INTEGRATED PEST MANAGEMENT OF SOYBEAN APHID (APHIS GLYCINES) IN NORTH DAKOTA

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

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ABSTRACT

Hochhalter, Julie; M.S.; Department of Entomology; College of Agriculture, Food Systems, and Natural Resources; North Dakota State University; January 2010. Integrated Pest Management of Soybean Aphid (*Aphis glycines*) in North Dakota. Major Professor: Dr. Marion O. Harris.

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybean (*Glycine max* L.). This aphid is a native of Asia and was first discovered in the United States in Wisconsin in July 2000, and in North Dakota in summer 2001. Management of the soybean aphid varies across the geographical range of the pest. The impact of the soybean aphid has been less in North Dakota compared to many areas of the Midwest. One reason is environmental conditions in North Dakota limits population growth of the soybean aphid. Another is that until recently the area of soybean hectares in North Dakota has been limited. But now production has increased and growers are expecting integrated pest management programs designed specifically for North Dakota conditions. This research addresses how insecticides and resistant soybean cultivars might be used to control North Dakota populations of the soybean aphid.

The objective of the first study was to determine efficacy of foliar and seed treatments for controlling the soybean aphid. Effects on beneficial insects were also determined. The foliar insecticide lambda-cyhalothrin (Warrior) was applied to soybean at different plant growth stages. The seed treatment thiamethoxam (Cruiser Maxx) was applied alone and in combination with the foliar insecticide lambda-cyhalothrin (Warrior). A foliar application of lambda-cyhalothrin (Warrior) applied at the economic threshold of 250 aphids per plant was the most effective control method. Seed treatments were not

effective, probably because insecticidal effects had declined by the time aphids were invading the crop.

The objective of the second study was to evaluate experimental soybean lines for resistance to soybean aphid. The first experiment was conducted in the greenhouse and involved 436 soybean lines. The second experiment included 30 susceptible lines and 25 resistant soybean lines at two field sites. The third experiment included the same lines that were evaluated in the field, but this screening was conducted in the greenhouse and involved caging aphids. In general, ranking of the lines for resistance was consistent between the first greenhouse experiment and the field experiment, suggesting that greenhouse screening is an effective method for scoring soybean lines for resistance and can be used to accelerate progress in soybean breeding programs. Five experimental lines, known to have the *Rag1* gene that confers resistance to soybean aphid, maintained aphid levels below the economic injury level. The economic injury level is 674 aphids per plant when the plant is at the reproductive stages. The third experiment, which caged aphids on leaves of susceptible and resistant lines in the greenhouse, was not an effective method for scoring resistance.

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CHAPTER 1. INTRODUCTION

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybean (*Glycine max* L.). It is a native of Asia and was first discovered in the United States in Wisconsin in July 2000. In the summer of 2001, it was found in North Dakota. Management of soybean aphid varies across the geographical range of the pest. The impact of the soybean aphid has been less in North Dakota compared to many areas of the Midwest. One reason is that environmental conditions in North Dakota limit population growth of the soybean aphid. Another is that until recently, the area of soybean production in North Dakota has been limited. But now it is on the rise and growers are expecting integrated pest management programs designed specifically for North Dakota conditions. This research addresses how insecticides and resistant soybeans might be used to control North Dakota populations of soybean aphids.

Systematics

Soybean aphid, *Aphis glycines* Matsumara, belongs to the family Aphididae in the order Hemiptera. The order is divided into four suborders, the Sternorryncha, Auchenorrhyncha, Coleorhyncha and the Heteroptera. The family Aphididae belongs to the suborder Sternorrhyncha, which also includes scale insects, psyllids, and whiteflies. Unlike the other three suborders, the suborder Sternorrhyncha contains species that have the rostrum arising from between the fore coxae and 1 or 2 segmented tarsi (Blackman and Eastop 1984). Aphids are more closely related to scales than psyllids and whiteflies. The soybean aphid belongs to the genus *Aphis*. *Aphis* contains more than 400 species of small- to medium- sized aphids that are mostly found in the Northern Hemisphere (Blackman and Eastop 1984).

Aphids as Agricultural Pests

Soybean aphid is not the only agricultural pest in the family Aphididae. Other important agricultural pests include: Russian wheat aphid (*Diuraphis noxia* Mordvilko), potato aphid (*Macrosiphum euphorbiae* Thomas), green peach aphid (*Myzus persicae* Sulzer), bird cherry-oat aphid (*Rhopalosiphum padi* Linnaeus), and greenbug (*Schizaphis graminum* Rondani). Other members of Aphididae are important pests of fruit and vegetable crops and are distributed throughout the world (Blackman and Eastop 1984).

Features of aphid biology allow the location and exploitation of host plants (Powell et al. 2006). Hosts are selected based on a sequence of chemical and physical cues including chemical and physiological (Powell et al. 2006). Some aphid species can reproduce without males for part of their life cycle, giving them a reproductive advantage over sexually reproducing individuals (Dixon 1992).

Aphids are often compared to other insects in terms of rapid turnover of generations and buildup of populations. Generation doubling time is an average of 6.8 days in the field (Ragsdale et al. 2007), with several generations being produced during a growing season. In controlled environments with ideal weather conditions and no natural enemies, soybean aphid populations can double in as little as 1.5 days (McCornack et al. 2004).

Distribution of Soybean Aphid

The center of origin for the soybean aphid is China. However, it is now found across Asia in China, Japan, the Philippines, South Korea, Indonesia, Malaysia, Thailand, Vietnam, and Russia (Wu et al. 2004). In China, the soybean aphid is the most serious insect threat to soybean production and has been studied extensively since the 1960's (Wu et al. 2004).

Soybean aphid was first discovered in the United States in July 2000 in Wisconsin (Alleman et al. 2002) and by the end of the 2000 growing season had been discovered in 10 states (Venette 2004). By 2003, soybean aphid was found in 21 states and three Canadian provinces (Venette 2004). The soybean aphid was first discovered in North Dakota in August 2001 (Glogoza 2004).

Reproduction

Aphids are classified as either monoecious, feeding on one host plant species, or heteroecious, feeding on two host plant species during different parts of the year (Helle 1989). The heteroecious species have a primary host, which serves as an overwintering host. A secondary host serves as a summer host (Helle 1989).

The life cycle of the soybean aphid is heteroecious with sexual reproduction occurring during a small part of its life cycle (McCornack et al. 2005). Soybean aphids have an egg stage that is cold-hardy, allowing them to overwinter in North Dakota (Crompton 2007). Adult females lay eggs on various buckthorn (*Rhamnus*) species at various locations within the shrub, but most commonly at the interface between the bud and twig (Ragsdale et al. 2004). *Rhamnus* is a common shrub in shelterbelts throughout North Dakota (Voegtlin et al. 2004). Aphid eggs hatch in the spring. After hatching, soybean aphids go through 2-3 generations of sexual reproduction on buckthorn (Voegtlin et al. 2004). The winged females, alates, start moving into soybean fields in June. These females reproduce parthenogenetically; they bear live female young clones (Gullan and Cranston 2000). Until crowding occurs and/or plant quality deteriorates, the young that are produced are wingless. Thereafter, winged females are produced, with these females moving to attack higher quality plants.

In the aphid species *Megoura viciae* Buckton, a major factor in producing winged forms of aphids is crowding with contact stimuli being more frequent in a crowded population (Lees 1967). In late summer, winged females change their behavior, now moving out of soybean fields to find buckthorn. These females can produce males or females. Males and females mate and females produce eggs (females) and oviposit on buckthorn. It is these eggs that overwinter (Ragsdale et al. 2004). The production of males by parthenogenetic females is done by the parent female losing a set of X-chromosomes in the course of the reproduction process (Helle 1989).

Host Range

Most aphids live on one plant species or a small number of plant species within a single plant genus (Eastop 1973). About 10% have a primary host plant where they spend fall, winter, and spring, and then spend the summer on a secondary host plant. The secondary host plant is rarely related to the primary host plant (Helle 1989). Most aphids show a high degree of host specificity.

The secondary host of soybean aphid is the cultivated soybean, *Glycine max* L. Merr. Soybean aphids are attracted by the odor of the soybean plant, but repelled by odors of nonhost plants (Du et al. 1994). Han and Yan (1995) found that stylet penetration and sucking behavior of the soybean aphid were significantly different on soybean than on other plants. Other hosts are members of the plant family Fabaceae, including wild soybean, *Glycine* Benth f. *lanceolate* Makino. However, soybean aphid has also been recorded on varieties of *Pueraria phaseoloides* (kudzu) (Venette and Ragsdale 2004) and *Desmodium intortum* (Wang et al. 1962). A host range study in Wisconsin found that soybean aphid was able to successfully colonize and reproduce on some clover species including red clover, (*Trifolium pratense* L.), Egyptian clover (*T. alexandrinum* L.), crimson clover (*T. incarnatum* L.), and Kura clover (*T. ambiguum* M. Bieb) (Alleman et al. 2002). Soybean aphids are able to feed, but reproduction is low on other clover species (white clover, *T. repens* L., white sweetclover, *Melilotus alba* Medikus, and yellow sweetclover, *M. officinalis* L. Lam.), snap beans (*Phaseouls vulgaris* L.), and alfalfa (*Medicago sativa* L.) (Alleman et al. 2002).

Status of Soybean Aphid

Soybean aphid causes significant yield losses by feeding on plant sap. This results in reduced pod set, plant stunting, and leaf distortion (Hill et al. 2004). When plants are colonized in the early vegetative growth stages, a yield loss of more than 50% can occur (Ostlie 2002; Wang et al. 1994). In 2001, a Wisconsin study showed a yield loss of 20% due to soybean aphid infestations during the reproductive growth stages that reached 800 aphids per plant (Myers et al. 2005).

In Asia, soybean aphid transmits soybean mosaic virus, a disease that reduces seed quality and causes significant yield losses (Wu et al. 2004). The soybean aphid is known to vector several other viruses: soybean stunt virus, soybean dwarf virus, abaca mosaic, beet mosaic, tobacco vein-banding mosaic virus, bean yellow mosaic virus, mungbean mosaic virus, peanut mottle virus, and peanut mosaic virus (Iwaki 1979).

In the United States, soybean aphid transmits soybean mosaic virus and alfalfa mosaic virus (Hill et al. 2001). More recently, soybean aphid has been known to successfully vector bean yellow mosaic virus (Wang et al. 2006). Currently in the United States, several viruses that could potentially be transmitted by the soybean aphid are being studied.

Biological Control

In Asia, soybean aphids are attacked by a number of natural enemies. In China and South Korea, natural enemies of soybean aphid include a number of parasitoids, predators, and pathogens (Wu et al. 2004). The importance of natural enemies in control of soybean aphid in Asia provides insight into their potential in integrated pest management programs in North America (Rutledge et al. 2004). Soybean aphid outbreaks in China occur sporadically in some growing regions. Therefore, when aphid populations occur in small numbers, natural enemies provide adequate control and an insecticide treatment is not needed.

In North America, natural enemies are an important source for aphid mortality (Fox et al. 2004, Liu et al. 2004, Costamanga and Landis 2006). Several experiments have demonstrated that the existing predator community suppresses soybean aphid populations (Fox et al. 2004, Rutledge et al. 2004). In a no-choice feeding trial, Asian ladybeetle, *Harmonia axyridis* (Pallas) caused an 86-88 percent reduction to a soybean aphid population in a 24-hour period (Rutledge et al. 2004). Some natural enemies of soybean, including predators and pathogens, follow the soybean aphid from soybean fields to its overwintering host buckthorn (Yoo et al. 2005, Nielsen and Hajek 2005) and continue to reduce aphid populations on the overwintering host well past soybean harvest. Fox et al. (2002) determined that Asian ladybeetle, *Harmonia axyridis* (Pallas) and minute pirate bug, *Orius insidiosus* (Say) were the most numerous predators to attack soybean aphids in Michigan field conditions. In Iowa, dominant natural enemies were *Orius insidiosus* Say, ladybeetles (coccinellids), and green lacewings (*Chrysoperla* spp.), and hoverflies (*Toxomerus* spp.) (Schmidt et al. 2008). Although *Chrysoperla* spp. are considered to be primarily predators of aphids, their ability to suppress aphid populations is limited (Rosenheim et al. 1993). The natural enemies of North Dakota populations of soybean aphid have not been characterized.

Chemical Control

In Asia, the most common method for management of high populations of soybean aphids is a well-timed foliar insecticide. Numerous insecticides have been tested for control of soybean aphid (Wu et al. 2004). Growers in Asia may apply insecticides up to four times in one growing season to prevent yield loss from soybean aphid (Dai and Fan 1991). Many of these insecticides are highly toxic, broad spectrum chemicals.

Extensive use of insecticides has led to development of resistance in many insect species, including aphids. For example, the damson-hop aphid, *Phorodon humuli* (Schrank), uses only the hop, *Humulus lupulus,* as its summer host, so a majority of the population in a hop-growing region will come in contact with insecticides used on this crop (Muir 1979). However, many of the insecticides used on hops are from the same insecticide class, which resulted in the aphids developing resistance and overcoming the insecticide. In many cases changing the class of insecticide used or the crop cultivar appears to have by-passed the resistance problem (Helle 1989).

Before the introduction of soybean aphid, few insect pests were present in soybean; therefore, insecticides were rarely applied. Use of insecticides in United States soybean fields has increased since 2000, with the increase attributed to the introduction of the soybean aphid (NASS USDA 2007). In 2001, less than one percent of soybean hectacres received an insecticide application of chloropyrifos and lambda-cyhalothrin. However, by

2008, 11% of U.S. soybean acres received at least one insecticide application (NASS USDA 2007).

Integrated pest management programs recommend that insecticides only be applied when pest populations reach the economic threshold. The threshold for soybean aphid is 250 aphids per plant on 80% of the field during the reproductive growth stages (Ragsdale et al. 2007). Insecticide treatment at this stage will prevent yield loss and permanent injury to the plant that occurs when aphid populations reache the economic injury level of 674 aphids per plant (Ragsdale et al. 2007).

Insecticides applied as seed treatments are an alternative method of chemical control. Seed treatments registered for control of soybean aphid are from the neonicotinyl-based insecticides. Neonicotinoids are an insecticide class that is generally used for systemic control (Tomizawa and Casida 2003).

Host Plant Resistance

Host plant resistance strategies are safe for the environment and can reduce the financial input of growers (Li et al. 2004). Plant resistance is controlled by one or more genes and can be modified by physical, chemical, and biological factors (Helle 1989). Morphological plant characteristics that may play a part in resistance include foliage size, shape, color, pubescence, tissue thickness, and nutritional value (Helle 1989).

Painter (1951) defined resistance of plants to an insect attack as the relative amount of heritable qualities possessed by the plant that influence the ultimate degree of damage done by the insect. Painter (1951) proposed three general mechanisms for plant resistance to insect damage: antixenosis, antibiosis, and tolerance. Antixenosis affects the behavior of the insect. Antibiosis affects physiology when the insect chooses the resistant host plant.

Tolerance is the ability of the plant to recover and support a population of an insect similar to the susceptible host.

The ultimate choice of a breeding method to incorporate a new trait into a plant depends on the reproduction of the plant and the inheritance of the trait to be introduced (Helle 1989). Plant breeding methods for control of aphids depend on the host plant involved. Breeding methods for cross-pollinated crops differ from self-pollinating crops on their sources for insect resistance (Helle 1989). Soybean is a self pollinated crop, and in self-pollinating crops, inbreeding usually does not result in a decrease in yield or vigor.

Dominant genes are usually involved in aphid resistance (Auclair 1989). Examples of the monogenetic dominant resistance to aphids include the Russian wheat aphid, (*Diuraphis noxia)* in wheat (*Triticum spp.*) (Liu et al. 2001) and the greenbug, (*Schizaphis graminum)* in barley (*Hordeum vulgare L.)* (Porter and Mornhinweg 2004).

Evolution of Virulence to Plant Resistance

There are many examples of effective plant resistance to aphids. Wheat germplasm with resistance to the Russian wheat aphid has been identified (Smith et al. 1991) and the gene *Mi* in tomato confers resistantce to the potato aphid (Magdalena et al. 1998). Different genotypes occur frequently among aphids and help overcome resistance (Helle 1989). Due to their reproductive biology, aphids impose a selection pressure in favor of overcoming plant resistance.

The use of cultivars with a single gene for aphid resistance encourages the rapid selection of aphid genotypes that may overcome resistance (Kim et al. 2008). Genotypes of the Russian wheat aphid were found to overcome resistance genes that were monogenetic (Burd and Porter 2006).

Recent studies indicate that some populations of soybean aphids can survive on soybean expressing the *Rag1* gene (Kim et al. 2008). In 2008, Kim et al. used soybean lines containing the *Rag1* gene to test aphids from Ohio and Illinois. The aphids from Ohio were able to colonize plants carrying *Rag1*; however, soybean aphids from Illinois were not able to colonize plants carrying *Rag1*. They concluded soybean aphid from Ohio can overcome the resistance of *Rag1* (Kim et al 2008). As a result, Kim et al. (2008) concluded there are different genotypes of the soybean aphid that differ in their susceptibility to plant resistance conferred by the *Rag1* gene.

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CHAPTER 2. EVALUATING FOLIAR AND SEED TREATMENTS FOR CONTROL OF SOYBEAN APHID

First discovered in the United States in 2000, soybean aphids have become a major pest of soybean. Populations of soybean aphid increase rapidly and spread quickly (Wang et al. 1998). Populations of over 1000 soybean aphids per plant before flowering have been shown to reduce plant height and pod number; thus resulting in a reduction in yield (Dai and Fan 1991, Lin et al. 1993, Wang et al. 1996). In China, yield losses of 50% have been reported due to high populations (Wang et al. 1994, 1998). Soybean aphids also cause indirect harm by excreting honeydew and promoting the growth of sooty mold, (*Cladosporium* spp.) which decreases photosynthetic rates (Macedo et al. 2003).

The most common method used to manage the soybean aphid is a well-timed foliar insecticide. In China, numerous insecticides have been tested and used for control (Wu et al. 2004) with growers applying insecticides up to four times in one growing season to prevent yield loss from the soybean aphid (Dai and Fan 1991). Many of these insecticides are highly toxic, broad spectrum chemicals. A challenge for controlling aphids using these broad spectrum chemicals is that insect populations can rebound after treatment (Myers et al. 2005). Therefore, any individuals that survive treatment have the potential to reproduce rapidly. Insecticides also kill the natural enemies, contributing to the rapid rebound. Before introduction of the soybean aphid to the United States, few insect pests were present in soybean and insecticides were rarely applied.

Myers et al. (2005) showed that yields can be increased by as much as 31% when multiple applications of λ-cyhalothrin or chlorpyrifos are applied during the plant's reproductive growth stages. More recently, the control recommendation is for a foliar insecticide to be applied at the economic threshold (Ragsdale et al. 2007). This threshold is 250 aphids per plant on 80% of the field during early flowering to full pod set (Ragsdale et al. 2007). Insecticides are applied at the economic threshold to prevent insects from reaching the economic injury level (674 aphids per plant) a level at which significant yield loss and other permanent injury occurs (Ragsdale et al. 2007).

Insecticides applied as seed treatments are an alternative method of chemical control. Seed treatments are a systemic control method and as a result, can provide protection that lasts longer than a foliar spray (Nault et al. 2004). Seed treatments have been used in Minnesota to control bean leaf beetle on snap beans, (*Phaseolus vulgaris*) (Koch et al. 2004), and also have been suggested for control of soybean aphid (Magalhaes et al. 2008, McCornack and Ragsdale 2006). Seed treatments registered for control of the soybean aphid are included in the neonicotinyl-based insecticide group and include thiamethxom (Cruiser MAXX, Syngenta Crop Protection Inc., Greensboro, NC) and imidacloprid (Gaucho, Bayer Crop Science LP., Research Triangle Park, NC). McCornack and Ragsdale (2006) showed that thiamethoxam seed treatment did not significantly increase yield in three of four experiments, and did not provide adequate control of late aphid infestations in Minnesota soybean. The advantage of seed treatments is that they slow aphid population growth early in the growing season. In Nebraska, concentrations of thiamethoxam and imidacloprid decreased after 40 days, allowing insects to start colonizing treated plants (Magalhaes et al. 2008). Thus, when late season aphid outbreaks occur, a foliar insecticide application may be needed (McCornack and Ragsdale 2006, Magalhaes et al. 2008).

My overall research objective was to compare insecticide treatments for control of soybean aphid in North Dakota. A small plot study and a grower study were conducted to compare a foliar application of lambda-cyhalothrin (Warrior) to two seed treatments: one with the active ingredient of thiamethoxam (Cruiser Maxx) and the other an experimental seed treatment (Valent). The first objective was to determine if the seed treatments would require a foliar insecticide application for control of the soybean aphid. Even though similar studies have been done in Nebraska and southern Minnesota (Magalhaes et al. 2009 and McCornack et al. 2006), chemical companies and producers sponsored my research, asking whether similar results would occur in North Dakota. Table 2.1 outlines the experiments that were conducted to determine the efficacy of the insecticide treatments. Tables 2.2 and 2.3 list the insecticide treatments included in the small plot study and the grower study, respectively. The second objective was to determine if a commonly used foliar insecticide (Warrior) would control aphid outbreaks when applied at different growth stages. A small plot study was conducted to determine the efficacy of applications at different plant growth stages with the economic threshold treatment of 250 aphids per plant on 80% of the field in the R1-R5 growth stages (Ragsdale et al. 2007). The third objective was to determine whether natural enemies are negatively impacted by insecticide treatments. Sweep net sampling and sticky cards were used to determine the natural enemies present in each treatment. Natural enemies found included members of the insect families: Nabidae, Coccinelidae, Geocoridae, Hemerobiidae, Chysopidae, and Syrphidae. Samples were also examined for arachnids.

Table 2.1. Experiments conducted for determining efficacy of the seed treatment thiamethoxam and an experimental seed treatment with and without an additional application of lambda-cyhalothrin (Warrior). Treatments are described in Table 2.2 for the small plot study and in 2.3 for the grower study.

Experiment	Dates and Location	Size of Study
Small Plot Study	2007- Prosper, ND	eight treatments/replicate
	2007 Glyndon, MN[1]	four replicates/site
	2008- Prosper, ND	
	2008- Johnson, MN	
Grower Study	2007 Mapleton, ND[1]	four treatments/replicate
	2007- Johnson, MN	three replicates/site
	2008- Johnson, MN	

[1]Abandoned due to flooding during the growing season.

Table 2.2. Seed and foliar treatments included in the small plot study.

Category	Treatment
Untreated seed	Control
Seed Treatments	Cruiser MAXX
	Valent experimental
Foliar Treatments	Warrior: economic threshold[1]
	Warrior: R3
	Warrior: R3, R4, R5
Seed Treatment plus Foliar Treatment	Cruiser MAXX plus Warrior: ET[1]
	Valent experimental plus Warrior: ET[1]

[1]The economic threshold used was 250 aphids per plant on 80% of the field during the reproductive growth stages (Ragsdale et al. 2007)
ET=economic threshold

Table 2.3. Seed and foliar treatments included in the grower study.

Category	Treatment
Untreated Seed	Control
Seed Treatment	Cruiser MAXX
Foliar Treatment	Warrior: economic threshold[1]
Seed Treatment plus Foliar Treatment	Cruiser MAXX plus Warrior: ET[1]

[1]The economic threshold used was 250 aphids per plant on 80% of the field during the reproductive growth stages
ET=economic threshold

Materials and Methods

Small Plot Experiment

Experiments were established at the North Dakota State University Research site near Prosper, ND in 2007 and 2008. Experiments were also establishes at a grower cooperator site near Johnson, MN in 2008. Treatments were assigned to experimental units using a randomized complete block design with a split plot in time arrangement with four replicates. Soybeans plots were planted using a plot planter (Almaco, Nevada IA) at a rate of 432400 live seeds ha^{-1}. Figure 2.1 shows the layout of the experiment and Table 2.2 lists the treatments included in this experiment. Plots were 1.98 m wide and 7.62 m long (15.09 sq. m). Each plot was six rows spaced 30.48 cm apart. All plots were planted to the Roughrider Genetics 600 Round-up Ready cultivar (Roughrider Genetics, North Dakota State University Research Foundation, Fargo, ND). This cultivar was chosen because it has a 0.0 maturity and is adapted to North Dakota growing conditions (http://www.roughridergenetics.com/RG600RR.htm). A border plot of untreated seed of the same cultivar was planted between each plot to help minimize insecticide drift from foliar applications. Plot maintenance was done on the dates listed in Tables 2.4 and 2.5.

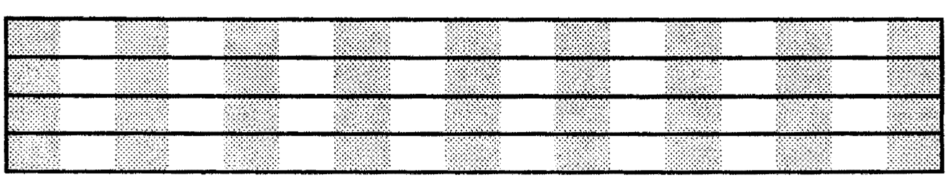

Figure 2.1. Small plot experimental layout. The dark shaded areas are the border plots of untreated seed and the white area represents the plot area. Replicates are indicated by the dark borders.

Table 2.4. Dates of 2007 plot maintenance work. Aphid populations never reached economic threshold in 2007.

Date	Location	Small Plot Study	Grower Study	Description of Activity
25 May	Johnson		X	Seed sown
29 May	Prosper	X		Seed sown
29 May	Glyndon	X		Seed sown
23 June	Johnson		X	Field sprayed with Round-up and Pursuit for weed control
3 July	Prosper	X		Field sprayed with Round-up for weed control
18 July	Prosper	X		Warrior applied to the R3 growth stage treatment and the first application of the treatment receiving three applications of Warrior
27 July	Prosper	X		Second application of Warrior applied to the treatment receiving three applications. Warrior was also applied to the following treatments in spite of the low aphid counts: economic threshold, Cruiser Maxx plus Warrior at economic threshold, Valent plus Warrior at economic threshold
9 August	Prosper	X		Third application of Warrior applied to the treatment requiring three applications.
9 August	Johnson		X	A commercial applicator applied Warrior to the economic threshold treatment and to the Cruiser Maxx plus Warrior at the economic threshold treatment in spite of low aphid numbers.
4 October	Johnson		X	Plots were harvested by a commercial grower. Yield was measured by a weigh wagon.
25 October	Prosper	X		Plots were harvested.

Table 2.5. Dates of 2008 plot maintenance work. Aphid populations reached economic threshold in 2008.

Date	Location	Small Plot Study	Grower Study	Description of Activity
21 May	Prosper	X		Seed sown
22 May	Johnson	X		Seed sown
28 May	Johnson		X	Seed sown
18 July	Johnson	X		First application of Warrior applied to the treatment requiring three applications.
20 July	Prosper	X		First application of Warrior applied to the treatment requiring three applications.
26 July	Prosper	X		The treatment requiring an application of Warrior at ET was sprayed.
1 August	Prosper	X		The following treatments were sprayed with Warrior: R3 growth stage, Cruiser Maxx plus warrior at ET, Valent plus Warrior applied at ET. The second application of Warrior was also applied to the treatment requiring three applications.
7 August	Johnson	X		The following treatments were sprayed with Warrior: R3 growth stage, ET, Cruiser Maxx plus Warrior at ET, and Valent plus Warrior at ET. The second application of Warrior was applied to the treatment requiring three applications.
7 August	Johnson	X		A commercial applicator sprayed the following treatments: Warrior at ET, and Cruiser plus Warrior at ET.
14 August	Johnson	X		The third application of Warrior was applied to the treatment requiring three applications.
15 August	Prosper	X		The third application of Warrior was applied to the treatment requiring three applications.
20 September	Johnson	X		Harvest plots.
26 September	Johnson		X	Plots were harvested by a commercial grower.
1 November	Prosper	X		Harvest plots.

ET= Economic Threshold

Each week, plant growth stages and node counts were recorded. The plant growth stages are described in Table 2.6. Aphid densities were determined using destructive whole-plant counts. Six plants per plot were selected from R2 through R6 (full seed) and removed from the plot at random and bagged. Sampling dates are listed in Tables 2.7 and 2.8. In 2007, 960 plants were sampled at Prosper, ND. In 2008, 768 plants were sampled at the Prosper, ND, location and 576 plants were sampled at the Johnson, MN, location. Bags were transported back to the laboratory where they were stored at 5° C, a temperature at which aphids survive, but do not reproduce. Since aphid populations were extremely high in 2008, plants with approximately 1000 aphids or more were recorded as 1000. Plots were harvested at harvest maturity using a small plot combine (Almaco, Nevada, IA). Yield was measured by the plot combine. Oil and protein content was measured by researchers at the North Central Research Extension Center located in Minot, ND using the near-infrared spectroscopy method.

Table 2.6. Soybean plant growth stages (Fischer and Fanta 2004).

Description	Growth Stage
Emergence	VE
Cotyledon stage	VC
First trifoliate	V1
Second trifoliate	V2
Third trifoliate	V3
Nth trifoliate	V(n)
Flowering will soon start	V6
Beginning bloom, first flower	R1
Full bloom, flower in top 2 nodes	R2
Beginning pod, 3/16" pod in top 4 nodes	R3
Full pod, 3/4" pod in top 4 nodes	R4
1/8" seed in top 4 nodes	R5
Full size seed in top 4 nodes	R6
Beginning maturity, one mature pod	R7
Full maturity, 95% of pods are mature	R8

Table 2.7. 2007 dates and growth stages for beneficial insect sampling and aphid counts.

Date	Location	Plant Growth Stage	Small Plot Study	Grower Study	Aphid Counts	Beneficial Insect Sweeps
27 June	Prosper	V4	X			X
28 June	Johnson	V6		X		X
2 July	Prosper	R1	X			X
5 July	Johnson	R1		X		X
9 July	Prosper	R2	X		X	X
12 July	Johnson	R2		X	X	X
16 July	Prosper	R3	X			X
17 July	Prosper	R3	X		X	
19 July	Johnson	R3		X	X	X
24 July	Prosper	R4	X		X	X
27 July	Johnson	R4		X	X	X
31 July	Prosper	R4	X		X	
3 August	Johnson	R5		X	X	X
9 August	Johnson	R5		X	X	X
13 August	Prosper	R5-R6	X		X	X
16 August	Johnson	R6		X	X	X

Table 2.8. 2008 sampling dates and growth stages for natural enemies and aphid counts.

Date	Location	Plant Growth Stage	Small Plot Study	Grower Study	Aphid Counts	Beneficial Insect Sweeps	Beneficial Insect Cards
10 July	Johnson	R1	X	X		X	X
17 July	Prosper	R1	X		X	X	
18 July	Johnson	R1	X	X		X	X
23 July	Prosper	R2	X		X		
25 July	Johnson	R2-R3	X	X		X	X
25 July	Prosper	R2-R3	X			X	
30 July	Prosper	R3	X		X	X	
2 August	Johnson	R3	X	X	X	X	
2 August	Prosper	R3-R4	X			X	
6 August	Prosper	R4	X			X	
7 August	Johnson	R4	X	X	X	X	
14 August	Johnson	R4-R5	X	X	X	X	
15 August	Prosper	R5	X		X		
20 August	Johnson	R5-R6	X	X	X	X	
25 August	Prosper	R5-R6	X				

Foliar insecticide applications of lambda-cyhalothrin (Warrior) were applied (Table 2.2) at 180 mL ha^{-1}, 275.79 kPa and 75.71 L ha^{-1} using a carbon dioxide hand sprayer (R & D Sprayers, Opelousas, Louisiana). In 2007, aphid populations did not reach the economic threshold, but foliar applications were applied at the R5 growth stage. The economic level threshold for insecticide applications occurred when 80% of the plants in a plot had 250 aphids per plant in 2008. This occurs when aphid populations typically are actively increasing and plants are in the R1-R5 growth stage (Ragsdale et al. 2007). Foliar applications based on the growth stage were applied using the growth stages described in Table 2.6. The Cruiser MAXX seed treatment was provided by Syngenta Crop Protection Inc., Greensboro, NC. The active ingredients are 22.61% thiamethoxam, 1.70% fludioxonil, and 1.12% mefenoxam. An experimental seed treatment was provided by Valent (Dublin, California). The active ingredients cannot be listed here due to confidentiality agreements.

A 38 cm sweep net was used to take 180° sweeps at a rate of 25 per plot for each of the treatments listed in Table 2.2. Tables 2.7 and 2.8 list the dates and locations of the sweep sampling. In 2007, a total of 5600 sweeps were taken at the Prosper, ND, location. In 2008, a total of 4800 sweeps were taken at the Prosper, ND, location and 5600 were taken at the Johnson, MN, location. Insects collected by sweeping were transferred to 30 cm Ziploc bags (Racine, Wisconsin), taken back to the laboratory and frozen. In 2007, members of the following insect families were scored: Nabidae, Coccinelidae, Geocoridae, Hemerobiidae, and Chysopidae. In 2008, members of the following insect families were examined: Nabidae, Coccinelidae, Geocoridae, Hemerobiidae, Chysopidae, and Syrphidae. In 2008, samples were also examined for arachnids.

Treatments effects on yield, oil and protein differences were estimated using analysis of variance (ANOVA) and Fisher's Protected LSD at $P = 0.05$ level (SAS Institute, 2002). The main effects were compared using analysis of variance (ANOVA). Error (a) (replicate x treatment) was used as the denominator of the F-test for treatment and replicate, error (b) (replicate x time) was used as the denominator for time, and error (c) (residual) was used as the denominator for the treatment*time interaction. If found to be significantly different, treatments were also compared for aphid counts across time using analysis of variance (ANOVA) and Fisher's Protected LSD at P = 0.05 level (SAS Institute, 2002). F-tests were considered significant at $P \leq 0.05$.

Grower Study Experiment

Grower studies were conducted in 2007 and 2008 near Johnson, MN at the Allen Gronfeld farm. Each grower study was a large field plot study done in cooperation with a local grower. Treatments were assigned to experimental units using a randomized complete block design with a split plot in time arrangement with three replicates. Seeds were sown using a commercial John Deere (Moline, Illinois) seeder with a plant population of 333600 seeds ha^{-1}. The treatments included in this experiment are listed in Table 2.3. Experimental units were 708.05 m long and 9.14 m wide (6471.58 sq. m). Since the treatments requiring a foliar application were to be sprayed by a commercial applicator, a border plot of untreated seed was sown between plots to help reduce the effects of insecticide drift. All plots were sown in 76.2 cm rows using the Pioneer 90M60 cultivar (Pioneer Hi-bred International, Inc., Johnston, Iowa).

Each week, plant growth stages (Table 2.6) were recorded on the plants sampled for aphid counts. Aphid densities were determined using destructive whole-plant counts on the dates listed in Tables 2.7 and 2.8. On each sampling date, 25 plants per plot were selected randomly at approximately 30 paces throughout teach plot and removed and bagged from R2 through R6 (full seed). In 2007, a total of 1800 plants were sampled. In 2008, a total of 1200 plants were sampled. Bags were transported back to the laboratory and counts were taken as previously described. Plots were harvested at R8 (full maturity) using a commercial John Deere 9600 combine (Moline, Illinois). Yield was determined using a weigh wagon supplied by Pioneer Hi-Bred International, Inc. located in Wheaton, Minnesota. Oil and protein content were determined using the method previously described.

A foliar insecticide application of lambda-cyhalothrin (Warrior) was applied as listed in Table 2.3 at 180 mL ha^{-1} using a tractor mounted sprayer 275.79 kPa and 140.3-187.0 L ha^{-1}. The economic level threshold was determined using the method previously described. The Cruiser MAXX seed treatment was applied by the seed supplier at Pioneer Hybrid International in Wheaton, MN using the active ingredients and rates as previously described.

A 38 cm sweep net was used to take 100 180°-sweeps per plot at Johnson in the treatments listed in Table 2.3. In 2007, a total of 9600 sweeps were collected and in 2008 a total of 7200 sweeps were collected. Samples were collected and stored as previously described. In 2007, members of the following insect families were scored: Nabidae, Coccinelidae, Geocoridae, Hemerobiidae, and Chysopidae. In 2008, members of the following insect families were scored: Nabidae, Coccinelidae, Geocoridae, Hemerobiidae, Chysopidae, and Syrphidae. In both years, all other insects were not counted. In 2008, samples were also scored for arachnids.

In 2008, twelve 7.62 cm by 12.7 cm yellow sticky strips (Great Lakes IPM, Inc., Vestaburg, Michigan) were placed in the center row of each plot. Six pieces of 1.8 meter conduit piping were placed in the ground and two yellow sticky strips were then attached to each pipe. The sticky strips were positioned at the height of the soybean canopy. Sampling was conducted for three weeks with the cards being replaced weekly. A total of 432 yellow sticky strip samples were collected.

Treatments effects on yield, oil and protein differences were estimated using analysis of variance (ANOVA) and Fisher's Protected LSD at $P = 0.05$ level (SAS Institute, 2002). The main effects were compared using analysis of variance (ANOVA). Error (a) (replicate x treatment) was used as the denominator of the F-test for treatment and replicate, error (b) (replicate x time) was used as the denominator for time, and error (c) (residual) was used as the denominator for the treatment*time interaction. If found to be significantly different, treatments were also compared for aphid counts across time using analysis of variance (ANOVA) and Fisher's Protected LSD at P = 0.05 level (SAS Institute, 2002). F-tests were considered significant at $P \leq 0.05$.

Results

Small Plot Experiment

In 2007 and 2008, densities of soybean aphids were determined on the dates and locations listed (Tables 2.7 and 2.8). Soybean aphid populations were low in 2007 and higher in 2008 (Figure 2.2). Data were analyzed to see if there was a significant treatment by time interaction (Tables 2.9, 2.10, and 2.11). Since the interaction was significant at the Prosper location in both 2007 and 2008, treatments also were analyzed for each time

(Tables 2.12 and 2.13). In 2008 at the Johnson location, the time by treatment interaction was not significant (Table 2.11).

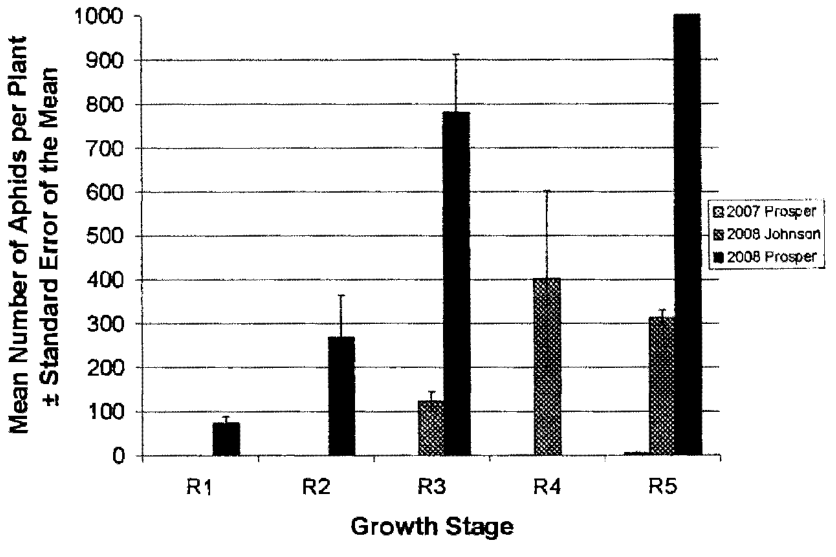

Figure 2.2. Mean number of aphids per plant ± standard error of the mean in the untreated control treatment compared across years and locations.

Table 2.9. Sources of variation, degrees of freedom, mean squares, and the results of *F*-tests for soybean grown in the small plot insecticide study in 2007 near Prosper, ND.

Source of Variation	Degrees of Freedom	Mean Squares
Replicate	3	2.19*
Treatment	7	2.56*
Error (a)	21	0.83
Time	4	39.60**
Error (b)	12	5.41**
Treatment*Time	28	1.66**
Error (c)	84	0.63

*, ** Significant at the $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 2.10. Sources of variation, degrees of freedom, mean squares, and the results of *F*-tests for soybean grown in the small plot insecticide study in 2008 near Prosper, ND.

Sources of Variation	Degrees of Freedom	Mean Squares
Replicate	3	51453.39
Treatment	7	448171.39**
Error (a)	21	31600.07
Time	3	1055924.32**
Error (b)	9	24033.78
Treatment*Time	21	289963.07**
Error (c)	63	26050.27

*, ** Significant at the $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 2.11. Sources of variation, degrees of freedom, mean squares, and the results of *F*-tests for soybean grown in the small plot insecticide study in 2008 near Johnson, MN.

Sources of Variation	Degrees of Freedom	Mean Squares
Replicate	3	12858.70
Treatment	7	87941.87*
Error (a)	21	28281.18
Time	2	75686.95
Error (b)	6	17523.48
Treatment*Time	14	66632.30
Error (c)	32	39138.16

*, ** Significant at the $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

At Prosper, aphid levels were unpredictable and varied between the two sampling years. In 2007, aphid levels remained at low levels throughout the summer and never reached the economic threshold of 250 aphids per plant on 80% of the plants (Table 2.12). In spite of this, a foliar application of lambda-cyhalothrin (Warrior) was applied to the required treatments on 27 July at the R3 growth stage. In 2008, aphid invasion occurred about mid-July and the population built rapidly, but somewhat unevenly in the experimental plots (Table 2.13). Warrior was sprayed on two different dates: 26 July and 1 August (Table 2.5). After all foliar insecticide treatments were sprayed; these treatments had fewer aphids than with the untreated control, Cruiser Maxx, and Valent treatments.

Table 2.12. Effect of seed and foliar treatments on mean aphid counts for soybean grown in the small plot insecticide study in 2007 near Prosper, ND.

Treatment	9 July R2	16 July R3	24 July R4	31 July R4	13 August R5-R6
Untreated Control	0.05 ± 0.05a	0.18 ± 0.18a	1.08 ± 0.46a	0.60 ± 0.14a	5.03 ± 0.99a
Seed Treatments					
Cruiser MAXX	0.00 ± 0.00a	0.05 ± 0.05a	0.05 ± 0.05b	1.43 ± 0.88a	4.15 ± 1.66a
Valent experimental	0.00 ± 0.00a	0.05 ± 0.05a	0.13 ± 0.13b	1.65 ± 0.75a	2.75 ± 0.91a
Foliar Treatments					
Warrior: ET	0.05 ± 0.05a	0.00 ± 0.00a	0.43 ± 0.22ab	0.10 ± 0.06a	1.93 ± 1.24a
Warrior: R3	0.00 ± 0.00a	0.13 ± 0.13a	0.05 ± 0.05b	0.13 ± 0.08a	1.88 ± 0.78a
Warrior: R3, R4, and R5	0.30 ± 0.30a	0.10 ± 0.06a	0.00 ± 0.00b	0.13 ± 0.08a	1.45 ± 0.47a
Seed Treatment plus Foliar Treatment					
Cruiser MAXX plus Warrior: ET	0.05 ± 0.05a	0.05 ± 0.05a	0.13 ± 0.08b	0.08 ± 0.08a	4.15 ± 1.66a
Valent plus Warrior: ET	0.00 ± 0.00a	0.13 ± 0.08a	1.05 ± 0.49a	0.35 ± 0.22a	1.55 ± 0.82a

Means within a column followed by the same letter are not significantly different as determined using a Fisher's protected LSD test (P=0.05).

The Warrior: R3, R4, and R5 treatment was sprayed on 18 July, 27 July, and 9 August.

27 July the following treatments received an application of Warrior: Warrior: ET, Warrior: R3, Cruiser MAXX plus Warrior: ET, and Valent plus Warrior: ET.

Table 2.13. Effect of seed and foliar treatments on mean aphid counts for soybean grown in the small plot insecticide study in 2008 near Prosper, ND.

Treatment	17 July R1	23 July R2	30 July R3	15 August R5
Untreated Control	73.50 ± 14.31ab	267.96 ± 95.27a	780.80 ± 131.56a	1000.00 ± 0.00a
Seed Treatments				
Cruiser MAXX	14.83 ± 2.85c	103.42 ± 19.42a	374.92 ± 140.03abc	1000.00 ± 0.00a
Valent	20.96 ± 6.65c	141.50 ± 28.61a	396.46 ± 140.95abc	1000.00 ± 0.00a
Foliar Treatments				
Warrior: ET	118.67 ± 40.90a	346.04 ± 160.28a	30.21 ± 14.04c	75.54 ± 0.99b
Warrior: R3	33.54 ± 7.92bc	272.67 ± 79.90a	715.95 ± 144.96ab	99.38 ± 45.97b
Warrior: R3, R4, and R5	31.04 ± 9.94bc	3.42 ± 0.75a	29.79 ± 10.98c	18.67 ± 9.77b
Seed Treatment plus Foliar Treatment				
Cruiser MAXX plus Warrior: ET	6.96 ± 2.21c	102.84 ± 22.94a	555.75 ± 274.59ab	80.21 ± 0.67b
Valent plus Warrior: ET	16.92 ± 3.78c	159.92 ± 23.89	319.34 ± 123.59bc	31.21 ± 1.00b

Means within a column followed by the same letter are not significantly different as determined using a Fisher's protected LSD test (P=0.05).

The Warrior: R3, R4, and R5 treatment was sprayed on 20 July, 1 August, and 15 August.

26 July the Warrior: ET treatment was sprayed. 1 August the following treatments received an application of Warrior: Warrior: R3, Cruiser MAXX plus Warrior: ET, and Valent plus Warrior: ET.

At the Johnson site in 2008, aphid infestation occurred in late July and the population built up very rapidly on the control. Treatments requiring a foliar application of lambda-cyhalothrin (Warrior) were sprayed on 7 August because the economic treatment level had been attained.

The effects of the seed and foliar treatments on yield, oil, and protein were determined. At Prosper and Johnson in 2007, no significant differences among treatments were found for yield, oil, and protein (Table 2.14). At Prosper in 2008, no significant differences among treatments were found for oil content (Table 2.15); however, for protein, the treatments receiving a foliar application of Warrior had significantly lower protein than the untreated control and the seed treatments alone (Table 2.15). In 2008 at the Johnson site, no significant differences among treatments were found for protein content. Yields for the Warrior: R3 and the Warrior: R3, R4, and R5 growth stage treatments were 125% and 123% higher than those of the Valent experimental seed treatment (Table 2.16).

The treatments were compared for total number of natural enemies. In 2007 at Prosper, aphids and natural enemies occurred in low numbers (Table 2.17). In 2008 at Johnson, aphid populations were higher, but insecticide treatments did not affect the natural enemies and no significant differences among treatments were found (Table 2.18). However, at Prosper in 2008, the treatments receiving a foliar application of Warrior had significantly lower numbers of natural enemies that the untreated control and the seed treatments (Table 2.19). Due to low numbers, natural enemies were calculated by treatment by combining all insects across all individual sampling dates.

Table 2.14. Effect of seed and foliar treatments on yield, oil content, and seed protein of soybean grown in the small plot insecticide study in 2007 near Prosper, ND.

Treatment	Yield (T ha^{-1})	Oil (%)	Protein (%)
Untreated Control	1.73 ± 0.11a	20.98 ± 0.16a	31.58 ± 0.24a
Seed Treatments			
Cruiser MAXX	1.71 ± 0.08a	20.95 ± 0.20a	31.55 ± 0.21a
Valent experimental	1.67 ± 0.03a	20.90 ± 0.21a	31.38 ± 0.26a
Foliar Treatments			
Warrior: ET	1.75 ± 0.03a	20.71 ± 0.15a	31.60 ± 0.25a
Warrior: R3	1.85 ± 0.07a	20.94 ± 0.12a	31.48 ± 0.49a
Warrior: R3, R4, and R5	1.79 ± 0.02a	21.03 ± 0.09a	31.18 ± 0.14a
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	1.81 ± 0.05a	21.08 ± 0.31a	31.55 ± 0.21a
Valent experimental plus Warrior: ET	1.82 ± 0.05a	20.79 ± 0.11a	31.73 ± 0.25a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.15. Effect of seed and foliar treatments on yield, oil content, and seed protein of soybean grown in the small plot insecticide study in 2008 near Prosper, ND.

Treatment	Yield (T ha^{-1})	Oil (%)	Protein (%)
Untreated Control	1.77 ± 0.29a	20.70 ± 0.06a	36.00 ± 0.23a
Seed Treatments			
Cruiser MAXX	2.06 ± 0.28a	21.05 ± 0.03a	35.48 ± 0.18ab
Valent experimental	1.77 ± 0.09a	20.80 ± 0.17a	35.95 ± 0.17a
Foliar Treatments			
Warrior: ET	2.31 ± 0.13a	20.83 ± 0.16a	35.23 ± 0.05b
Warrior: R3	2.12 ± 0.12a	21.05 ± 0.18a	35.33 ± 0.22b
Warrior: R3, R4, and R5	2.32 ± 0.08a	20.63 ± 0.26a	35.23 ± 0.32b
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	1.90 ± 0.07a	20.93 ± 0.11a	35.40 ± 0.12b
Valent experimental plus Warrior: ET	2.18 ± 0.08a	21.00 ± 0.33a	35.18 ± 0.27b

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.16. Effect of seed and foliar treatments on yield, oil content, and seed protein for soybean grown in the small plot insecticide study in 2008 near Johnson, MN.

Treatment	Yield (T ha^{-1})	Oil (%)	Protein (%)
Untreated Control	2.85 ± 0.09abc	20.57 ± 0.10abc	36.88 ± 0.06a
Seed Treatments			
Cruiser MAXX	2.76 ± 0.07bc	20.28 ± 0.10bc	37.17 ± 0.12a
Valent experimental	2.51 ± 0.14c	20.70 ± 0.03ab	37.10 ± 0.15a
Foliar Treatments			
Warrior: ET	2.78 ± 0.14abc	20.84 ± 0.11a	36.98 ± 0.25a
Warrior: R3	3.14 ± 0.07a	20.71 ± 0.13ab	36.65 ± 0.12a
Warrior: R3, R4, and R5	3.10 ± 0.07ab	20.81 ± 0.15a	36.75 ± 0.13a
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	2.72 ± 0.22c	20.44 ± 0.27abc	36.78 ± 0.24a
Valent experimental plus Warrior: ET	2.89 ± 0.19abc	20.25 ± 0.20c	36.98 ± 0.21a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.17. Effect of seed and foliar treatments on natural enemies for soybean grown in the small plot study in 2007 near Prosper, ND.

Treatment	Natural enemies[1]
Untreated Control	19.00 ± 0.82bc
Seed Treatments	
Cruiser MAXX	19.75 ± 3.94bc
Valent experimental	31.50 ± 3.33a
Foliar Treatments	
Warrior: ET	22.00 ± 2.20bc
Warrior: R3	20.00 ± 1.58bc
Warrior: R3, R4, and R5	15.75 ± 1.38c
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	16.75 ± 1.49bc
Valent experimental plus Warrior: ET	23.25 ± 2.43b

[1]Natural enemies included the following insect families: Nabidae, Chrysopidae, and Coccinellidae and members of the arachnid order.
Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.18. Effect of seed and foliar treatments on natural enemies for soybean grown in the small plot insecticide study in 2008 near Johnson, MN.

Treatment	Natural enemies[1]
Untreated Control	15.25 ± 1.93a
Seed Treatments	
Cruiser MAXX	11.50 ± 2.10a
Valent experimental	13.75 ± 2.66a
Foliar Treatments	
Warrior: ET	11.75 ± 1.11a
Warrior: R3	13.75 ± 1.89a
Warrior: R3, R4, and R5	8.25 ± 2.10a
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	13.25 ± 1.03a
Valent experimental plus Warrior: ET	11.50 ± 1.50a

[1]Natural enemies included the following insect families: Nabidae, Chrysopidae, and Coccinellidae and members of the arachnid order.
Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.19. Effect of seed and foliar treatments on natural enemies for soybean grown in the small plot insecticide study in 2008 near Prosper, ND.

Treatment	Natural enemies[1]
Untreated Control	52.00 ± 6.79a
Seed Treatments	
Cruiser MAXX	29.00 ± 6.34bc
Valent experimental	32.25 ± 5.84b
Foliar Treatments	
Warrior: ET	7.00 ± 1.29d
Warrior: R3	18.50 ± 5.85cd
Warrior: R3, R4, and R5	6.00 ± 1.47d
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	11.75 ± 1.55d
Valent experimental plus Warrior: ET	14.00 ± 2.08d

[1]Natural enemies included the following insect families: Nabidae, Chrysopidae, Syrphidae, and Coccinellidae and members of the arachnid order.
Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Grower Study Experiment

In 2007, aphid densities were very low and never reached the economic treatment threshold for the treatments requiring a foliar insecticide application (Table 2.21). In spite of this, a foliar application of lambda-cyhalothrin (Warrior) was applied to the required treatments on 9 August. The interaction of time by treatment for aphid density was not significant (Table 2.20) so the treatments were not analyzed by time for significant differences. However, the mean aphid counts by time are still presented in Tables 2.21 and 2.22. The time main effect was significant; thus means for each time, arranged across treatments are presented in Table 2.21.

Table 2.20. Sources of variation, degrees of freedom, mean squares, and the results of *F*-tests for soybean grown for the grower study in 2007 near Johnson, MN.

Sources of Variation	Degrees of Freedom	Mean Square
Treatment	3	242.46
Replicate	2	163.37
Error (a)	6	63.77
Time	5	2484.81**
Error (b)	10	39.72
Treatment*Time	15	78.21
Error (c)	30	62.84

*, ** Significant at the $P \leq 0.05$ and $P \leq 0.01$ level of probability, respectively.

Table 2.21. Effect of seed and foliar treatments on aphid counts in soybean grown in the grower study in 2007 near Johnson, MN.

Treatment	12 July R2	19 July R3	27 July R4
Untreated Control	0.47 ± 0.23a	0.63 ± 0.09a	6.70 ± 2.55a
Seed Treatments			
Cruiser MAXX	0.50 ± 0.10a	0.43 ± 0.09a	4.13 ± 0.70a
Foliar Treatments			
Warrior: ET	0.23 ± 0.03a	1.03 ± 0.27a	4.20 ± 1.01a
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	0.10 ± 0.10a	0.60 ± 0.21a	3.57 ± 0.69a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.22. Effect of seed and foliar treatments on aphid counts in soybean grown in the grower study in 2007 near Johnson, MN.

Treatment	3 August R5	9 August R5	16 August R6
Untreated Control	38.80 ± 4.11a	42.83 ± 13.95a	19.27 ± 6.50a
Seed Treatments			
Cruiser MAXX	22.47 ± 1.56a	22.86 ± 8.62a	13.40 ± 2.90ab
Foliar Treatments			
Warrior: ET	26.07 ± 7.21a	40.67 ± 1.70a	5.07 ± 1.31b
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	27.03 ± 7.20a	25.87 ± 6.36a	3.20 ± 1.51b

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.
Warrior was applied to the Warrior: ET and the Cruiser MAXX plus Warrior: ET on 9 August.

In 2008, aphid populations built up rapidly in early August and reached the economic treatment threshold and the required treatments were sprayed with a foliar application of Warrior on 7 August. The interaction of time*treatment was significant (Table 2.23) so the treatments were analyzed by time for significant differences (Table 2.24). The pre-spray count on 7 August showed no differences in the aphid populations among treatments, indicating aphid populations were evenly distributed across the treatments. The aphid population declined in the treatments receiving the foliar application of Warrior: Warrior at ET and Cruiser Maxx plus Warrior at ET, but continued to increase in the untreated control and the Cruiser Maxx seed treatment on 14 August and 20 August (Table 2.24).

Table 2.23. Sources of variation, degrees of freedom, mean squares, and results of F-tests for soybean grown in the grower study in 2008 near Johnson, MN.

Sources of Variation	Degrees of Freedom	Mean Square
Treatment	3	1141084.75^{**}
Replicate	2	1090.25
Error (a)	6	2359.98
Time	2	150880.48^{**}
Error (b)	4	1466.01
Treatment*Time	6	349260.25^{**}
Error (c)	12	2362.33

*,** Significant at the $P \leq 0.05$ and $P \leq 0.01$ level of probability, respectively.

Table 2.24. Effect of foliar and seed treatments on aphid counts for soybean grown in the grower study in 2008 near Johnson, MN.

Treatment	7 August R4	14 August R4-R5	20 August R5-R6
Untreated Control	310.47 ± 30.55a	1007.93 ± 4.49a	1000.00 ± 0.00a
Seed Treatments			
Cruiser MAXX	279.21 ± 64.11a	991.99 ± 12.41a	1000.00 ± 0.00a
Foliar Treatments			
Warrior: ET	328.72 ± 36.95a	54.28 ± 10.65b	29.15 ± 4.66b
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	391.22 ± 40.90a	54.03 ± 2.34b	33.60 ± 5.38b

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.
7 August the Warrior: ET and the Cruiser MAXX plus Warrior: ET treatments were sprayed.

The treatments were analyzed for yield, oil, and protein content. In 2007, no significant differences among treatments were found for yield and protein (Table 2.25); however, differences were found for oil content. The untreated control had significantly less oil content than the Cruiser Maxx seed treatment and the Warrior foliar application. In 2008, the untreated control and the Cruiser Maxx seed treatment had significantly lower yield than the Cruiser Maxx plus Warrior at ET and Warrior applied at ET treatments (Table 2.26). No significant differences among treatments were found for oil and protein content (Table 2.26).

Table 2.25. Effect of seed and foliar treatments on yield, oil, and seed protein for soybean grown in the grower study in 2007 near Johnson, MN.

Treatment	Yield (T ha^{-1})	Oil (%)	Protein (%)
Untreated Control	3.11 ± 0.19a	18.83 ± 0.03c	29.73 ± 0.28a
Seed Treatments			
Cruiser MAXX	3.28 ± 0.05a	19.07 ± 0.03ab	29.67 ± 0.18a
Foliar Treatments			
Warrior: ET	3.23 ± 0.02a	19.13 ± 0.07a	29.50 ± 0.06a
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	3.16 ± 0.11a	18.97 ± 0.07bc	29.80 ± 0.25a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.26. Effect of seed and foliar treatments on yield, oil, and seed protein for soybean grown in the grower study in 2008 near Johnson, MN.

Treatment	Yield (T ha^{-1})	Oil (%)	Protein (%)
Untreated Control	2.39 ± 0.06b	19.20 ± 0.10a	37.47 ± 0.13a
Seed Treatments			
Cruiser MAXX	2.53 ± 0.02b	18.93 ± 0.03a	38.10 ± 0.35a
Foliar Treatments			
Warrior: ET	2.97 ± 0.05a	19.33 ± 0.12a	37.50 ± 0.31a
Seed Treatment plus Foliar Treatment			
Cruiser MAXX plus Warrior: ET	2.96 ± 0.04a	19.30 ± 0.12a	36.90 ± 0.23a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

The treatments were also compared for natural enemies. In 2007, significant differences among treatments were found (Table 2.27). The untreated control had significantly more natural enemies that the other treatments. Also the Cruiser Maxx plus Warrior applied at economic threshold had significantly fewer natural enemies than the other treatments. In 2008, there were no significant differences among treatments (Table 2.28).

In 2008, yellow sticky cards also were used to sample natural enemies. The cards were placed throughout the plots for three weeks. However, on the first sampling date, a high wind blew some of the cards onto the ground. As a result 30% of the cards did not provide meaningful data. The means from the other two sample dates are presented in Table 2.29.

Discussion

Soybean aphid populations are unpredictable, making the damage done from the soybean aphid variable among years and planting dates (Myers et al. 2005). When aphid populations are low, as was the case in 2007, it is difficult to determine the efficacy of the

Table 2.27. Effect of seed and foliar treatments on natural enemies sampled by the sweep net method for soybean grown in the grower study in 2007 near Johnson, MN.

Treatment	Natural enemies[1]
Untreated Control	183.33 ± 12.00a
Seed Treatments	
Cruiser MAXX	124.33 ± 15.62bc
Foliar Treatments	
Warrior: ET	143.00 ± 12.66b
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	105.00 ± 4.00c

[1]Natural enemies included the following insect families: Nabidae, Chrysopidae, Geocoridae, and Coccinellidae.

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.28. Effect of seed and foliar treatments on natural enemies sampled by the sweep net method for soybean grown in the grower study in 2008 near Johnson, MN.

Treatment	Natural enemies[1]
Untreated Control	29.67 ± 8.25a
Seed Treatments	
Cruiser MAXX	29.00 ± 7.81a
Foliar Treatments	
Warrior: ET	19.00 ± 7.21a
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	37.33 ± 3.53a

[1]Natural enemies included the following insect families: Nabidae, Chrysopidae, Syrphidae, and Coccinellidae and members of the arachnid order.

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 2.29. Mean number of natural enemies collected by the sticky card method in soybean grown in the grower study in 2008 near Johnson, MN.

Treatment	Natural enemies[1]
Untreated Control	16.00 ± 1.53
Seed Treatments	
Cruiser MAXX	11.33 ± 4.33
Foliar Treatments	
Warrior: ET	24.67 ± 6.06
Seed Treatment plus Foliar Treatment	
Cruiser MAXX plus Warrior: ET	16.00 ± 1.73

[1]Natural enemies included the following insect families: Syrphidae, Chrysopidae, and Coccinellidae.

seed treatments and foliar insecticides. Indeed, no significant differences were found among insecticide treatments for yield, oil, and protein (Table 2.14). However, in 2008, when aphid populations were much higher, significant differences among treatments were found (Table 2.16).

The results from the grower study indicate that in 2008, when aphid populations were high, a single application of Warrior applied at the economic threshold provided a significant yield increase over the untreated check (Table 2.25). In Asia, foliar insecticides are widely used to control soybean aphid, but the recommendations on when to apply them vary considerably. Dai and Fan (1991) reported producers in China may apply insecticides up to four times a year to prevent yield loss. Another report from China indicates that a foliar application be applied in late June (Wang et al. 1996) and another report indicates that a foliar insecticide should be applied at early flowering (Lin et al. 1992). Recently, in the Unites States, the economic threshold for treating soybean aphids with a foliar insecticide was defined as 250 aphids per plant on 80% of the field during the R1-R5 growth stages (Ragsdale et al. 2007). However, in the small plot experiments, this was not always the case. In 2008, when aphid populations were above the treatment threshold, insecticides applied at the economic threshold produced yield differences at the Johnson location (Table 2.16), but not at the Prosper location (Table 2.15). This may have been the result of uneven aphid infestation and diverse environmental conditions. A late harvest at the Prosper location resulted in shattering of the pods and seed loss, which may have impacted the yield results for this location.

In contrast to the foliar insecticide, the Cruiser Maxx seed treatment did not provide a significant yield increase over the untreated check (Tables 2.14, 2.15, 2.16, 2.25, and

2.26). Possibly the protection offered by the seed treatment may have disappeared by the time the aphid populations reached economic threshold. In Minnesota, the protection provided by thiamethoxam seed treatments was gone 49 days after planting (McCornack and Ragsdale 2006). In Nebraska, thiamethoxam seed treatments were depleted 40 days after planting (Magalhaes et al. 2008). In 2007, because aphids did not reach economic threshold, I did not expect to see a significant difference between the control untreated plants and the Cruiser Maxx seed treatment (Tables 2.12, 2.21, and 2.22). In 2008, aphids did reach economic threshold, but did so more than 50 days after planting (Tables 2.13 and 2.24) at a time when other studies show insecticide efforts of Cruiser Maxx to be declining. Since the Cruiser Maxx seed treatment did not provide a yield advantage over the untreated check, it cannot be recommended as a method for soybean aphid control under North Dakota growing conditions.

The Cruiser Maxx seed treatment plus an application of Warrior foliar insecticide applied at economic threshold was compared to Warrior alone. In 2007, due to the low aphid populations, there was no yield advantage to applying Warrior (Tables 2.14 and 2.26). In 2008, no significant yield advantage was found between the Cruiser Maxx seed treatment with Warrior applied at the economic threshold and the Warrior alone (Tables 2.15, 2.16, and 2.25). These data indicate that there was no advantage to applying a seed treatment in addition to a foliar application of Warrior and recommend that a single foliar application of insecticide can prevent yield loss and provide adequate protection (Meyers et al. 2005).

The yield of the experimental Valent seed treatment did not provide a significant increase over the untreated check (Tables 2.14, 2.15, and 2.16). The Valent seed treatment with a foliar application of Warrior applied at the economic threshold was not significantly different than an application of Warrior applied to untreated seed at the economic threshold (Tables 2.14, 2.15, and 2.16). These data follow the trend of the results of the Cruiser Maxx seed treatments and indicate that in the growing region of eastern North Dakota and western Minnesota aphid populations build up at a time when seed treatments lose their efficacy.

My study of natural enemies did not allow strong conclusions to be made about the impact of insecticides. Due to the small numbers of natural enemies that were found, it is difficult to make conclusions on how seed treatments and foliar insecticides affect these populations. However, in 2008 when aphid populations were high, no significant differences among treatments were found in the two studies at the Johnson location (Tables 2.18 and 2.28). At the Prosper location in 2008 when aphid populations were high, the untreated control had significantly more natural enemies than the other treatments (Table 2.19). At the Prosper location in 2007 when aphid populations did not reach economic thresholds, significant differences were found among treatments for natural enemies (Tables 2.17 and 2.27). The small plot experiments could have been improved by increasing the sampling area. More insects were collected at the larger grower study experiment than in the small plot study experiments (Tables 2.17, 2.18, 2.19, 2.27, and 2.28). Additional sampling methods such as destructive whole plant counts and field counts may help provide insight into other natural enemies present. In an experiment in Iowa, four methods were used to sample natural enemies (Schmidt et al. 2008). These additional sampling methods provided different life stages of insects to be sampled.

Schmidt et al. (2008) also found that using more than one sampling method allowed more taxa to be sampled.

The unpredictability of the soybean aphid outbreaks in North Dakota makes it difficult for producers to make good decisions about insecticide treatments (Figure 2.3). Since our populations peak in late July and early August, North Dakota producers will likely benefit from actively scouting fields and applying a foliar insecticide only when populations reach the economic threshold. More research could be done to determine the period of time that seed treatments provide adequate control of soybean aphid. Here, leaf bioassays (Magalhaes et al. 2008) are conducted to determine how long protection lasts. More research is also needed on the effects of seed treatments and foliar insecticide applications on the beneficial insect populations. Multiple sampling methods could provide more information about the life stages of natural enemies and as a result provide more information about the vulnerability of these different life stages to various insecticide treatments.

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CHAPTER 3. EVALUATING EXPERIMENTAL SOYBEAN LINES FOR THE *RAG1* GENE FOR SOYBEAN APHID RESISTANCE

Host plant resistance can be an effective means of controlling insect crop pests (Pedigo 2002). Resistant cultivars are safe for the environment while reducing financial inputs of growers (Pedigo 2002). Problems of host plant resistance include a lack of effective resistance traits and a lack of breeding programs to incorporate resistant traits into adapted elite cultivars. A further problem is that insect pest sometimes adapt to the plant resistance trait, with the adaptive trait spreading through the pest population and compromising control of the pest. A final problem is that the resistance may be so effective that natural enemy specialists are decimated and therefore will not be present if and when the pest adapts to plant resistance.

In 2004, Hill et al. discovered plant resistance to the soybean aphid in the soybean germplasm 'Dowling,' 'Jackson' and 'PI 71506.' Further research (Hill et al. 2006) studied the genetics of resistance in a single cultivar 'Dowling' and found that it is controlled by a single dominant gene which was named *Rag1*. Resistance in 'Dowling' limits the survival, longevity, fecundity, and development of the soybean aphid (Li et al. 2004). Hill et al. (2006) concluded that this monogenic dominant nature of *Rag1* resistance would enable breeders to rapidly convert existing susceptible cultivars using backcrossing procedures.

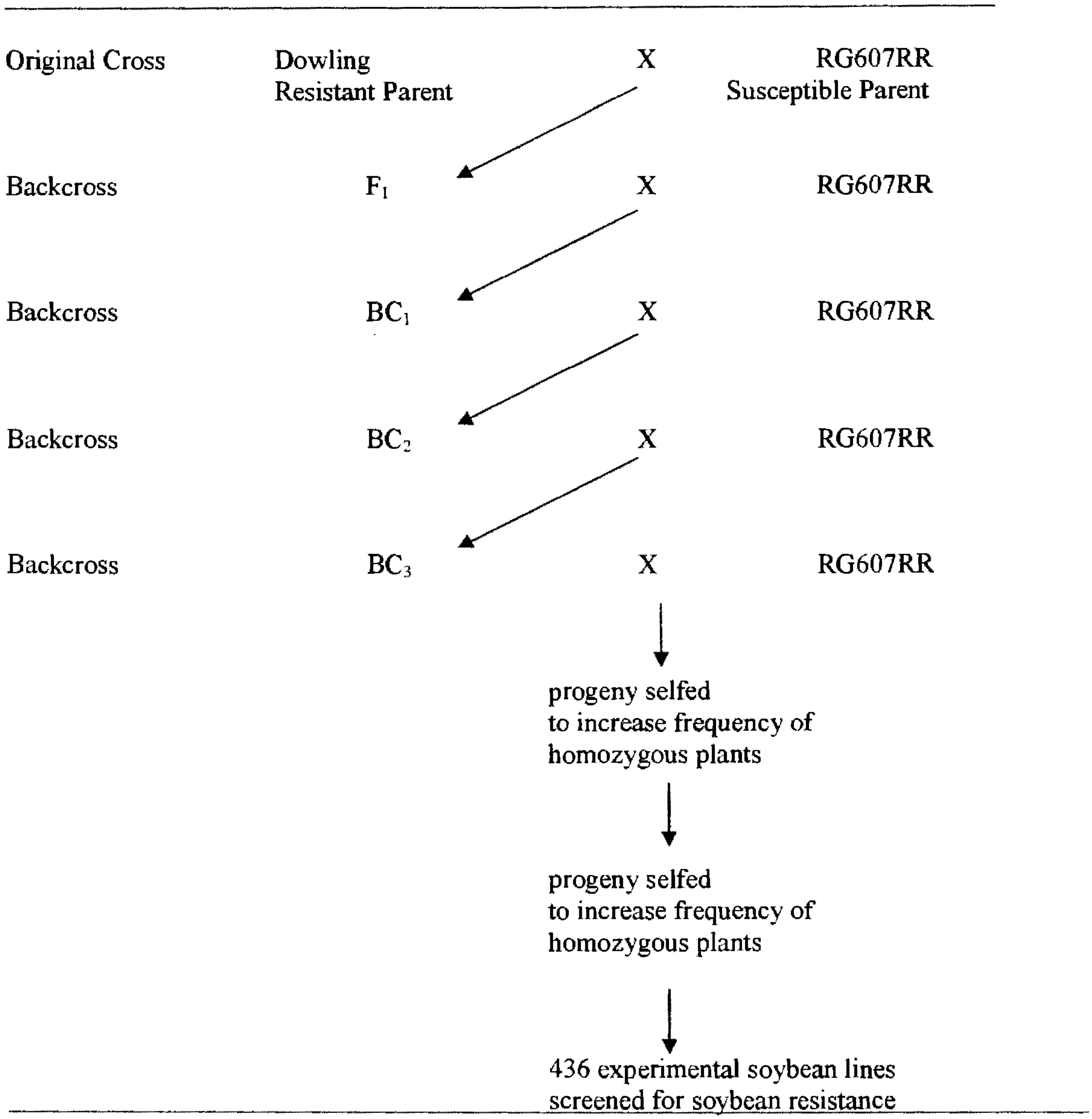
The main objective was to determine the resistance of 436 experimental North Dakota soybean lines for soybean aphid resistance. Experimental lines were provided by Dr. Ted Helms, the soybean breeder in the Department of Plant Sciences at North Dakota State University. 'Dowling' was crossed into the RG607RR soybean cultivar, with the progeny of this cross backcrossed three times to RG607RR. The plants were then selfed for two generations to produce experimental soybean lines adapted for North Dakota (Figure 3.1). The 436 lines were tested in the greenhouse. As a result of this preliminary screening, 56 lines were chosen for further evaluation in the greenhouse and field. Table 3.1 explains the various experiments while Table 3.2 provides a description of each experiment. These experimental lines were not screened for soybean aphid resistance until after the final cross was completed.

Materials and Methods

Initial Greenhouse Screening

Experimental soybean lines (n = 436) were sown in 20.3 cm pots in the greenhouse to determine resistance or susceptibility to the soybean aphid. The soybean lines were developed using the process outlined in Figure 3.1.

After seeds germinated, each pot was thinned to no more than two to five plants per pot. Soybean plants were grown under a 16 hour light: 8 hour dark photoperiod at 24° C. Plants were watered as needed. Each experimental line was infested using live aphids from a soybean aphid colony. Plants were screened at 2 to 3 trifoliate leaves. This was achieved by placing a single aphid infested leaf on the soil near the soybean stem. The aphids were allowed to naturally colonize the plants for 3 days before the first scoring. After this, plants were scored three, seven, and ten days after infestation. A rating system based on the number of aphids on the first trifoliate leaf was used to determine resistance or susceptibility. A rating of 1 meant there was less than 25 total aphids, a rating of 2 meant there was 26-75 aphids, a rating of 3 meant there was 75 to 100 aphids, and a rating of 4 meant there were more than 100 aphids. A rating of 1 or 2 indicated the plant was resistant



Experimental soybean lines were not tested for resistance to soybean aphid throughout this process. All screening occurred after the final cross was completed.

Figure 3.1. Outline of plant breeding methods used to create experimental *Rag1* soybean lines developed at North Dakota State University.

Table 3.1. Experiments conducted for evaluation of experimental *Rag1* soybean lines developed at North Dakota State University for soybean aphid resistance.

Experiment	Dates and Location	Size of Study
Initial Greenhouse Screening	2008: January to May	436 experimental lines; one replicate (pot) per line
Advanced Greenhouse Screening	2008: May to June (failed due to high humidity)	30 susceptible and 26 resistant lines/replicate 2 replicates
Field Study	2008: Prosper, ND 2008: Johnson, MN	30 susceptible and 25 resistant lines/replicate 3 replicates/site
Advanced Greenhouse Screening	2009: January to February	30 susceptible and 26 resistant lines/replicate 2 replicates

to soybean aphid. A rating of 3 or 4 indicated that the plant was susceptible to soybean aphid. Each plant was given a rating and then the ratings for a line were averaged across replicates to assign each experimental line an overall rating. Previous studies involving aphid resistance screening had used various methods to determine resistance (Hill et al. 2004, Diaz-Montano et al. 2006, Li et al. 2004). Hill et al. (2004) used a rating system based on colonization and plant damage, while Li et al. (2004) counted nymphs produced by a single alate female. The method used for this experiment was developed based on the number of aphids that colonized 'Dowling' in previous experiments. After the three-day count, pots were covered with a 42 by 50 cm Delnet bag (DelStar Technologies, Inc., Middletown, DE) to reduce aphid migration. Dates of the initial greenhouse screenings are listed in Table 3.3.

Table 3.2. Description of experiments conducted for evaluation of experimental *Rag1* soybean lines developed at North Dakota State University for soybean aphid resistance.

Experiment	Description
Initial Greenhouse Screening	Screened 436 experimental lines plus the susceptible parent RG607RR for soybean aphid resistance in the greenhouse. A soybean leaf infested with soybean aphid was placed on the soil of each pot containing a single soybean line. Aphids moved to infest the plants. Plants were evaluated for resistance by a rating system of 1 = less than 25 aphids; 2 = 26 to75 aphids; 3 = 76 to 100 aphids; 4= more than 100 aphids. Dowling was not included as a check because seed was not available.
Field Studies	
2008: Prosper, ND 2008: Johnson, MN	30 susceptible and 25 resistant lines were sown near Johnson, MN, and 30 susceptible and 16 resistant lines were sown near Prosper, ND. Lines were selected based on the initial greenhouse screening results. The susceptible lines were selected based on high aphid ratings during the initial greenhouse screening and the resistant lines were selected based on low aphid ratings. The number of soybean aphid and nodes on 3 plants per plot was counted three times during the growing season at Prosper and four times at Johnson. No resistant check was available because Dowling is not adapted for North Dakota. Natural enemies were collected by sweep net. Plots were harvested for yield.
Advanced Greenhouse Screening	30 susceptible and 25 resistant lines were compared to the resistant parent 'Dowling' for resistance to soybean aphid. RG607RR was included as the susceptible check. Five soybean aphids were caged on the first trifoliate leaf of each plant. Aphids were counted 3, 7, and 10 days after infestation.

Aphid Rearing

The soybean aphid colonies were maintained at the joint NDSU/ USDA Research Greenhouse facilities and at North Dakota State University Department of Entomology in Fargo, ND. Two separate colonies were maintained to foster healthy aphid populations. The colony was obtained in January 2008 from Jonathan Lundgren at the North Central

Table 3.3. 2008 initial greenhouse screening dates.

Date	Planting	Aphid Transfer	Aphid Counts
31 January	X		
13 February	X		
19 February		X	
22 February	X		X
26 February			X
29 February	X		X
8 March		X	
10 March	X		X
14 March		X	
15 March			X
17 March			X
18 March			X
21 March			X
24 March	X		X
28 March	X	X	
31 March			X
4 April		X	
7 April			X
14 April	X		
17 April	X		X
21 April	X		
25 April			X
28 April			X
2 May			X
5 May			X
14 May			X
23 May			X
27 May			X

Agricultural Research Laboratory of the USDA-ARS in Brookings, SD. For the initial greenhouse screening, aphids were used from the soybean aphid colony located at the USDA Research Greenhouse facilities. For the advanced greenhouse screening, aphids were collected in August 2008 from a soybean field at the North Dakota Agricultural Experiment Station Research site near Prosper, ND. Soybean aphids from the colony located at the North Dakota State University Department of Entomology were used for the advanced greenhouse screening. Aphids were maintained on seedlings of the susceptible cultivar 'Prosoy' (NDSU, Fargo, ND). Fresh plants with 2 to 3 trifoliate leaves were added at weekly intervals to maintain the colony.

Field Screening

Thirty susceptible and twenty-five resistant lines from the greenhouse trial were selected for a field plot study in 2008 (Tables 3.4 and 3.5). The susceptible lines selected had the highest ratings during the initial greenhouse screening. The resistant lines were the only lines with adequate seed amounts that remained after Dr. Ted Helms selected lines for his experiments. Soybean seeds were sown using a plot seeder (ALMACO, Nevada, Iowa) at a rate of 432400 seeds ha^{-1}. Plots were sown at the North Dakota Agricultural Experiment Station Research site near Prosper, ND, and at a producer's farm near Johnson, MN. Genotypes were assigned to experimental units using a randomized complete block design with a split plot in time arrangement with three replicates (Figure 3.2). A split plot in time arrangement was used since data were collected from the same experimental units over the growing season. Experimental units (i.e. plots) were 0.91 m wide and 7.62 m long (6.93 sq. m). Each plot had three rows spaced 30.5 cm apart. A border plot of untreated seed of RG600RR (Roughrider Genetics, Fargo, ND) was sown between each treatment plot within a range to encourage soybean aphid infestation throughout the plots. RG607RR was used as the susceptible check. No resistant check was used because Dowling is not adapted to North Dakota and no other resistant North Dakota cultivars have been developed.

A 38 cm diameter sweep net was used to collect samples of natural enemies from resistant plots (marked with red colored flags) and susceptible plots (marked with white colored flags). Ten susceptible and ten resistant plots were selected at random per replicate and each plot had ten sweeps taken for a total of 100 sweeps per replicate per type. Sweeps were combined to result in one resistant and one susceptible sample per replicate. Insects collected by sweeps were placed in bags (Ziploc, Racine Wisconsin), taken back to the laboratory and frozen. Sweeps were done weekly when plants were in the R2-R6 growth stages (Fischer and Fanta 2004). At Prosper, 1800 sweeps were collected and at Johnson 2400 sweeps were collected. Only natural enemies in the insect families Syrphidae, Chrysopidae, Nabidae, and Cocinnellidae were recorded. All other insect were not recorded.

Plant growth stages were recorded weekly. The plant growth stages are described in Table 3.6. Aphid counts were determined by using destructive whole plant counts. Destructive whole plant counts involve removing the whole plant including the roots. The whole plant was then examined for aphids. Three plants per plot were collected and individually bagged from the R2 through R6 (full seed) growth stages. At the Prosper location, 1485 plants were sampled and at the Johnson location, 1980 plants were sampled. Bags were transported back to the laboratory where they were stored at 5° C. Later, counts were taken of the number of aphids per plant. Individual plant counts reached as high as 3000 aphids per plant, so to reduce time and effort, counts estimated to be more than 1000 aphids per plant were recorded as 1000. Plots were harvested at harvest maturity by a small plot combine (Almaco, Nevada, Iowa; Kincaid, Haven, Kansas). Yield was measured by the plot combine. Dates aphid counts and natural enemies were sampled and are presented in Table 3.7.

Table 3.4. Final ratings[1] for *Rag1* Lines determined to be resistant in the initial greenhouse screening and used for the field experiments.

Line	Determination	Final Rating
18400	Resistant	1.0
18628	Resistant	1.0
18663	Resistant	1.4
18358	Resistant	1.5
18569	Resistant	1.5
18587	Resistant	1.5
18633	Resistant	1.6
18379	Resistant	1.8
18556	Resistant	1.8
18647	Resistant	1.8
18357	Resistant	2.0
18380	Resistant	2.0
18408	Resistant	2.0
18639	Resistant	2.0
18644	Resistant	2.0
18541	Resistant	2.2
18597	Resistant	2.2
18346	Resistant	2.4
18588	Resistant	2.4
18306	Resistant	2.7
18350	Resistant	2.7
18445	Resistant	2.7
18509	Resistant	2.7
18584	Resistant	2.7
18585	Resistant	2.7

Final rating based on the average rating of three to five individual plants per pot.
[1]A rating of less than 3.0 determined the line resistant and a rating greater than 3 determined the line susceptible

Advanced Greenhouse Experiment

Twenty-five resistant and twenty-nine susceptible experimental $BC_3F_{4:6}$ soybean lines (Tables 3.4 and 3.5) were sown in 20.3 cm pots in the greenhouse to further evaluate resistance or susceptibility to soybean aphid. One pot of the susceptible cultivar

Table 3.5. Final ratings[1] for *Rag1* Lines determined to be susceptible in the initial greenhouse screening and used for the field experiments.

Line	Determination	Final Rating
18309	Susceptible	4.0
18369	Susceptible	4.0
18375	Susceptible	4.0
18382	Susceptible	4.0
18384	Susceptible	4.0
18387	Susceptible	4.0
18388	Susceptible	4.0
18391	Susceptible	4.0
18397	Susceptible	4.0
18399	Susceptible	4.0
18405	Susceptible	4.0
18407	Susceptible	4.0
18423	Susceptible	4.0
18428	Susceptible	4.0
18429	Susceptible	4.0
18430	Susceptible	4.0
18435	Susceptible	4.0
18436	Susceptible	4.0
18439	Susceptible	4.0
18440	Susceptible	4.0
18452	Susceptible	4.0
18459	Susceptible	4.0
18463	Susceptible	4.0
18468	Susceptible	4.0
18478	Susceptible	4.0
18485	Susceptible	4.0
18488	Susceptible	4.0
18489	Susceptible	4.0
18545	Susceptible	4.0

Final rating based on the average rating of three to five individual plants per pot.
[1]A rating of 3.0 or less determined the line resistant and a rating greater than 3 determined the line susceptible.

'RG607RR' and one pot of the resistant cultivar 'Dowling' were used as checks in each replicate. After seed germination, each pot was thinned to three to five plants per pot. Soybean plants were grown under a 16 hour light: 8 hour dark photoperiod at 24° C. Plants

Table 3.6. Soybean plant growth stages (Fischer and Fanta 2004).

Description	Growth Stage
emergence	VE
cotyledon stage	VC
first trifoliate	V1
second trifoliate	V2
third trifoliate	V3
nth trifoliate	V(n)
flowering will soon start	V6
beginning bloom, first flower	R1
full bloom, flower in top 2 nodes	R2
beginning pod, 3/16" pod in top 4 nodes	R3
full pod, 3/4" pod in top 4 nodes	R4
1/8" seed in top 4 nodes	R5
full size seed in top 4 nodes	R6
beginning maturity, one mature pod	R7
full maturity, 95% of pods are mature	R8

Table 3.7. Dates of 2008 plot maintenance work for field studies.

Date	Location	Beneficial Insect Sampling	Aphid Counts
July 10	Johnson	X	
July 17	Prosper	X	X
July 18	Johnson	X	
July 23	Prosper		X
July 25	Prosper	X	
July 30	Prosper	X	X
August 2	Johnson		X
August 7	Johnson	X	X
August 14	Johnson		X
August 19	Johnson	X	
August 20	Johnson		X

were watered as needed to maintain a healthy plant. Genotypes were assigned to experimental unit (i.e. pots) using a randomized complete block design. Sowings at two different time periods represented two replicates. Each plant within a pot represented a sampling unit. Each generation of seed was screened using live aphids from the soybean aphid colony. To determine response to the soybean aphid, five aphids were placed on the first trifoliate leaf on each plant at the 2-3 leaf stage (V2-V3). The first trifoliate soybean leaf on each plant was caged with a modified 150 mm Petri dish (VWR International, West Chester, Pennsylvania) cage to reduce migration. Aphid no-choice tests using sticky cages (Diaz-Montano et al. 2006) and clip cages (Li et al. 2004) were reviewed. Since these tests did not seem to fit our research goals, we developed our own cage and method for screening in the greenhouse. Since potential damage to the leaf could occur and aphid populations could overwhelm the cage in the 10 days the cages were on the plant; we developed a cage using a Petri dish. Potentially, since segregation of the experimental soybean lines for *Rag1* could still be occurring, each Petri dish cage on each of the five plants was individually marked with letters A through E. This was done so each individual plant could be assessed.

The modified 150 mm Petri dish insect cage was created by cutting six 3 cm circles, three on the top lid and three on the bottom dish (Figure 3.2). A piece of nylon mesh was glued in place to cover each hole. This allowed airflow over the leaf and as a result, helped reduce condensation in the cage. A small hole was cut in the side of the dish for the petiole. The hole was surrounded by weather stripping foam to cushion the stem and also help reduce aphid migration. The five aphids were placed on the top of the first trifoliate soybean leaf with a 12/0 Angular Shader fine haired paintbrush and supported by a wire frame (Figure 3.3). The Petri dish cage was then closed with three pieces of tape. Fans placed nearby provided ventilation for the plants and helped to reduce condensation in the Petri dishes.

At three and seven days after infestation, the number of winged, adult, and nymph aphids per leaf were counted without opening the Petri dish cage. For the final aphid count ten days after initial infestation, the Petri dish cage was opened and the number of winged, adult, and nymph aphids were counted. Dates when advanced screenings were conducted are presented in Table 3.8.

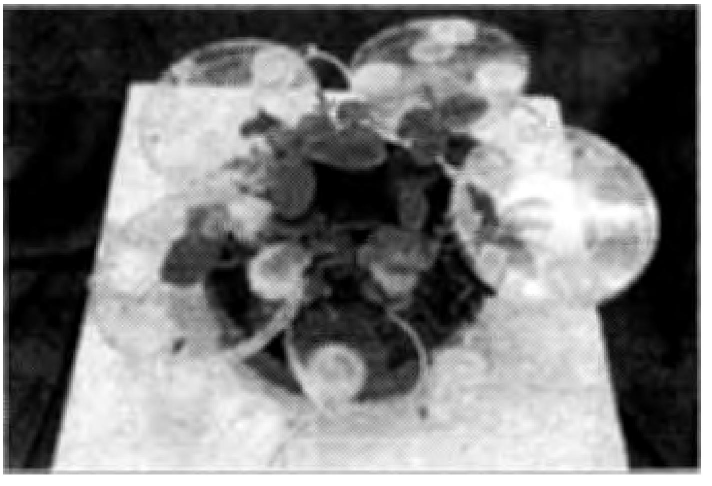


Figure 3.2. Petri dish cages used for resistance screening.

Table 3.8. 2009 advanced greenhouse screening dates.

Date	Replicate	Planting	Aphid Transfer	Aphid Counts
January 12	1	X		
January 23	2	X		
February 2	1		X	
February 5	1			X
February 9	1			X
February 12	1			X
February 13	2		X	
February 16	2			X
February 20	2			X
February 22-23	2			X

Statistical Analysis

Data from all experiments were analyzed using a randomized complete block design with a split plot arrangement using Analysis of Variance (ANOVA) and Fisher's Protected LSD at P=0.05 (SAS Institute, 2002). Error (a) was used as the denominator of the F-test for line, error (b) was used as the denominator for time, and error (c) was used as the denominator for line*time. If the F-test was significant for plant line effects, mean aphid counts of lines were compared for aphid counts at each time using analysis of variance (ANOVA) and Fisher's Protected LSD at $P = 0.05$ level (SAS Institute). Due to small sampling sizes, beneficial insect counts were summed over the growing season and compared by type using Analysis of Variance (ANOVA) and Fisher's Protected LSD at $P = 0.05$ level. If there was missing data, the SAS procedure Proc GLM was used (SAS Institute, 2002). F-tests were considered significant at $P \leq 0.05$.

Results

Initial Greenhouse Screening

The results of the initial greenhouse testing for the experimental lines selected for further experiments are listed in Tables 3.4 and 3.5. Of the 436 lines, 72 lines were determined to be resistant and 364 were determined susceptible to soybean aphid.

Field Study

Fifty-Five lines selected from the greenhouse screening based on their score were sown in the field experiments where the number of aphids and natural enemies were determined. Aphid counts were taken on 2 August, 14 August, and 20 August at Johnson and 17 July, 23 July, and 30 July at Prosper (Table 3.7). Aphid count data were analyzed for main effects and interaction between line and time (Tables 3.9 and 3.10). Since the interaction was significant at the Prosper location, treatments were also analyzed for each time (Table 3.11). At the Johnson site, there was no interaction between line and time (Table 3.10), but since the effect of line was significant, mean aphid counts for each date are presented in Table 3.12.

Four lines with the lowest mean aphid counts were selected as potential resistant lines from Tables 3.11 and 3.12. Mean aphid counts for these lines are shown at the Prosper and Johnson field locations and the advanced greenhouse screening. These lines with their average aphid counts are shown in Figure 3.3. RG607RR was used as the susceptible control and is also included. Figure 3.4 compares four of the susceptible lines to RG607RR for susceptibility to soybean aphid.

Table 3.9. Sources of variation, degrees of freedom, mean squares, and results of F-tests for soybean grown in the *Rag1* study in 2008 near Prosper, ND.

Sources of Variation	Degrees of Freedom	Mean Square
Line	31	101451.49**
Replicate	2	121149.64*
Error (a)	62	26186.00
Time	2	1537948.04**
Error (b)	4	29719.88
Line*Time	62	52595.07**
Error (c)	100	27968.67

*, ** Significant at the $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

Table 3.10. Sources of variation, degrees of freedom, mean squares, and results of F-tests for soybean grown in the *Rag1* study in 2008 near Johnson, MN.

Sources of Variation	Degrees of Freedom	Mean Square
Line	48	124156.74**
Replicate	2	19223.18
Error (a)	96	33115.82
Time	2	988270.69**
Error (b)	4	20966.23
Line*Time	96	32233.00
Error (c)	150	26304.62

*, ** Significant at the $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

Table 3.11. Effect of soybean line on aphid counts for soybean grown in the *Rag1* study in 2008 near Prosper, ND.

Line	Classification Based on Greenhouse Screening	July 17	July 23	July 30
18306	Resistant	41.44 ± 13.14a	530.78 ± 288.46ab	1000.00 ± 0.00a
18309	Susceptible	71.44 ± 31.99a	767.33 ± 256.94a	1000.00 ± 0.00a
18350	Resistant	19.55 ± 1.35a	212.11 ± 55.24bc	761.00 ± 0.00ab
18357	Resistant	18.56 ± 11.91a	12.22 ± 1.64c	118.00 ± 0.00cdef
18358	Resistant	21.78 ± 20.61a	50.50 ± 33.83c	82.67 ± 50.48fe
18369	Susceptible	91.78 ± 47.72a	367.45 ± 166.16bc	183.34 ± 119.34bcdef
18375	Susceptible	41.33 ± 20.33a	164.11 ± 34.26bc	432.89 ± 189.57abcdef
18379	Resistant	1.11 ± 0.95a	14.11 ± 10.28c	20.67 ± 19.67f
18380	Resistant	3.78 ± 1.73a	4.44 ± 1.44c	24.22 ± 10.08f
18387	Susceptible	18.66 ± 13.64a	95.84 ± 52.84c	238.56 ± 67.95bcdef
18388	Susceptible	35.11 ± 7.89a	227.78 ± 80.67bc	213.89 ± 40.59bcdef
18397	Susceptible	27.67 ± 9.67a	303.89 ± 201.69bc	145.34 ± 64.99cdef
18399	Susceptible	21.56 ± 16.39a	95.56 ± 11.65c	142.78 ± 36.65cdef
18400	Resistant	0.67 ± 0.51a	30.67 ± 26.07c	41.45 ± 6.74f
18405	Susceptible	27.55 ± 19.78a	279.22 ± 79.32bc	369.56 ± 315.41bcdef
18407	Susceptible	35.00 ± 23.68a	156.95 ± 33.76c	369.67 ± 163.03bcdef
18430	Susceptible	30.33 ± 13.78a	333.22 ± 135.12bc	307.67 ± 0.00bcdef
18435	Susceptible	5.89 ± 0.67a	228.89 ± 88.13bc	147.25 ± 63.25cdef
18436	Susceptible	102.33 ± 48.14a	250.67 ± 120.64bc	691.34 ± 114.67abcd
18440	Susceptible	16.33 ± 0.69a	98.44 ± 13.46c	520.56 ± 198.60abcdef
18445	Resistant	35.45 ± 14.70a	193.89 ± 78.99bc	699.33 ± 211.23abc
18452	Susceptible	113.00 ± 94.75a	229.00 ± 102.51bc	292.45 ± 70.21bcdef
18459	Susceptible	42.56 ± 19.09a	306.11 ± 68.15bc	211.78 ± 70.27bcdef
18468	Susceptible	12.67 ± 6.08a	196.22 ± 61.94bc	443.22 ± 282.11abcdef
18488	Susceptible	24.44 ± 8.57a	211.44 ± 53.49bc	621.00 ± 83.67abcdef
18489	Susceptible	8.67 ± 3.17a	159.84 ± 77.84c	137.17 ± 69.71cdef
18545	Susceptible	34.00 ± 21.32a	88.66 ± 37.69c	660.33 ± 207.95abcde
18556	Resistant	51.00 ± 17.49a	188.45 ± 72.70bc	647.11 ± 189.91abcde
18584	Resistant	0.00 ± 0.00a	26.78 ± 11.89c	71.67 ± 36.83f
18587	Resistant	0.67 ± 0.19a	22.17 ± 19.50c	302.17 ± 205.84bcdef
18588	Resistant	4.33 ± 2.03a	23.00 ± 2.00c	100.66 ± 21.36cdef
RG607RR	Susceptible	50.67 ± 23.62a	117.00 ± 38.47c	97.00 ± 0.00def

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Treatments were analyzed for yield. At the Johnson location, no significant differences were found (Table 3.13). At the Prosper location, there were significant differences among treatments for yield (Table 3.14). Figure 3.5 shows the mean number aphids and the resulting yield. Soybean at Johnson generally yielded higher than soybean at Prosper. This may have been the result of late harvesting at the Prosper location, which resulted in seed loss from shattering.

Treatments were also analyzed for the total number of natural enemies collected. Natural enemies were collected based on resistant or susceptible type. Due to the small number of insects collected over the growing season, the analysis was conducted based on the total number of natural enemies collected over the growing season by type. No significant differences were found (Tables 3.15 and 3.16).

Advanced Greenhouse Screening

The lines (25 resistant and 30 susceptible) selected during the initial greenhouse screening (Tables 3.4 and 3.5) were evaluated further in the greenhouse using insect cages. The lines were analyzed and found to not differ significantly (Table 3.17). The mean aphid counts are presented in Table 3.18.

Discussion

Interest in host plant resistance to soybean aphid has intensified in recent years. Recent studies have identified soybean germplasm with resistance to soybean aphid (Hill et al. 2004; Li et al. 2004; Mensah et al. 2005; Hesler et al. 2007; Diaz-Montano et al. 2006). Experimental lines with resistance to soybean aphid were developed at North Dakota State University and experiments in this chapter evaluated them for potential soybean aphid resistance.

The four lines that consistently had low aphid counts in the field and the advanced greenhouse studies also had low aphid counts during the initial greenhouse screening experiment (Table 3.19). The economic injury level when yield loss will occur from soybean aphid damage is 674 aphids per plant (Ragsdale et al. 2007) and the mean aphid counts were below this level.

Table 3.12. Effect of line on aphid counts for soybean grown in the *Rag1* study in 2008 near Johnson, MN.

Line	Classification Based on Greenhouse Screening	August 2	August 14	August 20
18306	Resistant	189.78 ± 20.66abcd	254.67 ± 22.67abcde	217.67 ± 175.67abcdef
18309	Susceptible	185.11 ± 51.79abcde	227.22 ± 111.50abcde	534.22 ± 95.71abcde
18346	Resistant	133.67 ± 40.97abcdefgh	284.56 ± 104.55abcde	114.67 ± 73.34cdef
18350	Resistant	126.56 ± 11.89abcdefgh	361.89 ± 218.71abcde	496.67 ± 256.94abcdef
18357	Resistant	39.11 ± 16.97defgh	26.11 ± 7.09c	41.78 ± 30.86ef
18358	Resistant	24.89 ± 20.73fgh	446.67 ± 264.20abcde	55.00 ± 30.00ef
18369	Susceptible	57.45 ± 30.67cdefgh	288.78 ± 72.74abcde	233.67 ± 53.39abcdef
18375	Susceptible	166.44 ± 57.09abcdefg	393.33 ± 90.18abcde	587.22 ± 221.34abcd
18379	Resistant	11.67 ± 8.47gh	22.17 ± 1.17e	18.33 ± 8.88f
18380	Resistant	20.89 ± 18.56fgh	9.84 ± 5.84e	24.84 ± 17.17ef
18382	Susceptible	210.44 ± 88.16abc	533.44 ± 233.75ab	441.00 ± 205.67abcdef
18384	Susceptible	123.00 ± 19.01abcdefgh	413.50 ± 152.83abcde	605.11 ± 22.50abc
18388	Susceptible	96.55 ± 47.53bcdefgh	408.56 ± 56.57abcde	445.78 ± 30.88abcdef
18391	Susceptible	171.84 ± 138.17abcdef	194.50 ± 133.83abcde	89.33 ± 34.00def
18397	Susceptible	145.78 ± 40.30abcdefgh	169.89 ± 83.79abcde	274.33 ± 10.00abcdef
18399	Susceptible	136.22 ± 47.19abcdefgh	424.22 ± 297.03abcde	467.00 ± 270.33abcdef
18400	Resistant	12.00 ± 11.34gh	33.33 ± 5.34de	13.89 ± 5.47f
18405	Susceptible	233.56 ± 61.32ab	285.00 ± 0.00abcde	500.00 ± 276.67abcdef
18407	Susceptible	117.33 ± 43.69abcdefgh	273.50 ± 35.50abcde	178.00 ± 0.00bcdef
18408	Resistant	58.22 ± 45.24cdefgh	72.84 ± 38.17cde	134.22 ± 83.32bcdef
18423	Susceptible	165.11 ± 53.23abcdefg	414.78 ± 87.71abcde	499.34 ± 369.34abcdef
18428	Susceptible	165.56 ± 21.86abcdefg	335.34 ± 30.67abcde	635.67 ± 170.68ab
18429	Susceptible	139.89 ± 75.87abcdefgh	507.00 ± 90.48abc	638.67 ± 0.00ab
18430	Susceptible	130.78 ± 20.32abcdefgh	468.50 ± 92.17abcd	631.89 ± 15.75ab
18435	Susceptible	119.56 ± 55.35abcdefgh	398.67 ± 6.34abcde	223.11 ± 54.94abcdef

(Table 3.12 continued)

18439	Susceptible	127.33 ± 13.54abcdefgh	324.78 ± 94.59abcde	444.33 ± 114.18abcdef
18445	Resistant	94.44 ± 70.82bcdefgh	146.56 ± 30.36bcde	69.44 ± 22.82ef
18459	Susceptible	268.00 ± 86.23a	545.11 ± 142.23ab	490.33 ± 0.00abcdef
18463	Susceptible	142.78 ± 48.16abcdefgh	365.33 ± 184.00abcde	612.33 ± 220.99abc
18468	Susceptible	192.00 ± 51.89abcd	588.67 ± 0.00a	419.00 ± 198.77abcdef
18478	Susceptible	143.67 ± 59.18abcdefgh	319.22 ± 128.33abcde	345.45 ± 130.41abcdef
18485	Susceptible	210.78 ± 44.38abc	356.67 ± 101.69abcde	250.11 ± 106.01abcdef
18489	Susceptible	163.55 ± 45.17abcdefg	367.78 ± 16.11abcde	517.50 ± 34.50abcdef
18509	Resistant	111.22 ± 57.65bcdefgh	84.45 ± 17.90cde	264.17 ± 100.50abcdef
18541	Resistant	104.34 ± 44.68bcdefgh	131.00 ± 46.64bcde	161.22 ± 57.62bcdef
18556	Resistant	100.22 ± 47.04bcdefgh	142.11 ± 24.21bcde	326.78 ± 119.18abcdef
18569	Resistant	62.56 ± 19.18cdefgh	229.78 ± 70.99abcde	129.67 ± 47.93bcdef
18584	Resistant	4.22 ± 1.82h	12.78 ± 2.54e	28.50 ± 9.83ef
18585	Resistant	10.00 ± 9.01gh	30.67 ± 1.67e	50.67 ± 14.96ef
18587	Resistant	32.22 ± 25.50efgh	18.78 ± 2.79e	28.34 ± 19.34ef
18588	Resistant	23.67 ± 9.90fgh	28.78 ± 2.23e	56.00 ± 5.86ef
18597	Resistant	176.67 ± 34.01acbdef	259.56 ± 86.85abcde	75.00 ± 15.94ef
18628	Resistant	21.22 ± 8.73fgh	233.67 ± 210.07abcde	29.00 ± 7.12ef
18633	Resistant	64.55 ± 48.73cdefgh	187.22 ± 112.62abcde	118.84 ± 18.17cdef
18639	Resistant	148.22 ± 22.16abcdefgh	109.22 ± 36.45bcde	66.84 ± 20.17ef
18644	Resistant	158.33 ± 22.96abcdefgh	160.22 ± 44.13abcde	350.78 ± 212.98abcdef
18647	Resistant	182.11 ± 25.37abcde	47.67 ± 22.83de	66.84 ± 34.17ef
18663	Resistant	133.00 ± 30.99abcdefgh	133.00 ± 124.33bcde	372.78 ± 313.63abcdef
RG607RR	Susceptible	17.78 ± 41.06abcdefgh	439.17 ± 128.50abcde	724.84 ± 275.17a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

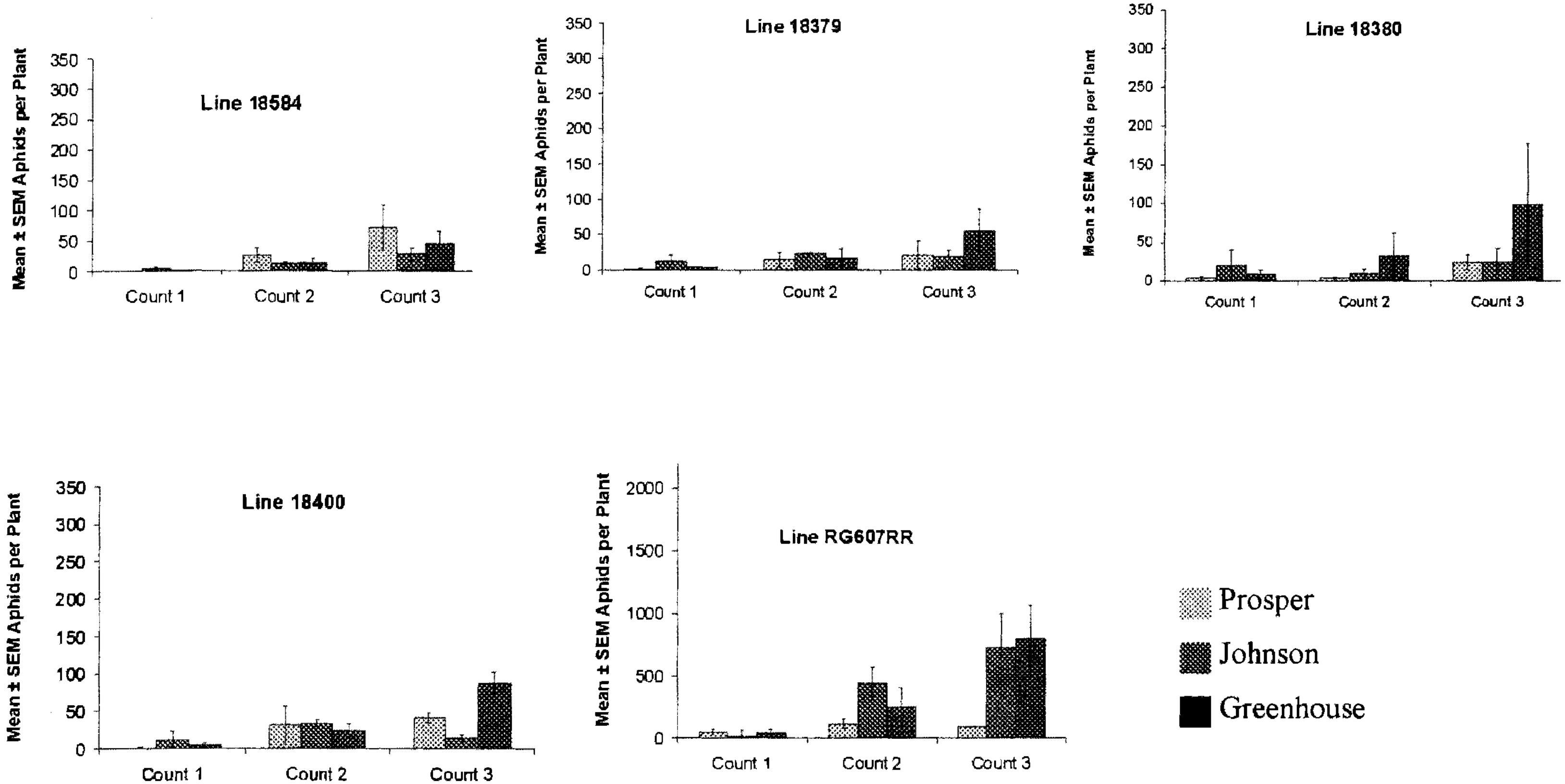


Figure 3.3. The average number of aphids per plant on four selected lines during the three experiments. Because of their low ratings, the lines were determined to be resistant during the initial greenhouse screening. Line RG607RR was the susceptible control.

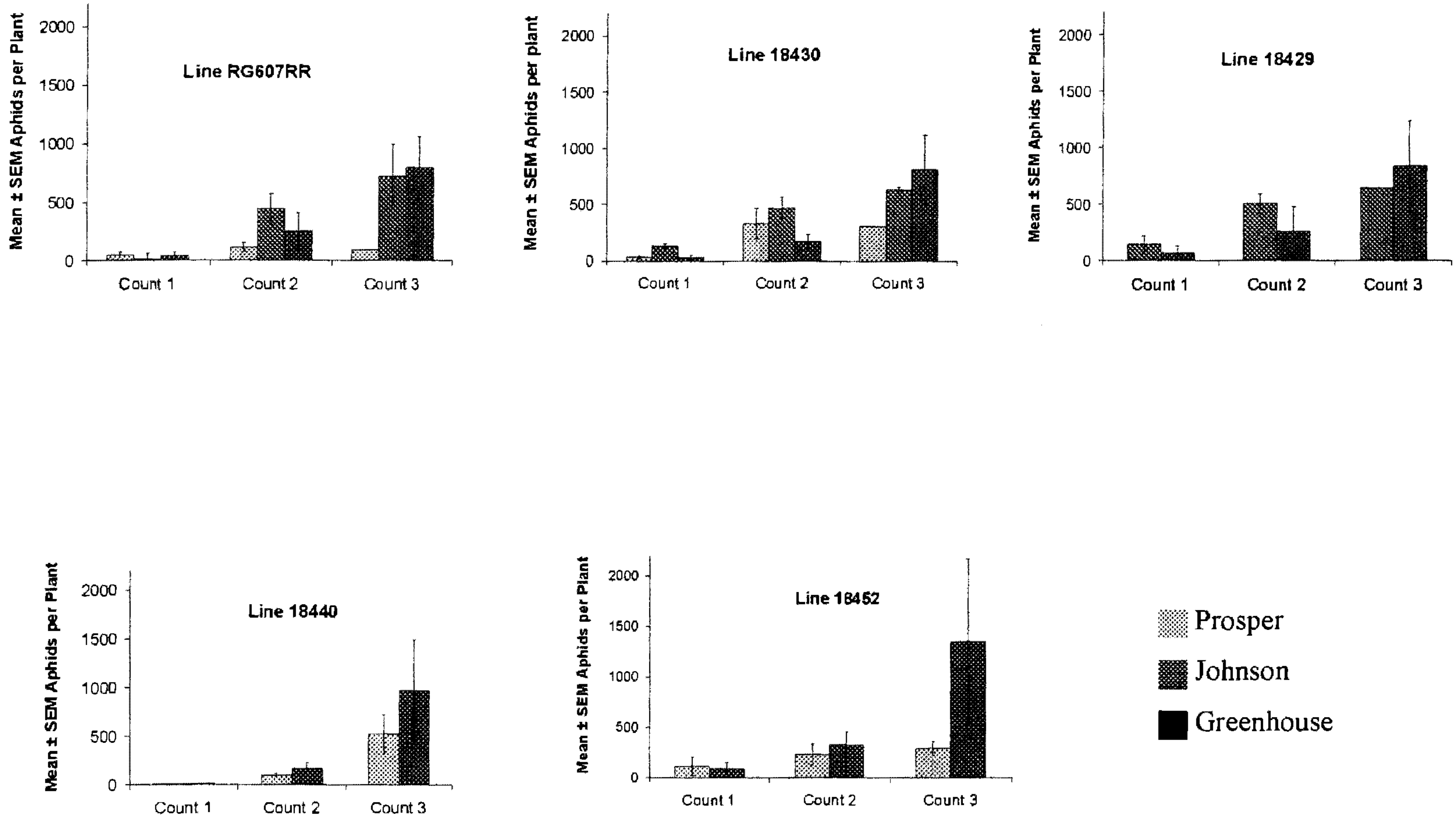


Figure 3.4.The average number of aphids per plant on five selected lines during the three experiments. Because of their high ratings, the lines were determined to be susceptible during the initial greenhouse screening.

Table 3.13. Effect of line on yield for soybean grown in the *Rag1* study in 2008 near Johnson, MN.

Line	Classification Based on Greenhouse Screening	Yield (T ha^{-1})
18306	Resistant	2.47 ± 0.21a
18309	Susceptible	2.59 ± 0.19a
18346	Resistant	2.62 ± 0.23a
18350	Resistant	2.54 ± 0.01a
18357	Resistant	2.75 ± 0.25a
18358	Resistant	2.93 ± 0.25a
18369	Susceptible	2.68 ± 0.09a
18375	Susceptible	2.81 ± 0.22a
18379	Resistant	2.92 ± 0.27a
18380	Resistant	2.95 ± 0.18a
18382	Susceptible	2.84 ± 0.21a
18384	Susceptible	3.25 ± 0.10a
18388	Susceptible	2.89 ± 0.33a
18391	Susceptible	2.57 ± 0.23a
18397	Susceptible	2.79 ± 0.18a
18399	Susceptible	2.86 ± 0.02a
18400	Resistant	3.05 ± 0.23a
18405	Susceptible	2.90 ± 0.07a
18407	Susceptible	2.85 ± 0.02a
18408	Resistant	2.77 ± 0.26a
18423	Susceptible	2.62 ± 0.32a
18428	Susceptible	2.75 ± 0.14a
18429	Susceptible	2.91 ± 0.31a
18430	Susceptible	2.89 ± 0.23a
18435	Susceptible	2.67 ± 0.08a
18439	Susceptible	2.59 ± 0.29a
18445	Resistant	2.53 ± 0.18a
18459	Susceptible	3.01 ± 0.05a
18463	Susceptible	2.59 ± 0.25a
18468	Susceptible	2.79 ± 0.15a
18478	Susceptible	2.68 ± 0.11a
18485	Susceptible	2.71 ± 0.20a
18489	Susceptible	3.07 ± 0.07a
18509	Resistant	2.45 ± 0.10a
18541	Resistant	2.76 ± 0.22a
18556	Resistant	2.87 ± 0.18a
18569	Resistant	2.95 ± 0.18a
18584	Resistant	3.07 ± 0.45a
18585	Resistant	3.25 ± 0.39a
18587	Resistant	2.83 ± 0.35a
18588	Resistant	3.18 ± 0.37a

(Table 3.13 continued)

18597	Resistant	2.69 ± 0.11a
18628	Resistant	3.15 ± 0.28a
18633	Resistant	2.78 ± 0.26a
18639	Resistant	2.68 ± 0.22a
18644	Resistant	2.83 ± 0.25a
18647	Resistant	2.78 ± 0.13a
18663	Resistant	2.90 ± 0.32a
RG607RR	Susceptible	3.32 ± 0.32a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

Table 3.14. Effect of line on yield for soybean grown for the *Rag1* study in 2008 near Prosper, ND.

Line	Classification Based on Greenhouse Screening	Yield (T ha^{-1})
18306	Resistant	1.32 ± 0.26hij
18309	Susceptible	1.43 ± 0.19fghij
18350	Resistant	1.18 ± 0.16ij
18357	Resistant	1.62 ± 0.14abcdefghi
18358	Resistant	2.05 ± 0.18a
18369	Susceptible	1.45 ± 0.09efghij
18375	Susceptible	1.44 ± 0.25efghij
18379	Resistant	1.95 ± 0.14abc
18380	Resistant	1.68 ± 0.08abcdefgh
18387	Susceptible	1.89 ± 0.09abcde
18388	Susceptible	1.52 ± 0.13cdefghij
18397	Susceptible	1.86 ± 0.06abcdef
18399	Susceptible	1.91 ± 0.09abcd
18400	Resistant	1.98 ± 0.28ab
18405	Susceptible	1.64 ± 0.28abcdefgh
18407	Susceptible	1.50 ± 0.18defghij
18430	Susceptible	1.51 ± 0.04cdefghij
18435	Susceptible	1.53 ± 0.05bcdefghij
18436	Susceptible	1.55 ± 0.18bcdefghij
18440	Susceptible	1.44 ± 0.15efghij
18445	Resistant	1.39 ± 0.07ghij
18452	Susceptible	1.43 ± 0.21fghij
18459	Susceptible	1.16 ± 0.09j
18468	Susceptible	1.18 ± 0.05ij
18488	Susceptible	1.66 ± 0.12abcdefgh
18489	Susceptible	1.50 ± 0.14defghij
18545	Susceptible	1.34 ± 0.28hij

(Table 3.13 continued)

18556	Resistant	1.62 ± 0.16abcdefghi
18584	Resistant	2.01 ± 0.31a
18587	Resistant	2.05 ± 0.09a
18588	Resistant	1.80 ± 0.18abcdefg
RG607RR	Susceptible	1.45 ± 0.19efghij

Means within a column followed by the same letter are not significantly different at $P \leq$ 0.05 as determined using a Fisher's protected LSD test.

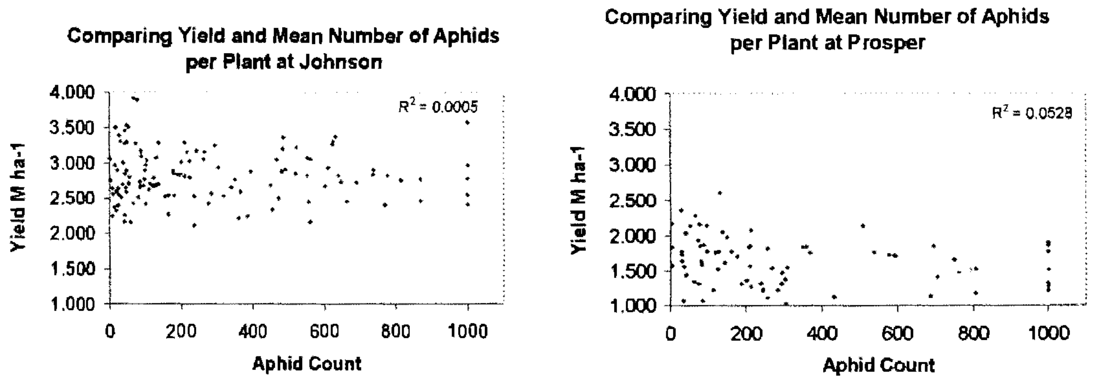


Figure 3.5. Comparing yield with the mean number of aphids at Prosper and Johnson.

Table 3.15. Effect of resistant and susceptible Rag1 lines on natural enemies using the sweeping method in 2008 near Prosper, ND.

Score from initial Greenhouse Trial	Natural Enemies
Resistant	38.33 ± 3.93a
Susceptible	33.67 ± 2.85a

Means within a column followed by the same letter are not significantly different at $P \leq$ 0.05 as determined using a Fisher's protected LSD test.

Table 3.16. Effect of resistant and susceptible Rag1 lines on natural enemies using the sweeping method in 2008 near Johnson, MN.

Score from initial Greenhouse Trial	Natural Enemies
Resistant	58.67 ± 9.53a
Susceptible	71.33 ± 4.33a

Means within a column followed by the same letter are not significantly different at $P \leq$ 0.05 as determined using a Fisher's protected LSD test.

Table 3.17. Sources of variation, degrees of freedom, mean squares, and f-tests for soybean grown in 2008 during the advanced greenhouse screening.

Sources of Variation	Degrees of Freedom	Mean Square
Line	55	70125.65
Replicate	1	42730.67
Error (a)	55	79174.12**
Time	2	5405746.23**
Error (b)	2	3808.60
Line*Time	110	31331.29
Error (c)	110	38237.35

*, ** Significant at the $P \leq 0.05$ and $P \leq 0.01$ levels of probability, respectively.

Table 3.18. Effect of line on aphid counts for soybean grown in 2008 during the advanced greenhouse screening for the *Rag1* gene for soybean aphid resistance.

Line	Classification Based on Greenhouse Screening	3 Day Count	7 Day Count	10 Day Count
18306	Resistant	5.67 ± 1.34a	82.17 ± 14.50a	526.50 ± 40.50a
18309	Susceptible	7.17 ± 0.17a	47.84 ± 40.84a	358.67 ± 328.67a
18346	Resistant	23.60 ± 20.40a	127.30 ± 73.70a	558.10 ± 276.90a
18350	Resistant	13.30 ± 0.70a	86.20 ± 42.40a	435.10 ± 175.50a
18357	Resistant	6.00 ± 3.20a	31.30 ± 12.30a	222.30 ± 49.50a
18358	Resistant	8.08 ± 0.68a	45.73 ± 2.53a	212.93 ± 39.33a
18369	Susceptible	9.84 ± 5.17a	91.50 ± 84.50a	447.50 ± 421.17a
18375	Susceptible	34.20 ± 28.20a	165.90 ± 107.90a	824.40 ± 536.40a
18379	Resistant	3.75 ± 0.25a	15.63 ± 12.88a	55.38 ± 29.63a
18380	Resistant	8.70 ± 5.30a	31.73 ± 29.53a	98.65 ± 77.85a
18382	Susceptible	17.50 ± 11.50a	52.38 ± 42.88a	306.00 ± 267.00a
18384	Susceptible	7.47 ± 1.87a	33.47 ± 17.87a	220.90 ± 166.10a
18387	Susceptible	25.50 ± 9.50a	145.50 ± 40.50a	571.40 ± 294.40a
18388	Susceptible	12.40 ± 1.80a	100.20 ± 5.20a	429.40 ± 107.60a
18391	Susceptible	15.25 ± 8.50a	80.63 ± 57.88a	295.13 ± 215.13a
18397	Susceptible	7.30 ± 0.50a	47.10 ± 24.90a	303.60 ± 191.00a
18399	Susceptible	33.40 ± 25.60a	105.38 ± 51.38a	446.85 ± 168.65a
18400	Resistant	5.21 ± 1.54a	23.75 ± 9.75a	86.92 ± 14.59a
18405	Susceptible	49.20 ± 26.80a	66.20 ± 27.80a	671.00 ± 125.80a
18407	Susceptible	11.40 ± 7.40a	75.78 ± 56.03a	335.58 ± 125.83a
18408	Resistant	24.20 ± 18.80a	168.90 ± 79.10a	578.47 ± 245.87a
18423	Susceptible	84.18 ± 70.43a	165.48 ± 142.73a	451.40 ± 296.40a
18428	Susceptible	49.84 ± 17.84a	59.13 ± 12.13a	521.75 ± 77.75a
18429	Susceptible	65.60 ± 60.40a	259.58 ± 211.18a	836.00 ± 403.00a
18430	Susceptible	28.63 ± 20.63a	169.75 ± 60.75a	808.75 ± 305.75a
18435	Susceptible	28.00 ± 13.60a	169.80 ± 4.00a	552.70 ± 72.70a
18436	Susceptible	42.80 ± 36.80a	175.40 ± 126.40a	612.74 ± 254.07a

(Table 3.18 continued)

18439	Susceptible	49.38 ± 43.63a	203.67 ± 176.67a	722.71 ± 546.96a
18440	Susceptible	16.63 ± 2.38a	169.38 ± 60.63a	971.75 ± 515.25a
18445	Resistant	9.67 ± 1.34a	49.17 ± 10.17a	330.75 ± 137.25a
18452	Susceptible	88.00 ± 56.00a	328.00 ± 126.00a	1353.40 ± 819.60a
18459	Susceptible	12.25 ± 4.25a	61.00 ± 35.00a	466.80 ± 389.20a
18463	Susceptible	28.24 ± 23.44a	88.50 ± 80.50a	481.67 ± 367.67a
18468	Susceptible	12.20 ± 3.80a	51.35 ± 12.15a	316.00 ± 105.00a
18474	Susceptible	5.50 ± 0.50a	41.63 ± 28.88a	425.38 ± 345.88a
18485	Susceptible	41.50 ± 13.50a	141.88 ± 15.88a	691.84 ± 204.84a
18488	Susceptible	4.40 ± 0.60a	24.87 ± 3.47a	219.10 ± 27.90a
18489	Susceptible	10.20 ± 4.00a	59.20 ± 32.60a	364.80 ± 288.00a
18509	Resistant	6.84 ± 1.84a	49.09 ± 25.59a	382.34 ± 145.34a
18541	Resistant	19.80 ± 16.00a	127.90 ± 51.70a	463.30 ± 63.10a
18545	Susceptible	19.75 ± 14.25a	67.88 ± 31.13a	594.75 ± 426.50a
18556	Resistant	6.25 ± 3.25a	42.53 ± 29.73a	267.43 ± 246.83a
18569	Resistant	15.30 ± 9.30a	99.80 ± 87.00a	338.00 ± 323.00a
18584	Resistant	2.20 ± 0.00a	14.40 ± 7.00a	44.68 ± 21.08a
18585	Resistant	28.63 ± 25.38a	79.38 ± 57.13a	328.75 ± 162.75a
18587	Resistant	13.38 ± 9.63a	52.88 ± 29.13a	216.00 ± 110.75a
18588	Resistant	7.40 ± 4.80a	50.60 ± 34.60a	136.70 ± 114.90a
18597	Resistant	11.18 ± 7.43a	91.60 ± 32.60a	637.58 ± 22.83a
18628	Resistant	25.40 ± 18.60a	135.05 ± 86.45a	332.53 ± 198.73a
18633	Resistant	8.94 ± 5.27a	64.60 ± 39.60a	362.04 ± 167.37a
18639	Resistant	7.34 ± 3.34a	62.17 ± 24.50a	272.84 ± 113.17a
18647	Resistant	8.68 ± 6.08a	43.00 ± 34.00a	179.40 ± 125.60a
18663	Resistant	5.79 ± 3.54a	29.25 ± 11.75a	269.88 ± 127.13a
18664	Resistant	11.25 ± 7.25a	117.00 ± 106.50a	479.50 ± 310.75a
Dowling	Resistant	7.03 ± 3.23a	21.08 ± 15.68a	69.58 ± 40.18a
RG607RR	Susceptible	45.47 ± 32.87a	249.47 ± 160.87a	794.67 ± 268.67a

Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ as determined using a Fisher's protected LSD test.

When evaluating all the lines that were determined to be resistant during the initial greenhouse screening compared to the other studies, the highest mean aphid counts were usually seen during the advanced greenhouse screening (Table 3.20). This may be attributed to the absence of natural enemies (Rutledge et al. 2004). Two of the lines, 18350 and 18306, had higher mean aphid counts at Prosper and during the advanced greenhouse screening compared to the initial greenhouse screening. These lines could potentially be susceptible to the soybean aphid and could have been incorrectly determined as resistant during the initial greenhouse screening experiment.

No differences among treatments for yield were observed at the Johnson location (Table 3.13). However, at the Prosper location, significant differences were found (Table 3.14). This may have been attributed to late harvesting that resulted in shattering and seed loss of the soybean. So no conclusions from these results can be attained. The economic injury level when yield loss is likely to occur from soybean aphid damage is 674 aphids per plant (Ragsdale et al. 2007).

Table 3.19. Initial greenhouse rating compared to the final mean aphid counts of the two field experiments and the advanced greenhouse screening in five lines determined to be resistant and the susceptible control. RG607RR.

Line	Initial Greenhouse Rating	Prosper	Johnson	Advanced Greenhouse Screening
18379	1.8	21	18	55
18380	2.0	25	25	99
18400	1.0	42	14	87
18584	2.7	72	29	45
18585	2.7	--	51	329
RG607RR	4.0	97	725	795

-- Line not planted at Prosper due to shortage of seed.

The beneficial insect populations were not significantly different between susceptible and resistant types of lines (Tables 3.15 and 3.16). In lab studies, soybean lines with Dowling as the *Rag1* resistant parent, reduced predator performance by reducing the life span of adult *Harmonia axyridis* (Lundgren et al. 2008). Since a small number of natural enemies were sampled, it is difficult to make a conclusion based on these results. Additional problems with this experiment include the potential segregation of the experimental lines and two of the lines determined resistant during the initial greenhouse screening appeared to be susceptible during the field studies and the advanced greenhouse

Table 3.20. Initial greenhouse screening rating on resistant lines compared to the final mean aphid counts ± standard error of the Prosper and Johnson field studies and the advanced greenhouse screening.

Line	Initial Greenhouse Rating	Prosper	Johnson	Advanced Greenhouse Screening
18400	1.0	41.45 ± 6.74	13.89 ± 5.47	82.92 ± 14.59
18628	1.0	--	29.00 ± 7.12	332.53 ± 198.73
18663	1.4	--	372.78 ± 313.63	269.88 ± 127.13
18358	1.5	82.67 ± 50.48	55.00 ± 30.00	212.93 ± 39.33
18569	1.5	--	129.67 ± 47.93	338.00 ± 323.00
18587	1.5	302.17 ± 205.84	28.34 ± 19.34	216.00 ± 110.75
18633	1.6	--	118.84 ± 18.17	362.04 ± 167.37
18379	1.8	20.67 ± 19.67	18.33 ± 8.88	55.38 ± 29.63
18556	1.8	647.11 ± 189.91	326.78 ± 119.18	267.43 ± 246.83
18647	1.8	--	66.84 ± 34.17	179.40 ± 125.60
18357	2.0	118.00 ± 0.00	41.78 ± 30.86	222.30 ± 49.50
18380	2.0	24.22 ± 10.08	24.84 ± 17.17	98.65 ± 77.85
18408	2.0	--	134.22 ± 83.32	578.47 ± 245.87
18639	2.0	--	66.84 ± 20.17	272.84 ± 113.17
18644	2.0	--	350.78 ± 212.98	479.50 ± 310.75
18541	2.2	--	161.22 ± 57.62	463.30 ± 63.10
18597	2.2	--	75.00 ± 15.94	637.58 ± 22.83
18346	2.4	--	114.67 ± 73.34	558.10 ± 276.90
18588	2.4	100.66 ± 21.36	56.00 ± 5.86	136.70 ± 114.90
18306	2.7	1000.00 ± 0.00	217.67 ± 175.67	526.50 ± 40.50
18350	2.7	761.00 ± 0.00	496.67 ± 256.94	435.10 ± 175.50
18445	2.7	699.33 ± 211.23	69.44 ± 22.82	330.75 ± 137.25
18584	2.7	71.67 ± 36.83	28.50 ± 9.83	44.68 ± 21.08
18585	2.7	--	50.67 ± 14.96	328.75 ± 162.75

-- Lines not grown at the Prosper location due to shortage of seed.

screening. The experimental lines should not have been grouped by type based on the initial greenhouse screening, but rather evaluated separately for the number of natural enemies. Using this method would have allowed for further analysis of each line for the number of natural enemies compared with the number of aphid counts. Increasing the plot size would have provided more habitats for the natural enemies and increased the sampling area.

This research should provide plant breeders with an effective method to screen experimental soybean lines with *Rag1* for aphid resistance. To save time and effort screening lines for aphid resistance should be conducted after each cross. Even though the experimental lines used in this research are no longer being advanced in a breeding program, they are still being used for additional soybean aphid studies. More research should be conducted in additional years with varying aphid populations to determine the lines best suited for producers. In years with low aphid pressures, neither the resistant cultivars nor an insecticide application may be necessary. Therefore, more research is needed to determine if the benefits of the resistant cultivars will outweigh the costs.

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