Integrating soybean aphid and soybean cyst nematode management

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Entomology

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2014

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ABSTRACT

Soybean aphid, *Aphis glycines*, and soybean cyst nematode (SCN), *Heterodera glycines*, can interact through the soybean plant resulting in increased SCN reproduction on both SCN-resistant and SCN-susceptible varieties. The management of SCN is heavily reliant on the planting of PI 88788-derived SCN-resistant varieties to limit yield loss to SCN in the current year and future years. Virulence to PI 88788 is increasing in SCN field populations due to its extensive use. Therefore, it is increasingly important to manage any factor that increases SCN reproduction on SCN-resistant varieties. Here I examined management tactics including host-plant resistance and insecticidal seed treatments to limit soybean aphid populations and disrupt the interaction between soybean aphids and SCN. Neither host-plant resistance incorporating a single resistance gene nor insecticidal seed treatments were able to prevent yield loss from soybean aphids. Furthermore, host-plant resistance incorporating a single resistance gene also failed to disrupt soybean aphid-SCN interactions. Host-plant resistance incorporating a pyramid of two resistance genes was, however capable of limiting yield loss to soybean aphids. The pyramid line also limited aphid population densities to below levels where we would expect to observe soybean aphid-SCN interactions. Future research will need to investigate the ability of a pyramid line to disrupt soybean aphid-SCN interactions in the field and the potential consequences for yield and long-term sustainable SCN population management.

CHAPTER 1. GENERAL INTRODUCTION AND LITERATURE REVIEW

Dissertation Organization

The research presented in this dissertation seeks to further our knowledge of current soybean aphid host-plant resistance in the hope of increasing its sustainability and integration with soybean cyst nematode management. The dissertation is organized into eight chapters. Chapter one contains a general review of the pertinent literature and an outline of the research presented in subsequent chapters. Chapter two will report on the ability of host-plant resistance and biological control to reduce soybean aphid populations. Chapter three will report on the ability of insecticidal seed treatments and host-plant resistance to reduce soybean aphid population growth across the growing season. Chapter four will report on the performance of single gene resistance and a pyramid of two resistance genes for soybean aphid management across the Midwestern United States. Chapter five will investigate insect resistance management tactics and their ability to delay the buildup of virulence alleles to host-plant resistance in soybean aphid populations. Chapter six reports on a greenhouse study elucidating the effect of soybean aphid feeding on soybean cyst nematode reproduction. Chapter seven investigates the effect of host-plant resistance and pest density on the interaction between soybean aphid and soybean cyst nematode. A brief summary of the findings and implications of the research presented in this dissertation is presented in Chapter eight.

Introduction and Literature Review

Soybean aphid ecology and management

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive pest of soybean in the Midwestern United States. The soybean aphid has a heteroecious holocyclic lifecycle, with sexual reproduction occurring on buckthorn, *Rhamnus* spp., in the fall (Ragsdale et al. 2004). The principal primary host of the soybean aphid in North America is European buckthorn, *Rhamnus cathartica* L. Sexual reproduction and successful overwintering can also take place on *Rhamnus lanceolata* Pursh and *Rhamnus alnifolia* L. Gynoparae (winged, asexual females) and oviparous (winged, sexual females) nymphs have also been identified on glossy buckthorn, *Frangula alnus*, but it is unclear whether this plant can serve as an acceptable overwintering host (Hill et al. 2010, Voegtlin et al. 2005).

Eggs are deposited around the buds of buckthorn in the fall and serve as the overwintering stage of the aphid. Eggs hatch on buckthorn in the spring and asexual reproduction occurs for three to four generations before alate females appear (Ragsdale et al. 2004). Alate females will leave the primary host in search of the secondary host plants, primarily soybean, *Glycine max* L. It has been proposed that this early spring colonization of soybean creates a genetic "bottleneck" (Ragsdale et al. 2004). This bottleneck is created by both the spatial and temporal disjunction between buckthorn and soybean. First, the distribution of buckthorn is concentrated in the northern region of the soybean aphid distribution in the United States (Voegtlin et al. 2005), but the current soybean aphid distribution extends to 30 states and three Canadian provinces (Ragsdale et al. 2011). Colonization of

soybean fields in which local populations of buckthorn do not exist requires long-distance migrations or secondary infestations from initially colonized fields. A temporal disjunction also occurs between buckthorn and soybean in that spring migrants can be found on buckthorn well before most cultivated soybean has emerged (Ragsdale et al. 2004). Michel et al. (2009) found evidence for an early season genetic bottleneck. Regional sampling of aphid populations found that more genetic variability among soybean aphid populations could be explained by grouping the populations by time rather than geography (Michel et al. 2009).

Soybean aphids reproduce asexually on soybean during the summer completing ten to fifteen generations per year (Ragsdale et al. 2004). In the fall, a decrease in photoperiod, declining host quality and a drop in temperature triggers the production of winged asexual females or gynoparae and winged sexually reproducing males (Wu et al. 2004). The gynoparae will migrate to buckthorn where they will give rise to wingless sexual females termed oviparae. The males will migrate back to buckthorn in search of oviparae to mate.

Soybean aphid management in the United States has primarily relied on the use of both foliar and seed-applied insecticides. Upon the discovery of the soybean aphid in 2000, research was undertaken to determine the optimum strategy for insecticidal control of the soybean aphid. Seed-applied insecticides were found to provide inconsistent and insufficient soybean aphid control (McCornack and Ragsdale 2006, Johnson et al. 2008, 2009, Megalhaes et al. 2009). This is likely due to the limitations of the residual activity of seed treatments. McCornack and Ragsdale (2006) found the residual activity of thiamethoxam seed treatments to last

up to the R2 soybean growth stage (Fehr and Caviness 1977) or 49 days after planting. After that time, any residual activity fails to negatively impact soybean aphid populations. In the Midwest, soybean aphid populations typically do not reach economically damaging levels until the R2-R4 growth stages (Ragsdale et al. 2007, Johnson et al. 2008), which occur on average 50 to 70 days after planting (Pedersen 2004).

For foliar applications of insecticide, Ragsdale et al. (2007) established an economic threshold of 250 aphids per plant between the R1 to R5 growth stages. Johnson et al. (2009) validated this threshold as more profitable than insecticidal seed treatments and prophylactic growth-stage-based applications of insecticide (Myers et al. 2005, Johnson et al. 2009).

Beginning in 2010, soybean aphid resistant cultivars were released for commercial production. Currently these cultivars employ a single gene, *Rag1* (**r**esistance to ***A****phis* ***g****lycines*), providing antibiosis-based resistance to the soybean aphid (Hill et al. 2006). Although *Rag1* is currently the only resistance gene commercially available, three other host-plant resistance genes have been identified and characterized: *Rag2* (Mian et al. 2008), *Rag3* (Zhang et al. 2009), and *rag4* (Zhang et al. 2010). It is unclear if single gene resistant cultivars will provide sufficient soybean aphid control (Chiozza 2009) or whether foliar insecticide applications may still be warranted.

Questions surround whether host-plant resistance will serve as a sustainable management tool or if virulence to the genes will develop rapidly in the soybean aphid population leading to a decrease in the utility of the host-plant resistance

genes. Rapid development of virulence to host-plant resistance has been documented for other aphid species (Smith 1989, Puterka et al. 1992, Burd and Porter 2006, Lombaert et al. 2009) Soybean aphid biotypes capable of overcoming the *Rag1* (Kim et al. 2008) and *Rag2* (Hill et al. 2010) genes have already been identified. The distribution and abundance of these biotypes remains to be determined. The ability of different management strategies, including refuges, pyramiding resistance genes, and enhancing fitness costs, to delay the development of virulence remains relatively unexplored for a parthenogenic species. Crowder and Carriere (2009) found increasing fitness costs, refuge size, and the recessiveness of virulence all delayed the development of virulence in parthenogenic insects. Their results, however, were limited to obligate pathenogenic species and do not necessarily apply to species with holocyclic or heteroecious lifecycles, such as the soybean aphid.

Soybean cyst nematode ecology and management

The soybean cyst nematode, *Heterodera glycines* Ichinohe is an invasive pest in North America first discovered in the United States in 1954. It is distributed throughout all major soybean-producing states in the United States and is considered the most economically damaging soybean pathogen worldwide (Schmitt et al. 2004).

The soybean cyst nematode is a sexually dimorphic obligate endoparasite that infects the roots of soybean plants (Niblack et al. 2006). Soybean cyst nematode eggs hatch in the spring releasing second-stage (J2) or infective juveniles. Infective juveniles enter the roots of soybean plants. In the root tissue the

nematode will establish a specialized multi-nucleated feeding cell known as a syncytium in or near the vascular tissue (Jones and Northcote 1972, Johnson et al. 1993). The syncytium is formed through the joining of neighboring cells via the widening of plasmodesmata in the cell wall (Golinowski et al. 1997). The syncytium serves as the sole food source for the duration of the life cycle. At this stage the J2 is sexually undifferentiated with sex determined by epigenetic factors including environmental conditions and food quality (Mugniéry and Fayet 1981, 1984, Mugniéry and Bossis 1985, Betka et al. 1991, Grundler et al. 1991). The developing nematode will complete three more molts (to J3, J4, adult) before reaching maturity. At maturity, males cease feeding and exit the soybean plant to locate females. Adult females are swollen and incapable of movement. The females will remain attached to their feeding site for the remainder of their lives. After mating, females continue to swell with the development of eggs, and eventually rupture onto the outside of the roots. Under ideal conditions a single female can produce up to 600 eggs, with fecundity reduced in response to field factors and host-plant resistance. Approximately a third of eggs produced by females will be secreted in a gelatinous mixture with the remaining eggs protected within the cyst created by the female nematode body. Eggs secreted in the gelatinous mixture are most likely to hatch during the same season, with two to four generations possible in a single year (Schmitt et al. 2004). The eggs contained within the female cyst serve as a method for long-term survival, with eggs remaining viable for as long as 11 years (Niblack et. al 2006).

Soybean cyst nematode management requires a long-term program, as high population densities may persist in the soil for several years. Current management relies on host-plant resistance and rotation to non-host crops. The soybean cyst nematode has a wide genetic diversity between and within populations, allowing it to readily adapt to resistant soybean cultivars (Niblack et al. 2002). The short crop rotations present in the north central region of the United States increases the potential for adaptation to host-plant resistance. Hundreds of soybean cyst nematode resistant cultivars have been developed. These cultivars are derived from three soybean source lines of resistance: PI 88788, PI 437654 and Peking. Of the numerous resistant cultivars available, over 95% contain PI 88788-derived resistance (Tylka et al. 2013). The high reproductive capacity of the nematode coupled with its ability to maintain high populations in the soil for multiple years requires long-term management programs focusing on both in-season control to ensure high yields and on maintaining low egg populations for sustainable production in the future. Host-plant resistance offers the most efficient and effective management strategy, but the heavy reliance on a single resistance source makes nematode adaptation to host plant resistance a realistic concern for growers (Niblack et al. 2006).

Soybean aphid and soybean cyst nematode interactions

The soybean aphid and soybean cyst nematode feed on distant parts of the vascular tissue of the plant, aphids feeding on the leaves and stems of the plant and nematodes attacking the roots. Despite the lack of direct contact between the two herbivores potential for interactions exist via the soybean plant. Both herbivores

are sedentary, biotrophic feeders, meaning that they create and draw nourishment from a single feeding site composed of living tissue for an extended period of time. In the case of the soybean aphid, a single feeding site will be maintained for around an hour (Crompton and Ode 2010). A female soybean cyst nematode will maintain one syncytium for the duration of life (≈28 days) (Niblack et al. 2006). The establishment and prolonged maintenance of these feeding sites for both herbivores involves the secretion of saliva-carrying effector molecules (Will et al. 2007, Davis et al. 2008, Will et al. 2009, Abad et al. 2010, Giordanengo et al. 2010). These effector molecules are essential for the manipulation of the host plant's primary metabolism and the evasion and manipulation of plant defenses (Zhu-Salzman et al. 2004, 2005, Thompson and Goggin 2006). Recent molecular studies have shown common hormonal signaling pathways are involved in host-plant resistance to both herbivores (Li et al. 2008, Klink et al. 2010). Through the systemic manipulation of primary metabolism and common plant defense pathways, it is possible for the two herbivores to interact (Kaplan et al. 2008).

Previous studies found soybean plant infection by the soybean cyst nematode deters plant selection by alate soybean aphids (Hong et al. 2010, 2011). In another study, analyzing the impact of multiple-pest infections on the performance of individual pests, evidence was found for an indirect interaction between the soybean aphid and soybean cyst nematode. In the study, plants were co-infected with soybean aphid, soybean cyst nematode and the fungal pathogen *Cadophora gregata* Harrington and McNew, the causal agent of brown stem rot. Soybean aphid performance was reduced on plants infected with soybean cyst

nematode and *C. gregata* compared to plants infected with only the soybean aphid. In the same study, soybean cyst nematode performance was enhanced on plants co-infected with soybean aphid and *C. gregata* compared to plants infected with soybean cyst nematode alone (McCarville et al. 2012).

Aboveground-belowground herbivore interactions

Research on aboveground-belowground herbivore interactions in other systems demonstrated that systemic effects on plant defenses and primary metabolism could affect the outcome of such interactions (Johnson et al. 2012, Soler et al. 2013, Wondafrash et al. 2013). Aspects of each herbivore's biology are likely to affect the outcome of aboveground-belowground interactions, including herbivore arrival time, population density, and feeding guild. Aspects of the host-plant, including to nutrient status and resistance to herbivores, are also likely to influence the outcome of the interaction (Huang et al. 2012, Kutyniok et al. 2014). Finally, aspects of the study of interactions can also affect the result, including the location of the study (i.e. field or greenhouse/lab) and the response variable measured (i.e. population growth rate, survival, fecundity, etc.) (Johnson et al. 2012).

Aboveground-belowground interactions involving aphids and plant-parasitic nematodes have been poorly studied. A small body of literature has investigated the effect of plant-parasitic nematodes on aphid performance (Table 1). A pattern has emerged, however, as the presence of plant-parasitic nematodes has generally reduced the performance of aphids. However, the available observations are largely derived from greenhouse or lab studies that are more prone to observing negative

interactions between aboveground and belowground herbivores (Johnson et al. 2012).

Fewer studies have investigated the reciprocal effect aphid feeding has on nematode performance (Table 2). In the few studies available, contrasting results are present. It is therefore difficult to draw any broad conclusions about how aphid feeding affects nematode performance. However, the results of Kaplan et al. (2009) highlight the importance of taking a broader view of plant-parasitic nematode communities. Researchers have already identified the importance of the insect's feeding guild in aboveground-belowground interactions, but the ecology of plant-parasitic nematodes has been largely ignored. Very few studies have considered the effects of aboveground feeding separately on endoparasitic versus ectoparasitic nematodes or migratory versus sedentary nematodes (Kaplan et al. 2009, Wondafrash et al. 2013).

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Table 1. Plant-parasitic nematode effects on aphids

Nematode	Aphid	Host Plant	Outcome	Performance Metric	Source
Heterodera glycines	*Aphis glycines*	*Glycine max*	**-**	alate preference	Hong et al. 2010, 2011
			0	seasonal abundance	Heeren et al. 2012
			-		McCarville et al. 2012[a]
Heterodera schachtii	*Myzus persicae*	*Beta vulgaris*	**-**	population growth rate	Gera Hol et al. 2010
Heterodera schachtii	*Brevicoryne brassicae*	*Brassica oleraceae*	**-**	population growth rate	Gera Hol et al. 2010, 2013
Heterodera schachtii	*Brevicoryne brassicae*	*Arabidopsis thalania*	**0**	population density	Kutyniok and Müller 2012
			0		Kutyniok and Müller 2013
Heterodera schachtii	*Myzus persicae*	*Arabidopsis thalania*	**-**	population density/preference	Kutyniok et al. 2014
Meloidogyne incognita	*Myzus persicae*	*Nicotiana tabacum*	**-**	growth rate/fecundity	Kaplan et al. 2011
Meloidogyne incognita	*Myzus persicae*	*Nicotiniana attenuata*	**-/0**[b]	population density	Kaplan et al. 2009
Meloidogyne sp.	*Metopolophium dirhodum*	*Avena sativa*	**-**	mortality/fecundity	Sell and Kuo-Sell 1990
Ditylenchus dipsaci	*Acyrthosiphon pisum*	*Medicago sativa*	**-**	aphid density	Ramirez unpublished
Pratylenchus penetrans	*Myzus persicae*	*Plantago lanceolata*	**-**	fecundity	Wurst and Putten 2007
Nematoda[c]	*Schizaphis rufula*	*Ammophila arenaria*	**-**	aphid density	Vandegehuchte et al. 2010
Nematoda[d]	*Rhopalosiphum padi*	*Anthoxanthum oduratum*	**-**	fecundity	Bezemer et al. 2005
Nematoda[d]	*Rhopalosiphum padi*	*Agrostis capillaris*	**-**	fecundity	Bezemer et al. 2005
Nematoda[e]	*Brevicoryne brassicae*	*Brassica oleracea*	**-**	aphid density	Kabouw et al. 2011

[a] Plants were exposed to *Aphis glycines* and the biotrophic fungus *Cadophora gregata* which infects the plant's vascular tissue.
[b] Nematode presence reduced aphid densities in July, but had no effect in August.
[c] Diverse community of nematodes including *Pratylenchus brzeskii* (53.75%), *Tylenchorhynchus ventralis* (15.62%), *Meloidogyne* sp. (15.62%), *Heterodera* sp. (12.5%), *Paratylenchus* sp. (4.25%), *Rotylenchus* sp. (3.125%), *Filenchus* sp. (3.125%), other species (2%)
[d] Diverse community of nematodes including, plant associates, plant feeders, bacterial feeders, fungivores, and omni-carnivores. Plant feeders composed of Paratylenchidae (48%), Pratylenchidae (28%), and Dolichodoridae (9%).
[e] Diverse community of nematodes with dominant species belonging to the bacterivorous families, but also including plant parasitic nematodes.

Table 2. Aphid effects on plant-parasitic nematodes

Aphid	Nematode	Host Plant	Outcome	Performance Metric	Source
Aphis glycines	*Heterodera glycines*	*Glycine max*	**+**	reproduction factor	McCarville et al. 2012[a]
Myzus persicae	*Meloidogyne incognita*	*Nicotiana attenuate*	**0**	fecundity	Kaplan et al. 2011
Brevicoryne brassicae	*Heterodera schachtii*	*Arabidopsis thaliana*	**-**	population density[b]	Kutyniok and Müller 2012
			-/+[c]		Kutyniok and Müller 2013
Brevicoryne brassicae	*Heterodera schachtii*	*Brassica oleracea*	**0**	population density	Gera Hol et al. 2013
Myzus persicae	*Tylenchorhynchus spp.*	*Nicotiniana attenuata*	**0**	reproduction factor	Kaplan et al. 2009
Myzus persicae	*Helicotylenchus spp.*	*Nicotiniana attenuata*	**0**	reproduction factor	Kaplan et al. 2009

[a] Plants were exposed to *Aphis glycines* and the biotrophic fungus *Cadophora gregata* which infects the plant's vascular tissue.
[b] Density of *H. schachtii* was assessed three days after plants were infested with *H. schachtii* and *B. brassicae*.
[c] Dependent on the fertilization level of plants, with positive outcomes at high nitrate level and negative effects at low nitrate level.

CHAPTER 2. MEASURING THE BENEFIT OF BIOLOGICAL CONTROL FOR SINGLE GENE AND PYRAMIDED HOST PLANT RESISTANCE FOR SOYBEAN APHID, *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) MANAGEMENT

A paper published in the Journal of Economic Entomology 105: 1835-1843.

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Abstract

The soybean aphid is an economically important pest in the North Central United States. In the state of Iowa, economically damaging populations occurred in seven of eleven growing seasons from 2001 to 2011. The high frequency and economic impact of the soybean aphid makes it an ideal candidate for management using host plant resistance. We compared an aphid susceptible line to near-isolines that contain *Rag1* and *Rag2*, both alone and pyramided together, to suppress aphid populations and protect yield. Each of four near-isolines, was artificially infested with aphids and grown in small plots in which the exposure to natural enemies was controlled by the use of cages, resulting in following treatment groups: Natural Enemy Free (only aphids), Biocontrol (both aphids and natural enemies), and Aphid Free (no aphids or natural enemies). The seasonal accumulation of aphids and the population growth rates were measured for each line and an estimate of yield was measured at the end of the season. Soybean aphid population growth rate was

reduced 20% by natural enemies alone, 44% by pyramided resistance and 63% by the combination of natural enemies and pyramided resistance. This reduction in population growth rate resulted in a 99.3% reduction in the pyramid line's seasonal exposure to aphids. In the presence of natural enemies, all three resistant lines maintained aphid populations below the economic injury level and prevented yield loss. This study demonstrates the compatibility of biological control with soybean aphid host plant resistance and its utility, especially for single resistance gene lines.

Keywords: host plant resistance, gene pyramid, *Aphis glycines*, soybean

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the most economically important pest of soybean in the North Central United States (Ragsdale et al. 2011). From 2003-2017 an estimated $2.3 to $3.7 billion dollars may be lost due to the soybean aphid (Song and Swinton 2009). In the United States, a suite of natural enemies attacks the soybean aphid (Nielsen and Hajek 2005, Schmidt et al. 2008), slowing the growth rate of aphid populations with the potential to prevent populations from reaching economically damaging levels (Fox et al. 2004, Costamagna and Landis 2006, Schmidt et al. 2007). However, the biological control offered by these natural enemies is inconsistent between years and locations. For example, large-scale migrations of aphids into soybeans can rapidly increase aphid populations beyond densities at which natural enemies can suppress aphid population growth (Desneux et al. 2006, Brosius et al. 2007, Schmidt et al. 2010). This can overwhelm the natural enemies present in a soybean field (Rutledge and O'Neil 2005, Desneux and O'Neil 2008). Several other factors can

contribute to outbreaks, including a landscape that does not contribute sufficient amounts of natural enemies to embed soybean fields (Gardiner et al. 2009, Noma et al. 2010).

Due in part to this inconsistent biological control, current management relies heavily on the use of both foliar and seed applied insecticides. Seed-applied insecticides are toxic to soybean aphids but do not prevent outbreaks later in the growing season (McCornack and Ragsdale 2006, Johnson et al. 2009). When insecticides are applied to foliage based on an economic threshold and economic injury level they protect yield (Ragsdale et al. 2007). However, the timing of these applications is critical for farmers to achieve the full economic return of this input (Johnson et al. 2009) and many farmers apply insecticide based on growth stage or calendar dates (Olson et al. 2008), which does not provide optimal economic returns (Johnson et al. 2009).

Host plant resistance offers the potential for inexpensive and effective pest management without negative environmental effects (Pedigo and Rice 2008). To date, this resistance is available commercially primarily as a single gene (*Rag1*) (McCarville et al. 2012). The *Rag1* gene reduces aphid population growth without negatively affecting agronomic factors (Mardorf et al. 2010, Kim and Diers 2009). However, the level of control provided by these cultivars can be inconsistent among locations and years, with economically significant populations capable of developing (Hodgson and VanNostrand 2012, Chiozza et al. 2010). The inconsistent performance of these cultivars may be due to the occurrence of soybean aphid biotypes capable of overcoming the *Rag1* gene (Kim et al. 2008). At least three

other soybean aphid resistance genes have been identified, *Rag2* (Mian et al. 2008), *Rag3* (Zhang et al. 2009), and *rag4* (Zhang et al. 2010). A resistant aphid biotype capable of overcoming the *Rag2* gene has already been identified (Hill et al. 2010).

Host plant resistance may not always be compatible with biological control. Previous studies have found instances in which host plant resistant cultivars can have either direct or indirect negative effects on predators or parasitoids (Kaplan and Thaler 2011, van Emden 1995). In the case of the *Rag1* gene, Lundgren et al. (2009) measured the performance of *Orius insidiousus* Say (Anthocoridae: Hemiptera) and *Harmonia axyridis* Pallas (Coccinelidae: Coleoptera) on resistant and susceptible soybean cultivars in the lab. *Orius insidiousus* and *H. axyridis* feed on plant material in addition to aphids (Armer et al. 1998, Moser and Obrycki 2009). Using *Ephestia kuehniella* eggs as surrogate prey, Lundgren et al. (2009) found no effect of resistant cultivars on *O. insidiosus* nymphs and adults and *H. axyridis* larvae. They did however find reduced longevity and survival for adult *H. axyridis*. Chacon et al. (2012) observed reduced fecundity in *Binodoxys communis* (a classical biological control agent released for soybean aphid management) when developing on soybean aphids feeding on soybean plants with *Rag1*. The overall outcome of the *Rag1* gene and other soybean aphid resistance genes on the soybean aphid natural enemy community and subsequent aphid mortality remains to be determined.

Pyramiding single sources of resistance may improve both the protection conferred by the genes, when compared to a single source of resistance, and contribute to preventing the occurrence of biotypes (Gould 1998). Wiarda et al. (2012) used cages that excluded natural enemies to measure the rate of aphid

population growth on soybeans with the *Rag1* gene, *Rag2* gene, and both genes in combination (i.e. a pyramid). In this setting the pyramid experienced significantly lower aphid populations than the lines with a single resistant gene. It is not clear if suppression of aphid population growth by a pyramid line would be further reduced when aphids experience mortality from natural enemies. Therefore the goal of this study was to examine the interaction of soybean aphid host plant resistance, both single gene and pyramided resistant lines, and biological control and its effect on aphid population suppression and soybean yield protection. We also modeled the effect of a large immigration of soybean aphids on the ability of both host plant resistance and biological control to maintain aphid populations below economically damaging levels.

Materials and Methods

In 2011, we conducted a field experiment at the Iowa State University's Field Extension Education Farm in Boone Co., Iowa, in which soybean lines with varying resistance to the soybean aphid were grown in small plots (micro-plots). These lines were developed by the soybean breeding program at Iowa State University and their development is described by Wiarda et al. (2012). Briefly, the near iso-lines were $BC_1F_{2:6}$ lines derived from the *Rag1* donor A08-123074 and *Rag2* donor LD08-89051a parent lines. The recurrent parent in the backcross was IA3027, an aphid-susceptible line. At the F_2 generation four lines from the same backcross family were selected based on their genotype for the *Rag1* and *Rag2* genes. The genotypes of the four lines selected were *Rag1Rag1Rag2Rag2* (referred to throughout as

pyramid), *Rag1Rag1rag2rag2* (*Rag1*), *rag1rag1Rag2Rag2* (*Rag2*), and *rag1rag1rag2rag2* (susceptible).

These isolines were exposed to varying amounts of soybean aphids in a factorial design comprised of the four soybean lines (pyramid, *Rag1, Rag2*, and susceptible) and four aphid treatments. The four aphid treatments used for this study were (1) soybean plants artificially infested with aphids and exposed to natural enemies (referred to as the Biocontrol treatment), (2) plants infested with aphids but caged to limit exposure to natural enemies (Natural Enemy Free), (3) caged plants infested with aphids and later uncaged after a uniform population was reached (Immigration), and (4) plants kept caged and free of aphids (Aphid Free). All four soybean lines were exposed to all four aphid treatments to create sixteen total treatments (16 treatments with 6 reps each, 96 total plots). Combinations of soybean line and aphid treatment were assigned to microplots arranged in a randomized complete block design with six replications.

These four aphid treatments allowed us to test the following hypotheses. First, that aphid abundance will vary across the four isolines, both in the absence and presence of biological control (i.e. comparing aphid abundance among the four lines in the Biocontrol and Natural Enemy Free treatments). Secondly, that a sudden aphid immigration event can overcome both aphid resistance and biological control (Immigration treatment). Finally, by comparing yield estimates measured in the Biocontrol, Natural Enemy Free, and Immigration treatments to the Aphid Free treatment we can assess the ability of host plant resistance and biological control, alone and together to protect against yield loss due to the soybean aphid.

The four near iso-lines were grown in microplots consisting of a single row 51 cm in length. Twenty-two seeds were sown in each plot on 19 May. After planting, cage frames were placed over all plots. Cage frames were constructed of 2.5 cm diameter thin-walled PVC pipe (Charlotte Pipe, Charlotte, NC). Cage frames measured 1.1 m by 0.8 m by 0.8 m (height x length x width). When plants reached the VC growth stage, each plot was thinned to ten evenly spaced plants.

When the third trifoliate leaf expanded (i.e. V3 stage per Fehr and Caviness 1977) the first three aphid treatments (Natural Enemy Free, Biocontrol, and Immigration) were infested with 10 aphids per plant. Soybean aphids were obtained from a laboratory colony at Iowa State University and were classified as biotype 1 (i.e. avirulent to *Rag1* and *Rag2*) (Kim et al. 2008). All ten plants within a plot were infested by using a paper clip to attach soybean leaf tissue containing 10 mixed-age aphids to the underside of the middle leaflet of the second trifoliate leaf. Plots assigned to the Biocontrol treatment were not enclosed within nets, allowing the access of predators and parasitoid wasps for the entire growing season. This treatment allowed for a measurement of the impact of natural enemies on soybean aphid population growth rates across the four soybean lines.

After infesting, plots assigned the Natural Enemy Free and Immigration treatments were enclosed within nets. Nets were used to exclude predators and parasitoid wasps from these treatments and prevent aphids from immigrating into plots. The Natural Enemy Free treatment allowed for an assessment of the impact of the *Rag1* and *Rag2* host plant resistance genes alone and in combination on the population growth rate of soybean aphids.

The goal of the Immigration treatment was to simulate a large immigration of aphids into a field. This would allow for a measurement of the ability of natural enemies with and without the assistance of host plant resistance to prevent immigration driven outbreaks from occurring. For this purpose, an equal density of aphids was artificially created inside each cage across the four lines. This was accomplished by waiting for aphid populations to reach a density of 100 aphids plant^{-1}. This occurred on 7 July, at which time a second infestation was performed on all cages below 50 aphids plant^{-1}. The second infestation consisted of clipping soybean leaf tissue containing 50 aphids to the underside of the top-most fully expanded trifoliate leaf of each plant within a plot. Soybean aphid populations in the Immigration treatment were then allowed to increase to an average of approximately 250 aphids plant^{-1} (an economic threshold for soybean aphids, see Ragsdale et al. 2007). On 11 July, nets were removed from plots assigned the Immigration treatment. By removing nets we modeled the impact of natural enemies and immigration on soybean aphid populations across the four lines.

The Aphid Free treatment, consisted of plots that were kept free of aphids for the entire season by enclosing plants within the nets to prevent colonization by aphids. Nets were placed over Aphid Free plots at the V3 stage (i.e. the same time as the Natural Enemy Free and Immigration treatments). This treatment allowed for the measurement of the yield potential of each line in the absence of aphid herbivory.

Aphid populations were tracked in all plots throughout the season by counting all aphids (nymphs and adults) on three randomly selected plants in each

plot (i.e. whole plant counts). Counts were conducted twice per week until populations exceeded 1,000 aphids plant^{-1} on the susceptible line in the Natural Enemy Free treatment. Counts were then conducted once per week until populations declined on all four lines in every treatment.

Yield was estimated based on the average seed weight for each plot. This estimate was determined at the end of the season by harvesting all plants within a plot (Fehr 1991, Wiarda et al. 2012). Plants were threshed with a rotary tooth thresher and seed weight and moisture content was measured. The seed weight of each plot was corrected for 13% moisture.

Statistical analysis.

Data were analyzed to address our hypotheses that (1) host plant resistance would reduce aphid populations in both the presence and absence of biological control, (2) biological control with the assistance of host plant resistance can prevent a large, sudden increase in soybean aphid population (i.e. immigration) from reaching economically damaging populations, and (3) host plant resistance and biological control can prevent yield loss from the soybean aphid.

Effect of biological control and host plant resistance. For the first hypothesis, we initially analyzed the impact of biological control and host plant resistance on the seasonal exposure of soybeans to aphids (i.e. cumulative aphid days). For this analysis only plots assigned to the Natural Enemy Free and Biocontrol treatments were used. The Aphid Free treatment was excluded from this analysis because it was kept successfully free of aphids. The Immigration treatment was also excluded because aphid populations in this treatment were manipulated to

have equal densities up to 250 aphids plant^{-1}. Cumulative aphid days were calculated for each plot in the Natural Enemy Free and Biocontrol treatments. Cumulative aphid days are a measure of the season-long aphid pressure experienced by a plant (Hanafi et al. 1989). The effects of soybean line and aphid treatment on CAD were analyzed using a two-way ANOVA (PROC GLM, SAS 2001).

A separate analysis was conducted to determine the ability of host plant resistance to reduce aphid populations in both the absence and presence of biological control at single points in time. Cumulative aphid days were not used in this analysis as it is a measure of the seasonal exposure of plants to aphids and may not account for differences among soybean lines that occur at unique points during the season. For example populations that increase and crash during a short period of time could accumulate the same CAD as a population that builds up more slowly for a longer period of time. Therefore, to further analyze the effect of soybean line, aphid counts were analyzed by date for the Natural Enemy Free and Biocontrol treatments. Aphid counts measured at each sampling date were log transformed to reduce heteroscadascity. The PROC MIXED procedure was used to fit a repeated measures model for this analysis (SAS 2001). The model included the fixed effects of block, soybean line, and the interaction of block and soybean line. The repeated variable in the model was sampling date. Akaike Information Criterion was used to determine a compound symmetry covariance structure provided the best-fit model.

Results from analysis of aphid populations indicated that the aphid populations crashed on the aphid susceptible line, likely due to a reduction in host-plant quality and/or density-dependent effects after 1 August. We analyzed the

effects of host plant resistance and biological control on population growth rates from 27 June through 14 July, the period of time during which aphid populations grew exponentially. Populations were well established in all plots by 27 June and populations were under 1,500 aphids plant^{-1} at 14 July, a population density that has been observed in commercial soybean fields (Hodgson and VanNostrand 2012). Population growth rates were calculated separately for each plot by log transforming the number of aphids per plant and graphing them over the date after infestation. The slope of the line was considered the rate of growth for each plot. The growth rates were analyzed with the main effects of block, soybean line, aphid treatment, and their two-way interactions using PROC GLM (SAS 2001).

Effect of biological control and host plant resistance in Immigration treatment. We compared the aphid populations on the Immigration treatments after cages were removed to test the hypothesis that the combination of biological control and host plant resistance can prevent an outbreak of soybean aphids due to an immigration event. Aphid counts on the four soybean lines in the Immigration treatment were analyzed by date to determine if differences occurred within a sampling period. The same repeated measures model described above for comparing soybean lines within the Predator Free and Biocontrol treatments was used for this analysis of the Immigration treatment.

Ability of biological control and host plant resistance to protect yield. For the third hypothesis yield was compared for each of the four aphid treatments within each soybean line. We estimated yield by measuring the average weight of seed from each plot. Soybean breeding has used this technique to select high

yielding early progeny within small plot conditions (Fehr 1991). Yield data were analyzed using the PROC MIXED procedure (SAS 2001). The model included the fixed effects of block, treatment, soybean line, and the two-way interactions between block, treatment, and soybean line.

Results

Soybean aphids were successfully established on all lines in the Natural Enemy Free, Biocontrol and Immigration treatments. Furthermore, we excluded aphids from plots assigned to the Aphid Free treatment. Soybean aphid natural enemies were regularly observed feeding on aphids in un-caged plots. These natural enemies included spiders and adults and larvae of the families Coccinellidae, Syrphidae, Anthocoridae, Chrysopidae, and mummies belonging to parasitoid wasps in the Aphelinidae family. Members of these families are commonly found within Iowa soybean fields and compose a large percentage of the natural enemy community (Rutledge et al. 2004, Schmidt et al. 2008). Although we did observe ants occasionally tending aphids, this was not a common phenomenon observed throughout the growing season.

Effect of biological control and host plant resistance. We observed a significant effect of both soybean line ($F = 10.90$; df = 1, 15; $P = 0.0005$) and aphid treatment ($F = 89.85$; df = 1, 15; $P < 0.0001$) on plant exposure to aphids (i.e. CAD) for the Natural Enemy Free and Biocontrol treatments. The interaction between aphid treatment and soybean line ($F = 1.92$; df = 3, 15; $P = 0.1701$) was not significant. Regardless of the soybean line, natural enemies significantly decreased plant exposure to aphids. Overall CAD was reduced by an average of 89% across the

four lines in the Biocontrol treatment compared to the Natural Enemy Free treatment (Figure 1).

Despite the strong impact of natural enemies on soybean aphid populations we were able to observe a significant effect of soybean line. Estimate statements using Student's *t*-tests were used to evaluate the effectiveness of the single gene lines compared to the susceptible and pyramid lines. The lines containing a single aphid-resistant gene accumulated significantly fewer CAD compared to the susceptible line ($t = 3.54$; df = 15; $P = 0.0030$). Cumulative aphid days were reduced by 38% compared to the susceptible line. The single gene lines accumulated significantly more CAD than the pyramid line ($t = 2.98$; df = 15; $P = 0.0093$). The pyramid accumulated 82% fewer CAD than the single gene lines, and 89% fewer CAD than the susceptible line.

Soybean aphid populations drastically declined on the susceptible line after 1 August (Table 1), two to three weeks prior to populations declining on lines containing a single aphid-resistance gene. Therefore, the CAD analysis indicating no significant differences among the susceptible and single gene lines within the Natural Enemy Free treatment (Figure 1) could be misleading.

Analysis of aphid populations at individual sampling dates for the Natural Enemy Free treatment showed a significant effect of soybean line ($F = 3.33$; df = 3,15; $P = 0.0483$). The interaction between soybean line and date was highly significant ($F = 20.93$; df = 52,260; $P < 0.0001$). Analyses were then performed by date. We did not observe a significant difference in aphid populations among any of the soybean lines during the first two sampling dates (Table 1). After 15 d post

infestation (8 July), there was a significant effect of soybean line. Soybean line significantly affected aphid populations for the remainder of our sampling period.

Analysis of aphid counts for the Biocontrol treatment showed a significant effect of soybean line ($F = 7.59$; df = 3, 15; $P = 0.0026$). The interaction between soybean line and date was highly significant ($F = 12.98$; df = 52, 260; $P < 0.0001$). Analyses were then performed by date with the effect of soybean line assessed using least squares means (Table 2). We did not observe a significant difference in aphid populations among the four lines until 18 d after infestation. We observed significant differences in aphid densities from 18 d after infestation through the final sampling date.

Analysis of growth rates indicated that both soybean line ($F = 14.04$; df = 3, 47; $P = 0.0001$) and aphid treatment ($F = 64.80$; df =1, 47; $P < 0.0001$) significantly affected population growth rates. The interaction between soybean line and treatment was non-significant ($F = 1.15$; df = 3, 47; $P = 0.3621$). Across all lines, the Biocontrol treatment reduced population growth rates by an average of 64.8% compared to the Natural Enemy Free treatment. To determine the effect of host plant resistance on soybean aphid growth, the growth rates were compared on each of the four lines in the Natural Enemy Free treatment (Table 3). The highest population growth rate was observed on the susceptible line. The population growth rate was reduced by 37.8% and 43.6% on the *Rag1* and *Rag2* lines, respectively. Population growth was reduced by 59.1% on the pyramid line. The combination of both aphid resistance genes and biological control were able to

reduce soybean aphid population growth by 89.1% (comparison of pyramid line in Biocontrol treatment to susceptible line in Natural Enemy Free treatment).

Effect of biological control and host plant resistance in Immigration treatment. Nets covering plots assigned the Immigration treatment were removed from cages on 11 July. We did not observe a significant difference in aphid populations among the four lines on 8 July ($F = 0.30$; df = 3,23; $P = 0.6042$) and 11 July ($F = 0.66$; df = 3,23; $P = 0.7676$). Therefore, we were successful at reaching a consistent population within cages across the four soybean lines in the Immigration treatment (Table 4). Aphid populations for all sampling dates after 11 July were analyzed. We observed a significant effect of soybean line ($F = 13.19$; df = 3,15; $P = 0.0002$) and a significant interaction between soybean line and sampling date ($F = 11.45$; df = 24,120; $P < 0.0001$). Analysis of soybean aphid populations were then performed individually for each sampling date. Aphid populations did not differ among the four soybean lines until 14 d (25 July) after cages were opened for the Immigration treatment. Aphid populations were significantly greater on the susceptible line than the *Rag2* and pyramid lines for the remaining five sampling dates (29 day span). The *Rag1* line had significantly fewer aphids than the susceptible line for three out of five sampling dates, but significantly greater aphids than the *Rag2* and pyramid lines for three out of five sampling dates (Table 4).

Ability of biological control and host plant resistance to protect yield. There was a significant effect of both aphid treatment ($F = 42.98$; df = 3, 44; $P < 0.0001$) and soybean line ($F = 23.30$; df = 3, 44; $P < 0.0001$) on yield. The interaction of aphid treatment by soybean line ($F = 5.18$; df = 9, 44; $P < 0.0001$) was also

significant. Due to the interaction between aphid treatment and soybean line, further analyses were performed by soybean line to determine if the yield varied by aphid treatment within each line. Aphid treatment significantly affected the yield for the susceptible (F = 25.03; df = 3,15; P < 0.0001), *Rag1* (F = 21.93; df = 3, 14; P < 0.0001), and *Rag2* (F = 10.42; df = 3, 15; P = 0.0006) lines, but did not affect yield for the pyramid line (F = 0.64; df = 3 ,15; P = 0.6033). Least squared means analysis was used to determine differences among aphid treatments within each soybean line (Fig. 2). Compared to the Aphid Free treatment, yield was significantly reduced in the Natural Enemy Free treatment for the susceptible (t = -6.27; df = 15; P < 0.0001), *Rag1* (t = -5.07; df = 14; P = 0.0009), and *Rag2* (t = -4.91; df = 15; P = 0.001) lines. However, yield in the Natural Enemy Free treatment was not significantly reduced for the pyramid line (t = -1.01; df = 15; P = 0.7459). For the Biocontrol and Immigration treatments, yield was not significantly different compared to the Aphid Free treatment for any of the four soybean lines.

The previous yield analysis was performed by soybean line, with the Aphid Free treatment serving as a control within each soybean line. The Aphid Free treatment was grown inside a cage and therefore maybe subjected to cage effects not present in the Biocontrol and Immigration treatments. Therefore yield data for the Biocontrol and Immigration treatments was analyzed by aphid treatment. This allowed for comparisons among soybean lines in the Biocontrol and Immigration treatments. However, no observable effect of soybean line was present for both the Biocontrol (F = 1.79; df = 3,14; P = 0.1949) and Immigration (F = 1.78; df = 3,15; P = 0.1946) treatments.

Discussion

Host plant resistance has the potential to significantly improve current soybean aphid management through reductions in chemical inputs and decreases in the frequency of economic outbreaks of soybean aphids. Before deploying host plant resistance at a large scale, an assessment of the impact of these varieties on the current soybean aphid system is prudent. The goal of our study was to analyze the compatibility of host plant resistance with biological control with an emphasis on the impact of these two sources of mortality on aphid population growth and subsequent effects on plant yield.

The economic injury level (EIL) is the point at which yield loss exceeds the cost of control measures (Stern et al. 1959). The economic injury level for soybean aphids has been estimated at 674 aphids plant^{-1}, when a foliar insecticide application is being considered (Ragsdale et al. 2007). This EIL provides an appropriate measurement for assessing the efficacy of the various treatments in this study. In our study, biological control alone was unable to prevent soybean aphid populations from exceeding the EIL, as evidenced by the high aphid populations on the susceptible line in the Biocontrol treatment. This observation is consistent with field observations and previous studies, which demonstrate that biological control can be insufficient to prevent aphid outbreaks under high aphid pressure conditions (Hodgson and VanNostrand 2012).

Rag genes significantly reduced soybean aphid populations compared to the susceptible line. This was true in both the presence and absence of biological control. However, only the pyramid line was able to maintain populations below the

EIL in the Natural Enemy Free treatment. This observation was mirrored in the yield data. Only the pyramid line was able to prevent significant yield loss in the Natural Enemy Free treatment. This is an important observation for the management and the utility of a pyramid line. The Natural Enemy Free treatment represents a "worst-case scenario" in which aphid populations arrive early in the season (plants were artificially infested in June) and biological control is non-existent. Although these conditions are unlikely to occur in the field, regional variation in the density of natural enemies found within soybean fields has been observed at the landscape level (Gardiner et al. 2009), with lower densities and subsequently less biological control in landscapes dominated by corn and soybean production. Furthermore, Landis et al. (2008) suggested that increased production of corn has reduced the biological control of soybeans aphids. Given the genetic composition of the aphids we employed (i.e. biotype), the pyramid line has the potential to effectively maintain aphid populations below the EIL when biological control is non-existent (i.e. soybeans grown in a cage).

Currently farmers are able to purchase soybeans with a single gene for aphid resistance. Since the single gene resistant lines alone were incapable of maintaining aphid populations below the EIL, it will be important for host plant resistance to be compatible with biological control. The single gene lines (i.e. *Rag1* and *Rag2* alone) by themselves were incapable of maintaining aphid populations below the EIL in the Natural Enemy Free treatment. With the addition of the mortality from aphid predators and parasitoids in the Biocontrol treatment, the single gene lines were able to maintain soybean aphids below the EIL. This inconsistent soybean aphid

control by single gene lines is consistent with field observations for the *Rag1* line (O'Neal and Hodgson 2009). This result highlights the important role biological control will play in any integrated pest management program in which single gene resistant lines are used. This conclusion is further emphasized in the Immigration treatment, in which only the resistant lines were able to prevent aphid populations from exceeding the EIL.

We did not observe a difference in yield between the Biocontrol treatment and Immigration treatment across any of the four soybean lines. We anticipated that yield loss would occur when aphid populations exceeded the EIL, as it did on 1 August in both the Biocontrol (Table 2) and Immigration treatment (Table 4). Yield loss may have occurred, but may have been undetected due to a large amount of variability common when soybean yield is estimated from micro-plots (Fehr 1991). We also failed to show a difference in yield for the susceptible line between, either the Biocontrol and Immigration treatments compared to the Aphid Free treatment. This lack of a significant difference could be due to either the variability for a micro-plot study or a potential cage effect. The Aphid Free and Natural Enemy Free treatments were both grown inside mesh cages for the duration of the season

Many of the micro-plots were caged for many weeks and this could affect the growth of the soybeans. In our experiment, a cage effect is best measured by comparing yield in the Aphid Free (grown in a cage) and Biocontrol (grown outside a cage) treatments for the pyramid line. This is an ideal comparison due to the extremely low aphid populations on the pyramid line in the Biocontrol treatment. We were unable to measure a significant difference between the Aphid Free and

Biocontrol treatments for the pyramid line suggesting that any effect of the cage on soybean growth were likely minimal.

Future adoption of aphid resistant soybeans will influence the frequency of virulent biotypes. Ideally, an insect resistance management (IRM) plan should be in place for farmers and agribusiness to limit the occurrence of these biotypes so that the benefits of the *Rag*-genes can be realized for as long as possible (Smith et al. 2004, Bates et al. 2005). One possible component of an IRM plan is the use of a pyramid (Gould 1998, Zhao et al. 2003); to what extent a pyramid alone can limit the development of biotypes is unclear. Several factors will need to be considered, including the frequency of biotypes within the soybean aphid population, the fitness cost of virulence to the aphid, and the impact natural enemies have on the rate at which virulence genes increase in the soybean aphid population. Gould et al. (1991) suggest that natural enemies can, under certain situations, increase the frequency of virulence. Our experiments suggest that with the biotype used within our study, natural enemies reduce the overall population of aphids and the plant's seasonal exposure to aphids equally across both aphid-resistant and susceptible soybean lines. Future research is required to determine if this impact is consistent among various soybean aphid biotypes and how this might affect the creation of an IRM plan for *Rag*-genes.

Host plant resistance holds the potential to increase the efficiency and effectiveness of soybean aphid management. The research presented in this paper demonstrates both the compatibility and importance of biological control for host plant resistance. This research also reinforces initial findings by Wiarda et al.

(2012) on the increased efficacy obtained by pyramiding *Rag1* and *Rag2* together in a single soybean line. Future research will need to focus on the performance of a pyramid line across the larger North Central region where it can potentially be exposed to naturally occurring resistant soybean aphid biotypes.

Acknowledgements

We would like to thank Dr. Walter Fehr and his laboratory for developing the soybean lines used for this project. We also thank Brent Pringnitz for preparing and maintaining the field site. This research was funded in part by the Iowa Soybean Association and Soybean Checkoff.

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Table 1. Soybean aphid populations on four lines in the absence of natural enemies

Soybean Line[c]	24 Jun[b]	30 Jun	8 Jul	14 Jul	18 Jul	25 Jul	1 Aug	8 Aug	17 Aug	23 Aug
Susceptible	7[c] a[d]	19 a	276 a	1,456 a	3,011 a	5,004 a	5,929 a	423 b	97 b	357 b
Rag1	4 a	7 a	42 b	177 b	354 b	857 b	2,333 b	3,702 a	2,616 a	1,340 ab
Rag2	11 a	59 a	171 b	478 b	540 b	871 b	1,313 b	1,863 a	3,124 a	2,572 a
Pyramid	7 a	9 a	29 b	64 b	167 b	204 b	352 c	318 b	422 b	571 ab

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes, either alone or combined.
[b] Aphid predators were excluded from plots using no-see-um mesh fabric (i.e. Natural Enemy Free treatment).
[c]Number of aphids per plant averaged from six plots and three plants per plot.
[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

Table 2. Soybean aphid populations on four lines in the presence of natural enemies

Soybean Line[a]	24 Jun[b]	30 Jun	8 Jul	14 Jul	18 Jul	25 Jul	1 Aug	8 Aug	17 Aug	23 Aug
Susceptible	5[c] a[d]	3 ab	16 a	31 a	90 a	359 a	891 a	521 a	202 a	107 a
Rag1	2 a	1 b	3 b	5 bc	15 b	13 b	29 b	16 b	34 b	14 a
Rag2	4 a	4 a	6 ab	13 b	30 b	42 b	109 b	113 ab	305 ab	59 a
Pyramid	4 a	2 ab	3 b	3 c	9 b	14 b	25 b	15 b	14 b	51 a

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes, either alone or combined.
[b] Plots were left open to allow predators access to aphid populations (i.e. Biocontrol treatment).
[ac]Number of aphids per plant averaged from six plots and three plants per plot.
[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

Table 3. Soybean aphid population growth rates

Soybean Line[a]	Natural Enemy Free[b]	Biocontrol[c]
Susceptible	0.188[d] ± 0.0128a[e]	0.1495 ± 0.0152bc
Rag1	0.1523 ± 0.019b	0.0753 ± 0.0134c
Rag2	0.1438 ± 0.0187b	0.0794 ± 0.0128c
Pyramid	0.105 ± 0.0190bc	0.0704 ± 0.0105c

[a] Near-isolines selected for combinations of *Rag1* and *Rag2* genes, either alone or combined.
[b] Aphid predators excluded from plots using mesh fabric.
[c] Plots left open allowing predators access to aphid populations.
[d] Growth rate from 27 June to 14 July, reported as an average ± SEM.
[e] Letters represent significant differences at $P < 0.05$.

Table 4. Soybean aphid populations on four lines in the Immigration Treatment

Soybean Line[a]	8 Jul	11 Jul[b]	14 Jul	18 Jul	25 Jul	1 Aug	8 Aug	17 Aug	23 Aug
Susceptible	134[c]a[d]	676a	685a	673a	565a	700a	413a	123a	95a
Rag1	145a	174a	197a	170a	141b	158b	85a	63b	60a
Rag2	173a	172a	235a	176a	70b	78b	10b	15c	6b
Pyramid	170a	198a	217a	167a	80b	43b	11b	15c	11b

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes.
[b] An immigration event was modeled by artificially infesting plants and covering plots with fabric until 11 July. After 11 July plots were opened to allow natural enemies access to aphid populations.
[c] Number of aphids per plant averaged from six plots and three plants per plot.
[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

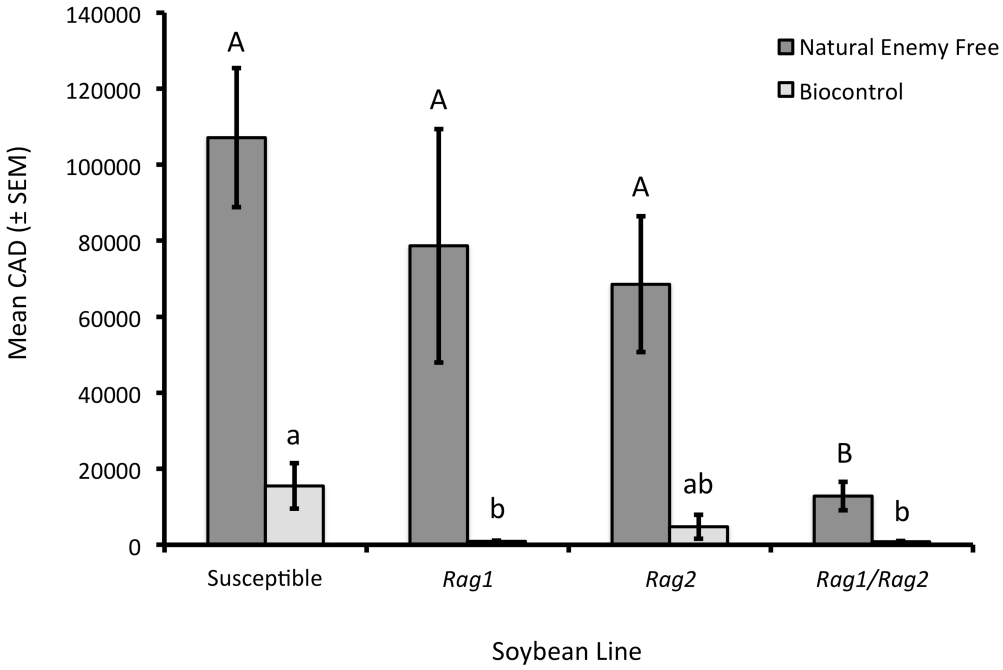

Figure 1. Mean ± SEM cumulative aphid days (CAD) for the four lines exposed to the Natural Enemy Free and Biocontrol treatments. Cumulative aphid days were significantly higher in the Natural Enemy Free treatment compared to the Biocontrol treatment ($P < 0.0001$). Letters represent significant differences at the $P < 0.05$ level among lines within an aphid treatment.

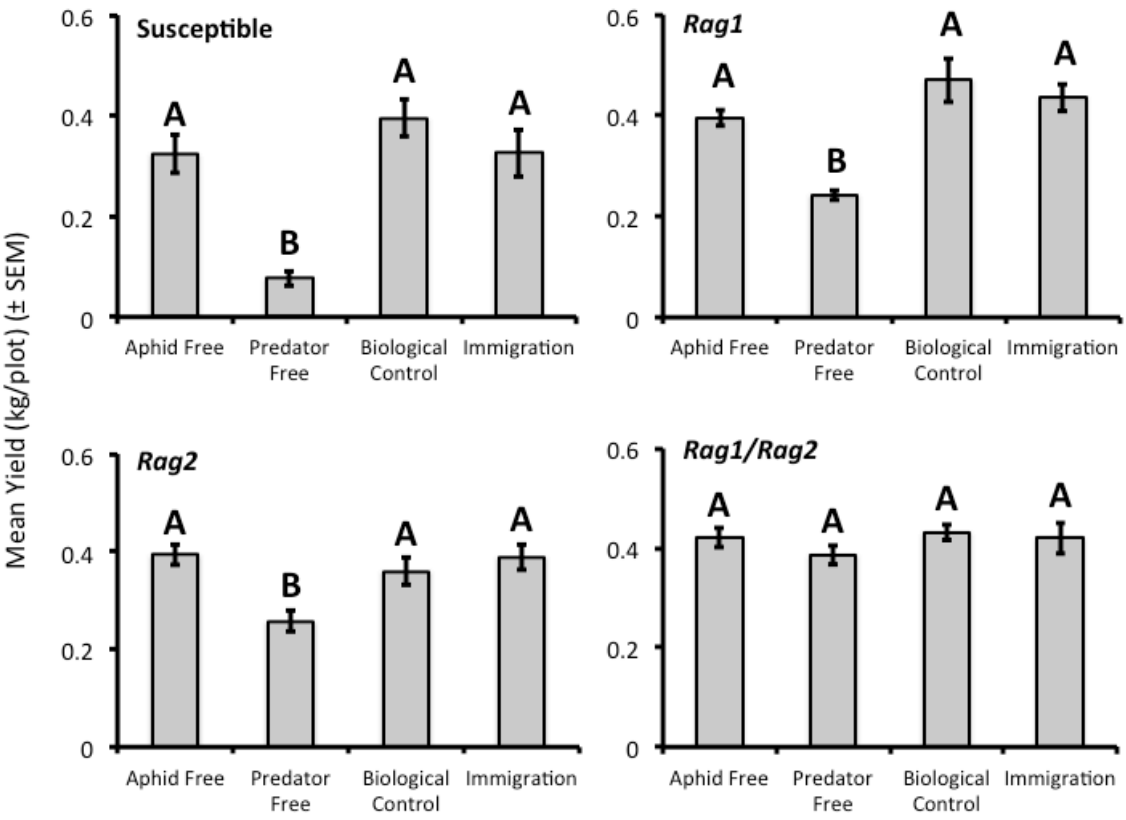

Figure 2. Yields (Mean ± SEM g/plot) of four soybean lines exposed to the four aphid treatments. Yield data was analyzed using a test for least significant differences. Letters signify significant differences at $P < 0.05$.

CHAPTER 3. SOYBEAN APHID (APHIDIDAE: HEMIPTERA) POPULATION GROWTH AS AFFECTED BY HOST PLANT RESISTANCE AND AN INSECTICIDAL SEED TREATMENT

A paper published in the Journal of Economic Entomology 106: 1302-1309.

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Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a significant soybean pest in the north central United States. Insecticidal seed treatments and host plant resistance are two commercially available management tools. Here we investigate the efficacy of both management tools throughout the season. Soybean lines containing the soybean aphid resistance genes *Rag1*, *Rag2*, or both *Rag1* + *Rag2* were compared to a near-isogenic aphid-susceptible line. Each line was grown in field plots both with and without thiamethoxam applied to the seed. Individual plants from each plot were caged and infested with soybean aphids to measure the efficacy and potential interaction of aphid resistance and thiamethoxam. Aphid population growth rate was measured for each caged plant for 9 - 12 d after infestation. New cages were established each week from 34 days after planting (dap) to 92 dap to track seasonal variations in efficacy. Thiamethoxam reduced population growth only at the 42 dap time point and only for the

susceptible, *Rag1*, and *Rag2* lines. The lack of an effect of thiamethoxam on the *Rag1*+*Rag2* line was likely due to already high mortality from two resistance genes. Aphid resistance alone reduced population growth compared to the susceptible line at least til 55 dap for single-gene resistance and 63 dap for the two genes combined. Aphid resistance provided suppression of soybean aphid population growth throughout the season unlike the insecticidal seed treatment.

Keywords: integrated pest management, thiamethoxam, *Aphis glycines*

Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is an invasive pest that causes economic damage to soybean in the north central United States (Ragsdale et al. 2011). Initial management recommendations focused on the use of foliar insecticides to prevent yield losses of up to 47% due to soybean aphid feeding (Ragsdale et al. 2007). Ragsdale et al. (2007) recommended a foliar application of insecticide when aphids reach an economic threshold of 250 aphids plant^{-1}. Johnson et al. (2009) noted that this threshold was more profitable than either the application of insecticides based on plant growth stage or the use of an insecticidal seed treatment. Insecticidal seed treatments have increased in use, especially in Iowa (Hodgson et al. 2012), despite inconsistent impacts on soybean aphid populations (McCornack and Ragsdale 2006, Johnson et al. 2008, Magalhaes et al. 2009). The inconsistent efficacy of insecticidal seed treatments for soybean aphid management is largely due to the seasonal variability in their efficacy (McCornack and Ragsdale 2006, Seagraves and Lundgren 2012) and variability in the timing of soybean aphid migration and field colonization (Hodgson et al. 2005, Schmidt et al.

2012). In general, insecticidal seed treatments provide protection to soybean from insect pests early in the growing season. For soybean aphids, the efficacy of an insecticidal seed treatment is lost ≤55 days after planting (McCornack and Ragsdale 2006), which corresponds to middle to late July for much of the soybean producing area of the north central United States (Pedersen 2004). Soybean aphid outbreaks typically occur in late July and August for this region (Ragsdale et al. 2011). Therefore, insecticidal seed treatments have a limited capacity to protect soybeans from soybean aphid colonization and subsequent population growth.

Host plant resistance may offer season–long protection from soybean aphids. At least four different soybean genes conferring resistance to the soybean aphid have been identified (Hill et al. 2006, Mian et al. 2008b, Zhang et al. 2009, 2010). Currently, commercially available soybean aphid-resistant varieties incorporate a single resistance gene, the *Rag1* gene, or two soybean aphid resistant genes, the *Rag1* gene and the *Rag2* gene (McCarville et al. 2012b). Experiments using artificially infested plants indicate that combining two soybean aphid resistance genes into a single soybean line increases resistance to the soybean aphid (McCarville and O'Neal 2012, Wiarda et al. 2012). Despite the increased resistance displayed by a pyramid line, varieties containing a single resistance gene will likely continue to be grown for at least the near future (McCarville et al. 2013).

Soybean aphids are capable of reaching populations that exceed the economic threshold on varieties containing a single aphid-resistance gene (McCarville and O'Neal 2012, Wiarda et al. 2012). High, potentially economically damaging aphid populations on these varieties could be due to several factors

including but not limited to (1) limited efficacy of these single genes (Wiarda et al. 2012), (2) the presence of virulent aphid biotypes capable of overcoming individual resistance genes (Kim et al. 2008, Hill et al. 2010), (3) declining efficacy of the resistance genes late in the season (Klun and Robinson 1969), or (4) induced susceptibility (Baluch et al. 2012) or density-dependent expression of resistance (i.e. resistance overcome by larger populations of aphids on a plant).

Given the limited efficacy of single gene lines and the presence of resistant biotypes capable of overcoming these genes, there may be a benefit to pairing insecticidal seed treatments with soybean aphid host plant resistance to improve soybean aphid management. Our goal was to measure soybean aphid mortality due to host plant resistance (i.e. *Rag1* and *Rag2*) and an insecticidal seed treatment (i.e. thiamethoxam) both alone and together and to determine if these two sources of mortality provide improved management of the soybean aphid during both vegetative and reproductive stages of soybean development.

Materials and Methods

We conducted our study during 2011 and 2012 in fields located within Story County, Iowa. The four soybean lines used for our experiment were developed using the backcross method (Fehr 1991), in which a desired gene(s) is introduced from the donor parent to the recurrent parent, offspring are backcrossed to the recurrent parent, and the BC_1F_1 progeny are selfed. The desired BC_1F_2 genotypes are selected and lines, which do not differ significantly from the recurrent parent in any desired traits, are bulked to create the new line. Additional details of the development of the lines used in our experiment are outlined in Wiarda et al.

(2012). Briefly, each experimental soybean line was a bulk of 10 $BC_1F_{2:5}$ (2011) or 10 $BC_1F_{2:6}$ (2012) lines derived from a cross of LD08-89051a (a line containing *Rag2*) and the recurrent parent A08-123074 (containing *Rag1*). Therefore, each individual line contains 75% of its genes from the recurrent parent. Plants heterozygous for the *Rag1* and *Rag2* genes were selected at the BC_1F_1 generation. Their progeny were evaluated to select four distinct genotypes: 'Susceptible' (*rag1rag1rag2rag2*), '*Rag1*' (*Rag1Rag1rag2rag2*), '*Rag2*' (*rag1rag1Rag2Rag2*), and '*Rag1* + *Rag2*' (*Rag1Rag1Rag2Rag2*). Ten lines of each genotype were bulked to form the four experimental soybean lines used for the experiment reported here.

We utilized a split-plot design with whole plots arranged in a randomized complete block design with three blocks. The whole plot treatment was one of four soybean lines planted at a rate of 346,000 seeds $hectare^{-1}$ using standard farming practices (i.e. tillage and pre-emergent weed control). Whole plots (soybean lines) were 15.25 m in length and comprised of 12 0.76 m-wide rows. Two treatments were assigned to a whole plot, so that each block consisted of eight split-plots. The two treatments were seed-treated and untreated. Soybean seed of each line assigned to the seed-treated treatment was shipped to Syngenta Seed Care (Stanton, MN) and treated with thiamethoxam (Cruiser 5 FS®, Syngenta Seeds) at a rate of 0.0756 mg/seed. Soybean seed assigned to untreated split-plots were kept free of pesticides and planted as naked seed. Plots were planted on 24 May and 15 May in 2011 and 2012, respectively.

Plants were artificially infested with soybean aphids to measure the efficacy of aphid resistance genes and a seed treatment throughout the course of the

growing season. Un-infested plants were randomly selected within the first and sixth row of each untreated and seed-treated split-plot for each soybean line. The first plants were selected 34 days after planting (dap) when plants reached the early third trifoliate vegetative stage (i.e. V3, per rating scale used by Fehr and Caviness 1977). Each week new plants were selected, caged and infested with aphids. The remaining sets of cages were established at 42 dap (R1 in 2011, V7 in 2012), 48-49 dap (R2 in 2011, R1 in 2012), 55-56 dap (R3 in 2011, R2 in 2012), 62-63 dap (R3 in 2011 and 2012), and 69 dap (R4 in 2011 and R3 in 2012). Cages at 69 dap were deployed only in untreated split-plots because there was no evidence of mortality due to the insecticidal seed treatment in the previous two cage sets (i.e. 55-56 and 62-63 dap). We stopped caging plants when un-infested plants were no longer available. In 2011 we stopped caging plants after 69 dap due to high populations of soybean aphids (>100 aphids per plant with >95% of plants infested on the susceptible line) making it unfeasible to locate non-infested plants within the field. In 2012, populations of aphids were much lower 69 dap (<5 aphids per plant with <5% of plants infested on the susceptible line), therefore, three additional sets of cages were established (77 dap, 84 dap, and 91 dap). As with the 69 dap cage sets in 2011 and 2012, cages were established in only the untreated split-plots due to the ineffectiveness of a seed treatment at prior sampling periods (i.e. 55-56 and 62-63 dap).

For the purpose of these experiments, each cage was considered an experimental unit. Since we visited each split-plot frequently to estimate soybean aphid populations, we varied the location from which plants were selected and

artificially infested to avoid damaging adjacent plants. In 2011, only rows one and six in each split-plot in blocks one and two were used for the first three sets of cages (34 dap, 42 dap, and 49 dap). This was done as plant emergence in block three was delayed and reduced compared to blocks one and two. However, block three was used for the rest of 2011. Rows one and six of blocks one, two, and three were used for the rest of 2011 (55-56 dap and 62-63 dap) and all of 2012. This resulted in fewer sampling points per treatment for the first three time points in 2011 in comparison to all other sampling dates.

Prior to infestation, each plant was inspected for soybean aphids and any aphids found were manually removed. A tomato cage was then placed over each selected plant. Tomato cages were covered with a white no-see-um mesh net (Quest Outfitters, Sarasota, FL), which was buried in the ground and closed at the top. Nets prevented the immigration and emigration of soybean aphids and prevented aphidophagous predators from consuming aphids within the cage (Costamagna et al. 2006). This limited mortality or population growth measured for each infested plant to only the host plant (i.e. its resistance gene(s)) or insecticidal seed treatment.

Plants were artificially infested with soybean aphids from a laboratory colony of biotype-1 soybean aphids (i.e. avirulent to all *Rag* genes) maintained at Iowa State University. Five, mixed-age apterous aphids were manually transferred with a fine camel hairbrush (Winsor & Newton, Piscataway, NJ) to the underside of the uppermost fully expanded trifoliate (McCarville et al. 2012a). Aphid populations inside cages were allowed to increase for 9 to 12 d with populations assessed every

3 to 4 d. Aphid populations were assessed by opening nets and counting all aphids (both nymphs and adults) on each caged plant.

Statistical Analyses.

The abundance of soybean aphids on caged plants was used to estimate the efficacy over time of both thiamethoxam and the aphid resistant lines. Aphid counts for each caged plant were log transformed and graphed over time (days after infestation). A linear regression was performed to estimate the slope of the line, which was considered the rate of population growth for each aphid population (i.e. each cage) (Ragsdale et al. 2007). The rate of population growth was analyzed using an analysis of variance (SAS, PROC Mixed, Cary, NC). Population growth data were analyzed to address two questions, (1) does the impact of the seed treatment on soybean aphid populations vary across the four soybean lines at any point in time and (2) does the efficacy impact of either the *Rag1* or *Rag2* genes (alone or combined) on soybean aphid populations vary across the season. To address the first question, each time set of cages (i.e. 34 dap or 42 dap) was analyzed separately to determine the effect of an insecticidal seed treatment on aphid populations across the four soybean lines. A mixed effects model was used, in which a significant seed treatment*soybean line interaction would indicate that the impact of the seed treatment varied among the four lines. The model included the main effects of year, soybean line, and seed treatment and the interactions of year*soybean line, seed treatment*soybean line, year*seed treatment and year*soybean line*seed treatment. The model also included the random factors of block, year*block, cage*soybean line, and year*block*soybean line.

A second analysis was conducted to assess the seasonal efficacy of the *Rag1* and *Rag2* genes. For this second analysis, only data from the untreated split-plots was analyzed to assess if either the *Rag1* or *Rag2* gene varied in efficacy across the growing season. These data were analyzed in two steps to account for variation in the number of cage sets (i.e. time points) between 2011 and 2012; in both steps a significant effect of soybean line would indicate aphid population growth rate differed among the soybean lines. If the effect of soybean line is not consistent across all time points, this would indicate variability in the seasonal efficacy of one or more of the *Rag* genes. In the first step of this analysis, data from all time points replicated in both 2011 and 2012 were analyzed in a mixed effects model which included the fixed effects of year, dap, soybean line and the interactions year*dap, year*soybean line, dap*soybean line, and year*dap*soybean line. The model also included the random effect block and the interaction terms year*block, soybean line*block, dap*block, year*dap*block, year*soybean line*block, and dap*soybean line*block. In total this analysis included all measurements taken prior to 77 dap.

In the second step, data from 2012 were analyzed separately. In this analysis data from all cage sets in 2012 including 77 dap, 84 dap, and 92 dap were analyzed. The model included the main effects of soybean line, dap and the interaction of soybean line*dap. The model also included the random effects of cage, and the interactions of block*soybean line and block*dap.

Results

In both 2011 and 2012, despite using a population of soybean aphid comprised of biotype-1 (i.e. avirulent to both *Rag1* and *Rag2*) we were able to

maintain soybean aphid populations on the aphid susceptible and aphid resistant soybean lines. The average aphid populations 9-12 days after receiving five aphids for the untreated split-plots (averaged across all time points and both years of the study) were 62.6, 7.9, 3.8, and 0.6 aphids plant^{-1} for the susceptible, *Rag1*, *Rag2*, and *Rag1* + *Rag2* lines, respectively. These results are consistent with previous studies demonstrating biotype-1 soybean aphids as capable of surviving on both the *Rag1* and *Rag2* lines, though with a reduction in fecundity and survival (Wiarda et al. 2012, McCarville and O'Neal 2012).

Although we consistently observed positive population growth rates in the untreated split-plot of the susceptible line, we did observe negative growth rates for the untreated split-plot of the susceptible line at 34, 70, and 84 dap in 2012. During these periods of 2012, we experienced high temperatures (61% of daily high temperatures for these time points were above the soybean aphid's developmental optimum) and dry conditions, which are not conducive for soybean aphid development (McCornack et al. 2004, Ragsdale et al. 2011). This observation is consistent with the extremely low aphid populations observed throughout Iowa during 2012 (Hodgson and VanNostrand 2012).

Interaction of insecticidal seed treatments and host plant resistance.

At all time points (i.e. dap) soybean line significantly affected aphid population growth (Table 1). Although the effect of year and the interaction of year*soybean line was occasionally significant, the interactions of year*seed treatment and year*soybean line*seed treatment did not significantly affect soybean aphid populations. Only during the 42 dap time point did the seed treatment

significantly affect aphid population growth (F = 33.75; df = 1, 30; P < 0.0001) (Figure 1). Also, only during this time point did the interaction of soybean line*seed treatment significantly affect aphid populations (F = 4.68; df = 3, 30; P = 0.0085). This interaction is observed in the reduction in aphid population growth rates by a seed treatment on *Rag1* (F = 8.00; df = 1,7; P = 0.0255), *Rag2* (F = 10.91; df = 1, 8; P = 0.0108), and susceptible (F = 16.31; df = 1, 8; P = 0.0037) lines, but not on the *Rag1+Rag2* line (F = 2.71; df = 1, 7; P = 0.1438).

Seasonal efficacy of Rag genes.

Aphid population growth on untreated soybean varied by year (F =36.01; df = 1, 3; P = 0.0093) and soybean line (F = 45.39; df = 3, 9; P <0.0001) for time points replicated in both 2011 and 2012 (i.e. 34 dap through 69 dap). In addition to the main effects, the interactions of year*soybean line (F = 7.14; df = 3, 9; P = 0.0094), and year*dap*soybean line (F = 2.03; df = 15, 42; P = 0.0365) both significantly affected aphid population growth. The main effect of dap (F = 2.32; df = 5, 15; P = 0.0947) and the interactions of year*dap (F = 2.26; df = 5, 15; P = 0.101) did not significantly affect aphid populations. The interaction of dap*soybean line (F = 1.75; df = 15, 86; P = 0.0551) had a marginally significant impact on soybean aphid populations. The significant interaction of year*dap*soybean line indicated that there may have been variability in the efficacy of one or more of the *Rag* genes between the two years of our study. Therefore, data were analyzed separately for 2011 and 2012.

In 2011, the factor of soybean line (F = 27.88; df = 3, 15; P <0.0001) significantly affected soybean aphid growth rates (Figure 2). We observed

consistently higher population growth rates for aphids on the susceptible line compared to the three aphid-resistant lines. We did not observe a significant effect of plant growth stage (as defined by differences in dap) on aphid population growth rates (F = 2.09; df = 5, 59; P = 0.1114), however the interaction of dap*soybean line (F = 1.85; df = 15, 53; P =0.0518) was marginally significant indicating possible variations in the efficacy of one or more of the *Rag* genes. Data were then analyzed by soybean line to determine if soybean aphid population growth varied across the season by soybean line. Soybean aphid growth rates did not vary significantly across the 6 different points in time for either the *Rag1*+*Rag2* line (F = 0.86; df = 5, 18; P = 0.5282) or the susceptible line (F = 1.24; df = 5, 18; P = 0.3333). Days after planting however, significantly affected soybean aphid growth rates on the *Rag2* line (F = 2.84; df = 15, 18; P =0.0461) and had a marginally significant effect on the *Rag1* line (F = 2.66; df = 15, 18; P = 0.0570), thus indicating potential temporal variability in the efficacy of both the *Rag1* and *Rag2* genes.

In 2012, the effect of soybean line (F = 23.50; df = 3, 15; P <0.0001) again significantly impacted soybean aphid population growth rate (Figure 3). The main effects of dap (F = 2.13; df = 5, 25; P = 0.0949) and the interaction of dap*soybean line (F = 1.79; df = 15, 75; P =0.0512) were marginally significant. Soybean aphid growth rates did not vary significantly over time for either the *Rag1* line (F = 1.07; df = 5, 25; P = 0.4020), *Rag2* line (F = 1.82; df = 5, 25; P = 0.1455) or the susceptible line (F = 1.98; df = 5, 25; P = 0.1166). Days after planting however, did significantly impact soybean aphid growth rates on the *Rag1*+*Rag2* line (F = 2.90; df = 15, 25; P

=0.0339), thus indicating potential temporal variability in the efficacy of the *Rag1* + *Rag2* line.

The 2012 data were then analyzed again, this time including the last three time points (77 dap, 84 dap, and 92 dap). When analyzing the complete data set, the effect of soybean line (F = 28.56; df = 3, 15; P < 0.0001) was still found to significantly impact soybean aphid growth rates (Figure 3). However, soybean aphid growth rates did not vary significantly for the main effects of dap (F = 1.51; df = 8, 40; P = 0.1851) and the interaction of dap*soybean line (F = 1.08; df = 24, 120; P = 0.3723).

Discussion

In this study we measured variability in the temporal efficacy and potential interaction of two soybean aphid mortality factors, an insecticidal seed treatment and the soybean aphid resistance genes *Rag1* and *Rag2*. In previous studies, McCornack and Ragsdale (2006) and Seagraves and Lundgren (2012) both investigated the efficacy of insecticidal seed treatments against soybean aphids at different time points during the season. While previous studies have successfully used soybeans at both vegetative growth stages (Li et al. 2007, Mian et al. 2008a, Hesler et al. 2012) and reproductive stages (Wiarda et al. 2012) to evaluate resistance, we are unaware of any previous experiments investigating the temporal efficacy of soybean aphid resistance genes across the growing season or their interaction over time with insecticidal seed treatments. In our study we found both insecticidal seed treatments and soybean aphid host plant resistance to significantly decrease soybean aphid populations at one or more times during the season.

Previous studies investigating thiamethoxam seed treatments against soybean aphids have used different methods to measure the efficacy of the seed treatment over varying lengths of time. McCornack and Ragsdale (2006) measured survival and nymph production over a 48-hour period in both detached leaf and field assays, Seagraves and Lundgren (2012) measured aphids present after 7 d in a detached leaf assay, while we measured population growth over a 10-12 d period in the field. Despite differences in assay methods and durations, similar efficacy results were obtained across the three studies. McCornack and Ragsdale (2006) observed soybean aphid mortality due to a thiamethoxam seed treatment was low during early vegetative stages (i.e. 28 dap), peaked at 35 dap, declined by 48 dap, and was absent by 55 dap. Seagraves and Lundgren (2012) found thiamethoxam and imidacloprid seed treatments increased aphid mortality from 26 dap to 40 dap, but had no effect by 46 dap. In our study, the thiamethoxam seed treatment significantly reduced population growth at only one time period (42 dap). In our experiment the peak for seed treatment efficacy is encompassed in the 42 dap time point in which aphids were placed on soybean plants 42 dap and population growth was tracked for 12 days (i.e. to 54 dap). This corresponds to McCornack and Ragsdale's 42 and 49 dap time points and Seagraves and Lundgren's 42 dap time point. Combined these studies suggest that the efficacy of thiamethoxam is low early in the season (i.e. 28 dap) and peaks at 42 to 48 dap before decreasing, indicating these observations are robust to a diversity of environmental and experimental variables.

In examining the interaction between thiamethoxam and aphid-resistance we found evidence for unequal effects of the seed treatment across the four soybean lines. At 42 dap, when the seed treatment significantly reduced soybean aphid growth rates on the susceptible line, we were unable to measure any impact of the seed treatment on soybean aphid populations on the *Rag1+Rag2* pyramid. The lack of an effect of the seed treatment on the *Rag1+Rag2* line was likely due to the design of our assay and the already high efficacy of the pyramid line, which resulted in almost complete soybean aphid mortality from the pyramid line in the absence of a seed treatment. Aphid population growth however was still significantly reduced on both single gene lines. This result suggests that while insecticidal seed treatments may provide protection to soybean aphid-susceptible varieties, and also soybean aphid-resistant varieties carrying a single resistance gene, they are likely unnecessary at this time for varieties with multiple resistance genes. This conclusion may not be valid in the future if with increased use of *Rag* genes, the frequency of virulent soybean aphid biotypes increase in the environment.

As for aphid resistance, we observed significantly reduced population growth compared to the susceptible line that extended to at least 55 dap for single-gene resistance and 63 dap for the two genes combined. In addition, at only three sampling periods between 2011 and 2012 was there a positive rate of population growth measured on a resistant soybean line (42 dap, 62 dap, and 69 dap in 2011) and at none of these three sampling points was there a positive rate of growth on the *Rag1+Rag2* pyramid line. However, in both 2011 and 2012, we observed temporal variability in the performance of at least one resistant line. In 2011, both

the *Rag1* and *Rag2* lines displayed significant temporal variability in soybean aphid population growth rates, while in 2012 the *Rag1*+*Rag2* pyramid displayed temporal variability. In neither year did soybean aphid population growth rates significantly vary with time on the susceptible line, indicating that the variations observed on the resistant lines occurred independently of overall host plant quality. In 2011, the variation in efficacy for the *Rag1* line and *Rag2* line was due to elevated soybean aphid growth rates at the 63 dap (*Rag1* and *Rag2*) and 70 dap (*Rag2*) time points. In 2012, the variation in efficacy for the *Rag1*+*Rag2* pyramid was due to exceedingly high rates of soybean aphid mortality at the 42 dap, 63 and 70 dap time points. Combined the two years of this study suggests there is little seasonal variation in resistance expression for either the *Rag1* or *Rag2* genes.

As a whole this study shows soybean aphid-resistance to provide greater and more consistent soybean aphid control throughout the growing season when compared to an insecticidal seed treatment. These results also suggest insecticidal seed treatments could be used in addition to single gene resistance to improve early season soybean aphid control. The management tactic of pairing host plant resistance with insecticidal seed treatments may have unintended consequences though. Seagraves and Lundgren (2012) showed thiamethoxam seed treatments can reduce the overall population of generalist predators in the field. McCarville and O'Neal (2012) demonstrated the large impact pyramided resistance has on plant exposure to aphids when paired with biological control. In their experiment the soybean plant's seasonal exposure to aphids was reduced by over 99% when soybean aphids were exposed to a *Rag1*+*Rag2* pyramid and natural enemies

compared to a natural enemy free susceptible plant. Therefore, care should be taken when pairing insecticidal seed treatments with soybean aphid host plant resistance.

How the reductions in soybean aphid population growth we measured affect soybean aphid abundance in the field specifically related to the current economic threshold and economic injury level will depend on factors such as soybean aphid arrival time and immigration rates, the occurrence of virulent biotypes, and natural enemy populations. All of these factors are likely to vary among locations and years within the north central United States; therefore a multi-location regional field study is warranted to investigate the effects of both host plant resistance and insecticidal seed treatments on soybean aphid abundance.

Acknowledgements

We thank Kent Burns for assistance with planting and field preparation. We also thank Syngenta for applications of thiamethoxam seed treatments. This research was funded by the Iowa Soybean Association, the North Central Soybean Research Program and Soybean checkoff.

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Table 1. Seasonal effect of insecticidal seed treatment on aphid populations

Effect	df	F statistic	*P*-value
34 days after planting			
year	1,3	4.55	0.1225
soybean line	3,9	23.84	0.0001*
year*soybean line	3,9	6.52	0.0123*
seed treatment	1,32	0.02	0.9009
soybean line*seed treatment	3,32	0.02	0.9946
year*seed treatment	1,32	2.47	0.1258
year*line*seed treatment	3,32	1.16	0.3416
42 days after planting			
year	1,3	3.36	0.1640
soybean line	3,9	11.24	0.0021*
year*soybean line	3,9	0.80	0.5250
seed treatment	1,30	33.75	<0.0001*
soybean line*seed treatment	3,30	4.68	0.0085*
year*seed treatment	1,30	1.76	0.1945
year*line*seed treatment	3,30	0.37	0.7781
48-49 days after planting			
year	1,3	14.70	0.0313*
soybean line	3,9	28.73	<0.0001*
year*soybean line	3,9	8.31	0.0058*
seed treatment	1,32	0.02	0.8876
soybean line*seed treatment	3,32	1.00	0.4052
year*seed treatment	1,32	0.04	0.8472
year*line*seed treatment	3,32	2.07	0.1235
55-56 days after planting			
year	1,3	5.27	0.1054
soybean line	3,8	17.46	0.0007*
year*soybean line	3,8	0.55	0.6618
seed treatment	1,34	0.06	0.8109
soybean line*seed treatment	3,34	0.36	0.7842
year*seed treatment	1,34	0.16	0.6908
year*line*seed treatment	3,34	0.04	0.9883
62-63 days after planting			
year	1,3	14.02	0.0332*
soybean line	3,9	7.86	0.0069*
year*soybean line	3,9	0.87	0.4907
seed treatment	1,40	2.94	0.0940
soybean line*seed treatment	3,40	1.33	0.2792
year*seed treatment	1,40	2.45	0.1254
year*line*seed treatment	3,40	0.76	0.5231

* Effect significantly impacted soybean aphid population growth at $P<0.05$.

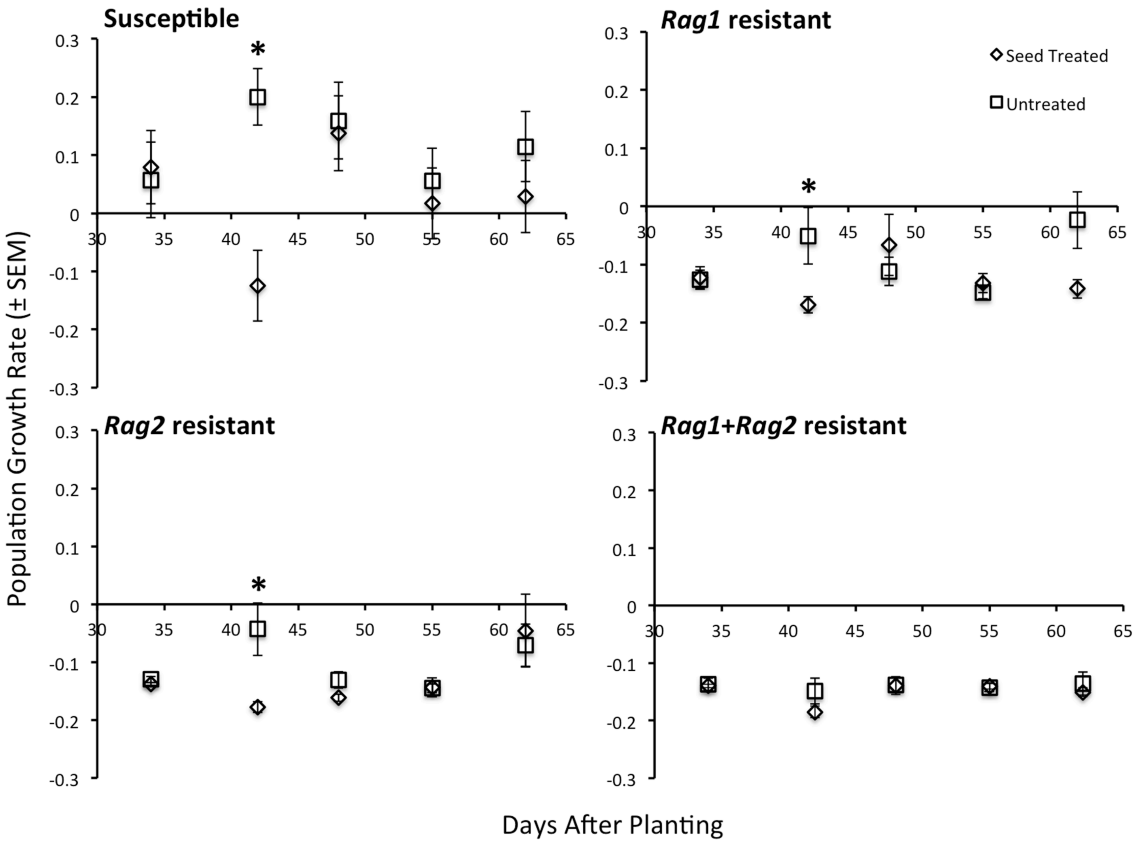

Figure 1. Soybean aphid population growth rates from both 2011 and 2012 as affected by a thiamethoxam seed treatment over the course of the growing season on four soybean lines. Soybean lines were near-isolines including a soybean aphid susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34 days after planting through 62 days after planting and population growth was tracked for nine to twelve days after infestation. Asterisks indicate significant differences ($P < 0.05$) between treatments within a soybean line.

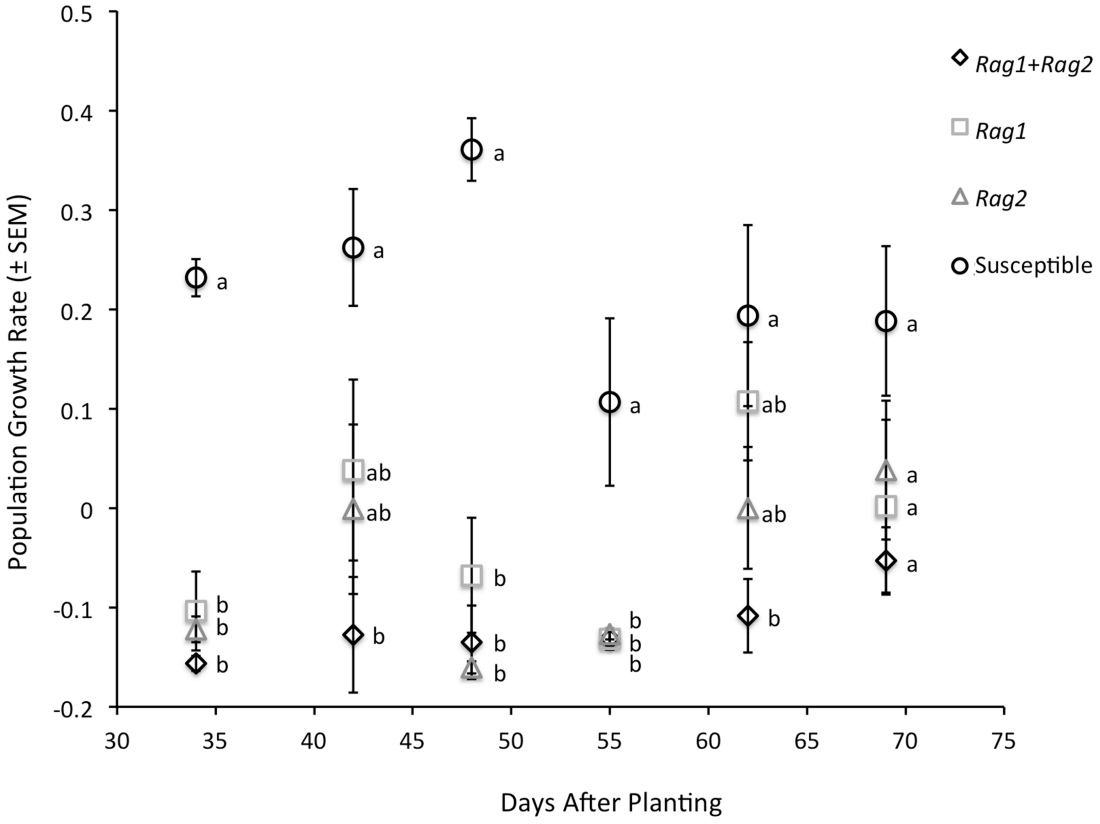

Figure 2. Soybean aphid population growth rates in 2011 as affected by four near-isoline soybean lines, a susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34 days after planting through 69 days after planting and population growth was tracked for nine to twelve days after infestation. Letters indicate significant differences ($P < 0.05$) among soybean lines within a single sampling point.

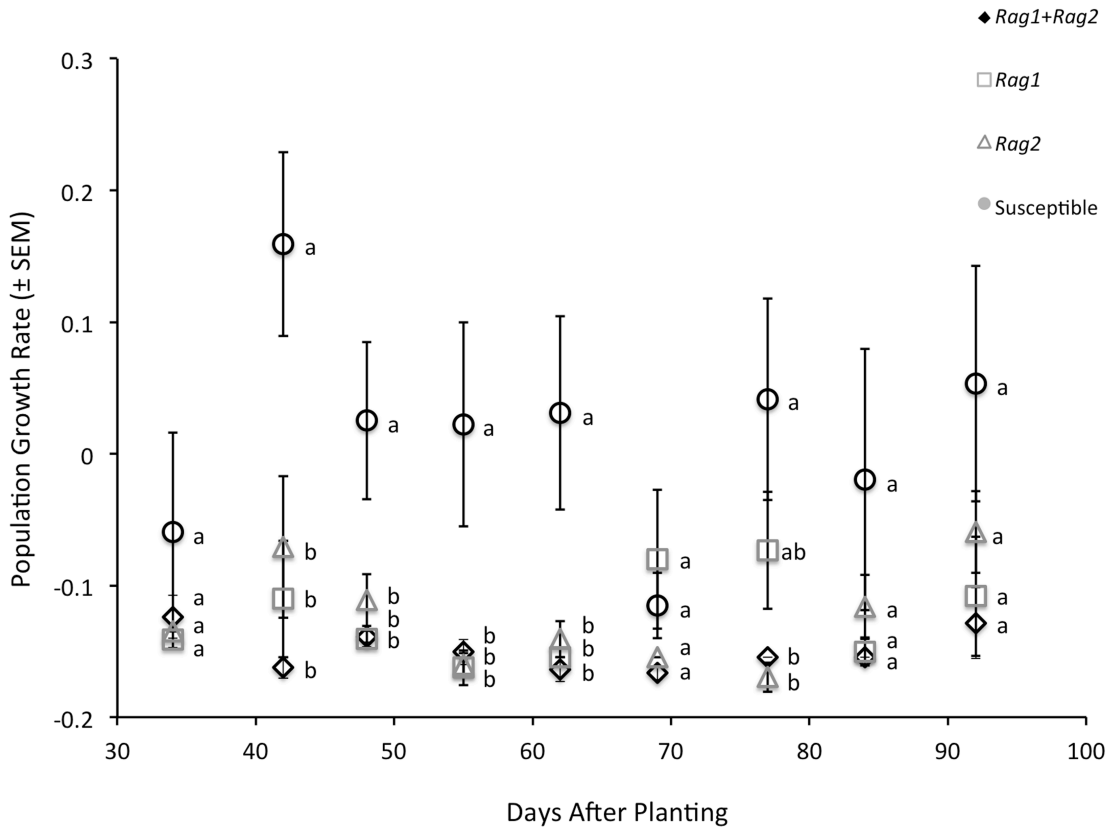

Figure 3. Soybean aphid population growth rates in 2012 as affected by four near-isoline soybean lines, a susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34 days after planting through 69 days after planting and population growth was tracked for nine to twelve days after infestation. Letters indicate significant differences ($P < 0.05$) among soybean lines within a single sampling point.

CHAPTER 4. ONE GENE VERSUS TWO: A REGIONAL STUDY ON THE EFFICACY OF SINGLE GENE VERSUS PYRAMIDED RESISTANCE FOR SOYBEAN APHID MANAGEMENT

A paper submitted to the Journal of Economic Entomology.

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Abstract

The soybean aphid (*Aphis glycines* Matsumura) is a threat to soybean production in the Midwestern United States. Varieties containing the *Rag1* soybean aphid resistance gene have been released with limited success in reducing aphid populations. Furthermore, virulent biotypes occur within North America and challenge the durability of single-gene resistance. Pyramiding resistance genes has the potential to improve aphid population suppression and improve resistance gene durability. Our goal was to determine if a pyramid could provide increased aphid

population suppression across a wide range of environments. We conducted a small-plot field experiment across seven states and three years. We compared soybean near-isolines for the *Rag1* or *Rag2* gene, and a pyramid line containing both genes to reduce plant exposure to aphids and protect yield compared to a susceptible line. These lines were evaluated both with and without a neonicitinoid seed treatment. All aphid-resistant lines significantly decreased plant exposure to aphids at all locations but one. The pyramid line experienced less exposure to aphids than both single gene lines at eight of 23 location-years. Soybean aphids significantly reduced soybean yield for the susceptible line and for both single gene lines, however no significant yield decrease was observed for the pyramid line. The neonicitinoid seed treatment reduced plant exposure to aphids across all soybean lines, but did not provide significant yield protection for any of the lines. These results demonstrate that pyramiding resistance genes can provide sufficient and consistent yield protection from soybean aphid in North America.

Keywords: host-plant resistance, integrated pest management, IPM

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an economically damaging, invasive pest throughout the North Central United States and southern Canada (Ragsdale et al. 2011). Since its discovery in Wisconsin in 2000, yield losses of up to 40% have been documented in the field (Ragsdale et al. 2007). Insecticide-based management options include neonicitinoid seed treatments and foliar insecticides (Hodgson et al. 2012). Applying foliar insecticides according to an economic threshold is the most profitable of these strategies,

however, this approach only provided farmers a 69 to 85% chance of at least recovering the cost of the insecticide application (Johnson et al. 2009).

As an alternative to insecticide applications, several research groups are exploring host-plant resistance for soybean aphid management. At least three soybean aphid resistance genes have been identified, with eight resistance genes proposed to date (Hill et al. 2012, Hesler et al. 2013). The *Rag1* and *Rag2* genes (both alone and together) appear to have no detrimental effects on agronomic performance (Kim and Diers 2009, Mardorf et al. 2010, Brace and Fehr 2012). The efficacy of these two genes was investigated in field cages with avirulent aphid populations (biotypes 1); alone, the two genes provided equivalent levels of population suppression, while plants containing both genes provided significantly greater population suppression, both in the absence (Wiarda et al. 2012) and presence of biological control (McCarville and O'Neal 2012). Economically damaging soybean aphid populations developed on both of the single gene lines in field cages when predators were excluded (McCarville and O'Neal 2012). However, even in the absence of predators, soybean aphid populations remained below economically damaging levels on the pyramid line.

Multiple field studies have investigated the efficacy of plant introduction lines and experimental lines carrying one or more of the *Rag* genes (reviewed by Hill et al. 2012 and Hesler et al. 2013). In these studies, researchers found virulent populations of soybean aphids that were capable of overcoming either the *Rag1* gene (biotype-2), the *Rag2* gene (biotype-3), or both genes (biotype-4) (Kim et al. 2008, Hill et al. 2010, Alt and Ryan-Mahmutagic 2013). Notably, these virulent

biotypes existed prior to the commercial release or large scale planting of any of the *Rag* genes and therefore, were not selected in response to the use of *Rag* genes. It is still unclear how prevalent these virulent biotypes are in the environment or how they will influence soybean aphid management that relies on *Rag* genes. Early experimental information estimates the incidence of biotype-2 could range from 0 to 40% for a given field, with regional incidence possibly as high as 20% (Michel et al. 2011). Fluctuations in the incidence of virulent biotypes are one possible explanation for the variability in *Rag1* efficacy observed across multiple years and locations (Hesler et al. 2013).

The availability of soybean aphid-resistant varieties is still limited commercially (McCarville et al. 2012, 2013), with almost all available aphid-resistant varieties incorporating the *Rag1* gene alone. These varieties are marketed to organic as well as conventional soybean producers. Aphid-resistant soybean varieties marketed towards conventional soybean producers are commonly sold with an insecticidal seed treatment, yet the benefit of such seed treatments for soybean aphid management is inconsistent and unreliable (McCornack and Ragsdale 2006, Johnson et al. 2009). The value of adding seed-applied insecticides to single-gene soybean aphid-resistant varieties are likely minimal (Hodgson and VanNostrand 2012, McCarville and O'Neal 2013), and it is unclear what benefit, if any, they can provide to a pyramid (McCarville and O'Neal 2013).

To explore the management benefits of *Rag* genes, we addressed four hypotheses that will be important for the successful release and adoption of host-plant resistance targeting soybean aphid. Specifically, we addressed if (1) the *Rag1*

and *Rag2* genes provide equivalent soybean aphid population suppression across multiple environments, (2) a two-gene pyramid (*Rag1* + *Rag2*) provides increased population suppression compared to either single gene alone, (3) soybean aphid-resistant lines (*Rag1*, *Rag2*, and *Rag1* + *Rag2*) require foliar insecticides for maximum yield, and (4) insecticidal seed treatments provide significant aphid population suppression and yield protection to soybean aphid-resistant lines (*Rag1*, *Rag2*, and *Rag1* + *Rag2*).

Materials and Methods

We used four soybean lines developed at Iowa State University (Wiarda et al. 2012). The four lines were developed from a cross between the parent lines A08-1243074 and LD08-89051a. The line A08-1243074 was the recurrent parent containing *Rag1* and LD08-89051a was the *Rag2* donor. At the BC_1F_2 generation four genotypes were selected *rag1rag1rag2rag2* (Susceptible), *Rag1Rag1rag2rag2* (*Rag1*), *rag1rag1Rag2Rag2* (*Rag2*), and *Rag1Rag1Rag2Rag2* (*Rag1* + *Rag2*). Ten plants of each genotype were identified and advanced. Each of the four soybean lines used for this experiment were a bulk of these ten lines at the $BC_1F_{2:5}$ generation. Therefore the four experimental lines used for this experiment were near-isolines sharing 75% genetic identities among lines.

We conducted a regional field plot study to address our four hypotheses. The study included seven locations in 2011 and eight locations in 2012 and 2013, for a total of 23 location-years (Table 1). We utilized a split-plot design, in which the main plot treatment was soybean line and the split-plot treatment was insecticide. It was necessary to have multiple field sites over a large region to best address our first

hypothesis of whether the *Rag1* or *Rag2* gene differs in performance across a larger region due to regional variations in the virulence of the soybean aphid population. We adjusted plot sizes to fit the space and resources available at each study location. We utilized a soybean row spacing of 76 cm and a planting density of 345,800 seeds hectare^{-1} at all locations. We used a standard split-plot size of 6 rows × 15.2 m (length × width) with exceptions made for the Lamberton, MN, Rock Springs, PA, Prosper ND, and Arlington, WI location-years. In Lamberton, MN and Prosper, ND split-plots were only 9.1 m in length. Split-plots were 10 rows × 6.1 m in Rock Springs, PA. The 2012 and 2013 Arlington, WI locations utilized 4 row × 15.2 m split-plots, while the 2011 Arlington, WI location utilized the standard split-plot size.

We utilized three insecticide treatments to address our four hypotheses, although not every location-year included all of these treatments. All location-years included an 'untreated' control. This treatment never received any insecticide (foliar or seed applications) and served as a measure of the aphid population suppression offered by each soybean line. The second treatment was designated as 'aphid-free'. If aphid populations reached a density of 50 aphids plant^{-1} in any of the aphid-free split-plots, all of the split plots in this treatment received a foliar application of λ-cyhalothrin (Warrior II with Zeon Technology®, Bayer CropScience), bifenthrin (Tundra® EC, Winfield Solutions) or chlorpyrifos (Lorsban® Advanced, Dow AgroSciences) according to the full label rate. Insecticide selection varied by location-year and was based on the presence of other pests (e.g. twospotted spider mite, *Tetranychus urticae* Koch). Although we considered the lines near-isolines, the

25% genetic difference among lines may produce differences in yield potential, complicating measurements of treatment effects on yield. The aphid-free treatment therefore allowed us to estimate the yield potential of each line in absence of aphid injury. We included the aphid-free treatment at all locations except the Rock Springs, PA and the Prosper, ND locations. It was excluded from the Rock Springs, PA due to space limitations and historically low aphid populations that rarely reach economically damaging densities. The aphid-free treatment was excluded from the Prosper, ND location as the lines were unlikely to reach physiological maturity before of a killing frost.

The final insecticide treatment was a neonicitinoid insecticide applied to the seed (insecticidal seed treatment). Thiamethoxam (Cruiser 5 FS®, Syngenta Crop Protection, Inc.) was applied to seed at a rate of 0.0756 mg seed^{-1}. Due to limited seed availability, field space, and planting equipment constraints, we only included this treatment at the two Iowa locations in 2011. In 2012 and 2013 we added the insecticidal seed treatment to the Volga, SD, Prosper, ND, and Arlington, WI locations.

We performed field preparation, planting, and weed management for each location according to local practices. Planting dates varied by location-year, but occurred between mid-May to early-June, with the exception of the Scandia, KS (Table 1). At Scandia, KS, soybeans were planted in mid-July after winter wheat was harvested. This is a common practice in Kansas and we chose it for this experiment as late planted or double-cropped soybeans in Kansas typically experience greater soybean aphid populations than early-planted or single-crop soybean.

We estimated soybean aphid populations throughout the growing season by counting all soybean aphids including alates, apterous adults, and nymphs for entire plants. We conducted counts at least once per month during the vegetative growth stages, and weekly from the R1 growth stage (i.e. beginning flowering, Fehr and Caviness 1977) until plant senescence. At each sampling date, we selected a minimum of five to a maximum of 20 plants from one of the middle rows of each split-plot. The number of plants we sampled was consistent across all split-plots within a location-year at a given sampling date. However, due to time limitations the number of plants we sampled differed among sampling dates and location-years.

We measured yield by harvesting the middle four rows of each six-row split-plot after plants reached physiological maturity. We harvested all four split-plot rows in Arlington, WI for 2012 and 2013, and we harvested the middle eight rows in Rock Springs, PA. We corrected grain moisture to 13% and report yield in kg hectare^{-1}.

Statistical Analyses.

We analyzed soybean aphid population data and yield data to address our four specific hypotheses. To address hypotheses one (do *Rag1* and *Rag2* provide equivalent aphid population suppression across multiple environments) and two (does a two-gene pyramid increase aphid population suppression), we used soybean aphid population data to calculate cumulative aphid-days (CAD). Cumulative aphid days are a summary statistic that measures the plant's seasonal exposure to aphids (Hanafi et al. 1989). We analyzed the response variable CAD using an analysis of variance (ANOVA). To address the first two hypotheses, we

analyzed data from only the untreated split-plots in a mixed-effects model (Proc mixed, SAS Institute, Cary, NC) (Table 2). This model included the fixed effects of location-year, and soybean line. The first hypothesis was addressed by the effect of soybean line, the two-way interaction of soybean line and location-year, and a mean separation test of the *Rag1* line and *Rag2* line. The second hypothesis was addressed by the effect of soybean line and a mean separation test of the three aphid-resistant soybean lines.

We analyzed yield data from the untreated and aphid-free split-plots to address our third hypothesis, whether soybean aphid-resistant varieties require foliar insecticides for optimal yield. To address this hypothesis, we first identified the study locations that experienced economically damaging populations of aphids in the untreated split-plot of the susceptible line. Ragsdale et al. (2007) estimated the economic injury level for soybean aphids to be approximately 5,200 CAD, therefore we used only location-years where populations exceeded 5,200 CAD in the untreated split-plot of the susceptible line to test this hypothesis. We again used a mixed-effects model to analyze these data with the fixed effects of insecticide treatment, and the interaction of soybean line and insecticide treatment used to test our hypothesis. The interaction of block nested within location-year and soybean line was considered a random variable and served as the whole-plot error term.

Our final hypothesis regarding the utility of an insecticidal seed treatment for aphid-resistant varieties was addressed using data from only location-years that included an insecticidal seed treatment. We analyzed CAD data from the untreated and seed treated split-plots using the same mixed-effects model as the previous

yield analysis. We also tested yield protection from soybean aphid provided by the insecticidal seed treatment with this mixed-effects model. Data for the yield analysis were drawn from location-years where the average CAD measured exceeded 5,200 CAD for the untreated susceptible split-plot. We tested our hypotheses regarding both aphid suppression and yield protection with the fixed effects of insecticide treatment, the two-way interaction of soybean line and insecticide treatment, and the three-way interaction of soybean line, insecticide treatment, and location.

Results

Soybean aphid populations varied greatly among the three years of the study and among the locations within each year. In general, populations were greater in 2011 and 2013 compared to 2012. The 2012 and 2013 Scandia, KS location-years were dropped from all analyses, as soybean aphids were never detected.

Effect of host-plant resistance on aphid populations.

Cumulative aphid-day data were analyzed to address our first two hypotheses. Soybean aphid populations in untreated split-plots varied significantly among location-years and soybean lines (Table 2). The significant interaction of location-year and soybean line indicates that the performance of the *Rag1* and *Rag2* genes differed across the larger geographic region of this study. Least squared means analysis revealed trends in the suppression provided by the *Rag1* and *Rag2* genes alone and in combination. In general, the single-gene lines had fewer aphids than the susceptible line and more than the pyramid, however these differences were not always significant (Table 3). During 2012, when aphid populations were the lowest, only at two of the seven locations were all of the resistant lines

significantly different from the susceptible line; these two locations experienced the greatest aphid populations during 2012. Significant differences between the *Rag1* and *Rag2* lines in CAD occurred at five of the 21 location-years included in the analysis, indicating the relative performance of the *Rag1* and *Rag2* genes varied among location-years. Among the resistant lines, in 15 of the 21 location-years, the pyramid line provided significantly greater aphid population suppression than at least one of the single gene lines, and the pyramid line provided significantly greater aphid population suppression than both single gene lines at eight location-years.

Yield protection provided by host-plant resistance.

Economically damaging populations of soybean aphids were present at the 2011 Lamberton, MN, and 2011 and 2013 Volga, SD, and Nashua, IA location-years. For these location-years, the main effects of location-year, soybean line and insecticide treatment affected yield (Table 2). A significant interaction of soybean line and insecticide treatment indicated yield loss due to soybean aphid feeding did not occur equally across the four soybean lines. Estimate statements were used to evaluate the effect of a foliar insecticide on yield for each of the four soybean lines. The greatest difference in yield was observed between the untreated and aphid-free treatments for the susceptible line (t = 5.28; df = 59; P < 0.0001), where 73.9 kg/ha of yield was protected by the application of a foliar insecticide (Figure 1). When data were pooled across both single-gene lines (i.e. both the *Rag1* line and the *Rag2* line) a foliar insecticide had a significant effect on the yield of the single-gene lines (t = 25.8; df = 59; P = 0.0125) protecting 25.3 kg/ha of yield. We did not observe a

difference in yield when the pyramid received a foliar insecticide application (t = 0.8; df = 59; P = 0.9546).

We repeated our yield analysis using data from 2012 Lamberton, MN, Volga, SD and Nashua, IA. These are the same locations included in the previous yield analysis, but a year in which economically damaging populations did not develop at the locations. This follow-up yield analysis was done to confirm the documented yield loss on the susceptible line and both single-gene lines were due to soybean aphids and not some other factor affected by insecticide application. Soybean line (F = 0.13; df = 3, 27; P = 0.9424), insecticide treatment (F = 1.17; df = 1, 36; P = 0.2874), and their interaction (F = 0.29; df = 3, 36; P = 0.8315) did not significantly affect yield in these low aphid pressure location-years.

Effect of an insecticidal seed treatment.

Soybean aphid populations varied significantly among location-years, soybean lines, and the presence of insecticidal seed treatment (Table 2). Similar to the analysis of only untreated split-plots, the effect of soybean line was not consistent across locations. However, the effect of insecticidal seed treatment on soybean aphid population suppression did not vary across soybean lines or location-years. Across all soybean lines, the insecticidal seed treatment reduced CAD by 38% compared to the untreated split-plots (Figure 2). For the yield analysis (aphid-free split-plots also included) the effect of insecticide treatment (including both foliar and seed-applied insecticides) significantly affected yield (F = 7.50; df = 2, 68; P = 0.0011). The effect of insecticide treatment, however varied significantly across the four soybean lines (F = 2.73; df = 6, 68; P = 0.0194), due primarily to

differences between untreated and aphid free split-plots. Yield of insecticidal seed treatment split-plots was not significantly different from untreated split-plots for any of the soybean lines (Figure 3). Therefore, the increased soybean aphid suppression provided by the insecticidal seed treatment did not result in significant yield protection for any of the soybean lines.

Discussion

Our goal was to assess the utility of host-plant resistance, specifically a two-gene pyramid, for soybean aphid management in the north central United States. We took care to limit the genetic variability among our test lines to ensure differences in aphid control among lines was due to the aphid-resistance genes and not plant maturity, health or other agronomic performance issues. During this experiment a wide range of aphid population densities developed across both locations within a year and years within locations. This is consistent with regional observations from previous experiments (Ragsdale et al. 2007, Johnson et al. 2009). The genetic relatedness of the test lines and the large number of locations and aphid pressures present during this study provided a robust test of our hypotheses.

The aphid population data presented here provides valuable information for current and future deployment of soybean aphid-resistant varieties. First, we observed significant variation between the *Rag1* and *Rag2* genes with respect to aphid suppression within five of the location-years in our study, but we need to interpret these results carefully. The *Rag1* and *Rag2* genes suppressed aphid populations to similar levels in caged settings using biotype-1 soybean aphids (Wiarda et al. 2012, McCarville and O'Neal 2012); therefore, differences in aphid

suppression for the *Rag1* and *Rag2 genes* within a given location-year may be due to the presence of virulent biotypes. Virulent soybean aphid biotypes are present in North America (Kim et al. 2008, Hill et al. 2010, Alt and Ryan-Mahmutagic 2013) and appear to be widespread (Michel et al. 2011), but soybean aphids in North America compose one large interbreeding population (Michel et al. 2009). Therefore, geographical differences in the efficacy of either gene in a particular year may be inconsistent in future years. For example, *Rag1* provided significant aphid population suppression in Nashua, IA in 2011 but not in 2013.

Gould (1986) predicted that a two-gene pyramid could provide improved pest population suppression and increased durability to insect virulence. Two-gene pyramids can demand increased time and resources for a breeding program to produce; therefore, a two-gene pyramid must provide a significant benefit for management to justify their production (Porter et al. 2000). Soybean aphid populations were significantly lower on the two-gene pyramid than at least one of the single-gene lines at 15 of the 21 locations. Included in these 15 location-years were the five locations that experienced economically damaging soybean aphid populations. The relevance of this observation can be seen in our yield analysis presented in Figure 1. Yield loss due to soybean aphid feeding was approximately 14% for the susceptible line and 5% for the two-single gene lines, whereas no yield loss was observed for the *Rag1* + *Rag2* two-gene pyramid. The use of a pyramid for soybean aphid management, therefore, could decrease need for insecticides, resulting in both monetary savings and less frequent disturbances to the natural-enemy community (Ohnesorg et al. 2009, Seagraves and Lundgren 2012). Natural

enemies contribute to regulating soybean aphid populations on soybean aphid-resistant varieties, reducing plant exposure to aphids by 89% (McCarville and O'Neal 2012).

Our study also examined the utility of insecticidal seed treatments for soybean aphid-resistant varieties. Previous studies demonstrated that for susceptible lines, insecticidal seed treatments provide inconsistent and often insufficient yield protection from soybean aphids (McCornack and Ragsdale 2006, Johnson et al. 2008, 2009; Magalhaes et al. 2009, Seagraves and Lundgren 2012). In cage and field settings, insecticidal seed treatments had similar efficacy on single-gene soybean aphid-resistant lines compared to soybean aphid-susceptible lines (Hodgson and VanNostrand 2012, McCarville and O'Neal 2013).

Insecticidal seed treatment reduced plant exposure to aphids by approximately 32% across the four soybean lines (Figure 2). These results indicate that in the field, insecticidal seed treatments provide similar protection to both single-gene and two-gene soybean aphid-resistant varieties as they do for susceptible varieties. Insecticidal seed treatments could, therefore, provide some management benefits, particularly for single-gene resistant varieties, which still experience yield loss due to soybean aphid feeding. These benefits, however, were not observed in our study and insecticidal seed treatments appear unnecessary for two-gene pyramids.

Our study demonstrates that a two-gene pyramid comprising *Rag1* and *Rag2* can significantly improve soybean aphid management in the field. The adoption of soybean aphid-resistant varieties has been slow by farmers as evidenced by their

availability (McCarville et al. 2012, 2013). This may be a product of their limited availability from commercial seed producers, limited availability in genetic backgrounds containing other desired agronomic traits, or the potentially insufficient efficacy of single-gene varieties as documented here. Two-gene pyramids have the potential to increase the efficacy and consistency of soybean aphid control provided by aphid-resistant varieties, potentially also increasing their adoption by farmers.

In addition to the benefits provided to soybean aphid management, two-gene pyramids may be useful for the management of virulent soybean aphid biotypes. Resistance pyramids can delay the development of insect virulence in other systems (Onstad and Meinke 2010, Zhao et al. 2003), however this remains to be investigated for insects with a heteroecious, holocyclic lifecycle, where multiple generations will experience the selection pressure before sexual reproduction occurs. Transgenic crop plants targeting insect pests have relied on the high-dose refuge strategy to delay virulence development. Virulence, however, has developed in insect populations in which the toxins deployed do not meet the high-dose requirement of this strategy (Tabashnik et al. 2013). Resistance pyramids, which incorporate at least two unique modes of action (i.e. cross resistance between resistance traits does not occur), can still help delay the evolution of virulence in these cases (Roush 1998). Transgenic corn expressing Bt toxins targeting western corn rootworm, for example, are low-dose in nature, which allowed virulence to develop within 10 years of initial commercial release of these Bt toxins (Gassmann et al. 2011). Resistance pyramids are now being used to target western corn

rootworm in hopes of overcoming the issue of low-dose traits through the redundant killing provided by resistance pyramids.

In addition to insect resistance management, future research will need to investigate the efficacy of both pyramiding other soybean aphid resistance genes (i.e. other combinations of two-gene pyramids) and of pyramiding three or more soybean aphid resistance genes. Eight possibly different soybean aphid resistance genes have been identified to date (Hill et al. 2012, Hesler et al. 2013). In this study, we were only able to investigate the efficacy of one possible two-gene pyramid. The results of the study presented here along with the additional soybean aphid-resistance genes available provide reason to believe host-plant resistance can be a valuable and sustainable part of an integrated pest management program for the soybean aphid.

Acknowledgements

We thank the many farm managers, research associates, and student workers for assistance with planting, harvesting, applying insecticides, and collecting data. We also thank Syngenta for applications of thiamethoxam seed treatments. This research was funded by Soybean Checkoff through grants provided by the North Central Soybean Research Program, Iowa Soybean Association, and South Dakota Soybean Research and Promotion Council.

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Table 1. Experiment locations within each year and insecticide treatments included at each location

Location	Sub-plot Size	Treatments Included[a]	Planting Date
2011			
Volga, SD	6 rows x 15.2 m	AF, UT	19 May
Lamberton, MN	6 rows x 9.1 m	AF, UT	2 June
Arlinton, WI	6 rows x 15.2 m	AF, UT	23 May
Nashua, IA	6 rows x 15.2 m	AF, UT, ST	17 May
Ames, IA	6 rows x 15.2 m	AF, UT, ST	24 May
Rock Springs, PA	10 rows x 6.1 m	UT	31 May
Scandia, KS	6 rows x 15.2 m	AF, UT	12 July
2012			
Prosper, ND	6 rows x 9.1 m	UT, ST[b]	6 June
Volga, SD	6 rows x 15.2 m	AF, UT, ST	15 May
Lamberton, MN	6 rows x 9.1 m	AF, UT	18 May
Arlington, WI	4 rows x 15.2 m	AF, UT, ST	24 May
Nashua, IA	6 rows x 15.2 m	AF, UT, ST	17 May
Ames, IA	6 rows x 15.2 m	AF, UT, ST	15 May
Rock Springs, PA	10 rows x 6.1 m	UT	25 May
Scandia, KS	6 rows x 15.2 m	AF, UT	28 June
2013			
Prosper, ND	6 rows x 9.1 m	UT, ST[b]	13 June
Volga, SD	6 rows x 15.2 m	AF, UT, ST	5 June
Lamberton, MN	6 rows x 9.1 m	AF, UT	31 May
Arlington, WI	4 rows x 15.2 m	AF, UT, ST	4 June
Nashua, IA	6 rows x 15.2 m	AF, UT, ST	18 June
Ames, IA	6 rows x 15.2 m	AF, UT, ST	15 June
Rock Springs, PA	10 rows x 6.1 m	UT	7 May
Scandia, KS	6 rows x 15.2 m	AF, UT	18 July

[a] AF = aphid-free, UT = untreated, ST = insecticidal seed treatment

[b] Aphid-free treatment not included as soybean lines would not reach physiological maturity at this location before first frost.

Table 2. Analysis of variance tables of treatment effects on cumulative aphid-days and yield

Effect	Fixed/Random	df	F statistic/ χ^2 [a]
Cumulative aphid day analysis of untreated split-plots			
location-year	F	20, 63	122.68***
block(location-year)	R	1	0.4
soybean line	F	3, 189	144.38***
location×soybean line	F	60, 189	3.05***
Yield analysis of high aphid pressure locations[b]			
location-year	F	4, 15	26.20***
block(location-year)	R	1	12.3***
soybean line	F	3, 45	4.20*
location×soybean line	F	12, 45	0.87
block(location-year)×soybean line	R	1	7.3*
insecticide treatment	F	1, 59	20.28***
soybean line×insecticide	F	3, 59	4.86**
location×soybean line×insecticide	F	16, 59	0.94
Cumulative aphid day analysis of untreated and seed treated split-plots			
location-year	F	11, 36	290.28***
block(location-year)	R	1	2.8
soybean line	F	3, 108	114.15***
location×soybean line	F	33, 108	6.56***
block(location-year)×soybean line	R	1	5.8*
insecticide treatment	F	1, 144	28.16***
soybean line×insecticide	F	3, 144	1.63
location×soybean line×insecticide	F	44, 144	0.84

* Significant effect at $P<0.05$, ** $P<0.01$, *** $P<0.0001$

[a] An F statistic was used to test for the significance of fixed effects, while a χ^2 test was used for random effects.

[b] Only includes location-years where the untreated split-plot of the susceptible line exceeded 5,200 cumulative aphid days, the economic injury level for soybean aphid.

Table 3. Effect of soybean line on cumulative aphid-days for untreated split-plots

Location[a]	Susceptible	*Rag1*	*Rag2*	*Rag1* + *Rag2*
2011				
Volga, SD	25,949 ± 4,205a[b]	2,498 ± 331b	5,001 ± 1,185b	850 ± 229c
Lamberton, MN	25,100 ± 2,791a	882 ± 240bc	5,544 ± 1,305b	1,440 ± 1,269c
Arlington, WI	845 ± 172a	563 ± 130ab	594 ± 149ab	415 ± 80b
Nashua, IA	8,281 ± 1,560a	2,490 ± 1,169b	1,137 ± 254b	287 ± 85c
Ames, IA	5,506 ± 1,315a	1,023 ± 272b	963 ± 175b	242 ± 54c
Rock Springs, PA	434 ± 75a	162 ± 25b	116 ± 31c	61 ± 15d
Scandia, KS	332 ± 54a	20 ± 8b	57 ± 31b	11 ± 3b
2012				
Prosper, ND	13 ± 3a	2 ± 1b	6 ± 3ab	4 ± 3b
Volga, SD	276 ± 113a	14 ± 7c	147 ± 56b	24 ± 22c
Lamberton, MN	2,409 ± 1,659a	559 ± 529bc	293 ± 102b	13 ± 6c
Arlington, WI	6 ± 5a	2 ± 2a	0 ± 0a	3 ± 3a
Nashua, IA	6 ± 4a	0 ± 0b	1 ± 1ab	0 ± 0b
Ames, IA	51 ± 29a	25 ± 14a	1 ± 1b	1 ± 1b
Rock Springs, PA	152 ± 47a	61 ± 15ab	54 ± 21bc	17 ± 2c
Scandia, KS	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a
2013				
Prosper, ND	593 ± 111a	127 ± 23c	292 ± 77b	102 ± 13c
Volga, SD	11,322 ± 5,211a	812 ± 154b	1,575 ± 325b	242 ± 72c
Lamberton, MN	2,059 ± 1337a	210 ± 148b	93 ± 20b	15 ± 5c
Arlington, WI	842 ± 98a	646 ± 210ab	425 ± 19b	345 ± 104b
Nashua, IA	24,361 ± 4,059a	12,776 ± 2,831ab	6,860 ± 2,263b	268 ± 102c
Ames, IA	789 ± 101a	99 ± 23c	223 ± 76b	28 ± 6d
Rock Springs, PA	4,309 ± 966a	2,256 ± 402ab	1,473 ± 524bc	989 ± 498c
Scandia, KS	0 ± 0a	0 ± 0a	0 ± 0a	0 ± 0a

[a] Cumulative aphid-days data from the untreated split-plot are shown by location for each of the four soybean lines.

[b] Different letters represent significant differences ($P < 0.05$) among soybean lines within a location.

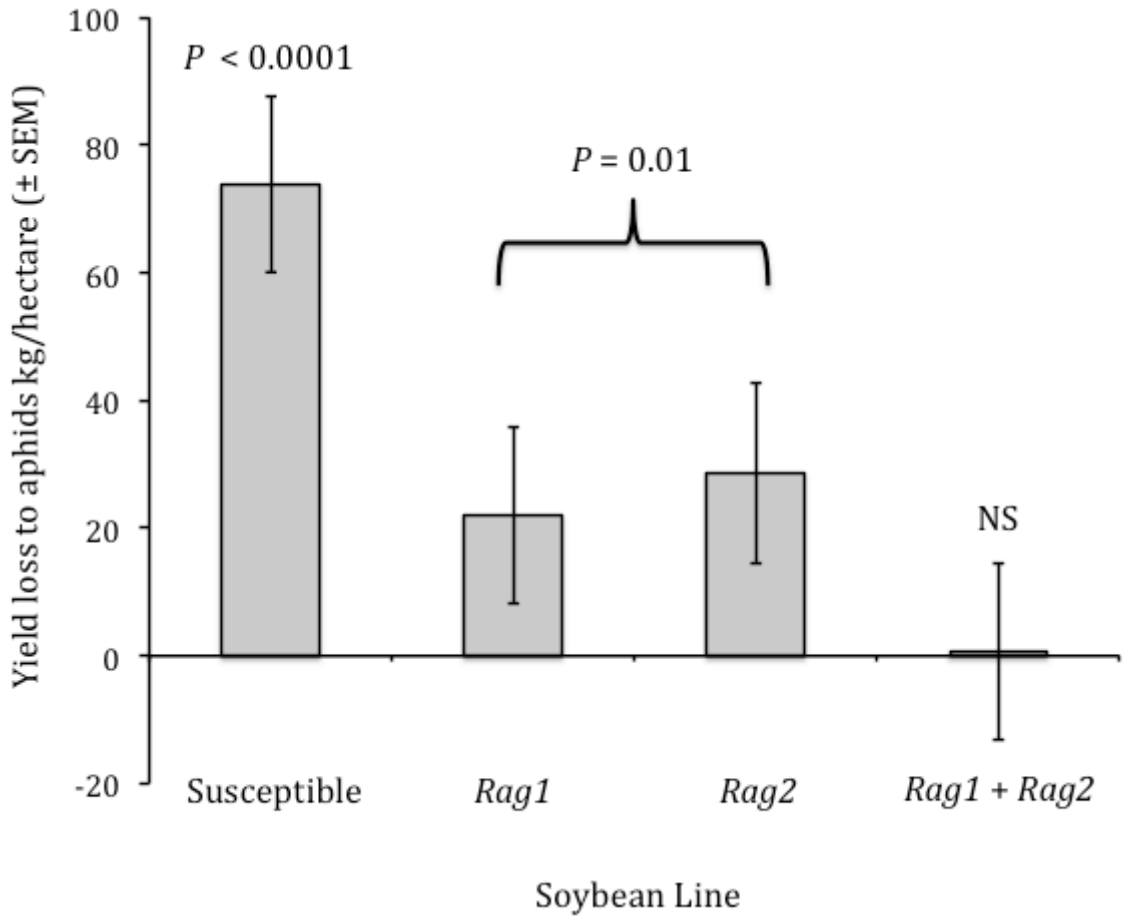

Figure 1. Yield loss attributed to soybean aphid for each of the four experimental soybean lines. Yield loss was calculated using a *t*-test to determine the yield difference between the aphid-free and untreated split-plots of each soybean line. Yield data was combined from the five location-years that experienced economically damaging soybean aphid populations (2011 Lamberton, MN; 2011 and 2013 Volga, SD; and 2011 and 2013 Nashua, IA).

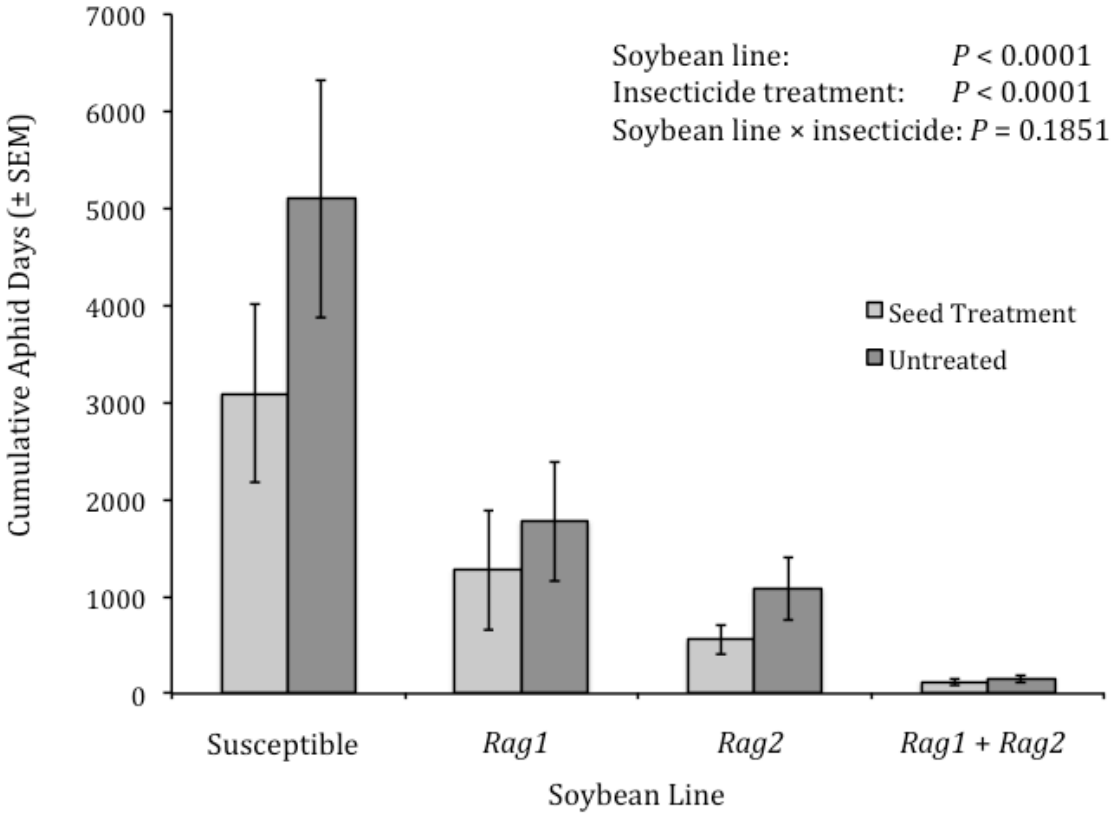

Figure 2. Cumulative aphid-day (CAD) data from the 12 location-years that included an insecticidal seed treatment split-plot. The thiamethoxam seed treatment significantly reduced plant exposure to aphids equally across each of the four soybean lines by an average of 38%.

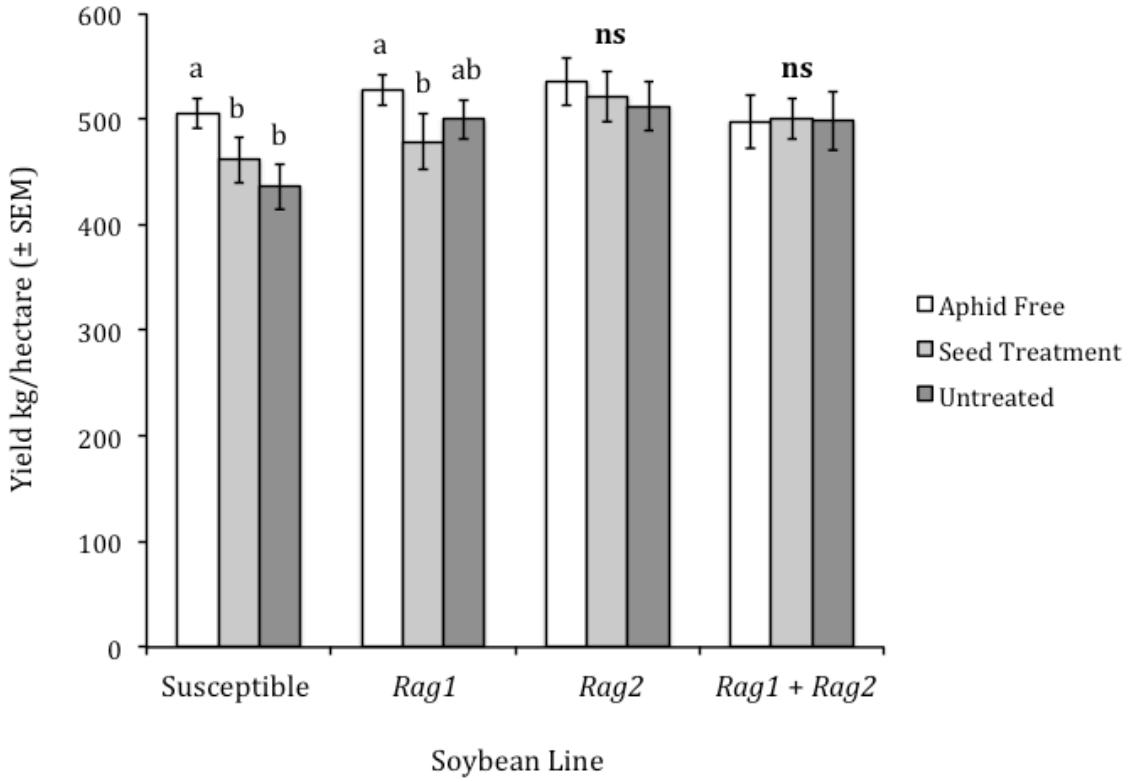

Figure 3. Yield data for each insecticidal treatment and soybean line combinations. Yield data was compared from the three location-years that included seed treatment split-plots and experienced economically damaging soybean aphid populations (2013 Volga, SD and 2011 and 2013 Nashua, IA). Letters represent significant differences among insecticide treatments within a soybean line.

CHAPTER 5. INVESTIGATING FACTORS INFLUENCING THE DEVELOPMENT OF *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) VIRULENCE TO HOST-PLANT RESISTANCE

A paper to be submitted to the Journal of Economic Entomology.

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Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybean in the United States. Host-plant resistance genes conferring resistance to the soybean aphid were recently commercialized. The durability of host-plant resistance for soybean aphid management will be greatly affected by the unique lifecycle of the aphid. Deterministic genetic models were used to evaluate factors likely to affect the rate that virulence alleles increase in the population. These factors included the dispersal rate of the aphid population, dominance of virulence, efficacy of resistance genes, deployment strategy for resistance genes, and use of insecticides (both foliar and seed-applied). Results demonstrated both the dominance of virulence, as well as the aphids high potential for field-to-field dispersal within the season, greatly affect the size of refuge required to delay the development of virulence. Management tactics deployed for soybean aphid control also affected virulence development. The deployment of resistance genes, as single

gene cultivars or as resistance pyramids also affected the development of virulence, with the release of a pyramid by itself providing the longest delay in the development of virulence. Foliar and seed applied insecticides both affected virulence development. Applications of foliar insecticides to the refuge increased the rate at which virulence developed due to the killing of susceptible individuals in the refuge. Insecticidal seed treatments applied to only the refuge increased the rate at which virulence developed, while if applied to only resistant soybean, the development of virulence could be delayed

Keywords: integrated pest management, *Rag* genes, insecticidal seed treatment, insect resistance management

Introduction

Host plant resistance (HPR) can be a powerful management tool reducing pest populations on crop plants with minimal negative environmental impacts. Perhaps the largest hurdle to deploying effective HPR is the ability of insects to develop virulence (resistance) in relatively short spans of time. Insect adaptation to HPR has been the subject of a large body of research, recently reviewed by Onstad and Knolhoff (2008). Strategies to prolong the effectiveness of a HPR crop have been explored, including the deployment of a refuge (Tabashnik 1994), varying the deployment of resistance traits (Gould 1986, Roush 1998), and the compatibility of chemical control with HPR (Bates et al. 2005, Pan et al. 2011). The overwhelming majority of this literature explores these concepts within the context of sexually reproducing organisms with few generations in a year (Crowder and Carriére 2009).

Aphids (Aphididae: Hemiptera) are major crop pests, removing plant nutrients as well as transmitting plant viruses (Smith and Boyko 2007). Within the family Aphididae, there are a diversity of life history strategies, ranging from anholocyclic obligate parthenogenesis on a single host (autoecious) or multiple hosts (heteroecious) to holocyclic, heteroecious lifecycles including parthenogenesis on a secondary host during much of the season with sexual reproduction isolated to a primary host (Dixon 1977). These life history strategies can have large effects on the rate that allele frequencies change within a year (Crowder and Carriére 2009). In the case of some aphid species this has led to the rapid development of resistance to both HPR traits and pesticides (Puterka et al. 1992, Nauen and Denholm 2005, Burd and Porter 2006).

The soybean aphid (*Aphis glycines,* Matsumura) is a significant insect pest of soybean (*Glycine max*, L.) in the North Central United States. The soybean aphid has a heteroecious, holocyclic lifecycle with sexual reproduction occurring on the primary host, buckthorn (*Rhamnus spp.*) and parthenogenesis occurring on the secondary host soybean (Voegtlin et al. 2004). In the spring, eggs hatch on buckthorn, with European buckthorn (*Rhamnus cathartica* L.) being the most common primary host (Ragsdale et al. 2004). European buckthorn is an invasive woody perennial plant that is widely distributed throughout the northern and central regions of the United States (Venette and Ragsdale 2004). After egg hatch, three to four generations of asexual reproduction occur on buckthorn (Ragsdale et al. 2004). The third generation and any subsequent generations produced on buckthorn consist of winged female alates that migrate in search of soybean.

Once on soybean, parthenogenesis occurs for approximately 15 generations (Wang et al. 1962). Under artificial rearing conditions the average apterous female is capable of producing between 20 and 63 offspring, dependent on temperature (Hanafi et al. 1995, McCornack et al. 2004). In the field, soybean aphid fecundity is much lower, likely due to abiotic factors that affect aphid reproduction and growth including rain, wind, and fluctuating temperatures (Dixon 1977), as well as biotic factors including varying host quality (Myers and Gratton 2006) and mortality from natural enemies (Fox et al. 2004, Desneux et al. 2006, Schmidt et al. 2007, Gardiner et al. 2009). Each generation on soybean may also give rise to asexual winged alates, which serve as within-season dispersal agents. Alates are capable of moving both within fields and between fields both locally and regionally. The percentage of offspring that are alates can vary among generations due to factors including host plant nutrition and crowding (Lu and Chen 1993, Hodgson et al. 2005). Field studies have recorded alates composing anywhere from 0% to 19% of the population (Hodgson et al. 2005, Donaldson et al. 2007).

Multiple factors, including decreasing daylight, colder temperatures, and plant senescence, trigger the production of gynoparae (fall winged asexual females), and winged males, which migrate back to the primary host. On buckthorn, the gynoparae give rise to ovipara (wingless sexual females), which the males seek out for mating. After mating, the ovipara will lay fertilized eggs on buckthorn, which will serve as the overwintering stage (Ragsdale et al. 2004).

Soybean aphid management has relied primarily on the use of broad-spectrum insecticides, however host-plant resistant (HPR) cultivars are available.

Insecticides commonly used for soybean aphid include foliar applications of pyrethroids (esfenvalerate, cyfluthrin, bifenthrin, deltamethrin, zeta-cypermethrin, lambda-cyhalothrin) and organophosphates (dimethoate, chlorpyrifos) and seed-applied neonicitinoids (thiamethoxam, imidacloprid) (Olson et al. 2008, Hodgson et al. 2012a). Host-plant resistance for soybean aphids became commercially available in 2010 (Hodgson et al. 2012b). Initial release was on a small scale with all commercially available HPR cultivars incorporating the *Rag1* gene (McCarville et al. 2012) conferring antibiosis-based resistance to the soybean aphid (Hill et al. 2012). At least four other genes have been identified, *Rag2*, *rag3*, *rag4,* and *Rag5* (Hill et al. 2012), of which only *Rag2* is commercially available. To date, *Rag2* has only been available as half of a pyramid with the *Rag1* gene (McCarville et al. 2012, 2013). Initial field studies with soybean cultivars containing *Rag* genes have found soybean aphid control to vary by year and location, with economically damaging populations building in some location-years (Hesler et al. 2013, McCarville et al. 2014). This may be due to the presence of virulent biotypes, which exist in North America. Biotypes virulent to *Rag1*, termed biotype 2 (Kim et al. 2008), or *Rag2*, termed biotype 3 (Hill et al. 2010), or both *Rag1* and *Rag2*, termed biotype 4 (Alt and Ryan-Mahmutagic 2013) have been isolated from the field. The abundance and distribution of these biotypes in North America is unknown.

Despite the occurrence of multiple virulent biotypes in North America, Michel et al. (2009) concluded that the soybean aphid population in North America has limited genetic diversity and likely represents a single interbreeding population with high within year dispersal capabilities. Soybean aphids also experience a

potential genetic bottleneck at the primary host plant. The genetic bottleneck occurs from the low success of aphids reaching buckthorn, finding a mate, their eggs successfully overwintering, and the resulting progeny producing clones that later successfully locate soybean in the spring. Therefore, only a small, potentially genetically homogenous population colonizes soybean fields in the spring. This occurs despite the large genetically diverse population that leaves soybean fields in the fall.

Host-plant resistance offers the potential for more sustainable and less disruptive management of the soybean aphid. The discovery of virulent biotypes, and dispersal capacity of soybean aphids, however requires an understanding of the factors likely to affect the buildup of virulence in the soybean aphid population if we hope to realize the potential of HPR. Therefore, our goal is to use a simple deterministic model to assess how both environmental factors and management strategies may affect the increase of virulence alleles in the soybean aphid population within North America.

Materials and Methods

Conditions for model.

We used a two-locus deterministic analytical model to calculate changes in the frequency of alleles controlling virulence to host-plant resistance in the soybean aphid population. The model is spatially implicit, in that buckthorn stands and soybean fields both HPR fields and refuges were assumed to be randomly distributed throughout the environment. Density-dependent mortality was not included in the model. Density-dependent mortality due to herbivore over-crowding

or increased attraction of predators can affect the development of virulence (Onstad 2008). Mortality or reductions in population growth rate due to over-crowding are less likely to occur for soybean aphid given its ability to disperse within and among fields. Soybean aphid predators do respond to changes in aphid population densities (Donaldson et al. 2007), but the effect of predation on development of virulence can be affected by multiple variables including the dispersal rate of the herbivore, which herbivore genotype arrives first to a field, and the availability of alternate prey (Wilhoit 1991, Mallampalli et al. 2005, Heimpel et al. 2005). The effect of predation was outside the considerations of our current model.

The model assumed soybean aphids complete 18 generations per year (Wang et al. 1962, Ragsdale et al. 2004), with the first three generations occurring on buckthorn (Ragsdale et al. 2004) and all subsequent generations developing on soybean. In-season field-to-field dispersal was assumed be density-independent and take place in each generation occurring on soybean except generation 18, which migrates back to buckthorn. We assumed dispersal occurred randomly and was not influenced by aphid genotype or plant genotype, therefore if the refuge size were 20%, the refuge would receive 20% of all dispersing aphids, regardless of aphid genotype. The model allowed for the rate of dispersal to be manipulated separately for each generation.

The genetic basis for virulence is not known for any of the soybean aphid biotypes currently identified. We assumed virulence to each resistance gene to be conferred by a single gene with two alleles, one allele conferring avirulence and another conferring virulence. A single gene-two allele model would lead to the most

rapid increase in virulence and therefore provide a conservative estimate of the longevity of current *Rag* genes. Virulence to *Rag1* was assumed to be conferred by the allele $G1_{vir}$ with avirulence conferred by $G1_{avr}$. Virulence to *Rag2* was assumed to be conferred by the allele $G2_{vir}$ with avirulence conferred by $G2_{avr}$. The G1 and G2 genes were assumed to occur at separate loci that were not linked. The initial frequency of each allele conferring virulence was assumed to be either 0.01 or 0.10. These frequencies were selected to provide a contrast to investigate the effect of initial allele frequency. The actual frequency of virulent individuals in the soybean aphid population is not known, however an initial allele frequency of 0.01 to 0.10 is within reason given the limited surveys available (Michel et al. 2011, 2010). The initial population was 100,000 aphids for each run of the model.

Each aphid genotype was assumed to have a reproductive potential of four offspring on buckthorn. Migration from buckthorn to soybean occurs randomly for each genotype. Therefore, the number of individuals of each aphid genotype beginning the fourth generation in a patch of soybean of a given plant genotype (e.g. *Rag1*) was calculated as

$$I_{xRag1} = A_x P_{Rag1}$$

Where I_{xRag1} is the number of individuals of genotype "x" in *Rag1* soybean fields, A_x is the abundance of genotype "x" in the total landscape, and P_{Rag1} is the proportion of the total soybean crop planted to *Rag1* containing soybeans.

Once on soybean each individual was assumed to have a maximum reproductive potential of 32 offspring, which represents approximately a 33% reduction from the estimates of soybean aphid fecundity in growth chambers

(Hirano et al. 1995, McCornack et al. 2004). The 33% reduction provides an approximation of mortality from abiotic factors, which can have a significant effect on the rate of soybean aphid population growth (McCarville et al. 2011).

Estimates of the efficacy of *Rag* genes were taken from a field cage study that measured the population growth rate of biotype-1 soybean aphids on experimental *Rag1*, *Rag2*, *Rag1* + *Rag2* pyramid, and susceptible near-isolines in the absence of natural enemies (McCarville and O'Neal 2012). Population growth rates were reduced by 41% on *Rag1* and *Rag2* lines and 59% on the *Rag1* + *Rag2* pyramid line compared to the susceptible line. We assumed that fitness costs did not exist for virulent individuals. The fitness of soybean aphid biotype-2 and biotype-3 on susceptible plants grown in the greenhouse was not statistically different from biotype-1 (Kim et al. 2008, Hill et al. 2010). Therefore, the increase in individuals on *Rag1* for a generation for a given genotype was calculated as

$$\Delta I_{xRag1} = I_{xRag1}(32W_{xRag1})$$

Where I_{xRag1} is the amount of individuals of genotype "x" on *Rag1* containing soybean and W_{xRag1} is the fitness of genotype "x" (i.e. 0.59) on *Rag1* containing soybean.

At the conclusion of the final generation, soybean aphids were assumed to migrate back to buckthorn. All genotypes had an equal rate of migration to buckthorn and chance of locating a mate. We assumed a low success rate for locating a mate. We assumed only 100,000 viable eggs would be produced for the subsequent year, this relatively low number, compared to the end-of-season population was used to prevent populations from rapidly increasing from year to

year. Soybean aphid populations fluctuate between years but have not increased consistently over the past decade (Ragsdale et al. 2011, Schmidt et al. 2012). The large reduction in population occurring on soybean presents a potential genetic bottleneck where chance occurrences could lead to genetic drift, however our model did not include stochastic effects and we therefore focus on the average progression of virulence evolution. The frequency of each virulence allele was determined at the end of season. For example the frequency of $G1_{vir}$ was calculated as

$$Freq_{G1vir} = G1_{vir}/(G1_{vir} + G1_{avr})$$

Where $G1_{vir}$ and $G1_{avir}$ are the total number of virulence and avirulence alleles, respectively for G1 present across all genotypes in the population.

Once on buckthorn mating was assumed to be random and follow the Hardy-Weinberg equilibrium principle. Each model was run for 25 years with changes in gene frequency assessed at the end of each season prior to random mating (i.e. on buckthorn prior to mating). Virulence was determined to have developed if the frequency of a virulence allele reached 0.25.

Model validation.

The conditions of our model were first set to those described by Crowder and Carriére (2009) for insects with parthenogenic reproductions. This included a constant rate of 100% field-to-field dispersal, an efficacy of 100% for the HPR gene, and an initial virulence allele frequency of 0.01. We then attempted to validate our model by reproducing the results for the evolution of virulence in parthenogenic individuals displayed in Table 2 of Crowder and Carriére (2009).

Dominance of expression and refuge size.

The effects of the dominance of virulence expression and refuge size were explored for a scenario in which one host plant resistance gene (*Rag1)* was deployed in the landscape. In-season dispersal was excluded from the model for this scenario. Virulence to *Rag1* was controlled by the allele $G1_{vir}$. The model was run considering three modes of expression for allele $G1_{vir}$, recessive, dominant, and additive, in which fitness of the heterozygote was set at 0.59, 0.79, and 1.00, respectively. The amount of landscape planted to a refuge of susceptible plants was also varied from 0% to 35%. A refuge size of 35% was used as the maximum size as this represents the percentage of corn planted to non-Bt corn hybrids in 2011 in Illinois, Iowa, Michigan, Minnesota, Nebraska, South Dakota, and Wisconsin (USDA-ERS 2011). A 35% refuge size therefore is a reasonable estimate of potential regional grower adoption for soybean aphid HPR cultivars.

Effect of field-to-field dispersal.

Two rates of dispersal were considered, static and dynamic. The static rate of dispersal was set at a constant of 5% of the total population in each field type. A rate of 5% was taken from estimates of alate production measured by Hodgson et al. (2005) and Donaldson et al. (2007). The fluctuating rate of dispersal was based on the estimates of alate production throughout the season published by Hodgson et al. (2005).

Effect of resistance gene release strategy.

We next assessed the development of virulence to *Rag1* resistance under a variety of deployment strategies. Release strategy was assessed with the inclusion of a dynamic rate of field-to-field dispersal. The release strategies analyzed included

(1) the release of one source of resistance (i.e. *Rag1* cultivars only), (2) the release of two sources of resistance in the form of two single gene cultivars (i.e. *Rag1* cultivars and *Rag2* cultivars), (3) the release of two sources of resistance in the form of only pyramid cultivars (i.e. *Rag1* + *Rag2* pyramid cultivars only), (4) the release of one single gene cultivars and pyramid cultivars (i.e. *Rag1* cultivars and *Rag1* + *Rag2* pyramid cultivars), and (5) the release of two single gene cultivars and pyramid cultivars (i.e. *Rag1* cultivars, *Rag2* cultivars, and *Rag1* + *Rag2* pyramid cultivars). The effect of each release strategy on the frequency of $G1_{vir}$ allele was explored for both an initial allele frequency of 0.01 and 0.10, and all three modes of inheritance. For additive inheritance, the fitness of individuals heterozygous for a single virulence allele was 0.47 on the pyramid cultivar. While individuals heterozygous for both virulence alleles had a fitness of 0.62 on the pyramid cultivar.

The release strategies were investigated for each of the refuge sizes explored in the previous analyses. For all refuge size by release strategy combinations investigated the non-refuge area was evenly divided among all cultivar types deployed (Table 2). For example, in the pyramid and two single gene cultivars release strategy with 25% refuge, the non-refuge would comprise 75% of the landscape and be divided as 25% *Rag1*, 25% *Rag2*, and 25% *Rag1* + *Rag2* pyramid. Evenly dividing the non-refuge area among all cultivar types may not accurately reflect actual adoption rates, but allows for comparisons among release strategies.

Effect of insecticidal seed treatments.

The model was amended to include a temporally variable density-independent mortality factor simulating an insecticidal seed treatment.

Measurements for mortality due to seed treatments were taken from McCornack and Ragsdale (2006) and McCarville and O'Neal (2013). Both studies measured mortality from seed treatments from the early vegetative, V3 stage to the mid-reproductive R3 stage of soybean growth (Fehr and Caviness 1977). Mortality was included in the model so that, at any time point in which significant mortality from seed treatments was observed in either study, the estimate of percent mortality was combined from both studies. This method resulted in four temporal periods of mortality, 32.5% for generation 4, 95% for generation 5, 43% for generation 6, and 0% for generations 7 thru 18. Based on results from McCarville and O'Neal (2013), seed treatments and soybean HPR were acted in an additive manner to reduce fecundity in the model. Therefore, the increase in individuals of a specific genotype in the presence of an insecticidal seed treatment on the *Rag1* cultivar was calculated as

$$\Delta I_{xRag1} = I_{xRag1}[32W_{xRag1}(1 - M_{stg(y)})]$$

Where I_{xRag1} is the amount of individuals of genotype 'x' on *Rag1* containing soybean and W_{xRag1} is the fitness of genotype 'x' (i.e. 0.60) on *Rag1* containing soybean, $M_{stg(x)}$ is mortality due to an insecticidal seed treatment at generation 'y'.

The effect of insecticidal seed treatments on the frequency of the $G1_{vir}$ allele was assessed under the pyramid and one single gene cultivar release strategy with a 35% refuge. This release strategy was selected as it represents the current commercial availability of the *Rag1* and *Rag2* genes (McCarville et al. 2013). The model was run for each combination of the three modes of inheritance and two initial allele frequencies (i.e. 0.01 and 0.10).

Effect of foliar insecticide application to the refuge.

The model was amended to include a density-independent mortality factor intended to simulate the application of a foliar insecticide to the refuge. Farmers not planting a resistant cultivar would be more likely to protect their crop using a foliar insecticide, thereby potentially decreasing the effect of the refuge. A foliar application of insecticides was simulated as a 99.99% mortality factor applied to a portion of the refuge at the eleventh generation. The 99.99% mortality was selected as an estimate of the efficacy of the common insecticides used against the soybean aphid (Hodgson and VanNostrand 2012, Olson et al. 2008). The eleventh generation was selected as it approximates the R3 growth stage of soybean, a time when soybean aphid outbreaks commonly occur and growth-stage-based applications are made (Hodgson et al. 2012b). Three insecticide use strategies were investigated, full prophylactic or preventative use, semi-prophylactic, and integrated pest management (IPM) use.

Full prophylactic use was simulated by applying the mortality factor to 80% of the refuge area every year. This approximates the usage of foliar insecticides on soybean in Iowa (Hodgson et al. 2012a), and its application every year is consistent with a preventative tank mixed application of insecticides with fungicides, which has increased in recent years (Johnson et al. 2009, Hodgson et al. 2012b). Semi-prophylactic use was simulated by again applying the mortality factor to 80% of the refuge area, but only on a biennial basis. This simulates the prophylactic use of insecticides during 'outbreak' years, or those in which there is an increased probability of an economic infestation of soybean aphids occurring. A biennial basis

was settled upon for the semi-prophylactic use to simulate the odd-even year soybean aphid outbreak phenomenon that has been observed in several states (Ragsdale et al. 2011). Finally, IPM use was simulated by applying the mortality factor to 40% of the refuge area on a biennial basis. Therefore, the frequency of use was held constant with semi-prophylactic use, however only half as much of the refuge was treated with an insecticide to simulate the application of insecticides to only fields that surpass the economic threshold. This degree of application is lower but similar to observations by Johnson et al. (2008), who observed soybean aphids reaching economically damaging levels in 33% of their study's location-years. Similar to insecticidal seed treatments, the effect of foliar insecticide applications was evaluated for the pyramid and one single gene cultivar release strategy, with the inclusion of a 35% refuge. Again the model was run for each combination of the three modes of inheritance and two initial allele frequencies.

Effect of increased efficacy of HPR traits.

The efficacy of current *Rag* genes is relatively low (41% reductions in population growth) compared to the efficacy rates displayed by current transgenic traits available and explored in other genetic models. In the future, researchers may develop transgenic HPR traits with greater efficacy for soybean aphids or aphid pests with similar lifecycles. Therefore, we decided to explore the effect of these traits on the development of virulence. Three efficacy rates were compared, 41%, 96%, and 99% reductions in population growth rates. The efficacy rates of 41%, 96% and 99% were selected as they represent approximate values for the efficacy of current *Rag* genes targeting soybean aphid, Bt toxins targeting western corn

rootworm (Meihls et al. 2008), and Bt toxins targeting European corn borer (Burkness et al. 2001), respectively. The effect of the three efficacy rates on virulence development was explored with a refuge size of 35%, and an initial virulence allele frequency of 0.01. We considered each efficacy rate released as a single gene cultivar, or as a part of a pyramid including a second *Rag* gene.

Results

Model validation.

We obtained identical results as Crowder and Carrière (2009). We therefore determined our model to be valid and proceeded to investigate various factors, which may affect the frequency of virulence alleles in a soybean aphid population.

Dominance of expression and refuge size.

For all refuge sizes, virulence developed fastest and most often with dominant expression (Figure 1). None of the refuge sizes were able to prevent virulence from developing when the initial frequency of the $G1_{vir}$ allele was 0.10, or if its expression was dominant. Only if the initial frequency of $G1_{vir}$ was 0.01, it was expressed as an additive or recessive trait, and the refuge size was 35% did virulence fail to develop in under 25 years when the *Rag1* resistance gene was released as a single gene cultivar.

Effect of field-to-field dispersal.

The addition of field-to-field dispersal within a season increased the rate that the frequency of $G1_{vir}$ allele increased for all modes of inheritance (Figure 2). The increase in the rate of virulence development was due to a greater proportion of the population experiencing the selection pressure due to avirulent individuals

migrating from the refuge to resistant cultivars. The increased rate of virulence development was most pronounced when the initial allele frequency was low (i.e. 0.01), and virulence was expressed as an additive or recessive trait (Figure 2). A dynamic rate of dispersal led to faster increases in the frequency of the $G1_{vir}$ allele compared to a static dispersal rate, however this difference was minimal compared to the effect of including either type of field-to-field dispersal.

Effect of resistance release strategy.

The effectiveness of a resistance release strategy at preventing virulence from developing was dependent upon the size of the refuge, the mode of inheritance, and the initial allele frequency (Table 3). If virulence was inherited as a dominant trait, no combinations of refuge size or release strategy was capable of preventing virulence from developing in under 25 years. If the initial allele frequency of $G1_{vir}$ was high (i.e. 0.10), only the combination of recessive expression of $G1_{vir}$, a pyramid alone release strategy and a relatively large refuge (25% or 35%) prevented virulence from developing in under 25 years.

In all scenarios investigated, a pyramid alone release strategy was most effective at delaying the development of virulence. The addition of one single gene cultivars accelerated the rate at which frequency of the $G1_{vir}$ allele increased. The addition of two single gene cultivars also accelerated the rate of increase of the $G1_{vir}$ allele, however to a lesser degree than one single gene cultivar. However, the pyramid and two single gene cultivars release strategy also placed equal selection pressure on the $G2_{vir}$ allele, whereas the pyramid and one single gene cultivar release strategy did not put additional selection pressure on the $G2_{vir}$ allele.

Effect of insecticidal seed treatments.

For all modes of inheritance and both initial allele frequencies, the way that insecticidal seed treatments were used had an effect on the rate at which virulence developed. For simplicity, only results for recessive inheritance of the $G1_{vir}$ allele are depicted (Figure 3). The general trend displayed was when insecticidal seed treatments were used on all cultivars there was little effect on the rate at which the frequency of virulence alleles increased. A seed treatment on the refuge only accelerated the rate at which the $G1_{vir}$ allele increased. However, a seed treatment to only the resistant cultivars was able to delay the rate at which the frequency of the $G1_{vir}$ allele increased.

Effect of foliar insecticide application to the refuge.

The use of foliar insecticides on the refuge accelerated the rate at which the $G1_{vir}$ allele increased (Figure 4). Only slight differences existed between the IPM and semi-prophylactic usages, however a full prophylactic usage differed greatly from both. A full prophylactic usage greatly accelerated the rate at which $G1_{vir}$ increased, to the extent that virulence developed in >25 years even when inheritance was recessive and the initial allele frequency was low. The difference in the full prophylactic usage compared to the IPM and semi-prophylactic usages was less pronounced when the initial allele frequency was high due to the limited ability of the refuge to delay the evolution of dominant virulence alleles.

Effect of increased efficacy of HPR genes.

Increasing the efficacy of the HPR genes considered increased the rate at which virulence increased in the soybean aphid population (Table 3). In all cases

pyramiding a single HPR gene with a second *Rag* gene delayed the development of virulence. Increased efficacy of transgenes has a negative relationship with the dominance of virulence, with higher efficacy more likely to be associated with recessive virulence genes. The most likely scenarios of inheritance therefore, are 99% efficacy transgenes associated with recessive inheritance and 96% efficacy transgenes associated with additive inheritance. The 96% efficacy transgene with additive inheritance of virulence required a second *Rag* gene to prevent virulence from developing in under 25 years.

Discussion

Researchers estimate that up to one billion dollars could be spent on efforts to control the soybean aphid over a ten-year period (Song and Swinton 2009). Host-plant resistance offers a potentially environmentally friendly and cost-effective management tactic. The development of effective strategies to delay the evolution and proliferation of virulent soybean aphid biotypes is essential to realize the potential of HPR. The soybean aphid will provide a new challenge given its multiple asexual generations, high reproductive capacity and high rate of dispersal.

Results of our model highlight the importance of considering variables that accurately depict the biology of the aphid (field-to-field migration) and other management tactics (foliar insecticide applications) that affect the evolution of virulence. Without the inclusion of these factors an overly optimistic view of the durability of a management strategy can be depicted.

Currently, the only commercially available HPR genes for soybean aphid control are *Rag1* and *Rag2* (McCarville et al. 2013). The models presented here

demonstrate that the widespread release of either of these resistance genes by themselves is unlikely to be a durable management strategy, even if a relatively large refuge (35%) is deployed. The issues of durability coupled with the moderate levels of soybean aphid control these cultivars provide (McCarville et al. 2014), make it unlikely that this strategy can be deployed successfully at a large scale. The release of a pyramid was more successful at delaying virulence and a *Rag1* + *Rag2* pyramid can provide consistent yield protection without the need for chemical insecticides (McCarville et al. 2014). The durability of a pyramid in our model was reduced by the release of single gene cultivars carrying genes present in the pyramid.

The addition of density-independent mortality, mimicking foliar insecticide applications, to our model resulted in an increase in the rate at which virulence alleles accumulated in the population. Currently foliar applications of broad-spectrum insecticides are the most common management tactic used to control soybean aphid populations. Olson et al. (2008) found the majority of growers treated for soybean aphid with broad-spectrum insecticides and most could identify the importance of scouting reports and applying according to integrated pest management guidelines (i.e. economic threshold). However, not all growers followed the integrated pest management guidelines. Our model emphasizes the benefits of limiting insecticide applications to the refuge. For this reason, continued grower education about the use of insecticides according to an economic threshold could limit unnecessary applications to the refuge increasing the durability of HPR.

Insecticidal seed treatments, a management strategy that has been deployed at a large scale, could have large effects on the durability of HPR cultivars. Pan et al. (2011) found seed treatments could have large effects on the durability of *Bt*-corn for western corn rootworm control depending on the efficacy of the seed treatment and its interaction with insect virulence. A seed treatment was applied to only HPR cultivars could delay the build-up of soybean aphid virulence alleles. This strategy may be difficult to implement as insecticidal seed treatments can be used for the control of multiple pests and are often sold in combination with fungicide-seed treatments. A more likely scenario is that seed treatments may be withheld from HPR cultivars due to the view that they are unnecessary for effective soybean aphid control (McCarville and O'Neal 2013) and could negatively affect natural enemies (Moser and Obrycki 2009, Seagraves and Lundgren 2012). The use of seed treatments on only the refuge however, resulted in an increase in the rate of virulence evolution.

In the future, transgenic traits for the control of soybean aphid may become commercially available. Our demonstrate the ability of pyramiding to delay the development of virulence to any future transgenic traits (Table 3). The development of transgenic insect control traits is both time and resource intensive making the release of pyramids with two novel transgenic genes unlikely. Therefore, pyramiding existing *Rag* genes with any future transgenic traits will likely be a successful strategy for delaying virulence. The increasing number of *Rag* genes identified but not yet released in commercial cultivars (Hill et al. 2012) make the production of a pyramid with a transgenic trait and a novel *Rag* gene possible.

The model presented here provides direction for any future large-scale release of HPR soybean cultivars. It is clear from both this model and early studies on the performance of resistant lines (McCarville and O'Neal 2012, McCarville et al. 2014) that a pyramid cultivar can provide improved soybean aphid control while also delaying the adaptation of soybean aphids to this management strategy. With pyramided resistant cultivars the area-wide suppression predicted by Painter (1951) and observed for other pest insects (Hutchinson et al. 2010) may be possible for soybean aphids given the superior control provided by these cultivars. This will however depend on both the rate of adoption and the refuge size necessary to slow the development of virulence. Therefore, research should focus on identifying the current frequency of virulent biotypes in the environment and determining the genetic basis of virulence in the biotype-2 and biotype-3 colonies previously collected from North America.

Acknowledgements

This research was supported in part by the Soybean Checkoff through a grant from the Iowa Soybean Association.

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Table 1. Parameter values used for simulation models

Parameter	Value	Citation
Generations on buckthorn	3	Ragsdale et al. 2004
Generations on soybean	15	Wang et al. 1962, Ragsdale et al. 2004
Initial virulence allele frequency	0.01, 0.10	Michel et al. 2011
Initial population	100,000	
Reproductive potential (buckthorn)	4	
Reproductive potential (soybean)	32	Hirano et al. 1995, McCornack et al. 2004, McCarville et al. 2011
Refuge size	0, 5, 10, 35%	USDA-ERS 2011
Fixed Dispersal rate	5%	Hodgson et al. 2005, Donaldson et al. 2007
Variable Dispersal Rate[a]	19, 2.5, 3, 4, 4, 5, 6, 5, 15.5, 4, 5, 5, 5%	Hodgson et al. 2005
Efficacy of foliar insecticide	99.99%	
Seed treatment efficacy[b]	32.5, 95, 43, 0%	McCornack and Ragsdale 2006, McCarville and O'Neal 2013

[a] Dispersal rates for generations 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 thru 16.
[b] Percent mortality for generations 4, 5, 6, and 7 thru 17.

Table 2. Resistance gene release strategy effects on virulence development

Relative Inheritance	Recessive		Additive		Dominant	
Allele Frequency[a]	0.01	0.1	0.01	0.1	0.01	0.1
Release Strategy[b]						
5% Refuge						
1 Single Gene	6 [c]	1	3	1	2	1
2 Single Genes	11	2	6	2	2	1
Pyramid	>25 (0.01)[d]	10	>25 (0.02)	6	4	1
Pyramid + 1 Single	10	2	5	2	2	1
Pyramid + 2 Single	15	2	8	2	2	1
10% Refuge						
1 Single Gene	9	2	5	1	2	1
2 Single Genes	19	3	8	2	3	1
Pyramid	>25 (0.01)	17	>25 (0.02)	9	5	1
Pyramid + 1 Single	18	2	8	2	2	1
Pyramid + 2 Single	>25 (0.10)	3	12	3	3	1
25% Refuge						
1 Single Gene	22	3	8	2	3	1
2 Single Genes	>25 (0.02)	5	16	2	4	2
Pyramid	>25 (0.01)	>25 (0.16)	>25 (0.02)	15	10	2
Pyramid + 1 Single	>25 (0.02)	4	15	3	4	1
Pyramid + 2 Single	>25 (0.02)	6	22	5	5	2
35% Refuge						
1 Single Gene	>25 (0.04)	3	10	2	3	1
2 Single Genes	>25 (0.02)	7	22	5	5	3
Pyramid	>25 (0.01)	>25 (0.13)	>25 (0.02)	20	14	3
Pyramid + 1 Single	>25 (0.02)	6	20	5	5	2
Pyramid + 2 Single	>25 (0.01)	9	>25 (0.13)	6	6	2

[a] Initial frequency of $G1_{vir}$ allele.
[b] Deployment strategy of G1 and G2 resistance genes.
[c] Years until the $G1_{vir}$ frequency exceeded 0.25
[d] For scenarios in which the frequency of $G1_{vir}$ did not exceed 0.25 in the first 25 years, the allele frequency after 25 years is displayed in parenthesis.

Table 3. Resistance gene efficacy effect on virulence development

Relative Inheritance	Recessive	Additive	Dominant
Release Strategy			
Rag gene	>25[a] (0.04)[b]	11	3
Rag gene + *Rag* gene	>25 (0.01)	>25 (0.02)	14
96% Efficacy Transgene	>25 (0.09)	15	3
96% Efficacy Transgene + *Rag* gene	>25 (0.01)	>25 (0.04)	12
99% Efficacy Transgene	>25 (0.09)	15	3
99% Efficacy Transgene + *Rag* gene	>25 (0.01)	>25 (0.04)	12

[a] Years until the $G1_{vir}$ frequency exceeded 0.25.

[b] For scenarios in which the frequency of $G1_{vir}$ did not exceed 0.25 in the first 25 years, the allele frequency after 25 years is displayed in parenthesis.

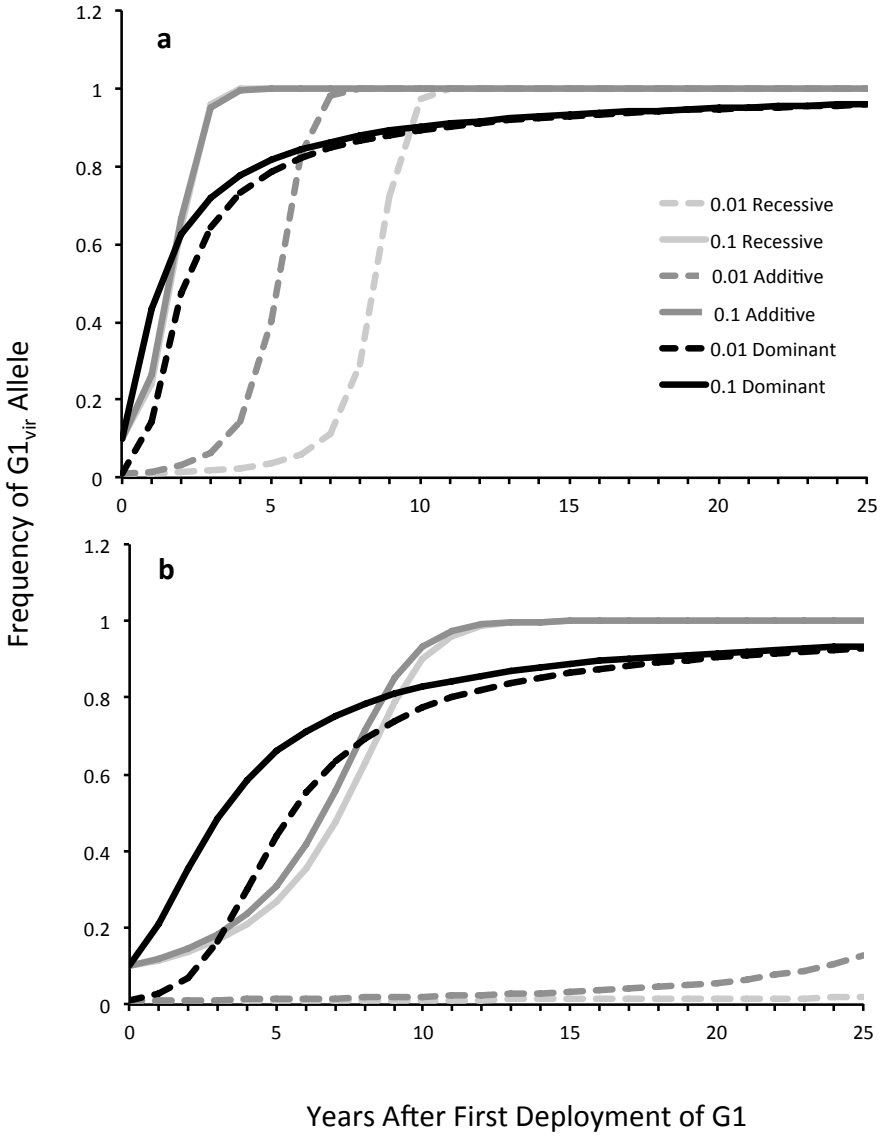

Figure 1. The frequency of the $G1_{vir}$ allele as affected by dominance of inheritance and initial allele frequency. The G1 resistance gene was released as a single gene cultivar grown with either a (a) 5% refuge or (b) 35% refuge. The y-axes are extended beyond 1.0 to allow the reader to discern the endpoints of each line.

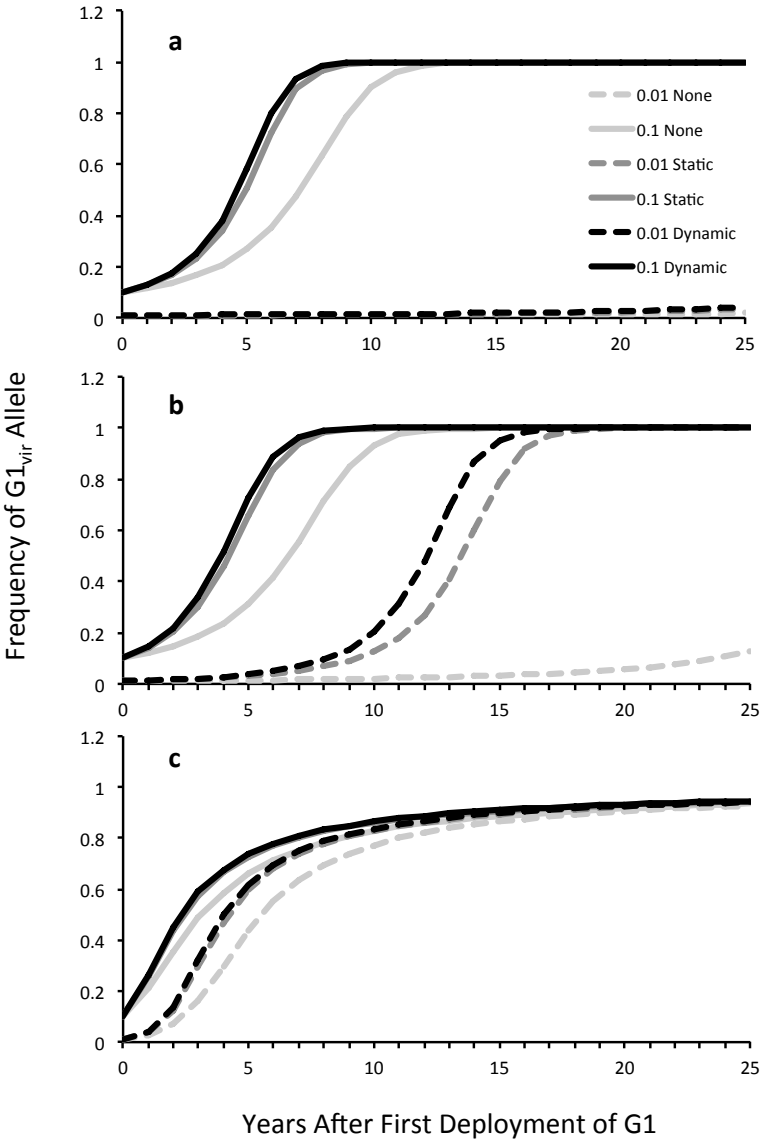

Figure 2. The effect of within season field-to-field dispersal on the frequency of the $G1_{vir}$ allele is depicted. Three rates of dispersal were analyzed, no dispersal, a static rate of 5%, and a dynamic rate that fluctuated from 2.5% to 19%. The resistance gene *Rag1* was released as single gene cultivars grown with a 35% refuge. Dominance of virulence was varied as (a) recessive, (b) additive, (c) or dominant. The y-axes are extended beyond 1.0 to allow the reader to discern the endpoints of each line.

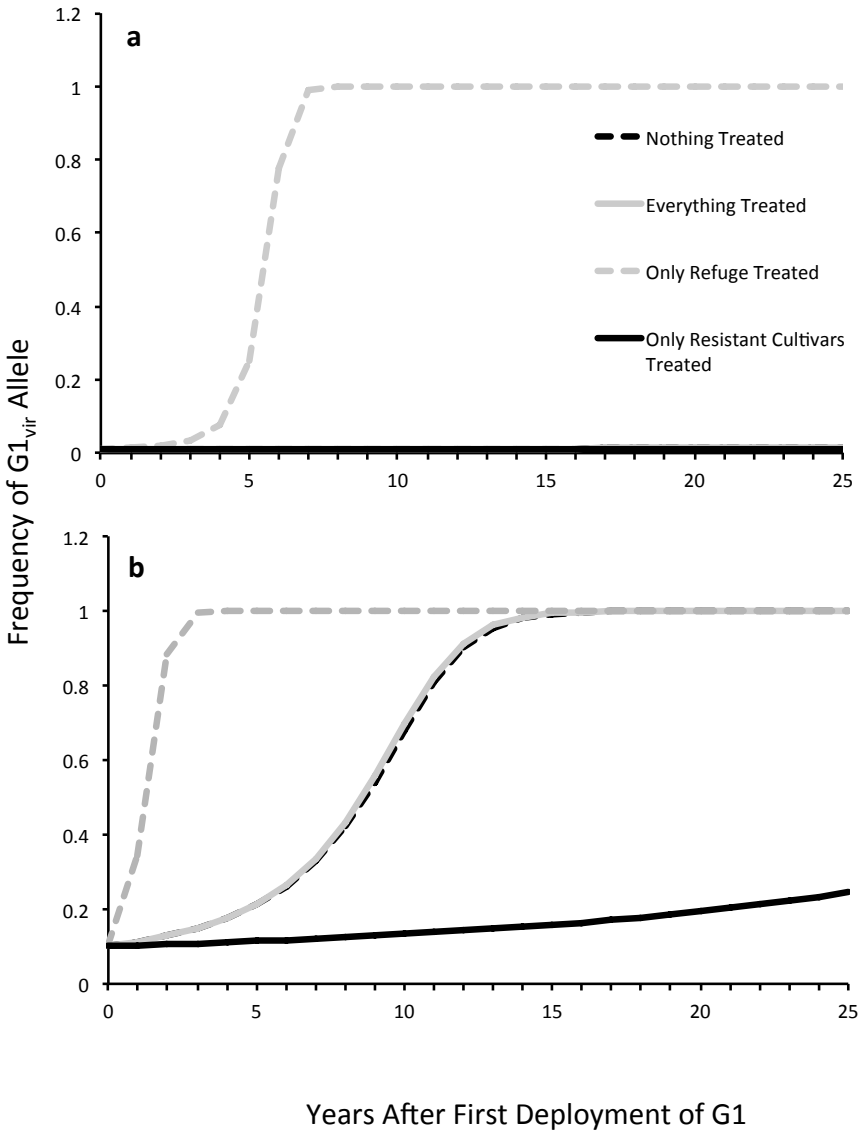

Figure 3. The effect of insecticidal seed treatments on the frequency of $G1_{vir}$ allele. The G1 resistance gene was released in both a pyramid with the G2 gene and as a single gene cultivar, with 35% of the crop land devoted to a refuge. Inheritance of the $G1_{vir}$ allele was recessive with an initial allele frequency of (a) 0.01 or (b) 0.1. Seed treatments were either not used, applied to both the resistant cultivars and refuge, the refuge only, or only the resistant cultivars. The y-axes are extended beyond 1.0 to allow the reader to discern the endpoints of each line.

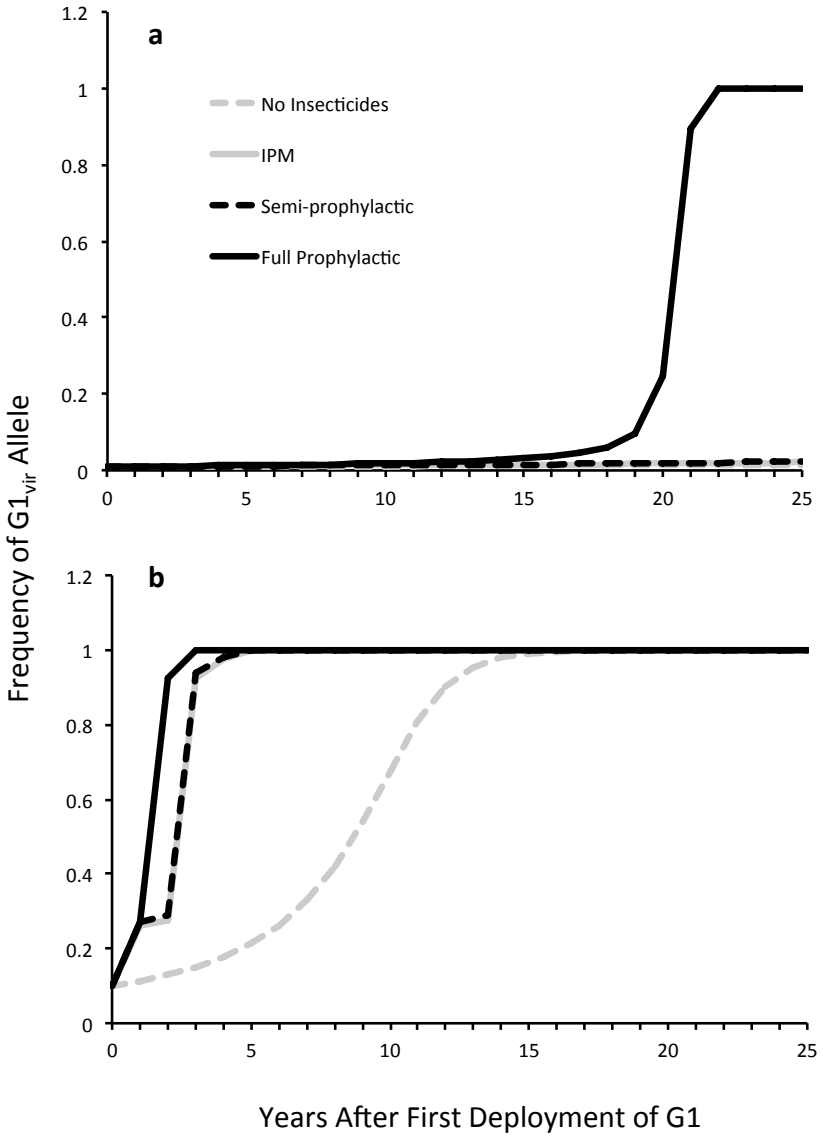

Figure 4. The effect of foliar insecticide use on the refuge crop on the frequency of the $G1_{vir}$ allele. The G1 resistance gene was released in a pyramid cultivar and a the $G1_{vir}$ allele was recessive with an initial allele frequency of (a) 0.01 or (b) 0.1. The refuge was left untreated (no insecticides), 40% of it treated every second year (IPM), 80% of it treated every second year (semi-prophylactic), or 80% treated every year (full prophylactic). The y-axes are extended beyond 1.0 to allow the reader to discern the endpoints of each line.

CHAPTER 6. ABOVEGROUND FEEDING BY SOYBEAN APHID, *APHIS GLYCINES*, AFFECTS SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, REPRODUCTION BELOWGROUND

A paper published in the journal *PLOS ONE* 9: e86415.

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Abstract

Heterodera glycines is a cyst nematode that causes significant lost soybean yield in the U.S. Recent studies observed the aphid *Aphis glycines* and *H. glycines* interacting via their shared host, soybean, *Glycine max*. A greenhouse experiment was conducted to discern the effect of *A. glycines* feeding on *H. glycines* reproduction. An *H. glycines*-susceptible cultivar, Kenwood 94, and a resistant cultivar, Dekalb 27-52, were grown in *H. glycines*-infested soil for 30 and 60 d. Ten days after planting, plants were infested with either zero, five, or ten aphids. At 30 and 60 d, the number of *H. glycines* females and cysts (dead females) and the number of eggs within were counted. In general, *H. glycines* were less abundant on the resistant than the susceptible cultivar, and *H. glycines* abundance increased from 30 to 60 d. At 30 d, 33% more *H. glycines* females and eggs were produced on the resistant cultivar in

the ten-aphid treatment compared to the zero-aphid treatment. However, at 30 d the susceptible cultivar had 50% fewer *H. glycines* females and eggs when infested with ten aphids. At 60 d, numbers of *H. glycines* females and cysts and numbers of eggs on the resistant cultivar were unaffected by *A. glycines* feeding, while numbers of both were decreased by *A. glycines* on the susceptible cultivar. These results indicate that *A. glycines* feeding improves the quality of soybean as a host for *H. glycines*, but at higher herbivore population densities, this effect is offset by a decrease in resource quantity.

Keywords: integrated pest management, soybean, plant-mediated interactions

Introduction

Crop production is at risk for yield loss from both aboveground and belowground herbivores that can occur concurrently and interact through a shared host plant [1], [2]. Recent reviews of aboveground-belowground herbivore interactions have hypothesized that both plant nutrients and common plant defense pathways are important mediators of herbivore interactions [3]-[5]. General hypotheses are proposed including the importance of study location (i.e. field versus greenhouse) [1], feeding guild similarity [3], herbivore arrival time [1], and infestation intensity [3] in determining the outcome of the interaction for each herbivore.

Belowground plant-parasitic nematodes are important yield-reducing pathogens of all major field crops produced in the U.S. [6]. However, their effect on aboveground insects has been sparingly studied, and the reciprocal effect of aboveground insects on nematodes is even less well studied [1]. Johnson *et al.* [1] conducted a meta-analysis that included 123 observations that investigated the

interaction between aboveground and belowground herbivores, of which only 11 observations included nematodes. Overall, plant-parasitic nematodes had no observable effect on the performance of aboveground insects, and the reciprocal effect (aboveground herbivores on nematodes) was not examined in the meta-analysis. To what extent the general pattern of above- and belowground herbivores predicts the interaction between nematodes and aboveground herbivores is not known.

Aboveground-belowground herbivore interactions are of particular importance for soybean, *Glycine max* (L.) Merrill, because the crop is challenged by a belowground herbivore, the soybean cyst nematode, *Heterodera glycines* Ichinhoe, and a diverse community of aboveground insect herbivores. *Heterodera glycines* is the leading yield-reducing pathogen of soybean both in the U.S. and worldwide [7], [8]. In the U.S., *H. glycines* is widely distributed throughout all major soybean-producing regions and causes an estimated yield loss of $1.8 billion each year [7]. The population density of *H. glycines* eggs in the soil at the beginning of the growing season is the strongest predictor of yield loss [9]-[12]. Population densities are managed by growing non-hosts (i.e. crop rotation) or *H. glycines*-resistant soybean cultivars. However, crop rotations often consist of two-year rotations, and *H. glycines*-resistant soybean cultivars are mostly derived from a single source of resistance, PI 88788 [13]. Therefore, populations of *H. glycines* persist within the agroecosystem, with infestations of *H. glycines* occuring in 47-83% of fields in the major soybean-producing region of the Midwestern U.S. [14].

Previous research observed that aboveground lepidopteran herbivores were capable of increasing *H. glycines* reproduction on soybean [15], [16]. These studies, however, were conducted using only *H. glycines*-susceptible cultivars and lepidopteran herbivores, which are rarely economic soybean pests in the Midwestern U.S. [17]. In this region, the invasive soybean aphid, *Aphis glycines* Matsumura, colonizes fields during early vegetative stages of soybean development with population densities increasing through the reproductive stages of the crop, leading to yield losses of up to 40% in outbreak years [18]. The co-occurrence of *H. glycines* and *A. glycines* is an intriguing system to study as plant-parasitic nematode and aphid infestations both result in changes in induced defense responses and primary plant metabolites [19].

Research exploring the co-occurrence of *A. glycines* and *H. glycines* on soybean suggests that an interaction may occur, but the results of these studies have been incomplete [11], [20] - [22]. *Heterodera glycines* infections are proposed to increase [22], decrease [20], and have no effect on *A. glycines* populations [11], [21]. Furthermore, *H. glycines* infections reduce *A. glycines* alate preference for soybean plants [11], [22]. In addition, *A. glycines* infestations are suggested to both increase [20] and have no effect [21] on *H. glycines* reproduction. Discrepancies in these reports may be due to differences in field versus laboratory settings of the experiments [11], [22], pest population densities, and the inclusion of other pest species in the experimental treatments [20], [21].

McCarville *et al.* [20] and Heeren *et al.* [21] used similar field micro-plots to investigate the effect of *A. glycines* feeding on *H. glycines* reproduction over the

course of the entire season. McCarville *et al.* [20] measured *H. glycines* reproduction on soybean infected with either *H. glycines* alone or with *H. glycines, A. glycines,* and *Cadophora gregata* Harrington and McNew, the causal agent of brown stem rot disease. They observed a 500% increase in *H. glycines* reproduction on soybean infested with all three pests. This increase was observed on both *H. glycines*-susceptible cultivars and *H. glycines*-resistant cultivars with the PI 88788 source of resistance. However, McCarville *et al.* [20] did not include a treatment in which plants were exposed to only *H. glycines* and *A. glycines*, and, therefore, could not discern whether the increase in *H. glycines* reproduction was due solely to *A. glycines* feeding.

Heeren *et al.* [21] included treatments in which soybean plants were exposed to *H. glycines* alone or to both *H. glycines* and *A. glycines*, thus making direct observations on the interaction between *A. glycines* and *H. glycines* possible. They did not observe an effect of *A. glycines* feeding on *H. glycines* reproduction. However, their study utilized much lower pest population densities than McCarville *et al.* [20], and in the case of *H. glycines*, densities were often below the limit of detection. Given the discrepancies in pest treatments studied (*A. glycines* alone or in combination with *C. gregata*) and pest population densities utilized between McCarville *et al.* [20] and Heeren *et al.* [21], our goal was to determine whether *A. glycines* feeding by itself could affect *H. glycines* reproduction. In addition we explored whether the population densities of both *A. glycines* and *H. glycines*, which vary widely across the North Central U.S., affect the outcome of the interaction.

Materials and Methods

In a greenhouse, we manipulated the density of *H. glycines* populations through the use of resistant and susceptible soybean cultivars and *A. glycines* populations through the use of different initial infestation densities. In addition to *H. glycines*-resistant and susceptible cultivars and differential *A. glycines* infestation densities (both described below), pest densities examined were also manipulated by conducting the experiment for different lengths of time. Half of all plants were harvested at 30 d to measure treatment effects on a single generation of *H. glycines* reproduction, and the remaining plants were harvested at 60 d to measure treatment effects after two generations of *H. glycines* reproduction.

For these experiments, a modified version of the Standard Cyst Evaluation-2008 (SCE-08) protocol was utilized [23], in which 125-ml cone-tainers (Stuewe & Sons, Tangent, OR) were arranged in 7.5-l sealed plastic buckets filled with construction sand. The buckets were kept in a water bath to maintain a constant soil temperature between 26.7°C and 28.9°C, which allows for the completion of a single generation of *H. glycines* in approximately 25 d [24].

Cone-tainers were filled with 100 ml of a soil-sand mixture created by adding construction sand to *H. glycines*-infested Eolian Sand type soil. The *H. glycines* population was HG type 0, which is defined by having less than 10% reproduction on all published sources of *H. glycines* resistance (i.e. avirulent to all *H. glycines* resistance genes) [25] and was chosen for its limited ability to reproduce on the PI 88788-derived resistant cultivar utilized in our experiment. Eolian Sand type soil (a fine silt type soil with a high sand content) was used as it consistently permits

high *H. glycines* reproduction in the field [26] and is easily washed from soybean roots permitting efficient collection of *H. glycines* females and cysts. The soil was diluted with construction sand to obtain a soil-sand mixture with an approximate population density of 1,000 eggs 100 ml^{-1} of soil. This population density was selected to reduce the likelihood of competition among *H. glycines* females for the nutritional resources of soybean plants. Plants were grown under natural lighting supplemented with 16:8 (L:D) 400 W high-pressure sodium growth lamps and watered as needed.

Two soybean cultivars were used for the experiment, Kenwood 94 and Dekalb 27-52. Kenwood 94 is a *H. glycines*-susceptible cultivar and Dekalb 27-52 is a PI 88788-derived *H. glycines*-resistant cultivar that was used in the field experiment by McCarville *et al.* [20]. In addition to the two soybean cultivars, we used three initial aphid population densities in both the 30 d and 60 d time periods. Aphid treatments were defined by the initial population of *A. glycines* added to each plant (zero aphids, five aphids, and ten aphids). The treatment factors of soybean cultivar and aphid density were fully crossed to create six total treatment combinations per time period. These treatments were arranged in a split-plot design, with the whole plots arranged in a randomized complete block design. It was possible to prevent *A. glycines* from moving between buckets but not between cone-tainers within a bucket, so the whole plot was an individual 7.5-l bucket, with the treatment factor of aphid density assigned to the whole plot. Each bucket contained eight cone-tainers, four per soybean cultivar. Each of the four cone-tainers per soybean cultivar was randomly assigned to one of the two time periods (i.e. 30 d and 60 d). Data were

analyzed separately for each time period. Therefore, the split-plot was considered a group of two cone-tainers from the same time point containing the same soybean cultivar, with each individual cone-tainer considered a subsample (two subsamples per split-plot). We conducted three separate runs of the experiment with eight blocks in each of the first two runs, and four blocks in the third. In the third run, all eight cone-tainers in each bucket were allocated to the 30 d group as sufficient statistical power had been achieved in the first two runs of the experiment to test our hypotheses involving the 60-d treatments.

Aphid-density treatments were applied to whole plots when plants reached the first trifoliate or V1 stage [27], which occurred 10 d after planting. Mixed-aged apterous *A. glycines* were transferred from a greenhouse biotype-1 colony (i.e. avirulent to all known *A. glycines* resistance genes, Hill *et al.* [28]) to each plant assigned to the five-aphid and ten-aphid treatments. Each whole plot bucket was then covered with a modified paint strainer (Trimaco, Morrisville, NC) to prevent the movement of aphids among whole plots. *Aphis glycines* populations were then allowed to increase for the remainder of the experiment.

Cone-tainers in the 30-d group were harvested from each whole plot at 30 d after planting, and data were collected as described below. Plants assigned to the 60-d group were transferred with all the soil within their respective 125-ml cone-tainers to 650-ml cone-tainers (Stuewe & Sons, Tangent, OR) after 30 d. The new cone-tainers then were filled to 650 ml with the addition of *H. glycines*-infested soil-sand mixture and placed back into the water bath. These larger cone-tainers prevented soybean roots from becoming tangled and pot bound before the plants

were harvested at 60 d, allowing for easier extraction of *H. glycines* females and cysts from roots.

All *A. glycines*, both nymphs and adults, were counted for each plant before the root mass of each plant was soaked in water to dislodge the soil. Roots were sprayed with pressurized water to dislodge *H. glycines* females and cysts, which were captured on a 250-µm-pore sieve positioned below a 850-µm-pore sieve. The total number of females and cysts recovered from each plant was counted under a dissecting microscope. Females and cysts were then ground on a 250-µm-pore sieve using a motorized rubber stopper [29], and released eggs were recovered on a 25-µm-pore sieve nested below a 75-µm-pore sieve. Eggs were suspended in 100 ml of water, and the number of *H. glycines* eggs present in a representative 1-ml sample of solution was counted under a dissecting microscope. The total number of *H. glycines* eggs recovered from each plant was calculated.

Data Analyses

Data collected from the 30-d and 60-d groups of plants were analyzed separately using analysis of variance (ANOVA) with a mixed effects model. The model included the fixed effects of experimental run, block, aphid density, and soybean cultivar. The interactions of run*block, run*aphid density, block*aphid density, and aphid density*soybean cultivar were included as fixed effects. The whole-plot error term of run*block*aphid density was included as a random effect, along with the effect of subsample (i.e. plant nested within aphid density*soybean cultivar). This model allowed us to assess the effects of soybean cultivar, aphid

density, and their interaction on the total number of *H. glycines* females and cysts and eggs produced plant^{-1}.

The number of *H. glycines* females and cysts plant^{-1} and eggs plant^{-1} were log transformed to meet the assumptions of ANOVA (non-transformed data are presented in all figures). These data were analyzed to determine if soybean cultivar, aphid density, or their interaction affected the number of *H. glycines* females and cysts present or the number of eggs they produced.

Based on the results of our initial analyses, we hypothesized that the effect of *A. glycines* on *H. glycines* reproduction varied with the population density of *H. glycines*. Despite our attempts to limit competition by using an initial low *H. glycines* population density, the numbers of *H. glycines* measured at 30 d and 60 d were high enough to suggest that competition may have occurred among *H. glycines* females. We hypothesized that the competition among *H. glycines* females would be increased with the addition of *A. glycines*. To test this hypothesis, we plotted the effect of *A. glycines* feeding on *H. glycines* population densities across the average *H. glycines* population density in the three aphid treatments. We calculated the effect of *A. glycines* feeding as the percent change in *H. glycines* population densities between the ten-aphid treatment mean and zero-aphid treatment mean for each combination of cultivar and time period (4 total data points). The ten-aphid treatment was selected because it generally represented the strongest effect of *A. glycines* feeding on *H. glycines* reproduction. We plotted the *H. glycines* females and cysts plant^{-1} and eggs plant^{-1} data separately.

Results

Aphis glycines populations

Mean *A. glycines* population densities plant^{-1} (± SEM) among the ten-, five-, and zero-aphid density treatments were 278 ± 24, 225 ± 16, and 3 ± 1, respectively for the 30-d group. Upon transfer of the 60-d group plants from the 125-ml cone-tainers to the 650-ml cone-tainers, there were only a few aphids on plants in the zero-aphid treatment. These aphids were removed before the nets were placed back over the buckets. At the conclusion of the 60-d group, *A. glycines* population densities in the ten- and five-aphid treatments had declined to 54 ± 10 aphids plant^{-1} and 99 ± 14 aphids plant^{-1}, respectively.

Heterodera glycines population density at 30 d

Numbers of *H. glycines* females plant^{-1} for the 30-d group varied significantly by experimental run (F = 6.27; df = 2,33; P = 0.0049), cultivar (F = 619.06; df = 1,18; P <0.0001), and the interaction of aphid density*cultivar (F = 6.18; df = 2,18; P = 0.0090). Consequently, the analysis was performed by cultivar to discern the effect of aphid density on the number of *H. glycines* females plant^{-1}. On the *H. glycines*-susceptible cultivar, the aphid density treatment factor had a marginally significant effect (F = 3.59; df = 2,9; P = 0.0715) on numbers of females plant^{-1} with *H. glycines* population densities decreasing with increasing aphid density (Fig. 1). On the resistant cultivar, numbers of females plant^{-1} varied significantly by experimental run (F = 11.06; df = 2,11; P = 0.0023) and by aphid density (F = 4.57; df = 2,9; P = 0.0428). The number of *H. glycines* females plant^{-1} increased as aphid density

increased on the resistant cultivar, with a 28% increase in numbers of females between the zero-aphid density and ten-aphid density treatments (Fig. 1).

The number of *H. glycines* eggs plant^{-1} for the 30-d group responded similarly to the treatment effects as the number of females plant^{-1}. Eggs plant^{-1} varied significantly by experimental run (F = 15.55; df = 2,33; P <0.0001), cultivar (F = 1,129.61; df = 1,18; P <0.0001), and the interaction of aphid density*cultivar (F = 7.06; df = 2,18; P = 0.0055). For the susceptible cultivar, numbers of *H. glycines* eggs plant^{-1} varied significantly by experimental run (F = 4.68; df = 2,11; P = 0.0338) and the variation in numbers was marginally significant for aphid density (F = 3.41; df = 2,9; P = 0.0790), with the number of *H. glycines* eggs plant^{-1} decreasing with increasing aphid density (Fig 1). For the resistant cultivar, numbers of eggs plant^{-1} varied by experimental run (F = 17.64; df = 2,11; P = 0.0004) and by aphid density (F = 4.25; df = 2,9; P = 0.0502), with the number of *H. glycines* eggs plant^{-1} increasing with increasing aphid density. We observed a 34% increase in eggs on resistant plants initially infested with 10 aphids compared to those assigned to the zero-aphid treatment (Fig. 1).

***Heterodera glycines* population density at 60 d**

Numbers of *H. glycines* females and cysts plant^{-1} were affected by cultivar (F = 121.76; df = 1,8; P <0.0001), and there was a significant aphid density*cultivar interaction (F = 4.60; df = 2,8; P = 0.0469) for the 60-d group. Consequently, the analysis was performed separately for each cultivar. Aphid density had a significant effect on the numbers of *H. glycines* females and cysts on the susceptible cultivar, with fewer *H. glycines* females and cysts plant^{-1} produced with increasing aphid

density (F = 5.36; df = 2,8; P = 0.0333) (Fig. 2). Aphid density did not affect the number of females and cysts produced on the resistant cultivar (F = 0.77; df = 2,8; P = 0.4950).

Results from the analysis of *H. glycines* eggs plant^{-1} at 60 d were similar to those obtained from the analysis of females and cysts plant^{-1}. The number of eggs plant^{-1} varied significantly by cultivar (F = 128.72; df = 1,8; P < 0.0001), but not significantly by aphid density (F = 1.71; df = 2,8; P = 0.2414). The interaction of aphid density*cultivar was marginally significant (F = 3.56; df = 2,8; P = 0.0784). The number of eggs plant^{-1} on the susceptible cultivar varied marginally with aphid density (F = 3.94; df = 2,8; P = 0.0643), but did not vary by aphid density on the resistant cultivar (F = 0.45; df = 2,8; P = 0.6521). Overall, the number of eggs plant^{-1} decreased with increasing aphid density on the susceptible cultivar at 60 d (Fig. 2).

Effect of *Heterodera glycines* population density

Our data summary analyses of both numbers of *H. glycines* females and cysts plant^{-1} and eggs plant^{-1} revealed that the effect of *A. glycines* feeding on *H. glycines* reproduction was highly dependent on the population density of *H. glycines* (Fig. 3). The trend suggested that as *H. glycines* population densities increased due to either soybean cultivar (susceptible versus resistant) or number of generations (60 d versus 30 d), increasingly negative effects of *A. glycines* feeding on *H. glycines* reproduction were observed. However, at the lowest *H. glycines* population (resistant cultivar at 30 d), *A. glycines* feeding increased *H. glycines* reproduction.

Discussion

In our experiment, *A. glycines* feeding significantly affected reproduction of

H. glycines. However, the outcome of this interaction varied significantly with the cultivar and length of experiment. In the 30-d experiment, we observed increased *H. glycines* reproduction on the *H.* glycines-resistant cultivar and decreased reproduction on the susceptible cultivar in response to *A. glycines* feeding. In the 60-d experiment, we again observed decreased *H. glycines* reproduction in response to *A. glycines* feeding on the susceptible cultivar, however we did not observe any effect on the resistant cultivar. We believe the differences in the effect of *A. glycines* feeding on *H. glycines* reproduction to be due to differences in overall pest population densities as mediated by soybean cultivar and experiment length. Support for this conclusion can be found both in the results of the final regression analyses and the *A. glycines* population density data. Our summary analyses (Fig 3.) indicate that higher numbers of *H. glycines* females increased the severity of competition experienced by females upon the addition of *A. glycines* to plants. Competition for limited plant resources may also explain the decline in *A. glycines* population densities from 30 d to 60 d in both the five- and ten-aphid treatments. Soler *et al.* [3] predicted that the population density of herbivores, especially phloem-feeders, a feeding guild that includes aphids and nematodes, would affect the outcome of interactions with other herbivores. More specifically, Soler *et al.* [3] predicted that facilitation would occur at lower herbivore densities and competition at higher densities. The results of this experiment provide evidence supporting this hypothesis. In contrast, Johnson *et al.* [30] found that increasing durations of aphid infestations, and therefore increasing population densities, did not diminish the positive effect of aphid feeding on belowground wireworms. This discrepancy may

be a result of the aphid population densities in the Johnson *et al.* [30] experiment not reaching a threshold to induce competition among the wireworms, or it may be due to a difference in how belowground chewing herbivores (i.e. wireworms) and belowground piercing-sucking herbivores (i.e. *H. glycines*) respond to increasing aphid population densities.

Aboveground lepidopteran herbivores are reported to affect belowground plant-parasitic nematodes in soybean [15], [16], [31], with the strength of the effect influenced by insect population density [16], [31]. Generally, these studies reported increasing nematode reproduction for both *H. glycines* and root-knot nematodes (*Meloidogyne* spp.) in response to increasing insect density or damage. This effect is counter to our observation of a variable response of the nematode to increasing aphid population density. This difference in trends may be due to differences in pest population densities in the experiments and the magnitude of their subsequent effect on plant quality, or it may be due to a difference in the resources utilized by the different insect feeding guilds. Both *A. glycines* and *H. glycines* feed from vascular plant tissue, increasing the likelihood for resource competition to occur, whereas lepidopteran herbivores feed on foliage. Therefore *H. glycines* and *A. glycines* could affect each other's performance both through the removal of shared nutritional resources and activation of related defense pathways [32], [33].

In more recent research, conflicting results concerning the effect of *A. glycines* feeding on the reproduction of *H. glycines* are reported. McCarville *et al.* [20] found that simultaneous infestations of *A. glycines* and the causal agent of brown stem rot disease, *C. gregata*, increased *H. glycines* reproduction. However,

Herren *et al.* [21] reported that *H. glycines* reproduction was unaffected by the presence of *A. glycines*. Both of these experiments used small, field micro-plots to measure *H. glycines* reproduction in response to artificial infestations of *A. glycines.* Therefore, it is worth comparing these two experiments to frame the results of our current greenhouse experiment.

McCarville *et al.* [20] observed *H. glycines* reproduction to be 5.24x greater on both *H. glycines*-resistant and susceptible cultivars when plants were also co-infected with *A. glycines* and *C. gregata* compared to plants infected with *H. glycines* alone. This observation was taken from soybean plants infected with *C. gregata* at planting and later infested with *A. glycines* at the early vegetative V3 stage and then comparing end-of-season *H. glycines* egg population densities to beginning-of-season population densities. Therefore, this increase in *H. glycines* reproduction was measured across an entire growing season. In our current experiment, we measured *H. glycines* egg production to be 1.34x greater in the presence of *A. glycines* on the resistant cultivar after 30 d. The 30-d period was a measurement of a single generation of *H. glycines* reproduction. In the U.S., *H. glycines* can complete three to six generations per year [34]. If the 1.34x increase we observed after 30 d occurred across all six generations in the field, we would expect to see a 5.79x increase for the entire year, which is consistent with the findings reported by McCarville *et al.* [20], suggesting that *A. glycines* feeding was primarily or solely responsible for the observed increase in *H. glycines* reproduction in that field micro-plot experiment. It is also noteworthy that, although *H. glycines* resistant and susceptible cultivars supported significantly different *H. glycines* populations in McCarville *et al.* [20],

these populations responded similarly to *A. glycines* feeding (i.e. *H. glycines* population densities increased). Therefore, *A. glycines*-mediated competition for resources with *H. glycines* may not occur in the field due to the lower *H. glycines* population densities present in field environments. Supporting this conclusion are the *H. glycines* egg population densities we observed in the current experiment, 55,941 and 91,209 eggs 100cc soil^{-1} in the 30-d and 60-d SCN-susceptible cultivar treatments, respectively, and the average end-of-season *H. glycines* egg population densities in Iowa soybean fields, 2,438 eggs 100cc soil^{-1} (maximum 34,975 eggs 100cc soil^{-1}) [13], [35]-[41]). This conclusion is consistent with the findings of Johnson *et al.* [1], specifically that negative effects of aboveground herbivores on belowground herbivores are more likely to be observed in laboratory studies than field studies.

Heeren *et al.* [21] manipulated the population densities of both *H. glycines* and *A. glycines* using a full factorial treatment arrangement of resistant and susceptible lines (i.e. susceptible to both, resistant to both, resistant to *A. glycines*, and resistant to *H. glycines*). They did not detect an effect of *A. glycines* feeding on *H. glycines* reproduction on any of the soybean lines. This result may be due, at least in part, to the extremely low pest population densities present in their study, including <100 *H. glycines* eggs 100cc soil^{-1} and <100 cumulative aphid days (i.e. <10 aphids plant^{-1} for <10 d) for some soybean lines.

Given the results of our current experiment and the previous results of McCarville *et al.* [20] and Heeren *et al.* [21], we propose the following model to explain the effect of *A. glycines* on *H. glycines* reproduction. *Aphis glycines* feeding

increases the quality of soybean as a host for *H. glycines* through the manipulation of plant defenses [33] and/or a change in nutrient content [42]. An estimated 28-56% of *H. glycines* juveniles that penetrate susceptible plants reach adulthood [43], [44]. We propose that *A. glycines* feeding increases the percentage reaching adulthood irrespective of the cultivar's resistance to *H. glycines*. At the 30-d time point in our experiments, *H. glycines* females which reached adulthood would have established their feeding site before aphids were added to plants. Therefore, *A. glycines* did not affect juvenile *H. glycines* penetration or feeding site establishment. *Aphis glycines* increased numbers of *H. glycines* females and eggs, but had no effect on fecundity or eggs female^{-1} (analysis not shown). Therefore, the effect of increased *H. glycines* reproduction observed in our experiment was likely due to an increased number of females. This increase could be through an increased ability of the nematodes to obtain nutrients from the feeding site (i.e. change in nutrient content) or to sustain the feeding site (i.e. change in plant defenses). If an increase in numbers of *H. glycines* females is due to a change in plant defense, this is likely due to a suppression by *A. glycines* of a broad-based, general plant defense to nematodes that is not mediated by *rhg* genes. This interaction, however, is density dependent, with *A. glycines* increasing *H. glycines* reproduction at all pest densities except at very low *A. glycines* population densities (<10 aphids plant^{-1}), where aphid feeding has no effect on *H. glycines* reproduction (see [21]), or at high pest population densities (see Fig. 3), where *A. glycines* and *H. glycines* compete for limited nutritional resources.

Going forward, it will be essential to determine under what range of field conditions *A. glycines* feeding leads to an increase in *H. glycines* reproduction or competition with *H. glycines*. It is also necessary to determine whether abiotic factors, such as drought, soil pH, or soil nutrient content can affect the outcome of the interaction indirectly by mediating host plant quality. Finally, given the widespread distribution of both *A. glycines* and *H. glycines* and the economic significance of both pests, it will be important to explore the need for an integrated management approach that mitigates yield reductions that occur both from *A. glycines* removing plant nutrients and from increasing the population density of *H. glycines*. Therefore, a multi-location field study is warranted to investigate this potentially significant aboveground-belowground interaction across a diversity of aphid population densities and infestation timings, nematode population densities, and abiotic conditions.

Acknowledgements

We thank Kayla Mills, Taylor Mordhorst, Tom Klunker, Cody Kuntz, and Adam Varenhorst for assistance with planting and estimating pest densities. We also thank Drs. Ian Kaplan and Scott Johnson for their helpful edits and suggestions, which improved this manuscript.

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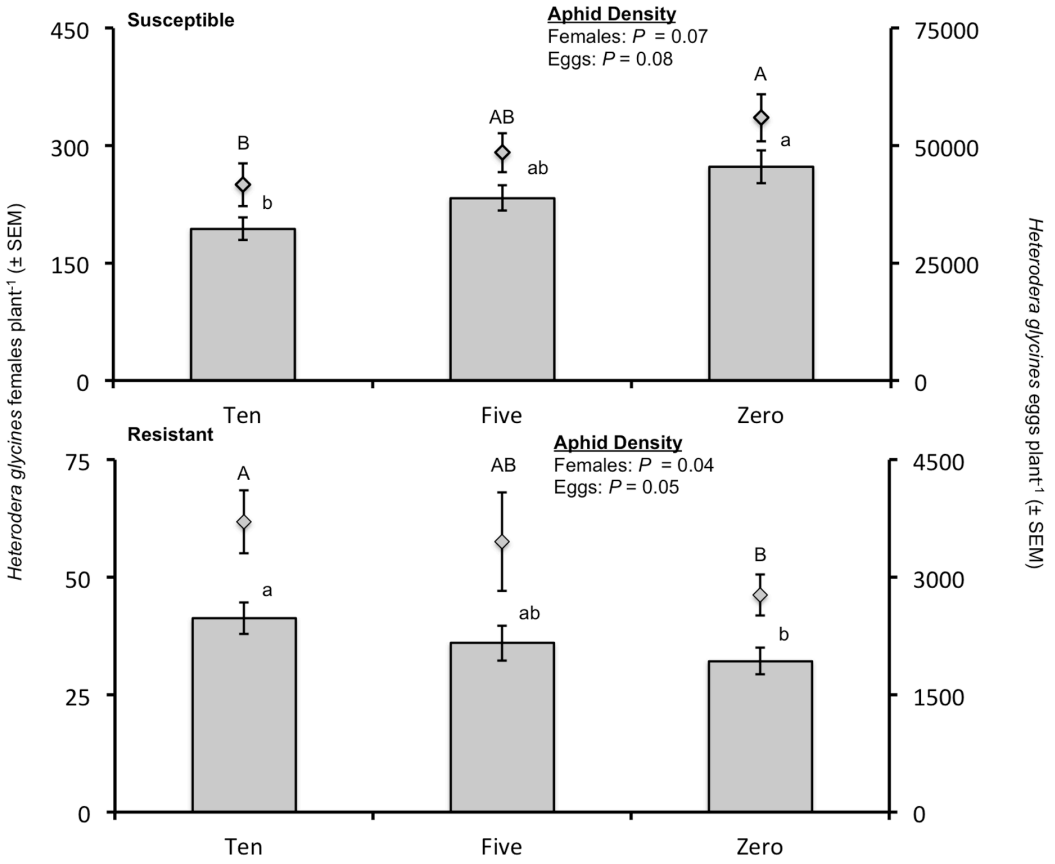

Figure 1. Numbers of *Heterodera glycines* females and eggs recovered plant^{-1} from the *H. glycines*-susceptible soybean cultivar Kenwood 94 and the resistant cultivar Dekalb 27-52 after 30 d. Numbers of females are represented by bars with numbers of eggs represented by boxes above the bars. Note the difference in scales used for the two graphs. Three aphid density treatments were established by artificially infesting plants with zero, five, or ten *Aphis glycines* plant^{-1} 10 d after seed was planted. Letters represent significant differences among aphid densities ($P < 0.10$), with capital letters assigned to eggs plant^{-1} and lower case letters assigned to females plant^{-1}.

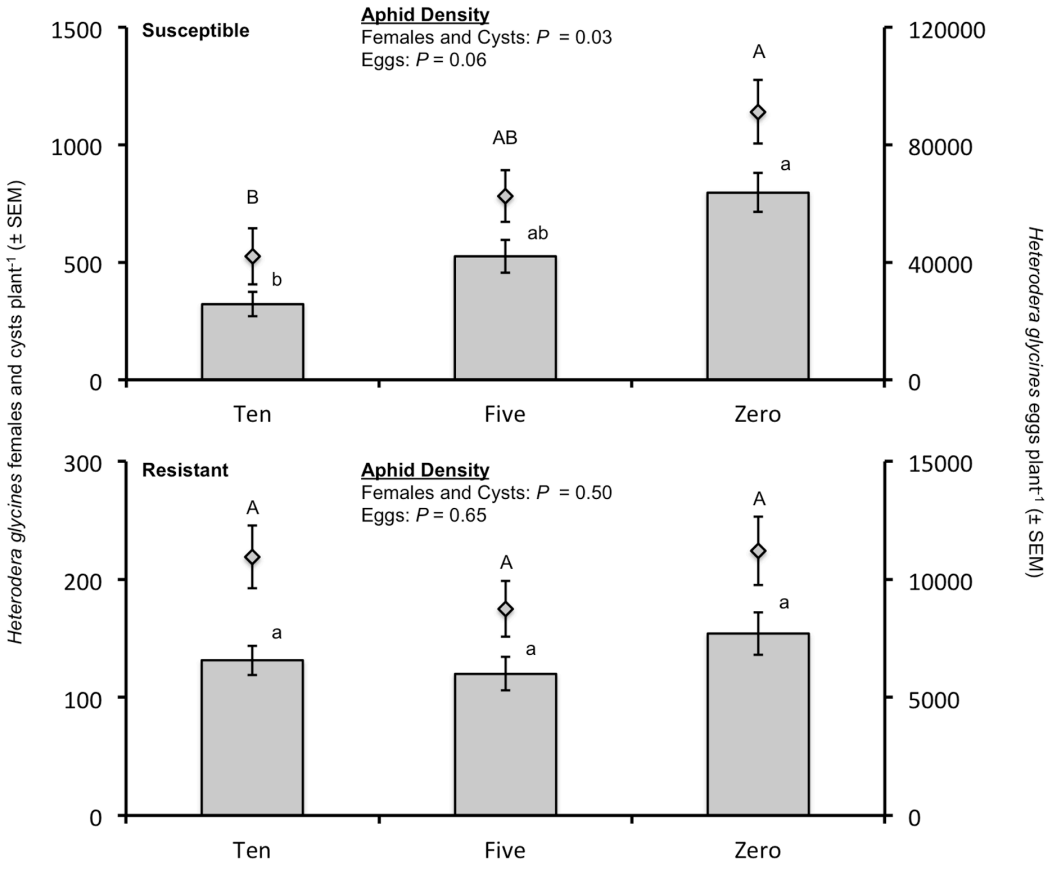

Figure 2. Numbers of *Heterodera glycines* females and cysts and eggs plant^{-1} on the *H. glycines*-susceptible soybean cultivar Kenwood 94 and resistant cultivar Dekalb 27-52 after 60 d. Numbers of females and cysts are represented by bars and numbers of eggs represented by boxes above the bars. Note the different scales used for the two graphs. Three aphid density treatments were established by artificially infesting plants with zero, five, or ten *Aphis glycines* plant^{-1} 10 d after seed was planted. For the susceptible cultivar, aphid density significantly affected the number of *H. glycines* females and cysts plant^{-1} and had a marginally significant effect on numbers of eggs plant^{-1}. Letters represent significant differences among aphid densities ($P < 0.10$), with capital letters assigned to eggs plant^{-1} and lower case letters assigned to females and cysts plant^{-1}.

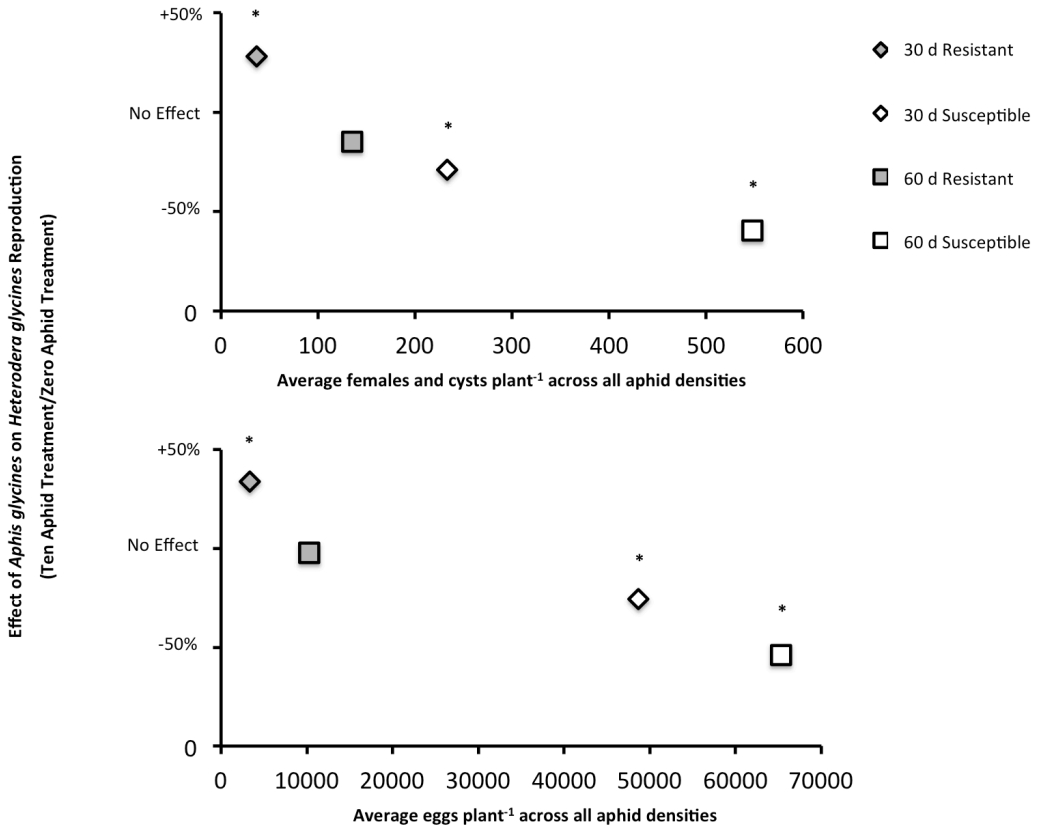

Figure 3. Effect of *Aphis glycines* feeding on numbers of *Heterodera glycines* (a) females and cysts plant^{-1} and (b) eggs plant^{-1} as affected by *H. glycines* population density. The effect of *A. glycines* on *H. glycines* reproduction was calculated as the ratio of the mean of the ten-aphid treatment divided by the zero-aphid treatment. The average number of *H. glycines* females and cysts plant^{-1} and eggs plant^{-1} was calculated as the average of the ten-, five-, and zero-aphid treatment means. *Aphis glycines* increased *H. glycines* reproduction at the lowest *H. glycines* population density, with competition occurring at higher population densities. Asterisks denote data points in which the effect of aphid density was significant at $P = 0.10$ (see Figures 1 and 2).

CHAPTER 7. INTERACTIONS BETWEEN AN ABOVEGROUND AND A BELOWGROUND HERBIVORE CHALLENGE CROP PRODUCTION

A paper to be submitted to Pest Management Science.

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Abstract

Interactions between aboveground and belowground herbivores have the potential to be an important mediator of ecological communities. Their importance in agricultural production and pest management is not well understood. We sought to investigate the ability of the aboveground feeding aphid, *Aphis glycines*, to affect the reproduction of the root-feeding nematode, *Heterodera glycines*, in a field setting. We investigated the ability of host-plant resistance to mediate pest population density, both *A. glycines* and *H. glycines*, and affect the outcome of the interaction between the two herbivores. In a micro-plot experiment, *A. glycines* affected the reproduction of *H. glycines*; however, the outcome of the interaction was affected by

pest density, which was mediated by host-plant resistance. Increases in *H. glycines* reproduction in response to *A. glycines* feeding occurred at the lower pest populations observed, while decreases occurred at the higher pest populations observed. In a companion field-plot experiment, *A. glycines* increased *H. glycines* reproduction on *H. glycines*-susceptible cultivars by 34%. However, *H. glycines* reproduction was unaffected by *A. glycines* feeding on *H. glycines*-resistant cultivars. *Aphis glycines* population densities were below economically damaging levels in the field-plot experiment, and therefore did not affect yield by themselves. *Aphis glycines* also did not affect yield through increased *H. glycines* reproduction, however yield was negatively correlated with *H. glycines* egg population densities at harvest.

Keywords: integrated pest management, integrated disease management, plant-mediated interactions, nematode, aphid, insect

Introduction

Host-plant mediated interactions between aboveground and belowground herbivores are suspected to be important mediators of ecological communities.[1] Herbivore arrival time, feeding guild, and population density are all proposed as important mediators of the outcome and strength of these interactions.[2,3] The importance of arrival time and feeding guild suggests induced changes in plant defenses or nutritional quality are responsible for mediating these interactions.

Host-plant resistance can involve the induction and regulation of plant defenses leading to herbivore population suppression. The ability of host-plant resistance to affect the outcome of aboveground-belowground herbivore

interactions is not well understood. Host-plant resistance mediated the outcome of interactions between brown planthopper (*Nilaparvata lugens* Stål) and plant-parasitic nematodes on rice. Positive effects of aboveground feeding on plant-parasitic nematode population densities occurred on a *N. lugens*-susceptible cultivar, while negative effects occurred on a *N. lugens*-resistant cultivar.[4,5] In the soybean aphid (*Aphis glycines* Matsumura)-soybean cyst nematode (*Heterodera glycines* Ichinohe) system an opposite effect was observed when investigating *H.* glycines-susceptible and resistant soybean cultivars.[6] Positive effects of *A. glycines* on *H. glycines* reproduction were observed on a *H. glycines*-resistant cultivar, while negative effects were observed on a susceptible cultivar. This observation however was correlated to the density of *H. glycines*, suggesting negative effects were due to competition for limited nutritional resources.

The importance of aboveground-belowground interactions for agro-ecosystems is not well understood. Limited studies have investigated the importance of these interactions for agricultural production, with very few investigating the outcome in the field where impacts on yield can be measured, thus making their inclusion into sustainable pest management programs difficult.[2,3] The soybean system provides an opportunity to investigate aboveground-belowground herbivore interactions and their consequences for agricultural production. *Heterodera glycines* is a consistent pest of soybean, causing yield losses exceeding $1.5 billion annually in the U.S. Alston et al. (1993) found *Helicoverpa zea* Boddie, feeding increased *H. glycines* reproduction with the two pests acting in an additive manner to suppress soybean yield. *Helicoverpa zea* is a significant pest of soybean in

the southern United States, but is not a regular pest of soybean in the Midwestern U.S. where much of the soybean is produced in the U.S. *Aphis glycines* is a sporadic pest of soybean, reaching economically damaging levels across the Midwestern U.S. in approximately half the years since its introduction to the U.S.[7] At the county level, *A. glycines* and *H. glycines* co-occur across approximately 22.3 mil hectares of soybean, representing approximately 77% of the soybean crop with an annual value around $29 billion (Fig. 1).[8,9,10]

Population densities are an especially important consideration for *H. glycines* management as an important predictor of yield loss during current years and in future years.[11,12] *Heterodera glycines* eggs can remain dormant for >10 years,[9] therefore *A. glycines* effects on *H. glycines* populations could reduced yield in the current growing season and have legacy effects reducing yield for multiple years in the future. Previous studies demonstrated *A. glycines* feeding could increase, decrease, or have no effect on *H. glycines* reproduction.[6,14,15] Herbivore density, both *A. glycines* and *H. glycines*, are proposed to affect the outcome of the interaction, with positive effects on *H. glycines* reproduction occurring at lower pest densities and decreases occurring at higher pest densities.[6]

Our goal was to test the hypothesis that *A. glycines* feeding affects *H. glycines* reproduction in the field, and that the outcome of the interaction is dependent on pest density, which can be affected by host-plant resistance. We sought to test our hypothesis in both caged micro-plots and small agricultural field-plots where yield can be measured more accurately. In both settings, *A. glycines* and *H. glycines* were manipulated through the use of host-plant resistance, allowing us to investigate the

ability of host-plant resistance to affect the interaction between *A. glycines* and *H. glycines* through the manipulation of pest population densities.

Materials and Methods

Micro-plot Experiment.

The micro-plot cage experiment was conducted in 2012 and 2013 at an Iowa State University research farm in Story, Co., Iowa. A total of 312 plots were established and arranged in a randomized complete block design. Due to the large size of the experiment, two fields were selected approximately 60 m apart. These fields were selected for the limited potential for existing *H. glycines* populations, as neither field was planted to soybean within the previous 10 years.[16] The eastern field contained eight blocks and was previously planted to a long-term stand of *Medicago sativa* L., while the western field contained the other five blocks and was previously planted to turfgrass.

Plots were spaced 76 cm apart and consisted of a 51 x 28 x 21 cm (l x w x h) bottomless rectangular plastic tote driven into field soil (Quantum Storage Systems Miami, FL). The distance between plots and the totes prevented soil and *H. glycines* from being washed among plots during heavy rainstorms. Plots were covered with no-see-um mesh netting (Quest Outfitters Sarasota, FL) stretched over a PVC-pipe rectangular frame, which prevented the movement of alate *A. glycines* among plots.[17] Plots were planted on 11 May and 14 May, in 2012 and 2013 respectively. A single row of 22 soybean seeds was planted lengthwise in the center of each plot. Plots were thinned to 10 evenly spaced plants after emergence.

We created a total of 24 treatments to address our hypothesis that *A. glycines* feeding affects *H. glycines* reproduction. The 24 treatments were randomly assigned to *H. glycines*-infested plots in 2011 and again in 2012. Each treatment was a combination of two factors, soybean cultivar and the presence of *A. glycines*. Twelve different commercial soybean cultivars were used, with three cultivars for each of four resistance categories (Table 1). The four categories were *H. glycines*- and *A. glycines*-susceptible, *H. glycines*-susceptible and *A. glycines*-resistant, *H. glycines*-resistant and *A. glycines*-susceptible, and *H. glycines*- and *A. glycines*-resistant. The 12 cultivars were not genetically related, allowing us to assess both the effect of pest resistance genes (both *H. glycines* and *A. glycines*) and background cultivar genetics on the interaction between the two herbivores. Two *A. glycines* infestation levels were used, plots were either kept free of *A. glycines* (- aphids), or infested with *A. glycines* (+ aphids).

Plots were infested with *H. glycines* inoculum prepared in the greenhouse. *Heterodera glycines* inoculum was added to each plot prior to planting in 2011. Inoculum was obtained by growing a standard *H.* glycines-susceptible cultivar (Williams 82) in field collected *H. glycines* infested soil in the greenhouse. The *H. glycines* population present in the field soil was characterized as HG type 0 (i.e. <10% reproduction on all seven sources of *H. glycines* resistance). After one generation of *H. glycines* increase in the greenhouse, all soybean root tissue was separated from the soil using a 1-cm-pore sieve. The soil was thoroughly mixed using a cement mixer. The root tissue was cut into 0.5 cm lengths and mixed with construction sand. Micro-plots were then infested by mixing 480 ml of the field soil

infested with *H. glycines* at a density of 11,650 eggs per 100 cc of soil and 60 ml of the construction sand infested with a density of 23,350 eggs per 100 cc of sand. The end result was each micro-plot was infested with approximately 69,930 *H. glycines* eggs, thus obtaining an initial population density of ≈300 eggs per 100cc of soil. This density is in the lower range of *H. glycines* infestations commonly observed in soybean production fields in the state of Iowa.[18]

Plots assigned to '+ aphid' treatments were infested with 10 mixed-age biotype-1 *A. glycines* (avirulent to all *Rag* genes) from a greenhouse colony at the third trifoliate stage according to the methods of McCarville et al. (2011). *Aphis glycines* populations were allowed to increase for the duration of the growing season.

In 2012, treatments were randomized again to plots. Plots that were assigned to *H. glycines*-susceptible cultivars contained 10.6x greater *H. glycines* egg population denisties. In an attempt to disentangle the effects of pest density and cultivar resistance,[6] treatments with an *H. glycines*-susceptible cultivar were randomized to plots that had a treatment with an *H. glycines*-resistant cultivar the previous year, and vice-versa. This resulted in *H. glycines*-susceptible cultivars being planted in plots with lower *H. glycines* population densities, and *H. glycines*-resistant cultivars being planted in plots with higher *H. glycines* population densities.

Field-plot Experiment.

We conducted a field-plot experiment to investigate whether our results from the micro-plot experiment could be replicated in an agricultural setting. In an agricultural field-plot setting *A. glycines* population densities were expected to be

lower than in the micro-plot experiment, due to the presence of natural enemies. *Heterodera glycines* populations were also expected to be more variable both within and among plots, due to the larger plot size and more variable soil conditions across the field.

An *H. glycines*-infested field was selected on the same farm as the micro-plot experiment. The field selected had been planted to soybean for 10+ years and contained a high population density of *H. glycines* (≈7,500 eggs per 100 cc soil). Eight treatments were planted in a split-plot design with eight replicates. Each whole plot measured eight rows wide (76 cm row spacing) x 5.3 m in length, with the split-plots consisting of four rows. The whole plot treatment factor was soybean cultivar with the split-plot treatment factor being presence of *A. glycines*. Four commercial cultivars were used, an *H. glycines*- and *A. glycines*-susceptible cultivar, a *H. glycines*-susceptible and *A.* glycines-resistant cultivar, a *H. glycines*-resistant and *A. glycines*-susceptible cultivar, and a *H. glycines*- and *A. glycines*-resistant cultivar (Table 1). Each split-plot was either kept free of *A. glycines* (- aphids) using foliar applications of a pyrethroid insecticide (Warrior II with Zeon Technology, Syngenta, Greensboro, NC), or infested with *A. glycines* beginning at the V3 growth stage (+ aphids). Field-plots were planted on 24 May 2012 at a seeding rate of 395,370 seeds per hectare. Split-plots assigned to '+ aphid' treatments were artificially infested with *A. glycines* from a greenhouse colony at the V3, R1, and R3 growth stages. Each infestation was accomplished by placing 10 soybean plants infested with ≈ 1,000 mixed-age *A. glycines* in the middle two rows of each '+ aphid' split-plot.

Data Collection.

Heterodera glycines populations were estimated each year from spring and fall *H. glycines* egg population densities. Six soil cores measuring 2 cm x 6-8 cm (d x l) were taken from each plot prior to planting and after harvest. The six cores were taken from the planting row of each micro-plot. In field-plots, soil cores were collected from the center two rows of each split-plot in a zig-zag pattern. For each plot, the six cores were combined, dried, and mixed. *Heterodera glycines* cysts were then extracted from a 100cc sub-sample by gravity sieving with a 710-um-pore sieve over a 180-um-pore sieve.[19] The extracted cysts were crushed using a rubber pestle and the eggs were recovered on a 25-um-pore sieve, stained with acid fuchsin, and counted using direct microscope counts.[20]

Aphis glycines populations were estimated using weekly whole plant counts. For the micro-plot experiment, three plants per plot were randomly selected each week and all *A. glycines* present were counted. Whole plant counts were conducted from the V3 stage until plant senescence. The average number of *A. glycines* per plant for each date was used to calculate cumulative aphid days for each plot. Cumulative aphid days estimate a plant's seasonal exposure to aphids and have a strong correlation to soybean yield loss and therefore plant stress.[21,22] *Aphis glycines* population data were collected in the identical manner for the field-plot experiment, with the exception that five plants per plot were counted each week.

Yield was measured for all micro-plots by harvesting all plants within each cage. Plants were threshed with a small bundle thresher (Almaco, Nevada, IA) and all soybean seed was recovered and weighed. Yield was measured for all field-plots

by harvesting the middle two rows of each split-plot with a small plot combine (Almaco, Nevada, IA). All yield measurements were adjusted to 13% moisture and are reported as either g/plot (micro-plots) or kg/ha (field-plots).

Data Analyses.

Heterodera glycines egg population density and cumulative aphid day data were log transformed for analyses, unless otherwise stated. For both experiments, egg population density data from the '- aphid' treatment and cumulative aphid day data from the '+ aphid' treatment were analyzed using an ANOVA with a mixed effects model to confirm each cultivar's resistance or susceptibility to *H. glycines* and *A. glycines*. For the micro-plot experiment we were interested in the fixed effects of *A. glycines* resistance and *H. glycines* resistance. We were also interested in the effect of cultivar (i.e. the effect of different genetic backgrounds), which was treated as a nested variable within the interaction of *A. glycines* resistance and *H. glycines* resistance. For the field-plot experiment, we were interested in the effects of *A. glycines* resistance and *H. glycines* resistance. In the field-plot experiment the effect of cultivar was measured by the interaction of *A. glycines* resistance and *H. glycines* resistance, as there was only one cultivar per combination of *A. glycines* and *H. glycines* resistance or susceptibility.

Heterodera glycines egg population density data for both the '- aphid' and '+ aphid' treatments were analyzed to discern the effect of *A. glycines* colonization on *H. glycines* populations. For both experiments, we were interested in the effects of *A. glycines* density, *H. glycines* resistance, *A. glycines* resistance, and the two- and three-way interactions of the effects. In the micro-plot experiment, we were also

interested in the effect of cultivar and its interaction with the effect of *A. glycines* density.

A regression analysis was conducted to assess the importance of pest density on the interaction between *A. glycines* and *H. glycines.* In the micro-plot experiment a separate relative pest pressure measurement was made for each cultivar in each year. Pest pressure was estimated using the cumulative aphid day measurement in the '+ aphid' treatment and the egg population density measurement in the '- aphid' treatment. Relative pest pressure was estimated as

$$\text{relative pest pressure} = (P_{fab} / P_{fm} + CAD_{ab}/CAD_{m}) / 2$$

where P_{fab} is the final egg population density for cultivar a in year b, P_{fm} is the highest average final egg population density measured for any cultivar in the experiment, CAD_{ab} is the average cumulative aphid days for cultivar a in year b, and CAD_{m} is the highest cumulative aphid days measured for any cultivar in the experiment. The effect of *A. glycines* feeding on *H. glycines* reproduction was estimated as the ratio of egg population density in the '+ aphid' treatment and the '- aphid' treatment for a cultivar. This ratio was regressed across the cultivar's relative pest pressure measurement.

Yield data were analyzed to discern the effect of *A. glycines* treatment, *A. glycines* resistance, and *H. glycines* resistance on yield. We were interested in whether *H. glycines* populations caused significant yield loss in each study and whether the presence of *A. glycines,* by increasing or decreasing *H. glycines* populations, indirectly affected yield. Data were first analyzed by aphid treatment, with the '- aphid' treatment analyzed to discern the effect of *H. glycines* resistance

and therefore *H. glycines* populations on yield. Data were then pooled from both *A. glycines* treatments and analyzed together to discern whether the presence of *A. glycines* resulted in significant yield reductions and whether the effect of *H. glycines* resistance was consistent across both *A. glycines* treatments.

Results

Micro-plot experiment.

Heterodera glycines egg population density data from the '- aphid' treatment and CAD data from the '+ aphid' treatment confirmed *H. glycines*-resistant cultivars supported lower *H. glycines* reproduction (F = 179.42; df = 1, 228; P <0.0001) and *A. glycines*-resistant cultivars supported lower *A. glycines* populations (F = 466.32; df = 1, 261; P <0.0001) (Figure 2). The difference in pest populations between resistant and susceptible cultivars varied significantly by year for both *H. glycines* populations (F = 35.72; df = 1, 228; P <0.0001) and *A. glycines* populations (F = 33.86; df = 1, 261; P <0.0001). In both cases, this was due to a lesser, but still significant difference between resistant cultivars in 2012 than 2013. In the case of *H. glycines*, higher egg population densities were present on *H. glycines*-resistant cultivars in 2013 than 2012 and in the case of *A. glycines,* lower CAD were experienced by *A. glycines*-susceptible cultivars in 2013 than 2012. *Heterodera glycines* egg population densities were 10.6x and 2.0x greater on *H. glycines*-susceptible cultivars than on *H. glycines*-resistant cultivars in 2012 and 2013, respectively. Similarly, CAD were 6.8x and 2.6x greater on *A. glycines*-susceptible cultivars than on *A. glycines*-resistant cultivars in 2012 and 2013, respectively.

Heterodera glycines egg population density data were analyzed across both the '- aphid' and '+ aphid' treatments. Egg population densities varied significantly by aphid treatment (F = 20.02; df = 1, 462; P <0.0001), however the effect of aphid treatment was not consistent across cultivar groups (i.e. combinations of *H. glycines* and *A. glycines* resistance) (F = 5.26; df = 1, 462; P = 0.0223). The effect of aphid treatment only significantly affected egg population densities for the *H. glycines*-susceptible, *A. glycines*-susceptible cultivar group (F = 29.91; df = 1, 94; P <0.0001), where the presence of *A. glycines* reduced *H. glycines* population densities by 65% (Figure 3).

The relative pest pressures ranged from 0.95 to 0.07, representing a wide-range of *H. glycines* population densities (11,464 to 400 eggs/100cc soil) and CAD (209,007 to 15,495 CAD). A significant relationship existed between relative pest pressure and the effect of *A. glycines* feeding on *H. glycines* reproduction (F = 19.36; df = 1, 23; P = 0.0002), with pest pressure explaining 46.81% of the variability in the *H. glycines* reproduction ratios observed. In general, positive to no effect measurements occurred at lower pest pressures, while no effect to negative effect measurements occurred at higher pest pressures (Figure 4).

Yield data for the '- aphid' treatment was not affected by *H. glycines*-resistance (F = 0.07; df = 1, 231; P = 0.7887), *A. glycines*-resistance (F = 2.25; df = 1, 231; P = 0.1349), or their interaction (F = 2.40; df = 1, 231; P = 0.1230). Therefore, *H. glycines* populations did not reach high enough population densities to reduce yield by themselves. Yield, however, was significantly affected by cultivar (F = 2.36;

df = 8, 231; *P* = 0.0185), indicating baseline differences in yield potential existed among the cultivars.

When data from both aphid treatments were analyzed together yield was significantly affected by aphid treatment (*F* = 277.67; df = 1, 465; *P* < 0.0001), *A. glycines*-resistance (*F* = 151.71; df = 1, 465; *P* < 0.0001), cultivar (*F* = 1.97; df = 8, 465; *P* = 0.0489), and the interactions between aphid treatment and *A. glycines*-resistance (*F* = 98.81; df = 1, 465; *P* < 0.0001), and aphid treatment and cultivar (*F* = 2.33; df = 8, 465; *P* = 0.0187). Yield was not affected by *H. glycines*-resistance (*F* = 0.05; df = 1, 465; *P* = 0.8167) or the interaction between aphid treatment and *H. glycines*-resistance (*F* = 0.10; df = 1, 465; *P* = 0.7497). Overall yield was most affected by the presence of *A. glycines*, which reduced yield on *A. glycines*-susceptible cultivars by 80% compared to only 19% on *A. glycines*-resistant cultivars.

Field-plot experiment.

Heterodera glycines egg population density data from the '- aphid' treatment and CAD data from the '+ aphid' treatment confirmed *H. glycines*-resistant cultivars supported lower *H. glycines* reproduction (*F* = 33.11; df = 1, 7; *P* = 0.0007) and *A. glycines*-resistant cultivars supported lower *A. glycines* populations (*F* = 14.85; df = 1, 7; *P* = 0.0063). Egg population densities were 2.3x greater on *H. glycines*-susceptible cultivars than *H. glycines*-resistant cultivars. Cumulative aphid days were 1.8x greater on *A. glycines*-susceptible cultivars than *A. glycines*-resistant cultivars (Figure 5).

When *H. glycines* egg population density data from both the '- aphid' and '+ aphid' treatment were analyzed, egg populations varied significantly by *H. glycines*-resistance (F = 31.56; df = 1, 3; P = 0.0112). Aphid treatment had a marginally significant effect on *H. glycines* egg population densities (F = 2.91; df = 1, 7; P = 0.0993), however the effect of aphid treatment appeared to vary with *H. glycines*-resistance (F = 2.85; df = 1, 28; P = 0.1026). The '+ aphid' treatment significantly increased *H. glycines* reproduction on *H. glycines*-susceptible cultivars (F = 6.00; df = 1,28; P = 0.0208), but had no effect on *H. glycines* reproduction on *H. glycines*-resistant cultivars (F = 0.00; df = 1, 28; P = 0.9903) (Figure 6).

Yield was significantly affected by *H. glycines*-resistance (F = 13.07; df = 1, 7; P = 0.0086) and *A. glycines*-resistance (F = 0.03; df = 1, 28; P = 0.0124), but was not affected by aphid treatment (F = 0.03; df = 1, 28; P = 0.8622), the interaction of aphid treatment and *H. glycines*-resistance (F = 0.37; df = 1, 28; P = 0.5464) or the interaction of aphid treatment and *A. glycines*-resistance (F = 0.68; df = 1, 28; P = 0.4172). The effect of *A.* glycines-resistance was consistent across aphid treatments and did not have a significant relationship with CAD, signifying that the effect of *A. glycines*-resistance on yield was due to differences in yield potential and not pest pressure (Figure 7). *Heterodera glycines*-resistant cultivars yielded 14% more than *H. glycine*-susceptible cultivars, and the average yield of each cultivar x aphid treatment was significantly correlated to the *H. glycines* population density present (F = 5.80; df = 1, 7; P = 0.0528; R^2 = 0.4913).

Discussion

In the experiments presented here, we observed that feeding by the aphid, *A. glycines* could affect reproduction by the nematode *H. glycines* in the field. We also observed that the effect of *A. glycines* feeding on *H. glycines* reproduction was dependent upon the density of both pest populations. At the higher pest population densities tested in the micro-plot experiment *A. glycines* feeding decreased *H. glycines* reproduction. Our results suggest that management could benefit from an integrated approach that considers not only yield losses due to *A. glycines* removing nutrients from the plant, but also *A. glycines* effects on *H. glycines* populations.

Our micro-plot field study obtained results similar to those of McCarville et al. (2013). Specifically, the outcome of the interaction is dependent upon the population density of both *A. glycines* and *H. glycines*, which can be manipulated by host-plant resistance. Population densities of *A. glycines* in Midwestern farm fields are <20% of the maximum density present in our micro-plot experiment.[22] Likewise, population densities of *H. glycines* in the field are commonly <30% of the maximum density present in the micro-plot experiment.[23] Therefore, interactions between *A. glycines* and *H. glycines* in the field likely exist at 0.25 or below along the regression line in Figure 4. Therefore, positive effects to no effect of *A. glycines* feeding on *H. glycines* reproduction would be expected in farm fields. The results of our field-plot experiment are in agreement with this conclusion. *Aphis glycines* feeding increased *H. glycines* reproduction by 34% on *H. glycines*-susceptible cultivars and had no effect on *H. glycines* reproduction on *H.* glycines-resistant

cultivars in the field-plot experiment. Pest population densities in the field-plot experiment would fall between 0.07 and 0.19 on the pest pressure regression in Figure 4.

In our current field-plot experiment, *A. glycines* populations were only present at population densities ≥ 2 aphids plant^{-1} for approximately 30 d. A single generation of *H. glycines* reproduction takes 25-35 d in the field. Therefore, our current results are in agreement with the greenhouse results of McCarville et al. (2013), where *A. glycines* feeding increased *H. glycines* population densities by 34% after a single generation. The arrival time of *A. glycines* in soybean fields varies among geographic regions and years. Therefore, increases in *H. glycines* reproduction may greatly exceed 34% in areas where *A. glycines* colonize soybean early and persist throughout the growing season. McCarville et al. (2012) found that when both pests co-occurred for the entire growing season, *A. glycines* feeding could increase *H. glycines* reproduction by ≈500%.

We were not able to observe an effect on yield of increased *H. glycines* reproduction due to *A. glycines* feeding. However, it is well documented that yield loss increases in response to increasing *H. glycines* egg population densities at planting[11,12] and increasing *H. glycines* reproduction within a season.[11] Therefore, *A. glycines* effects on *H. glycines* populations are likely to impact both yield within a season, and in future seasons.[12] Understanding how aboveground-belowground herbivore interactions shape pest populations and impact yield will be important for designing more effective integrated management programs. This is especially

true for the management of plant-parasitic nematodes, which can build up to high population densities in a single year and persist in the soil for long periods of time.

Acknowledgements

We thank Greg Gebhart and Stith Wiggs for assistance with planting and harvesting field plots, David Soh for assistance with preparation of *H. glycines* inoculum. This research was funded by the Soybean Checkoff through a grant from the Iowa Soybean Association.

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Table 1. Soybean cultivars planted in experiments

Cultivar	*Heterodera glycines* Resistance	*Aphis glycines* Resistance
Micro-plot experiment		
AG 2431	Susceptible	Susceptible
NK S24-M5	Susceptible	Susceptible
DK 28-52	Susceptible	Susceptible
AG 2232	PI 88788[a]	Susceptible
Kruger 2102	PI 88788	Susceptible
DK 27-52	PI 88788	Susceptible
NK S21-E4	Susceptible	*Rag1*[b]
NK S25-F2	Susceptible	*Rag1*
NK S17-D2	Susceptible	*Rag1*
AG 2131	PI 88788	*Rag1*
Renze 82599RRcn	PI 88788	*Rag1*
Kruger 1701	PI 88788	*Rag1*
Field-plot experiment		
NK S24-K2	Susceptible	Susceptible
NK S23-P8	PI 88788	Susceptible
NK S25-F2	Susceptible	*Rag1*
NK S21-Q3	PI 88788	*Rag1*

[a] The resistance in PI 88788 is polygenic therefore it is likely that the cultivars used in this experiment do not contain all of the same *H. glycines* resistance genes.
[b] The *Rag1* resistance gene is a single dominant gene, therefore these *A.* glycines-resistant cultivars all contain the same resistance gene.

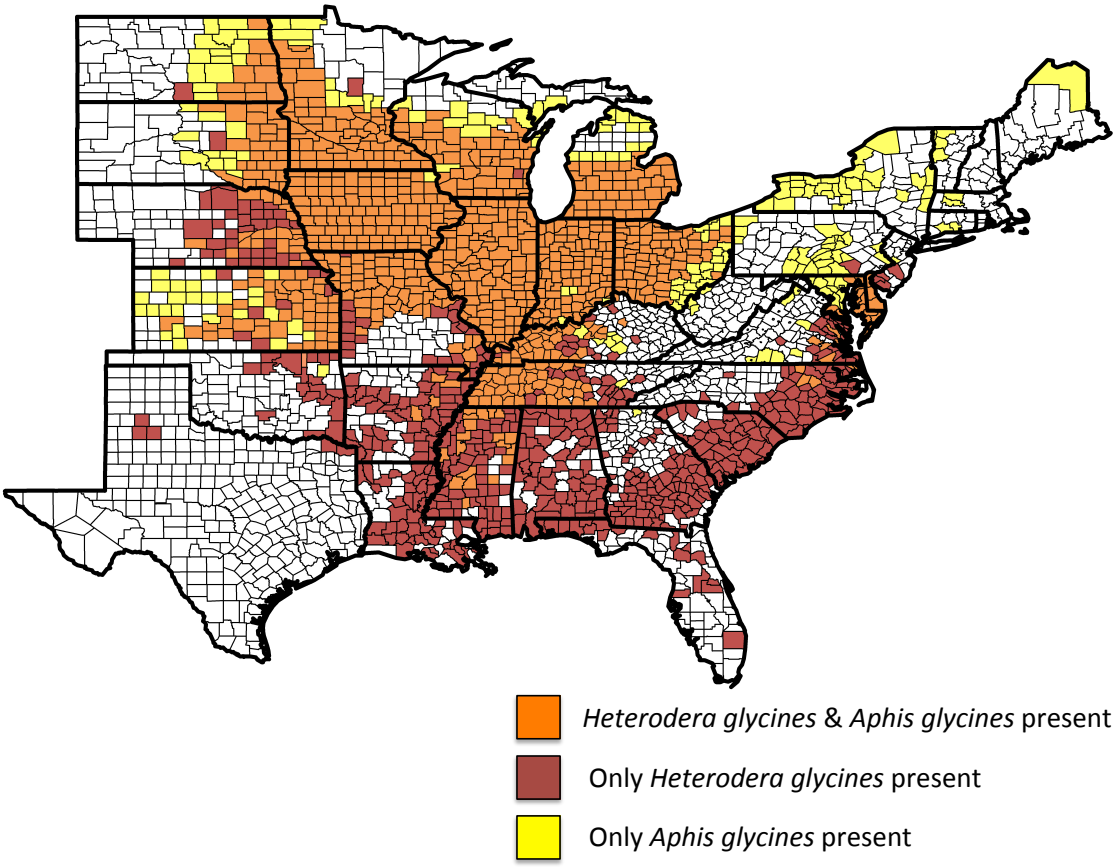

Figure 1.

Map of the distributions of *Aphis glycines* and *Heterodera glycines* in the United States. Counties where only *A. glycines* occurs are represented in yellow, counties where only *H. glycines* occurs are represented in red, and counties where both occur are represented in orange.

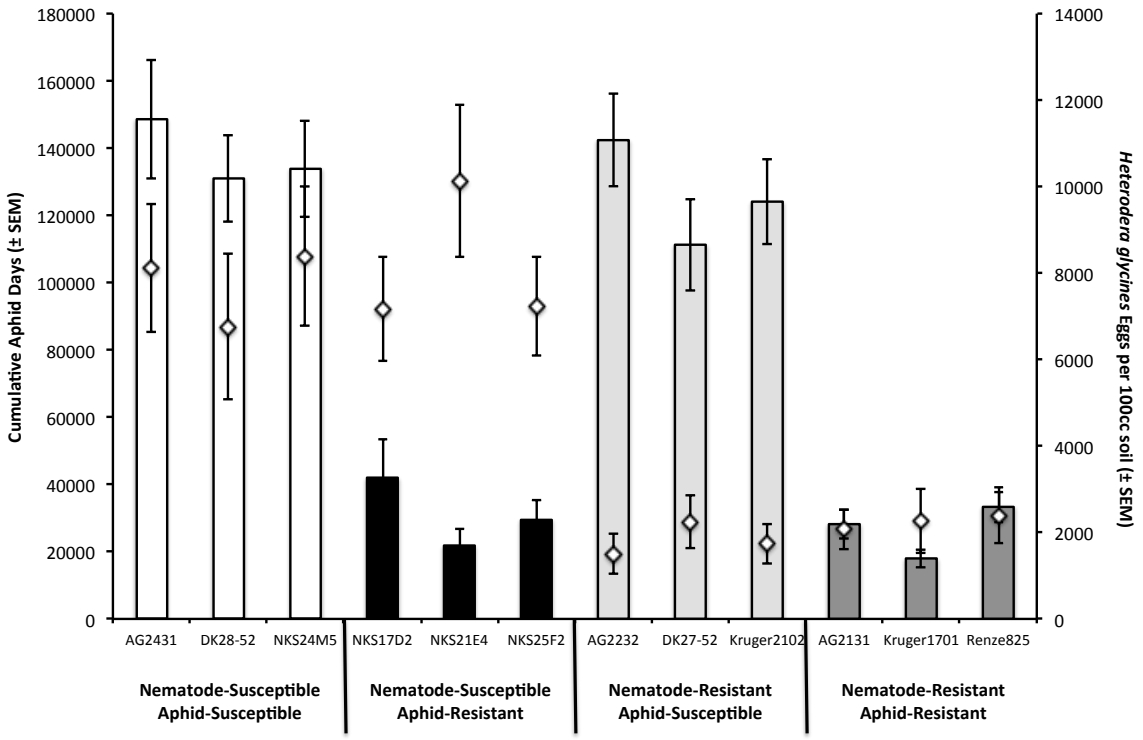

Figure 2.

Pest population densities in the micro-plot study as affected by soybean variety. *Aphis glycines* populations measured in cumulative aphid days are represented by bars. *Heterodera glycines* populations are represented as diamonds. *Aphis glycines* populations were significantly lower on *A. glycines*-resistant cultivars as compared to susceptible cultivars. *Heterodera glycines* populations were also significantly lower on *H. glycines*-resistant cultivars as compared to susceptible cultivars.

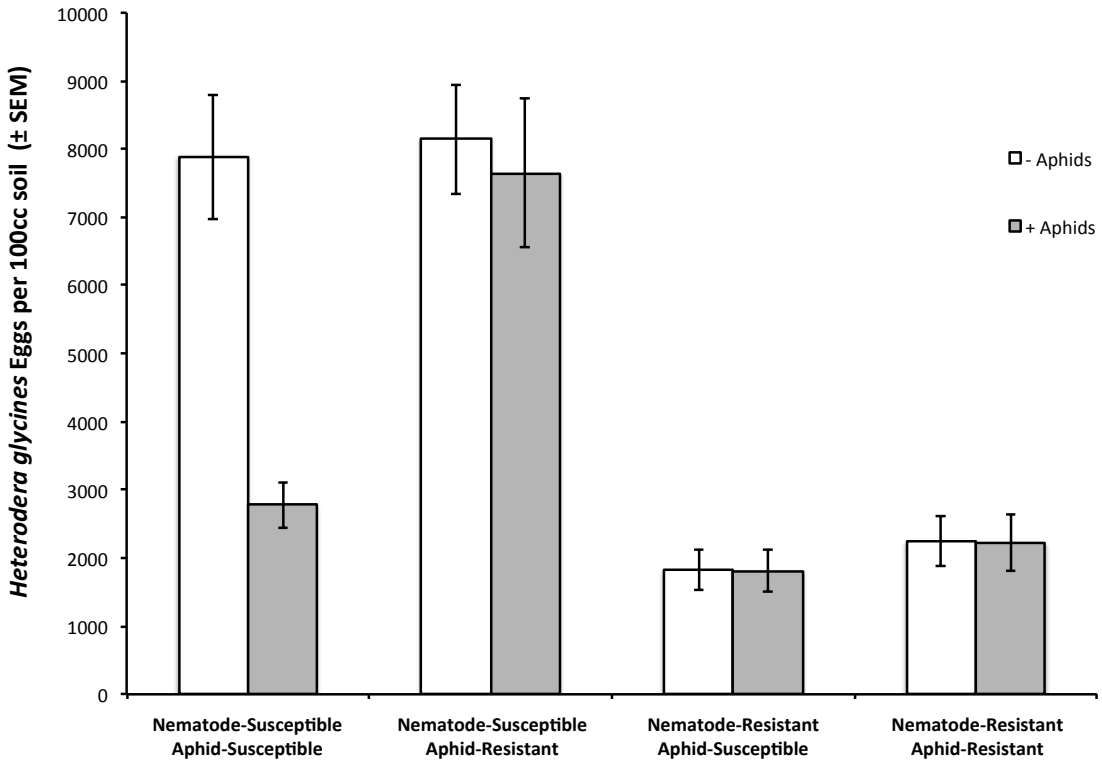

Figure 3.

Heterodera glycines egg population densities in the micro-plot experiment were affected by host plant resistance and the presence of *A. glycines*. Egg population densities were significantly affected by *A. glycines* only on cultivars susceptible to both *H. glycines* and *A. glycines*.

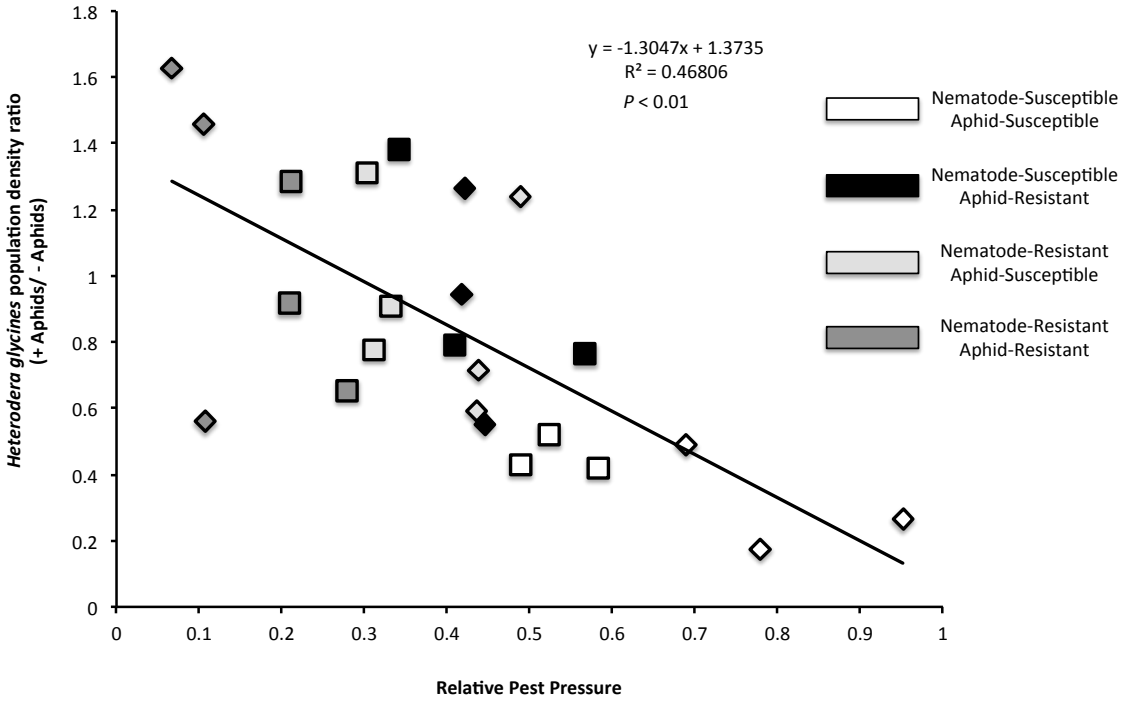

Figure 4.

The effect of *Aphis glycines* on *Heterodera glycines* egg population densities in the micro-plot experiment was influenced by pest population density. Pest population densities of both *H. glycines* and *A. glycines* were affected by host plant resistance, which affected the outcome of the interaction between *A. glycines* and *H. glycines*. Diamonds represent data from 2012, while squares represent data from 2013.

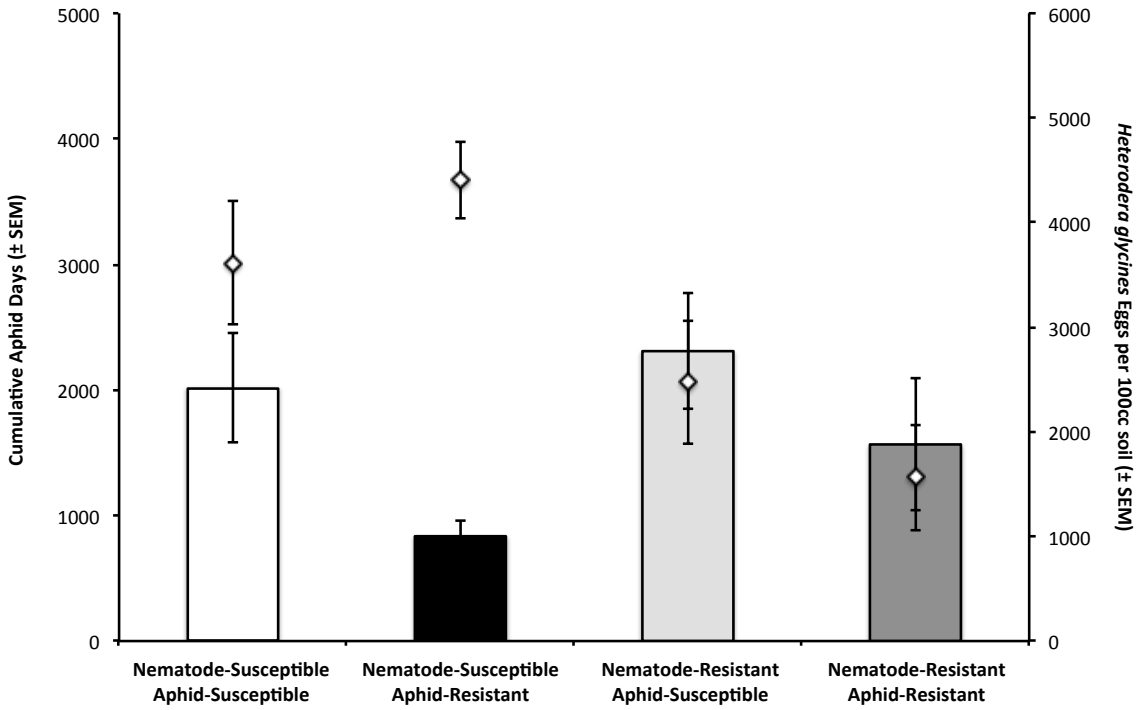

Figure 5.

Pest population densities in the field-plot study as affected by soybean variety. *Aphis glycines* populations measured in cumulative aphid days are represented by bars. *Heterodera glycines* populations are represented as diamonds. *Aphis glycines* populations were significantly lower on *A. glycines*-resistant cultivars as compared to susceptible cultivars. *Heterodera glycines* populations were also significantly lower on *H. glycines*-resistant cultivars as compared to susceptible cultivars.

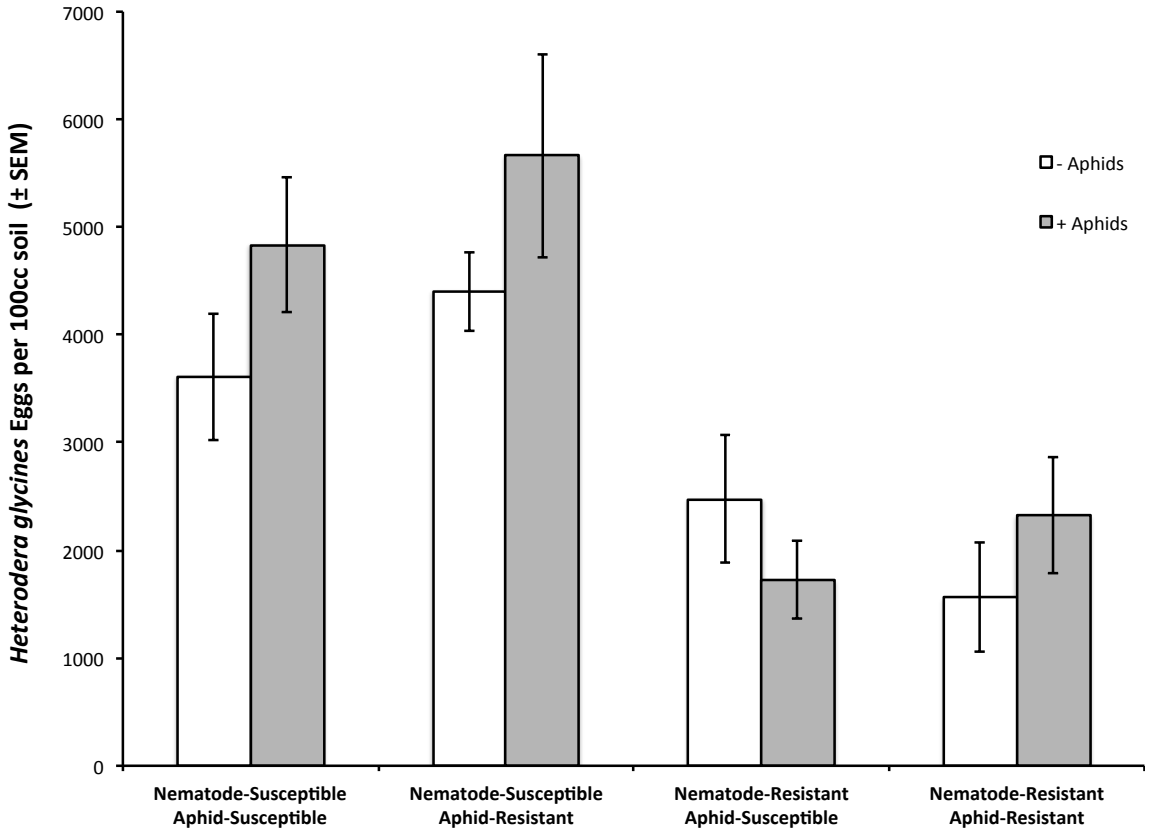

Figure 6.

Heterodera glycines egg population densities in the field-plot experiment were affected by host plant resistance and the presence of *A. glycines*. Egg population densities were 2.3x greater on *H. glycines*-susceptible cultivars compared to *H. glycines*-resistant cultivars. The presence of *A. glycines* significantly increased egg population densities by 34% on *H. glycines*-susceptible cultivars, but had no effect on population densities on *H. glycines*-resistant cultivars.

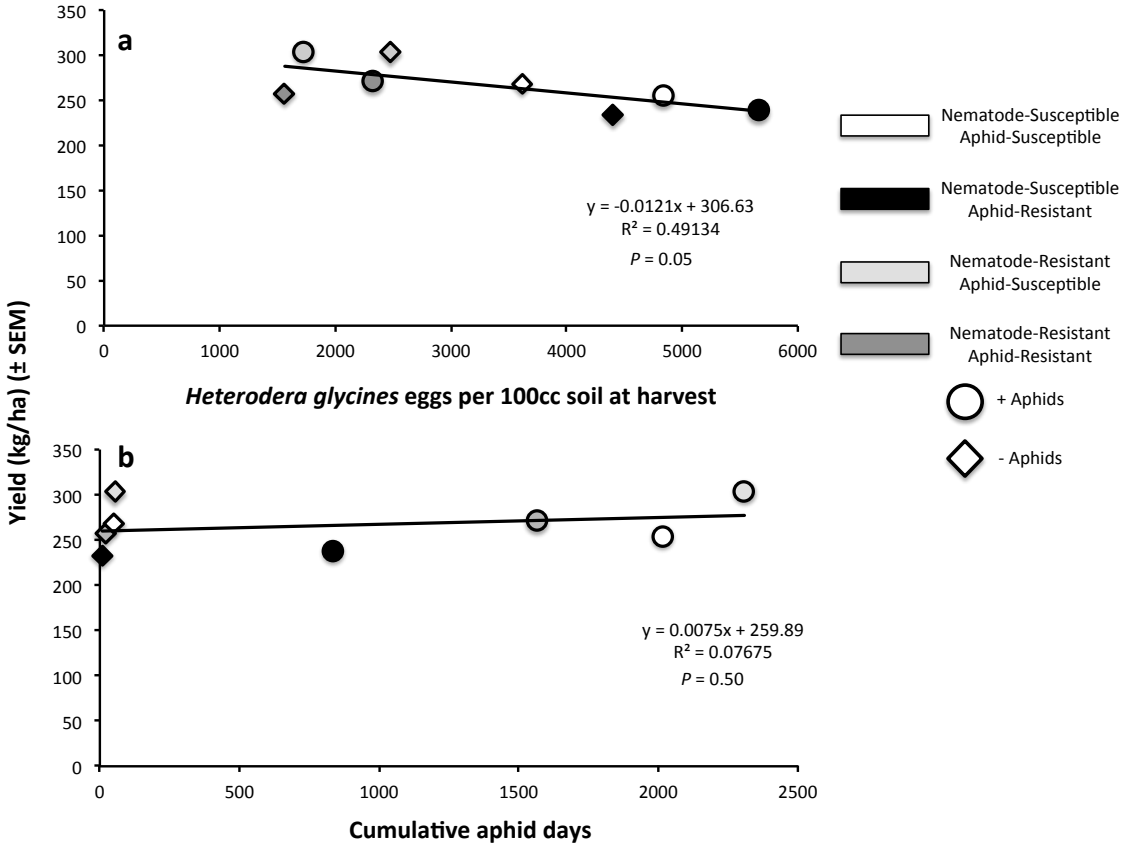

Figure 7.

Soybean yield was significantly correlated to (a) *Heterodera glycines* egg population densities at harvest, but not to (b) cumulative aphid days. A regression analysis indicated yield decreased 12.1 kg/ha or 4% for every 1,000 eggs per 100cc of soil at harvest.

CHAPTER 8. GENERAL CONCLUSIONS

The soybean aphid became an economically damaging pest shortly after its initial detection in the United States. The soybean aphid remains an economically damaging pest throughout much of the north central U.S. over a decade after its original discovery. Synthetic insecticides, specifically foliar applications of pyrethroid and organophosphate insecticides, have allowed farmers to prevent economic losses due to the soybean aphid. Synthetic insecticides however, are an added expense for soybean production, and a potential disruptor of the important soybean aphid natural enemy community. Host-plant resistance offers a potentially more cost-efficient and less disruptive management option for soybean aphid.

Farmer adoption has been slow for soybean aphid-resistant varieties. The slow adoption is likely due to the limited availability of soybean-aphid varieties and the limited efficacy of these varieties. Commercially available aphid-resistant varieties primarily incorporate a single gene, *Rag1*. Our results indicate single gene lines provide inadequate soybean aphid control and therefore still require insecticides for optimal yield. Resistance pyramids of *Rag1* and *Rag2* provide increased aphid resistance, eliminating the need for foliar insecticides. Resistance pyramids therefore, have the potential to increase adoption of aphid-resistant varieties. In addition, resistance pyramids could also facilitate sustainable adoption of aphid-resistant varieties.

Soybean aphid interactions with soybean cyst nematode also have the potential to drive adoption of aphid-resistant varieties. Soybean aphids, by themselves are estimated to cost the U.S. $2.3 - $3.7 billion over a fifteen-year

period. Soybean cyst nematode poses a much greater economic threat in the U.S. with annual losses of over $1.5 billion per year. Resistance pyramids may be of increased value if they can limit soybean aphid and soybean cyst nematode interactions. Our interaction research determined soybean aphid could increase SCN reproduction by 33% even at very low soybean aphid population densities (>100 aphids plant^{-1}). Current insecticide management options do not maintain aphid populations below 100 aphids plant^{-1}. Results from our research indicate only soybean aphid varieties containing two *Rag* genes could maintain aphid populations at such low densities.

An integrated management approach could provide more efficient SCN management if it considers effective soybean aphid management as a component. In this manner, the challenge posed by the aboveground-belowground interaction could provide an opportunity to promote the adoption of more sustainable and less environmentally disruptive management tactics for soybean aphid control.

ACKNOWLEDGEMENTS

I would like to thank my advisors Drs. Matthew O'Neal and Gregory Tylka for their guidance and support throughout my graduate education. I would also like to thank my committee members Drs. Erin Hodgson, Aaron Gassmann, and Gustavo MacIntosh for their advice and assistance with my Ph.D. program. I would be remiss if I did not thank Iowa soybean farmers, who through the Iowa Soybean Association, funded my research and graduate education.

I owe a special thanks to my fellow graduate students (at one time or another) Adam Varenhorst, Kelly Gill, Nick Schmidt, Kevin Johnson, Cody Kuntz, Joe Wheelock, Mike Dunbar, Missy Rynerson, Ryan Keweshan, and Eric Clifton for their friendship and help. I also need to thank the many lab technicians and postdocs who planted and harvested plots, collected soil cores, and counted aphids with me, or simply gave me advice. This large group includes Felicitas Avendano, Greg Gebhart, Chris Marett, Stith Wiggs, Mark Mullaney, David Soh, and Greg VanNostrand.

Finally I want to thank my family for supporting me throughout my six years of graduate school. I want to thank my parents, who without their support, quite literally (they paid my first month's rent in Ames), I would not have made it to or through graduate school. I also thank my siblings for the fun times we have had over breaks and vacations. Lastly, my wife Julia, who I owe so much, without her time, patience and support, I would not be where I am today, or as happy as I am today (also thanks to Miles the wonder dog for reminding me, above all else, life is fun).