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Physiological and behavioural response of the soybean aphid (Aphis glycines Matsumura) to susceptible and resistant soybean

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PHYSIOLOGICAL AND BEHAVIOURAL RESPONSE OF THE SOYBEAN APHID (*APHIS GLYCINES* MATSUMURA) TO SUSCEPTIBLE AND RESISTANT SOYBEAN

by

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ABSTRACT

The soybean aphid, *Aphis glycines* Matsumura, is an invasive pest which causes damage and yield loss to soybean plants in North America. One method of soybean aphid control involves the use of soybean varieties that negatively impact aphid physiology and behaviour. Mean aphid fecundity measured at different stages of soybean growth showed differences between resistant and susceptible plant varieties as well as differences between the growth stages. Mean aphid longevity on susceptible and resistant plants at different growth stages showed a similar trend. Movement from resistant plants was higher than movement from susceptible plants amongst 1^{st}-2^{nd} instars, apterous adults, and adult alates. Aphids left host plants without the knowledge of an alternative host plant indicating random dispersal. Adult apterous aphids were repelled by resistant plant leaves when given a choice between odour free air and resistant plant odours in a Y-tube olfactometer.

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General Introduction

In 2001, Ontario's soybean growers experienced a substantial yield loss in soybean, *Glycine max* (L.) Merr. due to the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). This was the first year that this invasive pest, native to Asia, was recognized in Ontario (Hunt et al. 2003). Costs associated with insecticide use for soybean aphid control are significant, estimated at $5 to $24 per acre with approximately 15 million acres sprayed in the United States in 2005 (Ragsdale et al. 2006). Other stressors of soybean include the japanese beatle and the bean leaf beatle; however, these pests do not decrease yield to the extent that the soybean aphid does. Aphids can be found in the thousands on a single plant. The economic threshold for soybean aphid control is 273 ± 38 aphids per plant, afterwhich higher populations result in reduced yield and reduced market values (Ragsdale et al. 2007). Planting resistant soybean cultivars against the soybean aphid is a potential control strategy that is currently under investigation as previous studies have demonstrated that some soybean varieties have negative effects on the biology and host preferences of the soybean aphid (Diaz-Montano et al. 2006; Hill et al. 2004; Li et al. 2004; Mensah et al. 2005). The objective of this thesis was to investigate physiological and behavioural responses of soybean aphid to potential resistant soybean cultivars with the goal of assisting soybean aphid control.

Invasive Species

Invasive species are species that have populations which have a demonstrable ecological or economic impact (Lockwood et al. 2007). Many species are introduced into

novel areas accidentally through the transportation of goods; however, intentional introductions also occur for environmental enhancement, farming, biocontrol, conservation and research (Lockwood et al. 2007). Some species that are newly introduced will die before they are noticed. Others, especially with multiple introductions, will become rampant. Characteristics of successful invasive species may include high-intrinsic growth rates, self-fertilization, phenotypic plasticity, competitive ability, and high dispersal rates (Sakai et al. 2001). These are all generalizations and no one particular trait is necessary for the establishment of an invasive species. Rather, the traits associated with a newly introduced species, the traits of the species in the area, and the characteristics of the area itself, all lead to the success or lack of establishment of an introduced species (Sakai et al. 2001). The costs of invasive species due to their damage and associated control can be extensive, such as in Texas where the cost due to fire ant damage is estimated to be $300 million per year (Pimentel et al. 2005).

Aphids have many characteristics that make them good candidates for becoming invasive species. They have high reproductive rates, short development times, reproduce asexually, and are good dispersers. Aphids have decreased the time required for reproduction by removing mating from a portion, or all, of their life cycle (Powell et al. 2006). In the soybean aphid, mating occurs for the production of overwintering eggs, which are produced on the primary host Buckthorn, *Rhamnus spp.* (Rhamnaceae), and is absent throughout the summer season (Ragsdale et al. 2004). Buckthorn is mostly abundant in the North Central United States north of the 41st parallel (Ragsdale et al. 2004). Although not abundant in Ontario, Buckthorn was reported with soybean aphid eggs in Guelph (Welsman et al. 2007). Development times have also been decreased by

parthenogenesis, a process by which asexual reproduction allows nymphs to start developing young before they themselves have been born (Powell et al. 2006). When conducting research in the field, I observed that newly born soybean aphid nymphs became adults and were able to start producing offspring within 5 days. Adult aphids can produce up to 12 nymphs per day (Ragsdale et al. 2006) and have 18 generations per year (Wang et al. 1962). The soybean aphid has 4 instars which are identifiable by the shape of the cauda and the number of antennal segments. Adult aphids have two forms, winged (alates) and non-winged (apterae). The energy requirements for the production of alates are higher than that of apterae. To avoid unnecessary energy expenditure, the production of alates is induced by crowding and for migration to Buckthorn (Powell et al. 2006). All of these traits aid in the rapid population increase and dispersal of the soybean aphid. In 2000, the first report of the soybean aphid in the United States was received from Wisconsin (Alleman et al. 2002). Before the end of the summer season, the soybean aphid had been reported to be in 10 states (Venette 2004). By 2003, reports had expanded to include 21 U.S. states and 3 Canadian provinces (Venette 2004).

Plant Resistance

There are three categories of mechanisms by which plants protect themselves from insect damage: antibiosis, antixenosis and tolerance (Smith, 2005). Antibiosis resistance occurs when a plant adversely affects the physiology of an arthropod that is trying to use the plant as a host, either by chemical or morphological plant defenses. Antixenosis resistance occurs when a plant affects the behaviour of an insect, such as causing an insect to select an alternative host. Tolerance is a plant trait which allows the

plant to withstand or recover quickly from arthropod damage. These categories are not exclusive; plants may have any combination of these defense mechanisms. The phenotypic expression of these mechanisms has been linked to specific genes in resistant plants that are not present in non-resistant plants (Smith, 2005). The Rag 1 gene, which is responsible for plant resistance to the soybean aphid in the cultivar Dowling, is expressed as both antibiosis and antixenosis resistance (Hesler et al. 2007; Hill et al. 2006).

The specific mechanism of resistance in plants can take the form of morphological enhancements such as cuticular waxes, tissue thickness or the number of trichomes (plant hairs) (Smith 2005). Simple trichomes can interfere with an arthropods ability to walk or feed on the plant, whereas hooked or glandular trichomes may trap or impale an arthropod's body. Cuticular waxes may make adhesion to the plant surface difficult or deter feeding. Some plants add a protective layer within their tissue which prevents insects with penetrating mouthparts from reaching the desired site of nutrition. As well as morphological features, chemical defense can also be employed as a means of plant resistance (Smith, 2005). Defensive volatiles emitted from the plant repel herbivores. Defensive toxins within plant tissues can deter herbivore feeding, inhibit growth, and kill arthropods.

Plant resistance may reduce or eliminate the need for farmers to spray pesticides for controlling pest species. Although there are benefits to using plant resistance, there may also be some risks. Plant resistance can both aid and debilitate biological control through positive or negative impacts on predators and parasitoids. The potential risks to natural enemies of pest species that feed on resistant plants have been pointed out by

several authors (Bottrell et al. 1998; Schuler et al. 1999). It is important to study the effects of altered plant attributes on both pest and beneficial insects (Cortesero et al. 2000). Plant resistance may affect a herbivore's development time, fecundity, survival, and behaviour. Effects on herbivores that feed on resistant plant material may carry over to the natural enemies of those herbivores. As well, plant physical characteristics that inhibit arthropod feeding may also inhibit natural enemies. For example, plant trichomes may reduce the searching efficiency of predators and parasitoids (Bottrell et al. 1998).

There is a co-evolutionary cycle between plant resistance and arthropod adaptation to plant resistance. Genotypic plasticity is a necessary evolutionary trait that allows plants to adapt to arthropod damage, disease, and environmental pressures. Utilizing plant resistance has become a method for herbivore control within the agricultural industry. It is recommended that when plant resistance is used as a tactic for herbivore control that it is kept at a moderate level. It is generally expected that strong resistance will speed the rate of adaptation to resistance in herbivores compared to moderate resistance, even with the presence of natural enemies (Gould et al. 1991). Additionally, the expression of resistance may vary due to plant characteristics such as plant tissue age, arthropod characteristics such as gender and infestation level, and environmental conditions, such as soil nutrients and the amount of available light (Smith 2005). Moderate resistance against arthropods is also suggested to maintain the presence of natural enemies. Natural enemies which are unrewarded when they search through plants with few pests may not return. Females of the parasitic wasp *Leptopilina heterotoma* (Thomson) (Hymenoptera: Eucoilidae) preferred a novel microhabitat type after being unrewarded in a former microhabitat type (Papaj et al. 1994).

The co-evolution of plant resistance and insect counter-resistance is an ongoing cycle of which plant breeders, the agriculture industry and entomologists are aware. In an attempt to slow the process of counter-resistance in pest species, evolutionary biologists are working on producing strategies to decrease the rate of counter-resistance. One strategy that has been developed is the high-dose/refuge strategy (Rausher, 2001). This strategy involves planting a mix of susceptible and highly toxic resistant plants so that breeding amongst pests will keep the counter-resistance gene recessive. This strategy is not feasible for haploid pests such as bacteria and viruses, or insects, such as aphids, which undergo asexual reproduction.

There is a general assumption that the energy put into plant resistance is at a cost to the energy that would otherwise be put towards functions such as plant growth and reproduction (Bergelson and Purrington 1996). The presence of increased trichome density (morphological defense) and production of glucosinolate (chemical defense) in the annual plant *Arabidopsis thaliana* decreased herbivory damage but resulted in a fitness cost (Mauricio 1997). Although plant resistance often incurs fitness costs, this is not always the case (Bazzaz et al. 1987). Resistance to the flea beatle *Chaetocnema confinis* Crotch (Coleoptera: Chrysomelidae) did not involve fitness costs in the annual morning glory *Ipomoea purpurea* Roth (Convolvulaceae) (Simms and Rausher 1987). The allocation of resources is dependent on the individual plant as well as the plant species, the amount of resources available to a plant, the environment and the amount of photosynthesis taking place (Bazzaz et al. 1987).

Soybean is an annual plant that begins as a seed which germinates in the soil and then proceeds through vegetative and reproductive stages, as described by Fehr et al.,

1971. After seed germination, new growth pushes its way to the soil surface, referred to as emergence (VE). Exposure of young leaves from unfolding cotyledons denotes the cotyledon stage (VC). After this stage, vegetative growth is characterized by the uppermost leaf node which has leaves on the node above it that have unrolled so that the edges of the leaves are no longer touching (Anon 1988). For example, if the fourth leaf node has young leaves that have unrolled so that their edges are no longer touching, the plant is in the 3rd-node or V3 stage of soybean development. When a plant begins reproductive growth, reproductive stages are usually used to identify the stage of development. The first reproductive stage of soybean development is the beginning bloom (R1) stage, characterized by the presence of one or more open flowers on the plant. When a plant has an open bloom on one of the two uppermost nodes, it is in the full bloom (R2) stage (Anon 1988). The beginning pod (R3) stage begins when a 5 mm pea pod can be found on one of the four uppermost nodes with a fully developed leaf (Anon 1988). When one of these pea pods grows to 2 cm, the full pod (R4) stage has been reached (Anon 1988). In the beginning pod (R5) stage, seed within a pea pod on one of the four uppermost nodes with a fully developed leaf is 3 mm long (Anon 1988). A green seed within one of these pods indicates development at the full seed (R6) stage. When at least one pea pod on the main stem turns brown or tan in colour the plant is in the beginning maturity (R7) stage and when 95% of the pods on a soybean plant are their mature colour, the plant is in the last stage of reproductive growth, the full maturity (R8) stage (Anon 1988).

There are many reasons why soybean resistance may vary at different stages of soybean growth. One reason is that the energy requirements for plant growth and

reproduction may take away from the energy that would otherwise be used for resistance. I hypothesize that there may be differences in the levels of resistance at different soybean growth stages due to the energy costs of resistance expression and that these differences may affect soybean aphid fecundity and mortality. The first trifoliate (V2), beginning bloom (R1), beginning seed (R3), and full seed (R6) stages of soybean growth were selected for this study as I expect there to be differences in fecundity and mortality between these growth stages. I predict that the most critical stages for reproductive growth will be the beginning bloom (R1) and the beginning seed (R3) stages and that a plant should invest more energy for resistance at these stages. Thus, aphid fecundity will be lower in the beginning bloom (R1) and beginning seed (R3) stages than the first trifoliate (V2) or full seed (R6) stages. I also predict that the energy expenditure for resistance will be greater in the first trifoliate (V2) stage than the full seed stage (R6) since the full seed stage is more tolerant to damage and damage at this stage has little affect on yield. Thus, the first trifoliate (V2) stage should support lower aphid fecundity than the full seed (R6) stage of soybean development.

Host Plant Selection

Host plant selection is a process by which insects use sensory mechanisms to locate and accept or reject potential host plants. This process includes finding the most suitable host within the appropriate plant species (Bernays and Chapman 1994). Host selection by aphids has been characterized into six stages: pre-alighting behaviour, initial plant contact and assessment of surface cues before stylet insertion, probing the epidermis, stylet pathway activity, sieve element puncture and salivation, and phloem

acceptance and sustained ingestion (Powell et al. 2006). At any point in the selection process, an aphid may choose to leave an unsuitable host in search of a suitable host.

The behaviour of insects attracted by an odour plume is described by Bernays and Chapman (1994). Once an insect detects odour from a host plant, the insect must be able to orient and proceed towards the odour source. Pockets of odour are released into the air by a host plant and are directed by wind. The breadth of the odour plume becomes larger as it travels farther from its source. As well, concentration of the plant odour decreases as the odour plume travels farther from the plant. Insects are able to orient towards the odour source by facing against the wind which is carrying the stimulus. By moving in and out of the odour plume, insects can follow a decreasing breadth and increasing concentration of plant odours towards the host plant.

Aphids are weak fliers and can only make headway in low wind speeds (Kennedy and Thomas 1974). For this reason, skepticism has arisen as to whether or not aphids can direct their flight towards host plants even if host plant odours can be detected. This line of thought is supported by the fact that very small percentages of dispersed aphids locate host plants. In a study by Ward et al. (1998), less than 1% of the autumn migrants of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), located their primary host plant, bird cherry trees, *Prunus padus* L. There is evidence, however, that aphids have the ability to locate host plants. Aphids are visually attracted to the green colour domain (Döring and Chittka 2007) and are attracted to host plant odours (Visser 1986).

It was observed by Kennedy et al. (1959) that host selection is determined by aphid settling as opposed to aphid landing, as aphids are just as likely to land on non-host plants than host plants. Aphid landing involves an aphid making contact with the leaf

surface and may involve the aphid leaving the plant soon afterwards, whereas aphid settling involves an aphid staying on the plant surface after the ingestion of phloem. Once an insect has made contact with a potential host plant, the insect conducts a series of behaviours where chemosensory and gustatory cues are encountered. The behaviours include exploring the leaf surface, antennation, probing of the stylet into plant tissue and ingestion of plant sap (Chapman and Bernays 1989; Powell et al. 2006). The host plant selection process ends with phloem acceptance and feeding (Powell et al. 2006).

There are many reasons that aphids leave host plants. A common behaviour amongst aphids is dropping from a host plant. This behaviour occurs in response to predators, alarm pheromone, or disturbance (Gish and Inbar 2006; Losey and Denno 1998; Montgomery and Nault 1978; Roitberg 1979). Random movement of aphids between plants has also been observed (Hodgson 1991). No matter what the reason for leaving a host plant, the decision to leave a host plant is dependent on many factors. Aphids are more likely to leave a host plant in moist cool conditions than in hot dry conditions (Dill et al 1990), likely due to high risk of mortality due to desiccation during hot dry conditions. The age class of an aphid may also dictate the likelihood of abandoning a host plant. Adult and late instars aphids are more likely to disperse from a host plant than immature aphids (Gish and Inbar 2006; Hodgson 1991; Roitberg et al. 1979). As well, aphids disperse more from poor quality host plants than good quality host plants (Dill et al 1990; Hodgson 1991; Losey and Denno 1998). I hypothesize that if soybean aphid make choices between host plants, that they would choose a good quality host (a susceptible plant) over a poor quality host (a resistant plant). Furthermore, I

hypothesize that the soybean aphid would abandon resistant plants by walking or dropping in favour of movement to susceptible plants.

Host Plant Volatiles

Host plant volatiles are chemicals emitted by a plant, usually with the escape of water vapour, when plant stomata (pores) are open (Bernays and Chapman 1994) or with plant damage. When herbivores begin to feed, plants can emit volatiles which are attractive to the natural enemies of those herbivores (Dicke et al. 1990) or to the herbivores themselves. Artificial plant volatiles have been used to attract predators and parasitoids of plant herbivores. Synthetic methyl salicylate, (Z)-3-hexenyl acetate, and (E)-4,8-dimethyl-1,3,7-nonatriene attracted a variety of predators and parasitoids in a field study by James (2003). Soybeans infested with the soybean aphid were found to emit the plant volatile methyl salicylate (Zhu and Park, 2005). In this same study, traps containing the volatile methyl salicylate were found to be highly attractive to the soybean aphid predator, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Using synthetic volatiles as a method of managing herbivore populations has been considered by several authors (Chapman et al. 1981; James 2003). However, the constant presence of synthetic plant volatiles, instead of the presence of natural plant volatiles released by herbivore feeding, may reduce the occurrence of predators or parasitoids due to a lack of reward if herbivores are not present (Bottrell et al. 1998).

There are several methods used to test olfaction in insects. The electroantennograph (EAG) tests the ability of an insect's antenna to detect odours. This test involves inserting an electrode into each end of an antenna. The detection of an

odour stimulus is then recorded by the change in action potential (Bernays and Chapman 1994). An insect's ability to detect an odour does not infer an insect's behavioural reaction to that odour. The olfactometer tests an insect's behavioural reaction to an odour stimulus. Movement towards an odour stimulus shows an insect's attraction to the odour (Bernays and Chapman 1994). Movement away from an odour stimulus demonstrates repulsion (Bernays and Chapman 1994). Of course, there may not be any behavioural reaction to the odour stimulus.

There are different types of olfactometer tests. The "Pettersson" olfactometer involves four odour zones for aphids to choose from (Pettersson and Stephansson 1991). Tests using either a Y-tube or a T junction are examples of linear track olfactometers. In both of these tests, aphids walk in a line until they are required to choose one of two odour sources which extend in opposite directions. Wind tunnel experiments are used to determine if an insect will orient upwind and move towards an odour stimulus (odour-conditioned anemotaxis). Odour conditioned anemotaxis was observed by apterous adults of the aphid *Cryptomyzus korschelti* Börner (Hemiptera: Aphididae) in a wind tunnel experiment conducted by Visser and Taanman (1987). Olfactometer tests conducted on the soybean aphid have demonstrated that the soybean aphid is attracted to soybean and that non-host odours can mask the attractive volatiles emitted by soybean (Du et al. 1994) but that is the current state of knowledge regarding soybean aphid olfaction. Since the soybean aphid has a preference for susceptible soybean over resistant soybean, I hypothesize that soybean aphid can detect the difference between resistant and susceptible soybean cultivars based on their odour plume and that the preferred attraction to susceptible soybean may be due to repulsive odours or the lack of attractive odours

from resistant plants. Thus, I predict that in y-tube choice tests, soybean aphids would choose susceptible soybean odours over resistant soybean odours.

Summary

Aphids are widespread pests that threaten agricultural crops. As aphids disperse into novel areas, damage incurred by crops and costs associated with aphid control will increase. It is important that research is conducted to provide a greater understanding of how to prevent the spread of aphids into novel areas and how to keep them at a manageable level. Plant resistance is just one method proposed to prevent heavy infestation, plant damage and aphid dispersal; however, other strategies exist and all strategies should be considered to provide a plan of action.

In the second chapter, I test the hypothesis that resistance varies throughout the different stages of soybean development. I predict that the beginning bloom (R1) and beginning seed (R3) stages will have the highest level of resistance and hence the lowest aphid fecundity, that the first trifoliate (V2) stage will have an intermediate level of resistance, and that the full seed (R6) stage will have the lowest level of resistance and hence the highest aphid fecundity. To test this hypothesis, I measured soybean aphid fecundity on susceptible and resistant soybean plants in the first trifoliate, beginning bloom, beginning pod, and full seed stages of soybean development. In the third chapter, I test the hypothesis that soybean aphids can assess and choose hosts, and that they exhibit this choice based on movement of the aphid between hosts. Thus, I predict that soybean aphids would abandon (walk or drop from) a poor quality host (resistant soybean) in favour of a good quality host (susceptible soybean). This hypothesis was

tested by placing aphids on either a resistant or susceptible soybean plant and allowing 24 h for travel to an alternative host plant. In the fourth chapter, I test the hypothesis that soybean aphid can detect suitable hosts by their odour. I predict that soybean aphid is repelled by resistant soybean odours. To test this hypothesis, aphids were given a choice between two odour sources in a dual choice Y-tube olfactometer. Odour preference was selected between resistant plant leaves versus odour-free air, resistant plant leaves versus susceptible plant leaves, and susceptible plant leaves versus odour-free air.

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Chapter 2

Variation in resistance across different growth phases of soybean aphid resistant soybean as measured by aphid fecundity

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is responsible for significant economic loss in soybean yield and quality in Canada and in the United States. First reports of the soybean aphid in the United States began with Wisconsin in 2000 (Alleman et al. 2002) followed by Michigan, Indiana, Illinois, Missouri, Iowa, Ohio, West Virginia, Kentucky, and Minnesota in the same year (Venette and Ragsdale 2004). In 2001, the spread of this invasive pest reached Canada (Hunt et al. 2003). Aphid feeding can transmit plant viruses such as the soybean mosaic virus (Hill et al. 2001) and can also cause additional plant damage such as stunting and reduced vigor from the loss of nutrients (Gill and Sanderson 1998). Significant yield loss due to reduced pod set can occur when aphid population numbers are overwhelming during the reproductive stages of soybean development (Sun et al. 1990). In China, a yield loss in excess of 50% resulted when infestation occurred on plants in the early vegetative stage of growth (Wang et al. 1994).

Growing plants resistant to soybean aphid has been suggested as a means of controlling aphid populations and maintaining soybean yield in commercial production. Plant resistance, which results in lower aphid populations on resistant than susceptible plants, may delay aphid doubling times and increase the time required for the aphid population to reach economic injury levels (Ragsdale et al. 2007). The benefits of using

plant resistance also include maintaining natural methods of controlling pest populations as these resistant cultivars have little impact on beneficials or non-pest species. Plant resistance to soybean aphid has been identified as the expression of a single gene in Dowling (Rag 1) (Hill et al. 2006a) and a single gene in Jackson (Rag) (Hill et al. 2006b). Resistance was demonstrated in non-choice tests on Dowling, Jackson, and Palmetto (Hill et al. 2004). Palmetto is a parent cultivar of Jackson (Hill et al. 2004). The specific mechanism of resistance to the soybean aphid takes many forms including increased aphid mortality (Hill et al. 2004; Li et al. 2004) and decreased fecundity (Diaz-Montano et al. 2006; Hill et al. 2004; Yan et al. 2004). Population fluctuations of the soybean aphid during particular stages of plant growth have been noted in several studies (Beckendorf et al. 2008; Ragsdale et al. 2007). As well, the expression of plant resistance can vary according to the age of a plant or the age of plant tissues (Smith 2005). It is probable that plant compensation for aphid feeding affects resistance levels at varying stages of soybean development. Not all factors affecting the degree of resistance in soybean are known.

Researchers have acknowledged that resistance occurs throughout plant growth (Hill et al. 2004); however it is not known how resistance changes through the stages of growth for resistant plant cultivars. Depending on the soybean growth stage, considerable energy is directed towards plant growth, and in this study, I hypothesized that differential resistance expression between stages may occur due to the differences in energy required for plant development or seed formation. The stages of soybean development chosen in this study, described by Fehr et al., 1971, include the first trifoliate (V2), beginning bloom (R1), beginning pod (R3), and full seed (R6) stages. I

predicted that the highest levels of resistance would occur at the most critical stages for reproductive success of a soybean plant and that at these stages higher resistance would result in lower aphid fecundity. The highest resistance levels should occur at the beginning bloom (R1) and the beginning seed (R3) stages of growth followed by the first trifoliate (V2) stage and then the more tolerant of the chosen stages to plant damage, the full seed (R6) stage. If the energy requirements for growth in the beginning bloom (R1) and beginning seed (R3) stages takes away from the energy required for resistance, then aphid fecundity should be highest in these stages. A decrease in resistance at these stages may result in aphid numbers reaching the economic threshold of 250 aphids per plant (NCSRP 2007) resulting in a decrease in yield. Thus, the objective of my study was to measure the fecundity of aphids individually caged on resistant and susceptible cultivars during different growth stages. Aphid fecundity and the number of days an aphid lived was tested as I believed this to be the best measure for indicating the differences between the growth of aphid populations between the resistant and susceptible cultivars.

Materials & Methods

Trials were conducted in two separate fields in 2007 at the Greenhouse and Processing Crops Research Centre (Agriculture and Agri-Food Canada) in Harrow, ON. 'J' field contained three trial plots with one plot per tested plant variety. The plant varieties tested in 'J' field included a Dowling cross containing the Rag 1 resistant gene (Hill et al. 2006a), Palmetto, an ancestral variety of Jackson containing the resistance gene (Rag), and Harovinton used as a susceptible control. The resistance gene in Jackson (Rag) has the same antibiosis-type resistance as Dowling and has been mapped to a

similar location (Hill et al. 2006b; Li et al. 2007). In a study by Hill et al. (2004), aphid populations were similar on Palmetto and Jackson and were lower than the tested susceptible cultivars. The Dowling cross used as one of the resistant varieties in 'J' field was produced by Vaino Poysa (AAFC-GPCRC) and Elroy Cober (AAFC-ECORC). The second field, 'S' field, also contained three trial plots with one plot per tested variety. One of the plant varieties tested included a variety provided by Michigan State University which showed resistance in choice and no-choice tests (Mensah et al. 2005). This variety was given the name 'MSU' for this study. A second variety tested in 'S' field was provided by Brian Diers from the University of Illinois, the variety is from the Dowling background and contains the Rag 1 resistance gene (Hill et al. 2006a). This variety was given the name 'Dowling, IL' for this study. Again, Harovinton was tested in a third plot to be used as a susceptible control variety.

Soybean aphids were collected from a colony that was formed in 2004 using several field aphids. The colony was maintained within a growth cabinet with a 26°C (day) to 24°C (night) and 16L:8D photoperiod. Aphids were reared on the soybean variety Mycogen 5261. Colony plants were hand watered daily and fertilized once weekly with soluble NPK fertilizer. It is estimated that each pot was provided with 250 to 500 mL of the fertilizer solution.

To test for a difference in resistance expression throughout the various growth stages, an antibiosis (non-choice) test was conducted. Four days prior to the commencement of each trial, 3rd to 4th instar aphids from the colony were placed in nursery cages on susceptible plants within a growth cabinet (16L:8D and 26°C(day):24°C(night)). The base of the nursery cages consisted of a 5 cm hard plastic

disc with foam insole glued onto the top to provide protection for the leaflet. The base was placed on the top of a trifoliate leaf. The top of the nursery cages were made from clear plastic Falcon™ petri dish lids (Model 3001, 4cm diameter by 5mm deep). The lids had a 2.5 cm diameter circle removed which was covered with a 100μm screen. The top of the nursery cage was placed on the underside of a trifoliate leaflet, the base of the nursery cage was placed on the top of the same leaflet, and the two pieces were secured with a 3cm long ACCO™ binder clip. On day 0, the first day of each trial, the nursery cages were clipped off the plant, placed in a cooler and brought into the field. Using a camel hair paint brush, one first instar aphid was removed from a nursery cage and placed onto the underside of one of the highest fully developed trifoliate leaflets on an experimental plant. A cork cage was then placed over the aphid.

The cork cages comprised of a 3cm by 3cm by 0.5cm piece of cork with a 1.9 cm diameter circle punched through the middle. On the top and bottom surface of the cage, Cantech™ double-face cloth carpet tape was placed to attach the cage to a leaf and to hold a piece of screen on the top. Newborn nymphs were not able to pass through the screen. On each date that data was recorded, the screen was lifted back so that the contents of the cage could be observed. Afterwards, the screen was replaced. When needed, the test aphid could be placed onto a new leaf and a new cage could be placed over the aphid. This occurred one to two times, if at all, per aphid. Aphids were transferred to a new cage if the leaf area within the cage showed signs of yellowing or if the screen on the cage was no longer affixing firmly to the tape on the cage. During cage transfer, the aphid would be removed from the old cage with a moistened camel hair paint brush, placed on the underside of a fresh leaflet, and a new cage would be placed over the

aphid. Throughout the summer, it was observed that the cork cages had little effect on the leaves that they were placed on. Each experimental plant held one caged aphid. There were a total of fifty experimental plants per plot which contained a single plant variety. For each of the 4 growth stages, 150 caged aphids were evaluated in each of the fields totaling 1200 test aphids over the course of the summer. The growth stages and dates of each trial for 'J' field in 2007 were as follows: V2- June 25th to July 27th, R1- July 16th to August 10th, R3- August 4th to August 29th, and R6- August 26th to September 17th. For 'S' field in 2007, the growth stages and dates for each trial included the following: V2- June 30th to July 27th, R1- July 14th to August 8th, R3- August 2nd to August 27th, and R6- August 27th to September 17th.

Four days were allotted for the aphids to mature and begin reproduction. On day 4 and every second or third day after day 4, it was recorded whether or not the test aphid was alive, the number of nymphs that had been produced, whether or not the test aphid had been transferred to a new cage, and the growth phase of the plants in the experimental plot. After the number of nymphs was recorded, nymphs were removed from the cage using a paint brush and culled. When test aphids were found dead or could not be found, the leaf containing the cage was clipped from the plant and brought into the laboratory for microscopic visualization. Dead aphids were confirmed to be dead once observed under the microscope. Any aphid found to be alive in the laboratory was returned to the field and placed within a new cage in the proper plot. Some aphids were recorded as dead when they became stuck on the edge of the tape which affixed the screening to the cages and the cages to leaves. Aphids that could not be found were designated as missing.

At the beginning of the trial in 'J' field at the first trifoliate (V2) stage of plant development, a rainstorm caused dirt and sand to splash up into the cages. The next morning, all of the cages had to be changed and some of the aphids were found dead. To prevent this from re-occurring, a layer of row cover (thin white material) was secured to the ground around the experimental plants to prevent splashing. The experiment continued with the remaining aphids which included 45 aphids for the susceptible variety Harovinton, 31 aphids for the resistant variety Palmetto and 35 aphids for the resistant Dowling cross.

Natural colonization of field plants and the test plots did occur. The only plot which became heavily infested was the susceptible plot in 'J' field in the beginning seed (R3) stage. For the full seed (R6) stage the susceptible test plot was changed to a different plot in J field with a lower natural infestation of soybean aphid. All of the other test plots, including the susceptible plot in 'S' field had little to no aphids present throughout all of the growth phases.

Temperature was recorded every 15 minutes using an outdoor hobo data logger in both 'J' field and 'S' field (HOBO ProSeries Temp, RH (C) 1998 ONSET). The mean daily high temperatures were calculated by taking the highest temperature reached each day and averaging them per soybean growth phase and field. The mean daily high temperature for J and S field were combined.

Degree days were calculated for each aphid using the base temperature of 8.6°C (McCornack et al. 2004). Degree days were calculated by subtracting the base temperature from the mean daily temperature. The mean daily temperature was the average temperature calculated from all the recorded temperatures per day. The value for

each day that an aphid was living was added to obtain the accumulated degree days for that aphid. Accumulated degree days were obtained for aphids in 'J' field using the temperature data collected in J field and for aphids in S field using temperature data collected in S field.

A pairwise comparison of mean (±SE) aphid fecundity was made between the resistant plant varieties and field to determine if the resistant varieties could be pooled.

The mean number of nymphs produced per day (fecundity) was calculated for each aphid and used as the response variable. The mean number of accumulated degree days was used as a covariate to account for temperature differences between the soybean growth phases. An ANCOVA with the factors field, susceptible or resistant and plant life stage was conducted. Estimated marginal means with a Bonferroni correction was used for means separation. Temperature was tested separately against plant life stage in a one-way ANOVA and Tukeys HSD was used for means separation.

Every 2 to 3 days aphid mortality was recorded. Aphid mortality was used as a response variable. An ANCOVA with the factors field, susceptible or resistant and plant life stage was conducted using the mean number of accumulated degree days as the covariate. Estimated marginal means with a Bonferroni correction was used for means separation.

A secondary trial in 2008 was conducted using the resistant Dowling cross provided by Vaino Poysa (AAFC-GPCRC) and Elroy Cober (AAFC-ECORC) and the susceptible variety (Mycogen 5261) at 3 locations in the same field. This was conducted to determine if field differences, such as soil properties, could have affected the plants enough to affect aphid fecundity. The planting date was June 9. Plots 1, 2, and 3

contained the susceptible variety and plots 4, 5, and 6 contained the resistant variety. Each susceptible plot was paired with a resistant plot. Plots 2 and 5 were 12.9 meters away from plots 1 and 4. Plots 3 and 6 were 52.0m from plots 2 and 5 and 64.8m from plots 1 and 4. Soil samples A, B, and C were taken from each set of susceptible and resistant plots. Soil for each sample was taken at 5 different places between each set of plots. The soil samples were sent to the University of Guelph laboratory services for analysis of soil type, total nitrogen, total carbon, extractable phosphorus, extractable magnesium, extractable potassium, pH and buffer pH. A 1-way ANOVA with plot as a factor and fecundity as a response variable was conducted and a Tukeys HSD was used for means separation. Daily high temperatures were recorded for the study period to determine if any temperatures reached the aphid upper developmental threshold of 34.9°C (McCornack et al. 2004).

Results

Within J field, mean (±SE) aphid fecundity did not differ between the resistant Dowling cross and the resistant variety Palmetto (Table 2-1). Within S field, mean (±SE) aphid fecundity did not differ between the resistant cross 'Dowling (IL)' and the resistant variety 'MSU'. Because mean aphid fecundity did not differ between resistant varieties within a field, these results were pooled for subsequent analysis.

There was no 3-way interaction of mean aphid fecundity between field, resistance level and plant life stage ($F_{3,1183}$=1.14, P=0.33). There was also no interaction between field and resistance level ($F_{1,1183}$=3.43, P=0.064) as in both fields, aphid fecundity was lower on resistant than susceptible cultivars. For the susceptible plant cultivar, aphid

fecundity did not differ between S and J field (Table 2-2, Figure 2-1). However, for resistant plant cultivars, aphid fecundity was lower in J field than S field.

Field interacted with plant life stage to produce differences in aphid fecundity ($F_{3,1183} = 8.51$, $P<0.0001$). Aphid fecundity was highest on the R6 plant life stage, regardless of field, and not different from the V2 stage in S field (Table 2-3, Figure 2-1). Aphid fecundity was higher for the V2 plant life stage in S field compared with J field. The lowest aphid fecundity was on the R3 plant life stage, regardless of field.

Resistance level and plant life stage interacted to produce differences in aphid fecundity ($F_{3,1183} = 28.44$, $P<0.0001$). In nearly all cases, the resistant cultivars showed a decrease in aphid fecundity and that fecundity differed between the growth phases (Figure 2-1). The highest aphid fecundity was observed in the R6 stage of plant development in the susceptible cultivar (Table 2-4). The V2 and R1 plant life stages were similar in the susceptible cultivars and had the second highest aphid fecundity. The lowest aphid fecundity was on the R3 stage in the resistant cultivars, followed by the R3 stage in the susceptible cultivar and the R1 stage in the resistant cultivars, which were not different from each other. The resistant cultivars in the V2 and R6 stages were not different from each other in aphid fecundity and were higher than the susceptible cultivar in the R3 stage.

There was a 3-way interaction between susceptibility or resistance, plant life stage, and field which produced differences in aphid mortality ($F_{3,1183}=6.34$, $P<0.0001$). Aphids lived the longest on susceptible plants in 'S' field in the V2 and R6 stages, and on susceptible plants in 'J' Field in the R6 stage (Figure 2-2). Aphid longevity on plants in

beginning bloom (R1) and beginning pod (R3) stages were similar regardless of resistance or susceptibility and field.

Temperature experienced by the aphids differed between growth phases ($F_{3,206}$ = 7.32, P<0.0001). The mean (±SE) daily high temperature during the V2, R1, and R3 stages did not differ at 27.37°C ± 0.41, 28.22 ± 0.42, and 27.23 ± 0.53 respectively. However, the mean daily high temperature during the R6 stage was lower than during the other three stages, at 25.00°C ± 0.56.

For the 2008 trial, aphid fecundity was similar within susceptible or resistant plots, but differed between resistant and susceptible plots ($F_{5,294}$=32.92, P<0.0001), with the highest fecundity in the susceptible plots (Table 2-5). The mean (±SE) daily high temperature was 30.7°C ± 0.47. All three soil samples had a similar soil type and total Nitrogen content (Table 2-6). Sample C had the highest amount of total Carbon whereas sample B had the lowest. There were some differences between the soil samples in the amount of extractable phosphorus, magnesium, and potassium. The pH and buffer pH between the three samples was similar. Overall, the three soil samples appear to be similar.

Discussion

Differences in mean aphid fecundity between the tested growth phases support the hypothesis that the expression of soybean resistance varies throughout plant growth. The expression of plant resistance can be affected by plant features, such as plant life stage, plant density, plant height, and disease, as well as factors associated with the pest species, such as density and duration of infestation (Smith 2005). From visual observation of the

field plants in our study, differences between the resistant and susceptible varieties could easily be seen based on aphid densities of naturally colonizing aphids. Resistant plants had low densities of aphids for all growth stages and appeared to be healthy. The susceptible plants in J field had higher aphid densities than the susceptible plants in S field. The highest number of aphids was observed on the susceptible plants in J field in the R3 stage of soybean growth.

However, there is another potential explanation for these observations. Overall, the V2 (first trifoliate) and R6 (full seed) stages of soybean development supported higher aphid fecundity levels than the R1 (beginning bloom) and R3 (beginning pod) stages. The high fecundity observed in the V2 stage may be attributed to young foliage which contains more nitrogen than the R1 and R3 stages (Hikosaka et al. 1994). Soybean aphid densities peaked in the vegetative stages of development in many farmers' fields in Indonesia (van den Berg et al. 1997). Beckendorf et al. (2008) had higher soybean aphid densities in the fourth trifoliate (V5) stage of soybean development compared to the full bloom (R2) stage. As well, Rutledge and O'Neil (2006) found more aphids on the youngest plants early in the season and on the oldest plants late in the season. High fecundity in the first trifoliate (V2) stage may also have been the result of fewer defense compounds in the young leaves. In a study by Coley (1988) the defense compounds tannin and lignin accumulated over the lifetime of a leaf and was greater in older leaves than younger leaves.

The R6 stage of soybean development had the highest fecundity overall for both the susceptible and resistant cultivars. During data collection for the R6 development stage it was observed that soybean plants were starting to senesce and many of the leaves

were yellowing. During plant senescence there is a higher concentration of soluble nitrogen available to aphids and this would have led to an increase in aphid fecundity (White 1984).

In a previous study by Rutledge and O'Neil (2006), soybean aphid intrinsic rate of increase, fecundity, and survivorship within different growth stages of susceptible plants did not change. There was also no difference found in the expression of resistance due to physiological age of soybean in studies by Hill et al. (2004) and Li et al. (2004), however neither of these studies tested aphids on different plant life stage. Instead, they noted that resistance was effective in all plant life stages, as also found in the current study. As I have suggested, it is possible that the difference in aphid fecundity seen at different growth stages in this study was not due to changes in resistance expression but due to other factors such as changes in plant nutrients at different growth stages. Plant nutrients such as nitrogen and soil nutrients such as potassium have affected aphid fecundity in other studies (White 1984; Myers and Gratton 2006; Kindler and Staples 1970; Barker and Tauber 1951). It is possible that resistance in soybean does not change but that other factors such as seasonal changes in a plant, plant nutrients, soil nutrients, and environmental stress play a more significant role in the rise and fall of fecundity in soybean aphid. Without chemical analysis of the leaves and determination of the specific biochemical resistance mechanism that reduces aphid fecundity, this study cannot distinguish between the two potential hypotheses.

It has been suggested that higher aphid numbers can be tolerated in later growth stages without much affect on soybean yield (NCSRP 2007; Ragsdale et al. 2006). Soybean is most at risk when aphid numbers exceed 250 per plant in the early

reproductive stages (NCSRP 2007). Another study found an economic threshold of 273 ± 38 aphids per plant which is valid from the beginning bloom (R1) to the beginning seed (R5) stages (Ragsdale et al. 2007). Although fecundity was lowest during the R1 and R3 stages in the current study, high fecundity during the V2 stage would result in high aphid numbers present on plants at the beginning of the reproductive stages of soybean growth with continued reproduction adding to this population despite the lower fecundity during these reproductive stages. This likely explains the observation of abundant aphid populations in the R1 and R3 stages found in several fields in the study by Ragsdale et al. (2007). Aphid migration and dispersal onto soybean plants before the full pod (R4) stage may also add to abundant populations in the R1 and R3 stages of soybean development. High infestation levels in the reproductive stages of soybean development can lead to reduced yield due to reduced pod set (Sun et al. 1990). In a study by Ragsdale et al. (2007), 18 out of 19 yield loss experiments over six states in the North Central region had peak aphid densities in the R3 to R5 stages with a majority reaching peak densities at the R1 stage at the time of 80% infestation.

Aphid mortality could have had an effect on aphid fecundity due to differences in the number of offspring produced at different stages of an aphid's life. Aphid longevity was generally greater on susceptible plants and in the first trifoliate (V2) and full seed (R6) stages of soybean development. Aphid fecundity followed a similar pattern. The shortest longevity occurred in the beginning bloom (R3) stage. The beginning bloom stage also showed lower levels of aphid fecundity.

Aphid fecundity in S field was higher than the aphid fecundity in 'J' field during the same first trifoliate (V2) stage of soybean development. The aphids in 'J' field may

have been affected by a storm which splashed sand into the cages. The storm occurred after the cages were placed in 'J' field but before cages were placed in 'S' field. After the storm, aphids in sandy cages were transferred to new cages. The lower aphid fecundity in 'J' field compared to 'S' field could have been due to the storm or the process of transferring aphids into new cages stressing the aphids and resulting in a shorter longevity as mean aphid longevity was shorter in the V2 stage in 'J' field than in 'S' field.

Since the growth phases in this study occurred sequentially, the results are confounded with environmental factors that changed throughout the growing season. Mean temperatures were similar between the V2, R1 and R3 growth stages but, were slightly cooler for the R6 stage. Temperature differences were accounted for by using accumulated degree days. Other environmental factors, such as the amount of rainfall in each developmental stage, were not considered in this study. Mean daily high temperatures never reached the upper developmental threshold, 34.9°C, for soybean aphid (McCornack et al 2004).

In conclusion, varying levels of soybean aphid fecundity at the first trifoliate (V2), beginning bloom (R1), beginning pod (R3), and full seed (R6) stages of soybean development indicate that resistance levels may change throughout soybean growth; however, aphid fecundity can be affected by many factors and resistance levels may have been unchanging. Differences in fecundity between resistant and susceptible plants suggest that plant resistance is an effective measure against soybean aphid. Since aphids were able to live and reproduce on the tested resistant cultivars, it is believed that the resistance was moderate. Resistance was observed in all plant life stages. Fecundity was lower on resistant plants than on susceptible plants for each soybean development stage

studied. Although high levels of fecundity were reached in the R6 stage, older soybean plants have a higher tolerance for aphid damage without much effect on yield. Fecundity was lowest in the most critical stages of soybean growth for pod development. Plant resistance, as well as natural enemies and soybean aphid management strategies, can reduce the likelihood of reaching economic thresholds and would prove valuable to increasing soybean yield potential.

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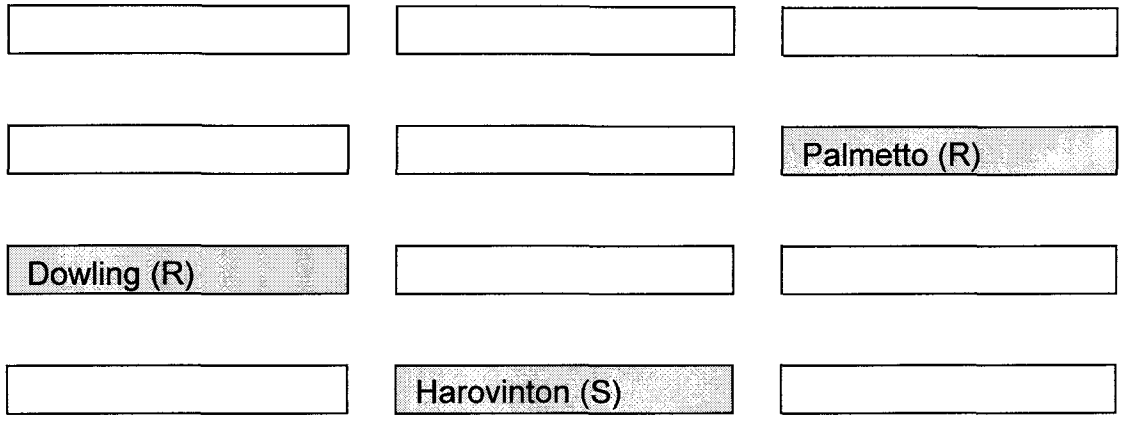
'J' Field



'S' Field

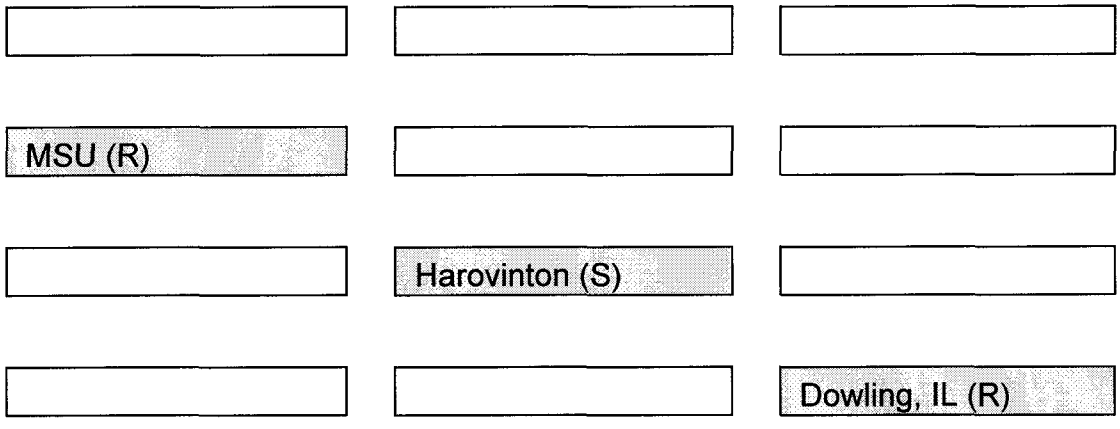


Figure 2-1. Field set-up for 'J' field and 'S' field in the summer of 2007. Each rectangular box represents one plot of 50 plants with one cage per plant containing a test aphid. Resistant soybean varieties are designated as (R) and the susceptible soybean variety is designated as (S).

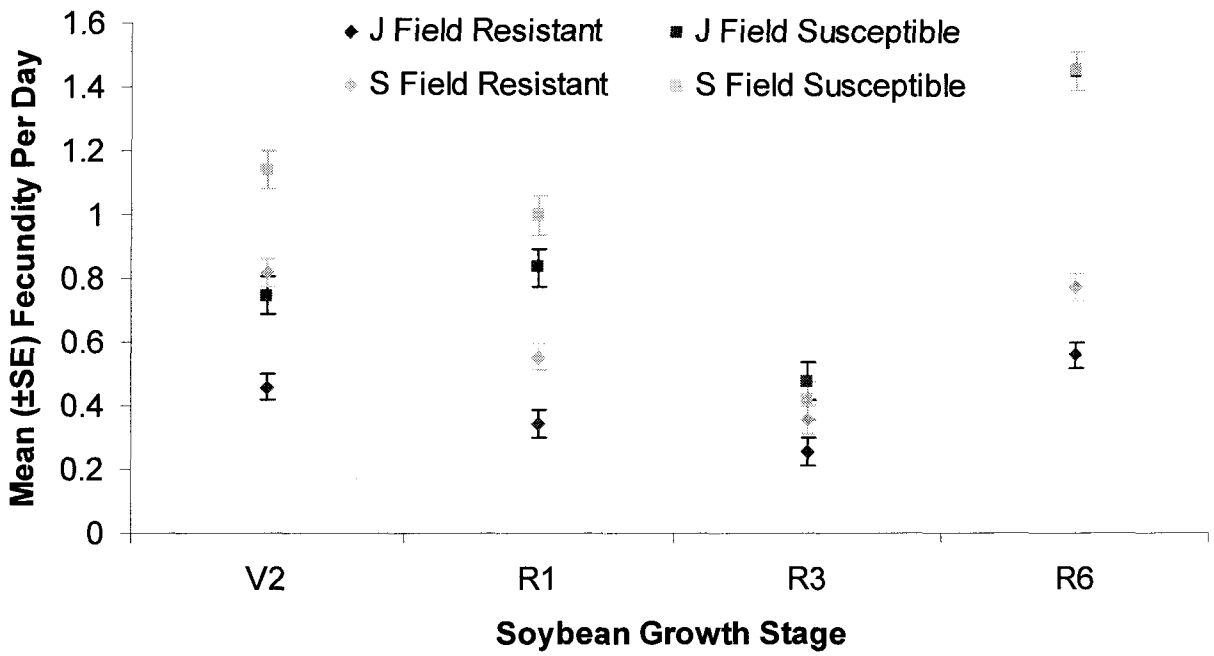


Figure 2-2. The mean daily fecundity (± SE) per day of *A.glycines* in the soybean growth stages V2-first trifoliate, R1- beginning bloom, R3- beginning pod, and R6- full seed in 'J' field (N=100 per growth phase for resistant cultivars (50 per cultivar) and N=50 per growth phase for susceptible cultivar).

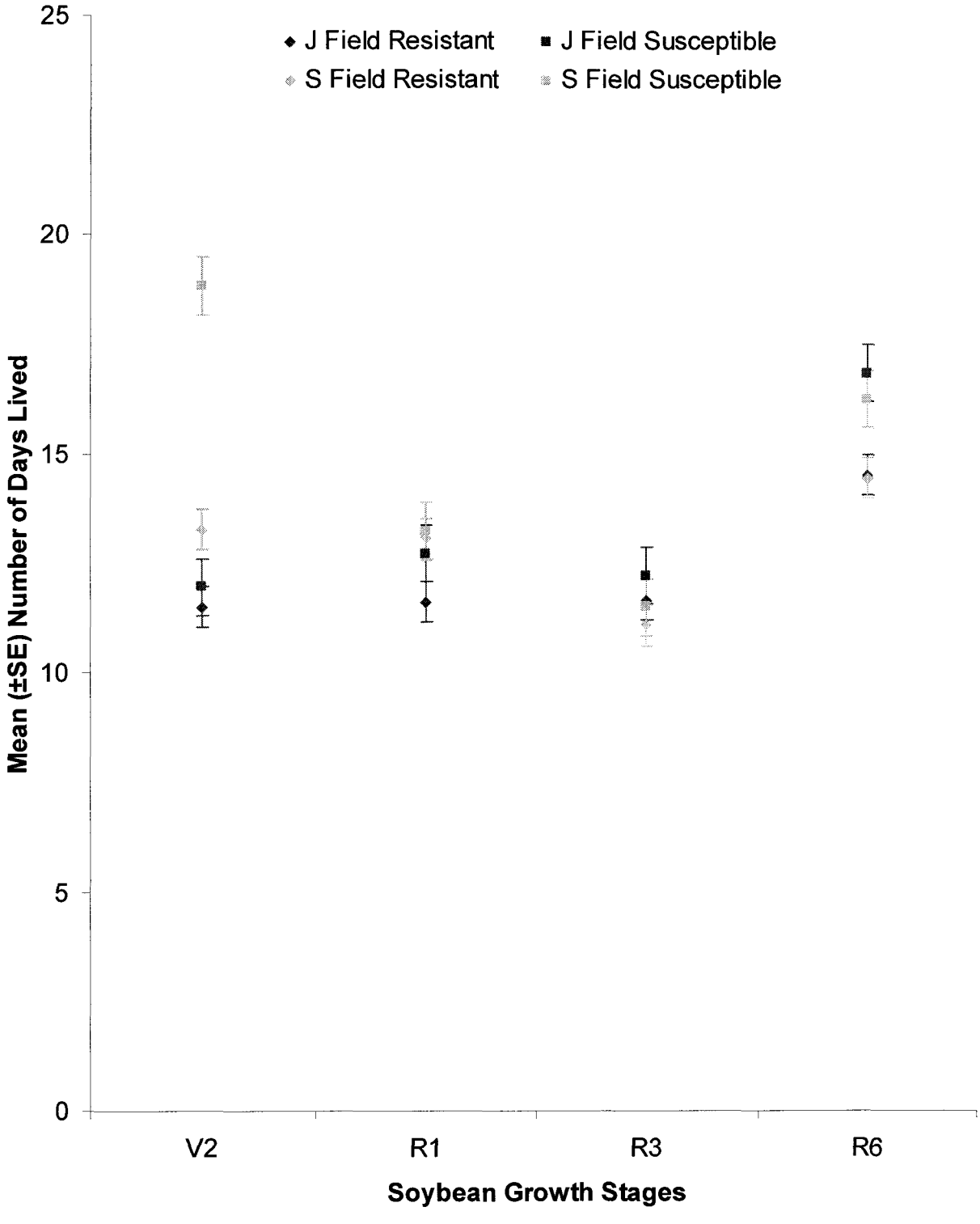

J Field Resistant
J Field Susceptible
S Field Resistant
S Field Susceptible
25
20
15
10
5
0
Mean (±SE) Number of Days Lived
V2
R1
R3
R6
Soybean Growth Stages

Figure 2-3. The mean number of days lived (± SE) by *A. glycines* in the soybean growth stages V2-first trifoliate, R1- beginning bloom, R3- beginning pod, and R6- full seed in 'S' field (N=100 per growth phase for resistant cultivars (50 per cultivar) and N=50 per growth phase for susceptible cultivar).

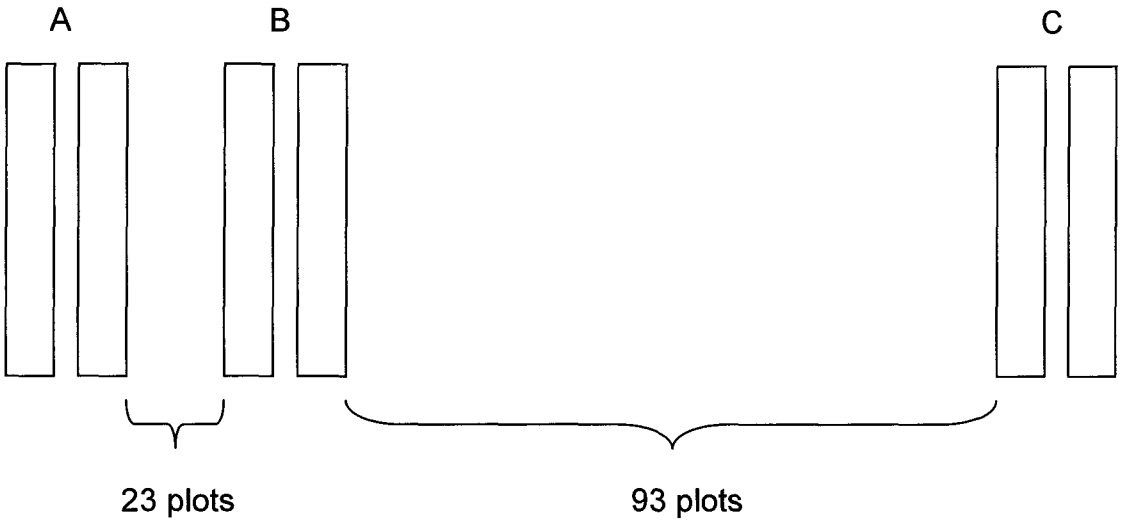


Figure 2-4. Field set-up for each pair of plots A, B, and C for the summer 2008 trial. Each rectangular box represents one plot of 50 plants (1.79 m wide) with one cage per plant containing a test aphid. The susceptible variety Mycogen 5261 was planted on the left side of each pair of test plots and the resistant variety, a Dowling cross, was planted on the right side of each pair of test plots.

Table 2-1. Mean (±SE) daily *A. glycines* fecundity between resistant plant varieties and field.

Field	Resistant varieties	Mean (±SE)
J	Dowling (Rag 1)	0.02 ± 0.03 a
	Palmetto	0.05 ± 0.03 ab
S	Dowling (Rag 1), IL	0.22 ± 0.04 b
	MSU	0.17 ± 0.03 b

Means followed by the same letter are not different (P>0.05).

Table 2-2. Mean (±SE) daily *A. glycines* fecundity between susceptible or resistant cultivars in two fields across all soybean growth phases.

Field	Susceptible/Resistant cultivars	Mean (±SE)
S	S	1.00 ± 0.03 a
J	S	0.88 ± 0.03 a
S	R	0.63 ± 0.02 b
J	R	0.41 ± 0.02 c

Means followed by the same letter are not different (P>0.05).

Table 2-3. Mean (±SE) daily *A. glycines* fecundity between plant life stages and fields.

Field	Plant life stage	Mean (±SE)
J	V2	0.60 ± 0.04 c
	R1	0.59 ± 0.04 c
	R3	0.37 ± 0.04 d
	R6	1.01 ± 0.04 a
S	V2	0.98 ± 0.04 a
	R1	0.78 ± 0.04 b
	R3	0.39 ± 0.04 d
	R6	1.12 ± 0.04 a

Means followed by the same letter are not different (P>0.05).

Table 2-4. Mean (±SE) daily *A. glycines* fecundity between susceptible or resistant cultivars at different plant life stages.

Susceptible/Resistant cultivars	Plant Life Stage	Mean (± SE)
S	V2	0.94 ± 0.04 b
	R1	0.92 ± 0.04 b
	R3	0.45 ± 0.04 d
	R6	1.46 ± 0.04 a
R	V2	0.64 ± 0.03 c
	R1	0.45 ± 0.03 d
	R3	0.31 ± 0.03 d,e
	R6	0.67 ± 0.03 c

Means followed by the same letter are not different (P>0.05).

Table 2-5. Mean (±SE) aphid fecundity on susceptible or resistant cultivars across each plot.

Soil Sample ID	Plot	Susceptible/Resistant Cultivars	Mean (±SE)
A	1	S	0.87 ± 0.07 a
B	2	S	0.94 ± 0.07 a
C	3	S	0.92 ± 0.08 a
A	4	R	0.27 ± 0.04 b
B	5	R	0.30 ± 0.04 b
C	6	R	0.31 ± 0.04 b

Means followed by the same letter are not different (P>0.05).

Table 2-6. Results of the soil analysis conducted on dry soil by University of Guelph Laboratory services for each of the three plots tested in the summer of 2008.

Sample ID	Soil Type	Total Carbon	Total Nitrogen	Extractable phosphorus	Extractable magnesium	Extractable Potassium	pH	Buffer pH
A	Loamy fine sand	0.70%	0.06%	50mg/L	99mg/L	140mg/L	5.8	6.9
B	Loamy fine sand	0.65%	0.07%	54mg/L	92mg/L	150 mg/L	5.7	6.8
C	Loamy sand	0.82%	0.07%	48mg/L	100mg/L	130 mg/L	5.8	6.9

Chapter 3

Movement of apterous and alatoid soybean aphids between resistant and susceptible plants

Introduction

Movement between plants is common in most insect species. Foragers move between plants to search for prey, oviposition sites, refuge, and host plants. Movement of insects between plants may benefit plants. For example, bumble bee movement from plant to plant is beneficial for pollination. As well, predators and parasitoids help rid plants of arthropods which feed on plants causing nutrient deficiencies, stunting, cessation of growth, and reduced plant fecundity or yield (Gill and Sanderson 1998; Hodgson 1991; Sun et al. 1990). The dispersal of herbivorous arthropods onto neighbouring plants also helps relieve herbivore-induced stresses of the plant from which the insects dispersed (Hodgson 1991). Alternatively, dispersal can also benefit herbivorous species and incur fitness costs on plants. Herbivore dispersion to neighbouring plants allows herbivores to avoid overcrowding and to reap the benefits of healthier plants. Plants can also provide places of refuge for herbivorous species to hide from predators. For example, leaf domatia, small cavities located on the undersurface of leaves where primary and secondary veins meet, provide shelter for many species of mites and mite eggs (Cortesero et al. 2000). One of the most devastating results of herbivore dispersal is the transmission of plant diseases. Through stylet penetration, herbivores feeding on diseased plants can spread disease when they move to a new plant and begin to feed (Hill et al. 2001).

Studies on the movement between plants by aphids usually involve a stimulus, such as a predator, disturbance or pheromone to cause the dispersal behaviour (Gish and Inbar 2006; Losey and Denno 1998; Montgomery and Nault 1978; Roitberg 1979). These stimuli usually cause aphids to drop from the plant at which point they either return to the host plant or find a new host plant. Roitberg et al. (1979) found that predators were the main cause of aphid dispersal in the pea aphid *Acyrthosiphon pisum* (Harris). Previous studies have also found that aphids are more likely to drop from a low quality host plant in the presence of a predator (Losey and Denno 1998) or in the presence of alarm pheromone (Dill et al. 1990).

The soybean aphid is an invasive pest originating from Asia (Blackman and Eastop 2000). In 2000, this pest abruptly and quickly made its way throughout many parts of North America (Venette and Ragsdale 2004). By 2001, the spread reached Ontario, Canada (Hunt et al. 2003). The soybean aphid overwinters on its primary host Buckthorn, *Rhamnus spp.* (Rhamnaceae), and migrates to soybean, *Glycine max* (L.) Merr, in the spring (Ragsdale et al. 2004). Migration to alternative fields and plant host species is associated with aphid alates in most aphid species. Small scale dispersal to neighbouring plants is usually due to the dispersal of apterous late instar nymphs and young adult aphids (Hodgson 1991). The soybean aphid is parthenogenic, females aesexually produce females, throughout the summer season (Arnett 2000). This ability allows them to reproduce and disperse quickly. Crowding triggers the production of alates which are then randomly wind dispersed and may end up on less crowded host plants (Ragsdale et al. 2004). The dispersal of the soybean aphid is responsible for the transmission of plant diseases such as the soybean mosaic virus (Hill et al. 2001). Once

populations increase on soybean, stunting, flaccidity, and reduced pod set may occur (Gill and Sanderson 1998; Sun et al. 1990). As well, the honeydew excretions of the aphid lead to the formation of sooty mould which may reduce photosynthesis (Li et al. 2004).

In order to control soybean aphid populations in fields, soybean breeders have located soybean genotypes which are resistant to the soybean aphid (Hill et al. 2004) by affecting either aphid physiology, aphid behaviour, or both. Knowledge of the dispersal ability of the soybean aphid in relation to susceptible and resistant plants may aid in intercropping decisions. Intercropping susceptible and resistant plants may be valuable in reducing the spread of counter-resistance, if counter-resistance develops in the soybean aphid. Trap plants may also be useful by reducing the amount of aphid dispersal. Since apterous dispersal occurs mostly in patches of plants which are side by side, trap plants may minimize the amount of dispersal by interrupting the patchiness of soybean. The objective of this study was to determine whether the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), would move from resistant soybean (a poor host) to susceptible soybean (a good host). This study was carried out using immature nymphs, apterous adults and alatoid adults.

Methods & Materials

Soybean Cultivars

The soybean varieties used were the susceptible variety SDO1-76R and a resistant variety with a Dowling background. Dowling germplasm has an antibiosis-type and antixenosis-type resistance to the soybean aphid (Hill et al. 2006), causing increased aphid mortality and reduced aphid fecundity (Li et al. in 2004). Seeds were produced and

provided by Vaino Poysa (AAFC-GPCRC) and Elroy Cober (AAFC-ECORC). Seeds were placed into petri dishes with wet paper towel and held in a growth cabinet at 25°C (± 1°C) for germination. Small seedlings were planted into trays in a 4:1:1 ratio of steam sterilized soil, peat moss, and vermiculite. Plants were grown in a greenhouse nursery and watered daily. No fertilizer was used in the nursery as only young plants were kept in the nursery and fertilizer was not required.

Soybean aphid rearing

Soybean aphids were collected from a laboratory colony initiated in 2004 with several field aphids. The colony was reared on the soybean variety Mycogen 5261 in a 16:8 h L:D growth cabinet set at 26°C (L) and 24°C (D). Each pot in the colony is hand watered daily with water and weekly with ca. 250-500 mL of soluble NPK fertilizer solution. The colony is maintained to contain healthy plants with low levels of crowding and few alatoid aphids.

Experimental setup

Plants with one to two trifoliates were used for the experiment. Plants tested within the same arena were matched to be of similar height, growth stage, and quality. Two soybean plants separated by 15 cm were placed within an 18 cm by 35 cm arena made from white bristle board. The arena sat on top of the 10cm pots in which the soybeans were planted. Slits were cut into each end of the bristle board to allow the stems of the plants into the arena. The plants and arena were placed within a tray of water to prevent aphid escape. A cotton pad was placed around the base of the plant stem on top of the arena to cover the hole at the end of each slit made for the stems. The cotton was wet with distilled water to prevent aphids from getting stuck in the cotton fibers.

The experiment was conducted using 1st -2nd instars, apterous adults, and adult alates. Apterous aphids were transferred directly from colony plants to experimental plants. Since alates were hard to find in the colony and were few in numbers, alates and aphids with wing buds, darkened protrusions at the sides of the body from which wings develop, were first collected into containers containing a moist filter paper and a susceptible soybean leaf. This ensured that I would have enough alates ahead of time to run an experiment and I was also able to keep extras for the next day. The stem of the soybean leaf was placed into a small plastic vial containing water. Aphids that had been collected into containers were kept in a growth cabinet set at 25°C until they were used.

At the start of each experiment, aphids were placed on the top leaves of the first plant, or start plant, in each treatment and the trial was allowed to run for 24 h. Forty aphids were placed on the start plant of each treatment in trials conducted with 1st to 2nd instars and adults. Due to low numbers of alates in the colony, only 12 alates were placed on the first plant in each treatment. The six treatments consisted of: a susceptible plant and no plant, two susceptible plants, two resistant plants, a resistant plant and no plant, a susceptible plant with a resistant plant, and a resistant plant with a susceptible plant. Five replicates of each treatment were conducted using 1st -2nd instars, 7 replicates for the apterous adults, and 16 replicates were conducted using alates.

To avoid the influence of aphid movement from the odours of other treatments, three separate rooms were used for the experiment with one room containing only susceptible plants, one room containing only resistant plants and one room with both. There were a total of 2 arenas per room when 1 replicate was run and 4 arenas per room when 2 replicates were run. Rooms were assigned at the start of each day based on a

series of coin tosses. Lights in the 3 rooms remained on from 7:00 to 17:30h daily. Temperatures for all three rooms had a mean of 21°C ± 1°C.

Statistical analysis

For each aphid type, a 2 way ANOVA was conducted to determine the effect of both the start plant type and the second plant type on the number of aphids which remained on the start plant. A planned comparison of the treatments plants vs no plants and susceptible plants vs. resistant plants was conducted for the second plant type.

Results

More $1^{st} - 2^{nd}$ instars remained on the start plant when the start plant was susceptible ($F_{1,24}$=58.63, P<0.0001) (Figure 3-2). There was no effect of the second plant type on the number of 1^{st}- 2^{nd} instars that remained on the start plant ($F_{1,24}$=1.62, P=0.22) (Figure 3-3). There was no effect on the number of 1^{st}-2^{nd} instars that remained on the first plant depending on whether or not a second plant was present ($F_{1,24}$=0.048, P=0.828) or if the second plant was susceptible or resistant ($F_{1,24}$=3.20, P=0.086). The number of aphids that remained on the start plant did not depend on an interaction between the start plant type and the second plant type ($F_{2,24}$=0.002, P=1.00).

More apterous adults remained on the start plant when the start plant was susceptible ($F_{1,36}$=79.08, P<0.0001) (Figure 3-2). The second plant type had no effect on the number of apterous adults that remained on the start plant ($F_{2,36}$=2.09, P=0.14) (Figure 3-3). The number of apterous adults that remained on the first plant was not affected by the presence or absence of a second plant ($F_{1,36}$=4.08, P=0.051); however, there is a strong trend whereby aphids are more likely to remain on the start plant if there

is no second plant. The susceptibility or resistance of the second plant had no effect on the number of apterous adults that remained on the first plant ($F_{1,36}$=0.10, P=0.75). The start plant and the second plant did not interact to affect the number of aphids which remained on the start plant ($F_{2,36}$=0.131, P=0.878).

Aphid alates also remained on the first plant in greater numbers when the first plant was susceptible ($F_{1,90}$=90.53, P<0.0001) (Figure 3-2), but there was no effect of the second plant type ($F_{2,90}$=1.51, P=0.23) (Figure 3-3). The presence or absence of a second plant did not affect the number of adult alates that remained on the first plant ($F_{1,90}$=1.29, P=0.26). Whether the second plant was resistant or susceptible had no effect on the number of adult alates which remained on the start plant ($F_{1,90}$=1.72, P=0.19). The number of aphids which remained on the start plant was not affected by an interaction between the start plant type and the second plant type ($F_{2,90}$=0.17, P=0.85).

Discussion

More aphids left the first plant when the first plant was resistant. This behaviour was observed for each soybean aphid morph. Movement away from resistant plants is likely due to poor host quality of the resistant plant material. The resistant plant cultivar Dowling affects aphid behaviour (antixenosis-type resistance) as well as physiology (antibiosis-type resistance) (Diaz-Montano et al. 2006), and my research has demonstrated lower fecundity of soybean aphid on Dowling (Chapter 2). Thus, I expected aphids to also exhibit behavioural changes such as being more willing to leave resistant plants than susceptible plants. Li et al. (2004) found that aphids left plants 8 to 24 h after placement on resistant plant leaves. In a study by Diaz-Montano et al. (2007),

the soybean aphids feeding behaviour was affected by plant resistance. Soybean aphids took a longer amount of time to penetrate the leaf surface, 7.5 h compared to 3.5 h, on the resistant cultivars K1639, Pioneer® 95B97, Dowling and Jackson. As well, the amount of time spent probing plant tissue was significantly reduced, 2 to 7 min compared to an hour, in the resistant cultivars. Other studies have also found that host quality plays a role in an insect's decision to leave a host plant. In a study by Dill et al. (1990), Pea aphids, *Acyrthosiphon pisum* (Harris), were more likely to leave low quality host plants in response to alarm pheromone than when alarm pheromone was absent (Losey and Denno 1998). I believe that the reduced time spent feeding, observed by Diaz-Montano et al. (2007), and reduced quality of the food source, observed by Dill et al. (1990), are reasons that would have led to aphids leaving resistant plants in this study.

Movement, either by walking or dropping, from the first plant was not dependant on the second plant type. This suggests that aphids were leaving their host plant without the knowledge of an alternative food source. It is likely that some amount of random dispersal occurs without a trigger such as crowding or poor host quality. This would explain why aphids left susceptible plants when crowding or poor host quality shouldn't have been an issue. In a study by Hodgson (1991), several experiments showed random dispersion of young adult apterous and alatoid aphids when small numbers of aphids were placed on a center plant within a circle of plants. Since there was no difference between the number of aphids that left the first plant based on the second plant type, it is likely that aphids did not return to their abandoned food plant when an alternative plant was not found. Otherwise, it would have appeared that fewer aphids left the first plant when there

was no second plant. Roitberg et al. (1979) observed that pea aphids often did not return to feeding sites from which they previously dropped.

Once aphids left the first plant, they should have tried to locate a new host plant. Aphids may locate host plants using visual cues, odours from the host plant, odours from fallen plant material, or aggregation pheromones emitted by aphids on a host plant (Gish and Inbar 2006). A greater proportion of aphids were found on the second plant when the second plant was susceptible. I was unable to discern whether or not this was due to host plant finding mechanisms or random movements. Aphids that located resistant plants after abandoning the first plant may have also abandoned the second plant. There is also the possibility that a greater proportion of aphids on resistant plants, whether they were on the first plant or the second plant, died as a result of the antibioisis effect of the resistance. This would have decreased the number of aphids found on resistant plants.

There are several studies that confirm some host selection ability by aphids using both visual and chemical stimuli. In a study by Gish and Inbar (2006) host location by the aphid *Macrosiphoniella artemisiae* (Boyer de Fonscolombe) (Hemiptera: Aphididae) was thought to be primarily due to visual cues. Only 17% of aphids were able to find a host plant in the dark compared to 96% host location in sunlight. As well, aphids would climb cardboard models of a host plant branch in the absence of host plants. When aphids were dropped in the center of an empty arena, they showed signs of negative phototaxis. No known studies have been conducted on host location by soybean aphids using visual cues alone. However, it has generally been found that aphids are attracted to the green domain of the colour spectrum (Döring and Chittka 2007).

Du et al. (1994) found that soybean produced plant volatiles attractive to the soybean aphid. It was also found that non-host odours could mask the attractiveness of soybean to the soybean aphid by changing the overall volatile profile. An important next step would be to test resistant and susceptible plant material in an olfactometer to see if soybean aphid is attracted to resistant soybean and if the two soybean genotypes can be distinguished by an olfactory preference for one over the other.

It is also possible that soybean aphids are not able to distinguish susceptible from resistant plants based on plant volatiles. The inability of the soybean aphid to distinguish between resistant and susceptible plants would be in congruence with the theory proposed by Stephens and Krebs (1986) on incomplete information. In this model, foragers trying to exploit a resource may be able to distinguish a resource type, such as patches of plants that appear similar. However, they are not likely able to distinguish sub-types within the resource due to incomplete information. For instance, a predator may be able to distinguish plant patches, the resource type, but is not able to distinguish the amount of prey contained within each patch, the resource sub-type. Applying the concept to this study, aphids would be able to distinguish soybean as a type, but would not be able to distinguish the sub-type, resistant or susceptible. Further exploration of a plant would be required to make a decision as to whether or not the plant is an acceptable host.

There were differences observed in the amount of movement made by each type of morph, with immature nymphs having the least success in locating the second plant when that plant was susceptible, alatoid adults being intermediate and apterous adults having the greatest success. This pattern was also consistent for when the second plant was resistant, although fewer aphids were found on the resistant second plant. As

previously suggested, the fewer numbers of aphids locating resistant plants may have been due to secondary abandonment or death due to the resistant nature of the plant. Other studies have also observed greater movement from adults than immature nymphs (Gish and Inbar 2006; Hodgson 1991; Roitberg et al. 1979). Hodgson (1991) found increased dispersal to neighbouring plants by young apterous adults compared with aphid alates. The differences in the amount of movement made between apterae and alates may be due to the morphological differences between the two. Walking by alates is difficult since alates are equipped with wings and are mainly dispersed by wind, whereas apterae are better suited for walking and commonly walk to nearby plants.

There were also differences in the amount of dispersal observed on the first plant. Immature aphids that were placed onto a susceptible plant likely stayed where they were placed and did not venture past the leaflet onto which they were placed. Adult aphids were likely to disperse to other leaves as well as the stem and areas of new growth. There are several reasons why immature nymphs do not disperse to the extent of adults. Immature nymphs are less likely to locate a new host plant than adult aphids (Dill et al. 1990; Losey and Denno 1998). Due to their small size, immature nymphs are more likely to die from desiccation if they leave a host plant (Losey and Denno 1998). They are also limited in movement due to the small size of their legs (Hodgson 1991). I also infer that immature nymphs are at greatest risk of predation due to the lack of ability to escape and that immature aphids are safer in aggregates where they would be able to detect alarm pheromone and not be isolated in the presence of a predator.

In conclusion, 1-2nd instars, apterous adults, and alatoid adult soybean aphids left resistant plants in greater numbers than susceptible plants. Movement away from

susceptible plants indicates that some amount of random dispersal occurs. Because there was no difference between the number of aphids that left the first plant based on the second plant type or the absence of a second plant, it is likely that aphids abandoned host plants without knowledge of the existence of an alternate host plant. A greater proportion of aphids left resistant plants and ended up on susceptible plants. This result was only different from the other treatments with the adult apterous aphids. It could not be detected if plant location was due to random movements or chemical and/or visual stimuli. Generally, a greater proportion of movement was observed from the apterous adults than alate adults or immature nymphs, which showed the least amount of movement.

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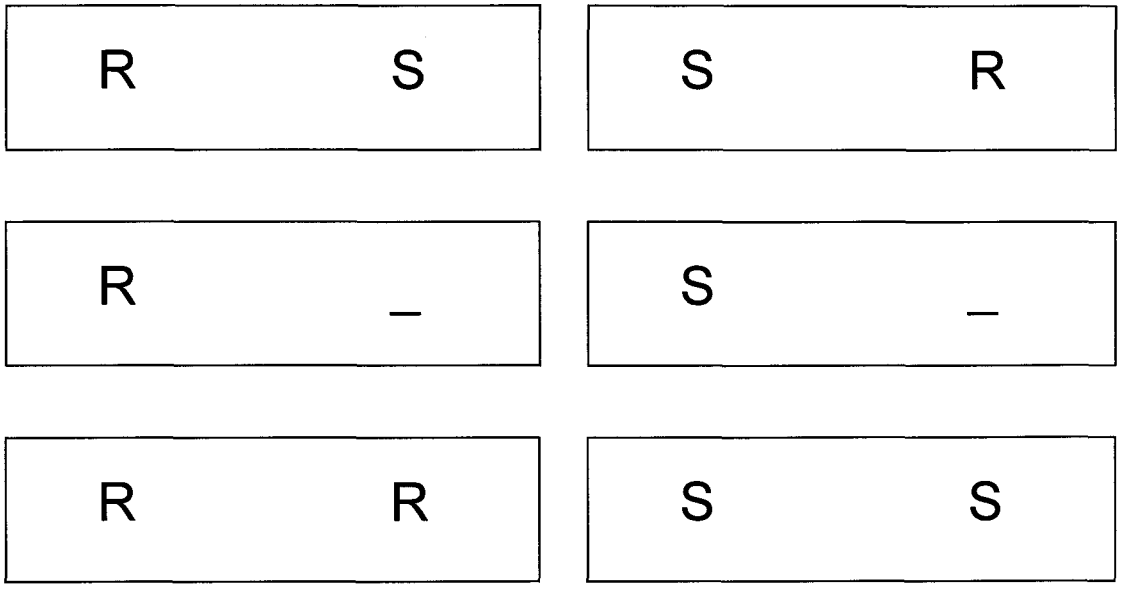

Figure 3-1. A schematic of all treatments where R represents a resistant soybean variety with a Dowling background, S represents the susceptible soybean variety SD01-76R, and _ represents the absence of a soybean plant. The first or start plant, where aphids were placed at the beginning of each trial, is represented by the letter on the left-hand side of each pair of letters.

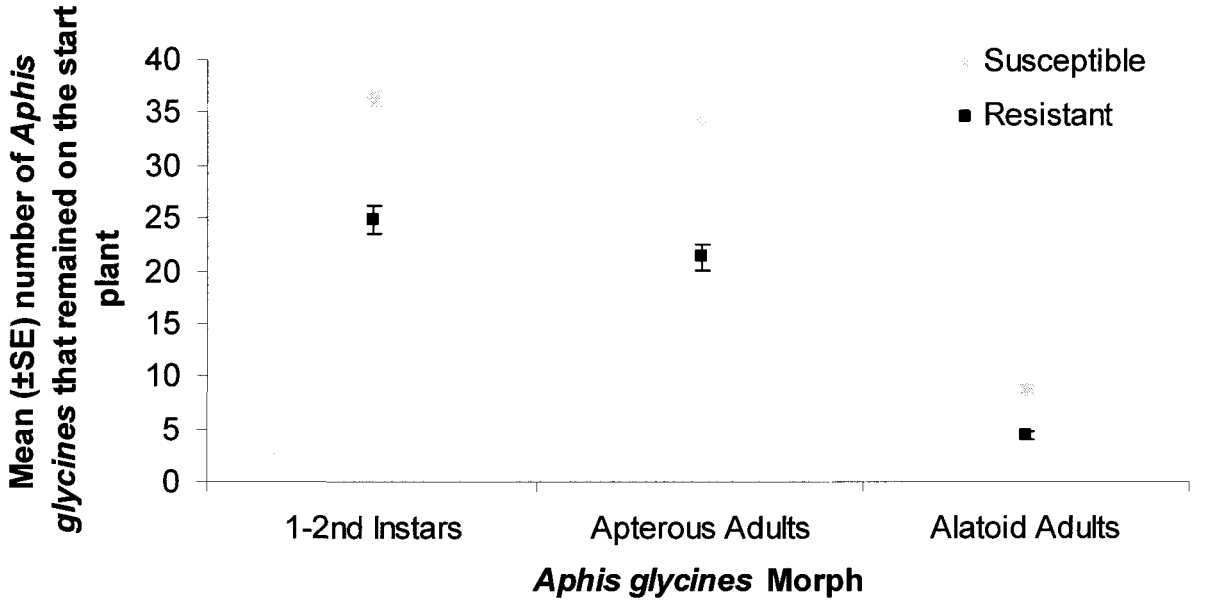

Figure 3-2. The mean number of 1-2nd instars, apterous adults and alatoid adults that remained on the start plant when the start plant was susceptible or resistant (1-2nd instars: 5 reps of 40 aphids/rep; apterous adults: 7 reps of 40 aphids/rep; alatoid adults: 16 reps of 12 aphids/rep).

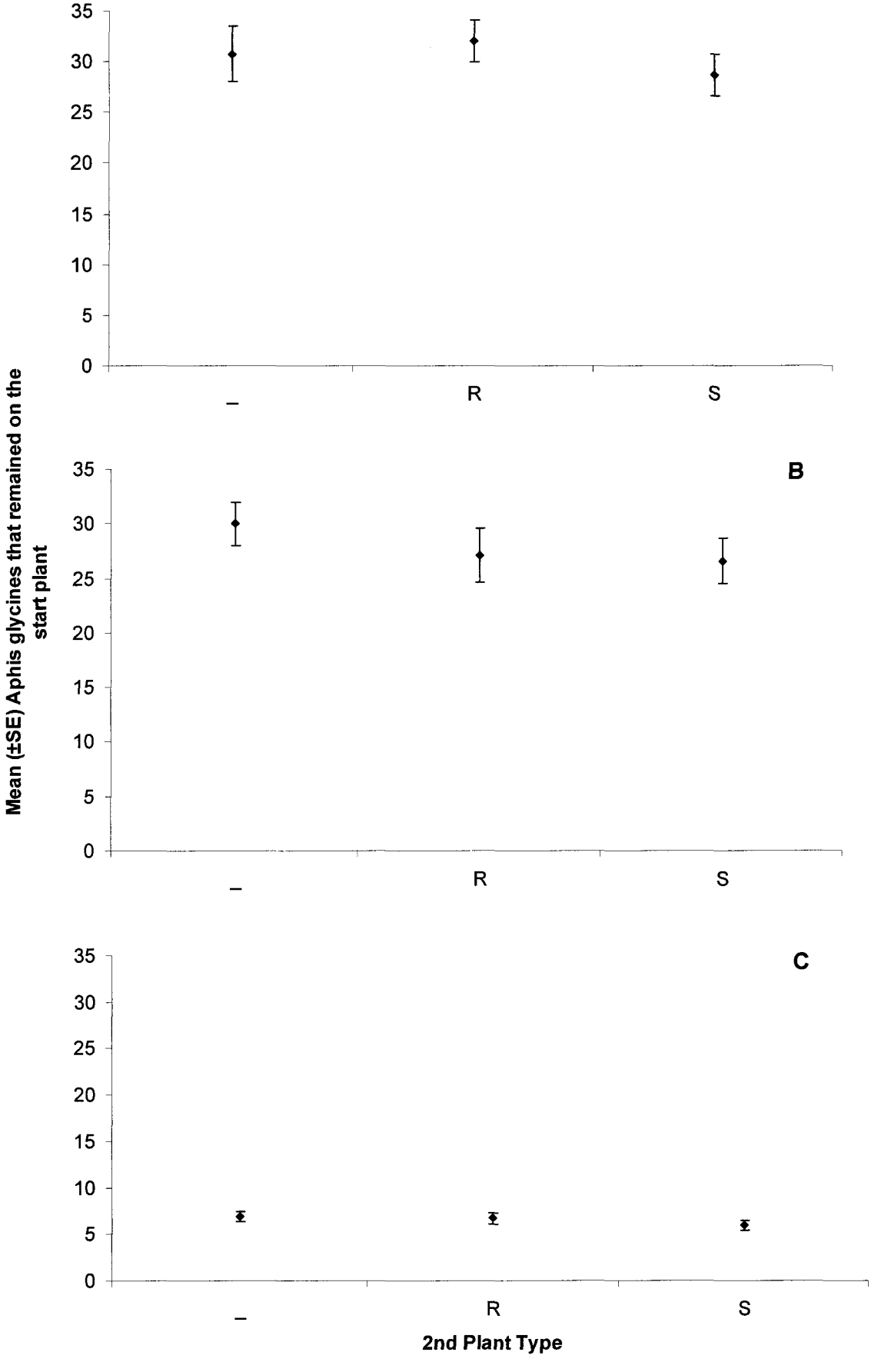
Mean (±SE) Aphis glycines that remained on the start plant
35
30
25
20
15
10
5
0
–
R
S
B
C
2nd Plant Type

Figure 3-3. Mean (±SE) of *Aphis glycines* Matsumura that remained on the start plant based on the 2nd plant type for A. 1st-2nd instars; B. apterous adults; C. adult alates.

Chapter 4

Olfactory orientation of the soybean aphid to susceptible and resistant soybean

Introduction

Host plant selection is the process whereby an insect uses plant cues to detect whether or not a plant is suitable for feeding or reproduction. It is uncertain whether aphids are able to locate host plants based on host plant volatiles since aphids are weak fliers (Kennedy and Thomas 1974) and land on host and non-host plants equally (Kennedy et al. 1959). As well, less than 1% of aphids are successful in host location (Ward et al. 1998). Despite these facts, many agree that some olfaction is involved in host plant selection (Pickett et al. 1992; Powell and Hardie 2001; Powell et al. 2006; Quiroz et al. 1999; Visser 1986).

Insects make oriented movements towards chemical attractants and make oriented movements away from chemical repellents (Bernays and Chapman 1994). Evidence has shown that aphid species can detect host plant odours and are attracted to host plant odours (Bernasconi et al. 1998; Chapman et al. 1981; Du et al. 1994; Nottingham et al. 1991; Visser and Taanman 1987). Aphids use several cues to locate host plants. Chemical cues detected from plant volatiles, epicuticular waxes, trichome exudates, gustatory cues, substrate texture and colour can influence host selection before stylet penetration (Powell et al. 2006). Some aphids may depend mostly on cues encountered through stylet penetration to determine the suitability of a host plant (Powell and Hardie 2001) whereas others may be sensitive to odours and be influenced by attractive or repellent volatiles. In a study by Storer et al. (1996), aphids that landed on suitable host

plants flew away from the plant after landing due to the non-host plant volatile 1-heptanonitrile which was permeated in the air directly above the leaf surface.

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is an invasive pest originating from Asia. It was first noticed in the United States in Wisconsin in 2000 (Alleman et al. 2002). That year, the soybean aphid was also reported for the first time in nine other states including Michigan (Venette and Ragsdale 2004). In 2001, the soybean aphid reached Ontario, Canada (Hunt et al. 2003). The soybean aphid is responsible for economic loss in soybean yield and costs incurred due to its control are extensive (Ragsdale et al. 2007). Resistant soybean varieties are being produced in the hopes to more effectively control aphid populations and decrease the costs associated with insecticide use. The soybean aphid has been found to show an attraction to soybean (Du et al. 1994); however, it is not known if the plant volatiles from resistant soybean have an effect on soybean aphid behaviour. Thus, the objective of this study was to determine if resistant plant odours have an effect on the orientation behaviour of the soybean aphid and if resistant plant odours change the overall volatile profile inhibiting the attractiveness of susceptible soybean to the soybean aphid.

Materials and Methods

Soybean aphid rearing.

Soybean aphids were collected from a laboratory colony that was initiated in 2004 with several aphids that had been collected from a field. Colony aphids were reared on the soybean cultivar Mycogen 5261 in a growth cabinet set at 26°C (L) and 24°C (D) and 16:8 h L:D. Each week, plants are hand watered with a solution of NPK fertilizer. The

colony is maintained by removing and adding soybean plants as needed to sustain a supply of soybean aphids.

Soybean cultivars

The soybean cultivars used in this study include susceptible genotypes and resistant genotypes of the Dowling background. Resistance in Dowling has been attributed to the presence of the gene Rag 1 (Hill et al. 2006). Soybean seed was produced and provided by Vaino Poysa (AAFC-GPCRC) and Elroy Cober (AAFC-ECORC). Seeds were placed into petri dishes with wet paper towel to stimulate germination. The petri dishes were placed into a growth cabinet at a temperature of 25°C (± 1°C). Seedlings were carefully planted into trays and placed into a greenhouse nursery. The media used for planting contained a 4:1:1 mixture of steam sterilized soil, peat moss, and vermiculite. Plants were checked daily and watered as needed. Fertilizer was not used.

Experimental set-up

In order to obtain apterous adults that would be less than 24 h old, third to fourth instar nymphs were collected into containers so that their development could be monitored daily. Third to fourth instar nymphs with wingbuds, darkened protrusions on the sides of the body from which wings will develop, were also collected into containers in order to obtain adult alates that were less that 24 h old. All aphids were collected from colony plants and placed onto susceptible leaves, variety SD01-76R, within clear plastic containers (10 cm diameter x 4 cm high) with a fine mesh screen lid (6 cm diameter). Within each container, a leaf was placed onto moistened filter paper and the leaf stem was inserted into a 2 ml plastic pipette tube containing distilled water. Collected aphids

inside their containers were held in a growth cabinet set at 25°C with a 16L:8D h diel cycle. Each morning, aphids that had developed into adults were removed from the containers and placed into smaller Millipore petri dishes (5 cm diameter x 1 cm height) containing only moistened filter paper (43 mm) for a 1 h starvation pre-treatment prior to the start of the experiment.

The experimental Y-tube olfactometer consisted of air flowing into 2 Whatman® activated carbon filter devices with pleated HEPA filters at a rate of approximately 800 ml/min. This odour free air then passed through 2-125ml Pyrex® flasks containing distilled water at room temperature to moisten the air. Odour free moist air flowed into both sides of the top of the Y-tube and then the 2 streams of air met and flowed out the bottom of the Y-tube. The Y-tube was held within a black wooden box (10.5 cm long x 6.5 cm wide x 9.5 cm high) and held at an upright angle of 45° to allow aphids to walk upwards as aphids have a natural tendency to walk upwards. The top of the black box was absent, instead a piece of plastic cut from a white plastic cup was placed over the opening to provide diffuse lighting from a fluorescent light placed above the top of the box as the sole source of light in the room. Aphids were introduced into the base of the Y-tube in a hole at the bottom of the black box and were then directed upwards towards the arms of the Y-tube with the odour sources and diffuse lighting. For the odor sources, soybean plants at the unifoliate stage were cut just above the cotlydon. The stem was placed into a 2 ml pipette tube containing distilled water. Treatments consisted of susceptible plants versus odour-free air, resistant plants versus odour-free air, and susceptible plants versus resistant plants with 83, 91, and 85 aphids tested respectively for the apterous adults and 71, 75, and 79 aphids tested respectively for the alatoid adults.

All three treatments were run on each day of the experiment consecutively. A series of coin tosses determined the order of the treatments and which arm of the Y-tube would contain which odour source. Between treatments, the apparatus was cleaned with Bio-Green™ biodegradable soap and water and fresh plants were obtained.

Aphids were placed one at a time into the Y-tube and were given 8 minutes to move. Aphids were tested individually to prevent bias occurring from aphids following each other so that at any given time, there was only one aphid within the olfactometer. I decided that an aphid had 'chosen' a side when an aphid walked through one arm of the Y-tube and into a tube extending past each arm and the top of the box, at which point the aphid was removed from the olfactometer by tapping the tube. Minimum and maximum temperatures were recorded from a digital indoor/outdoor thermometer after each aphid was placed into the base of the Y-tube. The minimum temperature was taken from outside the range of the light above the olfactometer. The maximum temperature was taken from below the light which was positioned above the olfactometer.

Statistical analysis

The number of aphids that walked towards each odour source was scored accordingly. Treatments were not compared due to differences between the sample numbers for each treatment. The amount of time it took for aphids to 'choose' an odour source and the number of aphids that did not 'choose' an odour source appeared to be similar between treatments but were not statistically compared due to the differences in the sample numbers for each treatment. A chi-square likelihood ratio test was used to test whether the number of aphids that chose each odour source within each treatment was similar assuming a hypothetical probability of 0.5.

Results

The mean (± SE) minimum temperature was 22.76°C ± 0.04 and the mean (± SE) maximum temperature was 23.50°C ± 0.04 for the duration of the experiment.

The only treatment amongst the apterous adults that showed a difference between the odour sources was the treatment involving resistant soybean seedlings and odour-free air (Figure 4-1). A greater number of apterous adults chose odour-free air over resistant soybean seedlings ($\chi^2_{1,91} = 6.96$, P = 0.0083). There was no difference between susceptible soybean seedlings and odour-free air ($\chi^2_{1,83} = 0.11$, P = 0.74), or resistant and susceptible soybean seedlings ($\chi^2_{1,85} = 0.29$, P = 0.59).

There were no differences seen in any of the treatments involving alatoid adult aphids (Figure 4-2). The number of adult alates that chose susceptible soybean seedlings was similar to the number that chose odour-free air ($\chi^2_{1,71} = 0.13$, P = 0.72). The number of adult alates that chose resistant soybean seedlings was similar to the number that chose odour-free air ($\chi^2_{1,75} = 0.01$, P = 0.91), and the number of adult alates that chose resistant soybean seedlings was similar to the number that chose susceptible soybean seedlings ($\chi^2_{1,79} = 0.62$, P = 0.43).

Discussion

Adult alates showed no attraction to susceptible plant odours, resistant plant odours, or odour free air in any of the treatments. As well, adult alates were not repelled by susceptible plant odours, resistant plant odours, or odour-free air in any treatment. A previous study by Du et al. (1994) found that soybean aphid was attracted to soybean plant volatiles. The difference between the results found by Du et al. (1994) and the

results found here may be due to the test aphids having a learned preference for the colony cultivar, Mycogen 5261, over the tested cultivar, the susceptible soybean variety SD01-76R. Apterous aphids also did not show an attraction towards the Dowling cultivar.

Plant cues on the surface of a plant leaf as well as cues detected through stylet penetration are important for host determination in many aphid species (Powell et al. 2006). Previous evidence has shown that preference for spindle leaf by winged autumn migrants of the black bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae) was shown after probing plant tissue and that factors detected during stylet penetration either inhibited take-off or did not (Powell and Hardie 2000). Powell et al. (2006) placed probing of plant tissue as an important stage in host selection. It is likely that soybean aphids require further cues from the host plant, such as stylet penetration or chemicals detected on the leaf surface to determine acceptability of a host plant. These cues are absent in an olfactometer experiment.

Apterous aphids did not show a preference for either resistant or susceptible plant volatiles when given a choice between susceptible and resistant plant volatiles. Due et al. (1994) found that non-host odours were able to mask the attractiveness of susceptible host plant odours by changing the overall volatile profile. It is possible that odour from both plant sources changed the overall volatile profile masking the individual plant volatiles from the resistant and susceptible plant leaves. It is not known why resistant plant volatiles repelled apterous adults and not adult alates. One likely reason is the morphological differences between the alates and the apterae. Alates are equipped for

wind dipersal and would have a difficult time walking with wings whereas apterae are better suited for walking.

There are morphological differences in the antenna between alate and apterous aphids. Alatoid aphids have a greater number of secondary rhinaria, scent detectors, than apterous aphids (Du et al. 1995). It is possible that this difference could have affected the results. However, it has been shown that the primary rhinaria, present in both morphs, are responsible for the detection of plant volatiles and that this detection is similar for both morphs (Park and Hardie 2002). Secondary rhineria did not detect plant volatiles but did detect sex pheromones in males and gynoparae (Park and Hardie 2002).

In conclusion, soybean aphid alates did not show any attraction and were not repelled by susceptible or resistant plant volatiles when presented a choice between the two or individually against odour-free air. Virginoparae alates are thought to be responsible for host location when migrating from the primary host and when dispersing to other fields. It is believed that stylet penetration or other plant cues may be important factors in choosing a host plant. These results do not indicate if alates were able to detect plant volatiles. An electroantennogram test would be required to test the detection of odours by the soybean aphid. To my knowledge, an electroantennogram has not been conducted on the soybean aphid. Apterous adults were repelled by resistant plant volatiles. Intercropping resistant and susceptible plants may prevent dispersion from plant to plant by apterous individuals. Further research on sensory cues and movement by soybean aphids between plants is necessary to understand host selection by these aphids.

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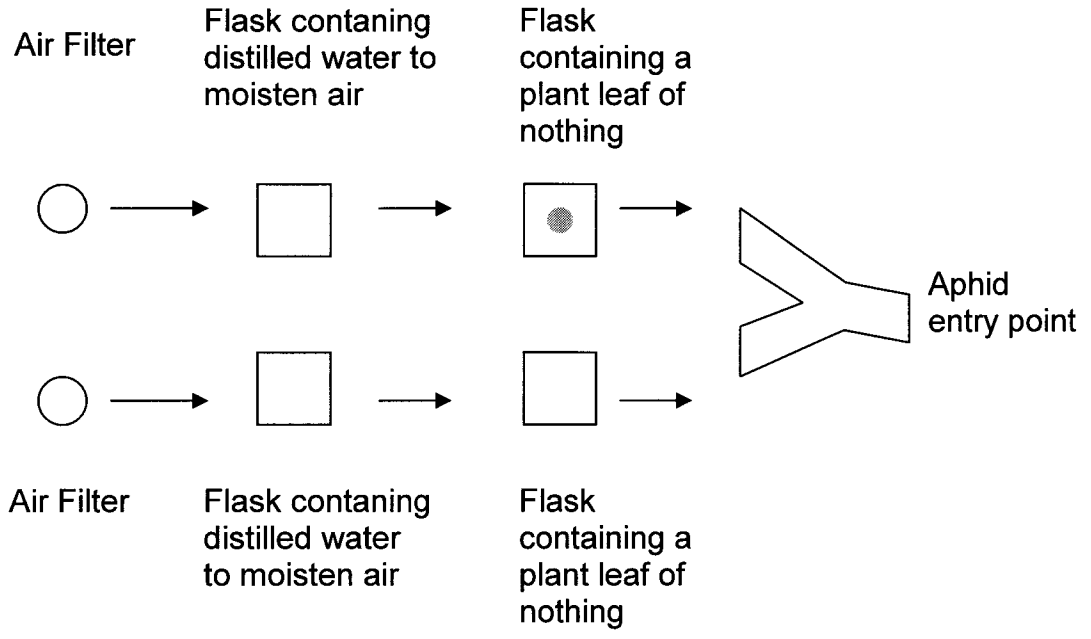

Figure 4-1. Filtered moist air picks up the odour of a soybean leaf, if present, before entering one arm of the Y-tube towards a test aphid. Test aphids that walk to the end of one of the arms of the Y-tube is recorded as having 'chosen' the odour source which flowed through that arm. Arrows show the direction of air flow.

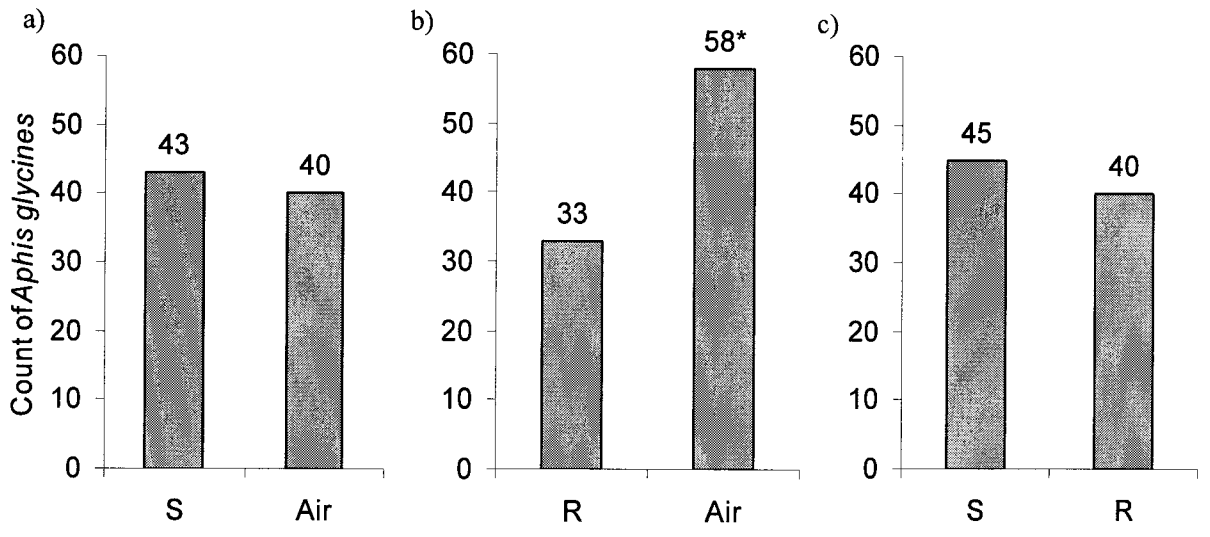

Figure 4-2. Total number of apterous adult *Aphis glycines* which walked towards an odour source when given a choice between a) susceptible soybean seedlings (S) and odour free air (Air), b) resistant soybean seedlings (R) and odour free air, and c) susceptible and resistant soybean seedlings. Numbers above each bar indicate the number that 'chose' each category. The sample numbers for treatments a), b) and c) are 83, 91, and 85 respectively. Numbers with asteriks are different from each other (*$P<0.05$).

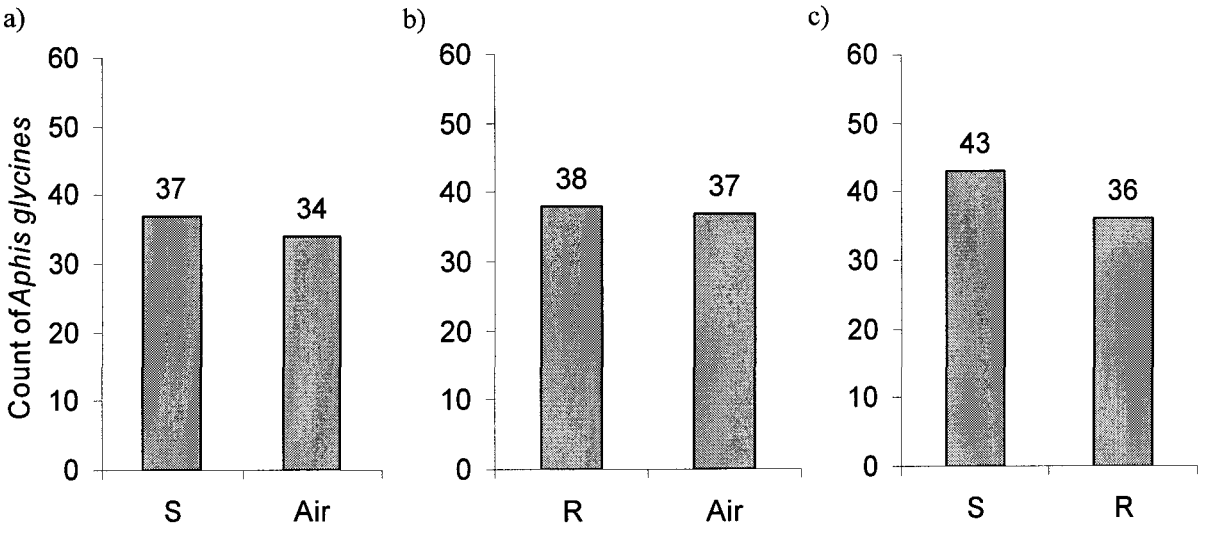

Figure 4-3. Total number of adult alate *Aphis glycines* which walked towards an odour source when given a choice between a) susceptible soybean seedlings (S) and odour free air (Air), b) resistant soybean seedlings (R) and odour free air, and c) susceptible and resistant soybean seedlings. Numbers above each bar indicate the number that 'chose' each category. The sample numbers for treatments a), b) and c) are 71, 75, and 79 respectively. No differences were observed between each set of categories.

Chapter 5

General discussion and conclusions

Aphids are a widespread and costly pest in agricultural systems. Aphid characteristics, such as asexual reproduction, parthenogenesis and alary clonal polyphenism, allow aphids to quickly reproduce, develop, and disperse. The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), has been an invasive pest in North America since 2000 (Alleman et al. 2002). The soybean aphid presents a threat to soybean, *Glycine max* (L.) Merr., yield by reducing plant nutrients (Gill and Sanderson 1998) and reducing pod set if infestation occurs during the reproductive stages of soybean development (Sun et al. 1990). By feeding on diseased plants followed by healthy plants, the soybean aphid spreads plant disease (Blackman and Eastop 2000; Hill et al. 2001). Like other sap sucking insects, the soybean aphid excretes a sticky substance known as honeydew. Honeydew provides nutrients for the growth of sooty mould (Li et al. 2004) which can inhibit photosynthesis.

Soybean aphid populations may be naturally managed by rainfall, low humidity, extreme heat, extreme cold (which can kill overwintering eggs), and natural enemies. Drought and mild temperatures throughout the winter and summer seasons may lead to soybean aphid outbreaks. Once aphids reach a high level of infestation, they can be difficult to quickly control. To date, insecticide use remains the most common method for reducing high populations of soybean aphid. The North Central Soybean Research Program (NCSRP) recommends using insecticides when aphids reach the economic threshold of 250 per plant in the early reproductive stages of soybean development (2007).

An alternative measure for reducing aphid populations in soybean fields is using plant resistance. Resistant plants have genetic qualities which result in less plant damage by pests than plants lacking the genetic qualities (Smith 2005). There are three categories of plant resistance mechanisms: antibiosis resistance, antixenosis resistance, and tolerance (Smith 2005). Antibiosis-type resistance affects the biology of the soybean aphid by decreasing fecundity (Diaz-Montano et al. 2006; Hill et al. 2004; Li et al. 2004) and increasing mortality (Hill et al. 2004; Li et al. 2004). Antixenosis-type resistance affects the behaviour of soybean aphid. In choice tests, the soybean aphid accumulates more on susceptible cultivars than those with antixenosis-type resistance (Mensah et al. 2005). Tolerance is a term used to explain a plant's ability to withstand or recover from plant damage caused by insect pests (Smith, 2005).

In my second chapter, I hypothesized that resistance may vary in different stages of soybean development and that this variation may lead to differences in soybean aphid fecundity between the different growth stages. To test my hypothesis, I recorded soybean aphid fecundity across the first trifoliate (V2), beginning bloom (R1), beginning pod (R3), and full seed (R6) stages of soybean growth. My results showed that resistance did vary across the different growth stages; however, this difference is likely due to other factors that affected fecundity and not due to a difference in resistance levels. There are two reasons for this final conclusion. First, it has been found that the fecundity of aphids on susceptible cultivars does not change across different growth stages (Rutledge and O'Neil 2006). In my study, the fecundity recorded for the resistant cultivars increased and decreased in synchrony with the susceptible cultivars throughout soybean growth. If the levels of resistance varied, I would have expected a change in the rise and fall of the

fecundity between the resistant and susceptible cultivars. Second, aphid fecundity can be affected by plant nutrients. The amount of nitrogen in plant leaves can change due to factors such as soil potassium deficiency (Walter and DiFonzo 2007) and plant stress (White 1984). The first trifoliate and full seed stages of soybean development had the highest overall fecundity amongst both susceptible and resistant cultivars in both fields. Young foliage (Hikosaka et al. 1994) and senescing plants (White 1984) typically have a greater abundance of nitrogen available in plant phloem for aphid feeding than plants in the early reproductive stages. Higher levels of nitrogen available to aphids in the first trifoliate and full seed stages of soybean growth would have also led to higher aphid fecundity in these stages. Soil samples and plant tissue samples were not analyzed for the different soybean growth stages in this study.

After completing this study, it was pointed out that differences in the soil properties between the different plots may have affected aphid fecundity. The following summer, aphid fecundity was measured on three plots in different field locations. Soil samples were analyzed from each of the plots and were found to be similar. Aphid fecundity between the plots was also similar.

Overall, there was lower aphid fecundity on resistant cultivars at each growth phase compared with susceptible cultivars. Aphid fecundity was highest in the V2 and R6 stages of soybean development and was lowest in the R1 and R3 stages. Studies which have measured soybean aphid population densities have found higher aphid populations in the R1 and R3 stages of soybean development than the V2 and R6 stages. There are a few reasons to explain the higher population densities during these stages. The arrival time of aphids can have an effect on population densities and aphids

sometimes do not arrive until the V2 stage or later. Once aphids arrive at their host plant, they require some time to increase in population size. If aphids arrive at or before the V2 stage of development, then the population accumulated by the R1 and R3 stages would be larger than the V2 stage. Population densities would begin to decrease around the R6 stage of soybean development with the migration of aphids to the primary host plant, Buckthorn, *Rhamnus spp.* (Rhamnaceae).

An important aspect of resistance is the effect of the plant on soybean aphid behaviour. Behaviour is an important aspect to host location, and studying the effects of resistance on aphid behaviour is an essential step in determining how resistance works against the soybean aphid and how to refine the resistance if necessary (Bernays and Chapman 1994). Knowledge of how soybean resistance affects host location by the soybean aphid will also help determine other strategies for soybean aphid control. Chapters 3 and 4 focused on soybean aphid behaviour in response to plant resistance.

It has been seen in other aphid species that dispersal from plant to plant is a common and natural event for apterous aphids (Hodgson 1991) and that predators sometimes aid in the dispersal behaviour (Roitberg et al. 1979). Dispersal by aphids has both positive and negative effects on field plants. Since aphids multiply quickly, dispersal can lead to many field plants being affected by nutrient loss, reduced yield, and possibly disease in a short period of time. However, if populations are kept to a manageable level by natural enemies, environmental conditions, and aphid control strategies, dispersal relieves population pressures on the plants the aphids disperse from and can help keep aphid populations at a manageable level (Hodgson 1991). Aphids may leave plants due to a negative stimulus such as a predator or alarm pheromone (Losey and

Denno 1998; Roitberg 1979) and are more likely to leave a host plant in cool moist conditions than in hot dry conditions (Dill et al. 1990). Aphids are also more likely to leave poor quality host plants than good quality host plants (Dill et al. 1990; Hodgson 1991; Losey and Denno 1998). In the third chapter, I hypothesized that if aphids were able to choose a host plant, they would choose a susceptible plant over a resistant plant and that they would abandon a poor quality host in favour of a good quality host. To test this hypothesis, aphids were placed on either a resistant (poor quality) or a susceptible (good quality) plant within an arena and the number of aphids found on that plant and a second plant in the arena, if present, was counted 24 h later. Treatments included all combinations of susceptible and resistant plants as well as a susceptible plant with no second plant and a resistant plant with no second plant. More aphids left resistant plants than susceptible plants, likely due to the poor host quality of the resistant plants. Since the type or presence of the second plant had no effect on the number of aphids leaving the initial plant, it was concluded that aphids will leave a plant without knowing if a second plant is available. This finding supports the finding by Hodgson (1991) in which random dispersal of apterous and alatoid aphids occurred. Random dispersal would also explain movement of aphids from susceptible plants. When the second plant was susceptible and the initial plant was resistant, more aphids were retained on the susceptible plant after 24 h than in any other treatment likely due to more aphids leaving the initial resistant plant and the presence of a suitable host preventing the aphids from continuing to disperse. This finding was only significant amongst the adult apterous aphids and may be due to a greater amount of movement by the apterous adults compared to the immature nymphs and adult alates. Adult apterous aphids have shown a greater amount of movement by

walking than immature nymphs and adult alates in other studies (Gish and Inbar 2006; Hodgson 1991; Roitberg et al. 1979).

Prior to this study, it was unknown whether the decision to select a susceptible plant over a resistant plant was due, in part, to plant odours. Since the soybean aphid has a non-preference for resistant soybean, I hypothesized that the non-preference may be due to resistant plant volatiles and that the soybean aphid may be repelled by resistant plant volatiles. To test this hypothesis, I used a Y-tube olfactometer to test the preference of the soybean aphid to resistant soybean leaves versus odour free air, susceptible soybean leaves versus odour free air, and susceptible soybean leaves versus resistant soybean leaves. Neither attraction nor repulsion was observed in any of the treatments involving adult alates. Resistant plant volatiles, however, repelled apterous adults.

An attraction to susceptible soybean has previously been observed in a study by Du et al. (1994). A possible explanation for the lack of attraction seen here may be due to a preference by the aphids to the colony cultivar (Mycogen 5261) over the test cultivar (SDO-76R). Although adult apterous aphids were repelled by resistant plant material, no distinction was made between the odours from susceptible and resistant plants when these odours were tested together. This may have been due to a change in the overall volatile profile from the two plant odours mixing. Du et al. (1994) showed that non-host plant odours masked the attractiveness of host plant odours. Alates were not repelled by the resistant plant material. Although there are differences in the structure of olfactory sensilla between alate and apterous aphids (Du et al. 1995), it has been shown that host plant cues are detected by the primary rhinaria which are similar between the alate and apterous morphs (Park and Hardie 2002). It is possible that soybean aphid alates were

able to detect odours but required other cues from the host plant, such as probing, to determine preference.

Plant resistance to the soybean aphid provides an alternative measure to insecticide use. Although plant resistance can be less costly and hazardous than insecticides, there are still potential problem which need to be addressed. In fields, natural enemies are a benefit to farmers and help control pest populations. When using control methods against pests, it is important to consider the effects of the control measure on natural enemies (Cortesero et al. 2000). It is not known if plant resistance has a negative effect on soybean aphid predators and parasitoids. There is also the possibility of the development of counter-resistance in the soybean aphid. A study by Kim et al. (2008), found that there are at least two soybean aphid biotypes in North America.

Overall, I suggest that soybean resistance would be an effective control measure against the soybean aphid. It is recommended that plant resistance be kept to a low or moderate level, which is the current situation in Canada, as high resistant levels can increase the speed of counter-resistance by aphid species (Gould et al. 1991). Future research should investigate intercropping resistant and susceptible plants. Intercropping may slow the rate of dispersal from plant to plant and may decrease the likelihood of counter-resistance being developed in the soybean aphid.

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