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EFFECTS OF FERTILIZATION ON THE SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA AND THE EFFECTS OF SOYBEAN APHIDS AND FERTILIZATION ON SOYBEAN PLANTS

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# EFFECTS OF FERTILIZATION ON THE SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA AND THE EFFECTS OF SOYBEAN APHIDS AND FERTILIZATION ON SOYBEAN PLANTS

By

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ABSTRACT

EFFECTS OF FERTILIZATION ON THE SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA AND THE EFFECTS OF SOYBEAN APHIDS AND FERTILIZATION ON SOYBEAN PLANTS

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Soybean aphid, *Aphis glycines* Matsumura, is an invasive agricultural pest first reported in North America in 2000. Soybean aphid populations appeared to be higher in areas where plants exhibited symptoms of potassium deficiency. In order to determine whether soil potassium deficiency affected aphid population levels, the effect of soybean aphids and soil potassium level on growth and yield characteristics of soybeans, and a possible mechanism for the soybean aphid-soil potassium interaction, field surveys and controlled cage studies were conducted. In both types of studies, soybean aphid populations were higher on plants with a lower level of potassium nutrition, and this was due to an increase in individual aphid fecundity on deficient plants. Phloem asparagine content was also negatively correlated with soil potassium level, and corresponded to aphid populations. Changes in asparagine content are suggested as the nutritional mechanism for the soybean aphid-soil potassium interaction. Plants with high soybean aphid populations had fewer leaves, pods, and nodes than uninfested plants; the effect of soil potassium on plant characteristics was variable. When soybean aphid populations were low, they were unaffected by foliar nutrient sprays.

To Vicki

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## Introduction

Soybean aphid (*Aphis glycines* Matsumura) is an invasive agricultural pest that first appeared in the United States in 2000 (Venette and Ragsdale 2004). It is the most important insect pest of soybean in Chine, where it originated (Wu et al. 2004), an it has rapidly risen to become the most important insect pest of soybeans in North America. There are currently no control strategies for soybean aphids in North America except for the application of chemical insecticides. Other control strategies are sorely needed so that soybean aphid control can be accomplished in the context of integrated pest management.

In general, aphids are greatly affected by the nutritional quality of their host plant (Honek 1991, Douglas 1993, Havlickova and Smetankova 1998, Ponder et al. 2000, Cisneros and Godfrey 2001, Nevo and Coll 2001, Jansson and Ekborn 2002, Koyama et al. 2004). This can be manipulated through a variety of cultural practices, most notably fertilization.

## Distribution, Life History, and Phenology

The soybean aphid originated in China, where it cycles between species of *Rhamnus* as the primary host and soybean, *Glycine max*, and wild relatives in the *Glycine* genus such as *Glycine soja*. It is currently distributed in China, Japan, the Philippines, South Korea, Indonesia, Malaysia, Thailand, Vietnam, Russia, Australia, the United States, and Canada (Wu et al. 2004).

Soybean aphid has a host-alternating life cycle typical of *Aphis* species. The primary hosts are woody plants in the genus *Rhamnus*. In North America, the only species known to be suitable for soybean aphid are *Rhamnus cathartica* L. and *Rhamnus alnifolia* L'Hértier (Voegtlin et al. 2004). The eggs overwinter on the primary hosts, hatching into fundatrices in the spring. The fundatrices have several generations on *Rhamnus* with each subsequent generation giving birth to proportionately more fundatrigeniae that migrate to soybean. Many generations of alate and apterous soybean aphids follow on soybean. Aphid populations on soybean can increase very rapidly, doubling every two to three days in a predator free environment (Program 2004). In the fall, soybean aphids on soybean give rise to gynoparae and winged males and then fly to the primary host. The gynoparae give birth to oviparae that mate with the males and oviposit eggs.

**Population Dynamics**

Soybean aphid was first discovered in North America in 2000. In 2000 and 2001, soybean aphid infestations were severe, causing up to 40% yield reduction in different parts of Michigan (DiFonzo 2002). In 2002, soybean aphid populations were low. There was an aphid outbreak again in 2003 with yield loss up to 50% in unsprayed fields in some parts of Michigan (DiFonzo, personnel communication). In 2004 aphid populations were again very low. In China the soybean aphid is also a sporadic pest. Factors that may induce a soybean aphid outbreak in China include higher temperatures, high precipitation while soybean aphid is on buckthorn, low precipitation when soybean aphid is on soybean, low humidity, overwintering, planting date, soybean variety, the presence

of natural enemies especially coccinellids, and the synchronization of soybean and soybean aphid phenology (Wu et al. 2004).

**Agricultural Impact and Control**

Soybeans are an important world crop, and especially important in the North Central Region of North America, which has been severely impacted by the soybean aphid. The North Central Region produces approximately 40% of the world's soybean crop (Anonymous 2005). The value of the 2003 U.S. soybean crop exceeded $18.4 billion (Anonymous 2004b). In 2003 Michigan planted 809,000 ha of soybean, producing 1.46 metric tones of beans with a value of US $387 million (Anonymous 2004b). In China severe infestations of soybean aphid can lead to 30% (Wang et al. 1996, Sun et al. 2000) to 70% (Wu et al. 2004) yield reduction, so the potential for yield loss in North America is very great.

Since its initial discovery in North America in 2000, soybean aphid spread rapidly in the United States and Canada and now affects much of the soybean-producing region of North America. Predictive modeling suggests that it is likely to spread to all or nearly all U.S. soybean-producing regions (Venette and Ragsdale 2004). In 2001 and 2003, unsprayed fields in Michigan suffered yield losses of up to 40-50%. During 2003 an estimated 770,000 acres or 36% of soybean acreage was sprayed with a chemical insecticide in Michigan (Difonzo, personnel communication). Prior to the occurrence of soybean aphid, soybeans were rarely treated with insecticide in Michigan, so these numbers reflect a huge increase in the amount of insecticides being released into the environment. Currently, there are no strategies for control of soybean aphid other than

chemical application. Organic production, an increasing market in Michigan, is also severely impacted by the lack of non-chemical control options for soybean aphid.

The soybean aphid may damage the soybean beginning in the vegetative stages all the way through seed set. In their review of the Chinese literature, Wu et al. reported that soybean aphid may cause leaf distortion early defoliation, and stunting of the plant. Soybeans with a severe aphid infestation have fewer branches, pods, seeds, and a lower weight per seed than uninfested plants (Wu et al. 2004). Other studies have shown that soybean aphid infestation reduces the number of pods per soybean plant and the total weight of beans produced but does not affect individual bean weight (Wang et al. 1996). In addition, sooty mold may grow on the honeydew excreted by soybean aphids. The presence of honeydew on foliage may reduce the amount of light that penetrates the foliage (Wood et al. 1988, Sparks and Yates 1991). In soybean, this shading effect has been shown to reduce yield and seed quality (Wu et al. 2004).

Xibei et al. (year unknown) have published a soybean aphid threshold of 500 soybean aphids per 100 plants and a 35% colonization rate. However, the North American experience shows that the threshold may be very different on this continent. Extension specialists in the North-Central Region recommend chemical control of soybean aphid when the population reaches 250 aphids per plant on 90% of the plants examined, and when the population is actively increasing. Chemical control should take place prior to the R6 (full seed) stage of soybeans (Anonymous 2004a). The preferred method for chemical control is through foliar insecticide sprays, although seed treatments may offer early season protection (Anonymous 2004a). Although there are efficacy differences among foliar insecticides, all of the products so far investigated offer the

same level of yield protection with the exception of dimethoate (DiFonzo 2002). All of the conventional sprays would kill beneficial insects as well as soybean aphids, so that biological control later in the year may be impacted. Also, certain organic products containing pyrethrum may fail to control soybean aphids or even cause a spike in soybean aphid populations. This is probably due to the high activity of these products against natural enemies.

## Aphid Nutrition

Aphids feed by tapping into sieve elements and ingesting the phloem sap that is being translocated through them (Srivastava 1987). Thus, they have access to only those plant compounds that may be translocated in the phloem sap. Phloem is known to contain water, sugars, alcohols, proteins, free amino acids and amides, plant hormones (including steroids), ATP, auxins, gibberellins, cytokinins, certain herbicides (such as 2,4-D), vitamins (such as thiamine, niacin, pantothetic acid, $B_6$-complex vitamins, myo-inositol, and ascorbic acid), some viruses, and elemental potassium, phosphorus, magnesium, iron, manganese, copper, molybdenum, cobalt, and titanium (Ziegler 1975). The pH of phloem sap is 7.2-8.5 (Ziegler 1975).

### *Essential Nutrients*

Aphids are prodigious feeders, ingesting their own weight in phloem sap every day as adults and many times their own weight per day as nymphs (Dixon 1998). Yet they are also remarkably efficient feeders. Even on a nutritionally poor diet, they are able to sustain not only their own lifelong embryogenesis but, because of their unique

telescoped generations, the beginning stages of embryogenesis in their progeny (Dixon 1998). In order to accomplish this, every aphid must be able to extract enough nutrients from the phloem sap ingested each day to feed three generations.

Aphids have a respiratory quotient of 1.0, indicating that they utilize carbohydrates for respiration. The main carbohydrate used by aphids is sucrose. Individuals of the alate morph require more sucrose then their apterous counterparts (Klingauf 1987). This is probably because the alatae must first build and maintain wings and the associated musculature, and must store enough food to embark on migrations that may take several days and cover hundreds of kilometers. Thus, their energy requirements are very different than those of apterae, which are assured of a constant energy rich food source and do not develop or maintain wing muscles.

Although the exact nutritional requirements of soybean aphids are not known, there are a number of nutrients commonly required by aphid species (Table 1) (Dadd et al. 1967, Srivastava 1987, Nation 2001).

*Amino Acids and N-limitation*

The acquisition of nitrogenous compounds is the main challenge facing aphids feeding on their natural host plants (Terra 1988). There is only one instance of a proteinase (a cathepsin-I-like cysteine proteinase) occurring in the gut of an aphid, *Acyrthosiphon pisum* (Harris). However, this enzyme probably plays a role in detoxification of ingested material rather than a role in the nutrition of the aphid (Cristofoletti et al. 2003). In general, aphids are not thought to use proteinases as part of their nutritional digestion. This is probably because the high levels of proteinase

inhibitors combined with the extremely low protein concentration typically encountered in phloem sap make plant proteins a poor nitrogen source (Sandstrom and Moran 2001). Furthermore, many of the proteins transported in the phloem are defensive proteins. Often these proteins must be cleaved by herbivore proteinases in order to become active. Thus, by not cleaving phloem proteins, aphids may circumvent this plant defense. Aphids are thought to obtain all of their dietary nitrogen from amino acids being translocated in the phloem sap. *Rhopalosiphum padi* (L.) have growth rates directly correlated to concentration of amino acids in the phloem of their host plants (Weibull 1987).

There is evidence that the growth of aphid embryos, and hence the growth of an aphid population, is limited by the availability of essential amino acids. Aphids have a unique mode of reproduction in which aphid embryos receive nutrients from the mother's hemocoel until they are larviposited as first instars. When the mother has a good quality diet, nearly all of the essential amino acids phenylalanine, threonine, and lysine in her hemolymph are taken up by her embryos. Larval development quickens and slows with the maternal supply of these essential amino acids (Wilkinson and Ishikawa 1999).

Aphids are among the most versatile animals in respect to nitrogen nutrition because they possess a primary intracellular symbiotic bacterium, *Buchnera* as well as secondary gut symbionts, which are able to manufacture certain essential amino acids from nonessential amino acids in the aphid diet (Moran et al. 2003). *Buchnera* may be able to provide their hosts with synthesized essential amino acids at a higher rate than aphids are able to bring these substances across the gut wall (Douglas 1998). For that reason, not every aphid species requires all of the essential amino acids; the amino acids

that are required varies between aphid species and populations because of differences in their symbionts (Srivastava 1987).

Among the amino acids, several are especially important to aphids. Dadd and Kreiger (1968) showed that methionine has an important phagostimulatory effect that increases host plant acceptance and reduces the tendency of aphids to leave a feeding site (restlessness). They also found that without one of the non-essential amino acids glutamine, glutamic acid, asparagine, aspartic acid, alanine, or serine almost no aphid growth occurred (Dadd and Krieger 1968). *Buchnera* manufacture essential amino acids from non-essential amino acids that occur more commonly in their hosts' diets. Good evidence exists that glutamate is the principal nitrogenous compound used by *Buchnera* to synthesize essential amino acids (Douglas 1998, Wilkinson and Ishikawa 1999). Indeed, glutamate and aspartate are known to be transported across the mycetome membrane from aphid hemocoel to *Buchnera* cells (Moran et al. 2003). On oats and barley, *R. padi* growth is greatest at the times in plant development when the most asparagine is being transported in the phloem (Weibull 1987).

**Nitrogen in the Soybean**

In order to understand the nitrogen nutrition of soybean aphids, it is important to understand the way nitrogen moves in a soybean plant. In nodulated soybeans, a symbiosis occurs between the soybean plant and one of four genera of Rhizobiaceae, usually *Rhizobium* or *Bradyrhizobium* (Schubert 1995). The bacterium supplies fixed nitrogen to the plant and receives fixed carbon. Nitrogen fixation by the bacteria can supply up to ninety-five percent of the plant's nitrogen demand (Unkovich and Pate

2000). The process of nitrogen fixation is energetically very expensive; respiration by the bacterial symbiont may account for 70% of total root respiration (Walsh et al. 1998). For this reason, it is advantageous to the plant to slow or stop nitrogen fixation under environmental stress or when the plant reaches a growth stage where additional fixed nitrogen is no longer required. This is accomplished via signaling between the soybean plant and the bacteria. The signaling compound is the amino acid asparagine (Bacanamwo and Harper 1997).

Ammonium or ammonia travel from the symbiosome (bacteroid and surrounding membrane) to the cytosol of the soybean plant (Whitehead et al. 2001). Inside the nodule soybean cells, this nitrogen is converted to ureides, which are transported to the rest of the plant via the xylem (Whitehead et al. 2001). Ureides are broken down into amino acids, including asparagine, in the shoot and loaded into the phloem (Vadez et al. 2000) or made into proteins. Asparagine that arrives at the nodules is broken down into aspartate and glutamate which can then enter the bacteroid and inhibit nitrogen fixation (Bacanamwo and Harper 1997). When the plant requires a reduced rate of nitrogen fixation because of an environmental stress or the age, higher levels of asparagine are loaded into the phloem. Higher levels of this signaling compound translate into reduced nitrogen fixation in the nodules, but can enhance the quality of phloem sap as a soybean aphid food source.

Potassium status may affect the way that nitrogen is stored in a plants; an increase in potassium levels leads to a decrease in soluble nitrogen, a correlate of phloem sap amino acid concentration, in brussels sprouts (Van Emden 1966). Potassium is a cofactor of the enzyme responsible for stringing amino acids into proteins (Mengel and Kirkby

2001). Thus, under potassium deficiency, amino acids may build up in the foliage. This could trigger a soybean plant to slow down or shut down its nitrogen fixation by increasing phloem loading of the nodule signaling compound asparagine.

**Role of Nutrition in Aphid Control**

There are several examples of aphid populations that are affected by the nutritional value of their food. Nitrogen fertilization has been shown to increase soybean aphid populations (Wang and Ba 1998). High-input cotton cultivation practices common in California have coincidentally elevated *Aphis gossypii* Glover from a secondary to a primary pest. *A. gossypii* is more fecund, has a shorter development time, and occurs at higher densities on cotton plants with high tissue nitrogen levels as a result of nitrogen fertilization (Cisneros and Godfrey 2001, Nevo and Coll 2001). The effects of nitrogen fertilization appear to be stronger for nymphs than adults (Nevo and Coll 2001).

*A. gossypii* is not the only aphid to exhibit a response to nitrogen fertilization of the host plant. Nitrogen fertilization of wheat and barley results in increased fecundity of the cereal aphid, *Metopolophium dirhodum* (Walker), which feeds from sieve elements located on the leaves of the plants (Awmack and Leather 2002). A positive correlation has also been found between foliar nitrogen concentrations in corn and population levels of the corn leaf aphid, *Rhopalosiphon maidis* (Fitch) (Morales et al. 2001). A related species, *R. padi*, has a much reduced intrinsic growth rate when it is cultured on nitrogen deficient barley (Ponder et al. 2000). These studies, like many studies of the relationship between plant fertilization and aphid populations measured foliar nitrogen concentration rather than phloem exudate samples (Morales et al. 2001). However, foliar nitrogen

concentration is thought to follow the same general pattern as phloem sap amino acid concentration, so a plant with high levels of foliar nitrogen is assumed to translocate high concentrations of amino acids in its phloem sap (Douglas 1993). It is important to note that nitrogen fertilization does not have predictable effects on the amino acid composition of phloem sap (Prosser and Douglas 1992).

A few fertilization studies examined the effect of nitrogen fertilization on amino acid concentration in the phloem sap, and these studies may shed some light onto the mechanisms of this relationship. Nitrogen fertilization of oats and barley changes both the total amount and proportions of amino acids present in the phloem sap. Most amino acids remain at the same levels in the phloem sap following fertilization, but glutamic acid and aspartic acid increase (Weibull 1987). Since these are the amino acids that *Buchnera* most frequently use to manufacture essential amino acids and are the amino acids most easily passed across the aphid gut wall (Douglas 1998), aphid nitrogen nutrition probably improves dramatically following nitrogen fertilization of these crops.

Magnesium fertilization increases the fecundity of *R. padi* on barley. Magnesium fertilization increases chlorophyll synthesis and presumably photosynthesis rates in plants (Havlickova and Smetankova 1998). This may lead to an increased rate of assimilate production, and thus increased assimilate loading in the phloem. This in turn would increase total phloem flux through osmotically generated pressure flow and more total phloem would be delivered to feeding aphids.

Potassium fertilization can affect aphids. Many sucking insects respond negatively to potassium fertilization (Waring and Cobb 1989). Potassium fertilization reduces lifespan and rate of reproduction of *R. padi* feeding on barley. In addition, *R.*

*padi* have lower landing rates on barley grown in potassium rich than barley grown in potassium deficient soil (Havlickova and Smetankova 1998). In low potassium soils, fewer aphids were observed on potatoes receiving potassium fertilization in years when aphid populations were high (Broadbent et al. 1952). An interaction with potassium has been observed with soybean aphid at high populations. In 2000 and 2001, fields or parts of fields with potassium deficiency appeared to have higher soybean aphid populations and greater soybean damage (DiFonzo 2002). However, potassium fertilization has also been shown to contribute to higher soybean aphid populations, although the beginning fertility of the field was not reported (Wang and Ba 1998).

Lower concentrations of phloem amino acids may be a mechanism for plant resistance to aphids. Peas that are resistant to *Acyrthosiphon pisum* (Harris) have a decreased concentration of free amino acids in their phloem as compared to susceptible cultivars (Weibull 1987). Wheat resistant to *Diuraphis noxia* Mordvilko decreases the amount of translocated amino acids in response to infestation (Telang et al. 1999). Oat and barley resistance to *R. padi* is not related to a change in total concentration of amino acids, but resistant barley has a decreased concentration of one particularly important amino acid, asparagine (Weibull 1988).

**Conclusion**

Aphids live in very intimate association with their host plants, and any factor that causes internal changes to the host plant, such as fertilization, will also affect the aphids living on that plant. Many studies show that these relationships do occur, and these effects may exacerbate pest problems. It is critically important to understand not only the

gross effects of practices such as fertilization on soybean aphid populations, but also the mechanisms. This information provides targets for plant breeders, facilitates the movement of knowledge across systems, and allows agronomists to plan control strategies in such a way as to minimize interference between the different strategies. This knowledge may allow the design or alteration of cultural practices for control of soybean aphid within an Integrated Pest Management system.

Table 1.1. Nutritional requirements of aphids (Dadd et al. 1967, Srivastava 1987, Nation 2001).

| Nutrient Type | Nutrient |
|---|---|
| Elemental Nutrients | C |
| | N |
| | K |
| | P |
| | Mg |
| | Fe |
| | Zn |
| | Mn |
| | Cu |
| | S |
| | Co |
| | Mo |
| | B |
| | Ca |
| Vitamins | Ascorbic Acid |
| | Calcuim Pantothenate |
| | Folic Acid |
| | Meso-inositol |
| | Pyridoxine |
| | Nicotinic Acid |
| | Thiamine |
| | Niacin |
| | Lipogenic Factors |
| | Biotin |
| | Choline |
| | Riboflavin |
| Other | Sterols |

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## Chapter 2—Soil Potassium Influences Soybean Aphid Population Size in Soybean

### Introduction

Soybean aphid (*Aphis glycines* Matsumura) is an invasive agricultural pest that was first discovered in the United States in 2000 (Venette and Ragsdale 2004). It is the most important insect pest of soybean in China (Wu et al. 2004) and has rapidly risen to become the most important insect pest of soybeans in North America. The current control strategy for soybean aphid in North America is the application of chemical insecticides. Organic soybean production, an increasing market in Michigan, is severely impacted by the lack of approved organic control options for soybean aphid. Other strategies, such as biological and cultural controls, are sorely needed so that soybean aphid infestations can be managed in the context of integrated pest management.

A severe infestation of soybean aphids impacts the soybean plant in a variety of ways. In a review of the Chinese literature on the soybean aphid, Wu et al. (2004) reported that severely infested plants exhibited distorted foliage, early defoliation, and stunting and had fewer branches, pods, and beans per plant as well as a lower individual seed weight. In another study, a sever soybean aphid infestation reduced the number of pods per plant and the weight of beans produced per plant but did not affect individual bean weight (Wang et al. 1996).

During the first two seasons of soybean aphid infestation in Michigan (2000 and 2001), an apparent interaction between soybean aphid and soil potassium deficiency was noted (DiFonzo 2002). Soybean aphid was found at higher populations in parts of fields

where soybeans exhibited an unusual top-down potassium deficiency symptom. It was hypothesized that soybean aphid achieved greater populations on potassium deficient plants, but no mechanisms were suggested. Two non-exclusive mechanisms could explain this effect: migrating soybean aphids could preferentially settle on the yellowed plants or soybean aphid fecundity could be higher on potassium-deficient soybeans, probably because of improved nutrition.

Several studies support the first hypothesis. The appropriate/inappropriate landings hypothesis states that migrating insects will first be attracted to an area by host plant volatiles, then make a landing decision based on visual stimuli (Finch and Collier 2000). Aphids are known to be attracted to yellow pan traps over green pan traps (Boiteau 1990). When studying a number of cereal aphids, De Barro (1991) showed that yellow is always in the most preferred group of colors. Specifically, *Rhopalosiphon maidis and Sitobion* nr *fragariae* prefer yellow and bright green to other colors, including a specially designed green tile that mimicked the color of their host plant, wheat. *Rhopalosiphon padi* and *Metopolophium dirhodum* prefer yellow to any other color (De Barro 1991). Higher levels of migration to yellowing, deficient plants could account for higher aphid populations of those plants.

The plant stress hypothesis supports the idea that soybean aphid fecundity should be higher on potassium-deficient plants (Waring and Cobb 1989). It states that herbivorous insects should perform better on host plants that are under some type of environmental stress. However, experimental studies have yielded conflicting results. It has been noted, however, that phloem feeders are particularly prone to exhibit a negative response to potassium fertilization (Waring and Cobb 1989).

There are several examples of aphid populations being affected by fertilization of their host plants. Nitrogen fertilization has been shown to contribute to higher populations of the soybean aphid (Wang and Ba 1998). High-input cotton cultivation practices, which include high nitrogen inputs, have become common in California and have coincidentally elevated *A. gossypii* from a secondary to a primary pest. *A. gossypii* is more fecund, has a shorter development time, and occurs at higher densities on cotton plants with high tissue nitrogen levels as a result of nitrogen fertilization (Cisneros and Godfrey 2001, Nevo and Coll 2001). The effects appear to be stronger for nymphs than adults (Nevo and Coll 2001). Nitrogen is not the only plant nutrient that affects aphids: magnesium fertilization increases reproduction of the bird-cherry oat aphid, *Rhopalosiphon padi* (L.) on barley (Havlickova and Smetankova 1998).

Aphid populations can also be affected by potassium. Potassium fertilization reduces lifespan and rate of reproduction of *R. padi* feeding on barley. Barley grown in potassium rich soil is less attractive to *R. padi* as measured by landing rates than barley grown in potassium deficient soil (Havlickova and Smetankova 1998). In low potassium soils, potatoes fertilized with potassium had fewer aphids per plant in years when aphid populations were high (Broadbent et al. 1952). An interaction was also observed in Michigan soybean fields between soil potassium levels and soybean aphid at high populations. In 2000 and 2001, fields with potassium deficiency appeared to have higher soybean aphid populations and greater yield loss than non-deficient fields (DiFonzo 2002). However, a Chinese study of a variety of cultural practices reported that fertilization with potassium could actually benefit soybean aphids (Wang and Ba 1998). The initial potassium fertility of the field used in this study was not reported.

The objectives of the study were to A) determine whether soybean aphid population sizes differed on potassium deficient and potassium sufficient plants and B) determine the type and magnitude of plant damage caused by soybean aphids and potassium deficiency during the growing season and at harvest. This was done with a combination of field surveys and cage studies over two years.

## Methods

### *Field Surveys*

Field surveys were undertaken to examine the effect of soil potassium fertility on soybean aphid populations and plant characteristics in commercial soybean fields. Surveys took place on August 13-14 2003 and August 17-23 2004. The 2003 survey was conducted in eight fields in Van Buren, Calhoun, and Kalamazoo Counties, Michigan; the 2004 survey was conducted in five fields in Van Buren County and Calhoun County, Michigan (Table 2.1). In each year, commercial soybean fields with patches of visual symptoms of potassium deficiency were chosen. Three paired samples were selected in each field and soil, soybean aphid populations, and plant characteristics were sampled (each year of the survey included one field where only two paired samples were collected). One site in each pair was in the center of an area of severe potassium deficiency (stunted plants with chlorosis and necrosis around the outside of their leaves), the other was a nearby topographically similar location without deficiency symptoms (green foliage).

Soil samples were taken by extracting 25 cm soil cores from the center of each site (1 core per sample in 2003, 3 cores per sample in 2004). The samples were

submitted to the Michigan State University Soil and Plant Nutrient Laboratory for analysis.

For the aphid and plant characteristic counts, three plants were randomly chosen and pulled from each site. These plants were then placed in coolers with ice packs that maintained the temperature at approximately 4° C. Coolers were returned to the laboratory and stored in a cold room at 4° C overnight. The next day, the following plant characteristics were counted on each of the plants removed from the field: primary leaves (leaves on the mainstem), secondary leaves (leaves on branching stems), flowers, unfilled pods, filling pods (beans could be felt in the pod), full pods, and empty nodes.

In 2003, the number of soybean aphids per plant was extremely high, so plants were placed in whirl-pack (Nasco, Fort Atkinson, WI) bags filled with 70% ethanol and stored in the cold room until the number of aphids could be counted under a dissecting microscope. When the number of aphids was counted, the proportion of alates in the first hundred aphids counted was also recorded. In 2004, aphid numbers were much lower and the aphids were counted the day following field sampling. In 2004, no alates were observed.

On 15 October 2004, GPS coordinates were used to return to five of the paired sampling sites in two fields. Five plants per site were collected from a total of ten sites. Nodes per plant, pods per plant, pods per node, beans per plant, beans per pod, total weight of beans, and hundredweight of beans were measured.

To determine the differences between paired sites, the difference between averages of the green plants and deficient yellow plants of each site were calculated. These were analyzed via the probt option of PROC MEANS in SAS version 8.2 (Institute

1999). This option ran a T-test to determine the probability that the differences measured were significantly different from zero ($p<0.05$).

*Controlled Cage Studies*

In 2003 and 2004, cage trials were conducted to test the interaction between soil potassium levels and soybean aphid population size in a controlled manner. The studies were conducted in potassium-deficient commercial soybean fields in Van Buren County, Michigan.

The 2003 study site (N 42 ° 7.55' W 85 ° 79.95') had a beginning soil potassium level of 75 ppm as measured by a soil test prior to planting. Three replicated strips (5.1 m x 40.5 m) were established for each of three potassium treatments. On 9 May, Full (recommended by MSU soil nutrient laboratory), Half, and no potassium amendments were done with potash fertilizer (0-0-62) and were as follows: 196, 98, and 0 kg per ha. The fertilizer was broadcast prior to planting and not incorporated. On 8 July 2003, four field cages in each strip were set up at approximately 10 m intervals in the plots.

Field cages were constructed of no-see-um mesh (Venture Textiles, Inc., Braintree, MA). The cages consisted of a 1 $m^2$ frame of 1.88cm (3/4 in) diameter PVC tubing connected to 4 1.5m legs of the same material via PVC 3-way corner connectors (PlumbingStore.com). The legs of the cage were buried 0.5 m in the soil. The frame was covered with the mesh cage, which was buried about 0.25 m in the soil. On one side of the cage, a strip of Velcro™ hook and loop fasteners was used as a door (Figure 2.1). Cages were sampled by opening the door and leaning into the cage, then resealing the door when finished.

Two of the four cages (sacrifice cages) were sampled weekly by removing and counting the number of soybean aphids and plant characteristics on five plants. The remaining two cages (yield cages) were not disturbed until harvest. In each pair of sacrifice or yield cages, one cage was infested with aphids while the other served as an aphid-free control. This resulted in a factorial design with three levels of potassium amendment and two levels of aphid infestation. On 9 July, counts of the soybean plants in each cage were taken and the cages were assigned to a soybean aphid treatment. Infested cages were inoculated by tapping an aphid-infested soybean plant above the plants in the cage for approximately 30 s. Beginning on 29 July, five plants per week were removed from the sacrifice cages, placed in a cooler at approximately 4 ° C, and transported back to the laboratory. The coolers were stored overnight in a cold room at the same temperature. The following day, the same plant characteristics measured in the field surveys were counted and the plants were preserved in whirl-paks as described above. On 12 August, soil samples were taken from each cage as described above. One core was taken from each cage.

In 2004, the study site (N 42 ° 7.50' W 85 ° 80.00') had an initial soil potassium level of 67 ppm as measured by a soil test just after planting (Table 2.2). Two strips in each of five replicates were established in the field and randomly assigned to unfertilized or fertilized treatments. Fertilization took place on 13 May by broadcasting 256.8 kg/ha potash fertilizer (0-0-62) (Mason Elevator, Mason, MI). In 2004, fertilization took place after planting. Two cages per strip were set up on 13 May 2004, when soybean germination was almost complete. On 28 May 2004, the soybeans in the cages were thinned to 13 plants per cage. One cage in each strip was infested with one soybean

aphid per plant on ten plants on 28 May 2004 and the second cage was maintained aphid-free to serve as a comparison. This resulted in a 2x2 factorial design, with the presence or absence of potash fertilizer and soybean aphids as factors. The source of the soybean aphids was a laboratory colony established from a single field-collected soybean aphid in 2003, and maintained at Michigan State University (27° C, 24 hour photoperiod). Soil samples (three cores) from each cage were taken on 4 June 2004.

The number of soybean aphids per plant and plant characteristics were counted two or three times a week from 28 May until 15 July. From 28 May till 30 June, all ten plants in each cage were counted; five plants per cage were sampled on 2 July and three plants per cage were sampled from 6 July till the end of the study.

At harvest in both years, the number of nodes per plant, pods per plant, pods per node, beans per plant, beans per pod, weight of beans per cage, and hundredweight of beans was measured. In 2003, yield data were collected on 30 September from plants in the yield cages. In 2004, ten plants per cage were harvested on 12 October; these were the same plants counted for aphid number and plant characteristics during the field season.

Because actual soil potassium levels did not correspond to the Full, Half, and none categories, 2003 data were regressed to measure the effect of potassium and contrasted by ANOVA for the effects of aphids. Because of this, exploring possible interactions between aphids and potassium was impossible. The 2003 data were analyzed via PROC REG in SAS version 8.2 (Institute 1999) for the effects of potassium, and using PROC MIXED in SAS version 8.2 for the effects of aphids. In 2004, the data were analyzed via PROC MIXED in SAS version 8.2 (Institute 1999) with potassium

treatment, date, and the potassium-date interaction as fixed factors and replicate as a random factor.

*Clip Cages*

Clip cage experiments were conducted to determine the effect of soil potassium levels on individual aphids. Clip cages were constructed using the following method. A 1.88 cm diameter PVC pipe was cut into lengths of approximately 1cm, and the ends were sanded until smooth. A piece of the no-see-um mesh (used to make the field cages described above) was glued over one end of the PVC piece. A hair roller clip (Discount Beauty Supply, Mesquite, TX) was bent to enlarge the opening then the PVC piece was glued to one side. The clip cages were suspended from wooden field stakes using fishing line; when a cage was attached to a leaflet the fishing line suspended the cage so it did not weigh down the leaf.

Clip cage experiments took place in the same field as the 2004 cage study. The experiment was conducted two separate times (10 June and 14 July). In each case, four clip cages per strip (4 cages/strip x 2 strips/replicate x 5 repetitions = 40 cages in each of three experiments) were put out. Because birth order and maternal nutrition can have an effect on aphid performance, the following procedure was used to control for maternal effects. Adult aphids were removed from the colony maintained at Michigan State University and placed on excised soybean leaves in Petri dishes. Every few hours, the newly deposited nymphs were removed from these dishes. Groups of approximately 10 nymphs were placed on excised soybean leaves with the petiole inserted into a 1 mL eppendorf tube filled with water and sealed with Parafilm. Individual leaves were kept in

Petri dishes and maintained in a growth chamber at 4° C with a 24 h photoperiod. After three to five days, the aphids were removed from these leaves and placed onto identically treated fresh soybean leaves in groups of one to three aphids per leaf. The aphids were checked daily. When the first nymph appeared (usually after 7 days), the mother was removed and the Petri dish was sealed with laboratory tape. Petri dishes containing nymphs less than 24 hours old were placed into coolers at approximately 4° C and transported to the field. In the field, individual nymphs were removed from the excised leaves using a fine camel hair paintbrush and placed onto the undersides of the second fully-expanded leaf from the top of the plant. The clip cages were monitored two to three times per week until live aphids were no longer present.

The first set of forty clip cages was infested with soybean aphid nymphs on 10 June 2004. In these cages, the aphid first placed in the cage was allowed to remain for the duration of its lifetime. On each sample date, the number of nymphs she produced was recorded and the nymphs were removed from the clip cage and killed.

Another set of forty clip cages was started on 14 July 2004. In these cages, the aphid first placed in the cage was allowed to remain until she had deposited five nymphs. That aphid was then removed and her five offspring were evaluated for the remainder of study. As in the first trial, on each sample date, the number of nymphs produced by the five sisters was counted and nymphs were removed and killed.

Clip cage data were analyzed using the PROC MIXED procedure of SAS version 8.2 (Institute 1999) with potassium treatment as the fixed factor and replicate as the random factor. Mean comparisons were conducted using Fisher's protected LSD ($p<0.05$).

## Results

*Field Surveys*

Sample sites selected based on green (less deficient) or yellow (more deficient) plants had significantly different levels of soil potassium in both years (Tables 2.3 and 2.4); in 2004 soil magnesium was also different within pairs. In 2003, three of the sample sites had already been invaded by entomopathogenic fungus, causing the aphid population to crash. These fields were excluded from the analysis of aphid number and proportions of alate aphids. In 2003, there was no difference in aphid number between green healthy and yellow deficient plants, but the yellow plants had significantly fewer leaves than plants from green areas. When expressed as the number of soybean aphids per leaf, aphid populations were significantly greater on the yellow plants (Table 2.3), showing that soybean aphids had a higher population density on potassium deficient plants than on nearby less deficient plants. In 2004, no difference in total aphid number or density was detected (Table 2.4). However, whereas 2003 was a soybean aphid outbreak year (up to 17,000 aphids per plant), aphid numbers were very low in 2004 (usually less than 100 aphids per plant with a maximum of 500 aphids per plant).

In 2003, the proportion of soybean aphids with wings or wing pads in the first hundred aphids counted on each plant was also measured. There was no difference in the proportion of aphids with wings (yellow plants = 1.3%, green plants = 1.8%, $t = 1.11$, $p = 0.30$) or wing pads (yellow plants = 6.9%, green plants =5.4, $t = -0.61$, $p = 0.55$) . In 2004, aphid numbers were very low and no alates were observed.

In 2003, unfilled pods, flowers, secondary leaves, total leaves, total nodes, and total pods were significantly higher in the green plants than the yellow plants (Table 2.3). Green plants had about 50% more total leaves and total nodes and three times as many pods as yellow plants.

In 2004, secondary leaves, filling pods, and total leaves were all significantly greater in the green plants, but there was no difference in yield based on a five-pair sample (Table 4). Once again, green plants had 50% more leaves and nodes than yellow plants. In 2004 green plants had only about 30% more pods, and the difference was nonsignificant.

*2003 Controlled Cage Studies*

In both years, fertilizing strips with potash produced a range of soil potassium levels in the study fields (Tables 2.5 and 2.6). Generally, the soil potassium levels in 2004 were lower than those in 2003.

Because the actual soil potassium levels in my cages did not always match the treatment goal (i.e. some of the cages receiving the full rate of potassium had the lowest actual potassium levels) (Table 2.5), 2003 plant characteristic and yield data were regressed against actual soil potassium levels. Cages with and without aphids were regressed separately to avoid the confounding effects of soybean aphid damage.

Cages in the 2003 study were sampled on four dates: 29 July, 5 August, 19 August, and 28 August. In 2003, the cages were inoculated late with an inconsistent number of aphids, and no differences were detected in the number of aphids per plant at different soil potassium levels.

In both soybean aphid-infested and uninfested plants, differences in plant characteristics based on soil potassium level did not appear until 28 August. In the aphid cages, full pods (df = 39, F = 9.04, slope = 0.04, $r^2$ = 0.0922, p = 0.0047), filling pods (df = 39, F = 10.72, slope = 0.04, $r^2$ = 0.2201, p = 0.0023), secondary leaves (df = 39, F = 4.90, slope = 0.02, $r^2$ = 0.1038, p = 0.0426), total leaves (df = 39, F = 7.23, slope = 0.03, $r^2$ = 0.1598, p = 0.0106), total pods (df = 39, F = 11.98. slope = 0.09, $r^2$ = 0.2397, p = 0.0013), and total nodes (df = 39, F = 4.40, slope = 0.03, $r^2$ = 0.1038, p = 0.0426) increased with soil potassium. In cages without aphids, only the number of unfilled pods (df = 39, F = 4.45, slope = 0.04, $r^2$ = 0.1049, p = 0.0415) increased with soil potassium. At harvest, there was no relationship between yield and soil potassium levels.

Aphids affected a number of plant characteristics during the last two sampling dates (Table 2.7). On 19 August, the presence of aphids significantly decreased the number of secondary leaves, unfilled pods, filling pods, total leaves, total pods, and total nodes. On 28 August, the presence of aphids significantly decreased the number of primary leaves, secondary leaves, filling pods, filled pods, total leaves, total pods, and total nodes.

At harvest, the effect of potassium was evaluated separately for aphid-infested and uninfested plants because of the large differences between these plants. For both types of plants, soil potassium level did not affect the total number of nodes, pods or beans at harvest or the total weight or hundredweight of the beans in a cage ($p>0.15$).

The presence of soybean aphids affected a number of yield parameters (Table 2.8). Aphids decreased the total number of nodes, pods, and beans per plant as well as the total weight of beans produced per cage.

*2004 Controlled Cage Study*

Aphid numbers were first evaluated on 1 June then evaluated two to three times a week until 15 July. Aphid and plant characteristic counts were also taken on 3 August and 1 September. By 30 June, the average number of soybean aphids per plant in the unfertilized cages was significantly higher than in the fertilized cages (Figure 2.2). This significant difference lasted at least until 15 July, when the counting ended. The numbers of aphids per plant had exceeded 22,000 in some cages and counting aphids was no longer feasible.

Plant characteristics were evaluated on the same dates as the aphid counts. Beginning on 9 June, plants in unfertilized, uninfested cages had more primary leaves than those in fertilized, uninfested cages. This lasted until 1 September, when the trend reversed. This same pattern was seen in infested cages, i.e. more primary leaves per plant were found on unfertilized versus fertilized plants after 10 June. This pattern may be due to compensatory growth. Beginning on 28 June, cages with no aphids had more primary leaves than cages with aphids if both cages received potassium. This lasted through the entire season. In the unfertilized cages, the uninfested plants had more primary leaves on 3 August through 1 September (Table 2.9, 2.10, 2.11, 2.12).

In secondary leaves, the date x potassium x soybean aphid interaction was non-significant. Thus, aphid treatments were pooled to compare potassium effects and potassium treatments were pooled to compare aphid effects. Beginning on 8 July, unfertilized cages had more secondary leaves than amended cages. On 1 September, aphid-free plants had more secondary leaves than aphid cages (Table 2.13, 2.14).

On 8 July aphid-infested plants had more flowers than uninfested plants regardless of potassium treatment. However, after this date uninfested plants had more flowers than aphid-infested plants for the rest of the season. Also on 8 July, plants with no potassium and aphids had more flowers than plants with potassium and aphids. This pattern continued for the rest of the season. The same pattern was seen for aphid-free cages on 14 July and 3 August. By 1 September, unamended cages had more flowers per plant within soybean aphid treatments but fewer within the uninoculated cages (Table 2.9, 2.10, 2.11, 2.12).

In both potassium treatments, aphid-free plants had more unfilled pods per plant than aphid plants on 8 July. On 14 July, aphid-infested plants had more unfilled pods than aphid-free plants in the unamended treatment and there were no effects in the ammended treatment. On 3 August and 1 September, aphid-free plants had more unfilled pods than aphid plants when both plants received the same fertilizer treatment. From 14 July onward, unfertilized had more unfilled pods than fertilized plants in the aphid treatments. On 3 August and 1 September the same pattern appeared in the uninfested plants (Table 2.9, 2.10, 2.11, 2.12).

The date x potassium x soybean aphid interaction was non-significant for filling pods. Thus, aphid treatments were pooled to compare potassium treatments and potassium treatments were pooled to compare aphid treatments. Unamended treatments had more filling pods on 3 August and 1 September. Aphid-free treatments had more filling pods per plant on the same dates (Table 2.13, 2.14).

On 1 September, fertilized, aphid-infested plants had more full pods than unfertilized, aphid-infested plants. On the same date, unfertilized, uninfested plants had fewer full pods than unfertilized, aphid-infested plants (Table 2.10, 2.11).

At harvest, fertilized plants had significantly fewer nodes per plant and more beans per pod than unfertilized plants. Uninfested plants had more nodes per plant, pods per plant, beans per plant, and beans per pod than infested plants. The total weight and hundredweight of beans produced in uninfested cages was greater than that of infested cages (Table 2.15, 2.16). Soybean aphids and soil potassium had only one interaction. Plants in fertilized, aphid-infested cages produced more beans per cage than plants in unfertilized, aphid-infested cages (fertilized, infested = 21.6 beans, unfertilized, infested = 49.6 beans, $t = -2.11$, $p = 0.0360$).

*Clip Cages*

For the first run of the clip cage experiment (10 June), the lifespan of the aphid first placed in the cage, age of the mother at first reproduction, total number of nymphs produced, and the number of nymphs produced per day during the reproductive period of the aphid was measured and the data were analyzed by cage. The number of nymphs produced per day during the mother's reproductive lifespan was significantly different between treatments (Figure 2.3). Aphids caged on unfertilized plants produced on average more nymphs per day then their counterparts on fertilized plants. However, this was very early in the season.

In the later trial (July 14), the performance of the second generation of soybean aphid in the clip cages was evaluated to further compensate for maternal effect. In these

cages, the lifespan of the second generation, total number of nymphs produced by the adult aphids in the cage, nymphs produced per day, and the nymphs produced per mother per day were evaluated. The mothers began to produce nymphs at an earlier age ($p=0.0282$) and the total number of nymphs produced per cage was higher ($p=0.0150$) in the clip cages placed in unfertilized strips (Figure 2.4).

**Discussion**

The results of the 2003 survey confirmed the field observations that were made in 2000 and 2001. In commercial soybean fields, areas of lower potassium fertility had smaller plants with a higher density of soybean aphids than nearby areas with better potassium nutrition. 2003 was a soybean aphid outbreak year; the aphids had escaped environmental population regulations such as natural enemies, and host plant effects probably had a large influence on the population. In 2004, aphid populations were much lower, and there was no effect of soil potassium on aphid populations. This may be because other controls (such as predation) suppressed populations enough that host plant mediated effects were no longer distinguishable. It should be noted that all of the fields included in this study had soil potassium levels far below the level of 150 ppm recommended for Michigan (Vitosh et al. 1995).

There are two possible hypotheses to explain the results of the survey. Migrating soybean aphids may be more attracted to yellowing plants than to healthier green plants. In the 2003 survey, there was no difference in the proportion of soybean aphids with wings or wing pads on the green and yellow plants. Thus, increased numbers of soybean aphids on yellowed, potassium deficient plants was not due to increased immigration

from migrating aphids. However, if the immigration occurred far before our sampling the winged immigrants responsible for the increased populations may have already died and would not be counted in this type of sampling. Also, if the immigrants had a large number of babies, the proportions of immigrants on green and yellow plants might be similar even though the total number of immigrants would be higher on the yellow plants. More research in this area is needed to definitely determine whether soybean aphids discriminate between yellowing and green soybean plants at the field level.

The cage studies were designed to test the second hypothesis, that soybean aphid populations increase at a higher rate on potassium deficient soybeans. Although the 2003 cage study did not yield usable results because of the faulty aphid infestation, the 2004 study clearly showed that in the absence of human or environmental control, soybean aphid populations were higher on soybeans growing in soils with lower potassium fertility up until the aphid populations overwhelmed the host plant and crashed. The results of the clip cage experiments show that this effect is probably due to higher individual fecundity of soybean aphids on plants with a lower potassium status. In different repetitions of the study, this was due to an earlier age of first reproduction or a faster rate of nymph production once reproduction had started.

In order to understand the relevance of the plant characteristic counts taken during the season, it is necessary to look at them in the context of the final yield. This is impossible in the 2003 survey since no yield data were taken. In the 2004 survey, there were no differences in any of the yield parameters measured. Thus, one can only assume that, in years such as 2004 when soybean aphid populations are very low, there are no differences between potassium-deficient and healthy appearing plants at the levels

measured in terms of yield. It was assumed that the yellowed plants compensated for the differences in the numbers of leaves and pods that were detected in mid-August.

In the 2003 cage study, only the presence or absence of soybean aphids affected yield; aphid free cages had more nodes per plant, more pods per plant, more beans per plant, greater weight of beans, and a greater hundredweight of beans. Although the mid-season and yield data were taken from two separate sets of cages, it was assumed that both would have experienced the same effects of soybean aphids and potassium. Therefore, although the regression results appeared to show that pod set was lower or delayed in the plants growing at lower potassium levels, especially when they were also being attacked by aphids, it seems that the plants must have been able to compensate for this effect between late August and harvest, possibly be reaching full maturity at a later date. It was also observed that aphid-free plants had greater numbers of those traits that contribute to yield, and that these effects appeared earlier or later in the season depending on when the traits first appear in the growing season.

In the 2004 cage study, both soybean aphids and potassium amendment affected mid-season growth and final yield. During the season, soybean aphids decreased the number of leaves, flowers, and pods. At harvest, plants in cages infested with soybean aphids had fewer nodes, pods, beans, beans per pod, total weight of beans, and hundredweight of beans than uninfested plants. The potassium amendment also affected soybean plants. During the season, potassium amendment reduced soybean aphid populations, leaves, flowers, and pods. At harvest, the total number of beans, weight of beans, and hundredweight of beans were the same in fertilized and unfertilized cages. However, fertilized cages had more nodes per plant and beans per pod than unfertilized

cages. Since weight of beans probably correlates best with commercial soybean yield, potassium fertilization did not reduce soybean yield. However, it also did not improve it in this study. The reasons for these unexpected potassium results in our mid-season counts are unknown, although they may be due to the small size of our cages and the compounding effect of the soybean aphids. The benefits of proper soil fertility are well established (Mengel and Kirkby 2001), and this study does not show that potassium amendment is without benefit. It is more likely that the mid-season potassium effects are some sort of experimental artifact, possibly due to compensatory growth.

Although potassium amendment did not improve soybean yield in either cage study, it is important to note that the green, non-deficient plants in both field surveys had higher mid-season characteristics than deficient plants. This probably represents a more realistic picture of what would happen in the field. In 2004, the only year of the survey when yield data was taken, these differences did not carry through to yield. However, the deficient plants lagged behind the other plants in mid-August. If the late summer had poor conditions for soybean growth, these plants may have yielded less at harvest. In 2003, the soybean aphid out break year, only the non-deficient plants had filling pods. In that year, it did not appear that the yellow plants were likely to set seed at all, so in that year, potassium level and it's interaction with soybean aphid populations probably had a large effect on final yield.

**Conclusion**

In both field surveys and controlled exclusion cages, soybean growing at lower levels of potassium nutrition supported higher levels of soybean aphid populations.

Limited data did not support the hypothesis that migrating soybean aphids land preferentially on yellowed soybean plants. However, studies in large exclusion cages and clip cages supported the hypothesis that soybean aphids have a greater rate of increase on potassium-deficient soybean plants. Maintenance of proper potassium fertility levels will result in a slower rate of increase and lower populations of the soybean aphid during outbreak years. This has important management implications and is ideal for use in the context of integrated pest management.

It is also interesting to note that the differences in plant characteristics between green and yellow plants in 2003 were greater than in 2004. Specifically, green and yellow plants had different numbers of pods, the trait that most directly translates to yield. The low soybean aphid populations in 2004 probably did not affect on the soybean plants. In 2003, both green and yellow plants were well above the aphid population level required for plant damage. It would appear that in 2003, aphid-infested and deficient plants had more severe damage than aphid-infested and less-deficient plants. Thus, maintenance of proper soil potassium nutrition may help to alleviate part of the damage caused by soybean aphids during outbreaks.

These results could have important implications for soybean aphid management. Currently, soybean populations reach outbreak levels into the tens of thousands in some years and in others the population remains at extremely low levels. Thus, there is the potential for environmental control of this pest, but it is not achieved every year. Proper potassium fertility will not by itself keep soybean aphid populations below damaging levels. However, it will result in a slower increase of the population. This will allow time for some other factor, such as predation, to provide soybean aphid control.

Furthermore, slower rates of population increase will be beneficial when resistant or tolerant cultivars are introduced because it will result in a lower level of pest pressure and the resistance will be less likely to fail. Finally, a slower rate of population increase is beneficial in the event that chemical control is employed. Slower increase of soybean aphid populations will allow decision makers more time to determine whether a field will need to be sprayed and also will allow more time between the decision and the application. In some areas, a lower level of aphid population rebound after chemical control is applied may prevent the need for multiple applications.

As a cultural control, maintenance of proper fertility fits very will into many Integrated Pest Management schemes. It is compatible with most if not all other forms of aphid population control including the use of resistant cultivars, biological control, or chemical control. Thus, there will be no trade-offs between different control measures, as in when an investment in biological control is lost when chemical control must be undertaken. In addition, maintaining soil at the recommended potassium levels has a number of other long-term and short-term benefits. Even if soybean aphid control is not needed in a given year, an investment in preventative control by fertilization need not be lost. There are benefits both for the crop being produced and also in long-term soil maintenance.

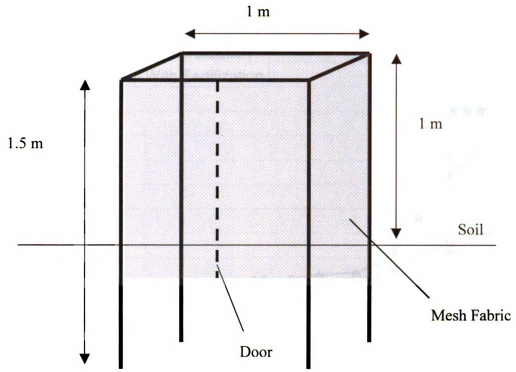


Figure 2.1. Schematic of field cages used in the 2003 and 2004 controlled cage experiments. Cages consisted of a PVC frame (dark bars) covered by a cage of no-see-um mesh (gray shading) with a Velcro™ door (dotted line).

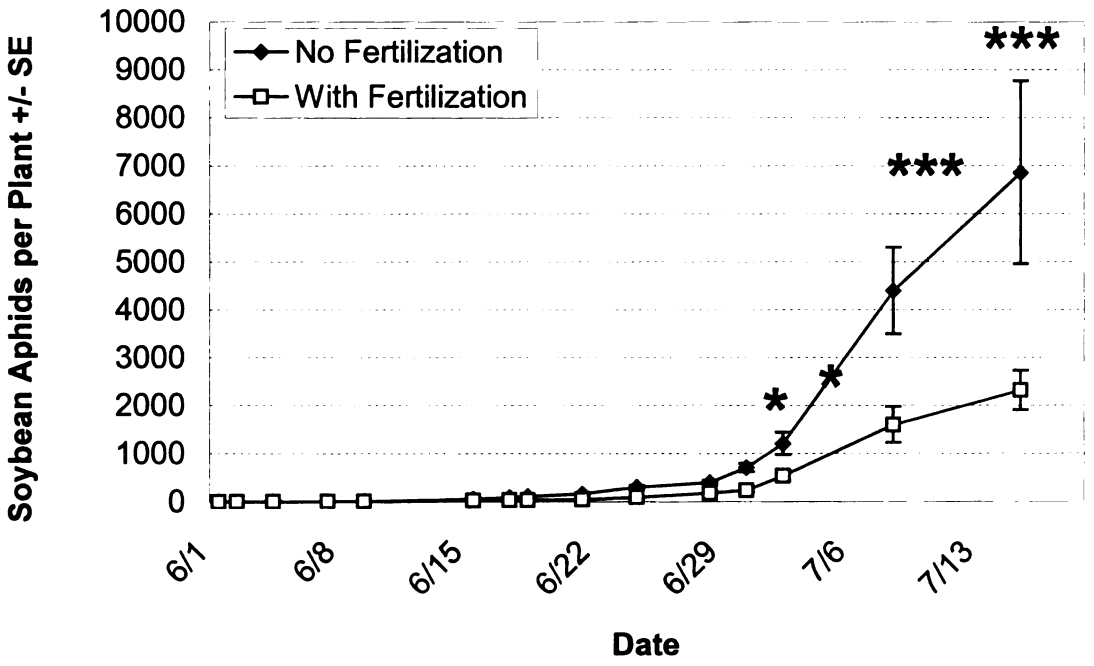


Figure 2.2. Soybean aphids per plant during the 2004 controlled cage study on plants with and without potassium fertilization in a field with an initial potassium level of 67 ppm. * Denotes sample dates where the treatments were different at $p<0.05$. *** Denotes sample dates where the treatments were different at $p<0.0001$.

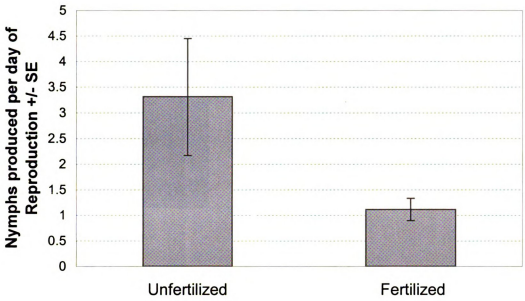


Figure 2.3. Soybean aphid nymphs produced per mother per day of reproduction on potassium fertilized and unfertilized plants in a field with an initial soil potassium level of 67 ppm in the earliest run of the clip cage experiment (June 10). The results are significantly different in an ANOVA. $p = 0.0271$

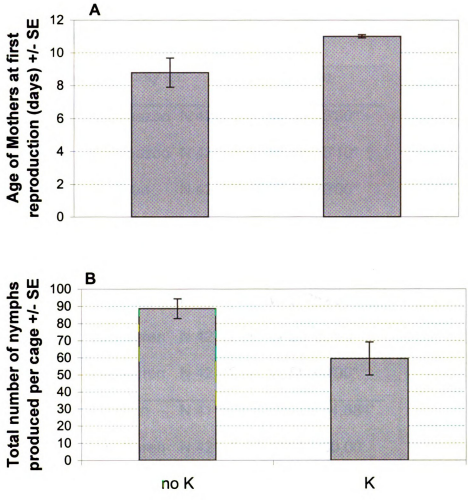


Figure 2.4. Response of soybean aphids to potassium soil amendment, expressed as A) age of aphids at first reproduction (days) and B) total number of young produced per cage on potassium fertilized and unfertilized plants in a field with an initial soil potassium level of 67 ppm for the clip cage study begun on July 14, 2004. Both are significantly different in an ANOVA with $p<0.05$.

Table 2.1: Location of potassium deficient commercial fields surveyed 14-15 August 2003 and 17-23 August 2004, Southwest Michigan.

| Survey Year | County | Field Location |
|---|---|---|
| 2003 | Kalamazoo | N 42°10'00" W 85°15'30" |
| | Kalamazoo | N 42°10'60" W 85°15'10" |
| | Calhoun | N 42°10'10" W 85°30'00" |
| | Van Buren | N 42°08'00" W 85°52'80" |
| | Van Buren | N 42°11'00" W 85°52'80" |
| | Van Buren | N 42°13'00" W 85°52'90" |
| | Van Buren | N 42°12'90" W 85°52'90" |
| | Van Buren | N 42°12'90" W 85°60'00" |
| 2004 | Calhoun | N 41°59.952' W 85°34.884' |
| | Van Buren | N 42 ° 7.50' W 85 ° 80.00' |
| | Van Buren | N 42°10.254' W 86°2.271' |
| | Van Buren | N 42°11.237' W 86°10.105' |
| | Van Buren | N 42°7.797' W 86°11.248' |

Table 2.2. Initial soil test values for potassium deficient fields used in controlled cage studies, 2003 and 2004, Van Buren County, Michigan

| | 2003 | 2004 |
|---|---|---|
| pH | 5.4 | 6.3 |
| LimeIndex | 67 | 69 |
| Phosphorus (ppm) | 180 | 14 |
| Potassium (ppm) | 75 | 67 |
| Calcium (ppm) | 150 | 1173 |
| Magnesium (ppm) | 5 | 161 |
| Cation Exchange Capacity (me/100g) | 4.6 | 8.6 |

Table 2.3. Comparisons of soil test levels, aphid number, and plant characteristics from putative potassium deficient (yellow) and less deficient (green) pairs of sample sites in eight commercial soybean fields, Southwest Michigan, 14-15 August 2003. p values based on a t-test to determine whether differences were significantly different from zero.

| | Green Plant Mean | Yellow Plant Mean | t | p |
|---|---|---|---|---|
| Total Soybean Aphids | 1561 | 1760 | -0.66 | 0.54 |
| Soybean Aphids/Leaf | 150 | 104 | -2.57 | 0.03 |
| Percentage of Winged Aphids | 1.83% | 1.28% | 1.11 | 0.3 |
| Percentage of Aphids with wing pads | 6.86% | 5.40% | -0.61 | 0.55 |
| Primary Leaves | 8.46 | 7.39 | 2.1 | 0.05 |
| Secondary Leaves | 6.59 | 2.71 | 3.55 | 0.002 |
| Empty Nodes | 2.87 | 2.79 | 0.24 | 0.82 |
| Flowers | 33.97 | 25.33 | 2.27 | 0.04 |
| Unfilled Pods | 20.8 | 7.39 | 4.23 | 0.0005 |
| Filling Pods | 1.09 | 0 | 1.72 | 0.10 |
| Full Pods | 0 | 0 | 1.00 | 1.00 |
| Total Leaves | 15.05 | 10.10 | 3.34 | 0.003 |
| Total Nodes | 17.92 | 12.88 | 3.46 | 0.003 |
| Total Pods at Survey | 21.89 | 7.39 | 4.58 | 0.0002 |
| Soil pH | 6.13 | 6.05 | 0.44 | 0.67 |
| Soil Phosphorus | 35.23 | 27.43 | 0.34 | 0.74 |
| Soil Potassium | 42.33 | 29.05 | 3.4 | 0.004 |
| Soil Magnesium | 138.00 | 104.00 | 1.98 | 0.07 |
| Soil Calcium | 860.00 | 747.38 | 1.20 | 0.25 |

Table 2.4. Comparisons of soil test levels, aphid number, plant characteristics, and harvest characteristics (two fields) from putative potassium deficient (yellow) and less deficient (green) pairs of sample sites in five commercial soybean fields, Southwest Michigan, 2003. Surveys took place 17-23 August and harvest took place in October. p values based on a t-test to determine whether differences were significantly different from zero.

| | Green Plant Mean | Yellow Plant Mean | t | p |
|---|---|---|---|---|
| Total Soybean Aphids | 42.3 | 18.8 | 1.57 | 0.1414 |
| Soybean Aphids/Leaf | 3.7 | 1.4 | 1.81 | 0.0935 |
| Primary Leaves | 8 | 6.7 | 1.78 | 0.0985 |
| Secondary Leaves | 6.9 | 3.5 | 2.27 | 0.0405 |
| Empty Nodes | 6 | 6.8 | -1.11 | 0.289 |
| Flowers | 9.7 | 11 | -0.81 | 0.4326 |
| Unfilled Pods | 24.1 | 18.1 | 1.73 | 0.1071 |
| Filling Pods | 24.1 | 9.5 | 3.83 | 0.0021 |
| Full Pods | 0 | 0 | 0 | 1 |
| Total Leaves | 14.9 | 10.2 | 2.41 | 0.0318 |
| Total Nodes | 48.2 | 27.6 | 3.01 | 0.01 |
| Total Pods | 21 | 17 | 2.12 | 0.0538 |
| Nodes/Plant at Harvest | 25.7 | 16.2 | 1.49 | 0.211 |
| Pods/Plant at Harvest | 35.7 | 9.7 | 2.01 | 0.114 |
| Beans/Plant at Harvest | 73 | 13.8 | 2.25 | 0.087 |
| Weight of Beans | 62.5 | 6.1 | 2.37 | 0.077 |
| Hundredweight of Beans | 16.3 | 11.6 | 0.54 | 0.615 |
| Soil pH | 7.4 | 7.2 | 1.51 | 0.1552 |
| Soil Phosphorus | 136.1 | 178.2 | -1.51 | 0.1554 |
| Soil Potassium | 53.3 | 33.5 | 5.48 | 0.0001 |
| Soil Magnesium | 115.2 | 74.1 | 2.81 | 0.0149 |
| Soil Calcium | 647.1 | 496.8 | 1.89 | 0.0806 |

Table 2.5. Soil potassium levels inside the cages in the 2003 controlled cage study.

| Replicate | Potassium Treatment | Aphid Treatment | Sacrifice | Soil Potassium (ppm) |
|---|---|---|---|---|
| 1 | Full Rate | Yes | Yes | 56 |
| 1 | Full Rate | Yes | No | 108 |
| 1 | Full Rate | No | Yes | 100 |
| 1 | Full Rate | No | No | 62 |
| 1 | Half Rate | Yes | Yes | 131 |
| 1 | Half Rate | Yes | No | 134 |
| 1 | Half Rate | No | Yes | 155 |
| 1 | Half Rate | No | No | 103 |
| 1 | No Ammendment | Yes | Yes | 82 |
| 1 | No Ammendment | Yes | No | 137 |
| 1 | No Ammendment | No | Yes | 115 |
| 1 | No Ammendment | No | No | 165 |
| 2 | Full Rate | Yes | Yes | 218 |
| 2 | Full Rate | Yes | No | 100 |
| 2 | Full Rate | No | Yes | 114 |
| 2 | Full Rate | No | No | 116 |
| 2 | Half Rate | Yes | Yes | 114 |
| 2 | Half Rate | Yes | No | 53 |
| 2 | Half Rate | No | Yes | 54 |
| 2 | Half Rate | No | No | 82 |
| 2 | No Ammendment | Yes | Yes | 77 |
| 2 | No Ammendment | Yes | No | 62 |
| 2 | No Ammendment | No | Yes | 103 |
| 2 | No Ammendment | No | No | 108 |
| 3 | Full Rate | Yes | Yes | 104 |
| 3 | Full Rate | Yes | No | 131 |
| 3 | Full Rate | No | Yes | 196 |
| 3 | Full Rate | No | No | 100 |
| 3 | No Ammendment | Yes | Yes | 46 |
| 3 | No Ammendment | Yes | No | 110 |
| 3 | No Ammendment | No | Yes | 119 |
| 3 | No Ammendment | No | No | 94 |

Table 2.6. Soil potassium levels inside the cages in the 2004 controlled cage study after treatment. Fertilization took place on 13 May and soil was sampled on 4 June.

| Replicate | Potassium Treatment | Aphid Treatment | Timing | Soil Potassium (ppm) |
|---|---|---|---|---|
| 1 | Yes | Yes | Early | 78 |
| 1 | Yes | No | Early | 115 |
| 1 | No | Yes | Early | 57 |
| 1 | No | No | Early | 59 |
| 2 | Yes | Yes | Early | 104 |
| 2 | Yes | No | Early | 52 |
| 2 | No | Yes | Early | 43 |
| 2 | No | No | Early | 31 |
| 3 | Yes | Yes | Early | 54 |
| 3 | Yes | No | Early | 46 |
| 3 | No | Yes | Early | 23 |
| 3 | No | No | Early | 27 |
| 4 | Yes | Yes | Early | 51 |
| 4 | Yes | No | Early | 71 |
| 4 | No | Yes | Early | 38 |
| 4 | No | No | Early | 45 |
| 5 | Yes | Yes | Early | 39 |
| 5 | Yes | No | Early | 52 |
| 5 | No | Yes | Early | 27 |
| 5 | No | No | Early | 23 |

Table 2.7. Significant differences in plant characteristics based on the presence of soybean aphids in the 2003 controlled cage study. Cages were infested on 8 July and sampling took place from 29 July through 28 August. t values and probabilities are based on Fisher's protected LSD.

| | Date | No Aphid Plant Counts | Aphid Plant Counts | t | p |
|---|---|---|---|---|---|
| Primary Leaves | 28 August | 6.8 | 4.3 | -4.73 | <0.0001 |
| Secondary Leaves | 19 August | 7.4 | 3.3 | -4.10 | <0.0001 |
| | 28 August | 7.6 | 3.3 | -4.33 | <0.0001 |
| Total Leaves | 19 August | 15.6 | 10.5 | -4.82 | <0.0001 |
| | 28 August | 14.4 | 7.6 | -6.61 | <0.0001 |
| Total Nodes | 19 August | 19.2 | 15.2 | -3.72 | 0.0002 |
| | 28 August | 19.1 | 14.0 | -4.78 | <0.0001 |
| Unfilled Pods | 19 August | 17.4 | 9.7 | -5.52 | <0.0001 |
| Filling Pods | 19 August | 19.5 | 9.0 | -8.12 | <0.0001 |
| | 28 August | 15.4 | 6.5 | -7.07 | <0.0001 |
| Filled Pods | 28 August | 7.9 | 5.4 | -3.81 | 0.0002 |
| Total Pods | 19 August | 36.8 | 18.6 | -7.27 | <0.0001 |
| | 28 August | 30.0 | 15.9 | -5.73 | <0.0001 |

Table 2.8. Significant differences in soybean yield parameters in the 2003 controlled cage study based on the presence of absence of soybean aphids. Results are based on an ANOVA with aphids as a fixed factor and replicate as a random factor.

| | No Aphids | Aphids | F | p |
|---|---|---|---|---|
| Nodes per Plant | 13.8 | 7.4 | 33.90 | <0.0001 |
| Pods per Plant | 24.1 | 9.2 | 48.61 | <0.0001 |
| Beans per Plant | 52.2 | 18.7 | 44.35 | <0.0001 |
| Total Weight of Beans per Cage (g) | 226.5 | 72.0 | 24.47 | 0.0002 |

Table 2.9. Significant differences in plant characteristics of uninfested soybean plants based on potassium fertilization in the 2004 controlled cage study. The field had an initial soil potassium level of 67 ppm. Results are based on Fisher's protected LSD.

| | Date | Fertilized Uninfested Plants | Unfertilized Uninfested Plants | t | p value |
|---|---|---|---|---|---|
| Primary Leaves | 9-Jun | 2.1 | 2.4 | 2.02 | 0.0435 |
| | 15-Jun | 2.7 | 3.4 | 3.85 | 0.0001 |
| | 18-Jun | 3.2 | 3.8 | 3.40 | 0.0007 |
| | 21-Jun | 3.6 | 4.3 | 4.19 | <0.0001 |
| | 24-Jun | 3.9 | 4.6 | 4.18 | <0.0001 |
| | 28-Jun | 4.5 | 5.5 | 5.35 | <0.0001 |
| | 30-Jun | 4.8 | 5.7 | 4.86 | <0.0001 |
| | 1-Jul | 5.3 | 6.4 | 4.32 | <0.0001 |
| | 14-Jul | 8.1 | 9.1 | 3.31 | 0.0010 |
| | 3-Aug | 12.3 | 13.7 | 4.34 | <0.0001 |
| | 1-Sep | 12.4 | 11.6 | -2.94 | 0.0033 |
| Flowers | 14-Jul | 14.9 | 19.3 | 4.44 | <0.0001 |
| | 3-Aug | 28.1 | 33.6 | 5.61 | <0.0001 |
| | 1-Sep | 9.5 | 7.2 | -2.78 | 0.0055 |
| Unfilled Pods | 3-Aug | 13.7 | 19.1 | 6.55 | <0.0001 |
| | 1-Sep | 13.1 | 21.8 | 12.34 | <0.0001 |
| Total Pods | 3-Aug | 27.0 | 42.4 | 7.12 | <0.0001 |
| | 1-Sep | 82.6 | 101.3 | 9.94 | <0.0001 |

Table 2.10. Significant differences in plant characteristics of soybean aphid-infested soybean plants based on potassium fertilization in the 2004 controlled cage study. The field had an initial soil potassium level of 67 ppm. Results are based on Fisher's protected LSD.

| | Date | Fertilized Infested Plants | Unfertilized Infested Plants | t | p |
|---|---|---|---|---|---|
| Primary leaves | 10-Jun | 2.6 | 2.1 | 2.55 | 0.0107 |
| | 15-Jun | 3.2 | 2.7 | 2.71 | 0.0067 |
| | 17-Jun | 3.7 | 3.1 | 3.22 | 0.0013 |
| | 18-Jun | 3.8 | 3.1 | 3.78 | 0.0002 |
| | 21-Jun | 4.3 | 3.2 | 5.54 | <0.0001 |
| | 24-Jun | 4.6 | 3.5 | 5.55 | <0.0001 |
| | 28-Jun | 5.4 | 4.0 | 7.71 | <0.0001 |
| | 30-Jun | 5.8 | 4.3 | 8.06 | <0.0001 |
| | 1-Jul | 6.5 | 4.9 | 5.86 | <0.0001 |
| | 8-Jul | 7.6 | 5.9 | 4.84 | <0.0001 |
| | 14-Jul | 8.5 | 7.2 | 3.96 | <0.0001 |
| | 3-Aug | 10.5 | 8.6 | 5.32 | <0.0001 |
| Flowers | 8-Jul | 12.3 | 7.5 | 4.64 | <0.0001 |
| | 14-Jul | 13.5 | 8.7 | 4.72 | <0.0001 |
| | 3-Aug | 12.4 | 8.6 | 3.56 | 0.0004 |
| | 1-Sep | 6.7 | 3.8 | 2.75 | 0.0060 |
| Unfilled Pods | 14-Jul | 8.8 | 6.1 | 3.25 | 0.0012 |
| | 3-Aug | 13.5 | 6.2 | 8.20 | <0.0001 |
| | 1-Sep | 8.3 | 4.1 | 4.66 | <0.0001 |
| Full Pods | 1-Sep | 3.7 | 6.9 | -10.95 | <0.0001 |
| Total Pods | 3-Aug | 19.0 | 10.9 | 3.39 | 0.0004 |

Table 2.11. Significant differences in plant characteristics of soybean plants based on soybean aphid infestation when the soil was not fertilized with potassium in the 2004 controlled cage study. Plants were infested on 28 May and the aphid population was allowed to increase without control. The initial soil potassium level of the field was 67 ppm. Results are based on Fisher's protected LSD.

| | Date | Unfertilized Uninfested Cages | Unfertilized Infested Cages | t | p value |
|---|---|---|---|---|---|
| Primary Leaves | 3-Aug | 13.7 | 10.5 | 9.26 | <0.0001 |
| | 1-Sep | 11.6 | 8.8 | 8.05 | <0.0001 |
| Flowers | 8-Jul | 5.6 | 12.3 | -6.45 | <0.0001 |
| | 14-Jul | 19.3 | 13.5 | 5.68 | <0.0001 |
| | 3-Aug | 33.6 | 12.4 | 19.96 | <0.0001 |
| Unfilled Pods | 8-Jul | 9.5 | 2.1 | 8.56 | <0.0001 |
| | 14-Jul | 4.8 | 8.9 | -4.83 | <0.0001 |
| | 3-Aug | 19.1 | 13.5 | 6.25 | <0.0001 |
| Full Pods | 1-Sep | 0.3 | 3.7 | -12.30 | <0.0001 |
| Total Pods | 8-Jul | 9.5 | 2.0 | 3.26 | 0.0008 |
| | 3-Aug | 42.4 | 19.0 | 9.94 | <0.0001 |
| | 1-Sep | 101.3 | 36.6 | 28.18 | <0.0001 |

Table 2.12. Significant effects of soybean aphid infestation on soybean plant characteristics of potassium fertilized plants in the 2004 controlled cage study. Soybean aphids infestation took place on 28 May and the population was allowed to increase without control. The field had an initial soil test level of 67 ppm potassium and was fertilized with 267 kg/ ha 0-0-62 fertilizer. Results are based on Fisher's protected LSD.

| | | Fertilized | Fertilized | | |
|---|---|---|---|---|---|
| | Date | Uninfested Plants | Infested Plants | t | p |
| Primary Leaves | 28-Jun | 4.5 | 4.0 | 3.05 | 0.0023 |
| | 30-Jun | 4.8 | 4.3 | 3.01 | 0.0027 |
| | 8-Jul | 6.8 | 5.9 | 2.69 | 0.0073 |
| | 14-Jul | 8.1 | 7.2 | 2.69 | 0.0073 |
| | 3-Aug | 12.3 | 8.6 | 11.41 | <0.0001 |
| | 1-Sep | 12.4 | 8.3 | 14.30 | <0.0001 |
| Flowers | 8-Jul | 4.2 | 7.5 | -3.35 | 0.0008 |
| | 14-Jul | 14.9 | 8.7 | 6.36 | <0.0001 |
| | 3-Aug | 28.1 | 8.6 | 19.67 | <0.0001 |
| | 1-Sep | 9.5 | 3.8 | 6.51 | <0.0001 |
| Unfilled Pods | 8-Jul | 8.3 | 2.7 | 6.88 | <0.0001 |
| | 3-Aug | 13.7 | 6.2 | 9.14 | <0.0001 |
| | 1-Sep | 13.1 | 4.1 | 12.23 | <0.0001 |
| Total Pods | 8-Jul | 8.3 | 2.7 | 2.59 | 0.0097 |
| | 3-Aug | 27.0 | 11.0 | 7.31 | <0.0001 |
| | 1-Sep | 82.6 | 27.2 | 28.40 | <0.0001 |

Table 2.13. Significant differences in soybean plant characteristics of caged plants based on the presence of soybean aphids for characteristics without an aphid x potassium interaction in the 2004 controlled cage study. Plants were infested on 28 May. Results are based on Fisher's protected LSD.

| | Date | Infested Plants | Uninfested Plants | t | p value |
|---|---|---|---|---|---|
| Secondary Leaves | 1-Sep | 23.1 | 7.8 | 31.72 | <0.0001 |
| Filling Pods | 3-Aug | 18.3 | 5.1 | 10.31 | <0.0001 |
| | 1-Sep | 74.0 | 20.4 | 44.85 | <0.0001 |
| Total Leaves | 28-Jun | 5.6 | 4.8 | 2.28 | 0.0201 |
| | 30-Jun | 6.0 | 5.3 | 1.99 | 0.0427 |
| | 3-Aug | 15.0 | 11.6 | 5.46 | <0.0001 |
| | 1-Sep | 35.1 | 16.4 | 32.31 | <0.0001 |
| Total Nodes | 3-Aug | 17.0 | 13.7 | 4.75 | <0.0001 |
| | 1-Sep | 41.4 | 27.0 | 22.65 | <0.0001 |

Table 2.14. Significant differences in soybean plant characteristics based on potassium amendment for those traits without a significant aphid x potassium interaction in the 2004 controlled cage study. Initial soil potassium level was 67 ppm and aphid infestation took place on 28 May. Results are based on Fisher's protected LSD.

| | Date | Fertilized Plants | Unfertilized plants | t | p |
|---|---|---|---|---|---|
| Secondary Leaves | 8-Jul | 2.0 | 3.3 | 2.48 | 0.0133 |
| | 14-Jul | 2.5 | 4.4 | 3.76 | 0.0002 |
| | 3-Aug | 1.5 | 2.9 | 2.56 | 0.0105 |
| | 1-Sep | 13.4 | 17.5 | 8.43 | <0.0001 |
| Filling Pods | 3-Aug | 9.0 | 14.4 | 4.23 | <0.0001 |
| | 1-Sep | 42.6 | 51.9 | 7.80 | <0.0001 |
| Total Leaves | 18-Jun | 3.2 | 3.8 | 1.99 | 0.041 |
| | 21-Jun | 3.5 | 4.4 | 2.80 | 0.004 |
| | 24-Jun | 3.8 | 4.8 | 2.90 | 0.0029 |
| | 28-Jun | 4.7 | 5.8 | 3.18 | 0.0011 |
| | 30-Jun | 4.9 | 6.3 | 4.07 | <0.0001 |
| | 1-Jul | 5.5 | 7.3 | 3.84 | <0.0001 |
| | 14-Jul | 10.1 | 13.1 | 5.00 | 0.0038 |
| | 3-Aug | 12.0 | 14.6 | 5.00 | <0.0001 |
| | 1-Sep | 23.8 | 27.7 | 6.79 | 0.0036 |
| Total Nodes | 21-Jun | 3.5 | 4.4 | 2.44 | 0.0134 |
| | 24-Jun | 3.9 | 4.8 | 2.41 | 0.0147 |
| | 28-Jun | 4.9 | 5.8 | 2.56 | 0.0095 |
| | 30-Jun | 5.1 | 6.4 | 3.55 | 0.0003 |
| | 1-Jul | 5.8 | 7.5 | 3.13 | 0.0015 |
| | 8-Jul | 8.7 | 11.0 | 3.44 | 0.0005 |
| | 14-Jul | 11.8 | 15.1 | 4.98 | 0.0023 |
| | 3-Aug | 14.1 | 16.6 | 3.69 | 0.0002 |
| | 1-Sep | 31.0 | 37.4 | 10.06 | <0.0001 |

Table 2.15. Significant differences in soybean yield parameters based on soybean aphid infestation in the 2004 controlled cage study. Infestation took place on 28 May, and the soybean aphid population was allowed to increase without control. The field had an initial soil potassium level of 67 ppm and was fertilized with 267 kg /ha of 0-0-62 fertilized. Results are based on Fisher's protected LSD.

| | Uninfested | Infested | F | p |
|---|---|---|---|---|
| Nodes per Plant | 44.8 | 24.1 | 105.03 | <0.0001 |
| Pods per Plant | 70.4 | 18.7 | 174.98 | <0.0001 |
| Beans per Plant | 149.0 | 35.6 | 162.67 | <0.0001 |
| Beans per Pod | 2.1 | 1.9 | 40.10 | <0.0001 |
| Weight of Beans per Cage (g) | 205.5 | 37.3 | 30.21 | 0.0001 |
| Hundredweight of Beans (g) | 14.7 | 12.9 | 12.81 | 0.0027 |

Table 16. Significant differences in soybean yield parameters based on potassium fertilization in the 2004 controlled cage study. Infestation took place on 28 May, and the soybean aphid population was allowed to increase without control. The field had an initial soil potassium level of 67 ppm and was fertilized with 267 kg /ha of 0-0-62 fertilized. Results are based on Fisher's protected LSD.

| | Fertilized | Unfertilized | F | p |
|---|---|---|---|---|
| Nodes per Plant | 31.0 | 37.9 | 9.82 | 0.0020 |
| Beans per Pod | 2.0 | 1.9 | 11.92 | 0.0007 |

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## Chapter 3—Improved nitrogen nutrition for the soybean aphid leads to higher aphid populations on potassium deficient soybean

### Introduction

The acquisition of nitrogenous compounds is the main challenge facing aphids feeding on their natural diets (Terra 1988, Douglas 1993, Dixon 1998, Sandstrom 1999, Sandstrom and Moran 2001, Douglas 2003). Aphids are thought to obtain all of their dietary nitrogen from amino acids translocated in the phloem sap. Many species, such as the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), have growth rates directly correlated to concentration of amino acids in the phloem of their host plants (Weibull 1987). Proteinases have not been detected in the gut or saliva of any aphid species (Srivastava 1987, Foissac 2002), probably because the high levels of proteinase inhibitors, combined with the extremely low protein concentration typically encountered in phloem sap, make plant proteins a poor nitrogen source. In addition, many of the proteins transported in the phloem are defensive chemicals that need to be cleaved in order to become toxic. Since aphids do not cleave these proteins, they avoid the toxic effects.

Aphids are among the most versatile animals in respect to nitrogen nutrition because they possess a primary intracellular symbiotic bacterium, *Buchnera* as well as secondary gut symbionts, which are able to manufacture certain essential amino acids from nonessential amino acids in the aphid diet (Moran et al. 2003). *Buchnera* may be able to provide their aphid hosts with synthesized essential amino acids at a higher rate than aphids are able to bring these substances across the gut wall (Douglas 1998). For this reason, not all aphid species require all of the essential amino acids, and the amino

acids that are required vary between aphid species and populations (Srivastava 1987, Wilkinson and Douglas 2003).

Among the amino acids, several stand out as having particular importance to aphids. Dadd and Kreiger (1968) showed that methionine has an important phagostimulatory effect that can influence host plant acceptance and restlessness in aphids. Dadd and Kreiger (1968) also showed that, without one of the non-essential amino acids glutamine, glutamic acid, asparagine, aspartic acid, alanine, or serine, almost no aphid growth occurred. This is because *Buchnera* can manufacture essential amino acids from non-essential amino acids that occur more commonly in aphid diets. Glutamate may be the principle nitrogenous compound used by *Buchnera* to synthesize essential amino acids (Douglas 1998, Wilkinson and Ishikawa 1999). Asparagine levels increase the population size of *R. padi* on oat and barley (Weibull 1988).

Potassium fertilization is a good candidate for cultural control of soybean aphid, *Aphis glycines* Matsumura. Potassium-deficient soybeans support higher soybean aphid populations in the field, and cage studies showed a higher rate of soybean aphid increase on soybean plants growing in soils with low potassium levels (Chapter 2). Potassium fertilization reduces aphid lifespan and rate of reproduction, and this has been quantified in the case of *R. padi* feeding on barley (Havlickova and Smetankova 1998). In addition, *R. padi* land on barley growing in potassium-rich soil less often then barley growing in potassium-poor soil (Havlickova and Smetankova 1998). Van Emden (1966) found that an increase in potassium fertilization of Brussels sprout leads to a decrease in soluble nitrogen, a correlate of phloem sap amino acid concentration. Thus, it is hypothesized that the potassium nutrition of soybean affects the free amino acids being transported in

the phloem, and that potassium-deficient soybean are of higher nutritional quality for the soybean aphid.

The objectives of this study were A) to compare the free amino acid profiles of the phloem sap of soybeans growing in potassium sufficient and deficient soils in commercial fields and in artificially fertilized plots and B) to define the relationship between phloem amino acid profiles with the soybean aphid populations on plants.

## Methods

### *Surveys*

Field surveys were undertaken to examine the effect of potassium fertility on soybean aphid populations and soybean phloem amino acids in commercial fields. Surveys took place on August 13-14 2003 and August 17-23 2004. The 2003 survey was conducted in eight fields in Van Buren, Calhoun, and Kalamazoo Counties, Michigan; the 2004 survey was conducted in five fields in Van Buren County and Calhoun County, Michigan (Table 1). In each year, commercial soybean fields with patches of visual symptoms of potassium deficiency were chosen. Three paired samples of soil, aphid populations, and phloem sap were taken in each field (each year of the survey included one field where only two pairs were sampled). One site in each pair was in the center of an area of severe potassium deficiency (stunted plants with chlorosis and necrosis around the outside of their leaves), the other was a nearby topographically similar location without deficiency symptoms (green foliage).

Soil samples were taken by extracting 25 cm soil cores from the center of each site (1 core per sample in 2003, 3 cores per sample in 2004). The samples were

submitted to the Michigan State University Soil and Plant Nutrient Laboratory for analysis.

For the aphid counts, three plants were randomly chosen and pulled from each site. These plants were then placed in coolers with ice packs that maintained the temperature at approximately 4° C. Coolers were returned to the laboratory and stored in a cold room at 4° C overnight. In 2003, the number of soybean aphids per plant was extremely high, so plants were placed in whirl-pack (Nasco, Fort Atkinson, WI) bags filled with 70% EtOH and stored in the cold room until the number of aphids could be counted under a dissecting microscope. When the number of aphids was counted, the proportion of alates in the first hundred aphids counted was also recorded. In 2004, aphid numbers were much lower and the aphids were counted the day following field sampling. In 2004, no alates were observed.

*Controlled Cage Studies*

In 2003 and 2004, we conducted cage trials to test the interaction between soil potassium levels and soybean aphid population size in a controlled manner. The studies were conducted in potassium-deficient commercial soybean fields in Van Buren County, Michigan.

The 2003 study site (N 42 ° 7.55' W 85 ° 79.95') had a beginning soil potassium level of 75 ppm as measured by a soil test prior to planting. Three replicated strips were established for each of three potassium treatments. Full (recommended by MSU soil nutrient laboratory), Half, and no potassium amendments were done with potash fertilizer (0-0-62) and were as follows: 196, 98, and 0 kg per ha. On 8 July 2003, four field cages

in each strip were set up. Two of the four cages (sacrifice cages) were sampled weekly by removing ad counting the number of soybean aphids and plant characteristics on five plants. The remaining two cages (yield cages) were not disturbed until harvest. In each pair of sacrifice or yield cages, one cage was infested with aphids while the other served as an aphid-free control. This resulted in a factorial design with three levels of potassium amendment and two levels of aphid infestation. On 9 July, stand counts of the soybean plants in each cage were taken and the cages were assigned to a soybean aphid treatment. Infested cages were inoculated by tapping an aphid-infested soybean plant above the plants in the cage for approximately 30 s.

Beginning on 29 July, five plants per week were removed from the sacrifice cages, placed in a cooler at approximately 4 ° C, and transported back to the laboratory. The coolers were stored overnight in a cold room at the same temperature. The following day, the same plant characteristics measured in the field surveys were counted and the plants were preserved in whirl-paks as described above. On 12 August, soil samples were taken from each cage as described above. One core was taken from each cage. In addition, one phloem sample per cage was taken from the 2003 study. Sampling for repetitions one and two took place on 5 September 2003 between 14:00 and 16:00. Sampling for repetition three took place on 9 September 2003 between 12:00 and 14:00. All phloem sampling took place while the plants were at the R6-R7 (full seed-beginning maturity) growth stages.

In 2004, the study site (N 42 ° 7.50' W 85 ° 80.00') had an initial soil potassium level of 67 ppm as measured by a soil test just after planting (Table 2). Two strips in each of five replicates were established in the field and either left unfertilized, or else

fertilized by broadcasting 256.8 kg/ha potash fertilizer (0-0-62) (Mason Elevator, Mason, MI) on 13 May 2004. Two cages per strip were set up on 13 May 2004, when soybean germination was almost complete. On 28 May 2004, the soybeans in the cages were thinned to 13 plants per cage. One cage in each strip was infested with one soybean aphid per plant on ten plants on 28 May 2004 and the second cage was maintained aphid-free to serve as a comparison. This resulted in a 2x2 factorial design, with the presence or absence of potash fertilizer and soybean aphids as factors. The source of the soybean aphids was a laboratory colony established from a single field-collected soybean aphid in 2003, and maintained at Michigan State University (27° C, 24 hour photoperiod). Soil samples (three cores) from each cage were taken on 4 June 2004. The number of soybean aphids per plant was counted two or three times a week from 28 May until 15 July.

In 2004, three phloem samples per cage were taken from three uninfested plants in the cages receiving the aphid infestation (cages had 13 non-touching plants, only 10 were infested) and three plants in the uninfested cages on 18 June 2004 when the soybeans were in the late vegetative or R1 (beginning flower) growth stages. A second set of 3 phloem samples per cage was taken from two remaining sets of uninfested cages on 15 July 2004 when the soybeans were in the R2 (full flower) growth stage. All 2004 phloem samples took place between 11:00 and 15:00.

*Phloem sampling and analysis*

Phloem samples were taken using a modification of the phloem exudation method of King and Zeevart (1974). A 10mM solution of ethylenediaminetetraacetate (EDTA)

was prepared and adjusted to pH 7.0 with 1 N NaOH. When a plant stem or petiole with a cut sieve tube is immersed in this solution, the EDTA binds the calcium in the sieve tube. Calcium acts as a signaling molecule that initiates callose formation. Thus, immersing a cut sieve tube in EDTA solution prevents the formation of callose, and phloem sap contained in both the cut sieve tube and connected phloem vessels exudes into the solution. The entire solution is then analyzed as an indirect measure of phloem contents. Five mL aliquots of the EDTA solution were placed in 2 dram vials that were then sealed with Parafilm. In the field, a slit was cut in the Parafilm covering of each vial. A soybean leaf (the second fully expanded leaf counting down from the top of the plant) was cut off at the petiole in a dish of the EDTA solution. It was then immediately transferred to one of the EDTA vials by passing the petiole through the slit in the Parafilm. Vials were placed in a cooler at approximately 4° C, returned to the lab, and placed in a cool room at 4° C. Twenty-four hours after the samples were taken, leaves were removed from the vials and the vials were sealed with a lid. The length and width of the central leaflet of each soybean leaf was then recorded.

The concentration of potassium as well as the physiological amino acids was determined for each phloem sample. The potassium concentration (ppm) of the exudate in each vial was determined by measuring the potassium content of a 0.5 mL aliquot of the exudate solution using a Cardy Potassium $K^+$ Meter (Spectrum Technologies, Plainfield, IL). The samples were then stored at –80° C until free amino acid analysis was performed. Free amino acid analysis was performed via high precision liquid chromatography (HPLC) using a Waters system (Waters 2690 Separations Module, Waters 474 Scanning Flourescence Detector, Waters Temperature Control Module,

Millennium Software Package, and the Acc-Q tag reagent kit, Waters Corportation, Milford, MA). HPLC took place at the Michigan State University Macromolecular Structure Facility. HPLC analysis included the following amino acids: alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine.

*Statistical Analysis*

Because phloem exudation is an indirect measure of phloem sap amino acid contents, it is not possible to directly evaluate the concentration of amino acids in the phloem. Instead, the profile of amino acids, as defined by the proportion of each amino acid in all the free amino acids of the exudate, was compared among treatments. In the surveys and the 2003 cage study, proportions of individual amino acids in the phloem profile and soybean aphid number per plant were regressed against soil nutrient levels using PROC REG in SAS version 8.2 (The SAS Institute, 1999). For the 2004 cage study, amino acid proportions and soil nutrient levels were regressed using Proc Reg; because of a strong replicate effect soybean aphid numbers were analyzed via Proc Mixed in SAS version 8.2 (The SAS Institute, 1999). For the field surveys, any p value less than 0.10 was considered significant when regressing proportions of amino acids. This is appropriate because of the large amount of variability encountered in the survey results and because there were many factors that could not be measured or controled in the field surveys (variety, planting date, soil type, moisture levels, etc.). Amino acids such as asparagine act as plant signaling molecules. Thus, they could be affected by a wide range of environmental factors besides soil potassium. In 2003, phloem samples

were taken from plants in cages with and without aphids. Because some species of aphids are able to influence the amino acid composition of their food source (Telang et al. 1999, Sandstrom 2000, Sandstrom and Moran 2001), samples taken from cages with and without soybean aphids were analyzed separately.

## Results

### *Surveys*

In 2003, one amino acid, asparagine, had a significant relationship with soil potassium (Figure 3.1). The proportion of asparagine in the phloem amino acid profile was negatively correlated with the concentration of potassium in the soil where the plants were growing. The average number of soybean aphids per leaf (20-200 aphids) was positively correlated with the proportion of asparagine in the phloem (Figure 3.2.). In 2004, 11 amino acids were correlated with soil potassium (Table 3.1), including a negative correlation between soil potassium concentrations and proportion of asparagine in the phloem profile (Figure 3.3). Other amino acids negatively correlated with soil potassium level were histidine, threonine, valine, isoleucine and lysine. Aspartic acid, glutamic acid, serine, glycine, and alanine were positively correlated with the soil potassium level. There was no correlation between soybean aphid population and amino acids, but soybean aphid populations were extremely low (fewer than 100 per plant).

### *Controlled Cage Studies*

The amino acid profiles of infested and uninfested plants were analyzed separately in the 2003 cage study. In plants from infested cages in the 2003 study,

glycine was the only amino acid significantly correlated with soil potassium (slope = 0.0024, r-squared =0.35, p = 0.0131). In plants from uninfested cages, aspartic acid (slope = 0.0035, r-squared = 0.46, p = 0.0078) and glutamic acid (slope = 0.0015, r-squared = 0.33, p = 0.0333) were positively correlated with soil potassium levels.

In 2004, two sets of phloem samples were taken. All 2004 phloem samples were taken from uninfested plants to avoid any effects of the aphids on phloem amino acids. Early in the season (18 June), no amino acid had a significant relationship with soil potassium. At that time, soybean aphid populations were also not affected by soil potassium levels. By 15 July, eleven amino acids were significantly correlated with soil potassium (Table 3.2). Once again asparagine was negatively correlated with soil potassium (Figure 3.4). The amino acids positively correlated with soil potassium included aspartic acid, glutamic acid, histidine, proline, tyrosine, valine, isoleucine, leucine, lysine, and phenylalanine. Because all phloem samples were taken from uninfested plants, it was not possible to directly assess the relationship between soybean aphid population level and phloem amino acids. However, soybean aphid populations were significantly higher on plants growing in lower potassium soil from 30 June until aphid populations crashed.

**Discussion**

In the 2003 survey, 2004 survey, and 2004 cage study, asparagine was negatively correlated with soil potassium level. Also in the 2003 survey and 2004 cage study, aphid population size was negatively correlated with soil potassium. The 2003 survey took place during a soybean aphid outbreak (up to 17,000 aphids per plant) and the 2004 cage

study also represents outbreak conditions since no biological or chemical control was present in the field cages. The 2004 survey, however took place at extremely low aphid populations (usually fewer than 100 aphids per plant). Therefore, the 2004 survey probably represents a situation where the soybean aphid population was limited by factors other than nutrition (i.e. predation) while during the 2003 survey and 2004 cage study, the soybean aphid population was probably constrained by nutrition. Of those two studies, soybean aphid population and asparagine may only be directly compared in the 2003 survey, and they are indeed positively correlated.

The 2003 cage study did not to follow this pattern. Neither soybean aphid population nor phloem asparagine was correlated with soil potassium. However, this should not be taken to contradict the patterns in the other studies. In 2003, cages were infested by tapping an infested plant over the plants in the cage. Thus, aphid populations were not standardized as they were in 2004. The aphid populations that were measured probably reflect differences in initial infestation rather than a host-plant mediated nutritional relationship. In addition, the phloem samples were taken at a late stage of plant maturity after the soybean aphid population crash. Since asparagine acts as a signaling molecule within the soybean plant (Bacanamwo and Harper 1997), it is possible that it was no longer needed at that stage of maturity and thus did not vary with soil potassium at the time that the plants were sampled. An interesting observation in the 2003 cage phloem samples is that different amino acids varied with potassium depending on the presence of soybean aphids. Other species of aphids, such as *Diuraphis noxia* Mordvilko are known to modify the amino acid profile of their host plants (Telang et al. 1999), and it is possible that the soybean aphid does this as well. This is particularly

intriguing in view of the fact that aspartic acid and glutamic acid, which were positively correlated with soil potassium in the uninfested plants, may be repellant to some aphids (Abisgold et al. 1994).

Asparagine may vary with soil potassium level because of its role as a signaling molecule between the shoots and nodules of the soybean plant. In nodulated soybeans, a symbiosis occurs between the soybean plant and one of four genera of Rhizobiaceae, usually *Rhizobium* or *Bradyrhizobium* (Schubert 1995). The bacterium supplies fixed nitrogen to the plant and receives fixed carbon. The process of nitrogen fixation is energetically expensive; respiration by the symbiont may account for 70% of total root respiration (Walsh et al. 1998). Thus, it is advantageous for the plant to slow or stop nitrogen fixation under environmental stress or when the plant reaches a growth stage where additional fixed nitrogen is no longer required. The signal to slow nitrogen fixation is asparagine (Bacanamwo and Harper 1997).

The mechanism for increasing phloem asparagine under conditions of potassium deficiency is as follows. Nitrogen is fixed and converted to ureides in the nodule and transported to the rest of the plant via the xylem (Whitehead et al. 2001). In the shoots, ureides are broken down into amino acids and loaded into the phloem (Vadez et al. 2000) or made into proteins. Asparagine arriving at the nodules is broken down into aspartate and glutamate which can enter the bacteroid and inhibit nitrogen fixation (Bacanamwo and Harper 1997). Potassium deficiency is likely to result in inhibition of nitrogen fixation because the enzyme responsible for converting amino acids into proteins requires potassium as a cofactor (Mengel and Kirkby 2001). When the plant is deficient in potassium, amino acids are especially likely to build up in the foliage (Mengel and

Kirkby 2001), causing increased levels of asparagine to be transported in the phloem in order to slow nitrogen fixation.

Through this mechanism, soil potassium deficiency is likely to increase levels of phloem asparagine when nitrogen fixation is occurring in soybean. Aphids are severely nitrogen limited (Terra 1988), and asparagine is one of the most important nitrogen sources in the aphid diet (Dadd and Krieger 1968) because it can be converted to essential amino acids lacking in the aphid diet (Douglas 1998). In both field surveys and controlled cage studies, soybean aphid populations or density and phloem asparagine levels are negatively correlated with soil potassium levels. The increased levels of phloem asparagine encountered by soybean aphids feeding on potassium deficient soybean plants may alleviate part of their dietary nitrogen limitation, allowing for greater soybean aphid populations.

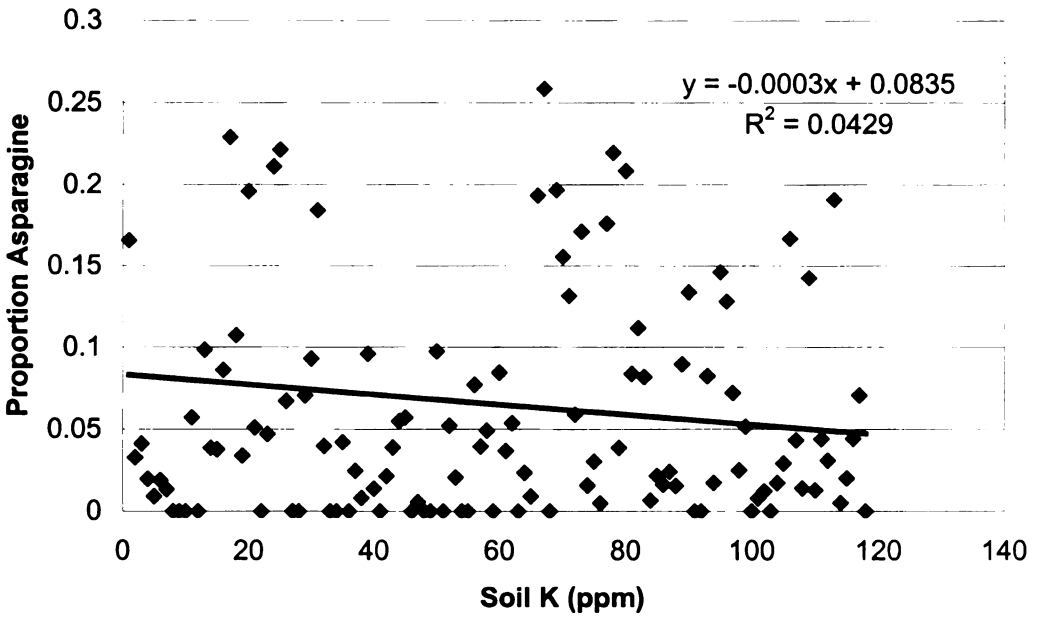


Figure 3.1. Proportion of asparagine in the phloem amino acid profile versus soil potassium level in eight commercial soybean fields in the 2003 Survey ($p = 0.03$). Samples were taken during a soybean aphid outbreak when aphid populations were commonly over 10,000 per plant. Samples were taken August 13-14 2003, just as the soybean were beginning to set seed.

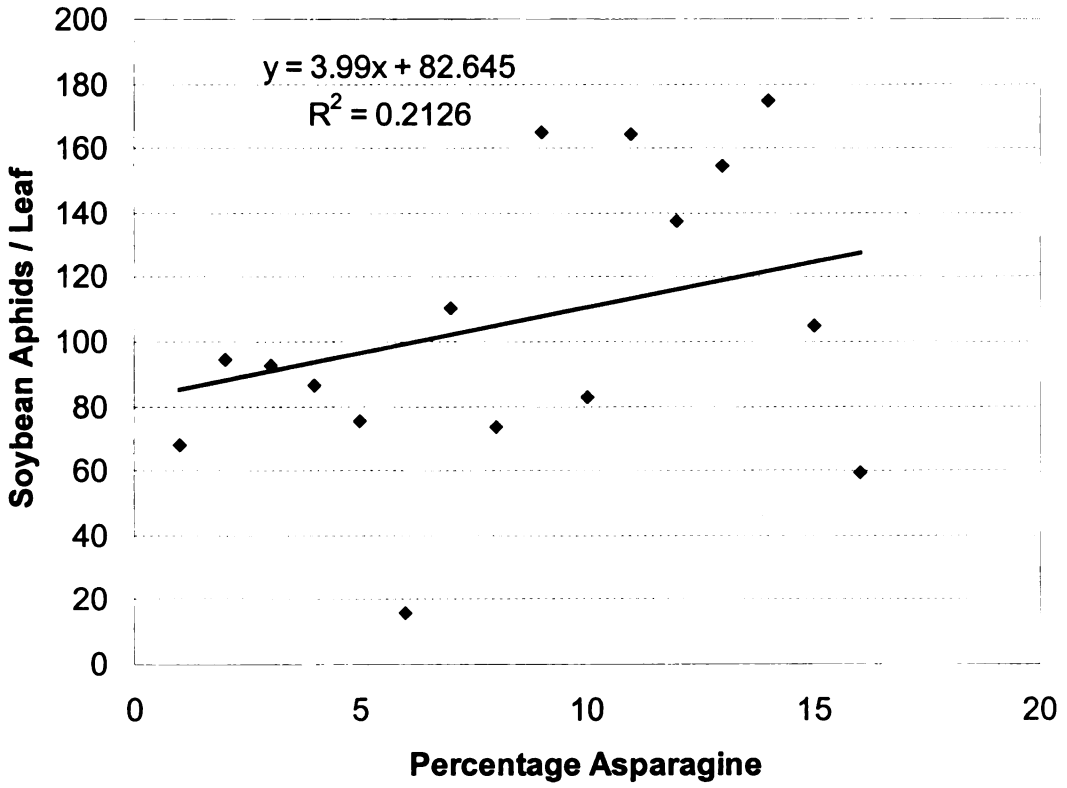


Figure 3.2. Soybean aphids per leaf versus the proportion of asparagine in the phloem free amino acids in three commercial soybean fields with active soybean aphid populations in the 2003 field survey ($p = 0.0722$).

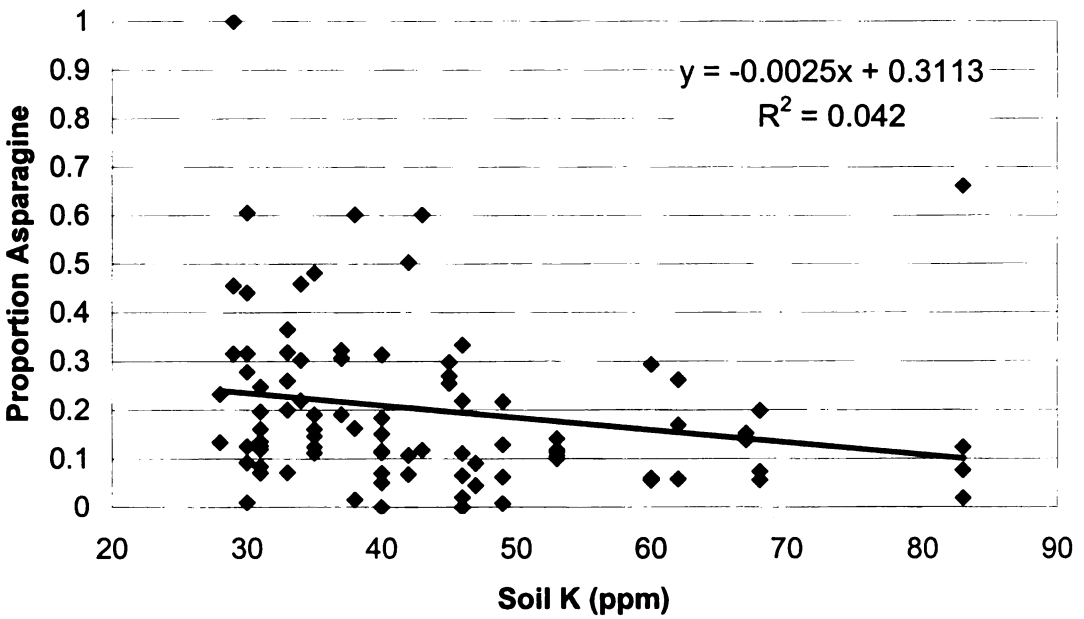


Figure 3.3. Proportion of asparagine in the phloem amino acid profile versus soil potassium level in five commercial soybean fields in the 2004 Survey ($p = 0.055$). Samples were taken 17-23 August 2004 during soybean seed set. Aphid populations were extremely low.

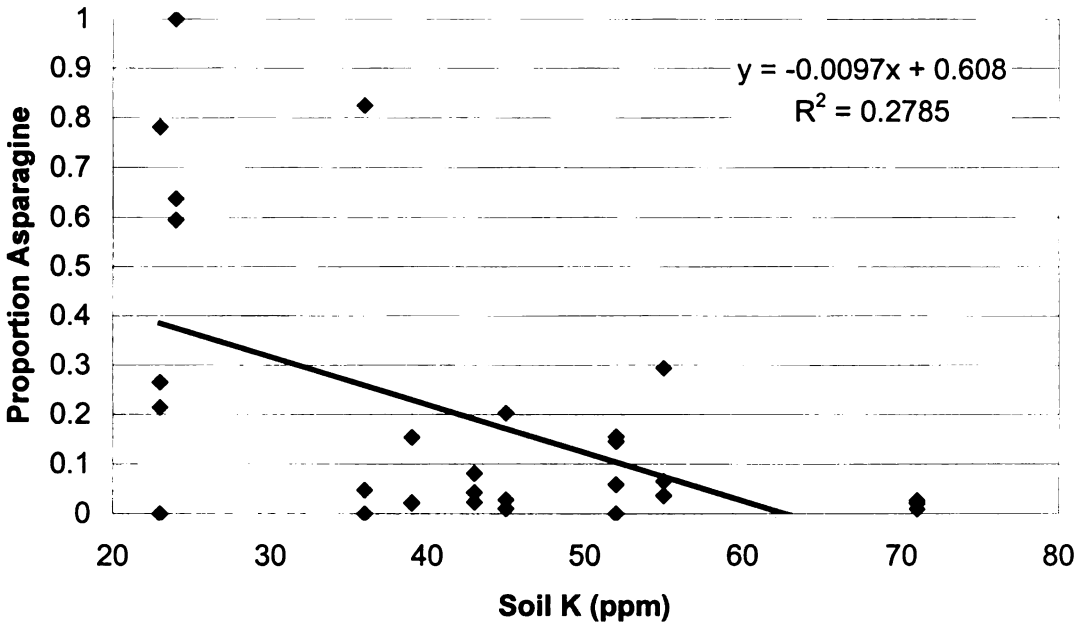


Figure 3.4. Proportion of asparagine in the phloem amino acid profile versus soil potassium level in the 2004 Cage Study on the July 15 sampling date ($p = 0.002$). Soybeans were in full flower. All samples were taken from uninfested plants.

Table 3.1. Slope, r-square value, and p value of the amino acids significantly correlated with soil potassium level five commercial soybean fields in the 2004 field survey on 17-23 August. Soybeans were setting seed and the soybean aphid populations were extremely low.

| Amino Acid | slope | $R^2$ | p |
|---|---|---|---|
| Aspartic Acid | 0.002 | 0.0949 | 0.0035 |
| Glutamic Acid | 0.0005 | 0.0394 | 0.0687 |
| Serine | 0.0016 | 0.0553 | 0.0275 |
| Asparagine | -0.0025 | 0.042 | 0.0553 |
| Glycine | 0.0012 | 0.043 | 0.0526 |
| Histidine | -0.0001 | 0.0815 | 0.007 |
| Threonine | -0.0002 | 0.0478 | 0.0406 |
| Alanine | 0.0005 | 0.0313 | 0.099 |
| Valine | -0.0003 | 0.0531 | 0.0437 |
| Isoleucine | -0.0003 | 0.0731 | 0.0109 |
| Lysine | -0.0009 | 0.159 | 0.0001 |

Table 3.2. Slope, r-square value, and p value of the amino acids significantly correlated with soil potassium level in the 2004 cage study 15 July sampling date. Soybean were in full flower. All phloem samples were taken from uninfested plants.

| Amino Acid | slope | $R^2$ | p |
|---|---|---|---|
| Aspartic Acid | 0.0045 | 0.1786 | 0.0179 |
| Glutamic Acid | 0.0021 | 0.1631 | 0.0269 |
| Asparagine | -0.0097 | 0.2785 | 0.0023 |
| Histidine | 0.0002 | 0.1928 | 0.0135 |
| Proline | 0.0009 | 0.3837 | 0.0002 |
| Tyrosine | 0.0001 | 0.1881 | 0.0148 |
| Valine | 0.0011 | 0.3571 | 0.0004 |
| Isoleucine | 0.0004 | 0.2741 | 0.0025 |
| Leucine | 0.0013 | 0.5623 | <0.0001 |
| Lysine | 0.0004 | 0.2335 | 0.0059 |
| Phenylalanine | 0.0002 | 0.1439 | 0.0354 |

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## Chapter 4—The impact of foliar fertilization on soybean aphids and soybeans

### Introduction

The purpose of foliar fertilization is to provide plants with nutrients at a critical growth stage. In soybeans, foliar fertilizers are used at both early vegetative stages (Haq and Mallarino 1998, 2000, Mallarino et al. 2001) and reproductive stages (Poole et al. 1983). Most of the foliar fertilizers used contain nitrogen, phosphorus, potassium, and micronutrients. The impact of foliar fertilization on soybean yield is variable (Poole et al. 1983, Haq and Mallarino 1998, 2000, Mallarino et al. 2001).

The soybean aphid was first discovered in North America in 2000 (Venette and Ragsdale 2004). It is the most severe insect pest of soybeans, and may reduce yield by up to 40% (DiFonzo 2002). Currently, there are very few alternatives to chemical insecticides for soybean aphid control. Some Michigan growers have been employing foliar fertilization during the reproductive stages of soybean as a method of aphid control. However, there are no controlled studies verifying this method.

Because foliar fertilizer contains a number of nutrients, the anticipated effect on aphids is unclear. Nitrogen fertilization of wheat increased the abundance of a leaf-feeding aphid, probably by increasing the amount of nitrogen available in the phloem (Honek 1991). However, nitrogen fertilization can depress nitrogen fixation in legumes (Bacanamwo and Harper 1997). If this resulted in a net decrease in the amount of nitrogen translocated in the phloem, it could slow soybean aphid population increase. The effects of the other nutrients on herbivorous insects are variable (Waring and Cobb 1989).

When used for aphid control, foliar fertilizers are applied to plants that are not thought to be deficient in any nutrient. However, it is possible that a hidden micronutrient deficiency may be alleviated in some cases. The plant stress hypothesis states that plants under stress are more susceptible to herbivores (Waring and Cobb 1989). Thus, if a foliar fertilizer reduced plant stress, it could allow the plant to better combat soybean aphids, reducing aphid populations and/or aphid damage.

Finally, the foliar fertilizer may act directly on the aphids. The foliar spray could coat the aphids. As it evaporates, the salty spray could draw water out of the bodies of the insects, putting them under osmotic stress. This stress could slow aphid reproduction or even kill the aphids, thus providing aphid control.

The objective of this study was to determine the effects of foliar fertilization of soybeans on foliar nutrient level, soybean yield, and soybean aphid population.

**Methods**

The study took place during the 2004 growing season at four locations in Michigan: on the Michigan State University Entomology Research Farm in Ingham County, on the Michigan State University Bean and Beet Research Farm in Saginaw County, and at two commercial soybean fields in Gratiot and Calhoun Counties.

At all locations, plots were established in existing soybean fields. The strips had two treatments, with and without Alpine Fortified Liquid Foliar Fertilizer (Alpine) (10-10-10 plus 0.1% Boron, 0.1% Iron, 0.05% Manganese, 0.05% Zinc, and 0.0006% Molybdenum). The foliar fertilizer was applied according to label directions: 4.94 l per ha of product at R3 (beginning seed) and again at R5 (full seed). See Table 1 for plot size

and treatment details. At one location (the Calhoun County site), an additional treatment of a half rate of foliar fertilizer (2.5 l per ha) was included.

*Aphid Counts*

At the university farms sites, the number of aphids per plant was counted weekly on five plants per plot. At the Ingham County site, counting began on 7 July 2004 and lasted until 23 August 2004. At the Saginaw County site, counting began on 28 June 2004. The site was not evaluated again until 5 August 2004. It was then evaluated weekly through 23 August 2004. In the commercial fields, cooperating extension agents noted the presence of aphids, but did not conduct weekly counts.

*Foliar Nutrient Sampling and Analysis*

Foliar leaf samples were taken seven days after each foliar fertilizer application. In each plot, the uppermost fully-unfurled leaf of 30 randomly selected plants was cut off at the base (petioles were not removed). The leaves from each plot were combined and placed a paper bag. Within 24 hours, the leaves were washed by placing them in a soapy water bath (Joy™ phosphate-free dish soap) then rinsed thoroughly and patted dry. The leaves were returned to the paper bags and dried in an oven at 32° C for three to four days. The dried samples were stored until 21 October. The samples were sent to A&L Labs (Fort Wayne, IN) and analyzed for their content of the following elements: nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, zinc, manganese, iron, copper, boron, aluminum, and sodium.

*Yield*

All plots were mechanically harvested for yield. Plot area harvested depended on the location and equipment (Table 1). All yield results were standardized to 13.5% moisture prior to analysis.

*Statistical Analysis*

Nutrient content and yield data were analyzed using PROC GLM in SAS version 8.2 (The SAS Institute, 1999). The soybean aphid counts did have a normal distribution, so they were analyzed with a Kruskall-Wallis test using PROC NPAR1WAY in SAS version 8.2 (The SAS Institute, 1999).

**Results**

*Aphid Counts*

Aphids were present at all locations throughout the season, but numbers were extremely low. The highest populations observed were 120 aphids on a plant at the Ingham County site (19 July). This single plant accounted for about half of the aphids observed for the entire season at that site. At the Saginaw County site, the highest aphid count was 73 aphids per plant (18 August). Aphid numbers in the fertilized and unfertilized treatments did not significantly differ at either site on any sampling date ($p>0.05$) (Figure 4.1).

*Foliar Nutrient Sampling and Analysis*

There were no significant differences in the concentrations of the selected foliar nutrients between the fertilized and unfertilized plots at any location and sampling date ($p>0.05$).

*Yield*

Although there were differences in yield between sites, there was no within-site difference in yield between fertilized and unfertilized plots ($p>0.05$) (Figure 4.2).

**Discussion**

In 2004, foliar fertilization did not significantly impact the soybean aphid populations at either the Saginaw County or the Ingham County sites. However, the soybean aphid populations at these sites were extremely low, and the results do not definitely predict the effects of foliar fertilizer on outbreak populations of soybean aphid. Although this does not appear to be a promising method for aphid control at this point, additional replication under conditions of higher aphid pressure is needed to definitely determine the effects of foliar fertilizer on soybean aphid populations.

We hypothesized that any effects on soybean aphids in this study would be due to changes in the amount of nitrogen available to them. Aphids in general are very sensitive to the amount of nitrogen in their diets (Dadd and Krieger 1968, Douglas 1993, Ponder et al. 2000, Koyama et al. 2004), and if foliar fertilization treatments affected phloem nitrogen, they could also affect aphid populations. There are several reports in the literature of aphid populations increasing when their host plants are fertilized with

nitrogen (Honek 1991, Ponder et al. 2000, Cisneros and Godfrey 2001, Nevo and Coll 2001, Jansson and Ekborn 2002). However, it is not known whether the small amount of foliar fertilizer used in these treatments could affect the phloem nitrogen content of soybean plants enough to affect the aphid population. Since soybeans are legumes, and can slow or stop nitrogen fixation if other nitrogen sources become available (Bacanamwo and Harper 1997), it is also not clear that if foliar fertilization affected phloem nitrogen that it would result in a net nitrogen increase. Thus changes in phloem nitrogen content due to foliar fertilization could positively or negatively affect soybean aphid populations.

The results of both the foliar nutrient analysis and yield comparison indicated that the foliar fertilizer did not affect the plant in any way. All four of our sites were nutritionally adequate fields because this study was designed to test the effects of supplemental fertilizer as aphid control, not as a rescue treatment for deficient plants. Nonetheless, we had expected differences in the foliar nutrient analysis that would indicate that the fertilizer had physiological effects on the plant; it would appear that it does not.

The results of the foliar nutrient analysis indicate that if foliar fertilization had any affect on soybean aphid populations, it is unlikely to be mediated by the host plant. It appears that any possible effects of foliar fertilizer on soybean aphid populations is due to the direct action of the fertilizer on the aphids, most likely by acting as a desiccating salt. Although there is no evidence in the literature for foliar fertilization or any other treatment to control aphid in this way, it is possible that it occurs. Spraying sugar solutions on fields had also been suggested as an alternative to aphid control through

chemical insecticides. It is possible that both methods could work through the same mechanism.

Foliar fertilization does not show great promise for soybean aphid control. However, it has not been tested in a year when high populations of the pest were present. Additional testing under higher aphid pressure is needed before the efficacy of this method can be definitely determined.

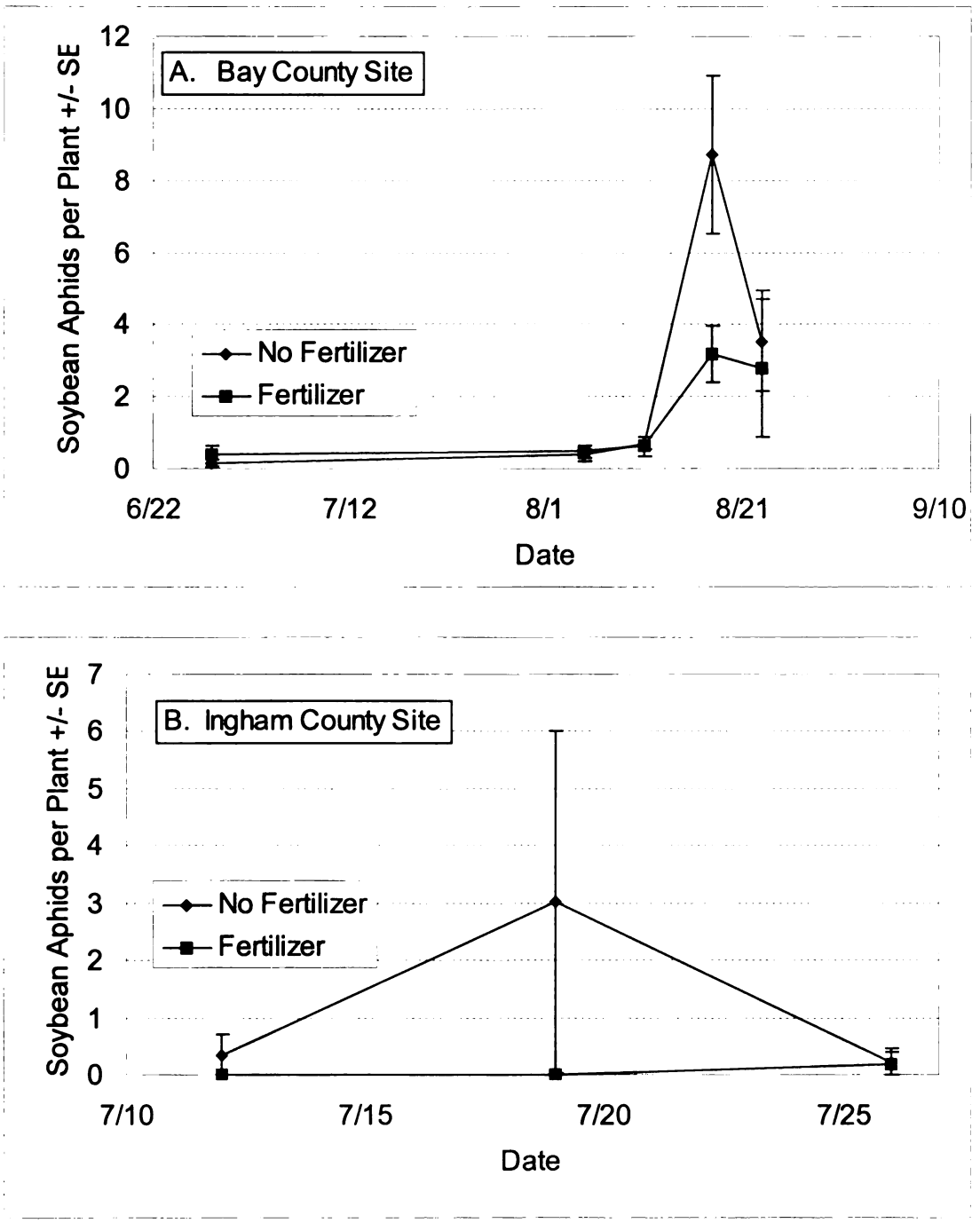


Figure 4.1. Aphid counts of fertilized and unfertilized plants at university farms sites during the 2004 field season. Counts are not significantly different at either site on any sampling date ($p>0.05$).

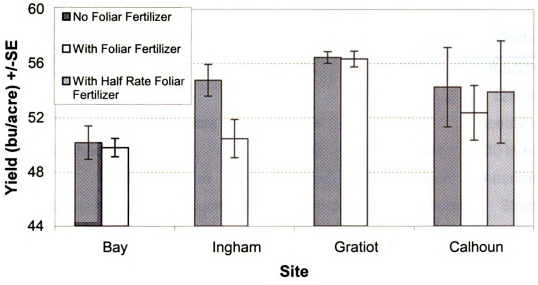


Figure 4.2. Yield of soybean at four experimental sites where foliar fertilizer was applied. In an ANOVA, there were no differences at $p<0.05$.

Table 4.1. Plot details and maintenance dates for the 4 sites in the study.

| | County | | | |
|---|---|---|---|---|
| | Ingham | Saginaw | Calhoun | Gratiot |
| Planting Date | 5/19/2004 | 5/7/2004 | 6/8/2004 | 6/5/2004 |
| Planting Rate | 80-90 lbs/acre | 148,000 seeds/acre | ** | 125,000 seeds/ acre |
| Planting Method | Drilled | 30 in rows | 30 in rows | 30 in rows |
| Study Design | Randomized Complete Block | Randomized Complete Block | Randomized Complete Block | Alternating Strips |
| Plot Size | 50 ft x 20 ft | 4 rows x 50 ft | 4 rows x 35 ft | 6 rows x 70 ft |
| Replications | 4 | 4 | 4 | 4 |
| Foliar Fertilzer Application--R3 | 8/16/2004 | 7/23/2004 | 7/29/2004 | 8/9/2004 |
| Foliar Fertilzer Application--R5 | 9/2/2004 | 8/11/2004 | 8/24/2004 | 8/27/2004 |
| Chemical Application Method | CO2 sprayer | CO2 sprayer | CO2 sprayer | commercial applicator |
| Chemical Application Rate | 17.4 gallons/acre | 17.4 gallons/acre | 13 gallons/acre | ** |
| Chemical Application Pressure | 30 psi | 30 psi | 24 psi | ** |
| Chemical Application Nozzle Type | flat fan | flat fan | ** | ** |
| Harvest Date | 10/26/2004 | 10/26/2004 | 11/8/2004 | 10/13/2004 |
| Harvest Area | 5 x 50 ft | 2 rows x 50 ft | 2 rows x 35 ft | 4 rows x 70 ft |
| Harvest Method | Plot Combine | Plot Combine | Plot Combine | Combine + Weigh Wagon |

** = unknown.

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## Chapter 5 -- Experiments on the effects of sooty mold on soybeans.

### Introduction

Sooty mold is a saprophytic fungus species or group of species that grow on the foliage of plants where aphids have deposited honeydew. Sooty mold may block up to 98% of light penetration to the leaf surface of pecan, reducing net photosynthesis up to 70% (Wood et al. 1988). This reduction in assimilate production could have large consequences on the production of energy-rich organs such as seeds and on yield.

During periods of high soybean aphid infestation, sooty mold was observed on soybean leaves (DiFonzo 2002). Even after the aphids were no longer present because of either chemical application, migration, or entomopathogenic fungus, the sooty mold persisted for the rest of the season. Because of the potential of sooty mold to shade the foliage, there was concern that sooty mold could be causing reductions in soybean yield apart from the effects of the soybean aphids. Soybeans are especially sensitive to shading in during pod set (R3-R5) (Board et al. 1990), which corresponds to the time period when aphid-infested soybeans are likely to have high levels of sooty mold.

The objective of these studies was to determine whether sooty mold alone could impact soybean yield and, if so, the relationship between mold coverage and yield reduction.

### Methods

Experiments took place over two years. In both years, the goal of the experiments was to stimulate the growth of sooty mold on soybean foliage in the absence of aphids by

applying a honeydew-like substance to soybean plants. We hoped to achieve a range of sooty mold coverage, which we could then use to determine the relationship between sooty mold coverage and soybean yield reduction independent of aphids.

*2002 Field Experiment*

A large-scale field experiment took place at the Michigan State University Bean and Beet Research farm in Saginaw County, Michigan. On 25 June, 2002, 12.2 m x 30.5 m plots were cut out of a soybean field. The field was planted using a seed drill to assure close plant spacing and full canopy coverage as early in the year as possible. On 11 July, the following treatments were applied for the first time: 1) molasses, 2) molasses and Asana (esfenvalerate) insecticide, 3) molasses and Microsperse (elemental sulphur) fungicide, 4) molasses, Asana, and Microsperse, and 5) an untreated control. Plots were treated weekly with molasses and biweekly with the pesticides. Initially, plots were treated with 4 gallons of molasses as a sugar source in 32 gallons of water, Asana at 5.4 oz/acre, and Microsperse at 12 oz/acre. On 8 August, molasses treatments were increased to 6 gallons in 32 gallons of water and Asana treatments were increased to 9.6 oz/acre of Asana. Microsperse treatments ceased on that day because of extremely low mold infestations. The object of the treatments were to have natural soybean aphid populations with natural sooty mold levels (untreated), natural soybean aphid populations with enhanced sooty mold levels (molasses only), natural soybean aphid populations without sooty mold (molasses and Microsperse), no soybean aphids with sooty mold (molasses and Asana), and no soybean aphid populations with no sooty mold (molasses, Asana, and Microsperse). Molasses was included in Microsperse treatments in order to

control for the effect of molasses application on the soybeans. This experiment was replicated four times.

Soybean aphids and mold were sampled weekly. Soybean aphid populations were sampled by counting the number of soybean aphids on one leaf from the top third of the plant on 30 plants per plot. Mold populations were evaluated by visually estimating the percent of mold coverage on one leaf from the middle third of the plant on ten plants per plot.

Neither the mold coverage data nor the aphid data met assumptions of normality. Therefore, the data were analyzed with the Kruskal-Wallis test in the NPAR1WAY procedure of SAS version 8.2 (The SAS Institute, 1999).

*2002 Shade Experiments*

The second experiment in 2002 measured the effects of artificial shading on potted soybeans. On 16 July, tomato rings were placed over potted soybeans with one true leaf (V2 stage) placed outdoors. Knitted shade cloth (Gothic Arch Greenhouses, Mobile, AL) was then fitted over the tomato ring to achieve 30, 50 70, or 90 percent shading. An uncovered plant inside a tomato cage was also included as a control, and the experiment was replicated four times. Plant characteristics (number of nodes, leaves, flowers, pods, and pod fill stage) were monitored weekly until plant maturity. When plants had senesced, the number of pods per node, beans per pod, and total weight of beans per plant were measured.

Data were analyzed via Fisher's protected LSD using the GLM procedure of SAS version 8.2 (The SAS Institute, 1999).

*2003 Cage Experiment*

In 2003, a study was performed on the Michigan State University Entomology Research Farm in Ingham County, Michigan. The field was planted on 23 May using a seed drill with Pioneer variety 92B13 soybeans at a rate of 175,000 seeds/acre. On 18 June, 42-1$m^2$ exclusion cages were placed in the field. See chapter two for a description of the cages. Cages were assigned to each of the six treatments: 1) negative control, 2) aphids only, 3) sugar only, 4) fungicide only, 5) sugar and fungicide, and 6) aphids and fungicide. The treatments were selected to provide planned contrasts measuring the effect of soybean aphid in the absence of sooty mold, the effect of sooty mold in the absence of soybean aphid, and the effect of both together. The experiment was replicated seven times. Five plants per cage were removed each week for counting of plant characteristics from the beginning of reproduction until late August. The fungicide used was Penncozeb (ionic manganese 15.0%, ionic zinc 1.9%, ethylenebisdithiocarbamate 58.1%) at 1.1 kg/ha. Sugar treatments were 31.25 g α–D glucose and 1mL liquid fertilizer (27-0-0 plus 1% S) per cage applied in 300 mL per cage of water. Treatments began on 1 July. Before treatment on the same day, soybean aphids were added to aphid cages at the rate of one aphid per plant (unless the plant was already infested with aphids) and the no aphid treatments were sprayed with Asana (esfenvalerate) at a rate of 19.2 oz/acre. Fungicide was applied weekly and sugar was applied twice a week. On 16 July, the liquid fertilizer was cut to 0.25 mL per cage because the plants exhibited burning symptoms. On 21 July, the sugar rate was adjusted to 93.75 g α–D glucose per cage to

encourage mold growth. Sugar treatments ceased on 1 August because of low mold growth.

Between 30 June and 7 July, the number of aphids present per plant on five plants per cage was counted four times *in situ*. At this time, the mold coverage on those same five plants was visually estimated. At the beginning of soybean reproduction (R1 stage), 5 plants a week were removed from the sacrifice cages. Plant traits were counted and sooty mold coverage was estimated. Although the plants and their aphids were preserved, soybean aphid populations were not evaluated in view of the other experimental results.

**Results**

*2002 Field Experiment*

Aphid numbers were extremely low in 2002, and very little sooty mold was observed in plots with either natural aphid infestations or molasses sprays. When molasses, insecticide, and fungicide (elemental sulphur) were evaluated for their effect on aphid numbers, only insecticide had a significant effect on aphids (season wide average with insecticide = 2.37 aphids per leaf, untreated = 10.62 aphids per leaf, $p<0.0001$). Molasses (with molasses = 0.4% mold coverage, untreated = 0.3% mold coverage, $p<0.0001$) and fungicide (with fungicide = 0.42% mold coverage, untreated = 0.38% mold coverage, $p = 0.0061$) significantly increased mold coverage, while insecticide decreased mold coverage (with insecticide = 0.3% mold coverage, untreated = 0.4% mold coverage, $p = 0.0018$). Mold infestations were extremely low, and any spots leaf with

dark spots that may have been sooty mold was recorded as 1% coverage; the differences in mold coverage could easily be due to artifacts of this practice.

*2002 Shade Experiments*

Shading had a significant effect of soybean growth and reproduction, especially when shading was greater than 50% (Table 5.1). The no shade treatment took less time to flower than the 70% and 90% treatments. The 30% and 50% treatments also took less time than the 90% treatment. The 50% shade treatment set more flowers and pods than the 70% or 90% treatments; the no shade and 30% treatments set more flowers and pods than the 90% treatment (Table 5.1). The percent of flowers aborted per plant did not vary by shade treatment. The lower-shade treatments aborted a lower percentage of pods than the 70% treatment and all of the treatments aborted a lower percentage of pods than the 90% treatment (Table 5.1). In the 90% treatment all pods aborted. The 90% shaded treatment also had fewer nodes than the less-shaded plants (Table 5.1).

At harvest, plants shaded by 50% had more pods yielding beans than the 30%, 70% or 90% treatments (Table 5.1). The no shade treatment had more than the 70% or 90% treatments, and the 70% treatment had more than the 90% treatment. The no shade, 30%, and 50% treatments had a higher number of beans per plant and weight of beans and thus a higher yield than the 70% and 90% treatments; the 70% treatment was significantly greater than the 90% treatment (Table 5.1). In almost all measures of plant growth, reproduction, and yield, there was a critical point for damage between 50% and 70% shading.

*2003 Cage Experiment*

In the early season aphid counts, neither date nor fungicide significantly affected aphid populations ( date Chi-square = 5.19, df = 3 p=0.1587, fungicide Chi-square = 0.01, df = 1, p = 0.9164). Although plants from the remainder of the experiment were preserved, they were not counted in view of the other results from this study. Sooty mold did not establish in the sugar treatments during the 2003 season.

The presence of aphids, sugar, and fungicide in the cages affected soybean growth. Aphid-free plants had more primary leaves than infested plants from 30 July through the last sample on 15 August (Table 5.2). Plants treated with sugar had a reduced number of primary leaves from 7 August onward (Table 5.3). Aphid-infested plants had fewer secondary leaves, flowers, and unfilled pods from 30 July through the end of the experiment (Table 5.2). On 15 August, aphid-infested plants had fewer filling pods per plant (Table 5.2) and, within the soybean aphid treatment, infested, fungicide-treated plants had fewer filling pods than infested untreated plants (15 August aphid plants with fungicide = 4.3, 15 August aphid plants without fungicide = 10.9, t = 3.95, $p<0.0001$). Aphid-infested plants had fewer leaves per plant from 30 July through the final sampling date, while plants receiving sugar had fewer leaves on 7 August and after (Table 5.2, 5.3). Soybean aphid-infested plants had fewer nodes from 30 August through the final sampling date while sugar treated plants had fewer on 7 August only (Table 5.2, 5.3). The total number of pods per plant was lower on aphid infested plants from 30 July onward; within the soybean aphid treatments, fungicide treated plants had fewer pods on 15 August (15 August aphid plants with fungicide = 5.15, 15 August aphid plants without fungicide = 29.8, t = 3.06, p = 0.0023).

**Discussion**

The effects of sooty mold on soybean growth and yield were not determined in the 2002 field or 2003 cage experiment. With the two sugar sources and weekly or biweekly applications, sooty mold was not successfully established in the absence of aphids. A different type of sugar, more frequent applications of sugar, or a constant supply of moisture may be needed to establish this fungus.

The 2002 shade experiment showed that if the primary effect of sooty mold is by shading of the plants, at least 70% total shading is necessary for yield reduction to occur. Soybean plants are partially shaded by their neighbors; thus less than 70% coverage by sooty mold may reduce yield, but enough sooty mold would be necessary to bring the amount of shading up to 70%.

Since these experiments began, an economic injury level of about 1,000 aphids per plant through the R6 stage of soybean has been set by a committee of specialists from the North Central Region (Anonymous 2004). This same committee recommends chemical control at a level of 250 aphids per plant. At these population levels, sooty mold is unlikely to occur as a result of aphid infestation. Therefore, if the soybean aphid population is properly controlled, additional yield loss as a result of sooty mold growing on aphid honeydew is not a concern.

The 2003 cage study showed the impacts that high soybean aphid populations can have on soybean development. Although yield was not evaluated, soybean aphids reduced the number of leaves, flowers, pods, and nodes. Since all of these characteristics

may be affected, soybean aphids have the potential to injure soybeans from the vegetative stages of soybean through at least seed fill.

There were also some cases where the use of fungicide affected soybean plants. More importantly, aphid-infested fungicide-treated plants had reduced filling pods and total pods on 15 August. Soybean rust has recently been detected in North America, and agronomists are anticipating the need for fungicide sprays in certain years throughout much of the North American soybean producing region. Although the results of small scale cages do not necessarily translate into effects that will be seen at the field level, the effects of fungicides on pod set and seed set should be monitored.

Table 5.1. Plant characteristics of potted soybeans receiving various levels of shading in the 2002 shade experiment. Numbers in the same row followed by the same letter are not statistically significant at $p<0.05$ in a Fisher's protected LSD.

| | Shading | | | | |
|---|---|---|---|---|---|
| | 0% | 30% | 50% | 70% | 90% |
| Days to Reach Flowering | 24.3 a | 26.5 ab | 25.0 ab | 31.0 bc | 33.0 c |
| Flowers Set per Plant | 87.5 ab | 83.5 ab | 95.3 a | 70.3 b | 10.5 c |
| Pods set per Plant | 36 ab | 37.5 ab | 46.0 a | 32.0 b | 5.8 c |
| Percent of Pods Aborted | 46.9 a | 47.9 a | 49.2 a | 71.7 b | 100.0 c |
| Nodes per Plant | 42.3 a | 32.5 b | 36.8 ab | 29.8 b | 6.5 c |
| Pods Yielded per Plant | 18.8 ab | 19.0 bc | 23.8 a | 9.0 c | 0.0 d |
| Beans per Plant | 40.0 a | 42.0 a | 52.3 a | 18.3 b | 0.0 c |
| Weight of Beans per Plant | 4.8 a | 5.6 a | 6.5 a | 2.3 b | 0.0 c |

Table 5.2. Effect of aphid infestation of plant characteristics throughout the season in the 2003 cage study t values and p values are the result of Fisher's protected LSD.

| | Date | No Aphids | Aphids | t | p |
|---|---|---|---|---|---|
| Primary Leaves per Plant | 30-Jul | 9.1 | 7.3 | 5.85 | <0.0001 |
| | 5-Aug | 9.0 | 7.3 | 7.17 | <0.0001 |
| | 15-Aug | 10.3 | 7.6 | 8.74 | <0.0001 |
| Secondary Leaves per Plant | 30-Jul | 7.6 | 4.2 | 2.69 | 0.0074 |
| | 5-Aug | 8.4 | 2.5 | 2.93 | 0.0035 |
| | 15-Aug | 7.7 | 5.6 | 5.54 | <0.0001 |
| Flowers per Plant | 30-Jul | 38.4 | 29.9 | 2.60 | 0.0097 |
| | 5-Aug | 27.5 | 17.1 | 4.10 | <0.0001 |
| | 15-Aug | 15.2 | 8.7 | 2.82 | 0.0050 |
| Unfilled Pods per Plant | 30-Jul | 18.2 | 8.1 | 3.99 | <0.0001 |
| | 5-Aug | 38.7 | 26.2 | 6.48 | <0.0001 |
| | 15-Aug | 24.7 | 14.9 | 4.14 | <0.0001 |
| Filling Pods per Plant | 15-Aug | 1.7 | 0.5 | 20.18 | <0.0001 |
| Total Leaves per Plant | 30-Jul | 16.8 | 11.5 | 3.80 | 0.0002 |
| | 5-Aug | 16.7 | 12.9 | 4.34 | <0.0001 |
| | 15-Aug | 18.7 | 10.1 | 7.00 | <0.0001 |
| Total Nodes per Plant | 30-Jul | 16.8 | 11.5 | 3.68 | 0.0003 |
| | 5-Aug | 18.7 | 16.9 | 2.45 | 0.0147 |
| | 15-Aug | 23.0 | 16.8 | 4.70 | <0.0001 |
| Total Pods per Plant | 30-Jul | 16.8 | 11.5 | 3.27 | 0.0011 |
| | 5-Aug | 40.4 | 26.6 | 11.18 | <0.0001 |
| | 15-Aug | 53.6 | 22.5 | 5.73 | <0.0001 |

Table 5.3. Effect of sugar application on plant characteristics throughout the season in the 2003 cage study t values and p values are the result of Fisher's protected LSD.

| | Date | No Sugar | Sugar | t | p |
|---|---|---|---|---|---|
| Primary Leaves per Plant | 7-Aug | 8.7 | 7.9 | 5.59 | <0.0001 |
| | 15-Aug | 9.3 | 9.6 | 3.58 | 0.0004 |
| Total Leaves per Plant | 7-Aug | 16.2 | 13.8 | 3.77 | 0.0002 |
| | 15-Aug | 15.4 | 16.6 | 2.74 | 0.0063 |
| Total Nodes per Plant | 7-Aug | 18.8 | 16.6 | 2.66 | 0.0082 |

*Literature Cited*

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Appendices

## Appendix 1.1

### Voucher Specimen Data

Page 1 of 1 Pages

| Species or other taxon | Label data for specimens collected or used and deposited | Number of: Eggs | Larvae | Nymphs | Pupae | Adults ♀ | Adults ♂ | Other | Museum where deposited |
|---|---|---|---|---|---|---|---|---|---|
| *Aphis glycines* | MI, Van Buren Co., Keeler Twp.<br>31 August 2004 | | | | | | | ## | MSU |

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Abigail Jan Walter

Date 12 May 2005

Voucher No. 2005-03

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

Curator 12 May 2005

Date

Appendix 1

Record of Deposition of Voucher Specimens*

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

Voucher No.: 2005-03

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

EFFECTS OF FERTILIZATION ON THE SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA AND THE EFFECTS OF SOYBEAN APHIDS AND FERTILIZATION ON SOYBEAN PLANTS

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

Entomology Museum, Michigan State University (MSU)

Other Museums: