

PLANT RESISTANCE

One Gene Versus Two: A Regional Study on the Efficacy of Single Gene Versus Pyramided Resistance for Soybean Aphid Management

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ABSTRACT The soybean aphid (*Aphis glycines* Matsumura) is a threat to soybean production in the Midwestern United States. Varieties containing the *Rag1* soybean aphid resistance gene have been released with limited success in reducing aphid populations. Furthermore, virulent biotypes occur within North America and challenge the durability of single-gene resistance. Pyramiding resistance genes has the potential to improve aphid population suppression and increase resistance gene durability. Our goal was to determine if a pyramid could provide improved aphid population suppression across a wide range of environments. We conducted a small-plot field experiment across seven states and three years. We compared soybean near-isolines for the *Rag1* or *Rag2* gene, and a pyramid line containing both genes for their ability to decrease aphid pressure and protect yield compared with a susceptible line. These lines were evaluated both with and without a neonicotinoid seed treatment. All aphid-resistant lines significantly decreased aphid pressure at all locations but one. The pyramid line experienced lower aphid pressure than both single-gene lines at eight of 23 location-years. Soybean aphids significantly reduced soybean yield for the susceptible line by 14% and for both single-gene lines by 5%; however, no significant yield decrease was observed for the pyramid line. The neonicotinoid seed treatment reduced plant exposure to aphids across all soybean lines, but did not provide significant yield protection for any of the lines. These results demonstrate that pyramiding resistance genes can provide sufficient and consistent yield protection from soybean aphid in North America.

KEY WORDS host-plant resistance, integrated pest management (IPM), insect resistance management, insecticidal seed treatment, areawide management

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an economically damaging, invasive pest throughout the north central United States and southern Canada (Ragsdale et al. 2011). Since its discovery in Wisconsin in 2000, yield losses of up to 40% have been documented in the field (Ragsdale et al. 2007). Insecticide-based management options include neonicotinoid seed treatments and foliar insecticides (Hodgson et al. 2012). Applying foliar insecticides according to an economic threshold is the most profitable of these strategies; however, this approach only provided farmers a 69–85% chance of

recovering the cost of the insecticide application (Johnson et al. 2009).

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

Multiple field studies have investigated the efficacy of plant introduction lines and experimental lines car-

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rying one or more of the *Rag* genes (reviewed by Hill et al. 2012 and Hesler et al. 2013). In these studies, researchers found virulent populations of soybean aphids that were capable of overcoming either the *Rag1* gene (biotype-2), the *Rag2* gene (biotype-3), or both genes (biotype-4; Kim et al. 2008, Hill et al. 2010, Alt and Ryan-Mahmutagic 2013). Notably, these virulent biotypes existed before the commercial release or large-scale planting of any of the *Rag* genes and therefore, were not selected in response to the use of *Rag* genes. It is still unclear how prevalent these virulent biotypes are in the environment or how they will influence soybean aphid management that relies on *Rag* genes. Early experimental information estimates the incidence of biotype-2 could range from 0 to 40% for a given field, with regional incidence possibly as high as 20% (Michel et al. 2011). Fluctuations in the incidence of virulent biotypes are one possible explanation for the variability in *Rag1* efficacy observed across multiple years and locations (Hesler et al. 2013).

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

Here we explored the utility of host-plant resistance and insecticidal seed treatments for soybean aphid management. We used near-isogenic advanced breeding lines and field plots, which allowed us to take more meaningful measurements on aphid population suppression and yield. We addressed four hypotheses that will be important for the successful release and adoption of host-plant resistance targeting soybean aphid. Specifically, we addressed if 1) the *Rag1* and *Rag2* genes provide equivalent soybean aphid population suppression across multiple environments, 2) a two-gene pyramid (*Rag1* + *Rag2*) provides increased population suppression compared with either single gene alone, 3) soybean aphid-resistant lines (*Rag1*, *Rag2*, and *Rag1* + *Rag2*) require foliar insecticides for maximum yield, and 4) insecticidal seed treatments provide significant aphid population suppression and yield protection to soybean aphid-resistant lines (*Rag1*, *Rag2*, and *Rag1* + *Rag2*).

Materials and Methods

We used four soybean lines developed at Iowa State University. The development of these lines is detailed

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

Location	Subplot size	Treatments included ^a	Planting date
2011			
Volga, SD	6 rows × 15.2 m	AF, UT	19 May
Lamberton, MN	6 rows × 9.1 m	AF, UT	2 June
Arlinton, WI	6 rows × 15.2 m	AF, UT	23 May
Nashua, IA	6 rows × 15.2 m	AF, UT, ST	17 May
Ames, IA	6 rows × 15.2 m	AF, UT, ST	24 May
Rock Springs, PA	10 rows × 6.1 m	UT	31 May
Scandia, KS	6 rows × 15.2 m	AF, UT	12 July
2012			
Prosper, ND	6 rows × 9.1 m	UT, ST ^b	6 June
Volga, SD	6 rows × 15.2 m	AF, UT, ST	15 May
Lamberton, MN	6 rows × 9.1 m	AF, UT	18 May
Arlington, WI	4 rows × 15.2 m	AF, UT, ST	24 May
Nashua, IA	6 rows × 15.2 m	AF, UT, ST	17 May
Ames, IA	6 rows × 15.2 m	AF, UT, ST	15 May
Rock Springs, PA	10 rows × 6.1 m	UT	25 May
Scandia, KS	6 rows × 15.2 m	AF, UT	28 June
2013			
Prosper, ND	6 rows × 9.1 m	UT, ST ^b	13 June
Volga, SD	6 rows × 15.2 m	AF, UT, ST	5 June
Lamberton, MN	6 rows × 9.1 m	AF, UT	31 May
Arlington, WI	4 rows × 15.2 m	AF, UT, ST	4 June
Nashua, IA	6 rows × 15.2 m	AF, UT, ST	18 June
Ames, IA	6 rows × 15.2 m	AF, UT, ST	15 June
Rock Springs, PA	10 rows × 6.1 m	UT	7 May
Scandia, KS	6 rows × 15.2 m	AF, UT	18 July

^a AF, aphid-free; UT, untreated; ST, insecticidal seed treatment.

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

elsewhere, but we will briefly describe it here (Wiarda et al. 2012). The four lines were developed from a cross between the parent lines A08-1243074 and LD08-89051a. The line A08-1243074 was the recurrent parent containing *Rag1*, and LD08-89051a was the *Rag2* donor. At the BC₁F₂ generation four genotypes were selected *rag1rag1rag2rag2* (Susceptible), *Rag1Rag1rag2rag2* (*Rag1*), *rag1rag1Rag2Rag2* (*Rag2*), and *Rag1Rag1Rag2Rag2* (*Rag1* + *Rag2*). Ten plants of each genotype were identified and advanced. Each of the four soybean lines used for this experiment were a bulk of these 10 lines at the BC₁F_{2,5} generation. Therefore, the four experimental lines used for this experiment were near-isolines that shared 75% of the *Rag1* recurrent parent's genotype.

We conducted a regional field plot study to address our four hypotheses. The study included seven locations in 2011 and eight locations in 2012 and 2013, for a total of 23 location-years (Table 1). We used a split-plot design, in which the main plot treatment was soybean line and the subplot treatment was insecticide. It was necessary to have multiple field sites over a large region to best address our first hypothesis of whether the *Rag1* or *Rag2* gene differs in performance across a larger region due to regional variations in the virulence of soybean aphid populations. We selected study locations to cover the north central soybean-producing region. We adjusted plot sizes to fit the space and resources available at each study location. We used a soybean row spacing of 76 cm and a planting density of 345,800 seeds per hectare at all locations.

We used a standard subplot size of 6 rows \times 15.2 m (length \times width) with exceptions made for the Lamberton, MN, Rock Springs, PA, Prosper, ND, and Arlington, WI, location-years. In Lamberton, MN, and Prosper, ND, subplots were only 9.1 m in length. Subplots were 10 rows \times 6.1 m in Rock Springs, PA. The 2012 and 2013 Arlington, WI, locations used 4 row \times 15.2 m subplots, while the 2011 Arlington, WI, location used the standard subplot size.

We used three insecticide treatments to address our four hypotheses, although not every location-year included all of these treatments. All location-years included an "untreated" control. This treatment never received any insecticide (foliar or seed applications) and served as a measure of the aphid population suppression offered by each soybean line. The second treatment was designated as "aphid-free." If aphid populations reached a density of 50 aphids per plant in any of the aphid-free subplots, all of the subplots in this treatment received a foliar application of λ -cyhalothrin (Warrior II with Zeon Technology, Bayer CropScience, Durham, NC), bifenthrin (Tundra EC, Winfield Solutions, Oklahoma City, OK), or chlorpyrifos (Lorsban Advanced, Dow AgroSciences, Indianapolis, IN) according to the full label rate. Insecticide selection varied by location-year and was based on the presence of other pests (e.g., twospotted spider mite, *Tetranychus urticae* Koch). Although we considered the lines near-isolines, the 25% genetic difference among lines may produce differences in yield potential, complicating measurements of treatment effects on yield. The aphid-free treatment therefore allowed us to estimate the yield potential of each line in absence of aphid injury. We included the aphid-free treatment at all locations except the Rock Springs, PA, and the Prosper, ND, locations. It was excluded from the Rock Springs, PA, due to space limitations and historically low aphid populations that rarely reach economically damaging densities. The aphid-free treatment was excluded from the Prosper, ND, location, as the lines were unlikely to reach physiological maturity before a killing frost.

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

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

ence greater soybean aphid populations than early-planted or single-crop soybean.

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

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

Statistical Analyses. We analyzed soybean aphid population data and yield data to test our four specific hypotheses. To test hypotheses one (do *Rag1* and *Rag2* provide equivalent aphid population suppression across multiple environments) and two (does a two-gene pyramid increase aphid population suppression to single-gene lines), we used soybean aphid population data to calculate cumulative aphid-days (CAD). CAD are a summary statistic that measures the plant's seasonal exposure to aphids (Hanafi et al. 1989). We analyzed the effect of treatments on the response variable CAD using an analysis of variance. To test the first two hypotheses, we analyzed data from only the untreated subplots in a mixed-effects model (Proc mixed, SAS Institute 2001, Cary, NC; Table 2). This model included the fixed effects of location-year and soybean line. Block was considered a random variable nested within location-year. We tested for the significance of block (and all other random effects in subsequent models) using a log-likelihood ratio statistic ($-2\text{RES Log Likelihood}$). The log-likelihood statistic follows an approximate χ^2 distribution with one degree of freedom (Littell et al. 2002). The first hypothesis was tested by the effect of soybean line, the two-way interaction of soybean line and location-year, and a mean separation test of the *Rag1* line and *Rag2* line. The second hypothesis was addressed by the effect of soybean line and a mean separation test of the three aphid-resistant soybean lines.

We analyzed yield data from the untreated and aphid-free subplots to test our third hypothesis, whether soybean aphid-resistant cultivars require foliar insecticides for optimal yield. To test this hypothesis, we first identified the study locations that experienced economically damaging populations of aphids in the untreated subplot of the susceptible line. Ragsdale et al. (2007) estimated the economic injury level for soybean aphids to be $\approx 5,200$ CAD; therefore, we used only location-years where populations ex-

Table 2. Analysis of variance tables of treatment effects on CAD and yield

Effect	Fixed/ random	df	F statistic/ χ^2_{2a}
CAD analysis of untreated split-plots			
Location-year	F	20, 63	122.68***
Block (location-year)	R	1	0.4
Soybean line	F	3, 189	144.38***
Location × soybean line	F	60, 189	3.05***
Yield analysis of high aphid pressure locations ^a			
Location-year	F	4, 15	26.28***
Block (location-year)	R	1	12.3***
Soybean line	F	3, 45	4.24*
Location × soybean line	F	12, 45	0.88
Block (location-year) × soybean line	R	1	7.3*
Insecticide treatment	F	1, 59	20.14***
Soybean line × insecticide	F	3, 59	4.84**
Location × soybean line × insecticide	F	16, 59	0.94
CAD analysis of untreated and seed-treated split-plots			
Location-year	F	11, 36	290.28***
Block (location-year)	R	1	2.8
Soybean line	F	3, 108	114.15***
Location × soybean line	F	33, 108	6.56***
Block (location-year) × soybean line	R	1	5.8*
Insecticide treatment	F	1, 144	28.16***
Soybean line × insecticide	F	3, 144	1.63
Location × soybean line × insecticide	F	44, 144	0.84

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

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

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

ceeded 5,200 CAD in the untreated subplot of the susceptible line to test this hypothesis. We again used a mixed-effects model to analyze these data with the fixed effects of insecticide treatment, and the interaction of soybean line and insecticide treatment used to test our hypothesis. Block was again considered a random variable nested within location-year. The interaction of block nested within location-year and soybean line was considered a random variable and served as the whole-plot error term.

Our final hypothesis regarding the utility of an insecticidal seed treatment for aphid-resistant varieties was addressed using data from only location-years that included an insecticidal seed treatment. We analyzed CAD data from the untreated and seed treated subplots using the same mixed-effects model as the previous yield analysis. We also tested yield protection from soybean aphid damage provided by the insecticidal seed treatment with this mixed-effects model. Data for the yield analysis were drawn from location-years where the average CAD measured exceeded 5,200 CAD for the untreated susceptible subplot. We tested our hypotheses regarding both aphid suppression and yield protection with the fixed effects of insecticide treatment, the two-way interaction of soybean line and insecticide treatment, and the three-way interaction of soybean line, insecticide treatment, and location.

Results

Soybean aphid populations varied greatly among the three years of the study and among the locations

within each year. In general, populations were greater in 2011 and 2013 compared with 2012. The 2012 and 2013 Scandia, KS, location-years were dropped from all analyses, as soybean aphids were never detected.

Effect of Host-Plant Resistance on Aphid Populations. CAD data were analyzed to test our first two hypotheses. Soybean aphid populations in untreated subplots varied significantly among location-years and soybean lines (Table 2). The significant interaction of location-year and soybean line indicated that the performance of the *Rag1* and *Rag2* genes differed across the locations of this study. In general, the single-gene lines had fewer aphids than the susceptible line and more than the pyramid; however, these differences were not always significant (Table 3). During 2012, when aphid populations were the lowest, only at two of the seven locations were all of the resistant lines significantly different from the susceptible line; these two locations experienced the greatest aphid populations during 2012. Significant differences between the *Rag1* and *Rag2* lines in CAD occurred at five of the 21 location-years included in the analysis, indicating the relative performance of the *Rag1* and *Rag2* genes varied among location-years. Among the resistant lines, in 15 of the 21 location-years, the pyramid line provided significantly greater aphid population suppression than at least one of the single-gene lines, and the pyramid line provided significantly greater aphid population suppression than both single-gene lines at eight location-years.

Yield Protection Provided by Host-Plant Resistance. Economically damaging populations of soybean aphids were present at the 2011 Lamberton, MN, and 2011 and 2013 Volga, SD, and Nashua, IA, location-years. For these location-years, the main effects of location-year, soybean line, and insecticide treatment affected yield (Table 2). A significant interaction of soybean line and insecticide treatment indicated yield loss due to soybean aphid feeding did not occur equally across the four soybean lines. Estimate statements (*t*-tests) were used to evaluate the effect of a foliar insecticide on yield for each of the four soybean lines. The greatest difference in yield was observed between the untreated and aphid-free treatments for the susceptible line ($t = 5.34$; $df = 75$; $P < 0.0001$), where 359 kg/ha (14%) of yield was protected by the application of a foliar insecticide (Fig. 1). When data were pooled across both single-gene lines (i.e., both the *Rag1* line and the *Rag2* line), a foliar insecticide had a significant effect on the yield of the single-gene lines ($t = 25.3$; $df = 75$; $P = 0.0127$) protecting 125 kg/ha (5%) of yield. We did not observe a difference in yield when the pyramid received a foliar insecticide application ($t = 0.05$; $df = 75$; $P = 0.9581$).

We repeated our yield analysis using data from 2012 Lamberton, MN, Volga, SD, and Nashua, IA. These are the same locations included in the previous yield analysis, but a year in which economically damaging populations did not develop at the locations. This follow-up yield analysis was done to confirm the documented yield loss on the susceptible line and both single-gene lines were due to soybean aphids and not

Table 3. Effect of soybean line on aphid populations (CAD) for untreated subplots

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

^a CAD data from the untreated subplot are shown by location for each of the four soybean lines.
^b Different letters represent significant differences ($P < 0.05$) among soybean lines within a location.
^c The 2012 and 2013 Scandia, KS, location-years were not included in statistical analyses but CAD are shown here for clarity.

some other factor affected by insecticide application. Soybean line ($F = 0.13$; $df = 3, 18$; $P = 0.9394$), insecticide treatment ($F = 1.17$; $df = 1, 36$; $P = 0.2874$), and their interaction ($F = 0.29$; $df = 3, 36$; $P = 0.8315$) did not significantly affect yield in these low aphid pressure location-years.

Effect of an Insecticidal Seed Treatment. Soybean aphid populations varied significantly among location-years, soybean lines, and the presence of insecticidal

seed treatment (Table 2). Similar to the analysis of only untreated subplots, the effect of soybean line was not consistent across locations. However, the effect of insecticidal seed treatment on soybean aphid population suppression did not vary across soybean lines or location-years. Across all soybean lines, the insecticidal seed treatment reduced CAD by 38% compared with the untreated subplots (Fig. 2). For the yield analysis (aphid-free subplots also included), the effect of insecticide treatment (including both foliar and seed-applied insecticides) significantly affected yield

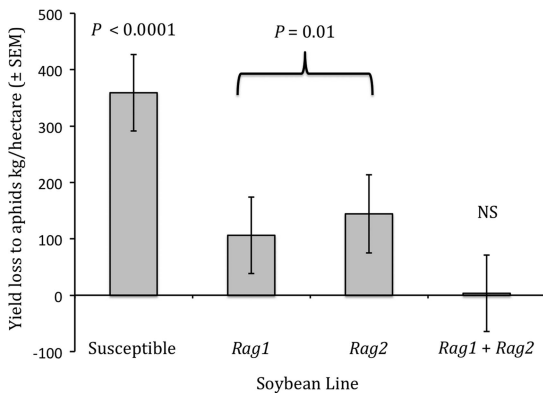


Fig. 1. Yield loss attributed to soybean aphid for each of the four experimental soybean lines. Yield loss was calculated using a *t*-test to determine the yield difference between the aphid-free and untreated subplots of each soybean line. Yield data were combined from the five location-years that experienced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

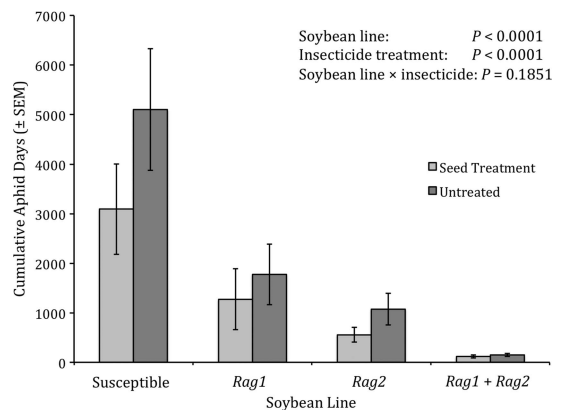


Fig. 2. CAD data from the 12 location-years that included an insecticidal seed treatment subplot. The thiamethoxam seed treatment significantly reduced plant exposure to aphids equally across each of the four soybean lines by an average of 38%.

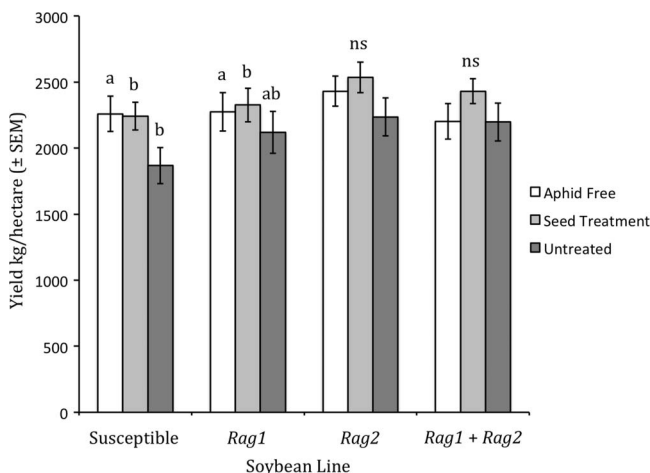


Fig. 3. Yield data for each insecticidal treatment and soybean line combinations. Yield data were compared from the three location-years that included seed treatment subplots and experienced economically damaging soybean aphid populations (2013 Volga, SD, and 2011 and 2013 Nashua, IA). Letters represent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

($F = 7.97$; $df = 2, 69$; $P = 0.0008$). The effect of insecticide treatment, however, varied significantly across the four soybean lines ($F = 2.76$; $df = 6, 69$; $P = 0.0182$), due primarily to differences between untreated and aphid free subplots. Yield of insecticidal seed treatment subplots was not significantly different from untreated subplots for any of the soybean lines (Fig. 3). Therefore, the increased soybean aphid suppression provided by the insecticidal seed treatment did not result in significant yield protection for any of the soybean lines.

Discussion

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

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

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

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

bean aphid-resistant varieties, reducing plant exposure to aphids by 89% (McCarville and O'Neal 2012).

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

Insecticidal seed treatment reduced plant exposure to aphids by $\approx 38\%$ across the four soybean lines (Fig. 2). These results indicate that in the field, insecticidal seed treatments provide similar protection to both single-gene and two-gene soybean aphid-resistant varieties as they do for susceptible varieties. Insecticidal seed treatments could, therefore, provide some management benefits, particularly for single-gene resistant varieties, which still experience yield loss due to soybean aphid feeding. These benefits, however, were not observed in our study and insecticidal seed treatments appear unnecessary for two-gene pyramids.

Our study demonstrates that a two-gene pyramid comprising *Rag1* and *Rag2* can significantly improve soybean aphid management in the field. The adoption of soybean aphid-resistant varieties has been slow by farmers as evidenced by their availability (McCarville et al. 2012, 2013). This may be a product of their limited availability from commercial seed producers, limited availability in genetic backgrounds containing other desired agronomic traits, or the potentially insufficient efficacy of single-gene varieties as documented here. Two-gene pyramids have the potential to increase the efficacy and consistency of soybean aphid control provided by aphid-resistant varieties, potentially also increasing their adoption by farmers.

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

commercial release of these Bt toxins (Gassmann et al. 2011). Resistance pyramids are now being used to target western corn rootworm in hopes of overcoming the issue of low-dose traits through the redundant killing provided by resistance pyramids.

In addition to insect resistance management, future research will need to investigate the efficacy of both pyramiding other soybean aphid resistance genes (i.e., other combinations of two-gene pyramids) and of pyramiding three or more soybean aphid resistance genes. Eight potentially different soybean aphid resistance genes have been identified to date (*Rag1*, *rag1b*, *rag1c*, *Rag2*, *Rag3*, *rag3*, *rag4*, and *Rag5*; Hill et al. 2012, Hesler et al. 2013). In this study, we were only able to investigate the efficacy of one possible two-gene pyramid. The results of the study presented here along with the additional soybean aphid-resistance genes available provide reason to believe host-plant resistance can be a valuable and sustainable part of an integrated pest management program for the soybean aphid.

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