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HOST PLANT RESISTANCE AND INSECTICIDE RESISTANCE
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DESMI INDUMALI CHANDRASENA

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of the requirements for the

M.S. degree in ENTOMOLOGY

Christ DiFonzo
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HOST PLANT RESISTANCE AND INSECTICIDE RESISTANCE STUDIES FOR SOYBEAN APHID *Aphis glycines* Matsumura

By

Desmi Indumali Chandrasena

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ABSTRACT

HOST PLANT RESISTANCE AND INSECTICIDE RESISTANCE STUDIES FOR SOYBEAN APHID *Aphis glycines* Matsumura

By

Desmi Indumali Chandrasena

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) causes serious damage to soybean, [*Glycine max* L. (Merrill)], which is a leading field crop and a top agricultural export in the United States. It continues to infest soybean fields across many states causing significant yield loss. To proactively address this problem, the Michigan State University (MSU) soybean-breeding program developed a series of aphid-resistant lines. E06901, E06905, and E06906 with *rag1b* and *rag3* soybean aphid-resistant genes were evaluated in a two-year field study in two counties in Michigan. E06901, E06905, and E06906 had lower Cumulative Aphid Days (CAD), and fewer plants infested compared to susceptible lines. Natural aphid pressure in all sites were below the economic threshold (250 aphids/plant) thus there were no significant yield losses caused by soybean aphid on susceptible and resistant lines when not protected by an insecticide. There was no added benefit from natural enemies on MSU aphid-resistant lines. Field and laboratory assessments of Japanese beetle feeding on E06901, E06905, E06906, with an aphid-resistant *(Rag1)* line, and susceptible cultivars showed elevated Japanese beetle feeding on MSU aphid-resistant lines. We developed a reliable aphid-dip bioassay technique, and tested susceptibility of soybean aphid to five insecticides. This method provides a baseline for comparing aphid-susceptibility to insecticides.

DEDICATION

This thesis is dedicated to my loving parents Mr. and Mrs. Chandrasena, my sister Madhumali, and to my husband Rasanga, for their love, encouragement, and constant support.

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CHAPTER 1

Review of literature

Soybean: Origin, history, production, and uses

Soybean [*Glycine max* L. (Merrill)] is a cultivated crop, native to East Asia. It has been grown in China for more than 5,000 years as a food and as a component of drugs (Wu et al. 2004). Linnaeus originally introduced the genus *Glycine* in 1737, in his first edition of *Genera Plantarum*. The cultivated soybean first appeared in the *Species Plantarum* by Linnaeus, under the name *Phaseolus max* L. The combination *Glycine max* was proposed by Merrill in 1917.

Soybeans spread to other Asian countries nearly 2,500 years ago (Wu et al. 2004). They were first brought to America in mid-1770s, by trading ships from Asia (Smith 1994). By 1898, the United States Department of Agriculture (USDA) began introducing new varieties of soybeans from Asia. Soybean became an important field crop beginning in the 1940s (Smith 1994). Currently, 31 states in the United States grow soybeans. The top three states in 2008 were Iowa, Illinois, and Minnesota (NASS 2009). In 2008, Soybean was the leading agricultural export from the United States (ERS 2009). In 2008, 75.7 million acres of soybean were grown in the United States, yielding 2.96 billion bushels (NASS 2009).

Soybeans are high in nutrition and are an important source of vegetable protein; beans contain on average 38% protein. About 18% of the bean consists of oil (0.5% lecithin), which is rich in polyunsaturated fatty acids (54% linoleic acid, 22% oleic acid, and 7.5% linolenic acid) and contains no cholesterol. The rest of the bean consists of moisture (14%), soluble carbohydrate (15% sucrose, stachyose, raffinose, others), and insoluble carbohydrate or dietary fiber (15%), (Singh et al. 2008).

Nearly all soybean grown in the United States are used for producing oil and as feed for livestock (Smith 1994). Another important product of soybean oil is biodiesel. Biodiesel is a clean-burning, alternative fuel made from vegetable oils that can be used in compression-ignition (diesel) engines. Since soybean oil is the top oil produced in the United States, the development of biodiesel has mainly focused around soy oil. One bushel of soybean produces about 1.5 gallons of biodiesel (NBB 2009).

Soybean aphid biology and ecology

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native of Asia, and is one of the most serious insect pests of soybean (Yu et al. 1989, Wang et al. 1996, Wu et al. 1999, Sun et al 2000, Hill et al. 2004, Ragsdale et al. 2004). It has been a soybean pest for many years in China, Japan, South Korea, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and Russia (Wu. et al. 2004). Soybean aphid causes heavy yield losses; yield reduction up to 70% was reported in China when infestations occurred

(He et al. 1995, Wang et al. 1996). In recent years, the soybean aphid was discovered in Australia (1999), Canada (2000), and the United States (2000) (Wu et al. 2004). By 2004, it was found in 24 states in the United States, including Michigan, and in three Canadian provinces (Ragsdale et al. 2004, Rutledge and O'Neil 2006).

The morphological characteristics of the soybean aphid were described in detail, by Chen and Yu (1988) and Takahashi et al. (1993). A combination of characters such as body color, black cornicles, and host range distinguish it from other *Aphis* species (Voegtlin et al. 2004.). Similar to other common *Aphis* species, soybean aphid nymphs have four instars; wing development occurs in the third and fourth instars (Zhang 1988). Adults may be winged (alate) or non-winged (apterous). Both of these forms can produce offspring. In general, nymphs range from 0.58 - 1.4 mm in length. Winged viviparous females generally have a long-ovoidal form, are 0.96 - 1.52 mm in length, have red-brown compound eyes and a black head. The wingless viviparous females have an ovoid form, and are 0.95 -1.29 mm in length. (Wu et al. 1999). The soybean aphid can reproduce parthenogenically or sexually depending on the time of the year to complete their life cycle (Figure 1.1).

Life cycle of soybean aphid

In China, soybean aphid alternates between its primary host buckthorn, *Rhamnus davurica* Pall., and secondary host(s) which are primarily cultivated soybean *G. max* and wild glycine species, *Glycine soja* Sieb.& Zucc. (Wang et al. 1962). There are two confirmed overwintering hosts in North America; *Rhamnus cathartica* L., common buckthorn, an invasive woody plant of European origin and *Rhamnus alnifolia* L'Héritier, the native alder leaf buckthorn (Voegtlin et al. 2004, Ragsdale et al. 2004). The secondary host of soybean aphid in North America is chiefly cultivated soybean, *G. max.*

The soybean aphid is heteroecious holocyclic, spending life on different hosts with sexual reproduction during a portion of its life cycle (Figure 1.1; Wang et al. 1962, Zhang and Zhong 1982, Ragsdale et al. 2004). The life cycle starts in spring, when eggs hatch and develop into wingless fundatrices. These fundatrices produce a second generation of wingless females. On the primary host *Rhamnus*, the third and subsequent generations of primarily winged morphs are generated, who emigrate in search of a secondary host in summer, typically cultivated soybean. Throughout the season, many overlapping generations of both wingless and winged morphs are produced on soybean. Later in autumn, under reduced photoperiod and temperature, winged gynoparae are produced on soybeans that move in search of *Rhamnus* again. On *Rhamnus*, they produce nymphs that develop into oviparae. The gynoparae also form males, on soybean, that later emigrate to *Rhamnus* in search of oviparae. Once the males and oviparae mate, their overwintering eggs are deposited on *Rhamnus* (Ragsdale et al. 2004).

The development time of soybean aphid depends on temperature and nutrition. Sun et al. (2000) found that between 20-25°C, only five to seven days were required for nymphs to develop to adults under suitable nutritional conditions. Average temperatures between 22-25°C and relative humidity less than 78%, were optimal for the development of soybean aphid in the field in China (Wang et al. 1962). Hirano et al. (1996) reported that optimal reproduction and longevity occurred at 22 °C. In the United States, aphids reproduced longer and produced more progeny at 20 and 25°C than at 30 or 35°C; populations doubled in only 1.5 days at 25°C, but took 1.9 days at 20 and 30°C (McCornack et al. 2004).

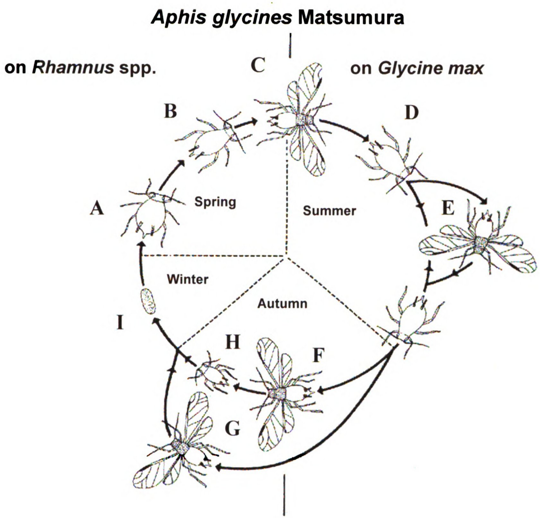

Figure 1.1. Life cycle of the soybean aphid (Reproduced from Ragsdale et al. 2004)

(A) Fundatrix on *Rhamnus*. (B) Apterous viviparous female on *Rhamnus*. (C) Alate viviparous female, spring migrant from *Rhamnus* to soybean. (D) Apterous viviparous female on soybean. (E) Alate viviparous female, summer migrant. (F) Gynopara, fall migrant from soybean to *Rhamnus*. (G) Male migrates from soybean to *Rhamnus*. (H) Ovipara, on *Rhamnus*. (I) Overwintering egg on *Rhamnus*.

Damage and economic impact

Soybean aphid reduces yield directly via plant feeding and indirectly through reduction in seed protein content (Wang et al. 1994). Plants with heavy infestations show wrinkled and distorted foliage, early defoliation, stem and leaf stunting, reduction in number of pods and seed weight, and even plant death (Wang et al. 1962, Wang et al.1996, Lin et al. 1992, 1994; He et al. 1995, Wu et al. 1999, DiFonzo and Hines 2002, Wu et al. 2004, Diaz-Montano et al. 2006). Honeydew excreted by aphids builds up on foliage, and supports the growth of sooty mold, affecting plant photosynthesis, yield, and seed quality (Chen and Yu 1988).

Significant yield loss can occur due to feeding damage by soybean aphid. In China, yield was reduced up to 52% when soybeans in the early vegetative stage (first node stage) were inoculated with 220 aphids per plant (Wang et al. 1994). Soybean aphid feeding results in reduction of seed yield, and also reduction in seed quality (e.g., discoloration, deformation) which could be a major concern for food-grade soybean growers and consumers (Mian et al. 2008a).

In addition to yield loss from the direct feeding, another threat posed by the aphid is its ability to transmit plant viruses to soybean (alfalfa mosaic virus, soybean dwarf virus, soybean mosaic virus) and other crops (Iwaki et al. 1980, Hill et al. 2001, DiFonzo 2006, DiFonzo and Agle 2008). In Michigan, soybean aphid outbreaks often coincide with high virus levels in cucumber (*Cucumis sativus* L.), squash (*Cucurbita* spp.), pumpkin

(*Cucurbita* spp.), and dry beans (*Phaseolus* spp.) (DiFonzo 2006, DiFonzo and Agle 2008). Since soybean aphid is a relatively recent pest to colonize soybean in the United States, its full consequences as a virus transmitter to soybeans and other crops is still unknown (Mian et al. 2008a).

Management of soybean aphid

Several factors affect soybean aphid populations on soybean, including environmental factors (e.g., temperature, precipitation, and humidity), number of overwintering aphid eggs, cultural practices (e.g. planting time and soybean variety), and natural enemies (Wu et al. 1999, Wu et al. 2004). Soybean aphid can be controlled by a number of distinct tactics including biological control, chemical control, and host plant resistance. These control options can be used individually or together.

In Asia, the complex of natural enemies attacking soybean aphid includes the predators *Propylaea japonica* (Thunberg), *Harmonia axyridis* (Pallas), and *Harmonia. arcuata* (Fabricius) (Coleoptera: Coccinellidae), and several species of syrphid and lacewing larvae (Van den Berg et al. 1997, Wu et al. 2004). In addition, 10-53% parasitism by the parasitoid *Lysiphlebia japonica* (Ashmead) was reported in China in a biological control program (Wu et al. 2004). In North America, the dominant soybean aphid natural enemies are mainly generalist predators, such as lady beetles (Coccinellidae), green lacewings (Chrysopidae) and, pirate bugs (*Orius* spp.). *Orius insidiosus* Say is present in the field prior to the arrival of soybean aphid, due to its ability to feed on alternative small prey and on the soybean plant itself (Costamagna et al. 2008). Studies also show that lady beetles play an important role in suppressing soybean aphid population (Fox et al. 2004, Costamagna and Landis 2006, 2007; Costamagna et al. 2008).

Reports of field parasitism of soybean aphid in North America indicate generally very low levels, with only a few reports with less than 10% parasitism (Nielsen and Hajek 2005, Costamagna and Landis 2006, Baute 2007, Noma and Brewer 2008). In a study in Michigan, six species of established parasitoids (Hymenoptera: four Braconidae and two Aphelinidae) parasitized aphids on infested soybeans (Noma and Brewer 2008). Due to the lack of effective parasitoids, efforts are currently underway to introduce parasitoids from Asia (Wyckhuys et al. 2009).

During outbreak years, cultural practices and biological control are not sufficient to keep soybean aphids under control, thus growers currently rely on chemical control. Numerous pesticides have been tested and applied to manage soybean aphid in China, including cyhalothrin, fenvalerate, omethoate, aldicarb, carbofuran, imidacloprid, pirimicarb, chloromethiuron, phosalone, deltamethrin, phorate and sumi-alpha (Chen and Yu 1988, Huang et al. 1998, Wu et al. 1999, Sun et al. 2000).

In North America, thè most commonly used foliar insecticides are chlorpyrifos, acephate, esfenvalerate, permethrin, and λ-cyhalothrin (NASS 2006). Many of these insecticides are highly toxic and have a broad spectrum of activity. In 1999, prior to the discovery of soybean aphid, less than 1% of the soybean acreage in Michigan was treated with insecticides (NASS 2000). In 2005, an outbreak year, 42% of Michigan soybean acres were treated, indicating the rapid increase in insecticide use since the discovery of this pest. Similar increases were observed in many North Central states (NASS 2000; 2006).

Unfortunately, the increase in acreage sprayed has both economic and environmental costs. Although use of insecticides can be a quick, easy way to control soybean aphid, frequent applications of broad-spectrum insecticides can lead to build up of aphid-resistance resulting in more chemicals being used with potentially severe environmental side effects. Secondary pests such as spider mites can also increase in fields after insecticide treatments (Steffey 2005). Another obvious consequence of insecticide use is the reduction of biodiversity in agricultural systems (Gao et al. 1993, Sun et al. 2000, Wu et al. 2004). In addition, spraying soybean fields with insecticides to control aphids can kill beneficial insects, and may cause environmental pollution (Sun et al. 2000).

Significant insecticide costs have been inevitable with soybean aphid control since its introduction in the North central States. Song et al. (2006) estimated a total yield loss exceeding 350 million bushels in the north-central states, if soybeans were left untreated. In 2004, Michigan soybean growers have reported spending $8-12/acre for insecticide application (Song et al. 2006).

Ragsdale et al (2007) developed an economic threshold (ET) to reduce unnecessary insecticide applications against soybean aphid. The average ET over all control costs, market values, and yield was 273 ± 38 aphids per plant. This ET provided a 7-d lead-time before soybean aphid populations exceeded the economic injury level (EIL) of 674 ± 95 aphids per plant (Ragsdale et al. 2007).This ET currently does not take into consideration, factors that may influence soybean aphid populations such as, weather conditions, and natural enemy populations. To date, use of insecticides is the only cost effective method to manage soybean aphid outbreaks in field. However, chemical control of soybean aphid is not widely accepted by organic soybean growers and consumers (Mian et al. 2008a).

Insecticide susceptibility of soybean aphid

Increased insecticide use raises the probability of developing resistance to insecticides. Thus, monitoring insecticide susceptibility is essential to effectively manage soybean aphid in the future. The Insecticide Resistance Action Committee (IRAC) defines resistance to insecticides as "a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species".

Pyrethroids and organophosphates were the first groups of insecticides used against soybean aphid, soon after its discovery in the United States (NASS 2001). In 2005, which was an outbreak year in the United States, a survey conducted in 17 states with 64.8 million soybean acres reported that the top two insecticides used were λ-cyhalothrin (Figure 1.2), a pyrethroid (6% of area applied) and chlorpyrifos (Figure 1.3), an organophosphate (5% of area applied) (NASS 2006). One or two foliar applications of λ-cyhalothrin or chlorpyrifos, at early reproductive stages (R2 to R3 stages) have shown to minimize yield loss (Myers et al. 2005).

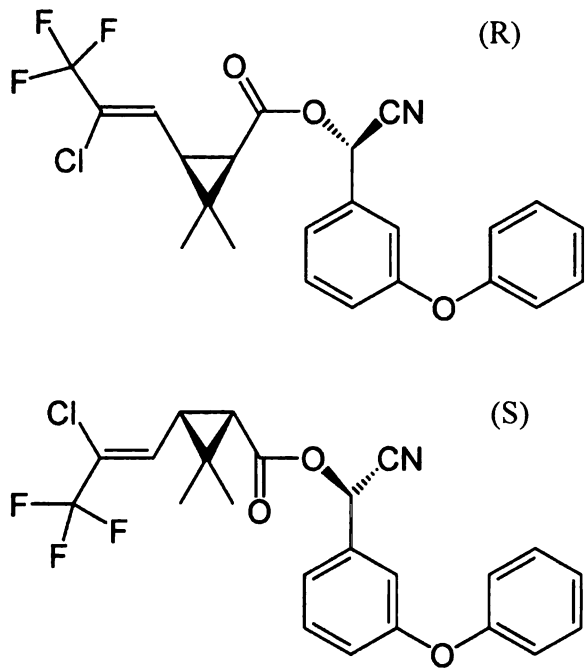

Figure 1.2. Chemical structure of λ- cyhalothrin [(RS)-alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3, 3, 3-trifluoropropenyl)-2,2,-dimethylcyclopropanecarboxylate (12)].

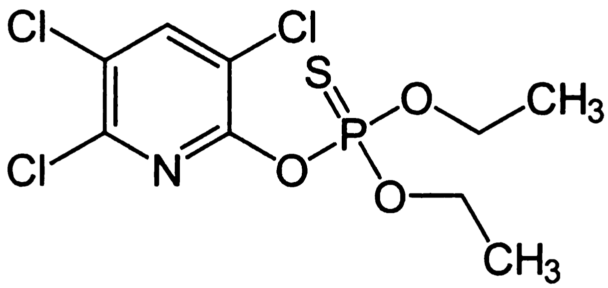

Figure 1.3. Chemical structure of chlorpyrifos [*O, O*-diethyl *O*-3, 5, 6-trichloro-2-pyridyl phosphorothioate].

Pyrethrins are natural compounds derived from plants in chrysanthemum family. Although they have a quick effect (knock-down) against insects, due to their instability in the environment, these may not last long enough to kill the insect. Pyrethroids such as λ-cyhalothrin and esfenvalerate (Figure 1.4) are synthetic versions of pyrethrins, which are specifically designed to be more stable in the environment thus provide longer control. Pyrethroids act on sodium channels on nerve membranes causing excitation of the neurons. Continuous nerve impulse caused by pyrethroids, prevent the sodium channels from closing thus can eventually cause death (Brown 2006).

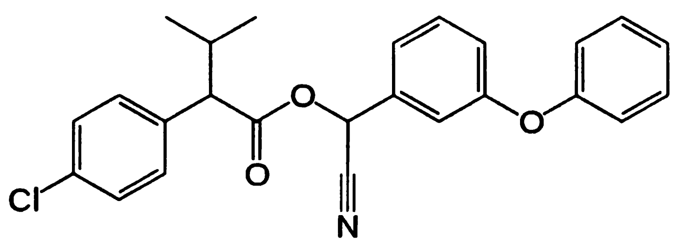

Figure 1.4. Chemical structure of esfenvalerate [(*S*)-α-cyano-3-phenoxybenzyl (*S*)-2-(4-chlorophenyl)-3-methylbutyrate].

Organophosphate insecticides are cholinesterase inhibitors. They bind to acetylcholine esterase, an enzyme that is responsible for the breakdown of acetylcholine (ACh) after the message has passed through the synapse. Cholinesterase inhibitors such as organophosphates, does not allow ACh to breakdown, thus the neuron continues to fire in the poisoned insect. Finally, this uncontrolled overstimulation causes insect death (Brown 2006). Chlorpyrifos is very effective for soybean aphid control due to its fuming action on hot days. Dimethoate (Figure 1.5) is an organophosphate with poor efficacy against soybean aphid, but is one of the few general use products remaining for growers, not requiring certification of application by the state Department of Agriculture.

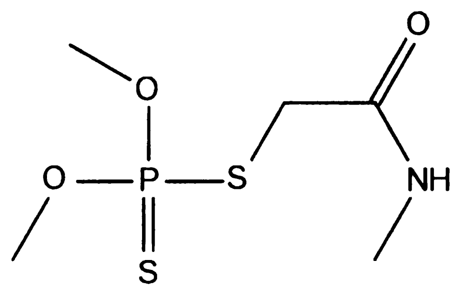

Figure 1.5. Chemical structure of dimethoate [*O,O*-dimethyl *S*-methylcarbamoylmethyl phosphorodithioate].

Although currently there are no reports of development of soybean aphid resistance to these five insecticides there are numerous reports of insecticide resistance in a closely related species, the cotton/ melon aphid, *Aphis gossypii* Glover (Whalon 2009). Herron and Powis (2005) documented chlorpyrifos resistance in 40% of the tested population of Australian field-collected *A. gossypii* in a bioassay. *A. gossypii* collected from cotton fields in Pakistan, showed resistance (resistance ratio of 41 at LC_{50}) to dimethoate in

1998, but reported a decline afterwards. Resistance ratios reported ranged from 1- 41 to dimethoate over the years of 1996-2004 (Ahmad and Arif 2008).

The insecticide fenvalerate was extensively used to control *A. gossypii* and other pests on cotton and other crops for decades, in many countries but more particularly in Asian countries (Wang et al. 2002). *A. gossypii* has become highly resistant to fenvalerate, with survival at extremely high concentrations (Zil'bermints and Zhuravleva 1984, Thayumanavan et al. 1993, Sun et al. 1994). Although not identical, fenvalerate and esfenvalerate are conformational isomers. Populations of *A. gossypii* from Hawaii showed a 390-fold resistance to esfenvalerate (Hollingsworth et al. 1994). *A.gossypii* populations collected in central Pakistan from 1997 to 2000 showed high resistance to seven pyrethroid insecticides with resistance ratios ranging from 205 -723 to λ-cyhalothrin (Ahmad et al. 2003).

Neonicotinoids are a relatively new class of insecticides that are similar in structure to nicotine. Both nicotine and nicotinoids are agonists of ACh receptor, and the result is similar to that caused by cholinesterase inhibitors – overstimulation of neurons causes poisoning and eventually death. Neonicotinoids closely mimic the insect ACh than human ACh, giving more specificity for insects and less ability to poison humans (Brown 2006).

Seed treatments using two neonicotinoids, imidacloprid (Figure 1.6) and thiamethoxam, are registered for soybean aphid control, and their use is becoming widespread (Magalhaes et al. 2008). Imidacloprid is also used as a foliar treatment for soybean aphid control. Megalhaes et al. (2008) developed methods to evaluate susceptibility of soybean aphids to imidacloprid and thiamethoxam. They found both lethal and sublethal effects that affected reproductive capacity and survivorship of soybean aphid. They attributed such effects not only to direct insecticide toxicity but also to possible anti-feedant behavior caused by neonicotinoids (Megalhaes et al. 2008).

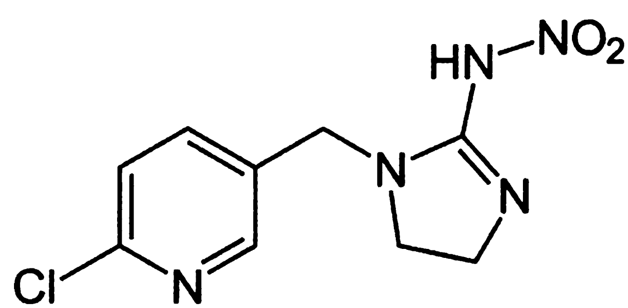

Figure 1.6. Chemical structure of imidacloprid [(*E*-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine].

To date, there are no published reports of field failures with neonicotinoids for soybean aphids. Megalhaes et al. (2008) in their bioassays found that both imidacloprid and thiamethoxam were toxic to soybean aphid. Another *Aphis* species, *A. gossypii* resistance to imidacloprid was reported from the laboratory. In China, Wang et al. (2002) reported an eight-fold increase in the resistance ratio after 13 generations of selection. Thus far, *A. gossypii* resistance to imidacloprid in field has not been confirmed.

An alternative to insecticides is the use of host plant resistance. In attempts to effectively manage soybean aphid using non-chemical approaches, soybean lines with aphid resistance have been identified by several breeding programs in the United States (Hill et al. 2004, Li et al. 2004, Mensah et al. 2005, Diaz-Montano et al. 2006, Hesler et al. 2007, Hesler and Dashiell 2008, Mian et al. 2008).

Host plant resistance

One way to reduce the dependence on insecticides is to grow soybean cultivars with aphid resistance. However, no aphid-resistant cultivar is commercially available to date. The first step to develop a resistant cultivar is to identify the sources of resistance. The three main modes of plant resistance to insects are antibiosis, antixenosis, and tolerance (Painter 1951). Plants with antibiosis resistance affect insect maturity, fecundity, and longevity. Plants with antixenosis resistance are less preferred by insects (Smith 1989). Tolerant genotypes can withstand the same level of colonization that occurs on susceptible genotypes without significant loss in yield.

Chinese scientists first reported resistance to soybean aphid in *G. max* (Fan 1988, Sun et al. 1991) and in *G. soja* (Yu et al. 1988; 1989). Fan (1988) selected two highly resistant varieties from 181 soybean accessions screened during studies in 1984-1986. Yu et al. (1988, 1989) found three highly resistant strains from nearly 1000 strains of wild soybean *G. soja.* These strains demonstrated higher resistance than other susceptible soybean varieties selected from cultivation. He et al. (1995) reported that resistant cultivars had lower aphid populations and were less preferred for feeding than susceptible varieties. Hu et al. (1992) reported that soybean varieties with higher nitrogen content were more susceptible to aphid damage, while high lignin content inhibited infestation.

After the discovery of soybean aphid in North America, several research groups started to search for aphid-resistant germplasm. The first soybean aphid-resistant lines in the United States were reported by Hill et al. (2004). They found nine resistant genotypes after screening >1500 soybean accessions originating from China. Genotypes ‘Dowling’, ‘Jackson’ and PI (Plant Introduction) 71506 had strong resistance in choice and no-choice tests, while four other accessions, ‘Sugao Zarai’, Sato’, ‘T260H’ and PI 230977 showed resistance only in choice tests (Hill et al. 2004). The effects of three resistant soybean genotypes (Dowling, Jackson, and PI 200538-Sugao Zarai’) were compared for fecundity, mortality, and maturation of soybean aphid with susceptible ‘Pana’ and ‘Loda’. Aphid fecundity was lower on Dowling and Jackson while mortality was higher on all three resistant genotypes (Li et al. 2004).

Other resistant genotypes were identified by several university breeding programs. In Michigan, Mensah et al. (2005) screened 2147 soybean accessions in maturity group (MG) 0-III, originally from northern China. In greenhouse and field studies, she found four MG III accessions from Shandong province resistant to soybean aphid. PI 567543C and PI 567597C exhibited antixenosis while PI 567541B and PI 567598B exhibited antibiosis (Mensah et al. 2005, 2008).

In Kansas after comparing 240 entries for aphid reproduction, two genotypes (K1639 and Pioneer 95B97) displayed antibiosis and antixenosis while nine other entries displayed antibiosis only (Diaz-Montano et al. 2006). Hesler et al. (2007) found that Jackson was a strong source of resistance while additional new lines named 'Cobb', 'Tie-feng8' and 'Braxton' were also confirmed as sources of resistance. Hesler et al. (2007) also found a strong source of resistance in PI 230977 and PI 595099. Hesler & Dashiell (2008) later reported three additional resistant lines ('Perrin', 'Tracy-M', and D75-10169).

In Ohio, nearly 200 soybean genotypes (cultivars, breeding lines, and PIs) were screened for resistance to soybean aphid in a greenhouse choice test using aphids collected from the field. Three PIs (PI 243540, PI 567301B, and PI 567324) were identified as resistant while six other PIs were identified as moderately resistant. These were re-confirmed through further field and greenhouse tests. PI 243540 displayed strong antibiosis resistance while the other two resistant PIs displayed mainly antixenosis resistance (Mian et al. 2008b).

Thus far, soybean aphid-resistance in soybean appears to be controlled by one or few genes. A single dominant gene (*Rag1*) controls antibiosis resistance in Dowling (Hill et al. 2006a). Another single gene *(Rag),* which also controls antibiosis, was found in Jackson (Hill et al. 2006b), which is not genetically related to Dowling. However, *Rag1* and *Rag* mapped in the same linkage group, 'M' (Li et al. 2007). Mian et al. (2008b) mapped a different resistant gene in PI 243540 to a different linkage group, 'F', and this gene was named *Rag2*. In contrast, resistance identified by Mensah et al. (2005) in two PIs was controlled by recessive genes that act epistatically. These genes were named *rag1c* (on linkage group M) and rag4 (on linkage group F) for PI 567541B (Zhang et al. 2009), and *rag1b* (on linkage group M) and *rag3* (on Linkage group J) for PI 567598B (Dechun Wang pers. comm.).

Host plant resistance can be overcome by the rise of new biotypes (Auclair, 1989, Smith 1989). If only a single gene is responsible for antibiosis resistance (such as *Rag1*) there is high probability for soybean aphid to overcome this resistance in a relatively short time. In a recent study that tested two soybean aphid biotypes from Ohio and Illinois, *Rag1* resistance was not effective against the Ohio biotype, and these soybean aphids were able to colonize breeding lines with *Rag1* (Kim et al. 2008). Therefore pyramiding of multiple resistance genes, particularly with different modes of action, in the same cultivar has great potential for providing higher and more durable resistance against soybean aphid (Mian et al. 2008a). This concept of 'gene pyramiding' could be a valuable addition to numerous efforts made by soybean breeders to develop aphid-resistant cultivars with long lasting resistance.

Literature Cited

Ahmad, M., M. I. Arif, and I. Denholm. 2003. High resistance of field populations of the Cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) to pyrethroid insecticides in Pakistan. J. Econ. Entomol. 96(3): 875-878.

Ahmad, M., and M. I. Arif. 2008. Susceptibility of Pakistani populations of cotton aphid *Aphis gossypii* (Homoptera: Aphididae) to endosulfan, organophosphorus and carbamate insecticides. Crop Prot. 27: 523–531.

Auclair, J. L. 1989. Host plant resistance, pp. 225–265. In A.K. Minks and P. Harrewijn (eds), Aphids: Their biology, natural enemies, and control. Elsevier, New York.

Baute, T. 2007. Let the scouting begin-soybean aphids are here. Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, Ontario. Available: http://www.omafra.gov.on.ca/english/crops/field/news/croppest/2007/08cpo07a1.htm (Aug 2007)

Brown, A. E. 2006. Mode of action of structural pest control chemicals, Pesticide information leaflet No. 41. Pesticide Education and Assessment Programs, University of Maryland, College Park, MD.

Chen, Q. H., and S. Y. Yu. 1988. Aphids and control. Shanghai Science and Technology Press, Shanghai, China.

Costamagna, A. C., and D. A. Landis. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecol. Appl. 16:1619-1628.

Costamagna, A. C., and D. A. Landis. 2007. Quantifying predation on soybean aphid through direct field observations. Biol. Cont. 42:16-24.

Costamagna, A. C., D. A. Landis, and M. J. Brewer. 2008. The role of natural enemy guilds in *Aphis glycines* suppression. Biol. Cont. 45:368-379.

Diaz-Montano, J. 2006. Components of soybean resistance to the soybean aphid. M.S. thesis, Kansas State University, Manhattan, KS.

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

DiFonzo, C.D. 2006. Soybean aphid and potato viruses - are they related? Michigan Potato Newsline 18(1), Michigan Potato Industry Commission.

DiFonzo, C. D., and K. Agle. 2008. Soybean aphid development on, and BCMV transmission to, Otebo dry bean, *Phaseolus vulgaris*. Crop Management.:10.1094/CM-2008-0916-01-RS.

DiFonzo, C.D., and R. Hines. 2002. Soybean aphid in Michigan: Update from the 2001 season. Extension Bulletin E-2748. Michigan State University, East Lansing, MI.

(ERS) Economic Research Service. 2009. Top 10 U.S. export markets for wheat, corn, soybeans, and cotton, by volume. Available: http://www.ers.usda.gov/Data/FATUS/DATA/ExportCmd.xls (26 May 2009).

Fan, Y. H. 1988. Screening for soybean varieties resistant to the soybean aphid. Soybean Sci. 7: 167-169.

Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. DiFonzo. 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environ. Entomol. 33:608-618.

Gao, J. F., G. X. Zhang, Y. C. Qin, K. Yu, M. H. Li, and Y. Qin.1993. Field survey on overwintering syrphids in Changbai Mountain and artificial protection of overwintering syrphids. Chin. J. Biol. Control 9: 142-143.

Guo, Y. C., S. X. Zhang, L. J. Zhao, and G. F. Chu. 1996. Studies on resistance trends of cotton aphid and cotton bollworm to fenvalerate in fields. Acta Agric. Univ. Henanensis 30: 284-287.

Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D.W. Onstad, J. T. Shaw, L. F. Solter, D. J. Voegtlin, C. J. D'Arcy, M. E. Gray, K. L. Steffey, S. A. Isard, and P. L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Available: www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/ Plant Health Progr. DOI:10.1094/PHP-2001-0205-01-HN (March 2009).

He, F. G., X. D. Liu, F. Y. Yan, and Y. Q. Wang. 1995. Soybean resistance to the soybean aphid. Liaoning Agric. Sci. 4: 30-34.

Herron, G., and K. Powis. 2005. Insecticide resistance of field-collected cotton aphid. Avaialable: http://www.cottoncrc.org.au/files/45711191-ec67-4e5e-9506-9a6b00b7a290/Herron1.pdf (15 July 2009).

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

Hesler, L. S., K. E. Dashiell, and J. G. Lundgren. 2007. Characterization of resistance to *Aphis glycines* in soybean accessions. Euphytica 154:91-99.

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

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

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

Hill, J. H., R. Alleman, D. B. Hogg, and C. R. Grau. 2001. First report of transmission of soybean mosaic virus and alfalfa mosaic virus by *Aphis glycines* in the New World. Plant Dis. 85:561.

Hirano, K., K. Honda, and S. Miyai. 1996. Effects of temperature on development, longevity and reproduction of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). Appl. Entomol. Zool. 31:178-180.

Hollingsworth, R.G., B.E. Tabashnik, D.E. Ullman, M.W. Johnson, and R. Messing. (1994). Resistance of *Aphis gossypii* (Homoptera: Aphididae) to insecticides in Hawaii: spatial patterns and relation to insecticide use. J. Econ. Entomol. 87(2), 293-300.

Hu, Q., W. Q. Zhang, Y. X. Yao, and S. Q. Yan. 1992. The relationship between the nitrogen content in soybean leaves and occurrence degree of *Aphis glycines* Matsumura. J. Jilin Agric. Univ. 14: 103-104 (Translated).

Huang, C. D., J. F. Zhou, and D. Yang. 1998. Experiments on the control of soybean aphid with imidacloprid. Pesticides. 37: 44-45 (Translated).

(IRAC) Insecticide Resistance Action Committee. 2009. Available: http://www.irac-online.org/resistance/overview.asp (14 May 2009).

Iwaki M., M. Roechan, H. Hibino, H. Tochihara, and D.M. Tantera. 1980. A persistent aphid-borne virus of soybean, *Indonesian* soybean dwarf virus, transmitted by *Aphis glycines*. Plant Dis. 64:1027–1030.

Kim, K. S., C. B. Hill, G. L. Hartman, M. A. R. Mian, and B. W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48:923-928.

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

Li, Y., C. B. Hill, S. R. Carlson, B. W. Diers, and G. L. Hartman. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding. 19: 25-34.

Lin, C. L., Z. S. Xun, L. T. Li, Y. P. Wang, and G. X. Zhang. 1992. Control threshold of the soybean aphid in the field. Soybean Sci. 11: 318-321.

Lin, C. L., Z. S. Xun, L. T. Li, H. K. Zhang, G. X. Zhang, and Y. P. Wang. 1994. Population dynamics and control stage of the soybean aphid in Jining prefecture, China. Shandong. Agric. Sci. 4: 44.

Lu, L. H. and R. L. Chen. 1993. Production of the soybean aphid alatae, *Aphis glycines.* Acta Entomol. Sinica 36:143-149.

Magalhaes, L.C., T. E. Hunt, and B. D. Siegfried. 2008. Development of methods to evaluate susceptibility of soybean aphid to imidacloprid and thiamethoxam at lethal and sublethal concentrations. Entomol. Exp. Appl. 128(2): 330-336.

McCornack, B. P., D. W. Ragsdale, and R. C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. J. Econ. Entomol. 97: 854-861.
Mensah, C., C. DiFonzo, R. L. Nelson, and D. C. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45: 2228-2233.

Mensah, C., C. DiFonzo, and D. C. Wang. 2008. Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. Crop Sci. 48:1759-1763.

Mian, M. A. R., R. B. Hammond, and S. K. S. Martin. 2008a. New plant introductions with resistance to the soybean aphid. Crop Sci. 48: 1055-1061.

Mian, M. A. R., S. T. Kang, S. E. Beil, and R. B. Hammond. 2008b. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor. Appl. Gent. 117: 955-962.

Myers, S. W., D. B. Hogg, and J. L. Wedberg. 2005. Determining the optimal timing of foliar insecticide applications for control of soybean aphid (Hemiptera: Aphididae) on soybean. J. Econ. Entomol. 98: 2006–2012.

(NASS) National Agricultural Statistics Service. 2000. Agricultural chemical usage 1999 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2001. Agricultural chemical usage 2000 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2006. Agricultural chemical usage 2005 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2007. Agricultural chemical usage 2006 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2009. National statistics for soybeans. Available: http://www.nass.usda.gov/QuickStats/index2.jsp (19 May 2009).

(NBB) National Biodiesel Board. 2009. Biodiesel education network. Available: http://www.biodiesel.org/askben/top10/. (May 2009).

Nielsen, C. and, A. E. Hajek. 2005. Control of invasive soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. Environ. Entomol. 34:1036-1047.

Noma, T., and M. J. Brewer. 2008. Seasonal abundance of resident parasitoids and predatory flies and corresponding soybean aphid densities, with comments on classical biological control of soybean aphid in the Midwest. J. Econ. Entomol. 101: 278-287.

Painter, R. H. 1951. Insect Resistance in Crop Plants. Macmillan, New York.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97: 204-208.

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

Rutledge, C. E., and R. J. O'Neil. 2005. *Orius insidiosus* (Say) as a predator of the soybean aphid, *Aphis glycines* Matsumura. Biol. Contr. 33: 56–64.

Rutledge, C. E., and R. J. O'Neil. 2006. Soybean plant stage and population growth of soybean aphid. J. Econ. Entomol. 99: 60-66.

Singh P., R. Kumar, S. N. Sabapathy, and A. S. Bawa. 2008. Functional and edible uses of soy protein products. Comprehensive Reviews in Food Science and Food Safety (7):14-28.

Smith, C.M. 1989. Plant resistance to insects: a fundamental approach. John Wiley & Sons, New York.

Smith, K. 1994. Importance of Soybeans. pp 3. In L.G Higley and D.J Boethel (eds), Handbook of Soybean Insects. Entomological Society of America, Lanham, MD.

Song, F., M. S. Swinton, C. DiFonzo, M. O'Neal, and D. W. Ragsdale. 2006. Profitability analysis of soybean aphid control treatments in three North-central States. Department of

Agricultural Economics Staff paper 2006- 24. Michigan State University, East Lansing, MI.

Steffey, K. 2005. Overview of insects and mites in Illinois soybean fields. The Bulletin 18, article 2. University of Illinois Extension, Urbana-Champaign, IL.

Sun, Z. Q., P. Z. Tian, and J. Wang. 1991. Utilization of aphid resistant character in wild soybean, aphid resistant performance of F2. Soybean Sci. 10: 98-103.

Sun, Y. Q., G. L. Feng, J. G. Yuan, and K. Y. Gong. 1994. Insecticide resistance of cotton aphid in North China. Entomol. Sin. 1: 242-250.

Sun, B., S. B. Liang, and W. X. Zhao. 2000. Outbreak of the soybean aphid in Suihua prefecture in 1998 and its control methods. Soybean Bull. 1: 56-58.

Takahashi, S., M. Inaizumi, and K. Kawakami. 1993. Life-cycle of the soybean aphid *Aphis glycines* Matsumura, in Japan. Japanese Journal of Appl. Entomol. and Zool. 37:207-212. (Translated).

Thayumanavan, B., S. Uthamasamy, V. Sumathi, and S. Saroja. 1993. Glutathione S-transferase and carboxyl esterase activities in aphid (*Aphis gossypii* Glover) fed on cotton leaves treated with synthetic pyrethroids. Indian J. Exp. Biol. 31: 788-789.

Van den Berg, H., D. Ankasah, A. Muhammad, R. Rusli, H. A. Widayanto, H. B. Wirasto, and I. Yully. 1997. Evaluating the role of predation in population fluctuations of the soybean aphid *Aphis glycines* in farmers' fields in Indonesia. J. Appl. Ecol. 34: 971-984.

Voegtlin, D. J., R. J. O'Neil, and W. R. Graves. 2004. Tests of suitability of overwintering hosts of *Aphis glycines*: Identification of a new host association with *Rhamnus alnifolia* L'Heritier. Ann. Entomol. Soc. Am. 97: 233-234.

Wang, C. L., L. Y. Xiang, G. X. Zhang, and H. F. Chu. 1962. Studies on the soybean aphid, *Aphis glycines* Mastumura. Acta Entomol. Sinica. 11: 31–44 (Translated).

Wang, S. Y., X. Z. Bao, Y. J. Sun, R. L. Chen, and B. P. Zhai. 1996. Study on the effect of population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. Soybean Science. 15: 243-247 (Translated).

Wang, X. B., Y. H. Fang, S. Z. Lin, L. R. Zhang, and H. D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Plant Protection. 20: 12-13 (Translated).

Wang, Y. Z. and F. Ba. 1998. Study on optimum control of the soybean aphid. Acta. Phyt. Sinica. 25: 152-155 (Translated).

Wang, Y. Z., L. Ma, J. Z. Wang, X. Z. Ren, and W. L. Zhu. 2000. Systematic optimum control of diseases and insect pests in summer soybean. J. Ecol. 20: 502-509.

Wang, K.Y., T. X. Liu, C. H. Yu, X. Y. Jiang, and M. Q. Yi. 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to fenvalerate and imidicloprid and activities of detoxification enzymes on cotton and cucumber. J. Econ. Entomol. *95*: 402-407.

Whalon, M. 2009. Athropod pesticide resistance database. Available: http://www.pesticideresistance.org/search/12/0/218/0/ (July 9, 2009).

Wu, X. B., W. J. Ni, and P. J. Liu. 1999. Occurrence and control of the soybean aphid, *Aphis glycines* Matsumura. Chinese J. Biol. Control. 6: 20. (Translated).

Wu, Z. S., D. Schenk-Hamlin, W. Y. Zhan, D. W. Ragsdale, and G. E. Heimpel. 2004. The soybean aphid in China: A historical review. Ann. Entomol. Soc. Am. 97:209-218.

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

Yu, D. R., S. G. Guo, and Y. L. Shan. 1988. Preliminary report on evaluation technique and method of soybean aphid resistance on the wild soybean. Jilin Agric. Sci. 3:1-3.

Yu, D. R., S. G. Guo, and Y. L. Shan. 1989. Resistance of wild soybean *Glycine soja* to *Aphis glycines.* I. Screening for resistant varieties. Jilin Agric. Sci. 3: 15-19.

Zhang, G. R., C. H. Gu, and D. C Wang. 2009. Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor. Appl. Gent. 118(3): 473-482.

Zhang, G. X., and T. S. Zhong. 1982. Experimental study on some aphid life-cycle patterns. Sinozoologica. 2: 7-17.

Zhang, X. R. 1988. Study on the instars of soybean aphid, *Aphis glycines* Matsumura, in Jilin province. Journal of Jilin Agric. Sci. 10: 15-17. (Translated).

Zil'bermints, I.V., and L. M. Zhuravleva. 1984. Response of melon and greenhouse aphids to ambush and actellic. Khim. Sel'sk. Khoz. 3: 37-40.

CHAPTER 2

Assessing soybean aphid (*Aphis glycines* Matsumura) resistance in three Michigan-bred soybean breeding lines

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Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native of Asia, and is one of the most serious insect pests of soybean [*Glycine max* L. (Merrill)] (Yu et al. 1989, Wang et al. 1996, Wu et al. 1999, Sun et al 2000, Hill et al. 2004, Ragsdale et al. 2004). It has been a soybean pest in China, Japan, South Korea, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and Russia for many years (Wu. et al. 2004). In recent years, the soybean aphid was discovered in Australia (1999), Canada (2000), and the United States (2000) (Wu et al. 2004). By 2004, it was found in 24 states in the United States, including Michigan, and in three Canadian provinces (Ragsdale et al. 2004, Rutledge and O'Neil 2006).

Soybean aphid reduces yield directly via plant feeding and indirectly through reduction in seed protein content (Wang et al. 1994). Heavily infested plants show wrinkled and distorted foliage, early defoliation, stem and leaf stunting, reduction in number of pods and seed weight, and even plant death (Wang et al. 1962, Wang et al.1996, Lin et al. 1992, 1994; He et al. 1995, Wu et al. 1999, DiFonzo and Hines 2002, Wu et al, 2004, Diaz-Montano et al. 2006). Honeydew excreted by aphids builds up on foliage, and supports the growth of sooty mold, affecting plant photosynthesis, yield, and seed quality (Chen and Yu 1988). Significant yield loss can occur due to feeding damage by soybean aphid. Yield reduction up to 70% was reported in China when infestations occurred (He et al. 1995, Wang et al. 1996). In China, yield was reduced up to 52% when soybeans in the early vegetative stage (first node stage) were inoculated with 220 aphids per plant (Wang et al. 1994). In addition to yield loss from direct feeding, another threat posed by soybean aphid is its ability to transmit plant viruses such as alfalfa mosaic virus, soybean dwarf virus, and soybean mosaic virus to soybean and other crops (Iwaki et al. 1980, Hartman et al. 2001, DiFonzo and Agle 2008).

Soybean aphid can be managed by several approaches including biological control, chemical control, and host plant resistance. These control options can be used individually or together for effective management (Brosius et al. 2007). Lady beetles (*Coccinellidae*), green lacewings (*Chrysopidae)* and minute pirate bugs (*Orius* spp.) are among the predators that feed on soybean aphid in the United States (Fox et al. 2004, Rutledge and O'Neil 2005). Predators are a significant factor in regulating soybean aphid populations (Fox et al. 2004). Introduction of parasitoids or use of already established parasitoids is another approach recently attempted for controlling soybean aphid. Reports of field parasitism of soybean aphid in North America indicate generally very low levels, with only a few reports with less than 10% parasitism (Nielsen and Hajek 2005, Costamagna and Landis 2006, Baute 2007, Noma and Brewer 2008). In Michigan, six species of established parasitoids (Hymenoptera: four Braconidae and two Aphelinidae) parasitized aphids on infested soybeans (Noma and Brewer 2008). However, due to the lack of effective parasitism, efforts are currently underway to introduce parasitoids from Asia (Wyckhuys et al. 2009).

Biological control is not always sufficient to keep soybean aphid populations below damaging levels. Soybean aphid outbreaks in the Midwestern United States occurred almost every other year since 2001 and there are reports of localized outbreaks every season (Chris DiFonzo pers. comm.). To date, insecticides are the primary means to manage these outbreaks in the field. Insecticides are a quick, effective way to control soybean aphid. However, frequent applications of broad-spectrum insecticides can increase the possibility of developing insecticide resistance in aphids (Gao et al. 1993, Sun et al. 2000, Wu et al. 2004). Application of broadspectrum insecticides also kill natural enemies that can lead to increase of non-target pests such as spider mites in soybean fields. Significant insecticide costs are inevitable with soybean aphid control. In 1999, prior to the discovery of soybean aphid, less than 1% of the soybean acreage in Michigan was treated with insecticides (NASS 2000). In 2005, an outbreak year, 42% of the acres were treated, illustrating the rapid increase in insecticide use since the discovery of this pest. Similar increases were observed in many North Central states (NASS 2000 and 2006). In 2004, Michigan soybean growers have reported spending $8-12/acre for insecticide application (Song et al. 2006). Song et al. (2006) also estimated a total yield loss exceeding 350 million bushels in north-central states, if soybeans were left untreated.

One way of reducing the dependence on insecticides is to grow soybean cultivars with aphid resistance. However, no aphid-resistant cultivar is commercially available to date. The first step to develop a resistant cultivar is to identify the sources of resistance. The three main modes of plant resistance to insects are antibiosis, antixenosis, and 'tolerance'

(Painter 1951). Plants with antibiosis resistance affect insect maturity, fecundity, and longevity. Plants with antixenosis resistance are less preferred by insects (Smith 1989). 'Tolerant' genotypes withstand the same level of colonization that occurs on susceptible genotypes without significant loss in yield.

Chinese scientists reported resistance to soybean aphid in cultivated soybean, *G. max* (Fan 1988, Sun et al. 1991) and in wild soybean, *G. soja* (Yu et al. 1988 and 1989). Fan (1988) selected two highly resistant varieties from 181 soybean accessions screened in 1984-1986. Yu et al. (1988, 1989) found three highly resistant strains from nearly 1000 strains of *G. soja* and these strains demonstrated higher resistance than other varieties selected from cultivated soybean. He et al. (1995) reported that resistant cultivars had lower aphid populations and were less preferred for feeding than susceptible varieties. Hu et al. (1992) reported that soybean varieties with higher nitrogen content were more susceptible to aphid damage, while high lignin content inhibited infestation.

After the discovery of soybean aphid in North America, several research groups started to search for aphid-resistant germplasm. The first soybean aphid-resistant lines in the United States were reported by Hill et al. (2004). They found nine resistant genotypes after screening >1500 soybean accessions originating from China. Genotypes 'Dowling', 'Jackson', and PI (Plant Introduction) 71506 had strong resistance in both choice and no-choice tests (Hill et al. 2004). When three resistant soybean genotypes (Dowling, Jackson, and PI 200538-Sugao Zarai) were compared to susceptible 'Pana' and 'Loda' for fecundity, mortality, and maturation of soybean aphid, aphid fecundity was lower on

Dowling and Jackson while mortality was higher on all three resistant genotypes (Li et al. 2004).

Other resistant genotypes were identified by several university breeding programs. In Michigan, Mensah et al. (2005) screened 2147 soybean accessions in maturity group (MG) 0-III, originally from northern China. In greenhouse and field studies, she found four MG III accessions from Shandong province resistant to soybean aphid. PI 567543C and PI 567597C exhibited antixenosis while PI 567541B and PI 567598B exhibited antibiosis (Mensah et al. 2005, 2008).

In Kansas after comparing aphid reproduction in 240 entries, two genotypes (K1639 and Pioneer 95B97) displayed antibiosis and antixenosis while nine other entries displayed antibiosis only (Diaz-Montano et al. 2006). Hesler et al. (2007) found that Jackson was a strong source of resistance while the new lines named 'Cobb', 'Tie-feng8' and 'Braxton' were also confirmed as sources of resistance. Hesler et al. (2007) also found a strong source of resistance in PI 230977 and PI 595099. Hesler and Dashiell (2008) later reported three additional resistant sources ('Perrin', 'Tracy-M', and D75-10169).

In Ohio, nearly 200 soybean genotypes (cultivars, breeding lines, and PIs) were screened for resistance to soybean aphid in a greenhouse choice test using aphids collected from the field. Three PIs (PI 243540, PI 567301B, and PI 567324) were identified as resistant while six other PIs were identified as moderately resistant. These were re-confirmed through further field and greenhouse tests. PI 243540 displayed strong antibiosis resistance while the other two resistant PIs displayed mainly antixenosis resistance (Mian et al. 2008a).

Thus far, soybean aphid-resistance in soybean appears to be controlled by one or few genes. Single dominant genes control antibiosis resistance in Dowling (*Rag1*) and in Jackson *(Rag),* (Hill et al. 2006a, and 2006b). Both Rag1 and *Rag* mapped in the same linkage group, 'M' (Li et al. 2007). Mian et al. (2008b) mapped a different resistant gene *Rag2*, in PI 243540 to a different linkage group, 'F'. In contrast, resistance identified by Mensah et al. (2005) in two PIs was controlled by recessive genes that act epistatically. These genes were named *rag1c* (on linkage group M) and *rag4* (on linkage group F) for PI 567541B (Zhang et al. 2009), and *rag1b* (on linkage group M) and *rag3* (linkage group J) for PI 567598B (Dechun Wang pers. comm.).

Host plant resistance can be overcome by the rise of biotypes (Auclair, 1989, Smith 1989). If only a single gene is responsible for antibiosis resistance (such as *Rag1*) there is high probability for soybean aphids to overcome this resistance in a relatively short time. In a recent study that tested two soybean aphid biotypes from Ohio and Illinois, *Rag1* resistance was not effective against the Ohio biotype, thus these soybean aphids were able to colonize breeding lines with *Rag1* (Kim et al. 2008). Therefore pyramiding of multiple resistance genes, particularly with different modes of action, in the same cultivar has great potential of providing a higher and more durable resistance against soybean aphid

(Mian et al. 2008a). This concept of 'gene pyramiding' could be a valuable addition to numerous efforts made by soybean breeders to develop aphid-resistant cultivars with long lasting resistance.

To further confirm the effectiveness of *rag1b* and *rag3*, PI 567598B was crossed with a susceptible commercial cultivar (Titan RR) to develop a series of aphid-resistant breeding lines. This study reports field experiments on three lines (E06901, E06905 and E06906) which inherited aphid-resistance from PI 567598B. The objectives of this study were to compare natural aphid infestation on, and yield of, these aphid-resistant lines with an aphid-susceptible line. The comparisons were done in the presence or absence of natural enemies and with or without an insecticide.

Materials and methods

Study sites

Studies were conducted at the Michigan State University (MSU) Entomology Research Farm, East Lansing, MI (2007, 2008) and at the MSU Saginaw Valley Sugar Beet and Dry Bean Research Farm, Saginaw, MI (2008). Each site consisted of 15.2 m x 9.1 m (12 row) plots arranged in a randomized complete block design with four replications. The treatments were three aphid-resistant lines (E06901, E06905, and E06906) and an aphid-susceptible commercial line (2007-Dekalb 27-53, 2008-Titan RR). A 4.6 m cultivated border was maintained between each replicate. In 2007, soybeans were planted in East Lansing on 15 May at a rate of 253,669 seeds/ha. In 2008, soybeans were planted on 13 May in East Lansing at 253,669 seeds/ha and on 16 May in Saginaw at 365,807 seeds/ha. Weeds were controlled using a combination of pre-emergence herbicides (Pursuit DG (102.2 ml/ha, BASF® Corporation, Florham Park, NJ) and Dual Magnum (105.12 ml/ha, Syngenta, Wilmington, DE) on 30 May 2007, and a combination of Dual Magnum (225 ml/ha Syngenta, Wilmington, DE) and Authority® First DF (292 ml/ha, FMC Agricultural products, Philadelphia, PA) was used on 13 May 2008, in East Lansing site.

Soybean aphid sampling on resistant and susceptible lines

To compare natural aphid infestation among lines, whole-plant soybean aphid counts, and number of plants infested were sampled (ten plants per plot) from mid-June until end of field season. Plant stages (V and R) were also recorded on each sampling date. As a measure of seasonal aphid abundance, Cumulative Aphid Days (CAD) were calculated as outlined by Hanafi et al. (1989) (Equation 1) where n is the number of sampling days, x_i is the mean number of aphids per plant on sample day i, x_{i-1} is the mean number of aphids per plant on the previous sample day, and t_i is the number of days since the previous sampling date. CAD represents the area under the aphid population curve, providing a single number that describes aphid density over a period of time (Ragsdale et al. 2006).

$$\text{CAD} = \sum_{i=1}^{n}\left(\frac{x_{i-1}+x_i}{2}\right)\times t_i \quad \text{........................Equation (1)}$$

Predator-proof cage study on resistant and susceptible lines

Predator-proof cages were used to compare the impact of natural enemies on aphids infesting aphid-resistant and aphid-susceptible lines. The cage consisted of a 1m commercial tomato frame covered by a mesh bag (no-see-um netting, 1.8 mm mesh size, Venture Textiles, Braintree, MA) (Figure 2.1). Prior to aphids colonizing the field, two randomly selected plants in the untreated half of each plot were caged to keep them aphid-free (15 June 2007, 3 June 2008 in East Lansing, and 5 June 2008 in Saginaw). The legs of the frame were pushed into the soil, and then a tube made of mesh was placed over the frame. The bottom edges of the bag were buried in soil and top was tied to prevent entry of insects.

The plants remained caged until the R1 stage (29 June 2007, 14 June 2008 in East Lansing, and 17 June 2008 in Saginaw). Each caged plant was artificially infested by carefully placing 10 adult aphids on the youngest fully-opened trifoliate using a fine paint brush. Aphids for infestation were collected from a neighboring soybean field. One plant per plot was randomly selected to remain caged for the rest of the experiment (14 days). The mesh cage was removed from the second plant to leave the open frame. A yellow sticky trap (Great Lakes IPM, Vestaburg, MI) was hung at canopy-level in the middle each plot capture natural enemies. After14 days, the total number of aphids on each plant (caged and open) were recorded. In addition, separate counts of three most abundant natural enemies (lady beetles, green lacewings, and minute pirate bugs) in each plot was recorded 14 days after placing the sticky traps. All cages still present were removed from the plants, leaving only the frames so plants could be identified. In 2008 at the end of the season, each plant was individually harvested and number of pods per plant and weight of 100 seeds per plant were recorded. East Lansing site in both years had four replications of predator- cages per treatments, while Saginaw site in 2008 had only three replications of predator- cages per line.

Figure 2.1. Natural predator- proof cage in East Lansing, MI 2008. Plants were caged at early vegetative stage (v3-v4) prior to natural aphid colonization in the field.

Yield response of resistant and susceptible lines with and without insecticide

To compare the yield response of lines with and without insecticide protection, half of each plot (six rows) was kept aphid-free with applications of λ-cyhalothrin (233.6 ml/ha

Warrior with Zeon Technology, Syngenta, Wilmington, DE) in both 2007 and 2008. Applications were made using a twin jet nozzle at 40 psi at 49.42 gallons/ha. Once insecticide treatment began (In 2007 - 22 July and 1 August at East Lansing, In 2008 -31 July at East Lansing, and 30 July at Saginaw) whole-plant aphid counts were taken separately from treated and untreated areas until the last sampling date. In 2007, plots were harvested on 15 October (East Lansing). In 2008 plots were harvested on 24 October (East Lansing) and on 20 October (Saginaw). Yield was taken from the two middle rows in treated and untreated halves of each plot. Beans were cleaned, weighed, and yield was corrected to 13% moisture.

Statistical analyses

Aphid count data were analyzed with 1-way Analysis of Variance (ANOVA) in PROC MIXED (SAS 9.3.1., SAS Institute Inc., and 2002-2003). Predator-proof cage study data were analyzed using 2-way analysis of Variance (ANOVA) in PROC MIXED. Agronomic performance data (yield components, treated and untreated yield) were analyzed with 1-way Analysis of Variance (ANOVA) in PROC MIXED. Data were transformed using suitable transformations (square root or arcsine) when normality assumptions were not met (all figures are presented with untransformed data). Mean separations were computed by Tukey's Honest Significant Difference Test (HSD) at 5% probability level. All mean comparisons were conducted using adjusted p values (Tukey-Kramer) at 5% probability level (SAS 9.3.1., SAS Institute Inc., 2002-2003).

Results and discussion

Soybean aphid sampling on resistant and susceptible lines

In 2007, soybean aphid populations in Michigan were low in general. However, differences among breeding lines at East Lansing site were still apparent between aphid resistant lines (E06901, E06905, E06906), and an aphid-susceptible commercial variety (Dekalb 27-53). In 2007, the aphid-resistant lines had very low CAD (1, 4, and 5 respectively) while the susceptible variety, Dekalb 27-53 reached 170 CAD at the end of field season (Figure 2.2).

The average number of aphids per plant in 2007 from East Lansing site did not show significant differences until the week of 25 July (Figure 2.3). Lines appeared to have significant differences in aphid numbers on 25 July ($F = 67.52$; $df = 3, 9$; $P<0.0001$). E06901 ($P<0.0001$), E06905 ($P< 0.0001$) and E06906 ($P<0.0001$) had significantly fewer aphids than the susceptible Dekalb- 2753 when means were compared, but showed no significant differences among them. Number of aphids on Dekalb 27-53 dropped on 3 August, thus showed no significant difference from others ($F =1.18$; $df =3, 9$; $P = 0.3693$). On the last sampling date of 17 August, the differences of aphid abundance became more apparent as aphid numbers peaked ($F =95.12$; $df =3, 9$; $P< 0.0001$). Despite the differences, the aphid populations in the susceptible plots never reached the economic threshold of 250 aphids per plant (Ragsdale et al. 2007). The highest number of aphids counted on a plant was reported from Dekalb 27-53 which was 57. E06901 ($P<0.0001$), E06905 (P< 0.0001) and E06906 (P<0.0001) had significantly fewer aphids than the susceptible Dekalb 27-53 when means were compared, but showed no significant differences among them.

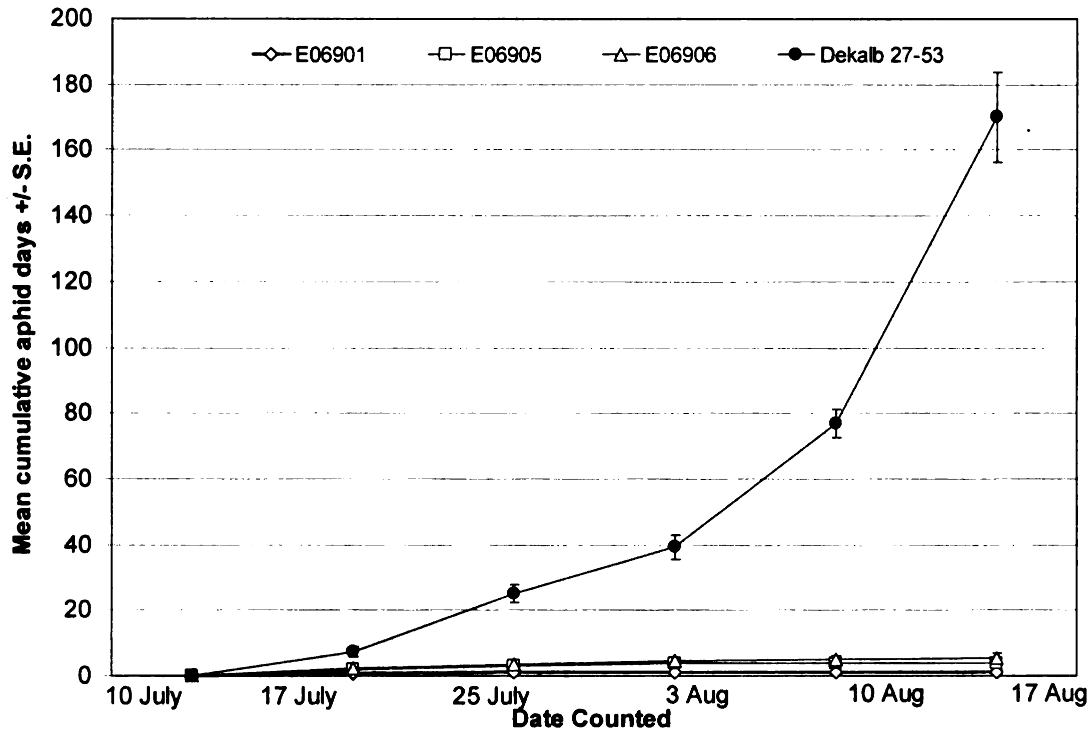

Figure 2.2. Mean cumulative aphid days for soybean aphid-resistant and aphid-susceptible lines East Lansing, MI, 2007.

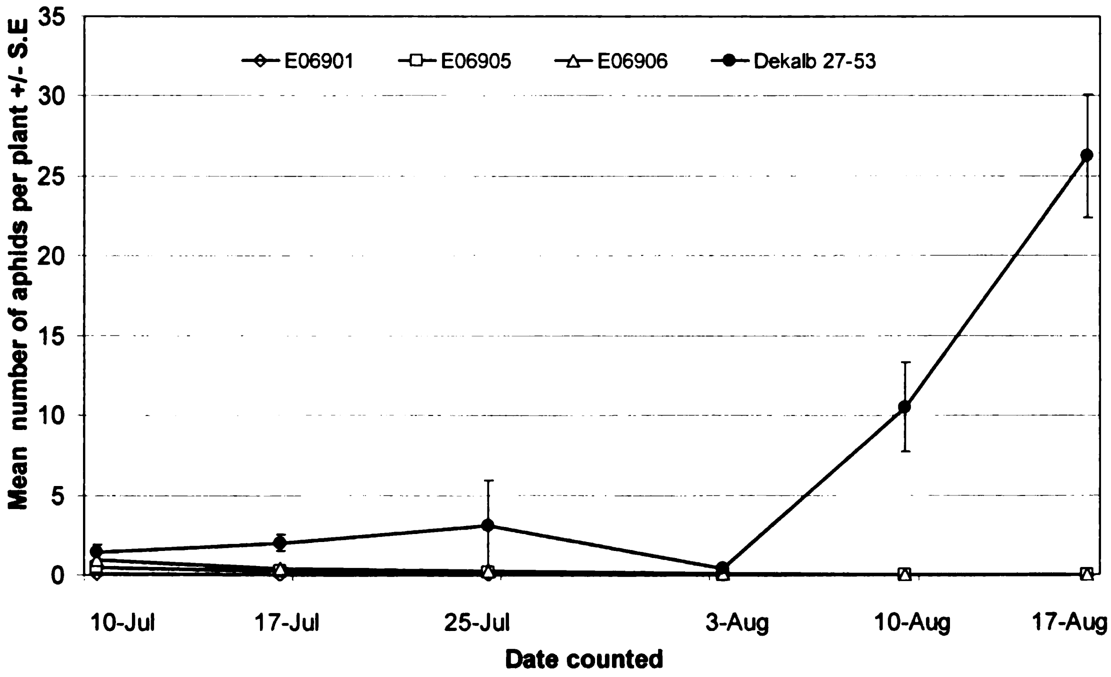

Figure 2.3. Mean number of aphids per plant on aphid-resistant and aphid-susceptible lines, East Lansing, MI, 2007.

In 2008, soybean aphid populations were again low across Michigan including the study sites. However, as in 2007, differences in aphid infestation were highly apparent between aphid resistant lines (E06901, E06905, E06906) and a aphid susceptible parent (Titan RR).

When CAD was calculated, Titan RR had the most, which reached 178 CAD. The three resistant lines E06901, E06905, and E06906 continued to show very low CAD of 1, 2, and 4 respectively (Figure 2.4)

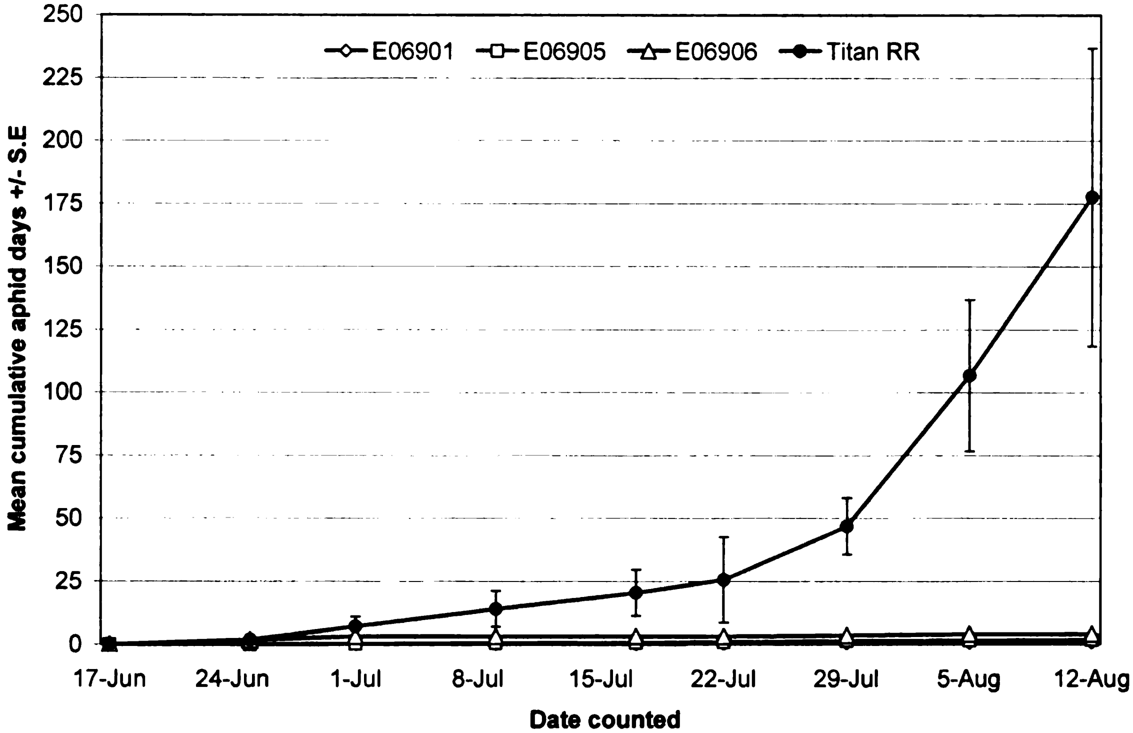

Figure 2.4. Mean cumulative aphid days for aphid-resistant and aphid-susceptible lines, East Lansing, MI, 2008.

There were no significant differences in mean number of aphids per plant on resistant and susceptible lines until July. At East Lansing site, the peak season lasted for about two weeks and numbers gradually decreased towards the end of second week of August. When aphids were sampled on 29 July, differences among lines were obvious and mean aphids per plant was statistically significant on lines ($F = 18.62$; $df = 3, 9$; $P < 0.001$).When means were separated it showed that all three resistant lines had fewer aphids than susceptible Titan RR. On 5 August there were varietal differences again ($F = 7.62$; $df = 3, 9$; $P < 0.0077$). E06901 ($P < 0.0159$), E06905 ($P < 0.0159$) and E06906 ($P < 0.0151$), had significantly fewer aphids than Titan RR. The maximum number of aphids seen on one plant at this site was observed on 5 August on Titan RR, which was 109. Aphid numbers on the MSU aphid-resistant lines continued to show no aphids throughout the season and showed no significant difference among them through the peak period (Figure 2.5).

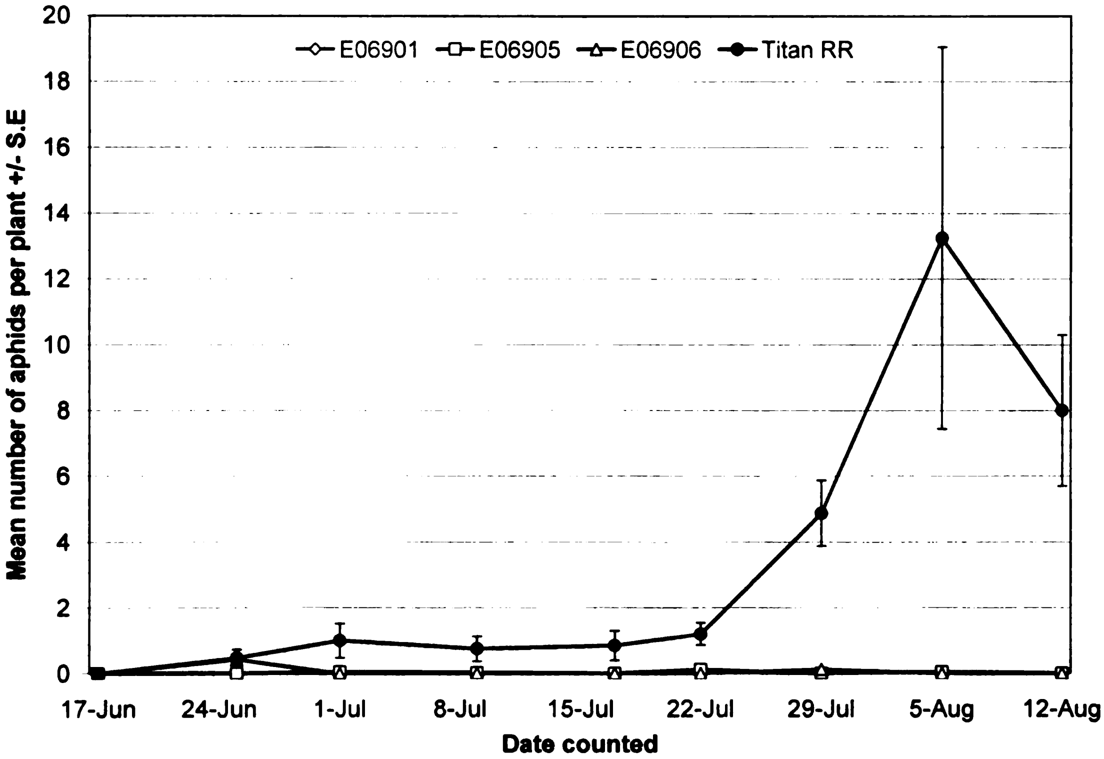

Figure 2.5. Mean number of aphids per plant on aphid-resistant and aphid-susceptible lines, East Lansing, MI, 2008.

In Saginaw site, Titan RR had the most CAD which reached 259 CAD while the three MSU resistant lines had comparatively very low CAD (3, 5, and 5 respectively) similar to observations in East Lansing site (Figure 2.6)

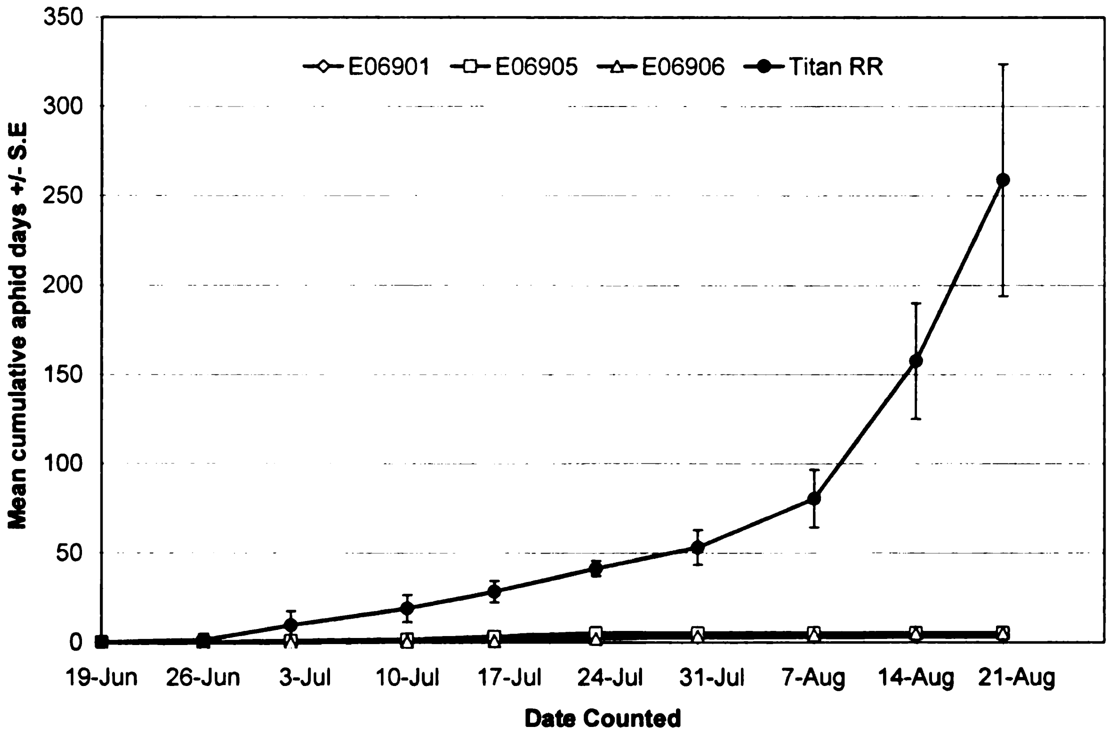

Figure 2.6. Mean cumulative aphid days for soybean aphid-resistant and aphid-susceptible lines in Saginaw site, MI. 2008.

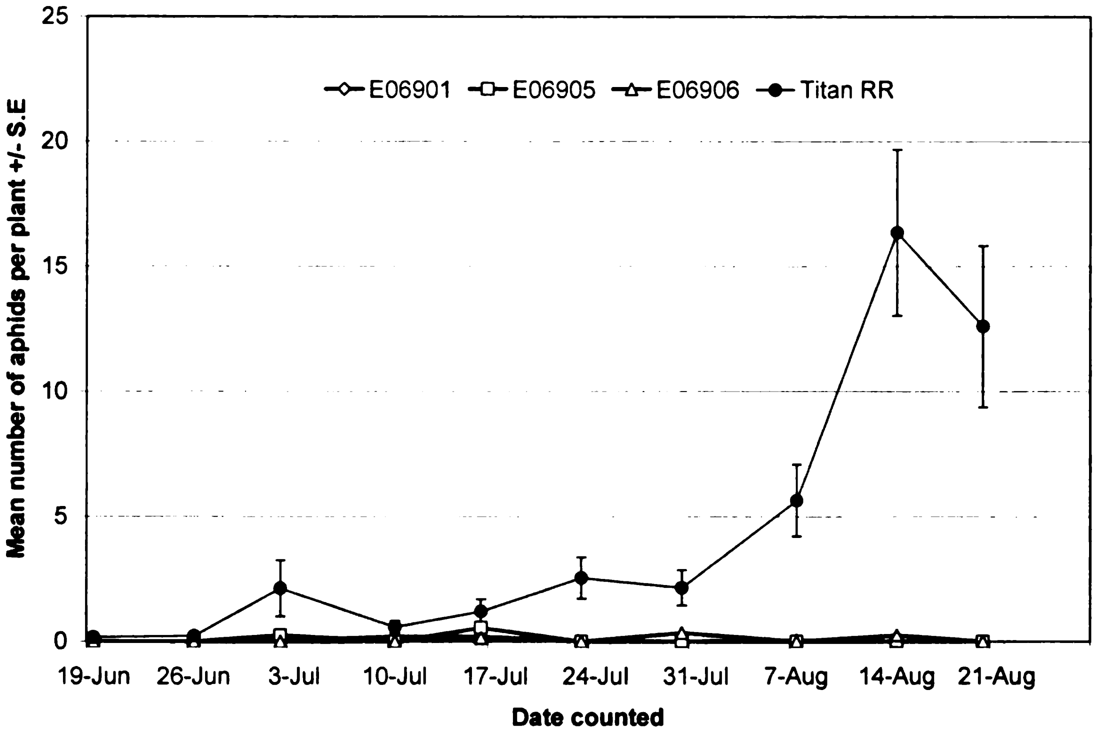

Figure 2.7. Mean number of aphids per plant on soybean aphid-resistant and aphid-susceptible lines, Saginaw, MI, 2008.

The peak week at Saginaw valley research farm was from 7 Aug to 14 August. Although we observed a gradual increase in the week before, were no significant differences in aphid abundance until 7 August. After the peak week the numbers gradually faded around 21 August (Figure 2.7). Significant differences in aphid numbers among lines were observed on 7 Aug ($F = 11.56$; $df = 3, 9$; $P = 0.0019$). 14 Aug ($F = 25.41$; $df = 3, 9$; $P < 0.0001$) 21 Aug ($F = 18.63$; $df = 3, 9$; $P = 0.0003$). On 7 August, E06901 ($P = 0.0032$), E06905 ($P = 0.0032$) and E06906 ($P = 0.014$), and on 14 August E06901 ($P = 0.0002$), E06905 ($P = 0.0002$) and E06906 ($P = 0.0004$), had significantly fewer aphids than Titan RR but showed no difference among them. On 14 August, mean number of aphids per plant increased on Titan RR whilst numbers remained low on the resistant lines. The highest mean number of aphids per plant was reported from Titan RR on 14 August which was 16 aphids per plant.

We also calculated percent plants infested on each resistant and susceptible line in 2007 and 2008. For East Lansing site in July of 2007, mean percent plants infested on all four lines were significantly different between resistant and susceptible lines ($F = 63.19$; $df = 3, 11$; $P < 0.0001$) (Figure 2.8). When means were separated MSU aphid-resistant lines had significantly lower plants infested (E06901with 4%, E06905 with 3%, and E06906 with 13% plants infested) while the commercial susceptible line, Dekalb 27-53 showed 60% infestation ($P<0.0001$). In August MSU aphid-resistant lines had significantly fewer or no plants infested (E06901with 0%, E06905 with 0%, and E06906 with 3% plants infested) while the commercial susceptible line, Dekalb 27-53 showed 57% infestation ($F = 85.81$; $df = 3, 10.9$; $P<0.0001$). Dekalb 27-53 had the heaviest infestation in all plots, which was 100% on 17 August.

For East Lansing site in June 2008, mean percent plants infested on all four lines did not show any significant differences among them ($F = 1.37$; $df = 2, 10$; $P = 0.2971$) (Figure 2.9). Changes in infestation became clearer in July and August and we observed statistically significant differences ($F = 31.62$; $df = 3, 9$; $P<0.0001$). When means were separated E06901, E06905 and E06906 grouped together with very low infestations while percent plants infested in Titan RR was significantly higher than the resistant lines. In August when soybean aphid infestation significantly peaked ($F = 68.89$; $df = 3, 9$; $P<0.0001$) Titan RR had the heaviest infestation of 72.5% (Figure 2.9). These three MSU resistant lines clearly differentiated from the susceptible line in July and August. No significant differences in soybean aphid infestation were observed among tested lines in June at Saginaw, MI in 2008 (F =10.32; df =2, 2.26; P = 0.073). Number of plants infested significantly changed among lines in July and August (F =55.63, df = 3, 12; P<.0.0001; F =11.855, df =2, 10; P = 0.0.0023 respectively) (Figure 2.10).

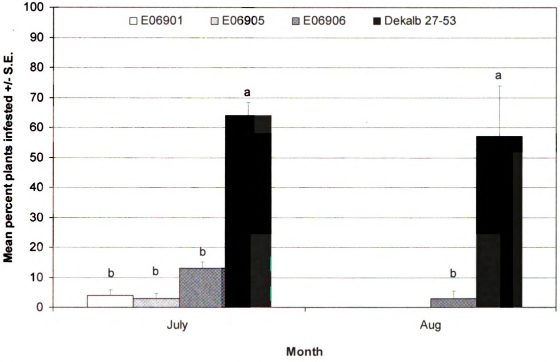

Figure 2.8. Percent soybean aphid infestation on aphid-resistant and aphid-susceptible lines, East Lansing, MI, 2007. Means followed by the same letter are not significantly different within that month (Tukey's HSD test, P = 0.05).

Changes in infestation became clearer in July and August and we observed statistical differences (F =31.62; df =3, 9, P<0.0001). When means were separated E06901, E06905 and E06906 grouped together with very low infestations while percent plants infested in

Titan RR was significantly higher than the resistant lines. In August when soybean aphid infestation significantly peaked ($F = 68.89$; df $= 3, 9$; $P < 0.0001$) Titan RR had the heaviest infestation which was 72.5% (Figure 2.9). These three MSU aphid-resistant lines clearly differentiated from other resistant and susceptible when soybean aphids peaked in July and August. No significant differences in soybean aphid infestation were observed among tested lines in June at Saginaw, MI in 2008 ($F = 10.32$; df $= 2, 2.26$; $P = 0.073$). Number of plants infested significantly changed among lines in July and August ($F = 55.63$, df $= 3, 12$, $P < .0.0001$; $F = 11.855$, df $= 2, 10$, $P = 0.0.0023$ respectively) (Figure 2.10).

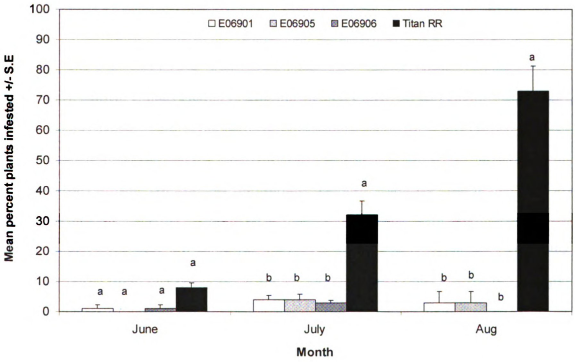

Figure 2.9. Percent soybean aphid infestation on aphid-resistant and aphid-susceptible lines, East Lansing, MI, 2008. Means followed by the same letter are not significantly different within that month (Tukey's HSD test, P = 0.05)

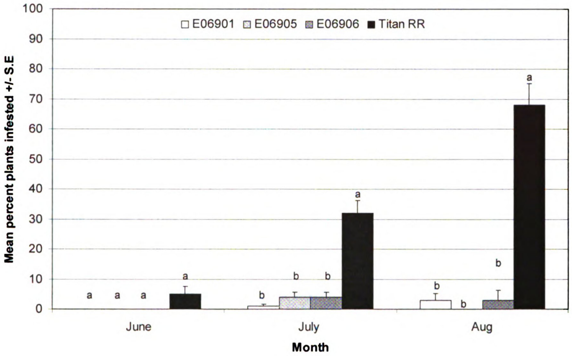

Figure 2.10. Percent soybean aphid infestation on aphid-resistant and aphid-susceptible lines Saginaw, MI, 2008. Means followed by the same letter are not significantly different within that month (Tukey's HSD test, P = 0.05).

Both 2007 and 2008 were low aphid years across many regions in Michigan. Confirming this, none of our sites reached the economic threshold of 250/aphids per plant (Ragsdale et al. 2007). However, we were able to clearly differentiate between resistant and susceptible lines based on aphid abundance. We observed comparatively lower CAD for the resistant lines in all occasions. When aphid outbreaks are present, CAD could rise as above 4000 CAD, although we did not experience such high numbers on susceptible lines.

Our results were further confirmed when number of plants infested were counted from both locations. In 2007 during peak season percentage of plants infested on susceptible lines peaked to a 100% in East Lansing site while E06901, E06905, and E06906 plants had percent infestations as low as 5-20%. In 2008 during peak season percentage of plants infested on susceptible lines peaked to a 72.5% and 68% respectively in East Lansing and Saginaw sites while E06901, E06905, and E06906 plants had percent infestations as low as 5-10%.

Predator-proof cage study on resistant and susceptible lines

In 2007 at East Lansing, MI, the two main effects, line and state of plant (caged or open) showed statistical significances independent of their interaction suggesting presence of differences in soybean aphid abundance based on the breeding line and the state (caged or open) alone, (for line $F = 10.32$; $df = 3,21$; $P < 0.001$; for state $F = 73.24$; $df = 1,21$, $P < 0.001$). The interaction between line and state was highly significant (for interaction $F = 16.18$; df 3,21; $P < 0.0001$) indicating that there was significant differences in aphid numbers between caged and open plants in at least one of the treatments. When mean number of aphids per plant were separated using Tukey's Honest Significance Difference (HSD) test, number of aphids per plant was significantly high on caged plants than on the open plant for Dekalb 27-53 ($P < 0.0001$). However in resistant lines E06901, E06905, and E06905 soybean aphid numbers were not significantly different between caged and open plants ($P = 0.2006$, 0.7964, and 0.1682 respectively) (Figure 2.11). Mean number of soybean aphids reported from a susceptible caged plant was 455 compared to none on the open plant.

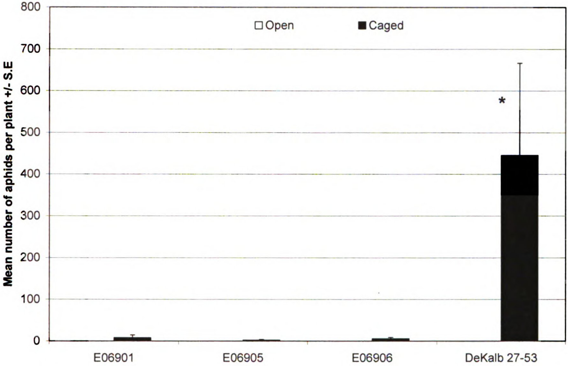

Figure 2.11. Mean number of soybean aphids on open and caged plants, 14 days after infestation in East Lansing, MI 2007. Mean separations were conducted using Tukey's HSD (P = 0.05). * Denotes significant differences between the open (with predator) and caged (predator-free) treatment within a line.

In East Lansing site in 2008, we carried out the same predator- proof cage study with E06901, E06905, E06906, and Titan RR. The interaction between the two main effects (line and state) showed high significance indicating that there was at least one line with significant differences in number of aphids per plant in caged and open plants (for line $F = 19.32$; $df = 3, 21$; $P < 0.001$; for state $F = 35.70$; $df = 1,21$; $P < 0.001$; for interaction $F = 7.34$; $df = 3,21$; $P = 0.0015$). Similar to our observations in 2007, the susceptible Titan

RR showed significant differences in aphids per plant between the caged and open plant in HSD test (P< 0.001) while no differences in aphid abundance were indicated between caged and open plants for E06901 (P = 0.6683), E06905 (P = 0.9904) and E06906 (P = 0.2537) when means were separated (Figure 2.12). Mean number of aphids reported from a susceptible caged plant was 412 compared to none on the open plant.

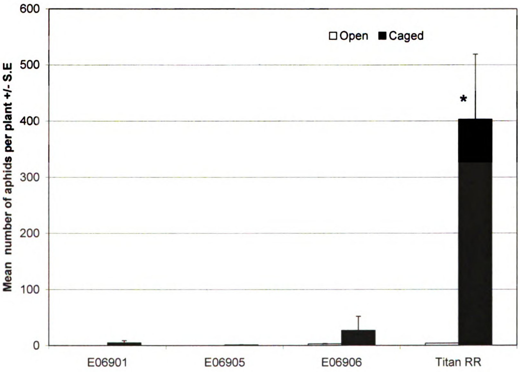

Figure 2.12. Mean soybean aphids on open and caged plants, 14 days after infestation in East Lansing, MI, 2008. Mean separations were conducted using Tukey's HSD (P = 0.05). * Denotes significant differences within a line between the open (with predator) and caged (predator-free) treatment.

In the same year at Saginaw site, we observed similar results. The two fixed effects (line and state) and their interaction was significant at 5% significance level (for line F =

26.16; df = 3, 14; P<0.001; for state F = 23.95; df =1, 3.84; P = 0.0090; for interaction F = 11.96; df = 3, 14; P = 0.0012). The interaction between line and state was highly significant indicating that there were significant differences in aphid numbers between caged and open plants in at least one of the lines. When mean aphids per plant were compared, caged Titan RR plants had statistically higher number of aphids than the open plants (P<0.0038) while other comparisons within resistant lines were not significant [E06901 (P = 1.0000), E06905 (P = 1.0000) and E06906 (P = 0.6177)] (Figure 2.13).

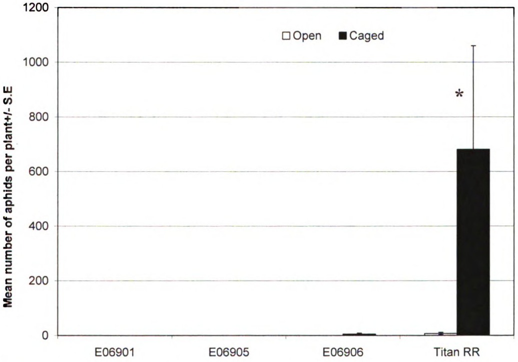

Figure 2.13. Mean soybean aphids on open and caged plants, 14 days after infestation in East Lansing site, MI, 2008. Mean separations were conducted using Tukey's HSD (P = 0.05). * Denotes significant differences within a line between the open (with predator) and caged (predator-free) treatment.

In all locations, caged susceptible plants had significantly more aphids than their corresponding open plants showing natural predators (most abundant were lady beetles) controlling soybean aphids. Abundance of three natural enemies, lady beetles (Coccinellidae), green lacewings (Chrysopidae) and, pirate bugs (*Orius* spp.) on each line was recorded using yellow sticky traps (Table 2.1). Aphids did not succeed on caged resistant lines due to presence of antibiosis resistance that affected their fecundity. There was no additional benefit of natural enemy predation on all three resistant lines, thus the antibiosis resistance was as or more effective than natural predators in controlling the aphid populations.

Table 2.1. Yellow sticky trap data of three natural enemies on susceptible and resistant lines after 14 days

E. Lansing 2007	**Average # of insects per sticky trap per line**		
Line	**Lady beetles (Coccinellidae)**	**Green lacewings (Chrysopidae)**	**Pirate bug (Orius spp.)**
E06901	0.5	0	0
E06905	0	0.7	0
E06906	0	0	0
Dekalb 27-53	2.2	0	2.0
E. Lansing 2008			
E06901	1.2	0	0.2
E06905	2.0	0.2	0.5
E06906	2.5	0	0
Titan RR	3.2	1.2	0
Saginaw 2008			
E06901	1.7	0.3	0.3
E06905	1.0	0	0
E06906	3.0	0	0
Titan RR	3.3	0.3	0.3

To investigate the impact of soybean aphid pressure on yield components in the absence and presence of natural enemies, caged and open plants were individually harvested from all lines in October, and yield components (number of pods per plant, weight of 100 seeds per plant) were measured. In East Lansing, the caged plants of Titan RR had significantly fewer number of pods per plant than the open plants (Table 2.2, $P = 0.0174$). However the weight of 100 seeds on caged and open Titan RR plants were not significantly different at a significance level of 0.05 ($P= 0.0649$). The aphid numbers on caged Titan RR were significantly higher than on the open plant of the same line ($P<0.001$). Natural enemies played an important role in reducing aphid numbers on open Titan RR plants, thus their yield was protected (Table 2.1). There was no significant difference in number of pods per plant, and 100 seed weight (g) between caged and open plants of E06901, E06905, and E06906 in East Lansing site (Table 2.2). Both caged and open plants had no or very low (less than 10) aphids per plant in lines E06901, E06905 and E06906 due to the strong antibiosis resistance. Thus, the presence or absence of natural enemies did not affect aphid numbers.

However, in Saginaw the differences between yield components in caged and open Titan RR plants were not significant (Table 2.2, $P = 0.2967$). The resistant lines, E06901 and E06906 had no significant differences between caged and open plants for both yield components, also no significant differences were observed in weight of 100 seeds of caged and open E06905 plants, again supporting the theory that antibiosis resistance was as or more effective than natural predators in controlling the aphids thus aphid populations had no significant effect on yield. However, we saw significantly lower number of pods on open E06905 than the caged E06905 plant (Table 2.2). This difference may have occurred because data from only 2 replications could be used for analyses of yield components due to mechanical errors in collecting yield (less number of replications).The analysis of # of pods per plant and weight of 100 seeds were unbalanced, due to loss of few bags with individual plants from some lines, thus not all lines had equal number of replications (E06905 and E06901 had only 2 replications each, at Saginaw site, and E06901 had only 2 replications at East Lansing site).

Table 2.2. Effect of predation on # of pods per plant, and seed weight of aphid-resistant and aphid-susceptible lines

Location: East Lansing

	Aphids per plant			# pods per plant			weight (g) of 100 seeds		
Line	**open**	**caged**	**p-value**	**open**	**caged**	**p-value**	**open**	**caged**	**p-value**
E06901	0	5	0.6683	210	170	0.537	13.20	11.41	0.6257
E06905	0	1	0.9904	135	128	0.3807	11.38	12.53	0.3003
E06906	3	27	0.2537	96	111	0.4313	13.27	13.82	0.4556
Titan RR	4	403	<0.0001	127	66	0.0174*	16.47	14.32	0.0649

Location: Saginaw

	Aphids per plant			# pods per plant			weight (g) of 100 seeds		
Line	**open**	**caged**	**p-value**	**open**	**caged**	**p-value**	**open**	**caged**	**p-value**
E06901	0	0	1.0000	142	208	0.6166	11.10	11.42	0.8305
E06905	0	0	1.0000	53	155	0.0374*	14.43	10.9	0.2916
E06906	0	5	0.6177	107	106	0.9642	12.76	14.07	0.4113
Titan RR	7	681	0.0038	102	88	0.1052	14.40	12.24	0.2967

* Indicates significant differences in yield components between caged and open plant within a line

Yield response of resistant and susceptible lines with and without an insecticide

There could be many factors contributing to differences in agronomic performances of resistant and susceptible varieties. The ultimate goal of breeding for a resistant variety is mainly to retain the crop's valuable agronomic traits while new traits are introduced to confer pest resistance. In addition to genetic components, many external factors such as climate, soil micro-conditions, changes in location, and damage by other insects or diseases could play important roles in determining yield. With this study, we aimed to study general agronomic performance of the MSU aphid-resistant lines when compared to a commercial variety and mainly to investigate presence of any yield differences in resistant and susceptible lines with and without insecticides. If the impact of resistance was equal or more effective than insecticide treatments, we expected to see no significant yield loss in resistant lines when untreated.

In 2007 at East Lansing site, there were no significant yield differences between treated (1149 kg/ha) and untreated (1049 kg/ha) plots of resistant E06901 ($F = 4.6$, $P = 0.1213$) (Figure 2.14). Similarly, there were no significant yield differences between treated (1019 kg/ha) and untreated (996 kg/ha) plots of resistant E06905 ($F = 0.25$, $P = 0.6528$). When mean yield from treated (1509 kg/ha) and untreated (1304 kg/ha) plots within the same line were compared for susceptible commercial line DeKalb 27-53, it did not show any significant yield difference ($F = 4.51$, $P = 0.1238$) (Figure 2.14).These observations were not surprising given the low aphid abundance far below the economic threshold of 250 aphids per plant (Ragsdale et al. 2007). However, for E06906, untreated yield was significantly lowered by 330 kg/ha than treated yield (F= 13.58, P = 0.0346).

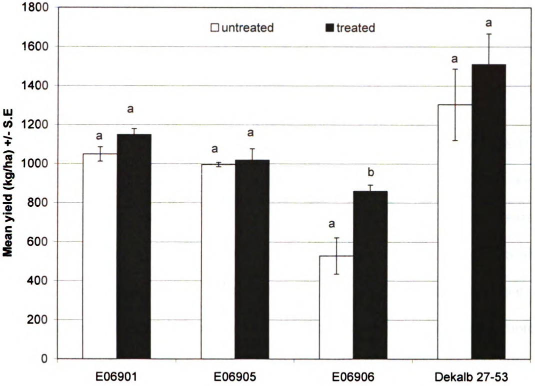

Figure 2.14. Comparison of yield in treated and untreated area in aphid-resistant and aphid-susceptible lines, from field plots in East Lansing, MI in 2007. Means within lines followed by the same letter are not significantly different (P = 0.05). Mean comparisons were done with adjusted P values (Tukey-Kramer).

In 2008 at East Lansing site, statistical analyses with mean comparisons were done for treated and untreated plots of aphid-resistant E06901, E06905, E06906, and aphid-susceptible Titan RR (Figure 2.15). There were no significant yield differences between treated (1326 kg/ha) and untreated (1096 kg/ha) plots of resistant E06901 (F = 3.45, P=

0.3145) (Figure 2.15). Similarly, there were no significant yield differences between treated (1150 kg/ha) and untreated (1095 kg/ha) plots of resistant E06905 (F = 2.73, P= 0.2403). When mean yield from treated (1238 kg/ha) and untreated (1053 kg/ha) plots within the same line were compared for susceptible commercial line Titan RR did, not show any significant yield differences most probably due to low aphid infestation on site (F=2.61, P=0.2376).

Similarly to 2007, in 2008 significantly lower yield was observed in untreated (800 kg/ha) plots when compared to insecticide treated (1013 kg/ha) plots of E06906 (F= 37.27, P=0.0088). In East Lansing for both years (2007, 2008) yield in aphid-resistant line E06906 was significantly less in untreated plots compared to insecticide treated plots. Yield was reduced by 330 kg/ha in 2007 and by 213 kg/ha in 2008 when not protected by an insecticide. A possible reason for this observation could be the more severe damage to E06906 caused by the Japanese beetle, *Popillia Japonica* Newman, which moved into the study site from a nearby asparagus field. Japanese beetles heavily defoliated all three MSU aphid-resistant lines than other soybean aphid-resistant and susceptible lines in the same site (see chapter 3). In a field assessment, E06906 had the greatest amount of feeding (86% of leaflets damaged) in 2007 and 53 % defoliation on three most-damaged trifoliates in 2008 (see chapter 3). We believe that Insecticide treatments for soybean aphid in this study reduced defoliation by Japanese beetle.

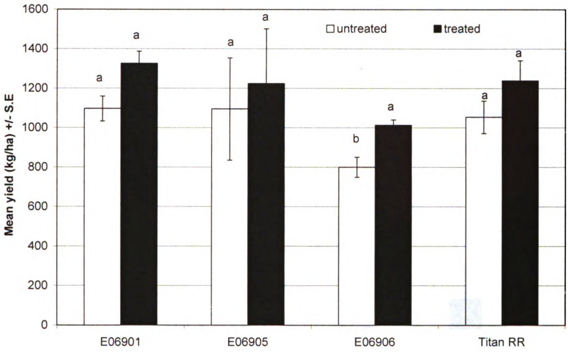

Figure 2.15. Comparison of yield in treated and untreated area in aphid-resistant and aphid-susceptible lines, collected from field plots in East Lansing, MI in 2008. Means within lines followed by the same letter are not significantly different (P = 0.05). Mean comparisons were done with adjusted P values (Tukey-Kramer).

In 2008 in Saginaw, there were no significant yield differences between treated (1218 kg/ha) and untreated (1099kg/ha) plots of resistant E06901 (F = 1.88, P = 0.2637) (Figure 2.16). Similarly, there were no significant yield differences between treated (1348 kg/ha) and untreated (1091 kg/ha) plots of resistant E06905 (F = 1.03, P = 0.3840). There were no significant yield differences between untreated (1295 kg/ha) plots and treated (1395 kg/ha) plots of E06906 either (F = 3.61, P = 0.1534) (Figure 2.16).When mean yield from treated (1573 kg/ha) and untreated (1574 kg/ha) plots within the susceptible commercial line Titan RR was compared, it did not show any significant yield differences most probably due to low aphid infestation on site (F=0.00, P=0.9908). This study site did not suffer defoliation by Japanese beetle. We believe that E06901, E06905, and E06906 would exhibit powerful aphid-resistance even under high natural aphid pressure in the field, whether insecticide treated or untreated, thus would not show any significant yield differences within treated and untreated areas.

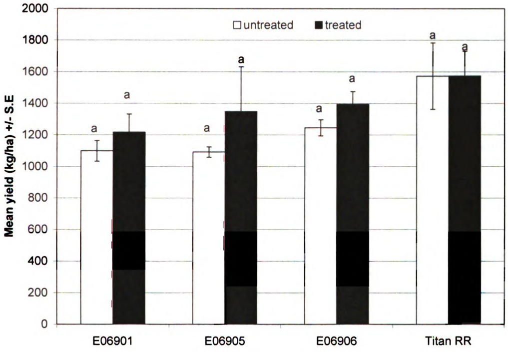

Figure 2.16. Comparison of yield in treated and untreated area in aphid-resistant and aphid-susceptible lines, collected from field plots in Saginaw site, MI in 2008. Means within lines followed by the same letter are not significantly different (P = 0.05). Mean comparisons were done with adjusted P values (Tukey-Kramer).

Most new aphid-resistant sources are identified by preliminary screening for aphid abundance in contained environments such as greenhouses or field cages with artificial infestation of soybean aphids (Hill et al. 2004, Mensah et al. 2005, Diaz-Montano et al. 2006, Mian et al. 2008a). Hill et al. (2004) tested three resistant sources (Dowling, Jackson, PI 71506) with five susceptible lines in field plots confined in a cage after artificial infestation and found three resistant sources had significantly lower aphid indices (0-9 scale) compared to most susceptible lines. They also studied per plant yield attributes such as height, dry mass, and number of pods, and 100-seed weight with and without imidacloprid treatment (Hill et al. 2004). Under heavy artificial infestation, Dowling had no significant differences in yield components between insecticide treated and untreated plants ($P = 0.05$) suggesting successful resistance which was later identified as antibiosis (Hill et al. 2006a).

However, as a further step, conducting field trials of these resistant lines under natural aphid pressure enable breeders and entomologists to discover vital information such as efficacy of resistance under field conditions, yield response, and need to integrate other management tools such as natural predators and insecticide treatments with aphid-resistant lines for more effective control.

Indentifying the importance of field evaluations, a multi-state study evaluated many aphid-resistant breeding lines in replicated field plots including E06901, E06905, E06906, and aphid-susceptible lines and following the same design. In 2007, a multivariate analysis across different locations in six north-central states with 18 soybean lines revealed three groups of breeding lines based on aphid infestation level (log CAD). E06901, E06905, and E06906 were termed 'group 1' with most resistance, where all other lines grouped into either 'group 2' or 'group 3' exhibiting lower or no resistance than group 1 in a cluster analysis, done with data collected from all participating states (IA, IL, MI, WI, SD, and MN) (M. O'Neal, Iowa State University. pers. comm.).

The results of our two-year study confirmed the expression of aphid-resistant genes (*rag3* and *rag1b*) from the parent source (PI 567598B) that confers effective resistance in E06901, E06905, and E06906 lines, against soybean aphid in the field. Although high aphid pressure would have served ideally to test the performance of these resistant lines, we expect to see similar behavior in E06901, E06905, and E06906 with powerful aphid resistance under high aphid pressure in the field. This assumption can be validated with results obtained in the multivariate study conducted in collaboration with many north-central states under varying degrees of aphid infestation (M. O'Neal pers. comm.).

We conclude that E06901, E06905, and E06906 aphid-resistant lines have the potential to agronomically compete with higher yielding susceptible cultivars under high aphid pressure. More importantly if an outbreak occurs, we believe that their inherent resistance will win over the susceptible counterparts giving higher yield with minimum insecticide application, if defoliation by Japanese beetle can be successfully overcome. The results of this study will assist the university breeding programs and seed companies in successful establishment of these lines as commercial cultivars.

Acknowledgements

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Literature Cited

Auclair, J. L. 1989. Host plant resistance, pp. 225–265. In A.K. Minks and P. Harrewijn (eds), Aphids: Their biology, natural enemies, and control. Elsevier, New York.

Baute, T. 2007. Let the scouting begin-soybean aphids are here. Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, Ontario. Available: http://www.omafra.gov.on.ca/english/crops/field/news/croppest/2007/08cpo07a1.htm (Aug 2007)

Brosius T.R., L. G. Higley., and. T. E Hunt. 2007. Population dynamics of soybean aphid and biotic mortality at the edge of its range. J. Econ. Entomol. 100(4): 1268-1275.

Chen, Q. H., and S. Y. Yu. 1988. Aphids and control. Shanghai Science and Technology Press, Shanghai, China.

Costamagna, A. C., and D. A. Landis. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecol. Appl. 16:1619-1628.

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

DiFonzo, C. D., and R. Hines. 2002. Soybean aphid in Michigan: Update from the 2001 season. Extension Bulletin E-2748. Michigan State University, East Lansing, MI.

DiFonzo, C. D., and K. Agle. 2008. Soybean aphid development on, and BCMV transmission to, Otebo dry bean, *Phaseolus vulgaris*. Crop Management.:10.1094/CM-2008-0916-01-RS.

Fan, Y. H. 1988. Screening for soybean varieties resistant to the soybean aphid. Soybean Sci. 7: 167-169.

Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. DiFonzo. 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environ. Entomol. 33:608-618.

Gao, J. F., G. X. Zhang, Y. C. Qin, K. Yu, M. H. Li, and Y. Qin. 1993. Field survey on overwintering syrphids in Changbai Mountain and artificial protection of overwintering syrphids. Chin. J. Biol. Control 9: 142-143.

Hanafi, A., E. B. Radcliffe, and D.W. Ragsdale. 1989. Spread and control of potato leaf roll virus in Minnesota. J. Econ. Entomol. 82: 1201-1206.

Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D.W. Onstad, J. T. Shaw, L. F. Solter, D. J. Voegtlin, C. J. D'Arcy, M. E. Gray, K. L. Steffey, S. A. Isard, and P. L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control.
Available: www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/ Plant Health Progr. DOI:10.1094/PHP-2001-0205-01-HN (March 2009).

He, F. G., X. D. Liu, F. Y. Yan, and Y. Q. Wang. 1995. Soybean resistance to the soybean aphid. Liaoning Agric. Sci. 4: 30-34.

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

Hesler, L. S., K. E. Dashiell, and J. G. Lundgren. 2007. Characterization of resistance to *Aphis glycines* in soybean accessions. Euphytica 154:91-99.

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

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

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

Hu, Q., W. Q. Zhang, Y. X. Yao, and S. Q. Yan. 1992. The relationship between the nitrogen content in soybean leaves and occurrence degree of *Aphis glycines* Matsumura. J. Jilin Agric. Univ. 14: 103-104 (Translated).

Iwaki M., M. Roechan, H. Hibino, H. Tochihara, and D.M. Tantera. 1980. A persistent aphid-borne virus of soybean, Indonesian soybean dwarf virus, transmitted by *Aphis glycines*. Plant Dis. 64:1027–1030.

Kim, K. S., C. B. Hill, G. L. Hartman, M. A. R. Mian, and B. W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48:923-928.

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

Li, Y., C. B. Hill, S. R. Carlson, B. W. Diers, and G. L. Hartman. 2007. Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding. 19: 25-34.

Lin, C. L., Z. S. Xun, L. T. Li, Y. P. Wang, and G. X. Zhang. 1992. Control threshold of the soybean aphid in the field. Soybean Sci. 11: 318-321.

Lin, C. L., Z. S. Xun, L. T. Li, H. K. Zhang, G. X. Zhang, and Y. P. Wang. 1994. Population dynamics and control stage of the soybean aphid in Jining prefecture, China. Shandong Agric. Sci. 4: 44.

Mensah, C., C. DiFonzo, and D. C. Wang. 2008. Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. Crop Sci. 48:1759-1763.

Mensah, C., C. DiFonzo, R. L. Nelson, and D. C. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45: 2228-2233.

Mian, M. A. R., R. B. Hammond, and S. K. S. Martin. 2008a. New plant introductions with resistance to the soybean aphid. Crop Sci. 48: 1055-1061.

Mian, M. A. R., S. T. Kang, S. E. Beil, and R. B. Hammond. 2008b. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor. Appl. Gent. 117: 955-962.

(NASS) National Agricultural Statistics Service. 2000. Agricultural chemical usage 1999 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2006. Agricultural chemical usage 2005 field crops summary. Agricultural Statistics Board, NASS, USDA.

Nielsen, C., and A. E. Hajek. 2005. Control of invasive soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. Environ. Entomol. 34:1036-1047.

Noma, T., and M. J. Brewer. 2008. Seasonal abundance of resident parasitoids and predatory flies and corresponding soybean aphid densities, with comments on classical biological control of soybean aphid in the Midwest. J. Econ. Entomol. 101: 278-287.

Painter, R. H. 1951. Insect Resistance in Crop Plants. Macmillan, New York.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97: 204-208.

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

Rutledge, C. E., and R. J. O'Neil. 2005. *Orius insidiosus* (Say) as a predator of the soybean aphid, *Aphis glycines* Matsumura. Biol. Contr. 33: 56–64.

Rutledge, C. E., and R. J. O'Neil. 2006. Soybean plant stage and population growth of soybean aphid. J. Econ. Entomol. 99:60-66.

SAS institute Inc., 2002. User's manual, v.9.1.3. SAS institute Inc, Cary, NC.

Smith, C.M. 1989. Plant resistance to insects: a fundamental approach. John Wiley and Sons, New York.

Song, F., M. S. Swinton, C. DiFonzo, M. O'Neal, and D. W. Ragsdale. 2006. Profitability analysis of soybean aphid control treatments in three North-central States. Department of Agricultural Economics Staff paper 2006- 24. Michigan State University, East Lansing, MI.

Sun, B., S. B. Liang, and W. X. Zhao. 2000. Outbreak of the soybean aphid in Suihua prefecture in 1998 and its control methods. Soybean Bull. 1: 56-58.

Sun, Z. Q., P. Z. Tian, and J. Wang. 1991. Utilization of aphid resistant character in wild soybean, aphid resistant performance of F2. Soybean Sci. 10: 98-103.

Wang, C. L., L. Y. Xiang, G. X. Zhang, and H. F. Chu. 1962. Studies on the soybean aphid, *Aphis glycines* Mastumura. Translation from Acta Entomol. Sinica. 11: 31–44. (Translated).

Wang, S. Y., X. Z. Bao, Y. J. Sun, R. L. Chen, and B. P. Zhai. 1996. Study on the effect of population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. Soybean Sci. 15: 243-247. (Translated).

Wang, X. B., Y. H. Fang, S. Z. Lin, L. R. Zhang, and H. D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Plant Protection. 20: 12-13. (Translated).

Wu, X. B., W. J. Ni, and P. J. Liu. 1999. Occurrence and control of the soybean aphid, *Aphis glycines* Matsumura. Chinese J. Biol. Control. 6: 20. (Translated).

Wu, Z. S., D. Schenk-Hamlin, W. Y. Zhan, D. W. Ragsdale, and G. E. Heimpel. 2004. The soybean aphid in China: A historical review. Ann. Entomol. Soc. Am. 97:209-218.

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

Yu, D. R., S. G. Guo, and Y. L. Shan. 1988. Preliminary report on evaluation technique and method of soybean aphid resistance on the wild soybean. Jilin Agric. Sci. 3:1-3 (Translated).

Yu, D. R., S. G. Guo, and Y. L. Shan. 1989. Resistance of wild soybean *Glycine soja* to *Aphis glycines.* I. Screening for resistant varieties. Jilin Agric. Sci. 3: 15-19 (Translated).

Zhang, G. R., C. H. Gu, and D. C Wang. 2009. Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor. Appl. Gent. 118(3): 473-482.

CHAPTER 3

An assessment of Japanese beetle (*Popillia japonica* Newman) defoliation on aphid-resistant and aphid-susceptible soybean breeding lines

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Introduction

Soybean, *Glycine max* (L.) Merrill is a host to many insect defoliators in North America. It can tolerate up to 40% defoliation before the pod set, and 30% after pods are formed with minimum or no yield loss (Lambert and Tyler, 1999). Major defoliators in North America include corn earworm, *Helicoverpa zea* (Boddie), soybean looper, *Pseudoplusia includens* (Walker), Mexican bean beetle, *Epilachna varivestis* (Mulsant), and bean leaf beetle, *Cerotoma trifurcata* (Forster). Japanese beetle *Popillia japonica* Newman, is increasing as a defoliator of soybeans in the eastern corn belt.

Japanese beetle is an introduced pest from Japan, and was first discovered in North America in 1916 in a nursery near Riverton, New Jersey (Fleming 1976). By 2009, it was established in 28 states (NAPIS 2009) and Canada.

Japanese beetle is a common destructive pest of turf and landscape plants, and adults feed on more than 300 species of wild and cultivated plants in 79 families (Potter and Held 2002). Japanese beetle was found in Michigan in the1930s, although it did not become established in Michigan until the early 1970s (Davis and Smitley 2007). The Japanese beetle is univoltine, completing its life cycle in one year throughout most of its range in the United States (Fleming 1972). However, in cooler climates, it can take up to two years to complete a single generation (Crocker et al. 1995).

Adult Japanese beetles feed on tissue between the leaf veins, leaving a characteristic lace-like skeletonized structure. Feeding usually begins at the top of the plant, regardless of height, on the upper and outermost leaves (Potter and Held 2002, Cook and Gray 2004). Severely injured leaves turn brown, die, and drop off the plant. Damage caused by foliar feeding of adult Japanese beetles occurs in soybean during the middle of the growing season (July and early August) when the plants are in the reproductive stages (Turnipseed and Kogan 1976, Cook and Gray 2004).

Effective control of Japanese beetle requires proper scouting and accurate estimates of defoliation. Measuring leaf area removed by insect-herbivores can give more accurate measurement that would yield better estimates of damage. This can also be very useful for evaluating host plant resistance. Early studies of defoliation assessments were done using visual scales (Stotz et al. 2000), hand tracing of leaves area, or leaf area meters (O'Neal et al. 2002). O'Neal et al. (2002) described a newer method to measure insect herbivory using a common desktop scanner and public domain software for digital image analysis. Although visual observations are still often used to estimate soybean defoliation, many believe that digital image analysis facilitates better estimates to the actual damage.

In 2000, the soybean aphid, *Aphis glycines* Matsumura, was discovered in North America and now is a serious pest of soybean in the Midwest. University breeding programs, including the soybean-breeding program at Michigan State University (MSU) identified many soybean accessions resistant to soybean aphid. Public institutions and seed companies are incorporating these resistant genes into commercial lines. Most commercial soybean lines in the United States have some resistance to defoliation by Japanese beetle (Hammond 1994). However, in 2007 and 2008, Japanese beetles in the field fed on soybean aphid-resistant lines developed by MSU, at a surprisingly high level. This study reports results from field and laboratory studies comparing Japanese beetle feeding on several aphid-resistant and aphid-susceptible soybean lines.

Material and methods

Visual estimates of Japanese beetle damage in the field

In 2007, Japanese beetle feeding was observed in late July in a soybean aphid study at the Michigan State University (MSU) Entomology Research Farm, East Lansing, MI. This site consisted of 15.2 m x 9.1 m (12 row) plots arranged in a randomized complete block design with four replications of six soybean lines. E06901, E06905, E06906 were developed by MSU and carried the aphid resistance genes *rag1b* and *rag3*. LD05-16060 was developed by the University of Illinois and carried the aphid resistance gene *Rag1*. SD01-76R was the aphid susceptible parent of LD05-16060, and Dekalb27-53 was an aphid-susceptible commercial line. Initial observations of differential feeding lead us to conduct a preliminary assessment on defoliation based on visual estimates. On August 24, 2007, four randomly selected plants per plot were assessed for the number of leaflets per plant fed on by Japanese beetle (irrespective of the severity of damage). The number of leaflets damaged was used to calculate the percentage of leaflets damaged per plant.

In 2008, Japanese beetles were again observed feeding at the same study site at the MSU Entomology Research Farm, East Lansing, MI. Once again, 15.2 m x 9.1 m (12 row) plots were arranged in a randomized complete block design with four replications of six soybean lines. As in 2007, the aphid-resistant lines were E06901, E06905, E06906, and LD05-16060. The aphid-susceptible lines were SD01-76R, the parent of LD05-16060 and Titan RR, the susceptible parent of the three MSU lines. In August 15 2008, four randomly selected plants per plot were assessed for Japanese beetle feeding by estimating the percent defoliation of the three most-damaged trifoliates on each plant. All ratings were done by the same observer. Since the majority of the defoliation at the top of the plant, this method enabled us to concentrate on the most damaged area of the plant. Mean defoliation on each selected plant (4 plants per plot) was calculated by averaging the percent defoliations on the three most-damaged trifoliates (nine leaflets).

Detached-leaflet assay

Choice test

After adult Japanese beetle emergence in 2008, a detached-leaflet choice test was designed to compare feeding on aphid-resistant (E06901, E06905, E06906, LD05-16060) and aphid-susceptible lines (Titan RR, SD01-76R). The third fully-expanded trifoliate from the top of a field-grown plant was selected and the middle leaflet was detached to be used in the choice test. Each leaflet was labeled with a unique identifier for scanning. The undamaged leaflets were scanned on a Hewlett-Packard HP Officejet Pro L7680 desktop scanner (Hewlett-Packard, Cupertino, CA) operating on a Dell Optiplex 755 computer.

The original images (in color) were saved as TIFF files for digital conversion to measure total leaf area.

After scanning, leaflet petioles were covered with a moist piece of cotton to retain the moisture. One leaflet from each of the six lines was randomly placed on a moist paper towel in a 20 cm x 30 cm aluminum cake pan with a plastic lid (Figure 3.1). Twelve Japanese beetles were placed on the moist paper towel (not on leaflets) in each pan. These beetles were collected from an asparagus field on the MSU campus (not from soybean), held in a cooler, and starved for 5-6 h prior to the experiment. Each pan was closed with a tight–fitting plastic lid to prevent escapes, and the pans were left at room temperature. There were ten replicates (pans) per trial, and two separate trials on 23 July (Trial1), and 28 July (Trial 2). After 48 h, individual leaflets were re-scanned and saved as TIFF files for digital conversion to measure leaf area removed by feeding.

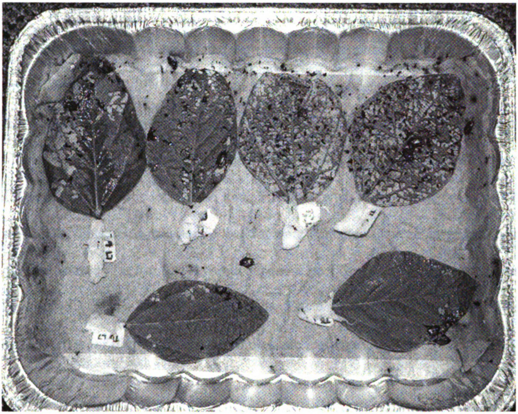

Figure 3.1. Experimental set up for choice test in the laboratory. Image shows an aluminum pan after Japanese beetles were placed.

No-choice test

A no-choice test was designed to determine if Japanese beetles fed on the non-preferred lines (LD05-16060, SD01-76R) in the absence of the preferred lines (E06906, Titan RR) lines. As in the choice test, on 28 July 2008 leaflets were collected from field-grown plants, labeled with a unique identifier, and scanned. Each detached leaflet was placed in 15 mm deep, 150 mm diam. Petri dish. There were ten leaflets tested per line, for a total of 40 Petri dishes. Adult Japanese beetles were collected from an asparagus field, held in a cooler, starved for 5-6 h. Two adult Japanese beetles were placed per Petri-dish and held for 48 h (figure 3.2). After 48h, the leaflets were re-scanned and images were saved as TIFF files for digital conversion to measure leaf area.

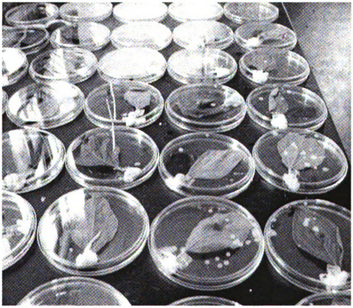

Figure 3.2. Experimental set up for no-choice test. Two beetles were confined to each leaflet in a Petri-dish.

Creating a digital image for measuring leaf area.

Following a similar method described by O'Neal et al. (2002) for creating digital images from scanned leaflets, the public domain software Scion Image 4.0.3 was downloaded from http://www.scioncorp.com, to measure the surface area of objects in a digital format. The 'before' images (TIFF) were converted from color to black and white, following the steps described by O'Neal et al. (2002). These converted images were then used to measure total leaf area in cm^2 from leaflets prior to Japanese beetle feeding .The amount of leaf area removed was measured from inverted black and white 'after' images in cm^2 as described by O'Neal et al. (2002). Percent leaf area removed was calculated using measurements of the total surface area before feeding, and leaf area removed after feeding, from each leaflet.

Data Analyses

All data were analyzed with Analysis of Variance (ANOVA) in PROC MIXED (SAS 9.3.1., SAS Institute Inc., 2002-2003). Mean separations were computed by Least Significant Difference Test (LSD) at 5% probability level (SAS 9.3.1., SAS Institute Inc., 2002-2003).

Results

Visual estimates of Japanese beetle damage in the field

In 2007, percentage of leaflets damaged by Japanese beetle differed among treatments (F = 151.32; df = 5, 15; P = <0.0001) (Figure 3.3). The three MSU aphid-resistant soybean lines had significantly higher percentages of leaflets fed on (50% on E06901, 59% on E06905, and 86 % on E06906) than *Rag1* line (11%) and aphid susceptible lines (15% on Dekalb 27-53, and 5% on SD01-76R). In addition, E06906 had significantly more leaflets damaged than E06905 and E06901. There were no significant differences between LD05-16060 *(Rag1)* and SD01-76R. In 2008, significant differences in defoliation were observed among treatments (F = 456.67; df = 5, 87; P = <0.0001) (Figure 3.4). The percent defoliation of the MSU aphid-resistant lines ranged from 49-53% and was significantly higher than their susceptible parent, Titan RR (34%). The *Rag1* aphid-resistant line LD05-16060, and its susceptible parent SD01-76R, had significantly less defoliation than other four lines (E06901, E06905, E06906, and Titan RR). Similar to 2007, E06906 had the heaviest defoliation in the field. These preliminary field assessments provided evidence of differential feeding on the MSU soybean aphid-resistant material.

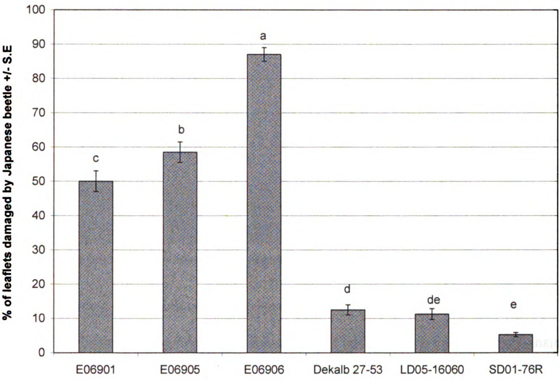

Figure 3.3. Mean percentage of leaflets fed on by Japanese beetle from six soybean lines in East Lansing, MI, 2007. Means were separated by Least Significant Difference Test (LSD) at 5% probability level.

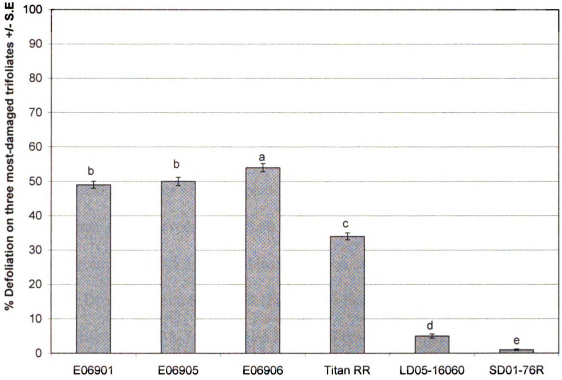

Figure 3.4. Percent defoliation caused by Japanese beetle on aphid-resistant and aphid-susceptible soybean breeding lines at the Entomology Field Research Farm at East Lansing, MI, 2008. Means were separated by Least Significant Difference Test (LSD) at 5% probability level.

Detached-leaflet assay

Choice test

When leaflets from different lines were exposed to feeding under choice conditions, significant differences in defoliation (Average leaf area removed) were observed after 48 h (Trial 1-F = 15.25; df = 18.3,5; P <0.0001 and Trial 2-F= 6.27; df = 5, 16.5; P = 0.0019) (Figure 3.5). In both trials, E06901, E06905, and E06906 had significantly more leaf area removed than LD05-16060 and SD01-76R (Figure 3.5). Titan RR did not significantly differentiate from all three MSU aphid-resistant lines in the first trial. However, Titan RR showed significantly less leaf area removed than E06906, and was not significantly different from LD05-16060 and SD01-76R in the second trial. In both trials, LD05-16060 and SD01-76R had significantly less feeding than MSU aphid-resistant lines and did not have significant differences in leaf area removed between them. Susceptible parent line of MSU aphid-resistant lines (Titan RR), was intermediate in amount of leaf area removed (Figure 3.6). In addition, these three aphid-resistant lines had higher percentage (100% or 90%) of leaflets fed on, by Japanese beetle when compared to LD05-16060 and SD01-76R (Figure 3.5).The choice test provided evidence that Japanese beetles chose to feed more on E06901, E06905, and E06906.

No-choice test

In a no-choice test, significant differences in feeding were observed ($F = 4.77$; $df = 3, 13.1$; $P = 0.0186$). The most fed breeding line after 48 h was E06906, and had significantly higher defoliation than SD01-76R and Titan RR (Figure 3.6). However, LD05-16060 did not differentiate from E06906 in this test. These tests provided sufficient evidence to believe that the MSU lines were most damaged by Japanese beetle feeding under choice and no-choice conditions, and that their least preferred line under choice conditions (SD01-76R), remained to be the least eaten even under no-choice.

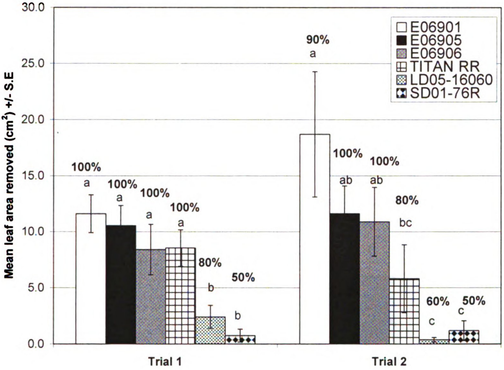

Figure 3.5. Mean leaf area removed by Japanese beetle on leaflets of aphid-resistant and aphid-susceptible soybean breeding lines under choice conditions. Percent values above bars indicate the percentage of leaflets fed on each line from ten leaflets. Means were separated by Least Significant Difference Test (LSD) at 5% probability level.

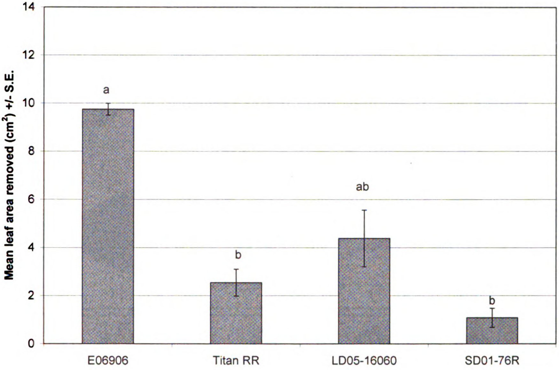

Figure 3.6. Mean leaf area removed by Japanese beetle on leaflets of aphid-resistant and aphid-susceptible soybean breeding lines under no-choice conditions. Means were separated by Least Significant Difference Test (LSD) at 5% probability level.

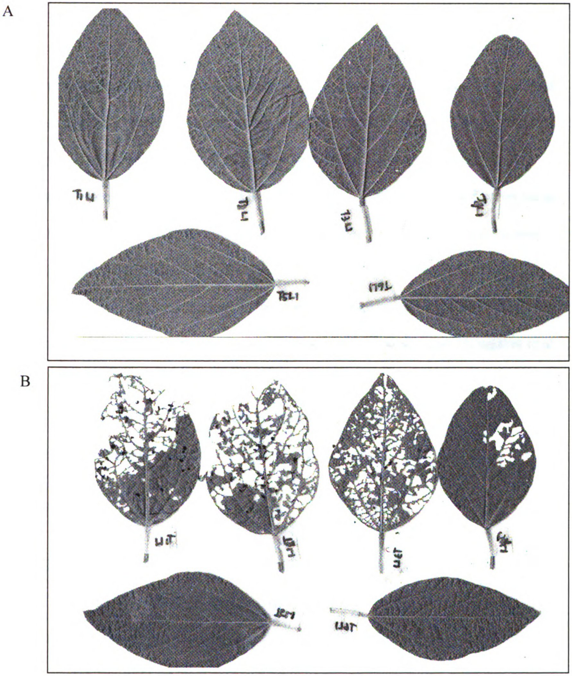

Figure 3.7. Images showing scanned leaflets from six soybean lines in choice test. E06901, E06905, E06906, and Titan RR (top row, from left to right), LD05-16060 (bottom row left) and SD01-76R (bottom row right) before (A) and 48 h after (B) exposure to Japanese beetles.

Discussion

Potter and Held (2002) suggested that increased feeding and/or increased attraction of Japanese beetles to certain varieties of the same host could be linked with presence of attractive volatile compounds or the absence of feeding deterrents such as stinging trichomes or latex secretions that make the plant less palatable. Japanese beetles locate hosts primarily by olfaction (Potter and Held 2002) thus, plants with greater feeding could have elevated levels of volatile compounds compared to less preferred plants. Some studies suggested that damaged leaves induce or attract more Japanese beetles by production of complex volatile substances in hosts such as *Malus* spp. (Loughrin et al. 1996). These complex mixtures induced by damaged leaves would inform beetles that they have a preferred plant in the vicinity, thus more and more beetles continue to feed on damaged plants versus undamaged plants with less complex blend of volatiles (Potter and Held 2002).

In addition, many sugars in plants are phagostimulants for Japanese beetles (Potter and Held 2002). Ladd (1988) evaluated sucrose (0.01-1 M) and 13 other naturally occurring sugars (0.1 M) on cellulose acetate-cellulose nitrate membrane filter disks as feeding stimulants for Japanese beetle larvae and concluded that sucrose, maltose, fructose, glucose, and trehalose stimulated larval feeding. All of the above sugars except trehalose have also been reported as strong phagostimulants to adult Japanese beetles (Ladd 1988).

Although Japanese beetle feeding in soybean has not become a severe threat in most soybean-growing regions in the United States, soybean varieties have been tested for defoliation as early as 1940s. Coon (1946) assessed Japanese beetle defoliation on 26 soybean varieties using a damage rating from 1-5 (lowest feeding to highest feeding) and concluded that all 26 varieties were susceptible. However, based on his ratings he confirmed that four varieties ('chief,' 'Wiking', 'Illini', and 'Wilson') indicated less susceptibility to Japanese beetle feeding than others. His studies further confirmed that increased beetle feeding resulted in decreased yield. Japanese PI229358 is one of the first defoliation-resistant soybean lines identified in the 1960s against Mexican bean beetle feeding (Hammond et al. 2001). This PI has served as a parent in conventional breeding programs aiming to develop defoliation-resistant soybean (Hammond et al. 2001). Several QTLs originating from PI229358 have been used to develop defoliation-resistant soybean (Zhu et al. 2007).

We made a general observation in the field that Japanese beetles were more abundant on E06901, E06905, and E06906, than on the *Rag1* aphid-resistant line, LD05-16060, and its aphid-susceptible parent SD01-76R in the same field. Since both the aphid-susceptible parent and the aphid-resistant lines bred in Michigan had increased feeding, it lead us to believe that all four MSU soybean lines were either moderately (Titan RR) or highly (E06901, E06905 and E06906) susceptible to Japanese beetle. This leads to the hypothesis that these aphid-resistant breeding lines may have inherited Japanese beetle susceptibility from their aphid-susceptible parent source, Titan RR. However this theory cannot be validated until the aphid-resistant parent source of these lines (PI 567598B) is assessed for Japanese beetle damage, which will be one of our future objectives.

We conclude that in the field, E06901, E06905 and E06906 had significantly more feeding (Number of leaflets damaged and % defoliation) by Japanese beetle compared to aphid-resistant LD05-16060, aphid-susceptible Dekalb 27-53, and SD01-76R, and their aphid-susceptible parent Titan RR. Furthermore, E06901, E06905, and E06906 had elevated defoliation in a choice test confirming Japanese beetle's special preference on MSU aphid-resistant lines. In addition, heavier defoliation on E06906 in the no-choice test indicated this line's high susceptibility to defoliation, even when Japanese beetles were not given a choice.

Identification of the underlying mechanism that leads to heavier defoliation by Japanese beetle would be the key component to mange defoliation on these MSU soybean lines, by breeding for lines with both aphid resistance and Japanese beetle resistance. It is also important to further investigate and address if the damage is specific to Japanese beetle or, whether all soybean defoliators can cause defoliation on MSU lines in general, and study the existence of any genetic linkage (if present) between aphid resistance and elevated susceptibility to Japanese beetle in MSU aphid-resistant lines.

Acknowledgements

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Literature Cited

Cook K. A., and M. E. Gray. 2004. Japanese Beetle. University of Illinois extension. Available: http://ipm.illinois.edu/hyg/insects/japanese_beetles/index.html (13 July 2009).

Coon B. F. 1946. Resistance of soybean varieties to Japanese beetle attack. J. Econ. Entomol. 39:510–513.

Crocker R. L., R. M. Marengo-Lozada, J. A. Reinert, and W. H. Whitcomb. 1995. Japanese Beetle, pp 66-69. In R. L. Brandenburg and M. G. Villani (eds), Handbook of Turfgrass Insect pests. Entomological Society of America, Lanham, MD.

Davis T., and D. Smitley. 2007. Turf tips for the home owner-Japanese beetle. Extension Bulletin E10TURF. Michigan State University, East Lansing, MI.

Fleming W. E. 1972. Biology of the Japanese beetle. USDA Tech. Bull. No.1449. 129-129.

Fleming W. E. 1976. Integrating control of the Japanese beetle-a historical review. USDA Tech. Bull. No. 1545. 65-65.

Hammond, R. B. 1994. Japanese beetle, pp. 64–65. In Handbook of Soybean Insect Pests, L.G. Higley and D.J. Boethel, (eds.), Entomological Society of America, Lanham, MD.

Hammond, R. B., P. Bierman, E. Levine, and R. L. Cooper. 2001. Field resistance of two soybean germplasm lines, HC95-15MB and HC95-24MB, against bean leaf beetle (Coleoptera: Chrysomelidae), western corn rootworm (Coleoptera: Chrysomelidae), and Japanese beetles (Coleoptera: Scarabaidae). J. of Econ. Entomol. 94:1594-1601.

Lambert, L. and J. Tyler. 1999. Appraisal of insect-resistant soybeans, pp. 131–148. In J.A. Webster & B.R. Webster (eds), Economic, Environmental, and Social Benefits of Insect Resistance in Field Crops. Thomas Say Publ., Entomol. Soc. of America, Lanham, MD.

Ladd T. L. Jr. 1988. Influence of sugars on feeding response of Japanese beetle (Coleoptera: Scarabaeidae) larvae. J. Econ. Entomol. 81:1390–1393.

Loughrin J. H., D. A. Potter, T. R. Hamilton-Kemp, and M. E. Byers. 1996. Volatile compounds from crabapple cultivars (*Malus* spp.) differing in susceptibility to the Japanese beetle (*Popillia japonica* Newman). J. Chem. Ecol. 22:1295–305.

(NAPIS) National Agricultural Pest Information Service. 2009. Available: http://pest.ceris.purdue.edu/searchmap.php?selectName=INBPAZANAPIS 2009 (18 June 2009).

O'Neal M. E., D. A. Landis, and R. Isaacs. 2002. An inexpensive, accurate method for measuring leaf area and defoliation through digital image analysis. J. Econ. Entomol. 95:1190–1194.

Potter, D. A., and D. W. Held. 2002. Biology and management of the Japanese beetle. Annu. Rev. Entomol. 47:175-205.

SAS Institute. 2002. PROC user's manual, version 9.1. SAS Institute, Cary, NC.

Stotz, H. U., B. R. Pittendrigh, J. Kroymann, K. Weniger, J. Fritsche, A. Bauke, and T. Mitchell-Olds. 2000. Induced plant defense responses against chewing insects ethylene signaling reduces resistance of *Arabidopsis* against Egyptian cotton worm but not diamondback moth. Plant Physiol. 124: 1007–1019.

Turnipseed, S. G., and M. Kogan. 1976. Soybean Entomology. Annu. Rev. Entomol. 21:247-282.

Zhu, S., D. R. Walker, H. R. Boerma, J. N. All, and W. A. Parrott. 2008. Effects of defoliating insect resistance QTLs and a cry1Ac transgene in soybean near-isogenic lines. Theor. Appl. Genet. 116: 455-463.

Chapter 4

A bioassay to evaluate baseline susceptibility of soybean aphid (*Aphis glycines* Matsumura) to pyrethroid, organophosphate, and neonicotinoid insecticides

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Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to Asia, and is one of the most serious threats to productivity among numerous insect pests of soybean, *Glycine max* (L.) Merr. (Yu et al. 1989, Wang et al. 1996, Wu et al. 1999, Sun et al. 2000, Hill et al. 2004, Ragsdale et al. 2004). It has been a soybean pest in China, Japan, South Korea, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and Russia for many years (Wu. et al. 2004). In recent years, soybean aphid was discovered in Australia, Canada, and the United States (Wu et al. 2004). After its discovery in the United States in 2000, it rapidly spread across the Midwestern regions (Ragsdale et al. 2004). It is now reported in 24 states in the United States and in three Canadian provinces (Ragsdale et al. 2004, Rutledge and O'Neil 2006).

Soybean aphid reduces yield directly via plant feeding and indirectly through reduction in seed protein content (Wang et al. 1994). Plants with heavy infestations show wrinkled and distorted foliage, early defoliation, stem and leaf stunting, reduction in number of pods and seed weight, and even plant death (Wang et al. 1962, Wang et al.1996, Lin et al. 1992, 1994; He et al. 1995, Wu et al. 1999, Wu et al, 2004, DiFonzo and Hines 2002, Diaz-Montano et al. 2006). Honeydew excreted by aphids builds up on foliage, and supports the growth of sooty mold, affecting plant photosynthesis, yield, and seed quality (Chen and Yu 1988). Significant yield loss can occur due to feeding damage by soybean aphid. In China, yield was reduced up to 52% when soybeans in the early vegetative stage (first node stage) were inoculated with 220 aphids per plant (Wang et al. 1994). In addition to yield loss from direct feeding, another threat posed by soybean aphid is its ability to transmit plant viruses such as alfalfa mosaic virus, soybean dwarf virus, and soybean mosaic virus to soybean and other crops (Iwaki et al. 1980, Hartman et al. 2001, Hill et al. 2001).

Control of soybean aphid involves a number of distinct approaches including biological control, chemical control, and host plant resistance (Wu et al. 2004). In North America, the dominant soybean aphid natural enemies are mainly generalist predators, such as lady beetles (Coccinellidae), green lacewings (Chrysopidae) and pirate bugs (*Orius* spp.). Studies also show that lady beetles play an important role in suppressing soybean aphid population (Fox et al. 2004, Costamagna and Landis, 2006, 2007; Costamagna et al. 2008). Introduction of parasitoids or use of already established parasitoids is another approach recently attempted for controlling soybean aphid. Reports of field parasitism of soybean aphid in North America indicate generally very low levels, with only a few reports of less than 10% parasitism (Nielsen and Hajek 2005, Costamagna and Landis 2006, Baute 2007, Noma and Brewer 2008). Furthermore, soybean aphid populations can change fast over time, doubling in less than two days under optimum conditions (McCornack et al. 2004). Therefore, suppression of soybean aphids by natural enemies is inconsistent and other approaches are required for effective control (Mian et al. 2008).

Another approach to control soybean aphid is the use of host plant resistance. In attempts to effectively manage soybean aphid using non-chemical approaches, soybean lines with aphid resistance were identified by several breeding programs in the United States (Hill et al. 2004, Li et al. 2004, Mensah et al. 2005, Diaz-Montano et al. 2006, Hill et al. 2006a and 2006b, Hesler et al. 2007, Hesler and Dashiell 2008, Mian et al. 2008). Since soybean aphid is a relatively recent soybean pest in the United States, no aphid-resistant cultivar is currently available. Until effective resistant varieties are commercially released, aphid control will rely primarily on insecticides.

For many locations in the United States, insecticide applications to soybean increased dramatically after the discovery of soybean aphid. In 1999, prior to the discovery of soybean aphid, <1% of the soybean acreage in Michigan was treated with insecticides (NASS 2000). In 2005, an outbreak year, 42% of Michigan soybean acres were treated (NASS 2006). Similar increases were observed in many North Central states (NASS 2000 & 2006). Unfortunately, the increase in acreage sprayed has both economic and environmental costs. Significant insecticide costs have been inevitable with soybean aphid control since its introduction in the North central States. Song et al. (2006) estimated a total yield loss exceeding 350 million bushels in the north-central states, if soybeans were left untreated. In 2004, Michigan soybean growers have reported spending $8-12/acre for insecticide application (Song et al. 2006).

To reduce insecticide applications against soybean aphid, Ragsdale et al. (2007) developed an economic threshold; if soybean aphid population were increasing and plants were in the late vegetative or early reproductive stages (R1–R4), an economic threshold of 250 aphids per plant is recommended to time insecticide applications against soybean aphid (Ragsdale et al. 2007).

Numerous insecticides have been applied for controlling soybean aphid in Asia, including fenvalerate, omethoate, aldicarb, carbofuran, pirimicarb, chloromethiuron, phosalone, and deltamethrin (Chen and Yu 1988, Wang and Ba 1988, Wu et al. 1999, Sun et al. 2000). Many of these insecticides have a broad spectrum of activity. In North America, the most commonly used foliar insecticides are chlorpyrifos, acephate, esfenvalerate, permethrin, and λ-cyhalothrin (NASS 2006).

Increased insecticide use raises the possibility of developing insecticide resistance in soybean aphids. Thus, monitoring insecticide susceptibility is essential to effectively manage soybean aphid in the future. The Insecticide Resistance Action Committee (IRAC) defined resistance to insecticides as 'a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species'.

So far, there is only one published report of laboratory measurement of soybean aphid susceptibility or resistance to commonly used insecticides in the United States. Magalhaes et al. (2008) provided information on soybean aphid susceptibility to two neonicotinoid insecticides used as seed treatments (imidacloprid and thiamethoxam) using a field colony from Nebraska. Additionally, the impact of different thiamethoxam concentrations on soybean aphid life-history characteristics was determined (Magalhaes et al. 2008).Thus far, there are no reports of insecticide resistance studies conducted using field collected soybean aphids from Michigan.

To proactively address the threat of aphid resistance, we developed a reliable aphid-dip bioassay protocol to test susceptibility of soybean aphid to several groups of foliar insecticides. Specifically, we

- developed an effective bioassay method and used it to determine baseline susceptibility of soybean aphids to selected pyrethroids, organophosphates, and neonicotinoids;
- bioassayed soybean aphid populations from commercial soybean fields with a history of insecticide compared to a laboratory (greenhouse) strain.

Materials and methods

Greenhouse strain

The greenhouse strain originated in August 2000, the month soybean aphid was confirmed in North America, from several fields in Ingham County, Michigan. The strain was first maintained at the former USDA-APHIS National Biological Control Laboratory in Niles, MI. In 2002, aphids from the Niles Laboratory were used to establish a greenhouse colony at Michigan State University. This strain was maintained on 'Williams 82' soybean plants at vegetative stages V4-V8. New plants were provided weekly to the colony, and aphids were transferred to new foliage weekly by placing infested leaf sections on uninfested plants. The colony was maintained at 27±5 °C, under photoperiod 16:8 (L:D) h. This strain originated from field collections made prior to insecticide-use for aphids in Michigan, and since its establishment, this colony has never (to our knowledge) been exposed to insecticides.

Field strains

In 2007, soybean aphids were collected in July-August from three fields in two Michigan counties. Each location had a history of insecticide use during aphid outbreaks in 2001, 2003, and 2005. They were a field at Michigan State University's Entomology Field Research Farm, East Lansing, MI (42^0 41" 29.38" N, 84^0 29" 22.96" W), a field at the Saginaw Valley Sugar Beet and Dry Bean Research Farm, Saginaw, MI (43^0 22" 41.38" N, 84^0 06" 42.68" W), and a commercial soybean field, Gera, MI (43^0 23" 13.78" N, 83^0 44" 18.58" W). These three strains were maintained in separate growth chambers at 22±5 °C, 16:8 (L:D) h photoperiod until testing was complete. In 2008, soybean aphids were collected only in August from a field at the Entomology Field Research Farm, East Lansing, MI only, and were maintained in growth chambers following the same procedure.

Developing a standard aphid-dip bioassay

We developed a protocol for an aphid-dip bioassay using a metal mesh (mesh size = 0.5 mm) tea strainer (Cost Plus Inc., Oakland, CA) to dip soybean aphids in insecticide solutions (Figure 4.1). Five apterous adults from the greenhouse strain were placed on the mesh of the tea strainer. The closed tea strainer was then dipped in a 200 ml beaker of de-ionized water (a control treatment) for 10 s. After 10 s, the strainer was removed from the solution and soybean aphids were blotted on clean, dry tissue to remove excess water. Finally, soybean aphids were transferred to a fresh-cut soybean leaf square (2 cm x 2 cm) on moist filter paper in a plastic 9 mm deep, 50 mm diam. Petri dish (#351006, Falcon®, Becton Dickinson Laboratories, NJ). Soybean aphid escapes were prevented by using a dish with a snap-on lid. Petri dishes were placed in an aluminum pan with a plastic lid, on a layer of moist paper towels to maintain high humidity. The pans were held in a growth chamber at 22°C, 16:8 (L:D) h photoperiod. After 48 h, soybean aphids were classified as dead or alive, and counted. Soybean aphids were considered dead when they did not move after multiple prodding with a fine-haired paintbrush. This protocol resulted in soybean aphid survival of 90% or greater (10% or less mortality) after 48 h, indicating that the aphid-dip method was reliable for conducting insecticide bioassays.

Aphid-dip insecticidal bioassay

Five commercial insecticides in three insecticide groups were tested in the bioassay (Table 4. 1). Organophosphates and pyrethroids were the first groups of insecticides used to manage soybean aphid after its discovery in the United States (NASS 2001). In 2005, an outbreak year in the United States, the top three insecticides used to control soybean aphid in the Midwest were chlorpyrifos, λ-cyhalothrin, and esfenvalerate (NASS 2006). These widely used products were very effective, so determining baseline susceptibility was important for early detection of future resistance. Dimethoate is one of the few general use products remaining for growers, not requiring pesticide applicator license. However, it has shown inconsistent efficacy against soybean aphid, for reasons unknown. Trimax Pro was recently introduced as a foliar spray on soybeans but its active ingredient imidacloprid is also available as a seed treatment. This raises concerns about development of resistance to imidacloprid.

Table 4.1. Insecticides used in aphid-dip bioassays, 2007-2008.

Common name	Brand name	Class (IRAC group*)	Manufacturer
chlorpyrifos	Lorsban 4E	organophosphate (group 1B)	Dow AgroSciences LLC, Indianapolis, IN
dimethoate	Dimethoate	organophosphate (group 1B)	Bayer CropScience, Research Triangle Park, NC
esfenvalerate	Asana XL	pyrethroid (group 3)	DuPont, Wilmington, DE
λ-cyhalothrin	Warrior with Zeon Tech.	pyrethroid (group 3)	Syngenta Crop Protection, Wilmington, DE
imidacloprid	Trimax Pro	neonicotinoid (group 4A)	Bayer CropScience, Research Triangle Park, NC

*Mode of action group, as given by the Insecticide Resistance Action Committee (IRAC).

Stock concentration for each insecticide in this study was 25% of the recommended application rate for soybean aphid control (DiFonzo 2007). Stock solutions were prepared by mixing de-ionized water with commercial formulations of each insecticide measured using a precision micro liter pipette (PIPETMAN®, Rainin Instrument Co, Inc.). A series of dilutions (Table 4.2) were then prepared from stock solutions using de-ionized water. Stock solutions were stored in a refrigerator, and used within a week from the date of preparation. From these, fresh dilutions were made daily prior to each trial. Once prepared, each fresh dilution series was used to test soybean aphids from field and greenhouse strains in a single trial.

As in the standard bioassay, individual groups of five adult apterous aphids were dipped for 10 s in stock solution or in a dilution, and then transferred to a fresh-cut soybean leaf square (2 cm x 2 cm) on moist filter paper in a plastic 9 mm deep, 50 mm diam. Petri dish (#351006, Falcon®, Becton Dickinson Laboratories, NJ) for 48 h. In an initial test, aphids poisoned but alive at 24 h were dead after 48 h, thus mortality in the remaining bioassays was assessed after 48 h. Groups of five aphids were also dipped in de-ionized water as control treatment. Multiple tea strainers were used, each assigned to a particular treatment, to prevent cross-contamination. Petri-dishes from the same aphid strain exposed to a given insecticide were placed in their own aluminum pan with a lid, on top of a layer of moist paper towels to maintain high humidity. The trays were held in a growth chamber at 22°C, 16:8 (L:D) h photoperiod. Control groups were maintained separately under the same conditions, but in another growth chamber to prevent exposure of these aphids to insecticides. After 48 h, dead and alive soybean aphids were counted.

Three replications of groups of five soybean aphids from each strain were tested per solution in a single trial. Four separate trials with serial dilutions were conducted per insecticide. Total number of aphids tested for each insecticide ranged from 346-600 (Table 4.2). In 2007, field-collected aphids from all three locations (East Lansing, Gera, and Saginaw) were compared to the greenhouse strain for chlorpyrifos and esfenvalerate. A single field strain from East Lansing was compared to the greenhouse strain for λ-cyhalothrin. In 2008, field-collected aphids from East Lansing were compared to the greenhouse strain for dimethoate and imidacloprid.

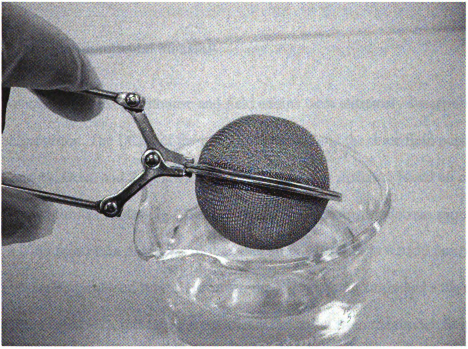

Figure 4.1. Round mesh tea strainer used to dip soybean aphids in insecticide solutions.

Data analyses

Mortality data analyses were conducted by probit analysis (Finney 1971) using PROC PROBIT (SAS Institute Inc. 2002-2003). If control survival was less than 100%, mortality was corrected using Abbott's formula (Abbott 1925). LC_{50}, LC_{90}, and 95% fiducial limits were calculated using PROC PROBIT. PROC PROBIT also calculated the slope of the logarithmic dose-response relationship for each combination of insecticide/location. A high slope value indicates less heterogeneity in population sensitivity to a particular insecticide (Georghiou and Metcalf 1961). In contrast, a shallow slope indicates high heterogeneity in sensitivity among individuals of a population.

Results and discussion

Aphid-dip insecticidal bioassay

Aphids from both greenhouse and field strains were extremely susceptible to chlorpyrifos. The LC_{50}s of the greenhouse strain and the three field populations (East Lansing, Gera, and Saginaw) were not significantly different based on overlapping fiducial limits (Table 4.2). The LC_{90} of the East Lansing strain was significantly higher (0.0743 ppm) than the LC_{90}s of the other strains (0.0124 to 0.0150 ppm) with wider non-over lapping fiducial limits. However, the East Lansing strain had a higher chi square value (Table 4.2), indicating a significant deviation from the model. Slope value for the Saginaw strain was low (0.76±0.22) compared to East Lansing (3.57) and Gera (3.58) populations. The highest value for slope was found for the greenhouse strain (5.55), which indicated the least heterogeneity in population sensitivity to chlorpyrifos. Despite these differences among populations, all populations were very sensitive to chlorpyrifos at much lower concentrations than its recommended application rate (~ 5248 ppm).

Soybean aphid strains from the greenhouse and East Lansing responded variably to dimethoate. A relatively large LC_{50} value was obtained for the greenhouse strain (4.05 ppm) compared to the field strain (0.71 ppm), and the fiducial limits did not overlap (Table 4.2). LC_{90} of the greenhouse strain was 263 ppm, while the LC_{90} was achieved at a significantly lower dose of 3 ppm for the East Lansing strain, with non-overlapping fiducial limits. This indicated that the greenhouse strain was more tolerant to dimethoate than the field strain. Relatively shallow slopes of both strains may be associated with high population heterogeneity in susceptibility to dimethoate in the greenhouse and East Lansing strain. This is in contrast to the response to chlorpyrifos, where the same two strains had much higher slopes (less population heterogeneity) and extremely low LC_{50} and LC_{90} Values. This finding supports observations from the field where chlorpyrifos is very effective against soybean aphid while dimethoate shows inconsistent and unsatisfactory control (Ragsdale et al. 2001). However, in the bioassay both strains were susceptible to dimethoate at lower concentrations than its application rate (~ 5248 ppm).

Aphids from all strains were highly susceptible to esfenvalerate. LC_{50} and LC_{90} fiducial limits for East Lansing and Saginaw strains overlapped (Table 4.2). Due to possible departure from the model, PROC PROBIT did not calculate fiducial limits for the greenhouse and Gera strains. A lower slope was calculated for the greenhouse strain compared to the other strains, indicating more heterogeneity in susceptibility to esfenvalerate. These results implied that all four strains were). However, LC_{50} and LC_{90} values for all populations (even though fiducial limits were not obtained for some) were low compared to recommend application rate of ~1888 ppm.

The second pyrethroid, λ- cyhalothrin was highly toxic to soybean aphid. The LC_{50} for the East Lansing strain (0.004 ppm) was not significantly different from the LC_{50} for the greenhouse strain (0.054 ppm). However, the LC_{90} values were significantly different and did not overlap. Both strains were highly susceptible when compared with the recommended application rate for λ-cyhalothrin (~1040 ppm). Wider fiducial limits and a relatively flat slope (Table 4.2) suggested that although susceptible, the greenhouse strain had more heterogeneity in sensitivity to λ- cyhalothrin than the East Lansing field population.

The neonicotinoid imidacloprid behaved differently from the other insecticides in the study. The LC_{50} value for the greenhouse strain was 2.55 ppm with fiducial limits of 0.18-162. Due to possible deviation from the model (P= 0.0008) fiducial limits were not calculated for the East Lansing strain, but the LC_{50} (0.45 ppm) fell within the fiducial limits of the greenhouse strain. Although fiducial limits again could not be calculated for the East Lansing strain, the LC_{90}s were relatively large for both the greenhouse strain (5178 ppm) and the field strain (362 ppm). These concentrations were near or above the recommended application rate of 484 ppm. Both strains had shallow slopes indicating high heterogeneity to susceptibility. This was observed directly in the bioassay, as active survivors were observed even at the stock concentrations. Overall, soybean aphids showed less susceptibility to imidacloprid than to other tested insecticides.

Table 4.2. Susceptibility of greenhouse and field soybean aphids exposed to five insecticides in aphid-dip bioassays

Insecticide with dose range (ppm)	Aphid Strain	n	Fit of Probit line: Slope ±S.E	χ^2 (df)	p value	LC_{50} ppm (95% F.L)	LC_{90} ppm (95% F.L)
chlorpyrifos	Greenhouse	597	5.55 ± 0.68	1.7 (4)	0.7966	0.0073 (0.0065-0.0081)	0.0124 (0.0108-0.0151)
1312-0.0026	E. Lansing	600	3.57 ± 0.38	45.6[b] (7)	<0.0001	0.0081 (0.0015-0.0120)	0.0743 (0.0274-5.1776)
	Saginaw	597	0.76 ± 0.22	2.0 (7)	0.9562	0.0065 (0.0057-0.0075)	0.0150 (0.0125-0.0191)
	Gera	594	3.58 ± 0.44	4.5 (6)	0.6074	0.0063 (0.0055-0.0073)	0.0145 (0.0117-0.0197)
dimethoate	Greenhouse	353	0.70 ± 0.07	1.8 (3)	0.6050	4.05 (2.22-7.11)	263 (116-815)
1302-0.1302	E. Lansing	350	0.54 ± 0.06	6.0 (3)	0.1139	0.71 (0.39-1.01)	3.01 (2.61-3.83)
	Greenhouse	535	0.76 ± 0.22	76.8[b] (6)	<0.0001	0.0093[a]	0.4590[a]
esfenvalerate	E. Lansing	528	4.41 ± 0.53	0.0 (6)	1.0000	0.0043 (0.0038-0.0049)	0.0086 (0.0072-0.0108)
470-0.0023	Saginaw	528	4.40 ± 0.55	5.7 (6)	0.4585	0.0049 (0.0044-0.0055)	0.0095 (0.0084-0.0124)
	Gera	531	3.59 ± 5.64	915.5[b] (6)	<0.0001	0.0034[a]	0.0076[a]
λ-cyhalothrin	Greenhouse	600	0.77 ± 0.18	10.1 (3)	0.0978	0.054 (0.000-0.307)	2.443 (0.417-4.456)
260-0.0013	E. Lansing	591	2.19 ± 0.26	12.1 (7)	0.0965	0.004 (0.003-0.006)	0.016 (0.011-0.035)
imidacloprid	Greenhouse	346	0.33 ± 0.06	6.8 (3)	0.0781	2.55 (0.81-162)	5178 (105-1.24 x 10^{13})
121-0.012	E. Lansing	353	0.45 ± 0.14	16.6[b] (3)	0.0008	0.45[a]	362.96[a]

[a] 95% Fiducial limits not generated by Proc Probit (SAS 9.1.3, SAS Institute Inc.).

[b] High chi-square values indicated a significant deviation from probit model $p < 0.05$.

Although currently there are no reports of development of soybean aphid resistance to the five insecticides tested in this study, there are numerous reports of insecticide resistance in a closely related species, the cotton/ melon aphid, *Aphis gossypii* Glover (Whalon 2009). Herron and Powis (2005) documented chlorpyrifos resistance in 40% of the tested population of Australian field-collected *A. gossypii* in a bioassay. *A. gossypii* collected from cotton fields in Pakistan, showed moderate resistance (resistance ratio of 41 at LC_{50}) to dimethoate in 1998, but reported a decline afterwards. Resistance ratios reported range from 1- 41 to dimethoate over the years of 1996-2004 (Ahmad and Arif 2008).

The insecticide fenvalerate was extensively used to control *A. gossypii* and other pests on cotton and other crops for decades, in many countries but more particularly in Asian countries (Wang et al. 2002). *A. gossypii* has become highly resistant to fenvalerate, with survival at extremely high concentrations (Zil'bermints and Zhuravleva 1984, Thayumanavan et al. 1993, Sun et al. 1994). Although not identical, fenvalerate and esfenvalerate are conformational isomers. Populations of *A. gossypii* from Hawaii showed a 390-fold resistance to esfenvalerate (Hollingsworth et al. 1994). *A. gossypii* populations collected in central Pakistan from 1997 to 2000 showed high resistance to seven pyrethroid insecticides including resistance ratios ranging from 205 -723 to λ-cyhalothrin (Ahmad et al. 2003). *A. gossypii* resistance to imidacloprid was also reported from the laboratory. In China, Wang et al. (2002) reported an eight-fold increase in the resistance ratio after 13 generations of selection. Thus far, *A. gossypii* resistance to imidacloprid in field has not been confirmed.

In this study, we found that the field aphid strains tested were susceptible to all five insecticides. However, the increase in insecticide use on soybean since the discovery of soybean aphid in North America increases the risk of developing resistance. In particular, the risk seems greatest for the neonicotinoids, which are used both as seed and foliar treatments on soybeans, potentially exposing aphids multiple times in the field season. To date there are no published reports of field failures with imidacloprid for soybean aphid. Recently, Megalhaes et al. (2008) studied baseline susceptibility for imidacloprid delivered systemically, as in a seed treatment. They found imidacloprid was very toxic to soybean aphid, and had both lethal and sublethal effects that affected reproductive capacity and survivorship. They attributed these effects not only to direct insecticide toxicity but also possible anti-feedant behavior (Megalhaes et al. 2008). In contrast, in our study the aphids were directly dipped in a series of imidacloprid dilutions thus we could not measure sub lethal anti-feedant effects, taken longer than 48 h to occur.

Numerous factors may have affected the outcome of these bioassays. Field strains were probably less adaptable to controlled environments than the well-established greenhouse strain. This may have affected the ability of the field strains to respond to toxic stress in the bioassay. Field-collected strains were temporarily reared in growth chambers for nearly three months until studies were completed. Therefore, if a field strain was less adaptable than the greenhouse strain to surviving in the growth chamber, it may have had reduced chance of surviving especially after being exposed to insecticides. Although control mortality for all strains were not higher than 10%, we made a general observation that the greenhouse strain showed better survival than field strains in control treatments.

Toxicity symptoms observed for all insecticides were similar in all strains. All insecticide-affected aphids had excessive secretions from their cornicles, which may be due to elevated secretion of alarm pheromone produced by many aphid species. Alarm pheromone is released with the cornicle secretions exuded by many aphid species, particularly in the subfamily Aphidinae, when disturbed (Hardie et al. 1999). The pheromone signals neighboring aphids to withdraw their stylets from the plant and move away from the pheromone source. van Toor et al. (2008) demonstrated that insecticide-resistant *Myzus persicae* Sulzer had a reduced alarm response compared to susceptible *M. persicae.* Our frequent observation of cornicle secretions by dead, insecticide-killed aphids could be associated with the susceptibility observed for all five insecticides. Excessive cornicle secretions were not observed from control aphids, which were only dipped in water. The few control aphids that died also turned red in color also implying that the redness was not associated with toxicity symptoms.

This aphid-dip bioassay method has its own advantages and drawbacks. The 10 s dip method had the benefit of producing results within 48 h and was sufficiently sensitive to detect toxicity at very low concentrations of insecticide. This method produced relatively consistent results in repeated trials for the same product, and allowed rapid assessment of mortality using visual observation. The method was repeatable, and used only simple laboratory equipment. The mesh tea strainer provided an easy-to-clean and inexpensive method to dip aphids. However, great care had to taken to avoid post-dip aphid deaths due to 'fuming' effect of chlorpyrifos. Therefore, aphids dipped in different doses were separated in the laboratory to avoid cross contamination. In conclusion, this study provides a method for testing susceptibility of soybean aphid to insecticides and provides baseline for comparison of aphid susceptibility for five insecticides. In the event of a field failure, the aphid populations involved can be tested with the greenhouse strain as a comparison to determine development of resistance.

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Literature Cited

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Ahmad, M., M. I. Arif, and I. Denholm. 2003. High resistance of field populations of the Cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) to pyrethroid insecticides in Pakistan. J. Econ. Entomol. 96(3): 875-878.

Ahmad, M., and M. I. Arif. 2008. Susceptibility of Pakistani populations of cotton aphid *Aphis gossypii* (Homoptera: Aphididae) to endosulfan, organophosphorus and carbamate insecticides. Crop Prot. 27: 523–531.

Baute, T. 2007. Let the scouting begin-soybean aphids are here. Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, Ontario. Available: http://www.omafra.gov.on.ca/english/crops/field/news/croppest/2007/08cpo07a1.htm (Aug 2007).

Chen, Q. H., and S. Y. Yu. 1988. Aphids and control. Shanghai Science and Technology Press, Shanghai, China.

Costamagna, A. C., and D. A. Landis. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecol. Appl. 16: 1619-1628.

Costamagna, A. C., and D. A. Landis. 2007. Quantifying predation on soybean aphid through direct field observations. Biol. Cont. 42:16-24.

Costamagna, A. C., D. A. Landis, and M. J. Brewer. 2008. The role of natural enemy guilds in *Aphis glycines* suppression. Biol. Cont. 45:368-379.

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

DiFonzo, C.D. 2007.Insect,Nematode and disease control in Michigan field crops. Extension Bulletin E-1582 .Michigan State University, East Lansing, MI.

DiFonzo, C.D., and R. Hines. 2002. Soybean aphid in Michigan: Update from the 2001 season. Extension Bulletin E-2748. Michigan State University, East Lansing, MI.

Finney D. J. 1971. Probit Analysis. Cambridge University Press, Cambridge, UK.

Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. DiFonzo. 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environ. Entomol. 33:608-618.

Georghiou, G. P., and R. L. Metcalf. 1961. A bioassay method and results of laboratory evaluation of insecticides against adult mosquitoes. Mosq. News 21: 328-337.

Hardie, J., J. A. Pickett, E. M. Poe, D. W.M. Smiley.1999. Aphids. pp. 227–249 in Hardie, J. & Minks, A.K. (Eds) Pheromones of non-lepidopteran insects associated with agricultural plants. Wallingford, Oxon, CABI Publishing.

Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D.W. Onstad, J. T. Shaw, L. F. Solter, D. J. Voegtlin, C. J. D'Arcy, M. E. Gray, K. L. Steffey, S. A. Isard, and P. L.

Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Available: www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/ Plant Health Progr. DOI:10.1094/PHP-2001-0205-01-HN (March 2009).

Herron, G., and K. Powis. 2005. Insecticide resistance of field-collected cotton aphid. Avaialable: http://www.cottoncrc.org.au/files/45711191-ec67-4e5e-9506-9a6b00b7a290/Herron1.pdf (15 July 2009).

Hesler, L. S., K. E. Dashiell, and J. G. Lundgren. 2007. Characterization of resistance to Aphis glycines in soybean accessions. Euphytica 154:91-99.

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

Hill, J. H., R. Alleman, D. B. Hogg, and C. R. Grau. 2001. First report of transmission of Soybean mosaic virus and Alfalfa mosaic virus by *Aphis glycines* in the New World. Plant Dis. 85:561.

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

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

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

Hollingsworth, R.G., B.E. Tabashnik, D.E. Ullman, M.W. Johnson, and R. Messing. (1994). Resistance of *Aphis gossypii (*Homoptera: Aphididae) to Insecticides in Hawaii: Spatial Patterns and Relation to Insecticide Use. J. Econ. Entomol. 87(2), 293-300.

Huang, C. D., J. F. Zhou, and D. Yang. 1998. Experiments on the control of soybean aphid with imidacloprid. Translation from Pesticides. 37: 44-45. Available: http://www.ksu.edu/issa/aphids/reporthtml/trans10.htm (May 2009).

(IRAC) Insecticide Resistance Action Committee. 2009. Available: http://www.irac-online.org/resistance/overview.asp (14 May 2009).

Iwaki M., M. Roechan, H. Hibino, H. Tochihara, and D.M. Tantera 1980. A persistent aphid borne virus of soybean, *Indonesian Soybean dwarf virus* transmitted by *Aphis glycines*. Plant Dis 64:1027–1030.

Landis, D. A., M. J. Brewer,and G. E. Heimpel. 2003. Soybean aphid parasitoid questionnaire 2003. NCR-125 Arthropod Biol. Cont.. Michigan State Report. Available: <http://www.cips.msu.edu.proxy2.cl.msu.edu/ncr125/StateRpts2003MI.htm> (March 2009).

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

Lin, C. L., Z. S. Xun, L. T. Li, Y. P. Wang, and G. X. Zhang. 1992. Control threshold of the soybean aphid in the field. Soybean Sci. 11: 318-321.

Lin, C. L., Z. S. Xun, L. T. Li, H. K. Zhang, G. X. Zhang, and Y. P. Wang. 1994. Population dynamics and control stage of the soybean aphid in Jining prefecture, China. Shandong. Agric. Sci. 4: 44.

Megalhaes, L. C., T. E. Hunt, and B. D. Siegfried, Development of methods to evaluate susceptibility of soybean aphid to imidacloprid and thiamethoxam at lethal and sub lethal concentrations. Entomol. Exp. Appl. 2008. 128(2): 330-336.

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

Mensah, C., C. DiFonzo, R. L. Nelson, and D. C. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45:2228-2233.

Mian, M. A. R., R. B. Hammond, and S. K. S. Martin. 2008. New plant introductions with resistance to the soybean aphid. Crop Sci. 48:1055-1061.

NASS) National Agricultural Statistics Service. 2000. Agricultural chemical usage 1999 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2001. Agricultural chemical usage 2000 field crops summary. Agricultural Statistics Board, NASS, USDA.

(NASS) National Agricultural Statistics Service. 2006. Agricultural chemical usage 2005 field crops summary. Agricultural Statistics Board, NASS, USDA.

Nielsen, C., and A. E. Hajek. 2005. Control of invasive soybean aphid, *Aphis glycines* (Hemiptera : Aphididae) populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. Environ. Entomol. 34:1036-1047.

Noma, T., and M. J. Brewer. 2008. Seasonal abundance of resident parasitoids and predatory flies and corresponding soybean aphid densities, with comments on classical Biol. Cont. of soybean aphid in the Midwest. J. Econ. Entomol. 101:278-287.

Ragsdale, D. W., K. Ostlie, and E. Hodgson. Insecticide Trial for Soybean Aphid, *Aphis glycines* Matsumura. 2 August 2001. Just for Growers. Available: http://www.soybeans.umn.edu/crop/insects/aphid/aphid_insecticide_trial.htm (July 15 2009).

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. Ann. Entomol. Soc. Am. 97:204-208.

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

Rutledge, C. E., and R. J. O'Neil. 2006. Soybean plant stage and population growth of soybean aphid. J. Econ. Entomol. 99:60-66.

SAS institute Inc., 2002. User's manual, v.9.1.3. SAS institute Inc, Cary, NC.

Song, F., M. S. Swinton, C. DiFonzo, M. O'Neal, and D. W. Ragsdale. 2006. Profitability analysis of soybean aphid control treatments in three North-central States. Department of Agricultural Economics Staff paper 2006- 24. Michigan State University, East Lansing, MI.

Sun, Y. Q., G. L. Feng, J. G. Yuan, and K. Y. Gong. 1994. Insecticide resistance of cotton aphid in North China. Entomol. Sin. 1: 242-250.

Sun, B., S. B. Liang, and W. X. Zhao. 2000. Outbreak of the soybean aphid in Suihua prefecture in 1998 and its control methods. Soybean Bull. 1: 56-58.

Thayumanavan, B., S. Uthamasamy, V. Sumathi, and S. Saroja.1993. Glutathione S-transferase and carboxyl esterase activities in aphid (*Aphis gossypii* Glover) fed on cotton leaves treated with synthetic pyrethroids. Indian J. Exp. Biol. 31: 788-789

van Toor, R. F., S. P. Foster, J. A. Anstead, S. Mitchinson, B. Fentonc, and L. Kasprowicz. 2008. Insecticide resistance and genetic composition of *Myzus persicae* (Hemiptera: Aphididae) on field potatoes in New Zealand. Crop Prot. 27:236-247.

Wang, C. L., L. Y. Xiang, G. X. Zhang, and H. F. Chu. 1962. Studies on the soybean aphid, *Aphis glycines* Mastumura. Acta Entomol. Sinica. 11: 31–44 (Translated).

Wang, S. Y., X. Z. Bao, Y. J. Sun, R. L. Chen, and B. P. Zhai. 1996. Study on the effect of population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. Soybean Science. 15: 243-247. (Translated).

Wang, K.Y., T. X. Liu, C. H. Yu, X. Y. Jiang, and M. Q. Yi. 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to Fenvalerate and Imidicloprid and Activities of Detoxification Enzymes on Cotton and Cucumber. J. Econ. Entomol. *95*: 402-407.

Wang Q., L. J. Han, X.L. Huang, Z. Y. Gu, X.-W. Qiao, and J. S. Zhu. 1995. Effects of imidacloprid in cotton aphid control and its toxicity. China Cottons 22: 17-18.

Wang, X. B., Y. H. Fang, S. Z. Lin, L. R. Zhang, and H. D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Translated from Plant Protection (Institute of Plant Protection, CAAS). 20: 12-13. Available: http://www.ksu.edu/issa/aphids/reporthtml/trans29.htm (May 2009).

Wang, Y. Z., and F. Ba. 1998. Study on optimum control of the soybean aphid. Acta Phyt. Sinica 25: 152-155

Whalon, M. 2009. Athropod pesticide resistance database. Available: http://www.pesticideresistance.org/search/12/0/218/0/ (July 9, 2009).

Wu, X. B., W. J. Ni, and P. J. Liu. 1999. Occurrence and control of the soybean aphid, *Aphis glycines* Matsumura. Translation from Chinese J. Biol. Control. 6: 20.

Wu, Z. S., D. Schenk-Hamlin, W. Y. Zhan, D. W. Ragsdale, and G. E. Heimpel. 2004. The soybean aphid in China: A historical review. Ann. Entomol. Soc. Am. 97:209-218.

Yu, D. R., S. G. Guo, and Y. L. Shan. 1989. Resistance of wild soybean *Glycine soja* to *Aphis glycines*, Screening for resistant varieties. Jilin Agric. Sci. 3: 15-19.

Zil'bermints, I.V., and L. M. Zhuravleva. 1984. Response of melon and greenhouse aphids to ambush and actellic. Khim. Sel'sk. Khoz. 3: 37-40.

Appendix 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2009-06

Title of thesis or dissertation (or other research projects):

HOST PLANT RESISTANCE AND INSECTICIDE RESISTANCE STUDIES FOR SOYBEAN APHID *Aphis glycines* Matsumura

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Desmi Indumali Chandrasena
Christina DiFonzo

Date 08-21-2009

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

Appendix 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Aphis glycines Matsumura	Greenhouse, Michigan State University collected 08-21-2009			11		13			Entomology Museum, MSU
Poppilia japonica Newman	Entomology Field Research Center Collins Road, East Lansing, MI					10	10		Entomology Museum, MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Desmi Indumali Chandrasena

Christina DiFonzo

Date 8/21/2009

Voucher No. 2009-06

Received the above listed specimens for deposit in the Michigan State University Entomology Museum

Curator Date 21 AUG 2009