

COMPONENTS OF SOYBEAN RESISTANCE TO THE SOYBEAN APHID, *Aphis glycines*  
Matsumura

by

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## ABSTRACT

The soybean aphid, *Aphis glycines* Matsumura, is a pest of soybean, *Glycine max* (L.) Merr. Studies to find control methods were initiated in 2000 when it was first detected in the United States. *Aphis glycines* can reduce yields by as much as 50%, and vectors several viral diseases. Plant resistance to *A. glycines* is one important component of integrated control. In the first study, reproduction of *A. glycines* was compared on 240 soybean entries. Eleven had fewer nymphs produced compared with two susceptible checks (KS4202 and Pioneer<sup>®</sup> 95B15). Antibiosis and antixenosis were assessed in no-choice and choice tests, respectively. Nine entries showed moderate antibiosis and the other two (K1639 and Pioneer<sup>®</sup> 95B97) showed strong antibiosis and antixenosis as categories of resistance to *A. glycines*. In the second study, chlorophyll loss was estimated in no-choice tests on infested and uninfested leaves of KS4202. The minimum combined number to detect significant chlorophyll loss was 30 aphids confined for 10 days. Using this number, seven resistant entries found in the first study were evaluated. There was no significant chlorophyll reduction between infested and uninfested leaves of five of the resistant entries (K1621, K1639, 95B97, Dowling and Jackson). Jackson and Dowling had a significantly lower percentage loss than the susceptible checks. In the third study, assessment of feeding behavior of *A. glycines* was compared and recorded for 9 h on four resistant entries and KS4202. The average time needed to reach the first sieve element phase by *A. glycines* was 3.5 h in KS4202 while in the resistant entries it was 7.5 h, and the total duration in this phase was longer than an hour in KS4202, and only two to seven minutes in the resistant entries. These data suggest that phloem tissues in the resistant plants change feeding behavior. However, aphids first reached the xylem phase and then the sieve element phase, and the time that aphids spent ingesting xylem sap was not different among all entries; therefore, it is possible that xylem sap in the resistant entries may contain toxic substances that alter aphid behavior and restrain further activities on the sieve element phase.

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## **DEDICATION**

I dedicate this thesis to the memory of my Father. Also to my Mother, Francia, my siblings, Libardo and Monica, and all other family members for their constant support and for always believing in me.

# CHAPTER 1 - Review of literature

## Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a native of Asia (Blackman and Eastop 2000) and a relatively new pest of soybean, *Glycine max* (L.) Merr., in North America. *Aphis glycines* was observed for the first time during 2000 in Wisconsin; later it was found in Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, and West Virginia (NCPMC 2000). In August of 2002, *A. glycines* was confirmed in five eastern Kansas counties (Sloderbeck et al. 2003), and by 2003 had spread to 17 Kansas counties (Sloderbeck et al. 2004). *Aphis glycines* is considered the main pest of soybeans (Takahashi et al. 1993), and is the only aphid that develops large populations on soybean in North America (Sloderbeck et al. 2003).

*Aphis glycines* populations grow up and spread very fast (Wang et al. 1998). Adults and nymphs not only feed on soybean plants causing severe damage and losses, they also vector viral diseases such as soybean mosaic virus (SMV) (Guo and Zhang 1989, Wang et al. 1998).

Chemical control is the most common method for controlling *A. glycines* (Wang et al. 1998, Ye et al. 1996), but this can lead to insecticide resistance (Ye et al. 1996). Natural enemies may provide biological control (Wang et al. 1998), but *A. glycines* populations in fields are

generally very high, so beneficial insects may not maintain populations below damaging levels. The use of resistant varieties reduces the application of insecticides and increases the number of natural enemies on the fields (Ye et al. 1996). Since *A. glycines* appeared in United States in 2000, universities, private companies and other entities have initiated studies on this insect and its possible control methods.

### **Taxonomy, Origin and Geographic Distribution of *A. glycines***

*Aphis glycines* belongs to the order Hemiptera, suborder Sternorrhyncha, superfamily Aphidoidea and family Aphididae.

*Aphis glycines*, a native of Asia, was first described by Matsumura in 1917 (Matsumura 1917). *Aphis glycines* has been found in Japan (Sakai 1949 cited by Takahashi et al. 1993); Korea, China, Taiwan, Thailand, Malay (Paik 1965 cited by Takahashi et al. 1993), the Philippines (Takahashi 1966 cited by Takahashi et al. 1993), India (Raychaudhuri et al. 1980 cited by Takahashi et al. 1993), Indonesia (Iwaki 1979 cited by CAB International 2001), Malaysia and North Borneo (Blackman and Eastop 1985 cited by CAB International 2001), Russia (D'yakonov 1975 cited by CAB International 2001), Vietnam (Waterhouse 1993 cited by CAB International 2001) and Australia (Fletcher and Desborough 2000).

It was found in United States of America in 2000 (NCPMC 2000) and in Canada in 2001 (OMAFRA 2002).

## **Morphology, Biology and Ecology of *A. glycines***

*Aphis glycines* is a small, greenish-yellow aphid with black “tailpipes” or cornicles near the tip of its abdomen (Sloderbeck et al. 2003).

Adults may be winged (alate) or non-winged (apterous). Both of these forms can produce offspring. Winged individuals are produced in spring or fall seasons, or in response to crowding on a plant. Their main function is to fly to new host plants and produce nymphs. Apterous females are baby-making machines whose job is to increase the colony (DiFonzo 2001).

Winged viviparous females generally have a long-ovoidal form, are 0.96 to 1.52 mm in length, have red-brown compound eyes and a black head. The wingless viviparous females have an ovoid form, and are 0.95 to 1.29 mm in length. Morphologically, adults and nymphs are very similar (Wu et al. 1999).

Aphid populations build rapidly, doubling every 2 to 3 days, and may reach several thousand aphids per plant at their peak in early August. The majority of adults are wingless. Some females develop wings and fly to other plants within the same or nearby fields (Ostlie 2002). Under suitable climatic conditions, nymphs will develop to the adult stage in 5 days, and about 15 generations can be developed in one year (Wang et al. 1998). *Aphis glycines* molts 2-3 times and has 3-4 instars in one generation. *Aphis glycines* has phenomenon of deformed paedogenesis (Zhang 1988).

*Aphis glycines* has a heteroecious holocyclic life cycle (spending life on different unrelated hosts with sexual reproduction during portion of its cycle). The biology of *A. glycines* in North America is similar to the biology observed in China and Japan (Ragsdale et al. 2004).

*Aphis glycines* overwinters on buckthorn (*Rhamnus* sp.) as eggs on buds or in branch cracks. During spring when temperatures reaches 10°C, fundatrices hatch from overwintering eggs, feed on sprouts of buckthorn and later reproduce 1-2 generations by thelytoky (production of females parthenogenetically). When buckthorn blooms, winged aphids develop and migrate to soybean fields and feed on soybean seedlings. The first generation in soybeans is apterous; some aphids of the second generation become alatae and disperse in the field (Wang et al. 1998).

During summer, *A. glycines* in the field are females reproducing by parthenogenesis (nymphs developed without fertilization). Females give live birth to female offspring which mature and give birth in a matter of days (DiFonzo 2001). Reproductive females can deposit 2-3 young per day (Ostlie 2002).

By August, populations decline as winged aphids migrate away from fields (Ostlie 2002). *Aphis glycines* feeds late into the fall until plants dry down. In September, a generation of males is produced. They mate with females, and females fly to an overwintering host to lay eggs (DiFonzo 2001).

In China Wang et al. (1962) reported that *A. glycines* eggs overwinters on *Rhamnus davuricus* of the buckthorn family. Consequently Zhang and Zhong (1982), cited by Takahashi et

al. (1993), established that 15 species in the genus *Rhamnus* found in China were hosts of *A. glycines*.

In the United States, *A. glycines* overwinter as eggs on woody shrubs (*Rhamnus* sp.) (Sloderbeck et al. 2003). *Rhamnus cathartica* (common buckthorn) was a confirmed overwintering host in 2000 (DiFonzo 2001). *R. canthartica*, *R. alnifolia* (Voegtlin et al. 2004) and *R. lanceolata* (Voegtlin et al. 2005) were found to be overwintering hosts of *A. glycines*.

The development time of *A. glycines* depends on temperature and nutrition. Between 20-25°C, 5-7 days were enough for *A. glycines* nymphs to develop to adults under suitable nutritious conditions, and aphids reproduced rapidly (Sun et al. 2000). Average temperatures between 22-25°C and relative humidity less than 78% were optimal for the development of *A. glycines* in the field (Wang et al. 1962). Higher reproduction and adult longevity occurred at 22 °C (Hirano et al. 1996). In United States McCornack et al. (2004) studied the temperature effect on *A. glycines*. Aphids reproduced longer and produced more progeny at 20 and 25°C than at 30 or 35°C, and populations doubled in 1.5 days at 25°C while populations doubled in 1.9 days at 20 and 30°C. McCornack et al. (2005) studied the supercooling point or temperature at which freezing occurs on different *A. glycines* life stages. Eggs had the lowest supercooling point (-34°C), while gynoparae and oviparae had the highest (-15°C).

*Aphis glycines* populations decrease gradually when soybean growing points cease to grow (senescence) or when high temperature or heavy rain occurs. High temperature and high humidity have been shown to be detrimental to *A. glycines*. When mean temperature for 5 days

was above 25°C and relative humidity up to 80%, a large number of aphids died (Wang et al. 1998).

## **Economic Importance, Habits and Damage**

*Aphis glycines* is a monophagous and migrating pest that spreads and infests as winged forms (Wu et al. 1999). It is the main sap-sucking pest on soybeans (Takahashi et al. 1993) and is the only aphid that develops large colonies on soybeans in North America (Sloderbeck et al. 2003). High populations can cause severe damages and losses up to 50% (Wang et al. 1998); however, the potential damage of *A. glycines* to soybeans in Kansas is still unknown (Sloderbeck et al. 2004).

Adults and nymphs of *A. glycines* grow on tender leaves and young stems sucking sap from soybean plants. Under heavy infestations, aphids cover entire leaves and stems, and they can also feed on young pods (Wang et al. 1998).

Crowding of apterous adults is the major factor causing formation of the alatae aphids in the next generation (Lu and Chen 1993). In addition to crowding, the production of winged nymphs is triggered by reduced host quality or decreasing day length (Ostlie 2002).

Studies on the spatial distribution patterns of aphid populations on infested soybean plants in the field showed an aggregated spatial distribution pattern (Huang et al. 1992, Su et al. 1996). Rutledge and O'Neil (2006) studied the population growth on different soybean stages

and there was not effect of planting date on the dynamics of *A. glycines*. Life history parameters of *A. glycines* did not show differences when feeding in the different growth stages of soybeans.

Adults and nymphs of *A. glycines* extract phloem sap (photosynthates) with their piercing-sucking mouthparts, and leave numerous brown-yellow spots on infested leaves. Heavy infestations may cause curling and premature loss of leaves, reduce numbers of branches and pods, or even leave bare stalks (Wu et al. 1999). Other effects include underdeveloped roots, stunted plants, decreased seed weights (Wang et al. 1998), flower shedding and fruit dropping (Lin et al. 1993). Soybean growing in low potassium soils may become chlorotic in upper leaves, an unusual deficiency symptom (Ostlie 2002).

*Aphis glycines* only digests 10% of the nutrients they take in. The rest is secreted out of their body as honeydew that sticks on the surface of leaves and acts as a substrate for development of sooty mold or mildew, which turns the leaves black and rubbery (OMAFRA 2002). This affects the photosynthetic activity of plants, and leads to a reduction in yield and quality (Lin et al. 1993).

Yield losses in Suihua District (China) in 1998 averaged 30% (Sun et al. 2000). Severely infested fields in southeast Minnesota in 2001 showed almost a 50% yield reduction (Ostlie 2002). When infestations are heavy, plants in the seedling stage will die, and yield reductions of up to 20-30% or over 50% can occur (Wang et al. 1998). Wang et al. (1994) reported yield reductions of up to 51.8%.



Different control thresholds have been suggested. (550 aphids per 100 plants and 35% of the plants colonized by *A. glycines*) (Wang et al. 1994), (1500 aphids per 100 plants and 50% of plants colonized) (Sun et al. 2000), (more than 25 aphids per leaflet, alate nymphs (nymphs with shoulder pads) are less than 50% of the population and soybean stage is R1 or R2) (Steffey 2002). Grau et al. (2003) suggested an economic threshold for different crop stages (full bloom 200 or more aphids per plant), (beginning pod 1000 or more per plant), and (full pod 1500 or more aphids per plant). Hodgson et al. (2005) recommended an economic threshold of 250 aphids per plant. Onstad et al. (2005) studied sampling of *A. glycines* in soybean fields and concluded that 50 plants must be counted per field (2 ha.) to obtain a reliable assessment of the population.

Macedo et al. (2003) studied the photosynthetic responses of soybean (Asgrow 0901) to *A. glycines* injury. Photosynthetic capacity was affected by densities greater than 20 aphids per leaflet, but *A. glycines* injury did not cause a significant reduction in chlorophyll on soybeans.

Guo and Zhang (1989) studied the vectors of *soybean mosaic virus* (SMV). There were several aphid species reproducing and damaging soybean fields, but *A. glycines* was the most important vector of SMV epidemic. Its number occupied 74% of all aphid vectors. Wang et al. (1998) also reported *A. glycines* as a vector of the SMV.

*Aphis glycines* is also able to transmit other viruses like *abaca mosaic*, *beet mosaic*, *tobacco vein-banding mosaic virus*, *peanut stripe potyvirus*, and *mungbean mosaic virus* (CAB International 2001). In the United States, *A. glycines* has been found to be a vector of *alfalfa mosaic virus*, *bean yellow mosaic virus*, *peanut stunt virus*, *tobacco ringspot virus* (Clark and

Perry 2002), SMV (Clark and Perry 2002, Burrows et al. 2005), and *potato virus Y* (Davis et al. 2005).

## **Control of *A. glycines***

Chemical control is the most common method for controlling *A. glycines* in the fields in China (Wang et al. 1998, Ye et al. 1996). Some insecticides, especially foliar sprays, provide temporary suppression (7 to 14 days) of *A. glycines* populations (Ostlie 2002).

A big problem when spraying insecticides is the potential for population rebounding. Aphids that survive are in a less crowded environment and proceed to rebuild their populations. This has been found to occur when a product does not reduce the aphid population by 95% or more (OMAFRA 2002).

In Heilongjiang province (China) the following pesticides are used: 10% wettable Imidacloprid<sup>®</sup> powder (200-300 g/ha), 40% Dimethoate (1.1-1.5 liter/ha), 50% wettable Pirimicarb powder (225-300 g/ha), pyrethroid pesticides (500 ml/ha). All these pesticides are sprayed with 450-600 liter water/ha (Wang et al. 1998).

Huang et al. (1998) studied the control of *A. glycines* with Imidacloprid<sup>®</sup>. Four treatments were evaluated (15.0, 22.5, 30.0 and 45.0 g a.i./ha). The average control at 3, 7, 14, 21 and 28 days after application in the four treatments were 85.0, 91.2, 92.8, and 94.6%, respectively.

In the United States, Myers et al. (2005) studied the optimal insecticide timing for *A. glycines* using Warrior ( $\lambda$ -cihalothrin, 33.6 g/ha) and Lorsban (chlorpyrifos, 560.4 g/ha) at different soybean plant stages and concluded that when aphid populations are high, applications at the R2 and R3 plant stages prevent yield loss.

Seed treatments with systemic activity are effective against *A. glycines*, but currently none are labeled in the United States (Ostlie 2002). Seed-coating chemicals are using in China. Five percent Phorate granules (23 kg/ha) can be applied with fertilizers when seeds are sown (Wang et al. 1998).

Insecticides can also kill natural enemies of *A. glycines* and promote the development of pest resistance. Additionally, insecticides are often too costly. Therefore, a high priority must be given to the research on soybean insects and non-chemical control methods (Ye et al. 1996).

Increasing natural enemies will significantly depress aphid density. Common natural enemies in China include the multicolored Asian lady beetle, *Harmonia axyridis*; the seven-spotted lady beetle *Coccinella septempunctata*; the thirteen-spotted lady beetle, *Hippodamia tredecimpunctata* and the lacewing, *Chrysopa septempunctata* (Wang et al. 1998). The braconid wasps *Aphidius cingulatus*, *Ephedrus persicae* and *Ephedrus plagiator* could be effective primary parasitoids against *A. glycines*. Among the hyperparasitoids, *Asaphes vulgaris* and *Ardilea convexa* might be dominant species to primary parasitoids of *A. glycines*. The life span of hyperparasitoid and primary parasitoids was estimated to be 3-29 days and 1-4 days, respectively (Chang et al. 1994). Investigations in 1985-1990 found that parasitism rates of

*Lysiphlebia japonica* were between 10.3-52.6% in the field. *L. japonica* could effectively control *A. glycines* in the early season while aphids developed normally, and significantly depress aphid densities of next generation (Gao 1994). Liu et al. (2004) found the parasitoid, *Lysiphlebus* sp., and the predators, *Propylaea japonica*, *Scymnus (Neopullus) babai*, and *Paragus tibialis* to be the most abundant natural enemies of *A. glycines* in soybean field experiments. Although there are many natural enemies (parasites, predators and pathogens), their use as a method for controlling soybean pests has not yet been adopted extensively in China, even though it will be effective (Ye et al. 1996).

In the United States, Rutledge et al. (2004) studied the interaction of *A. glycines* with natural enemies in soybean fields, *Orius insidiosus* and *Harmonia axyridis* were the most common predators. Fox et al. (2005) studied the impact of various predator communities on *A. glycines* in soybean fields. Carabid beetles (*Elaphropus anceps*, *Clavina impressifrons* and *Bembidion quadrimaculatum*) and spiders (Salticidae and Lycosidae families) were the most abundant in the experiments. Nielsen and Hajek (2005) found parasitoids (*Aphidius* sp. and two *Praon* sp.), predators (coccinelids, syrphids, and cecidomyiids), and entopathogenic fungi (*Pandora neoaphidis*, *Conidiobolus thromboides*, *Entomophthora chromaphidis*, *Pandora* sp., *Zoophthora occidentalis*, *Neozygites fresenii*, and *Lecanicillium lecanii*) attacking *A. glycines* in soybean fields.

Fan (1988) tested 181 soybean genotypes for resistance to *A. glycines* from 1983 to 1986. Only two varieties, Qingpi-pingdingxiang and Dulu-dou, showed high resistance in the year of a severe infestation of aphids. He et al. (1995) studied soybean resistance to *A. glycines* and found

populations much lower in resistant than in susceptible varieties. At the stage of flower bud differentiation, the average aphid population averaged 97.4 on the resistant varieties Guoyu 98-4 and Guoyu 100-4, while 640.4 on the susceptible varieties Amsoy, Tiefeng 20 and Wenfeng 5. Ten days later, an average of 243.2 and 1819.4 aphids was observed on resistant and susceptible varieties, respectively. The intrinsic rate of natural increase was higher in susceptible varieties than in resistant ones, 1.13% and 0.83%, respectively. In a free choice experiment most of the aphids moved to susceptible varieties and 72 h after inoculation, the number of aphids was higher in these varieties.

Hu et. al (1992) studied the relationship between the nitrogen content in soybean leaves and the occurrence of *A. glycines*. The soybean species Jinong 82 had not only the highest nitrogen content, but also the largest aphid population while in the species Jiling 21 the nitrogen content and the aphid populations were the lowest. They concluded that the nitrogen content on leaves is a food factor influencing the incidence of *A. glycines* that could impact resistance. Hu et al. (1993) noticed that levels of lignin in the leaves are involved in the chemical defense mechanism of soybean plants to *A. glycines*. Varieties with high levels of this substance were resistant to the insect. The cultivar Tiefeng 24 showed the highest lignin level in its leaves, the lowest infestation index and the least damage. In the variety Jinong 82 with the lowest lignin level, the infestation index was the highest.

In the United States, Hill et al. (2004a) studied *A. glycines* colonization on *Glycine* species and other legumes. Colonization occurred on *Glycine* species, but not on Hyacinth bean, *Lablab purpureus*; Lentil, *Lens culinaris*; common bean, *Phaseolus vulgaris*; pea, *Pisum sativa*,

*Vicia* sp. and *Vigna* sp. Hill et al. (2004b) tested more than 1500 soybean genotypes and found resistance in nine genotypes. The soybean entries Jackson, Dowling, and Palmetto were found to be highly resistant to *A. glycines*. Dowling and Jackson were found to have antibiosis as a category of resistance to *A. glycines* (Hill et al. 2004b, Li et al. 2004). A single dominant gene, named *Rag1*, controls resistance in Dowling (Hill et al. 2006). Mensah et al. (2005) evaluated 2147 soybean accessions, and four were found resistant to *A. glycines*. PI 567541B and PI 567598B had antibiosis and PI 567543C and PI 567597C possessed antixenosis.

A variety of cultural practices such as crop rotation, intercropping, interplanting, and burning or removal of crop residues, have been used for controlling soybean pests in China. And these practices must be combined with biological control and host-plant resistance strategies (Ye et al. 1996) for effective management.

## Objectives

Some studies on soybean resistance to *A. glycines* have been conducted. However, it is very important to continue with these studies in order to incorporate important components for the management of *A. glycines*. The use of host plant resistance to insects is a vital alternative method of control because it can reduce the application of insecticides and also maintain population of natural enemies in the field. The objectives of this research were:

1. To identify sources of resistance to *A. glycines* by comparing aphid reproduction on several soybean genotypes.
2. To characterize categories of resistance to *A. glycines* in selected soybean entries.
3. To determine the minimum number of days and the lowest number of aphids needed to cause chlorophyll loss in a susceptible soybean cultivar (KS4202).
4. To compare the reduction of chlorophyll on susceptible and resistant soybean entries in order to develop a bioassay for assessing chlorophyll loss resulting from *A. glycines* feeding.
5. To compare the feeding behavior of *A. glycines* on resistant and susceptible soybean genotypes using the electrical penetration graph (EPG) technique.

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## **CHAPTER 2 - Characterization of Antibiosis and Antixenosis to the Soybean Aphid (Hemiptera: Aphididae) in Several Soybean Genotypes**

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## Abstract

The soybean aphid, *Aphis glycines* Matsumura, is an introduced pest of soybean, *Glycine max* (L.) Merr., in North America, and may reduce yields by 50%. Since 2000, when *A. glycines* was first detected in the United States, studies of this insect and possible control methods have been initiated. Plant resistance to this aphid species is one important component of integrated control. Reproduction of *A. glycines* was compared on 240 soybean entries in a pesticide-free greenhouse. Eleven entries had fewer nymphs produced, compared with the susceptible checks, and were used in follow-up experiments to assess antibiosis and antixenosis. Antibiosis was estimated in true no-choice tests, in which adults were confined individually in double-sided sticky cages stuck to the upper side of leaves. Antixenosis was assessed in choice tests, in which all entries were planted in a single pot. Adult aphids were placed in the center of the pot, and 24 h later the number of adults on each plant was counted. Of the 11 entries evaluated, nine showed a moderate antibiotic effect to *A. glycines*, and the other two (K1639 and Pioneer<sup>®</sup> 95B97) showed not only a strong antibiotic effect, but were the only entries exhibiting antixenosis as a category of resistance to *A. glycines*. The resistant soybean entries found in this work are potential sources for *A. glycines* control.

**KEY WORDS** *Aphis glycines*, *Glycine max*, antibiosis, antixenosis



## Resumen (Spanish)

El áfido de la soya, *Aphis glycines* Matsumura, es una plaga introducida de la soya, *Glycine max* (L.) Merr., en Norte América y puede causar pérdidas en rendimiento hasta en un 50%. Se iniciaron estudios de este insecto y posibles métodos de control desde el año 2000, cuando fue detectado en los Estados Unidos. La Resistencia de plantas es un importante método de control en el manejo integrado contra este áfido. La reproducción de *A. glycines* fue comparada en 240 genotipos de soya en condiciones de invernadero libre de pesticidas. Once genotipos presentaron un número más bajo de ninfas que los testigos susceptibles y fueron usados en subsiguientes experimentos para detectar antibiosis y antixenosis. La antibiosis fue determinada por medio de experimentos de no-selección, donde adultos fueron confinados individualmente en pequeñas jaulas de doble faz pegadas sobre el haz de las hojas. La antixenosis fue estimada por medio de experimentos de libre selección, en los que diferentes genotipos fueron sembrados en un mismo recipiente. Los adultos fueron colocados en el centro del recipiente y el número de adultos en cada planta fue contado 24 horas después. De los 11 genotipos estudiados, nueve mostraron un moderado nivel de antibiosis al insecto, y los otros dos (K1639 y Pioneer<sup>®</sup> 95B97), no sólo presentaron un alto nivel de antibiosis, sino que fueron los únicos genotipos que exhibieron antixenosis como categoría de resistencia. Los genotipos resistentes encontrados en este trabajo son potenciales fuentes de resistencia para controlar *A. glycines*.

## Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native from Asia (Blackman and Eastop 2000), where it is the main pest in soybean, *Glycine max* (L.) Merr., fields (Takahashi et al. 1993). *Aphis glycines* is a new pest of soybean in North America since 2000, when it was observed for the first time in Wisconsin; later the same year it was found in Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio, and West Virginia (NCPMC 2000). *Aphis glycines* is a migrating pest that spreads and infests as winged forms (Wu et al. 1999), it is the main sap-sucking pest on soybeans fields (Takahashi et al. 1993), and it is the only aphid that develops large colonies on soybean in North America (Sloderbeck et al. 2003). *Aphis glycines* populations increase rapidly and spread quickly (Wang et al. 1998). High populations can cause severe damage; yield losses of as much as 50% have been reported in China (Wang et al. 1994, Wang et al. 1998) and North America (Ostlie 2002). Also, *A. glycines* can vector viral diseases such as soybean mosaic virus (SMV) (Guo and Zhang 1989, Wang et al. 1998). In the United States, Clark and Perry (2002) identified *A. glycines* as a vector of alfalfa mosaic virus, bean yellow mosaic virus, tobacco ringspot virus, and SMV. Chemical control is the most common method for controlling this aphid pest (Ye et al. 1996, Wang et al. 1998, Ostlie 2002), but high use of insecticides stimulates the development of insecticide resistance (Ye et al. 1996), and can reduce natural enemy populations that may provide biological control (Wang et al. 1998).

The use of resistant cultivars reduces the application of insecticides, thus helps to maintain natural enemies on the fields (Ye et al. 1996). Some investigations in soybean

resistance to *A. glycines* have been conducted. Fan (1988) screened 181 soybean genotypes for resistance to *A. glycines*, and two showed high levels of resistance. He et al. (1995) conducted studies of resistance to *A. glycines* in soybean fields and observed that resistant cultivars had much lower populations, were less preferred for feeding and habitat, and were more tolerant than susceptible varieties. High concentrations of lignin in soybean leaves are involved in the chemical defense mechanism to *A. glycines* in soybean plants (Hu et al. 1993), whereas leaves with the highest nitrogen content supported the largest aphid populations (Hu et al. 1992).

In the United States, Hill et al. (2004b) used clones of *A. glycines* to test more than 1500 soybean genotypes and found resistance in nine genotypes, Mensah et al. (2005) evaluated 2147 soybean accessions, and four were resistant to *A. glycines*, Li et al. (2004) studied the fecundity, mortality and maturation of *A. glycines* on resistant and susceptible soybean genotypes, Hill et al. (2004a) studied *A. glycines* colonization on *Glycine* species and other legumes, and Hill et al. (2006) determined the inheritance of resistance to *A. glycines* in a resistant soybean cultivar.

Three basic categories characterize plant resistance to insects: antibiosis, antixenosis, and tolerance (Painter 1951, Smith 2005). Although some successful studies in soybean plant resistance to the *A. glycines* have been conducted, resistance has been found only in a few soybean genotypes. For this reason we conducted no-choice tests to identify antibiosis, which affects the biology of the insect (Smith 2005), and choice tests to establish antixenosis (or nonpreference), in which the plant is a poor host to the insect (Smith 2005). The objectives of this work were to identify additional sources of resistance to *A. glycines* by comparing aphid reproduction on several soybean genotypes, and to characterize antibiosis and/or antixenosis as

categories of resistance to *A. glycines*. The genotypes Dowling, Jackson, and Palmetto found highly resistant to *A. glycines* by Hill et al. (2004b), were included as resistant checks in the no-choice and choice tests.

## **Materials and Methods**

### **Insect Culture**

*Aphis glycines* was originally collected from soybean fields in Geary County, Kansas, in August 2002 and the population was maintained on the soybean cultivar KS4202 in a pesticide-free greenhouse at 20 to 30°C, 20 to 40% RH, and with supplemental lights from high-pressure sodium lamps set for a photoperiod of 14:10 (L:D) h. Voucher specimen 180 for this colony is on file at the Kansas State University Museum of Entomological and Prairie Arthropod Research. Soybean plants were grown in pots (11.5 cm in diameter by 10 cm in height, with one to two plants per pot). All the experiments presented here were performed under the same greenhouse conditions as described earlier.

### **Screening Test of Soybean Entries**

A total of 240 soybean entries were screened for resistance to *A. glycines*. Reproduction of *A. glycines* was tested first on 196 soybean Pioneer<sup>®</sup> entries (named with a code for proprietary materials [PHIAG], followed by a number [001 thru 196]), and subsequently was

tested on 44 Kansas State University public lines (Table 1). Plants were grown separately in 3.8-cm-diameter by 21.0-cm-deep plastic Cone-tainers<sup>TM</sup> (Ray Leach Cone-tainer<sup>TM</sup>, Hummert International, Earth City, MO) containing steam-sterilized potting mix (Premier Promix<sup>®</sup>, Rivière-du-Loup, Québec, Canada). The 240 entries were evaluated in groups of 10 to 13 entries per experiment; each experiment included the susceptible check, KS4202, as a control. Each entry had five replicates in individual Cone-tainers<sup>TM</sup> that were placed on racks in a completely randomized design. The Cone-tainers<sup>TM</sup> were separated to avoid inter-plant aphid movements.

According to Hill et al. (2004b), soybean resistance to *A. glycines* was observed in all plant stages, and life history parameters were similar on the different growth stages of soybean plants (Rutledge and O'Neil 2006). Therefore, at 9 d after planting, or when soybean plants reached the V-1 stage (Fig. 1), with two fully developed leaves at unifoliate nodes (Fehr et al. 1971, Kilgore and Fjell 1997), plants were selected for infestation. Six adults were placed on the upper side of the leaves of each plant, by using a moist camel's hair paint brush (number 0), and were allowed to freely feed and reproduce. He et al. (1995) tested several soybean cultivars for resistance to *A. glycines* and found that susceptible genotypes have a higher number of nymphs, compared with resistant genotypes; therefore, in this study, nymph populations were used as an index to differentiate resistant and susceptible genotypes. Seven days after the infestation, the number of nymphs produced was counted on the entire plant. After testing the 240 soybean entries and using the same experimental design, those entries with nymph populations statistically lower than nymphs on the susceptible check, KS4202, in the five replicates, were retested to confirm potential resistance. To ensure greater accuracy in the experiments, the

soybean genotype Pioneer<sup>®</sup> 93B15 was included as a second susceptible check from this point forward.

### **Antibiosis or No-choice Tests**

Antibiosis as a category of resistance to *A. glycines* was studied on those soybean entries in which nymph populations were statistically lower than populations on the susceptible checks in the screening test. Hill et al. (2004b) showed that the genotypes Dowling, Jackson, and Palmetto were highly resistant to *A. glycines*; therefore, these three entries, along with the two susceptible checks, were also included in this experiment as resistant checks.

The selected soybean entries were planted following the protocol described for the screening test. When plants reached the V-1 stage, 10 plants per entry were selected for aphid infestation. The Cone-tainers<sup>™</sup> were placed separately in racks arranged in a completely randomized design. Two double-sided sticky cages (Converters, Inc., Huntingdon Valley, PA, USA), with an inner oval area of 1.2 cm<sup>2</sup>, were stuck to the upper side of each leaf, for a total of two cages per plant (Fig. 2). One adult aphid was placed inside each cage and the cage was immediately covered with a piece of organdy cloth slightly larger than the cage. Progeny were counted 4 d after infestation.

To reduce variability in reproduction of nymphs inside the cages, same-age adults were used. Several adults were placed on a soybean plant (KS4202) and were allowed to reproduce nymphs. Adults were removed 24 h later. According to McCornack et al. (2004), nymphs turn

into adults between 6.6 and 5.1 d at 20 and 30°C, respectively. Therefore, nymphs in this experiment were left for 7 d until they developed into adults before placing them inside the cages.

### **Antixenosis or Choice Tests**

Antixenosis was assessed on the same genotypes used in the antibiosis test. Two similar experiments were performed with different entries. Entries were planted together and arranged in a circle around a single pot (20-cm diameter by 20-cm height, with a distance of  $\approx 3.5$  cm between plants). When plants reached the V-1 stage, adults were released on a filter paper (11-cm diameter) placed at the center of the circle of plants (Fig. 3). The number of adults on each plant was counted 24 h later. In experiment I (100 adult aphids per pot) and experiment II (150 adult aphids per pot) pots were arranged in a completely randomized design with six and seven replicates (pots), respectively.

### **Statistical Analyses**

Analysis of variance for *A. glycines* population among entries was conducted by using Proc GLM. Multiple comparisons were computed by using Tukey's Studentized Range Test ( $P < 0.05$ ) (SAS Institute 1999).

## Results and Discussion

### Screening of Soybean Entries

Nineteen experiments (1-19) were performed to test the 196 soybean Pioneer<sup>®</sup> entries, but only eight entries had significantly ( $P < 0.05$ ) fewer nymphs than the susceptible check KS4202 did (Table 2). In Table 2, Pioneer<sup>®</sup> Commercial Designations are shown instead of property codes. When these eight entries were retested, they had significantly ( $F = 33.38$ ;  $df = 9, 40$ ;  $P < 0.001$ ) lower nymph populations, compared with the two susceptible checks (Fig. 4A), with the exception of Pioneer<sup>®</sup> YB59T03. The 44 Kansas State University soybean entries were evaluated in four experiments (I-IV), and four of these entries had significantly ( $P < 0.05$ ) lower nymph populations than the susceptible checks (Table 2). These cultivars were retested along with the susceptible checks in a separate test to corroborate their smaller numbers of nymphs. All four entries again sustained significantly ( $F = 36.11$ ;  $df = 5, 24$ ;  $P < 0.001$ ) fewer nymphs than did the susceptible checks (Fig. 4B).

Two entries, Pioneer<sup>®</sup> 95B97 (Fig. 4A) and K1639 (Fig. 4B), had significantly fewer nymphs than the other entries, suggesting that they are highly resistant to *A. glycines*, whereas the other entries could have intermediate level of resistance. The low populations of nymphs found on these 11 entries indicate that antibiosis and/or antixenosis could be conferring resistance to *A. glycines*.



## Antibiosis Tests

Antibiosis was tested on those entries that had significantly fewer nymphs produced than were on the susceptible checks in the previous experiment (screening test) (Fig. 4). Two no-choice tests were conducted; the no-choice test I included the seven Pioneer<sup>®</sup> entries with fewer nymphs produced in the screening test and the two susceptible checks (Fig. 5A). The no-choice test II included the four Kansas State University entries that had significantly lower nymph populations in the screening test, Pioneer<sup>®</sup> 95B97, the resistant checks (Dowling, Jackson, and Palmetto), and the two susceptible checks (Fig. 5B).

In all the entries evaluated, the average number of nymphs produced after 4 d of confinement for a single adult aphid was statistically (Figs. 5A [ $F = 5.19$ ;  $df = 8, 171$ ;  $P < 0.001$ ] and 5B [ $F = 66.35$ ;  $df = 9, 190$ ;  $P < 0.001$ ]) different than the nymph population produced on the susceptible checks KS4202 and Pioneer<sup>®</sup> 93B15. This indicated that all entries possess antibiosis as a category of resistance to *A. glycines*. The numbers of nymphs produced on the entries K1639, Pioneer<sup>®</sup> 95B97, Jackson, Dowling, and Palmetto were the lowest and were not significantly different from each other (Fig. 5B). This low level of reproduction indicates a strong antibiotic effect of these entries to *A. glycines*.

## Antixenosis Tests

Antixenosis was compared among the same entries from the antibiosis test by using two choice tests. In experiment I, Pioneer<sup>®</sup> 95B97 was the only entry with a statistically ( $F = 6.42$ ;  $df$

= 8, 45;  $P < 0.001$ ) lower number of adults than the number of adults found on the susceptible checks 24 h after aphid release (Table 3), indicating a strong antixenotic effect of this entry to *A. glycines*, whereas the numbers of adults found on the other entries were not different from those on the susceptible checks. In experiment II, Jackson, Dowling, Palmetto, and K1639 had significantly ( $F = 20.58$ ;  $df = 9, 60$ ;  $P < 0.001$ ) fewer adults than the susceptible checks, and their numbers were not different statistically from number of adults on Pioneer<sup>®</sup> 95B97 (Table 3), indicating that all of these entries exhibit antixenosis as a category of resistance to *A. glycines*.

Resistance is often found in a low percentage of the plant material evaluated (Smith 2005). Hill et al. (2004b) screened more than 1500 soybean genotypes, and only nine ( $\approx 0.6\%$ ) showed resistance to *A. glycines*, whereas Mensah et al. (2005) evaluated 2147 soybean accessions, and just four ( $\approx 0.2\%$ ) were resistant to the aphid. In our experiments, 240 entries were evaluated, and 11 ( $\approx 5\%$ ) showed different levels of resistance to *A. glycines*. In the screening test, we were looking for those entries with lower nymph populations; 240 entries were screened and 11 entries were found to have fewer nymphs than were on the susceptible checks. When these 11 entries were compared in the no-choice tests, all of them showed antibiosis at different levels. But when choice tests were performed, only two (K1639 and Pioneer<sup>®</sup> 95B97) of them showed antixenotic effects against *A. glycines*.

The soybean entries Jackson, Dowling, and Palmetto were found to be highly resistant to *A. glycines* as previously demonstrated by Hill et al. (2004b). These three entries, along with the entries K1639 and Pioneer<sup>®</sup> 95B97 found in this work, were confirmed to possess both antibiosis

and antixenosis as categories of resistance to *A. glycines*. Dowling and Jackson were confirmed to have antibiosis as a category of resistance to *A. glycines* (Li et al. 2004), and recently it was demonstrated that a single dominant gene, named *Rag1*, controls resistance in Dowling (Hill et al. 2006). Discover of high levels of resistance in K1639 and Pioneer<sup>®</sup> 95B97 will add a very important complement and component for sustainable management of *A. glycines*.

In this work, we developed new and simple methods that resulted in highly repeatable data to study soybean resistance to *A. glycines*. However, sometimes it is not easy to make a clear distinction between antibiosis and antixenosis (Smith 2005). In this study, the strong antixenosis found in K1639 and Pioneer<sup>®</sup> 95B97 may result in a reduction of antibiosis parameters. Therefore, we recommend that demographic and life-history parameters of *A. glycines* be studied on these two resistant genotypes.

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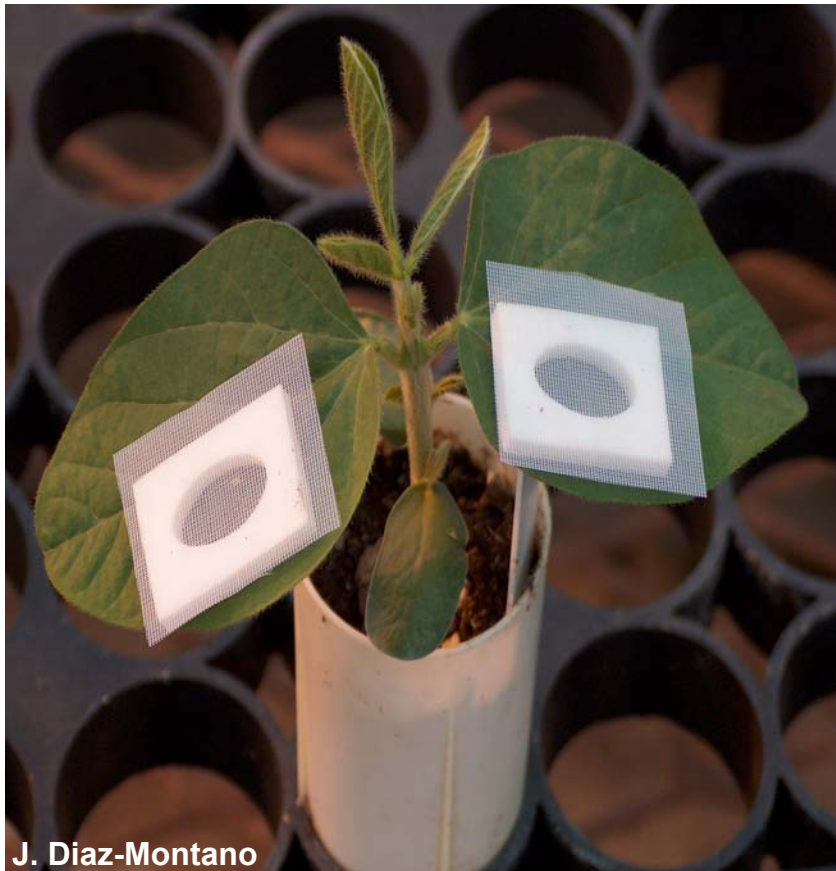
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**Figure 1. Soybean plant at the V1 stage.**



**Figure 2. Antibiosis or no-choice experimental setup. Aphids were placed inside cages on unifoliate leaves.**

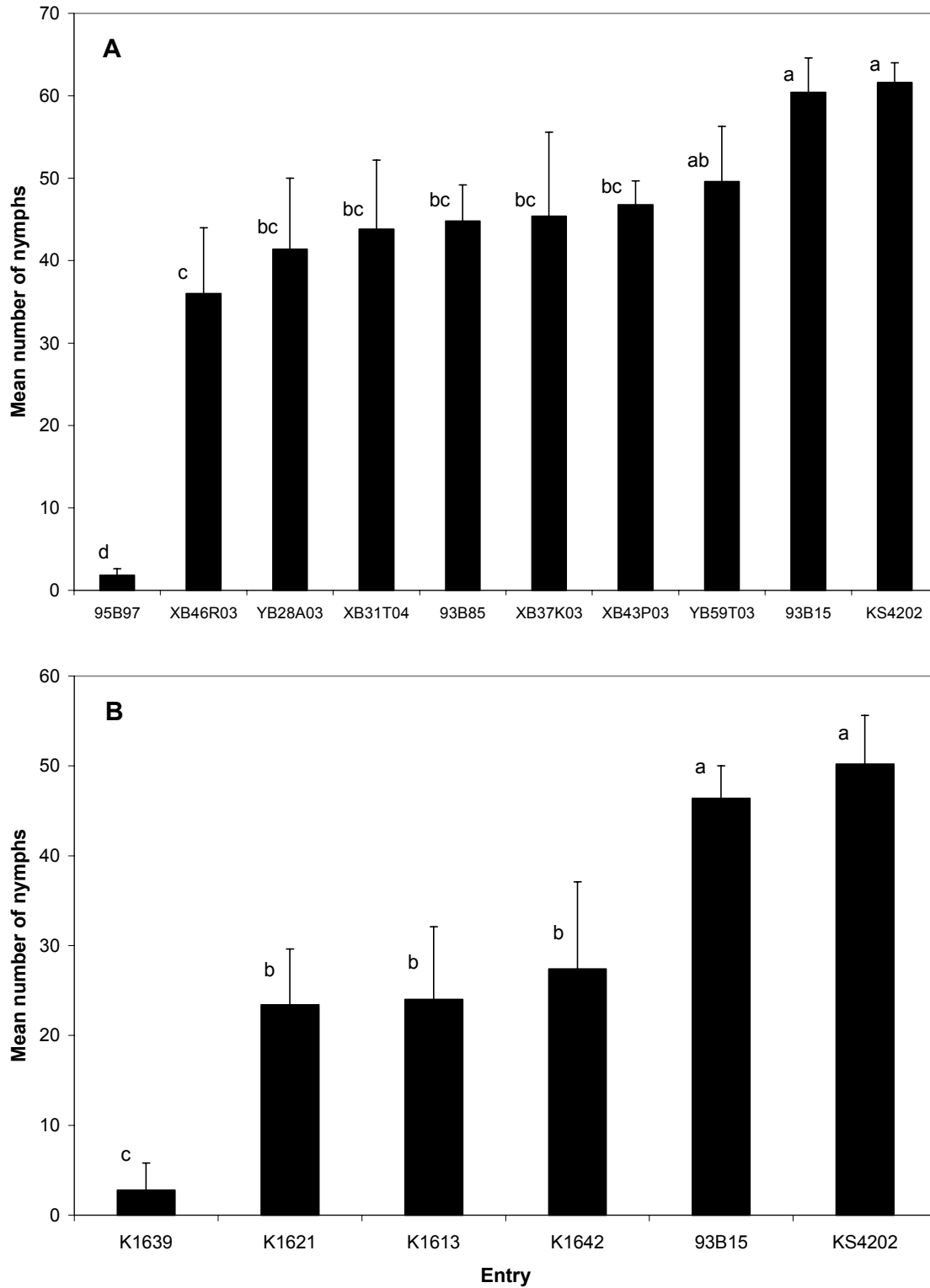


**Figure 3. Antixenosis or choice test experimental setup. Aphids were released on filter paper.**



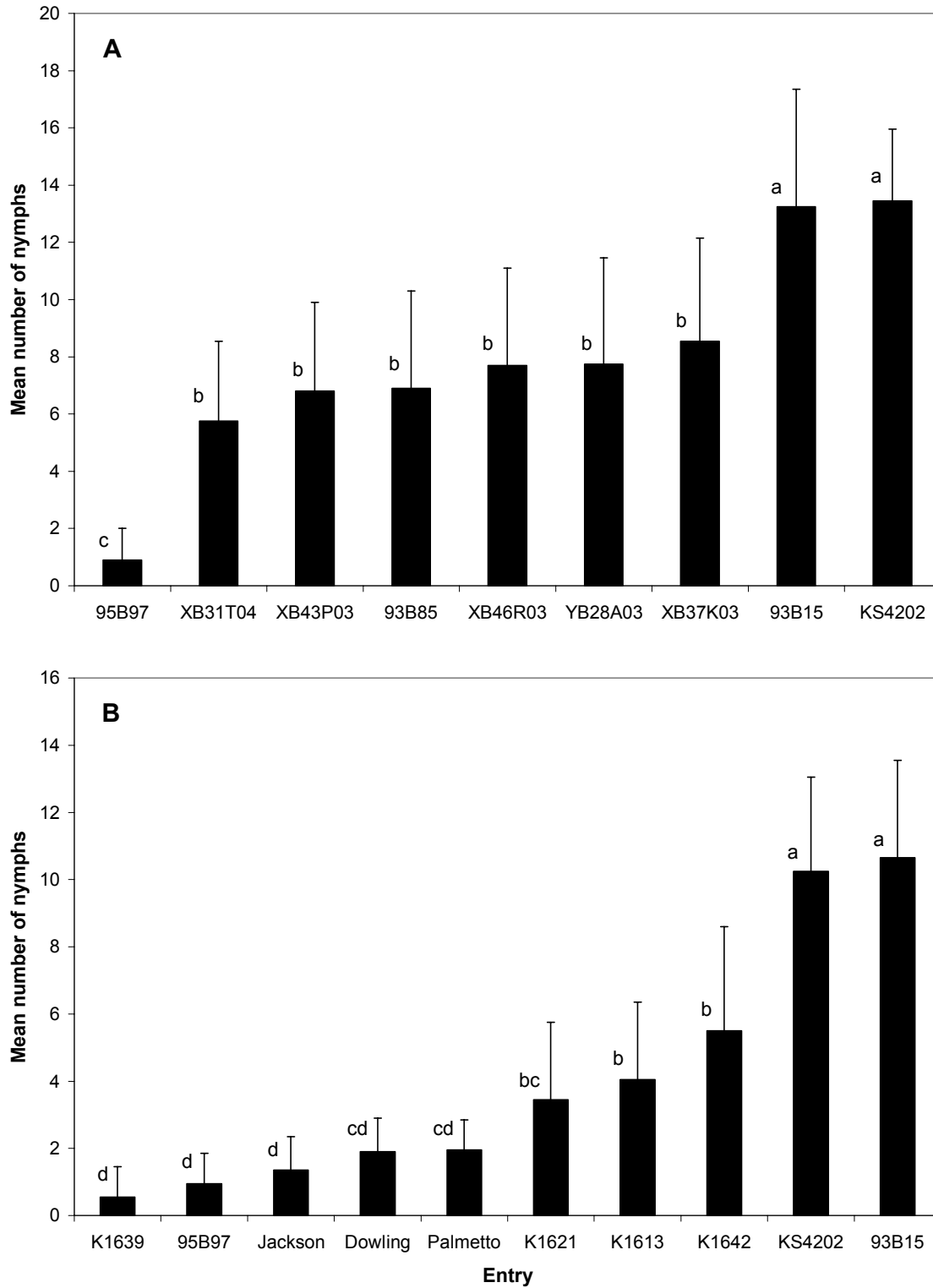
**J. Diaz-Montano**

**Figure 4. Number (mean  $\pm$  SE) of nymphs (screening retests) produced by six adults on different soybean entries 7 d after infestation.**



(A) Pioneer® entries and the two susceptible checks. (B) Kansas State University entries and the two susceptible checks. Bars with different letters are significantly different ( $P < 0.05$ , Tukey's test).

**Figure 5. Number (mean  $\pm$  SE) of nymphs (antibiosis tests) produced by one confined adult on different soybean entries 4 d after infestation.**



(A) No-choice test I: seven Pioneer® entries and the two susceptible checks. (B) No-choice test II: four Kansas State University entries, Pioneer® 95B97, resistant checks (Dowling, Jackson, Palmetto) and susceptible checks. Bars with different letters are significantly different ( $P < 0.05$ , Tukey's test)

**Table 1. List of Kansas State University soybean entries screened for resistance to *A. glycines***

<b>Entry</b>	<b>Pedigree</b>
K1599	Dekalb CX445 x Northrup King S46-44
K1607	Manokin x LN93-11586
K1613	CX1512-8 x K1218
K1614	Saturn x SS1386-5-2
K1619	K1364 x IA3010
K1620	K1370 x Pioneer <sup>®</sup> 9352
K1621	NTCPR94-5483 x Pana
K1622	NTCPR94-5483 x Pana
K1639	R93-174 x Northrup King S59-60
K1641	KS5502N x Pioneer <sup>®</sup> 9352
K1642	CX1512-8 x K1218
K1603RR	(KS4895(2)) x (Resnik(2) x 40-3-2)
K1623RR	Pioneer <sup>®</sup> 9352 x K97-132
K1624RR	U94-2306 x K97-132
K1625RR	U94-2306 x K97-132
K1626RR	Pioneer <sup>®</sup> 9352 x K97-132
K1627RR	U94-2306 x K97-132
K1628RR	Pioneer <sup>®</sup> 9352 x K97-132
K1629RR	IA3010 x K97-136
K1630RR	Pioneer <sup>®</sup> 9352 x K97-132
K1631RR	Pioneer <sup>®</sup> 9352 x K97-132
K1632RR	K1235 x K97-132
K1633RR	Delsoy 5500 x K97-132
K1634RR	Delsoy 5500 x K97-132
K1635RR	Delsoy 5500 x K97-132
K1636RR	Delsoy 5500 x K97-132
K1637RR	K1276 x K97-134
K1638RR	K1276 x K97-134
K1640RR	KS4997 x K97-138
KS4694	Sherman x Toano
KS4602N	Delsoy 4710 x KS4694
KS4895	Sherman x Bay
KS4997	Pioneer <sup>®</sup> 5482 x Asgrow 3127
KS5004N	KS5292 x SC91-2007
KS5502N	Hartwig x KS4895
KS4103sp	Flyer x Barc 6
KS4302sp	Hutcheson x Nattosan
KS4303sp	Jack x Mercury
KS4402sp	Hutcheson x Barc 6
KS4702sp	Saturn x Jack
KS5001sp	Hutcheson x SS201
KS5003sp	KS5292 x Mercury
KS5201sp	Camp x Sherman
KS5202sp	Hutcheson x Barc 9

**Table 2. Numbers of *A. glycines* nymphs (screening tests) on promising soybean entries (significantly lower nymph population), compared with the susceptible check KS4202 for that particular experiment 7 d after infestation**

Experiment	Entry (mean* $\pm$ SE) <sup>#</sup>	KS4202 (mean* $\pm$ SE) <sup>#</sup>	Degrees of freedom	<i>F</i>	<i>P</i>	
6	Pioneer <sup>®</sup> 93B85	14.4 $\pm$ 6.4b	45.8 $\pm$ 5.1a	10 <sup>+</sup> , 44 <sup>~</sup>	3.46	0.020
8	Pioneer <sup>®</sup> 95B97	2.2 $\pm$ 0.8c	52.6 $\pm$ 9.0ab	9, 40	10.93	< 0.0001
9	Pioneer <sup>®</sup> XB37K03	19.6 $\pm$ 10.9c	45.8 $\pm$ 9.7a	11, 48	3.47	0.0013
9	Pioneer <sup>®</sup> XB43P03	17.0 $\pm$ 12.7bc	45.8 $\pm$ 9.7a	11, 48	3.47	0.0013
9	Pioneer <sup>®</sup> XB46R03	13.6 $\pm$ 8.5bc	45.8 $\pm$ 9.7a	11, 48	3.47	0.0013
13	Pioneer <sup>®</sup> YB28A03	28.4 $\pm$ 7.1c	48.8 $\pm$ 12.4ab	10, 44	4.48	0.0002
14	Pioneer <sup>®</sup> XB31T04	29.0 $\pm$ 9.1c	61.6 $\pm$ 7.5ab	11, 48	4.80	< 0.0001
18	Pioneer <sup>®</sup> YB59T03	23.2 $\pm$ 5.0c	49.2 $\pm$ 5.2ab	11, 48	6.96	< 0.0001
I	K1613	65.4 $\pm$ 22.9bc	106.4 $\pm$ 22.0a	11, 48	16.31	< 0.0001
I	K1621	32.8 $\pm$ 8.7cd	106.4 $\pm$ 22.0a	11, 48	16.31	< 0.0001
I	K1639	3.6 $\pm$ 3.3d	106.4 $\pm$ 22.0a	11, 48	16.31	< 0.0001
II	K1642	39.0 $\pm$ 5.9b	57.6 $\pm$ 5.6a	12, 52	24.31	< 0.0001

\* Average of five replicates, nymphs produced by six adults per replicate 7 d after infestation

<sup>#</sup> Within a row, means followed by different letters are significantly different ( $P < 0.05$ , Tukey's test)

<sup>+</sup> Corrected number of entries evaluated

<sup>~</sup> Corrected number of replications

**Table 3. Number of adults in antixenosis tests found on different soybean entries in two choice tests after 24 h.**

Experiment I		Experiment II	
Entry	Adults (mean <sup>*</sup> ± SE) <sup>#</sup>	Entry	Adults (mean <sup>^</sup> ± SE) <sup>#</sup>
Pioneer <sup>®</sup> 93B15	12.3 ± 3.9a	KS4202	20.1 ± 5.7a
KS4202	9.8 ± 2.5ab	Pioneer <sup>®</sup> 93B15	19.0 ± 4.2a
Pioneer <sup>®</sup> YB28A03	8.7 ± 1.4ab	K1621	18.9 ± 5.4a
Pioneer <sup>®</sup> 93B85	8.3 ± 3.9ab	K1642	17.6 ± 6.2a
Pioneer <sup>®</sup> XB37K03	8.3 ± 2.5ab	K1613	15.3 ± 3.4a
Pioneer <sup>®</sup> XB31T04	7.2 ± 1.5abc	Jackson	8.4 ± 1.1b
Pioneer <sup>®</sup> XB46R03	6.5 ± 3.6bc	Dowling	7.6 ± 2.7b
Pioneer <sup>®</sup> XB43P03	5.0 ± 3.7bc	Palmetto	6.0 ± 1.2b
Pioneer <sup>®</sup> 95B97	1.8 ± 1.2c	Pioneer <sup>®</sup> 95B97	4.7 ± 1.5b
		K1639	4.4 ± 1.7b

\* Average of six replicates, 100 adults per replicate.

<sup>^</sup> Average of seven replicates, 150 adults per replicate.

<sup>#</sup> Within a column, means followed by different letters are significantly different ( $P < 0.05$ , Tukey's test)

Experiment I: seven Pioneer<sup>®</sup> entries and the two susceptible checks. Experiment II: four Kansas State University entries, Pioneer<sup>®</sup> 95B97, the resistant checks (Dowling, Jackson, and Palmetto), and the two susceptible checks



**CHAPTER 3 - Chlorophyll Loss Caused by Soybean Aphid  
(Hemiptera: Aphididae) Feeding on Soybeans**

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## Abstract

The soybean aphid, *Aphis glycines* Matsumura, is a worldwide pest of soybean, *Glycine max* (L.) Merr. Studies to find control methods were initiated in 2000 when it was first detected in North America. *Aphis glycines* can reduce yields by as much as 50% and vectors several viral diseases. *Aphis glycines* removes phloem sap from leaves, which can result in a reduction of chlorophyll content. Quantification of chlorophyll loss caused by *A. glycines* feeding on soybean is of vital importance. The SPAD-502 chlorophyll meter is a device that has been used to measure chlorophyll loss caused by nonchewing insects. Chlorophyll loss was studied in no-choice tests on the infested and uninfested leaves of a susceptible check (KS4202). The minimum combined number of days and aphids needed to detect significant chlorophyll loss was 30 aphids confined for 10 days. In a similar experiment, seven resistant entries and two susceptible checks were evaluated. There was no significant chlorophyll reduction between infested and uninfested leaves of five of the resistant entries (K1621, K1639, Pioneer<sup>®</sup> 95B97, Dowling, and Jackson). Percentage loss of the susceptible checks was around 40%; Jackson and Dowling had a significantly lower percentage loss (13 and 16%, respectively) than did the susceptible checks. The percentages loss of K1621, K1639 and Pioneer<sup>®</sup> 95B97 was not statistically different from the percentage loss of Jackson.

**KEY WORDS** *Aphis glycines*, *Glycine max*, chlorophyll losses

## Resumen (Spanish)

El áfido de la soya, *Aphis glycines* Matsumura, es una plaga de la soya, *Glycine max* (L.) Merr. En el año 2000 se encontró *A. glycines* en Norte América, y desde entonces, se iniciaron estudios para encontrar métodos de control. *Aphis glycines* reduce el rendimiento en un 50%, y transmite virus. *Aphids glycines* extrae el floema de las hojas lo cual puede tener efectos en la fisiología de la soya como la reducción de clorofila. Por lo tanto, la evaluación de las pérdidas de clorofila en soya causadas por *A. glycines* es de mucha importancia. El medidor SPAD-502 se usa para cuantificar pérdidas de clorofila causadas por insectos no masticadores. Se estudiaron las pérdidas en hojas infestadas y no-infestadas del testigo susceptible KS4202. Se descubrió que el número mínimo de áfidos necesario para observar pérdidas significativas de clorofila es de 30 áfidos confinados por 10 días. En un experimento análogo, siete genotipos resistentes y dos susceptibles fueron evaluados. De los genotipos resistentes, cinco (K1621, K1639, Pioneer<sup>®</sup> 95B97, Dowling y Jackson) no mostraron reducción en su contenido de clorofila en hojas infestadas y no-infestadas. El porcentaje de pérdida de los genotipos susceptibles fue del 40% aproximadamente; Jackson y Dowling tuvieron porcentajes de pérdida (13 y 16%, respectivamente) significativamente más bajos que los testigos susceptibles. Sin embargo, los porcentajes de K1621, K1639 y Pioneer<sup>®</sup> 95B97 no fueron estadísticamente diferentes al porcentaje de pérdida de Jackson.

## Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), a pest of soybean, *Glycine max* (L.) Merr., was first identified in the United States in 2000 (NCPMC 2000). *Aphis glycines* is the main sap-sucking pest in soybeans fields (Takahashi et al. 1993), and it is the only aphid that develops large colonies on soybean in North America (Sloderbeck et al. 2003). *Aphis glycines* populations grow and spread rapidly (Wang et al. 1998). High populations can cause severe damage and cause yield losses greater than 50% (Wang et al. 1994, Wang et al. 1998, Ostlie 2002). In addition to crop loss resulting from direct damage, this aphid also vectors several viral diseases, such as soybean mosaic virus (Clark and Perry 2002). Therefore, the potential losses caused by *A. glycines* on soybean are great. Adults and nymphs extract phloem sap (photosynthates) with their piercing-sucking mouthparts (Wang et al. 1998, Wu et al. 1999), and leave numerous brown-yellow spots on the infested leaves (Wu et al. 1999) that can affect soybean physiology. Reduction of chlorophyll due to herbivores populations may negatively affect the photosynthetic capacity of plants (Wang et al. 2004). Because *A. glycines* damage on soybean can cause reduction in chlorophyll content, quantification of chlorophyll loss is important. The use of the SPAD-502 chlorophyll meter is a rapid and nondestructive technique (Yadava 1986, Deol et al. 1997) that can be used to measure chlorophyll losses caused by nonchewing insects (Deol et al. 1997). Miller et al. (1994) and (Deol et al. 2001, Macedo et al. 2003b) used it to measure the loss of chlorophyll caused by the Russian wheat aphid on barley and wheat, respectively; Deol et al. (1997), Girma et al. (1998), and Nagaraj et al. (2002a, 2002b, 2005) used the SPAD technique to assess feeding damage by greenbug feeding on sorghum; and Deol et al. (2001) and Boina et al. (2005) used it to quantify chlorophyll losses caused by greenbugs on wheat leaves. These studies have shown that aphid damage induces measurable

changes in chlorophyll content. Macedo et al. (2003a), using a portable photosynthesis system and a chlorophyll fluorometer, studied the physiological responses of soybean to *A. glycines* feeding; photosynthetic rates were affected by densities greater than 20 aphids per leaflet, but *A. glycines* injury did not affect the chlorophyll contents of soybean.

The objectives of this study were 1) to determine the minimum number of days and the least number of aphids needed to cause chlorophyll loss on a susceptible soybean cultivar (KS4202), and 2) to compare the reduction of chlorophyll on susceptible and resistant soybean entries, in order to develop a bioassay for assessing chlorophyll loss resulting from *A. glycines* feeding.

## **Materials and Methods**

### **Insect Culture**

*Aphis glycines* was collected from soybean fields in Geary County, Kansas, in August 2002, and maintained on the soybean cultivar KS4202 in a pesticide-free greenhouse at 20 to 30°C, 20 to 40% RH, with supplemental lights from high-pressure sodium lamps set for a photoperiod of 14:10 (L:D) h. *Aphis glycines* voucher specimen was deposited at the Kansas State University Museum of Entomological and Prairie Arthropod Research-MEPAR (voucher collection number 180).

## Plant Material

Seven resistant and two susceptible entries were studied. Previously, we found five soybean entries resistant to *A. glycines* (Diaz-Montano et al. 2006). Three entries (K1613, K1621 and K1642) showed antibiosis as a category of resistance to *A. glycines*. Another KSU entry, K1639, and Pioneer<sup>®</sup> 95B97 were found highly resistant and displayed antibiosis as well as antixenosis as resistance categories. The other two resistant entries included were Dowling and Jackson, found highly resistant to *A. glycines* by Hill et al. (2004). The soybean genotypes KS4202 (KSU) and 93B15 (Pioneer<sup>®</sup>) were included as susceptible checks. Experiments were performed under the same greenhouse conditions described earlier.

## Timing and Infestation Rates

The susceptible check KS4202 was used to determine timing and infestation rates needed to find differences in chlorophyll losses caused by *A. glycines*. Plants were grown separately in a 3.8-cm-diameter by 21.0-cm-deep plastic cone-tainer<sup>™</sup> (Ray Leach Cone-tainer<sup>™</sup>, Hummert International, Earth City, MO) containing steam-sterilized potting mix (Premier Promix<sup>®</sup>, Canada). When soybean plants reached the V-1 stage, two fully developed leaves at unifoliate nodes (Fehr et al. 1971, Kilgore and Fjell 1997), a double-sided sticky cage (Converters, Inc., Huntingdon Valley, PA, USA), with an inner oval area of 1.2 cm<sup>2</sup>, was fixed to the upper side of each leaf by using steel curl clips (Goody<sup>®</sup>, USA). There were two cages per plant, one infested and the other uninfested; both cages were covered with a slightly larger piece of organdy cloth (Fig. 6). The upper side of the leaves was chosen in order to facilitate the set up of the cage. And,

Markwell et al. (1995) studied the relationship of soybean and maize (*Zea mays* L.) leaf chlorophyll content with the SPAD-502 chlorophyll meter and did not find significant differences in chlorophyll measurements taken from adaxial (upper) and abaxial (lower) surfaces.

Five experiments were performed with different numbers of mixed aphids (adults and nymphs) confined for different numbers of days. In experiment I and experiment II, the infested cages included 5, 10 and 20 aphids confined for 4 and 7 days, respectively. In experiment III, IV and V, 30 and 40 aphids were confined for 4, 7, and 10 days, respectively. There were five replications for experiments I and II, and three replications for experiments III, IV, and V. Replications in individual Cone-tainers<sup>TM</sup> were placed on racks in a completely randomized design.

After the cages and aphids were removed, the chlorophyll content was measured within the caged area on the upper side of infested and uninfested leaves, by using the soil plant analysis development (SPAD) 502 chlorophyll meter (Minolta Camera Co., Osaka, Japan) (Fig. 7). Three readings were taken from each site (infested caged area and uninfested caged area), and the mean was calculated.

### **Chlorophyll Losses on Different Soybean Entries**

Findings on the aphid number and time of infestation determined by the previous experiment were applied in a comparable experiment that was performed on seven soybean

aphid-resistant entries, K1613, K1621, K1639, K1642, Pioneer® 95B97, Dowling, and Jackson and two susceptible checks, KS4202 and Pioneer® 93B15. A total of nine entries were planted and infested according to the protocol described in the previous experiment. The Cone-tainers™ were placed separately in racks, arranged in a completely randomized design. When plants reached the V-1 stage, six plants per entry were selected for aphid infestation. A double-sided sticky cage was fixed to the upper side of each leaf; at the end of the experiment, the cages and insects were removed and chlorophyll contents were taken by using the SPAD-502 chlorophyll meter as explained in the previous experiment. To observe the percentage of chlorophyll loss caused by *A. glycines* on each soybean entry, a SPAD chlorophyll-loss index (Deol et al. 1997) was calculated according to the following formula: SPAD Index:  $(C - T)/C$ , where C is the SPAD measurement from the uninfested or control area and T is the SPAD measurement from the infested or treated area.

## **Statistical Analyses**

Analysis of variance for chlorophyll content (SPAD values) and chlorophyll loss (SPAD Index) were conducted by using Proc GLM. Multiple comparisons were computed with Tukey's Studentized Range Test ( $P < 0.05$ ) (SAS Institute 1999).



## Results

### Timing and Infestation Rates

Five experiments, each with different treatments, were carried out to find the minimum combined number of days and aphids that can cause a significant reduction in chlorophyll in the susceptible check KS4202. There were no statistical differences between the chlorophyll contents measured on the uninfested and infested leaves in experiment I (5, 10, and 20 aphids confined for 4 days), experiment II (5, 10, and 20 aphids restricted for 7 days) or experiment III (30 and 40 aphids confined for 4 days) (Table 4). In experiment IV, significant differences ( $P < 0.05$ ) were observed among the chlorophyll contents taken from leaves infested with 30 and 40 aphids confined for 7 days. In experiment V, in which 30 and 40 aphids were confined for 10 days, were significant differences ( $P < 0.05$ ) detected in the chlorophyll measured from the infested and uninfested leaves in both treatments (Table 4).

### Chlorophyll Losses on Different Soybean Entries

The treatment selected for this experiment was 30 aphids confined for 10 days. The chlorophyll content measured in the infested and uninfested leaves of KS4202, Pioneer<sup>®</sup> 93B15 (susceptible entries), K1642, and K1613 (intermediate-resistant entries) were statistically ( $F = 10.95$ ;  $df = 10, 95$ ;  $P < 0.001$ ) different (Fig. 8). The chlorophyll contents in infested and uninfested leaves of the other five resistant entries were not significantly different (Fig. 8).

The SPAD indices range from zero (no loss of chlorophyll) to one (total loss of chlorophyll, 100%) (Deol et al. 1997). The chlorophyll reduction caused by *A. glycines* on the susceptible entries KS4202 and Pioneer<sup>®</sup> 93B15 approximated 40% (Fig. 9). Only Dowling and Jackson had significantly ( $F = 4.68$ ;  $df = 8, 45$ ;  $P = 0.003$ ) less chlorophyll loss than the susceptible checks. But, the SPAD chlorophyll-loss indices on K1613, K1621, Pioneer<sup>®</sup> 95B97, and K1639 were not significantly different from Jackson, which showed the lowest SPAD chlorophyll-loss index (Fig. 9). The average SPAD chlorophyll-loss index of all the entries was 27% (Fig. 9).

## Discussion

Differences were detected in the chlorophyll content of infested and uninfested leaves of the susceptible genotype KS4202 when 30 and 40 aphids were confined for 10 days. Macedo et al. (2003a) reported that *A. glycines* feeding did not cause a significant reduction in chlorophyll, and they recommended studying photosynthetic responses by using different aphid densities. In our study, differences in chlorophyll content were not easily detected. It was necessary to conduct several experiments with increasing aphid densities and number of days to detect a reduction in chlorophyll content. Deol et al. (2001) found that chlorophyll content was affected on a susceptible wheat variety infested with five to seven greenbugs confined for only 4 days. In our study, 5, 10, 20, 30, and 40 *A. glycines*, confined for 4 days on a soybean leaf, did not significantly reduce chlorophyll content in any of the treatments. Therefore, we agree with Macedo et al. (2003a) that the chlorophyll content on soybean is not immediately affected by *A. glycines* feeding. In our study, the difference in chlorophyll content between infested and

uninfested leaves increased as the number of aphids and confinement time increased; this indicates that chlorophyll content can be reduced, and may affect the photosynthetic capacity of susceptible soybean when *A. glycines* populations increase through time.

When chlorophyll content was measured in all the entries, there was no significant reduction in chlorophyll between the infested (30 aphids) and uninfested leaves of the genotypes K1621, Pioneer<sup>®</sup> 95B97, K1639, Dowling, and Jackson. These five entries can develop normally under *A. glycines* infestations because not only were they resistant to *A. glycines* (Diaz-Montano et al. 2006) but their SPAD chlorophyll-loss indices found in this study were below the average SPAD index. Lack of differences in chlorophyll content in these five entries might be thought to assess tolerance, but the numbers of aphids “tolerated” by all entries has to be nearly identical. In our tests, even with 30 aphids per cages, it is possible that resistance, previously confirmed in the resistant entries (Diaz-Montano et al. 2006), may have reduced the number of aphids. So, although differences were not detected in the most resistant entries, we cannot call this a true test of tolerance. However, the accuracy of the SPAD chlorophyll technique has been proved, not only as an equivalent method, but also as a more rapid technique than other tolerance measurements, such as proportional dry weight change and tolerance index (Girma et al. 1998, Flinn et al. 2001, Boina et al. 2005).

Nagaraj et al. (2002a) found that greenbug feeding affected photosynthetic rate more than it affected chlorophyll content in sorghum. Therefore, it is possible that parameters other than chlorophyll reduction may be first severely affected by *A. glycines* feeding on soybean. Thus,

additional studies are required to quantify other photosynthetic parameters after *A. glycines* feeding has occurred on soybean.

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**Figure 6. Chlorophyll loss experimental setup. Aphids were caged on unifoliate leaves.**

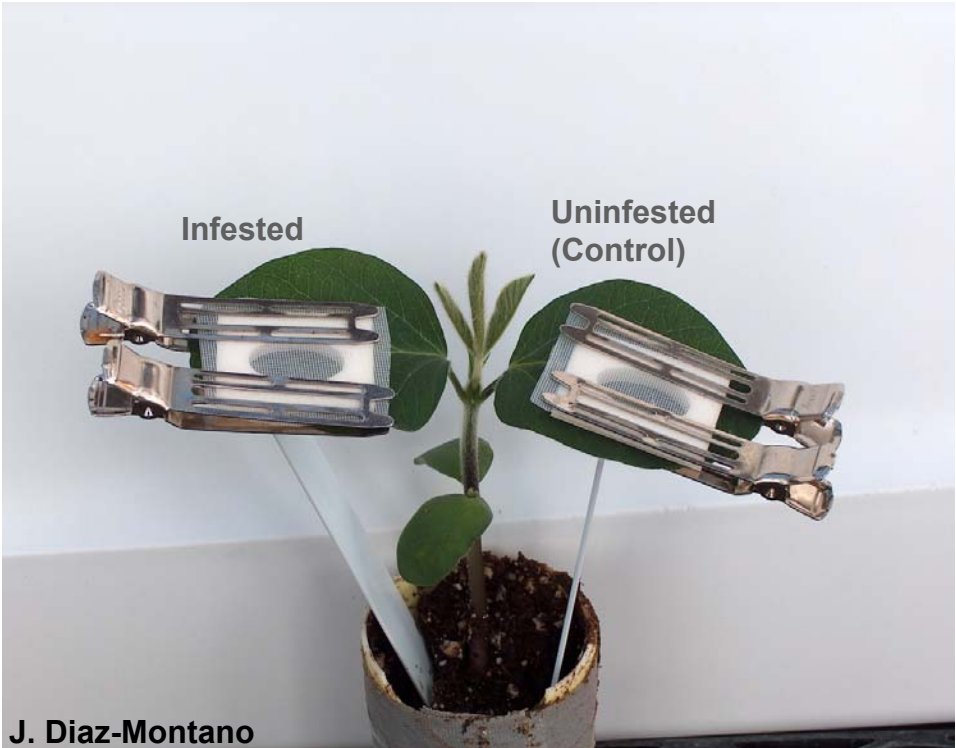
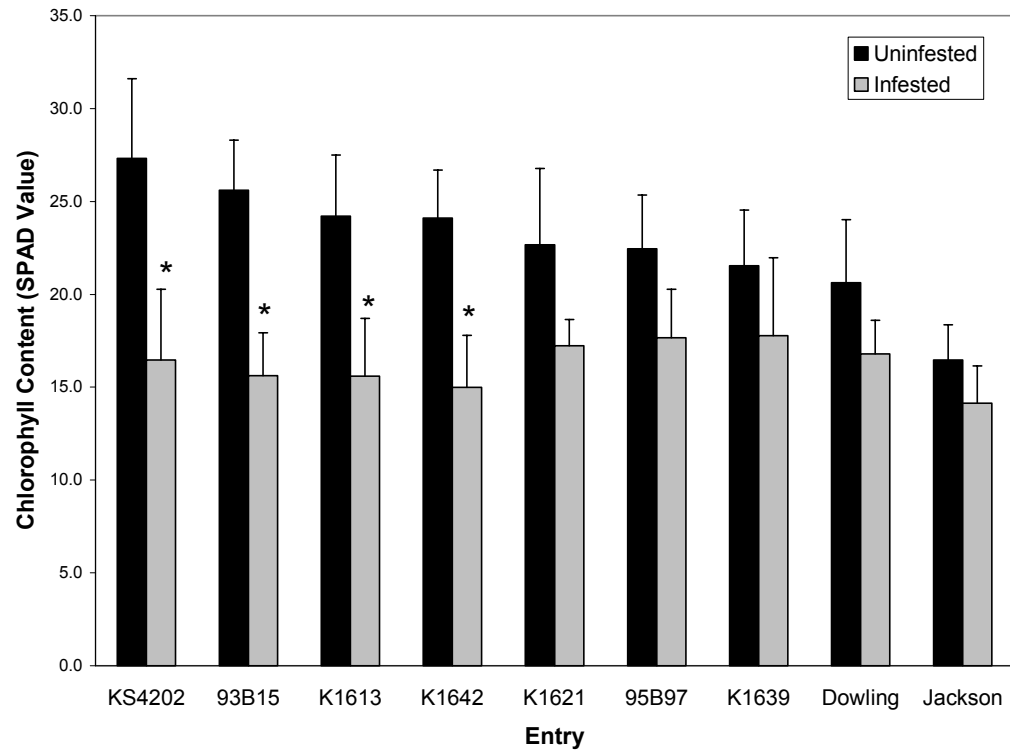


Figure 7. SPAD-502 chlorophyll meter.



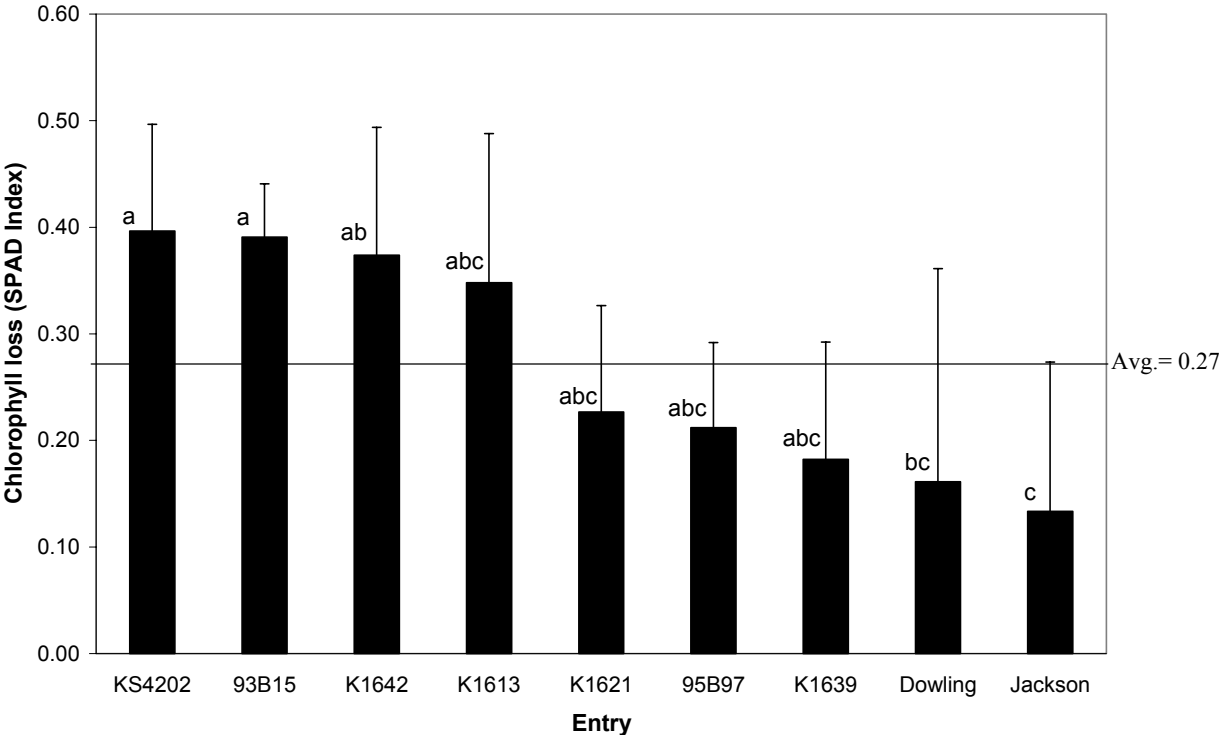
J. Diaz-Montano

**Figure 8. Chlorophyll content (mean  $\pm$  SE) in the infested (30 aphids confined for 10 days) and uninfested leaves of susceptible and resistant soybean entries.**



\*significantly different ( $P < 0.05$ , Tukey's test).

**Figure 9. Chlorophyll losses (mean  $\pm$  SE) caused by *A. glycines* on susceptible and resistant soybean entries.**



Bars with different letters are significantly different ( $P < 0.05$ , Tukey's test).

**Table 4. Chlorophyll content (mean  $\pm$  SE) in the infested and uninfested leaves of the susceptible entry KS4202**

Exp	Treatment	Chlorophyll content (SPAD Value)		df	<i>F</i>	<i>P</i>
		Uninfested	Infested			
I	5 aphids confined for 4 d	37.8 $\pm$ 1.1a*	36.8 $\pm$ 3.1a	5, 24	1.10	0.3847
	10 aphids confined for 4 d	36.3 $\pm$ 3.7a	34.7 $\pm$ 4.8a			
	20 aphids confined for 4 d	38.1 $\pm$ 2.0a	35.0 $\pm$ 1.5a			
II	5 aphids confined for 7 d	31.6 $\pm$ 5.2a	30.0 $\pm$ 5.5a	5, 24	0.90	0.4960
	10 aphids confined for 7 d	34.0 $\pm$ 0.6a	32.2 $\pm$ 1.6a			
	20 aphids confined for 7 d	33.3 $\pm$ 1.8a	31.5 $\pm$ 1.7a			
III	30 aphids confined for 4 d	30.4 $\pm$ 3.6a	28.0 $\pm$ 4.6a	3, 8	0.36	0.7826
	40 aphids confined for 4 d	28.8 $\pm$ 4.9a	26.7 $\pm$ 4.7a			
IV	30 aphids confined for 7 d	21.1 $\pm$ 1.4bc	17.5 $\pm$ 1.6c	3, 8	21.79	0.0003
	40 aphids confined for 7 d	26.8 $\pm$ 1.1a	24.4 $\pm$ 1.9ab			
V	30 aphids confined for 10 d	19.1 $\pm$ 4.0a	10.6 $\pm$ 2.6bc	3, 8	8.19	0.0080
	40 aphids confined for 10 d	17.0 $\pm$ 1.9ab	9.2 $\pm$ 2.8c			

\*Within an experiment, means followed by different letters are significantly different ( $P < 0.05$ , Tukey's test)

## **CHAPTER 4 - Feeding Behavior by the Soybean Aphid (Hemiptera: Aphididae) on Resistant and Susceptible Soybean Genotypes**

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## Abstract

The soybean aphid, *Aphis glycines* Matsumura, is a major pest of soybean, *Glycine max* (L.) Merr. Since 2000, when *A. glycines* was detected in the United States, several studies on this insect have been done in different areas; however, there is no report of any stylet penetration behavior studies of *A. glycines* on resistant and susceptible soybeans. Assessment of feeding behavior of this aphid species was compared on four resistant entries (K1639, Pioneer<sup>®</sup> 95B97, Dowling and Jackson) and a susceptible check (KS4202) using the electrical penetration graph (EPG) technique. Feeding behavior of *A. glycines* adults was recorded during a 9 h period. The average time needed to reach the first sieve element phase by *A. glycines* was 3.5 h in KS4202 while in the resistant entries it was 7.5 h. The total duration in the sieve element phase was longer than an hour in KS4202, and only two to seven minutes in the resistant entries. These results suggest that morphological or chemical factors in the phloem tissue of resistant plants affects stylet penetration activities of *A. glycines*. However, in the majority of the recordings, the aphid stylet reached the xylem phase before penetrating the sieve element, and the time that aphids spent ingesting xylem sap was not different among all entries. Therefore, it is possible that xylem sap in the resistant entries may contain toxic substances that change aphid behavior and affect further activities in the sieve element phase.

**KEY WORDS** *Aphis glycines*, *Glycine max*, feeding behavior



## Resumen (Spanish)

El áfido de la soya, *Aphis glycines* Matsumura, es una de las plagas más importantes de la soya, *Glycine max* (L.) Merr. Desde el año 2000, cuando *A. glycines* fue encontrado en los Estados Unidos, diferentes estudios se han realizado; sin embargo, no hay ningún estudio sobre el comportamiento alimenticio de *A. glycines* en genotipos de soya resistentes y susceptibles. Usando la técnica “electrical penetration graph (EPG)”, se comparó el comportamiento alimenticio de este insecto, durante un periodo de 9 h, en cuatro genotipos resistentes (K1639, Pioneer<sup>®</sup> 95B97, Dowling y Jackson) y un testigo susceptible (KS4202). El tiempo promedio para alcanzar el floema por *A. glycines* fue 3.5 h en KS4202 mientras que en los genotipos resistentes el tiempo fue de 7.5 h. El tiempo total en el floema fue mayor de una hora en KS4202, y únicamente de dos a siete minutos en los genotipos resistentes. Estos resultados indican que factores morfológicos o químicos presentes en el tejido del floema de los genotipos resistentes afectan la actividad de penetración de los estiletes de *A. glycines*. Sin embargo, en la mayoría de las repeticiones, los áfidos alcanzaron el xilema antes de penetrar el floema, y el tiempo que los áfidos ingirieron la savia del xilema no fue diferente entre todos los genotipos. Por lo tanto, es posible que el xilema en los genotipos resistentes contenga sustancias tóxicas que cambien el comportamiento del áfido y afecten su actividad posterior en el floema.

## Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a pest of soybeans, *Glycine max* (L.) Merr., and has been in the United States since 2000 (NCPMC 2000). *Aphis glycines* is considered the major pest of soybean (Takahashi et al. 1993) because it not only causes significant damage that can turn into yield losses greater than 50% (Wang et al. 1994, Wang et al. 1998, Ostlie 2002), but it also vectors several economically important viruses (e.g. soybean mosaic virus) (Clark and Perry 2002). *Aphis glycines* feeds on the phloem sap by penetrating soybean plants with its piercing-sucking mouthparts (Wang et al. 1998, Wu et al. 1999), which break through plant tissues until the vascular tissue is reached (Tjallingii and Hogen Esch 1993).

Stylet penetration by aphids is indispensable in host plant acceptance and rejection (Prado and Tjallingii 1997); in fact, every species of aphid we have tested, using the electrical penetration graph (EPG) technique (Fig. 10), will initiate probing on every species of non-host plant they have been offered (unpublished data). Since *A. glycines* appeared in North America, several studies on this insect have been done in diverse areas which include chemical control (Myers et al. 2005), biological control (Fox et al. 2005, Nielsen et al. 2005), plant viruses (Clark and Perry 2002, Burrows et al. 2005, Davis et al. 2005), ecology (McCornack et al. 2005, Voegtlin et al. 2005), and plant resistance (Hill et al. 2004a, 2004b, 2006; Li et al. 2004; Mensah et al. 2005; Diaz-Montano et al. 2006) among others. The only study reporting the use of the EPG technique for observing *A. glycines* feeding behavior was conducted in China by Han and

Yan (1995) where stylet activities on host (soybean) and nonhost plants (cotton, *Gossypium hirsutum*; cucumber, *Cucumis sativa*; and loofah, *Luffa cylindrical*) were examined.

The term EPG was first introduced and described by Tjallingii (1985). The EPG consists of a device that connects a wired aphid and a plant into an electrical circuit (Gabrys and Tjallingii 2002); when penetration of the stylets starts, the electrical circuit is completed and waveforms are observed and recorded; the different EPG waveforms (A, B, C, pd, E1, E2, F and G) characteristics and their correlations with the position of the stylet tips in the plant tissue were summarized by van Helden and Tjallingii (2000). The waveforms reveal different insect activities, such as mechanical stylet work, salivation, sap ingestion and position of the stylet tips within the plant (Tjallingii 2006). The waveforms are grouped into three main behavioral phases: pathway phase, phloem or sieve element phase, and xylem phase (Reese et al. 2000, Tjallingii 2006). The pathway phase (A, B, and C) constitutes multiple stylet penetration activities such as intercellular stylet insertion and withdrawal, periods of no stylet movement, and brief intracellular punctures by stylet tips (Jiang and Walker 2001), also known as potential drops or pds (Prado and Tjallingii 1994). The pathway phase is very important because during this phase the insect locates the sieve element (primary ingestion site), and accepts or rejects the host (Jiang and Walker 2001). The sieve element phase begins with a salivation period (E1) followed by phloem sap ingestion with continuous salivation (E2) (Tjallingii 2006). The xylem phase (G) is related to water intake (Spiller et al. 1990).

The EPG technique has been widely used to study the feeding behavior of sucking insects such as aphids; among some aphids studied using the EPG are: the bird cherry-oat aphid,

*Rhopalosiphum padi* (Prado and Tjallingii 1994, 1997, 1999); the black bean aphid, *Aphis fabae* (Spiller et al. 1990, Tjallingii and Hogen Esch 1993, Tjallingii 1994, Prado and Tjallingii 1997, 1999; Tosh et al. 2001, Powell and Hardie 2001, 2002); the blackberry-grain aphid, *Sitobion fragariae* (Ramirez and Niemeyer 2000); the cabbage aphid, *Brevicoryne brassicae* (Tjallingii 1985, Gabrys et al. 1997, Gabrys and Tjallingii 2002); the cowpea aphid, *Aphis craccivora* (Annan et al. 1997a, 1997b, 2000); the green peach aphid, *Myzus persicae* (Tjallingii 1985, Sauge et al. 2002); the pea aphid, *Acyrtosiphon pisum* (Tjallingii 1985, Gabrys and Tjallingii 2002); the soybean aphid, *A. glycines* (Han and Yan 1995); and the vetch aphid, *Megoura viciae* (Tjallingii 1985). However, in spite of the large number of studies performed in feeding behavior of different aphids on different crops using the EPG technique, there is no report of any study involving the feeding behavior of *A. glycines* on resistant soybean genotypes.

The objective of this work was to compare the feeding behavior of *A. glycines* on resistant and susceptible soybean genotypes using the EPG technique.

## **Materials and Methods**

### **Insect Culture and Plant Material**

*Aphis glycines* was first collected from soybean fields in Geary County, Kansas, in August 2002. The population was maintained on the soybean cultivar KS4202 in a pesticide-free greenhouse at 20 to 30°C, 20 to 40% RH with supplemental lights from high-pressure sodium

lamps set for a photoperiod of 14:10 (L:D) h. In this research, four resistant entries and one susceptible entry to *A. glycines* were used. The four resistant entries were found highly resistant to *A. glycines* in previous studies; antibiosis was found in Dowling and Jackson (Hill et al. 2004b, Li et al. 2004), and both antibiosis and antixenosis were confirmed in K1639, Pioneer<sup>®</sup> 95B97, Dowling, and Jackson (Diaz-Montano et al. 2006). The soybean genotype KS4202 was included as a susceptible check. Plants were grown separately in 3.8-cm-diameter by 21.0-cm-deep plastic Cone-tainers<sup>™</sup> (Ray Leach Cone-tainer<sup>™</sup>, Hummert International, Earth City, MO) containing steam-sterilized potting mix (Premier Promix<sup>®</sup>, Rivière-du-Loup, Québec, Canada). When soybean plants reached the V-1 stage ( $\approx$ 9 d after planting), with two fully developed leaves at unifoliate nodes (Fehr et al. 1971, Kilgore and Fjell 1997), plants were suitable for the experiments.

## **EPG Technique and Experimental Design**

EPG experiments were carried out in laboratory conditions at 21 to 24°C, 40 to 45% RH. Illumination was provided continuously by fluorescent ceiling-mounted lamps. Adult apterous aphids were starved for a 1-h period in a Petri dish. During this period, a gold wire electrode ( $\approx$ 12  $\mu$ m in diameter by 1-2 cm long) (Sigmund Cohn Corporation, Mount Vernon, NY) was attached to the dorsum of aphids with a small drop of high purity silver conductive paint (SPI Supplies, West Chester, PA). A copper wire (2 mm in diameter by 10 cm long), which serves as the plant electrode, was inserted into the plant soil. Both electrodes were connected to a Giga-8 DC EPG amplifier with  $10^9 \Omega$  input resistance and an adjustable plant voltage (Wageningen Agricultural University, Wageningen, the Netherlands). After the 1 h starving time period, the

aphids were cautiously lowered to one of the fully developed leaves (Fig. 11). The gain was set at 50x and the plant voltage source was adjusted at  $\pm 5$  V, so when stylets (Fig. 12) are inserted intercellularly the signal voltage is positive, and when inserted intracellularly the voltage is negative (Tjallingii 2006). Recordings were made at the same time on four plants (two always being the susceptible check, and the other two one of the resistant genotypes) placed at random in a faraday cage. For each genotype, 16 replications were done for a time period of 9 h. The feeding behavior of *A. glycines* was recorded using the EPG analysis PROBE 3.0 (Windows) software.

### **Feeding Behavior Parameters and Statistical Analyses**

One of the main purposes of this study was to compare total time *A. glycines* spent in the phloem phase or sieve element phase in susceptible and resistant entries. Therefore, waveforms E1 and E2 were labeled as waveform E in this study. Waveforms F (stylet penetration difficulties) were rarely found in the recordings, but when observed they were included in the pathway phase. The parameters recorded in each of the five entries were, the mean time from start of recording to first: probe or initiation of pathway phase, xylem phase (G), and sieve element phase (E); number of: potential drops (pds), pathway phases (A, B, C and F), xylem phases, and sieve element phases; total duration of: pathway phase, xylem phase, sieve element phase and non-probing; time left available after first sieve element phase; and percentage of time left available after first sieve element phase that was spent in sieve element phase.

According to Prado and Tjallingii (1997), when the sieve element phase is not reached during the entire experiment, the time from start of recording to first sieve element phase is considered the

same as the total time of EPG recording; in other words, it took at least that long, and probably much longer, to reach the sieve element. Therefore, in this study 9 h was given as the time to reach the first sieve element phase in those replications in which the aphids did not reach the sieve element during the record period (9h).

The different feeding behavior parameters were compared by the Kruskal-Wallis test ( $\alpha = 0.05$ ). Multiple comparisons were computed using the Tukey's Studentized Range Test ( $P < 0.05$ ) (SAS Institute 1999).

## Results

### Feeding Behavior Parameters

The time that *A. glycines* spent from start of recording to first probe was not different among all the entries tested (Parameter 1, Table 5). *Aphis glycines* spent significantly ( $P < 0.05$ ) more time to reach the first xylem phase in Jackson (Parameter 2, Table 5). The time used by *A. glycines* to reach the first sieve element phase in the susceptible check (KS4202) was significantly ( $P < 0.05$ ) less compared with the four resistant entries (K1639, Pioneer<sup>®</sup> 95B97, Jackson and Dowling) (Parameter 3, Table 5).

The total number of potential drops generated by the aphids on KS4202 was significantly ( $P < 0.05$ ) higher than the number on the resistant entries with the exception of Jackson

(Parameter 4, Table 5). Jackson had the highest number of pathway phases compared with the other entries, but this number was only significantly ( $P < 0.05$ ) different from K1639 (Parameter 5, Table 5). There were no significant differences in the number of xylem phases (Parameter 6), or total duration of: pathway phase (Parameter 8), xylem phase (Parameter 9), and non-probing (Parameter 10) among all the entries (Table 5). However, there were large significant ( $P < 0.05$ ) differences in the number of sieve element phases (Parameter 7, Table 5) and the total duration of sieve element phase ( $\chi^2 = 36.90$ ;  $df = 4$ ;  $P < 0.0001$ ) (Fig. 13) in the susceptible check, where *A. glycines* originated more phases and spent more time in the sieve element compared with all the resistant entries. There was significantly ( $P < 0.05$ ) more time left available after first sieve element phase (Parameter 10, Table 5) in KS4202 compared with the resistant entries; and the percentage of this time left that was spent in the sieve element by *A. glycines* was significantly ( $P < 0.05$ ) higher in KS4202 than the percentage used in the resistant entries except for Jackson (Parameter 12, Table 5), so even having finally reached a sieve element, there was something different happening.

Fifteen aphids out of 16 reached the sieve element on the susceptible check, but only two, three, four and five aphids reached it on Pioneer<sup>®</sup> 95B97, Dowling, Jackson, and K1639, respectively (Table 5).

## Discussion

The results of this EPG study indicate that antixenosis can be stronger than antibiosis in all four resistant genotypes. The parameters related to the sieve element phase, reflecting the



performance of *A. glycines* on the susceptible KS4202, were always significantly different compared with the resistant entries. For example, the time to reach the sieve element by *A. glycines* on the resistant entries was twice as much as the time on the susceptible check, the total duration in the sieve element phase by the aphid on KS4202 was longer than an hour, while when the aphids reached the sieve element on resistant entries, they remained for only a few minutes. In addition, 94% of the aphids reached the sieve element on the susceptible, compared to only 13 to 31% on the resistant entries. Therefore, it is possible that morphological or chemical factors in the resistant entries may be delaying the penetration of the sieve element, or may negatively change the behavior of the insect, specifically feeding behavior as a consequence of the presence of antixenosis in the resistant entries.

The strong antixenosis showed by K1639 and Pioneer<sup>®</sup> 95B97 may result in a reduction of antibiosis parameters such as reproduction (Diaz-Montano et al. 2006); however, sometimes antibiosis and antixenosis are difficult to differentiate from each other (Smith 2005). Phloem consumption involves nutrient ingestion (Spiller et al. 1990), and in our study it was clearly shown that aphids on the resistant entries were unable to spend much time in the sieve element phase. Low ingestion of nutrients may affect demographic parameters. Li et al. (2004) confirmed antibiosis in Dowling and Jackson by reporting an increase in mortality and a decrease in fecundity and longevity of *A. glycines*. Therefore, in spite of the results shown in this study that suggest a strong antixenosis over antibiosis, we cannot assure which of the two categories of resistance is more prevalent. And, these are just terms coined to categorize very complicated biological phenomena. But, behavior parameters in the sieve element, such as total duration in the sieve element phase by *A. glycines*, suggest that resistance in the entries tested in this study is

related to the phloem tissues. This was demonstrated by the fact that there were no differences between KS4202 and all four resistant entries tested in most of the parameters that are not related to the sieve element phase. It is interesting that *A. glycines* stayed in the xylem phase relatively the same time in all entries and that differences were observed only when *A. glycines* tried to penetrate and stay in the sieve element. Xylem ingestion is related to water intake (Spiller et al. 1990) in order to renovate and preserve a water balance (Spiller et al. 1990). Xylem sap may not provide aphids with adequate nutrients as compared with the phloem sap (Powell and Hardie 2002). Resistant plants may contain chemical substances that are toxic to insects (Smith 2005); and since the xylem phase is reached before the sieve element phase in all the entries tested here, it is possible that xylem sap in the resistant entries may have affected *A. glycines* after ingestion.

Another parameter that showed a distinction between KS4202 and the resistant entries was the higher number of potential drops recorded during the 9 h period. Potential drops are brief (5 s) punctures of many cells, including sieve element cells, during plant penetration which help the insect to recognize and accept the sieve element (Tjallingii and Hogen Esch 1993). Therefore, the higher number of potential drops in the KS4202 enabled the aphid to reach the sieve element phase faster.

In summary, the performance of *A. glycines* in the sieve element phase of the four resistant entries was significantly affected compared with the susceptible check. Therefore, resistance could be associated to phloem tissues, but it is also possible that substances ingested by aphids in the xylem sap of resistant entries may affect feeding behavior and their ability to reach the sieve element.

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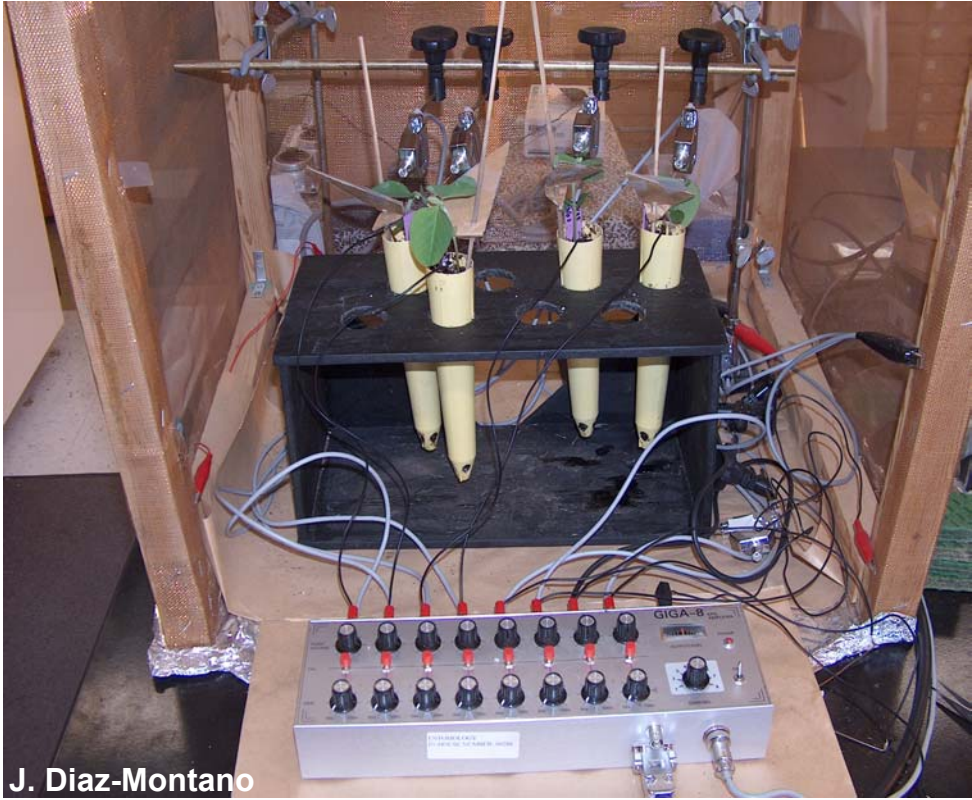
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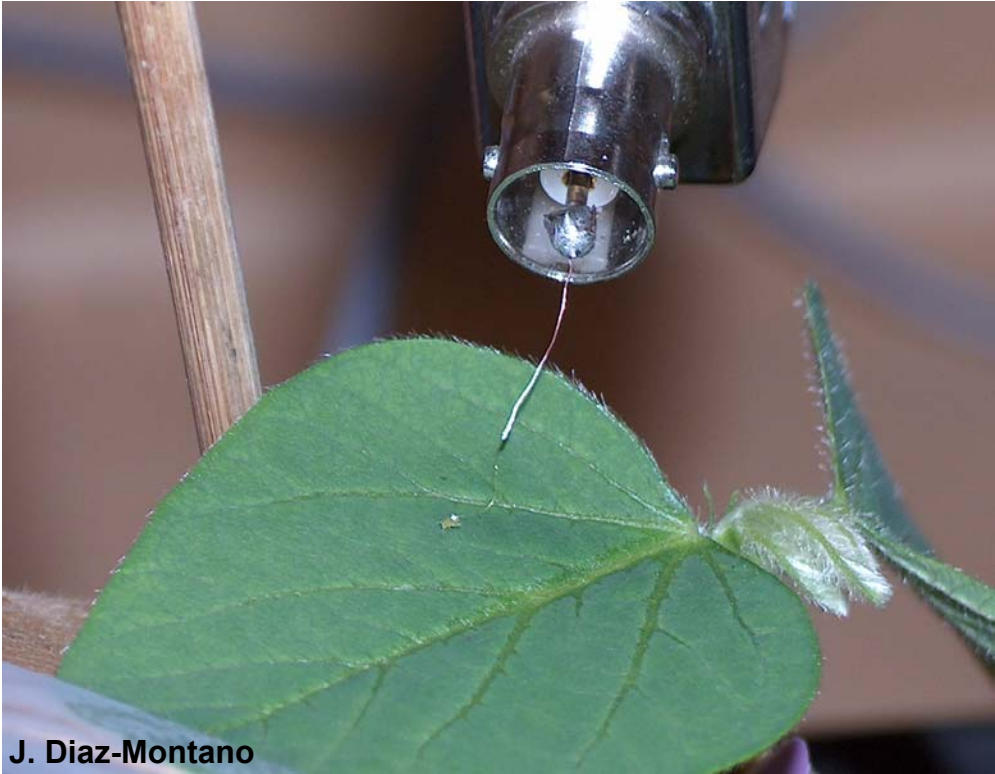


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Figure 10. Electrical penetration graph (EPG) device.

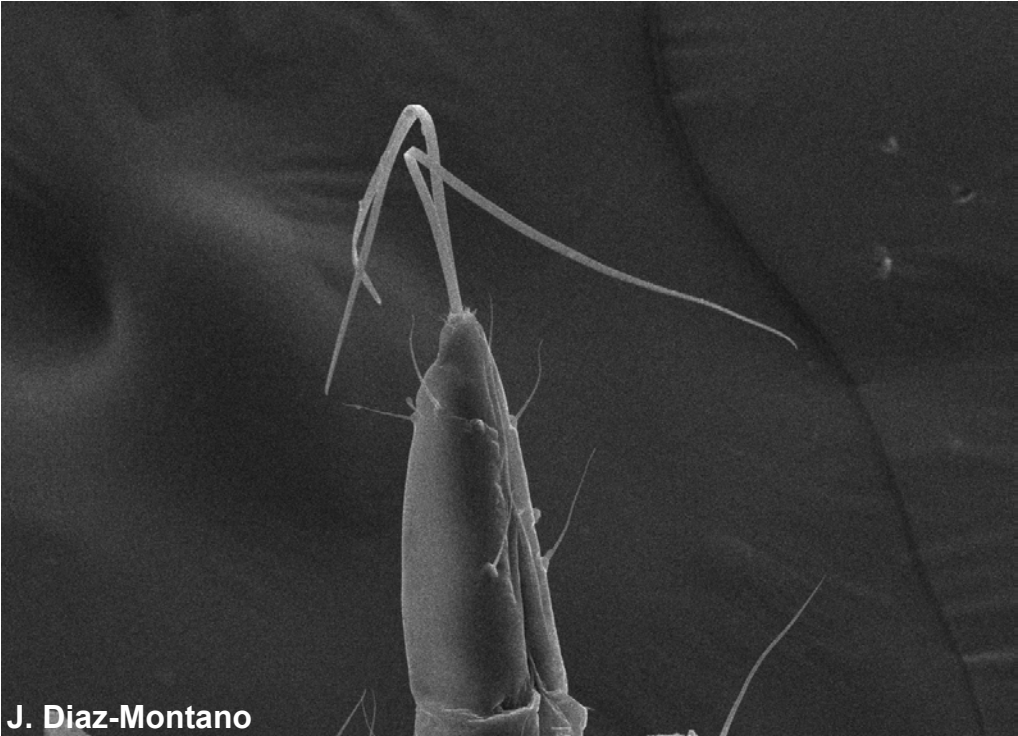


**Figure 11. Attached soybean aphid on a soybean leaf.**

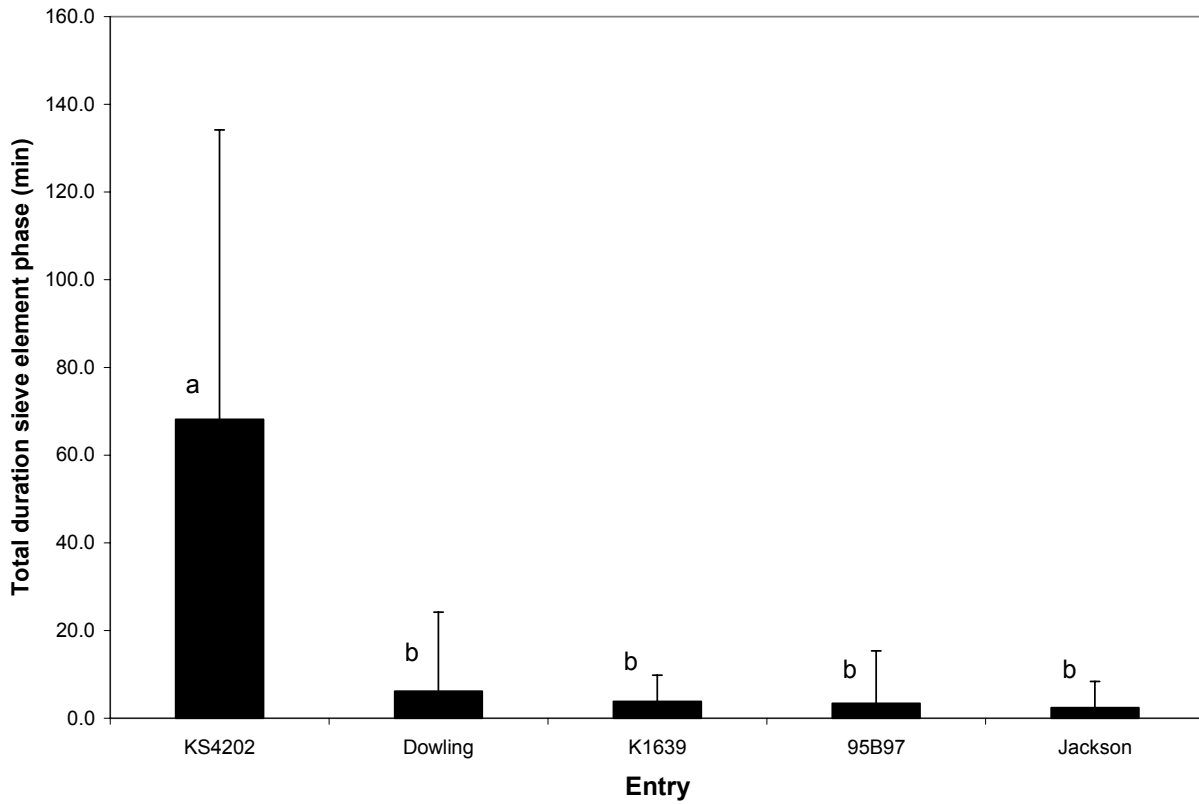


**J. Diaz-Montano**

**Figure 12. Stylets of the soybean aphid.**



**Figure 13. Total time (mean  $\pm$  SE) spent by *A. glycines* during a 9 h (540 min) period on the sieve element phase of the susceptible check KS4202 and the resistant entries K1639, Pioneer<sup>®</sup> 95B97, Jackson and Dowling.**



Bars with different letters are significantly different according to the Kruskal Wallis test ( $\alpha=0.05$ ) and multiple comparisons ( $P < 0.05$ , Tukey's test).

**Table 5. Feeding behavior (mean  $\pm$  SE of EPG parameters) of *A. glycines* during a 9 h (540 min) period on the susceptible check KS4202 and the resistant entries K1639, Pioneer<sup>®</sup> 95B97, Jackson and Dowling. Time in min**

Feeding behavior parameters	Soybean entries					$\chi^2$	df	P
	KS4202	K1639	Pioneer <sup>®</sup> 95B97	Jackson	Dowling			
1. Time from start of recording to first probe	14.9 $\pm$ 38.0	4.1 $\pm$ 5.1	25.7 $\pm$ 55.1	4.5 $\pm$ 8.0	3.3 $\pm$ 7.0	3.98	4	0.4089
2. Time from start of recording to first xylem phase	135.6 $\pm$ 88.2b	138.3 $\pm$ 98.9b	159.2 $\pm$ 119.7ab	266.1 $\pm$ 150.0a	96.2 $\pm$ 52.0b	11.05	4	0.0261
3. Time from start of recording to first sieve element phase	207.1 $\pm$ 133.9b	464.9 $\pm$ 134.8a	499.5 $\pm$ 125.6a	501.9 $\pm$ 85.4a	484.6 $\pm$ 124.2a	35.52	4	<0.0001
4. Number of potential drops	115.4 $\pm$ 58.5a	45.9 $\pm$ 57.5bc	38.1 $\pm$ 43.0c	93.7 $\pm$ 42.5ab	64.1 $\pm$ 52.3bc	28.76	4	<0.0001
5. Number of pathway phases	11.4 $\pm$ 6.1ab	8.7 $\pm$ 6.7b	12.8 $\pm$ 6.5ab	18.9 $\pm$ 10.2a	16.0 $\pm$ 8.9ab	14.11	4	0.0070
6. Number of xylem phases	1.5 $\pm$ 1.2	1.3 $\pm$ 0.7	1.5 $\pm$ 1.2	1.3 $\pm$ 0.9	1.6 $\pm$ 1.1	0.88	4	0.9269
7. Number of sieve element phases	2.4 $\pm$ 1.5a	0.4 $\pm$ 0.8b	0.4 $\pm$ 1.3b	0.3 $\pm$ 0.6b	0.3 $\pm$ 0.7b	34.25	4	<0.0001
8. Total duration pathway phase	276.8 $\pm$ 66.0	289.0 $\pm$ 108.0	312.1 $\pm$ 96.0	304.4 $\pm$ 78.0	303.8 $\pm$ 78.0	2.06	4	0.7242
9. Total duration xylem phase	59.9 $\pm$ 42.0	48.9 $\pm$ 42.0	67.6 $\pm$ 60.0	45.1 $\pm$ 36.0	71.0 $\pm$ 60.0	2.64	4	0.6193
10. Total duration non-probing	135.2 $\pm$ 72.0	198.3 $\pm$ 120.0	156.9 $\pm$ 96.0	188.1 $\pm$ 60.0	159.0 $\pm$ 84.0	4.78	4	0.3088
11. Time left available after first sieve element phase	332.9 $\pm$ 132.0a	75.1 $\pm$ 132.0b	40.5 $\pm$ 126.0b	38.1 $\pm$ 84.0b	55.4 $\pm$ 126.0b	35.52	4	<0.0001
12. Percentage of time left available after first sieve element phase that was spent in sieve element phase	18.9 $\pm$ 19.0a	2.4 $\pm$ 5.7b	1.0 $\pm$ 2.7b	7.0 $\pm$ 24.9ab	2.7 $\pm$ 8.3b	33.09	4	<0.0001
Total number of aphids that reach the sieve element phase	15	5	2	4	3			

Within a row, means followed by different letters are significantly different according to the Kruskal Wallis test ( $\alpha=0.05$ ) and multiple comparisons ( $P<0.05$ , Tukey's test)

## SUMMARY

The soybean aphid, *Aphis glycines* Matsumura, is a major pest of soybean, *Glycine max* (L.) Merr. *Aphis glycines* was detected in the United States in 2000, and since that time studies on this insect were initiated all around the country. Before it appeared in the United States, most of the investigations related to *A. glycines* had been done in China, where it is the main pest in soybean fields. Adults and nymphs extract the phloem sap using their piercing-sucking mouthparts. *Aphis glycines* populations grow and spread rapidly causing yield losses greater than 50%. In addition, this aphid vectors several viral diseases which include the *soybean mosaic virus*. Therefore, the impact of *A. glycines* on soybeans is very important.

Chemical control has been the most common method used to control *A. glycines*, but this can promote the development of insecticide resistance, and also can decrease natural enemies on the fields. Plant resistance to *A. glycines* is one important component of integrated control because it can reduce significantly the use of chemical insecticides, and maintain natural enemies in the fields. Although soybean resistance to *A. glycines* has been studied in China as well in the United States, few soybean genotypes, among the large amount evaluated, have been found highly resistant. For this reason, experiments were performed aimed to identify additional sources of resistance to *A. glycines*, and to characterize categories of resistance. Damage caused by *A. glycines* could lead to negative effects on soybean physiology and reduction in chlorophyll content, therefore chlorophyll loss caused by *A. glycines* were quantified on susceptible and resistant entries by using the SPAD-502 chlorophyll meter. Stylet penetration is very important to aphids in order to reject or accept the host plant, thus it was considered necessary to study the

feeding behavior of *A. glycines* on susceptible and resistant entries using the electrical penetration graph (EPG) technique.

To identify soybean resistance, reproduction of *A. glycines* was compared on 240 soybean entries. Eleven entries had fewer nymphs produced by adults during seven days, compared with the susceptible checks. These 11 entries were used in follow-up experiments to assess antibiosis and antixenosis. Antibiosis was estimated in true no-choice tests, in which adults were confined individually in double-sided sticky cages stuck to the upper side of leaves, and four days later the number of nymphs produced by each adult was counted. Antixenosis was assessed in choice tests, in which several entries were planted in a single pot. Adult aphids were placed in the center of the pot, and 24 h later the number of adults on each plant was counted. Of the 11 entries evaluated, nine showed a moderate antibiotic effect to *A. glycines*, and the other two (K1639 and Pioneer<sup>®</sup> 95B97) were highly resistant to *A. glycines* because they showed not only a strong antibiotic effect, but were the only entries exhibiting antixenosis as a category of resistance to *A. glycines*.

To quantify chlorophyll loss caused by *A. glycines*, no-choice tests on the infested and uninfested leaves of a susceptible check (KS4202) were performed. To determine the minimum combined number of days and aphids needed to detect significant chlorophyll loss, several experiments with different number of aphids (5, 10, 20, 30 and 40) confined for different days (4, 7, and 10) were conducted. The treatment to detect chlorophyll loss on KS4202 was 30 aphids confined for 10 days. Based on this, a similar experiment was conducted with seven resistant entries and two susceptible checks. There was not significant chlorophyll reduction between



infested and uninfested leaves of five resistant entries (K1621, K1639, Pioneer<sup>®</sup> 95B97, Dowling and Jackson). Percentage loss of the susceptible checks was 40%; Jackson and Dowling had a significantly lower percentage loss than the susceptible checks.

In order to observe feeding behavior of *A. glycines* stylet penetration activities were compared on four resistant entries (K1639, Pioneer<sup>®</sup> 95B97, Dowling and Jackson) and a susceptible check (KS4202) using the electrical penetration graph (EPG) technique. Recordings were done for a 9 h period. The average time needed to reach the first sieve element phase by *A. glycines* was 3.5 h in KS4202 while in the resistant entries it was longer than 7.5 h. The total duration in the sieve element phase was more than an hour in KS4202, while in the resistant entries it was only for two to seven minutes. These results suggest that morphological or chemical factors in phloem tissues of the resistant plants are affecting stylet penetration activities of *A. glycines*. However, in the majority of the recordings, the aphid stylet reached the xylem phase before penetrating the sieve element, and the time that aphids spent ingesting xylem sap was not different among all entries. Therefore, it is possible that xylem sap in the resistant entries may contain toxic substances that change aphid behavior and affect further activities in the sieve element phase.