Impact of host plant health and host plant resistance on Aphis glycines (Hemiptera: Aphididae)

Erika Rodbell

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/etd

Part of the Entomology Commons

Recommended Citation


https://lib.dr.iastate.edu/etd/17551

This Thesis is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Impact of host plant health and host plant resistance on *Aphis glycines* (Hemiptera: Aphididae)

by

Erika Adriana Rodbell

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Co-majors: Entomology; Sustainable Agriculture

Program of Study Committee:
Erin Hodgson, Co-major Professor
Matthew O’Neal, Co-major Professor
Matt Liebman

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University
Ames, Iowa
2019

Copyright © Erika Adriana Rodbell, 2019. All rights reserved.
DEDICATION

I would like to dedicate this work to my mother, Cecilia Maria Oballe, who passed away as I was working towards this degree. Her investment in my future was without question. I would also like to dedicate this work to my friends and family, whose support and encouragement helped me throughout this process.
# TABLE OF CONTENTS

ABSTRACT ....................................................................................................................................... iv

CHAPTER 1. GENERAL INTRODUCTION .............................................................................. 1
   Thesis Organization .................................................................................................................. 1
   Literature Review ................................................................................................................... 1
      Soybean aphid: biology, ecology, and life history ................................................................. 1
      Adverse effect of soybean aphid ......................................................................................... 2
      Biological control agents ..................................................................................................... 4
      Host plant resistance to soybean aphids and soybean aphid biotypes ......................... 5
      Effect of host plant quality on soybean aphid ................................................................. 7
      Host health and crop rotation ............................................................................................. 8
   Literature Cited .................................................................................................................... 10

CHAPTER 2. RESISTANCE TO APHIS GLYCINES GENE EFFICACY TRIAL .................. 17
   Abstract .................................................................................................................................. 17
   Introduction ............................................................................................................................ 18
   Materials and Methods ......................................................................................................... 20
   Results ................................................................................................................................... 23
   Discussion .............................................................................................................................. 24
   Acknowledgements ............................................................................................................... 25
   Literature Cited .................................................................................................................... 26
   Tables and Figures ............................................................................................................... 29

CHAPTER 3. ON-FARM TESTING OF APHID RESISTANT SOYBEAN IN IOWA ............. 36
   Abstract .................................................................................................................................. 36
   Introduction ............................................................................................................................ 37
   Materials and Methods ......................................................................................................... 40
   Results ................................................................................................................................... 43
   Discussion .............................................................................................................................. 44
   Acknowledgements ............................................................................................................... 45
   Literature Cited .................................................................................................................... 46
   Tables and Figures ............................................................................................................... 50

CHAPTER 4. EFFECT OF CROP ROTATION ON SOYBEAN APHID ................................. 56
   Abstract .................................................................................................................................. 56
   Introduction ............................................................................................................................ 57
   Materials and Methods ......................................................................................................... 59
   Results ................................................................................................................................... 65
   Discussion .............................................................................................................................. 70
   Acknowledgements ............................................................................................................... 73
   References Cited ................................................................................................................... 73
   Tables and Figures ............................................................................................................... 77

CHAPTER 5. GENERAL CONCLUSIONS ........................................................................... 86
   Literature Cited .................................................................................................................... 88
Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the most economically damaging insect pest of soybean, (*Glycine max* (L.) Merr.). Since soybean aphids were discovered in the United States in 2000, a substantial effort has been made to identify mechanisms for managing soybean aphid populations. Soybean aphid populations can be impacted by biocontrol, insecticides, and host plant resistance. Additionally, soybean susceptibility to soybean aphid can be influenced by soybean health and nutrient availability within phloem, which can be impacted through agricultural management practices. The focus of this research is three-fold. The first, was to evaluate how a three gene pyramid of *Rag* genes compared to four soybean varieties of varying resistance to soybean aphids. The second, was to demonstrate on a commercial scale, how soybean aphid populations and soybean yield differed between soybean aphid resistant and soybean aphid susceptible varieties on Iowa farms. The third, was to evaluate how crop rotation and soybean growth stage influenced soybean susceptibility to soybean aphid. We conducted our research in small plot cage studies, small plot studies, and on a commercial scale in central and northern Iowa. Cumulatively, our results suggest soybean aphid resistant varieties provide protection against soybean aphids with no consequence to yield, with host plant quality for soybean aphid being affected by crop rotation at the early reproductive growth stage.
CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

The purpose of the research presented in this thesis is to increase our knowledge of the interactions between soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) and its secondary host, soybean, *Glycine max* (L.) Merr. We evaluated the efficacy of soybean aphid-susceptible and soybean aphid-resistant soybean varieties in small-plot and on-farm studies. In addition, we studied the impact of three crop rotations on host plant quality for soybean aphid. This thesis is organized into five chapters. Chapter one is comprised of an introduction and literature review of how soybean aphid populations may be influenced by biological control agents, host plant resistance, soybean aphid biotypes, soil health, and nutrient availability. Chapter two reports on the performance of soybean varieties containing Resistance to *Aphis glycines* (*Rag*) genes in small plot cage studies. Chapter three reports the effect of soybean varieties, differing in their soybean aphid resistance and source, on naturally occurring soybean aphid populations and on yield on Iowa farms. Chapter four reports on the effect of crop rotation and soybean growth stage on soybean aphid population growth rate.

Literature Review

Soybean aphid: biology, ecology, and life history

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an insect pest of soybean, *Glycine max* (L.) Merr., and has been a source of yield loss in American soybean (Ragsdale et al. 2011). Soybean aphid is heteroecious, or host alternating, using common buckthorn (Rhamnaceae: *Rhamnus* spp.) during the non-summer months, and soybean during the
summer months (Ragsdale et al. 2004, Ragsdale et al. 2011). The life cycle of soybean aphid begins in the spring, when nymphs hatch on *Rhamnus* spp., and develop into wingless fundatrices. At this stage, reproduction is asexual, giving rise to the second generation, which predominantly consists of wingless females (Ragsdale et al. 2004). The third generation is alate, or winged, females (gynoparae), which disperse to soybean (Ragsdale et al. 2004, Ragsdale et al. 2011). Alate and wingless females emerge throughout the growing season, with alate aphids often triggered by a stressed host during the summer months (Ragsdale et al. 2004). In the fall, alate females emigrate to buckthorn. Once on buckthorn, the gynoparae produce nymphs that morph into a wingless oviparae. During this time, winged males are produced on soybean and search for the oviparae. Once a male finds the oviparae, they mate and generate a fertilized egg which over winters on buckthorn (Ragsdale et al. 2004).

**Adverse effect of soybean aphid**

Soybean aphids are native to eastern Asia but were discovered in Wisconsin in 2000 and subsequently spread to 30 states and three Canadian provinces (Hartman et al. 2001, Venette and Ragsdale 2004, Ragsdale et al. 2011). If soybean aphid populations remain unmitigated, they can reduce soybean yield though feeding. Feeding may result in smaller plants, reduced pod set and lower per pod seed quantity (Ragsdale et al. 2011). In addition, soybean aphids are vectors for plant pathogens, such as Soybean mosaic virus, Alfalfa mosaic virus, and Tobacco ringspot (Clark and Perry 2002, Ragsdale et al. 2011). The incidence of these pathogens is positively correlated with soybean aphid populations (Clark and Perry 2002).

Soybean aphid feeding damage can negatively affect the physiology of soybean by reducing photosynthetic rates of soybean by as much as 50% in leaflets (Macedo et al. 2003, Riedell et al. 2009). Soybean aphid feeding can reduce soybean nodule volume by 34% in turn
reducing nitrogen (N) fixation by 80% (Riedell et al. 2009). In addition, soybean aphids also produce honeydew, which in large enough quantities can serve as a substrate for sooty mold, which in turn can lead to reduced photosynthetic capacity of soybean (Fox et al. 2004, Lemos Filho and Paiva 2006).

Due to the adverse effects of large populations, soybean aphids are the most economically damaging insect pests of soybean, generating yield losses as high as 40% (Ragsdale et al. 2011). Depending on the cost of insecticide and the scale of the outbreak, soybean aphid was estimated to generate economic losses between US $3.6 to $4.9 billion cumulatively over a ten-year period (Kim et al. 2008b). To estimate when economic damage may occur, an economic injury level and economic threshold was established for soybean aphid. Economic injury level is the minimum pest population that can cause economic injury (Stern et al. 1959), which is 674 soybean aphids per plant (Ragsdale et al. 2007). The economic threshold is the pest population in which control mechanisms should be identified to maintain pest populations below the economic injury level (Stern et al. 1959). For soybean aphid, the economic threshold is 250 individuals per plant until the R5 growth stage (Ragsdale et al. 2007).

Prior to the discovery of soybean aphid in North America, less than 0.1% of soybean acreage in the north central United States was managed for insect pests. However, since its discovery up to 57% of a state’s soybean acreage may be treated with insecticides during an outbreak year in the United States (Ragsdale et al. 2011). The use of insecticides over vast stretches of agricultural land can have negative ecological consequences. Such consequences include environmental damage (Pimentel et al. 1992), insect pest population resurgence (Shepard et al. 1977, Johnson et al. 2008), insecticide resistance, and damage to beneficial insect populations (Flexner et al. 1986, Desneux et al. 2004, Desneux et al. 2007, Johnson et al. 2008).
In addition, insecticide use can also lead to altered development, developmental timing, and nerve damage for some beneficial insects (Ripper 1956, Theiling and Croft 1988). Foliar insecticides and insecticidal seed treatments have been implemented for soybean aphid population management (Ragsdale et al. 2011, Seagraves and Lundgren 2012).

Neonicotinoid seed treatments are available for use on soybean and can provide limited protection against soybean aphid (Seagraves and Lundgren 2012, Krupke et al. 2017). The systemic movement of neonicotinoid insecticides can protect soybean due to the bioactivity associated with the parent molecule as well as its break down products when they accumulate within various plant tissues, including phloem, and can target soybean aphids as they feed (Bonmatin et al. 2015). Although a neonicotinoid seed treatment can provide early-season protection from soybean aphid, optimal protection can be achieved by using foliar insecticides based on scouting and the economic threshold (Krupke et al. 2017).

**Biological control agents**

Generalist predators are the primary biocontrol agents for soybean aphid (Rutledge et al. 2004). Generalists can mitigate early season soybean aphid establishment and population growth on soybean (Fox et al. 2004, Rutledge et al. 2004, Rutledge and O’Neil 2005, Desneux et al. 2006, Donaldson et al. 2007, McCarville and O’Neal 2012). In the native range of eastern Asia, soybean aphids are considered a minor economic pest due to the abundance of natural enemies (Chang et al. 1994, Venette and Ragsdale 2004). In North America, biocontrol agents may mitigate soybean aphid from reaching economic injury level. However, the effectiveness of biocontrol agents is inconsistent between years (Schmidt et al. 2007). Aphidophagous biocontrol agents were found in North America decades prior to the discovery of soybean aphid (Venette
Despite predator establishment, soybean aphid spread over approximately 80% of U.S. soybean acreage (Venette and Ragsdale 2004, Schmidt et al. 2008).

Biocontrol agents have the most impact if their populations are established prior to soybean aphid populations exceeding the economic threshold (Xu et al. 2010). In North America, important aphid biocontrol agents include species from the families Carabidae, Coccinellidae, Anthocoridae, Cantharidae, Chamaemyiidae, Chrysopidae, Forficulidae, Hemerobiidae, Lampyridae, Nabidae and Syrphidae (Fox et al. 2004, Schmidt et al. 2008, Ragsdale et al. 2011).

In the north central United States, the most efficient biocontrol agents are members of the *Harmonia* and *Orius* genera, which is largely due to their efficiency as generalist predators and their population response to prey availability (Rutledge et al. 2004).

The impact that biocontrol agents have on soybean aphid populations within a field can be quantified by using predator exclusion cages. By sheltering plants from natural enemies, researchers can measure the interaction between soybean and soybean aphids in the field (Gardiner et al. 2009). Calculating the impact of biocontrol agents can be achieved by measuring the relative suppression of soybean aphid populations with and without protection from biocontrol agents (Gardiner et al. 2009).

**Host plant resistance to soybean aphids and soybean aphid biotypes**

(Zhang et al. 2017), rag4 (Zhang et al. 2009), Rag5 (Jun et al. 2012, Zhang et al., 2017), and Rag6 (Zhang et al. 2017). Rag genes vary in their dominance, those denoted with a capitalized “R” are dominant, those listed lacking capitalization are recessive (Ajayi-Oyetunde et al. 2016). These genes provide protection against soybean aphid through either antibiosis or antixenosis. Antibiosis affects the biology of soybean aphid, and antixenosis as non-preference for the host plant, with antibiosis being the predominant mechanism of soybean aphid protection (Teetes 1996). However, different populations, or biotypes, of soybean aphid vary in their virulence or ability to persist on resistant soybean (Hill et al. 2004a, Kim et al. 2008a, Pedigo and Rice 2009). Since the discovery of Rag genes, gene pyramids have been evaluated for soybean aphid resistance (McCarville and O’Neal 2012, Wiarda et al. 2012). Pyramiding Rag1 and Rag2 (Rag1+Rag2) genes increases efficacy against soybean aphid compared to resistant soybean varieties containing only one of the Rag genes in the absence of natural enemies (McCarville and O’Neal 2012, Wiarda et al. 2012). However, exposure of soybean aphid to resistant hosts coupled with their high reproductive rate increases the risk of selecting for virulent soybean aphids for a given resistant soybean variety (Kim et al. 2008a).

Biotypes of soybean aphid can be problematic in soybean, since some can be virulent on specific soybean aphid resistant varieties (Kim et al. 2008a). Soybean aphids virulent only on susceptible soybean plants are classified as biotype 1 (Hill et al. 2004a, 2004b, Ragsdale et al. 2011). Soybean aphids virulent on only susceptible and Rag1 soybean are classified as biotype 2 (Hill et al. 2009, Ragsdale et al. 2011). Soybean aphids virulent on susceptible and Rag2 soybean are classified as biotype 3 (Hill et al. 2010, Ragsdale et al. 2011). Finally, soybean aphids virulent on susceptible, Rag 1, Rag 2, or Rag1+Rag2 soybean are classified as biotype 4 (Ajayi-Oyetunde et al. 2016). New resistant soybean varieties are being produced to provide resistance
to biotype 4 (Alt and Ryan-Mahmutagic 2013). A pyramid containing $\text{Rag}^1+\text{Rag}^2+\text{Rag}^3$ has shown in laboratory assays sufficient resistance to biotype 4 (Ajayi-Oyetunde et al. 2016).

However, populations of soybean aphid have the potential to become virulent to $\text{Rag}$ genes and $\text{Rag}$ pyramids that may be utilized for future soybean aphid management (Kim et al. 2008a).

**Effect of host plant quality on soybean aphid**

Host plant quality for herbivores and phloem feeders, including soybean aphid, is correlated with the nutrient quality and nutrient availability of their host (Awmack and Leather 2002, Noma et al. 2010). Nutrient concentration within phloem is limited, with its main component being sucrose, making phloem a poor source of essential amino acids (Sandstöm and Moran 2001, Marschner 2011). Potassium (K) deficiency in soybean plants has an inverse relationship with the availability of N in the form of amino acids within soybean leaves (Yamada et al. 2002). Essential amino acids are attributed to increased soybean aphid fecundity and increased nymphs per mother, resulting in higher soybean aphid population growth rates on K deficient plants (Walter and DiFonzo 2007, Noma et al. 2010). Nitrogen and K concentrations within soybean leaflets can account for 25% and 27%, respectfully, of soybean aphid population variance in soybean fields (Noma et al. 2010).

Soybean aphids are N limited, however, when soybean is stressed it releases key amino acids into the phloem (Anderson et al. 2004). Amino acids in phloem act as a N storage molecule (Marschner 2011). Some amino acids, like asparagine, are vital for soybean aphid heath and fecundity (Walter and DiFonzo 2007). Asparagine accumulates within soybean phloem when the host plant is K deficient, making asparagine available for aphid consumption, in turn, alleviating part of the N limitation of soybean aphids (Walter and DiFonzo 2007). Thus, concentrations of
asparagine are positively correlated with greater soybean aphid fecundity and survivorship (Myers et al. 2005, Myers and Gratton 2006, Walter and DiFonzo 2007).

Host stress derived by environmental factors, such as drought, can increase soybean aphid growth rate (Nachappa et al. 2016). In addition, growth stage has been cited as impacting cereal aphid *Sitobion avenae* (Hemiptera: Aphididae) (Walters and Dixon 1982), colonization establishment on wheat, with the early growth stages of wheat having a higher population growth rate than later stages (Walters and Dixon 1982). Thus, growth stage of the host plant must be considered when comparing soybean aphid population growth rates.

Leaflet nutrient concentrations in soybean differs between soybean growth stage, in turn may influence nutrient availability for soybean aphids. Hanway and Weber (1971) found that N, P, and K concentrations in trifoliates were highest in the vegetative growth stage and declined as soybean progressed into reproductive growth stages. However, Bender et al. (2015) found that trifoliate nutrients in modern soybean varieties peaked at early reproductive growth stages and were lower in vegetative and reproductive growth stages for the majority of the 11 nutrients analyzed in their study. These findings indicate that the timing of nutrient translocation away from trifoliates in these two varieties may differ. Thus, host health, nutrient availability, and growth stage may have an impact on soybean aphid colony establishment and population growth.

**Host health and crop rotation**

Humans have utilized crop rotation for thousands of years to address soil productivity. Agriculture prior to crop rotation resulted in lower yields derived from continuous cropping (Karlen et al. 1994). In ancient civilizations, crop and fallow and crop rotation agricultural practices were the two common cropping systems (Karlen et al. 1994). After World War II, longer crop rotations diminished in the United States. In the north central U.S, longer rotations
were replaced by continuous corn or a corn-soybean rotation (Karlen et al. 1994, Padgitt et al. 2000).

Field crops such as maize, soybean, and sorghum, yield more when in rotation (Strickling 1950, Francis and Clegg 1990). Other benefits associated with crop rotation include improved water availability (Powers and Lewis 1930, Tilman et al. 2002), soil nutrient availability (Powers and Lewis 1930, McDaniel et al. 2014b), soil structure (Chan and Heenan 1996), soil microbial communities (Tiemann et al. 2015), decomposition rates (Powers and Lewis 1930, McDaniel et al. 2014a), weed control (Liebman and Dyck 1993), and growth promoting substances (Karlen et al. 1994). Crop rotation also impacts the relationship between a crop its associated pests. For example, crop rotation can control some insect pests such as nematodes as well as members of the genus *Diabrotica* and *Naupactus* (Dabney et al. 1988, Francis and Clegg 1990).

The benefits of crop rotation in regard to N use efficiency for plants can only occur if crop rotation increases the N pool in the soil, and only if the plant utilizes the available N more efficiently when compared to plants in the conventional corn-soybean rotation (Pierce and Rice 1988). Microbial activity is vital for N fixation, with more diverse rotations generating more microbial biomass and activity as well as bolstering microbial N and enzyme activity in more diverse rotations (Bolton et al. 1985, Pierce and Rice 1988). Enzyme activity is vital for nutrient cycling of carbon (C), N, phosphorous (P), and sulfur (S) within soils, with enzyme activity being bolstered by longer more diverse rotations compared to corn-soybean rotations (Dick 1984). Diversified crop rotations have been associated with increased organic N and/or reduced soil organic N loss (Pierce and Rice 1988). Crop rotation is correlated with efficient water use and increased soil organic matter, with crop rotation increasing water infiltration thus improving water availability (Unger and Stewart 1983, Pierce and Rice 1988).
The benefits associated with crop rotation may be influenced by the preceding crops, with more rotationally diverse cropping systems optimizing the benefits associated with crop rotation (Chan and Heenan 1996, Davis et al. 2012). The corn-soybean crop rotation that dominates the landscape of the north central United States may be leading to K deficiency within soybean if fields are not nutritionally managed by adding K fertilizers (Silva 2017). Given that crop rotation has been cited as increasing soil organic N as well as improving water infiltration and availability to crops, there is a possibility that crop rotations longer than two years may influence soybean host quality for soybean aphid.

**Literature Cited**


CHAPTER 2. RESISTANCE TO *APHIS GLYCINES* GENE EFFICACY TRIAL

Abstract

Host plant resistance of soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), conferred by host plant resistance genes in soybean, *Glycine max* (L.) Merr. germplasm is an effective management tool. However, soybean aphid biotypes have the ability to overcome some resistance (*Rag*) genes. We hypothesized soybean plants containing a *Rag1+Rag2+Rag3* gene pyramid would be effective against biotype-1 and biotype-4 soybean aphids in a field environment. The objectives were to evaluate soybean aphid resistant soybean varieties against their susceptible isolines to understand which soybean cultivar(s) were more efficacious at mitigating the establishment and population growth of both soybean aphid biotypes. We measured cumulative aphid days for each treatment combination to evaluate exposure of susceptible, *Rag1, Rag1+Rag2,* and *Rag1+Rag2+Rag3* plants to both soybean aphid biotypes with and without the presence of aphidophagous predators. We observed contradictory results where plants containing either *Rag1* or *Rag1+Rag2* genes had higher cumulative aphid days than susceptible soybean and soybean containing the *Rag1+Rag2+Rag3* pyramid regardless of the presence or absence of predators, indicating that further research is needed to understand how combinations of drought, heat, host health, and host quality impact biotype response to host plant resistance.

**Key words:** soybean aphid, host plant resistance, *Rag* genes, virulent, avirulent
Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is native to eastern Asia but was discovered in Wisconsin in 2000 and subsequently spread to 30 states and three Canadian provinces (Hartman et al. 2001, Venette and Ragsdale 2004, Ragsdale et al. 2011). Soybean aphid is one of the most economically damaging insect pests of soybean, generating yield losses as high as 40% (Ragsdale et al. 2011). Depending on the cost of insecticide and the scale of the outbreak, soybean aphids were estimated to cumulatively generate losses between US $3.6 to $4.9 billion since their discovery in North America (Kim et al. 2008b).

Soybean aphid populations may be suppressed by predation, host health, host plant resistance and chemical means (Anderson et al. 2004, Fox et al. 2004, Rutledge et al. 2004, Ragsdale et al. 2011, Hill et al. 2012). Insect biocontrol agents are effective at managing soybean aphid populations (Rutledge et al. 2004). Biocontrol agents include members of the families Anthocoridae, Aphelinidae, Braconidae, Cecidomyiidae, Chrysopidae, Coccinellidae, Hemerobiidae, Nabidae, and Syrphidae (Gardiner et al. 2009). However, the efficiency of biocontrol agents is inconsistent, leaving farmers to rely on foliar insecticides as a means of protecting yield (Ragsdale et al. 2007, Kim et al. 2008a). Host plant resistance to soybean aphid can reduce the amount of insecticides required to manage soybean aphid populations (Hill et al. 2004).

In the north central region of the United States host plant resistance can maintain soybean aphid populations below the economic threshold level of 250 aphids per plant for the region (Hill et al. 2004). Soon after the 2000 discovery of the soybean aphid in Wisconsin, researchers uncovered antibiosis and antixenosis sources of resistance naturally occurring within the soybean genome, which were subsequently labeled Resistance to *Aphis glycines* (*Rag*) genes (Hill et al.
Soybean aphid resistant genes include Rag1 (Kim et al. 2009), rag1b (Bales et al. 2013), rag1c (Zhang et al. 2009), Rag2 (Kim et al. 2010), rag3 (Bales et al. 2013), Rag3 (Zhang et al. 2010), Rag3b (Zhang et al. 2013), Rag3c (Zhang et al. 2017), rag4 (Zhang et al. 2009), Rag5 (Jun et al. 2012; Zhang et al., 2017), and Rag6 (Zhang et al. 2017). Rag genes vary in their dominance, those denoted with a capitalized “R” are dominant, those listed lacking capitalization are recessive (Ajayi-Oyetunde et al. 2016). Different populations, or biotypes, of soybean aphid vary in their virulence or ability to persist on resistant soybean (Hill et al. 2004, Kim et al. 2008a). Exposure of soybean aphid to resistant hosts coupled with soybean aphid’s high reproduction rate increases the risk of selecting for virulent soybean aphid (Kim et al. 2008a). Since the discovery of Rag genes, gene pyramids have been evaluated for their efficacy for soybean aphid resistance (McCarville and O’Neal 2012, Wiarda et al. 2012). Pyramiding Rag1 and Rag2 (Rag1+Rag2) genes have been shown to increase efficacy compared to resistant soybean varieties containing only one of the resistance genes in the absence of natural enemies (McCarville and O’Neal 2012, Wiarda et al. 2012).

Biotypes of soybean aphid can be problematic in soybean fields, since some biotypes can be virulent on specific soybean aphid resistant varieties (Kim et al. 2008a). Soybean aphids virulent only on susceptible soybean plants are classified as biotype 1 (Hill et al. 2004a, Hill et al. 2004b, Ragsdale et al. 2011). On the other extreme, soybean aphids virulent on susceptible, Rag1, Rag2, or Rag1+Rag2 soybean are classified as biotype 4 (Ajayi-Oyetunde et al. 2016). New resistant soybean varieties are being produced to provide resistance to biotype 4 (Alt and Ryan-Mahmutagic 2013). A pyramid containing Rag1+Rag2+Rag3 has shown in laboratory assays sufficient resistance to biotype 4 (Ajayi-Oyetunde et al. 2016). However, soybean aphid
populations have the potential to become virulent to \textit{Rag} genes and \textit{Rag} pyramids that may be utilized for future soybean aphid management (Kim et al. 2008a).

We evaluated soybean aphid resistant varieties against their susceptible isolines to understand which soybean cultivar(s) were more efficacious at mitigating the establishment and population growth of soybean aphid biotype-1 and biotype-4 in the field. We hypothesized that soybean containing the \textit{Rag}1+\textit{Rag}2+\textit{Rag}3 gene pyramid would be efficacious against both biotypes with and without predator exclusion.

**Materials and Methods**

Soybean aphids from Iowa State University were used in this experiment. Soybean aphid populations were identified based on their responses to soybean aphid resistant varieties. Aphids with an avirulent response to all \textit{Rag}-genes were considered biotype-1 and were maintained on soybean aphid susceptible soybean (LD12-15833R; Iowa State University) (Hill et al. 2004, Hill et al. 2004). Soybean aphids virulent on susceptible, \textit{Rag}1, \textit{Rag}2, or \textit{Rag}1+\textit{Rag}2 soybean cultivars are classified as biotype-4 and were maintained on \textit{Rag}1+\textit{Rag}2 soybean (LD12-15841Ra; Iowa State University) (Ajayi-Oyetunde et al. 2016).

In 2016 and 2017 we conducted small-plot experiments on two Iowa State University Research Farms in Story County, Iowa. Soybean varieties consisted of ISU Susceptible (LD14-8007), \textit{Rag}1 (LD14-8033), \textit{Rag}1+\textit{Rag}2 (LD14-8040), \textit{Rag}1+\textit{Rag}2+\textit{Rag}3 (LD14-8035). For both years, we used biotype-1 and biotype-4 soybean aphids.

In 2016, we had three treatments consisting of the four soybean varieties, the two soybean aphid biotypes, and the presence or absence of predator exclusion cages (caged and uncaged, respectively). Treated plants had predator exclusion cages constructed over them, however, caged plants had the mesh pulled up and sealed to prevent aphidophagous predators
from entering the cage (Gardiner et al. 2009). Uncaged plants had the cage constructed but cages were left unsealed after infestation.

The 2017 field season was divided into two plantings. The first planting contained the four seed varieties, the two soybean aphid biotypes, with all experimental units having predator exclusion cages sealed (Gardiner et al. 2009).

The second planting of 2017 contained the four seed varieties, the two soybean aphid biotypes, and the presence or absence of predator exclusion cages. There was a total of three treatment replicates per location. Treated plants had predator exclusion cages constructed over them and after infestation, mesh netting was pulled up and sealed. Uncaged plants, however, had the cage constructed but were left unsealed.

Experimental plots were 9.14 meters by 18.29 meters and were organized in a randomized complete block design, with each location containing one block. All plots were hand weeded. Plants were planted at both locations on 10 June 2016, 31 May 2017, and 17 June 2017. We randomly assigned treatment combinations with a random number generator. We planted three seeds 7.62 centimeters from each other. Plants were planted in rows 1.83 meters apart and the distances between plants within each row was 3 meters. Predator exclusion cages were constructed at planting. Every experimental unit received a predator exclusion cage. Cage construction required PVC pipes making a rectangular structure, rebar, zip-ties, twist ties, and no-see-um number 20 mesh (Quest Outfitters, Sarasota, FL). Rectangular PVC pipe structures were placed over each experimental unit. Cages were reinforced with two pieces of rebar placed on opposite sides of the cage and secured with zip ties. The mesh netting was placed over the cage with the bottom 15 centimeters of mesh buried to ensure predator exclusion. Cages were left unsealed until infestation occurred, and natural enemies were removed.
Once cages were constructed, we removed soybean plants leaving one soybean under each structure. We infested all treated soybean plants with the soybean aphid biotype randomly assigned to the experimental unit. All plants were infested at the V3 soybean growth stage, which occurred on 4 July 2016, 14 June 2017, and 19 July 2017, depending on planting date. Ten mixed aged soybean aphids were placed on the middle leaf of the youngest trifoliate of each plant using a fine tipped paint brush. Once the plant was infested caged plants had the No-see-um mesh netting pulled up, sealed, and secured with a twist tie. Uncaged plants remained unsealed.

After each experimental unit was infested, we estimated soybean aphid populations on each plant until alate (winged) soybean aphids were found on treated plants (Ragsdale et al. 2004). We measured populations for each experimental unit three times per week from July 8 to 1 August 2016, 27 June to 28 July 2017, and 20 July to 4 August 2017. We used cumulative aphid days (CAD) as a measure of soybean plant exposure to soybean aphid over time (Hanafi et al. 1989, Ragsdale et al. 2007). Cumulative aphid days calculated from each treated plant allowed us to generate mean plant exposure to soybean aphid for each treatment combination.

We utilized a four-factor experimental design in 2016 and the second phase of 2017, with soybean variety, soybean aphid biotype, presence and absence of predators, and location as fixed factors, resulting in 32 treatment combinations for 2016 and the second phase of 2017. For the first phase of 2017 there were 16 treatment combinations which included soybean variety, soybean aphid biotype, and location as fixed factors.

Cumulative aphid day data were power transformed to meet the normality assumptions of the analysis of variance (ANOVA). The statistical model included soybean variety, the presence or absence of predator exclusion cages, location, soybean aphid biotype, and their interactions as
fixed factors, with year as a random factor (Table 1). Location was not considered random as only one block was located within each location. We tested treatment significance using ANOVA on RStudio statistical software version 0.99.903 (RStudio Team 2009). Significant treatment effects were determined with a significance level of Alpha=0.05.

Results

We found no significant difference in CAD by the soybean variety and location interaction (F=1.212; df=5, 106; P=0.308) (Table 1). We found no significant difference in CAD by the soybean aphid biotype and the presence or absence of predator exclusion cage interaction (F=0.242; df=1, 106; P=0.624) (Table 1). We found no significant difference in CAD by the soybean aphid biotype and soybean variety (F=0330; df=5 106; P=0.894) (Table 1).

We observed biotype-1 soybean aphids infested on caged plants generated more CAD on Rag1+Rag2 plants than the other soybean varieties included in this study, however, no significant difference was observed (F=0.424, df=3, 20; P=0.737) (Fig. 1). We observed biotype-1 soybean aphids infested on uncaged plants generated significantly more CAD on Rag1 soybean plants than the other soybean varieties included in this study (F=2.44, df=3, 20; P=0.032) (Fig. 2). We observed biotype-4 infested on caged plants generated more CAD on Rag1 soybean plants than the other soybean varieties included in this study, however, no significant difference was observed (F=2.476, df=3, 20; P=0.075) (Fig. 3). We observed biotype-4 soybean aphids infested on uncaged plants generated more CAD on Rag1+Rag2+Rag3 soybean plants than the other soybean varieties included in this study, however, no significant differences were observed (F=1.2044, df= 3, 20; P=0.376) (Fig. 4). Across all treatments there was a significant difference in CAD between biotype-1 and biotype-4 soybean aphids (F=6.340; df=1, 106; P=0.013) (Fig. 5).
Cumulative aphid days were significantly affected by predator exclusion cage treatment \((F=15.656; \text{df}=1, 106; P<0.001)\), where caged soybean generated higher CAD than uncaged soybean (Fig. 6). There was an 80% reduction in mean CAD between the two treatments when predators were allowed to feed. These results indicate that biocontrol agents remain effective at managing soybean aphid populations.

**Discussion**

Soybean aphid resistant genes have been identified as a means for mitigating soybean aphid establishment on soybean and subsequent population growth (Hill et al. 2004, Hill et al. 2012). Pyramiding soybean aphid resistant genes has been shown to increase efficacy of soybean compared to resistant soybean varieties containing only one of the resistance genes (McCarville and O’Neal 2012, Wiarda et al. 2012). We hypothesized that soybean containing the \(Rag_{1+Rag_{2+Rag_{3}}}\) gene pyramid would be more efficacious against biotype-1 and biotype-4 soybean aphids than soybean lacking the \(Rag_{1+Rag_{2+Rag_{3}}}\) pyramid. Understanding how soybean aphid resistant soybeans affect soybean aphid populations across biotypes is important as it may aid in selecting which \(Rag\) genes will be included in future commercially available soybean varieties.

We observed that biotype-1 soybean aphids were more virulent on \(Rag_{1}\) soybean and \(Rag_{1+Rag_{2}}\) soybean regardless of the presence or absence of aphidophagous predators. Our findings support previous observations of the possible presence of a fitness cost associated with biotype-4 soybean aphid, as biotype-4 CAD was lowest on susceptible soybean and \(Rag_{1+Rag_{2+Rag_{3}}}\) soybean (Varenhorst et al. 2015, Varenhorst et al. 2017). Our results are contradictory to established literature that biotype-1 soybean aphids are only virulent on
susceptible soybean, yet we observed highest CAD values on *Rag1* soybean (Hill et al. 2004, Hill et al. 2004).


Results here suggest that soybean aphid virulence may have been influenced by abiotic and biotic factors over our data collection periods. Factors include temperature (Richardson 2011), drought (Nachappa et al. 2016), host stress (Myers et al. 2005), amino acid concentration within the phloem (Walter and DiFonzo 2007), and the stability of *Rag* gene expression at higher temperatures (Chirumamilla et al. 2015). This indicates that soybean aphid biotypes exposed to resistant hosts may be able to overcome hostplant resistance if a combination of abiotic and biotic factors allow for populations to establish and persist. Further research is needed to understand how combinations of drought, heat, and host health impact soybean aphid biotype responses to host plant resistance.

**Acknowledgements**

We would like to thank the North Central Region Sustainable Agriculture Research and Education Program for funding. Derek Welshhons, Greg VanNostrand, Ashley Dean, Todd Broadrick, Joseph Salaba, Meiling Wong, and Derek Burger, Shelby Pritchard, Ryan Wilcox,
Josh Rhoads, and Lyndi Freel for their assistance in aphid colony management, data collection, and/or field site preparation.

Literature Cited


Tables and Figures

Table 1. Analysis of variance table (ANOVA) of cumulative aphid days (CAD) in small plot studies in 2016 and 2017 experiments

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean variety</td>
<td>5</td>
<td>0.080</td>
<td>0.016</td>
<td>2.816</td>
<td>0.020</td>
</tr>
<tr>
<td>Aphid biotype</td>
<td>1</td>
<td>0.036</td>
<td>0.036</td>
<td>6.340</td>
<td>0.013</td>
</tr>
<tr>
<td>Caged/uncaged</td>
<td>1</td>
<td>0.089</td>
<td>0.089</td>
<td>15.656</td>
<td>0.0001</td>
</tr>
<tr>
<td>Location</td>
<td>1</td>
<td>0.041</td>
<td>0.041</td>
<td>7.197</td>
<td>0.008</td>
</tr>
<tr>
<td>Soybean variety and location</td>
<td>5</td>
<td>0.034</td>
<td>0.007</td>
<td>1.212</td>
<td>0.308</td>
</tr>
<tr>
<td>Aphid biotype and caged/uncaged</td>
<td>1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.242</td>
<td>0.624</td>
</tr>
<tr>
<td>Soybean variety and aphid biotype</td>
<td>5</td>
<td>0.009</td>
<td>0.002</td>
<td>0.330</td>
<td>0.894</td>
</tr>
<tr>
<td>Residuals</td>
<td>106</td>
<td>0.604</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Cumulative aphid days (CAD) ± standard error of the mean (SEM) of biotype-1 soybean aphids on caged soybean plants of various soybean aphid resistances. Susceptible depicts CAD of susceptible plants exposed to biotype-1 soybean aphids. *Rag*1 signifies CAD of soybean plants containing soybean aphid resistance gene *Rag*1 exposed to biotype-1 soybean aphids. *Rag*1+*Rag*2 signifies CAD of soybean plants containing soybean aphid resistance gene pyramid *Rag*1 and *Rag*2 exposed to biotype-1 soybean aphids. *Rag*1+*Rag*2+*Rag*3 signifies CAD of soybean plants containing soybean resistance gene pyramid *Rag*1, *Rag*2, and *Rag*3 exposed to biotype-1 soybean aphids. Treatment combinations did not yield significant differences \(F=2.816, \text{df}=5, 106; P=0.020\).
Figure 2. Cumulative aphid days (CAD) ± standard error of the mean (SEM) of biotype-1 soybean aphids on uncaged soybean plants of various soybean aphid resistances. Susceptible depicts CAD of susceptible plants exposed to biotype-1 soybean aphids. *Rag*1 signifies CAD of soybean plants containing soybean resistance gene pyramid *Rag*1, *Rag*2, and *Rag*3 exposed to biotype-1 soybean aphids. Treatment combinations did yield a significant difference ($F=2.44$, df=3, 20; $P=0.032$).
Figure 3. Cumulative aphid days (CAD) ± standard error of the mean (SEM) of biotype-4 soybean aphids on caged soybean plants of various soybean aphid resistances. Susceptible depicts CAD of susceptible plants exposed to biotype-4 soybean aphids. Rag1 signifies CAD of soybean plants containing soybean aphid resistance gene Rag1 exposed to biotype-4 soybean aphids. Rag1+Rag2 signifies CAD of soybean plants containing soybean aphid resistance gene pyramid Rag1 and Rag2 exposed to biotype-4 soybean aphids. Rag1+Rag2+Rag3 signifies CAD of soybean plants containing soybean resistance gene pyramid Rag1, Rag2, and Rag3 exposed to biotype-4 soybean aphids. Treatment combinations did not yield a significant difference ($F=2.476, \text{df}=3, 20; P=0.075$).
Figure 4. Cumulative aphid days (CAD) ± standard error of the mean (SEM) of biotype-4 soybean aphids on uncaged soybean plants of various soybean aphid resistances. Susceptible depicts CAD of susceptible plants exposed to biotype-4 soybean aphids. *Rag1* signifies CAD of soybean plants containing soybean aphid resistance gene *Rag1* exposed to biotype-4 soybean aphids. *Rag1*+*Rag2* signifies CAD of soybean plants containing soybean aphid resistance gene pyramid *Rag1* and *Rag2* exposed to biotype-4 soybean aphids. *Rag1*+*Rag2*+*Rag3* signifies CAD of soybean plants containing soybean resistance gene pyramid *Rag1*, *Rag2*, and *Rag3* exposed to biotype-4 soybean aphids. Treatment combinations did not yield a significant difference (F=1.204, df=3, 20; P=0.376).
Figure 5. Cumulative aphid days (CAD) ± standard error of the mean (SEM) between biotype-1 and biotype-4 soybean aphid. The right side of the graph illustrates biotype-1 CAD regardless of predator pressure and hostplant resistance. The left half of the figure illustrates biotype-4 CAD regardless of predator pressure and hostplant resistance. Treatment combinations yielded a significant difference ($F=6.340$; df=1, 106; $P=0.013$).
Figure 6. Mean Cumulative Aphid Days (CAD) ± standard error of the mean (SEM) on caged and uncaged plants. Caged plants had predator exclusion cages sealed, protecting soybean aphid populations from predation from natural enemies. Uncaged plants had predator exclusion cages but remained unsealed. Our results show that CAD was lowest on soybean that were exposed to natural enemies. Different letters indicate a significant difference ($F=15.656$; df=1, 106; $P<0.001$).
CHAPTER 3. ON-FARM TESTING OF APHID RESISTANT SOYBEAN IN IOWA

Abstract

Host plant resistance to soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), can suppress outbreaks of this invasive pest in North America, and provide yield protection. Substantial effort has been made to support host plant resistance genes within the soybean, *Glycine max* (L.) Merr., germplasm. Aphid resistance (i.e., *Rag* genes) can provide season long protection without impacting the agronomic performance of soybean cultivars. These genes are available through public and USDA soybean breeding programs and are commercially available in limited quantities and maturity groups. Despite their efficacy, farmer adoption of soybean aphid-resistant varieties is low. The purpose of this study was to demonstrate how these varieties preform alongside soybean aphid susceptible varieties on commercial farms when exposed to naturally-occurring soybean aphid populations in realistic growing conditions. We recorded soybean aphid populations and yield for five different varieties of soybean from commercial and university sources. Varieties consisted of three soybean aphid-resistant varieties, conferred by *Rag1+Rag2* genes, and two susceptible varieties. Despite low populations in 2017 and 2018, we observed soybean aphid-resistant varieties were effective at managing populations without consequence to yield. Indicating, the implementation of soybean aphid-resistant soybean in Iowa is not dictated by the performance of these varieties.

Key words: soybean aphid, host plant resistance, *Rag* genes, yield
Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native soybean [Glycine max (L.) Merr.] pest in eastern Asia. In 2000, soybean aphid was discovered in Wisconsin and has since spread to 30 states and three Canadian provinces (Hartman et al. 2001, Venette and Ragsdale 2004, Ragsdale et al. 2011). Soybean aphid is one of the most economically damaging insect pests of soybean in America, due to its potential to reduce yield up to 40% (Ragsdale et al. 2011). Across vegetative and reproductive soybean growth stages, soybean yield decreases as soybean exposure to soybean aphids increases (Beckendorf et al. 2008). Kim et al. (2008) modeled an economic loss of US$3.6 to $4.9 billion cumulatively over a ten-year period in North American soybean production due to increased production costs for soybean aphid management.


In the north central region of the United States, fields reach economic threshold when 80% of plants have a minimum of 250 aphids per plant at early pod development, specifically, the R1 to R4 soybean growth stages (Ragsdale et al. 2007). Foliar insecticides and insecticidal seed
treatments have been implemented for soybean aphid population management (Ragsdale et al. 2011, Seagraves and Lundgren 2012). Prior to the discovery of soybean aphid in North America, less than 0.1% of soybean acreage in the north central United States managed for insect pests. However, since the discovery of soybean aphid in the United States, up to 57% of a state’s soybean acreage may be treated with insecticides during an outbreak year (Ragsdale et al. 2011).

Neonicotinoid seed treatments are available for use on soybean and can provide limited protection against soybean aphid (Seagraves and Lundgren 2012, Krupke et al. 2017). The systemic movement of neonicotinoid insecticides can protect soybean as active ingredients and their metabolites accumulate within various plant tissues, including phloem, and target soybean aphids as they feed (Bonmatin et al. 2015). Although a neonicotinoid seed treatment can provide early-season protection from soybean aphid, optimal protection can be achieved by using foliar insecticides based on scouting and the economic threshold (Krupke et al. 2017).

In an effort to find alternatives to insecticides, plant breeders in North America found Resistance to *Aphis glycines* (*Rag*) within the soybean genome (Hill et al. 2004b). To date, *Rag* genes include, *Rag1* (Kim et al. 2009), *rag1b* (Bales et al. 2013), *rag1c* (Zhang et al. 2009), *Rag2* (Kim et al. 2010), *rag3* (Bales et al. 2013), *Rag3* (Zhang et al. 2010), *Rag3b* (Zhang et al. 2013), *Rag3c* (Zhang et al. 2017), *rag4* (Zhang et al. 2009), *Rag5* (Jun et al. 2012; Zhang et al. 2017), and *Rag6* (Zhang et al. 2017). Combining two genes conferring resistance to the soybean aphid in a pyramid (e.g. *Rag1+Rag2*) improves the protection when compared to varieties containing a single *Rag* gene (McCarville and O’Neal 2012, Wiarda et al. 2012). McCarville et al. (2014), found that pyramided soybean provided more consistent control against soybean aphid throughout the growing season than insecticidal seed treatments. These *Rag* genes provide protection against soybean aphid through either antibiosis or antixenosis. Antibiosis affects the...
biology of soybean aphid, and antixenosis as non-preference for the host plant, with antibiosis being the predominant mechanism of soybean aphid protection (Teetes 1996).

Long term efficacy of soybean aphid-resistant varieties is influenced by different populations, or biotypes, of soybean aphid that vary in their virulence towards soybean containing *Rag* genes (Hill et al. 2004a, Kim et al. 2008). To date, there are four known soybean aphid biotypes found in the north central United States (Hill et al. 2004a, Hill et al. 2004b, Hill et al. 2009, Hill et al. 2010, Ragsdale et al. 2011, Ajayi-Oyetunde et al. 2016). Biotype-1 soybean aphids are virulent only on susceptible soybean (Hill et al. 2004a, Ragsdale et al. 2011). Biotype-2 soybean aphids are virulent on susceptible and *Rag1* soybean (Hill et al. 2009, Ragsdale et al. 2011). Biotype-3 soybean aphids are virulent on susceptible and *Rag2* soybean (Hill et al. 2010, Ragsdale et al. 2011). Biotype-4 soybean aphids are virulent on susceptible, *Rag1*, *Rag2*, or *Rag1+Rag2* soybean (Ajayi-Oyetunde et al. 2016).

To date, there has been virtually no adoption of soybean aphid-resistant soybean by commercial seed producers (McCarville et al. 2012, Hesler et al. 2013). The commercial availability of soybean aphid-resistant soybean is relatively limited, with commercially available soybean aphid-resistant varieties being conferred by either *Rag1* or *Rag1+Rag2* (McCarville et al. 2012, Hanson et al. 2019).

Soybean farmers make many management decisions over the course of a year, including which soybean variety to plant in the spring (Naeve 2015). Soybean variety selection by farmers is often determined by soybean maturity group, yield potential, and resistance to pests and diseases (Naeve 2015). The limited commercial availability of soybean aphid-resistant varieties may prevent farmer adoption of this approach to management. Anecdotally, farmers may
perceive that there is a limited need for a novel form of aphid management, concerns for yield
drag associated with these novel traits and potential added costs.

We evaluated commercially and publicly available varieties that varied in their resistance
to the soybean aphid. This evaluation was conducted with participation from farmers in Iowa,
who compared these varieties to those they use within their production practices. Our objectives
included measuring performance of these varieties on a commercial scale to naturally occurring
infestations of soybean aphids, as well as noting natural enemy populations. We selected
soybean aphid susceptible and resistant varieties (both commercial and from a university
breeding program), with resistance conferred by soybean aphid resistance genes $Rag1+Rag2$.

Included in these comparisons was the documentation of yield, allowing us to determine if
soybean aphid resistance was associated with yield loss.

**Materials and Methods**

The experiment was conducted on three privately-operated farms in 2017 and 2018, and
in 2017 one Iowa State University Research Farm. In 2017, participating farms were located in
north central Iowa in proximity to Marble Rock, Osage, Iowa Falls, and Kanawha, Iowa.
Participating farms in 2018 were located in north central and northwestern Iowa in proximity to
Marble Rock, Iowa Falls, and Aurelia, Iowa. All soybeans were planted between 12 and 28 May
2017, and between 18 and 28 May 2018. All seed lacked insecticidal seed treatments and were
not herbicide tolerant (conventional). Herbicide applications were either spot or broadcast
applications at the vegetative growth stages of soybean development. All farm management
practices were independently determined by each farmer (Table 1).

Soybean aphid-resistant varieties were selected based on the presence of $Rag1+Rag2$
soybean aphid-resistant genes and their commercial availability or their availability from the
Iowa Research Foundation. Farmer’s choice soybean aphid susceptible varieties were selected by the participating farmer, with the Iowa State University soybean aphid susceptible variety being selected based on the lack of resistance genes and as well as its availability through the Iowa Research Foundation (Table 1). Four soybean varieties with varying resistance and genetic backgrounds were randomly planted in 0.809-hectare strips by participating farmers (Table 1). All plots were embedded within a field of soybean aphid susceptible soybean selected by the participating farmer. In 2017, soybean aphid susceptible varieties consisted of Iowa State University’s (ISU) susceptible line (IA 2102; Iowa State University) and a variety of the farmer’s choosing. Resistant varieties consisted of an ISU resistant line (ISU 2112 RA12; Iowa State University) and a commercially available resistant line (Viking O.2188AT12N). In 2018, the soybean aphid susceptible variety was the farmer’s choosing (IA3045LF, Acres Edge 23C79, or Channel 2108R2). Soybean aphid-resistant varieties consisted of an ISU resistant line (ISU 2112 RA12; Iowa State University) and two commercially available varieties (Viking O.2188AT12N and Viking O.2399AT12N).

At each location, in 2017, aphid populations for each soybean variety were estimated weekly by counting all aphids on 30 randomly selected plants from 20 June to 9 August, and in 2018 once a month from 12 June to 6 September. Plants were randomly selected by moving in a zig-zag pattern through each treated 0.809-hectare strip. For each randomly selected plant, natural enemy populations were noted. Data was taken from 120 randomly selected plants from each location, for 30 plants per treatment. Each randomly selected plant was visually inspected for natural enemies of all life stages and species with number of individuals per plant recorded.
Yield data for each year were reported from three of the participating farms. All yields were adjusted for moisture. Yield data was measured and documented by the participating farmers. These data were standardized to kilograms per hectare (kg/ha).

To determine if soybean aphid-resistant varieties were more effective at managing soybean aphid populations than susceptible varieties, we calculated cumulative aphid days (CAD) from the number of aphids recorded from each treated strip by using the formula outlined in Hanafi et al. (1989). We power-transformed these data to meet the normality assumptions of analysis of variance (ANOVA). To analyze our data across years, we standardized the treatments, as varieties used were not the same across years, we tested for significance in CAD within resistant varieties and within susceptible varieties. There was no significant difference in CAD within soybean aphid-resistant or within soybean aphid susceptible varieties (p>0.05), because of this, we then merged all resistant varieties as one treatment and all susceptible varieties as another. Resulting in two replicates for susceptible soybean and three replicates for resistant soybean on each farm. Our statistical model included host plant resistance (aphid susceptible or resistant), seed source (commercially available or university sourced), location, and their interactions as factors, with year as a random factor and tested for significance with ANOVA on RStudio statistical software (RStudio, Inc. 2016). Significant treatment effects were determined with a significance level of Alpha=0.05.

To determine if soybean aphid-resistant varieties yielded similarly to soybean aphid susceptible varieties across years, data were log transformed to meet the normality assumptions of ANOVA. Statistical model included location, soybean variety, soybean aphid resistance, and the soybean variety by location interaction as fixed factors, with year as a random factor. We
tested for significance with ANOVA using RStudio statistical software (RStudio, Inc. 2016).

Significant treatment effects were determined with a significance level of Alpha=0.05.

**Results**

In 2017, soybean aphids were first observed on 26 June; however, by 3 July the soybean aphid-susceptible varieties generated larger populations than the soybean aphid resistant varieties. Similarly, in 2018, soybean aphids were first observed on 12 June and by 17 July soybean aphid susceptible varieties generated larger populations than soybean aphid resistant varieties (Fig. 1). Overall, a month after soybean aphid establishment, susceptible varieties generated larger soybean aphid populations than resistant varieties (Fig. 1). Both years, soybean aphid populations were low and did not exceed the economic threshold of 250 aphids per plant (Fig. 1) (Ragsdale et al. 2007).

No significant differences in CAD were noted in the soybean aphid resistance, seed source, and location interaction (\(F=0.039; \text{df}=2, 9; P=0.961\)). Despite low soybean aphid populations, we observed a significant difference in CAD between soybean aphid susceptible and soybean aphid resistant varieties (\(F=39.864; \text{df}=1, 9; P<0.001\)). Cumulative aphid days on soybean aphid resistant varieties were significantly lower than on soybean aphid susceptible varieties (Fig. 2). Furthermore, no significant difference in CAD was noted between seed sourced from public sources (ie. university breeding programs) and commercial sources (\(F=1.711; \text{df}=1, 9; P=0.223\)).

In both years, there was no significant difference in soybean yield by the soybean variety and location interaction (\(F=3.583; \text{df}=7, 43; P=0.967\)). We observed no evidence of yield drag associated with varieties containing \(\text{Rag1}+\text{Rag2}\) compared to aphid susceptible varieties.
(F=0.198; df=1, 43; P=0.659) (Fig. 3). In addition, yield was not significantly impacted by soybean variety (F=1.245; df=2, 43; P=0.300) (Fig. 4).

Aphidophagous predators were present at all locations over the course of our data collection periods (Fig. 5). This indicates that natural enemies may have suppressed soybean aphid establishment and population growth on soybean.

Discussion

Soybean containing Rag1+Rag2 genes were effective at mitigating soybean aphid, confirming on a commercial scale, the findings of in-lab assays, small plot, and cage studies (McCarville and O’Neal 2012, Wiarda et al. 2012, Ajayi-Oyetunde et al. 2016). We found that CAD did not significantly vary by soybean variety or seed source. Meaning, across resistant soybean varieties and seed sources, soybean aphid resistant varieties are an effective source of protection from soybean aphid. Such consistent efficacy means that Iowa soybean producers can be confident in the protection their soybean aphid resistant soybean would provide. However, in a different aphid pressure environment differences in CAD between soybean varieties of the same resistance and differences in CAD between seed sources may arise.

Aphidophagous predators were present within the field across the data collection periods and may have mitigated soybean aphid population establishment and growth (Gardiner et al. 2009). Soybean aphid populations never exceeded the economic threshold level of 250 aphids per plant, as well as the economic injury level of 5,560 CAD, meaning that foliar insecticides were never applied and yield was not impacted by soybean aphid populations (Ragsdale et al. 2007, Hodgson 2017).

As there was no yield damage associated with soybean aphid populations, we were unable to test how aphid resistant soybean varieties protected yield in a soybean aphid outbreak...
event. However, we observed no evidence of yield loss associated with pyramiding \textit{Rag1+Rag2}, with soybean aphid susceptible and soybean aphid resistant varieties yielding similarly to each other. Further highlighting that commercially available resistant soybean varieties provide similar yield to soybean varieties already implemented by Iowa farmers.

Soybean aphid resistant genes \textit{Rag1+Rag2} may protect yield during a soybean aphid outbreak, reducing the need for foliar insecticides, providing financial and environmental benefits (Flexner et al. 1986, Hill et al. 2004b, Seagraves and Lundgren 2012). Meaning, that soybean aphid resistance is an important component in integrated pest management strategies to mitigate aphid outbreak and to protect yield.

We found that soybean aphid resistant varieties were efficacious at managing soybean aphid populations with no consequence to yield. Our results indicate that the implementation of resistant soybean varieties in Iowa is dictated not by the performance of these varieties but rather by factors not evaluated in this project. Future research should focus on the societal factors impeding the implementation of these varieties on Iowa farms. If resistant soybean varieties are implemented on a much larger scale, exposure of soybean aphids to resistant hosts coupled with their high reproduction rate increases the risk of selecting for virulent soybean aphids (Kim et al. 2008). Further research is needed to understand how this technology should be implemented to reduce selection pressure on soybean aphid populations and still provide yield protection during a soybean aphid outbreak event.

\textbf{Acknowledgements}

We would like to thank the North Central Region Sustainable Agriculture Research and Education Program for funding. We would like to thank contributing farmers for their participation in this study; and Derek Welshhons, Greg VanNostrand, Ashley Dean, Todd
Broadrick, Joseph Salaba, Meiling Wong, and Derek Burger for their assistance with data collection.

Literature Cited


Naeve, S. 2015. There’s more to seed selection than just yield. United Soybean Board, Chesterfield, Missouri.


Tables and Figures

Table 1. Soybean seed variety information. Soybean aphid resistance was conferred from soybean aphid resistance genes conferred by $Rag1+Rag2$, with susceptible plants lacking $Rag$ genes. Conventional tolerance to herbicides means that soybean varieties were not herbicide tolerant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variety</th>
<th>Soybean Aphid Tolerance</th>
<th>Herbicide Tolerance</th>
<th>Maturity Group</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa State University (ISU)</td>
<td>IA2102</td>
<td>Susceptible</td>
<td>Conventional</td>
<td>2.7</td>
<td>2017</td>
</tr>
<tr>
<td>ISU</td>
<td>IA2112RA12</td>
<td>Resistant</td>
<td>Conventional</td>
<td>2.7</td>
<td>2017 &amp; 2018</td>
</tr>
<tr>
<td>Commercially Available</td>
<td>Viking O.2188AT12N</td>
<td>Resistant</td>
<td>Conventional</td>
<td>2.1</td>
<td>2017 &amp; 2018</td>
</tr>
<tr>
<td>Commercially Available</td>
<td>Viking O.2399AT12N</td>
<td>Resistant</td>
<td>Conventional</td>
<td>2.5</td>
<td>2018</td>
</tr>
</tbody>
</table>
Figure 1. Mean soybean aphids per plant ± standard error of the mean (SEM) across all locations in 2017 and 2018. Solid lines indicate 2017 soybean aphid populations and dashed lined indicate 2018 soybean aphid populations. Grey lines indicate soybean aphid susceptible varieties and black lines indicate soybean aphid resistant varieties. Overall, a month after soybean aphid establishment, susceptible varieties generated larger soybean aphid populations than resistant varieties.
Figure 2. Mean cumulative aphid days (CAD) ± standard error of the mean (SEM) across all locations and years. Resistant soybeans contain soybean aphid resistant genes $Rag_1+Rag_2$ and susceptible soybeans lack $Rag$ genes. Different letters signify significant differences between treatments. Our results indicate that soybean aphid resistant varieties were effective at managing aphid populations compared soybean aphid susceptible varieties even in years of low aphid populations in Iowa ($F=39.864; df=1, 9; P<0.001$).
Figure 3. Mean soybean yield in kilograms per hectare (kg/ha) ± standard error of the mean (SEM) across locations and years. Resistant soybeans contain soybean aphid resistant genes *Rag*1+*Rag*2 and susceptible soybeans lack *Rag* genes. Yield was reported by three participating farmers per year, with no significant difference in yield between varieties being observed ($F=0.198; df=1, 43; P=0.659$).
Figure 4. Mean soybean yield in kilograms per hectare (kg/ha) ± standard error of the mean (SEM) by variety across locations and years. Farmer’s choice varieties were soybean aphid susceptible and were all commercially available. IA 2112 was an Iowa State University soybean aphid susceptible variety. IA 2112RA12 was an Iowa State University soybean aphid resistant variety. Viking 2188AT12N and Viking 2399AT12N were both soybean aphid resistant and are commercially available through Albert Lea Seed. Each year, yield data by variety was reported by three participating farmers, with no significant difference in yield between varieties being observed (Table 1) \( F=1.245; \text{df}=2,43; P=0.300 \).
Figure 5. Mean natural enemy population per plant ± standard error of the mean (SEM) across all locations and treatments. Black lines indicate 2017 natural enemy populations, with grey lines indicating 2018 natural enemy populations. Each data point represents data from 360 randomly selected plants across locations and treatments. These data indicate that natural enemies were present in the treated strips throughout the data collection period.
CHAPTER 4. EFFECT OF CROP ROTATION ON SOYBEAN APHID

*To be submitted to the Journal of Economic Entomology*

Erika A. Rodbell¹, Erin W. Hodgson¹, Matt Z. Liebman², and Matthew E. O’Neal¹

¹Department of Entomology, Iowa State University, Ames, IA 50010
²Department of Agronomy, Iowa State University, Ames, IA 50010

Abstract

Crop rotation alters the soil environment and has the potential to influence plant physiology, in turn influencing host quality for some agricultural pests. Soybean aphid *Aphis glycines* (Hemiptera: Aphididae) establishment and growth on soybean (*Glycine max* (L.) Merr.) is influenced by soybean health and nutrient availability. Thus, an agronomic change that influences these two factors may impact soybean aphid populations. Since 2002, agronomists at Iowa State University have investigated the impact of growing corn and soybean in a conventionally managed two-year (corn; soybean) rotation, and diverse three-year (soybean; oat and red clover; corn) and four-year (soybean; oat and alfalfa; alfalfa; corn) rotations. By utilizing these established plots, we investigated the impact of crop rotation at different soybean growth stages (vegetative, early reproductive, and reproductive) on soybean aphid populations. Soybean aphid populations grew at a higher rate on soybeans in the two-year rotation than the three-year rotation at the early reproductive growth stage. Soybean aphid population growth rates tended to be higher on soybean in the vegetative growth stage than in early reproductive or reproductive growth stages. In addition, we sampled soybean leaflets from each treatment and quantified eleven nutrient concentrations as an indicator of nutrient availability for soybean aphids at the date of infestation. Our results indicate that growth stage and crop rotation influence leaflet nutrient concentrations as well as soybean aphid population growth rates.

**Key words:** soybean aphid, crop rotation, soybean growth stage, nutrient concentrations
Crop rotation has been in practice for thousands of years to manage nutrient availability in agricultural settings (Karlen et al. 1994). The effect of crop rotation on soil may be influenced by preceding crops (Chan and Heenan 1996). Benefits of longer, more diverse rotations include reduced chemical inputs, decreased freshwater toxicity from pesticides, and decreased incidence of sudden death syndrome of soybean (*Glycine max* (L.) Merr.) (Davis et al. 2012, Leandro et al. 2018). Furthermore, crop rotation benefits include elevated water and soil nutrient availability (Powers and Lewis 1930, Tilman et al. 2002, McDaniel et al. 2014b), improved soil structure (Chan and Heenan 1996), bolstered soil microbial communities (Tiemann et al. 2015), elevated decomposition rates (Powers and Lewis 1930, McDaniel et al. 2014a), and improved weed control (Liebman and Dyck 1993). Crop rotation also impacts the relationship between a crop and its associated pests and can be an effective practice to manage insects with specific host ranges and short migration distances (Francis and Clegg 1990). For example, crop rotation is recommended for the management of some nematode species, including soybean cyst nematode (*Heterodera glycines* Ichinohe), as well as insect pests such as members of the genus *Diabrotica* and *Naupactus* (Dabney et al. 1988, Francis and Clegg 1990).

The quality of a host plant for herbivorous insects can be affected by defensive compounds in the plant (e.g. allelochemicals), micronutrients, and macronutrients like nitrogen (N) (Awmack and Leather 2002). Specifically, N within plants has a significant effect on both the development and fecundity of herbivorous insects, especially for members of the family Aphididae (Dixon 1998). Aphids feed from phloem, which is comprised primarily of sucrose with low concentrations of N (Dixon 1998, Sandstöm and Moran 2001, Marschner 2011). Aphids gather their dietary requirement of N by consuming free amino acids in phloem, and as the concentration of N varies within phloem, so too does aphid fecundity (Montllor 1991, Dixon...
1998, Awmack and Leather 2002). Free amino acids are divided into essential and unessential amino acids, with unessential amino acids being metabolized first by gut symbionts and subsequently by the aphid host (Dixon 1998). For soybean, nutrient concentrations vary temporally, with increasing concentrations of N, phosphorus (P), and potassium (K) in modern varieties beginning at the R1 growth stage and reaching their apex at the R5 growth stage (Bender et al. 2015). It is not known which specific soybean growth stage the soybean aphid (Aphis glycines Matsumura [Hemiptera: Aphididae]) achieves its highest population growth rate. However, the rate at which populations of cereal aphids (Sitobion avenae [Hemiptera: Aphididae]) increase can vary by the growth stage of the plant. Specifically, cereal aphid populations grew at a faster rate on plants in the vegetative than the reproductive growth stage (Walters and Dixon 1982).

The availability of nutrients in the soil for plants also contributes to the fecundity of aphids. For example, soybean grown in K-deficient soils experience an increase in amino acids within phloem, such as asparagine, resulting in higher soybean aphid fecundity on nutrient stressed plants (Myers et al. 2005, Myers and Gratton 2006, Walter and DiFonzo 2007). Concentrations of N and K within soybean leaflets can account for 25% and 27%, respectively, of the variation in soybean aphid populations in soybean fields (Noma et al. 2010). In general, nutrient concentration within phloem is influenced by water availability, in turn affecting aphid fecundity. Aphids can experience increased fecundity on plants that experience intermittent water-stress when compared to an unstressed plant (Huberty and Denno 2004). The specific response of soybean aphids to soybean experiencing drought stress is also influenced by the presence of Soybean mosaic virus, with non-viruliferous aphids experiencing reduced fecundity on drought stressed soybean (Nachappa et al. 2016). Improved water availability to plants and increased soil organic N are both agronomic benefits associated with longer, more diverse crop
rotations (Unger and Stewart 1983, Pierce and Rice 1988). Cropping systems, specifically crops preceding soybean, can influence soybean aphid populations but the extent to which this contributes to preventing aphid outbreaks is not known (Lundgren et al. 2017). In addition, the impact of crop rotations longer than two years on the relationship between soybean and soybean aphid are not well studied.

The goal of this study was to evaluate the impact of crop rotation on soybean aphid population growth rates throughout the growing season. We hypothesized that longer, more diverse crop rotations would alter soybean aphid population growth rates compared to a conventional two-year rotation. We also hypothesized that soybean aphid population growth rates would vary at three soybean growth stages due to variation in nutrient concentrations within soybean. To account for this potential impact, we quantified the concentration of 11 nutrients in soybean leaflets across crop rotation treatments and soybean growth stages as a potential indicator of host plant quality for soybean aphids. Together, these data suggest how crop rotation may influence the interaction between soybean and soybean aphids.

**Materials and Methods**

**Crop rotation experiment**

The experiment was conducted in 2016, 2017, and 2018 at Iowa State University’s Marsden Farm in Boone County, IA. Since 2002, three rotation schemes have been compared within a randomized complete block design containing four blocks, each comprised of nine plots (83.3 m by 18.3 m), with one plot per block representing every phase of each crop rotation scheme (i.e. treatment) (Davis et al. 2012). Consequently, every year each block contained a plot of soybean grown in each rotation treatment. The rotation treatments include a conventionally managed two-year (corn; soybean) rotation, a three-year (soybean; oat and red clover; corn)
rotation, and a four-year (soybean; oat and alfalfa; alfalfa; corn) rotation (Hunt et al. 2017, Hunt et al. 2019). Soybean always followed corn in each rotation scheme.

Soybeans (Latham 2758) were planted on 20 May 2016, 15 May 2017, and 17 May 2018, with soybean seeds treated with Latham® Soyshield™, a formulation consisting of three fungicides (pyraclostrobin, fluxapyroxad, and metalaxyl) and no insecticides. Soil fertility management among treatments differed as the two-year rotation treatment was managed exclusively with synthetic fertilizer inputs. In contrast, soil in the three-year and four-year rotations received applications of composted cattle manure prior to corn production and lower rates of mineral fertilizers (Hunt et al. 2017, Hunt et al. 2019). However, soil management practices in the fall to spring prior to soybean planting were uniform across treatments and blocks. All soybean plots were measured for nutrient concentrations in the fall prior to planting to determine base fertilizer requirements. Uniform fertilizer applications occurred in the spring, with all soybean treatments and plots receiving the same quantity of a triple superphosphate, potassium chloride, and sulfur fertilizer applied with a bulk spreader.

Plot management for weeds in soybean differed among years but not among rotation treatments within years. In 2016, plots were treated with a post-emergent application of glyphosate and acifluorfen. In 2017 and 2018, plots were treated with a broadcast pre-emergent application of flumioxazin, followed by a post-emergent application of glyphosate with acifluorfen. The plots were not cultivated for weed management.

**Estimating aphid population growth rates in the field**

Population growth rates of soybean aphids were estimated on individual soybean plants that were artificially infested with soybean aphids from a colony kept at Iowa State University. This soybean aphid population is avirulent to aphid-resistant soybeans (i.e., biotype-1),
susceptible to insecticides (Valmorbida et al. in preparation), and maintained throughout the year on an aphid-susceptible soybean variety (LD12-15833R) in a growth chamber (25°C, 16:8 L:D). Two soybean plants within each plot were randomly selected within the third row from each edge of every soybean plot, for a total of eight plants per rotation treatment. All selected plants were surrounded by a cage to exclude soybean aphid natural enemies and other herbivores. Due to flooding in June 2018, only three blocks were available, resulting in a total of six caged plants per crop rotation treatment at the V3 (vegetative) growth stage, however, all four blocks were included for the remaining growth stages evaluated in 2018. Cages were constructed of a tomato cage surrounded by a sleeve made of no-see-um netting (number 20 mesh; Quest Outfitters, Sarasota, FL). Tomato cages were placed over the selected plants and adjacent plants were removed. Tomato cages were reinforced with two pieces of rebar placed on opposite sides of the cage and secured to the cage with zip ties. The sleeve was placed over the cage with the bottom 15 cm buried to prevent entry of natural enemies and defoliators.

The date and growth stage at which soybean plants were selected, caged, and infested varied by year. In 2016, soybean plants were selected, caged, and infested on 11 July at the R1 (early reproductive) growth stage. In 2017, soybean plants were selected, caged, and infested on 22 June at the vegetative growth stage, and 1 August at the R4 (reproductive) growth stage. In 2018, soybean plants were selected, caged, and infested on 19 June at the vegetative growth stage, 12 July at the early reproductive growth stage, and 30 July at the reproductive growth stage. Before plants were infested with soybean aphids, all herbivores and natural enemies were removed from each caged plant. Caged soybean plants were infested with ten mixed-aged soybean aphids, placed on each plant using a fine tipped paint brush on the middle leaf of the youngest leaflet. Once plants were infested, cages were sealed by pulling up the mesh netting,
twisting it at the top and securing with a twist tie. The population of aphids was measured four
days after the initial infestation and then every three days for two weeks.

**Estimating population growth rate in the lab**

In 2018, in an effort to control for abiotic factors that influence soybean aphid survival
and fecundity, soil from each treatment was used to test the effect of crop rotation on the
relationship between soybean aphids and soybean within a growth chamber. Two liters of soil
were collected from each treatment and block combination. Soil from the same crop rotation
treatment was mixed together to limit block effect, and then stored at 4.4°C. Ten plastic flower
pots (15.24 cm diameter) per treatment were filled with their assigned soil and planted with three
seeds from a soybean aphid susceptible variety (Latham 2758). Once planted, all pots were
randomly placed within the same greenhouse bay until plants were caged and infested with
soybean aphids.

When soybean plants reached the vegetative growth stage, they were caged and infested
with soybean aphids. The cage consisted of a single wire bent over the plant and a covering of
no-see-um (number 20 mesh) covering both wire frame and pot, secured with a rubber band
around the base of the pot. Each treated plant was infested on the same date with ten mixed-aged
soybean aphids placed on each plant with a fine tipped paint brush on the middle leaf of the
youngest leaflet. Infested plants were randomly placed in the same growth chamber (25°C, 16:8
L:D). The population of aphids was measured every three days for a two-week period. This
experiment was replicated three times, beginning on 10 August, 22 September and 24 October
2018. Altogether, soil from each crop rotation treatment was used to grow 30 soybean plants (i.e.
replicants) that were artificially infested with soybean aphids.
Soybean leaflet tissue analysis

In 2018, soybean leaflets were collected to determine if nutrient concentrations varied by rotation scheme and growth stage. Six soybean plants were selected in each plot, for a total of 24 total samples per treatment at each infestation date in 2018. The youngest fully developed leaflet was cut at the node from each plant, with the whole leaflet included in each collected sample. These samples were collected when soybeans were artificially infested with aphids in the field (i.e. 19 June, 12 July, and 30 July 2018).

In the lab, plant samples were dusted to remove dirt and other contaminants. All plant tissue samples were placed on paper towels and allowed to dry in a fume hood for 4-5 days. Once dried, samples were sent to the University of Wisconsin’s Soil and Forage Laboratory (https://uwlab.soils.wisc.edu/). Samples from each individual treatment were separately ground to a fine dust, in which six subsamples were taken, to measure concentrations of zinc (Zn), P, iron (Fe), manganese (Mn), calcium (Ca), K, magnesium (Mg), boron (B), sulfur (S), copper (Cu). Estimates of N had fewer replicates because the process to estimate the percentage of N within a sample differed from that used for other nutrients. This process was repeated with new samples for every growth stage evaluated in 2018.

Statistical analysis

Soybean aphid population growth rates in the field and in the lab were estimated with linear regression generated from population data collected from each infested plant over a two-week period. The slope of each line developed by the regression provided the population growth rate for each treated plant.

To test the hypothesis that soybean aphid population growth rate in the field varies based on the rotation scheme in which soybean is grown, three statistical models were generated, one for each soybean growth stage. We ran three separate models because nutrient concentrations in
soybean leaflets change as the plant develops (Bender et al. 2015). In addition, the manner in which this experiment was designed did not allow for data collected from the three growth stages to be collected at the same time. The statistical models included crop rotation treatment, year, and their interaction as fixed factors with block as a random factor. All soybean aphid population growth rate data were power transformed to meet assumptions of normality for analysis of variance (ANOVA). Significant treatment effects were determined with a significance level of $P<0.05$.

Results of the three models suggest the presence of an interaction between crop rotation treatment and growth stage. To test for the presence of a crop rotation treatment and growth stage interaction we ran three models, one for each year the experiment was conducted. As 2016 was the only year that included the early reproductive growth stage, the statistical model included rotation treatment as a fixed factor with block as a random factor. The 2017 and 2018 statistical model included rotation treatment and growth stage and their interaction as fixed factors with block as a random factor. Growth stage was included in the 2017 and 2018 models as population data were collected within the same year, and data from each growth stage was collected on the same date. All soybean aphid population growth rate data were power transformed to meet assumptions of normality for ANOVA. Significant treatment effects were determined with a significance level of $P<0.05$.

The in-lab assay was used to determine if crop rotations affected soybean aphid population growth rate in more controlled environment with optimal temperatures for soybean aphid growth and reproduction. We power transformed soybean aphid population growth rate data from the lab to meet assumptions of normality for ANOVA. We used a statistical model that included crop rotation treatment as a fixed factor and repetition as a random factor. Significant treatment effects were determined with a significance level of $P<0.05$. 
To test for significant differences among nutrient concentrations by crop rotation and soybean growth stage, we ran a multivariate analysis of variance (MANOVA). To do so, we standardized all nutrient concentrations to parts per million (PPM) and included growth stage and crop rotation treatment as fixed factors with subsample as a random factor. Growth stage was included in the model as all samples were collected in 2018, and all samples within each growth stage were collected on the same day. To identify how each nutrient was impacted by both factors, we generated statistical models which included crop rotation treatment, growth stage, and their interaction as fixed factors with subsample as a random factor. All nutrient data were power transformed to meet assumptions of normality for ANOVA. Significant treatment effects were determined with a significance level of P<0.05.

**Results**

**Soybean aphid population growth rate by crop rotation treatment**

Soybean aphid population growth rate at the vegetative growth stage did not vary significantly by crop rotation treatment ($F=0.480; \text{df}=2, 29; P=0.624$), but did significantly vary by year ($F=18.071; \text{df}=1, 29; P<0.001$) (Fig.1). We did not observe a significant interaction between rotation treatment and year ($F=0.921; \text{df}=2, 29; P=0.410$) during the vegetative stage.

Soybean aphid population growth rate at the early reproductive growth stage did significantly vary by rotation treatment ($F=4.333; \text{df}=2, 37; P=0.020$) and year ($F=18.532; \text{df}=1, 37; P<0.001$). At the early reproductive growth stage, soybean aphids in the two-year rotation generated significantly higher population growth rates than soybean aphids in the three-year rotation, with soybean aphids in the four-year rotation not significantly varying between the two treatments (Fig. 2). We did not observe a significant interaction between rotation treatment and year ($F=2.760; \text{df}=3, 37; P=0.076$) during the early reproductive growth stage.
Soybean aphid population growth rate at the reproductive soybean growth stage, did not vary significantly by crop rotation treatment ($F=0.129; \text{df}=2, 37; P=0.878$), but did significantly vary by year ($F=197.849; \text{df}=1, 37; P<0.001$) (Fig. 3). We did not observe a significant interaction between rotation treatment and year ($F=0.382; \text{df}=2, 37; P=0.685$) during the reproductive growth stage.

**Soybean aphid population growth rate by year**

In 2016, soybean aphid population growth rate significantly varied by rotation treatment ($F=6.677; \text{df}=2, 17; P=0.007$). Soybean aphids in the two-year rotation generated higher population growth rates than soybean aphids in the three-year rotation, with soybean aphids in the four-year rotation not significantly varying between the other two treatments (Fig. 4).

In 2017, soybean aphid population growth rate did not significantly vary by rotation treatment ($F=0.379; \text{df}=2, 41; P=0.687$), but did significantly vary by growth stage ($F=64.098; \text{df}=1, 41; P<0.001$). Aphid infestations on soybeans in the vegetative growth stage had a higher rate of growth than those on soybeans in the reproductive growth stage. (Fig. 5). We did not observe a significant interaction between rotation treatment and growth stage ($F=0.176; \text{df}=2, 41; P=0.839$).

In 2018, soybean aphid population growth rate did not significantly vary by rotation treatment ($F=0.547; \text{df}=2, 54; P=0.582$), but did significantly vary by growth stage ($F=13.850; \text{df}=1, 54; P<0.001$). Aphid infestations on soybeans in the vegetative growth stage, had higher population growth rates than infestations on early reproductive and reproductive growth stage (Fig. 6). Soybean aphid population growth rate did not vary significantly by the crop rotation treatment and growth stage interaction ($F=0.547; \text{df}=2, 54; P=0.818$).
In-lab assay soybean aphid population growth rate

Estimates of soybean aphid population growth rates on plants in the vegetative growth stage did not vary significantly when plants were grown with soil collected from the rotation treatments ($F=3.051; \text{df}=2, 36; P=0.060$). The growth rates estimated from the two-year, three-year, and four-year crop rotation treatments generated similar population growth rates (Fig. 7).

Leaflet nutrient concentrations by treatments and growth stages

Results from the MANOVA indicated that nutrient concentrations in PPM significantly varied by both the rotation treatment ($F=6.006; \text{df}=2, 18; P<0.001$) and soybean growth stage ($F=230.967; \text{df}=2, 18; P<0.001$). Individual ANOVAs for each tested nutrient indicated that the direction and magnitude of change by crop rotation treatment and soybean growth stage were dependent upon the nutrient analyzed.

Boron concentrations significantly varied by the rotation treatment and soybean growth stage interaction ($F=2.918; \text{df}=4, 38; P=0.034$) (Table 1). However, significant differences among crop rotation treatments were only noted at the vegetative growth stage, with the two-year rotation treatment generating the highest concentration ($F=61.179; \text{df}=2, 6; P<0.001$). Boron concentrations did not significantly vary by rotation treatment at the early reproductive growth stage ($F=2.909; \text{df}=2, 24; P=0.074$) and at the reproductive growth stage ($F=1.075; \text{df}=2, 5; P=0.409$) (Table 1).

Calcium concentrations did not significantly vary by the rotation treatment and soybean growth stage interaction ($F=0.278; \text{df}=4, 38; P=0.890$). Calcium did not significantly vary by rotation treatment ($F=0.662; \text{df}=4, 38; P=0.522$), however, Ca significantly varied by soybean growth stage ($F=41.770; \text{df}=2, 38; P<0.001$) (Table 1). However, significant differences among rotation treatments were only noted at the vegetative growth stage with Ca being highest in the three-year rotation, intermediate in the two-year rotation and lowest in the four-year rotation.
Calcium concentrations were not significantly affected by rotation treatments at the early reproductive growth stage \( (F=3.015; \text{df}=2, 24; \ P=0.068) \) and at the reproductive growth stage \( (F=0.313; \text{df}=2, 5; \ P=0.745) \) (Table 1).

Copper significantly varied by the rotation and soybean growth stage interaction \( (F=4.526; \text{df}=4, 38; \ P=0.004) \). Significant differences among rotation treatments occurred at all three growth stages. At the vegetative growth stage Cu was highest in the four-year rotation, intermediate in the three-year rotation, and lowest in the two-year rotation \( (F=741.590; \text{df}=2, 6; \ P<0.001) \). At the early reproductive growth stage Cu was highest in the four-year rotation, intermediate in the three-year rotation, and lowest in the 2-year rotation \( (F=4.269; \text{df}=2, 24; \ P=0.026) \). At the reproductive growth stage Cu was highest in the two-year rotation and lowest in the three-year and four-year rotations \( (F=5.818; \text{df}=2, 5; \ P=0.049) \) (Table 1).

Iron significantly varied by the rotation treatment and soybean growth stage interaction \( (F=3.779; \text{df}=4, 38; \ P=0.010) \) (Table 1). Significant differences among rotation treatments occurred at the vegetative and early reproductive growth stages. At the vegetative growth stage Fe was highest at the four-year rotation, intermediate in the two-year rotation, and lowest in the three-year rotation \( (F=648.930; \text{df}=2, 6; \ P<0.001) \). At the early reproductive growth stage Fe was highest in the two-year rotation, and lowest in the early reproductive and reproductive growth stages \( (F=16.204; \text{df}=2, 24; \ P<0.001) \). At the reproductive growth stage Fe was not significantly affected by rotation treatment \( (F=2.030; \text{df}=2, 5; \ P=0.226) \) (Table 1).

Magnesium significantly varied by the rotation treatment and soybean growth stage interaction \( (F=776.22; \text{df}=4, 38; \ P<0.001) \) (Table 1). Significant differences among rotation treatments occurred at the vegetative and early reproductive growth stages. At the vegetative growth stage Mg was highest in the three-year rotation, intermediate in the four-year rotation, and lowest in the tow-year rotation \( (F=18469; \text{df}=2, 6; \ P<0.001) \). At the early reproductive growth stage Mg was highest in the three-year rotation, intermediate in the four-year rotation, and lowest in the tow-year rotation.
growth stage Mg was highest in the four-year rotation, intermediate in the two-year rotation, and lowest in the three-year rotation \((F=8.065; \text{df}=2, 24; P=0.002)\). At the reproductive growth stage Mg concentrations did not significantly vary by rotation treatment \((F=0.317; \text{df}=2, 5; P=0.742)\) \((\text{Table 1})\).

Manganese was not significantly affected by the rotation treatment and soybean growth stage interaction \((F=0.518; \text{df}=4, 38; P=0.723)\). Manganese was significantly affected by rotation treatment \((F=4.324; \text{df}=2, 38; P=0.020)\) and soybean growth stage \((F=20.717; \text{df}=2, 38; P<0.001)\) \((\text{Table 1})\).

Total N was not significantly affected by crop rotation treatment \((F=2.611; \text{df}=2, 18; P=0.101)\), however, it was significantly affected by growth stage \((F=28.541; \text{df}=2, 18; P<0.001)\) \((\text{Table 1})\).

Phosphorus was significantly affected by the rotation treatment and soybean growth stage interaction \((F=4.058; \text{df}=4, 38; P=0.008)\) \((\text{Table 1})\). Significant differences in P concentrations occurred among rotation treatments at the vegetative and early reproductive growth stages. At the vegetative growth stage P had similarly high concentrations in the two-year and three-year rotations, and low concentrations in the four-year rotation \((F=59.24; \text{df}=2, 6; P<0.001)\). At the early reproductive growth stage P was highest in the four-year rotation, intermediate in the two-year rotation, and lowest in the three-year rotation \((F=5.252; \text{df}=2, 24; P=0.013)\). At the reproductive growth stage P concentrations did not significantly vary by rotation treatment \((F=1.421; \text{df}=2, 5; P=0.325)\) \((\text{Table 1})\).

Potassium did not significantly vary by the rotation treatment and soybean growth stage interaction \((F=0.539; \text{df}=4, 38; P=0.708)\). Potassium did not significantly vary by rotation treatment \((F=0.903; \text{df}=2, 38; P=0.414)\), however, did significantly vary by soybean growth stage \((F=114.992; \text{df}=2, 38; P<0.001)\) \((\text{Table 1})\).
Sulfur did not significantly vary by the rotation treatment and soybean growth stage interaction ($F=1.534; \, df=4, \, 38; \, P=0.212$). Sulfur significantly varied by rotation treatment ($F=7.170; \, df=2, \, 38; \, P=0.002$) and growth stage ($F=110.254; \, df=2, \, 38; \, P<0.001$) (Table 1).

Zinc significantly varied by the rotation treatment and soybean growth stage interaction ($F=4.335; \, df=4, \, 38; \, P=0.006$). Significant differences in Zn concentrations occurred among rotation treatments at the vegetative growth stage, with Zn concentrations highest in the two-year rotation, intermediate in the four-year rotation, and lowest in the three-year rotation ($F=1191.500; \, df=2, \, 6; \, P<0.001$). No significant differences in Zn concentrations occurred among rotation treatments at the early reproductive growth stage ($F=2.064; \, df=2, \, 24; \, P=0.149$), and at the reproductive growth stage ($F=1.126; \, df=2, \, 5; \, P=0.395$) (Table 1).

Discussion

The field results suggest that soybeans grown within the three rotation schemes vary in their quality as a host for the soybean aphid. Furthermore, these results suggest that the effect of crop rotation on the soybean aphid may be dependent upon soybean growth stage. The growth rate of soybean aphid populations only varied significantly across the three crop rotation schemes when the plant was in the early reproductive growth stage. At this growth stage, the two-year (corn; soybean) rotation had a significantly higher population growth rate than the three-year (soybean; oat and red clover; corn) rotation. However, population growth rates measured in the four-year (corn; soybean; oat and alfalfa; alfalfa) rotation were intermediate and did not significantly differ between the two-year and three-year rotations.

In general, crop rotation can have an agronomic effect on soils which may be the source of the results we observed from the field experiment. Soil in our two-year, three-year, and four-year rotation treatments may have distinct structural and microbial communities that altered soil nutrient and water availability for soybean, which in turn impacted soybean aphid population
growth rate. In addition, crops preceding soybean, specifically, oat-pea and spring wheat, can influence soybean aphid populations (Lundgren et al. 2017). However, the impact of crop rotation was not observed when soil from the rotation schemes was used in the in-lab assay. To what extent the lab assay captured the soybean aphid’s response to soybeans grown in the field is not clear. The estimates of growth rates from the lab assay (Fig. 6) appear to be lower than what was observed in the field for plants of a similar growth stage (Fig. 1). The lab assay may have removed the capacity for the soils to alter this plant-insect relationship. Future exploration of these rotation schemes should consider at what amount and form the soil is in to potentially affect this plant-insect interaction. For example, if the water retaining ability of soils across the three rotation schemes varied, our lab assay was not designed to account for this. Given the general positive response of aphids to water stressed plants, variation in soil water retention that prevents intermittent drought stress could lead to soybean plants that produce lower populations of soybean aphids. In the lab assay, plants were watered such that they did not show signs of water stress. This did not mimic the field conditions, which relied solely on rainfall, preventing this specific mechanism from being accounted for in the lab assay.

Overall, we found that soybean aphid population growth rates were higher with infestation on plants in the vegetative growth stage than the early reproductive and the reproductive growth stages (Figs. 5, and 6). This may be related to nutrient concentration changes as soybean develop. To attempt to account for the response of soybean aphids to variation in rotation and plant growth stage, we recorded leaflet nutrient concentrations as a proxy for nutrients in the phloem. Overall nutrient concentrations were significantly affected by crop rotation and soybean growth stage. However, the direction and magnitude of change was dependent upon the nutrient analyzed. At the early reproductive growth stage, crop rotation had a significant effect on concentrations of Cu, Fe, Mg, Mn, N, P, and S, indicating that host plant
quality for soybean aphids may have been impacted as well. As we used soybean leaflet tissue as a proxy for phloem nutrient concentrations, further research could study how phloem nutrient concentrations may be influenced by crop rotation and growth stage.

The effect of growth stage on leaflet nutrient concentrations was evident in our study as N, P, and K concentrations peaked at the early reproductive growth stage. These results support observations that seasonal nutrient profile has changed in modern soybean varieties (Bender et al. 2015). These findings counter previous studies that analyzed leaflet nutrient concentrations in soybean varieties cultivated in the mid-twentieth century, in which N, P, and K concentrations peaked during the vegetative growth stage (Borst and Thatcher 1931, Hanway and Weber 1971). This comparison suggests that the timing of nutrient translocation away from leaflets in modern soybean varieties may differ from older varieties. The altered timing of peak N, P, and K concentrations in modern soybean varieties may influence plant-insect interactions, as increased nutrient concentrations in leaflets may improve the quality of a host plant for herbivorous insects. Improving the quality of a host for herbivorous insects at the early reproductive growth stage may negatively affect soybean yield, as the early reproductive growth stage is when yield is most vulnerable if soybean is stressed (McWilliams et al. 1999).

This study shows not all longer, more diverse crop rotations impact soybean aphid population growth rates. Counter to our hypothesis, the four-year crop rotation did not significantly reduce population growth rates from that of the two-year rotation or three-year rotation treatments. This suggests the type of crops included in the rotation may have an effect on how crop rotations influence soybean aphid population growth rate. The four-year cropping scheme included alfalfa, a crop that removes more K than corn and soybean (Vitosh et al. 1995). Although fertilizer was uniformly applied to meet the needs of the crops for yield, it may not have prevented the deficiencies that affect aphid fecundity. However, in the three-year crop
rotation treatment at the early reproductive growth stage had lower soybean aphid population
growth rates than the conventionally managed two-year crop rotation treatment. These results
indicate that both preceding crops and crop rotation influence soybean aphid populations. Further
research is needed to determine which crops included in a rotation are optimal for mitigating
soybean aphid population growth and subsequent outbreaks.

Acknowledgements

We would like to thank the North Central Region Sustainable Agriculture Research and
Education for funding this research. Iowa State University Marsden Farm is supported by grants
from the US Department of Agriculture National Research Initiative, the Leopold Center for
Sustainable Agriculture, and the Iowa Soybean Association. We would like to thank Matthew
Woods for managing Marsden Farm. Furthermore, we thank Derek Welshhons, Derek Burger,
Shelby Pritchard, Ryan Wilcox, and Lyndi Freel for their assistance in soybean aphid colony
management and data collection.

References Cited

Annu. Rev. Entomol. 47: 817-844.

Bender, R.R., J.W. Haegele, and F.E. Below. 2015. Nutrient uptake, partitioning, and

Bulletin 494. Ohio Agricultural Experiment Station, Wooster, OH.

Chan, K.Y., and D.P. Heenan. 1996. The influence of crop rotation on soil structure and soil

systems for soybean production. Agron. J. 80: 197-204.

cropping system diversity balances productivity, profitability, and environmental health.


Tables and Figures

**Table 1.** Nutrient concentrations at three growth stages by treatment. Nutrient data was analyzed by concentration, either by parts per million (PPM) or percent concentration (%). Capitalized bolded letters indicate significant differences between growth stages for each nutrient included in this study. Lower case bolded letters next to mean nutrient concentrations indicate significant differences by crop rotation treatment at specific growth stages. With “A” being the highest value and “C” being the lowest value for a specific nutrient concentration. The lack of letters in indicate no significant difference in nutrient concentration. This table includes mean nutrient concentrations ± standard error of the mean (SEM).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Growth Stage (Mean ± SEM)</th>
<th>Two Year Rotation (Mean ± SEM)</th>
<th>Three Year Rotation (Mean ± SEM)</th>
<th>Four Year Rotation (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boron (PPM)</strong></td>
<td>Vegetative:79.10±1.73(B)</td>
<td>82.89±0.26 (a)</td>
<td>76.40±0.38 (b)</td>
<td>78.00±0.59 (b)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:94.89±2.7(A)</td>
<td>94.73±3.93</td>
<td>92.10±1.52</td>
<td>97.83±2.79</td>
</tr>
<tr>
<td></td>
<td>Reproductive:47.10±1.88 (C)</td>
<td>48.67±2.33</td>
<td>48.50±2.25</td>
<td>44.50±0.65</td>
</tr>
<tr>
<td><strong>Calcium (%)</strong></td>
<td>Vegetative:1.49±0.03(A)</td>
<td>1.48±0.01 (b)</td>
<td>1.55±&lt;0.01 (a)</td>
<td>1.44±&lt;0.01 (c)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:1.16±0.02(B)</td>
<td>1.18±0.02</td>
<td>1.15±0.02</td>
<td>1.14±0.015</td>
</tr>
<tr>
<td></td>
<td>Reproductive:1.13±0.08 (B)</td>
<td>1.11±0.06</td>
<td>1.15±0.11</td>
<td>1.12±0.10</td>
</tr>
<tr>
<td><strong>Copper (PPM)</strong></td>
<td>Vegetative:10.61±0.48 (A)</td>
<td>9.68±0.03 (c)</td>
<td>10.88±0.05 (b)</td>
<td>11.63±0.02 (a)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:8.89±0.35(B)</td>
<td>8.73±0.10 (ab)</td>
<td>8.57±0.13 (b)</td>
<td>9.16±0.42 (a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:8.55±0.41(B)</td>
<td>10.61±0.48 (a)</td>
<td>8.88±0.35 (b)</td>
<td>8.55±0.41(b)</td>
</tr>
<tr>
<td><strong>Iron (PPM)</strong></td>
<td>Vegetative:886.28±47.45 (A)</td>
<td>932.15±3.09 (b)</td>
<td>777.39±4.07 (c)</td>
<td>949.28±3.91(a)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:133.33±16.00(B)</td>
<td>165.77±21.03(a)</td>
<td>122.20±2.60 (b)</td>
<td>112.00±1.86 (b)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:81.27±5.03 (B)</td>
<td>87.00±2.31</td>
<td>84.25±3.52</td>
<td>74.00±6.36</td>
</tr>
<tr>
<td><strong>Magnesium (%)</strong></td>
<td>Vegetative:1.49±0.03 (A)</td>
<td>&lt;0.01±7.6e-6(c)</td>
<td>0.42±&lt;0.01(a)</td>
<td>0.41± &lt;0.01 (b)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:0.39±0.01 (B)</td>
<td>0.39±0.01(ab)</td>
<td>0.38±&lt;0.01(b)</td>
<td>0.41±0.01(a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:0.34±0.01 (B)</td>
<td>0.34±0.01</td>
<td>0.34±0.01</td>
<td>0.34±0.01</td>
</tr>
<tr>
<td><strong>Manganese (PPM)</strong></td>
<td>Vegetative:61.34±4.26 (A)</td>
<td>60.76±0.38(b)</td>
<td>53.15±0.31(c)</td>
<td>70.10±0.39(a)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:46.22±1.60 (B)</td>
<td>46.91±1.90(a)</td>
<td>43.57±1.15(b)</td>
<td>48.20±1.38(a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:59.00±8.06 (AB)</td>
<td>56.67±7.13</td>
<td>57.25±9.72</td>
<td>62.5±9.5</td>
</tr>
</tbody>
</table>
Table 1 Continued. Nutrient concentrations at three growth stages by treatment. Nutrient data was analyzed by concentration, either by parts per million (PPM) or percent concentration (%). Capitalized bolded letters indicate significant differences between growth stages for each nutrient included in this study. Lower case bolded letters next to mean nutrient concentrations indicate significant differences by crop rotation treatment at specific growth stages. With “A” being the highest value and “C” being the lowest value for a specific nutrient concentration. The lack of letters in indicate no significant difference in nutrient concentration. The table includes mean nutrient concentrations ± standard error of the mean (SEM).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Growth Stage (Mean ± SEM)</th>
<th>Two Year Rotation (Mean±SEM)</th>
<th>Three Year Rotation (Mean±SEM)</th>
<th>Four Year Rotation (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Nitrogen</strong> (%)</td>
<td>Vegetative:3.73±0.16 (B)</td>
<td>3.42</td>
<td>3.97</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:5.03±0.14 (A)</td>
<td>4.73±0.08(b)</td>
<td>5.19±0.15(a)</td>
<td>5.17±0.09(a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:4.75±0.14 (AB)</td>
<td>4.51±0.1(b)</td>
<td>4.96±0.08(a)</td>
<td>4.73±0.15(ab)</td>
</tr>
<tr>
<td><strong>Phosphorus</strong> (%)</td>
<td>Vegetative:0.35±0.01(B)</td>
<td>0.36±&lt;0.01(a)</td>
<td>0.36±&lt;0.01(a)</td>
<td>0.33±&lt;0.01(b)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive: 0.5± 0.01(A)</td>
<td>0.49±0.01(ab)</td>
<td>0.48±0.01(b)</td>
<td>0.51±0.02(a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive: 0.43±0.03 (AB)</td>
<td>0.39±0.01</td>
<td>0.45±0.03</td>
<td>0.44±0.22</td>
</tr>
<tr>
<td><strong>Potassium</strong> (%)</td>
<td>Vegetative: 2.21±0.03(B)</td>
<td>2.25±0.01(a)</td>
<td>2.24±0.01(a)</td>
<td>2.14±&lt;0.01(b)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:2.99±0.09(A)</td>
<td>3.03±0.16</td>
<td>2.95±0.08</td>
<td>2.98±0.05</td>
</tr>
<tr>
<td></td>
<td>Reproductive:2.38± 0.06(AB)</td>
<td>2.21±0.04 (b)</td>
<td>2.99±0.09 (a)</td>
<td>2.38±0.06(ab)</td>
</tr>
<tr>
<td><strong>Sulfur</strong> (%)</td>
<td>Vegetative:0.26±&lt;0.01(C)</td>
<td>0.25±&lt;0.01(b)</td>
<td>0.26±&lt;0.01(a)</td>
<td>0.25±&lt;0.01(c)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive:0.39±0.01(A)</td>
<td>0.29±&lt;0.01(b)</td>
<td>0.31±0.01(a)</td>
<td>0.31±0.01(a)</td>
</tr>
<tr>
<td></td>
<td>Reproductive:0.32±0.01(B)</td>
<td>0.30±0.01</td>
<td>0.33±0.01</td>
<td>0.31±&lt;0.01</td>
</tr>
<tr>
<td><strong>Zinc</strong> (PPM)</td>
<td>Vegetative:25.31±0.92(B)</td>
<td>26.97±0.07 (a)</td>
<td>23.35±0.05 (c)</td>
<td>25.61±0.04(b)</td>
</tr>
<tr>
<td></td>
<td>Early Reproductive: 38.40±0.77(A)</td>
<td>37.60±0.69</td>
<td>38.57±0.58</td>
<td>38.99±1.18</td>
</tr>
<tr>
<td></td>
<td>Reproductive:35.09±2.08(AB)</td>
<td>31.67±1.76</td>
<td>37.00±2.83</td>
<td>35.75±0.75</td>
</tr>
</tbody>
</table>
Figure 1. Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment in 2017 and 2018 at the vegetative soybean growth stage. There were no significant differences between crop rotation treatments ($F=0.480; \text{df}=2, 29; P=0.624$).
Figure 2. Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment in 2016 and 2018 at the early reproductive growth stage. There was a significant difference between treatments indicating that crop rotation impacts soybean aphid population growth rates. Different letters indicate a significant difference between treatments ($F=4.333; \text{df}=2, 37; P=0.020$).
Figure 3. Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment in 2017 and 2018 at the reproductive soybean growth stage. There were no significant differences between treatments ($F=0.129; \text{df}=2, 37; P=0.878$).
Figure 4. Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment in 2016 at the early reproductive growth stage (R1). There was a significant difference between treatments indicating that crop rotation impacts soybean aphid population growth rates. Different letters indicate a significant difference between treatments ($F=6.677; df=2, 17; P=0.007$).
**Figure 5.** Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment and soybean growth stage in 2017. 2017 included soybean in the vegetative and reproductive growth stages. The vegetative growth stage represents soybean at the V3 growth stage, and the reproductive growth stage represents soybean at the R4 growth stage. There was a significant difference in soybean aphid population growth rate between soybean growth stages. Different letters indicate a significant difference between treatments ($F=64.098$; df=1, 41; $P<0.001$).
Figure 6. Mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment and soybean growth stage in 2018. 2018 included soybean in the vegetative early reproductive, and reproductive growth stages. The vegetative growth stage represents soybean at the V3 growth stage, the early reproductive growth stage represents soybean at the R1 growth stage, and the reproductive growth stage represents soybean at the R4 growth stage. There were significant differences in soybean aphid population growth rate between soybean growth stages. Different letters indicate a significant difference between treatments ($F=13.850; df=1, 54; P<0.001$).
Figure 7. In-lab assay mean soybean aphid population growth rate ± standard error of the mean (SEM) by crop rotation treatment in 2018. All soybean plants included in this study were at the vegetative growth stage. There were no significant differences among treatments ($F=3.051; df=2, 36; P=0.060$).
CHAPTER 5. GENERAL CONCLUSIONS

Host plant resistance conferred by Resistance to *Aphis glycines* (*Rag*) genes has been evaluated for their efficacy against soybean aphids and has been studied for over a decade. This effort has resulted in a consensus that *Rag* genes are efficacious against soybean aphids, with two or more genes providing improved protection when compared to a single gene (Hill et al. 2004a, McCarville and O’Neal 2012, Wiarda et al. 2012). Despite years of research indicating that *Rag* genes are effective at managing soybean aphid populations farmer use of these soybean varieties is low (McCarville et al. 2012). Low farmer use of these varieties may be due to associated concerns pertaining reduced yield and inconsistent protection from soybean aphid.

In the second chapter, we evaluated the effect of aphid resistant varieties exposed to two soybean aphid biotypes, biotype-1 and biotype-4, with and without the presence of natural enemies. Results of this study suggest that soybean aphid virulence may have been influenced by abiotic and biotic factors over our data collection periods. These factors may include temperature (Richardson 2011), drought (Nachappa et al. 2016), host stress (Myers et al. 2005), and amino acid concentration within the phloem (Walter and DiFonzo 2007). This indicates that soybean aphid biotypes exposed to resistant hosts may be able to overcome host plant resistance if a combination of abiotic and biotic factors allow for populations to establish and persist.

In addition, we evaluated commercially and publicly available soybean varieties that varied in their resistance to the soybean aphid on Iowa Farms in chapter 3. All soybean varieties included in the study were either soybean aphid susceptible or soybean aphid resistant, conferred with soybean aphid resistant genes *Rag1+Rag2*. This project was conducted with participation from Iowa farmers, with all varieties exposed to naturally occurring soybean aphid populations and biocontrol agents. Participating farmers documented yield, allowing us to determine if
soybean aphid resistant genes $\text{Rag}_1+\text{Rag}_2$ were associated with yield loss. Results of this study suggest soybean aphid-resistant varieties were effective at managing soybean aphid populations without impacting yield. Indicating, the implementation of resistant soybean varieties in Iowa may not be dictated by their performance but rather other factors not evaluated in this project.

As the majority of farmers select soybean aphid susceptible varieties more so than resistant varieties, understanding how soybean susceptibility to soybean aphids can be affected by agronomic management practices is important. In the fourth chapter, we evaluated the impact that crop rotation had on soybean nutrient concentrations and on soybean aphid population growth rate at three soybean growth stages.

Results of this study suggest that overall soybean nutrient concentrations were significantly affected by crop rotation and soybean growth stage, supporting established literature (Bender et al. 2015). However, the direction and magnitude of change by these two factors was dependent upon the nutrient analyzed. In addition, we found that the agronomic benefits of crop rotation extend to soybean aphid at the early reproductive growth stage. Furthermore, we found that soybean growth stage influenced soybean aphid population growth rate, with the vegetative growth stage generating higher population growth rates than the reproductive growth stages included in this study.

Overall, our findings indicate that $\text{Rag}_1+\text{Rag}_2$ soybean provide protection against soybean aphid on Iowa farms. However, our findings described in chapter 2 indicate that $\text{Rag}$ gene efficacy is not uniform, as our findings suggest soybean aphid may be able to overcome resistant hosts if the right combination of abiotic and biotic factors occur. Our findings indicate that the agronomic benefits of crop rotation influence soybean nutrient content, with soybean aphid population growth rates being impacted as well at the early reproductive growth stage.
Further research is needed to understand how combinations of drought, heat, and host health impact soybean aphid biotype response to host plant resistance. Research pertaining to how aphid resistant soybean varieties should be implemented to reduce selection pressure on soybean aphids as well as providing yield protection during a soybean aphid outbreak event should be conducted to aid in the effective implementation of these varieties in the North Central region of America. Based off of our findings discussed in chapters three and four, further research questions may focus on the potential synergistic relationship between crop rotation and soybean aphid resistant soybean varieties.

**Literature Cited**


