# Management options for insecticide-resistant soybean aphid: Effectiveness of a novel insecticide and implementation of host plant resistance

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# Dedication

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# Abstract

Management of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is threatened by a limited number of insecticide groups and evolution of resistant to one of the commonly used groups (i.e., pyrethroids). There is a need to evaluate additional chemical and non-chemical tools for management of soybean aphid. A novel insecticide, afidopyropen is available for soybean aphid management, but the baseline susceptibility of soybean aphid to this insecticide is unknown. Using a leaf-dip bioassay, susceptibility of several populations of soybean aphid was assessed during 2017 and 2018. Variation in susceptibility was found among populations with concentrations of afidopyropen necessary to kill 50% of a population ($LC_{50}$) for soybean aphid adults ranging from 0.0013 to 0.40 mg a.i. per liter. Concentrations of afidopyropen as low as 0.0285 mg a.i. per liter were able to kill 100% of soybean aphid nymphs after 6 days compared with control. Afidopyropen appears to be an effective tool for use against nymph and adult of soybean aphid. Host plant resistance is a promising strategy for management of soybean aphid, but further understanding on the potential implications of the adoption of aphid-resistant soybean on management of soybean insects is needed. A greenhouse experiment and field experiments were used to assess movement and spatial pattern of soybean aphid on aphid resistant plants with *Rag* genes. More movement of soybean aphid was found in aphid-resistant varieties (i.e., Rag1, Rag1+Rag2) than aphid-susceptible soybean. However, under field conditions, the spatial patterns of soybean aphid in plots with susceptible, *Rag1* or pyramided *Rag1+Rag2* varieties were all aggregated and did not differ among varieties in vegetative and reproductive growth stages. In addition, three-years of field experiments were used to access compatibility of aphid-resistant soybean with several non-target herbivores. Plant genotype (i.e., *Rag1+Rag2* genes and no *Rag* genes) affected densities of soybean aphid, with fewer soybean aphid on aphid-resistant soybean than aphid-susceptible soybean. Furthermore, plant genotype affected seasonal abundance of bean leaf beetle (*Cerotoma trifurcata* Forster), potato leafhopper (*Empoasca fabae* Harris) and green cloverworm (*Hypena scabra* Fabricius). Host plant resistance with *Rag* genes appears to be compatible with scouting recommendations for soybean aphid and management of other pests.  This thesis provides essential information for growers and scientists to incorporate these additional management tools into soybean aphid management programs.

**Key words**: soybean aphid, afidopyropen, *Rag* genes, spatial pattern

# Table of Contents

# List of Tables

# List of Figures

## Introduction

Soybean is an important crop in the U.S. with an estimate of 88.34 million acres of soybean harvested in 2018 (USDA-NASS 2018). Soybean is used mainly to produce oil and protein for human and animal consumption. Soybean provides protein for several animals' diets, due its high level of essential amino acids (Cromwell 2000). One of the many challenges faced in production of soybean in the U.S. is the attack by insect pests, such as soybean aphid (*Aphis glycines* Matsumura) (Hemiptera: Aphididae) (Hodgson et al. 2012b).

Soybean aphid, an invasive species from Asia, is the most important insect pest of soybean in the Midwest Region of the U.S. (Ragsdale et al 2007, 2011). The first detection of soybean aphid in the U.S. occurred in 2000 in Wisconsin (Alleman et al. 2002) with rapid spread of this pest across several states in the U.S. and part of Canada (Venette and Ragsdale 2004). The life cycle of soybean aphid is complex requiring two hosts (i.e., heteroecious) and having asexual and sexual reproduction (i.e. holocyclic) (Ragsdale et al. 2004). Soybean aphid uses several species of buckthorn (*Rhamnus* spp.) as its primary host (Voegtlin et al. 2004) and soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), as the main secondary host. In addition, Carolina horsenettle, *Solanum carolinense* Linneaus (Solanales: Solanaceae) and red clover, *Trifolium pratense* Linneaus (Fabales: Fabaceae), are possibly other secondary hosts (Ragsdale et al. 2004, Clark et al. 2006). Under favorable conditions, soybean aphid can have approximately 15 asexual generations on soybean (Li et al. 2000), with populations under field conditions able to double in 6-7 days (Ragsdale et al. 2007).

This pest is a piercing-sucking insect that removes sap from the phloem, consequently reducing plant height, pod number, seed number and seed size (Ragsdale et al. 2007, Rhainds et al. 2008, Beckendorf et al. 2008). In addition, this pest can transmit viruses such as Soybean mosaic virus and Alfalfa mosaic virus (Hill et al. 2001; Mueller and Grau 2007). High infestation levels of soybean aphid can reduce soybean yield by 40% or more (Ragsdale et al. 2011). A potential economic impact is estimated to be from $2.4 to 4.9 billion annually, due to yield losses and control cost (Song et al. 2006, Kim et al. 2008). To manage soybean aphid, it is recommended to use several management strategies in an integrated pest management (IPM) program that aims to reduce input cost and unnecessary insecticide application (Ragsdale et al. 2011, Hodgson et al. 2012a). Current management includes chemical control, biological control and host plant resistance (Ragsdale et al. 2011, Hodgson et al. 2012a).

The use of insecticides was the first method used to manage soybean aphid in the U.S. (Ragsdale et al. 2007) and until today is the main method to reduce soybean aphid infestations (Koch et al. 2018). In IPM programs, application of insecticides is determined by scouting and use of an economic threshold of 250 aphids per plant (Hodgson et al. 2004, Ragsdale et al. 2007, Koch et al. 2016). The insecticides used for soybean aphid management are broad-spectrum insecticides classified in three main groups: carbamates and organophosphate (Group 1A and 1B), pyrethroids (Group 3A), and neonicotinoids (Group 4A) (IRAC 2018, Koch et al. 2018). Organophosphates and pyrethroids are mainly used for foliar applications to control soybean aphid (Johnson et al. 2009, Koch et al. 2018). Neonicotinoids are mainly used in seed treatments (Magalhaes et al. 2009), but also for foliar applications (Koch et al. 2018). The preference for organophosphate insecticides is due to relatively low price and high efficacy against a challenging secondary pest, the two-spotted spider mite (*Tetranychus urticae* Koch) (Trombidiformes: Tetranychidae) in soybean fields (Ragsdale et al. 2011). The preference for pyrethroid insecticides is largely due to a higher persistence and efficacy at relatively low rates (Ragsdale et al. 2011).

The use of insecticides has increased greatly in soybean since the arrival of the soybean aphid in the U.S. (Koch et al. 2018). Before 2000, less than 0.1% of the soybean crop was treated with foliar insecticides (Ragsdale et al. 2011). In ten years after the appearance of soybean aphid in the U.S., there was an increase of 130-fold in insecticide application to soybean (Ragsdale et al. 2011). This increase in insecticide use may cause unwanted impacts, such as negative impacts to non-target insects (Ripper et al. 1951, Weinzerl 2009, Gentz et al. 2010, Torres and Bueno 2018), human poisoning (Yu 2008), environmental contamination (Carriger et al. 2006, Hussain et al. 2009, Bahlai et al. 2010), pest resurgence, pest replacement and insecticide resistance of pest populations (Pedigo and Rice 2009). The appearance of insecticide-resistant populations creates a challenge for management of soybean aphid, because there is already a limited number of groups of insecticide available to manage this pest (Koch et al. 2018). In 2015 and 2016, several populations of soybean aphid were detected to be resistant to the insecticides bifentrin and lamba-cyhalothrin, which are both pyrethroid insecticides (Hanson et al. 2017). In their study, the most resistant population expressed 39-fold decreased mortality compared to the laboratory susceptible population (Hanson et al. 2017). Rotation of insecticides groups is recommended to slow down resistance (Sparks and Nauen 2016, Koch et al. 2018). Therefore, there is a need to add more groups of insecticides to better manage soybean aphid using chemical control.

Afidopyropen is a new active ingredient coming from the fermentation of pyripyropene, which is produced by *Aspergillus fumigatus* Fresenius (Eurotiales: Trichocomaceae) (Gerwick and Sparks 2014, Sparks et al. 2017, Jeschke 2018). This insecticide is classified in a new mode of action: Group 9D (Chordotonal organ TRPV channel modulators) (IRAC 2018).  Afidopyropen acts by overstimulating and silencing the vanilloid-type transient receptor potential channels located in the chordotonal stretch receptor neurons (Kandasamy et al. 2017). Chordotonal receptors present in insects are a type of mechanosensor responsible to detect the body position, external movement and air-born vibration at articulations (Kavlie and Albert 2013). Afidopyropen received registration in 2018 in the U.S. to be used against several piercing-sucking insects, such as Aphididae (Leichter et al. 2013, Tejeda-Reyes et al. 2017, Vafaie and Grove 2017), Aleyrodidae (Kumar et al. 2018) and Liviidae (Solís-Aguilar et al. 2015, Chen et al. 2018, Stansly and Kostyk 2018). Afidopyropen has the potential to be used in IPM programs to manage soybean aphid. However, little is known about the susceptibility of soybean aphid to this new active ingredient. It is necessary to explore the effect of afidopyropen on adults and nymphs of soybean aphid under controlled conditions as well as test its efficacy against soybean aphid under field conditions.

In addition to chemical control, another promising strategy for soybean aphid IPM is host plant resistance (Wiarda et al. 2012, Hill et al 2012, Hesler et al. 2013). For more than a decade, the USDA soybean germplasm collection has been screened for sources of resistance against soybean aphid (Hill et al. 2006). Several genes that confer resistance against soybean aphid have been identified (Hill et al, 2004, Mensah et al. 2005, Mian et al. 2008a,b, Zhang et al. 2009, Hill et al. 2010, Hesler et al. 2013). *Rag* genes (i.e. *Rag1, rag1b, rag1c, Rag2, Rag3, rag3, rag4,* and *Rag5*) can confer resistance against soybean aphid through antixenosis (i.e., change in behavior), antibiosis (i.e., change in biology of the pest) or a combination of both categories (Hesler et al. 2013). Studies performed with *Rag1* and *Rag2* soybean lines have shown that *Rag1* and *Rag2* can efficiently reduce soybean aphid populations (Hill et al. 2006, 2009, McCarville and O'Neal 2012, 2013, Kim et al. 2013, Varenhorst et al. 2015). However, the availability of commercial varieties with aphid resistance remains low with just soybean varieties containing *Rag1* and *Rag1+Rag2* genes (Hanson et al. 2016, 2019). Also, soybean lines expressing the tolerance category of resistance have also been found, but are similarly limited in availability (Pierson et al. 2010, Prochaska et al. 2013, Marchi-Werle et al. 2014).

Management of soybean aphid using *Rag* genes may be jeopardized due to soybean aphid biotypes that are able to overcome the resistance conferred by *Rag* genes (Kim et al. 2008, Hill et al. 2012). Four biotypes of soybean aphid have been identified in North America and are named as biotypes 1 to 4 depending on ability to colonize *Rag1* and *Rag2* genes (Hill et al. 2004, Kim et al. 2008b, Hill et al. 2010, Alt and Ryan-Mahmutagic 2013). High variation in occurrence of biotypes of soybean aphid across years and locations in the Midwestern U.S. was reported by Cooper et al. (2015). This variation suggests that biotypes may impact the effectiveness of this strategy.

To better understand the mechanism of resistance behind the *Rag* genes (i.e. antibiosis and antixenosis), several studies have been performed and some major differences in plant quality, phloem, gene expression and aphid behavior have been found for aphid-resistant plants compared with susceptible plants (Diaz-Montano et al. 2007, Li et al. 2008, Chiozza et al. 2010, Whalen and Harmon 2012, Studham and MacIntosh 2013). Soybean containing the *Rag1* gene has lower levels of some amino acids than susceptible soybean (Chiozza et al. 2010). This difference in amino acids may help explain the lower ability of aphids to reproduce and survive on aphid-resistant plants. In addition, a difference in aphid ability to reach the phloem and time spent feeding were found for *Rag1* and *Rag2* soybean compared with susceptible soybean, with aphid spending 7.5 h to reach the phloem of *Rag1* soybean compared with 3.5 h for susceptible soybean (Diaz-Montano et al. 2007, Baldin et al. 2018). Despite the effect of the *Rag1* gene on behavior of soybean aphid being explored, there is a lack of studies about the impacts of other *Rag* genes on the behavior of soybean aphid and the resulting effect of these genes under field conditions. This knowledge is vital to understand the implications of adoption of this technology under field conditions. Alteration in behavior could lead to change in the spatial pattern of soybean aphid (Whalen and Harmon 2012), which could affect sampling plan recommendations (Southwood 1978, Hutchison et al. 1988, Binns and Nyrop 1992). Therefore, there is a need to investigate the effect of other resistance genes on the movement of soybean aphid and the impact of these changes on the spatial pattern of soybean aphid under field conditions.

Adoption of host plant resistance in the context of integrate pest management programs may depend on compatibility of this strategy with non-target insects (Smith 2005). Therefore, it is essential to understand the potential impact of aphid-resistant soybean on non-target herbivores. Some laboratory and caged field studies have explored potential effects (i.e., preference, survival and developmental time) of *Rag* genes on non-target soybean herbivores (Chandrasena et al. 2012, Bruner el al 2013, Rich and Koch 2015, Özsisli and Prischmann-Voldseth 2016). Among the invasive species examined, Japanese beetle preferred *rag1b*, *rag3* and susceptible soybean leaves compared with *Rag1* soybean leaves in a laboratory choice test (Chandrasena et al. 2012). In addition, brown marmorated stink bug preferred aphid-resistant soybean pods containing *Rag1* after 4 hours. Furthermore, lower mortality of brown marmorated stink bug were found in *Rag1* compared with no *Rag* genes. However, *Rag1* gene did not affect developmental time and weight of brown marmorated stink bug (Rich and Koch 2015). Among the native herbivores examined, adults of several beetles (i.e., northern corn rootworm, southern corn rootworm and bean leaf beetle), showed no preference between *Rag1* aphid-resistant and aphid-susceptible soybean were found. For caterpillars (i.e., fall armyworm, corn earworm and soybean lopper), negative impacts of the *Rag1* gene on conversion efficiency were found when caterpillars fed on aphid-resistant soybean (Bruner et al. 2013). Many of these studies were performed under laboratory conditions, so there is a lack of understanding about the possible impacts on non-target herbivores under field conditions. Understanding of the impact of this technology on other soybean pests will lead to better management of soybean using host plant resistance for soybean aphid.

Considering the challenges related to the reliance on pyrethroid and organophosphate insecticides for soybean aphid management, this thesis aims to provide better understanding about IPM options using a novel insecticide group and host plant resistance. In Chapter 1, I investigate the effect of *Rag1* and pyramided *Rag1+Rag2* aphid-resistant soybean varieties on movement of soybean aphid on plants under greenhouse conditions and the impact of such movement on the spatial pattern of soybean aphid among plants under field conditions. In Chapter 2, I investigate possible impacts of aphid-resistant soybean containing *Rag1+Rag2* genes on the abundance of non-target herbivores under field conditions. In chapter 3, I evaluate the baseline-susceptibility of soybean aphid populations to afidopyropen. Results from my thesis will provide information to improve the management of soybean aphid using chemical control and host plant resistance.

# Chapter 1: Impact of single gene and pyramided aphid-resistant soybean on movement and spatial pattern of soybean aphid (Hemiptera: Aphididae)

## Summary

Host plant resistance may be an effective option to manage soybean aphid, *Aphis glycines* (Matsumura), an important pest on soybean (*Glycine max* (L.) Merr.) in the U.S. Movement of soybean aphid may be altered by the presence of resistance (i.e., *Rag*) genes in soybean and changes in movement may affect the spatial pattern of a species. The present study aims to assess the effects of *Rag1* and pyramided *Rag1+Rag2* aphid-resistant varieties on movement of soybean aphid under laboratory conditions and to evaluate potential impacts of this movement on spatial pattern of soybean aphid under field conditions. Results from the greenhouse study showed more movement of soybean aphid on both aphid-resistant varieties than the susceptible variety when aphids were placed on unifoliate leaves and no statistically significant difference in movement between *Rag1* and pyramided *Rag1+Rag2* varieties. When aphids were placed on new growth, movement was greater on pyramided *Rag1+Rag2* than the *Rag1* and susceptible variety. However, under field conditions, the spatial patterns of soybean aphid in plots with susceptible, *Rag1* or pyramided *Rag1+Rag2* varieties were aggregated and did not differ among varieties in vegetative and reproductive growth stages. These results are of relevance because they suggest that aspects of soybean aphid management that may be sensitive to changes in spatial pattern of the pest (e.g., natural enemy efficacy and sampling plans) may not be impacted by implementation of varieties with these resistance genes for host plant resistance.

**Key words:** *Aphis glycines*, host plant resistance, pyramided resistance, sampling, Taylor's Power Law

## Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybean (*Glycine max* (L.) Merr., in the midwestern U.S. and southern Canada (Ragsdale et al. 2007, 2011). Since its appearance in 2000 (Alleman et al. 2002), soybean aphid spread to 21 U.S states and three Canadian provinces (Venette and Ragsdale 2004) causing an estimated economic impact of U.S.$2.4 - 4.9 billion annually (Song et al. 2006, Kim et al. 2008a). Chemical management with insecticide applications has been the primary method to manage this pest (Hodgson et al. 2010). After the detection of soybean aphid, there was a 130-fold increase in insecticide use in soybean (Ragsdale et al. 2011). With this increase in insecticide application due to presence of soybean aphid, there has been increased concern about collateral damage, such as negative impacts to non-target insects (i.e., pollinators and natural enemies) (Johnson and Tabashnik 1999, Johnson et al. 2008, O'Neil and Johnson, 2010) and development of insecticide resistance. The recently discovered resistance of soybean aphid populations to pyrethroids in several U.S. states and a Canadian province (Hanson et al. 2017, Koch et al. 2018) coupled with the limited number of effective insecticide groups currently available (IRAC 2017, Koch et al. 2018) imposes a challenge to management of this pest. Considering this scenario, it is necessary to integrate other management strategies for soybean aphid (Pedigo 1995).

An alternative strategy for soybean aphid management is host plant resistance. Host plant resistance can be defined as a "heritable character that some plants have that allows them to avoid, tolerate or recover from the attack of insects under conditions that would cause greater injury to other plants of the same species" (Painter 1951). Host plant resistance is a desirable strategy for soybean aphid management because it is often effective, relatively inexpensive, compatible with other management strategies and has the potential to reduce insecticide load and associated adverse impacts (Pedigo 1999, Smith 2005, Tilmon et al. 2011, Hodgson et al. 2012a). Several resistance genes (*Rag* [Resistance to *Aphis glycines*] genes) have been identified for use against soybean aphid (reviewed by Hesler et al. 2013). Aphid-resistant soybean varieties, which are currently commercially available for the northern U.S., have *Rag1* or *Rag1*+*Rag2* genes (McCarville et al. 2012b, Bhusal et al. 2016).

These *Rag* genes (e.g., *Rag1*, *Rag1*+*Rag2*) can affect life-history traits, such as survival, fecundity and developmental time (i.e., antibiosis), and/or reduce the preference of the pest for the host plant (i.e., antixenosis). Both *Rag1* and *Rag2* affect soybean aphids through antibiosis, but *Rag1* also affects soybean aphids through antixenosis (Hesler et al. 2013). Through antixenotic effects, aphid-resistant soybean may alter the movement of soybean aphid. Li et al. (2004) and Whalen and Harmon (2012) showed that aphid-resistant varieties containing the *Rag1* gene alter the movement of soybean aphid within and among soybean plants under laboratory conditions. However, it is unknown if other *Rag* genes or combinations of *Rag* genes alter soybean aphid movement.

Changes in aphid movement within and between plants could have important consequences for soybean aphid biology and management. For example, changes in aphid movement among plants could potentially increase the transmission rates of viruses among soybean plants (Clark and Perry 2002, Shaw et al. 2017) consequently increasing the threat posed by this pest. Furthermore, changes in aphid movement among plants could result in changes to the pest's spatial pattern (i.e., pattern of the individuals in space: aggregated, random or uniform) (Turchin 1991, Wiens et al. 1995). The spatial pattern of a species is often related to the species' behavior (Taylor 1984) and can inform pest management decisions. For example, changes in the spatial pattern of soybean aphid could impact natural enemy efficacy. *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), which is a predator of soybean aphid, had different predation rates when *Aphis citricola* (van der Goot) were in uniform versus aggregated spatial patterns, suggesting that spatial pattern of prey can affect *H. axyridis* foraging (Feng et al. 2018). In addition, knowledge of the spatial pattern of an insect is vital for creation of effective sampling plans (Iwao 1968, Shaw et al. 1983, Ho 1993, Hodgson et al. 2004). Sampling plans provide guidance for the number of sample units (e.g., plants) from which aphid densities should be counted to provide a precise estimate of field- or plot-wide aphid density (Naranjo and Hutchison 1997, Athanassiou et al. 2005). On aphid-susceptible soybean varieties, the spatial pattern of soybean aphid is aggregated, and a sampling plan has been developed for estimation of aphid densities (Hodgson et al. 2004, Hodgson et al. 2010). Under current recommendations for soybean aphid management, the sampling plan is used to estimate aphid density in fields, which is then compared to an economic threshold of 250 aphids per plant to determine if foliar application of insecticide is warranted for management of this pest (Ragsdale et al. 2007, Koch et al. 2016). Though effects of the *Rag1* gene on soybean aphid movement have been documented (Li et al. 2004, Whalen and Harmon 2012), little is known about the consequences of aphid movement on spatial pattern of soybean aphid on aphid-resistant soybean under field conditions. Potential changes in the spatial pattern of soybean aphid could have consequences for biological control and sampling recommendations for this pest.

To further prepare growers for implementation of host plant resistance for soybean aphid management, knowledge gaps related to potential effects of aphid-resistance genes on movement and in-field spatial pattern of soybean aphid must be addressed. Therefore, the present study aims to assess the effects of *Rag1* and pyramided *Rag1+Rag2* aphid-resistant varieties on: (1) within plant movement of soybean aphid under greenhouse conditions and (2) to evaluate impacts of aphid-resistant varieties on spatial pattern of soybean aphid under field conditions.

## Materials and methods

### Greenhouse experiments

Near-isolines of aphid-resistant and -susceptible soybean were used to assess aphid movement on individual plants in greenhouse experiments. The near-isolines consisted of two aphid-resistant lines (i.e., IA3027RA1 and IA3027RA12 containing *Rag1,* and *Rag1+Rag2*, respectively) and an aphid-susceptible line (IA3027 with no *Rag* genes) (Brace and Fehr 2012; Wiarda et al. 2012). The insects used for the experiments were from a laboratory colony of biotype 1 soybean aphids (i.e., population of soybean aphid unable to colonize aphid-resistant plants containing *Rag1* or *Rag2* gene) originally obtained from the University of Illinois. The aphids were reared on SD01–76R (i.e., aphid-susceptible) soybean plants in an environmental growth chamber at 25°C, approximately 70% humidity, and a photoperiod of 16:8 h (L:D) at the University of Minnesota, St. Paul, Minnesota, USA.

Pots (10×10×10 cm) were filled to a depth of approximately 8 cm with potting soil (Sunshine MVP, Sun Gro Horticulture Products, Agawam, MA) and three soybean seed of one isoline were sown at a depth of 2 cm in each pot. To minimize fungus gnat (Diptera: Sciaroidea) infestation, a 1-cm layer of sand was added to the top of the soil in each pot (Harris et al. 1996). Prior to experimentation, pots were maintained in environmental growth chambers (1.8×0.8×1.2 m) at 25°C, a photoperiod of 16:8 h (L:D) and approximately 70% humidity. Pots received water three times a week by adding water to the flats in which the pots were held (e.g., Hill et al. 2004). Soon after germination, all pots were thinned to one plant per pot to reduce competition and to maximize plant size uniformity. Plants were grown to the V1 growth stage (i.e., first trifoliate unrolled) (Fehr and Caviness 1977) for use in experiments. Potted plants were transferred to a greenhouse at the University of Minnesota in January and February 2017. Conditions in the greenhouse were 25±5°C and a photoperiod of 16:8 h (L:D).

Two experiments were performed using the IA3027 near-isolines. In one experiment, aphids were released on a unifoliate leaf of each plant, and in the other experiment aphids were released on the newly developing first trifoliate leaf of each plant. Each experiment was conducted as a randomized complete block design with two blocks over time and 10 replications per block for a total of 20 replications (i.e., plants) per soybean line. Apterous adult aphids (identified by their apparent, much extended cauda, dark cornicles and absence of wing pads [Voegtlin et al. 2004, Hodgson et al. 2005]) were transferred with fine-tipped (size 4/0) paint brushes from infested SD01-76R plants from the laboratory colony into 60×15-mm Petri dishes containing pieces of filter paper dampened with reverse osmosis water. During aphid transfer, care was taken to ensure that the mouthparts of the aphids were not injured by gently prodding the aphids with the paint brush and allowing them to withdraw their stylets from the leaf tissue before transfer. The transfer of aphids to Petri dishes prior to placement on experimental plants allowed for the assessment of any injury caused by removal of aphids from the infested plants.

Ten apterous adult aphids were transferred from the Petri dishes to the adaxial side of a single unifoliate leaf on each plant or onto the newly developing first trifoliate leaf of each plant, depending on experiment. At 6, 24 and 48 h post infestation, the number of aphids (adults and nymphs) and their locations on the plants were recorded for each plant. During this interval, it was possible to visually distinguish the adult aphids that were transferred to the plants from nymphs produced by those adults. For all observation times, locations of aphids on plants were recoded as initial leaf on which aphids were released (i.e., unifoliate or newly developing first trifoliate, depending on experiment) or elsewhere on the plant. For 6, 24 and 48-h observation times, we calculated the proportion of initially released aphids recovered (i.e., remaining anywhere on the plant) and the proportion of recovered aphids remaining on the initial leaf. In these experiments, the plants were spatially isolated to prevent aphid movement among plants by placing each potted soybean on top of an inverted pot inside a flat containing water. Pots within flats were arranged approximately 5 cm apart so canopies of adjacent plants were not touching.

Data from the greenhouse experiments were analyzed using R Development Core Team software (version 3.3.3, 2017). The first analysis focused on the proportion of adult soybean aphids recovered at each observation period. The second analysis focused on the proportion of recovered adult soybean aphids remaining on the initial leaf on which they were released. For both analyses, we used generalized linear mixed model with the function ‘glmer’ from the ‘lme4’ package (Bates et al. 2015) for a binomially distributed response variable and logit as the canonical link. The predictor variables were soybean genotype, time as a repeated measure, and the interaction of genotype and time. Random effects for block and plant nested with block were also included. Type II Chi-Square Wald Test for fixed effects (i.e., genotype and time) and their interaction were performed using ‘car’ package (Fox and Weisberg 2011). Means for each genotype across all time periods were separated using Tukey’s HSD post hoc test.

## Field experiments

To assess the impact of aphid-resistance genes on spatial pattern of soybean aphid under field conditions, we analyzed per-plant aphid count data from aphid-resistant and -susceptible plots from eight experiments conducted from 2011 to 2016 in Lamberton and Rosemount, Minnesota, U.S. In Lamberton, the experiments were conducted at the Southwest Research and Outreach Center in 2011-2013. In Rosemount, experiments were conducted at the University of Minnesota Rosemount Outreach, Research and Education Park in 2013-2016. In each experiment, plots of near-isolines of aphid-resistant and -susceptible soybean were planted in randomized complete block experimental designs with four replications of susceptible and resistant near-isolines. Plots from which aphid data were collected did not receive seed- or foliar-applied insecticides.

In Lamberton, in mid-May 2011, 2012 and 2013, near-isolines of aphid-resistant (i.e., containing *Rag1*, *Rag1*+*Rag2*) and -susceptible soybean (i.e., containing no resistance genes) were sown at a rate of 384,800 seeds per hectare with 76-cm row spacing. These near-isolines were developed from a cross between A08-1243074 and LD08-89051a (McCarville et al. 2014a). Each plot at Lamberton was 15.2 m long and 9.1 m (six rows) wide. In Rosemount, on 11 June 2013, 10 June 2014, 27 May 2015, near-isolines of aphid-resistant (i.e., IA3027RA1: containing *Rag*1; IA3027RA12: containing *Rag*1+*Rag*2) and -susceptible soybean (IA3027: containing no resistance genes) were sown at a rate of 370,650 seeds per hectare with 76-cm row spacing. In Rosemount, on 18 May 2016, near-isolines of aphid-resistant (IA2104RA12: containing *Rag*1+*Rag*2) and -susceptible soybean (IA2104: containing no resistance genes) were sown as in the other years at Rosemount. Each plot in Rosemount was 9.1 m long and 3.05 m (4 rows) wide.

To assess aphid densities, soybean plants were randomly selected from interior rows of each plot at approximately weekly intervals from vegetative soybean growth to plant senescence (Fehr and Caviness 1977). During vegetative growth stages, 20 plants were selected from each plot. During the reproductive growth stages, 5 and 10 plants were selected from each plot at Lamberton and Rosemount, respectively. On each sample date, the total number of aphids (i.e., nymphs, and alate and apterous adults) on each plant was recorded.

Data from the field experiments were analyzed using R Development Core Team software (version 3.3.3, 2017). All data sets with mean aphid densities equal to zero were removed. The mean densities and corresponding variances were transformed using base 10 logarithm. To compare the spatial pattern (i.e., interplant variability) of soybean aphids in plots of aphid-resistant and -susceptible soybean, we used Taylor's Power Law (Taylor 1984) to assess the relationship between log variance and log mean of per-plant aphid counts as described by: $s^2 = a\ m^b$, where m is the sample mean and $s^2$ is the sample variance, $a$ is the antilog of the intercept and $b$ is the slope of the linear regression. The slope ($b$) describes the spatial pattern of the species, such that $b = 1$, $b > 1$ and $b < 1$ indicate that the spatial pattern is random, aggregated and uniform, respectively (Taylor 1984, Boag et al. 1991). Because of general differences in sample sizes over the growing season, data sets were grouped into two categories representing vegetative (i.e., V1 to V6) and reproductive stages (i.e., R1 to R6) (Fehr and Caviness 1977). A separate Taylor's Power Law analysis was performed for each category of growth stages.

To find the intercept and slope of the regressions, we used a general linear mixed model using the 'lmer' function from the 'lme4' package (Bates et al. 2015). In the primary model, log variance was described as a function of fixed effects for log mean, genotype (i.e., susceptible, *Rag1*, and *Rag1+Rag2*), and the two- way interactions. The model also included random effects for year (i.e., 2011 to 2016), location (i.e., Lamberton and Rosemount), experiment within location, and block within experiment. Result from the mixed effect model analyses included REML estimates and approximate Wald test statistics for the variance components of the random effects. A type II Chi-Square Wald-Test for fixed effects (i.e., log mean, gene) and all their interactions were performed using the 'car' package (Fox and Weisberg 2011). Non-significant ($P > 0.05$) interactions of main effects were removed through backward selection. Akaike's Information Criterion (AIC) was used to determine which random effects to maintain in the final model. The best-fit model was the model with lowest AIC value (Akaike 1974). The slope ($b$) for each regression was compared with 1 (i.e., random spatial pattern) using two-tailed, one-sample t-test. Tukey tests ($P > 0.05$) were performed to find the variance (related to different intercepts) for each genotype. The variances were adjusted based on the overall mean of the aphid density.

Assumptions of constant variance, and independent and normally distributed errors were assessed for each regression by plotting the residuals against the explanatory variable using qqplot, plotting the residuals against the fitted values, and building a histogram of the residuals. There was no evidence of violation of the assumptions above. To determine what percent of the overall variance of our data was explained by the fixed effects, we calculated the marginal $R^2$ (Nakagawa and Schielzeth 2013) using the package ‘piecewiseSEM’ (Lefcheck 2016).

## Results

### Greenhouse experiments

The proportion of adult aphids recovered was significantly affected ($P < 0.05$) by soybean genotype, time, and their interaction when aphids were released on unifoliate leaves or newly expanding trifoliate leaves (Table 1.1). For both release locations, significantly more aphids were recovered across all three periods of time on the susceptible soybean genotype (i.e., IA3027) than on the resistant genotypes (i.e., IA3027RA1 and IA3027RA12), but the proportion of aphids recovered across all three periods of time did not differ between resistant genotypes (Figure 1.1A and C). The proportion of adult aphids remaining on the initial leaves on which they were released was significantly affected ($P < 0.05$) by soybean genotype, time, and their interaction when aphids were released on unifoliate leaves and by soybean genotype and time when aphids were released on newly expanding trifoliate leaves (Table 1.1). When aphids were released on the unifoliate leaves, significantly more aphids remained across all three periods of time on the initial unifoliate leaves of the susceptible soybean genotype (i.e., IA3027) than on those of the resistant genotypes (i.e., IA3027RA1 and IA3027RA12), but the proportion of aphids remaining across all three periods of time on the initial unifoliate leaves did not differ between resistant genotypes (Figure 1.1B and D). When aphids were released on the newly expanding trifoliate leaves, significantly more aphids remained across all three periods of time on the newly expanding trifoliate leaves of the susceptible soybean genotype (i.e., IA3027) than on those of the *Rag1+Rag2* soybean genotype (i.e., IA3027RA12), but the proportion of aphids remaining on the unifoliate leaves of the *Rag1* soybean genotype (i.e., IA3027RA1) did not differ from those of the susceptible or *Rag1+Rag2* soybean genotypes (Figure 1.1B and D).

### Field experiments

On aphid-susceptible soybean, peak soybean aphid densities ranged from 18.6 to 1140.0 aphids per plant (Table 1.2). Of the seven site-years, four site-years had soybean aphid densities exceeding the economic threshold of 250 aphids per plant. In contrast, peak soybean aphid densities on aphid-resistant soybean ranged from 1.0 to 242.0 aphids per plant (Table 1.2).

To assess the spatial pattern of soybean aphid on soybean in vegetative growth stages, log variance of aphid density was best described as a function of main effects for log mean of aphid density and soybean genotype, and random effects for year, location, and experiment within location (marginal $R^2 = 0.97$). The slope ($b$) of the Taylor’s Power Law relationship, represented by the effect of aphid density in this model, was significant ($\chi 2 = 5637$, $df = 1$, $P < 0.0001$), and did not differ among soybean genotypes, as indicated by the lack of an interaction between aphid density and soybean genotype ($P > 0.05$).

Furthermore, this slope was greater than 1 (t = 8.83, $P < 0.0001$), indicating an aggregated spatial pattern of soybean aphid across soybean genotypes. The effect of soybean genotype in this model was significant ($\chi^2 = 11.28$, $df = 1$, $P = 0.003$), indicating that the intercepts of the Taylor's Power Law relationship differed among soybean genotypes (i.e., separate, but parallel Taylor's Power Law regression lines for the soybean genotypes) (Table 1.3). In general, since the slopes were the same for all lines, the adjusted variance is correlated with the intercept of each line. The adjusted variance across aphid densities was greater for aphids on the *Rag1* soybean genotype and least on the *Rag1*+*Rag2* soybean genotype ($P < 0.05$) (Table 1.3).

The spatial pattern of soybean aphid on soybean in reproductive growth stages was best described using log variance of aphid density as function of main effects for log mean of aphid density and soybean genotype, and random effects for year, location and experiment within location (marginal $R^2 = 0.95$). Like the vegetative growth stages, the slope of the Taylor's Power Law relationship for reproductive soybean growth stages, was significant ($\chi^2 = 7474$, $df = 1$, $P < 0.0001$) and did not differ among soybean genotypes, as indicated by the lack of an interaction between aphid density and soybean genotype ($P > 0.05$). In addition, the slope was greater than 1 (t = 15.21, $P < 0.0001$), indicating an aggregated spatial pattern of soybean aphid across genotypes. As for the vegetative soybean growth stages, the effect of soybean genotype in this model was significant ($\chi^2 = 10.90$, $df = 1$, $P = 0.004$), which indicated that the intercepts of the Taylor's Power Law relationship differed among soybean genotypes (i.e., separate, but parallel Taylor's Power Law regression lines for the soybean genotypes) (Table 1.4). The adjusted variance across aphid densities was greater for aphids on the *Rag1* soybean genotype and least on the susceptible soybean genotype ($P < 0.05$).

## Discussion

Management of soybean aphid has relied primarily on use of insecticides (Ragsdale et al. 2011, Hodgson et al. 2012a); however, this pest is developing resistance to commonly used insecticides (Hanson et al. 2017, Koch et al. 2018). Sustainability of soybean production could be improved through integration of non-chemical management strategies. Host plant resistance holds promise as an additional strategy for effective management of soybean aphid (Hesler et al. 2013, McCarville et al. 2014a,b). However, availability of aphid-resistant soybean varieties remains low (McCarville et al. 2012b, Hanson et al. 2016). As further research is performed to identify new sources of aphid resistance and incorporate the resistance in to well-adapted soybean varieties (e.g., Wiarda et al. 2012, McCarville et al. 2012b, Hanson et al. 2016, 2018), an understanding of potential implications of deployment of aphid-resistant soybean is necessary. The research presented here provides further examination of the effect of aphid-resistant soybean on intra-plant movement of soybean aphid under controlled conditions and provides the first assessment of the potential effects of aphid-resistant soybean on the spatial pattern (i.e., interplant variability) of soybean aphid under field conditions.

In our greenhouse studies, we confirm previous findings that rates of soybean aphid movement are greater on aphid-resistant than -susceptible soybean (Whalen and Harmon 2012). After 48 h about 55% of the biotype 1 soybean aphids (i.e., avirulent to resistance genes) disappeared from the plants containing *Rag1* or *Rag1*+*Rag2*, and about 70% of those that were recovered on resistant plants had moved from the initial leaves on which they were released. Interestingly, movement caused by the pyramided soybean (i.e., *Rag1*+*Rag2*) did not differ from soybean containing the single *Rag1* gene (Figure 1.1). This lack of difference in soybean aphid movement on these two soybean genotypes could be related to the mechanisms of resistance conferred by the *Rag1* and *Rag2* genes. Specifically, both genes confer antibiosis, but the *Rag1* gene, which occurred in both varieties, may also confer antixenosis (Hesler et al. 2013). The antixenotic effect of *Rag1* may have been expressed similarly for both varieties.

The relative differences in the proportion of aphids remaining on the initial leaves (i.e., release locations) between experiments with unifoliate and newly expanding trifoliate leaves as release locations (Figure 1.1B and D) might be related to differences of resistances factors in leaves of different ages or soybean aphid preferences for feeding sites on plants. A proposed model for defense against soybean aphid on aphid-resistant plants suggests that several genes are triggered in aphid-resistant soybean containing *Rag1*, which then trigger jasmonic acid and other soybean plant mediators (Li et al. 2008). Saravitz and Siedow (1995) found variation on lipoxygenases isozymes on soybean leaves with different ages. They found higher levels of lipoxygenases isozymes in young leaves with a decrease in amount as leaves age. In addition, they found higher levels of minor lipoxygenase isozymes in intermediate-aged leaves than young or old leaves (Saravitz and Siedow 1995). Lipoxygenases isozymes are involved with biosynthesis of jasmonic acid, a plant defense mediator used against wounding and pathogen attack (Saravitz and Siedow 1995). Therefore, factors in leaves of different ages could help explain the difference in movement observed when aphids were released on unifoliate and newly expanding trifoliate leaves. In addition, differences in movement of soybean aphid on unifoliate versus newly expanding trifoliate leaves could be related to preferences in feeding sites by soybean aphid. On soybean in vegetative growth stages, soybean aphid prefers newly expanding trifoliate leaves (Tilmon et al. 2011). This behavior is probably related to the higher amount of nitrogen (i.e., a limiting factor to soybean aphid population growth) in the new growth compared with unifoliate leaves. It is probable that aphids initially located on unifoliate leaves moved to the new growth in search of better nutritional quality.

The differences in aphid movement on aphid-resistant and -susceptible varieties could be a response to factors such as differences in plant volatiles, nutritional quality, structures, or chemistry. It seems unlikely that the differences in movement of soybean aphid on resistant and susceptible soybean was due to plant volatiles, because Lamont (2010) showed no preferences of soybean aphid for resistant (i.e., *Rag1*) versus susceptible soybean in an olfactometer study. Further work should examine potential volatile effects associated with other *Rag* genes. Nutritional composition, especially nitrogen content, of the soybean could drive differences in movement of soybean aphid. *Rag1*-containing soybean have lower concentrations of important amino acids (i.e., A-aminobutyric acid, asparagine, glutamine and glutamic acid, histidine, proline, and serine) than aphid-susceptible soybean (Chiozza et al. 2010), which could cause soybean aphid to move away from resistant plants in search of plants with better nutritional quality. In addition, it is possible that aphid-resistant varieties have physical and/or chemical differences in the phloem that could affect aphid behavior and movement. On aphid-resistant soybean (i.e., K1639, Pioneer 95B97, Dowling (*Rag1* gene) and Jackson), soybean aphid takes longer to reach the phloem and feeds for less time compared with aphid-susceptible soybean (Diaz-Montano et al. 2007). Diaz-Montano et al. (2007) suggest that in resistant plants, morphological or chemical factors related to the phloem tissue may affect the stylet penetration of soybean aphid. Differences in gene regulation between susceptible and resistant plants with *Rag1* gene do exist (Studham and MacIntosh 2013) and could be responsible for differences in chemical compounds and/ or physical structure of the phloem. Resistance on *Rag1* soybean appears primarily constitutive, with aphid feeding suppressing phytohormones on susceptible, but not resistant plants (Studham and MacIntosh 2013). The resulting morphological or chemical factors (e.g., defensive compounds) could potentially affect the behavior of soybean aphid making with them move away from resistant plants.

Although aphid-resistant soybean increases movement of soybean aphids within plants (Figure 1.1, Table 1.1; Li et al. 2004, Whalen and Harmon 2012) and among plants (Whalen and Harmon 2012) under controlled conditions, there is a lack of literature on the effect of these behavioral changes or other factors related to *Rag* genes on spatial pattern of soybean aphid under field conditions. Results presented here indicate that mean-to-variance relanstionships for soybean aphid density, the slope of which is a measure of spatial pattern, were not affected by plant genotype. The spatial pattern of soybean aphid on aphid-resistant (i.e., *Rag1* or *Rag1*+*Rag2*) and -susceptible soybean was aggregated (i.e., slope ($b$) > 1) in both vegetative and reproductive soybean growth stages. Hodgson et al. (2004) also found an aggregated spatial pattern for soybean aphid in Minnesota soybean fields. Similarily, aggregated spatial patterns were found for *Myzus persicae* (Sulzer) in tobacco fields, *Nicotiana tabacum* L. (Athanassiou et al. 2005) and *Aphis gossypii* Glover in cotton field, *Gossypium arboreum* L. (Kapatos et al. 1996).

Similarity of spatial pattern across plant growth stages was also found for *Sitobion avenue* F. on winter wheat, *Triticum* spp. and *Brevicoryne brassicae* L. on *Brassica napus* L. (Ward et al. 1986, Nematollahi et al. 2014). Aggregated spatial patterns can be related to habitat factors (i.e. patchiness of habitat including differential predation), individual traits (i.e., limited dispersal ability and high reproductive rates, or aggregation behavior) and combination of both (Southwood 1978). For example, for cereal aphid species (*Sitobion avenae* (F.), *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* (L.)), the aggregated spatial pattern may be related to weather conditions and intrinsic biological factors (Tomanovic et al. 2008). In addition, agregrated spatial patterns may indicate dispersal limitation (Burslem et al. 2001). For example, for a majority of the growing season in the Midwest U.S., soybean aphid populations often predominantly consist apterous adults, with limited dispersal capability.

Changes to the spatial pattern (e.g., slope from Taylor's Power Law regression) of an insect population could result in changes in sampling recommendations (e.g., recommended number of sample units) (Southwood 1978, Hutchison et al. 1988, Binns and Nyrop 1992) and biological control (Turchin and Kareiva 1989). In addition, change in the intercept from Taylor's Power Law could also affect the recommended number of sample units. Though we found similar slopes for aphid-resistant and -suscptible soybean, there were difference among intercepts (Table 1.3 and 1.4). However, using Green's fixed precision sequential sampling plans ($n = a\,\bar{x}^{\,b-2}/c^2$, where $a$ is the antilog of the intercept and $b$ is slope from the Taylor's Power Law regression; $\bar{x}$ is the mean density of soybean aphid; and $c$ is the desired precision level (i.e., standard error / mean)) (Green 1970) we found that the average difference in recommended sample size across observed densities for soybean aphids on aphid-resistant and -susceptible varieties during vegetative or reproductive growth stages was less than 3 sample units (i.e., plants) (data not shown). This small difference in recommended sample size is not large enough to justify changes to sampling recommendations for aphid-resistant soybean. Therefore, current sampling recommendations for susceptible soybean (Hodsgon et al. 2004), can be used for aphid-resistant varieties with *Rag1* or *Rag1+Rag2*. Consequently, specific sampling plans for resistant varieties are not needed, which decreases potential complexity of management programs.

Furthermore, potential changes to the spatial pattern could affect natural enemy efficacy (e.g., lacewings, minute pirate bugs, lady beetles, parasitoids) (Hassell and May 1974, Croft el at. 1976, Nachappa et al. 2011, Tran and Koch 2017, Feng et al. 2018). Changes to the spatial pattern of the prey could potentially affect functional response of the predators to prey density by affecting attack rates and handling time by predators (Feng et al. 2018). Feng et al. (2018) showed that differences in prey spatial pattern (i.e., aggregated versus uniform) caused differences in foraging behavior and prey consumption by *H. axiridis*. In addition, Yasuda and Ishikawa (1999) found higher numbers of aphids consumed by this conccinallid with aphids in an aggregated spatial pattern than in a uniform spatial pattern. However, attack rates and handling times of *H. axiridis* were not significantly affected by change in *A. citricola* (van der Goot) spatial pattern (i.e., aggregated versus uniform) (Feng et al. 2018). Cappucino (1988) showed that difference in spatial pattern of *Uroleucon nigrotuberculatum* Olive and *U. tissoti* (Boudreaux) resulted in differences in vunerability of these species to predation by coccinellid larva and infection by the fungal pathogen, *Neozygites fresenii* (Nowak.). In addition, changes in spatial pattern could affect important dynamics parasitoids and their hosts. Optimal patch-use of a parasitoid is highly dependent on host spatial pattern and may favor highly aggregated hosts (Walde and Murdoch, 1988). For example, *Encarsia* spp. and *Nasonia* spp. had higher parasitism rates when hosts have aggregated spatial patterns (Walde and Murdoch, 1988). Interactions between prey and natural enemies can be very complex and other factors might need to be considered for a full understanding of interaction between soybean aphid and natural enemies on susceptible versus resistant soybean plants.

Aphid-resistant soybean provides promising additional tool for management of soybean aphid. However, the appearance of soybean aphid biotypes (i.e., populations of soybean aphid able to colonize resistant plants) might increase the challenge to manage soybean aphid aphid-resistant soybean. Until now, four biotypes of soybean aphid have been identified in the United States (Kim et al. 2008; Hill et al. 2010, 2012; Alt and Ryan-Mahmutagic 2013). Biotype 1 cannot colonize aphid-resistant plants containing *Rag1* or *Rag2* gene (Hill et al. 2010). Biotype 2 colonizes aphid-resistant plants containing *Rag1* but not *Rag2* genes (Kim et al. 2008b). Biotype 3 can colonize aphid-resistant plants containing *Rag2* genes and some other *Rag* genes (Hill et al. 2010, 2012). Biotype 4 can colonize aphid-resistant plants with both *Rag1* and *Rag2* genes (Alt and Ryan-Mahmutagic 2013). The biotype composition of soybean aphid varies over space and time (Cooper et al. 2015, Crossley and Hogg 2015). The high variability in virulence of soybean aphid to aphid-resistant soybean has the potential to reduce the effective life of *Rag* genes (Hill et al. 2010) leading to need for additional sources of resistance to manage biotypes of soybean aphid (Michel et al. 2011, Hesler et al. 2013, Hanson et al.

2018). In our field study, the biotypic profile of soybean aphid was unknown; however, results are representative of soybean aphid populations experienced in Minnesota. The presence of relatively high densities of soybean aphid on aphid-resistant soybean at Rosemount in 2014 and 2015 might indicate the presence of virulent biotypes (Table 1.2). However, it remains unknown what effect the presence of virulent biotypes (e.g., biotypes 2, 3, and 4) would have on spatial pattern of soybean aphid. Therefore, further, more-detailed work is needed to determine if variability of soybean aphid biotypes would affect movement and spatial pattern of soybean aphid on aphid-resistant soybean.

In conclusion aphid-resistant varieties containing *Rag1* or *Rag1*+*Rag2* altered the movement of biotype 1 soybean aphid on soybean plants under greenhouse conditions. However, the spatial pattern of soybean aphid on resistant plants did not differ from that on susceptible plants under field conditions with naturally occurring aphids. Because the field study presented here was performed in small plots, further study should be done in large-scale plots or fields to confirm applicability of these results to production fields. Overall, the results presented here are of relevance because they suggest that aspects of soybean aphid management that may be sensitive to changes in spatial pattern of the pest (e.g., natural enemy efficacy and sampling plans) may not be impacted by implementation of varieties with aphid-resistant genes for host plant resistance.

## Tables and Figures

**Table 1.1.** Effects of soybean genotype and time for soybean aphids released on unifoliate leaves or newly expanding trifoliate leaves of susceptible or resistant (i.e., *Rag1* or *Rag1+Rag2*) soybean varieties under greenhouse conditions.

| **Experiment** | **Effects** | $\chi^2$ | ***df*** | ***P*** |
|---|---|---|---|---|
| Infested unifoliate: Proportion recovered [a] | Plant genotype | 168.096 | 2 | < 0.0001 |
| | Time | 63.638 | 2 | < 0.0001 |
| | Plant genotype × Time | 12.611 | 4 | 0.013 |
| Infested unifoliate: Proportion remaining on the initial leaf [b] | Plant genotype | 136.154 | 2 | < 0.0001 |
| | Time | 29.545 | 2 | < 0.0001 |
| | Plant genotype × Time | 18.269 | 4 | 0.001 |
| Infested new growth: Proportion recovered [a] | Plant genotype | 93.986 | 2 | < 0.0001 |
| | Time | 44.358 | 2 | < 0.0001 |
| | Plant genotype × Time | 17.872 | 4 | 0.001 |
| Infested new growth: Proportion remaining on the initial leaf [b] | Plant genotype | 32.713 | 2 | < 0.0001 |
| | Time | 6.486 | 2 | 0.039 |
| | Plant genotype × Time | 17.872 | 4 | 0.989 |

[a] Proportion of initially released aphids recovered (i.e., remaining anywhere on the plant).
[b] Proportion of recovered aphids remaining on the initial leaf.

**Table 1.2.** Peak densities of soybean aphid in field experiments performed with aphid-susceptible and –resistant soybean varieties (i.e., *Rag1* or *Rag1+Rag2*).

| **Year** | **Location** | **Plant genotype** | **Aphids/plant (mean± SE)** |
|---|---|---|---|
| 2011 | Lamberton | Susceptible | 1140 ± 140 |
| | | *Rag1* | 7.09 ± 3.86 |
| | | *Rag1+Rag2* | 14.50 ±13.90 |
| 2012 | Lamberton | Susceptible | 105 ± 7.44 |
| | | *Rag1* | 42.3 ± 3.19 |
| | | *Rag1+Rag2* | 0.95 ± 0.65 |
| 2013 | Lamberton | Susceptible | 276 ± 99.90 |
| | | *Rag1* | 138 ± 84.10 |
| | | *Rag1+Rag2* | 20 ± 7.11 |
| 2013 | Rosemount | Susceptible | 228 ± 23.9 |
| | | *Rag1* | 3.87 ± 0.410 |
| | | *Rag1+Rag2* | 1.36 ± 0.26 |
| 2014 | Rosemount | Susceptible | 365 ± 44.40 |
| | | *Rag1* | 121 ± 13.20 |
| 2015 | Rosemount | Susceptible | 458 ± 45.09 |
| | | *Rag1* | 242 ± 25.97 |
| | | *Rag1+Rag2* | 207 ± 27.60 |
| 2016 | Rosemount | Susceptible | 18.61 ± 3.48 |
| | | *Rag1+Rag2* | 3.30 ± 0.619 |

**Table 1.3.** Taylor's power law regression parameters for mean-to-variance relationship of soybean aphid densities on aphid-susceptible and -resistant (i.e., *Rag1* or *Rag1+Rag2*) soybean varieties in vegetative growth stages in field experiments from 2011 to 2016.

| **Gene** | **N** | **Intercept (*a*) ±SE**** | **Slope (*b*) ± SE** | **Adjusted Variance ± SE**** |
|---|---|---|---|---|
| Susceptible | 149 | 0.647 ± 0.112 ab | 1.607 ± 0.021 | 1.369 ± 0.110 ab |
| *Rag1* | 86 | 0.736 ± 0.112 a | 1.607 ± 0.021 | 1.458 ± 0.112 a |
| *Rag1+Rag2* | 49 | 0.577 ± 0.115 b | 1.607 ± 0.021 | 1.299 ± 0.115 b |

**Letters separate the adjusted variances/intercepts based on Tukey's HSD post hoc test (*P* <0.05).

**Table 1.4.** Taylor’s power law regression parameters for mean-to-variance relationship of soybean aphid densities on aphid-susceptible and -resistant (i.e., *Rag1* or *Rag1+Rag2*) soybean varieties in reproductive growth stages in field experiments from 2011 to 2016.

| **Gene** | **N** | **Intercept (*a*) ± SE**** | **Slope (*b*) ± SE** | **Adjusted Variance ± SE **** |
|---|---|---|---|---|
| Susceptible | 238 | 0.431 ± 0.095 a | 1.697 ± 0.019 | 2.196 ± 0.092 a |
| *Rag1* | 168 | 0.557± 0.094 b | 1.697 ± 0.019 | 2.322 ± 0.093 b |
| *Rag1+Rag2* | 114 | 0.448 ± 0.096 ab | 1.697 ± 0.019 | 2.213 ± 0.097 ab |

**Letters separate the adjusted variances/intercepts based on Tukey’s HSD post hoc test ($P < 0.05$).

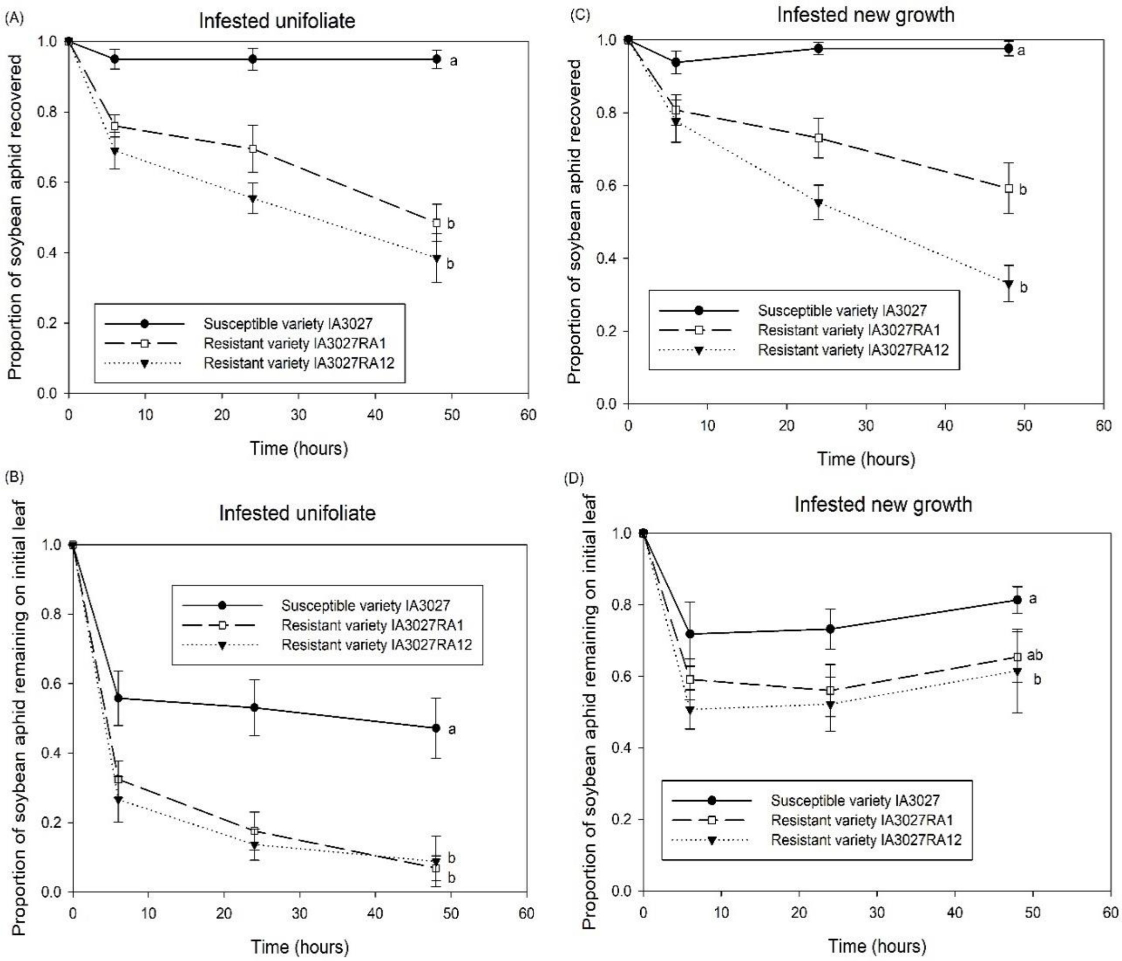

**Figure 1.1.** Proportion of adult soybean aphids recovered (mean ±SEM) and proportion of recovered aphids remaining on the initial leaf (mean ±SEM) for aphid-suscetible and aphid resistant (i.e., *Rag1* or *Rag1*+*Rag2*) soybean varieties under greenhouse conditions. Different letters indicate indicate significant differences among varieties across all periods of time based on Tukey's HSD post hoc test ($P < 0.05$).

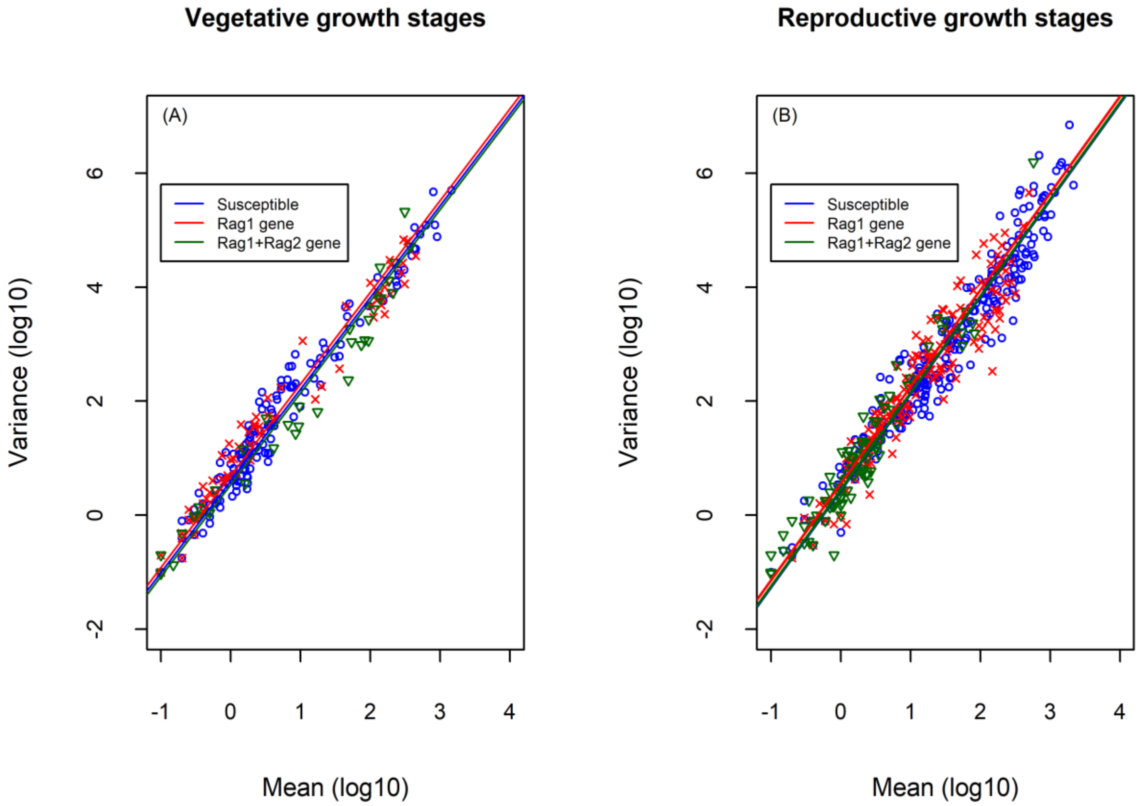

**Figure 1.2.** Taylor's power law regressions of soybean aphid densities on aphid-susceptible and -resistant (i.e., *Rag1* or *Rag1*+*Rag2*) soybean varieties in (a) vegetative and (b) reproductive growth stages. Blue circles represent sample points for the susceptible. variety, red stars represent sample points for the *Rag1* variety, and green triangles represent sample points for the *Rag1*+*Rag2* variety.

# Chapter 2: Effects of aphid-resistant soybean on non-target herbivores in Minnesota

## Summary

The use of resistant varieties containing *Rag* genes can provide an effective pest management strategy against soybean aphid (*Aphis glycines* Matsumura). However, the compatibility between *Rag* genes in soybean and the management of other potential soybean pests (i.e., non-target herbivores) is poorly understood. Therefore, our goal was to: 1) assess the efficacy of pyramided (*Rag1+Rag2*) aphid-resistant soybean for reduction of soybean aphid populations, and 2) determine if aphid-resistant soybean affects populations of non-target herbivores under field conditions. To accomplish these goals, field experiments were conducted in southeast Minnesota from 2015 to 2017 for a total of five site-years using whole-plant counts to assess soybean aphid densities and sweep-net sampling to estimate non-target herbivore densities. Plant genotype (i.e., *Rag1+Rag2* and no *Rag* genes) affected densities of soybean aphid with fewer soybean aphids on resistant varieties than susceptible near-isoline soybean. Furthermore, plant genotype affected seasonal abundance of bean leaf beetle (*Cerotoma trifurcata* Forster), green cloverworm (*Hypena scabra* Fabricius) and potato leafhopper (*Empoasca fabae* Harris). *Rag1+Rag2* resistant soybean was associated with increased seasonal abundance of bean leaf beetle and reduced the seasonal abundance of potato leafhopper and green cloverworm. The abundance of these herbivores was also associated with cumulative aphid days as a covariate. Results presented here confirm the efficacy of pyramided aphid-resistant soybean against soybean aphid and show that aphid-resistant soybean can influence the seasonal abundance of non-target herbivores in soybean. However, the magnitude of impacts of aphid-resistant soybean on non-target herbivores was relatively small and likely of little to no agronomic significance, which suggests compatibility of aphid-resistant soybean with management of other potential pests.

**Key words:** Host plant resistance, soybean aphid, IPM

## Introduction

Soybean (*Glycine max* (L.) Merr.) (Fabales: Fabaceae) is one of the main crops used worldwide to produce oil and dietary protein for humans and animals (Hartman et al. 2011), with U.S. harvested area estimated at 89.52 million acres of soybean in 2017 (USDA-NASS 2017) Among the challenges faced by soybean growers, the attack of soybean by invertebrate herbivores can significantly decrease soybean yield and, consequently, result in significant economic losses (Kogan and Herzog 1980, Higley and Boethel 1994, Boethel 2004, Steffey 2015). Many insect species are listed as pests of soybean (Kogan and Herzog 1980, Higley and Boethel 1994, Hodgson et al. 2012a, Steffey 2015). In the Upper Midwest U.S., insect pests of soybean include several Coleoptera, Lepidoptera, Hemiptera and Orthoptera (Hodgson et al. 2012a). Among these pests, the invasive soybean aphid (*Aphis glycines* Matsumura) (Hemiptera: Aphididae) remains the most threatening arthropod pest of this crop in the Upper Midwest U.S. (Ragsdale et al. 2011, Hurley and Mitchell 2017).Soybean aphid can cause yield losses higher than 40% during high infestations (Ragsdale et al. 2007) and is responsible for an increase of 130-fold in insecticide application to soybean since its invasion (Ragsdale et al. 2007, 2011). The economic impact from yield loss and insecticide applications as consequence of the attack of this pest are estimated between US$2.4-4.9 billion annually (Song et al. 2006, Kim et al. 2008).

Soybean aphid management relies mainly on threshold-based application of foliar insecticides (Ragsdale et al. 2011, Koch et al. 2016), with few insecticide groups available (Koch et al. 2018). The recent development and spread of insecticide resistance of soybean aphid to pyrethroids can jeopardizes current management programs for soybean aphid (Hanson et al. 2017, Koch et al. 2018). Therefore, management programs for soybean aphid need to advance beyond sole reliance on insecticides, to include multiple management strategies (i.e., integrated pest management) and thereby provide more sustainable soybean production (Pedigo 1995, Ragsdale et al. 2011, Hodgson et al. 2012c).

Host plant resistance is a promising option for soybean aphid management. Screening of germplasm banks has allowed scientists to identify several resistant genes in soybean named *Rag* (Resistance to *Aphis glycines*) (Mian et al. 2008, Zhang et al. 2009, Hesler et al. 2013). From the many genes identified, just *Rag1* and *Rag1+Rag2* are accessible in commercial varieties (Hanson et al. 2019). However, the potential for discovery of additional resistance genes is high (Hanson et al. 2018a). Two main categories are used to explain the resistance conferred by *Rag* genes, with antixenosis affecting the behavior of soybean aphid and antibiosis affecting biological traits of soybean aphid (i.e., survival, reproduction, developmental time, etc.) (Hesler et al. 2013). Resistant varieties containing *Rag* genes, especially when multiple resistance genes are pyramided into an individual soybean variety, can effectively reduce soybean aphid populations and protect soybean yields (Hill et al. 2006, Kim et al. 2009, McCarville and O’Neal 2012b, 2013, Varenhorst et al. 2015). Aphid-resistant varieties using *Rag* genes have the potential to be integrated with existing soybean aphid management programs

(Hodgson et al. 2012a, Hanson et al. 2018b, da Silva Queiroz et al. 2018) and may decrease the use of chemical control (Tilmon et al. 2011).

The adoption of resistant soybean varieties containing *Rag* genes is still insignificant in soybean production (Hodgson et al. 2012b), with a limited number of well-adapted *Rag* genes varieties available (Hanson et al. 2019). However, the management challenges caused by resistance of soybean aphid to insecticides (Koch et al 2018) may stimulate increased interest in and adoption of host-plant resistance for management of this pest. Overall, the prospect of wide-scale adoption of pest-resistant varieties may raise concern about potential effects of this management strategy on non-target organisms (e.g., soil organisms, local biodiversity, beneficial insects, and non-target herbivores) (Snow et al. 2005). Therefore, it is essential to evaluate the compatibility of *Rag* genes with other soybean herbivores. Ideally, management strategies for soybean aphid should be compatible with the management of other potential pests.

Previous research has shown variance in compatibility of host plant resistance with natural enemies (van Emden 1995, Lundgren et al. 2008, Kaplan and Thaler 2011, Chacon et al. 2012, McCarville and O’Neal 2012a, Hopper and Diers 2014). Furthermore, previous studies under laboratory and greenhouse conditions (Chandrasena et al. 2012, Bruner et al. 2013, Rich and Koch 2016, Özsisli and Prischmann-Voldseth 2016) and caged field conditions (Chandrasena et al. 2012) showed variable effects of *Rag* genes on non-target herbivores depending on factors such as the particular *Rag* gene and the herbivore species involved. Some studies explored the impact on invasive species. For example, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) had higher defoliation levels on aphid-resistant soybean containing *rag1b*, *rag3* and susceptible soybean compared with *Rag1* soybean in laboratory and caged field choice tests (Chandrasena et al. 2012). In addition, *Halyomorpha halys* Stal (Hemiptera: Pentatomidae) preferred *Rag1* soybean pods and experienced reduced mortality when feeding on this genotype compared to a susceptible near-isogenic soybean line (Rich and Koch 2016). Furthermore, the impact of *Rag* genes was investigated on native soybean defoliators. For beetles, *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), and *Cerotoma trifurcata* (Foster) (Coleoptera: Chrysomelidae) did not show any preference between *Rag1* soybean and susceptible soybean (Bruner et al. 2013). For caterpillars, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) had lower efficiency of converting food to insect body mass when fed *Rag1* soybean than susceptible soybean (Bruner et al. 2013). For *Tetranychus urticae* Koch (Trombidiformes: Tetranychidae), the *Rag1*, *Rag2* and *Rag1+Rag2* genes had no impact on the density of this pest under greenhouse or cage field conditions (Özsisli and Prischmann-Voldseth 2016).

The previously mentioned laboratory and caged field studies indicate potential for *Rag* genes to positively or negatively impact non-target herbivores of soybean. However, there is a lack of information on the effect of such aphid-resistant soybean varieties on non-target herbivore populations under field conditions. Our objectives were to: 1) assess the efficacy of pyramided (*Rag1+Rag2*) aphid-resistant soybean for suppression of soybean aphid populations, and 2) determine if this pyramided aphid-resistant soybean affects populations of non-target herbivores under field conditions. To achieve these goals, research was performed over five site-years of field experiments in southeast Minnesota. Results of this research will facilitate adoption of host plant resistance traits in integrated pest management (IPM) programs for soybean aphid.

## Materials and Methods

Five site-years of field trials were managed in southeast Minnesota from 2015 to 2017. At the University of Minnesota Research and Outreach Center in Rosemount, Minnesota, one experiment was conducted in each of 2015 and 2016, and two experiments in 2017. At the University of Minnesota Agricultural Experimental Station in Saint Paul, Minnesota, one experiment was conducted in 2017.

Experiments were conducted in randomized complete block designs with two treatments and four blocks. Each plot was 3.05 m wide and 9.10 m long with four rows and had a tilled alley of 1.52 m between treatments within blocks and 3.05 m between blocks. On 27 May 2015, 18 May 2016, and 11 May 2017 at Rosemount and on 15 May 2017 at Saint Paul, two near-isoline soybean varieties (i.e., no Rag genes and *Rag1+Rag2* genes) were sown at 384,800 soybeans per hectare with 76-cm row spacing. In 2015 and 2016, soybean varieties used as treatments were IA3027 and IA3027RA12. In 2017, soybean varieties used as treatments were IA2104 and IA2104RA12. In 2015, each block of the experiment had one replication (plot) of each treatment. However, in 2016 and 2017, each block of each experiment had two replications (plots) of each treatment. Weeds were controlled with pre-emergent herbicide application and insecticides or fungicides were not used.

Soybean aphid density was assessed weekly by performing nondestructive whole-plant counts of ten soybean plants in the two middles rows of each plot. The combined count of soybean aphid nymphs and winged and wingless adults was recorded for each plant. Whole-plant counts were performed from mid-June (i.e., approximately V3 growth stage) to late-August (i.e., approximately R5 growth stage) of each site-year (Fehr and Caviness 1977). In the middle rows of each plot, densities of non-target herbivores were assessed weekly by collecting one set of 20 sweeps (Varenhorst and O'Neal 2012). Sweep net sampling was performed from early July (i.e., approximately V5 growth stage) to early September (i.e., approximately R6 growth stage) of each year (Fehr and Caviness 1977). The insects captured in the sweep net from each plot were transferred to plastic zipper-locking bags and later identified in the laboratory. Non-target herbivores of interest in this study were *P. japonica*, *C. trifurcata*, *Empoasca fabae* (Harris) (Hemiptera:Cicadellidae), *Hypena scabra* (Fabricius) (Lepidoptera: Erebidae), *C. includens*, *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae), *Calomycterus setarius* Roelofs (Coleptera: Curculionidae), stink bugs (Hemiptera: Pentatomidae) and grasshoppers (Orthoptera: Acrididae), and were identified using morphological characteristics (Higley and Boethel 1994, Hodgson et al. 2012c).

All analyses were performed using R Development Core Team software (version 3.5.0, 2018). Cumulative insect days (CID) were calculated as a response variable to quantify the seasonal abundance for each herbivore using the formula modified from Hanafi et al. (1989):

$$\text{CID} = \sum_{i=1}^{n} \left[\frac{x_i + x_{i-1}}{2}\right] * (t_i - t_{i-1})$$

For whole-plant counts, $n$ was the number of sample dates, $x_i$ was the mean number of aphids per plant on sample date i, $x_{i-1}$ was the mean number of aphids per plant at the previous sample date, and $t_i - t_{i-1}$ was days since the previous sample dates. For sweep-net sampling, $x_i$ was the mean number of a given non-target taxon per 20 sweeps on sample date *i*, and, $x_{i-1}$ was the mean number of that non-target taxon on the previous sample date. The season-long CID for each herbivore and sampling method were transformed using $\log_{10}(x+1)$ to normalize variance. To compare differences in CID for soybean aphid between resistant variety containing *Rag1+Rag2* genes and susceptible variety, CID for soybean aphid were analyzed with analyses of variance (ANOVA) using a general linear model with function "lm" (Wilkinson and Rogers 1973). In this model, CID for soybean aphid was described as a function of plant genotype (i.e., *Rag1+Rag2* or no *Rag* genes). To compare differences in CID for each non-target herbivore between aphid-resistant and aphid-susceptible soybean using sweep-net sampling, general linear models were used to perform analyses of covariance with function "lm" (Wilkinson and Rogers 1973). Analysis of covariance was performed to allow comparison of CID for non-target herbivores adjusted for a potential confounding effect of differences in CID for soybean aphid. In these models, log-transformed CID for a given non-target herbivore was described as a function of main effects for plant genotype (i.e., *Rag1+Rag2* or no *Rag* genes) and log-transformed CID for soybean aphid from whole-plant counts (i.e., a covariate) and the two-way interaction. For aphids and non-target herbivores, non-significant ($P>0.05$) effects of site-year were removed from the models. Furthermore, for non-target herbivores, non-significant ($P>0.05$) interactions between plant genotype and CID for soybean aphid were removed from the models. Assumptions of normally distributed residuals, independence of error and constant variance were checked for each regression using qqplot, histograms of residuals, and plotting the residuals against fitted values.

## Results

Across the 5 site-years, the observed maximum density of *A. glycines* on any sample date was 206 and 950 aphids per plant in aphid-resistant and -susceptible soybean, respectively. CID for soybean aphid ranged from 17 to 545 and 89 to 4,012 in aphid-resistant and -susceptible soybean, respectively. Mean CID for *A. glycines* across the site-years, was significantly affected by plant genotype ($F_{1,70}$= 39.32, $P < 0.0001$) with greater CID for soybean aphid on aphid-susceptible than on aphid-resistant soybean (Figure 2.1).

Across the site-years, numerous taxa of non-target herbivores were detected using sweep-net sampling (Table 2.1). However, only adults of *C. trifurcata*, nymphs and adults combined of *E. fabae*, and larvae of *H. scabra* had high enough densities to permit analyses (Table 2.2). The other herbivores detected generally had extremely low densities or a high frequency of samples without those particular species (Table 2.1).

Across the 3 site-years in which *C. trifurcata* was detected, the maximum density observed was 7.0 and 7.9 *C. trifurcata* per 20 sweeps in aphid-susceptible and -resistant soybean, respectively (Table 2.1). CID for *C. trifurcata* ranged from 26 to 171 and 29 to 137 on aphid-resistant and -susceptible soybean, respectively (Table 1). Mean CID for *C. trifurcata* across the site-years was significantly affected by plant genotype ($F_{1,45}$= 22.81, $P < 0.0001$) (Table 2.2), with significantly greater CID for *C. trifurcata* on aphid-resistant than on -susceptible soybean (Figure 2.1). In addition, CID for *C. trifurcata* was associated with the covariate of CID for soybean aphid ($F_{1,45}$= 48.24, $P < 0.0001$) (Table 2.2), with CID for *C. trifurcata* increasing with increasing CID for soybean aphid on both plant genotypes (Figure 2.3a).

Across the 5 site-years in which *E. fabae* was detected, the maximum density observed was 69.9 and 74.9 *E. fabae* per 20 sweeps in aphid-resistant and -susceptible soybean (Table 2.1). CID for *E. fabae* ranged from 6 to 785 and 9 to 833 in aphid-resistant and -susceptible soybean, respectively (Table 1). Mean CID for *E. fabae* across the site-years was significantly affected by plant genotype ($F_{1,68}$= 19.71, $P < 0.0001$) (Table 2.2), with significantly greater CID for *E. fabae* on aphid-susceptible than on aphid-resistant soybean (Figure 2.2). In addition, CID for *E. fabae* was associated with the covariate of CID for soybean aphid ($F_{1,68}$= 77.10, $P < 0.0001$) (Table 2.2), with CID for *E. fabae* decreasing with increasing CID for soybean aphid in both plant genotypes (Figure 2.3b).

Across the 5 site-years in which *H. scabra* was detected, the maximum density observed was 1.1 *H. scabra* per 20 sweeps in aphid-resistant and -susceptible soybean (Table 2.1). CID for *H. scabra* ranged from 6 to 81 and 8 to 130 in aphid-resistant and -susceptible soybean, respectively (Table 2.1). Mean CID for *H. scabra* was significantly affected by plant genotype ($F_{1,69}$= 30.02, $P < 0.0001$) (Table 2.3), with significantly greater CID for *H. scabra* on aphid-susceptible than on aphid-resistant soybean (Fig. 2.2). In addition, CID for *H. scabra* was associated with the covariate of CID for soybean aphid ($F_{1,69}$= 78.65, $P < 0.0001$) (Table 2.2) with CID for *H. scabra* decreasing with increasing CID for soybean aphid in both plant genotype (Figure 2.3c).

## Discussion

The current management of soybean aphid relying mainly on foliar insecticide applications (Ragsdale et al. 2011, Koch et al. 2016) is not sustainable due to development of resistant population to insecticides (Hanson et al. 2017, Koch et al. 2018) and potential enviromental impacts (Carriger et al. 2006, Hussain et al. 2009, Bahlai et al. 2010, Sofo et al. 2012). Therefore, additional non-chemical management strategies, such as host plant resistance, are required to improve soybean aphid management (Ragsdale et al. 2011, Hodgson el al. 2012a). In this study, aphid-resistant soybean contaning *Rag1+Rag2* genes effectively reduced soybean aphid populations (Figure 2.1). Similar to this result, previous studies also showed efficacy of resistant varieties contaning *Rag* genes to decrease population of soybean aphid (Hill et al. 2006, Brace and Fehr 2012, McCarville and O'Neal 2012, 2013, Varenhorst el al. 2015). Aphid-resistant soybean could be a useful management option to maintain soybean aphid populations below economically significant levels (Ragsdale et al. 2007) and reduce need for foliar insecticide applications.

Implementation of host plant resistance may depend on compatibility of the strategy with the management of other organisms, such as natural enemies and non-target herbivores (Smith 2005). Nonetheless, effects on non-target organisms of pest-resistant crops in traditional host-plant resistance have received relatively little attention. Cultivars of soybean resistant to *Epilachna varivestis* Mulsant (Coleoptera: Coccinellidae) increased developmental time and mortality of nymphs of hemipteran predators (Rogers and Sullivan 1986). However, lines of wheat resistant to *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) did not affect population growth of a non-target aphid, *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) compared to susceptible wheat (Messina and Bloxham 2004). In addition to traditional host plant resistance, which relies on naturally occurring resistance in plants, use of transgenic pest-resistant plants can also be consider host plant resistance (Kennedy 2008). Impacts of transgenic pest-resistant crops on non-target organisms have been much more studied than traditional host plant resistance. In many cases, mainly with natural enemies, no negative impacts of transgenic crops were found on non-target insects (Yu et al. 1997, Naranjo 2005, Lawo et al. 2009, Naranjo 2009, Yu et al. 2011). However, possible negative impacts of transgenic pest-resistant crops were found on biological parameters (i.e., developmental time, weight and survival rates) of *Aphis gossypii* Glover and *Spodoptera littoralis* Boisduval (Li et al. 2005, Vojtech et al. 2005). Risk assessments of trangenic pest-resistant crops haven shown a general lack of risk to non-target insects in the majority of studies (Naranjo et al. 2009, Yu et al. 2011). More thorough risk assessment of traditional host plant resistance, such as *Rag* genes in soybean, are needed to more thoroughly evaluate potential negative or positive impacts on non-target insects (e.g., natural enemies and potential pests).

Understanding of the potetial impact of host plant resistance on non-target herbivores, is important for the implementation of this pest management strategy on a large scale (Kogan 1998). In this study, we explored the possible impacts of aphid-resistant soybean on non-target herbivores under open-field conditions with ambient pest populations. Numerous non-target herbivores were detected (Table 2.1), but most were not of sufficient densities to permit analyses (i.e., *C. includens, V. cardui*, Acrididae, *C. setarius* and Pentatomidae). These relatively low densities were not surprising for soybean in northern states (Hodgson et al. 2012a). Prior to the invasion by soybean aphid, less than 2% and 0.1% of acres in northcentral states were scouted and treated, respectively, for insect pests (Ragsdale et al. 2011). For the seasonal abundance of the non-target herbivores in sufficient abundance for analyses (i.e., *C. trifurcata, E. fabae* and *H. sacabra*), we found significant effects of plant genotype (Figure 2.2) and seasonal abundance for *A. glycines* as a covariate (Figure 2.3).

A wide range of potential effects of aphid-resistance genes on non-target herbivores of soybean have been documented in previous studies using mostly laboratory and caged field conditions (Chandrasena et al. 2012, Bruner et al. 2013, Rich and Koch 2016, Özsisli and Prischmann-Voldseth 2016), but may help explain some of the effects observed in the present study. Brunner et al (2013) did not find any impact of *Rag1* soybean on feeding preference of three Chrysomelidae (i.e., *D. undecimpunctata howardi*, *D. barberi*, and *C. trifurcata*) compared with aphid-susceptible soybean under laboratory conditions. The reported lack of effect of *Rag1* on *C. trifurcata* preferences may suggest that the increased abundance of *C. trifurcata* in plots of aphid-resistant soybean in the present study (Figure 2.2) may be due to effects of *Rag1* on the biology (e.g., survival, reproduction, etc.) or some unknown effect of *Rag2* on biology or preference. In addition, Bruner et al (2013) found a negative impact of *Rag1* on conversion efficiency of larvae of *S. frugiperda*, *H. zea* and *C. includens* when compared to aphid-susceptible soybean. A similar effect may have occurred with *H. scabra* in our study and resulted in the decreased abundance of this insect in aphid-resistant soybean (Figure 2.2). The decreased abundance of *E. fabae* observed in plots of aphid-resistant soybean may possibly be explained by the phylogenetic relatedness between potato leafhopper and the targeted aphid pest within the Hemiptera.

Specific mechanisms affecting the abundance of these nontarget herbivores remain unknown, but may be explained by the mechanisms documented for effects of resistant varieties containing *Rag* genes on soybean aphid. For example, differences in expression of genes related to plant defence have been documented between *Rag1* soybean and aphid-susceptible soybean (Sutham and MacIntosh 2013), and may play a role in some of the impacts observed on non-target herbivores. Furthermore, *Rag1* soybean can affect insect biology (e.g., survival, developmental time, etc.) through change in nutritional factors, such as amino acids. In early growth stages (i.e., V6 and R2), *Rag1* soybean, has lower levels of asparagine, α-aminobutyric acid, glutamine, serine, proline, histidine, glutamic acid, compared to *Rag* soybean varieties (Chiozza et al. 2010). Among these amino acids expressed at lower levels, histidine is an essential amino acid for Lepidoptera (Vanderzant 1958, Landry et al. 1986) and *E. fabae* (Dahlman 1953). Reduction of this essential amino acid in *Rag1* soybean could explain the negative effects observed on the abundance of *H. scabra* and *E. fabae* in the present study. In later growth stages of *Rag1* soybean, some of the amino acids such as histidine, glutamine and α-aminobutyric acid, that are essential to Chrysomelidae are at higher concentrations compared to aphid-susceptible soybean (Wardojo 1969, Chiozza et al. 2010). Increase of these essential amino acids in *Rag1* soybean may explain the greater abundance of *C. trifurcata* observed in the aphid-resistant soybean compared to aphid-susceptible soybean (Figure 2.2). Further work is needed to elucidate plant defensive chemicals and nutritional changes in aphid-resistant soybean as potential mechanisms affecting the abundance of some non-target herbivores.

Beside potential direct impacts of aphid resistant soybean on non-target herbivores, indirect impacts mediated by change in agricultural practices due to adoption of host plant resistance could also occur (Naranjo 2005, Williams 2006, Ho and Chu 2008, Naranjo 2009, Zhao et al. 2011). Such indirect effects could result from reduction in insecticide applications or reduced competition with the targeted pest. For example, the adoption of transgenic cotton in some situations led to increased abundance of stink bugs (Hemiptera: Pentatomidae), mirid bugs (Hemiptera: Miridae) and thrips (Thysanoptera: Thripidae), due to reduction of insecticide applications which were also previously supressing these pests (Lei et al. 2003; Wilson et al. 2006, Lu et al. 2010, Zeilinger et al. 2016,). Furthermore, in transgenic corn with the Cry1Ab protein, the abundance of and injury from western bean cutworm increased due to reduced populations of other competing Lepidoptera (Catangui and Berg 2006). If host plant resistance using *Rag* genes becomes well adopted for soybean aphid, it is possible that changes in agricultural practices, such as reduction in insecticide application, could potently result in increases of some secondary pests.

In our study, the abundance of soybean aphid also affected C. *trifurcata, E. fabae* and *H. scabra* (Figure 2.3). The coexistence of multiple insects on the same resource may result in positive, negative or neutral impacts among species (Tomlin and Sears 1992, Srinivas et al. 2001, Wimp and Whitham 2001, Nakamura and Ohgushi 2003, Mooney 2006, Kapplan and Denno 2007). Such interspecific effects can be driven by changes in plant chemistry, nutrition or morphology (Lynch et al. 2006, McArt et al. 2013). For example, populations of *A. gossypii* reduced survival of *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) by inducing defense proteins in cotton (Ramirez and Eubanks

2016). In addition, presence of *A. gossypii* attracted ladybeetles, which consequently increased predation of *S. exigua* (Ramirez and Eubanks 2016). Furthermore, high densities of *Brevicoryne brassicae* Linnaeus (Hemiptera: Aphididae) reduced growth rate of diamondback moth *Plutela xylostella* Linnaeus (Lepidoptera: Plutellidae) on *Arabidopsis thaliana* (L.) Heynh. (Brassicales: Brassicaceae) (Kroes et al. 2015). Feeding by soybean aphid on aphid-resistant soybean could lead to induction of synthesis of jasmonic acid and reactive oxygen species (i.e., $H_2O_2$) related signaling that might be harmful to other insects what could cause reduction in herbivory (Li et al. 2008). Further work is needed to better understand the negative associations observed between the abundance of soybean aphid and other herbivores in soybean.

In conclusion, this study provides the first assessment of potential population-level effects of aphid-resistant soybean on multiple non-target herbivores under field conditions. After correcting for seasonal abundance of soybean aphid, the pyramided (*Rag1+Rag2*) resistant soybean decreased the seasonal abundance on *E. fabae* and *H. scabra* and increased the seasonal abundance of *C. trifurcata*. However, the magnitude of these effects of aphid-resistance genes were relatively small and may be of little agronomic significance (Hodgson 2012a). Overall, the results presented here suggest that pyramided aphid-resistant soybean is compatible with managing other soybean pests in Minnesota. However, if such aphid-resistant varieties are widely adopted for soybean aphid management, populations of *C. trifurcata* should be monitored due to the potential for direct or indirect effects of aphid-resistant soybean on the population density of this pest.

## Tables and figures

**Table 2.1.** Mean cumulative insect days and mean density per 20 sweeps for sweep-net sampling for each soybean herbivore at Rosemount and Saint Paul from 2015, 2016, 2017

| **Species** | **Site-years detected** | **Plant genotype** | **Season-long CID** | | **Density (per 20 sweeps)** | |
|---|---|---|---|---|---|---|
| | | | Minimum | Maximum | Minimum | Maximum |
| *Cerotoma trifurcata* | 3 | *Rag1+Rag2* | 25.9 | 171.0 | 0.0 | 7.9 |
| | | No *Rag* | 29.1 | 136.7 | 0.0 | 7.00 |
| *Popillia japonica* | 5 | *Rag1+Rag2* | 16.5 | 1255.0 | 0.5 | 46.7 |
| | | No *Rag* | 9.0 | 1476.0 | 0.3 | 50.8 |
| *Empoasca fabae* | 5 | *Rag1+Rag2* | 6.4 | 785.0 | 0.38 | 69.9 |
| | | No *Rag* | 9.0 | 833.0 | 0.0 | 74.9 |
| *Hypena scabra* | 5 | *Rag1+Rag2* | 6.1 | 81.0 | 0.0 | 1.1 |
| | | No *Rag* | 7.7 | 130.0 | 0.0 | 1.1 |
| *Chrysodeixis includens* | 3 | *Rag1+Rag2* | 4.7 | 24.5 | 0.0 | 2.3 |
| | | No *Rag* | 6.4 | 23.7 | 0.0 | 1.3 |
| *Vanessa cardui* | 3 | *Rag1+Rag2* | 0.1 | 0.6 | 0.0 | 0.1 |
| | | No *Rag* | 0.3 | 1.3 | 0.0 | 0.3 |
| *Calomycterus setarius* | 5 | *Rag1+Rag2* | 3.0 | 123.0 | 0.0 | 2.4 |
| | | No *Rag* | 0.8 | 121.0 | 0.0 | 3.5 |
| Acrididae | 4 | *Rag1+Rag2* | 1.5 | 13.2 | 0.0 | 0.6 |
| | | No *Rag* | 0.9 | 11.1 | 0.0 | 0.4 |
| Pentatomidae | 2 | *Rag1+Rag2* | 5.9 | 8.3 | 0.0 | 0.1 |
| | | No *Rag* | 6.4 | 11.4 | 0.0 | 0.1 |
| *Colaspis brunnea* | 2 | *Rag1+Rag2* | 0.3 | 0.4 | 0.0 | 0.3 |
| | | No *Rag* | 0.3 | 0.6 | 0.0 | 0.3 |

**Table 2.2.** Effects of soybean genotype (i.e., aphid-resistant or susceptible) and seasonal abundance of soybean aphid (i.e., covariate) on seasonal abundance of non-target herbivores across multiple site-years in southeast Minnesota.

| **Insect** | **Site-years** | **Effects **** | **df** | **F** | **P** |
|---|---|---|---|---|---|
| *Cerotoma trifurcata* | 3 | Genotype | 1, 45 | 22.81 | <0.0001 |
| | | Cumulative aphid days | 1, 45 | 48.24 | <0.0001 |
| *Empoasca fabae* | 5 | Genotype | 1, 68 | 19.71 | <0.0001 |
| | | Cumulative aphid days | 1, 68 | 77.10 | <0.0001 |
| *Hypena scabra* | 5 | Genotype | 1, 69 | 30.02 | <0.0001 |
| | | Cumulative aphid days | 1, 69 | 78.65 | <0.0001 |

**Non-significant interactions were removed

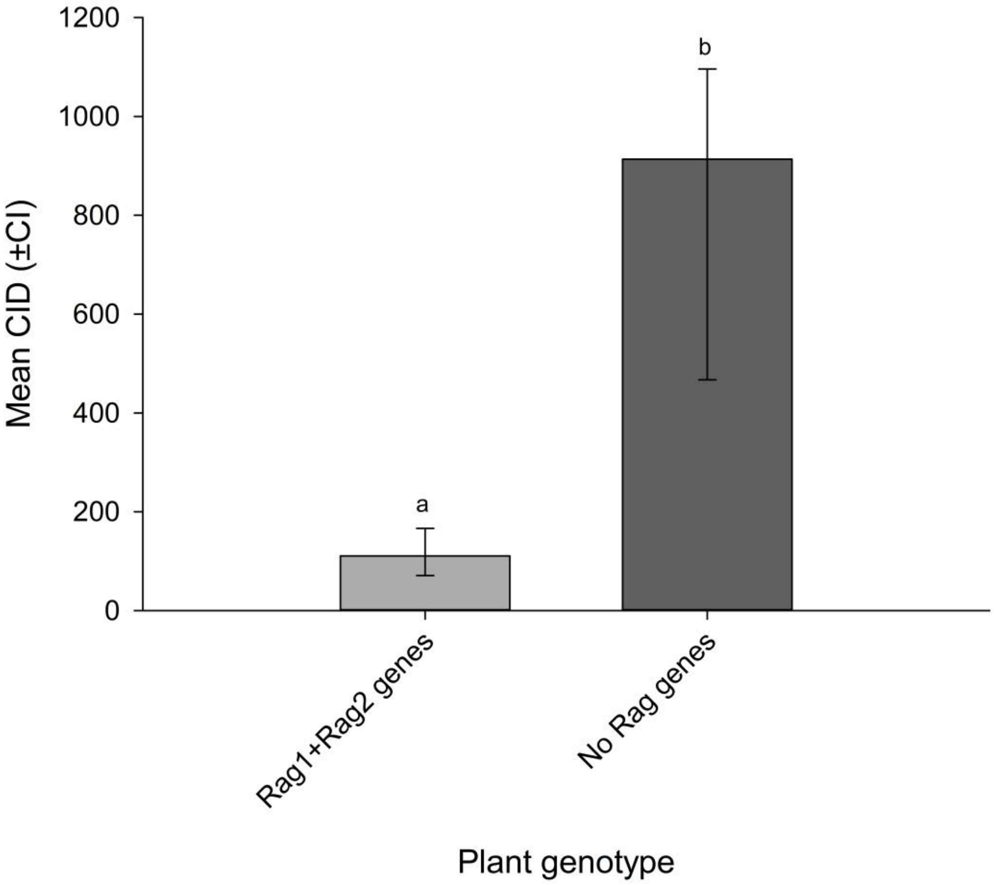

**Figure 2.1.** Least squares means of cumulative insect days (CID) (±CI) for soybean aphid on aphid-resistant (Rag1+Rag2 genes) and –susceptible (no Rag genes) soybean calculated from whole-plant counts at Rosemount and Saint Paul, Minnesota from 2015 to 2017. Data are back transformed from a base ten log distribution. Different letter indicates a significant difference between aphid-susceptible and -resistant varieties at $P < 0.05$

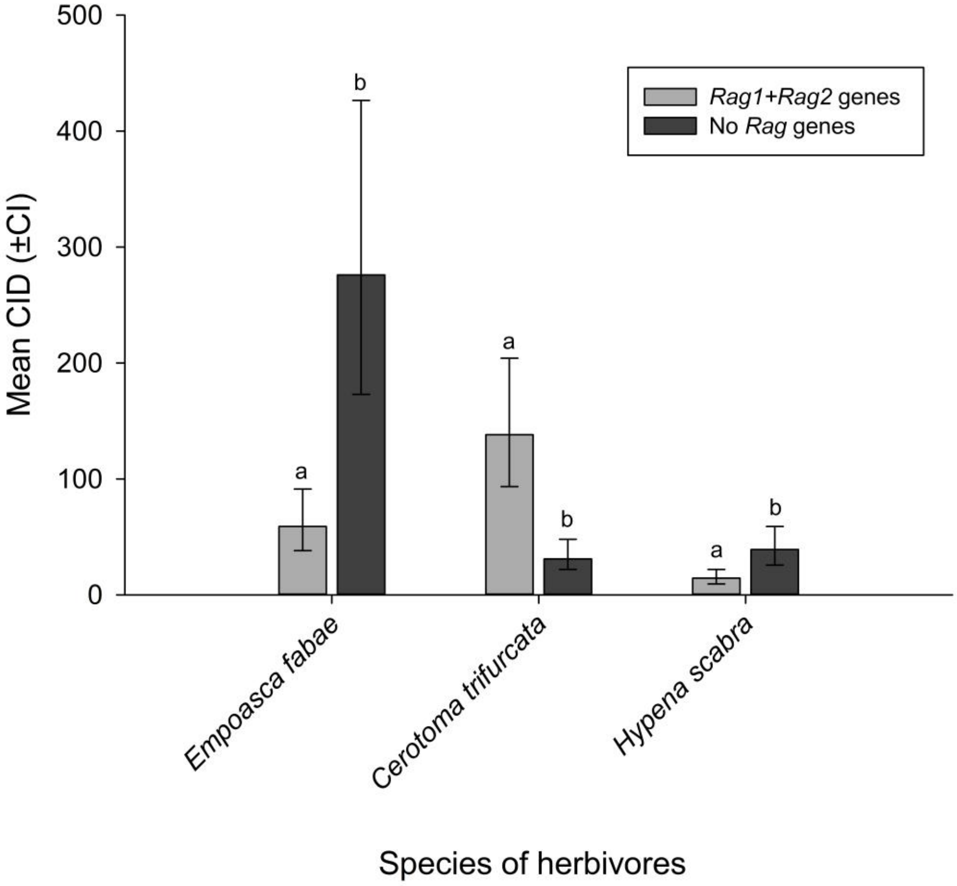

**Figure 2.2.** Least squares mean cumulative insect days (CID) (±CI) for non-target herbivores on aphid-resistant (Rag1+Rag2) and –susceptible (no Rag genes) soybean calculated from sweep-net sampling at Rosemount and Saint Paul, Minnesota from 2015 to 2017. Data are back transformed from a base ten log distribution. Different letters within each species indicate a significant difference between aphid-susceptible and -resistant soybean at $P < 0.05$. Least squares mean cumulative insect days for non-target herbivores account for effect of CID for aphids.

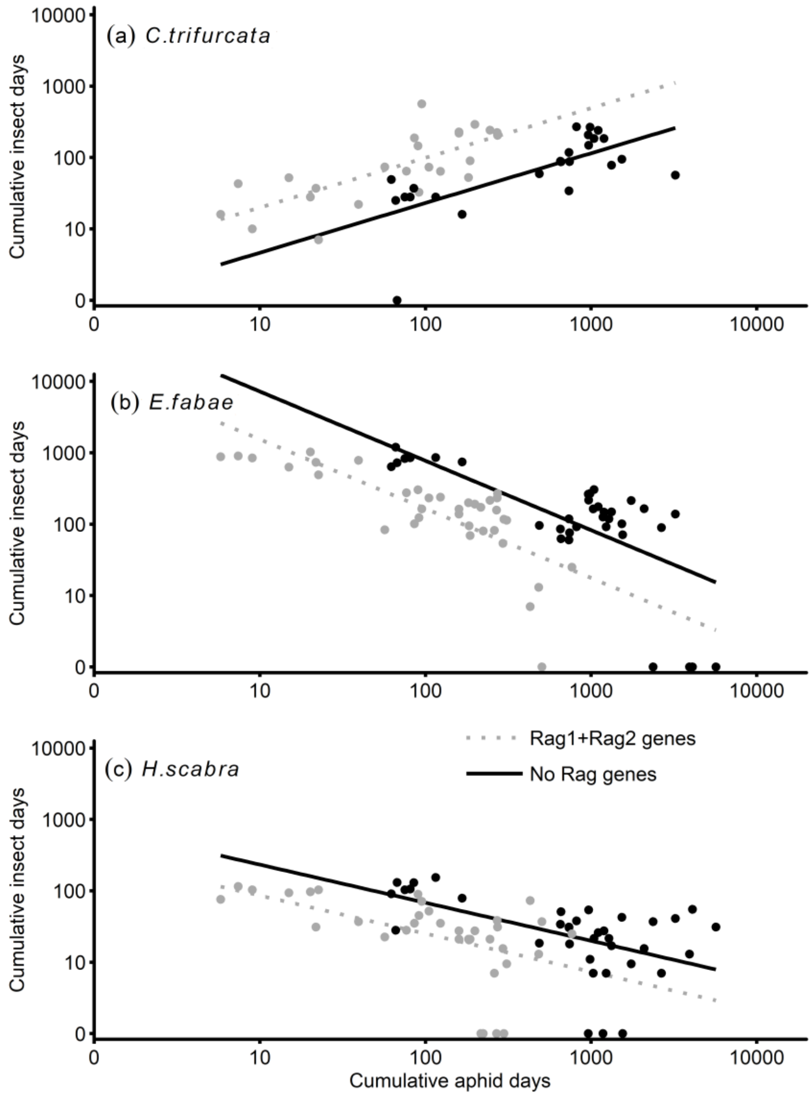

**Figure 2.3.** Relationship between cumulative insect days for non-target herbivores (sweep-net sampling) and cumulative insect days for soybean aphid (whole-plants counts) in aphid-resistant (*Rag1+Rag2*) and –susceptible (no *Rag* genes) at Rosemount and Saint Paul, Minnesota from 2015 to 2017. $R^2$ from regressions: *C.trifurcata* = 0.50, *E. fabae* = 0.59, and *H. scabra* = 0.25.

# Chapter 3: Baseline susceptibility to afidopyropen of soybean aphid (Hemiptera: Aphididae) from the north central United State

## Summary

Management of soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), rely primarily on threshold-based insecticide applications. Additional groups of insecticides are required for resistance management programs to slow further resistance evolution. The goal of this study was to evaluate baseline susceptibility of several populations of soybean aphid to afidopyropen (Sefina Inscalis ™ use), a novel group of insecticide (i.e., Group 9D) using leaf-dip bioassays in 2017 and 2018. Overall mortality of soybean aphid adults varied significantly among populations. Lethal concentrations necessary to kill 50% of a population ($LC_{50}$) for soybean aphid adults ranged from 0.0013 to 0.40 mg a.i. per liter of afidopyropen. Lethal concentrations necessary to kill 70% of a population ($LC_{70}$) of soybean aphid adults ranged from 0.136 to 13.1 mg a.i. per liter of afidopyropen. Afidopyropen was effective against populations resistant to a pyrethroids (i.e., Bifenthrin and lambda-cyhalothrin), suggesting a lack of cross-resistance between afidopyropen and pyrethroids. Such a lack of cross-resistance suggests afidopyropen could be an effective tool in management programs against pyrethroid-resistant soybean aphids. Furthermore, afidopyropen was effective against nymphs of the soybean aphid with concentrations ranging from 00285 to 54.45 mg a.i. per liter of afidopyropen causing 100% mortality by 6 days after exposure. Therefore, afidopyropen has potential to be used as an additional tool for the management of soybean aphid.

**Keywords:** *Aphis glycines*, leaf-dip bioassay, insecticides, resistance management

## Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), became one of the most problematic pests in soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), in the Midwestern U.S. and southern Canada since 2000 (Ragsdale et al. 2011). Feeding by soybean aphid can decrease plant height, pod size, seed number, seed size, and concentration of oil in seed (Beckendorf et al. 2008, Ragsdale et al. 2011). Furthermore, honeydew excreted by soybean aphid during feeding can be used as a substrate by sooty mold, which can interfere with photosynthesis (Wu et al. 2004). High infestation rates of soybean aphid can cause yield losses greater than 40% (Ragsdale et al. 2007). Due to losses and cost of control, soybean aphid can cause economic impacts between US$2.4-4.9 billion annually (Song et al. 2006, Kim et al. 2008)**.**

The current management of soybean aphid is primarily with threshold-based application of insecticides (Hodgson et al. 2012a, Koch et al. 2016), which has proven to be an effective management program (Johnson et al. 2009, Song and Swinton 2009, Krupke et al. 2017). However, the appearance of insecticide-resistant pest populations can jeopardize management programs based solely on insecticides (Pedigo and Rice 2009). Insecticide resistance is a genetically based decrease in susceptibility of a pest to an insecticide (Tabashnik et al. 2014). In 2015 to 2017, several populations of soybean aphid across several states/provinces (i.e., Minnesota, Iowa, South Dakota, North Dakota and Manitoba) were detected with resistance to pyrethroid insecticides (Hanson et al. 2017, Koch et al. 2018). Resistance of soybean aphid to pyrethroid insecticides compromises current management programs for this pest (Koch et al. 2018). To slow the evolution of insecticide resistance, alternation of insecticide groups is recommended

(Sparks and Nauen 2015, IRAC 2018). However, there has been a limited number of insecticide groups (Group 1: carbamates and organophosphates; Group 3: pyrethroids; and Group 4: neonicotinoids and butenolides) available for soybean aphid management (IRAC 2018, Koch et al. 2018, Knodel et al. 2018, Varenhorst and Wagner 2018). Due to the availability of relatively few insecticide groups and the evolution of insecticide resistance, access to additional insecticide groups with different modes of action will be necessary for resistance management programs to slow further resistance evolution (Sparks and Nauen 2015).

Afidopyropen is a new insecticide that is specific to piercing-sucking insects, such as Aphididae (Leichter et al. 2013, Vafaie and Grove 2018), Liviidae (Solís-Aguilar et al. 2015, Chen et al. 2018, Stansly and Kostyk 2018), and Aleyrodidae (Kumar et al. 2018). Afidopyropen represents a novel insecticide classified in Group 9D (IRAC 2018), which acts by overstimulating and silencing the vanilloid-type transient receptor potential channels located in the chordotonal stretch receptor neurons (Kandasamy et al. 2017). After exposure to afidopyropen, insects stop feeding and consequently starve, dry out and die (Gerwick and Sparks 2014, Leichter et al. 2013). Afidopyropen could provide an additional insecticide group to diversify insecticide alternations for management of soybean aphid. However, little is known about the susceptibility of soybean aphid to this new insecticide. Baseline susceptibility of an insecticide can be used to establish resistance monitoring programs (Roush and Miller 1986). The baseline susceptibility to insecticides has been established for various pests (Cahill et al. 1996, Wang et al. 2010, Armstrong et al. 2011, da Silva et al. 2012, Bird 2015). This study aimed to evaluate the baseline susceptibility of soybean aphid adults and nymphs to afidopyropen before this insecticide becomes used widely in soybean production.

## Materials and methods

### Leaf-dip bioassay for adults

**Insects.** As a reference population in this experiment, a laboratory colony of biotype 1 soybean aphid was obtained from the University of Illinois. This population has not been exposed to insecticides since detection in North America. The laboratory colony was reared on SD01-76R soybean plants in V3 to V5 growth stages (Fehr and Caviness 1977) in an environmental growth chamber at 70% humidity, 16L:8D hours photoperiod and approximately 25°C. Field populations of soybean aphid were collected from soybean fields from June to September in 2017 and 2018 (Table 3.1). Prior to being used in bioassays, each field population was established as a colony to ensure the aphids were not parasitized and not infected with entomopathogenic fungi. First, an average of ten to 15 soybean aphid adults were placed in soufflé cups (29.6 ml, Solo Cup Company, Lake Forest, IL) with an agar bed and leaf disk of soybean. The methods for preparation of leaf disks in cups are described in detail below. Those cups were placed in a growth chamber at 70% humidity, 16L:8D hour photoperiod and approximately 25°C. Aphids showing signs of parasitism or disease infection were removed from the cups. After one week, aphids from cups were placed on soybean plants under greenhouse conditions to establish colonies using the rearing protocol described above for the laboratory colony until enough soybean aphids were available to be used in the bioassay.

**Bioassays.** To assess the susceptibility of soybean aphid adults to afidopyropen, leaf-dip bioassays were performed (modified from IRAC 2016). Results of preliminary bioassays were used to determine a range of concentrations that would kill between 5 and 80% of the laboratory population of soybean aphid. Concentrations (i.e., treatments) used in this bioassay were 0.00000855, 0.000428, 0.0004, 0.021, 1.069, 53.54, 106.9 mg a.i. per L and a control, which consisted of reverse osmosis water. The concentration of 53.54 mg a.i. per L was an approximate field rate. The leaf-dip bioassay for each population was conducted as a randomized complete block design with nine concentrations and three independent replications of each concentration.

Leaf disks were cut from the middle leaflets of the second trifoliate leaves of soybean plants (SD01-76R) in the V5 growth stage using a 3.8-cm diameter hole punch (Fiskars, Helsinki, Finland). These soybean plants were grown in an environmental growth chamber at 70% humidity, 16L:8D h photoperiod and approximately 25°C. Leaf disks were submerged for ten second in one of the above mentioned concentrations of insecticide and then allowed to air dry with the adaxial side up on paper towels. While leaf disks were drying, agar beds were prepared to assure the longevity of the leaf disks. To create the agar beds, 3 grams of agar (Fisher Scientific Molecular Genetic Granulated Agar, Thermo Fisher Scientific, Waltham, MA) were put in 350 ml of reverse osmosis water and brought to a boil. The agar solution was cooled to 55°C and transferred to soufflé cups (29.6 ml; Solo Cup Company, Lake Forest, IL). The cups were filled to 10 mm of the top of each cup. When agar cooled to about 35°C (i.e., congealing point), the dipped and dried soybean leaf disks were placed abaxial side up in the cups. The leaf disks were gently pressed down onto the agar beds to ensure contact with the agar. After the cups were prepared, 10 apterous soybean aphid adults were transferred to each leaf disk. The identification of apterous adult of soybean aphids was based on their extended cauda, absence of wing pads and dark cornicles (Hodgson et al. 2005). During and after transfer, soybean aphids were inspected ensure that they were uninjured. The cups containing soybean aphids on leaf disks were covered with lids, which approximately 40 small pin-sized holes to allow ventilation. All cups were maintained in a growth chamber at 70% humidity, 16L:8D hours photoperiod and approximately 25°C.

The mortality of soybean aphids on leaf disks was assessed after five days. This duration was selected based on preliminary bioassay results. Aphid condition (i.e., live, dead or moribund) was assessed by prodding them with a fine-tipped camel-hair brush. Dead aphids were defined as those that failed to move after ten seconds. Live aphids were defined as those showing coordinated walking. Moribund aphids were defined as those showing a lack of coordinated movement. For analyses, moribund aphids were considered as dead aphids (Hanson et al. 2017).

**Analyses.** Mortality of soybean aphid adults from bioassays was analyzed using R Development Core Team software (version 3.5.0, 2018). Mortality of soybean aphids at 5 days for each concentration was corrected for control mortality using the Abbott correction (Abbott 1925). The concentration-response for each population of soybean aphid was estimated using a log-logistic function (Hanson et al. 2017). Prior to analyses, insecticide concentrations were transformed using a natural log to satisfy the assumption of normal distribution of residuals. We used a binomial response (dead or live) with a logit link function using the “glm” function. Mortality was described as fixed effects for log-transformed concentration, population and the two-way interaction. The overall difference in mortality was assessed using an analysis of variance (ANOVA) with the function "Anova" from package "car" (Fox and Weisberg 2011). In the model, mortality was described as a function of insecticide concentration (i.e., slope) and population (i.e., intercepts). Using the slopes and intercepts from the concentration-response models, we calculated lethal concentrations ($LC_{50}$ and $LC_{70}$) and the 95% confidence intervals (CIs) using the function "dose.p" from the package "MASS" (Venables and Ripley 2002). Differences in $LC_{50}$ or $LC_{70}$ values among populations were determined by nonoverlapping CIs (Faraggi et al. 2003).

### Leaf-dip bioassay for nymphs

**Insects.** Soybean aphids from the laboratory colony described above were used to evaluate the impact of afidopyropen on nymphs.

**Bioassay.** To assess the susceptibility of soybean aphid nymphs to afidopyropen, a leaf-dip bioassay was performed in October and November 2018 using methodology modified from that described above for the adult bioassays. The leaf-dip bioassay was conducted as a completely randomized design with four concentrations (i.e., treatments) and 11 independent replications of each concentration. Concentrations were an approximate field rate of afidopyropen (53.45 mg a.i. per liter), the $LC_{50}$ for adults from the laboratory population (0.057 mg a.i per liter), half the $LC_{50}$ for adults from the laboratory population (0.0285 mg a.i. per liter), and an untreated control (reverse osmosis water). After leaf disks were prepared and placed in the cups (as described above), 10 apterous adult soybean aphids were transferred to each cup. After 24 h, the adult soybean aphids were removed from the cups and the freshly deposited progeny of those adults were reduced 5 first instars per cup. The cups were covered with lids and maintained in growth chambers as described above. The condition of nymphs was assessed as described above at 4 and 6 days.

**Analyses.** Survival of soybean aphid nymphs at 4 and 6 days was analyzed using a generalized linear mixed effect model with binomial distribution (i.e., live or dead) with the function “glmer”. A separate model was used for each observation time. Proportion survival was described as a function of a fixed effect for concentration of afidopyropen and a random effect for replication. Mean separations were performed using Tukey’s HSD post hoc test using package “lsmeans” (Lenth 2016) and package “MulticompView” (Graves et al. 2015).

## Results

### Leaf-dip bioassay for adults

Overall control mortality for soybean aphid adults at 5 days using the leaf-dip bioassay averaged 5% (range: 0-20%) across all populations. Mortality of soybean aphid adults decreased significantly with increasing concentration of afidopyropen (effect of concentration: $\chi^2 = 325.59$, df =1, $P < 0.0001$). Overall mortality varied significantly among populations (effect of population: $\chi^2 = 23.12$, df =9, $P = 0.0059$), with the rate of response to concentrations varying among populations (two-way interaction: $\chi^2 = 33.91$, df = 9, $P <0.0001$) (Table 3.2). Lethal concentrations necessary to kill 50% of a population ($LC_{50}$) for soybean aphid adults ranged from 0.0013 to 0.40 mg a.i. per liter of afidopyropen (Table 3.3). $LC_{50}$s for field collected populations did not differ significantly from that of the laboratory population, except for soybean aphids from Morris (2017), Lamberton (2018) and Osnabrock (2017), which all required less insecticide to kill 50% of the populations (i.e., lower $LC_{50}$s) (Table 3.3).

Because of the significant interaction between population and concentration, an additional point estimate (i.e., LC value) was examined. Lethal concentrations necessary to kill 70% of a population ($LC_{70}$) for soybean aphid adults ranged from 0.136 to 13.1 mg a.i. per liter of afidopyropen (Table 3.4). $LC_{70}$s for field collected populations did not differ significantly from that of the laboratory population (Table 4). The $LC_{70}$ for Lamberton (2018) was less than Emerado (2018), Lamberton (2017) and Calumet (2018) (Table 3.4).

### Leaf-dip bioassay for nymphs

Overall control survival for soybean aphid nymphs at 6 days using the leaf-dip bioassay averaged 89%. Survival of soybean aphid nymphs decreased significantly with increasing concentration of afidopyropen at 4 days (effect of concentration: $\chi^2 = 63.31$, df =3, $P < 0.0001$). A concentration of 54.45 mg a.i. per liter had significantly lower survival compared with 0.057 mg a.i. per liter and the control at 4 days (Figure 3.1a). Survival of soybean aphid nymphs decreased significantly with increasing concentration of afidopyropen at 6 days (effect of concentration: $\chi^2 = 195.44$, df =3, $P < 0.0001$). By 6 days after exposure, survival was 0% in the three insecticide-containing treatments, which all differed significantly from survival in the control (Figure 3.1b).

## Discussion

Soybean aphid is managed primarily with threshold-based application of foliar insecticides (Hodgson et al. 2012a, Koch et al. 2016). However, evolution of resistance to pyrethroid insecticides (Hanson et al. 2017) and the availability of relatively few groups of insecticides (IRAC 2018, Koch et al. 2018) impose an increase in challenge to management programs for this pest. Additional insecticides groups with different modes of action are required in resistance management programs to slow further resistance evolution (Sparks and Nauen 2015). Afidopyropen could be an alternative insecticide for management of piercing-sucking insects, such as aphids (Leichter et al 2013, Vafaie and Grove 2018). Using a leaf-dip bioassay, we characterized the susceptibility to afidopyropen of soybean aphid adults from soybean fields in Minnesota, North Dakota and Iowa from 2017 and 2018. Susceptibility of soybean aphid adults to afidopyropen varied among populations, but $LC_{50}$s and $LC_{70}$s were below the approximate field rate of 53.45 mg a.i per liter of afidopyropen. $LC_{50}$ values typically occur below the recommended field rate for insecticides, because the concentration of insecticide to which the insects get exposed in the field is dependent of factors such as weather and plant canopy (Guillebeau et al. 1989). The baseline susceptibilities reported here will provide a foundation for development of resistance monitoring programs for this insecticide (Roush and Miller 1986).

Susceptibility (i.e., $LC_{50}$s or $LC_{70}$s) of soybean aphid to afidopyropen varied among field populations by up to two orders of magnitude from the most to least susceptible (Table 3.3 and Table 3.4). Susceptibility of piercing-sucking pests has been observed ranging from one to two orders among populations for other insecticides (i.e., buprofezin, pyriproxyfen, acephate, triazophos, indoxacarb, pyriproxyfen, flonicamid, pirimicarb and lambda-cyhalothrin) (Toscano et al. 2001, Lowery et al. 2006, Roditakis et al. 2014). For flonicamid, which also affects chordotonal organ TRPV channel (Kandasamy et al. 2017, IRAC 2018), susceptibility of cotton aphid, *Aphis gossypii* (Hemiptera:Aphididae) varied by up to two orders of magnitude (Gore et al. 2013). Even higher levels of variability in susceptibility were found among populations of diamondback moth exposed to Cry1Ab *Bt*-toxin (Kumar and Gujar 2005). The source of variability in susceptibility among populations of soybean aphid to afidopyropen remains unknown. The variability observed among populations may have been due genetic variation among field populations (Orantes et al. 2012). Other bioassay methodologies could be explored to begin to determine if variability observed among populations was due to populations or interactions of susceptibility to bioassay methodology.

Resistance to one group of insecticide can lead to loss of efficacy of other groups of insecticides (i.e., cross-resistance) (Tabashnik et al. 2014). However, there is no documentation of cross-resistance between afidopyropen and other insecticides. Several populations evaluated in the present study (i.e., Osnabrock 2017, Lamberton 2018, Hastings 2018 and Willmar 2018) showed resistance to pyrethroid insecticides in other bioassays (Koch, unpublished data). The susceptibility (i.e., $LC_{50}$ and $LC_{70}$) for all resistant populations were equal to or less than that for the laboratory susceptible population, suggesting a lack of cross-resistance between afidopyropen and pyrethroids for these populations of soybean aphid (Table 3.3). A lack of cross-resistance with other insecticide groups potentially makes afidopyropen an effective tool to be used in management programs against pyrethroid-resistant soybean aphids.

Afidopyropen at different concentrations was effective at killing soybean aphid nymphs after 6 days (Figure 3.1b). Toxicity of afidopyropen against nymphs has been studied for other piercing-sucking insects in laboratory (Solis-Aguilar et al. 2015, Chen et al. 2018) and field experiments (Kumar et al. 2018). Chen et al. (2018) found reduced nymph survival of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) at concentrations ranging between 0.01 and 100 ng per µL after 72 hours. Solis-Aguilar et al. (2015)

showed reduced survival of nymphs of *D. citri* after 7 days. Kumar et al. (2018) found a significant reduction in nymph populations of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) 3 weeks after using afidopyropen. Afidopyropen is effective against immature and adult stages of piercing-sucking pests, including soybean aphid.

In conclusion, susceptibility of soybean aphid to afidopyropen varied among populations, but $LC_{50}$s and $LC_{70}$s were below the approximate field rate, indicating that this insecticide could be effective against soybean aphid. In addition, afidopyropen was effective against populations of soybean aphid that showed resistance to pyrethroid insecticides in other studies. This finding may suggest a lack of cross-resistance between afidopyropen (Group 9D) and pyrethroids (Group 3A). Furthermore, afidopyropen was effective against nymphs of soybean aphid in all concentration tested after 6 days. Further work should be performed to evaluate efficacy of afidopyropen against soybean aphid populations under field conditions.

## Tables and figures

**Table 3.1.** Soybean aphid (*A. glycines*) populations used for leaf-dip bioassays with afidopyropen.

| **Year** | **State** | **City** | **Collection date** | **Bioassay date** |
|---|---|---|---|---|
| 2017 | Minnesota | Morris | 2 Aug. 2017 | 22 Sep. 2017 |
| 2017 | Minnesota | Lamberton | 10 Aug. 2017 | 06 Oct. 2017 |
| 2017 | North Dakota | Osnabrock | 26 Aug. 2017 | 20 Oct. 2017 |
| 2017 | - | Laboratory | - | 15 Dec. 2017 |
| 2018 | Iowa | Calumet | 21 Nov. 2017 | 19 Jan. 2018 |
| 2018 | Minnesota | Rosemount | 11 Jul. 2018 | 27 Jul. 2018 |
| 2018 | Minnesota | Willmar | 11 Jul. 2018 | 6 Aug. 2018 |
| 2018 | Minnesota | Lamberton | 1 Aug. 2018 | 24 Aug. 2018 |
| 2018 | Minnesota | Hastings | 28 Aug. 2018 | 9 Sep. 2018 |
| 2018 | North Dakota | Emerado | 27 Aug. 2018 | 28 Sep. 2018 |

**Table 3.2.** Regression results for adult mortality of soybean aphid (*A. glycines*) in leaf-dip bioassay from 2017 and 2018.

| Response | $\chi^2$ | Df | P > ($\chi^2$) |
|---|---|---|---|
| Concentration | 325.59 | 1 | <0.0001 |
| Population | 23.12 | 9 | 0.0059 |
| Concentration × Population | 33.91 | 9 | <0.0001 |

**Table 3.3.** Lethal concentration ($LC_{50}$) of multiple field populations and one laboratory population of adult of soybean aphid (*A. glycines*) to afidopyropen using leaf-dip bioassay in 2017 and 2018.

| Population | N | Intercept ± SE | Slope ± SE | $LC_{50}$ (95% CI) (Mg a.i./L) * | |
|---|---|---|---|---|---|
| Morris, MN (2017) | 240 | 0.99 ± 0.20 | 0.15 ± 0.02 | 0.0013 (0.00041–0.0044) | a |
| Lamberton, MN (2018) ** | 240 | 1.26 ± 0.22 | 0.20 ± 0.03 | 0.0045 (0.0014–0.014) | ab |
| Osnabrock, ND (2017) ** | 240 | 1.07 ± 0.26 | 0.15 ± 0.03 | 0.005 (0.0016–0.016) | ab |
| Rosemount, MN (2018) | 240 | 0.98 ± 0.21 | 0.21 ± 0.03 | 0.008 (0.0027–0.028) | abc |
| Lamberton, MN (2017) | 240 | 0.55 ± 0.18 | 0.14 ± 0.03 | 0.019 (0.0061–0.064) | bc |
| Calumet, IA (2018) | 240 | 0.47 ± 0.18 | 0.14 ± 0.02 | 0.036 (0.011–0.12) | bcd |
| Willmar, MN (2018) ** | 240 | 1.07 ± 0.26 | 0.35 ± 0.05 | 0.048 (0.015–0.15) | cd |
| Laboratory | 240 | 0.63 ± 0.20 | 0.22 ± 0.03 | 0.057 (0.018–0.18) | cd |
| Hasting, MN (2018) ** | 240 | 0.64 ± 0.22 | 0.28 ± 0.04 | 0.095 (0.029–0.31) | cd |
| Emerado, ND (2018) | 240 | 0.32 ± 0.22 | 0.34 ± 0.05 | 0.40 (0.12–1.29) | d |

*Different letters indicate differences in $CL_{50}$ based on nonoverlapping 95% confidence intervals

** Resistant populations of soybean aphid to Pyrethroid (group 3A)

**Table 3.4.** Lethal concentration ($LC_{70}$) of multiple field populations and one laboratory population of adult of soybean aphid (*A. glycines*) to afidopyropen using leaf-dip bioassay in 2017 and 2018.

| Population | N | Intercept ± SE | Slope ± SE | $LC_{70}$ (95% CI) (Mg a.i./L) * | |
|---|---|---|---|---|---|
| Lamberton MN (2018) ** | 240 | 0.99 ± 0.20 | 0.15 ± 0.02 | 0.136 (0.03– .61) | a |
| Osnabrock, ND (2017) ** | 240 | 1.26 ± 0.22 | 0.20 ± 0.03 | 0.330 (0.073–1.48) | ab |
| Morris, MN (2017) | 240 | 1.07 ± 0.26 | 0.15 ± 0.03 | 0.368 (0.08–1.65) | ab |
| Rosemount, MN (2018) | 240 | 0.98 ± 0.21 | 0.21 ± 0.03 | 0.537 (0.12–2.40) | ab |
| Willmar, MN (2018) ** | 240 | 0.55 ± 0.18 | 0.14 ± 0.03 | 0.519 (0.115–2.32) | ab |
| Hasting, MN (2018) ** | 240 | 1.07 ± 0.26 | 0.35 ± 0.05 | 2. 06 (0.46–9.25) | abc |
| Laboratory | 240 | 0.63 ± 0.20 | 0.22 ± 0.03 | 2.67 (0.59–11.9) | abc |
| Emerado, ND (2018) | 240 | 0.64 ± 0.22 | 0.28 ± 0.04 | 4.51 (1. 00– .25) | bc |
| Lamberton, MN (2017) | 240 | 0.32 ± 0.22 | 0.34 ± 0.05 | 7.67 (1.71–34.38) | bc |
| Calumet, IA (2018) | 240 | 0.47 ± 0.18 | 0.14 ± 0.02 | 13.1 (2.94–59.10) | c |

*Different letters indicate differences in $CL_{70}$ based on nonoverlapping 95% confidence intervals

** Resistant populations of soybean aphid to Pyrethroid (group 3A)

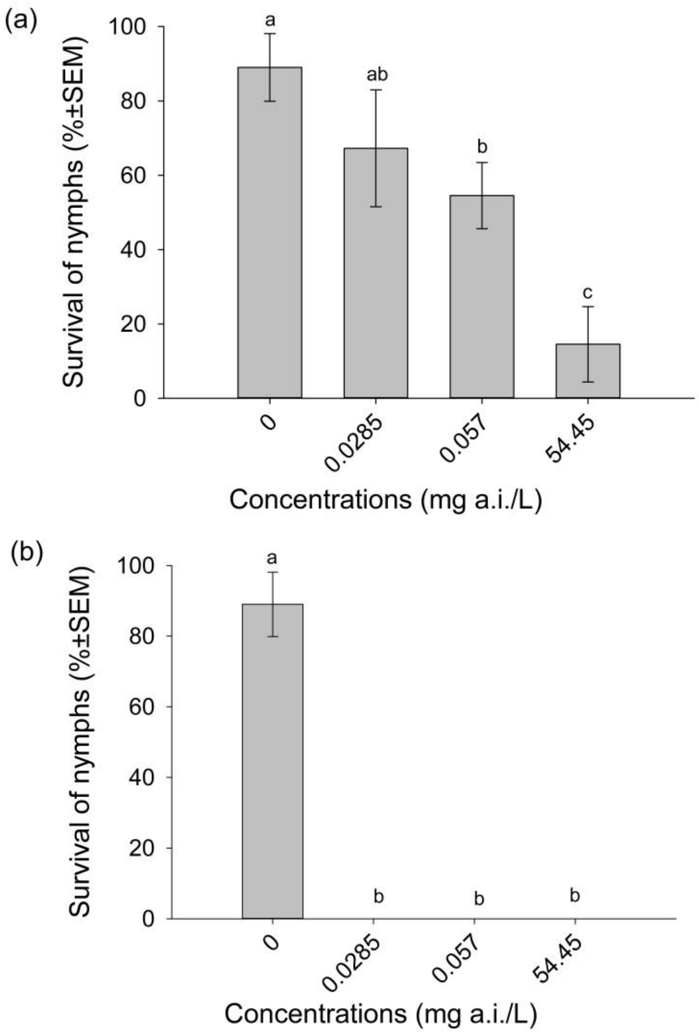

**Figure 3.1.** Nymph survival of soybean aphid (*A. glycines*) to different concentrations of afidopyropen at 4 days (a) and 6 days (b). Different letters indicate statistically significant differences among treatments.

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## Appendix 1: Efficacy of foliar insecticides for management of soybean aphid, 2018

The goal of this study was to evaluate insecticides for management of soybean aphid (SBA) in Minnesota in 2018. The insecticide trial was conducted at the University of Minnesota Outreach, Research and Education Park in Rosemount, MN. On 17 May 2018, soybean seed (variety Asgrow 'AG1435') was sown 1.5 inches (3.81 cm) deep with 30 inches (76.2 cm) row spacing at a seeding rate of 149,000 seeds/acre (368,187 seeds/ha). The design of the insecticide trial was a randomized complete block with seven treatments and four replications (i.e., plots) of each treatment. Each plot consisted of four rows 20 ft (6.10 m) long with separation of 10 ft (2.04  to 3.05 m) of fallow ground between plots. Treatments consisted of six insecticide treatments and an untreated check (Table A1).

Insecticides were sprayed on R5 soybean growth stage (i.e., 17 Aug 2018) using a $CO_2$-pressurized backpack sprayer with a 10-ft (3.05-m) boom and eight nozzles (XR-Teejet 8002 flat fan, with no screen) calibrated to deliver 15 gpa (140.3 liters/ha) at 30 psi (206.8 kPa). Conditions during application were: 75°F (23.9°C), 3-mph (4.83-km/h) winds, light dew on plants, dry soil surface, and no precipitation during or within 24 hours after application.  To estimate soybean aphid densities in each plot, visual whole-plant inspection of plants was performed prior to application of insecticides and at 6 and 13 DAT (i.e., 23 and 30 Aug, respectively). On each sample date, five plants were randomly selected from the middle two rows of each plot and the total number of SBA was recorded for each plant. On each sample date, SBA densities were averaged within plots. Data were analyzed on R software (R Development Core Team version 3.5.0).

Mean densities of SBA per plant were analyzed with analysis of variance (ANOVA) using a general linear mixed-effect model with treatment as a fixed effect and plot as random effect. Means were separated using Tukey's studentized range test (HSD) at α = 0.05. Prior to application of insecticides, SBA densities averaged 181 aphids per plant. At 6 DAT, all insecticides reduced populations of SBA compared to the untreated check, however, Warrior II reduced populations less than the other insecticides (Table A1). At 13 DAT, Sivanto Prime, Sefina and both rates of Argyle OD reduced populations of SBA compared to Warrior II and the untreated check. No yield differences were found among treatments (Table A1).

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**Table A1.** Average of soybean aphid per plant and yield treated with different insecticides at 7 and 14 days.

| Treatments/formulations | Rate/acre (fl oz) | 6 DAT | 13 DAT | |
|---|---|---|---|---|
| | | Aphids per plant | Aphids per plant | Yield (bu/ac) |
| Untreated Check | -- | 453.4 A | 83.3 A | 36.2 A |
| Warrior II 2.08CS | 1.6 | 246.4 B | 102.0 A | 38.2 A |
| Endigo ZC 2.06CS | 4.0 | 38.7 C | 43.3 AB | 35.0 A |
| Sivanto Prime 1.67SL | 14.0 | 42.9 C | 11.3 B | 39.4 A |
| Sefina 0.42DC | 3.0 | 80.0 C | 2.7 B | 35.0 A |
| Argyle OD 2.52SC | 5.0 | 6.1 C | 1.6 B | 38.8 A |
| Argyle OD 2.52SC | 8.0 | 0.7 C | 0.9 B | 37.4A |
| *P* | | <0.0001 | 0.0001 | 0.36 |

Means within columns followed by the same letter are not significantly different (*P*>0.05), ANOVA and Tukey’s studentized range test (HSD).