

MINI REVIEW

Performance and prospects of *Rag* genes for management of soybean aphid

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Abstract

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive insect pest of soybean [*Glycine max* (L.) Merr. (Fabaceae)] in North America, and it has led to extensive insecticide use in northern soybean-growing regions there. Host plant resistance is one potential alternative strategy for managing soybean aphid. Several *Rag* genes that show antibiosis and antixenosis to soybean aphid have been recently identified in soybean, and field-testing and commercial release of resistant soybean lines have followed. In this article, we review results of field tests with soybean lines containing *Rag* genes in North America, then present results from a coordinated regional test across several field sites in the north-central USA, and finally discuss prospects for use of *Rag* genes to manage soybean aphids. Field tests conducted independently at multiple sites showed that soybean aphid populations peaked in late summer on lines with *Rag1* or *Rag2* and reached economically injurious levels on susceptible lines, whereas lines with a pyramid of *Rag1* + *Rag2* held soybean aphid populations below economic levels. In the regional test, aphid populations were generally suppressed by lines containing one of the *Rag* genes. Aphids reached putative economic levels on *Rag1* lines for some site years, but yield loss was moderated, indicating that *Rag1* may confer tolerance to soybean aphid in addition to antibiosis and antixenosis. Moreover, no yield penalty has been found for lines with *Rag1*, *Rag2*, or pyramids. Results suggest that use of aphid-resistant soybean lines with *Rag* genes may be viable for managing soybean aphids. However, virulent biotypes of soybean aphid were identified before release of aphid-resistant soybean, and thus a strategy for optimal deployment of aphid-resistant soybean is needed to ensure sustainability of this technology.

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a principal insect pest of soybean [*Glycine max* (L.) Merr. (Fabaceae)] worldwide (Hartman et al., 2011). It is an important native pest of soybean in eastern Asia (Wang et al., 1996), and has been recognized since

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2000 as a serious invasive pest in northern soybean-production areas of North America (Ragsdale et al., 2011). Soybean aphid has a heteroecious, holocyclic life cycle that includes primary host plants, namely buckthorn (*Rhamnus* spp.), on which sexual reproduction and overwintering occur, and secondary hosts, principally soybean and wild relatives, on which several generations of asexual parthenogenetic populations occur throughout summer (Ragsdale et al., 2004). Soybean aphids colonize soybean in early summer, and their populations may grow exponentially, resulting in several hundred to many thousand aphids per plant (Mensah et al., 2005; Beckendorf et al., 2008; McCornack et al., 2008). Yield loss due to soybean aphids results from a soybean plant's cumulative exposure to aphids throughout a growing season (Ragsdale et al., 2007; Beckendorf et al., 2008). Seed yield and seed-oil concentration decline linearly as soybean aphid populations increase (Ragsdale et al., 2007; Beckendorf et al., 2008). In addition, soybean aphid can indirectly cause crop loss by transmitting plant-pathogenic viruses to soybean (e.g., *Soybean mosaic virus*, *Alfalfa mosaic virus*, and *Bean yellow mosaic virus*) and by facilitating sooty mold via deposition of honeydew on soybean leaves (Hill et al., 2001; Mensah et al., 2005; Wang et al., 2006).

Specific injury levels and action thresholds have been developed for soybean aphid. An economic injury level (EIL) of 674 aphids per soybean plant is generally used throughout north-central North America, and an economic threshold (ET) of 273 soybean aphids per plant (often rounded to 250 aphids per plant) is used to trigger foliar insecticide application, which is the management tactic used by most soybean producers in North America (Ragsdale et al., 2007, 2011; Hodgson et al., 2012a,b). This is a static EIL that is applied across multiple soybean reproductive stages, that is, from first bloom through beginning seed set (Ragsdale et al., 2007; Hodgson et al., 2012b). Alternatively, others have maintained that economic injury by soybean aphid varies by phenological stage of soybean (Catangui et al., 2009) and incidence of insect predators (Rhainds et al., 2007), and that such factors should be given greater consideration in determining action thresholds. Regardless, use of ET-based insecticide applications can protect yield and preclude unnecessary insecticide application (Johnson et al., 2009; Hodgson et al., 2012b), but despite this, soybean aphid is estimated to cost soybean producers in the USA between \$2 and \$5 billion annually in yield loss and insecticide use (Kim et al., 2008a; Song & Swinton, 2009).

Even though insecticides are the primary means of managing soybean aphid, alternative methods such as host plant resistance are being developed (Michel et al., 2011;

Hill et al., 2012). Several sources of cultivated and wild soybean have been identified with resistance to soybean aphid, but thus far the resistance has been found primarily in plant introductions, breeding lines, and older cultivars not adapted to the northern production areas of North America (Hill et al., 2012). Several studies in China found resistance to soybean aphid in some soybean lines (Fan, 1988; Hu et al., 1993; He et al., 1995; Wu et al., 2009; Meng et al., 2011), although none of these resistant sources is known to have been developed into a soybean aphid-resistant cultivar yet. Researchers in the USA have also identified many soybean aphid-resistant lines (Hill et al., 2012). The first soybean aphid-resistant lines discovered in the USA were characterized primarily with antibiosis and antixenosis resistance modalities (Michel et al., 2011; Hill et al., 2012), but later studies also identified the tolerance modality in at least one soybean line (Pierson et al., 2010).

Resistance to soybean aphid has been genetically characterized in eight lines (Table 1). Of these, eight individual *Rag* (Resistance to *Aphis glycines*) genes have mapped to four chromosomes, and been numbered from *Rag1* through *Rag5*, with three of the genes given provisional designations, depending on outcomes of future genetic tests (Hill et al., 2012). Four of the eight *Rag* genes act as single dominant genes (Hill et al., 2006a,b; Kang et al., 2008; Kim et al., 2008b; Mian et al., 2008b; Zhang et al., 2010; Jun et al., 2012a), and the remaining two pairs of genes are recessive (Mensah et al., 2008). In addition, soybean aphid resistance in PI 71506, though incompletely characterized, is due mainly to a single dominant gene that is distinct from *Rag1* (Carter et al., 2007; Van Nurden et al., 2010). Chinese researchers recently found resistance in two novel quantitative trait loci located on chromosomes 8 and 13, respectively, in soybean cultivar Zhongdou 27 that were strongly associated with high isoflavone content (Meng et al., 2011). In addition, an undetermined single, dominant gene mediated resistance to soybean aphid in the Chinese cultivar 'P746' (Wu et al., 2009; Xiao et al., 2012).

Identification and genetic characterization of *Rag* genes is key to development of aphid-resistant cultivars, and commercially available soybean aphid-resistant soybean cultivars in North America have been available in limited quantities since 2010 (Chiozza et al., 2010; Michel et al., 2011; McCarville et al., 2012a). At least 18 aphid-resistant soybean lines adapted to the Midwestern USA were commercially available through 2012, with 17 of the lines having *Rag1* only and one having a *Rag1+Rag2* pyramid (McCarville et al., 2012a). Statistics on the acreage planted to aphid-resistant soybean have not been published. Nevertheless, aphid-resistant cultivars face several

Table 1 Soybean genes conferring resistance to the soybean aphid, *Aphis glycines*

Gene	Chromosome (linkage group)	Resistance genes source(s)	Principal resistance modality	Reference(s)
<i>Rag1</i>	7 (M)	PI 548657 (cultivar Jackson)	Antibiosis and antixenosis	Hill et al., 2006a,b; Li et al., 2006; Kim et al., 2010
		PI 548663 (cultivar Dowling)	Antibiosis and antixenosis	Hill et al., 2006a,b; Li et al., 2006; Kim et al., 2010
<i>rag1b</i> (provisional)	– ¹	PI 567598B	Antibiosis	Mensah et al., 2008; Chandrasena et al., 2012
<i>rag1c</i>	7 (M)	PI 567541B	Antibiosis	Zhang et al., 2010
<i>Rag2</i>	13 (F)	PI 200538, PI 243540	Antibiosis	Kang et al., 2008; Mian et al., 2008b; Hill et al., 2009
<i>Rag3</i>	16 (J)	PI 567543C	Antixenosis	Mensah et al., 2005; Zhang et al., 2010
<i>rag3</i> (provisional)	– ¹	PI 567598B	Antibiosis	Mensah et al., 2008; Chandrasena et al., 2012
<i>rag4</i>	13 (F)	PI 567541B	Antibiosis	Zhang et al., 2010
<i>Rag5</i> (provisional)	13 (F)	PI 567301B	Antixenosis	Mian et al., 2008a; Jun et al., 2012b

Genes with capital letter are dominant, whereas the ones with only lower case letters are recessive.

¹Not determined.

potential constraints that are common to implementation of host plant resistance in various pest management systems (Stout & Davis, 2009). Three potential constraints are (1) selection for virulent genotypes (i.e., resistance-breaking biotypes) of soybean aphid; (2) reduction in yield and other desirable agronomic traits caused by linkage of resistance gene(s) (Lambert & Tyler, 1999; Strauss et al., 2002); and (3) variable regional performance due to genotype × environment interaction (Boethel, 1998). Several studies have addressed each of these constraints for *Rag* genes. The objectives of this article are to give an overview of several independent studies that have tested *Rag* genes against soybean aphid and in terms of agronomic performance, then present additional results on aphid resistance and agronomics from a comprehensive, regional study of the performance of *Rag* lines in the north-central USA, and finally discuss future use of *Rag* genes in soybean aphid management.

Rag genes in relation to virulent aphid biotypes

Arthropod pests are often able to adapt to and overcome management tactics used against them, and the tactic of host plant resistance is no exception (Gould, 1991; Rausher, 2001; Onstad & Knolhoff, 2008). Protection afforded by an arthropod-resistant cultivar may be overcome by virulent individuals of an arthropod pest species, whose genotype enables them to thrive on that particular cultivar (Smith, 2005). These virulent individuals, known as resistance-breaking biotypes, span at least 22 arthropod species (Smith, 2005; van Emden, 2007). Virulent arthropod biotypes are commonly associated with single-gene resistance in crop plants, and aphids are the most common type of

pest arthropod with virulent biotypes, having at least 14 species with two or more biotypes (Smith, 2005; van Emden, 2007).

Virulent biotypes of soybean aphid were identified in North America before commercial deployment of aphid-resistant soybean cultivars. To date, three soybean aphid biotypes are now known in relation to *Rag1* and *Rag2*. Biotype 1 is unable to colonize soybean plants containing *Rag1* or *Rag2* (Hill et al., 2010), biotype 2 is able to colonize *Rag1* but not *Rag2* plants (Kim et al., 2008b), and biotype 3 can colonize *Rag2* plants and also some plants with other *Rag* genes (Hill et al., 2010, 2012).

The ‘single-gene’ basis of aphid resistance identified thus far in soybean cultivars and preexistence of virulent biotypes begs the need for a strategy to optimally deploy *Rag* genes in North America. General strategies for deployment of crop cultivars with single, dominant, aphid resistance genes have been developed to prolong their utility in light of resistant-breaking biotypes. These strategies include (1) sequential release of resistance genes; (2) pyramiding two or more resistance genes in individual cultivars; (3) use of a multiline cultivar (in which individual plants vary in resistance gene carried); and (4) geographically varying deployment of resistance genes over a region (Smith, 2005; Khush, 2007). Depending on pest genetics, some strategies also require refuges of non-resistant crop (and sometimes non-crop) plants to ensure maintenance of susceptible pest individuals (Onstad & Knolhoff, 2008). Simulation models have been developed for several insect species to predict the optimal deployment strategy for various pest insect-crop situations, but outcomes have depended on specific pest and cropping system parameters (Gould et al., 2006).

To date, neither modeling to predict optimal *Rag* gene deployment nor a particular working strategy for deploying *Rag* genes has been developed despite commercial release of soybean aphid-resistant cultivars. Development of a predictive model for optimal *Rag*-gene deployment has been hindered because of limited knowledge about the geographic distribution and population genetics of virulent soybean aphid biotypes (Michel et al., 2011; Hill et al., 2012). Recently, Orantes et al. (2012) used six microsatellite markers to geographically characterize seasonal gene flow within soybean aphid populations in north-central North America. They determined that soybean aphid populations undergo genetic bottlenecks during spring colonization of soybean fields, but that genetic diversity is restored in late summer as aphid genotypes that have built up in individual fields migrate to other fields and effectively intermix with other genotypes. Presumably, genetic diversity is maintained as these intermixed populations migrate back to primary host plants in early autumn. Orantes et al. (2012) note implications for using aphid-resistant soybean lines, in that virulent biotypes may establish locally through early-season founder effects and then spread within a season over a larger geographic area.

The population genetics study by Orantes et al. (2012) used microsatellites not known to be associated with virulence to plant genotypes because these had not been identified at the time. However, studies have begun to identify and map virulence genes in soybean aphid (Bai et al., 2010; Jun et al., 2012a), and population genetics models for soybean aphid can be refined by adding genetic markers associated with virulence. Such models will provide an improved understanding of interaction between *Rag* genes and soybean aphid virulence genes, and may inform researchers and practitioners about optimal use of *Rag* genes in aphid-resistant soybean cultivars (Michel et al., 2011; Hill et al., 2012).

Individual field tests

Evaluations of aphid infestations on *Rag* lines

Despite lack of a model to predict optimal *Rag*-gene deployment, nine field studies conducted in individual states in the north-central USA since 2005 have evaluated various *Rag* genes, and these studies offer empirical evidence regarding *Rag*-gene performance. Eight studies will be reviewed herein, and the ninth will be presented later in this article. These studies include experiments in which single *Rag* genes were tested in individual lines at 1–2 locations (five studies), and ones in which pyramided lines of *Rag1* + *Rag2* were compared against lines with only one of the resistance genes and susceptible parent lines.

Among field studies that have evaluated single *Rag* genes, Carter et al. (2007) found that soybean aphids overcame *Rag1*-associated resistance by late summer (mid-August) of 2006 in South Dakota after inoculation with soybean aphids a few weeks earlier. Soybean aphids used to inoculate plants had been field-collected and then released onto soybean test plants within a large, walk-in field cage that largely excluded natural enemies. Hesler et al. (2012a) showed that non-caged, F₂-derived soybean selections subjected to a combination of natural infestation and supplemental inoculation exceeded 799 soybean aphids per plant (and thus the EIL) by late summer of 2006 in South Dakota. In Iowa, Chiozza et al. (2010) tested non-caged soybean plants and found that aphids on a *Rag1* line peaked at levels of a several hundred to a few thousand aphids per plant, whereas levels peaked at a few thousand to several thousand soybean aphids per plant on a related, susceptible, soybean line. O'Neal & Johnson (2010) also found that soybean aphid populations in Iowa peaked earlier and at a few thousand soybean aphids per plant on a susceptible line, whereas aphid populations rose gradually from midsummer to late summer and peaked at several hundred soybean aphids per *Rag1* plant. In 2008, Hesler et al. (2012b) found that soybean lines with the *Rag* or *Rag1* gene had infestations of <400 soybean aphids per plant, whereas *Rag2* plants ('Sugao Zairai') exceeded the EIL by late summer in 1 of 2 years under a combination of natural infestation and supplemental inoculation, but without caging. Non-cage tests conducted in 2011 by Bhusal et al. (2012) in South Dakota found that soybean aphid levels on lines with *Rag1* ('Dowling', PI 548663) or *Rag2* ('Sennari') did not differ from those on susceptible checks. In addition, lines with *Rag3* (PI 567543C) and *rag1c* + *rag4* (PI 567541B) had intermediate soybean aphid levels that differed neither from susceptible checks nor from a line (PI 603712) with strong, newly identified resistance to soybean aphid. Thus, these studies showed that aphid populations could exceed the EIL and potentially threaten yield on soybean lines carrying a single *Rag* gene, although none of them measured yield.

Two other field studies that evaluated aphid-resistant soybean each included a pyramid of *Rag1* + *Rag2*. The *Rag* genes in each of the respective studies were crossed with a common parent, which was also included in evaluations. In the first study, Wiarda et al. (2012) made 1–2 artificial infestations of soybean aphids in field cages to achieve target treatment levels of 675 soybean aphids per plant (based on the EIL; Ragsdale et al., 2007); 25 000 cumulative aphid-days (CAD, i.e., the number of aphids per plant accumulated over time; Hanafi et al., 1989); and 50 000 CAD in Iowa in 2010. The 25 000 and 50 000 CAD treatments represented maxima that had been used in

previous field-cage studies with soybean aphid (Ragsdale et al., 2007; Beckendorf et al., 2008). The susceptible line suffered yield loss of 19 and 27% at the 25 000- and 50 000-CAD levels, respectively. Nonsignificant yield reductions of 2, 12, and 5% corresponded to mean achievable maxima of 26 540 CAD, 23 845 CAD, and 761 soybean aphids per plant, respectively, on *Rag1*, *Rag2*, and *Rag1 + Rag2* soybean lines.

In the other pyramid study, Kandel (2012) tracked natural infestations of soybean aphids in South Dakota during 2011 in field plots of soybean breeding lines containing *Rag1*, *Rag2*, a pyramid of *Rag1 + Rag2*, and the common susceptible parent. All *Rag* lines accumulated fewer aphid-days than the susceptible line, but did not differ in CAD from one another. Soybean aphid populations diverged among lines from the 3rd week of July through the end of August, with soybean aphid populations on the susceptible line exceeding the ET (250 soybean aphids per plant; Ragsdale et al., 2007) during the 4th week of July. The *Rag2* line reached a maximum of 320 soybean aphids per plant in the 3rd week of August, whereas *Rag1* and *Rag1 + Rag2* lines never exceeded the ET. However, none of the *Rag* treatments differed in CAD.

Yield and other agronomic traits in *Rag* lines

Theoretically, defense against herbivores may be costly to soybean in terms of plant fitness, that is, yield (Strauss et al., 2002). Two studies addressed the issue of yield and other agronomic traits in *Rag1* lines, and two additional studies addressed it in lines pyramided with *Rag1 + Rag2* genes. First, Kim & Diers (2009) found no differences in yield and other agronomic traits under very low aphid levels between soybean lines with or without *Rag1*, except for a 2-day delay in maturity with *Rag1* lines in one elite background. Under aphid-free conditions, Mardorf et al. (2010) found no yield differences between commonly derived lines with or without *Rag1*, and small or nonsignificant differences in other agronomic traits; however, *Rag1* lines outyielded non-*Rag1* lines under aphid pressure. Similarly, Brace & Fehr (2012) compared soybean agronomic traits between pyramided lines and a susceptible recurrent parent under aphid-free conditions, and found that pyramided lines generally yielded less than the parental line and differed in several other agronomic traits. However, several individual pyramided lines did not differ from the parental line in agronomic traits, thus indicating it was possible to select for pyramided resistant lines with desirable agronomic traits. Kandel (2012) found resistant lines outyielded the susceptible parent, but observed no yield differences among lines with a single resistant gene or the *Rag1 + Rag2* pyramid. Collectively, these studies demonstrate the potential to develop cultivars containing *Rag1*

or *Rag1 + Rag2* that have agronomic performance comparable to elite susceptible cultivars, and thus free of yield constraints that could be associated with plant defense.

The apparent lack of cost to aphid resistance in soybean is contrary to theoretical predictions about trade-offs of plant defense, but consistent with several empirical studies showing no cost to plant defense of herbivores (Bergelson & Purrington, 1996; Strauss et al., 2002). Understanding the mechanism behind specific plant-defense responses may explain varying costs of plant resistance found in different plant-herbivore interactions (Bergelson & Purrington, 1996). To date, the specific mechanism(s) of resistance for any of the *Rag* genes have not yet been determined. In the case of *Rag1*-based resistance, Chiozza et al. (2010) found that soybean aphid failed to induce increased levels of several amino acids in resistant plants compared to susceptible plants during vegetative and early reproductive developmental stages of soybean, when aphid population growth is typically greatest and plants are most vulnerable to feeding injury (Beckendorf et al., 2008). Soybean aphids have been shown to be responsive to variation in plant physiology, specifically variation in the amino acid composition of phloem. For example, soybean grown in potassium-deficient soils has elevated concentrations of key amino acids that are essential for optimal soybean aphid population growth (Myers et al., 2005; Myers & Gratton, 2006; Walter & DiFonzo, 2007). The relationship observed by Chiozza et al. (2010) between the amino acid composition of aphid-susceptible and -resistant soybeans suggests that a mechanism of *Rag* resistance may include a constitutive response related to general host plant quality. Furthermore, higher aphid populations on susceptible plants were attributed to greater concentrations of several amino acids induced in susceptible plants during those developmental stages, but increases in aphid levels on *Rag1* plants corresponded with increased amino acid levels at a later reproductive (i.e., beginning bean) stage. Thus, aphid resistance in *Rag1* plants may also be a cost-saving mechanism by which failure to increase amino acid production during vegetative and early reproductive stages in response to aphid infestation conserves resources and manifests no measurable penalty to plants in terms of yield and quality.

Two studies have conducted large-scale transcriptional profiling of soybean responses of susceptible and *Rag1* lines to infestation by soybean aphid. Li et al. (2006) found differential transcription between Dowling and a susceptible cultivar ('Williams 82') over a 48-h period, with earlier and greater induction of three defense-related genes in Dowling. The induction declined after 24 or 48 h in Dowling but continued to increase in Williams 82 after 24 h. Transcriptional profiles of soybean by Studham &

MacIntosh (2012) showed a susceptible response to soybean aphids that involved upregulation of hundreds of transcripts, whereas only a single transcript changed in a *Rag1* line. This difference suggested that many defense-related transcripts are expressed constitutively in the *Rag1* line.

Regional test of *Rag* lines

Field studies with aphid-resistant soybean genotypes summarized above were each conducted independently at 1–2 nearby sites each within individual states, and those studies provided isolated examples of performance by

aphid-resistant soybean. However, degree of aphid resistance and yield of resistant genotypes may depend on environment where the crop develops and on interaction between crop genotype and environment (Boethel, 1998), and thus a regional study was appropriate to evaluate performance of soybean aphid-resistant soybean genotypes in terms of aphid infestations and yield protection across multiple sites in the north-central USA. Various soybean genotypes with different sources of *Rag* genes were planted at 10 sites in 2007 and 2008 (Table 2). Typically, three replications of each genotype were planted in a randomized complete block design, but in Michigan, five and four

Table 2 Soybean lines tested against naturally occurring infestations of soybean aphid in the north-central USA during 2007 and 2008

Line	Year	Site ¹	Description
E06901 ²	2007	1–7	Sister lines derived from different F ₃ plants that each trace to individual F ₂ plants of the cross Titan RR × PI 567598B. Titan RR was developed by backcrossing Titan (Diers et al., 1999) four times to F ₁ of Titan × Olympus RR. PI 567598B is resistant to soybean aphid (Mensah et al., 2008; D Wang, unpubl.)
	2008	1–3, 5–8	
E06905 ²	2007	1–7	Derived from F ₅ plant of PI 567597C × Titan. PI 567597C is resistant to soybean aphid (Mensah et al., 2005)
E06906 ²	2007	1–7	
E06902 ²	2007	1, 3, 5–7	R93–174 × Northrup King s59-60; resistant to soybean aphid (Diaz-Montano et al., 2006)
	2008	1–3, 5–8	
E07901 ²	2008	1–3, 5–8	KS5292 × SC91-2007
K1639 ³	2007	1–7	Dowling × Loda (4). BC ₃ F ₂ -derived line that does not have the <i>Rag1</i> allele
KS5004N ³	2007	1–7	
LD05-16519 ⁴	2007	1–3, 5–7	Dowling × Loda (4). BC ₃ F ₂ -derived line that carries the <i>Rag1</i> allele
	2008	1–3, 5–8	
LD05-16529 ⁴	2007	1–3, 5–7	(Dowling × Loda) × Dwight (3). BC ₃ F ₂ -derived line that carries the <i>Rag1</i> allele
	2008	1–3, 5–7	
LD05-16611 ⁴	2007	1–3, 5–7	(Dowling × Loda) × Dwight (3). BC ₃ F ₂ -derived line that does not have the <i>Rag1</i> allele
	2008	1–3, 5–8	
LD05-16621 ⁴	2007	1–3, 5–7	(Dowling × Loda) × SD01-76R(3). Carries <i>Rag1</i> allele
	2008	1–3, 5, 6–8	
LD05-16060 ⁴	2007	1–3, 6, 7	Loda was crossed with Dowling and then three crosses were made to SD01-76R. The line would be a backcross two (BC ₂) line. BC ₂ F ₂ -derived line that carries the <i>Rag1</i> allele
	2008	1–3, 5, 6–8	
SD(LD)05R-16137 ⁵	2008	1–3, 5–8	(Loda × Dowling) × SD01-76R (3). Selected as resistant
SDX04R-68-1-3 ⁵	2007	1–7	Line SD-824 was selected from Parker × Archer cross and then crossed 4 times with Resnik RR
SDX04R-68-1-8 ⁵	2007	1–7	Line SD93-828R was obtained from that cross and then crossed with IA2021. Line SDX00R-039-42 was selected and crossed with PI71506
SDX04R-68-1-9 ⁵	2007	1–7	F ₅ plant from the cross SDX00R-039-42 × PI 567541B. SDX00R-039-42 is an advanced soybean breeding line from South Dakota State University. PI 567541B is resistant to soybean aphid (Mensah et al., 2005)
E07906-2-2 ⁵	2008	1–3, 5–8	
SD01-76R ⁴	2007	1–3, 6, 7	(Stride × Resnik RR) × Stride; susceptible to soybean aphid
	2008	1–3, 5–8	

¹Site 1 = Iowa (Story County), 2 = Iowa (Floyd County), 3 = Illinois (Whiteside County), 4 = Illinois (Champaign County), 5 = Michigan (Ingham County), 6 = Wisconsin (Dane County), 7 = South Dakota (Brookings), and 8 = Minnesota (Redwood County).

Soybean line developed and seed provided by:

²Dechun Wang, Michigan State University.

³John Reese, Kansas State University.

⁴Brian Diers, University of Illinois.

⁵Roy Scott and Marci Green, South Dakota State University.

replications were planted in 2007 and 2008, respectively. All plots were planted at 30 seeds m^{-2} in four rows 0.76 m apart and 3.6 m long with standard agronomic practices, with no insecticides applied to seed or foliage.

Number of soybean aphids was recorded once per week throughout the growing season on a number of plants per plot that varied with infestation level, that is, 20 randomly selected plants per plot when less than 50% of plants were aphid infested, reduced to 10 plants per plot when 50–80% of plants were infested, and reduced further to five plants per plot when more than 80% of plants were aphid infested. Seasonal exposure to soybean aphid was reported in cumulative aphid-days (CAD), calculated as the sum of the average number of aphids per plant between consecutive sampling dates multiplied by the number of days between sampling (Hanafi et al., 1989). Cumulative aphid-days were log-transformed to achieve homogeneity of variances.

Methods: cluster analysis and group comparisons

A hierarchical cluster analysis (Williams, 1976) with a centroid method as the fusion criterion (Jobson, 1991) was used to group lines based on soybean aphid infestation levels within the site and soybean-genotype matrix. Dendrograms for aphid levels were constructed on the basis of the fusion criterion to determine patterns in performance among soybean lines and sites. Groups identified in cluster analysis were compared to each other to eliminate redundancy from pairwise comparisons of individual lines. Combinations of cluster analysis and group comparison have been applied to assess yield response of different crop genotypes among environments (Chapman et al., 1997; de la Vega et al., 2001).

The density of aphids was converted to logCAD and analyzed for 15 soybean lines at seven sites during 2007 and 11 soybean lines at seven sites during 2008 (Table 2). A separate cluster analysis was conducted in each year because not all lines were planted in both years. 'Aphid infestation' was the logCAD value of a given soybean line at a given site, and 'aphid pressure' was the average logCAD value of all soybean lines at a given site. Cluster methodology requires a complete dataset within each year. Because not all soybean lines were planted at all sites within a year, the aphid infestation value of these lines at a particular site was estimated by linear regression between aphid infestation and aphid pressure using sites and years where these lines were planted (analysis not shown). For instance, line E06902 in 2007 was not planted in Iowa (Floyd County) and Illinois (Champaign County). The logCAD values for this line at these two sites were estimated using the regression equation between aphid

infestation and aphid pressure at all the other sites where this line was planted in 2007 and 2008, thereby predicting performance of a line at sites where it was not planted and producing a more robust analysis, that is, high r^2 values for the regressions (data not shown).

After genotypes were grouped by cluster analysis, linear regression between aphid infestation in each group and aphid pressure at each site was used to evaluate relative performance among groups. Aphid infestation in each group was calculated as the average aphid infestation (logCAD) of lines that belong to each group. Aphid pressure by site was calculated as was stated before. Slope and intercept differences between groups in the linear regression were determined using Proc GLM (SAS Institute, 2005) with groups as fixed factors and lines within each group as replications or random effects.

Analysis of genotype \times environment interaction

We conducted an analysis for a genotype \times environment (G \times E) interaction in a subset of soybean lines that were planted in both years in the same environments. Five of all lines were planted in 2007 and 2008 (E06901, LD05–16519, LD05–16529, LD05–16611, LD05–16621) in the same six environments (Iowa, Story County and Floyd County; Illinois, Whiteside County; Michigan, Ingham County; South Dakota, Brookings County; Wisconsin, Dane County). Data were analyzed using Proc GLM (SAS Institute, 2005) with sites and lines as fixed factors and years and blocks as random factors.

Evaluation of *Rag1* across environments

To evaluate performance of *Rag1* across environments and its effect on yield, LD05-16060 and SD01-76R were planted from May to June in several sites each year. LD05-16060 is a backcross line with *Rag1* developed by using SD01-76R as susceptible recurrent parent (Table 2). In 2007, these lines were planted in five environments (Illinois, Whiteside County; Iowa, Story County; Minnesota, Redwood County; and Michigan, Ingham and Saginaw counties); in 2008, they were planted in six environments: (Illinois, Whiteside County; Iowa, Story County; Minnesota, Redwood County; South Dakota, Brookings County; and Michigan, Ingham and Saginaw counties). Thus, over both years, 11 site years were used to test effectiveness of *Rag1* on yield preservation.

A split-plot design with four blocks was used per each site and year, with soybean lines assigned to the whole plot and aphid exposure (manipulated with insecticide) assigned to subplots. Whole plots were planted at 30 seeds m^{-2} with 12 rows 0.76 m apart and 16 m long. Subplots (six rows wide and 16 m long) were randomly assigned within each whole plot and were either not

treated with insecticide ('natural aphid infestation' plots) or treated with lambda-cyhalothrin at 94.6 ml per acre when more than 50 soybean aphids per plant were observed ('aphid-free' plots) to preclude yield loss. Aphid-free subplots received 1–3 applications of insecticide per growing season, depending upon site. Soybean aphid infestation in all sub-plots was estimated as above, and analyzed as logCAD using Proc ANOVA (SAS Institute, 2005), with sites and lines as fixed factors and replications as random effects. Yield was also compared between resistant and susceptible lines in subplots.

Regression analysis was used to determine yield response (in terms of relative yield) of resistant and susceptible lines to different levels of aphid infestation. Relative yield (%) was calculated as the ratio between yield of naturally infested and aphid-free subplots. Yield (expressed on 13% moisture basis) was measured in four central rows per subplot. A bilinear model was used to fit the susceptible line using Table Curve 2D v5.01 software (Jandel Scientific, 1991). No yield estimates were obtained from Ingham County, Michigan, during 2007. In addition, although the yield at Ingham County during 2008 was estimated in the regression, it was excluded from the model due to severe infestation with Japanese beetles. *Popillia japonica* Newman (Chandrasena et al., 2012), as this may have confounded relationship between soybean aphid infestation and yield.

Results: cluster analysis and group comparisons

Cluster analysis grouped soybean lines with similar sources of resistance and pedigree. A dendrogram created from 2007 data revealed four groups of soybean lines (Figure 1A). Groups 1 and 2 shared four lines (E06901, E06905, E06906, E06902) with a common aphid-resistant source (PI 567598B). Group 3 included soybean lines (K1639, KS5004N, SDX04R-68-1-3, SD X04R-68-1-8, SD X04R-68-1-9) and *Rag1* genotypes (LD05-16060, LD05-16529, LD05-16611). Group 4 included non-resistant lines LD05-16519, LD05-16621, and SD01-76R.

The dendrogram created from 2008 data had three clusters (Figure 1B). Reduction in the number of groups in 2008 compared to 2007 was likely due to addition of new lines from Michigan (E07906-2 and E07901) and Illinois [SD(LD)05R-16137] and the removal of others (E06905, E06906, K1639, KS5004N, SDX04R-68-1-3, SDX04R-68-1-8, SDX04R-68-1-9). Lines E06901 and E07906-2 separated into different clusters, but we decided to include them in the same group, as both lines were highly resistant (i.e., very low seasonal infestation of aphids) at all sites and years, except E06901 reached 4 478 aphids per plant in Story County, Iowa, in 2008. This high aphid population

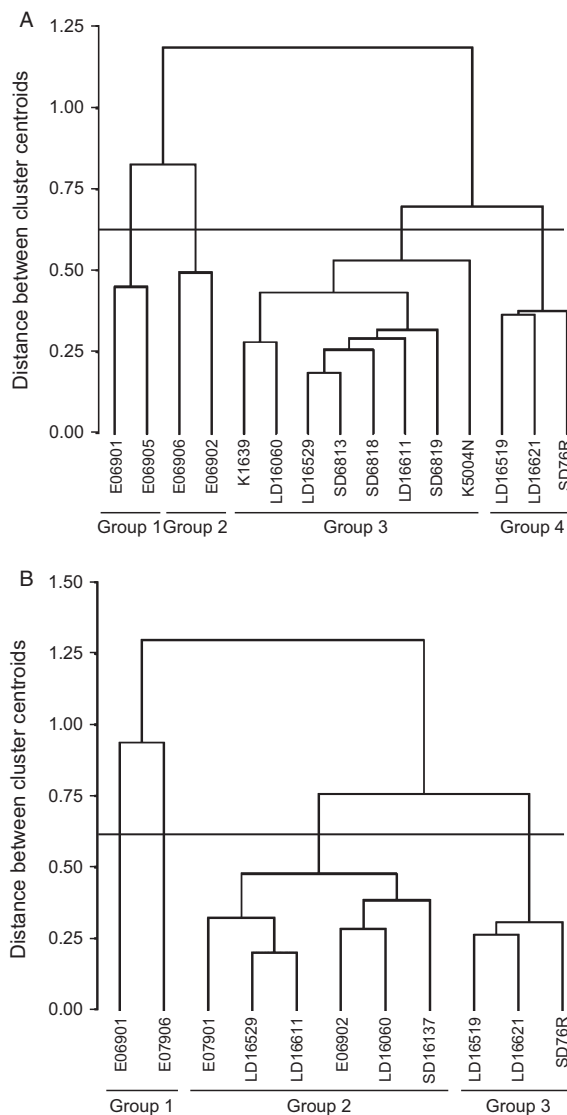


Figure 1 Classification pattern of naturally occurring infestation levels of soybean aphids on soybean lines grown in the north-central USA during (A) 2007 and (B) 2008. See Table 1 for entries and sites.

in Story County resulted in an average of 370 aphids per plant. If the Story County site is excluded, the mean aphids per plant equals 54; this value is similar to that for E07906-2 (48 aphids per plant). We accounted for this similarity in grouping from cluster analysis by including lines E06901 and E07906-2 into the same group (group 1). The second group (group 2) included lines carrying *Rag1* [LD05-16060, LD05-16529, LD05-16611, SD(LD)05R-16137] and two lines developed in Michigan (E07901, E06902). Group 3 contained lines without aphid resistance genes (LD05-16519, LD05-16621, SD01-76R).

To compare resistance to soybean aphids among groups, we conducted regression analyses in which aphid pressure at a given site (x-axis) was compared to pressure experienced by a group (y-axis). Regression analyses of the 2007 data revealed that all groups responded similarly to increasing levels of aphid pressure (Figure 2A). Differences in slope among the four groups were not significant. Despite similarity in slopes, y-intercepts differed among groups ($P = 0.01$). At similar slope, the y-intercept can be seen as an indication of the level of resistance of a group, with the most resistant groups presenting a y-intercept value lower than average, and more susceptible groups showing higher values. Based on aphid infestation, group 1 was the most resistant group (Figure 2A). Intercept differences between group 1 and the other three groups were all significant ($P_{\text{group 1 vs. 2}} = 0.01$; $P_{\text{group 1 vs. 3}} = 0.007$; $P_{\text{group 1 vs. 4}} = 0.002$). The cluster analysis suggested groups 2 and 3 as unique, but their intercepts did not differ from one another ($P_{\text{group 2 vs. 3}} = 0.42$). Intercept

differences between Groups 2 and 4 were significant ($P_{\text{group 2 vs. 4}} = 0.04$). Finally, group 4 was the most susceptible group, even though intercept differences between this group and group 3 were not significant (Figure 2A). Changes in group rankings across sites were not observed. For example, lines within group 1 experienced the lowest aphid populations at all sites.

For 2008, regression analyses of aphid pressure by site and aphid infestation by groups (Figure 2B) revealed that, as in 2007, all groups responded similarly to greater aphid pressure. Slopes did not differ among groups ($P = 0.17$), but intercept differences among groups were significant ($P = 0.01$). Group 1 had the lowest levels of aphid infestation. Intercept differed between this group and groups 2 and 3, respectively ($P_{\text{group 1 vs. 2}} = 0.04$; $P_{\text{group 1 vs. 3}} = 0.007$). Intercepts differed between groups 2 and 3 ($P_{\text{group 2 vs. 3}} = 0.03$). Finally, group 3 was the most susceptible group. In 2008, there was no change in group ranking across sites among different aphid pressures.

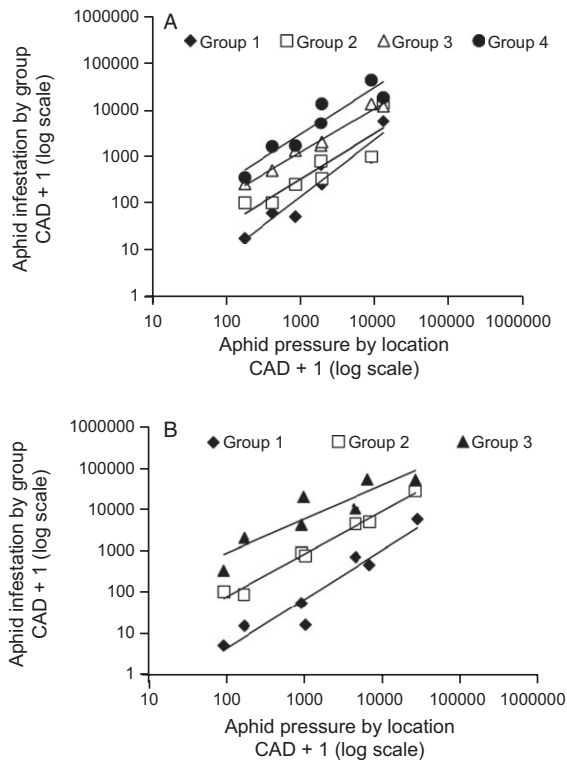


Figure 2 Linear regression between aphid infestation and aphid pressure for each group in (A) 2007 and (B) 2008. Groups represent multiple lines of soybeans (see Table 1) based on their performance at multiple environments in the north-central USA. In (A), groups 1, 2, and 3 include aphid-resistant lines, group 4 does not. In (B), groups 1 and 2 include aphid-resistant lines, group 3 does not.

Results of tests for G × E interaction

A total of five lines and six sites were used to determine whether a G × E interaction occurred in 2007 and 2008. We found a significant site*line*year interaction (Table 3) suggesting that the relative performance of the lines among sites varied depending on the year (Figure 3). In 2007, E06901 was the most resistant line at all sites except in Dane County, Wisconsin, where this line experienced similar aphid infestation levels to that of LD05-16611, LD16629, and LD05-16621. In two of the five states, lines with *Rag1* had lower levels of aphid infestation than lines that did not have the gene. In 2008, E06901 was the most resistant line in three of five states. In Story County, Iowa, E06901 had similar aphid infestation levels to LD05-16611, LD16629, and LD05-16621. In Floyd County, Iowa, the aphid infestation of E06901 was similar to that of the line LD05-16611 (Figure 3). As in 2007, *Rag1*-containing

Table 3 Impact of environment (site) and genotype (soybean line) on soybean aphid population levels on soybean plants, 2007 and 2008 [analysis of variance (Proc GLM): $P < 0.05$]

Source	d.f.	F	Pr>F
Year	1,27	4.06	0.054
Site	5,5	10.46	0.011
Line	4,4	38.07	0.002
Year*site	5,27	17.50	<0.0001
Year*line	4,108	4.81	0.001
Site*line	20,20	1.92	0.076
Year*site*line	20,108	3.48	<0.0001

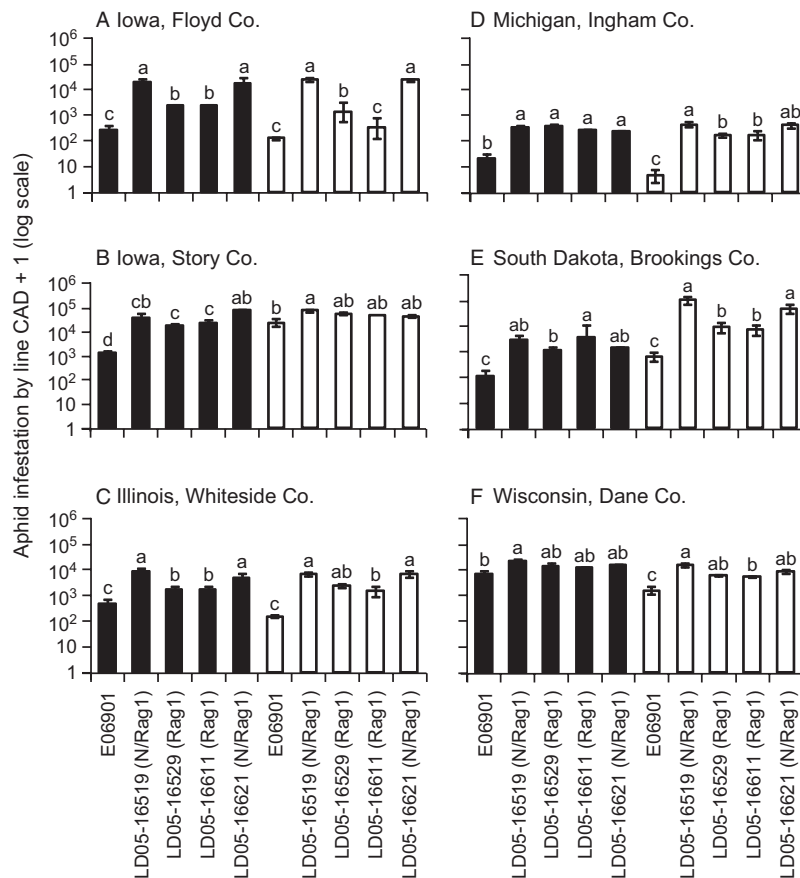


Figure 3 Performance (measured as cumulative aphid-days, CAD, mean \pm SE) of soybean lines among six sites in 2007 (black bars) and 2008 (white bars). Only lines planted in both years in the same sites were tested for genotype \times environment interaction (N/Rag1 = lines without the *Rag1* gene; Rag1 = lines with the *Rag1* gene). Different letters indicate significant differences between lines within a site year [analysis of variance (Proc ANOVA), $P < 0.05$].

lines had lower aphid infestation in two states and similar aphid infestation in three states when compared to lines that did not have the gene (Figure 3).

Rag1 performance across environments

Aphid infestation levels on the resistant (LD05-16060) line were significantly lower (lower logCAD) than on the susceptible (SD01-76R) line at most site-year combinations as revealed by the site-year \times line interaction ($P = 0.004$). However, this difference was not observed in Michigan during 2007 [Saginaw County (MI07/2)] and 2008 [Ingham County (MI08/1), Saginaw County (MI08/2)]. Although aphid populations were higher on the susceptible line at the Michigan sites (Figure 4), they were not significantly different from that on the resistant line. Aphid populations observed on both susceptible and resistant lines in Michigan during the 2 years were the lowest of any of the sites.

As aphid infestations increased, relative yield dropped significantly for the susceptible line (Figure 5, linear phase). A 42% decrease in yield was observed when CAD reached 151 355 aphids per plant (logCAD = 5.18). In contrast, we did not observe as great a yield reduction in the resistant line (site \times line interaction, $P = 0.0009$). In fact, the resistant line showed no changes in relative yield as aphid infestation increased; the slope in the linear regression was not significantly different from zero. However, relative yield of the susceptible line was similar to that of the resistant line when CAD were below 4 073 aphids per plant (logCAD = 3.61).

To determine whether yield drag was potentially associated with the presence of *Rag1*, yield potential in the resistant and susceptible lines was compared in subplots that were sprayed with insecticide. Yield differences were observed between these two lines for the South Dakota-2008 site year, contributing to a marginally significant

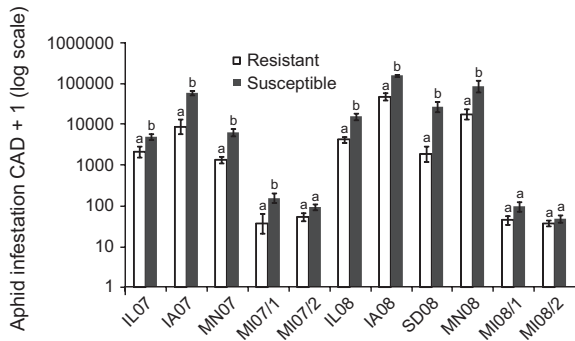


Figure 4 Soybean aphid infestation (mean numbers of aphids \pm SE) on aphid-resistant (LD05-16060) and aphid-susceptible (SD01-76R) lines across multiple sites and years (2007, 2008). Different letters depict significant differences between lines at each site [analysis of variance (Proc ANOVA) $P < 0.05$]. IL, Illinois (Whiteside County); IA, Iowa (Story County); MN, Minnesota (Redwood County); SD, South Dakota (Brookings County); MI/1, Michigan (Ingham County); and MI/2, Michigan (Saginaw County).

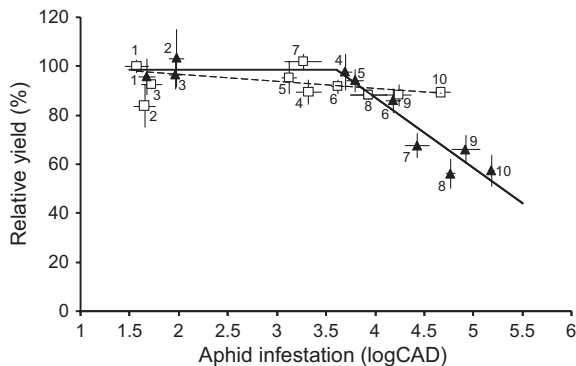


Figure 5 Linear regression between relative yield and soybean aphid infestation in resistant (LD05-16060; dashed line) and susceptible (SD01-76R; solid line) soybean lines across multiple sites and years. Each data point (white squares, resistant; black triangles, susceptible) indicates a combination of site and year with means (\pm SE) for aphid infestation (horizontal bars) and relative yield (calculated as the ratio between yield of naturally infested and aphid-free subplots; vertical bars): 1 = MI 2008 (Saginaw County), 2 = MI 2008 (Ingham County), 3 = MI 2007 (Saginaw County), 4 = IL 2007 (Whiteside County), 5 = MN 2007 (Redwood County), 6 = IL 2008 (Whiteside County), 7 = SD 2008 (Brookings County), 8 = IA 2007 (Story County), 9 = MN 2008 (Redwood County), and 10 = IA 2008 (Story County). The equation for the line created from the regression analysis for the resistant soybean 00 line was $y = -2.86x + 102.41$, $r^2 = 0.35$. For the susceptible line in the constant phase $y = 98.51$ and in the linearly decreasing phase $y = -28.8x + 202.48$, $r^2 = 0.92$.

site*line interaction ($P = 0.053$) (Table 4). However, average yield in the susceptible line was lower than that of the resistant line (3 260 vs. 3 692 kg ha⁻¹, respectively). Yield did not differ between susceptible and resistant lines in aphid-free subplots for other site years.

Discussion

Performance of aphid-resistant soybean genotypes was consistent across multiple sites and years. Line E06901 (*rag1b* provisional and *rag3* provisional) tended to have fewer aphids per plant than lines carrying *Rag1*, but the magnitude of that difference depended on site and year. Using cluster analysis, E06901, E06902, E06905, E06906, and E07906-2 were the most resistant lines across both years of evaluation. In 2008, some of these lines grouped with ones carrying *Rag1*, suggesting that *Rag1* lines performed better in 2008 than in 2007. For example, in four of six sites, aphid infestation (logCAD) was lower in 2008 than in 2007 for LD05-16060 and LD05-16611.

There was a wide range in aphid numbers (100–1 000 s of aphids per plant) across sites. Even so, performance of the different groups in both years, and thus lines comprising the groups, was consistent. Regression analyses among groups identified in cluster analysis supported this contention. In both years, there was no change in ranking of groups in response to increasing aphid pressure. The rate of increase (Figure 2) was similar for all groups as determined by cluster analysis. This suggests that resistant lines may not suffer localized outbreaks due to aphid biotypes that are present within a given region, although the risk may increase with selection for virulent biotypes over time.

Performance of *Rag1* was consistent across different site years. The resistant line was never aphid free, but had significantly lower soybean aphid populations than the corresponding susceptible line, except for sites that experienced low aphid pressure (MI07/2, MI08/1, MI08/2) (Figure 4). *Rag1* lines experienced significant aphid infestation at multiple sites. For example, during 2008 in Story County, Iowa, peak aphid population was 2 458 aphids per plant, well above the EIL. However, although we observed

Table 4 Impact of the *Rag1* gene on soybean yield in the absence of soybean aphids across sites, 2007 and 2008 [analysis of variance (Proc GLM), $P < 0.05$]

Source	d.f.	F	Pr>F
Site	8,27	34.46	<0.0001
Line	1,8	0.72	0.48
Site*line	8,27	1.32	0.053

significant yield losses on susceptible lines as aphid infestation exceeded EIL, there was no significant yield loss in *Rag1* soybeans. Based on regression analysis (Figure 5), only 35% of relative yield was explained by aphid pressure on resistant lines, whereas 92% of relative yield was explained by aphid pressure in the susceptible line. Thus, in addition to antibiosis, *Rag1* may also confer tolerance to aphid infestations. Whether this is due to *Rag1* or minor genes present in the specific line tested is not known. Given that population growth of aphids appears slower on the *Rag1* line (Chiozza et al., 2010), this tolerance may be a result of aphid outbreaks occurring on plants that are more mature and thus less susceptible to damage by aphid infestations.

Synthesis and future directions

In summary, soybean lines containing 1–2 *Rag* genes generally performed well across a range of infestation levels of soybean aphid at multiple sites in the north-central USA, although individual lines with *Rag1* or *Rag2* alone sometimes had aphid populations that on average exceeded the EIL. In the case of *Rag1*, concern about late-season increases in soybean aphid populations may be moderated by accompanying tolerance to yield loss demonstrated in the regional test. Analogous testing for tolerance in *Rag2* lines is needed.

Although individual soybean cultivars with *Rag1* or *Rag2* alone may harbor low population density of soybean aphid that occasionally rises to economically relevant levels, at least two factors favor the adoption of aphid-resistant soybeans as a proactive means of managing soybean aphid. First, the premium for aphid-resistant soybean lines has been lower than the cost of foliar insecticides (McCarville et al., 2012a,b). In addition, a large proportion of soybean fields in northern production regions are scouted for soybean aphid (Hodgson et al., 2012a). Moreover, lines with a pyramid of *Rag1* + *Rag2* may counter biotypes that are able to build up on lines with either *Rag* gene by itself, and the commercial availability of pyramided lines (McCarville et al., 2012a) may facilitate the adoption of host plant resistance as the primary management tactic against soybean aphid. Additional combinations of *Rag* genes in pyramided lines may be developed in the future to broaden the suite of plant resistance options for soybean producers (Onstad & Knolhoff, 2008; Hill et al., 2012). One strategy for managing *Rag* genes may be to pyramid lines that combine a *Rag* gene that confers antibiosis with another one that provides antixenosis to potentially reduce the proportion of antibiosis-breaking biotypes that colonizes lines (Hesler & Dashiell, 2011).

Finally, there was no yield penalty to soybean plants having *Rag1*, *Rag2*, or a pyramid of *Rag1* + *Rag2*. Corresponding studies are needed to test for yield drag or tolerance that may be associated with other *Rag* genes, including additional pyramided lines. Thus, the EIL for soybean aphid may need re-evaluation to determine whether resistant soybean can accommodate higher infestation levels given tolerance to soybean aphid in some lines (Hodgson et al., 2012b). If so, this may lead to reduced insecticide applications in this crop.

Identification and characterization of additional sources of resistance are also needed. Many soybean lines have now been identified with resistance to *A. glycines* (Hill et al., 2012), but further studies are needed to determine the genetics of resistance in uncharacterized lines and whether novel *Rag* genes may exist. The search for new sources of resistance with novel genetic bases is justified, given the presence of multiple North American biotypes of soybean aphid (Hill et al., 2009).

So far, there has been a laissez-faire approach to releases and sales of aphid-resistant soybean, with no overarching strategy regarding deployment of aphid resistance genes. Thus, there is a pressing need to develop a strategy for optimal deployment of *Rag* genes (McCarville & O'Neal, 2012). Given preexistence of virulent biotypes of soybean aphid in North America, one critical piece needed for developing a strategy is knowledge of the genetics of virulence to resistant lines (Hill et al., 2012), and such knowledge may soon be forthcoming from studies currently underway (Bai et al., 2010; Jun et al., 2012b). Upon its development, one possible challenge in implementing a deployment strategy will be coordinating release and use of various *Rag* genes in aphid-resistant lines by multiple public and private entities. Some resistance-gene management plans require a given proportion of acreage be planted to susceptible lines as refugia to maintain non-virulent pest genotypes (Onstad & Knolhoff, 2008), and research is needed to determine whether a *Rag* deployment strategy would require refugia for soybean aphid. Seed of soybean aphid-resistant lines is currently limited in availability, and so there is a de facto refuge. Thus, time remains for the development of a *Rag*-gene deployment strategy.

Host plant resistance is generally compatible with other strategies that may be integrated within an insect management program (Smith, 2005; Stout & Davis, 2009). Some research of this type has been conducted with soybean aphid. For instance, no observable negative impact on key generalist aphidophagous predators in soybean has been associated with the planting of *Rag* lines (Kandel, 2012; Tinsley et al., 2012), although mummy production of a soybean aphid parasitoid was diminished on soybeans

with *Rag1* due to abbreviated life span of parasitized vs. non-parasitized soybean aphids (Ballman et al., 2012). In the presence of various natural enemies, soybean plants with *Rag1*, *Rag2*, or a *Rag1* + *Rag2* pyramid held soybean aphid populations below the EIL and preserved yield (McCarville & O'Neal, 2012). Further research is also needed to ensure integration of *Rag*-based resistance with other aphid-management practices in soybean (McCarville et al., 2012b).

In addition, work is needed to make certain that development of host plant resistance against soybean aphid is compatible with management of other soybean pests. For instance, two other invasive insects, the Japanese beetle, *P. japonica*, and the brown marmorated stink bug, *Halyomorpha halys* (Stål), are pests in late vegetative to reproductive stages of soybeans in North America (Chandrasena et al., 2012; Leskey et al., 2012). Because their infestations may overlap temporally and geographically with that of soybean aphid, management of these three pests could conflict, particularly if insecticide applications against Japanese beetle or brown marmorated stink bug disrupted the complementary control of soybean aphid by natural enemies and plant resistance (McCarville & O'Neal, 2012). However, alternative means of managing Japanese beetle and brown marmorated stink bug may conserve aphid natural enemies and capitalize on the effectiveness of resistant lines against soybean aphid. For instance, some aphid-resistant lines suffer less damage from Japanese beetle than other lines (Chandrasena et al., 2012), and thus it may be possible to avoid insecticide applications and simultaneously manage both Japanese beetle and soybean aphid through host plant resistance. With marmorated stink bug, some management tactics, such as trap cropping, limiting insecticidal sprays to field perimeters, or applying insecticide at soybean stages (e.g., bean fill and later) when soybean aphid is not injurious, might fit with host plant resistance and other aphid-management approaches (Leskey et al., 2012).

Some aphid-resistant soybean cultivars have been stacked with other resistance traits, such as those to glyphosate herbicide, soybean cyst nematode, and fungal root rots (McCarville et al., 2012a). Correspondingly, genes for resistance to other insect pests might also be included in stacked cultivars (Lambert & Tyler, 1999). Future research may also include ensuring compatibility of resistant lines with natural enemies and determining effects of insecticidal seed treatments used in conjunction with aphid-resistant plants. Ultimately, various approaches may then be integrated with host plant resistance to optimize management of soybean aphid.

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