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EVALUATION OF BIOTYPE 4 *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) INDUCED  
SUSCEPTIBILITY ON SOYBEAN AND SOJA

BY

JAKOB DAVID HICKS

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Plant Science

South Dakota State University

2021

## THESIS ACCEPTANCE PAGE

Jakob David Hicks

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree.

Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Adam Varenhorst  
Advisor

Date

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Date

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## ABSTRACT

EVALUATION OF BIOTYPE 4 *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE)

INDUCED SUSCEPTIBILITY ON SOYBEAN AND SOJA

JAKOB HICKS

2021

Soybean aphids have been a significant pest of soybeans in North America since 2000. Before 2000, soybeans did not face significant insect pest pressure from any arthropods with piercing-sucking mouthparts. It is estimated that economic damage from soybean aphids range from \$1 billion to \$4.7 billion annually. Research efforts focused on the identification of host plant resistance genes in soybean and discovered many resistant to *Aphis glycines* genes (i.e., *Rag* genes) in soybean. However, the adoption of commercially released *Rag* soybean cultivars has been limited. The prospect of management with *Rag* genes was further complicated by the identification of three virulent soybean aphid biotypes. Currently, biotype 4 is the greatest threat to management using *Rag* genes because it can colonize soybean containing *Rag1*, *Rag2*, *Rag1+Rag2* or *Rag1+Rag2+Rag4* genes. Previous studies have determined that soybean aphids illicit an induced susceptibility response in soybean. For example, virulent soybean aphid biotypes can obviate the resistance provided by *Rag* genes thereby allowing for the colonization by otherwise avirulent biotypes. This phenomenon was initially documented for biotype 1 and biotype 2 but not for biotype 4. In the first study we examined biotype 4 on *Rag* genes and found induced susceptibility. We then performed a second study that was an induced susceptibility screen using three plant introductions of soja with identified



soybean aphid host plant resistance. We determined that induced susceptibility occurs on soja as well.

## CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

### Introduction

This thesis discusses the interactions between soybean aphid biotypes and their soybean and soja hosts. The objectives of this thesis are to determine if biotype 4 soybean aphids can produce an induced susceptibility effect on 1) soybean containing *Rag1* and *Rag2* genes and 2) soja plant introductions.

### Soybean Aphid Biology and Ecology

Soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae), are a significant pest of soybean in North America (Venette and Ragsdale 2004); however, it rarely reaches economically injurious levels in its native range of eastern Asia (Liu et al. 2004). Before the arrival of soybean aphids in the U.S. in 2000, soybean had few insect pests that required insecticidal management (Kogan and Turnipseed 1987). After their arrival, soybean aphids spread rapidly in North America to 23 states and three Canadian provinces. Subsequently, there was an increased use of broad-spectrum foliar insecticides in soybean (Ragsdale et al. 2011b, Yang and Suh 2015). Previous research has suggested that soybean aphids in North America originated from Japan, Korea, or perhaps separately from each area, as it is likely that more than one invasion occurred (Wenger and Michel 2013, Kim et al. 2016, Fang et al. 2018). In North America, soybean aphids exhibit a heteroecious holocyclic lifecycle (Ragsdale et al. 2004). Soybean aphids undergo parthenogenesis on their secondary host, soybean, and sexual reproduction on their primary host, buckthorn (*Rhamnus spp.*) (Voegtlin et al. 2004). Soybean aphids can

also use soja, a crop wild relative of soybean, as an alternative secondary host, however; soja is not cultivated in North America (Wu et al. 2004, Li et al. 2010).

During the fall, soybean aphids sexually reproduce and deposit eggs on buckthorn buds. The eggs are very hardy and can withstand temperatures as low as -34 °C (McCornack et al. 2005). In the spring, the eggs hatch into fundatrices (apterous females). Fundatrices asexually produce apterous fundatrigenia (Takahashi et al. 1993). Mature fundatrigenia have six antennal segments instead of the five segments that are present in mature fundatrices (Takahashi et al. 1993). Three or more generations of asexually reproducing fundatrigenia may occur on buckthorn (Ragsdale et al. 2004). These soybean aphids also produce alate fundatrigenia, which travel to soybean to reproduce. Throughout the spring and summer, apterous exule (female aphids), reproduce by thelytokous parthenogenesis on soybeans (Takahashi et al. 1993). As summer temperatures cool and photoperiod diminishes, gynoparae are produced on soybean plants (Ragsdale et al. 2004). Alate gynoparae seek out buckthorn and asexually reproduce apterous oviparae. Oviparae are considerably more durable and can withstand colder temperatures than gynoparae (McCornack et al. 2005). At roughly the same time on soybean, arrhenotokous parthenogenesis gives rise to winged males that will seek out the oviparae on buckthorn for sexual reproduction. The mated oviparae then deposit eggs on the buds of buckthorn, *Rhamnus spp.*, completing a season.

Soybean aphid reproduces on soybean at temperatures between 8.6 °C and 34.9 °C (McCornack et al. 2004). However, nymphs cannot develop above 30 °C and will perish in 11 days (McCornack et al. 2004). At an optimal temperature of approximately

27.8°C and in the absence of natural enemies, soybean aphid populations can double every 1.5 to 5 days (McCornack et al. 2004). However, in field settings the average population doubling time is 6.8 days (Ragsdale et al. 2007). Soybean aphids produce up to 15 generations asexually on soybean (McCornack et al. 2004). Soybean aphid feeding by large populations can result in short soybeans, a reduction of pods, and reduced seed size and quality (Beckendorf et al. 2008).

Soybean aphids are phloem feeders (Ragsdale et al. 2004) that have a stylet mouthpart that is used to probe the soybean epidermis (Minks and Harrewijn 1987). When a suitable host is selected the stylet will penetrate the epidermis and probe the apoplast in search of a vascular bundle from which to consume phloem sap (Li et al. 2008). This intercellular navigation causes little damage to plant leaves. Aphids release two types of saliva; the first type is a gel saliva that hardens to form a sheath from the apoplast to the vascular bundle (van Bel and Will 2016). Once the stylet has reached the phloem tissue, a second watery saliva is released containing proteins that are released to draw amino acids to the stylet insertion location (Tjallingii 2006, Elzinga and Jander 2013). Once feeding has been established, it may last for several hours if the host plant is susceptible, and conditions remain favorable (Prado and Tjallingii 1997).

Soybean phloem is high in sucrose and low in amino acids (Servaites et al. 1979). Because of this, soybean aphids excrete many of the sugars that are ingested (Ashford et al. 2000). This excrement is referred to as honeydew and is a food source for saprophytes, ants and parasitoid wasps (Schwartzberg et al. 2014). Aphids will occasionally consume

xylem contents for osmoregulation (Spiller et al. 1990). This feeding behavior can vary and is dependent on the quality of the host plant tissue.

### **Soja and Soybean**

As soybean was domesticated, it underwent three genetic bottlenecks (Hyten et al. 2006). Plant introductions of the wild relative, soja (*Glycine soja* Sieb. and Zucc.), are a source of genetic variability that can be incorporated into soybean germplasm. The theory of invigorating the cultivated gene pool with a wild progenitor species has been well applied in many crops including rice, wheat, tomato, potato and peanuts (Zhang et al. 2017a). Soja and soybean have the same number of chromosomes ( $2n = 40$ ), are cross-compatible, and exhibit normal meiotic chromosome pairing, which allows for efficient cross breeding (Carter et al. 2004). Three competing hypotheses have been proposed to explain soybean domestication and its relation to soja (Sedivy et al. 2017). The first hypothesis is that at a single time point soybean diverged from soja (Li et al. 2010). The second hypothesis is that over a period of time, multi-divergence resulted in multiple soybean lines that interbred with soja multiple times before completely diverging (Han et al. 2016). The third hypothesis is that a soybean-soja complex existed before a separation into soja and landrace soybean by human selection (Li et al. 2014).

Soja has been the source for many agronomic traits that can be incorporated or now are present in elite cultivars of domesticated soybean (Chang et al. 2016, Kofsky et al. 2018). For instance, soja plant introduction (PI) 88788 confers resistance to many races of soybean cyst nematode, *Heterodera glycines* Ichinohe, and is used in 95% of production soybean acres in Illinois (Kim et al. 2011). A beneficial trait reducing pod

shattering was also found in soja and has potential to be incorporated into soybeans (Dong et al. 2014). Soybean aphid resistance has been identified in multiple soja plant introductions (Hesler 2013, Hesler et al. 2017). More traits are yet to be discovered and utilized from soja, with genetic methodology becoming readily available (Zhou et al. 2015). Breeding soja traits into soybean could provide many beneficial traits, including resistance to soybean aphid.

### **Soybean Aphid Management**

Soybean aphid populations can significantly reduce the yield of soybeans and often require management. The economic threshold used for soybean aphids is 250 aphids per soybean plant (Ragsdale et al. 2007). Once pest populations have reached the economic threshold, an insecticidal treatment is recommended to prevent additional population growth that could cause observable yield losses (Ragsdale et al. 2007). The economic injury level for soybean aphids is 674 per plant (Ragsdale et al. 2007). Weekly scouting of a soybean field is recommended to monitor soybean aphid populations and to determine whether the economic threshold has been reached (Koch et al. 2016).

Soybean aphids are commonly managed using broad-spectrum foliar insecticides like pyrethroids and organophosphates (Olson et al. 2008, DiFonzo 2009, Ragsdale et al. 2011a, Hodgson et al. 2012, Hesler et al. 2013). One challenge associated with the use of insecticides is the development of pyrethroid-resistant soybean aphid populations in Iowa, Minnesota, North Dakota, and South Dakota (Koch et al. 2018). In addition, the use of broad-spectrum insecticides can have negative impacts on beneficial insects that are also present at the time of application (Ohnesorg et al. 2009, Varenhorst and O'Neal

2012). Reduced risk, or selective, insecticides have been evaluated against soybean aphids (Varenhorst and O'Neal 2012, Koch et al. 2019). However, the commercial availability of these products to soybean growers is limited and potentially cost prohibitive.

Aphidophagous predators are common in the environment and can have negative impacts on soybean aphid populations (Desneux et al. 2006, Meihls et al. 2010, Varenhorst and O'Neal 2012, Hesler 2014). Although there is the potential for a diverse and potentially abundant assemblage of aphidophagous predators in soybean, soybean aphid populations may still exceed the economic threshold (Schmidt et al. 2008). Soybean plants that are infested by soybean aphids produce signaling compounds that attract aphidophagous predators (Zhu and Park 2005). However, during optimal conditions the reproductive rate of large soybean aphid populations can exceed the predation rates of the natural enemies. The effectiveness of natural enemies can be increased when used in combination with another management strategy, such as host plant resistance (McCarville and O'Neal 2012).

### **Host Plant Resistance**

Host plant resistance in soybeans is currently being developed and deployed to combat soybean aphid populations (Hanson et al. 2018). Crop wild relatives and several soybean plant introductions have demonstrated resistance to soybean aphids (Hesler 2013, Hesler and Tilmon 2018). Host plant resistance can be classified into three modalities: antixenosis, antibiosis, and tolerance (Painter 1958, Kogan and Ortman 1978,

Panda and Khush 1995, Smith and Clement 2012). Antixenosis repels a pest or reduces a plant's attractiveness to the pest, but it does not directly affect the pest's biology (Price 2011). Antibiosis reduces fecundity, causes mortality, or otherwise negatively affects pest biology (Smith 2005). Tolerance is a host plant's ability to be colonized by an insect pest but produce yields comparable to uncolonized plants (Painter 1958, Mitchell et al. 2016). Combining modalities of host plant resistance increases the durability of the incorporated genes by reducing the selection pressure for virulent pest biotypes (Stenberg and Muola 2017). All three modalities of host plant resistance have been observed in soybean and wild relatives of soybean in the soybean aphid model (Baldin et al. 2018).

**R**esistance to *Aphis glycines* (*Rag*) genes have been documented and bred into soybean (Hesler et al. 2013). Multiple soybean plant introductions, landraces and cultivars share similar resistance quantitative trait locus (QTL). Soybean lines Dowling (PI 548663) and Jackson (PI 548657) expressed both antixenosis and antibiosis resistance and this QTL was mapped to chromosome 7 and was inherited by its offspring as a dominant phenotype (Hill et al. 2006, 2007). Originally, both Dowling and Jackson had been considered *Rag1*; however, populations of aphids respond differently to traits found in respective parent lines, leading Jackson to be referred to as *Rag* soybean (Kim et al. 2008). Dowling's QTL was mapped to two nucleotide binding domain-leucine rich repeat (NBS-LRR) proteins, that can interact with pathogen virulence proteins (Kim et al. 2010b). The *Rag2* gene was documented in PI 243540 and PI 200538 and expressed antixenosis and antibiosis resistance to soybean aphids. For both PI's, this QTL was traced to chromosome 13 (Rouf Mian et al. 2008, Kim et al. 2010a). PI 200538 was fine-



mapped and one candidate NBS-LRR was found. Mensah et al. (2005) discovered resistance in PI 567543C, PI 567597C, PI 567598B and PI 567541B. *Rag3* was found in PI 567543C and exhibited antixenosis with this QTL on chromosome 16 (Zhang et al. 2010). The *rag1b* and *rag3* were found in PI 567598B and express antibiosis (Bales et al. 2013). The *rag4* and *rag1c* were found in PI 567541B and expressed polygenic antibiosis, and this QTL was traced to Chromosome 7 and 13 respectively inheritance patterns of both QTL's was shown to be recessive (Zhang et al. 2008). More plant introductions have been fine mapped resulting in the discovery of *Rag5*, which was found in PI 567301B on Chromosome 13, with a minor locus on chromosome 8 near the *Rag2* locus (Jun et al. 2012). *Rag5* expresses antixenosis that codes for an NBS-LLR. *Rag6* and *Rag3c* (Zhang et al. 2017b), provisional *Rag* genes, found in a Soja 85-32 have also been mapped. *Rag6* was potentially proposed to be three unique NBS-LRR regions that may need all three NBS-LRR to be expressed for antibiosis to occur. *Rag3c* was proposed to have an LRR protein kinase or LLR protein lipase that would produce an antixenosis effect.

Previous research has evaluated the combination of multiple *Rag* genes into a pyramid (i.e., coupling multiple sources of resistance in the same host), through breeding or discovery of germplasm containing multiple *Rag* genes (Wiarda et al. 2012, Zhang et al. 2018). Pyramids have the benefit of being more robust and increase the durability of resistant genes when compared to the release of plant lines with single genes (Dogimont et al. 2010). A pyramid of *Rag1* and *Rag2* originally exhibited excellent management of soybean aphids and maintaining yield when compared related susceptible lines (Brace

and Fehr 2012, McCarville and O'Neal 2012, McCarville et al. 2014). Additional pyramids with three *Rag* genes have been evaluated (Chandrasena et al. 2015, Ajayi-Oyetunde et al. 2016, Varenhorst et al. 2017). Currently soybeans containing only *Rag1* and a pyramid of *Rag1+Rag2* are available commercially (Hanson et al. 2017). Soybean cultivars with *Rag* genes have had a low rate of adoption by U.S. farmers and have primarily been used in organic cropping systems (O'Neal et al. 2018).

### **Soybean Aphid Biotypes**

There is the possibility that host plant resistance traits will select for pests that are capable surviving on and colonizing on the resistant host (Panda and Khush 1995). There have been documented populations of soybean aphids that are able to feed and thrive on soybean containing one or more *Rag* genes (Kim et al. 2008, Hill et al. 2010, Alt and Ryan-Mahmutagic 2013). These populations are generally referred to as virulent biotypes (Claridge and Den Hollander 1983). The slow release and low adoption rates of *Rag* genes have not produced a large selection pressure on wild populations of soybean aphids in North America (Kim et al. 2008). In addition, there is evidence that soybean aphids underwent a genetic bottleneck when introduced into North America (Michel et al. 2009, Wenger et al. 2014).

Soybean aphids maintain genetic diversity by sexually reproducing on buckthorn, which could lead to a rapid development of widespread virulence within populations if the frequency of alleles for virulence is increased (Orantes et al. 2012). Genetic diversity could potentially be a contributing factor to the observed issues with the durability of *Rag* genes (O'Neal et al. 2018). Furthermore, there may be intrabiotypic variation based on

the geographical location of the biotypes and the rearing history if they have been kept in laboratory settings (Wenger and Michel 2013, Conzemius et al. 2019a). Wenger and Michel (2013) proposed that phenotypical plasticity could contribute to biotype virulence towards *Rag* genes.

Currently there are four documented biotypes of soybean aphid. Biotype 1 is avirulent (i.e., unable to colonize) to soybean plants with *Rag* genes. Biotype 2 is virulent to soybean containing the *Rag1* gene (Kim et al. 2008). Biotype 3 is virulent to soybean containing the *Rag2* gene (Hill et al. 2010). Biotype 4 is virulent to soybeans with *Rag1*, *Rag2*, a pyramid of *Rag1+Rag2*, and a pyramid of *Rag1+Rag2+Rag4* (Alt and Ryan-Mahmutagic 2013, Varenhorst et al. 2017).

### **Induced Susceptibility**

When insects feed on host plants the feeding may produce either a susceptible or resistance response from the host plant (Price et al. 2011, Pitino and Hogenhout 2013, Takemoto et al. 2013). The susceptible responses result in increased host plant suitability, where feeding by an initial population of insects increases the plant's suitability for subsequent populations (Rotem and Agrawal 2003, Giovanini et al. 2006, Chiozza et al. 2010, Price et al. 2011, Takemoto et al. 2013). These susceptible effects are referred to as induced susceptibility (Price et al. 2011, Varenhorst et al. 2015a, O'Neal et al. 2018). Two induced susceptibility effects include feeding facilitation and obviation of resistance (Varenhorst et al. 2015a).

Feeding facilitation is observed when initial feeding by an insect improves host suitability for subsequent populations of the same biotype (Rotem and Agrawal 2003,

Price et al. 2011, Varenhorst et al. 2015a). Obviation of resistance is observed when initial feeding by a virulent biotype increases suitability or an otherwise resistant host for subsequent populations that are not virulent to that host plant (Baluch et al. (2012). Rotem and Agrawal (2003) found that spider mites exhibited density dependent induced susceptibility. As the host plant size increased, more mite feeding was required to overcome the host plant resistance. Takemoto et al. (2013) observed that feeding by initial populations of pea aphids increased the host suitability for subsequent pea aphids. Natukunda et al. (2019) tested several soybean lines at high and low soybean aphid population levels and found that some resistant lines became susceptible at the high level of infestation and that at low infestation leaves showed resistance. Biotype 1 soybean aphids have demonstrated that feeding facilitation can happen on soybean and the interaction is density dependent (Varenhorst et al. 2015a).

If a virulent pest feeds on a resistant plant, it may cause obviation of resistance and allow for a susceptible pest to feed on the once resistant plant. Baluch et al. (2012) observed larvae of avirulent Hessian fly, *Mayetiola destructor* (Say), feeding on wheat that contained a vertical R gene. The effect occurred when virulent larvae initially infested the wheat and fed on it, making it suitable for the subsequent avirulent biotype (Baluch et al. 2012). Obviation of resistance was also observed in the peach aphid as avirulent aphids survival on resistance plants was increased after the resistant plant was fed on by virulent peach aphids (Sauge et al. 2006). Varenhorst et al. (2015b) found that initial populations of biotype 2 soybean aphids improved the host suitability of *Rag1* soybean for subsequent populations of biotype 1 soybean aphids.

## **Insect Resistance Management**

The durability of plant traits that resist herbivores is constantly at risk due to the selection pressure the traits impose on herbivore populations (Smith 2005). The discovery of three virulent soybean aphid biotypes could greatly reduce the durability of *Rag* genes (O'Neal et al. 2018). Parthenogenetic species like soybean aphids often result in the increased populations of the phenotype with the highest fitness, which eventually will become the most commonly observed phenotype (Crowder and Carriere 2009). This effect can be reduced by the incorporation of a refuge, which increases the population of avirulent individuals. However, for this to occur fitness costs must exist for the virulent populations of soybean aphids. Varenhorst et al. (2015b) determined that virulent soybean aphid biotypes experience fitness costs on susceptible soybean. However, some populations of virulent soybean aphids may not possess a fitness costs when they feed on susceptible cultivars (Conzemius et al. 2019a). If a resistant biotype does not have a fitness cost and incomplete virulence the use of refuge may not increase the durability of *Rag* genes (O'Neal et al. 2018). Induced susceptibility may promote the longevity of resistance traits by promoting avirulent soybean aphids to survive on resistant plants creating a refuge effect (Varenhorst et al. 2015a). Effects of induced susceptibility create a 'within plant' refuge that could decrease the frequency of virulent alleles in a soybean aphid population (O'Neal et al. 2018). Utilizing other management tactics like systemic insecticides or mid- to late-season insecticide treatments in addition to host plant resistance may increase the durability of a *Rag* genes.

## **Research Objectives**

Varenhorst et al. (2015a) documented that biotype 2 soybean aphids can obviate the resistance of *Rag1* soybean for biotype 1 soybean aphids. However, the ability of biotype 4 soybean aphids to obviate the resistance of *Rag1*, *Rag2*, or the two-gene pyramid *Rag1+Rag2* has not been evaluated. The first objective of this thesis project is to evaluate the potential for biotype 4 soybean aphids to obviate the resistance of *Rag1*, *Rag2* and *Rag1+Rag2* soybean for biotype 1 soybean aphids.

Conzemius et al. (2019b) tested soja plant introductions for host plant resistance against biotype 4 soybean aphids and found resistance. However, the potential induced susceptibility effects of obviation of resistance and feeding facilitation have not been evaluated for this biotype. PI 101404A performed well against biotype 1 soybean aphids, PI 549046 performed well in laboratory environment against biotype 4 soybean aphids and PI 522212B is a documented susceptible check for biotype 1 and 4 aphids (Hesler 2013, Hesler and Tilmon 2017, Conzemius et al. 2019b). All three soja lines underwent induced susceptibility screening adapted from (Varenhorst et al. 2015a). The second objective of this thesis is to perform induced susceptibility screening on these three soja lines using biotype 4 and biotype 1 soybean aphids.

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**CHAPTER 2: EVALUATING THE POTENTIAL FOR BIOTYPE 4 SOYBEAN  
APHIDS TO INDUCE SUSCEPTIBILITY OF *RAG1+RAG2* SOYBEAN**

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**Abstract**

On soybean, induced susceptibility was first observed with biotype 1 soybean aphid populations. The effect was further evaluated using both virulent (biotype 2) and avirulent (biotype 1) populations on *Rag1* soybean. The effects that were observed were determined to be feeding facilitation and obviation of resistance. Feeding facilitation occurs when biotype 1 soybean aphid populations improve the host suitability for subsequent biotype 1 populations on either susceptible or resistant soybean. Obviation of resistance occurs when biotype 2 populations improve host suitability for subsequent biotype 1 populations on *Rag1* soybean. Obviation of resistance results in indistinguishable populations of avirulent and virulent on resistant soybean. To date, no study has evaluated the potential for biotype 4 soybean aphids to obviate the resistance of *Rag1+Rag2* soybean for biotype 1 soybean aphids. The purpose of this study is to explore the potential that induced susceptibility is not unique to *Rag1* soybean. To do this, we used a susceptible (IA3027) and a resistant *Rag1+Rag2* (IA3027RA12) soybean cultivar. Inducer populations were either no soybean aphids, biotype 1 or biotype 4 soybean aphids. Inducer populations were allowed to feed for 24 hours before the



addition of the response populations. The response populations were either biotype 1 or biotype 4 soybean aphid. We observed that biotype 4 soybean aphids improved the host suitability of *Rag1+Rag2* for biotype 1 soybean aphids. These results suggest that induced susceptibility, specifically obviation of resistance, is not unique to individual biotypes and *Rag* gene combinations.

## Introduction

Soybean aphids, *Aphis glycines* Matsumura, have been a significant pest of soybean in North America since 2000 (Ragsdale et al. 2011). On soybean, the aphids reproduce asexually leading to rapid population growth and subsequent soybean yield losses. If populations, exceeding the economic threshold of 250 soybean aphids per plant, are left unmanaged, they may reduce soybean yields by as much as 40% or approximately 2.4 billion dollars in annual losses (Ragsdale et al. 2007, Tilmon et al. 2011). Soybean aphid populations are primarily managed using broad-spectrum foliar insecticides (Olson et al. 2008, Ragsdale et al. 2011). Although insecticides have been effective against soybean aphids, their broad-spectrum efficacy detrimentally impacts non-target insects. In addition, researchers have determined that there are populations of soybean aphids that are resistant to the pyrethroid class of insecticides (Koch et al. 2018). Specifically, soybean aphids were observed that have resistance to the active ingredients bifenthrin and lambda-cyhalothrin in Iowa, Minnesota, North Dakota and South Dakota (Koch et al. 2018). Host plant resistance has been investigated as an alternative management strategy for soybean aphids and Resistance to *Aphis glycines* (*Rag*) genes have been incorporated into production lines as early as 2010 (Hill et al. 2007, McCarville and O'Neal 2012, Hanson et al. 2017). However, the adoption of the initially released and subsequent *Rag* soybean cultivars has been slow (McCarville and O'Neal 2012).

The first *Rag* gene was discovered in the soybean cultivar 'Dowling,' which is referred to as *Rag1* (Hill et al. 2006). *Rag1* resistance has been incorporated into

commercial cultivars since 2010 (McCarville and O'Neal 2012, Hanson et al. 2017). The *Rag2* gene was discovered in the soybean PI 200538 and was briefly incorporated into commercial cultivars (Kim et al. 2010). A pyramid of *Rag1+Rag2* was developed and commercial soybean cultivars containing the genes are commercially available (Wiarda et al. 2012). Additional *Rag* genes have been discovered in soybean plant introductions. The most recent breeding efforts have been evaluating the effectiveness of *Rag1+Rag2+Rag3* and *Rag1+Rag2+Rag4* three gene pyramids (Varenhorst et al. 2017, Zhang et al. 2018). However, these pyramids have not been released commercially.

Host resistance to manage insect plant pests has been used effectively in other cropping systems (Smith 2005, Zhang et al. 2017). The benefit of using host plant resistance in agricultural production systems is that insecticide applications may be reduced while maintaining yields (Smith and Clement 2012). However, utilization of host plant resistance presents challenges in production agriculture. The first challenge is that deployment of most resistance genes imposes a high selection pressure on the insect pest populations (Price 2011). Without the implementation of a refuge, virulent individuals in the pest population will eventually represent the majority of the pest population (Crowder and Carriere 2009). The second challenge is the deployment strategy of the resistance genes. The sequential release of single genes could dramatically reduce the longevity of the resistance genes' efficacy (da Silva Queiroz et al. 2018). The third challenge is that even when deployed, adoption of cultivars containing the resistance genes may be limited (O'Neal et al. 2018).

The development and deployment of *Rag* genes has faced challenges in North America. For example, prior to the commercial release of the *Rag1* soybean, virulent soybean aphids were observed (Kim et al. 2008). The discovery of a virulent population resulted in the naming of the avirulent soybean aphids as biotype 1 and the soybean aphids virulent to *Rag1* as biotype 2 (Kim et al. 2008). Additional virulent biotypes have since been discovered. Biotype 3 is virulent to soybean containing the *Rag2* gene (Hill et al. 2010). Biotype 4 was first determined to be virulent to *Rag1*, *Rag2* and *Rag1+Rag2* pyramid (Alt and Ryan-Mahmutagic 2013). Varenhorst et al. (2017) later determined that biotype 4 was also virulent to *Rag 4* as well as the three gene pyramid *Rag1+Rag2+Rag4*.

Although there are challenges associated with the deployment of resistance genes, there are also factors that would reduce the impact that virulent biotypes would have on resistance gene deployment (Varenhorst et al. 2015b). For example, the presence of fitness costs for virulent biotypes would promote the longevity of released resistance genes. Varenhorst et al. (2017) determined that laboratory populations of biotype 2, biotype 3 and biotype 4 soybean aphids experienced fitness costs on susceptible soybean. However, Conzemius et al. (2019) observed a population of biotype 4 soybean aphids that did not experience a fitness cost on susceptible soybean. An explanation for these different results may be intrabiotypic variation (Alt et al. 2019). Intrabiotypic variation is observed when quantitative variation in virulence is detected on different host genotypes by isolates from different colonies of the same biotype (Pawlowski et al. 2015). These variations may be due to the duration a colony is maintained and potentially any stressors

that the colony is exposed to during extended laboratory conditions (Michel et al. 2010a, Conzemius et al. 2019).

Induced susceptibility also reduces the impact of virulent biotypes on *Rag* soybean (Varenhorst et al. 2015a). Induced susceptibility is defined by the positive interaction that occurs when initial insect feeding makes the host plant more suitable for subsequent populations (Price et al. 2011). The concept of induced susceptibility can be divided into different categories based on the virulence of the initial insect population. One such category is feeding facilitation, which occurs when feeding by avirulent individuals improves the host suitability for subsequent conspecific populations of either avirulent or virulent individuals. For example, Varenhorst et al. (2015a) observed an increase in biotype 1 soybean aphid populations on susceptible soybean and also *Rag1* soybean when the plants were initially infested with biotype 1 aphids. Feeding facilitation has been observed with other hosts and insect pests (Rotem and Agrawal 2003, Robert et al. 2012, Takemoto et al. 2013).

Another category of induced susceptibility is obviation of resistance, which occurs when feeding by initial virulent individuals improves the suitability of the resistant host for subsequent avirulent populations (Baluch et al. 2012). Varenhorst et al. (2015a) observed this effect when an initial population of biotype 2 soybean aphids improved the suitability of *Rag1* soybean for a subsequent biotype 1 population. Obviation of resistance may produce a within plant refuge due to the fact that both the avirulent and virulent populations are able to survive and successfully colonize the plant (O'Neal et al. 2018). In addition, the discovery of induced susceptibility effects in soybean suggests that

initial observations of soybean aphids on *Rag* soybean in field settings may have overestimated the population density of virulent soybean aphids. This is due to the potential that feeding facilitation could have allowed large populations of soybean aphids to successfully colonize single *Rag* gene lines (Varenhorst et al. 2015a).

Although Varenhorst et al. (2015a) evaluated induced susceptibility effects for biotype 1 and biotype 2 soybean aphids on *Rag1* soybean, no study has evaluated the potential for biotype 4 soybean aphids to induce susceptibility of the two gene pyramid *Rag1+Rag2*. Varenhorst et al. (2015b) observed fitness costs for biotype 2, biotype 3 and biotype 4 soybean aphids on susceptible soybean. It was also determined that biotype 1 soybean aphids could alleviate the fitness costs for biotype 2 and biotype 3 soybean aphids on susceptible soybean (Varenhorst et al. 2015b). The first objective of this study was to determine if biotype 4 soybean aphids could improve the host suitability of the *Rag1+Rag2* soybean for biotype 1 soybean aphids. The second objective of this study was to determine if biotype 1 soybean aphids can alleviate fitness costs for biotype 4 soybean aphids on susceptible soybean.

## **Material and Methods**

### **Aphid colonies and soybean cultivars**

The biotype 1 and biotype 4 soybean aphid colonies used for this experiment were obtained from colonies that were initially reared at The Ohio State University in 2012. The biotype 1 soybean aphids were characterized by their avirulence to *Rag1*, *Rag2* and *Rag1+Rag2* (Varenhorst et al. 2017), and the biotype 4 soybean aphids by their virulence to *Rag1*, *Rag2* and *Rag1+Rag2* (Alt and Ryan-Mahmutagic 2013). The biotype 1

colonies were originally founded from individuals that were collected in Illinois (biotype 1) and Wisconsin (biotype 4) (Kim et al. 2008, Alt and Ryan-Mahmutagic 2013). The biotype identity of each population was confirmed using detached leaf assays (Michel et al. 2010b). After confirmation of the biotype identification, the populations used for this experiment were derived from a single clone for each biotype. For rearing, the biotype 1 soybean aphids were raised on susceptible soybean (IA3027), and the biotype 4 soybean aphids were raised on a near-isogenic resistant soybean containing the *Rag1* and *Rag2* genes (IA3027RA12). These cultivars are near-isolines that are approximately 93.25% genetically identical (Wiarda et al. 2012). Each biotype was reared in a separate Percival E41L2C9 growth chamber (Percival Scientific, Incorporated, Perry, IA) using a 14:10 light dark cycle, a constant temperature of 27 °C and a relative humidity of 60%. Colonies were maintained on separate days to reduce the potential for contamination. The growth chambers for the biotype 1 and biotype 4 colonies were housed in the Science II building on the Iowa State University campus, Ames, IA.

### **Induced susceptibility experiments**

We hypothesized that that initial feeding by biotype 4 soybean aphids would increase host plant suitability for biotype 1 soybean aphids on *Rag1+Rag2* soybean (i.e., obviating of resistance). We also hypothesized that initial feeding by biotype 1 soybean aphids would increase the host plant suitability for biotype 4 on susceptible soybean (i.e., removal of fitness costs). We tested for obviating of resistance following the methods described by Varenhorst et al. (2015a). Briefly, the first trifoliolate leaf of a second vegetative stage (V2) soybean plant was initially infested with either 50 biotype 1 or 50

biotype 4 soybean aphids (i.e., the inducer treatment). Each infested leaf was covered with a custom sewn No-See-Um mesh net (Quest Outfitters) and sealed with a large metal paper clip and Tangle-Trap Sticky Coating (The Tanglefoot Company, Grand Rapids, MI). Each potted plant was covered using a custom No-See-Um mesh net (Quest Outfitters) to reduce the potential for plant-to-plant movement of the aphids. The inducer populations were allowed to feed for 24 hr. After 24 hr, the response populations were added to the second trifoliolate of the plants. The response populations could move freely on the plants except for the caged first trifoliolate. After 11 d, the response populations were counted.

For this experiment, we utilized 12 treatment combinations, which were derived from two soybean cultivars, three inducer population treatments, and two response population treatments. The two soybean cultivars were susceptible (IA3027) and *Rag1+Rag2* (IA3027RA12). The inducer treatments consisted of no soybean aphids, 50 biotype 1 soybean aphids, or 50 biotype 4 soybean aphids. The two response treatments were five biotype 1 or five biotype 4 soybean aphids. Soybean aphids used for infesting experimental plants were mixed age with a bias for late instars and adults. Soybean aphids were removed from infested leaves from colony plants and transferred to experimental plants using a fine tip 000 paintbrush. The experiment was conducted using a randomized complete block design with three blocks and was repeated three times to obtain a total of nine experimental units per treatment combination. Each experimental repetition was conducted in a Percival E41L2C9 growth chamber with conditions identical to those used for rearing the colonies. The growth chambers used for each



experimental repetition were housed in the Insectary on the Iowa State University campus.

### **Statistical Analyses**

This experiment was repeated three times using a randomized complete block design (RCBD) with 3 blocks per repetition (9 total experimental units per treatment). To address each of the hypotheses, we analyzed the number of aphids per plant at 11 days after infestation. Statistical analyses of the aphid count data from the induced susceptibility experiment were completed using RStudio (2020). Analysis of variance was performed on data from the experiments after aphid counts were transformed by taking the natural log of the aphid count plus 1 ( $\ln+1$ ) to account for heterogeneity of soybean aphid population densities due to exponential growth (Varenhorst et al. 2015a). Significant treatment differences were separated using a Tukey's test with a significance level of ( $P < 0.05$ ). Non-transformed data were used to create the graphical representations of the data.

### **Results**

We confirmed our hypothesis that the presence of biotype 4 soybean aphids would improve the host suitability for biotype 1 soybean aphids on *Rag1+Rag2* soybean (i.e., obviating of resistance occurs). We observed this by analyzing the data first for the significance of the main effects of block and treatment (i.e., cultivar, inducer population biotype, and response population biotype). The main effect block was not significant, but treatment was significant ( $F = 23.52$ ;  $df = 11, 94$ ;  $P < 0.001$ ). We next analyzed each factor of treatment (i.e., cultivar, inducer population biotype, and response population

biotype) as well as all interactions of the three factors. The main effect of soybean cultivar ( $F = 58.75$ ;  $df = 1, 94$ ;  $P < 0.001$ ) was significant as well as the interaction of soybean cultivar and response population biotype ( $F = 49.025$ ;  $df = 1, 94$ ;  $P < 0.001$ ) and the interaction of soybean cultivar and inducer population biotype was significant ( $F = 7.83$ ;  $df = 2, 94$ ;  $P < 0.001$ ). For this reason, the data were analyzed by soybean cultivar.

For the *Rag1+Rag2* soybean, the main effects inducer population biotype ( $F = 17.82$ ;  $df = 2, 46$ ;  $P < 0.001$ ), response population biotype ( $F = 53.37$ ;  $df = 1, 46$ ;  $P < 0.001$ ), and the interaction of inducer population biotype and response population biotype significantly affected the response population densities ( $F = 10.72$ ;  $df = 2, 46$ ;  $P < 0.001$ ). That is, the presence and virulence of the inducer population positively affected the population density of both the biotype 1 and biotype 4 response populations.

On the *Rag1+Rag2* soybean, the biotype 4 response population without an inducer population was 501% greater than the biotype 1 response population ( $P < 0.01$ ) (Fig. 1). Even when a biotype 1 inducer was present for the biotype 1 response population, the biotype 4 response population was still 127% greater ( $P < 0.01$ ) (Fig. 1). However, there were no differences between the biotype 4 response population with no inducer and the biotype 1 response population with a biotype 4 inducer. When both biotypes had a biotype 4 inducer population the biotype 4 response was 160% greater than the biotype 1 response (Fig. 1). In summary, the difference in the population density between the virulent and avirulent response populations diminished with the addition of a biotype 1 inducer population and was completely removed by the presence of a biotype 4 inducer population. However, the differences observed between the biotype 1 and biotype

4 response populations when both had a biotype 4 inducer may be due to the increased resistance present in the *Rag1+Rag2* soybean. The biotype 1 response population with a biotype 4 inducer population was 509% greater than the biotype 1 response population with a biotype 1 inducer population ( $P < 0.01$ ) (Fig. 1). The biotype 1 response with a biotype 4 inducer population was 5,065% greater than the biotype 1 response population with no inducer ( $P < 0.01$ ) (Fig. 1). This indicates that a biotype 4 inducer population significantly increased the population density of the biotype 1 response population and that obviation of resistance was observed on *Rag1+Rag2* soybean.

For the susceptible soybean, the main effect of inducer population biotype ( $F = 8.16$ ;  $df = 2, 46$ ;  $P < 0.001$ ) significantly affected the final population. The response population biotype or the interaction of inducer population biotype by response population biotype did not significantly affect the density of the response population density.

When no inducer populations were present, the biotype 1 response population was 160% greater than the biotype 4 response population on susceptible soybean (Fig. 2). This indicates that a fitness cost was observed for the biotype 4 colony that was used for this experiment. When the biotype 4 response population was added to soybean with a biotype 4 inducer population there was no significant difference between its population density and the biotype 1 response population without an inducer. The addition of a biotype 1 inducer population resulted in a biotype 4 response population that was 126% greater than the biotype 1 response population without an inducer (Fig. 2). However, it was not significantly different from the biotype 1 response population with a biotype 1

inducer (Fig. 2). The biotype 4 response population with a biotype 1 inducer population was 190% greater than the biotype 4 response with a biotype 4 inducer population (Fig. 2). and 313% greater than the biotype 4 response population without an inducer population (Fig. 2). These results indicate that biotype 1 can improve susceptible soybean for biotype 4 soybean aphids (i.e., alleviate observed fitness costs).

### Discussion

The results of our study indicate that the previously observed insect-host plant interaction of induced susceptibility and specifically obviation of resistance can be produced by biotype 4 soybean aphids on *Rag1+Rag2* soybean. In addition, we observed that biotype 1 soybean aphids can remove the fitness costs for biotype 4 on susceptible soybean. The interaction of virulent and avirulent biotypes on soybean was first observed by Varenhorst et al. (2015a). However, in that study only interactions between biotype 1 (avirulent) and biotype 2 (virulent) soybean aphids were examined on soybean with a single resistance gene (*Rag1*). This study was the first to explore the potential for a virulent soybean aphid biotype (i.e., biotype 4) to obviate the resistance of a two gene pyramided resistance source. Although Varenhorst et al. (2015a) determined that biotype 1 could alleviate fitness costs for biotype 2 and biotype 3 soybean aphids on susceptible soybean, no study has evaluated the potential for this to occur with biotype 4. The observation that the *Rag1+Rag2* soybean can be made suitable for biotype 1 soybean aphids and that susceptible soybean can be made suitable for biotype 4 soybean aphids indicates that there is a potential for indistinguishable populations in field settings.

Although obviating of *Rag1+Rag2* resistance may appear negative from an insect resistance management standpoint, O'Neal et al. (2018) suggests that induced susceptibility may result in an otherwise unsuitable host plant becoming a refuge for either avirulent or virulent soybean aphid biotypes. The results from Varenhorst et al. (2015a) and Varenhorst et al. (2015b) provided a framework for this hypothesis based on the obviating of *Rag1* resistance and the removal of fitness costs for biotype 2 and biotype 3, both of which result in increased host suitability. These findings suggest that the obviating of resistance could apply to all future virulent soybean aphid biotype discoveries and the respective *Rag* genes. A simple deterministic, single-locus, two compartment genetic model that was adapted for soybean aphids determined that induced susceptibility (i.e., obviating of resistance and feeding facilitation) reduced directional selection for virulence to released *Rag* genes by 25% to 40% within a season (Varenhorst et al. 2015a).

A common approach to managing virulent biotypes is the pyramiding of two or more resistance genes. For soybean, the first commercially available pyramid was *Rag1+Rag2* (Wiarda et al. 2012). The *Rag1+Rag2* pyramid is very effective at managing biotype 1 soybean aphids (McCarville et al. 2012), but the discovery of biotype 4 populations posed a threat to its efficacy. Currently *Rag* genes are deployed in a combined fashion. Adding more genes to a pyramid does increase the efficacy but will not increase the durability of the resistance traits (MacIntosh 2019). Recent studies have evaluated three gene pyramids (*Rag1+Rag2+Rag3*) that are effective at managing biotype 4 soybean aphid populations (Varenhorst et al. 2017, Zhang et al. 2018).

However, it may only be a matter of time until a virulent biotype capable of overcoming the combination is discovered.

A survey of soybean aphids populations from multiple states suggests that virulent soybean aphid biotypes are common in field populations (Cooper et al. 2015). This supports estimates by Michel et al. (2011) that approximately 20% of the soybean aphid population in North America is composed of biotype 2. However, the extent that induced susceptibility occurs in soybean fields is unknown. It is likely that there is biotypic diversity within fields. This diversity may vary as the season progresses. In addition, the non-genetic pathway created by induced susceptibility that allows an avirulent biotype to colonize a resistant host may result in an overestimation of virulence unless iso-female lines are tested in laboratory settings or genetic markers are used to determine the aphid population's biotype.

Biotypes are more common to move on hosts with resistance genes as soybeans mature (da Silva Queiroz et al. 2018). Soybeans that express the pyramid of *Rag1+Rag2* may be infested with biotype 4 aphids, but plants that have undergone induced susceptibility will have become suitable hosts for biotype 1 aphids. This could result in an environment where virulent populations of soybean aphids may be overestimated. The implications that these findings have for soybean breeding needs to be further explored. The results of our study indicate that induced susceptibility is not unique to the interaction between biotype 1 and biotype 2 soybean aphids on *Rag1* soybean. This suggests that the presence of biotype 4 soybean aphids do not indicate the complete failure of *Rag1+Rag2* soybean for production systems. However, the development of

more robust soybean cultivars with multiple sources of resistance should continue to be investigated to ensure the longevity of resistance genes in North America.

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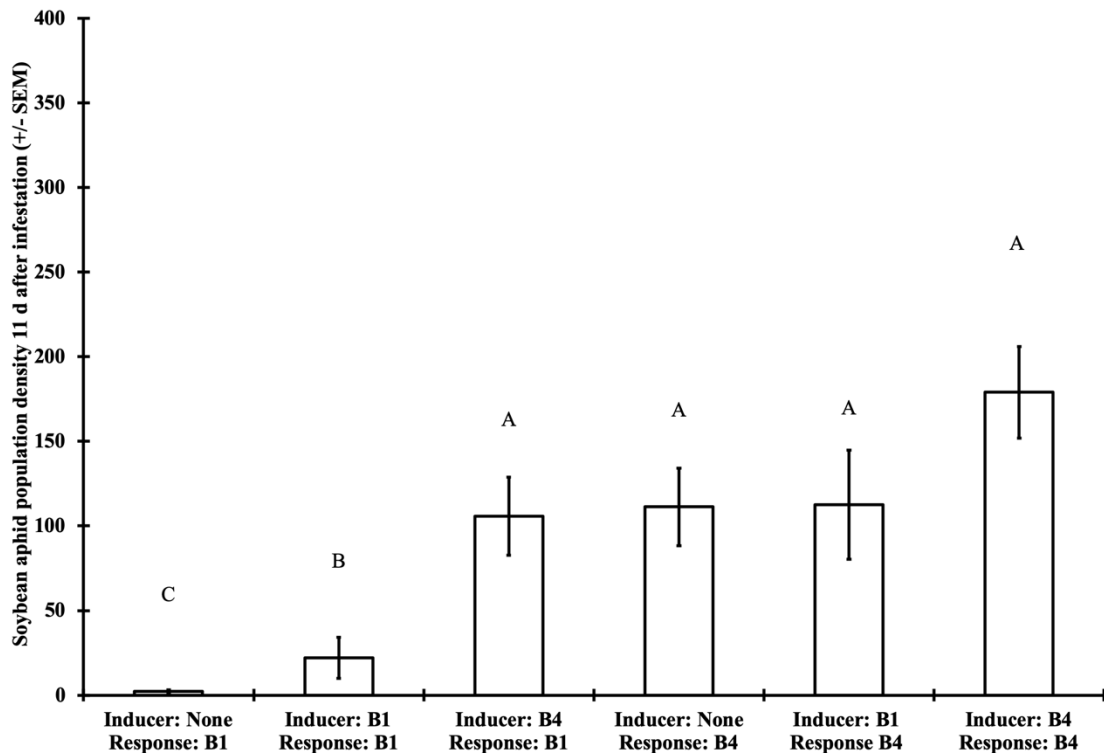


Figure 1. Determining if biotype 4 soybean aphids can obviate the resistance of

*Rag1+Rag2* soybean for biotype 1. For this experiment, soybean aphid biotype 1 and biotype 4 populations were examined 11 d after infestation on *Rag1+Rag2* soybean. Each biotype had either no inducer (Inducer: None), a biotype 1 inducer of 50 aphids (Inducer: B1) or an inducer population of 50 biotype 4 aphids (Inducer B2). For this experiment, the resistant soybean cultivar IA3027RA12 was used. Capital letters indicate significance among treatments ( $P < 0.05$ ). Data were transformed for analysis. Plotted values represent data prior to transformation.

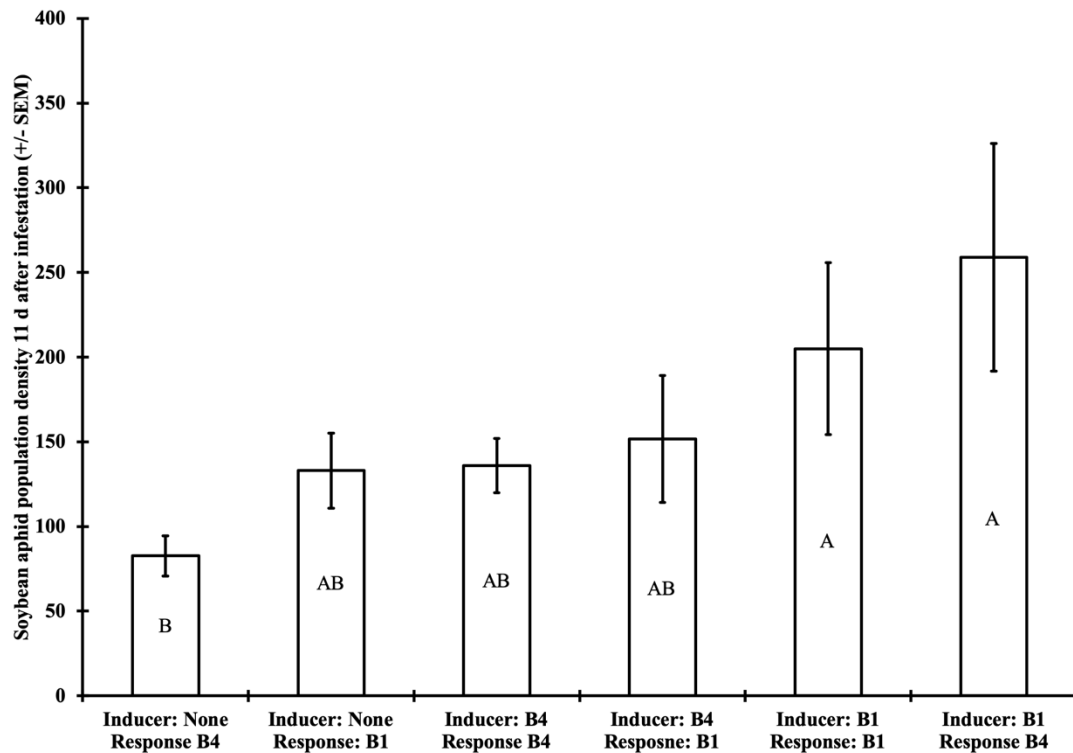


Figure 2. Determining if biotype 1 soybean aphids can obviate the fitness costs of susceptible soybean for biotype 4. For this experiment, soybean aphid biotype 1 and biotype 4 populations were examined 11 d after infestation on susceptible soybean. Each biotype had either no inducer (Inducer: None), a biotype 1 inducer of 50 aphids (Inducer: B1) or an inducer population of 50 biotype 4 aphids (Inducer: B2). For this experiment, the resistant soybean cultivar IA3027 was used. Capital letters indicate significance among treatments ( $P < 0.05$ ). Data were transformed for analysis. Plotted values represent data prior to transformation.

**CHAPTER 3 EVALUATION OF BIOTYPE 4 *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) INDUCED SUSCEPTIBILITY OF RESISTANT SOJA LINES**

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**Abstract**

The presence of virulent soybean aphid biotypes in North America has resulted in the search for additional robust resistance sources. In soybean, several genes that confer resistance to soybean aphids have been discovered but biotype 4 soybean aphids have been able to overcome several of them. However, soja, which is a wild crop relative of soybean, has previously been evaluated for its preserved diversity and potential sources of resistance to other soybean pests. Research has found many agronomically beneficial traits in soja that can be bred into the soybean germplasm. Previous research has determined that 135 soja lines have resistance to biotype 1 soybean aphids. More recently, researchers discovered soja lines that conferred resistance to biotype 4 soybean aphids. However, previous work on soybean has revealed that induced susceptibility effects result in the improvement of otherwise resistant soybean for avirulent populations. The purpose of this study was to evaluate resistant soja lines and determine if biotype 4 soybean aphids could elicit an induced susceptibility effect. We observed induced susceptibility occurring on both of the tested soja lines, which resulted in increased



population densities of biotype 1 soybean aphids. This suggests that that resistance sources present in soja may be different from those in soybean but have likely co-evolved with soybean aphids.

### **Introduction**

Soybean, *Glycine max* (L.) Merr., production in North America is negatively affected by soybean aphids, *Aphis glycines* Matsumura (Hemiptera: Aphididae), which can reduce yields by as much as 40% when left unmanaged (Ragsdale et al. 2011). Although the traditional management approach for soybean aphids has been the use of broad-spectrum foliar insecticides, host plant resistance represents a viable alternative (Olson et al. 2008, McCarville et al. 2014). Plant resistance to insects is the result of the host plant expressing traits that offset the balance in complex interactions between plants and insects, which benefit the plant. Implementation of host plant resistance as a management tool in combination with other insect pest management tools can provide options to construct a sustainable management system (Smith and Clement 2012).

Plant genes that provide resistance towards soybean aphids are referred to as **Resistance to *Aphis glycines*** (i.e., *Rag*) genes (Hill et al. 2006, Rouf Mian et al. 2008, Bales et al. 2013). The first *Rag* containing soybean were commercially released in 2010 (i.e., *Rag1* gene in soybean). However, Kim et al. (2008) observed virulent soybean aphids (i.e., capable of colonizing the resistant soybean) on *Rag1* soybean prior to the commercial release. In addition, Michel et al. (2009) documented that soybean aphids experienced a genetic bottleneck when being introduced into the U.S. Regardless of the fact that selection pressure and genetic diversity were not explanations for the

development of a virulent population, there are soybean aphid populations that are capable of overcoming *Rag* genes.

Classification of insect population based on their phenotypical ability to feed on resistant plants resulted in the development of biotype nomenclature (Claridge and Den Hollander 1983). The biotype nomenclature was adopted for describing soybean aphids based on their avirulence or virulence to different *Rag* genes. For example, biotype 1 soybean aphids are avirulent (i.e., unable to colonize) to all known *Rag* genes (Kim et al. 2008). Biotype 1 aphids are the most common in the North America (Michel et al. 2009, Crossley and Hogg 2015). Biotype 2 soybean aphids are characterized by their virulence (i.e., ability to colonize) towards soybean containing the *Rag1* gene (Kim et al. 2008). The biotype 3 soybean aphids are virulent to *Rag2* soybeans (Hill et al. 2010). Biotype 4 soybean aphids are virulent to *Rag1*, *Rag2*, the two gene pyramid of *Rag1+Rag 2* and the three gene pyramid of *Rag1+Rag2+Rag4* (Alt and Ryan-Mahmutagic 2013, Varenhorst et al. 2017). The presence of three virulent biotypes in North America presented a challenge for the development of soybean aphid-resistant soybean lines. However, Varenhorst et al. (2015c) constructed a model that suggests the presence of fitness costs, induced susceptibility and the use of pyramids could promote the longevity of deployed *Rag* genes.

Varenhorst et al. (2015a) determined that virulent soybean aphid biotypes (i.e., biotype 2, biotype 3 and biotype 4) experience fitness costs on susceptible soybean and negative cross resistance (i.e., biotype 2 and biotype 3) on soybean containing *Rag* genes that they are not virulent to. For asexual species like soybean aphids, fitness costs should

select for clonal populations that have reduced fitness costs and these populations can become the predominant clones within a single season (Crowder and Carriere 2009). In contrast to Varenhorst et al. (2015a), a recent study by Conzemius et al. (2019a) determined that three different biotype 4 colonies did not exhibit fitness costs on susceptible soybeans. Michel et al. (2010) observed that genetic diversity is greatly reduced within soybean aphid colonies that are maintained in laboratory settings for extended periods of time without the addition of field collected clonal populations. Differences observed in studies using different colony sources may be explained by the duration that clonal colonies are maintained.

Another aspect that could prolong the efficacy of *Rag* genes is the presence of induced susceptibility. Induced susceptibility is widely observed in many different plant-insect interactions (Price et al. 2011, Baluch et al. 2012). The effect is observed when insect feeding results in increased host suitability for either intraspecific or interspecific feeding (Takemoto et al. 2013). The induced susceptibility effect can be further evaluated by the specific effects that are being observed. For instance, feeding facilitation occurs when initial feeding by a population of insects increases the host suitability for additional individuals (Price et al. 2011). This results in increased population growth when compared to a smaller population feeding on the same host (Varenhorst et al. 2015a). Feeding facilitation is typically observed when insects are feeding on an already acceptable host (Rotem and Agrawal 2003).

Another effect of induced susceptibility is obviation of resistance (Baluch et al. 2012), which occurs when a virulent insect biotype promotes the population growth of an

avirulent biotype (Giovanini et al. 2006, Sauge et al. 2006). Varenhorst et al. (2015a) determined that virulent soybean aphids can promote the population growth of avirulent soybean aphids on soybean containing *Rag* genes. In addition, Varenhorst et al. (2015c) determined that avirulent soybean aphids can promote population growth of virulent biotypes (i.e., remove fitness costs) on susceptible soybean. This effect allows multiple biotypes to survive on the same plant and could represent genetic diversity within the population that would otherwise be solely composed of the virulent population (Varenhorst et al. 2015a). It is theorized that in field populations, obviating of resistance may create refuges of biotype 1 soybean aphids on soybean that are co-infested with both the biotype 1 and a virulent population (O'Neal et al. 2018). Intrabiotypic variability, fitness cost and induced susceptibility contribute to field failure of *Rag* traits in production agriculture. The lack of a fitness cost for virulent populations of aphids calls for most traditional insect resistance management techniques to be remodeled (O'Neal et al. 2018).

An alternative source of resistance to soybean aphids may be present in soybean's close relative. Soja, *Glycine soja* Siebold & Zucc., is the undomesticated relative of soybean that could be a source of unrealized diversity for soybean breeding. With a native range that spans eastern Russia through southern China, many different specimens have been identified and are classified by the plant introduction (PI) number provided by the U. S. Soybean Germplasm Collection (USSGC) in Urbana, IL. Soja and soybean have the same number of chromosomes ( $2n = 40$ ), are cross-compatible and exhibit normal meiotic chromosome pairing (Carter et al. 2004). Soja holds many traits that could be

identified and bred into elite soybean lines used in production today (Zhou et al. 2015). Of those traits, it is the potential source of insect resistance genes present in soja that could present many breeding opportunities and advancements for soybean aphid management (Hesler and Tilmon 2018).

Soja lines have undergone screening in no-choice and choice assays that explore the potential for host plant resistance to soybean aphids. Hesler (2013) used PI 522212B as a susceptible check and PI 549046 is a resistant check. Hesler and Tilmon (2018) found that PI 101404A exhibited host plant resistance to biotype 1 aphids. Conzemius et al. (2019b) evaluated 21 soja lines using three temporally or geographically unique biotype 4 populations and found that the Volga16 biotype 4 soybean aphids were virulent to PI 522212B but were avirulent to PI 101404A and PI 549046.

Although there are documented sources of virulent biotypes to *Rag* genes, there may be more durable sources of resistance present in soja lines (Kofsky et al. 2018). Conzemius et al. (2019b) determined that novel resistance traits in soja are effective against biotype 4 soybean aphids. However, no studies to date have evaluated if soja is resistant to the induced susceptibility effects that have been previously observed on soybean (Varenhorst et al. 2015a). The purpose of this experiment was to evaluate three soja lines (i.e., one susceptible and lines previously documented as resistant to both biotype 1 and biotype 4) for potential biotype 4 induced susceptibility effects, specifically feeding facilitation.

## **Material and Methods**

This experiment was conducted at the USDA-ARS North Central Agricultural Research Laboratory (NCARL) Brookings, SD. On-site aphid-free greenhouses were used to grow soybean plants for colony rearing of soybean aphid biotypes and also soja plants for the experiment. A mixture of soil (2:1:1 Vienna soil [fine-loamy, mixed Calcic Hapludolls], coarse vermiculite [Perlite Vermiculite Packaging, North Bloomfield, OH], and sphagnum peat moss [Sun Gro Horticulture Distribution Inc., Agawam, MA]) was used for all plantings. Greenhouse and growth chamber conditions were replicated from Conzemius et al. (2019b). Greenhouse plants were grown with a 16:8 (L:D) hr photoregime and approximately 23:18°C (L:D) temperature with a relative humidity of 50%. Colonies and the experiments were maintained in CMP4030 growth chambers (Conviron, Winnipeg, Canada).

#### **Soybean Aphid Colonies (Biotype 1 and Biotype 4)**

The biotype 1 soybean aphid colony was obtained from the University of Illinois. This colony was originally collected on soybean in Ohio and isolates were brought to Urbana, IL and were maintained at the University of Illinois. Once obtained, the biotype 1 colony used for the experiment was started at the NCARL as iso-female line. These aphids were maintained on susceptible soybean with approximately 10 plants per pot (6 cm top diameter × 4 cm bottom diameter × 5.7 cm height) (Myers Industries Inc., Earth City, MO). Soybean plants were grown in the greenhouse for four weeks. After that, they were transferred to the growth chamber and infested by the existing soybean aphid colony. After four weeks in the growth chamber, aphid infested plants were removed and placed into a freezer to eradicate aphids and prevent cross colony contamination. Isolates

from the biotype 1 collection were maintained under the same conditions as Hesler (2013).

The biotype 4 colony was collected on soybean cultivar LD12-15805Ra (*Rag1+Rag2*) in August 2016 near Volga, SD (Volga16) (Conzemius et al. 2019b). The biotype 4 soybean aphid colony was established as an iso-female and maintained in separate growth chambers at NCARL on IA2104RA12 (*Rag1+Rag2*). Approximately 10 plants per large pot were grown in the green house for four weeks and then transferred to a growth chamber and infested with the existing colony. After four weeks in the growth chamber, aphid infested plants were removed and placed into a freezer to eradicate aphids and prevent cross colony contamination. To prevent colony contamination, growth chambers containing different biotypes were never opened by the same person during the same day.

### **Soja Seed**

Seeds of the soja lines were acquired from the U.S. Soybean Germplasm Collection (Urbana, IL). Previous research has determined that PI 522212B is susceptible to aphid biotypes 4 and 1 and that PI 549046 and PI 1014041A are both resistant to biotype 1 and Volga16 biotype 4 (Hesler and Tilmon 2018, Conzemius et al. 2019a). Lines utilized in this trial were seed increased at NCARL. For each repetition of the experiment, soja seeds were scarified prior to planting using sulfuric acid ( $H_2SO_4$ , Fisher Chemical Catalog No. A300-212) (Lenis et al. 2011). For each line, two seeds were planted into a peat pellet (Jiffy-7® Horticultural Peat Pellet, Jiffy Products of America Inc., Tea, SD). Seven days after emergence, the soja plants were thinned to one soja per

peat pellet. Twenty-one days after planting the germination of soja was evaluated and first vegetative (V1) growth stage plants that possessed uniform shoot length and cotyledon size were selected for the trial and transplanted into the large pots (Fehr 1977).

### **Induced Susceptibility Trial**

We hypothesized that initial feeding by biotype 1 or biotype 4 soybean aphids would increase the host plant suitability of previously tested aphid resistant soja lines for subsequent populations (i.e., feeding facilitation occurs). Due to the avirulence of both biotypes to PI 101404A and PI 549046 we did not expect to observe obviation of resistance during this experiment. The protocol used for this experiment was adapted from Varenhorst et al. (2015a), which evaluated induced susceptibility in soybean. Unlike Varenhorst et al. (2015a), inducer populations were not maintained on a caged trifoliolate, but instead were allowed to freely move on the plant. This difference is due to the architectural differences between soybean and soja, as soja petioles are unable to support the netting and clip, and soja leaves are also much smaller.

This experiment was conducted using individually potted soja plants that were grown in large pots as previously described. Soybean aphids were transferred to soja plants from colony plants using a fine tip 000 paintbrush. To ensure that plant-to-plant movement during the experiment did not occur, exclusion cages were utilized. Each exclusion cage was constructed from a 0.6-cm thick clear extruded acrylic tube that had a 12.7 cm outer diameter and was 40.6 cm tall (Ridout Plastics Co. Inc., San Diego, CA). Each cage had two opposing ventilation holes that were 5.1-cm in diameter. To prevent soybean aphids from escaping, the holes and top of the tube were covered with no-thrips



mesh screen (screen hole size: 0.150 mm<sup>2</sup>, thread size: 15mm BioQuip, Rancho Dominguez, CA), which was glued into place.

For this experiment, we utilized a randomized complete block design with five blocks. The experiment was repeated twice for a total of 10 experimental units per treatment. In each block, we used a total of 18 treatments to test our hypothesis. Each treatment was a combination of three factors: soja PI, inducer population biotype and response population biotype. Three soja PI were used for this experiment that were PI 522212 B (susceptible), PI 549046 (resistant) and PI 1014041 A (resistant) (Hesler 2013, Hesler and Tilmon 2018, Conzemius et al. 2019b). For each cultivar, individual plants in each block were infested with an inducer population of either 15 biotype 1 or 15 biotype 4 soybean aphids at day 0 of the experiment. An exclusion cage was placed over the plants before the response populations were added (Varenhorst et al. 2015b). After 24 hr, soja plants were then infested with a response population of either no response aphids, five biotype 1 or five biotype 4 soybean aphids. Total soybean aphid populations present on the plant were counted 11 days after the response population was added. Therefore, the feeding effect of the inducer populations could be measured on the growth of the response population. However, due to the inability to restrict the inducer population on the plant, total population density was used to determine if induced susceptibility occurred in this experiment. For instance, induced susceptibility effects were confirmed if population densities of treatments with an inducer and response population were significantly greater than corresponding treatments that only received an inducer population.

## Statistical Analyses

This experiment was repeated twice using a randomized complete block design (RCBD) with 5 blocks per repetition (10 total experimental units per treatment). To address each of the hypotheses, we analyzed the number of aphids per plant at 11 days after infestation. Statistical analyses of the aphid count data from the induced susceptibility experiment were completed using RStudio (2020). Standard analysis of variance was performed on aphid counts after data were log transformed to correct for heterogeneity of the soybean aphid populations. Significant treatment effects were separated using Tukey's test with a significance level of ( $P < 0.05$ ). Non-transformed data were used to create the graphical representations of the data.

## Results

We confirmed our hypothesis that biotype 1 and biotype 4 soybean aphids would improve the host suitability for subsequent biotype 1 and biotype 4 soybean aphids on resistant soja plant introduction lines (i.e., induced susceptibility occurs). This was observed by analyzing the data first for the significance of the main effects of treatment, block, and secondly for soja plant introduction, inducer population, response population and the interaction main effects. The main effects treatment significantly affected the response aphid population ( $F = 127.91$ ;  $df = 17,158$ ;  $P < 0.001$ ). The main effect of response population biotype significantly affected the population density present on the soybean aphids ( $F = 115.47$ ;  $df = 2,158$ ;  $P < 0.001$ ), and the interaction of response population by inducer population also significantly affected the final population density

of soybean aphids ( $F = 15.22$ ;  $df = 2,158$ ;  $P < 0.001$ ). The main effect of the soja plant introduction ( $F = 858.79$ ;  $df = 2,158$ ;  $P < 0.001$ ), the interaction of soja plant introduction and response population biotype ( $F = 20.05$ ;  $df = 4,158$ ;  $P < 0.001$ ), and the interaction of soja plant introduction and inducer population biotype ( $F = 24.04$ ;  $df = 2,158$ ;  $P < 0.001$ ) significantly affected soybean aphid population density among resistant and susceptible soja lines. Therefore, data were analyzed by soja PI.

For the susceptible soja line PI 522212B, the main effects of inducer population biotype ( $F = 11.21$ ;  $df = 2,50$ ;  $P < 0.001$ ) and response population biotype ( $F = 13.00$ ;  $df = 2,50$ ;  $P = 0.001$ ) significantly affected the density of the response populations. The interaction of the two main effects was also significant ( $F = 11.38$ ;  $df = 1, 50$ ;  $P < 0.001$ ). The biotype 1 inducer: biotype 1 response treatment population density was significantly greater than the biotype 1 inducer: no response treatment ( $P < 0.001$ ). (Fig. 1). This indicated that feeding facilitation was occurring on the susceptible soja PI and resulted in a 233% total population increase for the biotype 1 inducer: biotype 1 response treatment. We did not observe fitness costs present for tested biotype 4 population on the susceptible soja PI. We also did not observe any evidence of interbiotypic induced susceptibility interactions occurring. We did observe a negative response for the biotype 4 inducer: biotype 4 response treatment when compared to the biotype 4 inducer: no response treatment.

For the resistant soja line PI 549046, the main effects of inducer population biotype ( $F = 21.96$ ;  $df = 1, 50$ ;  $P < 0.001$ ) and response population biotype ( $F = 81.97$ ;  $df = 1, 50$ ;  $P < 0.001$ ) both significantly affected the density of the response populations.

The interaction of the two main effects was also significant ( $F = 10.32$ ;  $df = 1, 50$ ;  $P < 0.001$ ). The biotype 1 inducer: biotype 1 response treatment had a significantly greater population density than the biotype 1 inducer: no response treatment ( $P < 0.001$ ) (Fig. 2). The population density of the biotype 1 inducer: biotype 1 response treatment was 334% greater than the biotype 1 inducer: no response treatment. We did not observe a significant difference between the biotype 4 inducer: biotype 4 response treatment and the biotype 4 inducer: no response treatment. This indicated that feeding facilitation was observed for biotype 1 on PI 549046 but not for biotype 4.

The population density of the biotype 4 inducer: biotype 1 response treatment was significantly greater than the biotype 1 inducer: no response ( $P < 0.001$ ) (Fig. 2), the biotype 4 inducer: no response ( $P < 0.001$ ) (Fig. 2), and the biotype 1 inducer: biotype 1 response treatments ( $P < 0.001$ ) (Fig. 2). The population density of the biotype 4 inducer: biotype 1 response treatment was 672% greater than biotype 1 inducer: no response, 293% greater than the biotype 4 inducer: no response, and 387% greater than the biotype 1 inducer: biotype 1 response treatments. The increased population density of the biotype 4 inducer: biotype 1 response treatment when compared to the biotype 1 inducer: biotype 1 response treatment indicated that interbiotypic feeding facilitation was occurring. These results also indicate that PI 549046 is more susceptible to the tested biotype 4 aphids than the biotype 1 aphids. This was determined based on the biotype 1 inducer: no response treatment population density being 57% lower than the biotype 4 inducer: no response population ( $P < 0.001$ ) (Fig. 2). This is the first observation of soybean aphids eliciting feeding facilitation on resistant soja. In addition, we observed an increased population of

the biotype 4 inducer: biotype 1 response treatment when compared to the biotype 1 inducer: biotype 1 response treatment. Previous research determined that biotype 4 is avirulent on PI 549046 (Hesler 2013, Conzemius et al. 2019b). It suggests that interbiotypic interactions on soja may result in increased host suitability when compared to intrabiotypic interactions.

For the resistant soja line PI 101404A, the main effect inducer population biotype was significant ( $F = 9.22$ ;  $df = 1, 50$ ;  $P < 0.003$ ). The main effect response population biotype significantly affected the density of the response population ( $F = 89.19$ ;  $df = 2, 50$ ;  $P < 0.001$ ). The interaction of the two main effects was also significant ( $F = 70.98$ ;  $df = 2, 50$ ;  $P < 0.001$ ). The population density of the biotype 4 inducer: biotype 4 response treatment was significantly greater than the biotype 4 inducer: no response treatment ( $P < 0.001$ ) (Fig. 3). This resulted in the population density of the biotype 4 inducer: biotype 4 response population being 282% greater than the population of the biotype 4 inducer: no response treatment. However, we did not observe any differences between the biotype 1 inducer: biotype 1 response treatment and the biotype 1 inducer: no response treatment. This indicated that feeding facilitation occurred for biotype 4 on PI 101404A but not for biotype 1.

The biotype 1 inducer: biotype 4 response treatment was significantly greater than the biotype 4 inducer: no response treatment ( $P < 0.001$ ), and the biotype 4 inducer: biotype 4 response treatment ( $P < 0.001$ ). This indicated that a biotype 1 inducer improved the host's suitability for the biotype 4 response population. This appears to be an interbiotypic feeding facilitation response. However, we also observed that the

population density for the biotype 4 inducer: biotype 1 response treatment was significantly greater than the biotype 1 inducer: no response treatment ( $P < 0.001$ ), the biotype 1 inducer: biotype 1 response treatment ( $P < 0.001$ ), and the biotype 1 inducer: biotype 4 response treatment ( $P < 0.001$ ). (Fig. 3). The population density for the biotype 4 inducer: biotype 1 response treatment was 85% greater than the biotype 1 inducer: biotype 4 response treatment. This finding suggests that biotype 4 also produced an interbiotypic feeding facilitation response of PI 101404A for biotype 1 soybean aphids.

Although the interbiotypic interactions occurring on the PI 101404A both resulted in significantly increased populations it appears that a biotype 4 inducer had the greatest impact in terms of improved host suitability. This is surprising, as PI 101404A was significantly more resistant to biotype 4 aphids than to biotype 1 aphids ( $P < 0.001$ ) and the biotype 1 inducer: no response treatment was 153% greater than the biotype 4 inducer: no response treatment.

### **Discussion**

Our results indicate that feeding by biotype 1 and biotype 4 soybean aphids can alter soja to make it more suitable for subsequent soybean aphid infestations (i.e., induced susceptibility occurred). The observed effects and which soybean biotype elicited them varied based on the soja PI line. Varenhorst et al. (2015a) describes induced susceptibility as a phenomenon in which host plant is physiologically transformed by the feeding of an arthropod herbivore, that results in increased host-plant suitability for subsequent colonization. This effect can be due to feeding on a susceptible host by the same biotype or different avirulent biotypes (i.e., feeding facilitation). Feeding facilitation has been

observed for soybean aphids on soybean (Varenhorst et al. 2015b). This study demonstrates that feeding facilitation can occur on soja and it can be induced by the same biotype or a different biotype.

We observed evidence that biotype 1 soybean aphids produce a feeding facilitation effect on the susceptible soja (PI 522212B) and one of the resistant soja PI lines (PI 549046) (Fig. 1 and Fig. 2). We also observed that biotype 4 soybean aphids produced a feeding facilitation effect on one of the resistant soja PI lines (PI 101404A) (Fig. 3). Although the tested biotype 4 population was not virulent to either of the resistant soja PI lines (Conzemius et al. 2019b), we observed it increasing the host suitability for biotype 1 soybean aphids on both PI 549046 and PI 101404A. This response is considered feeding facilitation because biotype 4 is classified as avirulent to the resistant soja PI lines. However, the significant improvement of the host for the biotype 1 population after biotype 4 feeding suggests that the biotype 4 induced interbiotypic feeding facilitation on resistant soja is greater than that caused by biotype 1.

Previous research has found that soybean aphids that are reared in growth chamber conditions may be affected by continued coloanal amplification (Michel et al. 2010). Similar studies have found that biotype populations with the same origin but maintained in separate facilities for extended periods of time can have different responses to the same hosts (Conzemius et al. 2019a). However, the biotype 4 colony that was used for this experiment had limited population growth on both PI 549046 (Fig. 2) and PI 101404A (Fig. 3), which was similar (i.e., PI 549046) or even less (PI 101404A) than

biotype 1 population growth. These findings prevent the conclusion that the tested biotype 4 population has increased virulence to the resistant soja lines.

The results from this study suggest that interbiotypic interactions on soja may result in different responses than those observed on soybean. Additional research is necessary to determine additional effects of interbiotypic induced susceptibility responses on soja. The results of this study suggest the virulence mechanisms employed by soybean aphids to overcome resistant soybean are likely similar to those used to overcome resistance in soja. Although soja represents a great resource for resistance genes for managing soybean aphids, it appears that soybean aphids are already well adapted for overcoming those sources of resistance.

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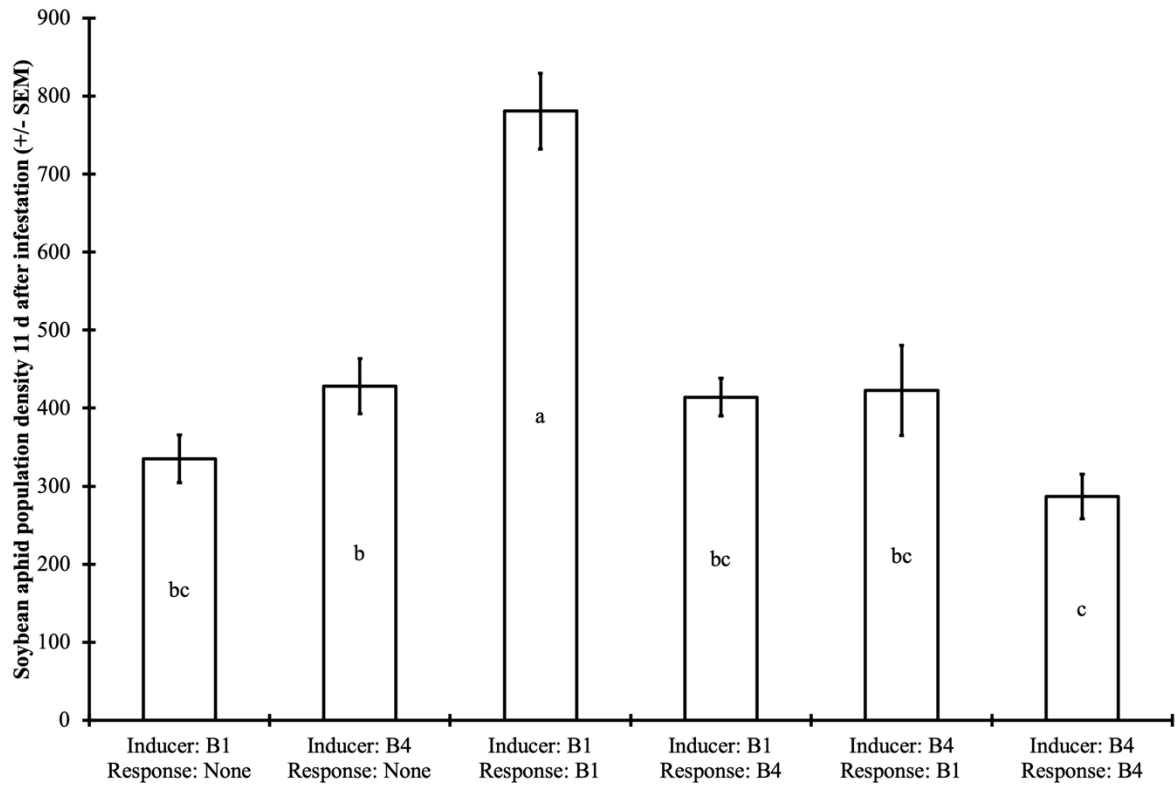
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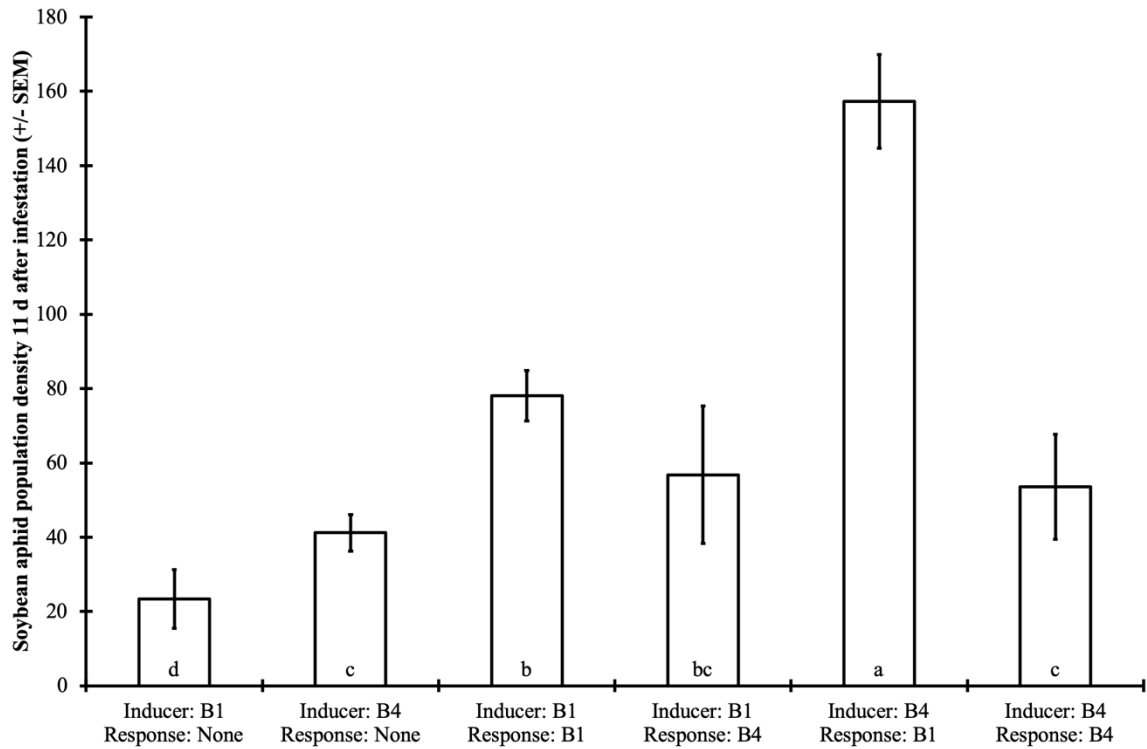
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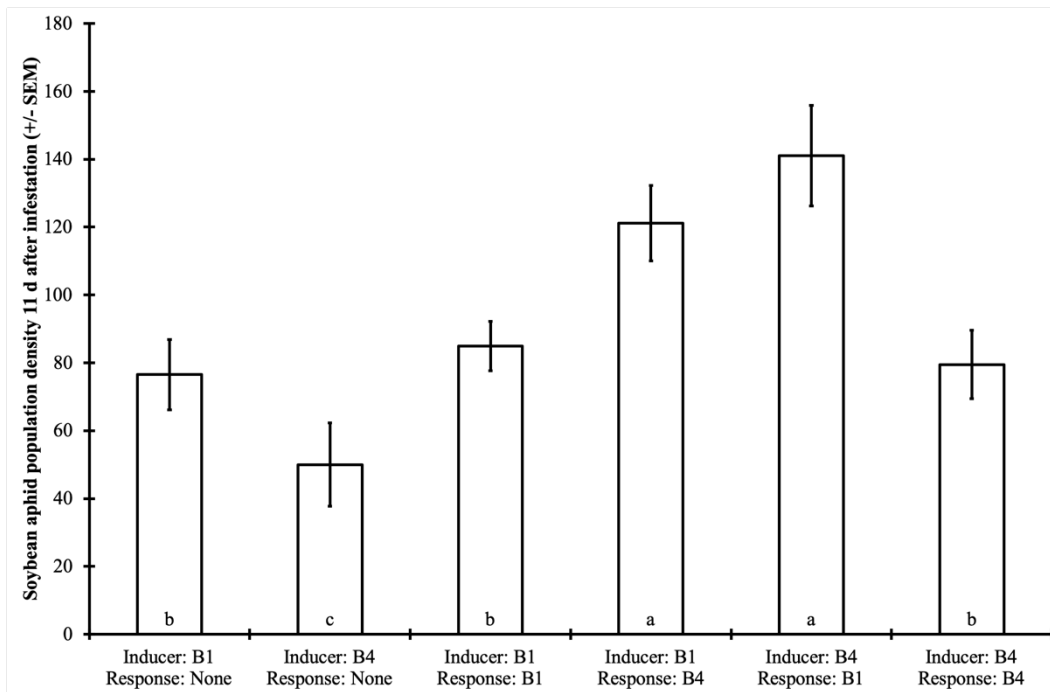


**Figure 1.** A comparison of the effects produced by varying inducer population biotypes (biotype 1: B1 and biotype 4: B4) on varying response populations of no aphids (None), biotype 1 (B1) and biotype 4 (B4) on a susceptible soja line (PI 522212B). Letters denote significant differences among treatments ( $P < 0.05$ ). Data were log transformed for analysis, but plotted values represent the data prior to transformation.



**Figure 2.** A comparison of the effects produced by varying inducer population biotypes (biotype 1: B1 and biotype 4: B4) on varying response populations of no aphids (None), biotype 1 (B1) and biotype 4 (B4) on a resistant soja line (PI 549046). Letters denote significant differences among treatments ( $P < 0.05$ ). Data were log transformed for analysis, but plotted values represent the data prior to transformation.





**Figure 3.** A comparison of the effects produced by varying inducer population biotypes (biotype 1: B1 and biotype 4: B4) on varying response populations of no aphids (None), biotype 1 (B1) and biotype 4 (B4) on a resistant soja line (PI 101404A). Letters denote significant differences among treatments ( $P < 0.05$ ). Data were log transformed for analysis, but plotted values represent the data prior to transformation.

## CHAPTER 4: GENERAL CONCLUSIONS

Since their first detection in 2000, soybean aphids continue to be a significant pest of soybeans in North America. Traditional management strategies that rely heavily on the uses of insecticides have resulted in the development of pyrethroid resistant soybean aphid populations. Incorporating host plant resistance into management strategies will help reduce the reliance of soybean aphid management on foliar insecticides. One caveat is that the host plant resistance must be durable and deployed in a manner that encourages longevity of the selected genes.

It seems as fast as a host plant resistance source is identified; a virulent biotype emerges that can overcome the host plant resistance. Although soybean aphids underwent a genetic bottleneck when they were introduced into North America, virulent biotype populations have been detected. It may be that the high selective pressure on a phenotypically flexible species like the soybean aphid results in fast adaptation to the soybean defenses. Or it may be that diversity preemptively exists in the soybean aphid populations. Our findings show that mixed biotype populations of aphids may provide an advantage over uniform biotype populations. At this point, screening of soybean aphid populations shows that virulent biotypes are widespread and intermixed.

The resistance identified in soybean's wild relative soja has been demonstrated to be very durable against many populations of soybean aphids. Perhaps this resistance may be the key to developing long term host plant resistance that can be incorporated into the soybean germplasm. The diversity within soja presents some hindrances in the effectiveness of directly crossing specific traits into traditional soybean lines.

Nevertheless, this difficult path may provide the most comprehensive management solution for soybean aphids.

Insect resistance management is important to prolong the durability of host plant resistant germplasm. Proper deployment of *Rag* traits utilizing the best stewardship practices is essential for successful soybean aphid management. The use of refuge has not been effectively utilized when previous *Rag* traits have been released into production. Refuge in asexual insect species like soybean aphids is difficult to develop. The within plant refuge effect of induced susceptibility in soybean host pest interactions complicates the refuge strategy.

As the global demand for soybeans continues to rise, durable production methods for soybeans will have more value. A benefit of using traditionally bred host plant resistance, compared to incorporating genetically modified insecticidal proteins, is that germplasm can be used in organic cropping systems.