Measuring the Benefit of Biological Control for Single Gene and Pyramided Host Plant Resistance for *Aphis glycines* (Hemiptera: Aphididae) Management

M. T. MCCARVILLE[1,2] AND M. E. O'NEAL[1]

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ABSTRACT The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an economically important pest in the north central United States. In the state of Iowa, economically damaging populations occurred in seven of 11 growing seasons from 2001 to 2011. The high frequency and economic impact of the soybean aphid makes it an ideal candidate for management by using host plant resistance. We compared an aphid-susceptible line to near-isolines that contain *Rag1* and *Rag2*, both alone and pyramided together, to suppress aphid populations and protect yield. Each of four near-isolines, were artificially infested with aphids and grown in small plots in which the exposure to natural enemies was controlled by the use of cages, resulting in the following treatment groups: natural enemy free (only aphids), biocontrol (both aphids and natural enemies), and aphid free (no aphids or natural enemies). The seasonal accumulation of aphids and the population growth rates were measured for each line and an estimate of yield was measured at the end of the season. Soybean aphid population growth rate was reduced 20% by natural enemies alone, 44% by pyramided resistance, and 63% by the combination of natural enemies and pyramided resistance. This reduction in population growth rate resulted in a 99.3% reduction in the pyramid line's seasonal exposure to aphids. In the presence of natural enemies, all three resistant lines maintained aphid populations below the economic injury level and prevented yield loss. This study demonstrates the compatibility of biological control with soybean aphid host plant resistance and its utility, especially for single resistance gene lines.

KEY WORDS host plant resistance, gene pyramid, *Aphis glycines*, soybean

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the most economically important pest of soybean [*Glycine max* (L.) Merrill] in the north central United States (Ragsdale et al. 2011). From 2003 to 2017 an estimated US$2.3 to 3.7 billion dollars may be lost because of the soybean aphid (Song and Swinton 2009). In the United States, a suite of natural enemies attacks the soybean aphid (Nielsen and Hajek 2005, Schmidt et al. 2008), slowing the growth rate of aphid populations with the potential to prevent populations from reaching economically damaging levels (Fox et al. 2004, Costamagna and Landis 2006, Schmidt et al. 2007). However, the biological control offered by these natural enemies is inconsistent between years and locations. For example, large-scale migrations of aphids into soybeans can rapidly increase aphid populations beyond densities at which natural enemies can suppress aphid population growth (Desneux et al. 2006, Brosius et al. 2007, Schmidt et al. 2010). This can overwhelm the natural enemies present in a soybean field (Rutledge and O'Neil 2005, Desneux and O'Neil 2008). Several other factors can contribute to outbreaks, including a landscape that does not contribute sufficient amounts of natural enemies to embed soybean fields (Gardiner et al. 2009, Noma et al. 2010).

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

Host plant resistance offers the potential for inexpensive and effective pest management without negative environmental effects (Pedigo and Rice 2008). To date, this resistance is available commercially primarily as a single gene (*Rag1*) (McCarville et al. 2012). The *Rag1* gene reduces aphid population growth with-

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[1] Department of Entomology, Iowa State University, Ames, IA 50011.

[2] Corresponding author: Michael McCarville, 113 Insectary, Iowa State University, Ames, IA 50011 (e-mail: mikemcc@iastate.edu).

out negatively affecting agronomic factors (Kim and Diers 2009, Mardorf et al. 2010). However, the level of control provided by these cultivars can be inconsistent among locations and years, with economically significant populations capable of developing (Chiozza et al. 2010, Hodgson and VanNostrand 2011). The inconsistent performance of these cultivars may be because of the occurrence of soybean aphid biotypes capable of overcoming the *Rag1* gene (Kim et al. 2008). At least three other soybean aphid resistance genes have been identified, *Rag2* (Mian et al. 2008), *Rag3* (Zhang et al. 2009), and *rag4* (Zhang et al. 2010). A resistant aphid biotype capable of overcoming the *Rag2* gene already has been identified (Hill et al. 2010).

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

Pyramiding single sources of resistance may improve both the protection conferred by the genes, when compared with a single source of resistance, and contribute to preventing the occurrence of biotypes (Gould 1998). Wiarda et al. (2012) used cages that excluded natural enemies to measure the rate of aphid population growth on soybeans with the *Rag1* gene, *Rag2* gene, and both genes in combination (i.e., a pyramid). In this setting the pyramid experienced significantly lower aphid populations than the lines with a single resistant gene. It is not clear if suppression of aphid population growth by a pyramid line would be reduced further when aphids experience mortality from natural enemies. Therefore, the goal of this study was to examine the interaction of soybean aphid host plant resistance, both single gene and pyramided resistant lines, and biological control and its effect on aphid population suppression and soybean yield protection. We also modeled the effect of a large immigration of soybean aphids on the ability of both host plant resistance and biological control to maintain aphid populations below economically damaging levels.

Materials and Methods

In 2011, we conducted a field experiment at the Iowa State University's Field Extension Education Farm in Boone County, IA, in which soybean lines with varying resistance to the soybean aphid were grown in small plots (microplots). These lines were developed by the soybean breeding program at Iowa State University and their development is described by Wiarda et al. (2012). Briefly, the near-isolines were $BC_1F_{2:6}$ lines derived from the *Rag1* donor A08–123074 and *Rag2* donor LD08–89051a parent lines. The recurrent parent in the backcross was IA3027, an aphid-susceptible line. At the F_2 generation four lines from the same backcross family were selected based on their genotype for the *Rag1* and *Rag2* genes. The genotypes of the four lines selected were *Rag1Rag1Rag2Rag2* (referred to throughout as pyramid), *Rag1Rag1rag2rag2* (*Rag1*), *rag1rag1Rag2Rag2* (*Rag2*), and *rag1rag1rag2rag2* (susceptible).

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

These four aphid treatments allowed us to test the following hypotheses: First, that aphid abundance will vary across the four isolines, both in the absence and presence of biological control (i.e., comparing aphid abundance among the four lines in the biocontrol and natural enemy free treatments). Second, that a sudden aphid immigration event can overcome both aphid resistance and biological control (immigration treatment). Finally, by comparing yield estimates measured in the biocontrol, natural enemy free, and immigration treatments to the aphid free treatment we can assess the ability of host plant resistance and biological control, alone and together to protect against yield loss because of the soybean aphid.

The four near-isolines were grown in microplots consisting of a single row 51 cm in length. Twenty-two seeds were sown in each plot on 19 May. After planting, cage frames were placed over all plots. Cage frames were constructed of 2.5-cm-diameter thin-walled PVC pipe (Charlotte Pipe, Charlotte, NC). Cage frames measured 1.1 m by 0.8 m by 0.8 m (height × length × width). When plants reached the VC growth stage, each plot was thinned to 10 evenly spaced plants.

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

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

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

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

Aphid populations were tracked in all plots throughout the season by counting all aphids (nymphs and adults) on three randomly selected plants in each plot (i.e., whole plant counts). Counts were conducted twice per week until populations exceeded 1,000 aphids plant^{-1} on the susceptible line in the natural enemy free treatment. Counts then were conducted once per week until populations declined on all four lines in every treatment.

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

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

Effect of Biological Control and Host Plant Resistance. For the first hypothesis, we initially analyzed the impact of biological control and host plant resistance on the seasonal exposure of soybeans to aphids (i.e., cumulative aphid days). For this analysis only plots assigned to the natural enemy free and biocontrol treatments were used. The aphid free treatment was excluded from this analysis because it was kept successfully free of aphids. The immigration treatment also was excluded because aphid populations in this treatment were manipulated to have equal densities up to 250 aphids plant^{-1}. Cumulative aphid days were calculated for each plot in the natural enemy free and biocontrol treatments. Cumulative aphid days are a measure of the season-long aphid pressure experienced by a plant (Hanafi et al. 1989). The effects of soybean line and aphid treatment on cumulative aphid days (CAD) were analyzed using a two-way analysis of variance (ANOVA) (PROC GLM, SAS 2001).

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

Results from analysis of aphid populations indicated that the aphid populations crashed on the aphid susceptible line, likely because of a reduction in host-plant quality, density-dependent effects after 1 August, or both. We analyzed the effects of host plant resistance and biological control on population growth rates from 27 June to 14 July, the period of time during which aphid populations grew exponentially. Populations were well established in all plots by 27 June and populations were under 1,500 aphids plant^{-1} at 14 July, a population density that has been observed in commercial soybean fields (Hodgson and VanNostrand 2011). Population growth rates were calculated separately for each plot by log transforming the number of aphids per plant and graphing them over the date after infestation. The slope of the line was considered the rate of growth for each plot. The growth rates were analyzed with the main effects of block, soybean line, aphid treatment, and their two-way interactions using PROC GLM (SAS Institute 2001).

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

Ability of Biological Control and Host Plant Resistance to Protect Yield. For the third hypothesis yield was compared for each of the four aphid treatments within each soybean line. We estimated yield by measuring the average weight of seed from each plot. Soybean breeding has used this technique to select high yielding early progeny within small plot conditions (Fehr 1991). Yield data were analyzed using the PROC MIXED procedure (SAS Institute 2001). The model included the fixed effects of block, treatment, soybean line, and the two-way interactions between block, treatment, and soybean line.

Results

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

Effect of Biological Control and Host Plant Resistance. We observed a significant effect of both soybean line ($F = 10.90$; df = 1, 15; $P = 0.0005$) and aphid treatment ($F = 89.85$; df = 1, 15; $P < 0.0001$) on plant exposure to aphids (i.e., CAD) for the natural enemy free and biocontrol treatments. The interaction between aphid treatment and soybean line ($F = 1.92$; df = 3, 15; $P = 0.1701$) was not significant. Regardless of the soybean line, natural enemies significantly decreased plant exposure to aphids. Overall CAD was reduced by an average of 89% across the four lines in the biocontrol treatment compared with the natural enemy free treatment (Fig. 1).

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

Soybean aphid populations drastically declined on the susceptible line after 1 August (Table 1), 2–3 wk before populations declining on lines containing a single aphid-resistance gene. Therefore, the CAD analysis indicating no significant differences among the susceptible and single gene lines within the natural enemy free treatment (Fig. 1) could be misleading.

Analysis of aphid populations at individual sampling dates for the natural enemy free treatment showed a significant effect of soybean line ($F = 3.33$; df = 3,15; $P = 0.0483$). The interaction between soybean line and date was highly significant ($F = 20.93$; df = 52,260; $P < 0.0001$). Analyses then were performed by date. We did not observe a significant difference in aphid populations among any of the soybean lines during the first two sampling dates (Table 1). After 15 d postinfestation (8 July), there was a significant effect of soybean line. Soybean line significantly affected aphid populations for the remainder of our sampling period.

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

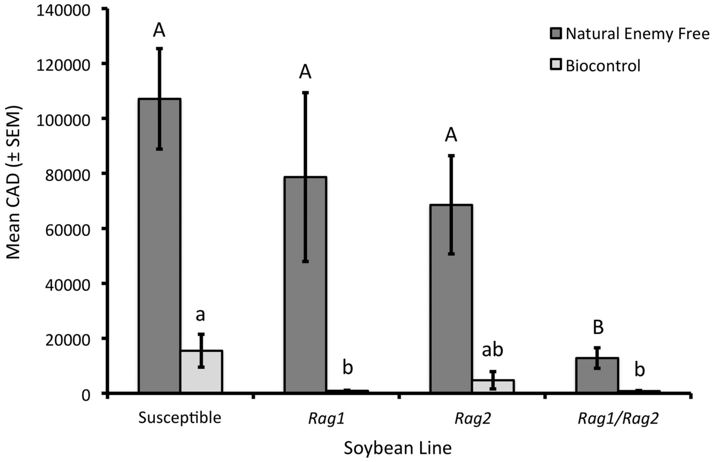

Fig. 1. Mean ± SEM cumulative aphid days (CAD) for the four lines exposed to the natural enemy free and biocontrol treatments. Cumulative aphid days were significantly higher in the natural enemy free treatment compared with the biocontrol treatment ($P < 0.0001$). Letters represent significant differences at the $P < 0.05$ level among lines within an aphid treatment.

from 18 d after infestation through the final sampling date.

Analysis of growth rates indicated that both soybean line ($F = 14.04$; df = 3, 47; $P = 0.0001$) and aphid treatment ($F = 64.80$; df = 1, 47; $P < 0.0001$) significantly affected population growth rates. The interaction between soybean line and treatment was nonsignificant ($F = 1.15$; df = 3, 47; $P = 0.3621$). Across all lines, the biocontrol treatment reduced population growth rates by an average of 64.8% compared with the natural enemy free treatment. To determine the effect of host plant resistance on soybean aphid growth, the growth rates were compared on each of the four lines in the natural enemy free treatment (Table 3). The highest population growth rate was observed on the susceptible line. The population growth rate was reduced by 37.8 and 43.6% on the *Rag1* and *Rag2* lines, respectively. Population growth was reduced by 59.1% on the pyramid line. The combination of both aphid resistance genes and biological control were able to reduce soybean aphid population growth by 89.1% (comparison of pyramid line in biocontrol treatment to susceptible line in natural enemy free treatment).

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

Ability of Biological Control and Host Plant Resistance to Protect Yield. There was a significant effect of both aphid treatment ($F = 42.98$; df = 3, 44; $P <$ 0.0001) and soybean line ($F = 23.30$; df = 3, 44; $P <$

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

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

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes, either alone or combined.

[b] Aphid predators were excluded from plots using no-see-um mesh fabric (i.e., natural enemy free treatment).

[c] Number of aphids per plant averaged from six plots and three plants per plot.

[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

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

Soybean line^{a}	24 June^{b}	30 June	8 Jul	14 July	18 July	25 July	1 Aug.	8 Aug.	17 Aug.	23 Aug.
Susceptible	5a^{c,d}	3ab	16a	31a	90a	359a	891a	521a	202a	107a
Rag1	2a	1b	3b	5bc	15b	13b	29b	16b	34b	14a
Rag2	4a	4a	6ab	13b	30b	42b	109b	113ab	305ab	59a
Pyramid	4a	2ab	3b	3c	9b	14b	25b	15b	14b	51a

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes, either alone or combined.

[b] Plots were left open to allow predators access to aphid populations (i.e., biocontrol treatment).

[c] Number of aphids per plant averaged from six plots and three plants per plot.

[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

0.0001) on yield. The interaction of aphid treatment by soybean line ($F = 5.18$; df = 9, 44; $P < 0.0001$) was also significant. Because of the interaction between aphid treatment and soybean line, further analyses were performed by soybean line to determine if the yield varied by aphid treatment within each line. Aphid treatment significantly affected the yield for the susceptible ($F = 25.03$; df = 3,15; $P < 0.0001$), *Rag1* ($F = 21.93$; df = 3, 14; $P < 0.0001$), and *Rag2* ($F = 10.42$; df = 3, 15; $P = 0.0006$) lines, but did not affect yield for the pyramid line ($F = 0.64$; df = 3 ,15; $P = 0.6033$). Least squared means analysis was used to determine differences among aphid treatments within each soybean line (Fig. 2). Compared with the aphid free treatment, yield was significantly reduced in the natural enemy free treatment for the susceptible ($t = -6.27$; df = 15; $P < 0.0001$), *Rag1* ($t = -5.07$; df = 14; $P = 0.0009$), and *Rag2* ($t = -4.91$; df = 15; $P = 0.001$) lines. However, yield in the natural enemy free treatment was not significantly reduced for the pyramid line ($t = -1.01$; df = 15; $P = 0.7459$). For the biocontrol and immigration treatments, yield was not significantly different compared with the aphid free treatment for any of the four soybean lines.

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

Table 3. Soybean aphid population growth rates

Soybean line^{a}	Natural enemy free^{b}	Biocontrol^{c}
Susceptible	0.188 ± 0.0128a^{d,e}	0.1495 ± 0.0152bc
Rag1	0.1523 ± 0.019b	0.0753 ± 0.0134c
Rag2	0.1438 ± 0.0187b	0.0794 ± 0.0128c
Pyramid	0.105 ± 0.0190bc	0.0704 ± 0.0105c

[a] Near-isolines selected for combinations of *Rag1* and *Rag2* genes, either alone or combined.

[b] Aphid predators excluded from plots using mesh fabric.

[c] Plots left open allowing predators access to aphid populations.

[d] Growth rate from 27 June to 14 July, reported as an average ± SEM.

[e] Letters represent significant differences at $P < 0.05$.

Discussion

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

The EIL is the point at which yield loss exceeds the cost of control measures (Stern et al. 1959). The EIL for soybean aphids has been estimated at 674 aphids plant^{-1}, when a foliar insecticide application is being considered (Ragsdale et al. 2007). This EIL provides an appropriate measurement for assessing the efficacy of the various treatments in this study. In our study, biological control alone was unable to prevent soybean aphid populations from exceeding the EIL, as evidenced by the high aphid populations on the susceptible line in the biocontrol treatment. This observation is consistent with field observations and previous studies, which demonstrate that biological control can be insufficient to prevent aphid outbreaks under high aphid pressure conditions (Hodgson and VanNostrand 2011).

Rag genes significantly reduced soybean aphid populations compared with the susceptible line. This was true in both the presence and absence of biological control. However, only the pyramid line was able to maintain populations below the EIL in the natural enemy free treatment. This observation was mirrored in the yield data. Only the pyramid line was able to prevent significant yield loss in the natural enemy free treatment. This is an important observation for the management and the utility of a pyramid line. The natural enemy free treatment represents a "worst-case scenario" in which aphid populations arrive early in the season (plants were infested artificially in June) and biological control is nonexistent. Although these conditions are unlikely to occur in the field, regional

Table 4. Soybean aphid populations on four lines in the immigration treatment

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

[a] Near-isolines selected for presence and absence of *Rag1* and *Rag2* genes.

[b] An immigration event was modeled by artificially infesting plants and covering plots with fabric until 11 July. After 11 July plots were opened to allow natural enemies access to aphid populations.

[c] Number of aphids per plant averaged from six plots and three plants per plot.

[d] Letters represent significant differences among soybean lines within a sampling date at $P < 0.05$ using test for least significant differences.

variation in the density of natural enemies found within soybean fields has been observed at the landscape level (Gardiner et al. 2009), with lower densities and subsequently less biological control in landscapes dominated by corn and soybean production. Furthermore, Landis et al. (2008) suggested that increased production of corn has reduced the biological control of soybeans aphids. Given the genetic composition of the aphids we employed (i.e., biotype), the pyramid line has the potential to effectively maintain aphid populations below the EIL when biological control is nonexistent (i.e., soybeans grown in a cage).

Currently farmers are able to purchase soybeans with a single gene for aphid resistance. Because the single gene resistant lines alone were incapable of maintaining aphid populations below the EIL, it will be important for host plant resistance to be compatible with biological control. The single gene lines (i.e., *Rag1* and *Rag2* alone) by themselves were incapable of maintaining aphid populations below the EIL in the natural enemy free treatment. With the addition of the mortality from aphid predators and parasitoids in the biocontrol treatment, the single gene lines were able to maintain soybean aphids below the EIL. This inconsistent soybean aphid control by single gene lines is consistent with field observations for the *Rag1* line (O'Neal and Hodgson 2009). This result highlights the important role biological control will play in any integrated pest management (IPM) program in which single gene resistant lines are used. This conclusion is further emphasized in the immigration treatment, in which only the resistant lines were able to prevent aphid populations from exceeding the EIL.

We did not observe a difference in yield between the biocontrol treatment and immigration treatment across any of the four soybean lines. We anticipated that yield loss would occur when aphid populations exceeded the EIL, as it did on 1 August in both the biocontrol (Table 2) and immigration treatment (Table 4). Yield loss may have occurred, but may have been undetected because of a large amount of variability common when soybean yield is estimated from

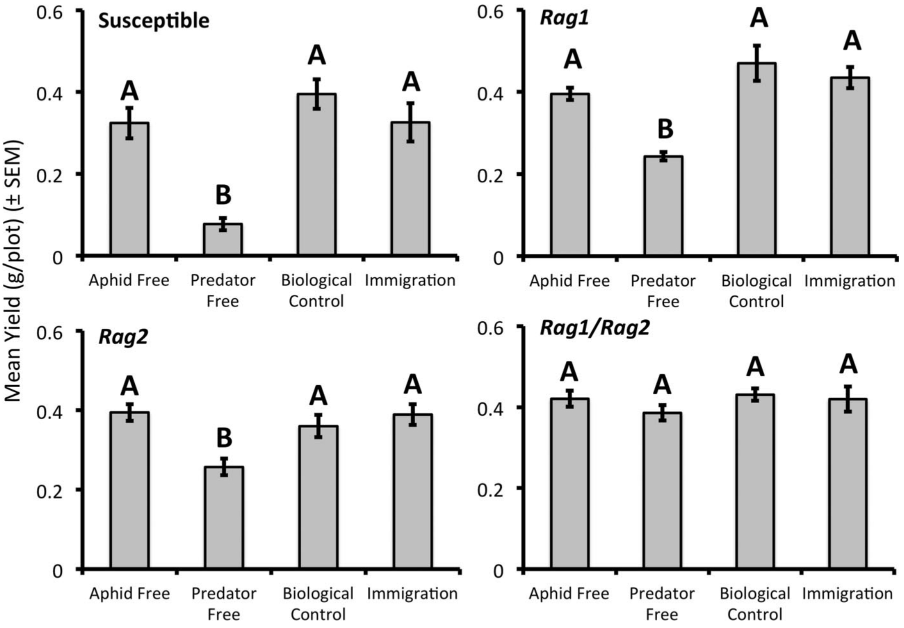

Fig. 2. Yields (Mean ± SEM g/plot) of four soybean lines exposed to the four aphid treatments. Yield data were analyzed using a test for least significant differences. Letters signify significant differences at $P < 0.05$.

micro-plots (Fehr 1991). We also failed to show a difference in yield for the susceptible line between, either the biocontrol and immigration treatments compared with the aphid free treatment. This lack of a significant difference could be because of either the variability for a micro-plot study or a potential cage effect. The aphid free and natural enemy free treatments were both grown inside mesh cages for the duration of the season.

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

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

Host plant resistance holds the potential to increase the efficiency and effectiveness of soybean aphid management. The research presented in this paper demonstrates both the compatibility and importance of biological control for host plant resistance. This research also reinforces initial findings by Wiarda et al. (2012) on the increased efficacy obtained by pyramiding *Rag1* and *Rag2* together in a single soybean line. Future research will need to focus on the performance of a pyramid line across the larger North Central region where it can potentially be exposed to naturally occurring virulent soybean aphid biotypes.

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