible in these systems because their combination was better able to reduce herbivore populations than either method used alone. However, short-term reductions in pest populations should not be the only measure of plant resistance and biological control compatibility. Long-term successful compatibility will depend on the growth and sustainability of the natural enemy population.

The soybean aphid, *Aphis glycines* Matsamura (Hemiptera: Aphididae) is a recent invader of North American soybeans and is considered to be a serious pest with substantial

economic costs (Song and Swinton 2009). Both biological control and the use of aphid-resistant varieties of soybean are attractive methods for regulating soybean aphid populations (Rongcai *et al.* 1994, Fox *et al.* 2005, Rutledge *et al.* 2004, Ragsdale *et al.* 2007). There is some level of natural control provided by the generalist aphidophagous predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Han 1997, Landis *et al.* 2004, Fox *et al.* 2005), and resistant soybean plants have been discovered that deter aphids from feeding, decrease aphid survival, or both depending on the variety (Hill *et al.* 2004 a and b, Li *et al.* 2004, Diaz-Montano *et al.* 2006, Hesler and Dashiell 2007). While there may be some interaction between resistant soybean plants and *H. axyridis* (Lundgren *et al.* 2009), there is currently no information on addressing possible interactions between aphids reared on resistant soybean varieties and *H. axyridis*.

Coccinellids generally are not selective in what prey they feed on, and this can have an impact on their life history traits (Michaud 2000 and 2005, Evans and Gunther 2005). Non-selective feeding may expose coccinellid natural enemies to potentially toxic effects from aphid-resistant plant varieties. For example, *Hippodamia convergens* Guerin has increased larval development time, decreased larval survival, lower adult female weight, and higher adult male weight after being fed greenbug, *Schizaphis graminum* Rondani, raised on greenbug-resistant sorghum versus susceptible varieties (Rice and Wilde 1989). However, not all resistant plants have negative effects on predaceous coccinellids. *Propylaea japonica* Thunberg had a shorter development time and increased weight when fed aphids (*Aphis gossypii* Glover) reared on resistant cotton cultivars in comparison to susceptible cultivars (Du *et al.* 2004). The resistance was due to a high gossypol content reducing aphid longevity and fecundity, and aphids feeding on the cultivar accumulated a



high fatty acid content, which was then passed onto the lady beetle (Du *et al.* 2004). In this instance, plant resistance enhanced biological control. Francis *et al.* (2001) reported mixed results when *Adalia bipunctata* Linnaeus was fed aphids (*Myzus persicae* Sulzer) raised on *Brassica* plants producing high glucosinolate levels. Glucosinolates usually reduce herbivore performance, but in *M. persicae* it stimulates feeding (Francis *et al.* 2001). In *A. bipunctata*, it was shown to cause increased larval development time and larger pupal mass but also reduced fecundity and egg viability (Francis *et al.* 2001). Another study found no trophic effects of *Bt* transgenic potato plants on the lady beetle *H. convergens* when it fed on the aphid *M. persicae* (Dogan *et al.* 1996). Clearly, coccinellids can be influenced by a wide range of plant traits mediated by their herbivorous prey. However, the overwhelming majority of these previous studies do not make a clear prediction how changes in plant resistance will influence these predators.

We used a series of different resistant and susceptible soybean varieties in a laboratory study to determine if soybean aphid resistance in these lines affected the development, size, and fecundity of *H. axyridis*. The motivation for this study comes from the increasing interest in soybean aphid-resistant soybeans to help reduce or eliminate pesticide use. The recent determination of soybean aphid economic threshold levels (Ragsdale *et al.* 2007) helps reduce pesticide use. However, in using these strategies to control the pest, we must also evaluate the compatibility with biological control via the predatory insect, *H. axyridis*.

## Methods and Materials

### *Predator Colony*

A colony of *H. axyridis* was established using approximately 50 individuals caught in soybean fields near Minot, ND and Prosper, ND and on various flowers and trees (including *Rhamnus* sp., *Acer* sp., *Aster* sp.) in the vicinity of the North Dakota State University campus in Fargo, ND from July through September 2006. Lady beetles were kept in cages inside a rearing room at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , and a 16L:8D photoperiod. Field-collected ladybeetles were kept in separate cages from their offspring to prevent the spread of any bacterial or fungal pathogens between individuals. The cage for field-collected beetles was approximately 0.30 m square with plexi-glass on 4 of the 6 sides. Another side was covered with fine mesh (approximately 2 threads per mm) and the last side had a knit sock insert for access to the beetles. The cage for the offspring was approximately 0.61m square with fine mesh sides (openings approximately 1mm x 2 mm) and aluminum bottom. A knit sock was also set into a side for easy access to the beetles. Individual ovipositing females were held in separate Petri dishes to allow removal of eggs and to minimize cannibalism. Eggs were removed from Petri dishes daily. When a female no longer laid fertilized eggs, she was placed back into the colony with males to mate again (females mate multiple times; see Pervez and Omkar 2006) and subsequently was placed into a Petri dish. Eggs were kept in the dish and placed in the rearing room to hatch. Once hatched, individual larvae were held in separate Petri dishes to prevent cannibalism.

*Harmonia axyridis* larvae were fed primarily *A. glycines*, supplemented with eggs of *Trichoplusia ni* (Lepidoptera: Noctuidae). Adult lady beetles were given *T. ni* eggs (approximately 10-50 eggs per beetle per day), aphids (several infested leaves every other day; approximately 300+ aphids of mixed instars per leaf) and honey water (1 part honey to 2 parts water) in a 330-ml cup with a lid. The lid had a small hole (approximately 7 mm in

diameter) to accommodate a cotton wick of similar diameter to draw up honey water, preventing ladybeetles drowning in liquid. The cup was kept full of honey water or replaced when the cotton became dirty (i.e., contaminated with frass, dead aphids, mold, etc.). Ovipositing adult females held in Petri dishes were fed aphids and *T. ni* eggs only as Petri dishes could not accommodate honey cups.

### *Soybean Aphid Colony*

The main colony of soybean aphids came from North Dakota and was reared on a susceptible soybean variety (*Glycines max*; variety Asgrow 0801). The colony was kept in three metal-framed cages 0.61 m×0.61 m×0.61 m covered with fine mesh fabric (approximately 4 threads per mm) with a knit sock for easy access.

Two soybean seeds were planted in a 6” pot in Sunshine Mix #2 (SunGro Horticulture Canada Ltd (Vancouver, British Columbia, Canada) containing sphagnum peat moss, perlite, gypsum, dolomitic lime) potting soil and placed in the cage when the first full trifoliate leaf opened. Up to 10 pots were placed in a cage at one time. Plants were removed when pods developed. Trifoliate leaves were removed and placed on young plants to transfer aphids onto new colony plants or sectioned into appropriate amounts (i.e., single leaves to Petri dishes, larger portions to cages) to feed the colony of lady beetles.

### *Experimental Soybean Plants and Aphids*

To test how differentially aphid-resistant soybean varieties affect aphid predators, we chose seven varieties of soybeans that varied in their level of resistance to soybean aphid. Soybean aphids reared on each of the seven varieties were used in each of the seven

treatments of the experiment described below. Two of the chosen varieties (Jackson and Palmetto) are considered resistant, two are considered susceptible (Arksoy and Ralsoy), and three are considered moderately resistant (Curtis, Verde, and Wye) (Hill *et al.* 2004a). Seed from each line was obtained from the USDA Soybean Germplasm Collection at the University of Illinois in Urbana, IL. The level of aphid resistance was measured by Hill *et al.* (2004a,b) in these lines using a qualitative index that takes into account both the density of soybean aphids on a plant and their damage to the plant (aphid index (0-9) = aphid density (0-3) x aphid damage (0-3); where density range is scored: 0 = no aphids, 1 = low aphid population, 2 = moderate aphid population, 3 = high aphid population; damage range is scored: 0 = no damage, 1 = mild leaf damage, 2 = moderate leaf damage, 3 = severe damage (including plant death); overall aphid index is scored as following 0-3 = high resistance, 4-6 = moderate resistance, 7-9 = no resistance; Hill *et al.* 2004a,b). Aphid indices for each soybean variety were determined by Hill *et al.* (2004 a,b): Jackson 2.8, Palmetto 2.0, Wye 3.4, Verde 4.0, Curtis 4.3, Arksoy 9.0, and Ralsoy 9.0.

Soybean plants were planted individually in 15 cm pots with Sunshine mix #2 potting soil and grown in the greenhouse. Natural light was supplemented with standard sodium greenhouse overhead lights to maintain a 16L:8D photoperiod, and temperature was maintained at approximately 24°C. Plants were grouped according to variety and each group was spaced at least two feet away from any other variety to prevent apterous aphid movement between varieties. Plants inside each group varied in distance from pots touching to approximately 10 cm away from each other, depending upon plant size. However, leaves of each plant touched leaves from at least two other plants of the same variety to facilitate aphid searching for new feeding sites.

To establish aphids on each variety, up to 10 plants of each variety were placed on an isolated table and interspersed with the infested aphid colony plants until small aphid populations of at least 10 new individuals were found on each plant of the new variety. We further encouraged aphid colonization on all plants by gently placing two to three aphids on the trifoliolate leaf of each new plant using a small soft brush. Each new plant had at least one trifoliolate leaf before aphids were placed on it.

Plants were fertilized when needed (24 oz of Prolific™ 20-20-20, Terra International, Inc Sioux City, IA dissolved in 5 gallons water and applied with siphon hose at rate of 15 parts water: 1 part solution), watered as needed, and monitored for other soybean herbivores as well as aphid predators and parasitoids. Any aphid predators were manually removed. Herbivores, such as spider mites, were removed by cutting off infected leaves and isolating the plant for two days to monitor for additional spider mite infestation. If still infested, the plant was discarded.

#### *Experiment –H. axyridis development from first instar to adults*

A comparison of *H. axyridis* development times was conducted by providing soybean aphids (*A. glycines*) reared on one of the seven soybean varieties to individual *H. axyridis*. The following fitness parameters were measured for each *H. axyridis* individual: the duration of development, adult body weight, and fecundity of each female over the first 31 days of egg production.

#### *Larval development.*

Mated females from the main colony were placed individually in Petri dishes to allow oviposition. To increase the amount of variation between sibling groups, each *H.*

*axyridis* colony female only contributed one set of eggs (approximately 10-30 eggs) to the experiment. This increase in diversity in the experimental population is presumably more representative of lady beetle populations in the field. Larvae were collected within 1 day of hatching (first instar). Newly hatched larvae found feeding on unhatched eggs or larval siblings were not used in the experiment because those individuals could have had an advantage over siblings who had not fed. Larvae feeding on eggs or siblings develop faster and are larger than individuals not participating in sibling cannibalism (Osawa 2002).

Thirteen female *H. axyridis* produced the larvae used in the experiment. From each set of hatched eggs, individual first-instar larvae were placed into a Petri dish and randomly assigned to one of the seven soybean varieties. The number of larvae from each clutch varied from two to twelve, so not all treatments received a larva from each clutch and no treatment received more than two larvae from the same clutch. Each Petri dish was labeled according to the larva's sibling group and assigned plant variety for identification and mating purposes. Each beetle larva was fed aphids raised solely on plants from the assigned variety.

A total of 92 larvae were assigned to treatments (12 Arksoy, 17 Ralsoy, 12 Verde, 14 Wye, 9 Curtis, 17 Jackson, 11 Palmetto), and 78 successfully completed development to become adults. Of the 14 individuals that did not complete development, seven died before reaching adulthood, and seven were lost during development. Of the seven beetles lost, two Curtis larvae were removed from the experiment because Curtis aphid populations decreased to levels that could not support the number of beetles assigned to the variety. Later, two more individuals were removed from the study because each was the only remaining member of its clutch.

Aphids were collected each day using a small, soft brush and placed in small plastic containers with lids. Only 3<sup>rd</sup> and 4<sup>th</sup> instar aphids (primarily 4<sup>th</sup>) instars were collected and used to feed beetle larvae. Aphids were segregated into different containers according to the soybean variety on which they had fed. After aphids were collected, containers were brought from the greenhouse to the lab where aphids would be counted out and given to the developing lady beetle larvae.

The number of aphids fed to individuals daily depended on the instar of the larva. Ten, 20, 40, and 80 third- or fourth-instar aphids were fed per day to first-, second-, third-, and fourth-instar *H. axyridis*, respectively. Aphid numbers in each Petri dish were counted before and after feeding larvae to monitor larval aphid consumption.

We intended to feed aphids in excess so that they were not limited by food availability and preliminary experiments indicated that the above numbers of aphids would accomplish this. However in our experiment larvae consumed over 90% of the aphids available and there were no aphids remaining in the dish after 24 hours on over 70% of the days larvae were fed. Therefore we cannot claim that larvae were fed in excess, but all larvae were given the same number of aphids.

The duration of each instar was recorded in days to determine the overall length of development. Since there are many factors (*i.e.*, temperature, diet) that can change the rate of growth and size of the lady beetle, all lady beetles were reared in the same rearing room chamber with 25°C ± 2°C and 16L:8D photoperiod.

An ANOVA was performed on the number of days for larvae to develop into an adult using the JMP statistical package (SAS Institute 2000, version 4) to determine any effect from the varieties of soybean. Included in the statistical analysis model were the sex

and the clutch from which the larva came. We also performed planned contrasts to compare the two most susceptible varieties (Arksoy and Ralsoy) with the two most resistant varieties (Jackson and Palmetto) to determine any overall effects of plant resistance on larval development time.

#### Adult Mass.

The mass of each adult was recorded from the previous experiment within 24 hours after eclosion. Most adults were still relatively soft from emerging from the pupal exoskeleton but enough time had passed that adults were gaining color and no longer colorless. Newly emerged adults were not fed until after they were weighed.

ANOVA (JMP; SAS Institute 2000 version 4) was used to determine any effects of soybean variety on the mass of newly emerged adults. Beetle sex and the clutch of origin were included in the model. Planned contrasts between the two most susceptible varieties (Ralsoy and Arksoy) and two most resistant varieties (Jackson and Palmetto) were also performed to help determine any overall effects of plant resistance, and Tukey-Kramer HSD ( $\alpha = 0.05$ ) was used to determine differences in masses between soybean variety treatments.

#### Fecundity: Egg and First Instar Production.

Soon after adult eclosion, each beetle was identified to sex using characteristics stated in McCornack *et al.* (2007) (i.e., pigmentation is present in the labrum and prosternum in females and absent in males). Adult females were maintained with the aphids from the same soybean variety that they experienced as larvae. A total of 39 females were used across the seven treatments: four females on Jackson, five females on Palmetto, six females on Wye, seven females on Verde, six females on Curtis, seven



females on Arksoy, and three females on Ralsoy. One female died (Wye) without ever having laid any eggs and was excluded from the analysis. All adults were fed at least 80 aphids per day and all adults had aphids remaining after 24 hours. Petri dishes in which adults were kept were changed before feeding every day.

Before testing the fecundity of females reared on aphids from different varieties, each female was paired with a single male in a plastic Petri dish (60 mm diameter) for 24 hours to ensure mating. In most cases, we gave each female a mate from a different mother that was reared on aphids from the same variety; however, due to differences in availability, ten females were each paired with males from a different mother that were reared on aphids from a different soybean variety and nine females were mated with males from the main colony. All females were checked once daily for any eggs laid. If eggs were laid, they were counted and incubated to measure hatch success. This was repeated daily for each female to obtain an estimate of the effect of soybean variety on fecundity. The number of eggs laid was recorded daily over the course of the experiment, and eggs produced during the first 31 days after mating were used for the analysis.

The intention of this study was also to look for differences in the hatch rate of eggs across treatments. However, data from the production of first instars could not be included in the analysis because hatch rate was very poor across all treatments. Hatch rate was 0.27% of egg production. This hatch rate was much lower than what has been seen in other studies (e.g., Michaud 2000), and since it was across all treatments we felt that there was a problem with our experimental methods. Thus those results were excluded.

Statistical analysis (JMP; SAS Institute 2000, version 4) was performed on the first 31 days of oviposition using ANOVA. Egg data were square root transformed as the

residuals of untransformed data were skewed. The model included the age of the female when she was mated as well as clutch of origin. Planned contrasts were also used between the two most susceptible varieties (Ralsoy and Arksoy) and two most resistant varieties (Jackson and Palmetto) to help determine any overall effects of plant resistance on egg production.

## Results

### *Larval Development*

Despite the highly variable length of time for individual *H. axyridis* larvae to complete development (14 – 21 days; overall model fit:  $F_{17,57} = 6.74$ ,  $p < 0.0001$ ), there was no difference in larval development time as a function of beetles feeding upon aphids from the seven soybean varieties (partial- $F_{6,57} = 1.46$ ,  $P = 0.208$ , Table 2). Furthermore, larval

Table 2: Mean times in days for *H. axyridis* larvae to develop from first instar to adult when fed aphids reared on soybean lines varying in resistance to soybean aphid. Varieties are listed in order of increasing susceptibility to soybean aphids.

Variety	Resistance	Days to Adult Eclosion		n
		Least Squares Mean	Arithmetic Mean	
Palmetto	2	16.01 ± 0.27	15.90 ± 0.34	10
Jackson	2.8	16.70 ± 0.23	17.00 ± 0.35	14
Wye	3.4	16.69 ± 0.25	16.83 ± 0.37	12
Verde	4	16.39 ± 0.26	16.20 ± 0.29	10
Curtis	4.3	15.81 ± 0.33	15.57 ± 0.37	7
Arksoy	9	16.10 ± 0.27	16.00 ± 0.33	10
Ralsoy	9	16.21 ± 0.25	16.25 ± 0.41	12

development time did not differ between groups of *H. axyridis* larvae fed aphids from susceptible or resistant soybean varieties (t ratio = -0.80 p = 0.426).

Male and female *H. axyridis* larvae did not differ in the length of time it took to complete development to adulthood (partial- $F_{1,57} = 0.08$ ,  $P = 0.785$ ). However, significant differences in larval development time existed among the clutches produced by the 11 females (partial- $F_{10,57} = 8.84$ ,  $P < 0.001$ ). The average development time for larvae from different clutches ranged from 14 d to over 17 d. Since there were not equal numbers of larvae from each clutch assigned to each treatment, some treatments had more larvae from fast-developing clutches and other treatments had more larvae from slow-developing clutches. The least squares means from the full model allow us to account for this potential bias when looking for treatment differences. Table 2 lists both the least square mean and the arithmetic mean from the raw data.

#### *Adult Mass*

After accounting for the effects of sex and clutch of origin, the mass of adult *H. axyridis* was affected by the soybean variety on which their prey aphids fed (partial  $F_{6,57} = 2.56$ ,  $P = 0.029$ ). Beetles fed Curtis reared aphids weighed significantly less than those from either Arksoy or Wye reared aphid colonies. Mean adult mass ranged from 0.0187 to 0.0369 g (overall model fit:  $F_{17,57} = 7.53$ ,  $p < 0.0001$ ); however, there was not an obvious relationship between soybean resistance and mass of adult *H. axyridis* (Table 3). Mean adult weight of *H. axyridis* reared on aphids that developed on the two susceptible soybean varieties were not significantly different from those on two highly resistant soybean varieties (t ratio = 0.99,  $P = 0.333$ ).

Adult *H. axyridis* mass was affected by the sex of the predators as well as the clutch

Table 3: Mean mass (g) of newly emerged *H. axyridis* adults after being fed aphids reared on soybean lines that varied in resistance to soybean aphid. Soybean aphid resistance increases as the index number decreases. Varieties are listed in order of increasing susceptibility to soybean aphids.

Variety	Resistance	Adult Mass (g)		
		Least Squares Mean	Arithmetic Mean	n
Palmetto	2	0.0259 ± 0.0007	0.0263 ± 0.0008	10
Jackson	2.8	0.0267 ± 0.0006	0.0252 ± 0.0008	14
Wye	3.4	0.0277 ± 0.0007	0.0277 ± 0.0012	12
Verde	4	0.0272 ± 0.0007	0.0276 ± 0.0009	10
Curtis	4.3	0.0242 ± 0.0009	0.0265 ± 0.0010	7
Arksoy	9	0.0279 ± 0.0007	0.0285 ± 0.0011	10
Ralsoy	9	0.0261 ± 0.0007	0.0255 ± 0.0010	12

of eggs from which an individual came. Females weighed on average significantly more than males across all varieties (partial  $F_{1,57} = 44.14$ ,  $P > 0.001$ ; Table 4). Adult mass also

Table 4: Mean mass (g) of newly emerged *H. axyridis* adult male and females after being fed aphids reared on soybean lines of varying resistance to soybean aphid.

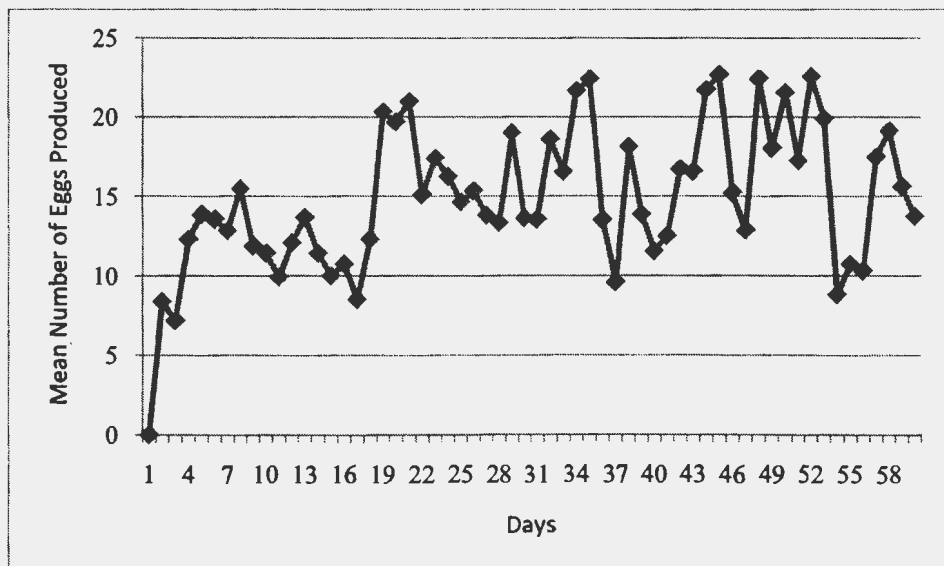
Sex	Adult Mass (g)		
	Least Squares Mean	Arithmetic Mean	n
Female	0.0284 ± 0.0004	0.0285 ± 0.0005	38
Male	0.0247 ± 0.0004	0.0248 ± 0.0004	38

differed significantly as a function of clutch of origin (partial  $F_{10,57} = 6.02$ ,  $P > 0.001$ ) with average adult mass varying from 0.0245 g to 0.0308 g.

*Egg Production within 31 Days*

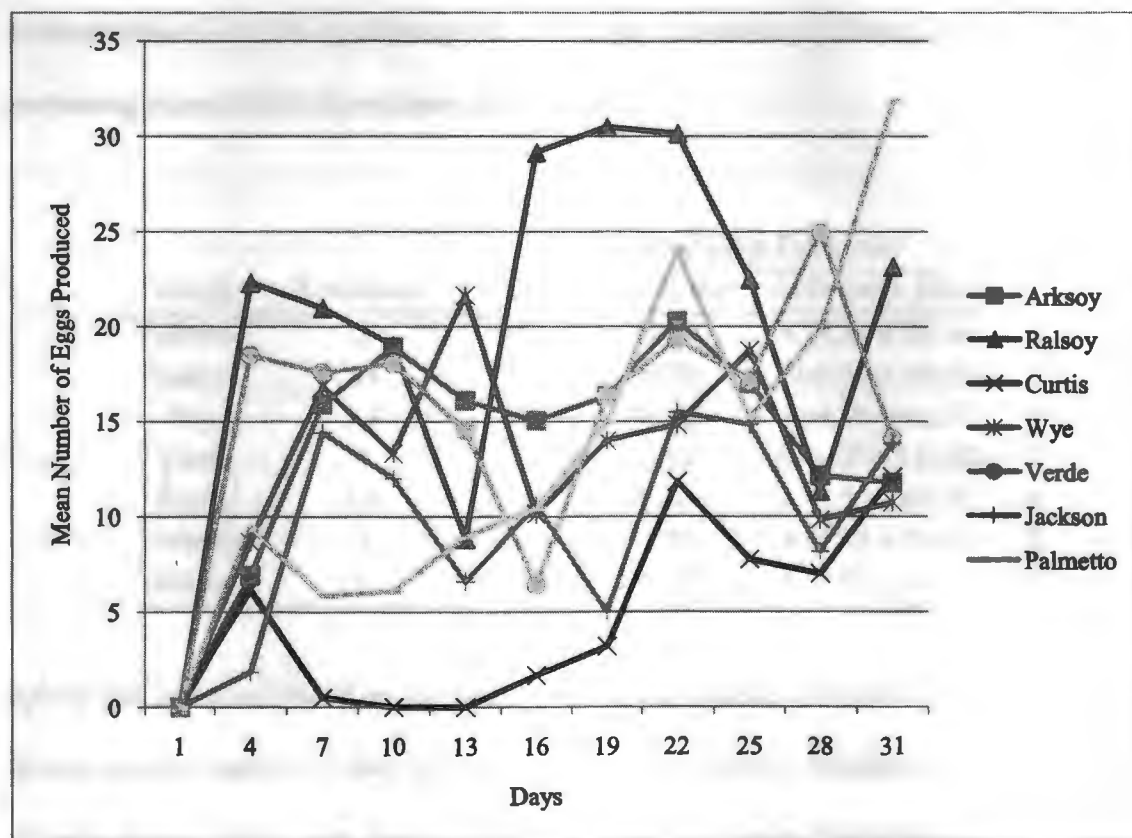
Overall, egg production increased as adult females aged and subsequently plateaued by one month (Figure 2), and although variation in amount of eggs laid was influenced by

Figure 2: Mean egg production of all beetles regardless of variety over the duration of the experiment.



soybean varieties (Figure 3), plant resistance level did not appear to be a strong explanation for the variation in eggs laid (overall model fit:  $F_{16,17} = 1.71$ ,  $p = 0.14$ ). Despite the overall model fit, the mean number of eggs laid over 31 days by adult *H. axyridis* females varied significantly as a function of the soybean variety on which their aphid prey fed (partial- $F_{6,17} = 3.16$ ,  $P = 0.029$ ; Figure 3). However, mean egg production was not obviously correlated with the aphid-resistance index of the seven soybean varieties (Table 5). A linear contrast comparing the egg production of the two susceptible lines ('Ralsoy' and 'Arksoy') and the two resistant lines ('Jackson' and 'Palmetto') found no significant differences ( $t$  ratio = -1.65,  $P = 0.117$ ). Neither clutch of origin ( $F_{9,17} = 1.51$ ,  $P = 0.223$ ) nor the age of the female

Figure 3: Mean number of eggs produced over time for female *H. axyridis* beetles fed aphids reared on differing soybean varieties during the first 31 days after mating.



when mated ( $F_{1,17} = 2.99$ ;  $P = 0.102$ ) were significant factors in the number of eggs laid over 31 days.

### Discussion

The variety of soybean on which soybean aphids were reared had an indirect effect on two fitness measures of the coccinellid, *H. axyridis*: adult weight and the number of eggs laid over 31 days. Soybean variety did not have an indirect effect on the time to develop from egg to adult in *H. axyridis*. Because *H. axyridis* were provided soybean

Table 5: Mean number of eggs produced by female *H. axyridis* over the first 31 days of egg production. Females had developed on and continued as adults to feed on aphids reared on soybean lines of various soybean aphid resistances. Varieties are listed in order of increasing susceptibility to soybean aphids.

Variety	Resistance	Number of Eggs Produced		n
		Least Squares Mean	Arithmetic Mean	
Palmetto	2	357.97 ± 118.82	475.24 ± 95.48	4
Jackson	2.8	230.74 ± 96.00	309.06 ± 69.62	4
Wye	3.4	309.76 ± 100.32	434.31 ± 62.52	6
Verde	4	454.12 ± 96.32	481.80 ± 130.82	7
Curtis	4.3	67.08 ± 54.22	157.50 ± 69.53	4
Arksoy	9	379.08 ± 91.51	457.53 ± 99.68	2
Ralsoy	9	626.00 ± 207.67	677.04 ± 38.51	7

aphids that had been reared on one of seven different varieties of soybeans in the absence of any soybean material, I was able to measure indirect effects of soybean variety on *H. axyridis* fitness parameters. In this sense, these findings complement the study of Lundgren et al. (2009) who examined direct effects of soybean varieties on *H. axyridis* by eliminating any host-mediated effects (see discussion below). Interestingly, the varieties that had the greatest effect on *H. axyridis* were not the varieties with the reported greatest resistance to soybean aphid. For instance, beetles fed aphids reared on Curtis (a moderately resistant variety) weighed less than either beetles fed aphids reared on Arksoy (a susceptible variety) or Wye (a moderately resistant variety). Beetles fed Curtis-reared aphids were also the lowest egg producers, whereas beetles fed Ralsoy-reared aphids produced the most number of eggs. These differences between the feeding treatments suggest that soybean varieties can have indirect effects on *H. axyridis*. However, a general

relationship cannot be made concerning the average reported soybean aphid resistance of a given soybean variety and *H. axyridis* fitness measures because *H. axyridis* performed better on aphids from both resistant and susceptible varieties than it did on some moderately resistant varieties. Since we did not retest soybean resistance levels, our conclusions rely on the overall averages found in Hill *et al.* (2004a,b). The actual amount of resistance expressed and therefore the effect of the plant on the aphid could have differed for the plants used in this experiment.

While we tested Jackson and Palmetto as our high aphid-resistant soybeans (Palmetto is a parent of Jackson [Hill *et al.* 2004a]), we only found intermediate levels of indirect effects on *H. axyridis* with these varieties. However, other varieties could still affect the quality of aphids as prey for coccinellids. Several genes have been identified as sources of soybean aphid resistance including a resistance gene in Jackson (Hill *et al.* 2006b), *Rag1* in Dowling (Hill *et al.* 2006a), in addition to others (Kim *et al.* 2008, Mian *et al.* 2008b, Zhang *et al.* 2009, Zhang *et al.* 2010). The Jackson resistance gene (*Rag*) was mapped to the same loci on chromosome 7 as *Rag1* in Dowling (Li *et al.* 2007) although it is not clear if these two genes are the same (Hill *et al.* 2006b, Li *et al.* 2007, Hesler and Dashiel 2008, Kim *et al.* 2008, Zhang *et al.* 2010). Each gene confers resistance differently (Kim *et al.* 2008) and so could in turn have differing indirect effects on natural enemies.

Resistance to soybean aphids can be in the form of plant chemistry, which likely has a genetic component. Lower aphid densities were found on plants with high levels of lignin, a soybean chemical defense mechanism (Hu *et al.* 1993). Also, low plant nitrogen content was associated with low aphid numbers on soybean plants, although this relationship was seen as more a predictor of aphid infestation rather than soybean



resistance (Hu *et al.* 1992). Similarly, high potassium was associated with lower aphid densities on soybean (Walter and DiFonzo 2007). Potassium has been observed as reducing free nitrogen in the plant and therefore reducing the amount of nitrogen available to aphids (Walter and DiFonzo 2007) similar to the pattern seen in Hu *et al.* (1992).

Not all mechanisms of resistance are genetically based. Dean *et al.* (2009) found that rhizobia (*Bradyrhizobium japonicum*) can facilitate soybean aphid resistance in soybean. This mutualism-dependant resistance mechanism is strain specific. That is, not all rhizobia strains can confer the same level of soybean aphid resistance. Also, naturally occurring strains are better able to reduce aphid population densities than commercially available strains or nitrogen fertilizer alone (Dean *et al.* 2009). This mechanism of resistance very likely would not confer the same level of resistance as the various *Rag* genes (aphid populations peaked at approximately 500 aphids per plant with naturally occurring rhizobia – about half the size of other treatments [Dean *et al.* 2008]), but it could help explain a mechanism of moderate resistance. Moderate levels of resistance can also be important because combined with biological control, they can form an effective soybean aphid control strategy (Hesler *et al.* 2007).

The findings of this study suggest that the compatibility between soybean varieties resistant to soybean aphids with biological control is not clear cut. Jackson and Palmetto (Jackson's parent [Hill *et al.* 2004a]) were used as our resistant varieties. Although Dowling and Jackson may not have the same resistance gene (Hill *et al.* 2006b, Li *et al.* 2006, Hesler and Dashiell 2008, Zhang *et al.* 2010), their resistance mechanism is thought to involve toxic compounds (Li *et al.* 2004). Soybeans with moderate resistance (Wye,

Verde and Curtis) could have different or multiple resistance mechanisms which might explain the variation in results.

In a study of direct effects of soybean resistance to aphids on aphid natural enemies, *H. axyridis* and *Orius insidiosus* (Say) were reared in the presence of resistant soybean leaves but fed a surrogate prey instead of soybean aphid to measure direct effects between resistant soybean and biological control (Lundgren *et al.* 2009). Resistant soybean lines used were descendants of Dowling (Lundgren *et al.* 2009). While both predators performed worse overall in the presence of soybean in comparison to morning glory leaves or no-leaf treatments, *H. axyridis* experienced reduced adult longevity with resistant soybean but *O. insidiosus* was not affected (Lundgren *et al.* 2009). The results of our study did not detect indirect effects in high resistant varieties (Jackson and Palmetto). These differences could simply be expression of different genes (*Rag* versus *Rag1*), or something more complex as the presence or absence of the plant and all its physical and chemical characteristics.

In the present study, certain varieties had an indirect impact on *H. axyridis* egg production. However, this study did not address possible effects this would have on aphid population dynamics. In soybean varieties where smaller numbers of *H. axyridis* larvae may hatch and, if confined to the same field in the absence of sufficient aphid numbers, individual beetles should be able to find other insect eggs, larvae, or even weaker siblings for consumption (Hodek and Honěk 1996).

From the results of the present study, we conclude that soybean aphid resistance is potentially compatible with the biological control agent *H. axyridis*. It is important to recognize that only compatibility of a physiological nature was measured since the effects

of resistance levels on the population dynamics between predator and pest were not measured. More varieties of resistant soybean should be studied to further explore the compatibility of soybean aphid resistance and *H. axyridis*.

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