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Soybean Aphid (Hemiptera: Aphididae) Development on Soybean with Rag1 Alone, Rag2 Alone, and Both Genes Combined

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Abstract
Aphis glycines Matsumura (Hemiptera: Aphididae) can reduce the yield of aphidsusceptible soybean (*Glycine max* (L.) Merrill) cultivars. The *Rag1* and *Rag2* genes confer resistance to some biotypes of *A. glycines*. These genes individually can limit population growth of *A. glycines* and prevent yield loss. The impact of these genes when combined is not known. We compared the development of *A. glycines* on soybean with Rag1 alone (R1/S2), *Rag2* alone (S1/R2), both genes combined (R1/R2), or neither gene (S1/S2). In addition, we determined the impact of different levels of aphid infestation on seed yield. The genotypes were grown in cages and artificially infested with *A. glycines* to achieve five treatment levels: aphid-free, 675 aphids per plant, 25,000 cumulative aphid days (CAD) (25K), 50,000 CAD (50K), and 75,000 CAD (75K). The S1/S2 line reached the 50K treatment, but did not reach the 75K treatment. Aphid development on R1/S2 and S1/R2 soybeans after two infestations reached a maximum of 25K. The maximum treatment reached on R1/R2 was only 675 aphids per plant after two infestations, at which there was no significant yield reduction when compared with the aphid-free treatment. The maximum yield reduction of S1/S2 was 27% at 50K treatment compared with 2% for R1/S2 and 12% for S1/R2 at the 25K treatment. Our results indicated that for *A. glycines* used in our study, cultivars with both Rag1 and Rag2 had less aphid exposure and less yield reduction than soybeans with only one resistant gene.

Keywords
host plant resistance, integrated pest management (IPM), antibiosis, Aphis glycines, soybean

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Soybean Aphid (Hemiptera: Aphididae) Development on Soybean With *Rag1* Alone, *Rag2* Alone, and Both Genes Combined

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ABSTRACT *Aphis glycines* Matsumura (Hemiptera: Aphididae) can reduce the yield of aphid-susceptible soybean (*Glycine max* (L.) Merrill) cultivars. The *Rag1* and *Rag2* genes confer resistance to some biotypes of *A. glycines*. These genes individually can limit population growth of *A. glycines* and prevent yield loss. The impact of these genes when combined is not known. We compared the development of *A. glycines* on soybean with *Rag1* alone (R1/S2), *Rag2* alone (S1/R2), both genes combined (R1/R2), or neither gene (S1/S2). In addition, we determined the impact of different levels of aphid infestation on seed yield. The genotypes were grown in cages and artificially infested with *A. glycines* to achieve five treatment levels: aphid-free, 675 aphids per plant, 25,000 cumulative aphid days (CAD) (25K), 50,000 CAD (50K), and 75,000 CAD (75K). The S1/S2 line reached the 50K treatment, but did not reach the 75K treatment. Aphid development on R1/S2 and S1/R2 soybeans after two infestations reached a maximum of 25K. The maximum treatment reached on R1/R2 was only 675 aphids per plant after two infestations, at which there was no significant yield reduction when compared with the aphid-free treatment. The maximum yield reduction of S1/S2 was 27% at 50K treatment compared with 2% for R1/S2 and 12% for S1/R2 at the 25K treatment. Our results indicated that for *A. glycines* used in our study, cultivars with both *Rag1* and *Rag2* had less aphid exposure and less yield reduction than soybeans with only one resistant gene.

KEY WORDS host plant resistance, integrated pest management (IPM), antibiosis

In the United States, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive pest of soybeans that has the potential to cause significant yield losses if left untreated (Ragsdale et al. 2007). The biology and management of *A. glycines* in the United States has been reviewed by Ragsdale et al. (2011). They indicated that insecticides are the primary tool for preventing yield loss because of *A. glycines* feeding on cultivars of soybeans that are susceptible to aphids. When insecticides are applied based on the economic threshold and economic injury level (EIL), they can reduce the economic damage caused by aphids (Johnson et al. 2009). The addition of soybean cultivars with aphid resistance to the United States agroecosystem could reduce the amount of insecticide used and the damage insecticides can cause to beneficial insects (Hill et al. 2006, Ohnesorg et al. 2009). In addition, organic farmers who do not use the broad-spectrum insecticides available to conventional soybean growers to control the *A. glycines* could grow aphid-resistant, non-genetically modified organisms (non-GMO) cultivars.

Genes that confer resistance to *A. glycines* through antibiosis have been identified in the soybean germplasm. The *Rag1* gene was found in the cultivar Dowling and the *Rag2* gene was identified in PI 200538 (Hill et al. 2006, 2009). In North America, the *Rag1* gene has been incorporated into both GMO and non-GMO cultivars that are sold commercially. However, biotypes of *A. glycines* that can survive on *Rag1* and *Rag2* have been found (Kim et al. 2008, Hill et al. 2010). While *Rag1* is still an effective source of resistance for Biotype 1, Biotype 2 was found to overcome this resistance. *Rag2* is an effective source of resistance to both Biotype 1 and Biotype 2 (Kim et al. 2008). Hill et al. (2010) identified Biotype 3 that has overcome both *Rag1* and *Rag2* resistance.

The *Rag1* gene has been found to reduce aphid development in soybean without negatively influencing their agronomic and seed traits (Li et al. 2004, Kim and Diers 2009, Mardorf et al. 2010). To date, no data have been reported on the influence of the *Rag2* gene alone or in combination with the *Rag1* gene on aphid development or agronomic performance of soybean. One objective of our research was to compare aphid development on soybean genotypes with the *Rag1* and *Rag2* genes together (R1/R2), the *Rag1* gene alone (R1/S2), the *Rag2* gene alone (S1/R2), or neither resistance gene (S1/S2). A second objective was to assess the influence

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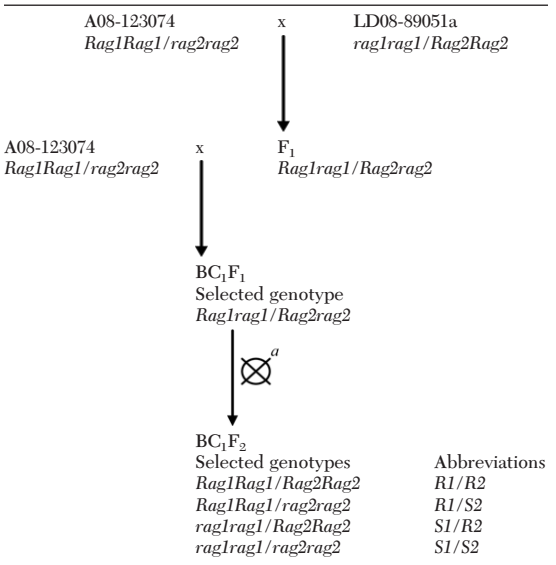
Table 1. The crosses made and genotypes selected for the field research during 2010



[a] Symbol representing the BC_1F_1 plants were allowed to self-pollinate to obtain the BC_1F_2.

of different levels of aphid infestation on seed yield of the four genotypes.

Materials and Methods

We developed four related soybean genotypes that varied in their susceptibility to soybean aphids. The lines differed in the resistant gene combinations. The breeding program that resulted in these genotypes is outlined in Table 1. The parent lines used to develop the backcross population for the study were A08-1243074 and LD08-89051a. A08-1243074 was a BC_2F_2-derived line with the *Rag1* gene developed at Iowa State University. The donor of the *Rag1* gene was LD05-16521 developed at the University of Illinois at Urbana-Champaign (UIUC). The recurrent parent in the backcross was IA3027, a cultivar developed at Iowa State University. The line LD08-89051a with the *Rag2* gene was developed by scientists at the USDA–ARS and the UIUC.

The cross of A08-123074 with LD08–89051a was made at Santa Isabel, PR, during March 2009. The F_1 seeds and seeds of A08-123074 were planted at the Agronomy and Agricultural Engineering Research Center near Ames, IA, during the summer of 2009. Three F_1 plants were confirmed as hybrids with the SSR marker Sct-033 by Brian Diers at UIUC. The F_1 hybrid plants were used as males for backcrossing to A08-123074 to obtain 35 BC_1F_1 seeds.

The BC_1F_1 seeds were planted at Santa Isabel, PR, in October 2009. Nine BC_1F_1 plants were found to be heterozygous for both *Rag1* and *Rag2* by Brian Diers at UIUC. The SSR marker Satt 540 was used to select for *Rag1* and Sct-033 was used to select for *Rag2* (Li et al. 2007, Hill et al. 2009). The double heterozygous plants were harvested individually to obtain BC_1F_2 seeds.

The BC_1F_2 seeds from the nine BC_1F_1 individuals were planted as families at Santa Isabel, PR, in January 2010. IA3027RA1, an Iowa State cultivar with the *Rag1* gene, and LD08-89051a were planted as checks for genotyping. A TaqMan assay was used to determine the presence of the *Rag1* gene (Kim et al. 2009) and *Rag2* gene (Kim et al. 2010) for each plant. The genotypic combinations selected were *Rag1Rag1/Rag2Rag2* (R1/R2), *Rag1Rag1/rag2rag2* (R1/S2), *rag1rag1/Rag2Rag2* (S1/R2), and *rag1rag1/rag2rag2* (S1/S2). The selected plants were harvested individually. Each of the four genotypic lines was prepared by bulking seed from the BC_1F_2 plants of seven families.

Each of the four genotypes was exposed to a range of aphid populations. The exposure to large populations was summarized by calculating cumulative aphid days (CAD) (Hanafi et al. 1989). The experiment was designed so that each of the genotypes would be exposed to five treatment levels of aphid populations. The five treatments were aphid-free, 675 aphids per plant, 25,000 CAD (25K), 50,000 CAD (50K), and 75,000 CAD (75K). The treatment of 675 aphids per plant was chosen because it represented the EIL as defined by Ragsdale et al. (2007). The treatments of 25K, 50K, and 75K exceeded the EIL. The 25K treatment represented the highest infestation found in experiments described by Ragsdale et al. (2007) that involved naturally occurring soybean aphid infestations. The 50K treatment represented the range of infestations obtained in a similar cage experiment as described by Catangui et al. (2009).

The four genotypes and five aphid treatments were organized in a randomized complete-block design with six replications. The experiment was planted on 7 June, 2010 at the Agronomy and Agricultural Research Center in Boone County, IA. The single row of each plot was 0.61 m long with a 1.02 m row spacing and a 1.15 m alley. There were 24 seeds planted in each plot. The plots were thinned to 10 plants on 18 June, when the soybeans reached the V1 stage (Fehr et al. 1971).

To reach the desired aphid populations for each treatment, each plot was enclosed with a cage ($0.76 \times 0.76 \times 1.1$ m). The cages were covered with a fine-mesh made from white no-see-um netting (Balson-Hercules, New York, NY). The cage frames were set up on 16 June, the nets were anchored in the soil on 25 June, and the nets were pulled over the frames to enclose the plants on 12 July.

A. glycines for this experiment were obtained from a colony maintained at Iowa State University. The colony was established from field-collected aphids found in multiple soybean fields in Jasper and Story counties in Iowa during 2008. Additional field-collected aphids were added in 2009 from Story County, IA. Aphids were maintained in a growth chamber under a 14:10 day–night cycle on Prairie Brand 2636NRR soybeans, an aphid-susceptible genotype. We increased the population of aphids for our experiment by transferring aphids from the growth chamber

in early June to an outdoor enclosure (4.5 × 2.4 × 2.4 m) covered in a fine mesh fabric. Within this enclosure were six 6.9 m rows of the susceptible cultivar IA3027 planted on 26 May.

All treatments, except the aphid-free, were initially infested on 12 July. A leaf with ≈50 aphids was attached to the top of the youngest fully expanded leaf on five plants in each plot. Aphids were first counted 3 d after infestation. All genotype and treatment combinations (120 cages) were counted at least once a week until the end of the experiment on 18 August. Because *A. glycines* can increase their populations rapidly in absence of predation, we measured aphid populations 2–3 d later during the same week for those treatment and genotype combinations (24 cages) that represented the lowest aphid population that had yet been reached. The mean population density of the six replications was used to determine when the treatment level was reached. Once a treatment level was reached for a genotype, the plots were sprayed with Warrior II (Lambda-cyhalothrin; Syngenta, Wilmington, DE). Whenever an insecticide was applied, the aphid-free treatment and all previously sprayed treatments also were sprayed.

As will be discussed in the results section, aphid population growth on the aphid resistant genotypes was significantly slower than on the susceptible line. To ensure that the aphid-resistant genotypes reached the desired populations, the plots assigned the 25K, 50K, and 75K treatments of the R1/R2, R1/S2, and S1/R2 genotypes were reinfested on 29 July. A leaf with ≈100 aphids was attached to the top of the youngest fully expanded leaf on five plants in each plot. By 18 August, the aphid populations had plateaued and started to decline on all of the plots. Therefore, all the plots were sprayed and no additional aphid counts were made.

The plots were harvested individually with a stationary plot thresher (ALMACO, Nevada, IA). Moisture of the seed was determined using an Infratec 1221 near-infrared whole grain analyzer (Tecator AB, Hooganas, Sweden). Seed yields of the plots were adjusted to 130 g/kg^{-1} moisture.

Data Analysis. We report the mean number of aphids per plant ± SEM for each genotype measured once a week for those treatments that did not receive an insecticide application until 18 August. We were unable to reach the targeted densities of aphids, as measured in CAD for each genotype despite the second infestation. Therefore, the number of replications used to calculate this mean varied by genotype and treatment. Data collected from all the initial treatment and genotype combinations are reported in the supplemental material (Supplemental material available online only, Table S1). We used a subset of these data for our analysis. For the S1/S2 line, six replications of the 75K treatment were used to calculate the mean number of aphids per plant. The aphid populations on all of the aphid resistant soybeans did not reach the 75K treatment; therefore, these treatment and genotype combinations were not included in our analyses but can be found in the supplementary information. For the R1/R2, R1/S2, and S1/R2 genotypes, 12 replications were used to calculate the mean number of aphids per plant, which included the six replications of both the 25K and 50K treatments that were not sprayed until the experiment was terminated on 18 August. These data were transformed by ln + 1 to determine if aphid densities varied across the genotypes. Aphid density was analyzed with a complete randomized repeated measures analysis of variance (ANOVA) using the mixed model procedure (SAS Institute 2008). We determined that a Heterogeneous First-order Autoregressive Covariance Structure resulted in the lowest Akaike's Information Criterion score when compared with other covariance structures. Block, genotypes, and dates were considered fixed effects. Tukey's honest significant difference (Tukey 1949) was used to determine if the number of aphids per plant measured on each genotype differed significantly by each sampling date.

The population growth rate (r) was estimated to determine the impact of the four genotypes on *A. glycines* population growth. We used a simple method for calculating r based on the methods of Myers and Gratton (2006) by calculating the slope of aphid density (transformed by ln + 1) over time for each plot. We calculated an average r (±SEM) for each genotype was reported. The first r value was calculated from the 675 aphids per day treatment for the range of time beginning 3 d after the initial infestation and ending when a plot had reached or exceeded 675 aphids per plant or when the population had reached a plateau: a plateau was based on population growth being stagnant for two consecutive sampling dates. The 25K and 50K treatments of the R1/R2, R1/S2, S1/R2 genotypes also were used to calculate a second r value from after the plants were infested for a second time (29 July) until the end of the experiment on 18 August. Population growth was analyzed as a randomized complete-block design using the mixed model procedure of SAS version 9.2 (SAS Institute, 2008). Block and genotypes were considered fixed effects. Tukey's honest significant difference (Tukey 1949) was used to determine if the r values differed significantly among the four genotypes.

Because the number of treatments obtained within each genotype was different, each genotype was analyzed separately to determine if the seed yield of each genotype varied across the treatments. Yield data were analyzed as a randomized complete-block design using the mixed model procedure (SAS Institute 2008). Blocks and treatments were considered fixed effects. The mean squares for the block × treatment interaction were used to test the significance of the main effect of treatments with an *F* test. Tukey's honest significant difference (Tukey 1949) was used to determine if the yield values for treatments differed significantly for each genotype.

Results and Discussion

We observed a significant effect of soybean genotype on the abundance of aphids (Table 2) that were derived from a colony of *A. glycines* collected in Iowa.

Table 2. Impact of soybean genotype on the abundance (aphids per plant) of *A. glycines* based on ANOVA

Source	df	*F*-value	Pr>F
Block	11	4.81	0.0004
Genotype (G)[a]	3	10.12	0.0001
Date (D)[b]	5	139.72	<0.0001
G*D	15	3.13	0.0001

[a] Four genotypes were used, which included an aphid susceptible line, a line containing *Rag1*, a line containing *Rag2* and a line containing both *Rag1* and *Rag2*.

[b] Aphid data analyzed for all four genotypes were from plots that were artificially infested on 12 July and were monitored once a week until plots were treated with insecticide on 18 Aug.

Table 4. Impact of soybean genotype on the pop growth rate of *A. glycines* based on ANOVA

Sampling period	Source	df	*F*-value	Pr>F
Initial infestation[a]	Block	5, 15	1.51	0.24
	Genotype[b]	3, 15	28.22	<0.0001
After second infestation[c]	Block	11, 28	1.73	0.13
	Genotype	2, 28	1.93	0.17

[a] Aphid data used for all four genotypes were log transformed and r was calculated for the time period between the initial infestation on 15 July until the pop reach 675 aphids/plant or reached a plateau.

[b] Four genotypes were used, which included an aphid susceptible line, a line containing *Rag1*, a line containing *Rag2* and a line containing both *Rag1* and *Rag2*.

[c] Aphid data used for the three resistant genotypes were log transformed and r was calculated for the time period between the second infestation at 29 July until 16 Aug.

From this colony it was possible to successfully infest and maintain populations on both the susceptible and resistant genotypes. However, we did observe significant differences in the abundance of aphids among the genotypes (Table 3). Starting on 26 July, a significant difference was observed in the number of aphids per plant on the S1/S2 line compared with the R1/R2 and S1/R2 genotypes. The S1/S2 genotype reached an average of 675 aphids per plant treatment on 26 July, 16 d after the initial infestation, and the R1/S2 and S1/R2 genotypes reached an average of 675 aphids per plant treatment on 5 August, 8 d later. The R1/R2 genotype only reached a maximum population of 388 ± 67 aphids per plant after the initial infestation. Because of the slow growth of aphid populations on the resistant genotypes, the 25K, 50K, and 75K treatments for the resistant genotypes were infested a second time to increase the likelihood of reaching the higher aphid exposure treatments. After this second infestation, we observed the largest mean population for the R1/R2 genotype (761 ± 264 aphids per plant) within the plots assigned to the 25K treatment. Therefore, the plots of the 25K treatment were used to represent the 675 aphids per plant treatment for subsequent evaluations of yield response to the aphids.

The S1/S2 genotype reached the 25K treatment on 5 August, 24 d after the initial infestation, and the 50K treatment on 12 August, 35 d later. The S1/S2 genotype never reached the 75K treatment. For the R1/S2 and S1/R2 genotypes, a second infestation was required to reach 25K, which occurred 18 d after the second infestation on 16 August.

We observed a significant effect of genotype on the population growth rate of *A. glycines* after the first infestation but not the second infestation (Table 4) The r value for the four genotypes was based on an estimate that began 3 d after the initial infestation on 12 July until 5 August, when populations reached 675 aphids per plant (S1/S2, R1/S2, S1/R2) or reached a plateau (R1/R2). The r value of the S1/S2 genotype was significantly greater than that of the resistant genotypes (Table 5). There was a significant difference in the r values calculated for the genotype containing both resistant genes compared with the genotypes with only one resistant gene. The population growth rates on the R1/R2, R1/S2, and S1/R2 genotypes from the date of the second infestation until the end of the experiment were not significantly different.

The lower rate of population growth between the aphid resistant genotypes prevented us from reaching all of the targeted aphid populations (i.e., treatments) that were part of our initial experimental design. By 18 August and with the second infestation we were able to reach a subset of our treatments (Table 6). This included reaching the 675 aphid/plant treatment for all genotypes, the 25K CAD treatment for R1/S2, S1/

Table 3. Soybean aphid populations on four genotypes measured once a week during 2010

Genotype[a]	Treatment[b]	Mean aphids per plant[c] (±SEM)					
		15 July	21 July	26 July	3 Aug.	9 Aug.	16 Aug.
R1/R2	25K + 50K	33 ± 5a[e]	62 ± 13a	175 ± 32a	430 ± 71a	481 ± 98a	505 ± 145a
R1/S2	25K + 50K	31 ± 7a	68 ± 12a	255 ± 48ab	1079 ± 225ab	1374 ± 233ab	1283 ± 278a
S1/R2	25K + 50K	22 ± 4a	49 ± 11a	126 ± 31a	730 ± 191a	1312 ± 215ab	1572 ± 281a
S1/S2	75K	30 ± 8a	113 ± 20a	561 ± 46b	3125 ± 384b	3409 ± 312b	2486 ± 387a

[a] R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1*; S2 = absence of *Rag2*.

[b] Treatments used to calculate the mean no. of aphids per plant.

[c] Aphid populations were established from a colony found in Iowa and manually infested on 12 July. The resistant genotypes were reinfested on 29 July.

[d] Number of aphids per plant reported were from plots that did not receive insecticide until 18 Aug. This included the combination of two treatments that were assigned to the original experimental design for the aphid resistant genotypes. The aphid susceptible line is represented by the 75K treatment.

[e] Values within a column with the same letter were not significantly different at the 0.05 probability level based on Tukey's honest significant difference (Tukey 1949).

Table 5. Aphid pop growth rate (r) of soybean aphids on four soybean genotypes during 2010

Genotype[a]	Mean r[b] ± SEM	
	Initial infestation[c]	After second infestation[d]
S1/S2	0.34 ± 0.023a	
S1/R2	0.18 ± 0.018b	0.06 ± 0.050a
R1/S2	0.16 ± 0.015b	0.01 ± 0.033a
R1/R2	0.13 ± 0.010c	−0.008 ± 0.032a

[a] R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1;* S2 = absence of *Rag2.*

[b] Means within a column with the same letter were not significantly different at the 0.05 probability level based on Tukey's honest significant difference (Tukey 1949).

[c] Aphid data were log transformed and r was calculated for the time period between the initial infestation on 15 July until the pop reach 675 aphids/plant or reached a plateau.

[d] Aphid data were log transformed and r was calculated for the time period between the second infestation until 16 Aug. for only the three resistant genotypes, because the susceptible genotype was not infested a second time.

Table 7. Impact of aphid abundance (treatment) on soybean yield based on ANOVA

Genotype[a]	Source	df	*F*-value	Pr>F
R1/R2	Block	5, 5	0.38	0.84
	Treatment[b]	1, 5	0.88	0.39
R1/S2	Block	5, 10	0.64	0.68
	Treatment	2, 10	1.41	0.29
S1/R2	Block	5, 10	1.71	0.22
	Treatment	2, 10	3.21	0.08
S1/S2	Block	5, 15	2.00	0.14
	Treatment	3, 15	9.42	0.0010

[a] R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1;* S2 = absence of *Rag2.*

[b] Treatment levels were based on reaching an economic injury level (675 aphids/plant), and two high populations (25K CAD and 50K CAD) that would be expected to reduce yield.

R2, and S1/S2. The highest treatment of 50K CAD was only reached by the S1/S2 genotype.

Yield varied only by the aphid treatment for the susceptible genotype (S1/S2) (Table 7) and only marginally varied ($P = 0.08$) with increasing aphid exposure on the genotype containing *Rag2* (S1/R2). As the exposure of the S1/S2 line to aphids increased, the difference in yield compared with the aphid-free treatment increased (Table 8). For the R1/S2 and S1/R2 genotypes, there was not a significant difference in yield between the aphid-free and 675 aphids per plant treatment. The mean yield of the 25K treatment was only 2% less than the aphid-free treatment for the R1/S2 genotype, while S1/R2 had 12 % lower yield at the same treatment level. For the R1/R2 genotype, there was no significant difference in the mean yield between the aphid-free and 675 aphids per plant treatment. Overall, the yield response by any of the genotypes in this experiment should only be considered as an estimate of how these genotypes would respond to an outbreak of *A. glycines* when grown under conventional agricultural methods. Components of the interaction between soybean and *A. glycines* that were not addressed in this study are the amount of time and the growth stages that the plants were exposed to aphids. The duration of the plants exposure to aphids could affect the yield loss that soybeans experience in two ways. The first way is indicated by the varying rates of aphid population growth that were observed on the four genotypes; *A. glycines* were on resistant plants for a longer period of time than the susceptible plants while they reached the treatment levels of our experiment. However, we did not observe significant yield loss within any of the resistant genotypes exposed to aphids. A second way that the varying exposure of aphids across the genotypes could affect yield loss is based on the growth stages at which the treatment levels occurred. As noted by Ragsdale et al. (2007), the yield loss of soybeans from aphid outbreaks can vary depending upon the growth stage of the plant. Yield lose by soybeans is reduced when plants have matured past the R5 stage when compared with plants in earlier stages (i.e., R1 up to R5). Although we did not track growth stages for the various genotypes, we did observe that all of the resistant genotypes reached the treatment levels later in the growing season than the aphid susceptible genotype. Therefore, we may not have observed significant yield loss in the resistant genotypes because the aphid treatment levels occurred after a period of plant growth that was susceptible to yield loss.

The plants in our study were exposed to a colony of *A. glycines* collected in Iowa. Given that the aphid abundance was lowest in the aphid resistant soybeans, it is likely that the composition of our colony is comprised of Biotype 1 that is susceptible to the *Rag1* and *Rag2* gene. However, we do not know the genetic composition of this colony and whether it contains

Table 6. Observed populations of soybean aphid within cages assigned four exposure levels for four genotypes varying in aphid resistance during 2010

Treatment[a]	Mean ± SEM of observed populations[b] (95% confidence interval)			
	R1/R2	R1/S2	S1/R2	S1/S2
Target pop				
Aphid-free	1 ± 1 (0–3)	0 ± 0 (0–0)	2 ± 1 (0–4)	17 ± 14 (0–44)
675 aphid/plant	761 ± 264 (243–1278)	745 ± 232 (290–1200)	804 ± 233 (347–1261)	1171 ± 240 (701–1641)
25K CAD	—	26450 ± 5998 (14694–38206)	23845 ± 5352 (13355–34335)	28479 ± 1530 (25480–31478)
50K CAD	—	—	—	50711 ± 8236 (34568–66854)

[a] Treatment levels were based on reaching an economic injury level (675 aphids/plant), and two high populations (25K CAD and 50K CAD) that would be expected to reduce yield.

[b] Genotypes are designated as follows R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1;* S2 = absence of *Rag2.*

Table 8. Mean seed yield of four soybean genotypes exposed to varying soybean aphid populations during 2010

Treatment	Genotype[a]							
	R1/R2[b]		R1/S2		S1/R2		S1/S2	
	g plot^{-1}	% diff[c]	g plot^{-1}	% diff	g plot^{-1}	% diff	g plot^{-1}	% diff
Aphid-free	571 ± 10a		570 ± 15a		516 ± 21a		573 ± 22a	
675 aphid/plant	540 ± 23a	−5	603 ± 20a	+6	465 ± 21a	−10	561 ± 20ab	−2
25K CAD	—		557 ± 16a	−2	455 ± 13a	−12	465 ± 35bc	−19
50K CAD	—		—		—		419 ± 19c	−27

[a] R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1;* S2 = absence of *Rag2.*

[b] Means within a column with the same letter were not significantly different at the 0.05 probability level based on Tukey's honest significant difference (Tukey 1949).

[c] Percentage difference from the aphid-free treatment.

biotypes that survive on *Rag*-containing soybeans (e.g., Biotype 2 and 3). Because we were able to establish and maintain populations of *A. glycines* on all three aphid resistant genotypes, it is possible that the colony we used is comprised of multiple biotypes. However, their putative presence did not reduce the effectiveness of the resistant genotypes in preventing a significant increase in aphid populations. Previous field experiments with genotypes of soybeans containing *Rag1* in central Iowa resulted in reduced populations of *A. glycines* compared with aphid susceptible genotypes (Chiozza et al. 2010, Madorf et al. 2010). Both this study and previous studies were conducted before all of these genotypes have been made commercially available. Over time, the capacity for these resistant genotypes to prevent soybean aphid outbreaks may be reduced with their increase used as the abundance of biotypes that can survive on *Rag*-containing soybeans increase. To what extent aphid growth and yield of aphid resistant soybeans vary when exposed to populations comprised of different biotypes is not known. In the future, similar studies should be conducted with biotypes that can survive on the R1/R2, R1/S2, and S1/R2 genotypes.

The results of the experiment suggested that soybean with resistance to *A. glycines* will be a useful component of its management. In general, the capacity of the *Rag* genes for preventing an aphid outbreak was improved when the two genes were combined. Alone or together, *Rag1* and *Rag2* were both capable of reducing aphid population growth when compared with a related susceptible line. However, only the R1/R2 line had aphid populations that, on average, did not exceed 675 aphids per plant after the initial infestation. Only after the second infestation did the aphid population on the R1/R2 line exceed the EIL for aphid-susceptible soybeans. For the R1/R2 line, the aphid population reached a plateau and stayed at this population after the initial and second infestations. There also was a negative rate of growth on the R1/R2 line after this second infestation. Recommendations for applying a foliar insecticide to aphid-susceptible soybean cultivars include a 7 d lag time between the economic threshold of 250 aphids per plant and the EIL of 675 aphids per plant based on aphid population growth that includes the impact of natural enemies. Our experiment did not include mortality from natural enemies. Therefore, the rate of aphid growth measured in our experiment likely was higher than what would be observed in an uncaged setting. As aphid-resistant cultivars that incorporate the *Rag1* and *Rag2* genes become more available to growers, the interval of 7 d between the economic threshold and the EIL developed for aphid-susceptible cultivars possibly could be extended. Overall, these results suggest that aphid resistance will improve the capacity for growers to manage *A. glycines* within Iowa and possibly most of the north central region of the United States that suffers from outbreaks of this sporadic pest.

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