

EFFECT OF PREVIOUS FEEDING ON ANTIBIOSIS LEVELS OF SOYBEANS

by

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Abstract

The soybean aphid, *Aphis glycines* is documented to have arrived in North America in mid 2000 and has ever since established itself as a formidable pest of soybeans, with the capacity to cause immense crop losses. This formidable pest with its complex life cycle and habits represents a current threat to soybean production. Host plant resistance is a promising avenue that can offer considerable control over the soybean aphid problem. Antibiosis being the most effective host plant resistance category, this study was aimed at attempting to understand the effects of induction on the antibiosis levels of soybeans. In the first set of experiments, different soybean genotypes and two soybean aphid biotypes were tested to comprehend if and how the genotypes and biotypes affected the survival and reproduction of the aphid. The experiments revealed mixed results that can be attributed to the genotypes tested and the biotypes used. While some genotypes showed no significant changes due to previous infestation, K1621 suggested signs of induced resistance to biotype 1 and PI567301B showed induced resistance to biotype 2, while K1639 pointed towards induced susceptibility to biotype 2. A follow up feeding behavior study with Electrical Penetration Graph (EPG) technique was carried out on PI567301B to elucidate if the induced resistance was tissue-specific, which could affect the feeding behavior of the aphid (biotype 2); but the results showed no appreciable differences in the feeding behavior of the aphids on clean vs. infested plants. Induced response studies shed light on how plants respond to herbivory and help us identify how changes in plant physiology affect the various herbivores that visit it for food and shelter. This knowledge can thus be applied to the development of superior varieties of crops that can defend themselves better against recurring infestations.

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Dedication

I dedicate this thesis to my parents, Viswanathan and Prema, who are more like best friends than parents. I cannot thank them enough for being so wonderful and understanding; letting me live my life as I choose, believing in my choices and standing by me through thick and thin.

Chapter 1 - Review of Literature

Introduction

Glycine max L. commonly called soybean is an annual herbaceous dicot plant belonging to the family, Fabaceae. It is an economically important crop that is the world's foremost provider of protein and oil, currently representing over 53 percent of the world's oilseed production. The United States produces 38 percent of the share with 30% of crop area in the country set aside for soybean cultivation. Soybean has wide range of uses in various food products for human and animal consumption. In 2009, the US accounted for about 46 percent of the world's soybean trade with the export revenue exceeding \$21 billion (Soy Stats[®] 2010). Soybean also has numerous industrial uses (Soy Stats[®] 2010). Being a highly valuable crop that is grown in more than 30 states, pests are a chief threat to soybean production. And hence, the soybean aphid, *Aphis glycines* Matsumura has attained great attention as a major pest of soybean since its arrival in the US in 2000 from its native home of Asia. And now there are even reports of the soybean aphid having invaded Australia (Fletcher and Desborough, 2000).

Taxonomy, Morphology, Origin and Distribution of *A. glycines*

The soybean aphid, *Aphis glycines* Matsumura belongs to the Order Hemiptera, Suborder Sternorrhyncha, Superfamily Aphidoidea and Family Aphididae. The soybean aphid is a small, greenish-yellow aphid with a pair of conspicuous black siphunculi (Blackman and Eastop, 1984) also called cornicles or 'tail pipes' near the distal tip of its abdomen (Sloderbeck et al. 2003). The alate females are 0.96 to 1.52mm in length identifiable from their yellow or yellow-green color, long-ovoidal body, red-brown compound eye and black head while the apterous females are also yellow or yellow-green but with a shorter ovoidal body and measuring 0.95-1.29mm in length. However, the nymphs and adults are morphologically similar (Wu et al. 1999). Taxonomic work by Voegtlin et al. (2004) provides a useful guide to identify the soybean aphid from other morphologically similar species.

This aphid is an invasive pest species that is native to Eastern Asia and was first described by Matsumura in 1917 (Matsumura, 1917). The soybean aphid has been documented in Southeast Asia and parts of Africa (Wang et al. 1962; Kobayashi et al. 1972; Hill, 1987 and Hirano and Fuji, 1993; Singh and Van Emden, 1979) such as Japan (Sakai, 1949), Thailand, Korea, Taiwan, China, Malaysia (Paik, 1965), India (Raychaudhari et al. 1980), Indonesia (Iwaki, 1979), Malaysia and North Borneo (Blackman and Eastop, 1985 as cited by CAB International, 2001), Russia (D'yakonov, 1975), Vietnam (Waterhouse, 1993) and Australia (Fletcher and Desborough, 2000).

The soybean aphid was not reported in North America before July 2000 (Hartman et al. 2001) and in 2001, the presence of the aphid was reported from Canada (OMAFRA, 2004). Presently, this invasive exotic pest has spread its range throughout the Midwestern United States and Southern Canada since the initial reports of its presence in North America.

Distribution and Spread of *A. glycines* in the US

The soybean aphid was first detected in the United States in the soybean fields of southern Wisconsin in July, 2000 (Ostlie, 2002; Alleman et al. 2002) and it has since then rapidly expanded its range infesting soybeans in 21 U.S. states and 3 Canadian provinces by the end of 2003 (OMAFRA, 2004). The soybean aphid was first detected in Kansas in small numbers confined to 5 eastern counties in August and September of 2002 (Sloderbeck et al. 2003). By 2003, it had spread to 17 counties in Kansas (Sloderbeck et al. 2004) and reached damaging population levels in 2004 facilitated by favorable environmental conditions (Whitworth, 2008).

Biotypes

Since its arrival in the US in 2000, the soybean aphid has, in a matter of a few years, adapted and developed resistance to the existing insecticides (organophosphates, carbamates and pyrethroids) and resistant varieties of soybean by evolving biotypes. The original biotype named the Illinois isolate and now renamed as Biotype 1 (Hill et al. 2009), was first reported by

Hartman et al. (2001) and then in the summer of 2005, the existence of the Ohio isolate [renamed Biotype 2 (Hill et al. 2009)] was established at the Ohio Agricultural Research and Developmental Center, Wooster OH and was found to be virulent to soybean varieties with the *Rag1* gene which confers resistance against the biotype 1 in field-cage experiments in Ohio (Kim et al. 2008). A new biotype of the soybean aphid, biotype 3 collected from *Frangula alnus* in Springfield Fen, IN in 2007 has been documented by Hill et al. (2010) with evidence of the biotype's ability to colonize plants with *Rag2* resistance.

Biology and Ecology of *A. glycines*

The soybean aphid has a heteroecious holocyclic life cycle (i.e) there is alternating sexual and asexual reproduction on alternating hosts. The primary host (overwintering host) of the soybean aphid in North America is the common buckthorn, *Rhamnus* spp. (Voegtlin et al. 2004, 2005) and soybean, *Glycine max* is its secondary host. In addition to cultivated soybean, the soybean aphid 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). A study of the biology of the soybean aphid in N. America by Ragsdale et al. (2004) shows that its life history is similar to that observed in China and Japan except for the primary hosts which are *R. davurica* Pallus and *R. japonica* Maxim respectively, in those countries..

The asexual apterae spend the summer on the secondary host (*G. max*), reproducing parthenogenetically and dispersing across to new hosts by producing winged forms. In late summer and fall, they produce winged forms (alate females called gynoparae and males). These then migrate to the primary host, where the gynoparae produce oviparae, which mate with the males and lay eggs. The overwintering stage of the soybean aphid is thus the egg. During early summer, the soybean aphids migrate back to the secondary host and the life cycle thus continues (Ragsdale et al. 2004).

In summer, though most of the individuals are wingless, winged females develop as a result of crowding serving the dispersal and spread of the population to other nearby plants

(Ostlie, 2002). Up to 15 generations can occur on soybean in one year under suitable climatic conditions (Wang et al. 1998) apart from the 2-3 generations on buckthorn. The nymphs undergo 2-3 moults. So, there are 3-4 instars and normally the nymphs develop into adults in 5 days. Hirano et al. (1996) studied the effects of temperature on the development, longevity and reproduction of the soybean aphid. The soybean aphid population growth rate is found to be highest at 25° C with the population doubling time of 1.5 days. Further increase in temperature retards the reproduction rate of the soybean aphid (McCornack et al. 2004). Further, McCornack et al. (2005) studied the overwintering potential of the soybean aphid to better estimate the aphid overwintering mortality and found that the eggs were the most cold-hardy with the lowest mean Super Cooling Point (SCP) of ~ -34°C while the gynoparae and oviparae had the highest mean SCP of ~ -15°C.

Habits, Damage and Economic Importance

A. glycines is the only species of aphid that forms large colonies on soybeans in North America (Sloderbeck et al. 2003). In general, three periods of damage can be recognized on soybean: i) from the seedling to blooming stage, when the aphid populations reach their peak and colonies concentrate on young growth, ii) during late July, when growth is completed, aphid colonies move to the lower leaves of the plant for feeding and, iii) from late August to early September when the aphids start to multiply again before migrating back to the winter host (Wang et al. 1962). But the different growth stages of soybean plants seem to have no significant impact on the population growth or dynamics of the soybean aphid and also on the life history characteristics of individual aphids (Rutledge and O'Neil, 2006).

In a 3-yr yield loss experiment carried out across 6 states by Ragsdale et al. (2007), the average ET level of soybean aphid was found to be 273 ± 38 aphids per plant (strongly supported during the R5 stage) with a population doubling time of $6.8d \pm 0.8d$. The EIL was found to be 674 ± 95 aphids per plant which gave a 7d lead time from the ET.

Plant Physiology

Being the main sap sucking pest in soybean fields (Takahashi et al. 1993), high soybean aphid populations reduce soybean yield directly by their feeding causing the stunting of the plant, leaf distortion, brown yellow spots on infested leaves (Wu et al. 1999) and reduced pod set (Sun et al. 1990). Visual symptoms like curling, wilting, yellowing and premature dropping of leaves can result from severe aphid infestation of soybean (DiFonzo and Hines, 2002). These symptoms, however, can be mistaken for symptoms of potassium and/or iron deficiencies in the soil.

Apart from seed yield, these aphids can reduce seed quality also with discoloration, deformation, etc., which is a concern to both farmers and consumers (Mian et al. 2008). In a 2-yr controlled infestation study measuring the feeding injury effects of the soybean aphid on V5 and R2 stages of soybean, Beckendorf et al. (2008) documented a linear decline in seed yield, yield components, and seed oil concentration with increasing aphid numbers per plant and maximum cumulative aphid days per plant. However, they found positive correlation between seed protein concentration and peak aphid numbers per plant.

The photosynthetic capacity of plants may be negatively affected with increase in herbivore populations (Wang et al. 1962). The consequential reduction in chlorophyll content could thus contribute to the economic loss. Macedo et al. (2003) determined photosynthetic responses of soybean (Asgrow 0901) to soybean aphid injury where the photosynthetic capacity was affected by densities greater than 20 aphids/leaflet.

Changes in the nitrogen and lignin content of soybean plants as a consequence of aphid infestation were reported by Hu et al. (1992, 1993). Aphid feeding can also cause water stress or drought stress in the plant that could facilitate further colonization of the pest on the host plant (Cabrera et al. 1995).

Diseases

A. glycines vectors a number of plant viruses. D'yakonov (1975) showed *A. glycines* to be a vector of soybean virus in Soviet Far East. Zhang and Zhong (1982) attempted to artificially infest soybean plants with alates of *A. glycines* and found that the incidence of the plant infection by the viral disease transmitted by this aphid reached to about 100%. Li and Pu (1991) reported 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, the most frequent vector being *A. glycines*.

Besides direct feeding damage to soybeans, the soybean aphid is also a vector for a number of plant viruses, including Soybean mosaic virus (SMV), Bean yellow mosaic virus, Peanut mottle virus, Alfalfa mosaic virus (AMV), and Peanut stunt virus (North Central Pest Alert Factsheet: Soybean Aphid, 2002). The ability of this aphid to vector viruses such as alfalfa mosaic virus and soybean mosaic virus has also been documented (Hartman et al. 2001; Hill et al. 2001). Davis et al. (2005) documented *A. glycines* as a new vector of three strains of the Potato Virus Y viz., PVY^o, PVY^N, and PVY^{NTN} in potato.

The development of sooty mold on leaves as a result of honeydew excretion by soybean aphids is another cause for further yield losses (Krupke et al. 2005).

Control Measures

The control of the soybean aphid gained importance after a 58% yield loss was reported in China (Wang et al. 1996) and also documentation of over 50% yield losses in the fields of Minnesota during 2001, just a year after its arrival in the US (Ostlie, 2002). Insecticides have been used widely to control the aphid with millions of dollars spent on it, as with the control of any insect pest. Landis et al. (2003) reported that over 7 million acres of soybean fields were sprayed for the soybean aphid in the North Central Region in 2003 with second treatment in over

500,000 acres. It costs between \$12 and \$15 per acre to apply insecticides for aphid control (Paul, 2004).

Insecticides like Phosalone, Pirimicarb, Omethoate and Fenvalerate were shown to be efficient at controlling aphid numbers (Wang et al. 1993). Also Huang et al. (1998) documented the effectiveness of Imidacloprid treatment in controlling the aphid. Li et al. (2000) demonstrated a greater than 90% control of soybean aphids in the seedling stage with the furrow application of Carbofuran or Aldicarb.

Although insecticides help suppress pests over the short term, they have ill effects on the environment, human and animal health over the long term. Also a prevalent problem with regard to pest control is that of pest resurgence and the pest population rebounding after insecticidal applications. Extensive use of chemicals in high doses for quick control is detrimental for the natural enemy populations and therefore conservation and safe manipulation of the beneficial organisms is highly essential. It is here that the concept of Integrated Pest Management gains significance.

Natural enemies used for biocontrol of the soybean aphid in the fields include Asian lady beetles (*Harmonia axyridis*, *Propylaea japonica*, *Coccinella septempunctata*, *Hippodamia tredecimpunctata*), lace wings (*Chrysopa formosa*, *C. septempunctata*), syrphids, hymenopteran parasitoids, chamaemyiids and entomophagous fungi etc. (Wang et al. 1998). A description of primary and hyperparasitoids of *A. glycines* was given by Chang et al. (1994) from collections in the Korean republic. He documented *Aphidius cingulatus*, *Ephedrus persicae* and *E. plagiator* are among the common primary parasitoids and *Asaphes vulgaris* and *Ardilea convexa* as the dominant hyperparasitoids.

More recently, Rutledge et al. (2004) reviewed the various findings on the soybean aphid predators found in the North Central soybean systems that could contribute to soybean aphid management and identified that *Orius insidiosus* (Say) and *Harmonia axyridis* (Pallas) as two potentially key predators that could be of use in the biological control of *A. glycines*. Two parasitoid species *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae) and

Lipolexis gracilis Förster (Hymenoptera: Braconidae) have been imported from Asia and have passed through quarantine (Heimpel et al. 2004). Liu et al. (2004), in a field survey in Northern China found that the aphid parasitoid *Lysiphlebus* sp., and the aphid predators *Propylaea japonica* (Thunberg), *Scymnus (Neopullus) babai* Sasaji, and *Paragus tibialis* (Fallén) were the main natural enemies of the soybean aphid. Fox et al. (2005) mentioned the significance of generalist predators like spiders along with specialists in controlling the establishment of soybean aphid colonies or populations on soybeans.

Cultural control practices like intercropping, crop rotation, barrier crops (sunflower), tilling and removal of crop residue and varying the planting times have been practiced in soybean to reduce the soybean aphid numbers (Quimio and Calilung, 1993). However, only a proper and effective combination of the various control measures will ensure successful control of the soybean aphid.

Host Plant Resistance

Host plant resistance (HPR) is a major component in integrated pest management ensuring minimal use of chemical insecticides or pesticides, thus bringing benefit to growers and also protecting the environment. Research on HPR involves the screening and selection of resistant (antibiotic, antixenotic and tolerant) varieties of plants which can be made commercially available to growers. This is made possible by understanding the mechanism that confers the resistance, i.e. the gene(s) involved, and incorporating them into varieties or cultivars that have better yield, crop quality and other advantageous characteristics which will in effect, benefit the crop production as a whole.

Immense amount of research has been and is being done on a vast number of economically important crop plants and their pests. Plant breeding programs have been in practice in China and Indonesia with the goal of developing aphid resistant soybean varieties since China is believed to have more than 90% of the soybean resources. Sama et al. (1974) reported the screening results of over 200 varieties in Indonesia. Fan et al. (1988) undertook

screening of 181 soybean varieties from 1983 to 1986, of which only two entries showed high resistance to the soybean aphid in a year of severe aphid infestation. Wang et al. (1962) put forth that the susceptible varieties have a significantly higher aphid density and harbored a younger aphid population compared to that of resistant varieties.

Hill et al. (2004) found six accessions of *G. soja* resistant to the soybean aphid that could be novel sources of resistance not found in *G. max*. Hill et al. (2004a) reported antibiosis and antixenosis type resistance against the soybean aphid in nine soybean germplasm accessions. From the above, the accessions Dowling and Jackson were found to contain the *Rag1* and *Rag* resistance genes respectively (Hill et al. 2006a, 2006b). 2147 accessions of soybean originally from Northern China were evaluated for resistance by Mensah et al. (2005) and resistance was found in the Plant Introductions (PI) - PI 567543C, PI 567597C, PI 567541B and PI 567598B with the former two showing antixenosis and the latter two, antibiosis type of resistance. Diaz-Montano et al. (2006) screened 240 entries under greenhouse conditions and found nine entries with moderate antibiotic resistance and two entries namely K1639 and Pioneer 95B97 to possess strong antibiosis and also antixenosis resistance against the Illinois biotype of the soybean aphid. Feeding behavior study and chlorophyll loss experiments were undertaken by Diaz-Montano et al. (2007a, 2007b) on biotype 1 of the soybean aphid in 4 resistant soybean genotypes (K1639, Pioneer[®] 95B97, Dowling, and Jackson) and a susceptible check (KS4202) using the Electrical Penetration Graph method and SPAD meter respectively and the results revealed that feeding of the aphids were deterred in the resistant entries compared to the susceptible check and a similar trend was observed with respect to chlorophyll loss also where the resistant entries showed no significant loss in chlorophyll. Hessler and Dashiell (2007), in their tests for sources of soybean aphid resistance reported the potential of the lines Tie-feng 8, Braxton, and Cobb as useful sources of resistance. Hessler and Dashiell (2008) found possible sources of resistance to *A. glycines* in the soybean lines Perrin, Tracy-M and D75-10169. Mensah et al. (2008) reported that inheritance of resistance in the PIs, PI 567541B and PI 567598B was controlled by two major recessive genes. Mian et al. (2008) screened nearly 200 soybean genotypes and found resistance trait in three PIs namely PI 243540 (antibiosis resistance), PI 567301B and PI 567324 (antixenosis resistance) and six others moderately resistant to *A. glycines* collected from Ohio, in greenhouse experiments. These PIs thus are promising sources of resistance against the more

virulent biotype 2 and also the biotype 1. Kang et al. (2008) reported that resistance in the PI 243540, resistant to the biotype 2, was controlled by a single dominant gene. PI 200538 is another genotype reported to possess *Rag2* gene conferring resistance against both biotypes of the soybean aphid (Hill et al. 2009). More recently, Zhang et al. (2010) identified a new resistance gene in the PI567543C that mapped on a chromosome different from the other earlier published soybean aphid resistance genes and designated this gene as *Rag3*. Pierson et al. (2010) tested six soybean genotypes during their reproductive stages for resistance and categorized KS4202, Asgrow 2703 and K-1639-2 with having moderate resistance.

While Host Plant Resistance has many advantages over chemical control, one disadvantage to this otherwise effective and environment friendly pest management tactic is the ability of the insect pests to overcome the resistance in a host plant through development of new virulence. This is particularly evident in aphids. In the case of the *A. glycines*, the original isolate documented in the US for the first time in 2000 has evolved into a more virulent biotype namely the biotype 2 detected in 2005 (Kim et al. 2008) and in April 2010, the presence of a third biotype, Biotype 3 was documented (Hill et al. 2010). The soybean aphid thus demonstrates its enviable potential for survival against adversities.

Induced Plant Defenses

In crops that are affected by perennial pests, an understanding of how recurring infestations can induce defense response in host plants is important since induction of resistance could facilitate to further minimize the extent of damage caused by herbivory. Although, the response induced may or may not be beneficial to the host plant, the recognition and understanding of those crop varieties and their underlying traits which make them more resistant or tolerant to repeated infestations by the pest has immense significance from the host plant resistance point of view.

Laboratory studies by Formusoh et al. (1992) on the fecundity of the greenbug, *Schizaphis graminum* (Rondani) biotype E on wheat, *Triticum aestivum* L., pre-conditioned by

Russian wheat aphid feeding, *Diuraphis noxia* and by greenbug biotype E, showed that 10 days of previous exposure of wheat to Russian wheat aphid may provide favorable environment for future infestation by the greenbug biotype E, while previous infestation by the greenbug itself did not induce any changes. However, no significant differences were found in the feeding behavior of greenbug biotype E conditioned by Russian wheat aphid. Cabrera et al. (1995) documented that greenbug feeding damage caused drought stress and lesser CO₂ assimilation in young barley plants. They found that greenbug nymphs reared on previously infested plants showed lower Mean Relative Growth Rate (MRGR) and developmental rate (1/D) compared to those reared on clean uninfested plants. Similar reduced MRGR and 1/D were observed in nymphs reared on drought-stressed plants compared to controls. Their study thus shows that aphid infestation produces changes similar to drought-stress in plants, which in turn impairs aphid growth and development.

Wool and Hayes (1996) examined how previous infestation of the cotton aphid, *Aphis gossypii* Glover affected the resistance of cotton seedlings to future recolonization and concluded from their results that the aphids on previously infested plants showed lower success mainly due to plant damage caused from the previous infestation rather than the increased production of secondary plant metabolites. Feeding behavioral changes brought about in *Aphis fabae* (black bean aphid) and *Rhopalosiphum padi* (bird cherry-oat aphid) as a consequence of previous infestation were studied by Prado and Tjallingii (1997) using the EPG technique. They demonstrated that previously infested leaves improved the host plant acceptance of black bean aphid as the aphids spent reduced time on the non-probing pathway phase and the salivation phase, reaching the sustained phloem ingestion sooner and continuing with the phloem ingestion (committed phloem ingestion-CPI) for a longer period of time. Previous infestation also increased the production of honeydew droplets when compared with clean plants. In contrast, previous infestation had no beneficial effects on bird cherry-oat aphid.

Williams et al. (1998) found that previous infestation by the green peach aphid *Myzus persicae* (Sulzer) or black bean aphid improved the host quality of sugar beet for subsequent infestation by the same species. The study also showed that nymphs grew more rapidly on infested plants than controls and those favorable conditions produced as a result of prior

infestation lasted for up to two weeks. Another finding in this study was that previous infestation made the plants more susceptible to viral diseases transmitted by aphids like the beet mild yellowing virus (BMYV), in the case of the sugarbeet. A field study by Agrawal (1998) on wild radish (*Raphanus sativus* L.) showed that previous infestation resulted in a 30% reduction in plant colonization by green peach aphids and other insect herbivores. This study also demonstrated that induced plants developed resistance to herbivore insect pests and had a lower mortality rate compared to the uninfested controls.

The host plant changes owing to previous attack by *Sipha flava* (Forbes) on *Sorghum halepense* (L.) was studied by Gonzales et al. (2002) and they found that previous infestation induced plant susceptibility where in the aphids spent significantly shorter total time on non-penetration activities during feeding and also that the nymphs exhibited significantly higher mean relative growth rate on the infested leaves when compared with the clean leaves. The settling behavior and reproduction of the green peach aphid as affected by previous infestation on two peach cultivars was examined by Sauge et al. (2002) and they showed that a 48h preconditioning induced resistance in the resistant cultivar Rubira while slightly enhancing susceptibility in the susceptible control GF305. Also previously infested GF305 were more readily accepted by the green peach aphids while in pre-infested Rubira, the aphids spent longer time in the pathway phase to reach a sieve element and also recorded a 4-fold reduction in phloem ingestion time.

The feeding preferences and performances of Russian wheat aphid and bird cherry-oat aphid on wheat as affected by previous infestations by the two species were examined by Messina et al. (2002). They found that previous infestation by the Russian wheat aphid did not produce any detrimental effects on subsequent population growth of either species while the same by bird cherry-oat aphid reduced its own subsequent population by 50% with little or no effect on Russian wheat aphid populations. Also both species preferred feeding on control plants to previously infested plants. From their results, they concluded that there is specificity in both the plant's response to the aphid species infesting it and the aphid's response to a particular modification in the host plant. In 2004, Messina and Bloxham tested several resistant lines of

wheat with Russian wheat aphid for induced response and only found a small decline in the performance of bird cherry-oat aphid as a consequence to previous infestation.

Studies pertaining to the resistance in *Medicago truncatula* Gaert. against the blue alfalfa aphid, *Acrythosiphon kondoi* by Klingler et al. (2005) showed that previous infestation enhanced resistance in the resistant variety, Jester compared to the susceptible line A17 both in terms of aphid feeding and reproductive performance on the host. EPG method indicated that the resistance was induced at the level of the phloem sieve element and with 4 days of prior infestation Jester showed a 7-fold decrease in aphid survival as compared to A17.

Weng et al. (2005) studied the induction of resistance in wheat with the green bug resistance gene *Gb3* and found that preconditioning induced systemic resistance in resistant varieties with intensities and effect varying in different parts of the plants. They showed that greenbug performance and the damage caused by their herbivory were reduced through host resistance gene-mediated induced resistance, thus supporting the optimal defense theory about the within-plant defense allocation.

Sauge et al. (2006) tested the effect of previous herbivory by green peach aphids on five genotypes of peach with regard to the development and feeding behavior of the same. Their results showed that even those genotypes falling under the same category of resistance responded differently to the previous infestation by the aphids, thus, showing that the induced responses elicited, differ with genotypes and is not dependent on the type of resistance involved. Dugravot et al. (2007) studied the local and systemic responses of potato (*Solanum tuberosum* L.) on the probing and feeding behavior of green peach aphids upon infestation by conspecifics and heterospecifics (here, *Macrosiphum euphorbiae* (Thomas)) and found that both kinds of previous infestation produced modification of green peach aphids feeding activities wherein previous infestation was beneficial for green peach aphids at the local level while being detrimental at the systemic level.

Franzen et al. (2008) studied the physiological changes occurring in wheat and barley when infested with green peach aphids and bird cherry-oat aphids. Pierson et al. (2011)

documented the physiological responses of a few reproductive stage soybean genotypes to aphid feeding by comparing infested and control treatments. They found that Asgrow 2703 showed notable changes in its photosynthetic capacity, carbon-linked dark reactions and peroxidase activity after 28 days of aphid infestation compared to their controls while KS4202 showed an up-regulation of peroxidase activity after 24 days of infestation.

Objectives

From the standpoint of the benefits of host plant resistance as an easy and environmentally friendly IPM tactic, research has been conducted on the resistance aspect of soybean against the soybean aphid since its arrival in 2000. However, the problem of the aphids overcoming resistance is ever present. An understanding of the effects of aphid infestation on subsequent colonization of the host plant by the same will provide an insight into how the aphids modify the host plant physiology and whether prior infestations are beneficial or detrimental to the aphids. The knowledge of the changes on the host plant at the genetic level can then be applied as an added tool in combating the aphids through the development of resistant varieties. Moreover, there have been no studies pertaining to induced resistance in the soybean-soybean aphid system. This research was, therefore, a venture to explore this existing avenue within host plant resistance for the control of the soybean aphid.

The objectives of this research were:

1. To identify any changes in the level of antibiotic resistance in the host soybean plants as a result of previous infestation by aphids. In other words, to recognize if previous infestation caused any changes in the plant physiology that were detrimental to the aphids and compare the same between clean and infested plants.
2. To determine if physiological changes in the host plants due to an early aphid infestation can cause changes in the aphid feeding behavior.

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Chapter 2 – Induced Response of Soybean to Soybean Aphid Biotypes

Abstract

The soybean aphid, *Aphis glycines*, has established itself as a formidable pest of soybean in North America since its arrival in mid 2000, with its ability to cause immense crop losses. Its complex lifecycle and habits aid this flexible insect to continually adapt itself to the different measures that are developed for its control. This particular host plant resistance study on the induced response of soybean genotypes to soybean aphid biotypes 1 and 2 was undertaken to understand how different genotypes might affect the soybean aphid (either favorably or detrimentally) when previously infested with conspecifics. The experiments revealed mixed results that can be attributed to the genotypes tested and the biotypes used. K1621 provided signs of induced resistance to biotype 1. The genotypes PI567301B, K1639-2, KS4202 and Asgrow2703 showed no significant response to biotype 1 feeding while biotype 2 feeding did produce significant differences in aphid number in the PI567301B (induced resistance) and K1639-2 (induced susceptibility). Knowledge of induced plant responses to aphid feeding will be an added benefit while developing new resistant varieties of soybean, thus helping us to circumvent those genotypes that could become susceptible to increased aphid attack upon initial infestation.

Introduction

Glycine max L. or soybean is a highly important economic crop, being the leading provider of protein and oil in the world. The soybean aphid, *Aphis glycines* Matsumura is an important pest of soybean with the potential to cause high levels of yield damage and loss in the crop. The aphid belongs to the Order Hemiptera, with its origin in Eastern Asia and was first described by Matsumura in 1917 (Matsumura 1917). An invasive pest, the soybean aphid has spread across several states in the United States and few provinces in Canada, rapidly within the span of a decade since the documentation of its arrival in the nation in mid 2000 (Hartman et al.

2001). The aphid was first reported from Canada in 2001 (OMAFRA, 2004). Apart from the ability of this pest to spread rapidly, the soybean aphid has also developed biotypes. There are now 3 biotypes of the soybean aphid reported from North America – biotype 1 from Wisconsin in 2000 (Hartman et al. 2001), biotype 2 from Ohio in 2005 (Kim et al. 2008) and most recently, biotype 3 from Indiana in 2007 (Hill et al. 2010) wherein the newer biotypes have evolved to overcome the resistance factors presently helping to control soybean aphid populations. Thus biotype 2 can colonize plants with the *Rag1* resistance gene (which confer resistance against biotype 1) (Kim et al. 2008) and biotype 3, those plants with *Rag2* gene (conferring resistance against biotype 2) (Hill et al. 2010).

The soybean aphid is a fast spreading insect with a heteroecious and holocyclic life cycle requiring two host plants to alternate between sexual and asexual forms and thus complete one full cycle. Common buckthorn, *Rhamnus* spp. is the primary as well as winter host (Voegtlin et al. 2004, 2005) and soybean, *Glycine max* is its secondary as well as summer host. The soybean aphid is the only aphid species that forms large colonies on soybeans in North America (Sloderbeck et al. 2003). There can be up to 15 generations on soybeans in a year (Wang et al. 1998) with winged females helping the spread and dispersal of the aphids to nearby plants (Ostlie, 2002). This piercing sucking pest can thus reduce soybean yields from direct feeding damage (Wu et al. 1999). Aphid infestation can adversely affect seed quality also (Mian et al. 2008, Beckendorf et al. 2008). Apart from these, soybean aphids are also capable of transmitting plant viruses to soybeans (North Central Pest Alert Factsheet: Soybean Aphid, 2002).

Control of the soybean aphid is of significance with regard to the extent of damage it can cause on soybean in more than one aspect. Until recently, chemical control was the only major control measure that was depended upon (Landis et al. 2003). The drawbacks of this control technique are many as in they are not environment friendly and only provide short-term relief, with the imminent danger of a rebound in the pest population in the future. Recurring use of the chemicals induces adaptive changes in the pest whereby new biotypes evolve. Chemical control is also not cost effective (Paul 2004). Although there are other control tactics in use like cultural and biological control, they have not been put to practice as extensively as chemical control for reasons like incompatibility of methods. Host Plant Resistance (HPR) has gained prominence as

an important Integrated Pest Management (IPM) tactic to reduce our dependence on chemicals to control pests and vast amount of research has been and is being undertaken to develop better soybean varieties that can control or withstand aphid invasions with less or no chemical intervention. Also this tactic is highly compatible with control measures like biological control, thus helping achieve the true sense of IPM. Soybean IPM is projected to bring immense benefit in terms of revenue to US soybean growers and consumers, with an estimate of net benefits ranging between \$0.6 and \$2.6 billion dollars as of 2005 (Song and Swinton, 2009).

Host plant resistance involves research that pertains to the screening and development of resistant plant varieties and research in this area has been ongoing for decades. Research on soybean aphid HPR in North America gained pace with Hill et al. (2004, 2004a) reporting new sources for soybean aphid resistance and with further identification of antibiosis and antixenosis type of resistances in some of those accessions. Several other resistant varieties of soybean have been reported which include sources of resistance genes such as, *Rag1*, *Rag2* (Hill et al. 2006a, 2006b, 2009) and *Rag3* (Zhang et al. 2010).

Resistance is categorized into antibiosis (where the biology of the insect is adversely affected), antixenosis or non-preference (where the behavior of the insect is affected) and tolerance (plant characteristic whereby the plant can withstand infestation levels as found on a susceptible). Further, antibiosis can be divided into constitutive (innately present traits like physical and chemical barriers) and induced resistance (response induced by injury/insect feeding damage).

Induced response studies, from a host plant resistance perspective, focus on whether herbivory makes a plant or crop variety more resistant or susceptible to recurring infestations. This understanding would be added knowledge in the breeding and development of resistant crop varieties whereby the plants can better defend themselves when faced with persistent attacks from the pest. Research on aphid-host plant systems show mixed responses from host plants with regard to an induction of resistance and the associated detrimental effects on the pest species (Formusoh et al. 1992, Cabrera et al. 1995, Wool and Hayes 1996, Williams et al. 1998, Agrawal 1998, Gonzalez et al. 2002, Sauge et al. 2002, Messina et al. 2002, Klinger et al. 2005, Weng et

al. 2005, Sauge et al. 2006). While no literature is available for induced response studies in the soybean-soybean aphid system, a recent study by Pierson et al. (2011) examined the physiological responses of the some soybean genotypes (KS 4202, K 1639-2, Asgrow 2703 and Jackson) in their reproductive stages to feeding by the soybean aphid and compared them with control plants at various points in time. The team documented significant changes in the photosynthetic capacity of Asgrow 2703 after 28 days of infestation. Aphid feeding brought about detrimental impacts on the carbon-linked dark reactions in Asgrow 2703 as well as an up-regulation in the peroxidase activity of Asgrow 2703 and KS4202 after 24 and 28 days of aphid infestation as compared with the respective controls.

In the present study, the effect of previous aphid infestation on the antibiosis levels of some soybean genotypes were tested.

Materials and Methods

Aphid Cultures. Two biotypes of the soybean aphid were utilized in experiments viz., biotype 1 and biotype 2. Biotype 1 was collected from soybean fields in Nebraska in 2008 and biotype 2 was obtained in the same year, from Brian W. Diers, Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801. Both biotypes were maintained on the susceptible soybean genotype KS4202, with a continuous supply of seedlings. Biotype 1 was kept under pesticide-free greenhouse conditions: temperature 20-30°C, relative humidity 23-40% and supplemented with sodium vapor lamps set to a photoperiod of 14:10 (L:D). Biotype 2 was maintained in the laboratory within a growth chamber (Percival[®] Model – 136LL, IA 50220) at 22-24°C and with photoperiod 11:13 (L:D). Both biotypes are on file at the Kansas State University Museum of Entomological and Prairie Arthropod Research. The voucher specimen number for biotype 1 colony is 180 and that of biotype 2 is 218. For experiments, the aphids were moved between the lab and greenhouse inside petri-dishes that were sealed securely with paraffin tape. After use in experiments, all plants and aphids were frozen to prevent accidental escape and spread of aphids.

Soybean Genotypes. Genotypes were selected to include a variety that showed resistance they possessed against either biotype 1 or 2 of the soybean aphid or both (Table 2.1). KS4202 and

Asgrow 2703 were used as susceptible checks. K1639-2 and K1621 are resistant and moderately resistant respectively, to biotype 1 (Diaz-Montano et al. 2006) while both are susceptible to biotype 2 (Chandran, 2011). The Plant Introduction, PI567301B and the Michigan entries, E06902 and E07906-2 are resistant to biotypes 1 and 2 (Mian et al. 2008, Chandran 2011).

Experimental Design. The induced response experiments were performed in a pesticide-free greenhouse [20-30°C, 20-40% RH, and a photoperiod of 14:10 (L:D)] with high pressure sodium lamps as supplementary lighting. Plants were grown in 3.8 cm-diameter by 21 cm deep plastic Cone-tainers™, Hummert International, Earth City, MO) containing steam-sterilized potting mix (Premier Promix™, Canada). One seed was sown per cone-tainer. Initially, V-1 stage plants and later V-2 stage plants were used in the experiments. V-1 is the stage in which there are two fully developed leaves at the unifoliate nodes (Fehr and Caviness, 1977) and V-2 stage is where there are two sets of fully developed trifoliate leaves. An entry represented a genotype of soybean. Eight to ten plants from an entry were randomly selected and subjected to two treatments viz., uninfested/clean and aphid-infested respectively. Each observation (one individual plant) comprised an experimental unit.

The preliminary tests were conducted with the purpose of establishing the optimal number of aphids and pre-infestation days required to bring about significant induced changes in the entries. The plants randomly selected for the aphid infested treatment at the V-1 stage were exposed to 10 or 25 aphids for 2, 4, 9, 10, 12 and/or 15 days respectively, with either biotype 1 or biotype 2 of the soybean aphid. The aphids were transferred onto the unifoliate leaves by hand using a moist camel's hair paint brush (number 0) and allowed to feed and reproduce on the plant during the infestation period. The clean plants were kept aphid-free during this period but underwent mock cleaning, so as to ensure that all plants in the experiment were handled similarly. At the end of the infestation period, the plants were cleaned from aphids and an antibiosis test (Diaz-Montano et al. 2006) was conducted to perceive effects of induced changes in the entries, if any. Antibiosis involves caging an adult aphid on a leaf for 4 days and counting of the number of nymphs produced during that time frame (Diaz-Montano et al. 2006).

Based on the results of the preliminary tests, the experimental design was modified slightly wherein the infestation was restricted to one leaflet in a trifoliate of a V-2 stage plant

using a small zip-loc bag, instead of infesting an entire V-1 stage plant. One leaflet each (in two trifoliates) was infested in each plant (Figure 2.2). The number of aphids used for the infestation was set at approximately 75-100 and the aphids were transferred on the plant by the introduction of an infested leaf. The infestation period was set at 10 days and an extra 24 hours were allowed for the plant to recover from or react to the infestation before antibiosis test was carried out in a leaflet adjacent to the one that was infested. With this modification, the focus of research was shifted to uncover any locally systemic induction of resistance or susceptibility.

All the plants in the different treatments and entries in each experiment were arranged in a completely randomized block design within the greenhouse and supplied with water reservoirs (Figure 2.1).

Statistical Analyses. The analyses of the aphid reproduction data between treatments within an entry were done using Proc GENMOD and the means and standard errors of aphid nymph population were calculated using Proc MEANS of the SAS[®] Program (SAS Institute 1999).

Results and Discussion

Plant response to insect feeding can result in induced resistance, induced susceptibility or neither. The response need not always be for the plant's benefit. Factors like cultivars or genotypes of the plant (Sauge et al. 2006) and the kind of pre-herbivory viz., conspecifics, heterospecifics or different biotypes (Formisoh et al. 1992) affect the outcome of the response elicited by the plant. In this present study on induced response, the induction responses of soybean genotypes to the two soybean biotypes observed have been mixed.

The preliminary sets of experiments were carried out on V-1 stage plants using biotype 1 aphids for both infestation and antibiosis tests over different periods of pre-infestation. Of the three genotypes tested, namely KS4202, K1621 and K1639-2, the moderately resistant K1621 showed differences in aphid numbers between clean and treated plants with statistically significant main effects for treatment and genotype ($\alpha=0.05$) when pre-infested for 2, 4 (trt:

DF=2; $\chi^2 = 16.87$; $p=0.0002$; ty: DF=1; $\chi^2 = 21.44$; $p= <.0001$) and 9 days (trt: DF=1; $\chi^2 =4.43$; $p=0.0353$; ty: DF=2; $\chi^2 =147.09$; $p= <.0001$), while the 12-day pre-infestation did not provide changes in aphid numbers with significant main effects or interaction effects (Figure 2.3 and Table 2.2). With the exception of the 9-day treatment, all other treatments showed a decline in aphid numbers in K1621. The 9-day treatment showed an increase in aphid nymphs (5.9 ± 1.39 and 9.2 ± 1.41). This cause of this anomaly in K1621, in an otherwise apparently consistent decrease in aphids through the different treatments, is unknown. Further experimentation would help clarify this incongruity and understand whether the 9th day of infestation has any particular effect on the plant physiology, making it briefly more susceptible to the aphids. K1639-2 is highly resistant to biotype 1 (Diaz-Montano et al. 2006) and this could have contributed to the negligible changes in aphid number in K1639-2 upon pre-infestation with biotype 1 (Figure 2.3 and Table 2.2). Two days of pre-infestation with 10 aphids demonstrated differences in aphid nymph numbers in KS4202 (13.1 ± 2.32 and 9.2 ± 2.08 ; $P < 0.0094$ at $\alpha=0.05$) with significant main effects (trt: DF=2; $\chi^2 = 16.87$; $p=0.0002$; ty: DF=1; $\chi^2 = 21.44$; $p= <.0001$) indicating induced resistance while the other treatments showed changes that were not statistically significant (Figure 2.3 and Table 2.2).

With whole plant pre-infestation using 25 aphids of biotype 2 in the V-1 stage for a period of 15 days, KS4202, a susceptible variety, showed signs of increased susceptibility with statistically significant treatment x genotype interaction effects (4.1 ± 1.15 and 8.2 ± 1.5 ; DF= 2; $\chi^2 = 7.88$; $P= 0.0195$ at $\alpha=0.05$) (Figure 2.4 and Table 2.3). To the contrary, the Michigan genotypes (developed by Department of Crop and Soil sciences, Michigan State University, MI48824) E06902 and E07906-2, which are highly resistant to both biotypes 1 and 2 (Chandran 2011), showed little or no change in the level of antibiosis resistance which is reflected in the number of aphid nymphs.

With the modification of the experiment design to understand the locally induced systemic changes in the soybean genotypes, where a leaflet in a trifoliate (total of two trifoliates in each plant) was infested with 75-100 aphids for a 10 day period, it was found that there is significant treatment main effect (DF=1; $\chi^2 = 17.27$; $p= <.0001$) with the PI567301B showing an induction of resistance, with significantly fewer nymphs in the treated plants (5.4 ± 0.83 and

2.0±0.84; P<0.0001 when $\alpha=0.05$) when pre-infested with biotype 2 and tested with biotype 2 (Figure 2.4 and Table 2.3). However, pre-infestation with biotype 1 produced no significant differences in the number of biotype 2 nymphs between treatments in PI567301B (Table 2.4) nor was there any main effect of the treatments. The PI567301B has been documented to possess antibiosis and antixenosis type resistance against the biotype 2 (Mian et al. 2008). Although Asgrow 2703 demonstrated a decline in the aphid nymph numbers when pre-infested with biotype 2, the change is not statistically significant. The genotype K1639-2 showed little or no change in the biotype 2 nymph numbers when pre-infested with biotype 1 while pre-infestation with biotype 2 indicated significant treatment and genotype main effects (trt: DF=1; $\chi^2 = 6.32$; p=0.0119; ty: DF=2; $\chi^2 = 30.84$; p= <.0001). It also indicated a tendency towards induced susceptibility with an increase in biotype 2 aphid nymphs in the antibiosis test (5.3±1.49 and 8.1±2.77; P<0.0164 at $\alpha=0.05$) (Figure 2.4 and Table 2.3). It has been shown that K1639-2 is susceptible to biotype 2 of the soybean aphid (Chandran 2011) and the induction experiment suggests an increase in the susceptibility of K1639-2 upon infestation, thus resulting in favorable conditions for aphid survival and reproduction.

Although no overall severe damage was noticed in any of the treatments in the experiments, loss in chlorophyll content was visibly apparent where aphid numbers were high (as in those leaflets where aphids were restricted). These indicate possible local as well as systemic physiological changes in the plant resulting from aphid feeding. Physiological changes brought about by aphid feeding in different host plant-aphid systems have been studied and documented (Cabrera et al. 1995, Franzen et al. 2008, Pierson et al. 2011). The dearth of literature in the soybean-soybean aphid system on induced plant resistance and physiology studies offers scope for further research in this area.

Conclusions

This particular host plant resistance study on the induced response of some soybean genotypes to the soybean aphid biotypes 1 and 2 were conducted to understand how different genotypes might affect the soybean aphid (either favorably or detrimentally) when previously

infested by conspecifics. Mixed results were observed in the experiments where K1621 showed signs of induced resistance to biotype 1, the genotypes PI567301B, K1639-2, KS4202 and Asgrow2703 demonstrated no significant response to biotype 1 feeding. Similarly biotype 2 feeding produced differences in aphid number in the PI567301B (induced resistance) and K1639-2 (induced susceptibility) that were statistically significant. Induced response of host plants to pest feeding is an area of research with great potential to benefit the development of newer resistant varieties of crops. Further research and added knowledge of soybean responses to soybean aphid feeding will assist in better selection of genotypes for breeding purposes, evading those that could become susceptible to increased aphid attack upon initial infestation.

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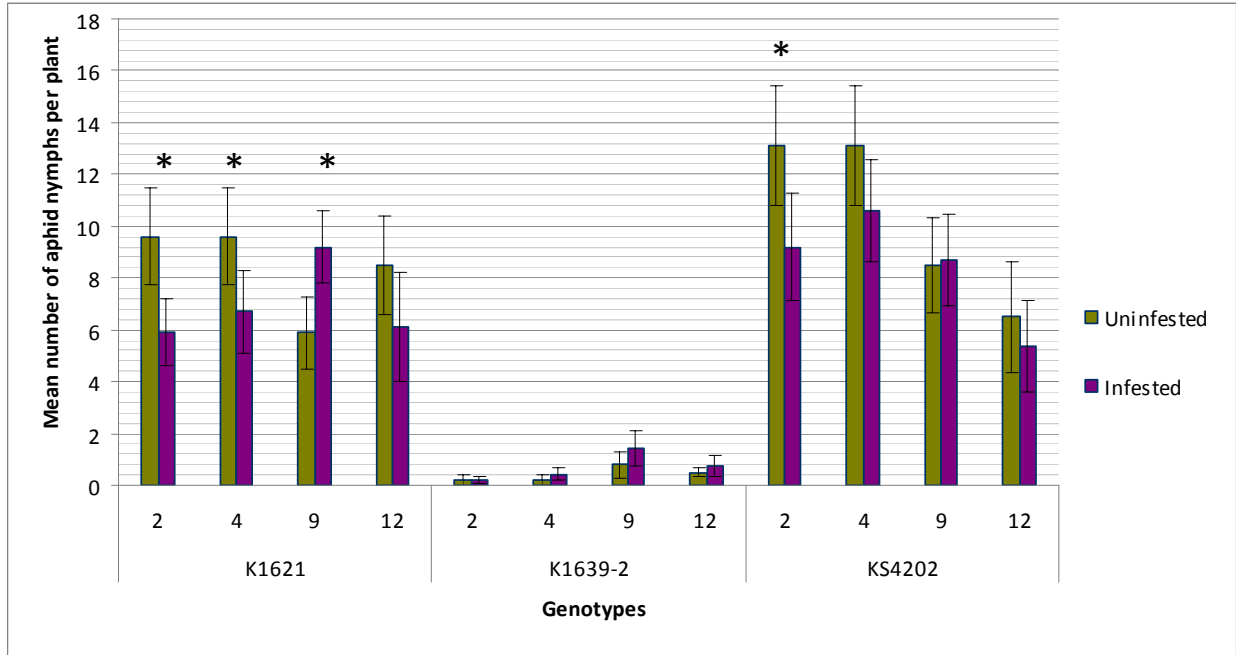
Figure 2.1. Experiment set up for Induced Response Tests



Figure 2.2. An experimental unit showing leaflets infested with aphids using zip-loc bags



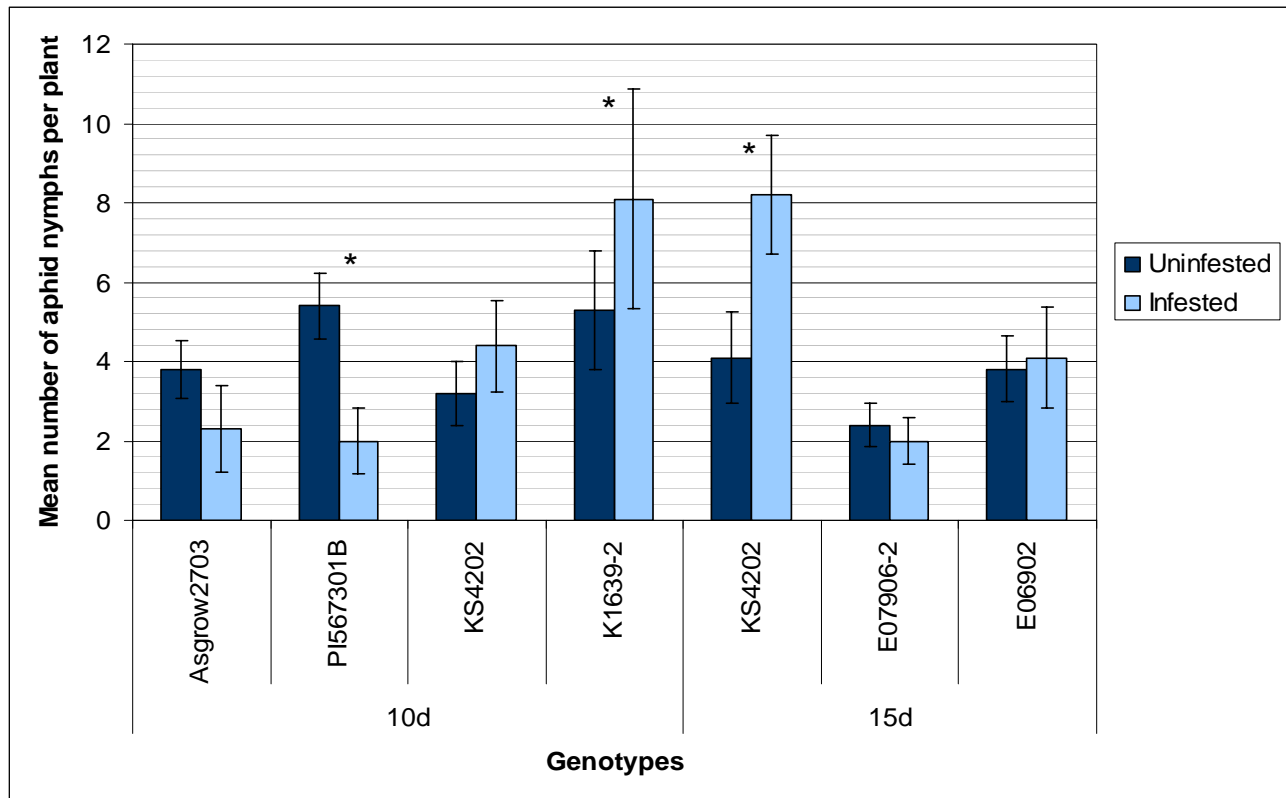
Figure 2.3. Mean \pm SE number of aphid nymphs (biotype 1) per plant in different genotypes infested for 2, 4, 9 and 12 days respectively with biotype 1



* indicates significant treatment x genotype effect for that particular combination of treatments in a genotype at $\alpha = 0.05$ in GENMOD analysis.

Note 1. The infestations were done on V-1 stage plants. Whole plants at the unifoliate leaf stage were infested with 10 aphids each, for 2, 4, 9 and 12 days respectively, following which Antibiosis test was conducted.

Figure 2.4. Mean \pm SE number of aphid nymphs (biotype 2) per plant in different genotypes infested for 10 and 15 days respectively with biotype 2



* indicates significant treatment x genotype effect for that particular combination of treatments in a genotype at $\alpha=0.05$ in GENMOD analysis.

Note 1. The 15d infestation was done on V-1 stage plants. Whole plants at the unifoliate leaf stage were infested with 25 aphids each, for 15 days following which Antibiosis test was conducted.

Note 2. The 10d infestation was done on V-2 stage plants. One leaflet in two trifoliate leaves was infested with 75-100 aphids for 10 days. Antibiosis was conducted in the adjacent leaflet in each of the trifoliate 24 hours after the infestation period.

Table 2.1. Genotypes of soybeans used for induction experiments and their level of resistance to the soybean aphid biotypes 1 and 2

Sl. No.	Genotype	Reaction to biotype 1	Reaction to biotype 2
1.	KS4202	S	S
2.	Asgrow2703	S	S
3.	K1639	R	S
4.	K1621	R	S
5.	E0 6902	R	R
6.	E0 7906-2	R	R
7.	PI 567301B	R	R

Table 2.2. Mean \pm SE number of aphid nymphs (biotype 1) per plant in different genotypes infested for 2, 4, 9 and 12 days respectively with biotype 1

Genotype	Length of Infestation (Days)	Uninfested	Infested	Significance at $\alpha= 0.05^*$
K1621	2	9.6 \pm 1.86	5.9 \pm 1.28	0.0033
	4	9.6 \pm 1.86	6.7 \pm 1.59	0.0239
	9	5.9 \pm 1.39	9.2 \pm 1.41	0.0077
	12	8.5 \pm 1.92	6.12 \pm 2.09	NS
K1639-2	2	0.22 \pm 0.22	0.22 \pm 0.15	NS
	4	0.22 \pm 0.22	0.44 \pm 0.24	NS
	9	0.8 \pm 0.51	1.4 \pm 0.68	NS
	12	0.5 \pm 0.18	0.75 \pm 0.41	NS
KS4202	2	13.1 \pm 2.32	9.2 \pm 2.08	0.0094
	4	13.1 \pm 2.32	10.6 \pm 1.94	NS
	9	8.5 \pm 1.83	8.7 \pm 1.79	NS
	12	6.5 \pm 2.15	5.37 \pm 1.77	NS

*indicates significant treatment x genotype effect for that particular combination of treatments and genotype at $\alpha= 0.05$ in GENMOD analysis.

Note. The infestations were done on V-1 stage plants. Whole plants at the unifoliate leaf stage were infested with 10 aphids each, for 2, 4, 9 and 12 days respectively, following which antibiosis test was conducted.

Table 2.3. Mean \pm SE number of aphid nymphs (biotype 2) per plant in different genotypes infested for 10 and 15 days respectively with biotype 2

Length of Infestation (Days)	Genotype	Uninfested	Infested	Significance at $\alpha= 0.05^*$
10	Asgrow2703	3.8 \pm 0.73	2.3 \pm 1.09	NS
	PI567301B	5.4 \pm 0.83	2.0 \pm 0.84	0.0001
	KS4202	3.2 \pm 0.8	4.4 \pm 1.15	NS
	K1639-2	5.3 \pm 1.49	8.1 \pm 2.77	0.0164
15	KS4202	4.1 \pm 1.15	8.2 \pm 1.5	0.0003
	E07906-2	2.4 \pm 0.54	2.0 \pm 0.58	NS
	E06902	3.8 \pm 0.83	4.1 \pm 1.28	NS

*indicates significant treatment x genotype effect for that particular combination of treatments and genotype at $\alpha= 0.05$ in GENMOD analysis.

Note 1. The 10d infestation was done on V-2 stage plants. One leaflet in two trifoliolate leaves was infested with 75-100 aphids for 10 days. Antibiosis was conducted in the adjacent leaflet in each of the trifoliolate 24 hours after the infestation period.

Note 2. The 15d infestation was done on V-1 stage plants. Whole plants at the unifoliolate leaf stage were infested with 25 aphids each, for 15 days following which antibiosis test was conducted.

Table 2.4. Mean \pm SE number of aphid nymphs (biotype 2) per plant in different genotypes infested for 10 days with biotype 1

Length of Infestation (Days)	Genotype	Uninfested	Infested	Significance at $\alpha=0.05^*$
10	Asgrow2703	3.9 \pm 0.90	3.5 \pm 0.86	NS
	PI567301B	2.4 \pm 0.61	2.6 \pm 0.66	NS
	KS4202	6.6 \pm 1.23	5.4 \pm 0.72	NS
	K1639-2	3.3 \pm 0.79	3.4 \pm 0.89	NS

*indicates significant treatment x genotype effect for that particular combination of treatments and genotype at $\alpha=0.05$ in GENMOD analysis.

Note. The 10d infestation was done on V-2 stage plants. One leaflet in two trifoliolate leaves was infested with 75-100 aphids for 10 days. Antibiosis was conducted in the adjacent leaflet in each of the trifoliolate 24 hours after the infestation period.

Chapter 3 - Effect of Induced Response on Soybean Aphid Feeding Behavior

Abstract

The soybean aphid, *Aphis glycines* is a serious pest of soybeans. It was first documented in North America in mid 2000 and owing to its composite lifecycle and habits has become a certain threat to soybean production. This Host Plant Resistance study was aimed at understanding the effect of previous infestation of soybeans on the soybean aphid (biotype 2) feeding behavior. The Electrical Penetration Graph (EPG) technique was carried out on PI567301B and Asgrow 2703 to elucidate if the induced response was tissue specific and thus identify the tissue level at which the resistance or susceptibility was evoked when pre-infested for 10 days. Although PI567301B showed signs of induced resistance resulting in reduced aphid reproduction, the results of EPG feeding parameters showed no appreciable differences in the feeding behavior of the aphids between clean and infested plants. Asgrow2703 also showed no changes in aphid feeding patterns upon infestation of the plants. Further work on additional genotypes may reveal interesting insights and information on feeding behavioral changes in soybean aphid as a consequence of previous infestation.

Introduction

Glycine max L. or soybean is the leading provider of protein and oil in the world and thus, an important economic crop. However, this crop has been afflicted with considerable yield damage and loss that can be attributed to its important pest, the soybean aphid, *Aphis glycines* Matsumura. Belonging to the Order Hemiptera, the soybean aphid has its origin in Eastern Asia and was first described by Matsumura in 1917 (Matsumura 1917). Since its arrival in North America in mid 2000, this invasive pest has spread across several states in the United States and few provinces in Canada with great rapidity within the span of a decade (Hartman et al. 2001). Being a highly adaptable invasive pest, the soybean aphid has also developed biotypes within a matter of a few years. Three biotypes of the soybean aphid have been documented from North

America – biotype 1 from Wisconsin in 2000 (Hartman et al. 2001), biotype 2 from Ohio in 2005 (Kim et al. 2008) and most recently, biotype 3 from Indiana in 2007 (Hill et al. 2010) wherein the newer biotypes have evolved to overcome the resistance factors presently helping to control soybean aphid populations. In other words, biotype 2 can colonize plants with the *Rag1* resistance gene (which confer resistance against biotype 1) (Kim et al. 2008) and biotype 3, those plants with *Rag2* gene (conferring resistance against biotype 2) (Hill et al. 2010).

With a heteroecious and holocyclic life cycle involving two host plants viz., common buckthorn, *Rhamnus spp.* as primary host (Voegtlin et al. 2004, 2005) and soybean as the secondary host, this piercing and sucking pest can significantly affect the quantity and quality of soybean yields from direct feeding damage (Wu et al. 1999, Mian et al. 2008, Beckendorf et al. 2008) as well as by vectoring various plant viruses (North Central Pest Alert Factsheet: Soybean Aphid, 2002). Until recently, chemical control was the only major control measure that was depended upon (Landis et al. 2003). But owing to its incompatibility with other IPM measures like cultural and biological control, host plant resistance (HPR) has gained prominence as an important Integrated Pest Management (IPM) tactic. Vast amount of research has been and is being undertaken to develop better soybean varieties that can control or withstand aphid invasions with little or no chemical intervention. Soybean IPM is projected to bring immense benefit in terms of revenue to US soybean growers and consumers, with an estimate of net benefits ranging between \$0.6 and \$2.6 billion dollars as of 2005 (Song and Swinton, 2009).

Host plant resistance (HPR) involves research that pertains to the screening and development of resistant plant varieties. Research on soybean aphid HPR in North America gained pace with Hill et al. (2004, 2004a) reporting new sources for soybean aphid resistance and with the identification of antibiosis and antixenosis type of resistances in some of those accessions. Several other resistant varieties of soybean have been reported which include sources of resistance genes such as *Rag*, *Rag1* and *Rag2* (Hill et al. 2006a, 2006b, 2009). Understanding the resistance mechanisms occurring in plants is fundamental to the process of developing better yielding resistant varieties of crops. Will et al. (2007) clearly demonstrated the role of aphid salivary secretion in the prevention of sieve tube plugging, a natural phloem wound response in the plant, which ensured the aphid unimpeded access to phloem. Feeding behavior related studies

have been undertaken in many pest-crop systems, especially in many aphid-crop systems using the Electrical Penetration Graph (EPG) technique. First developed and described by McLean and Kinsey (1964), electronic monitoring of feeding behavior provides insights into the complex but unobservable activities and interactions that occur in host plant tissues during the feeding of a piercing and sucking insect. Tjallingii (1978) described the direct current (DC) based Electrical Penetration Graph (EPG) which differed in its source of current (McLean and Kinsey's Electronic Monitoring System that used alternating current (AC)). Tjallingii (1978) described the varied patterns of electrical signals detected during aphid feeding behavior and their related aspects and Tjallingii and Esch (1993) recorded the finer structural details of the aphid stylet routes with plant tissues and documented their relation to the different EPG signals using Transmission Electron Microscopy (TEM).

Resistance in plants affecting the feeding behavior of the pest can be located using the EPG technique whereby the time spent on the different feeding phases provide an indication of the resistance or susceptibility of a plant to the feeding insect. It has been reported that phloem factors are major contributors towards aphid feeding-induced resistance (Prado and Tjallingii 2007). The EPG is, thus, a valuable tool aiding the furthering of our knowledge as regards to locating the resistance factors present in the plant tissues that hinder the feeding activity of the pest. There is a constant battle between plants and piercing-sucking pests where the latter are constantly seeking to overcome the allelochemicals and other plant metabolites that act as phagostimulants or deterrents, with aid from their salivary secretions in order to feed from the sieve tube elements (Tjallingii, 2006). The EPG technique facilitates investigation of how changes in host plant physiology from previous infestation by conspecifics or heterospecifics, mechanical damage, drought stress, etc. modifies the feeding behavior of a pest (Prado and Tjallingii 1997; Gonzalez et al. 2002; Sauge et al. 2002; Klingler et al. 2005; Ameline et al. 2007; Dugravot 2007; Prado and Tjallingii 2007) and help in the location of resistance or susceptibility factor present in the host plant tissues.

In this study, feeding behavior of the soybean aphid on previously infested vs. clean plants of Asgrow 2703 and PI567301B were recorded to follow up with the previous induced

response experiment and thereby elucidate what resistance factors were triggered at the tissue level, thus inducing resistance in PI567301B.

Materials and Methods

Aphid Culture and Soybean Genotypes. Biotype 2 was maintained on a continuous supply of susceptible soybean genotype KS4202 plants, within a growth chamber in the laboratory under temperature range of 22-24°C and with photoperiod 11:13 (L:D) (Percival[®] Model – 136LL, IA 50220). Biotype 2 was obtained in 2008 from Brian W. Diers, Dept. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801. Biotype 2 is on file at the Kansas State University Museum of Entomological and Prairie Arthropod Research and the voucher specimen number is 218. The genotypes that were tested for aphid feeding behavior study were PI567301B and Asgrow2703. The PI567301B was chosen as it showed possible induced antibiotic resistance upon pre-conditioning and testing with biotype 2 and Asgrow2703 was used as a susceptible check. All plants and aphids were frozen immediately following the completion of experiments, to prevent accidental escape and spread of the aphids.

Experimental Design and Recording of Electrical Penetration Graph.

The plants in each genotype were randomly assigned to one of the two treatments namely infested and uninfested or clean, arranged in a completely random fashion. At the V-2 stage (two fully emerged trifoliolate leaves), one leaflet each in each of the trifoliates of the plants assigned to the infested treatment was exposed to 75-100 soybean aphids of biotype 2. The movement of the aphids was restricted to within the selected leaflet using zip loc bags which were used to cover the leaflet entirely. The aphids were allowed to feed on the leaflet for a period of ten days, at the end of which, the plants were cleaned and allowed to stand one more day. Thus, after a total of 11 days (10+1), the plants were brought to the laboratory (Manhattan, KS) and Electrical Penetration Graph test was conducted under laboratory conditions (22-26°C, 35-45% RH with fluorescent ceiling mounted lamps for additional lighting) and aphid feeding behavior on both sets of plants (infested and clean) were recorded, 8 plants at a time (2 plants per treatment per entry). The aphids were allowed to feed on a leaflet adjacent to the one that was infested (in the

infested plants) and the feeding pattern was recorded for a period of 9 hours. Each treatment had 12-14 replications.

In this study, the more common and sensitive of the two existing EPG systems, DC-EPG system (Tjallingii, 2000) has been used to record aphid feeding behavior. The technique is to record the insect feeding behavior on a plant by making both the insect and the plant, part of an electrical circuit. This was accomplished as follows. The insect electrode consists of a copper nail, copper wire, very thin gold wire and the insect – all attached in the order mentioned. The copper wire was soldered to the copper nail. The insect, here an adult apterous soybean aphid of biotype 2, was carefully removed from the colony using a fine brush. The aphid was starved for one hour (Diaz-Montano et al. 2007) during which a thin gold wire (10-12 μ m diameter and 2-3cm length; from Johnson Matthey, Materials tech, U.K. Orchard Road, Royston, England) was glued to its dorsum using a tiny drop of highly conductive silver paint (SPI Supplies, P.O. Box 342, West Chester, PA)(Figure 3.2). The other end of the gold wire was glued to the copper wire (about 0.2mm thick and 1-2cm long) using the conductive silver paint. The plant electrode also consists of a copper wire (about 2mm in thickness and 10cm in length) which was inserted into the soil of the plant to be tested on (Tjallingii, 1988)(Figure 3.1). Both electrodes were plugged into a *Giga-8* model EPG amplifier with a $10^9 \Omega$ resistance (Wageningen Agricultural University, Wageningen, The Netherlands) and with provision to adjust any irregularities in voltage along the primary circuit. The whole set up was arranged within a Faraday cage to avoid interference from other electrical noises during the recording of the EPG signals. At the end of an hour, each aphid on the insect electrodes was placed on the upper side of a leaflet (which is adjacent to the infested leaflet in the trifoliate) and allowed to settle down and start feeding (Figure 3.1). The thin gold wire does not cause any hindrance to small movements of the aphids. Annan et al. (1997) concluded that it is cultivars and not wiring of aphids for purposes of EPG studies that affected aphid feeding behavior. The waveforms start to get recorded on the computer when the aphids insert their mouthparts into the plant (probe) thereby closing the circuit. The output signal voltage, amplified 50-100 times in the amplifier, is adjusted to be within a range of -5 and +5 V. The signals are such that when the stylets are intercellular in position, the signal voltage is positive and when intracellular, the signal voltage is negative (Tjallingii, 2006).

In this manner, 8 plants were tested at a time (2 replications from each treatment in each entry). The waveforms recorded over the 9-hr long period for each plant- aphid combination were analyzed using the software program, PROBE 3.0 (Windows).

Assessment of Feeding Parameters and Statistical Analyses

Stylet penetration by aphids and related hemiptera can be broadly categorized into pathway phase, phloem phase and xylem phase (Prado and Tjallingii, 1994) (Figure 3.3). The pathway phase (ABC) involves the intercellular probing of the plant tissues in search for the xylem or phloem cells with occasional intracellular puncturing of the cells with the stylets which are termed Potential Drops (PDs) (Tjallingii 2000). The phloem phase is further subdivided into E1 phase (salivation) and E2 phase (ingestion and salivation) (Prado and Tjallingii 1994). The xylem phase, G, represents water intake (Spiller 1986).

The parameters assessed in this study parallel those in previous studies by Diaz-Montano et al. (2007) and Chandran (2011). Among approximately 15 parameters that were recorded and analyzed, the observations of 12 of them have been drawn on to obtain an idea of the variation in feeding behavior of the biotype 2 aphids brought about by previous infestation in the two genotypes, Asgrow2703 and PI567301B. They include mean time taken to begin the pathway phase or first probe (f-P), mean time from the beginning of the recording to the Xylem Phase (f-XP), mean time from start of recording to the Sieve Element Phase (f-SEP), mean number of Potential Drops (n-PDs), Pathway Phases (n-PP), Xylem Phases (n-XP) and Sieve Element Phases (n-SEP) throughout the recording period of 9 hours, total duration or sum of duration of Pathway Phases (s-PP), Xylem Phases (s-XP), Sieve Element Phases (s-SEP) and Non-probing phases (s-NP). The percentage of time spent in the sieve elements feeding from phloem tissues since the first probe is also calculated (%SEP) as well as the number of aphids reaching the sieve elements during the 9 hour recording in each of the treatment-genotype combination is provided.

Since the feeding parameters were found not to be normally distributed with values ranging from zero or very small to very large as well values being excluded from analysis of certain parameters like f-SEP and %SEP, the comparison was made using Kruskal-Wallis test with $\alpha = 0.05$ and multiple comparisons between parameters of treatment x genotype

combinations was carried out with Tukey's studentized range test ($P < 0.05$). All analyses were made using SAS Program (SAS Institute 2003).

Results and Discussion

The aphid feeding behavior patterns offer a clear insight into the feeding activities of an aphid on its host plant. In this study, the soybean aphid (biotype 2) feeding behavior parameters were analyzed and tabulated for the clean and infested treatments of the genotypes PI567301B and Asgrow2703 (Table 3.1). These represent genotypes that are resistant and susceptible to biotype 2, respectively. They were chosen based on the previous induction experiments where PI567301B showed signs of induced antibiotic resistance while Asgrow2703 remained a susceptible check. However, from the data it was found that no feeding behavior parameter showed any statistical significance over its counterpart within either genotype when tested with Kruskal-Wallis test (at $\alpha = 0.05$) followed by Tukey's studentized range test ($P < 0.05$). This could be due to the sample sizes for each genotype x treatment combination being small (12-14) coupled with the variation in individual aphid responses. The mean time to first probe (f-P), first xylem phase (f-XP) were very similar within the two treatments in both genotypes as were the number of Potential Drops (n-PDs), Pathway Phases (n-PP), Xylem Phases (n-XP) and Sieve Element Phases (n-SEP) for both the genotypes (Table 3.1).

Despite there being no statistical significance in the mean time taken by the aphids to reach the sieve element for the first time from the start of the recording, we can notice a sizeable difference in time between that of Asgrow2703 clean and Asgrow2703 infested treatments (242.7 ± 96.7 and 326.64 ± 54.2) respectively. We could therefore assign biological significance to this feeding behavior parameter in Asgrow2703 and conclude that previous infestation increases the amount of time taken by the aphid to reach SEP. However, from the s-SEP and %SEP calculations, we notice that this delay does not reflect as a big difference in the total time spent in SEP and percentage of time spent in SEP between the two treatments of Asgrow2703. The infested PI567301B shows that the aphid spent 7.74 ± 4.18 minutes in SEP over all (s-SEP) compared to 0.25 ± 0.10 minutes on the clean plant which reflects in the slightly higher percentage of time spent in SEP by the aphid in infested PI567301B. This indicates improved

feeding conditions for the aphids upon infestation (signs of susceptibility) which can be further validated with increasing the sample size. Having shown signs of increased antibiotic resistance during induction experiments, this is an interesting outcome for PI567301B indicating that the resistance factors may not be present at the phloem level after all. It is known from previous studies that reduced SEP is a clean indicator of resistance factors being located at the sieve element level (Diaz-Montano et al. 2007; Crompton and Ode, 2010; Chandran, 2011). However, in the present study, the total sieve element phases (s-SEP) are in themselves quite small to be able to confirm resistance or induced resistance in the two genotypes with regard to feeding behavior as no prior EPG work has been done on them.

The mean total times spent in pathway and xylem phase is approximately same for both treatments of PI567301B while in Asgrow2703 the mean total pathway phase (s-PP) is longer by roughly thirty minutes and mean total xylem phase (s-XP) longer by about twenty minutes in the infested treatment compared to the clean (Table 3.1). Consequently, the total non-probing time (s-NP) is reduced proportionally in infested Asgrow2703 while s-NP is almost same in both treatments of PI567301B. The SEP being an important indicator of host plant acceptance, we find that the number of aphids that reached the sieve element phase in any of the treatment-genotype combinations (with 12-14 observations in each) ranged between 5 and 7 only.

While there have been reasonable numbers of EPG feeding behavior studies documenting induction of resistance or susceptibility in host plants against their aphid pests [Sauge et al. 2002 (*Myzus persicae*-peach system); Prado and Tjallingii 1997 (*Vicia faba*-*Aphis fabae* system) Klingler et al. 2005 (*Medicago truncatula* Gaert- *Acyrtosiphon kondoi* Shinji system); Gonzalez et al. 2002 (*Sorghum halepense* (L.) by *Sipha flava* (Forbes) system)], there have also been documentation of no response to previous infestation. One such study was by Prado and Tjallingii (1997) who reported that previous infestation of *Triticum aestivum* does not produce any significant changes in feeding behavior of *Rhopalosiphum padi* on the host plant.

A recent study by Pierson et al. (2011) documented the physiological changes brought about by soybean aphid feeding in certain genotypes of soybean including Asgrow2703. The study reported that Asgrow2703 showed significant changes in certain physiological parameters

like photosynthetic capacity, carbon-linked dark reactions and peroxidase activity after 28 days of pre-infestation. In the present study, the pre-infestation period was only for 10 days and it is possible that the genotypes require prolonged exposure to aphids in order to show appreciable changes which might in turn affect survival and/or feeding behavior of the aphid.

Conclusions

The Electrical Penetration Graph (EPG) technique was aimed at understanding the effect of previous infestation of soybeans on the soybean aphid (biotype 2) feeding behavior and was carried out on PI567301B and Asgrow 2703 to elucidate if there was any tissue specific induced response in these genotypes upon 10 days of pre-infestation. The results of EPG feeding parameters showed no appreciable differences in the feeding behavior of the aphids between clean and infested plants of both genotypes, although PI567301B showed signs of induced resistance resulting in reduced aphid reproduction. Further work with varying infestation levels and infestation periods on these as well as additional genotypes may reveal interesting insights and information on feeding behavioral changes in soybean aphid as a consequence of previous infestation.

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Figure 3.1. EPG set up showing plant electrode (V-2 stage plant) and insect electrode (wired aphid)



Figure 3.2. (a) Ventral view of an aphid showing its piercing and sucking mouthparts (Stylets)
(b) An aphid with gold wire glued to its dorsum.

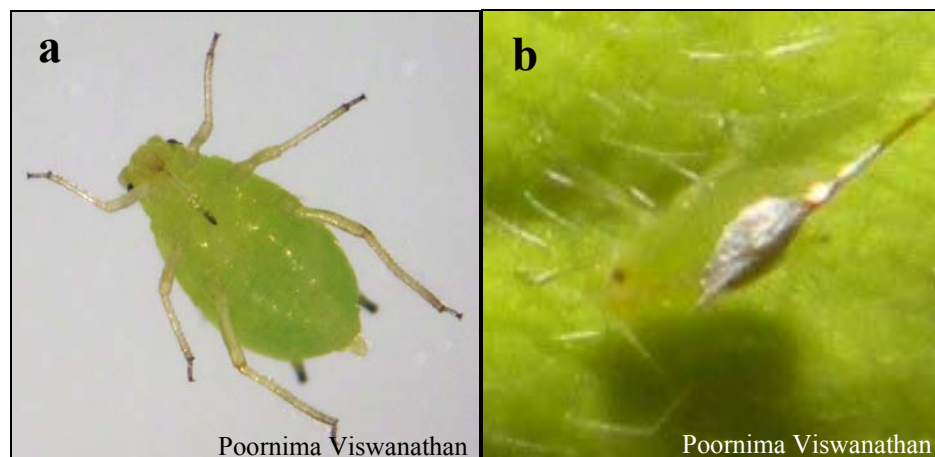


Figure 3.3. A one hour long Electrical Penetration Graph recording on an infested soybean genotype PI567301B depicting the waveform patterns corresponding to the different aphid feeding behaviors and phases.

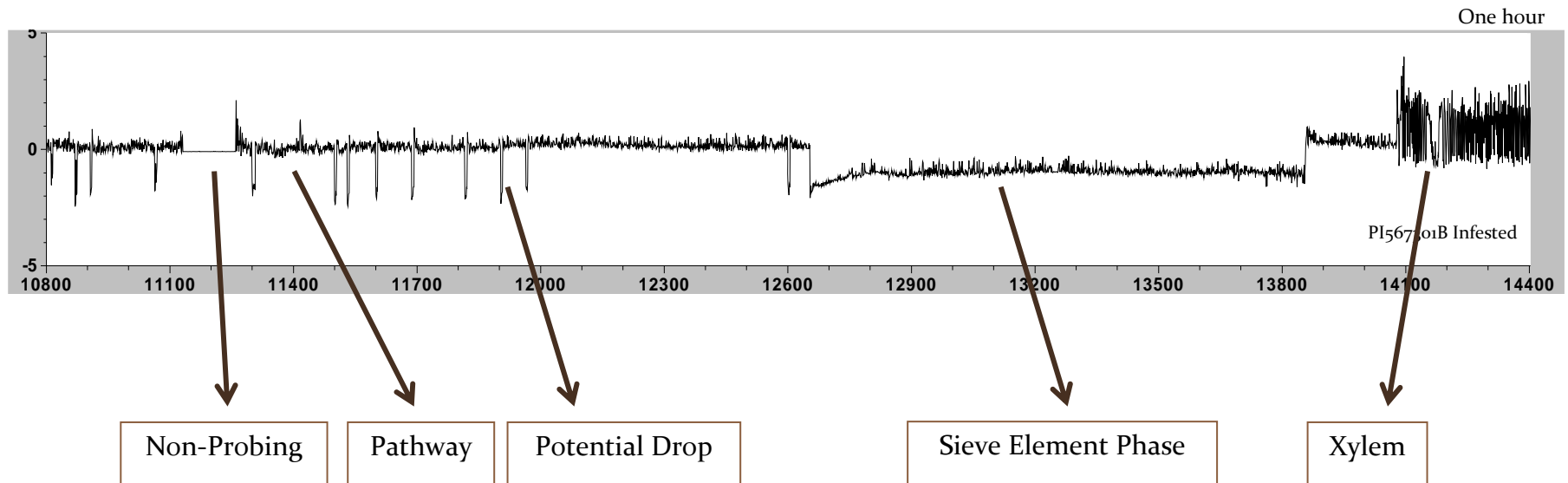


Table 3.1. Comparison of EPG feeding behavior parameters (Mean \pm SE) of biotype 2 on uninfested and previously infested (with biotype 2 aphids) Asgrow2703 and PI567301B over a period of 9 hours (540 minutes).

#	Feeding behavior parameter (minutes)	Asgrow2703 Uninfested (N=14)	Asgrow2703 Infested (N=14)	PI567301B Uninfested (N=13)	PI567301B Infested (N=12)
1	Time to First Probe (fP)	8.4 \pm 1.8	9.8 \pm 3.4	23.3 \pm 12.1	22.5 \pm 7.8
2	Time to First Xylem Phase (f-XP)*	276.5 \pm 48.42 (N=12)	256.8 \pm 41.4 (N=13)	213.3 \pm 33.7 (N=11)	243.8 \pm 42.5 (N=11)
3	Time to First Sieve Element Phase (f-SEP)**	242.7 \pm 96.7	326.64 \pm 54.2	221.7 \pm 49.3	212.0 \pm 47.2
4	No. of Potential Drops (n-PDs)	71 \pm 12.8	85.8 \pm 9.0	81.6 \pm 9.7	80.1 \pm 12.4
5	No. of Pathway Phases (n-PP)	92.7 \pm 15.3	111.4 \pm 9.1	112.1 \pm 12.8	101.5 \pm 13.8
6	No. of Xylem Phases (n-XP)	2 \pm 0.7	2 \pm 0.3	2 \pm 0.5	1.9 \pm 0.3
7	No. of Sieve Element Phases (n-SEP)	0.78 \pm 0.43	0.85 \pm 0.31	0.69 \pm 0.26	1.25 \pm 0.66
8	Total duration of Pathway Phase (s-PP)	192.6 \pm 15.2	225.7 \pm 25.3	224.1 \pm 20.3	212.9 \pm 16.6
9	Total duration of Xylem Phase (s-XP)	54.8 \pm 16.4	74.1 \pm 16.5	53.7 \pm 13.6	56.3 \pm 12.7
10	Total duration of Sieve Element Phase (s-SEP)	0.19 \pm 0.09	1.56 \pm 1.04	0.25 \pm 0.10	7.74 \pm 4.18
11	Total duration of Non-Probing (s-NP)	265.2 \pm 23.4	220.5 \pm 22.7	238.5 \pm 17.5	239.5 \pm 17.8
12	Percentage of available time spent in Sieve Element Phase (%SEP)**	0.098 \pm 0.03	0.58 \pm 0.36	0.10 \pm 0.02	2.86 \pm 1.34
13	No. of aphids that reached Sieve Element Phase	5	7	6	6

Note 1: Kruskal-Wallis test ($\alpha=0.05$) and Tukey's test for multiple comparisons ($P<0.05$) within treatments were carried out as well as Genmod analysis ($P<0.05$) was done for treatment x type interaction effects.

Note 2: Time is calculated in minutes.

*Replicates with no value for the parameter were excluded from the analyses.

** N corresponds to values in parameter 13.