

DIFFERENT SOURCES OF RESISTANCE IN SOYBEAN AGAINST SOYBEAN APHID
BIOTYPES

by

PREDEESH CHANDRAN

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Approved by:
Major Professor
John C. Reese

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Abstract

The soybean aphid, *Aphis glycines* Matsumura, arrived first to North America during the mid-2000s. It is a very fast spreading insect and causes a high yield loss of above 50% in most of the soybean growing tracts of United States. Another important economic threat is its ability to transmit some viruses to soybean. Studies to control this exotic pest started early during the year of its arrival. But a complete integrated pest management (IPM) approach that includes a combination of different control measures has yet to be completely developed. Host plant resistance is one component of integrated pest management and is more sustainable than any other control methods against this insect. In the first study, more than 80 genotypes were screened with two given aphid biotypes, biotype 1 and biotype 2. It was found that the genotypes that were earlier resistant to biotype 1 (K1639, K1642, K1613 K1621, Dowling and Jackson) were susceptible to the new biotype 2 with large populations developing on these genotypes. But we found three new Kansas genotypes that showed resistance only against biotype 1, but not against biotype 2. However, the two of the Michigan genotypes (E06902 and E07906-2) showed resistance to both biotype 1 and biotype 2. In second study, the feeding behavior analyses of aphid biotypes were done using the EPG, Electrical penetration graph, technique for a recorded 9 hrs probing time. The resistant and susceptible genotypes show significant differences in their EPG parameters, especially for the sieve element duration in both biotypes. Most of the aphids reached sieve element phase (> 90%) in susceptible genotypes, but only few (<30%) were reached in resistant genotypes. But, no differences were found in any other probing phases between resistant and susceptible genotypes, except the number of potential drops (PDs) in biotype 2. Thus, it is concluded that resistance is largely associated with phloem tissues and there could be some biochemical, physical or morphological factors that affect the stylet penetration in aphids.

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Dedication

I dedicate this thesis to my parents, Balachandran and Sudha, my loving wife, Sruthi, my brother Rajesh and all other family members for giving me the opportunity of education from the best institutions and for supporting me throughout the life.

Chapter-1. Literature Review

Introduction

The soybean aphid, *Aphis glycines* Matsumura., an exotic pest, was first reported in North America in 2000 (Hartman et al. 2001). During the summer and autumn of 2000, the soybean aphid was observed in number of states in US alone, that include Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, Ohio etc. (Ohio state soybean aphid monitoring, 2003). It has spread into most, if not all, soybean producing areas of the United States and Canada since its first report (Venette and Ragsdale., 2004). A low number of aphids were confined in five eastern Kansas counties in August and September of 2002 (Sloderbeck et al., 2003). However, it has been reported in 17 Kansas counties by 2003 (Sloderbeck et al., 2004). But the environmental conditions allowed them to reach damaging population levels by 2004 (Whitworth, 2008). When considering the centre of origin, it is native to eastern Asia that including China, Japan, Philippines, Indonesia, Korea, Vietnam and in some parts of eastern Russia (Ragsdale et al. 2004). And it is a recent invasive of Australia (Krupke et al., 2005).

Soybean is one of the most important cultivated crops in the world. Approximately 60% of the soybeans is used in animal feed. Other major uses include cooking oils, margarine, tofu and other human foods, as well as biodiesel. Since soybean oil is the dominant oil produced in the US, the development of biodiesel has focused around the soy oil. One bushel of soybean produces about 1.5 gallons of biodiesel (Soybean Extension and Research Program, ISU, 2007). The demand for soybean production is expected to continue to increase as world population increases to 8-9 billion by 2050.

The soybean aphid has been causing millions of dollars in losses to this legume crop. This is a very fast spreading aphid (Wang et al., 1998) and cause much damage even including virus transmission such as alfalfa mosaic virus, soybean dwarf virus and soybean mosaic virus (Sama et al., 1974, Hartman et al., 2001).

The soybean aphid is controlled mainly by chemical insecticidal application (Wang et al., 1998, Ye et al., 1996). An estimated 3 million hectares of field in the US were sprayed during 2003 for controlling this invasive pest (Landis et al., 2003). High insecticidal application produced soybean aphid resistance, an usual disaster co-related with insecticidal application. But the high insecticide usage was reduced by the introduction of aphid resistant varieties and natural enemies in the fields (Ye et al., 1996). Thus there carried out lots of studies in resistant varieties and natural enemies, all around the world. These studies and researches couldn't find any grip until 2004, when some scientists from both ARS, Illinois, Urbana and University of Illinois found successful in discovering one gene (named *Rag1*) in some soybean cultivars (Hill et al., 2006a, 2006b) as a long term solution for this invasive pest.

Taxonomy, Center of Origin and Geographical Distribution of Soybean Aphid

The soybean aphid is an invasive insect and belongs to the Order Hemiptera, suborder Sternorrhyncha, Superfamily Aphidoidea and Family Aphididae.

As already stated, the soybean aphid is native of eastern Asia and was first described by Matsumura in 1917 (Matsumura, 1917). Soybean aphid has been found first in Asia, especially in the temperate zones of Japan (Sakai 1949 by Takahashi et al., 1993), also observed in Southeast Asia and parts of Africa (Wang et al., 1962; Kobayashi et al., 1972; Singh and Van Emden, 1979, Hill, 1987 and Hirano and Fuji, 1993 cited by Hirano et al., 1996), Thailand, Korea, Taiwan, China, Malaysia (Paik 1965 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), Russia (D'yakonov 1975 cited by CAB International 2001), Vietnam (Waterhouse 1993 cited by CAB International 2001) and Australia (Krupke et al., 2005). The soybean aphid was not reported in North America before July 2000 (Hartman et al., 2001) and the aphid has found in Canada, 2001 (Baute, 2002). Now this pest spread throughout the Midwestern United States and Southern Canada since its first report (Venette and Ragsdale, 2004).

Earlier there were two soybean aphid biotypes were present in North America, biotype 1 (Hill et al., 2004a) and biotype 2 (Kim et al., 2008). The later one was established in the fields of Ohio and was found virulent to the 'Rag1' in field cage experiments. But in 2010, one more biotype was reported, biotype 3 (Hill et al. 2010), and found virulent to *Rag2* resistance gene

Morphology, Biology and Ecology of Soybean Aphid

The soybean aphid is a small, greenish-yellow aphid with black siphunculi (Blackman and Eastop, 1984) or projections called as cornicles near the tip of its abdomen (Sloderbeck et al., 2003).

Some biometric data of soybean aphid, including body sizes: 1.89mm for virgino-parous aptera, 1.75mm for virgino-parous alata, 2.02mm for gynopara, 1.5mm for ovipara, 1.68mm for alata males and 1.87mm for both fundatrix and apterous fundatrigenia. The adults and nymphs are morphologically very similar (Wu et al., 1999). The soybean aphid is a typical heteroecious holocyclic species (host-alternating with sexual reproduction during parts of its life cycle). The observed life history of soybean aphid in North America is similar to that observed in China and Japan, with exception of the primary host, those plant species used as overwintering hosts (Ragsdale et al., 2004).

The winged sexual forms were found to migrate from soybeans to the winter host, *Rhamnus davurica*, where they mate and produce eggs which overwinter there. They migrate back to *Glycine sp.* in early summer. These aphids colonized first on the stem apices and young leaves of growing soybean and later on the aphids are found on the underside of leaves of mature plants. In the late June to early July by 22-25⁰ C optimum range of temperatures and RH < 78% the aphid development is favored more (Wang et al., 1962).

Up to 15 generations may occur on soybean in the summer, ie out of the total 18 generations per year, 15 were on only soybean, in summer (Wang et al., 1962). The majority of soybean aphids are wingless, but some females develop wings and they fly to other plants within the same or nearby fields to lay eggs and then produce nymphs (Ostlie, 2002). These nymphs

will develop to adult aphids in 5 days and about 15 generations can develop in one year under the suitable climatic conditions (Wang et al., 1998). Soybean aphids usually molt 2-3 times, has 3-4 instar in one generation. Generally we can say that most of them molt 3 times, and have 4 instars in one generation.

In soybean aphids, crowding of apterae (wingless parthenogenetic females), the main factor in the production of alates (winged parthenogenetic females) was found on the summer host (Lu and Chen, 1993 cited by Takahashi et al., 1993). Alates are responsible for dispersion to secondary hosts.

In China and Japan, the most common overwintering hosts are *Rhamnus davurica* Pallus and *Rhamnus japonica* maxim (Takahashi et al., 1993). And in North America, various buckthorn *Rhamnus* species are used as primary hosts (Voegtlin et al., 2004). In addition to cultivated soybean, it has been found on wild *Glycine* species (Wang et al., 1962) and has also been recorded from *Pueraria phaseoloides* and *Desmodium intortum* (Blackman and Eastop, 1984).

Another evidence shows that apterous and alate virginopare of soybean aphid were attracted to volatiles of the winter host (*Rhamnus devurica*) and to a summer host (*Glycine max*) in a laboratory study (Du, 1992 cited by Takahashi et al., 1993). Takahashi et al. (1993) described the life cycle of soybean aphid in Japan, along with observations on Rhamnaceae occasionally used as alternative winter hosts, particularly *Rhamnus japonica*. *R. cantharica* and *R. alnifolia* as overwinter hosts of the soybean aphid in US (Voegtlin et al., 2004).

When comparing *A. glycines* and *A. solani*, the intrinsic rate of increase of *A. glycines* at 22⁰C was much higher than that of *A. solani* at 23⁰C (Okada and Nakasuji, 1980). The soybean aphid will develop under 20-25⁰C, 5-7 days including suitable nutritious conditions and they reproduced rapidly in the given conditions (Sun et al., 2000). Thus the development process of soybean aphid depends on temperature and nutrition. The soybean aphid has a higher gross fecundity at 22⁰C because of the longer reproductive period (Hirano et al., 1996).

The effects of different temperature on soybean aphid were studied in US by McCornack et al., (2004). Reproduction is much longer and the aphids produced more progeny at 20 and 25⁰ C than at 30 or 35⁰C .The soybean aphid populations decreased by senescence or when there is high temperature or heavy rain. It was found that, when the mean temperature is above 25⁰C and the relative humidity is upon 80% for a given period of 5 days, a large number of soybean aphids were killed (Wang et al., 1998). All of these studies thus stated that 20-25⁰ C is the optimum temperature range for the development of soybean aphids.

Economic importance, Habits and Damage

Soybean aphid is the main sap-sucking pest in soybean fields (Takahashi et al., 1993). High soybean aphid populations reduce soybean (*Glycine max* (L) merr.) yield directly when their feeding behavior causes stunting of plant, leaf distortion, brown-yellow spots on the infested leaves (Wu et al., 1999) and reduced pod set (Sun et al., 1990; Hill et al., 2004a). Sap feeding can cause yellowing, cupping and wilting of soybean leaves. Leaf yellowing can be confused with symptoms of potassium or iron deficiencies in the soil where the soybeans were planted. Sloderbeck et al. (2003) found that it is the only aphid sp. that produces large colonies on soybeans in North America. The yield losses of greater than 50% were attributed to the aphids in fields in Minnesota during 2001 (Ostlie, 2002). But the yield losses reported in China was about 58% (Wang et al., 1996).

There are three periods of damage on soybean that can be categorized i) is from the seedling to blooming stage, When the aphid populations reach their highest peak and colonies concentrate on young growth ii) during the late July, when the growth gets completed, the aphid colonies move lower down the leaves of the plant for feeding and iii) during late August to early September, the multiplications of aphids started again before migrating back to the winter host (Wang et al., 1962).

The increase in herbivore populations may negatively affect the photosynthetic capacity of plants (Wang et al., 1962) and thus, the reduction of chlorophyll has much influence on its economic loss. The use of the soil plant analysis development (SPAD)- 502 Chlorophyll meter is

a rapid and non-destructive technique (Yadava, 1986; Deol et al., 1997) that can be used to measure the chlorophyll losses caused by non-chewing insects (Deol et al., 1997). Photosynthesis responses of soybean (Asgrow 0901) to soybean aphid injury were determined by Macedo et al., (2003). Photosynthesis capacity was affected by densities greater than 20 aphids/leaflet. Deol et al., (1997) Girma et al., (1998) and Nagaraj et al., (2002a) used the SPAD technique to assess feeding damage by greenbug, *Schizaphis graminum* (Rondani), feeding on sorghum, etc.

Soybean aphid is a vector of a number of viruses. Li and Pu, (1991 as cited by Takahashi et al., 1993) found that epidemics of soybean mosaic potyvirus (SMV) in summer-sown soybean fields in Jiangsu, China, were closely related to the time of immigration of the aphid vectors, with soybean aphid the most frequent. Zhang et al. (1998) made an attempt to artificially infest soybean plants with alates of soybean aphid and the incidence of the virus disease transmitted by this aphid reached about 100% of the plant infection limit. (D'yakonov, 1975 cited by Takahashi et al., 1993) showed soybean aphid to be a vector of soybean virus in Soviet Far East.

The ability to transmit some of the viruses such as alfalfa mosaic virus, soybean dwarf virus, and soybean mosaic virus etc given by (Sama et al., 1974, Iwaki et al., 1980, Hartman et al., 2001). Honeydew excreted by soybean aphids on the leaves leads to the development of sooty mold, which results in further yield losses (Krupke et al., 2005)

Control Measures

It was realized a significant importance, since yield losses are greater than 50% were attributed to the aphid in most soybean fields (Ostlie, 2002, Wang et al., 1996). Results for different insecticides on seedling stages of soybean were reported in China (Wang et al 1993 cited by Takahashi et al. 1993). Phosalone and Fenvalerate were insecticides reported to cause less natural enemy mortality (Qu et al., 1987 cited by Takahashi et al., 1993). But other insecticides used in soybean field have different effects on natural enemy mortality (Wang et al., 1993 cited by Takahashi et al., 1993).

Nearly 3 million hectares of soybean fields in United States were sprayed to control the soybean aphid during 2003 (Landis et al., 2003) including \$ 9 to 12 million spent on insecticide applications in Illinois (Steffey, 2004).

Significant control was achieved with Phosalone, Pirimicarb, Omethoate and Fenvalerate (Wang et al., 1993 cited by Takahashi et al., 1993). The control of soybean aphid by Imidacloprid[®] was conducted by Huang et al., (1998). He carried out the experiments with four treatments 15, 22.5, 30 and 45 g a.i/ ha. The results showed that Imidacloprid[®] carries good control against soybean aphid and the average control effects of five observations (3, 7, 14, 21, and 28 days after application) in the four treatments were 85.0, 91.2, 92.8, 94.6% respectively.

There is a big problem of population rebounding after a chemical treatment in soybean aphid. This problem mainly occurred when an insecticide does not reduce the aphid population by 95 % or more in the applied field (Baute, 2002). DiFonzo (2001) conducted experiments with four different insecticides to control soybean aphid, and four of them seem to have a control greater than 95 % after ten days of spraying. Pymetrozine, a pyridine azomethine compound has a selective insecticidal activity against homopteran insects, especially on their feeding behavior (Harrewjin, 1997).

Successful pest management has been achieved on soybean using selective insecticides in conjunction with cultural control and resistant varieties (Wang et al., 1994). But the insecticides are very costly and have high chances of occurrence of insecticide resistance in aphids. Thus, high priority must be given to the research on soybean aphid and its non-chemical control methods (Ye et al., 1996). Even though some seed treatments with systematic activity show interesting results, but none of them is currently labeled against soybean in the US (Ostlie, 2002).

Natural enemies play a major role in aphid density in soybean fields. In the past, a number of natural enemies were reported, including the dominant Asian lady beetles (*Harmonia axyridis*, *Propylaea japonica*, *Coccinella septempunctata*, *Hippodamia tredecimpunctata*), lace wings (*Chrysopa formosa*, *C. septempunctata*), syrphids, parasitoids, chamaemyiids and

entomophagous fungi at the end of the season (Wang et al., 1998). However, recently the unreasonable application of highly toxic pesticides in large volumes kills natural enemies of soybean aphids and destroyed the ecological balance, resulting in high aphid density and heavy infestation.

Chang et al. (1994) described the primary parasitoids and hyperparasitoids of soybean aphid, from the collections made in the Korean Republic. From their aphid mummies collection, 27 % of adults emerging were primary parasitoids and 50 % were hyperparasitoids. Some of the most common primary parasitoids are *Aphidius cingulatus*, *Ephedrus persicae* and *E. plagiator*. Among the hyperparasitoids, *Asaphes vulgaris* and *Ardilea convexa* might be dominant species. The described studies on the braconid parasite *Cysiphlebia japonica* in jilin, China, where an average of 56 % of individuals of soybean aphid were found to be parasitized (Gao, 1994).

Orius insidiosus and *Harmonia axyridis* were the two common natural enemies found in the soybean fields of United States (Rutledge et al. 2004). Some of the other important natural enemies that attack soybean aphids and found common in soybean fields were the parasitoids (*Aphidius* sp. and *Praon* sp.), predators (coccinellids, cecidomyiids and syrphids), and entopathogenic fungi (*Pandora neoaphidis*, *Pandora* sp., *Entomophthora chromaphidis*, *Conidiobolus thromboides*, *Neozygites fresenii*, *Zoophthora occidentalis*, and *Lecanicillium lecanii*) (Nielson and Hajek, 2005)

The plant breeding programs for the development of soybean varieties resistant to soybean aphid exist in China and Indonesia. (Sama et al., 1974) reported results for over 200 varieties screened in Indonesia. Fan (1988) carried out screening of 181 soybean materials for resistance to *A. glycines* from 1983 to 1986. But only two from the above genotypes, Qinpi-Pingdingxiang and Dulu-dou, showed high resistance in the year of more severe infestation of aphids. Wang et al., (1962) found out that, compared with the resistant varieties, the susceptible varieties had 1) significantly higher aphid density and 2) younger aphid population. Hu et al., (1992, 1993) reported the effect of nitrogen and lignin content in soybean plants against aphid infestation.

Three highly resistant strains were selected from nearly 1,000 strains of the wild soybean, *G. soja* and these strains showed more resistance to soybean aphids than those collected from *G. max*. One hypothesis is that aphid resistance in wild soybean genotypes might be controlled by two independent recessive loci and also some other minor genes. It is also believed that China has more than 90 % of world's soybean resources.

Hill et al., (2004a) found two categories of resistance in soybeans. They found both antibiosis and antixenosis and they reported resistance to soybean aphid in nine soybean germplasm accessions. From the above germplasm they found *Rag1* and *Rag* genes in Dowling and Jackson, respectively. In 2005, Mensah et al., identified four sources of aphid resistance by screening 2147 soybean accessions. Another reports is from the genetic studies in PI 567541B and PI 567598B, for earlier resistance is controlled by quantitative trait loci (QTL) and for later, the resistance is controlled by two recessive genes (Chen et al., 2006; Mensah et al., 2006). Some accessions collected from China show resistance to the Ohio biotype (aphid collected in Ohio) and two of them shows resistance to Illinois biotype (Mian et al., 2008). Kim et al., (2008) reported that there are two soybean biotypes that occur in N. America. They are named as biotype 1 and biotype 2. PI 200538 and PI 567597C were resistant to both biotype 1 and biotype 2 and will be useful sources of resistance to both isolates. Recent studies showed some more PIs (PI 243540, PI 567301 and PI 567324) having antixenosis and antibiosis resistance to biotype 2 (Mian et al., 2008).

Feeding behavior difference of soybean aphid (Illinois biotype) between different soybeans entries were given by EPG method (Diaz-Montano et al., 2007b). Here the assessment of feeding behavior of aphid species was compared on four resistant entries (K1639, Pioneer 95B97, Dowling and Jackson) and a susceptible check (KS 4202) using Electrical Penetration Graph (EPG) technique. Another study related with the chlorophyll reduction between the resistant and Susceptible soybean entries with soybean aphid (biotype 1) (Diaz-Montano et al., 2007a). The results show that there was no significant chlorophyll reduction between infested and un-infested leaves of the resistant entries (K1621, K1639, Pioneer95B97, Dowling and Jackson). But there was a significantly major loss of chlorophyll in susceptible check (KS4202).

Cultural control practices that have been used in soybean against soybean aphid, include the planting of barrier crops (eg. sunflower), crop rotation, intercropping, inter-planting and burning or removal of crop residues, rouging of infected plants and varying planting dates, (Quimio and Calilung, 1993 as cited by Takahashi et al., 1993). The implementation of cultural practices that support biological control and host-plant resistance strategies might be the future control methods (Ye et al., 1996).

Although use of insecticides can be a quick and easy way to control soybean aphid, frequent applications of broad-spectrum pesticides can lead to the buildup of aphid resistance to chemicals, resulting in more chemicals being used with potentially severe environmental side effects. This made a switching over to resistant varieties rather than go for chemical pesticides for controlling soybean pests. Soybean breeding programs for producing resistant varieties have been going all around the world. This helps the breeders to incorporate more and more diversified sources of resistance to agronomically acceptable cultivars. In deed, among the control strategies available, host plant resistance has become widely recognized as the pivot of integrated pest management. These resistant cultivars help to reduce the frequencies of insecticide application and thus favor the conservation of natural enemies. In addition, resistant cultivars cost farmers nothing extra, nor does their adoption necessarily disrupt the farming system (Ye et al., 1996).

Objectives

Host plant resistance is an important alternative to other control methods in controlling insect pests. Furthermore, it is an environmental friendly, cheap and compatible method of pest control with other measures. The development of improved host-plant resistance techniques will help the farmers to control the insect pest efficiently without harming the environment. The following are the objectives of this study:

- 1) To compare the development of two soybean aphid biotypes population on different soybean genotypes.
- 2) To characterize the categories of resistance of selected soybean genotypes to the given two soybean aphid biotypes.
- 3) To analyze the EPG probing of different soybean genotypes with the above two aphid biotypes.

References

- Baute, T. 2002. Soybean aphids. Ontario Ministry of Agriculture and Food (OMAF).
www.gov.on.ca/OMAFRA/english/crops/facts/soybean_aphid/soybean_aphid.htm
- Blackman. R. L., Eastop.V.F.1984. Aphids on the worlds crops. An identification and information Guide. Chichester, UK: John wiley.
- CAB International. 2001. Crop protection compendium. CD-Rom. Source:
www.aphis.usda.gov/npb/soybean/aphisglycines.pdf
- Chang, Y. D., J.Y. Lee, and Y. N. Youn. 1994. Primary parasitoids and hyperparasitoids of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). Translation from the Korean J. Appl. Entomol. 33(2): 51-55.
- Chen, Y., C. Mensah, C. DiFonzo, and D. Wang. 2006. Identifi cation of QTLs underlying soybean aphid resistance in PI 567541B [CD-ROM]. 2006 Agronomy abstracts. ASA, Madison, WI.
- Deol, G. S., J. C. Reese, and B. S. Gill. 1997. A rapid, nondestructive technique for assessing chlorophyll loss from greenbug (Homoptera: Aphididae) feeding damage on sorghum leaves. J. Kans. Entomol. Soc. 70: 305D312.
- Diaz-Montano J, Reese J. C, Schapaugh W. T, et al. 2007. Chlorophyll loss caused by soybean aphid (Hemiptera : Aphididae) feeding on soybean. J. Econ. Entomol. 100: 1657–1662.
- Diaz-Montano J, Reese J. C, Louis J, et al. 2007. Feeding behavior by the soybean aphid (Hemiptera:Aphididae) on resistant and susceptible soybean genotype. J. Econ. Entomol.100: 984:989.

- DiFonzo, C. 2001. Soybean aphid - a recap of what we know. Field Crop Advisory Team Alert. Vol. 16, No. 13, July 12. Michigan State University. www.ipm.msu.edu/CAT01_fld/FC07-12-01.htm#1
- Fan, Y. H. 1988. Screening for soybean varieties resistant to soybean aphid. Translation from Soybean Science. 7: 167-169. www.ksu.edu/issa/aphids/reporthtml/trans63.htm
- Gao, J. F. 1994. Biological characteristics and control effect of *Lysiphlebia japonica* (Hymenoptera: Braconidae) on *Aphis glycines* (Homoptera: Aphididae). Translation from Chinese Journal of Biological Control. 10(2): 91-92. www.ksu.edu/issa/aphids/reporthtml/trans3.htm
- Girma, M., K. D. Kofoid, and J. C. Reese. 1998. Sorghum germplasm tolerant to greenbug (Homoptera: Aphididae) feeding damage as measured by reduced chlorophyll loss. J. Kans. Entomol. Soc. 71: 108-115.
- Harrewjin, P, Hartment K . 1997. Pymetrozine, a fast- acting and selective inhibitor of aphid feeding: Insitu studies with electronic monitoring of feeding behavior. Pesticid. sci. 149:130-140.
- Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D. W. Onstad, J.T. Shaw, L. F. Solter, D. J. Voegtlin, C. J. D'Arcy, M. E. Gray, K. L. Steffy, and P. L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybean in Illinois in 2000 and its Potential control. www.plantmanagementnetwork.org/php. Plant Health Progr. DOI 10.1094/php-2001-0205-01-HN.
- Hill, C. B., Y. Li, and G. L. Hartman. 2004a. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44: 98–106.
- Hill, C. B., Y. Li, and G. L. Hartman. 2006a. A single dominant gene for resistance to the Soybean aphid in the soybean cultivar Dowling. Crop Sci. 46: 1601–1605.

- Hill, C. B., Y. Li, and G. L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. *Crop Sci.* 46:1606–1608.
- Hill, C. B., Y. Li, and G. L. Hartman. 2004b. Resistance to *Glycine* species and various cultivated legumes to the soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 97: 1071– 1077.
- Hill, C. B., L. Crull, T. K. Herman, D. J. Voegtlin and G. L. Hartman. 2010. A new soybean aphid (Hemiptera:Aphididae) biotype identified. *J. Econ. Entomol.* 103: 509- 515
- Hirano, K., K. I. Honda, and S. Miyai. 1996. Effects of temperature on development, longevity and reproduction of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). *Appl. Entomol. Zool.* 31: 178-180.
- Huang, C. D., J. F. Zhou, D. Yang. 1998. Experiments on the control of soybean aphid with Imidacloprid. Translation from *Pesticides.* 37: 44- 45.
www.ksu.edu/issa/aphids/reporthtml/trans10.htm
- Hu, Q., J. W. Zhao, and J. W. Cui. 1993. The relationships between the level of lignin, the secondary metabolite in soybean plant and the aphid resistance in soybean. Translation from *Plant Protection* 19 : 8-9. www.ksu.edu/issa/aphids/reporthtml/trans46.htm
- Hu, Q., W. Q. Zhang, Y. X. Yao, and S. Q. Yan. 1992. The relationship between the nitrogen content in soybean leaves and occurrence degree of *Aphis glycines* Matsumura. Translation from *Journal of Jilin Agricultural University* 14 : 103-104.
<http://www.ksu.edu/issa/aphids/reporthtml/trans44.htm>
- Huang, C. D., J. F. Zhou, D. Yang. 1998. Experiments on the control of soybean aphid with Imidacloprid. Translation from *Pesticides.* 37 : 44-45.

www.ksu.edu/issa/aphids/reporhtml/trans10.htm

- Iwaki, M., M. Roechan, H. Hibino, H. Tochiara, and D.M. Tantera. 1980. A persistent aphid borne virus of soybean, Indonesian Soybean dwarf virus transmitted by *Aphis glycines*. *Plant Dis.* 64: 1027–1030.
- Kim, K. S, Hill.C. B, Diers.B.W, Hartman.G. L, and Rouf Mian.M. A. 2008. Discovery of Soybean Aphid Biotypes. *Crop Sci.* 48: 923–928.
- Krupke, C. H., J. L. Obermeyer, and L.W. Bledsoe. 2005. Soybean aphid. Available at www.entm.purdue.edu/entomology/ext/targets/e-series/EseriesPDF/E-217.pdf (verified 21 Mar. 2008). E-217-W. Purdue Extension, Purdue Univ., West Lafayette, IN.
- Landis, D., M. Brewer, and G. Heimpel. 2003. Soybean aphid parasitoid questionnaire 2003. Available at www.ncera125.ent.msu.edu/StateRpts2003MI.htm (verified 21 Mar. 2008). NCERA-125, Michigan State Univ., East Lansing.
- Li, Y., C. B. Hill, S. R. Carlson, B.W. Diers, and G. L. Hartman. 2007. Soybean aphid resistance in the soybean cultivars Dowling and Jackson map to linkage group M. *Mol. Breed.* 19:25–34.
- Mensah, C., C. DiFonzo, and D. Wang. 2006. Inheritance of soybean aphid resistance in PI 567541B and PI 567598B [CDROM]. 2006 Agronomy abstracts. ASA, Madison, WI.
- Macedo, T. B., C. S. Bastos, L. G. Higley, K. R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J. Econ. Entomol.* 96(1): 188-193.
- Matsumura, S. 1917. A list of the Aphididae of Japan, with descriptions of new species and new genera. *Journal of the College of Agriculture, Tohoku Imperial University, Sapporo* 7, 351–414.

- McCornack, B. P., D. W. Ragsdale, and R. C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. *J. Econ. Entomol.*
- Mian, M. A. R., R. B. Hammond, and S. K. St. Martin. 2008. New plant introductions with resistance to the soybean aphid. *Crop Sci.*48:1055-1061.
- Nagaraj, N. J., J. C. Reese, M. B. Kirkham, K. Kofoed, L. R. Campbell, and T. Loughin. 2002a. Effect of greenbug, *Schizaphis graminum*, biotype K on chlorophyll content and photosynthetic rate of tolerant and susceptible sorghum hybrids. *J. Kans. Entomol. Soc.* 75: 299-307.
- Nielsen, C., and A. E. Hajek. 2005. Control of invasive soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. *Environ. Entomol.* 34: 1036-1047.
- Ohio State Soybean Aphid Monitoring. 2003. Facts.www.vanwert.osu.edu/ag/aphid.htm
- Okada, Y. and F. Nakasuji.1980. *Jpn. J. Appl. Entomol.Zool.*24: 86-92 (in Japanese with English Summary)
- Ostlie, K. 2002. Managing Soybean Aphid. University of Minnesota.
www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm
- Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. *Ann. Entomol. Soc. Am.* 97: 204-208.
- Rutledge, C. E., R. J. O'Neil, T. B. Fox, and D. A. Landis. 2004. Soybean aphid predators and their use in integrated pest management. *Ann. Entomol. Soc. Am.* 97: 240-248.
- Sama, S., K. M. Saleh, and P. van Halteren. 1974. Research reports 1969–1974. p. 171–172. *In* Varietal screening for resistance to the aphid, *Aphis glycines*, in soybean. *Agricultural*

Cooperation, Indonesia, the Netherlands.

Sloderbeck, P. E., J. C. Reese, R. J. Whitworth, C. M. Smith, R. A. Higgins, W. T. Schapaugh, R. E. Wolf, and D. J. Jardine. 2003. The soybean aphid: A new pest in Kansas Soybeans, Kansas State University. June.

Sloderbeck, P. E., R. J. Whitworth, and R. A. Higgins. 2004. Soybean insect management 2004, Kansas State University. February. P. 4.

Soybean Extension and Research Program, Iowa State University. 2007.
www.extension.agron.iastate.edu/soybean/uses_biodiesel.htm, 2008

Steffey, K. L. 2004. Soybean aphids in Illinois. Available at www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/SoybeanAphidsInIllinois_Steffey.ppt (verified 21 Mar. 2008). Univ. of Illinois, Urbana-Champaign.

Sun, Z., P. Z. Tian, and J. Wang. 1990. Study on the uses of aphid-resistant character in wild soybean: I. Aphid-resistance performance of F2 generation from crosses between cultivated and wild soybeans. *Soybean Genet. Newsl.* 17: 43–48.

Sun, B., S. B. Liang, and W. X. Zhao. 2000. Outbreak of soybean aphid in Suihua District in 1998 and its control strategies. Translation from *Soybean Bulletin*. 8(1): 5.
www.ksu.edu/issa/aphids/reporthtml/trans40.doc

Takahashi, S., M. Inaizumi, and K. Kawakami. 1993. Life cycle of the soybean aphid *Aphis glycines* Matsumura, in Japan. Translation from *Japanese Journal of Applied Entomology and Zoology*. 37: 207-212. www.ksu.edu/issa/aphids/reporthtml/trans77.htm

Venette, R. C., and D. W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): Where will it end? *Ann. Entomol. Soc. Am.* 97: 219–226.

- Voegtlin, D. J., R. J. O'Neil, and W. R. Graves. 2004. Tests of suitability of overwintering hosts of *Aphis glycines*: identification of a new host association with *Rhamnus alnifolia* L' Heritier. *Ann. Entomol. Soc. Am.* 97: 233-234.
- Wang, C. L., L. Y. Xiang, G. X. Zhang, and H. F. Chu. 1962. Studies on the soybean aphid, *Aphis glycines* Matsumura. Translation from *Acta Entomol. Sinica*. 11: 31-44.
www.ksu.edu/issa/aphids/reporthtml/trans28.htm
- Wang, X. B., Y. H. Fang, S. Z. Lin, L. R. Zhang, and H. D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. Translation from *Plant Protection* (Institute of Plant Protection, CAAS). 20: 12-13.
www.ksu.edu/issa/aphids/reporthtml/trans29.doc
- Wang, C. R., J. G. Chen, Y. R. Guo, X. Y. Gong, Z. F. Xu, and C. Lin. 1998. Development and control of soybean aphid *Aphis glycines* in Heilongjiang Province. Translation from *Soybean Bulletin*. 6: 15.
www.ksu.edu/issa/aphids/reporthtml/trans12.doc
- Wang, S., X. Bao, Y. Sun, R. Chen, B. Zhai, S. Y. Wang, X. Z. Bao, Y. J. Sun, R. L. Chen, and B. P. Zhai. 1996. Study on the effects of the population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. *Soybean Sci.* 15: 243-247.
- Whitworth, J., 2008. Soybean aphid found in Kansas. www.southwestfarmpress.com/grains/Soybean_aphid
- Wu, X. B., W. J. Ni, and P. J. Liu. 1999. Occurrence and control of the soybean aphid *Aphis glycines* Matsumura. Translation from *Chinese Journal of Biological Control*. 6: 20.
www.ksu.edu/issa/aphids/reporthtml/trans15.htm
- Yadava, U. L. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. *Hort. Sci.* 21: 1449-1450.
- Ye, Z., Z. Liu, and D. Jin. 1996. Background information of cooperative IPM project: Soybean

and soybean insect pests in China. Translation from Unpublished Report. 6 p.
www.ksu.edu/issa/aphids/reporthtml/trans20.htm

Zhang, X. R. 1988. Study on the instars of soybean aphid, *Aphis glycines* Matsumura, in Jilin province. Translation from Journal of Jilin Agricultural University (*Acta Agriculturae Universitatis Jilinensis*). 10(3): 15-17, www.ksu.edu/issa/aphids/reporthtml/trans32.htm

CHAPTER-2. Characterization of Soybean Resistance to Soybean Aphid (Hemiptera: Aphididae) Biotypes.

Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybean, *Glycine max* L.(Merr.) and was first detected in Wisconsin in 2000. It has spread into most, if not all, soybean producing areas of the United States and Canada since it was first detected. Host plant resistance to insects is an important alternative to other controls and is more sustainable than any other control methods against this insect. Recent studies identified two soybean aphid biotypes during 2005 in just five years after its invasion. This study includes the entries from Kansas, Michigan, and Nebraska soybean genotypes. Also it is the first attempt to study the different Kansas soybean entries response to biotype 2. The plants were screened by infested at V1 stage with 6 aphids per plant and populations counted after 7 days. The results showed that the genotypes that were resistant to biotype 1 (K1639, K1642, K1613 K1621, Dowling and Jackson) were susceptible to the new biotype 2 with large populations developing on these entries. We found three new Kansas genotypes show resistance against biotype 1. And two of the Michigan genotypes (E06902 and E07906-2) showed resistance to both biotype 1 and biotype 2. Further characterization of resistance made clear that they showed antibiosis type of resistance with the two above biotypes. Thus, it is concluded that we found two genotypes resistant to both biotypes and the biotype 2 overcame the several different sources of resistance in previously found resistant genotypes.

Introduction

Soybean is one of the most important cultivated crops in the world. The soybean aphid, *Aphis glycines* Matsumura, is an invasive pest and belongs to the Order Hemiptera. It was first described by Matsumura in 1917 (Matsumura, 1917). The aphid was first reported in North America in Wisconsin (Macedo et al. 2003). It was not reported in North America until 2000 (Hartman et al. 2001). During the summer and autumn of 2000, the soybean aphid was observed

in some other states , that include Illinois, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri and Ohio (Ohio state soybean aphid monitoring, 2003). The aphid has been found in Canada in 2001 (Baute, 2002). It has spread into many of the soybean producing areas of the United States and emerged as an important pest of soybean in North America (Ragsdale et al. 2004). It has been a serious problem in many of the states of US and Canadian provinces. Now in North America, there are three soybean aphid biotypes are present. Earlier there were reported only two biotypes (Kim et al. 2008), the biotype 1 (Hill et al. 2004a) and the biotype 2. But in 2009, one more biotype was reported and named as biotype 3 (Hill et al. 2010). Originally it is native to temperate zones of Asia including Japan, China, Philippines, Indonesia, Korea, Vietnam and in some parts of eastern Russia, Africa, India and Indonesia and is a recent invasive of Australia (Krupke et al. 2005).

Soybean aphids spread very fast (Wang et al. 1998) and cause much damage even indirectly, as vectors for virus transmission such as alfalfa mosaic virus, soybean dwarf virus and soybean mosaic virus (Sama et al. 1974, Hartman et al. 2001). When large number of aphids feed on stem and leaves, it causes the wilting, curling, yellowing and even dropping of leaves from the plant and this mainly because of removal of water and nutrients from the plant (Mensah et al. 2005). Environmental conditions, climate, cultural practices, planting time, predators, pathogenic fungi, insecticide, host resistance and the synchronization of soybean and aphid development are the various factors that affect soybean aphid outbreaks in most of the soybean growing areas (Wu et al. 1999). In soybean, a reduction in photosynthetic capacity can result from soybean aphid feeding (Macedo et al. 2003). Some secretions from the soybean aphids, like honeydew, can cause development of sooty mold on the leaves, which results in more yield losses (Krupke et al. 2005) In North America, soybean aphid is the only aphid species that produces large colonies on soybeans (Sloderbeck et al. 2003). The soybean aphid has been causing millions of dollars in losses to this legume crop. In fields of Minnesota, an yield loss of more than 50% was reported during 2001 (Ostlie, 2002). During the heavy infestations an yield reduction of 50-70% have been reported from China (He et al. 1995). A large area of 3 million hectars soybean fields have been sprayed in USA in 2003 (Landis et al. 2003) and about 9 to 12 million dollars have been spent for controlling this pest in Illinois alone (Steffey, 2004)

The main available method to control the pest during the earlier years was chemical treatment (Hill et al. 2006b). There is a big problem of population rebounding after a chemical treatment in soybean aphid. This problem mainly occurred when a product did not cut down the aphid population by 95 % or more (Baute, 2002). One way to reduce the dependence on chemical control is to grow soybean varieties with aphid resistance. Development of soybean varieties and their breeding programs started long back in China and Indonesia and reported screening results for about 200 varieties came from Indonesia (Sama et al. 1974). Integrated pest management (IPM) is a systematic approach used widely against the control of soybean aphid. In US, economic threshold of IPM generated an economic net benefit of \$ 1.3 billion over the last 8 years since the IPM research started and about \$0.6- \$2.6 net benefit for growers and consumers during 2005 (Song and Swinton, 2009). Host plant resistance is one of the most important IPM strategy used against soybean aphid with some added merits like environmentally friendly, economical and compatible with other control measures. So many studies are going on in host plant resistance since Hill et al. (2004a) found antibiosis to be the main category of resistance in nine soybean genotypes accessions, through choice and no-choice studies in the greenhouse. Antibiosis is the category of resistance, found in Dowling (PI 548663), Jackson (PI 548657) and Sugao Zarai (PI 200538) (Li et al. 2004).

Another group of scientists found single dominant gene *Rag1* in Dowling and a similar gene in Jackson (Hill et al. 2006a, Hill et al. 2006b). Mensah et al. (2005) screened about 2147 soybean genotypes and got four resistant genotypes. Eleven more genotypes found resistant to soybean aphid and nine of them showed antibiosis effect and other two (K1639 and Pioneer 95B97) showed both antibiosis and antixenosis as category of resistance (Diaz-Montano et al. 2006). Some accessions collected from China show resistance to the biotype 2 (aphid collected in Ohio) and two of them show resistance to biotype 1 (collected from Illinois) (Mian et al. 2008). Kim et al. 2008 reported that there are two soybean aphid biotypes that occur in N. America, biotype 1 and biotype 2. PI 200538 and PI 567597C were resistant to both the biotype 1 and 2 and will be useful sources of resistance to both biotypes. Recent studies show some more PIs (PI 243540, PI 567301 and PI 567324) having antixenosis and antibiosis resistance to Ohio biotype (Mian et al. 2008). Thus, the objective includes the comparison of two soybean aphid

biotypes on more soybean genotypes and the characterization of soybean resistance to the above two biotypes.

Materials and Methods

Aphid cultures. Two biotypes used in the experiments are biotype 1 and biotype 2. Biotype 2 was obtained from Brian W. Diers, Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801 on July 2008 and biotype 1 from the fields of Nebraska on 2008. They were reared on the susceptible soybean genotype KS 4202. A continuous supply of seedlings maintained the two colonies properly, biotype 1 under pesticide free greenhouse conditions at 20 -30° C temperature, 23- 40 % relative humidity and supplemental high pressure sodium vapor lamps set for a photoperiod of 14:10 (L:D) h. The biotype 2 was maintained in lab conditions, inside a growth chamber (Percival Scientific, Inc. 505 Research Drive, Perry, IA 50220) with same environmental conditions as that of green house. The movement of aphids from lab to green house at the time of infestation was done by keeping the infested leaves in tightly closed petri-dish using parafilm rolls. The infested plants got freezed overnight to kill all the aphids after the experiments.

Screening of soybean genotypes. The experiments were carried out in pesticide-free greenhouse with same heating and cooling facilities as in insect culture. A total of 83 genotypes were screened against the two biotypes, starting with Kansas State University Public lines (K and KO₃), subsequently Nebraska genotypes (supplied by Thomas E. Clemente, University of Nebraska, Lincoln, NE 68588), PI genotypes (plant introductions) and Michigan genotypes (Department of Crop and Soil sciences, Michigan State University, MI48824). Soybean plants were grown separately in plastic cone-tainers having 3.8-cm-diameter and 21.0-cm-deep, (Ray Leach Cone-tainer, Hummert International, Earth City, MO), placed on low platform racks and filled with steam-sterilized potting mix (Premier Promix, Rivie`re-Loup, Que`bec, Canada). Two similar sets of plants were grown for the two aphid biotypes concurrently. Thus experiments were done separately for the two biotypes with five different genotypes and two susceptible checks (KS 4202 & KO₃ 4686), included for greater accuracy, as a control in each experiment and in a complete randomized design with five replications. The interplant aphid

movements were avoided by keeping the plants separated on the racks (Diaz-Montano et al. 2006).

It was found that soybean resistance had been expressed in all stages of plant growth (Hill et al. 2004b). The leaf dry matter is maximum at the V1-stage, with two fully developed leaves at unifoliate nodes (Fehr and Caviness, 1977), or 9 days after planting, the plants were found most suitable for infestation. The healthy plants were selected and 3 adult aphids were placed on the upper side of each unifoliate leaf using a moist camel's- hair paint brush (number 0), thus 6 aphids per plant. Aphids are allowed to freely feed and reproduce on the plants (Diaz-Montano et al. 2006). Disturbances were minimized by watering the plants from bottom using the pans. The aphid number was counted 7 days after infestation on the entire plant (Diaz-Montano et al. 2006) and soybean genotypes were compared for aphid population (both biotype 1 and biotype 2) in the above experiments separately. Genotypes with significantly lower number of aphids than the susceptible genotypes selected and carried out second sets of experiments with the same experimental design for confirmation (He et al. 1995, Diaz-Montano et al. 2006).

Antibiosis Tests or No-Choice tests. Antibiosis is a category of resistance and having adverse effect on the physiology of insects. The genotypes with significantly lower number of aphids than the susceptible checks were selected for this test from the screening results. The susceptible checks used were same as that of screening tests. But three resistant checks were included, PI 567597C, PI567598B (Mensah et al. 2005) and PI567301B (Mian et al. 2008). The planting protocols and greenhouse conditions were same as that of screening tests for the given genotypes. The aphids used were same aged adult aphids maintained on susceptible check, KS4202. For the synchronization of same aged aphids, several adults were placed on the susceptible check (KS4202) and were allowed to reproduce for 24hours. Then the adult aphids were removed (Li et al. 2004, Diaz-Montano et al. 2006) and the nymphs were allowed to develop into an adult for 7 days (McCornack et al. 2004).

Selected plants were arranged on the low platform racks in cone-tainers with a completely randomized design and the experiments were done separately for both biotypes as in screening. Healthy soybean plants were selected at the V-1 stage (Fehr et al. 1971) with five

replications per genotype. Three aphids were placed in double-sided sticky cages (Converters, Inc., Huntingdon Valley, PA) having an inner oval area of 1.2 cm² using a moist camel's brush (number 0) and closed the cage immediately with organdy cloth, for free air movements between cage inside and surroundings. The cages were stuck on each unifoliate leaf and thus a total of two cages per plant. Observations were recorded 4 days after infestation (Diaz-Montano et al. 2006). Aphid populations of the two biotypes were compared separately on the resistant and susceptible genotypes. The plants were watered from the bottom to avoid the disturbance to the cages.

Antixenosis Tests or Choice tests. Antixenosis is the category of resistance affecting the behavior of the insect and is assessed in choice tests (Hill et al. 2004a). Choice tests included the same genotypes from the no-choice tests (antibiosis tests) and carried out in same green house conditions as that of the above two tests. Single pots (20-cm diameter by 20-cm height) were used with 17-18 cm height of potting mixture. The selected genotypes planted along the sides of the pot with about 3.5 cm between plants and arranged in a circle (Diaz-Montano et al. 2006). At the V-1 stage of the plants, a filter paper (11-cm diameter) was kept at the centre of the pot (exactly in the middle of the circle of plants) with 100 adult aphids released on it. The aphid number on each of the plants in the pot was counted 24 hours later. Pots were arranged in a completely randomized design with ten replicates (pots) (Diaz-Montano et al. 2006). Separate experiments were performed with two biotypes.

Statistical analyses. Statistical analyses of soybean aphid populations (two biotypes) in different genotypes were done by using Proc. GLM of the SAS® Program (SAS Institute 1999). Multiple comparisons were done using Tukey's studentized range test.

Results and Discussions

Screening of soybean genotypes. A total of 83 genotypes were tested against both biotypes and 5 entries were found resistant against biotype 1 and 2 entries against biotype 2 with significantly lower ($P < 0.05$) number of aphids than the susceptible checks (KS4202 &

KO34686). The earlier resistant genotypes, K1639, K1642, K1613, K1621 (Diaz-Montano et al. 2006), Jackson and Dowling (Hill et al. 2004a) to biotype 1 were found highly susceptible (Table 2) to the new biotype 2. The numbers of aphids were less than 15 in these genotypes in case of biotype 1 but greater than 40 in case of biotype 2, 7 days after infestation. One of the important resistant genotype earlier found, K1639, showed susceptibility towards biotype 2. The above screening results showed that the biotype 2 is more virulent and can overcome the sources of resistances in the earlier genotypes those showed resistance against biotype 1. The fecundity of the biotype 2 was much greater than the biotype 1 for the given time period of infestation. The genotypes found resistant to biotypes, 1 and 2, were those supplied from Michigan (Tables 1 & 2). Their resistance to biotype 1 was stronger than that of K1639. This shows that the Michigan genotypes have some additional sources of resistance to the given two biotypes than the earlier existing ones. It is more clear from the table of results that the genotypes (K1639, K1642, K1613 K1621, Dowling and Jackson) found resistant to biotype 1 were significantly different ($P < 0.05$) from the susceptible genotypes (Table 1) and these genotypes were not significantly different from the susceptible genotypes with biotype 2 (Table 2). When the susceptible genotypes screened with biotype 2, the aphid numbers increased significantly ($P < 0.05$) higher than the biotype 1.

Antibiosis or No-Choice tests of selected genotypes. The selected genotypes with significantly lower number of aphids (Tables 1 & 2) than that of susceptible checks ($P < 0.05$) were tested for antibiosis test. There were two separate sets of experiments, one for each of the biotypes in the screening experiments. In the first set of experiments, the Michigan genotypes, E06902 and E07906-2, along with three PI'S (PI 567597C, PI567598B and PI567301B), susceptible checks and one Kansas entry (Fig 1) were included in tests against the biotype 2. Second set, including the Michigan and Kansas genotypes showed lower number of aphids than the susceptible checks (Fig 1), when screened against biotype 1.

The results showed that the Michigan genotypes (E06902 and E07906-2) and the PIs (resistant checks), except PI567597C, had statistically lower number of aphids ($P < 0.05$) compared with the susceptible checks (KS4202 and KO3-4686) against biotype 2. Thus these genotypes had good antibiosis type of resistance against the biotype 2. But the PI567597C was statistically different from the other resistant genotypes with high number of aphids. Hence had

both antibiotic and antixenosis type of resistance against the biotype 2. All the evaluated genotypes showed statistically lower number of aphids ($P < 0.05$) compared with the susceptible check against biotype 1. But the K1639 was susceptible for the biotype 2 and therefore no more categories of resistance against the biotype 2 with no statistical difference with the susceptible checks. But for biotype 1, it showed similar categories of resistances as in PIs and Michigan.

Antixenosis or Choice tests of selected genotypes. The same genotypes in the antibiosis tests were used for the choice test (antixenosis) tests. As that of no-choice test, there were two sets of experiments for the two biotypes separately (Fig 2). The genotypes were same as in no-choice tests for each set of experiments. The results showed that two of PIs resistant checks (PI567301B and PI567598B) had strong antixenotic resistance against both biotypes. Hence the Michigan genotypes had statistically higher number of aphids than the resistant checks; they were not showing any antixenotic resistance against the both biotypes. Also the K1639 genotype showed significantly lower number than the susceptible checks and thus expressed both antibiosis and antixenosis resistance against biotype 1.

From the results, it was confirmed that most the genotypes showing resistance to biotype 1 were found susceptible to the new biotype 2. Hence biotype 2 overcomes the sources of resistance in previous found resistant genotypes. During the middle of 2008 Kim et al. reported the occurrence of multiple biotypes in soybean aphid and found they can overcome the *Rag1* resistance. They found earlier resistant genotypes with *Rag1* gene, Jackson and Dowling overcame their resistance with new biotype. Almost the same period Mian et al., 2008 found more plant introductions (PIs) found resistant to biotype 2 and encouraged the development of other aphid resistant cultivars. Our results further more confirmed the new virulent biotype 2 reaction on different Kansas genotypes with their high susceptibility towards biotype 2. With the other newly discovered soybean genotypes, the Michigan genotypes, E06902 and E07906-2, we found add boost towards the aphid resistant genotype development programs in US. Also these studies helps the soybean breeders to give the opportunity for developing new soybean cultivars against the biotype 2 in Kansas and other areas in US, where have not yet reported any biotype 2 attack.

References

- Baute, T. 2002. Soybean aphids. Ontario Ministry of Agriculture and Food (OMAF).
www.gov.on.ca/OMAFRA/english/crops/facts/soybean_aphid/soybean_aphid.htm
- Diaz-Montano, J., J. C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid in several soybean genotypes. *J. Econ. Entomol.* 99: 1884–1889.
- Fehr, W. R., C. E. Caviness, D. T. Burmood, and J. S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. *Crop Sci.* 11:929–931.
- Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. *Spec. Rep. Coop. Ext. Serv. Agric. Home Econ. Exp. Stn., Iowa State Univ.* 80: 1-12.
- Hartman, G. L., L. L. Domier, L. M. Wax, C. G. Helm, D.W. Onstad, J.T. Shaw, L. F. Solter, D. J. Voegtlin, C.J. D’Arcy, M.E. Gray, K.L. Steff y, and P.L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybean in Illinois in 2000 and its potential control. Available at www.plantmanagementnetwork.org/php. *Plant Health Progr.* DOI 10.1094/php-2001-0205-01-HN.
- He, F. G.; X. D. Liu, F. Y. Yan; Y. Q. Wang. 1995. Study on soybean aphid resistance. Translation from *Liaoning Agricultural Science* 4: 30-34.
www.ksu.edu/issa/aphids/reporthtml/trans18.doc
- Hill, C. B., Y. Li, and G. L. Hartman. 2004a. Resistance to the soybean aphid in soybean germplasm. *Crop Sci.* 44: 98–106.
- Hill, C. B., Y. Li, and G. L. Hartman. 2004b. Resistance to *Glycine* species and various cultivated legumes to the soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 97: 1071– 1077.

- Hill, C. B., Y. Li, and G. L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. *Crop Sci.* 46: 1601–1605.
- Hill, C. B., Y. Li, and G. L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. *Crop Sci.* 46:1606–1608.
- Hill, C. B., L. Crull, T. K. Herman, D. J. Voegtlin and G. L. Hartman. 2010. A new soybean aphid (Hemiptera:Aphididae) biotype identified. *J. Econ. Entomol.* 103: 509- 515.
- Kim, K. S., C. B. Hill, B.W. Diers, G. L. Hartman, and M. A. Rouf Mian. 2008. Discovery of soybean aphid biotypes. *Crop Sci.* 48: 923–928.
- Krupke, C. H., J. L. Obermeyer, and L. W. Bledsoe. 2005. Soybean aphid. Available at www.entm.purdue.edu/entomology/ext/targets/e-series/EseriesPDF/E-217.pdf (verified 21 Mar. 2008). E-217-W. Purdue Extension, Purdue Univ., West Lafayette, IN.
- Landis, D., M. Brewer, and G. Heimpel. 2003. Soybean aphid parasitoid questionnaire 2003. Available at www.ncera125.ent.msu.edu/StateRpts2003MI.htm (verified 21 Mar. 2008). NCERA-125, Michigan State Univ., East Lansing.
- Li, Y., C. B. Hill, and G. L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 97:1106–1111.
- Matsumura, S. 1917. A list of the Aphididae of Japan, with descriptions of new species and new genera. *Journal of the College of Agriculture, Tohoku Imperial University, Sapporo* 7, 351–414
- Macedo, T. B., C. S. Bastos, L. G. Higley, K. R. Ostlie, and S. Madhavan. 2003. Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J. Econ.*

Entomol. 96(1): 188-193.

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

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

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

Ohio State Soybean Aphid Monitoring. 2003. Facts.www.vanwert.osu.edu/ag/aphid.htm

Ostlie, K. 2002. Managing Soybean Aphid. University of Minnesota.
www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm

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

Sama, S., K. M. Saleh, and P. van Halteren. 1974. Research reports 1969–1974. p. 171–172. *In* Varietal screening for resistance to the aphid, *Aphis glycines*, in soybean. Agricultural Cooperation, Indonesia, the Netherlands.

SAS Institute. 1999. SAS/STAT user’s guide, version 8. SAS Institute, Cary, NC.

Sloderbeck, P. E., J. C. Reese, R. J. Whitworth, C. M. Smith, R. A. Higgins, W. T. Schapaugh, R. E. Wolf, and D. J. Jardine. 2003. The soybean aphid: A new pest in Kansas Soybeans, Kansas State University. June.

Song, F., and S. M. Swinton. 2009. Returns to integrated pest management research and outreach for soybean aphid. *J. Econ. Entomol.*102: 2116- 2125.

Steffey, K. L. 2004. Soybean aphids in Illinois. Available at www.ipm.uiuc.edu/fieldcrops/insects/soybean_aphids/workshop/SoybeanAphidsInIllinois_Steffey.ppt (verified 21 Mar. 2008). Univ. of Illinois, Urbana-Champaign.

Wang, C. R., J. G. Chen, Y. R. Guo, X. Y. Gong, Z. F. Xu, and C. Lin. 1998. Development and control of soybean aphid *Aphis glycines* in Heilongjiang Province. Translation from Soybean Bulletin.6: 15 www.ksu.edu/issa/aphids/reporthtml/trans12.doc

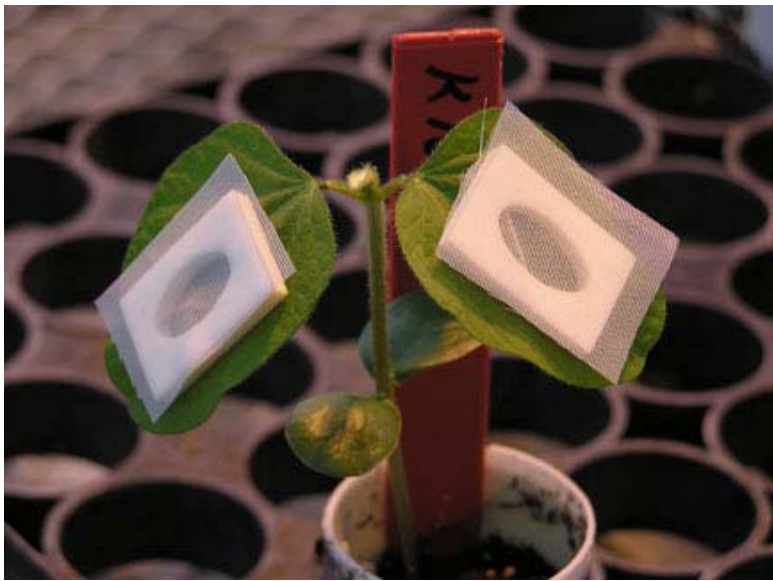
Wu, X. B., W. J. Ni, and P. J. Liu. 1999. Occurrence and control of the soybean aphid *Aphis glycines* Matsumura. Translation from Chinese Journal of Biological Control. 6: 20. www.ksu.edu/issa/aphids/reporthtml/trans15.htm

Figure 2.1 Experimental set up for screening test and soybean plant at V1 stage



PREDEESH

Figure 2.2. No-choice or Antibiosis test experimental setup



PREDEESH

Figure 2.3. Choice test or Antixenosis experimental setup



Figure 2.4. Antibiosis test. Number of aphids (Mean \pm SE) 4 d after infestation. Bars with different letters are significantly different within each biotype ($P < 0.05$), using Tukey's test.

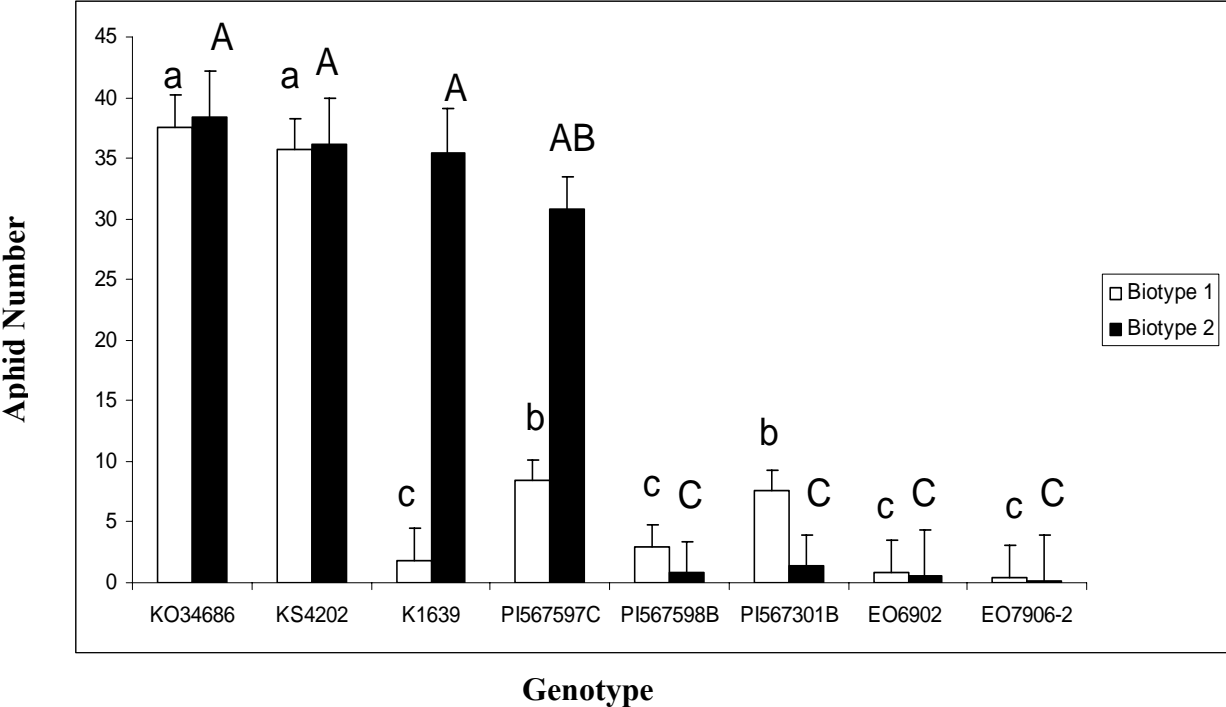


Figure 2.5 Antixenosis test. Number of aphids (Mean \pm SE) 24 h after infestation. Bars with different letters are significantly different within each biotype ($P < 0.05$), using Tukey's test

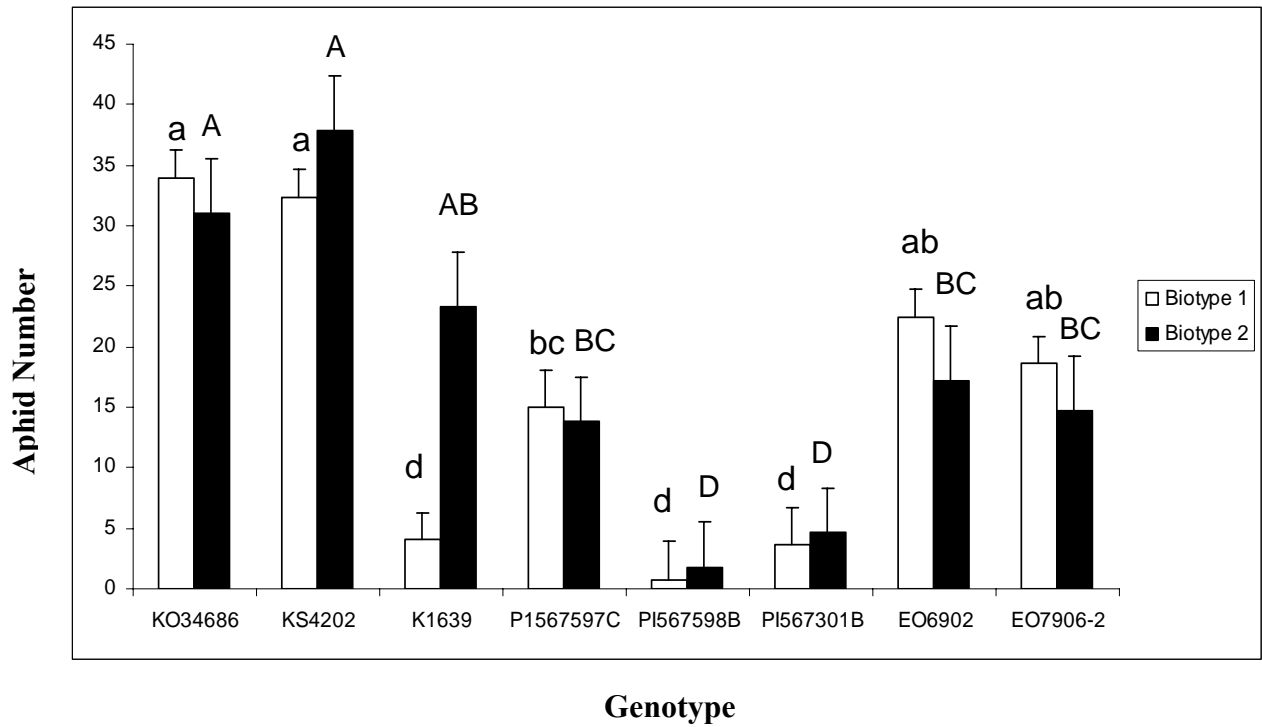


Table 2.1 List of soybean genotypes supplied for screening with their known reactions.

#	Genotype	Priority	Biotype 1 Reaction (known)	Biotype 2 Reaction (known)
1	K0 ₃ 4686rr	HP	Susceptible	Unknown
2	KS 4402	HP	Susceptible	Unknown
3	KS 4202sp	HP	Susceptible	Unknown
4	K1639	HP	Resistant	Unknown
5	K1642	HP	Resistant	Unknown
6	K1613	HP	Resistant	Unknown
7	K1621	HP	Resistant	Unknown
8	JACKSON	HP	Resistant	Susceptible
9	DOWLING	HP	Resistant	susceptible
10	PALMETTO	HP	Resistant	Unknown
11	PI 200538	HP	Resistant	Resistant
12	PI 567597C	HP	Resistant	Resistant
13	PI 5677541B	HP	Resistant	Moderately Resistant
14	PI567301B	HP	Resistant	Resistant
15	PI567324	HP	Resistant	Resistant
16	PI567541A	HP	Resistant	Susceptible
17	PI567542	HP	Resistant	Susceptible
18	PI567597A	HP	Resistant	Susceptible
19	PI567598B	HP	Resistant	Susceptible
20	K07-1016	HP	Unknown	Unknown
21	K07-1057	HP	Unknown	Unknown
22	K07-1135	HP	Unknown	Unknown
23	K07-1238	HP	Unknown	Unknown
24	K07-1527	HP	Unknown	Unknown
25	K07-1663	HP	Unknown	Unknown
26	K07-1741	HP	Unknown	Unknown

27	K07-1853	HP	Unknown	Unknown
28	K07-2025	HP	Unknown	Unknown
29	K07-2057	HP	Unknown	Unknown
30	K07-2389	HP	Unknown	Unknown
31	K07-2399	HP	Unknown	Unknown
32	K07-2417	HP	Unknown	Unknown
33	K07-2623	HP	Unknown	Unknown
34	K07-2862	HP	Unknown	Unknown
35	K07-3436	HP	Unknown	Unknown
36	K07-3474	HP	Unknown	Unknown
37	K07-3561	HP	Unknown	Unknown
38	K07-4031	HP	Unknown	Unknown
39	E06902	HP	Unknown	Unknown
40	E07906-2	HP	Unknown	Unknown
41	740-1,T1-5	HP	Unknown	Unknown
42	740-3, T1-2	HP	Unknown	Unknown
43	740-1, T1-2	HP	Unknown	Unknown
44	740-3, T1-7	HP	Unknown	Unknown
45	K1599	LP	Susceptible	Unknown
46	K1607	LP	Susceptible	Unknown
47	K1614	LP	Susceptible	Unknown
48	K1619	LP	Susceptible	Unknown
49	K1620	LP	Susceptible	Unknown
50	K1622	LP	Susceptible	Unknown
51	K1641	LP	Susceptible	Unknown
52	K1603rr	LP	Susceptible	Unknown
53	K1623rr	LP	Susceptible	Unknown
54	K1624RR	LP	Susceptible	Unknown
55	K1625RR	LP	Susceptible	Unknown
56	K1626RR	LP	Susceptible	Unknown

57	K1627RR	LP	Susceptible	Unknown
58	K1628RR	LP	Susceptible	Unknown
59	K1629RR	LP	Susceptible	Unknown
60	K1630rr	LP	Susceptible	Unknown
61	K1631rr	LP	Susceptible	Unknown
62	K1632rr	LP	Susceptible	Unknown
63	K1633RR	LP	Susceptible	Unknown
64	K1634rr	LP	Susceptible	Unknown
65	K1635RR	LP	Susceptible	Unknown
66	K1636RR	LP	Susceptible	Unknown
67	K1637RR	LP	Susceptible	Unknown
68	K1638rr	LP	Susceptible	Unknown
69	K1640rr	LP	Susceptible	Unknown
70	KS4694	LP	Susceptible	Unknown
71	KS4602N	LP	Susceptible	Unknown
72	KS4895	LP	Susceptible	Unknown
73	KS4997	LP	Susceptible	Unknown
74	KS5004N	LP	Susceptible	Unknown
75	KS5502N	LP	Susceptible	Unknown
76	KS4103sp	LP	Susceptible	Unknown
77	KS4302sp	LP	Susceptible	Unknown
78	KS4303sp	LP	Susceptible	Unknown
79	KS4702sp	LP	Susceptible	Unknown
80	KS5001sp	LP	Susceptible	Unknown
81	KS5003sp	LP	Susceptible	Unknown
82	KS5201sp	LP	Susceptible	Unknown
83	KS5202sp	LP	Susceptible	Unknown

Table 2.2 Soybean genotypes from the screening tests with significantly lower numbers of aphids ($P<0.05$), when compared with two susceptible checks (K03-4686 and KS4202) for a given experiment after 7d of infestation against biotype 1.

Expmnt	Genotype	(Mean ^a ± SE) ^b	KS4202	K03-4686	Comment
2b	K1613	25.6±6.9b	84.8 ±6.9a	76 ±6.9a	Moderately Resistant (Diaz-Montano et al. 2006)
2b	K1621	15 ±6.9b	84.8 ±6.9a	6 ±6.9a	Moderately Resistant (Diaz-Montano et al. 2006)
2b	K1639	2.2 ±6.9b	84.8 ±6.9a	76 ±6.9a	Resistant (Diaz-Montano et al. 2006)
3b	K1642	14.2 ±4.2b	72 ±2.69	63 ±2.69c	Moderately Resistant (Diaz-Montano et al. 2006)
5b	Dowling	1.6 ±2.69c	62 ±2.69a	57 ±2.69a	Resistant (Hill et al. 2004a)
5b	Jackson	1.7 ±2.69c	62±2.69a	57±2.69a	Resistant (Hill et al. 2004a)
8b	K07-3474	9.8 ±2.78d	40.6 ±2.78ba	48.6 ±2.78a	Moderately resistant
8b	K07-3436	3.2 ±2.78d	40.6 ±2.78ba	48.6 ±2.78a	Resistant
10b	K07-4031	4.2 ±6.75b	50.4 ±6.5a	49.2 ±6.5a	Resistant
12b	E06902	1.3 ±3.44b	44.2 ±3.44a	41 ±3.44a	Resistant
12ab	E079060-2	1.1 ±3.44b	44.2 ±3.44a	41 ±3.44a	Resistant

^a average 5 replicates, observations 7 d after infestation

^b different letters followed by the mean within the row are significantly different
($P<0.05$; Tukey's test)

Table 2. 3. Soybean genotypes from the screening tests with significantly lower numbers of aphids ($P<0.05$), when compared with two susceptible checks (K03-4686 and KS4202) for a given experiment after 7d of infestation against biotype 2.

Expmnt	Genotype	(Mean ^a ± SE) ^b	KS4202	K03-4686	Comment
2b	K1613	48.8 ±17.02a	62.2 ±17.02a	78.4 ±17.02a	Susceptible
2b	K1621	40.8 ±17.02a	62.2 ±17.02a	78.4 ±17.02a	Susceptible
2b	K1639	40 ±17.02a	62.2 ±17.02a	78.4 ±17.02a	Susceptible
3b	K1642	47.2 ± 13.2a	54 ±13.2a	49±13.2a	Susceptible
5b	Dowling	22.5 ±5.47c	60.8 ±5.47a	44 ±5.47ba	Susceptible (Kim et al. 2008)
5b	Jackson	23.4 ±5.47c	60.8 ±5.47a	44 ±5.47ba	Susceptible (Kim et al. 2008)
8b	K07-3474	56 ±6.85a	61 ±6.85ba	66.6 ±6.85a	Susceptible
8b	K07-3436	39.6 ±6.85a	61 ±6.85a	66.6 ±6.85a	Susceptible
10b	K07-4031	51.8 ±7.6a	58 ±7.6a	52 ±7.6a	Susceptible
12b	E06902	9.6 ±4.4b	70.4 ±4.4a	62 ±4.4a	Resistant
12b	E079060-2	7.8 ±4.4b	70.4 ±4.4a	62 ±4.4a	Resistant

^a average 5 replicates, observations 7 d after infestation

^b different letters followed by the mean within the row are significantly different ($P<0.05$; Tukey's test)

Chapter-3. Feeding behavior comparison of soybean aphid biotypes on different soybean genotypes

Abstract

The soybean aphid, *Aphis glycines* Matsumura, was first found in North America in 2000. It has become a serious pest of soybean, *Glycine max* L. (Merr.) throughout the northern regions during the following years. Three soybean aphid biotypes have been documented in United States in just 10 years after its invasion. So far, there were a few studies done on feeding behavior of soybean aphid in United States. Electrical Penetration Graph (EPG) technique is a convenient and successful tool to study the feeding behavior of piercing and sucking insects. It is the first attempt to study the feeding behavior differences of biotype 1 and biotype 2 on soybean genotypes using the EPG technique. This study includes both resistant and susceptible genotypes from Kansas and Michigan. Here, the wired aphids were attached to a probing system and the whole system in turn was attached to a computer. The aphids were placed on soybean plants at V1 stage and the circuit got completed when the aphids started probing. The experiments were run for 9 hours each for the given channels. The graphs were saved on the computer hard disk, with 8 channels at a time. The results showed that the susceptible genotypes had significantly greater duration of sieve element phase than resistant genotypes. Also the time taken to reach first sieve element phase in resistant genotypes was significantly greater than susceptible genotypes. Most of the aphids were reached sieve element phase (> 90%) in susceptible genotypes, but only a few (<30%) were reached in resistant genotypes for the 9hr recording period. However, no differences were found in any other probing phases between resistant and susceptible genotypes, except the number of potential drops (PD's) in biotype 2. Thus, the resistance was largely associated with phloem tissues and there could be some biochemical, physical or morphological factors that affect the stylet penetration in aphids.

Introduction

The soybean aphid (*Aphis glycines* Matsumura), is an exotic pest to North America from eastern Asia (Ragsdale et al. 2004), ranging from center of the United States to southern provinces of Canada. It is a major pest to soybean, *Glycine max* (L.) Merr., and causing a yield loss of greater than 50% in US and Chinese provinces (Wan et al., 1996, Ostlie, 2002). This aphid rapidly spread throughout the United States soybean growing fields and about 80% of the soybean fields in United States got infected with this aphid by 2004 (Venette and Ragsdale, 2004). Soybean yield loss was caused mainly by direct feeding on plant tissues, especially the vascular tissues and indirectly by the transmission of viruses during feeding and the reduction in seed and pod quality (Ragsdale et al.2007). Transmission of viruses is a serious threat associated with soybean aphid infection; soybean aphid mosaic virus and soybean dwarf virus are some examples (Clark and Perry 2002). It is found that up to \$ 5 billion could be lost in soybean production annually due to insecticidal application, which in turn depends on the size of soybean aphid outbreak and the price flexibility of soybean supply (Kim et al. 2008a).

The first important triumphs on research on resistant varieties of soybean were the discovery of *Rag1* and *Rag*, two single dominant genes, for controlling soybean aphid resistance in ‘Dowling’ and ‘Jackson’ genotypes, (Hill et al.2006a & 2006b). Until recently the biotypes of soybean aphids were not present or identified in North America. The existence of two soybean aphid biotypes was first identified from Illinois and Ohio soybean fields and reported as Illinois and Ohio biotype respectively based on the tests done on *Rag1* gene with the two given biotypes (Kim et al. 2008b). In 2009, these two biotypes, Illinois and Ohio, got renamed as biotype 1 and biotype 2 respectively (Hill et al.2009). Later in 2007, one new biotype, biotype 3, was identified from the aphids collected from Springfield Fen, IN, showing resistance to plants with *Rag 2* (Hill et al. 2010).

Aphids are piercing and sucking insects and cause injury by their direct feeding with needle like mouth parts (stylets) into the plant tissues, especially the vascular tissue (phloem) and thus removing the plant sap (Crompton and Ode, 2010). The stylet goes into plant tissues until it reaches vascular tissues (either xylem or phloem) and the pores made by the stylet can be intact for long without coagulating the phloem proteins (Tjallingi and Hogen Esch, 1993: Prado and

Tjallingii, 1994). Stylet probing can be influenced by different chemical components present in plant tissues (Gabrys et al. 1997, Xu et al. 1994). Electronic Monitoring System (EMS) has been used since 1964 for observing the resistance mechanism of plants against aphids (Reese et al. 2000). It was first described by McLean and Kinsey (1964) using the alternate current (AC) for recording the feeding behavior of aphids. It helps to overcome the technical difficulties to study the feeding characteristics of piercing and sucking insects. The term Electrical Penetration Graph technique (EPG) was first coined and described by Tjallingii (1978) using direct current (DC) as the source for monitoring the aphid behavior. Technique involves attachment of thin gold wire to dorsum of aphids and it gives direct observation of probing behavior of freely moving aphids on the host plant (Tjallingii, 1986). When the aphid probing starts, the electrical circuit gets completed and the waveforms related to aphid feeding are recorded in the computer. The waveforms produced by the changes in voltage across the stylet (input resistor) during its movement in host tissues, are amplified and concurrently recorded (Walker 2000). EPG can be used to reveal the stylet activities of aphids and its tip position during feeding on the plant tissues (Han and Chen 2000). It has been used for comparing the penetration of aphid mouthparts into plant tissue and host plant resistance (van Helden and Tjallingii, 1994). Some of the merits of this technique include effectiveness to find the plant resistance mechanism to aphids, its actual location and characterization of the probing behavior of aphids (Hunter and Backus 1989, Walker and Perring 1994, Montllor and Tjallingii 1989). This technique also helps in finding intracellular and intercellular locations of stylet without causing any damage to living cells in the plant tissue (Tjallingii, 1988). The technique has been reviewed extensively throughout the recent years (Ellsbury et al. 1994, Walker and Backus 2000).

The three important waveforms observed from EPG experiments are pathway phase, sieve element phase and xylem phase (Reese et al. 2000, Tjallingii 2006). The insect accepts or rejects the host plant and stylet penetration to the sieve element (ingestion of plant sap) is dependent on the pathway phase (Jiang and Walker 2001). The two sub phases in the sieve element phase are the E1 (salivation) and E2 (sap ingestion) (Tjallingii 2006). Sieve element phase is called as the most important wave form for studying the plant resistance in EPG (Han and Yan 1995). EPG is used for detailed studies of host plant selection, phloem finding by the stylet and sap feeding from the phloem during the course of probing (Tjallingii, 1988). Xylem

phase (G) is for water intake by the insects during their feeding on the host plant (Spiller et al. 1990). Another important waveform that observed is potential drop (pd) and is correlated with intracellular cell punctures by the aphid stylet in epidermal and vascular tissues (phloem) (Tjallingii 2000). Later, all the other related stylet activities with the pathway potential drop (pds), that includes salivation, was reported as ‘X’ waveform (Reese et al. 2000).

EPG is the most rigorous method of monitoring and quantifying the feeding behavior of insects with piercing and sucking mouth parts (Walker 2000). There are a number of studies associated with EPG and feeding behavior of insects. It is used to study leafhopper or plant hopper feeding, penetration of stylet through the tissues, salivary sheath branching and puncture of xylem cell (Backus et al. 2005). Will et al. (2007), demonstrated the unplugging of sieve tube by the aphid saliva with the molecular interactions of saliva proteins and calcium. Prado and Tjallingii, (2007) reported that phloem factors were the main cause for aphid- induced resistance and they also found the systematic nature of this kind of resistance in broccoli. It was established that horned aphids, using the feeding site vacated by earlier individual were more benefited by the rapid access to the phloem tissues than those probing fresh site for feeding (Morris and Foster 2008). Experiments with tea aphids in host and non-host plants revealed that the duration of sieve element phase was longer in host plant than in non-host plant (Han and Chen 2000). It was also found that the non-persistent virus transmission by aphids was related to their feeding behavior and the acquisition and inoculation of viruses that take place in the different sub-phases of potential drops (PDs) during feeding (Martin et al. 1997). Another study showed that plant resistance was associated with repeated probing and penetration of stylet without going to xylem or phloem tissues with several failures in the beginning to start the ingestion of plant sap (Caillaud et al.1994). E1 (Watery E1 saliva) and E2 (watery E2 saliva) are two different types of saliva secreted by aphids during phloem ingestion to prevent clogging of proteins in sieve elements and aphid stylets (Tjallingii, 2006).

Han and Yan (1995) first reported the use of EPG technique for monitoring feeding behavior of soybean aphid. They found that phloem ingestion in *Gossypium hirsutum*, *Cucumis sativa* and *Luffa cylindrica* (non-host plants) was lower than that in *Glycine max* (host plant). Later, Diaz-montana et al. (2007), carried out EPG studies on feeding behavior of soybean aphid

(*Aphis glycines*) on resistant and susceptible soybean genotypes. Their results showed that the duration of sieve element phase was long in susceptible genotypes and very short in resistant genotypes. In 2010, Crompton and Ode conducted EPG experiments with a resistant soybean genotype, ‘Dowling’ and susceptible soybean entry, ‘Glenwood’ and found that antibiotic resistance resides in the phloem tissue of the resistant genotype and not in any other tissues of the plant. In this study, we used four soybean genotypes (E06902, E07906-2, K1639 and KS4202) and two soybean aphid biotypes (biotype 1 and biotype 2). As we mentioned earlier, there were only a few EPG studies on soybean aphid with soybean as host plant. But this study is the first of that series, including two biotypes of soybean aphid with different resistant and susceptible genotypes. And this is the first EPG study with biotype 2 in United States since it was reported in 2006 (Kim et al. 2008b). The objective of this study was to compare the feeding behavior differences of soybean aphid biotypes using EPG technique on different resistant and susceptible soybean genotypes.

Materials and Methods

Aphid Cultures and Soybean Genotypes. The two aphid biotypes used in the experiments were biotype 1 and 2. In the beginning of 2008, biotype 1 was collected from fields of Nebraska and in July of 2008, we obtained biotype 2 from Brian W. Diers, Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801. The aphid colonies were maintained on susceptible soybean genotype, KS4202, in two different locations to avoid contamination. The biotype 1 colony was cultured in a cage with proper ventilation under pesticide free greenhouse conditions at 20 -30° C temperature, 23- 40 % relative humidity and supplemental high pressure sodium vapor lamps set for a photoperiod of 14:10 (L:D) h. The biotype 2 colony was located in laboratory; inside a growth chamber (Percival Scientific, Inc. 505 Research Drive, Perry, IA 50220) with similar environmental conditions as that of greenhouse. During the experiments, aphids from greenhouse (biotype 1) were transported to the laboratory in tight closed petri-dishes to avoid contamination from the environment and lab cultured aphids (biotype 2). After each experiment, both soybean plants and aphids were frozen to kill the aphids. Caution was taken by giving separate timing for experiments with the two aphid biotypes.

Stylet penetration activities were significantly affected by plant varieties rather than the wiring of aphids and EPG components in the recording (Annan et al. (1997a). So, it is important to select the appropriate plant for the experiments. Soybean genotypes used in the experiments include E06902, E079062, K1639 and KS4202. Genotypes, E06902 and E07906-2 were found resistant to biotype 2 and K1639 found resistant to only biotype 1 (unpublished data). In this experiment, the only susceptible soybean genotype to biotype 1 was KS4202 and the susceptible genotypes to biotype 2 were K1639 and KS4202 (Diaz-Montana et al. 2006). Soybean genotypes were grown separately in plastic cone-tainers, with dimensions, 3.8-cm-diameter and 21.0-cm-depth, (Ray Leach Cone-tainer, Hummert International, Earth City, MO), filled with steam-sterilized potting mix (Premier Promix, Rivie`re-du-Loup, Que`bec, Canada) and were kept under sodium vapor lamps in laboratory conditions. Plants used for the experiments were in V1 stage or nine days after planting (Diaz-Montano et al. 2007).

EPG Recording and Experimental Design

The experiments were done in the Reese laboratory in Manhattan, KS at room temperature (22-26°C), 35-45% relative humidity and with fluorescent ceiling-mounted lamps as source of illumination. The system used in the given study to record the feeding behavior of soybean aphid biotypes was DC based EPG system, the most common and sensitive type of EPG system (Tjallingii, 2000). At the beginning of the experiments, adult apterous aphids, collected from the respective aphid colonies using a moist camel's brush (number 0) to avoid any damage to its body, were kept starved in a petri-dish for 1 hour (Diaz-Montano et al. 2007). A thin gold wire was attached to the dorsum of aphids (10-12µm diameter and 2-3cm length) (Johnson Matthey, Materials tech, U.K. Orchard Road, Royston, England) using high purity conductive silver paint (SPI Supplies, P.O. Box 342, West Chester, PA). The opposite end of the gold wire was attached to a copper wire of 0.2 mm diameter and 1-2 cm length and this copper wire was soldered to a copper nail (1.6 mm X 19.0 mm). This served as an insect electrode. The plant electrode consist of a copper wire (2 mm thick and 10 cm length) inserted into the soil of potted plant in the rack (Tjallingii, 1988). The two electrodes (insect and plant) were attached to the *Giga-8* model, $10^9 \Omega$ resistance amplifier (Wageningen Agricultural University, Wageningen, The Netherlands), in their respective sites on the amplifier. It has an adjustable voltage source for

dealing with any irregularities with voltages in the primary circuit. The insects and plants were kept inside a Faraday cage to avoid electrical noises during the recording of EPG. After the above mentioned whole set up, 1 hour starved aphids (insect electrodes) were allowed to stand on the upper side of the fully developed unifoliate leaf. When the aphids started probing and the stylets were inserted into the leaves, the circuits got completed and the recording system recorded amplified (50-100) voltage fluctuations. Voltage source was used to adjust the output signal voltage in between +5 and -5 V; for intercellular stylet puncture, the signal voltage was positive and for intracellular puncture, the signal voltage was negative (Tjallingii 2006). Eight channels with eight soybean plants and insect electrodes were used for recording EPG using *Giga-8* DC amplifier.

Separate experiments were carried out for two biotypes to avoid contamination between biotypes. Each experiment included eight plants of the given four genotypes with two replications each. The genotypes were placed at random in Faraday cage in two stands with four plants each. For biotype 1, three resistant (E06902, E07906-2 and K1639) and one susceptible check (KS4202) were used in each experiment. But for biotype 2, two resistant (E06902 and E07906-2) and two susceptible (K1639 and KS4202) (unpublished data) checks were used. Characterization of full range of EPG waveforms were done by recording long duration experiments in host plants (Annan et al. 2000). In this experiment, EPG recorded continuously for 9 hours had acquired 15 replications from each genotype. Digitized signals, recorded on a computer hard disk using a software, PROBE 3.0 (Windows), later helped in the analysis of different waveforms that retrieved from the hard disk (Tjallingii, 1988, Wageningen Agricultural University, Laboratory of Entomology, The Netherlands).

Different feeding parameters and statistical analysis

Feeding parameters recorded in this experiment were almost the same as that of earlier study conducted by Diaz-Montano et al. (2007) on resistant and susceptible soybean entries. Recorded parameters included the mean time from beginning of recording to first pathway phase or first probe (FP), mean time from the beginning of recording to Xylem Phase (f-XP) and mean time from the beginning of recording to sieve element phase (f-SEP); mean number of potential

drops (n-PDs), mean number of pathway phases (n-PP), mean number of xylem phases (n-XP) and mean number of sieve element phases (n-SEP); sum of duration of pathway phase (s-PP), sum of duration of xylem phase (s-XP), sum of duration of sieve element phase (s-SEP) and sum of duration of non-probing (resting phase) (s-NP); finally the % of time that aphid spent in sieve elements since its first probing to phloem tissues (% SEP) and the number of aphids reached the sieve element during their full 9 h feeding. When comparing the sieve element phases of aphid feeding between resistant and susceptible host plants, it is better to consider both E1 and E2 sieve element sub phases as single E waveform (Annan et al. 2000, Diaz-Montana et al. 2007). Hence only E parameter (SEP) was analyzed in the given EPG study.

As the feeding behavior parameters were not normally distributed and some parameters ranged from zero or low duration to long durations (s-SEP) and for some other parameter, zero is not included in final statistical analysis (f-SEP), they were compared using Kruskal-Wallis test ($\alpha = 0.05$). Tukey's studentized range test ($P < 0.05$) was carried out for multiple comparison between the parameters. SAS Institute 2007, software was used for all statistical analysis.

Results

The feeding behavior parameters were analyzed and calculated separately for two biotypes (biotype 1 and biotype 2). It was shown that the first parameter (Table 1 and 2), time from the beginning of recording to the first pathway phase (first probe) (FP) was not significantly different ($P < 0.05$) in both biotypes. But the second parameter (Table 1 and 2), time from the beginning of recording to first or initiation of xylem phase (f-XP) was significantly ($P < 0.05$) more in three resistant entries (E06902, E07906-2 and K1639) for biotype 1 with E07906-2 having the longest duration. Biotype 2 spent significantly ($P < 0.05$) more time in the two resistant entries (E06902 and E07906-2) than in the two susceptible entries (K1639 and KS4202). Third parameter from the tables (1 and 2), time from the beginning of recording to the initiation of first sieve element phase (f-SEP) gave absolute significant difference ($P < 0.05$) between susceptible genotype (short duration) and resistant genotypes (long duration) for biotype 1 and the biotype 2 spent significantly ($P < 0.05$) less time in two susceptible entries (K1639 and KS4202) than in two resistant (E06902 and E07906-2) entries.

Comparing the mean number of two given phases [pathway (n-PP) and xylem (n-XP)] (Parameter 4 and 5, Table 1 and 2) with the two biotypes, there was no significant difference ($P<0.05$) in mean numbers for pathway and xylem phases in resistant and susceptible entries. But the sixth parameter (Table 1 and 2), the number of sieve element phases (n-SEP), showed large significant difference ($P<0.05$) between susceptible and resistant soybean entries with both biotypes. In susceptible entry, KS4202, the number of sieve element phases was more than that of resistant entries for biotype 1. Biotype 2 produced more number of sieve element phases in two susceptible entries (K1639 and KS4202) than in the two resistant entries. For the seventh parameter (Table 1 and 2), the mean number of potential drops (n-PD,s), there was no significant difference ($P<0.05$) between susceptible and resistant entries in case of biotype 1. However, two out of the three resistant genotypes (E06902 and E07906-2) showed more number of potential drops than the susceptible genotype (KS4202). K1639 produced less number of PDs in all the four genotypes tested. But in biotype 2, the mean number of potential drops (n-PDs) was significant larger in the two resistant entries than in the susceptible entries.

The sum of duration of the pathway, xylem and non-probing phases (Parameters eight, nine and ten, Table 1 and 2) was not significantly different ($P<0.05$) in susceptible and resistant entries with both biotypes. But the sum of duration of sieve element phase and the % time aphids spent in the sieve elements (Parameter ten and eleven, Table 1 and 2) were significantly ($P<0.05$) large in susceptible entries than in the resistant ones with both biotypes. However, biotype 2 produced long duration and percentage time than the biotype 1 in susceptible entries (Table 1 and 2). Finally, in the given fifteen replications of each entry, nearly all the aphids reached the sieve element phase (SEP) in susceptible plants, but less than five aphids reached that phase in resistant entries for both biotypes.

Discussion

EPG study conducted here could explain processes occurring in different tissues of soybean plant during aphid feeding and their influence on soybean aphid feeding behavior. The different feeding behavior parameters of soybean aphids and their comparison in susceptible and resistant soybean entries revealed some of the important factors related to resistance and

susceptibility in soybean plants. The most important waveform and key parameter that explains aphid feeding, sum of duration of sieve element phase, is a helpful factor in EPG study regarding plant resistance (Han and Yan, 1995). In this study, duration of sieve element phase and time from the beginning of recording to first sieve element phase were proved to be more important than the other parameters and were significantly different in susceptible and resistant genotypes with both biotypes. Out of the total time of recorded parameters, duration of sieve element phase was 2-4% in resistant genotypes and up to 14% in susceptible genotype, KS4202, with biotype 1 probing. Likewise, with biotype 2 probing, it was 4-6% in resistant (E06902 and E07906-2) and 15-20% in susceptible genotypes. The less duration of sieve element in resistant genotypes shows that phloem tissues are the major source tissues of resistance (Han and Yan, 1995, Prado and Tjallingii 2007). Here, K1639 showed resistance to biotype 1 with low SEP duration, but susceptibility to biotype 2 with long SEP duration. This result is in accordance with the finding of Tjallingii (2006); plants resistant to one species or biotype of aphids may not be resistant to other species or biotype, ie the resistance is very specific to aphid type. Similarly, time from the beginning of recording to first sieve element phase was significantly larger in resistant entries than in susceptible entries with both aphid biotypes. Annan et al. (2000) concluded that the factors for aphid resistance are located in or at least associated with phloem tissues and thus produce a lack or delay of access of aphid stylets to phloem tissues. But in this study it was shown that the time from the beginning of recording to first probing was not significantly different in soybean genotypes with the given biotypes. Thus it is clear that resistance factors are not located in epidermal or mesophyll layers, if so, it should have significantly different time of duration for the susceptible and resistant soybean genotypes (Crompton and Ode, 2010). In resistant genotypes, morphological or chemical factors may cause delay in the penetration of aphid stylet into sieve elements, or may change the feeding behavior of aphids negatively due to antixenosis (Diaz-Montana et al. 2007). Therefore, in this study only less than 5 aphids reached sieve element in resistant genotypes, but almost all aphids reached sieve element in susceptible genotypes.

As we discussed earlier, phloem phase has a significant role in plant resistance to aphid feeding. The difference in the duration and percentage of sieve element phase in the resistant and susceptible genotypes was the clear cut evidence for the presence of resistance factors in soybean

genotypes. For successful feeding of phloem sap, aphids require ability to overcome different plant properties and reactions that associated with phloem (Tjallingii 2006). Phloem phases, E1 and E2 are associated with watery salivation (to the sieve element) and sap ingestion (phloem sap to the food channel) respectively. During E1 phase, aphids inject saliva into the sieve elements and the valve for food channel in aphids gets closed, during E2 phase the valve for food channel gets opened, thus phloem sap gets mixed with the saliva, flows up through the food channel and there is no injection of saliva to the sieve element (Prado and Tjallingii, 1994). Phloem wound responses are influenced by some proteins in sieve elements and are Ca⁺⁺ triggered (Knoblauch and Van Bel, 1998, Knoblauch et al. 2001). Molecular interactions between salivary proteins and calcium help the aphids to get continuous supply of phloem sap without occluding the sieve elements during their feeding (Will et al. 2007). Watery salivation (E1) and phloem ingestion (E2) were identified by EPG. It was also found out that protein coagulation in plant sieve elements and aphid capillary food channel was prevented by watery saliva during the E1 and E2 phases (Tjallingii, 2006). It was shown that in resistant genotypes, the E2 phase had a threshold time not longer than 10 min and it was called as ‘phloem acceptance indicator (Tjallingii, 1990). Honey dew secretions in some aphids are followed by E2 stage (Phloem ingestion) (Li et al. 1998). These evidences show that the stylet penetration into the phloem tissues could be largely influenced by a number of factors; morphological, mechanical and chemical; those having a vital role in aphid feeding behavior changes during probing in the respective tissues. In this study, it was also found that resistant genotypes took longer time to reach the xylem phase than the susceptible genotypes. This may be due to some morphological or chemical factors in the resistant genotypes those affect the penetration of aphid stylet into xylem and phloem tissues initially (Diaz- Montana et al. 2007). The changes in the feeding behavior were mainly caused by the presence of antixenosis in resistant genotypes (Diaz- Montana et al. 2007).

Parameter 7, the number of potential drops (PD,s), was greater in resistant genotypes with biotype 1, but the difference was not significant. However, the number of PD,s was significantly ($P<0.05$) greater for resistant genotypes than susceptible genotypes with biotype 2. These potential drops are brief (5-10s) intracellular punctures by the stylets along their pathway and are also found in most sieve element tissues without going to phloem phases (E1 and E2) (Tjallingii

and Hogen Esch, 1993). During this process, aphids inject some watery saliva into the puncturing cells and collect some sap samples with the stylets (Martin et al. 1997). These samples may contain different chemical signals for continuous stylet penetration to plant tissues and thus the host plant acceptance by the aphids (Montllor, 1999). It was shown in wheat lines that even though there were many repeated stylet penetrations (attempts) into sieve elements in E1 sub phase (notably PD,s), aphids failed to start E2 sub phase in case of resistant genotypes (Caillaud et al. 1995). As a consequence, the greater number of potential drops (PD,s) observed in resistant entries showed its difficulty to reach the phloem phases and thus the host plant rejection by the aphids. Nevertheless, transmissions of non-persistent viruses were occurred by these intracellular punctures (PD,s) on the host plant during the aphid probing (Lopez Abella and Bradley, 1969; Powell, 1991). The inoculation of viruses was caused by saliva ejection, first sub-phase (II-1), through intracellular punctures in epidermal tissues and acquisition of viruses was caused by sap ingestion, last sub-phase (II-3), through intracellular punctures in inner tissues (Martin et al. 1997). Some aphids secreted watery saliva into the sieve elements and caused the transmission of persistent viruses to the host plant during the aphid feeding (Prado and Tjallingii, 1994). Thus, number of potential drops (PD,s) may be influenced by different plant factors and is very essential in aphid feeding.

Diaz-Montana et al. (2007), reported that the antibiosis and antixenosis were the categories of resistances present in sieve elements of resistant genotypes and they affected the behavior of aphids; but it was difficult to say which could be expressed more in most resistant genotypes. The presence of one category of resistance may be significantly affected by other and thus, the behavior of aphids to probe into the sieve elements tissues (Diaz-Montana et al. 2007). In conclusion, the factors of resistance could be mainly related with sieve element phase and the aphid feeding behavior may be affected during the probing especially in phloem tissues. The chemical analysis of plant saps, principally phloem sap and the molecular and biochemical aspects of insect-plant interactions would give more insight into the plant resistance mechanism to aphids.

References

- Annan, I. B., G. A. Schaefer, W. M. Tingey, and W. F. Tjallingii. 1997a. Effects of treatments for electrical penetration graph recordings on behaviour and biology of *Aphis craccivora* (Aphididae). *Physiol. Entomol.* 22: 95-101.
- Annan, I. B., W. M. Tingey, G. A. Schaefer, W. F. Tjallingii, E. A. Backus, and K. N. Saxena. 2000. Stylet penetration activities by *Aphis craccivora* (Homoptera: Aphididae) on plants and excised plant parts of resistant and susceptible cultivars of cowpea (Leguminosae).
- Backus, E.A., J. Habibi., F. Yan., and M. Ellersieck. 2005. Stylet Penetration by Adult *Homalodisca coagulata* on Grape: Electrical Penetration Graph Waveform Characterization, Tissue Correlation, and Possible Implications for Transmission of *Xylella fastidiosa*. *Ann. Entomol. Soc. Am.* 98(6): 787-813.
- Caillaud, C.M., J.S. Pierre, B. Chaubet and J.P. Di Pietro.1995. Analysis of wheat resistance to the cereal aphid *Sitobion avenae* using electrical penetration graphs and flow charts combined with correspondence analysis. *Entomologia Experimentalis et Applicata* 75: 9-18
- Clark, A.J., and K.L. Perry. 2002. Transmissibility of field isolates of soybean viruses by *Aphis glycines*. *Plant Dis.* 86:1219–1222.
- Crompton, D.S and P.J.Ode. 2010. Feeding behavior analysis of soybean aphid (Hemiptera:Aphididae) on resistant soybean ‘Dowling’. *J. Econ. Entomol.* 103(3): 648-653.
- Diaz-Montano, J., J. C. Reese, J. Louis, L. R. Campbell, and W. T. Schapaugh. 2007. Feeding behavior by the soybean aphid (Hemiptera: Aphididae) on resistant and susceptible soybean genotypes. *J. Econ Entomol.* 100: 984- 989.
- Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of

- antibiosis and antixenosis to the soybean aphid in several soybean genotypes. *J. Econ. Entomol.* 99: 1884–1889.
- Ellsbury, M. M., E. A. Backus, and D. L. Ullman. (eds.). 1994. History, development, and applications of an electronic monitoring system for insect feeding. Proceedings of the Thomas Say Publications in Entomology. Entomological Society of America, Lanham, Md.
- Gabrys, B., W. F. Tjallingii, and T. A. van Beek. 1997. Analysis of EPG recorded probing by cabbage aphid on host plant parts with different glucosinolate contents. *J. Chem. Ecol.* 23: 1661-1673.
- Han, B.y, and Z.m. Chen. 2000. Difference in probing behavior of tea aphid on vegetative parts of tea plant and non-host plants. *Entomologia Sinica.*7:337-343.
- Han, X. L., and F. S. Yan. 1995. Stylet penetration behavior of the soybean aphid, *Aphis glycines*, on host and non-host plants. Translation from *Acta Entomol. Sinica* 38: 278-283. (<http://www.k-state.edu/issa/aphids/reporthtml/trans104.htm>).
- Hill, C. B., K.-S. Kim, L. Crull, B. W. Diers, and G. L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. *Crop Sci.* 49: 1193-1200.
- Hill, C.B., Y. Li, and G.L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. *Crop Sci.* 46:1601–1605.
- Hill, C.B., Y. Li, and G.L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. *Crop Sci.* 46:1606–1608.
- Hill, C.B., L. Crull, T.K. Herman., D.J Voegtlin., and G.L. Hartman.2010. A New Soybean Aphid (Hemiptera: Aphididae) Biotype Identified. *J. Econ. Entomol.* 103(2): 509-515

- Hunter, W. B., and E. A. Backus. 1989. Mesophyll-feeding by the potato leafhopper, *Empoasca fabae* (Homoptera: Cicadellidae): results from electronic monitoring and thin-layer chromatography. *Environ. Entomol.* 18: 465- 472.
- Jiang, Y. X., and G. P. Walker. 2001. Pathway phase waveform characteristics correlated with length and rate of stylet advancement and partial stylet withdrawal in AC electrical penetration graphs of adult whiteflies. *Entomol. Exp. Appl.* 101: 233-246.
- Kim, K.-S., C. B. Hill, G. L. Hartman, M.A.R. Mian, and B. W. Diers. 2008b. Discovery of soybean aphid biotypes. *Crop Sci.* 48: 923-928.
- Kim, C., G. Schaible, L. Garrett, R. Lubowski, and D. Lee. 2008a. Economic impacts of the US soybean aphid infestation: a multi-regional competitive dynamic analysis. *Agric. Res. Econ. Rev.* 37: 227-242.
- Knoblauch, M., W. S. Peters, K. Ehlers, and A.J.E. van Bel. 2001. Reversible calcium-regulated stopcocks in legume sieve tubes. *Plant Cell* 13: 1221-1230.
- Knoblauch, M., and A.J.E. van Bel. 1998. Sieve tubes in action. *Plant Cell* 10: 35-50.
- Lopez Abella, D. and R. H. E. Bradley. 1969. Aphids may not acquire and transmit stylet-borne viruses while probing intercellularly. *Virology* 39 : 338-342.
- Martin, B., J. L. Collar, W. F. Tjallingii, and A. Fereres. 1997. Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *J. Gen Virol.* 78: 2701-2705.
- McLean, D. L., and R. B. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. *Nature (Lond.)*. 202: 1358-1359.
- Montllor, C. M., and W. F. Tjallingii. 1989. Stylet penetration by two aphid species on

susceptible and resistance lettuce. *Entomol. Exp. Appl.* 52: 103-111.

Morris, G, and W. A. Foster. 2008. Duelling aphids: electrical penetration graphs reveal the value of fighting for a feeding site. *Journal of Experimental Biology.* 211: 1490-1494.

Ostlie, K. 2002. Managing soybean aphid. University of Minnesota, St. Paul, MN.
(http://www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm).

Powell, G. 1991. Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. *Annals of Applied Biology* 119, 313-321.

Prado, E., and W. F. Tjallingii. 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. *Entomol. Exp. Appl.* 82: 189-200.

Prado, E, and W.F. Tjallingii. 2007. Behavioral evidence for local reduction of aphid-induced resistance. 2007. *Journal of insect science* 7:48, available online: [insectscience.Org/7.48](http://insectscience.org/7.48).

Ragsdale, D. W., B. P. McCornack, R. C. Venette, B. D. Potter, I. V. Macrae, E. W. Hodgson, M. E. O'Neal, K. D. Johnson, R. J. O'Neil, C. D. DiFonzo, et al. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 100: 1258-1267.

Ragsdale, D. W., D. J. Voegtlin, and R. J. O'Neil. 2004. Soybean aphid biology in North America. *Ann. Entomol. Soc. Am.* 97: 204-208. *Entomol.* 99:1884-1889.

Reese, J. C., W. F. Tjallingii, M. van Helden, and E. Prado. 2000. Waveforms comparisons among AC and DC electronic monitoring systems for aphid (Homoptera: Aphididae) feeding behavior, pp. 70-101. In G. P. Walker and E. A. Backus (eds.), *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Thomas say publications in Entomology, Entomological Society of

America Lanham, MD.

SAS Institute. 2003. SAS/STAT User's Guide, version 8. SAS institute, Cary, NC.

Spiller, N. J., L. Koenders, and W. F. Tjallingii. 1990. Xylem ingestion by aphids – a strategy for maintaining water balance. *Entomol. Exp. Appl.* 55: 101-104.

Tjallingii, W. F. 1978. Electronic recording of penetration behavior by aphids. *Entomol. Exp. Appl.* 24: 721-730.

Tjallingii, W. F. 1986. Wire effects on aphids during electrical recording of stylet penetration. *Entomol. Exp. Appl.* 40: 89-98.

Tjallingii, W. F. 1988. Electrical recording of stylet penetration activities, pp. 95-108. *In* A. K. Minks and P. Harrewijn (eds.), *Aphids: their biology, natural enemies and control*. World crop pests, Volume 2B. Elsevier, Amsterdam.

Tjallingii, W. F. 1990. Continuous recording of stylet penetration activities by aphids, pp. 89-101. *In* R. K. Campbell and R. D. Eikenbary [eds.], *Aphid-plant genotype interactions*. Elsevier, Amsterdam.

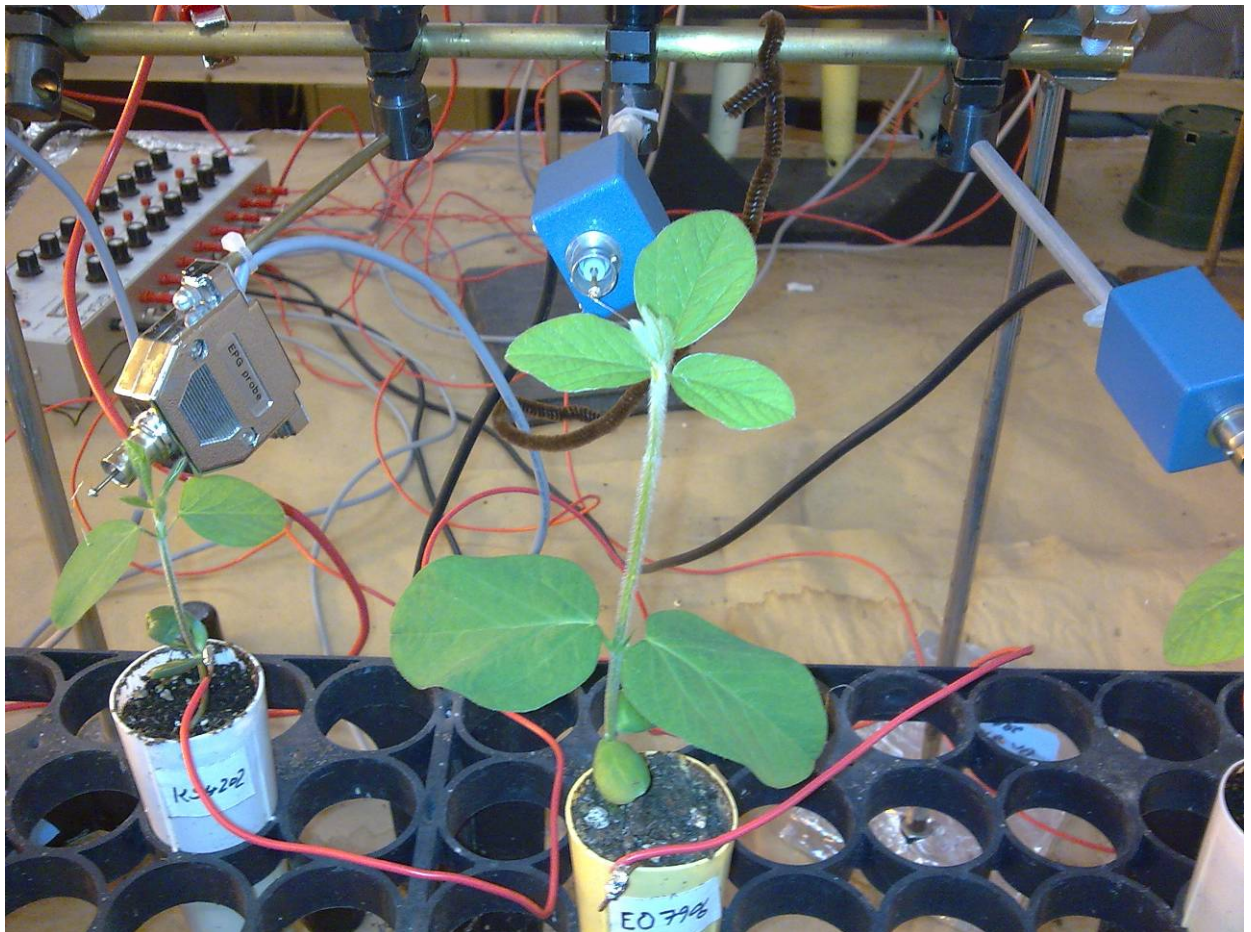
Tjallingii W.F. 2000. Comparison of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans. *In*: Walker GP, Backus EA, eds. *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. USA: Thomas Says Publications in Entomology: 41–69.

Tjallingii, W. F. 2006. Salivary secretions by aphids interacting with proteins of phloem wound responses. *J. Exp. Bot.* 57: 739-745.

Tjallingii, W. F., and T. Hogen Esch. 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiol. Entomol.* 18: 317-328.

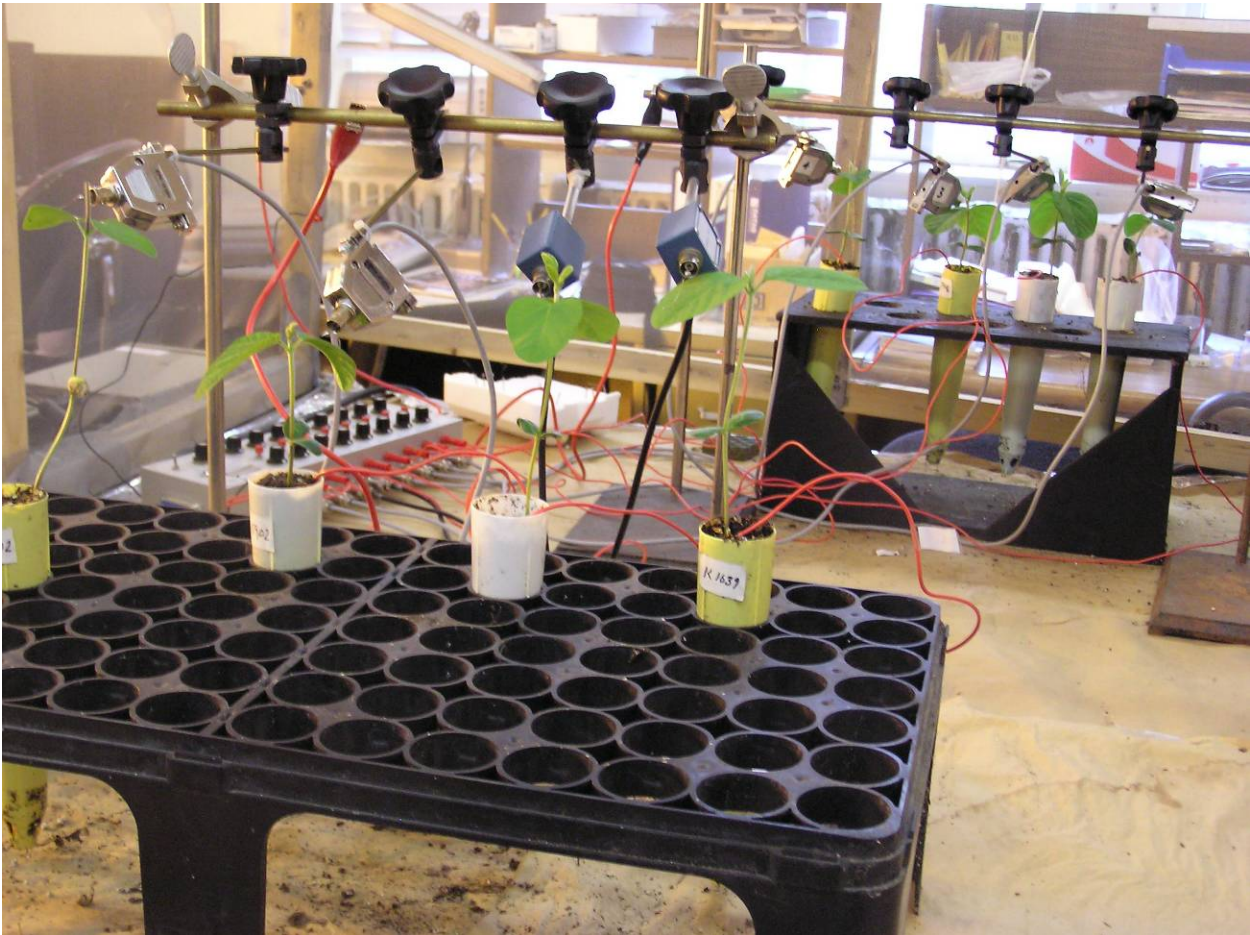
- Van Helden and W.F. Tjallingii. 1994. Phloem sap collection from lettuce (*Lactuca sativa*): chemical comparison among methods. *Journal of Chemical Ecology* 20: 3190–3206.
- Venette, R.C., and D.W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): Where will it end? *Ann. Entomol. Soc. Am.* 97:219–226.
- Wang, S., X. Bao, Y. Sun, R. Chen, B. Zhai, S.Y. Wang, X.Z. Bao, Y.J. Sun, R.L. Chen, and B.P. Zhai. 1996. Study on the effects of the population dynamics of soybean aphid (*Aphis glycines*) on both growth and yield of soybean. *Soybean Sci.* 15:243–247.
- Walker, G. P. 2000. A beginner's guide to electronic monitoring of homopteran probing behavior, pp. 14-40. *In* G. P. Walker and E. A. Backus (eds.), *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.
- Walker, G. P., and T. M. Perring. 1994. Feeding and oviposition behavior of whiteflies (Homoptera: Aleyrodidae) on young and mature lemon leaves. *Ann. Entomol. Soc. Am.* 87: 363-374. Walker, G. P., and E. A. Backus. 2000. *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Entomological Society of America, Lanham, MD.
- Will, T., W.F. Tjallingii., A. Thonnessen., and A.J.E. Van Bel. 2007. Molecular sabotage of plant defense by aphid saliva. *PNAS.* 104:10536-10541.
- Xu, R.M., Y.Zhang and W. R. Ma. 1994. The probing and feeding process of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood. *Entomologia Sinica.* 1(1): 67-76

Figure 3.1. Attached aphids on individual plants



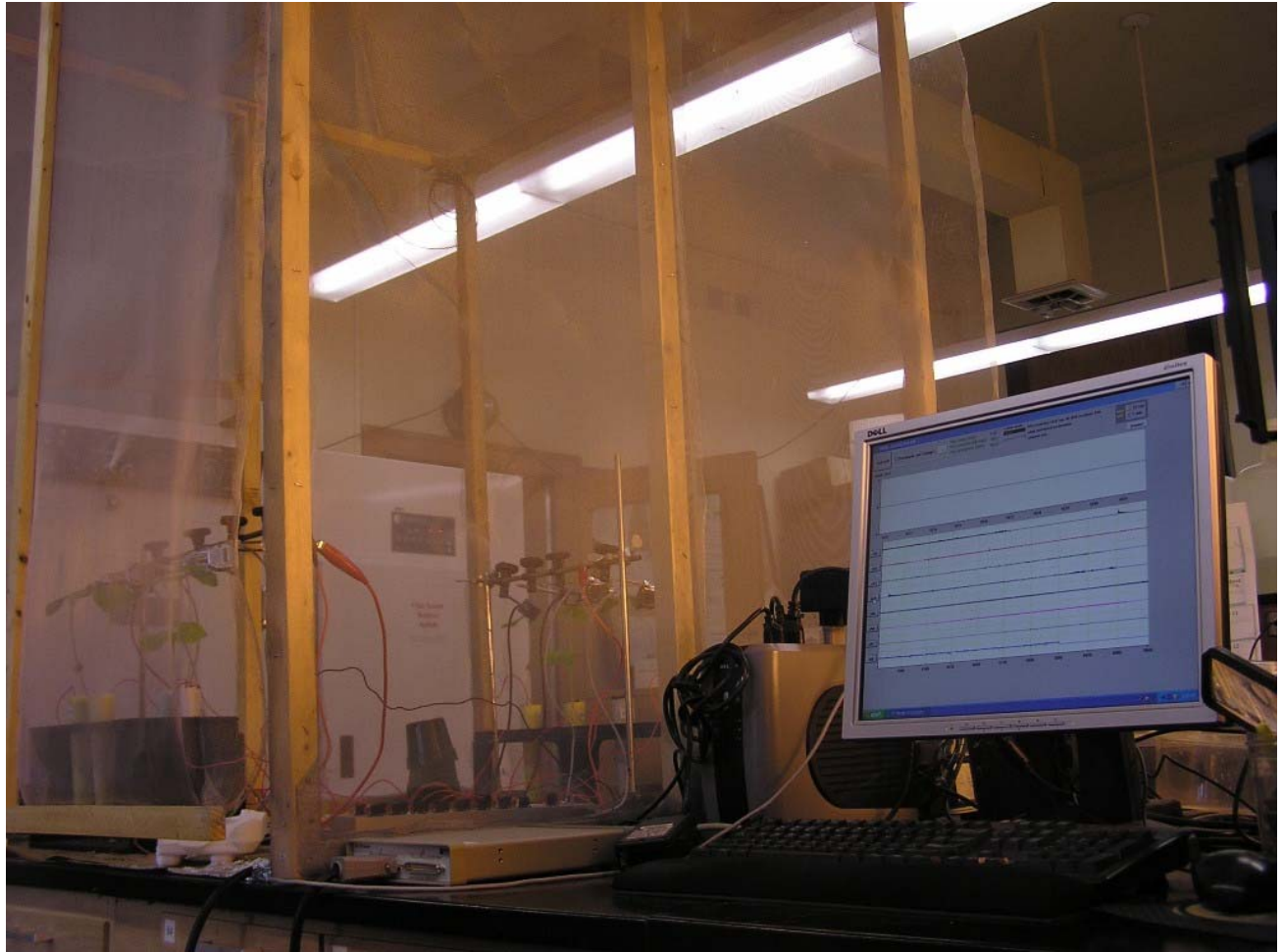
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Figure 3.2. Electrical Penetration Graph (EPG) Setup



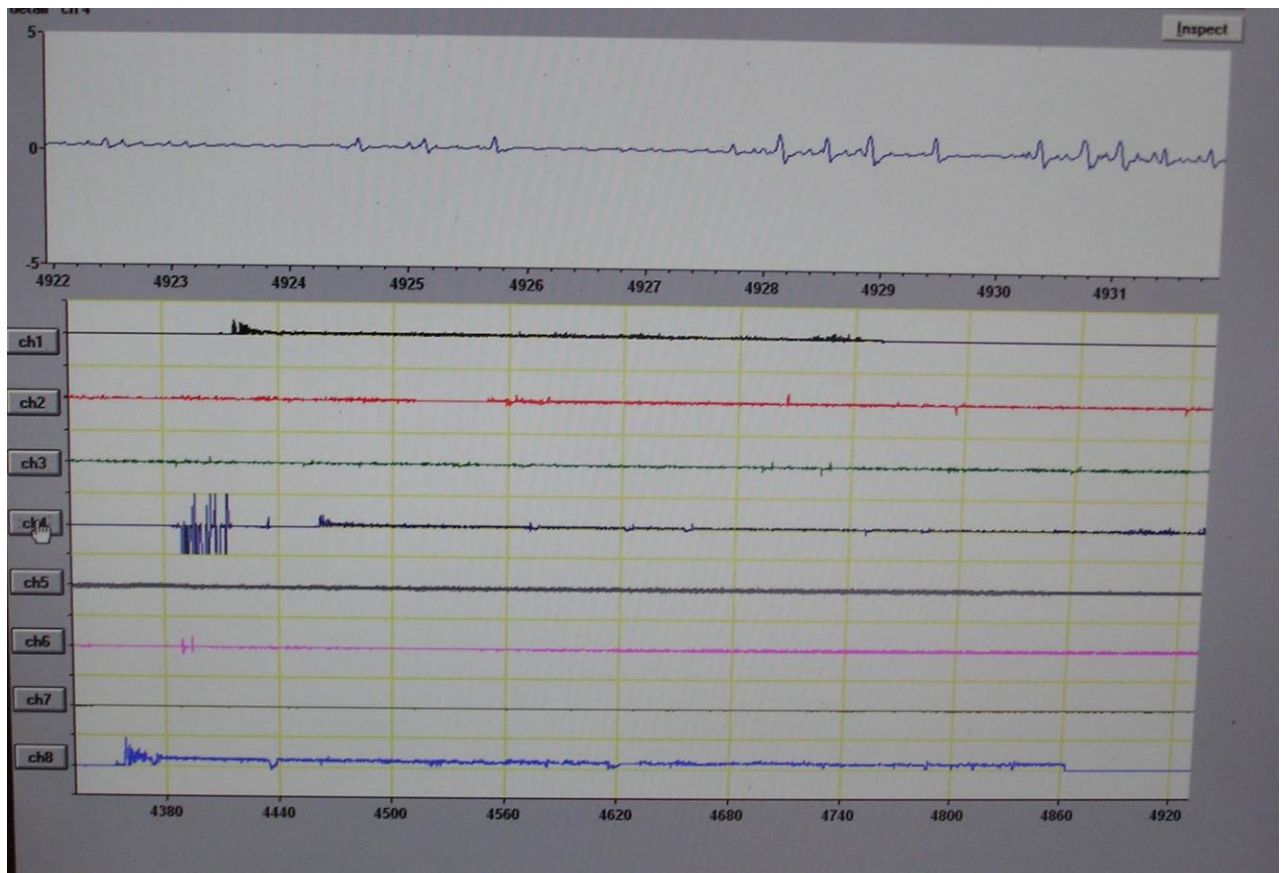
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Figure 3.3. Faraday cage with EPG system.



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Figure 3.4. Computer monitor with EPG recordings



PREDEESH

Figure 3.5. Comparison of time spent (mean \pm SE) by aphid biotypes on sieve element phase (SEP) of resistant and susceptible genotypes for a period of 9 h (540 min). Bars followed by different letters are significantly different according to the Kruskal-Wallis test ($\alpha = 0.05$) and multiple comparisons ($P < 0.05$; Tukey test).

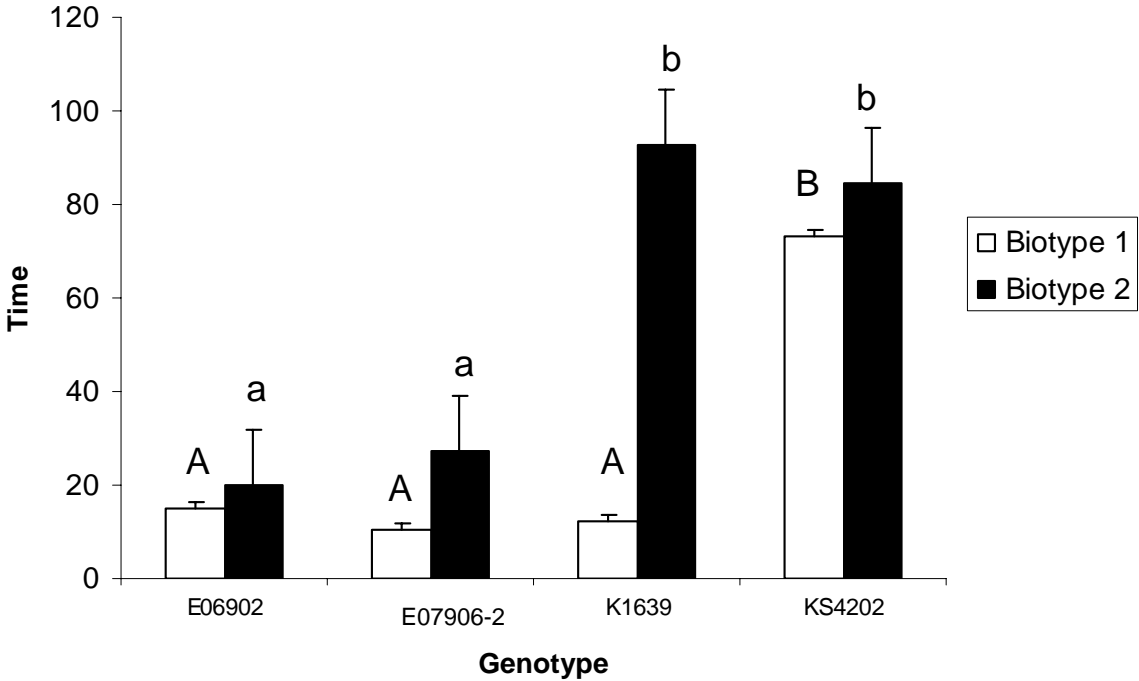


Figure 3.6. Comparison of no. of potential drops (n-PDs) by aphid biotypes on resistant and susceptible genotypes. Bars followed by different letters are significantly different according to the Kruskal-Wallis test ($\alpha = 0.05$) and multiple comparisons ($P < 0.05$; Tukey test).

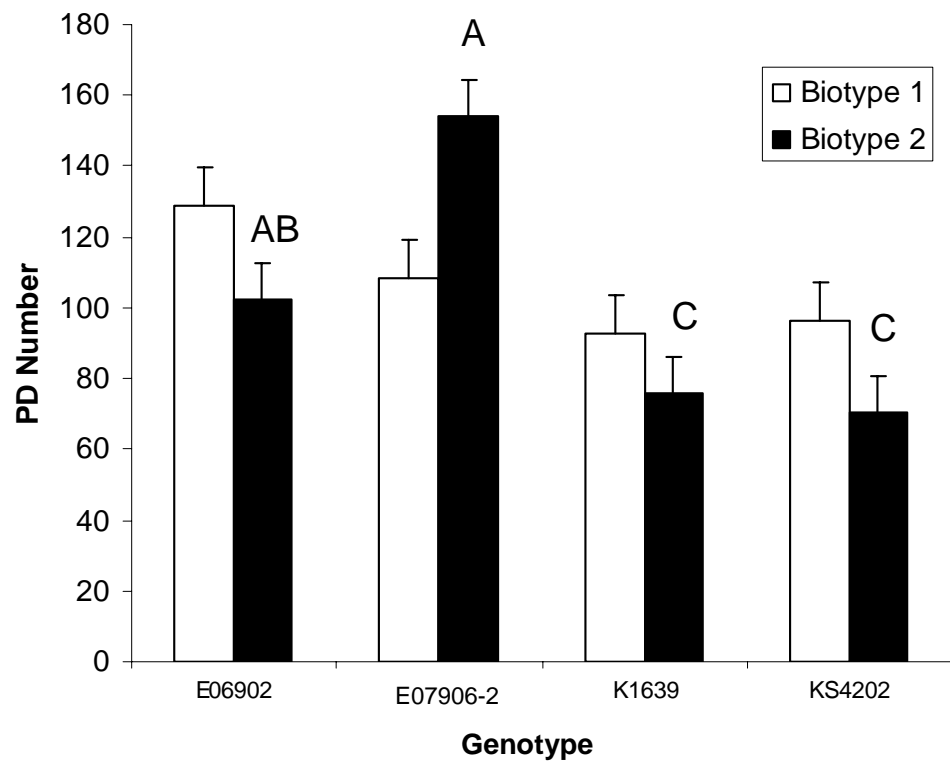


Table. 3.1. Comparison of EPG parameters (mean \pm SE) of resistant and susceptible soybean genotypes against soybean aphid (biotype 1) for a 9-h (540-min) time period.

#	Feeding behavior parameter	E06902	E07906-2	K1639	KS4202	χ^2	df	P
1	Time from beginning of recording to first probe (FP)	14.56 \pm 1.36	12.36 \pm 1.36	17.63 \pm 1.36	9.36 \pm 1.36	1.32	3	0.2798
2	Time from beginning of recording to first xylem phase (f-XP)	144.27 \pm 28.75ab	198.92 \pm 28.75a	160.50 \pm 28.75a	127.13 \pm 28.75ac	1.14	3	0.3444
3	Time from beginning of recording to first sieve element phase (f-SEP)*	302.48 \pm 13.31a	329.28 \pm 13.31a	284.32 \pm 13.31a	118.60 \pm 13.31b	43.45	3	<.0001
4	No. of pathway phases (n-PP)	15.98 \pm 1.87	20.00 \pm 1.87	17.32 \pm 1.87	18.44 \pm 1.87	0.84	3	0.4793
5	No. of xylem phases (n-XP)	2.00 \pm 0.25	2.5 \pm 0.25	1.58 \pm 0.25	1.67 \pm 0.25	2.80	3	0.0507
6	No. of sieve element phases (n-SEP)	0.34 \pm 0.31b	0.25 \pm 0.31b	.167 \pm 0.31b	2.00 \pm 0.31a	11.76	3	<.0001
7	No. of potential drops (n-PDs)	128.63 \pm 10.82	108.50 \pm 10.82	92.50 \pm 10.82	96.42 \pm 10.82	0.99	3	0.4067
8	Sum of duration of pathway phase(s-PP)	256.51 \pm 18.52	249.53 \pm 18.52	302.63 \pm 18.52	267.26 \pm 18.52	0.05	3	0.9868
9	Sum of duration of xylem phase (s-XP)	72.45 \pm 12.45	70.47 \pm 12.45	80.22 \pm 12.45	85.17 \pm 12.45	0.13	3	0.8237
10	Sum of duration of non-probing (s-NP)	191.23 \pm 20.3	181.44 \pm 20.3	140.3 \pm 20.3	160.22 \pm 20.3	0.19	3	0.9009
11	Sum of duration of sieve element phase (s-SEP)**	15.2 \pm 1.31b	10.5 \pm 1.31b	12.2 \pm 1.31b	73.3 \pm 1.31a	20.09	3	<.0001
12	% time aphid spent in sieve element phase after the first probe to sieve elements (% SEP)	2.8 \pm 0.21b	1.95 \pm 0.21b	2.2 \pm 0.21b	13.57 \pm 0.21a	39.30	3	<.0001
13	No. of aphids that reached the sieve element phase (n = 15)	3	2	5	15			

1. Values followed by different letters are significantly different according to the Kruskal-Wallis test ($\alpha = 0.05$) and multiple comparisons ($P < 0.05$; Tukey test), within a row.

2. Time is calculated in minutes

* Replicates with zero value are not included in final analyses, ** All replicates are included in final analyses

Table 3.2. Comparison of EPG parameters (mean \pm SE) of resistant and susceptible soybean genotypes against soybean aphid (biotype 2) for a 9-h (540-min) time period.

#	Feeding behavior parameter	E06902	E07906-2	K1639	KS4202	x^2	df	<i>P</i>
1	Time from beginning of recording to first probe (FP)	17.6 \pm 1.4	7.569 \pm 1.4	6.2 \pm 1.4	10.3 \pm 1.4	0.61	3	0.6098
2	Time from beginning of recording to first xylem phase (f-XP)	147.4 \pm 25.3ab	247.7 \pm 25.3a	103.6 \pm 25.3c	96.4 \pm 25.3c	3.49	3	0.0245
3	Time from beginning of recording to first sieve element phase (f-SEP)*	379.9 \pm 10.9a	412 \pm 10.9a	125.8 \pm 10.7b	146.3 \pm 10.7b	98.51	3	<.0001
4	No. of pathway phases (n-PP)	11.5 \pm 1.4b	19.3 \pm 1.4a	10.3 \pm 1.4bc	6.11 \pm 1.4c	15.28	3	<.0001
5	No. of xylem phases (n-XP)	1.6 \pm 0.5	2.1 \pm 0.5	2.5 \pm 0.5	1.8 \pm 0.5	0.64	3	0.5945
6	No. of sieve element phases (n-SEP)	0.3 \pm 0.4b	0.4 \pm 0.4b	2.4 \pm 0.4a	1.9 \pm 0.4a	6.90	3	0.0007
7	No. of potential drops (n-PDs)	102.1 \pm 10.2ab	154.1 \pm 10.2a	75.8 \pm 10.2c	70.5 \pm 10.2c	10.51	3	<.0001
8	Sum of duration of pathway phase (s-PP)	253.3 \pm 21.7	210.8 \pm 21.7	183.5 \pm 21.7	237.7 \pm 21.7	2.00	3	0.1274
9	Sum of duration of xylem phase (s-XP)	82.1 \pm 16.2	33.3 \pm 16.2	90.3 \pm 16.2	61.4 \pm 16.2	2.46	3	0.0756
10	Sum of duration of non-probing (s-NP)	159.2 \pm 20.5	183.6 \pm 20.5	162.4 \pm 20.5	177.9 \pm 20.5	0.33	3	0.8014
11	Sum of duration of sieve element phase (s-SEP)**	20.2 \pm 11.9a	27.2 \pm 11.9a	92.76 \pm 11.9b	84.69 \pm 11.9b	17.77	3	<.0001
12	% time aphid spent in sieve element phase after the first probe to sieve elements (% SEP)	4.5 \pm 2.3b	5.3 \pm 2.3b	19.6 \pm 2.3a	15.5 \pm 2.3a	19.96	3	<.0001
13	No. of aphids that reached the sieve element phase (n = 15)	5	4	13	15			

1. Values followed by different letters are significantly different according to the Kruskal-Wallis test ($\alpha = 0.05$) and multiple comparisons ($P < 0.05$; Tukey test) within a row.

2. Time is calculated in minutes

* Replicates with zero value are not included in final analyses, ** All replicates are included in final analyses