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# Geographic Distribution of Soybean Aphid Biotypes in the United States and Canada during 2008–2010

## Abstract

Soybean aphid (*Aphis glycines* Matsumura) is a native pest of soybean [*Glycine max* (L.) Merr.] in eastern Asia and was detected on soybeans in North America in 2000. In 2004, the soybean cultivar Dowling was described to be resistant to soybean aphids with the *Rag1* gene for resistance. In 2006, a virulent biotype of soybean aphid in Ohio was reported to proliferate on soybeans with the *Rag1* gene. The objective was to survey the occurrence of virulent aphid populations on soybean indicator lines across geographies and years. Nine soybean lines were identified on the basis of their degree of aphid resistance and their importance in breeding programs. Naturally occurring soybean aphid populations were collected in 10 states (Kansas, Illinois, Indiana, Iowa, Michigan, Minnesota, North Dakota, Ohio, South Dakota, and Wisconsin) and the Canadian province of Ontario. The reproductive capacity of field-collected soybean aphid populations was tested on soybean lines; growth rates were compared in no-choice field cages at each geographic region across 3 yr. The occurrence of soybean aphid biotypes was highly variable from year to year and across environments. The frequency of Biotypes 2, 3, and 4 was 54, 18, and 7%, respectively, from the 28 soybean aphid populations collected across 3 yr and 11 environments. Plant introduction (PI) 567598B, a natural gene pyramid of *rag1c* and *rag4*, had lowest frequency of soybean aphid colonization (18%). Several factors may have contributed to the variability, including genetic diversity of soybean aphids, parthenogenicity, abundance of the overwintering host buckthorn (*Rhamnus* spp.), and migratory patterns of soybean aphids across the landscape.

## Disciplines

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## RESEARCH

# Geographic Distribution of Soybean Aphid Biotypes in the United States and Canada during 2008–2010

Susannah G. Cooper,\* Vergel Concibido, Ronald Estes, David Hunt, Guo-Liang Jiang, Christian Krupke, Brian McCornack, Rouf Mian, Matthew O'Neal, Vaino Poysa, Deirdre Prischmann-Voldseth, David Ragsdale, Nick Tinsley, and Dechun Wang

## ABSTRACT

Soybean aphid (*Aphis glycines* Matsumura) is a native pest of soybean [*Glycine max* (L.) Merr.] in eastern Asia and was detected on soybeans in North America in 2000. In 2004, the soybean cultivar Dowling was described to be resistant to soybean aphids with the *Rag1* gene for resistance. In 2006, a virulent biotype of soybean aphid in Ohio was reported to proliferate on soybeans with the *Rag1* gene. The objective was to survey the occurrence of virulent aphid populations on soybean indicator lines across geographies and years. Nine soybean lines were identified on the basis of their degree of aphid resistance and their importance in breeding programs. Naturally occurring soybean aphid populations were collected in 10 states (Kansas, Illinois, Indiana, Iowa, Michigan, Minnesota, North Dakota, Ohio, South Dakota, and Wisconsin) and the Canadian province of Ontario. The reproductive capacity of field-collected soybean aphid populations was tested on soybean lines; growth rates were compared in no-choice field cages at each geographic region across 3 yr. The occurrence of soybean aphid biotypes was highly variable from year to year and across environments. The frequency of Biotypes 2, 3, and 4 was 54, 18, and 7%, respectively, from the 28 soybean aphid populations collected across 3 yr and 11 environments. Plant introduction (PI) 567598B, a natural gene pyramid of *rag1c* and *rag4*, had lowest frequency of soybean aphid colonization (18%). Several factors may have contributed to the variability, including genetic diversity of soybean aphids, parthenogenicity, abundance of the overwintering host buckthorn (*Rhamnus* spp.), and migratory patterns of soybean aphids across the landscape.

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**Abbreviations:** DI, damage index; LG, linkage group; PI, plant introduction.

THE SOYBEAN APHID, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a native soybean [*Glycine max* (L.) Merr.] pest in eastern Asia and also has become an important insect pest of soybean, *Glycine max* (L.), in the northcentral region of the United States over the last decade (Ragsdale et al., 2011). The first documented occurrence of soybean aphid in North America was in Wisconsin in 2000 (Hartman et al., 2001). Yield losses in soybean attributed to the soybean aphid have been reported as high as 50% (Wang et al., 1994; Ragsdale et al., 2007). Economic losses attributed to soybean aphid have been calculated between US\$2.4 and US\$4.9 billion annually due to yield losses and increased input costs, such as scouting and insecticide usage (Song et al., 2006; Kim

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et al., 2008a). Between 2000 and 2003 the soybean aphid spread to 21 states of the United States and three Canadian provinces, threatening 80% of North American soybean growing regions (Ragsdale et al., 2004). On soybeans, soybean aphids reproduce asexually and with ideal conditions can undergo up to 18 generations in a growing season (McCornack et al., 2004). Soybean aphid populations generally double every 6 to 7 d but can double in as little as 2 to 3 d; this means management must be timely and coupled with regular scouting programs (Hodgson 2013; Ragsdale et al., 2007). The primary control method for soybean aphids has been with foliar-applied insecticides, primarily using products from the pyrethroid and organophosphorus classes (Ragsdale et al., 2011; Ragsdale et al., 2007).

Host-plant resistance offers an additional complementary management tactic in an overall integrated pest management program. More than 3500 soybean accessions were screened, resulting in the identification of only 30 soybean accessions with either antixenosis- and/or antibiosis-type resistance to the soybean aphid (Hill et al., 2004a,b; Mensah et al., 2005; Diaz-Montano et al., 2006; Mian et al., 2008a; Jun et al., 2012; Bansal et al., 2013; Fox et al., 2014; Hesler et al., 2013; Jun et al., 2013; Kim et al., 2014; Xiao et al., 2013; Bhusal et al., 2014; Liu et al., 2014). Antixenosis-based resistance refers to a nonpreference of insects for behaviors like feeding and oviposition that can result in the poor establishment of an insect (Smith, 1989). Antibiosis-based resistance refers to an adverse effect of the host plant on the insect survival, development, and reproduction often exhibited by larval death or abnormal larval growth (Smith, 1989). A number of independent aphid resistance *Rag* (resistance to *Aphis glycines*) genes have been mapped and described, including: *Rag1* (Hill et al., 2006a,b; Li et al., 2008), *Rag2* (Kang et al., 2008; Mian et al., 2008b), *rag1c*, *rag4* (Zhang et al., 2009), and *Rag3* (Zhang et al., 2010). *Rag1* was identified in the soybean cultivar Dowling and provides strong antibiosis-type resistance; it is a single dominant gene and maps to soybean linkage group (LG) M or chromosome 7 (Li et al., 2004; Hill et al., 2006a; Li et al., 2008). An unnamed *Rag* gene was initially identified in cultivar Jackson; it is also a single dominant gene that maps to the same genomic region and is allelic to *Rag1* (Hill et al., 2012). Antibiosis-based resistance was also identified in plant introduction (PI) 567541B and PI 567598B and was found to be controlled by two recessive genes (Mensah et al., 2005; Mensah et al., 2008). The *rag1c* and *rag4* genes from PI 567541B were mapped to LG M and LG F (chromosome 13), respectively. *Rag1* and *rag1c* mapped to a similar region on LG M, but the allelic relationship is unknown. *Rag2* confers antibiosis and was discovered independently in two accessions, PI 243540 and PI 200538, and maps to LG F (Kang et al., 2008; Mian et al., 2008b; Hill et al., 2009). Meng (2010) identified

an unnamed gene, [*Rag*]<sub>K1621</sub>, on a similar region to *Rag2* on LG F. *Rag3* is a single dominant gene identified from PI 567543C and mapped to LG J or chromosome 16 (Zhang et al., 2010). Additionally, a major gene, *Rag5*, that provides antixenosis was identified in PI 567301B and mapped to a similar region as *Rag2* on LG F (Jun et al., 2012). More recently, a single dominant aphid resistance gene, *Rag6*, was identified in P203 and mapped on LG A2 or chromosome 8 (Xiao et al., 2013).

Subpopulations of insects capable of surviving on resistant plants occur in several crop systems, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), melon (*Cucumis* spp.), and apple (*Malus domestica*) (Berzonsky et al., 2003; Bus et al., 2008; Murugan et al., 2010; Thomas et al., 2012). These populations are often designated as biotypes and defined by their ability to survive and develop large colonies (i.e., virulence) in relation to plants with varying levels of resistance or with different resistance genes (i.e., soybean indicator lines). In the context of this study, virulence is defined by relative differences in aphid population growth and survival rates for an aphid population on a resistant and susceptible cultivar. The term biotype is defined as “populations that are able to reproduce and survive on cultivars developed for resistance to this insect” (Downie, 2010). Soybean aphids that are avirulent on any known *Rag* gene containing soybeans are considered Biotype 1. Five years before the release of commercial cultivars with *Rag1*, a soybean aphid biotype (Biotype 2) was identified in Ohio on the basis of its ability to colonize *Rag1* soybeans in fields in 2005 (Kim et al., 2008b). From field observations, Biotype 2 (*Rag1* virulent) was initially believed to be more predominant in eastern North America (Kim et al., 2008b). However, field tests across a multistate region indicate that survival by soybean aphid populations on *Rag1* soybeans was not limited to Ohio (Hesler et al., 2013). Since the discovery of Biotype 2, at least four biotypes have been identified in the United States with tolerance to other *Rag* genes with limited selection pressure, suggesting soybean aphids may have broad genetic variability and can rapidly adapt to many host plant resistance genes (Kim et al., 2008b). For example, Biotype 3 was collected in Indiana and is able to proliferate on soybean plants with *Rag2* but in lesser numbers than on *Rag1* genotypes (Hill et al., 2010). Additionally, Biotype 4 was collected in Wisconsin and reported to survive and reproduce on soybean plants with both *Rag2* and *Rag1* genotypes (Alt and Ryan-Mahmutagic, 2013). Most recently, Zhong et al. collected four soybean aphid populations from different provinces in China, evaluated the ability of the aphid populations to colonize soybean plants with *Rag1*, *Rag2*, *Rag3*, *Rag5*, or *Rag6*, and found that each soybean aphid population possesses a unique virulence profile (2014). The distribution of soybean-aphid-biotype soybean-growing regions is not well understood.

**Table 1. Soybean aphid indicator lines.**

Line	Maturity group	Descriptor	Gene	Linkage group	Reference
K1621	IV	Resistant	[ <i>Rag</i> ] <sub>K1621</sub>	F	Meng, 2010
PI 567598B	III	Resistant	<i>rag1b, rag3</i>	M, J	
PI 567541B	III	Resistant	<i>rag1c, rag4</i>	M, F	Zhang et al., 2009
'Dowling'	VIII	Resistant	<i>Rag1</i>	M	Hill et al., 2006a
'Jackson'	VII	Resistant	<i>Rag1</i>	M	Hill et al., 2006b
PI 243540	IV	Resistant	<i>Rag2</i>	F	Mian et al., 2008b
UGA-MON	VI	Resistant			
PI 200538	VIII	Resistant	<i>Rag2</i>	F	Hill et al., 2009
'CNS'	VII	Moderately Resistant			Hill et al., 2004b
'Wyandot'	II	Susceptible			

This study was conducted to better understand (i) the virulence frequency of soybean aphid populations on a panel of soybean indicator lines across geographies and years and (ii) compare the soybean aphid population development on soybean indicator lines artificially infested with field-collected soybean aphids from multiple geographies and years. Currently, over 30 soybean accessions with resistance to soybean aphid have been reported, but a select number of sources are currently used by public and private breeding programs (Hill et al., 2012; McCarrville et al., 2012). Therefore, nine aphid resistant soybean lines were selected on the basis of unique resistance and potential to be deployed in the field.

## MATERIALS AND METHODS

### Soybean Indicator Lines

One susceptible soybean line and nine aphid resistant soybean lines were selected on the basis of the source of resistance and potential for deployment in commercial soybean production fields (Table 1). A plant line is defined as progeny of a plant. In this study, a line may be a soybean cultivar or plant germplasm accession. The susceptible check 'Wyandot' was developed by the Ohio Agricultural Research and Development Center (OARDC) and released in 2006. Wyandot was chosen as the standard susceptible soybean line on the basis of performance in prior greenhouse and field screenings (Kang et al., 2008; Mian et al., 2008a). In addition, the Wyandot is used in calculating a damage index (DI, see description below) (Zhang et al., 2009). The soybean indicator lines vary in maturity from maturity groups II to VIII. The wide range in plant maturity was not expected to impact the assessment of soybean aphid colonization of the plant because the assessment was conducted during vegetative growth. Furthermore, soybean aphid growth is not affected by the planting date, maturity group, or physiological age of the plant (Hill et al., 2004b; Rutledge and O'Neil, 2006).

**Table 2. Soybean aphid populations and participating years.**

State or Province	Abbreviation	Institution	Participating years <sup>†</sup>		
			2008	2009	2010
Illinois	IL	University of Illinois		X	
Indiana	IN	Purdue		X	L
Iowa	IA	Iowa State University	X	X	X
Kansas	KS	Kansas State University	L	L	L
Michigan	MI	Michigan State University	X	X	L
Minnesota	MN	University of Minnesota	X	X	X
North Dakota	ND	North Dakota State University		X	X
Ohio	OH	USDA	X	L	X
Ontario	ON	Ag Canada	X	X	X
South Dakota	SD	South Dakota State University		X	L <sup>‡</sup>
Wisconsin	WI	University of Wisconsin, AgStat	X	X	L

<sup>†</sup>X = data used for hierarchical analysis; L = low population development, data was not used for hierarchical analysis.

<sup>‡</sup>Regionally collected laboratory strain.

### Soybean Aphid Field Populations

A total of 28 soybean aphid populations were collected from 2008 to 2010 from commercial soybean fields in selected regions in the United States and Canada (Table 2). Each location was considered to have a unique population of soybean aphids (hereafter referred to as populations) that is potentially comprised of a community of different biotypes. Plots of susceptible soybean cultivar Wyandot were planted at least a month before planting soybean indicator lines and used to develop a nursery population of soybean aphids for infesting soybean indicator lines. The planting date varied by region. Typically, Wyandot was planted in late June. When local field populations could not be collected in any particular year on time for the experiments, laboratory colonies originating from state collections the previous year were used (Table 2). The susceptible soybean plants were caged to reduce population loss due to predation, parasitism, and migration in each environment. Populations were allowed to build for 2 to 6 wk or until sufficient numbers were reached for infesting soybean indicator lines.

### Aphid Resistance Evaluation

Although biotypes may develop to overcome antibiosis and/or antixenosis resistance, this study focused on antibiosis using a no-choice design (Smith and Boyko, 2007). Indicator lines were exposed to populations of soybean aphids that potentially comprised a community of different biotypes. The survival of these putative biotypes was measured using a no-choice exclusion cage experiment where a single indicator line comprised of several plants was enclosed within an individual cage. The soybean indicator lines (Table 1) were planted in a single row at a density of 15 seeds per ~1-m row (3-ft row), thinned back to 10 plants per ~1 m, and each plot was individually caged (94 × 43.2 × 91.4 cm, L × W × H) with no-seeum mesh (Trivantage); soybean lines were caged soon after emergence to protect the plants from unintended insect pests and aphid predators. The cages of

artificially infested soybean indicator lines were established at 8 to 11 locations per year during 2008 to 2010 (Table 2). Each soybean plant was rated, and the average rating across the 10 soybean plants within the plot cage was reported. The experiment was replicated three times at each location in an individual year.

## Population Determination of Biotype

The soybean indicator lines were artificially infested with five aptarae aphids per plant when soybean plants produced the first fully expanded trifoliolate leaf (i.e., V2 stage.). The susceptible check Wyandot was monitored weekly for aphid population development. The entire experiment was rated when aphid populations reached an average of 800 aphids per plant on Wyandot. If the aphid population growth was delayed because of weather or other factors, then ratings were conducted before aphid populations reached at least 800 aphids per plant on Wyandot. Aphid resistance was visually rated for 10 plants in each cage using a 0 to 4 rating scale developed by Mensah et al. (2005, 2008), where plants scoring a “0” are considered resistant to soybean aphid and a score of “4” means that a plant is fully susceptible or aphids are totally virulent. An average rating was given for each plot of 10 plants. Three replicates were performed at each location in an individual year. The scale is as follows: 0 = no soybean aphids, plant is normal and healthy; 0.5 = less than 10 soybean aphids per plant, no colony formation; 1 = 11 to 100 soybean aphids per plant, plant appears normal and healthy; 1.5 = 101 to 150 soybean aphids per plant, mostly on the young leaves of the plant; 2.0 = 151– 300 soybean aphids per plant, mostly on the young leaves and the tender stem at top of plant, plant appears normal and healthy; 2.5 = 301–500 soybean aphids per plant, plant appears healthy; 3.0 = 501– 800 soybean aphids per plant, leaves slightly curly and shiny, young leaves and stems covered with aphids; 3.5 = more than 800 soybean aphids per plant, plants stunted, leaves curled, slightly yellow, no sooty mold, and few cast skins; and 4.0 = more than 800 soybean aphids per plant, plants stunted, leaves severely curled, yellow, covered with sooty mold, and cast skins.

The rate of soybean aphid population growth is influenced by several factors including temperature and humidity. This influence of abiotic factors on aphid populations was noticeable in our data because of the high level of variation by location for ratings taken from susceptible soybean check (Wyandot). To normalize the rating data across locations, we created a damage index (DI) that adjusts ratings by different soybean aphid population growth rates to a common scale, before data analysis. The data set is normalized by using the average scale value of Wyandot in the damage index as described below:

$$DI = \frac{\text{Scale value of indicator line}}{\text{Average scale value of Wyandot}} \times 100.$$

The classification is as follows: Resistance (-) = average DI less than 40%; Moderate-Resistance (+) = average DI is greater than or equal to 40% and less than or equal to 60%; Susceptible (++) = DI is greater than 60% (Mensah et al., 2005). The classification system was based on previous observations that a soybean genotype with a DI value less than 40% did not typically demonstrate symptoms of damage above the economic threshold of 250 aphids/plant until the end of the growing season (Mensah et al., 2005; Ragsdale et al., 2007).

## Data Analysis

All statistical analyses were conducted with R (R Core Team, 2013). Mean comparisons of the DI were conducted using the Fisher least significant difference procedures  $\alpha = 0.05$  within a location within a single year. The linear models procedure in R using the “Agricolae” package was used for statistical analysis (Mendiburu, 2014). Furthermore, the DI data sets were subjected to hierarchical cluster analysis using Euclidian distances and “heatmap 0.2” function in R (from the “gplots” package) to generate visual representations of all DIs in a clustergram (Warnes et al., 2013). The DI data was used to normalize across locations within a field season. The hierarchical clustering was used to (i) group together various soybean aphid populations that responded more similarly on the panel of soybean indicator lines and (ii) group together the soybean indicator lines that responded more similarly in different environments. It was anticipated that soybean aphid populations collected in adjacent states or provinces would respond to the panel of soybean indicator lines more similarly than soybean aphid populations collected more distantly. Similarly, it was anticipated that soybean indicator lines with aphid resistance genes mapped to similar regions in the soybean genome would provide protection to similar aphid populations compared with soybean indicator lines that differed in aphid resistance genes.

To perform the analysis, the average DI was transformed by (50-DI); the transformation allowed for ratings in the susceptible category to have a negative value and ratings in the resistant categories to have positive values. Hierarchical clustering is a form of cluster analysis that requires no prior specification of the number of clusters present in the data. Instead, it generates a hierarchy of relationships based on a distance function (Hastie et al., 2001). Heatmap 0.2 performs agglomerative clustering, which is a stepwise, “bottom-up” strategy that recursively groups the most similar soybean aphid populations’ response to the soybean indicator lines as well as similarity of the soybean indicator lines performance subjected to the soybean aphid populations. In addition, two dendrograms are produced that demonstrate the level of similarity between the soybean aphid population response and indicator soybean line performance; the shorter the path along the dendrogram between two responses, the more similar the correlation patterns. The output from the clustering algorithm provided a graphical display of similarity of the soybean aphid population response to the soybean indicator lines as well as similarity of the soybean indicator lines performance subjected to the soybean aphid populations.

## RESULTS AND DISCUSSION

### Virulence Profiling of Soybean Aphid Populations

Soybean aphid populations were collected from 28 soybean fields from 11 United States and a Canadian province across 3 yr. Indiana, North Dakota, and South Dakota were not included in the survey in 2008. In addition, soybean aphid populations were not collected from Illinois in 2008 and 2010 because of low aphid pressures and difficulties with finding a local isolate. The susceptible indicator line, Wyandot, did not reach a rating of 3 for a number of locations,

**Table 3. Frequency of populations colonizing indicator lines across all locations and years.**

Indicator line	Gene	Resistant	Mod-erately resistant	Suscep-tible	Number of observa-tions (n)
'CNS'		11%	11%	79%	28
'Dowling'	<i>Rag1</i>	29%	11%	61%	28
'Jackson'	<i>Rag</i>	36%	14%	50%	28
K1621	[ <i>Rag</i> ] <sub>K1621</sub>	25%	11%	64%	28
PI 200538	<i>Rag2</i>	61%	14%	25%	28
PI 243540	<i>Rag2</i>	26%	7%	67%	28
PI 567541B	<i>rag1c, rag4</i>	50%	14%	36%	28
PI 567598B	<i>rag1b, rag3</i>	64%	18%	18%	28
UGA-MON		43%	32%	25%	28

**Table 4. Soybean aphid virulence profiles on indicator lines in 2008.**

Line	Soybean aphid field populations <sup>†</sup>										
	ND	SD	KS	MN	IA	WI	IL	MI	IN	ON	OH
'CNS'	ND	ND	-	++	++	++	ND	++	ND	+	-
'Dowling'	ND	ND	-	-	++	+	ND	++	ND	++	++
'Jackson'	ND	ND	-	-	++	++	ND	++	ND	++	++
K1621	ND	ND	-	++	++	++	ND	++	ND	+	+
PI 200538	ND	ND	-	-	++	++	ND	+	ND	+	-
PI 243540	ND	ND	-	++	++	++	ND	++	ND	-	-
PI 567541B	ND	ND	-	++	++	++	ND	-	ND	-	-
PI 567598B	ND	ND	-	+	++	+	ND	-	ND	-	-
UGA-MON	ND	ND	-	-	++	++	ND	-	ND	-	-
'Wyandot'	ND	ND	++	++	++	++	ND	++	ND	++	++

<sup>†</sup> - indicates an average damage index (DI) less than 40%; + indicates an average DI is greater than or equal to 40% and less than or equal to 60%, ++ indicates a DI is greater than 60%; ND indicates no data.

including Indiana (2010), Kansas (2008–2010), Michigan (2010), Ohio (2009), South Dakota (2010), and Wisconsin (2010). Although the DI is reported, the above locations were not used for hierarchical analysis. The virulence reaction for Biotypes 1, 2, and 3 has not been reported to a number of the soybean indicator lines present in this survey (Kim et al., 2008b; Mian et al., 2008b; Hill et al., 2010). Thus, the 28 soybean aphid populations were assessed with an expanded panel of soybean indicator lines than previously designated biotypes. The frequency of populations virulent to individual soybean indicator lines across all geographies and years is depicted in Table 3. Furthermore, the soybean aphid virulence profiles of the populations for each location and year are listed in Tables 4–6. The DI index for each population is depicted in supplemental material in Tables S1–S3. Across all locations and years, 54% of the 28 soybean aphid populations were able to colonize soybeans with *Rag1* genotype, were not able to colonize soybeans with *Rag2* genotype, and could be classified as Biotype 2. Similarly, 18% of the 28 soybean aphid populations were able to colonize soybeans with *Rag2* genotype, were not able to colonize soybeans with *Rag1* genotype, and could be classified as Biotype 3. Biotype 4 is characterized as a

**Table 5. Soybean aphid virulence profiles on indicator lines in 2009.**

Line	Soybean aphid field populations <sup>†</sup>										
	ND	SD	KS	MN	IA	WI	IL	MI	IN	ON	OH
'CNS'	++	++	++	++	++	++	+	-	++	++	+
'Dowling'	-	-	-	+	+	++	++	-	++	-	-
'Jackson'	-	-	+	++	-	++	+	+	++	-	-
K1621	++	++	++	++	++	++	-	++	-	++	-
PI 200538	++	++	+	++	-	++	-	-	-	++	-
PI 243540	++	++	++	+	++	++	-	+	++	++	-
PI 567541B	+	+	+	++	++	++	-	-	-	++	+
PI 567598B	-	-	-	-	-	+	-	-	-	-	++
UGA-MON	+	+	-	++	++	++	-	-	++	+	-
'Wyandot'	++	++	++	++	++	++	++	++	++	++	++

<sup>†</sup> - indicates an average damage index (DI) less than 40%; + indicates an average DI is greater than or equal to 40% and less than or equal to 60%; ++ indicates a DI is greater than 60%.

**Table 6. Soybean aphid virulence profiles on indicator lines in 2010.**

Line	Soybean aphid field populations <sup>†</sup>										
	ND	SD	KS	MN	IA	WI	IL	MI	IN	ON	OH
'CNS'	++	++	++	++	++	++	ND	++	++	++	++
'Dowling'	++	++	++	++	++	++	ND	++	++	++	++
'Jackson'	++	+	++	++	-	++	ND	-	++	++	-
K1621	++	-	++	++	-	++	ND	-	++	++	-
PI 200538	-	-	+	-	-	-	ND	-	-	-	-
PI 243540	++	-	++	++	-	++	ND	++	++	++	-
PI 567541B	+	++	-	-	-	++	ND	-	-	-	++
PI 567598B	+	++	-	-	-	++	ND	-	-	-	++
UGA-MON	++	+	-	-	+	-	ND	+	++	++	+
'Wyandot'	++	++	++	++	++	++	ND	++	++	++	++

<sup>†</sup> - indicates an average damage index (DI) less than 40%; + indicates an average DI is greater than or equal to 40% and less than or equal to 60%; ++ indicates a DI is greater than 60%; ND indicates no data.

soybean aphid population that is able to colonize soybeans with *Rag1*, *Rag2*, or pyramid of *Rag1/Rag2* genotype; this study did not directly assess soybeans with a pyramid *Rag1/Rag2* genotype, and, therefore, the frequency of Biotype 4 cannot be directly determined. That being said, soybean aphid populations with the capacity to colonize soybeans with either *Rag1* or *Rag2* appeared rare, with only 7% of the 28 soybean aphid populations able to colonize soybean with *Rag1* and *Rag2* genotypes.

In general, the virulence profile varied within a region from year to year (Tables 4–6). Soybean aphid populations collected from Wisconsin frequently had the widest spectrum of virulence and readily colonized many of the soybean indicator lines across the 3 yr. Soybean aphids were initially discovered in Wisconsin and thus may have the larger initial genetic diversity compared with other regions (Hartman et al., 2001). Plant introduction 567598B had the broadest spectrum of protection across 3 yr, with only 18% of the soybean aphid populations able to develop on these plants (Table 3). Plant introduction 567598B is a

natural pyramid of two loci, *rag1b* and *rag3* (Mensah et al., 2005; Bales et al., 2013). Combining different insect resistance genes, assuming the insect is less likely to develop resistance to more than one gene simultaneously, can delay resistance development by orders of magnitude. This may be a contributing factor in the observation that PI 567598B, which is a natural stack, provides the broadest resistance (Mani, 1985). In contrast, 79% of the soybean aphid populations were able to colonize the soybean indicator line ‘CNS’ across the 3 yr. CNS was previously identified as having antixenosis-based resistance in a 21-d choice assay and medium level of resistance in a 7-d no-choice assay in the growth chamber (Hill et al., 2004b). Antixenosis-based resistance is often overcome in a no-choice situation (Van Emden and Harrington, 2007). It was expected that soybean aphids might colonize CNS in the absence of choice in a longer-duration assay.

### Hierarchical Clustering of Environmental Populations across Years

A clustergram was obtained using hierarchical cluster analysis of Euclidian distances and heatmap 0.2 function in R (Fig. 1a–c). The figure shows all soybean aphid populations by year on the basis of virulence reaction to soybean indicator lines. The clustering was based on the DI of the soybean aphid populations with no prior specification of the number of clusters present in the data. The analysis was performed to evaluate regional distribution of soybean aphid populations on the basis of virulence to the soybean indicator lines. It was expected that soybean aphid populations collected in adjacent regions would group more closely than soybean aphid populations collected more distantly. The soybean aphid populations collected from nearby regions, such as Ohio and Ontario or Wisconsin and Iowa, were often grouped within the same clade in 2008 (Fig. 1a). A similar trend was observed with soybean aphid populations collected from nearby regions being grouped within the same clade in 2009, with Wisconsin and Minnesota, South Dakota and North Dakota, Indiana and Illinois being grouped together (Fig. 1b). The trend was not observed in 2010, with soybean aphid populations collected from nearby regions often performing differently on the soybean indicator lines (Fig. 1c). The soybean aphid populations in 2008 and 2009 were collected early in the season to increase likelihood of the soybean aphid populations being two to three generations from buckthorn (*Rhamnus* spp.). Soybean aphid populations were generally low in 2010. Many of the soybean aphid populations were collected later in the season compared with previous years owing to difficulties in finding soybean aphid field populations. As a result, many of the soybean aphid populations collected in 2010 may have contained soybean aphid populations that did not originate from nearby buckthorn. Instead, the soybean aphid populations were likely derived

from migratory populations that originated from several regions within the study. Furthermore, the virulence of soybean aphid populations collected within a region was not uniform from year to year. ‘Dowling’ was susceptible to soybean aphid populations collected in Iowa, Michigan, Ohio, and Ontario in 2008, but it was resistant to soybean aphid populations collected in the same regions in 2009.

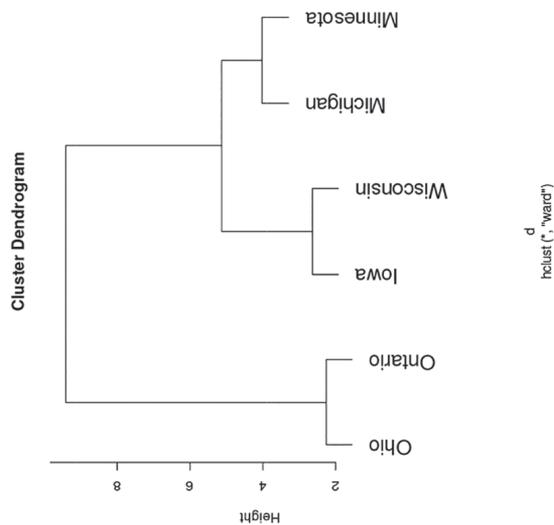
The unpredictable clustering and virulence of geographic populations may be attributed to the complicated life cycle and ability of soybean aphids to migrate large distances (Ragsdale et al., 2011; Schmidt et al., 2012). Soybean aphids have three distinct phases that influence the frequency of virulence within a population: spring migration from buckthorn to soybean, asexual parthenogenetic reproduction on soybean, and autumn migration from soybean to buckthorn, where sexual reproduction that includes an egg stage occurs (Michel et al., 2011). In the spring, nymphs hatch and produce three or more asexual generations on buckthorn before emigrating to soybean. Soybean aphids have many overlapping generations, both wingless and winged forms that occur on soybean throughout the growing season. Although soybean aphids are weak fliers, they are able to migrate large distances on prevailing winds (Schmidt et al., 2012). This is demonstrated by the rapid expansion of soybean aphids in North America. Soybean aphid was first detected in Wisconsin in 2000. By 2003, soybean aphid was detected in 23 states and provinces (Ragsdale et al., 2011). Furthermore, Michel et al. (2009) demonstrated that early soybean aphid populations were genetically distinct across geographies, likely because of the genetic bottleneck imposed by the soybean aphid sexual forms returning to buckthorn. By contrast, later season soybean aphid populations had insignificant amounts of genetic differentiation among aphid populations collected over 1500-km distances, suggesting expansive migration in the summer season or natural selection.

### Hierarchical Clustering of Soybean Aphid Population Colonization on Soybean Indicator Lines across Years

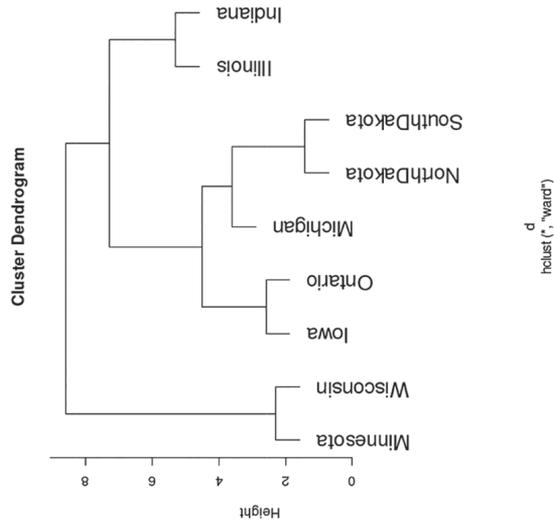
A clustergram was obtained by using hierarchical cluster analysis using Euclidian distances and heatmap 0.2 function in R (Fig. 1d–f). The figure shows all soybean populations by year based on virulence reaction to soybean indicator lines. The analysis was performed to evaluate the level of soybean aphid population virulence on soybean indicator lines and ascertain if soybean aphid populations virulent to particular soybean indicator lines were often resistant to the same indicators across geographies and years.

*Rag2* was independently mapped in two soybean indicator lines, PI 200538 and PI 243540 (Kang et al., 2008; Mian et al., 2008b; Hill et al., 2009). In previous studies, PI 200538 and PI 243540 possess a similar biotype virulence profile: Biotype 3 aphids are able to colonize

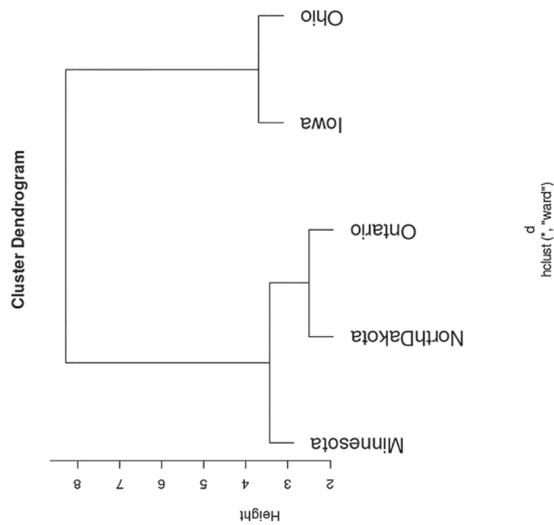
(a) 2008: Soybean Aphid Population Response



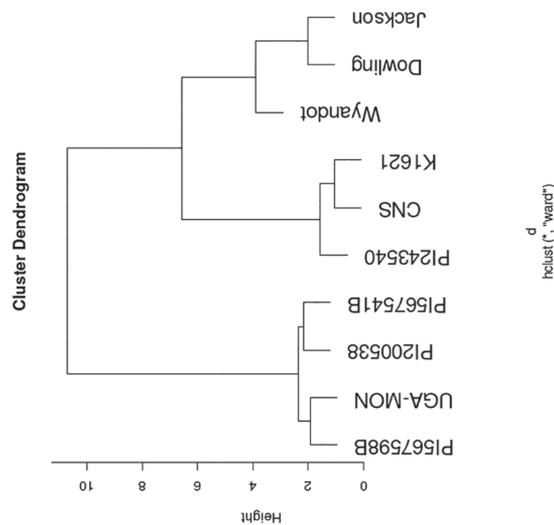
(b) 2009: Soybean Aphid Population Response



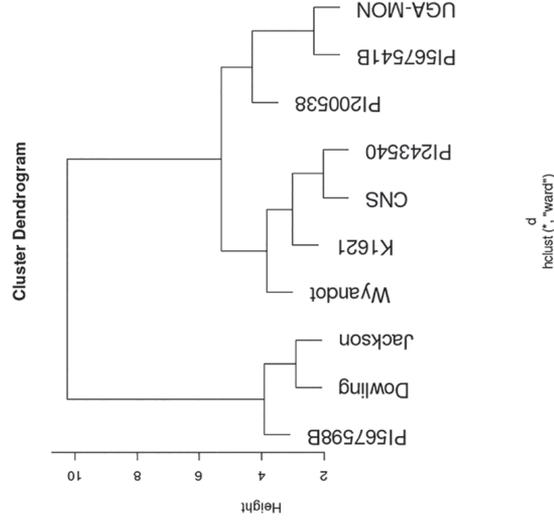
(c) 2010: Soybean Aphid Population Response



(d) 2008: Soybean Indicator Line Performance



(e) 2009: Soybean Indicator Line Performance



(f) 2010: Soybean Indicator Line Performance

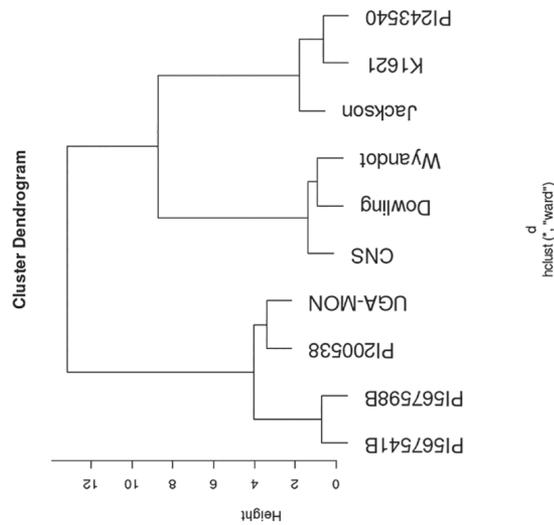


Figure 1. Data sets of the damage index estimated were subjected to hierarchical cluster analysis using Euclidian distances, and heatmap 0.2 function in R (from the gplots package) was used to generate visual representations in the form a clustergram. (a–c) Dendrogram demonstrating the level of similarity among the soybean aphid population response to soybean indicator lines in 2008, 2009, and 2010, respectively; (d–f) dendrogram demonstrating the level of similarity among the soybean indicator lines' performance to soybean aphid populations, 2008, 2009, and 2010, respectively.

both lines and Biotypes 1 and 2 are unable to colonize PI 200538 and PI 243540 (Hill et al., 2010). PI 200538 and PI 243540 did not cluster within the same clade across all 3 yr (Fig. 1d–f). This suggested that the different sources of *Rag2* gene may confer different spectra of resistance to soybean aphid populations used in this study. PI 243540 and K1621 clustered closely together across all 3 yr. Meng (2010) identified an aphid resistance quantitative trait locus in a similar region to *Rag2* from K1621. The soybean aphid population profile on PI 243540 and K1621 suggested that the soybean indicator lines confer plant protection to similar soybean aphid populations.

Dowling and 'Jackson' were delineated within the same subgroup in 2008 and 2009 (Fig. 1d, 1e). Furthermore, Dowling and Jackson had a similar distribution of soybean aphid populations that were classified as resistant, moderately resistant, and susceptible across the 28 populations. *Rag1* from Dowling and an unnamed *Rag* gene from Jackson were mapped to a similar region on LG M (Hill et al., 2006a; Hill et al., 2006b). In 2006, a soybean aphid able to colonize soybean plants with *Rag1* was identified in Ohio. Similarly, Kim et al. (2008b) observed that the Ohio population (Biotype 2) was able to colonize both Dowling and Jackson. Possibly, cross-resistance between *Rag1* and the unnamed *Rag* gene from Jackson could reduce the potential efficacy if the alternative unnamed *Rag* gene from Jackson was deployed. Currently, no commercial soybean cultivars are being marketed carrying the unnamed *Rag* gene from Jackson.

PI 567898B and PI 567541B often clustered closely together and within the same clade in 2008 and 2010 (Fig. 1d, 1f). Antibiosis-based resistance was also identified in PI 567541B and PI 567598B and found to be natural gene pyramids, controlled by two recessive genes (Mensah et al., 2005; Mensah et al., 2008). The soybean aphid resistance genes *rag1b* and *rag3* were identified in PI 567898B, while *rag1c* and *rag4* were identified in PI 567541B (Zhang et al., 2010). Resistance genes *rag1b* and *rag1c* map to similar regions, while *rag3* and *rag4* mapped to different regions and were different resistance genes (Zhang et al., 2010). Only 18 and 38% of the soybean aphid populations were able to colonize PI 567898B and PI 567541B, respectively, possibly because of resistance against a broader range of soybean aphid virulence genes rather than the similarity of genes conferring resistance (Table 3).

## CONCLUSIONS

Despite the limited use of *Rag* genes and the estimates of low soybean aphid genetic diversity, at least one of the 20 soybean aphid populations collected from 2008 to 2010 was identified to proliferate on all of the nine soybean indicator lines. This suggested that soybean aphid in North America has a high degree of virulence diversity. Virulent pest populations are often believed to arise from selection pressure

exerted by the overuse of hosts that are resistant to the pest. For the soybean aphid in North America, this explanation may not be appropriate. The soybean indicator lines (CNS, Dowling, Jackson, K1621, PI 200538, PI 243540, PI 567541B, PI 567598B, and UGA-MON) were selected because of their importance in private and public breeding programs. However, soybean-aphid-resistant cultivars have only been recently commercially available to farmers in North America (McCarville et al., 2012). During the period of this study, soybean aphids have been exposed to limited selection pressure from the resistance genes evaluated. Furthermore, the soybean aphid was an exotic species introduced into North America. It appeared to have a genetic bottleneck, limiting the amount of genetic diversity compared with the genetic diversity available in its native range of East Asia, which does not explain the virulence diversity found in this study (Michel et al., 2009).

Porter et al. (2000) observed a similar trend with greenbug resistance [*Schizaphis graminum* (Rondani)], where the biotypes were identified before large-scale deployment of resistant wheat, *Triticum aestivum* L., and sorghum, *Sorghum bicolor* (L.) Moench. In greenbug, sexual reproduction and populations proliferating on noncultivated grasses have been suggested to increase the genetic diversity and likelihood for development of new biotypes (Porter et al., 2007). Similar to greenbug, soybean aphid is holocyclic and has two major hosts, soybean and buckthorn (Ragsdale et al., 2004). The holocyclic life cycle, in conjunction with sexual reproduction on the return to the alternate host, common buckthorn, increases genetic diversity within populations and likelihood of new biotypes (Porter et al., 2007).

The duration of effectiveness of a particular soybean-aphid-resistant gene may be limited by soybean aphid virulence variability. Therefore, it is critical to deploy aphid resistance genes in a manner that maximizes the effectiveness and durability of resistance. Three strategies have been previously described by Dogimont et al. (2010) to limit outbreaks of aphids. First, continue to identify new resistance genes to control multiple aphid biotypes. Over 3500 soybean germplasm accessions have been screened for soybean aphid resistance, with only 30 soybean accessions being identified as resistant and the number of the resistance genes mapping to similar regions in the genome (Hill et al., 2004a; Hill et al., 2004b; Mensah et al., 2005; Diaz-Montano et al., 2006; Mian et al., 2008a; Jun et al., 2012; Bansal et al., 2013; Fox et al., 2014; Hesler, 2013; Jun et al., 2013; Kim et al., 2014; Xiao et al., 2013; Bhusal et al., 2014; Liu et al., 2014). This approach may not be feasible owing to the limited ability of finding new resistant soybean accessions, quick adaptation of soybean aphid, and the time needed to identify, map, and genetically characterize resistance genes and incorporate them into commercial cultivars via marker-assisted selection. Another

strategy is to increase the spectrum of resistance against multiple biotypes by pyramiding resistance genes. A modeling study suggested that combining more than one resistance gene in a single cultivar may be more durable than sequential releases of single resistance genes (Painter, 1958; Roush, 1998). Wiarda et al. (2012) and McCarville and O'Neal (2013) evaluated the rate of soybean aphid population growth of a single soybean aphid population on soybeans with either the *Rag1* and *Rag2* individually or both genes together and observed that the soybean line with *Rag1* and *Rag2* together had significantly lower soybean aphid populations than lines with either *Rag1* and *Rag2* alone. Although this study demonstrated increased efficacy, it did not address durability. Porter evaluated the efficacy of pyramiding resistance genes in wheat against greenbug (2000). Lines with resistance genes *Gb2*, *Gb3*, and *Gb6* and pyramided *Gb2/Gb3*, *Gb2/Gb6*, and *Gb3/Gb6* were tested for effectiveness against a number of biotypes, including E, F, G, H, and I. In this system, plants with two genes did not provide additional spectrum of control against biotypes compared with plants with one gene.

The third strategy is to develop cultivars with resistance controlled by multiple, quantitative, or recessive loci (Dogimont et al., 2010). In this study, PI 567541B and PI 567598B are natural gene pyramids, with resistance in each accession controlled by two recessive genes (Mensah et al., 2005; Mensah et al., 2008). Similar to the observations of Wiarda et al. (2012) and McCarville and O'Neal (2013), the soybean indicator lines with two genes, PI 567541B and PI 567598B, had the widest spectrum of resistance to soybean aphid populations.

Although soybean aphid populations within North America had the ability to colonize aphid resistant accessions in this study, the risk of aphid biotypes to the soybean grower is not well understood. Information on biotype frequency patterns, distribution, and migration is needed to determine the actual risk of aphid biotypes. Further nationwide sampling of soybean aphids and virulence characterization are needed to better understand the virulence variability, frequency, and geographic distribution of biotypes. Our data suggested that greater virulence variability of soybean aphid occurred in Wisconsin, the state where soybean aphid was first detected in 2000 (Ragsdale et al., 2004). More study of the virulence variability of migrant and local soybean aphids is needed.

Survival of soybean aphids on aphid-resistant soybeans may not be due to biotype virulence. Aphids have been observed to manipulate the quality of their host plant. For example, Sauge et al. (2006) observed that *Myzus persicae* feeding on peach (*Prunus persica*) increased the survivorship for conspecifics that later coinfecting a shared host plant. Similarly, Chiozza et al. (2010) observed changes in the amino acid profile of soybean (both cultivars susceptible and resistant to aphids) after colonization by

soybean aphids that presumably improved the nutritional quality of soybean for the aphid. Furthermore, Baluch et al. (2012) observed that virulent biotypes of Hessian fly improved the survival of avirulent biotypes when they shared a resistant wheat cultivar. By artificially infesting soybean plants, possibly with multiple biotypes within cages, we may have facilitated the survival of avirulent soybean aphids, which may have resulted in an overestimation of soybean aphid colonization. Determination of biotype virulence frequency with aphid genetic markers would greatly help increase the accuracy of the estimates of virulent soybean aphid biotypes.

## References

- Alt, J., and M. Ryan-Mahmutagic. 2013. Soybean aphid biotype 4 identified. *Crop Sci.* 53:1491–1495. doi:10.2135/cropsci2012.11.0672
- Bales, C., G. Zhang, M. Liu, C. Mensah, C. Gu, Q. Song, D. Hyten, P. Cregan, and D. Wang. 2013. Mapping soybean aphid resistance genes in PI 567598B. *Theor. Appl. Genet.* 126:2081–2091. doi:10.1007/s00122-013-2120-y
- Baluch, S.D., H.W. Ohm, J.T. Shukle, and C.E. Williams. 2012. Obviation of wheat resistance to the Hessian fly through systemic induced resistance. *J. Econ. Entomol.* 105:642–650. doi:10.1603/EC11329
- Bansal, R., M.A.R. Mian, and A.P. Michel. 2013. Identification of novel sources of host plant resistance to known soybean aphid biotypes. *J. Econ. Entomol.* 106:1479–1485. doi:10.1603/EC12453
- Berzonsky, W.A., H. Ding, S.D. Haley, M. Harris, R.J. Lamb, and R. McKenzie. 2003. Breeding wheat for resistance to insects. *Plant Breed. Rev.* 22:221–296.
- Bhusal, S.J., G.L. Jiang, L.S. Hesler, and J.H. Orf. 2014. Soybean aphid resistance in soybean germplasm accessions of maturity group I. *Crop Sci.* 54:2093–2098. doi:10.2135/cropsci2014.03.0205
- Bus, V.G.M., D. Chagne, H.C.M. Bassett, D. Bowatte, F. Calenge, J.M. Cleton, C.E. Durel, M.T. Malone, A. Patocchi, A.C. Rantunga, E.H.A. Rikkerink, D.S. Tustin, J. Zhou, and S.E. Gardiner. 2008. Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). *Tree Genet. Genomes* 4: 223–236.
- Chiozza, M.V., M.E. O'Neal, and G.C. MacIntosh. 2010. Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant soybean plants in response to the soybean aphid (Hemiptera: Aphididae). *Environ. Entomol.* 39:856–864. doi:10.1603/EN09338
- Diaz-Montano, J., J.C. Reese, W.T. Schapaugh, and L.R. Campbell. 2006. Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. *J. Econ. Entomol.* 99:1884–1889. doi:10.1093/jee/99.5.1884
- Dogimont, C., A. Bendahmane, V. Chovelon, and N. Boissot. 2010. Host plant resistance to aphids in cultivated crops: Genetic and molecular bases, and interactions with aphid populations. *C. R. Biol.* 333:566–573. doi:10.1016/j.crv.2010.04.003
- Downie, D.A. 2010. Baubles, bangels, and biotypes: A critical review of the use and abuse of the biotype concept. *J. Insect Sci.* 10:176. doi:10.1673/031.010.14136

- Fox, C.M., K.-S. Kim, P.B. Cregan, C.B. Hill, G.L. Hartman, and B.W. Diers. 2014. Inheritance of soybean aphid resistance in 21 soybean plant introductions. *Theor. Appl. Genet.* 127:43–50. doi:10.1007/s00122-013-2199-1
- Hartman, G.L., L.L. Domier, L.M. Wax, C.G. Helm, D.W. Onstad, J.T. Shaw, L.F. Solter, D.J. Voegtlin, C.J. D'Arcy, M.E. Gray, K.L. Steffey, S.A. Isard, and P.L. Orwick. 2001. Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. *Plant Health Progress*. <http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/> (accessed 11 Aug. 2015).
- Hastie, T., R. Tibshirani, and S. Friedman. 2001. *The elements of statistical learning; data mining, inference, and prediction*. Springer, New York.
- Hesler, L.S. 2013. Resistance to soybean aphid among wild soybean lines under controlled conditions. *Crop Prot.* 53:139–146. doi:10.1016/j.cropro.2013.06.016
- Hesler, L.S., M.V. Chiozza, M.E. O'Neal, G.C. MacIntosh, K.J. Tilmon, D.I. Chandrasena, N.A. Tinsley, S.R. Cianzio, A.C. Costamagna, E.M. Cullen, C.D. DiFonzo, B.D. Potter, D.W. Ragsdale, K. Steffey, and K.J. Koehler. 2013. Performance and prospects of *Rag* genes for management of soybean aphid. *Entomol. Exp. Appl.* 147:201–216. doi:10.1111/eea.12073
- Hill, C.B., Y. Li, and G.L. Hartman. 2004a. Resistance of *Glycine* species and various cultivated legumes to the soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 97:1071–1077. doi:10.1093/jee/97.3.1071
- Hill, C.B., Y. Li, and G.L. Hartman. 2004b. Resistance to the soybean aphid in soybean germplasm. *Crop Sci.* 44:98–106. doi:10.2135/cropsci2004.0098
- Hill, C.B., Y. Li, and G.L. Hartman. 2006a. A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. *Crop Sci.* 46:1601–1605. doi:10.2135/cropsci2005.11-0421
- Hill, C.B., Y. Li, and G.L. Hartman. 2006b. Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. *Crop Sci.* 46:1606–1608. doi:10.2135/cropsci2005.11-0438
- Hill, C.B., K.S. Kim, L. Crull, D.W. Diers, and G.L. Hartman. 2009. Inheritance of resistance to the soybean aphid in soybean PI 200538. *Crop Sci.* 49:1193–1200. doi:10.2135/cropsci2008.09.0561
- Hill, C.B., L. Crull, T.K. Herman, D.J. Voegtlin, and G.L. Hartman. 2010. A new soybean aphid (Hemiptera: Aphididae) biotype identified. *J. Econ. Entomol.* 103:509–515. doi:10.1603/EC09179
- Hill, C.B., A. Chirumamilla, and G.L. Hartman. 2012. Resistance and virulence in the soybean–*Aphis glycines* interaction. *Euphytica* 186:635–646. doi:10.1007/s10681-012-0695-z
- Hodgson, E.W. 2013. Scout crops for aphids. *Integrated Crop Management News*: 53.
- Jun, T.H., M.A.R. Mian, and A.P. Michel. 2012. Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B. *Theor. Appl. Genet.* 124:13–22. doi:10.1007/s00122-011-1682-9
- Jun, T.H., M.A.R. Mian, and A.P. Michel. 2013. Genetic mapping of three quantitative trait loci for soybean aphid resistance in PI 567324. *Heredity* 111:16–22. doi:10.1038/hdy.2013.10
- Kang, S., M.A.R. Mian, and R.B. Hammond. 2008. Soybean aphid resistance in PI 243540 is controlled by a single dominant gene. *Crop Sci.* 48:1744–1748. doi:10.2135/cropsci2007.12.0672
- Kim, C., G. Schaible, L. Garrett, R. Lubowski, and D. Lee. 2008a. Economic impacts of the US soybean aphid infestation: A multi-regional competitive dynamic analysis. *Agric. Econ. Res.* 37:227–242.
- Kim, K.-S., C.B. Hill, G.L. Hartman, M.A.R. Mian, and B.W. Diers. 2008b. Discovery of soybean aphid biotypes. *Crop Sci.* 48:923–928. doi:10.2135/cropsci2007.08.0447
- Kim, K.-S., A. Chirumamilla, C.B. Hill, G.L. Hartman, and B.W. Diers. 2014. Identification and molecular mapping of two soybean aphid resistance genes in soybean PI 587732. *Theor. Appl. Genet.* 127:1251–1259. doi:10.1007/s00122-014-2296-9
- Li, Y., C.B. Hill, and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). *J. Econ. Entomol.* 97:1106–1111. doi:10.1093/jee/97.3.1106
- Li, Y., J. Zou, M. Li, D.D. Bilgin, L.O. Vodkin, G.L. Hartman, and S.J. Clough. 2008. Soybean defense responses to the soybean aphid. *New Phytol.* 179:185–195. doi:10.1111/j.1469-8137.2008.02443.x
- Liu, G., H. Xing, Y. Diao, X. Yang, D. Sun, Q. Wang, N. Qi, and H. Lin. 2014. Identification of resistance to soybean aphid in early germplasm. *Crop Sci.* 54:2707–2712. doi:10.2135/cropsci2013.11.0735
- Mani, G.S. 1985. Evolution of resistance in the presence of two insecticides. *Genetics* 109:761–783.
- McCarville, M.T., E.W. Hodgson, and M.E. O'Neal. 2012. Soybean aphid-resistant soybean varieties for Iowa. Iowa State Univ. Extension and Outreach PM 3023.
- McCarville, M.T., and M.E. O'Neal. 2013. Soybean aphid (Aphididae: Hemiptera) population growth as affected by host plant resistance and an insecticidal seed treatment. *J. Econ. Entomol.* 106:1302–1309. doi:10.1603/EC12495
- McCornack, B.P., D.W. Ragsdale, and R.C. Venette. 2004. Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. *J. Econ. Entomol.* 97:854–861. doi:10.1093/jee/97.3.854
- Meng, J. 2010. Genetic analysis of soybean aphid resistance gene in soybean K1621. Doctoral diss. Kansas State Univ., Manhattan. <http://krex.k-state.edu/dspace/handle/2097/4599> (accessed 11 Aug. 2015).
- Mendiburu, F. 2014. agricolae: Statistical procedures for agricultural research. R package version 1.2–1. <http://CRAN.R-project.org/package=agricolae> (accessed 7 July 2015).
- Mensah, C., C. DiFonzo, R.L. Nelson, and D. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. *Crop Sci.* 45:2228–2233. doi:10.2135/cropsci2004.0680
- Mensah, C., C. DiFonzo, and D. Wang. 2008. Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. *Crop Sci.* 48:1759–1763. doi:10.2135/cropsci2007.09.0535
- Mian, M.A.R., R.B. Hammond, and S.K. St. Martin. 2008a. New plant introductions with resistance to the soybean aphid. *Crop Sci.* 48:1055–1061. doi:10.2135/cropsci2007.06.0357
- Mian, R.M., S.-T. Kang, S. Beil, and R. Hammond. 2008b. Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. *Theor. Appl. Genet.* 117:955–962. doi:10.1007/s00122-008-0835-y
- Michel, A.P., W. Zhang, J.K. Jung, S.T. Kang, and M. Mian. 2009. Population genetic structure of *Aphis glycines*. *Environ. Entomol.* 38:1301–1311. doi:10.1603/022.038.0442

- Michel, A.P., O. Mittapalli, and M.A.R. Mian. 2011. Evolution of soybean aphid biotypes: Understanding and managing virulence to host-plant resistance. In: A. Sudaric, editor, Soybean—Molecular aspects of breeding. Tech-Open Access Publisher, Rijeka, Croatia. <http://www.intechopen.com/articles/show/utle/evolution-of-soybeanaphid-biotypes-understanding-and-managing-virulence-to-host-plant-resistance> (accessed 7 July 2015).
- Murugan, M., S.A. Khan, P. Sotelo Cardona, G. Vargas Orozco, P. Viswanathan, J. Reese, S. Starkey, and C.M. Smith. 2010. Variation of resistance in barley against biotypes 1 and 2 of the Russian wheat aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 103:938–948. doi:10.1603/EC09396
- Painter, R.H. 1958. Resistance of plants to insects. *Annu. Rev. Entomol.* 3:267–290. doi:10.1146/annurev.en.03.010158.001411
- Porter, D.R., J.D. Burd, K.A. Shufram, and J.A. Webste. 2000. Efficacy of pyramiding greenbug (Homoptera: Aphididae) resistance genes in wheat. *J. Econ. Entomol.* 93:1315–1318. doi:10.1603/0022-0493-93.4.1315
- Porter, D.R., J. Burd, and D. Mornhinweg. 2007. Differentiating greenbug resistance genes in barley. *Euphytica* 153:11–14. doi:10.1007/s10681-006-9193-5
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/> (accessed 7 July 2015).
- Ragsdale, D., D.J. Voegtlin, and R.J. O’Neil. 2004. Soybean aphid biology in North America. *Ann. Entomol. Soc. Am.* 97:204–208. doi:10.1093/aesa/97.2.204
- Ragsdale, D.W., B.P. McCornack, R.C. Venette, D.B. Potter, I.V. MacRae, E.W. Hodgson, M.E. O’Neil, K.D. Johnson, R.J. O’Neil, and C.D. DiFonzo. 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). *J. Econ. Entomol.* 100:1258–1267. doi:10.1093/jee/100.4.1258
- Ragsdale, D.W., D.A. Landis, J. Brodeur, G.E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. *Annu. Rev. Entomol.* 56:375–399. doi:10.1146/annurev-ento-120709-144755
- Roush, R.T. 1998. Two-toxin strategies for management of insecticidal transgenic crops: Can pyramiding succeed where pesticide mixtures have not? *Philos. Trans. R. Soc. Lond., B* 353:1777–1786. doi:10.1098/rstb.1998.0330
- Rutledge, C.E., and R.J. O’Neil. 2006. Soybean plant stage and population growth of soybean aphid. *J. Econ. Entomol.* 99:60–66. doi:10.1093/jee/99.1.60
- Sauge, M.H., F. Mus, J.P. Lacroze, T. Pascal, J. Kervella, and J.L. Poëssel. 2006. Genotypic variation in induced resistance and induced susceptibility in the peach–*Myzus persicae* aphid system. *Oikos* 11:305–313. doi:10.1111/j.2006.0030-1299.14250.x
- Schmidt, N.P., M.E. O’Neal, P.F. Anderson, D. Lagos, D. Voegtlin, W. Bailey, P. Caragea, E. Cullen, C. DiFonzo, K. Elliott, C. Gratton, D. Johnson, C.H. Krupke, B. McCornack, R. O’Neil, D.W. Ragsdale, K.J. Tilmon, and J. Whitworth. 2012. Spatial distribution of *Aphis glycines* (Hemiptera: Aphididae): A summary of the suction trap network. *J. Econ. Entomol.* 105:259–271. doi:10.1603/EC11126
- Smith, C.M., and E.V. Boyko. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: Current status. *Entomol. Exp. Appl.* 122:1–16. doi:10.1111/j.1570-7458.2006.00503.x
- Smith, C.M. 1989. Plant resistance to insects: A fundamental approach. John Wiley & Sons, New York.
- Song, F., S.M. Swinton, C. DiFonzo, M. O’Neil, and D.W. Ragsdale. 2006. Probability analysis of soybean aphid control treatments in three north-central states. Staff paper no. 2006–24. Dep. of Agricultural, Food, and Resource Economics, East Lansing, MI.
- Thomas, S., C. Dogimont, and N. Boissot. 2012. Association between *Aphis gossypii* genotype and phenotype on melon accessions. *Arthropod-Plant Interactions* 6:93–101.
- Van Emden, H., and R. Harrington. 2007. Aphids as crop pests. CABI, London.
- Wang, X.B., Y.H. Fang, S.Z. Lin, L.R. Zhang, and H.D. Wang. 1994. A study on the damage and economic threshold of the soybean aphid at the seedling stage. *Plant Prot.* 20:12–13.
- Warnes, G.R., B. Bolker, L. Bonebakker, R. Gentleman, W. Huber, A. Liaw, T. Lumley, M. Maechler, A. Magnusson, S. Moeller, M. Schwartz, and B. Venables. 2013. gplots: Various R programming tools for plotting data. R package version 2.11.3. <http://CRAN.R-project.org/package=gplots> (accessed 7 July 2015).
- Wiarda, S.L., W.R. Fehr, and M.E. O’Neil. 2012. Soybean aphid (Hemiptera: Aphididae) development on soybean with *Rag1* alone, *Rag2* alone, and both genes combined. *J. Econ. Entomol.* 105:252–258. doi:10.1603/EC11020
- Xiao, L., Y. Hu, B. Wang, and T. Wu. 2013. Genetic mapping of a novel gene for soybean aphid resistance in soybean [*Glycine max* (L.) Merr.] line P203 from China. *Theor. Appl. Genet.* 126:2279–2287. doi:10.1007/s00122-013-2134-5
- Zhang, G., C. Gu, and D. Wang. 2009. Molecular mapping of soybean aphid resistance genes in PI 567541B. *Theor. Appl. Genet.* 118:473–482. doi:10.1007/s00122-008-0914-0
- Zhang, G., C. Gu, and D. Wang. 2010. A novel locus for soybean aphid resistance. *Theor. Appl. Genet.* 120:1183–1191. doi:10.1007/s00122-009-1245-5