Soybean Aphid (Aphididae: Hemiptera) Population Growth as Affected by Host Plant Resistance and an Insecticidal Seed Treatment

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ABSTRACT The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a significant soybean pest in the north central United States. Insecticidal seed treatments and host plant resistance are two commercially available management tools. Here we investigate the efficacy of both management tools throughout the season. Soybean lines containing the soybean aphid resistance genes *Rag1*, *Rag2*, or both *Rag1* + *Rag2* were compared with a near-isogenic aphid-susceptible line. Each line was grown in field plots both with and without thiamethoxam applied to the seed. Individual plants from each plot were caged and infested with soybean aphids to measure the efficacy and potential interaction of aphid resistance and thiamethoxam. Aphid population growth rate was measured for each caged plant for 9–12 d after infestation. New cages were established each week from 34 d after planting (dap) to 92 dap to track seasonal variations in efficacy. Thiamethoxam reduced population growth only at the 42 dap time point and only for the susceptible, *Rag1*, and *Rag2* lines. The lack of an effect of thiamethoxam on the *Rag1*+ *Rag2* line was likely because of already high mortality from two resistance genes. Aphid resistance alone reduced population growth compared with the susceptible line at least till 55 dap for single-gene resistance and 63 dap for the two genes combined. Aphid resistance provided suppression of soybean aphid population growth throughout the season unlike the insecticidal seed treatment.

KEY WORDS integrated pest management, thiamethoxam, *Aphis glycines*

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) is an invasive pest that causes economic damage to soybean in the north central United States (Ragsdale et al. 2011). Initial management recommendations focused on the use of foliar insecticides to prevent yield losses of up to 47% because of soybean aphid feeding (Ragsdale et al. 2007). Ragsdale et al. (2007) recommended a foliar application of insecticide when aphids reach an economic threshold of 250 aphids/plant. Johnson et al. (2009) noted that this threshold was more profitable than either the application of insecticides based on plant growth stage or the use of an insecticidal seed treatment. Insecticidal seed treatments have increased in use, especially in Iowa (Hodgson et al. 2012), despite inconsistent impacts on soybean aphid populations (McCornack and Ragsdale 2006, Johnson et al. 2008, Magalhaes et al. 2009). The inconsistent efficacy of insecticidal seed treatments for soybean aphid management is largely because of the seasonal variability in their efficacy (McCornack and Ragsdale 2006, Seagraves and Lundgren 2012) and variability in the timing of soybean aphid migration and field colonization (Hodgson et al. 2005, Schmidt et al. 2012). In general, insecticidal seed treatments provide protection to soybean from insect pests early in the growing season. For soybean aphids, the efficacy of an insecticidal seed treatment is lost ≤55 d after planting (McCornack and Ragsdale 2006), which corresponds to middle to late July for much of the soybean producing area of the north central United States (Pedersen 2004). Soybean aphid outbreaks typically occur in late July and August for this region (Ragsdale et al. 2011). Therefore, insecticidal seed treatments have a limited capacity to protect soybeans from soybean aphid colonization and subsequent population growth.

Host plant resistance may offer season–long protection from soybean aphids. At least four different soybean genes conferring resistance to the soybean aphid have been identified (Hill et al. 2006; Mian et al. 2008b; Zhang et al. 2009, 2010). Currently, commercially available soybean aphid-resistant varieties incorporate a single resistance gene, the *Rag1* gene, or two soybean aphid resistant genes, the *Rag1* gene and the *Rag2* gene (McCarville et al. 2012b). Experiments using artificially infested plants indicate that combining two soybean aphid resistance genes into a single soybean line increases resistance to the soybean aphid (McCarville and O'Neal 2012, Wiarda et al. 2012). Despite the increased resistance displayed by a pyramid line, varieties containing a single resistance gene

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will likely continue to be grown for at least the near future (McCarville et al. 2013).

Soybean aphids are capable of reaching populations that exceed the economic threshold on varieties containing a single aphid-resistance gene (McCarville and O'Neal 2012, Wiarda et al. 2012). High, potentially economically damaging aphid populations on these varieties could be because of several factors including but not limited to 1) limited efficacy of these single genes (Wiarda et al. 2012), 2) the presence of virulent aphid biotypes capable of overcoming individual resistance genes (Kim et al. 2008, Hill et al. 2010), 3) declining efficacy of the resistance genes late in the season (Klun and Robinson 1969), or 4) induced susceptibility (Baluch et al. 2012) or density-dependent expression of resistance (i.e., resistance overcome by larger populations of aphids on a plant).

Given the limited efficacy of single gene lines and the presence of resistant biotypes capable of overcoming these genes, there may be a benefit to pairing insecticidal seed treatments with soybean aphid host plant resistance to improve soybean aphid management. Our goal was to measure soybean aphid mortality because of host plant resistance (i.e., *Rag1* and *Rag2*) and an insecticidal seed treatment (i.e., thiamethoxam) both alone and together and to determine if these two sources of mortality provide improved management of the soybean aphid during both vegetative and reproductive stages of soybean development.

Materials and Methods

We conducted our study during 2011 and 2012 in fields located within Story County, IA. The four soybean lines used for our experiment were developed using the backcross method (Fehr 1991), in which a desired gene(s) is introduced from the donor parent to the recurrent parent, offspring are backcrossed to the recurrent parent, and the BC_1F_1 progeny are selfed. The desired BC_1F_2 genotypes are selected and lines, which do not differ significantly from the recurrent parent in any desired traits, are bulked to create the new line. Additional details of the development of the lines used in our experiment are outlined in Wiarda et al. (2012). Briefly, each experimental soybean line was a bulk of 10 $BC_1F_{2:5}$ (2011) or 10 $BC_1F_{2:6}$ (2012) lines derived from a cross of LD08-89051a (a line containing *Rag2*) and the recurrent parent A08-123074 (containing *Rag1*). Therefore, each individual line contains 75% of its genes from the recurrent parent. Plants heterozygous for the *Rag1* and *Rag2* genes were selected at the BC_1F_1 generation. Their progeny were evaluated to select four distinct genotypes: 'Susceptible' (*rag1rag1rag2rag2*), '*Rag1*' (*Rag1Rag1rag2rag2*), '*Rag2*' (*rag1rag1Rag2Rag2*), and '*Rag1* + *Rag2*' (*Rag1Rag1Rag2Rag2*). Ten lines of each genotype were bulked to form the four experimental soybean lines used for the experiment reported here.

We used a split-plot design with whole plots arranged in a randomized complete block design with three blocks. The whole plot treatment was one of four soybean lines planted at a rate of 346,000 seeds/ha using standard farming practices (i.e., tillage and preemergent weed control). Whole plots (soybean lines) were 15.25 m in length and comprised of 12 0.76 m-wide rows. Two treatments were assigned to a whole plot, so that each block consisted of eight split-plots. The two treatments were seed-treated and untreated. Soybean seed of each line assigned to the seed-treated treatment was shipped to Syngenta Seed Care (Stanton, MN) and treated with thiamethoxam (Cruiser five FS, Syngenta Seeds) at a rate of 0.0756 mg/seed. Soybean seed assigned to untreated split-plots were kept free of pesticides and planted as naked seed. Plots were planted on 24 May and 15 May in 2011 and 2012, respectively.

Plants were artificially infested with soybean aphids to measure the efficacy of aphid resistance genes and a seed treatment throughout the course of the growing season. Noninfested plants were randomly selected within the first and sixth row of each untreated and seed-treated split-plot for each soybean line. The first plants were selected 34 d after planting (dap) when plants reached the early third trifoliate vegetative stage (i.e., V3, per rating scale used by Fehr and Caviness 1977). Each week new plants were selected, caged, and infested with aphids. The remaining sets of cages were established at 42 dap (R1 in 2011, V7 in 2012), 48–49 dap (R2 in 2011, R1 in 2012), 55–56 dap (R3 in 2011, R2 in 2012), 62–63 dap (R3 in 2011 and 2012), and 69 dap (R4 in 2011 and R3 in 2012). Cages at 69 dap were deployed only in untreated split-plots because there was no evidence of mortality because of the insecticidal seed treatment in the previous two cage sets (i.e., 55–56 and 62–63 dap). We stopped caging plants when noninfested plants were no longer available. In 2011 we stopped caging plants after 69 dap because of high populations of soybean aphids (>100 aphids per plant with >95% of plants infested on the susceptible line) making it unfeasible to locate noninfested plants within the field. In 2012, populations of aphids were much lower 69 dap (<5 aphids per plant with <5% of plants infested on the susceptible line), therefore, three additional sets of cages were established (77, 84, and 91 dap). As with the 69 dap cage sets in 2011 and 2012, cages were established in only the untreated split-plots because of the ineffectiveness of a seed treatment at prior sampling periods (i.e., 55–56 and 62–63 dap).

For the purpose of these experiments, each cage was considered an experimental unit. Because we visited each split-plot frequently to estimate soybean aphid populations, we varied the location from which plants were selected and artificially infested to avoid damaging adjacent plants. In 2011, only rows one and six in each split-plot in blocks one and two were used for the first three sets of cages (34, 42, and 49 dap). This was done as plant emergence in block three was delayed and reduced compared with blocks one and two. However, block three was used for the rest of 2011. Rows one and six of blocks one, two, and three were used for the rest of 2011 (55–56 and 62–63 dap) and all of 2012. This resulted in fewer sampling points per

treatment for the first three time points in 2011 in comparison to all other sampling dates.

Before infestation, each plant was inspected for soybean aphids and any aphids found were manually removed. A tomato cage was then placed over each selected plant. Tomato cages were covered with a white no-see-um mesh net (Quest Outfitters, Sarasota, FL), which was buried in the ground and closed at the top. Nets prevented the immigration and emigration of soybean aphids and prevented aphidophagous predators from consuming aphids within the cage (Costamagna et al. 2006). This limited mortality or population growth measured for each infested plant to only the host plant (i.e., its resistance gene(s)) or insecticidal seed treatment.

Plants were artificially infested with soybean aphids from a laboratory colony of biotype-1 soybean aphids (i.e., avirulent to all *Rag* genes) maintained at Iowa State University. Five, mixed-age apterous aphids were manually transferred with a fine camel hairbrush (Winsor & Newton, Piscataway, NJ) to the underside of the uppermost fully expanded trifoliate (McCarville et al. 2012a). Aphid populations inside cages were allowed to increase for 9–12 d with populations assessed every 3–4 d. Aphid populations were assessed by opening nets and counting all aphids (both nymphs and adults) on each caged plant.

Statistical Analyses. The abundance of soybean aphids on caged plants was used to estimate the efficacy over time of both thiamethoxam and the aphid resistant lines. Aphid counts for each caged plant were log transformed and graphed over time (days after infestation). A linear regression was performed to estimate the slope of the line, which was considered the rate of population growth for each aphid population (i.e., each cage). The rate of population growth was analyzed using an analysis of variance (ANOVA) (SAS, PROC Mixed, Cary, NC). Population growth data were analyzed to address two questions, 1) does the impact of the seed treatment on soybean aphid populations vary across the four soybean lines at any point in time and 2) does the efficacy of either the *Rag1* or *Rag2* genes (alone or combined) on soybean aphid populations vary across the season. To address the first question, each time set of cages (i.e., 34 or 42 dap) was analyzed separately to determine the effect of an insecticidal seed treatment on aphid populations across the four soybean lines. A mixed effects model was used, in which a significant seed treatment*soybean line interaction would indicate that the impact of the seed treatment varied among the four lines. The model included the main effects of year, soybean line, and seed treatment and the interactions of year*soybean line, seed treatment*soybean line, year*seed treatment, and year*soybean line*seed treatment. The model also included the random factors of block, year*block, cage*soybean line, and year*block*soybean line.

A second analysis was conducted to assess the seasonal efficacy of the *Rag1* and *Rag2* genes. For this second analysis, only data from the untreated split-plots was analyzed to assess if either the *Rag1* or *Rag2* gene varied in efficacy across the growing season. These data were analyzed in two steps to account for variation in the number of cage sets (i.e., time points) between 2011 and 2012; in both steps a significant effect of soybean line would indicate aphid population growth rate differed among the soybean lines. If the effect of soybean line is not consistent across all time points, this would indicate variability in the seasonal efficacy of one or more of the *Rag* genes. In the first step of this analysis, data from all time points replicated in both 2011 and 2012 were analyzed in a mixed effects model that included the fixed effects of year, dap, soybean line, and the interactions year*dap, year*soybean line, dap*soybean line, and year*dap*soybean line. The model also included the random effect block and the interaction terms year*block, soybean line*block, dap*block, year*dap*block, year*soybean line*block, and dap*soybean line*block. In total, this analysis included all measurements taken before 77 dap.

In the second step, data from 2012 were analyzed separately. In this analysis data from all cage sets in 2012 including 77, 84, and 92 dap were analyzed. The model included the main effects of soybean line, dap, and the interaction of soybean line*dap. The model also included the random effects of cage, and the interactions of block*soybean line and block*dap.

Results

In both 2011 and 2012, despite using a population of soybean aphid comprised of biotype-1 (i.e., avirulent to both *Rag1* and *Rag2*) we were able to maintain soybean aphid populations on the aphid susceptible and aphid resistant soybean lines. The average aphid populations 9–12 d after receiving five aphids for the untreated split-plots (averaged across all time points and both years of the study) were 62.6, 7.9, 3.8, and 0.6 aphids/plant for the susceptible, *Rag1*, *Rag2*, and *Rag1* + *Rag2* lines, respectively. These results are consistent with previous studies demonstrating biotype-1 soybean aphids as capable of surviving on both the *Rag1* and *Rag2* lines, though with a reduction in fecundity and survival (McCarville and O'Neal 2012, Wiarda et al. 2012).

Although we consistently observed positive population growth rates in the untreated split-plot of the susceptible line, we did observe negative growth rates for the untreated split-plot of the susceptible line at 34, 70, and 84 dap in 2012. During these periods of 2012, we experienced high temperatures (61% of daily high temperatures for these time points were above the soybean aphid's developmental optimum) and dry conditions, which are not conducive for soybean aphid development (McCornack et al. 2004, Ragsdale et al. 2011). This observation is consistent with the extremely low aphid populations observed throughout Iowa during 2012 (Hodgson and VanNostrand 2012).

Interaction of Insecticidal Seed Treatments and Host Plant Resistance. At all time-points (i.e., dap) soybean line significantly affected aphid population growth (Table 1). Although the effect of year and the

Table 1. Seasonal effect of insecticidal seed treatment on aphid populations

Effect	df	F statistic	*P* value
34 dap			
Year	1,3	4.55	0.1225
Soybean line	3,9	23.84	0.0001[a]
Year*soybean line	3,9	6.52	0.0123[a]
Seed treatment	1,32	0.02	0.9009
Soybean line*seed treatment	3,32	0.02	0.9946
Year*seed treatment	1,32	2.47	0.1258
Year*line*seed treatment	3,32	1.16	0.3416
42 dap			
Year	1,3	3.36	0.1640
Soybean line	3,9	11.24	0.0021[a]
Year*soybean line	3,9	0.80	0.5250
Seed treatment	1,30	33.75	<0.0001[a]
Soybean line*seed treatment	3,30	4.68	0.0085[a]
Year*seed treatment	1,30	1.76	0.1945
Year*line*seed treatment	3,30	0.37	0.7781
48–49 dap			
Year	1,3	14.70	0.0313[a]
Soybean line	3,9	28.73	<0.0001[a]
Year*soybean line	3,9	8.31	0.0058[a]
Seed treatment	1,32	0.02	0.8876
Soybean line*seed treatment	3,32	1.00	0.4052
Year*seed treatment	1,32	0.04	0.8472
Year*line*seed treatment	3,32	2.07	0.1235
55–56 dap			
Year	1,3	5.27	0.1054
Soybean line	3,8	17.46	0.0007[a]
Year*soybean line	3,8	0.55	0.6618
Seed treatment	1,34	0.06	0.8109
Soybean line*seed treatment	3,34	0.36	0.7842
Year*seed treatment	1,34	0.16	0.6908
Year*line*seed treatment	3,34	0.04	0.9883
62–63 dap			
Year	1,3	14.02	0.0332[a]
Soybean line	3,9	7.86	0.0069[a]
Year*soybean line	3,9	0.87	0.4907
Seed treatment	1,40	2.94	0.0940
Soybean line*seed treatment	3,40	1.33	0.2792
Year*seed treatment	1,40	2.45	0.1254
Year*line*seed treatment	3,40	0.76	0.5231

[a] Effect significantly impacted soybean aphid pop growth at $P < 0.05$.

interaction of year*soybean line was occasionally significant, the interactions of year*seed treatment and year*soybean line*seed treatment did not significantly affect soybean aphid populations. Only during the 42 dap time point did the seed treatment significantly affect aphid population growth ($F = 33.75$; df = 1, 30; $P < 0.0001$; Fig. 1). In addition, only during this time point did the interaction of soybean line*seed treatment significantly affect aphid populations ($F = 4.68$; df = 3, 30; $P = 0.0085$). This interaction is observed in the reduction in aphid population growth rates by a seed treatment on *Rag1* ($F = 8.00$; df = 1,7; $P = 0.0255$), *Rag2* ($F = 10.91$; df = 1, 8; $P = 0.0108$), and susceptible ($F = 16.31$; df = 1, 8; $P = 0.0037$) lines, but not on the *Rag1*+ *Rag2* line ($F = 2.71$; df = 1, 7; $P = 0.1438$).

Seasonal Efficacy of Rag Genes. Aphid population growth on untreated soybean varied by year ($F = 36.01$; df = 1, 3; $P = 0.0093$) and soybean line ($F = 45.39$; df = 3, 9; $P < 0.0001$) for time points replicated in both 2011 and 2012 (i.e., 34–69 dap). In addition to the main effects, the interactions of year*soybean line ($F = 7.14$; df = 3, 9; $P = 0.0094$), and year*dap*soybean line ($F = 2.03$; df = 15, 42; $P = 0.0365$) both significantly affected aphid population growth. The main effect of dap ($F = 2.32$; df = 5, 15; $P = 0.0947$) and the interactions of year*dap ($F = 2.26$; df = 5, 15; $P = 0.101$) did not significantly affect aphid populations. The interaction of dap*soybean line ($F = 1.75$; df = 15, 86; $P = 0.0551$) had a marginally significant impact on soybean aphid populations. The significant interaction of year*dap*soybean line indicated that there may have been variability in the efficacy of one or more of the *Rag* genes between the 2 yr of our study. Therefore, data were analyzed separately for 2011 and 2012.

In 2011, the factor of soybean line ($F = 27.88$; df = 3, 15; $P < 0.0001$) significantly affected soybean aphid growth rates (Fig. 2). We observed consistently higher population growth rates for aphids on the susceptible line compared with the three aphid-resistant lines. We did not observe a significant effect of plant growth stage (as defined by differences in dap) on aphid population growth rates ($F = 2.09$; df = 5, 59; $P = 0.1114$); however, the interaction of dap*soybean line ($F = 1.85$; df = 15, 53; $P = 0.0518$) was marginally significant indicating possible variations in the efficacy of one or more of the *Rag* genes. Data were then analyzed by soybean line to determine if soybean aphid population growth varied across the season by soybean line. Soybean aphid growth rates did not vary significantly across the six different points in time for either the *Rag1*+ *Rag2* line ($F = 0.86$; df = 5, 18; $P = 0.5282$) or the susceptible line ($F = 1.24$; df = 5, 18; $P = 0.3333$). Days after planting, however, significantly affected soybean aphid growth rates on the *Rag2* line ($F = 2.84$; df = 15, 18; $P = 0.0461$) and had a marginally significant effect on the *Rag1* line ($F = 2.66$; df = 15, 18; $P = 0.0570$), thus indicating potential temporal variability in the efficacy of both the *Rag1* and *Rag2* genes.

In 2012, the effect of soybean line ($F = 23.50$; df = 3, 15; $P < 0.0001$) again significantly impacted soybean aphid population growth rate (Fig. 3). The main effects of dap ($F = 2.13$; df = 5, 25; $P = 0.0949$) and the interaction of dap*soybean line ($F = 1.79$; df = 15, 75; $P = 0.0512$) were marginally significant. Soybean aphid growth rates did not vary significantly over time for either the *Rag1* line ($F = 1.07$; df = 5, 25; $P = 0.4020$), *Rag2* line ($F = 1.82$; df = 5, 25; $P = 0.1455$), or the susceptible line ($F = 1.98$; df = 5, 25; $P = 0.1166$). Days after planting, however, did significantly impact soybean aphid growth rates on the *Rag1*+ *Rag2* line ($F = 2.90$; df = 15, 25; $P = 0.0339$); thus, indicating potential temporal variability in the efficacy of the *Rag1* + *Rag2* line.

The 2012 data were then analyzed again, this time including the last three time points (77, 84, and 92 dap). When analyzing the complete data set, the effect of soybean line ($F = 28.56$; df = 3, 15; $P < 0.0001$) was still found to significantly impact soybean aphid growth rates (Fig. 3). However, soybean aphid growth rates did not vary significantly for the main effects of dap ($F = 1.51$; df = 8, 40; $P = 0.1851$) and the inter-

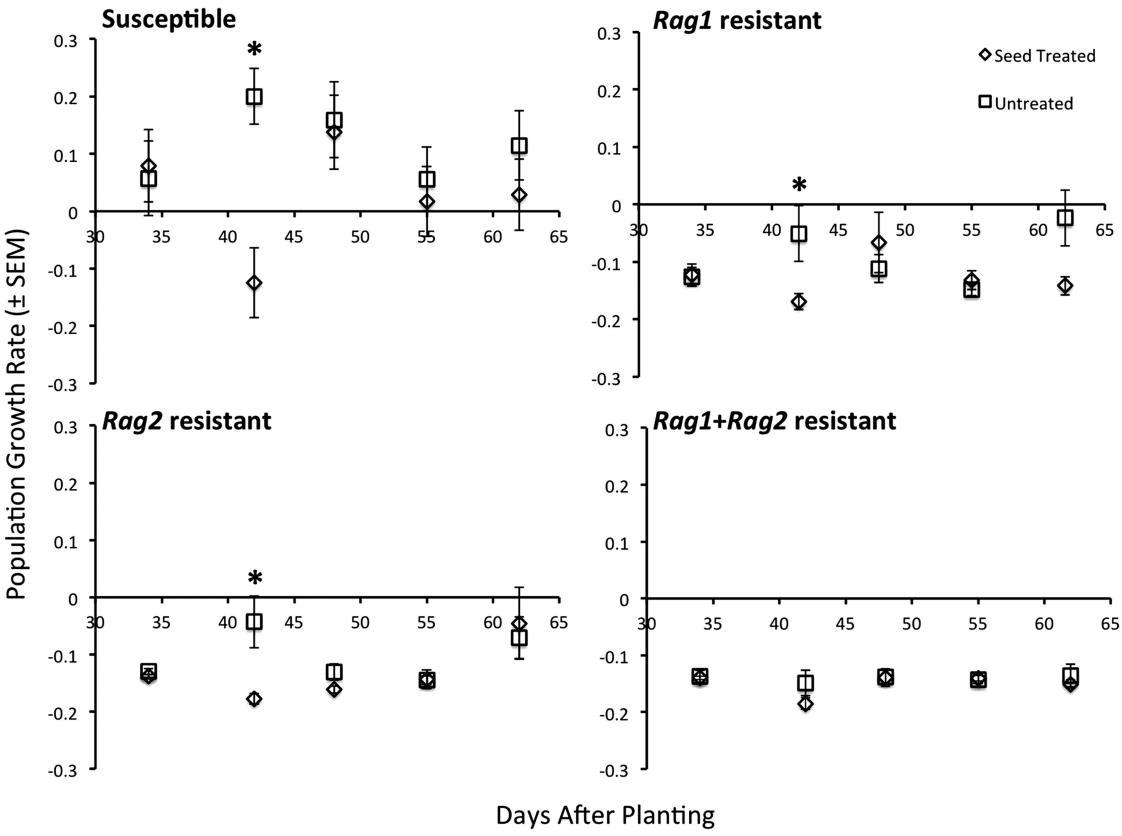

Fig. 1. Soybean aphid population growth rates from both 2011 and 2012 as affected by a thiamethoxam seed treatment over the course of the growing season on four soybean lines. Soybean lines were near-isolines including a soybean aphid susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34–62 dap and population growth was tracked for 9 to 12 d after infestation. Asterisks indicate significant differences ($P < 0.05$) between treatments within a soybean line.

action of dap*soybean line ($F = 1.08$; df = 24, 120; $P = 0.3723$).

Discussion

In this study, we measured variability in the temporal efficacy and potential interaction of two soybean aphid mortality factors, an insecticidal seed treatment and the soybean aphid resistance genes *Rag1* and *Rag2*. In previous studies, McCornack and Ragsdale (2006) and Seagraves and Lundgren (2012) both investigated the efficacy of insecticidal seed treatments against soybean aphids at different time points during the season. While previous studies have successfully used soybeans at both vegetative growth stages (Li et al. 2007, Mian et al. 2008a, Hesler et al. 2012) and reproductive stages (Wiarda et al. 2012) to evaluate resistance, we are unaware of any previous experiments investigating the temporal efficacy of soybean aphid resistance genes across the growing season or their interaction over time with insecticidal seed treatments. In our study, we found both insecticidal seed treatments and soybean aphid host plant resistance to significantly decrease soybean aphid populations at one or more times during the season.

Previous studies investigating thiamethoxam seed treatments against soybean aphids have used different methods to measure the efficacy of the seed treatment over varying lengths of time. McCornack and Ragsdale (2006) measured survival and nymph production over a 48-h period in both detached leaf and field assays, Seagraves and Lundgren (2012) measured aphids present after 7 d in a detached leaf assay, while we measured population growth over a 10–12 d period in the field. Despite differences in assay methods and durations, similar efficacy results were obtained across the three studies. McCornack and Ragsdale (2006) observed soybean aphid mortality because of a thiamethoxam seed treatment was low during early vegetative stages (i.e., 28 dap), peaked at 35 dap, declined by 48 dap, and was absent by 55 dap. Seagraves and Lundgren (2012) found thiamethoxam and imidacloprid seed treatments increased aphid mortality from 26 to 40 dap, but had no effect by 46 dap. In our study, the thiamethoxam seed treatment significantly reduced population growth at only one time period (42 dap). In our experiment the peak for seed treatment efficacy is encompassed in the 42 dap time point in which aphids were placed on soybean plants 42 dap and population growth was tracked for 12 d (i.e., to 54

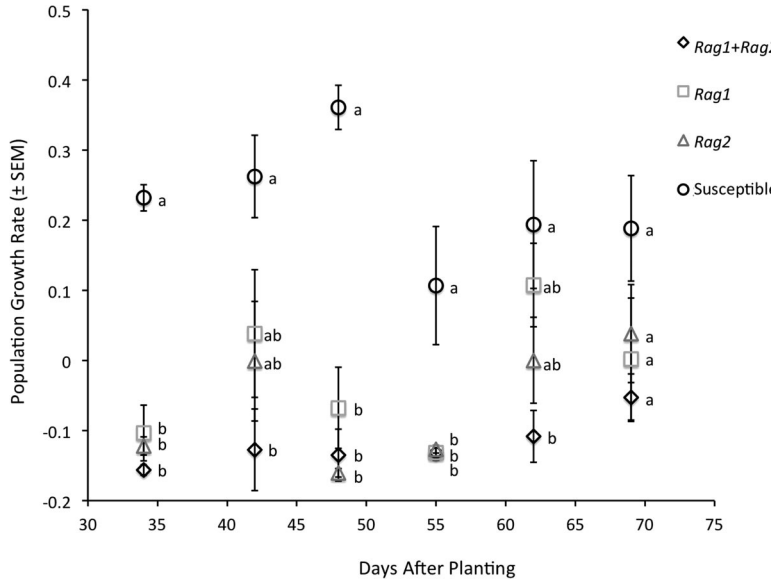

Fig. 2. Soybean aphid population growth rates in 2011 as affected by four near-isoline soybean lines, a susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34–91 dap and population growth was tracked for 9 to 12 d after infestation. Letters indicate significant differences ($P < 0.05$) among soybean lines within a single sampling point.

dap). This corresponds to McCornack and Ragsdale's 42 and 49 dap time points and Seagraves and Lundgren's 42 dap time point. Combined these studies suggest that the efficacy of thiamethoxam is low early in the season (i.e., 28 dap) and peaks at 42–48 dap before decreasing, indicating these observations are robust to a diversity of environmental and experimental variables.

In examining the interaction between thiamethoxam and aphid-resistance we found evidence for unequal effects of the seed treatment across the four soybean lines. At 42 dap, when the seed treatment

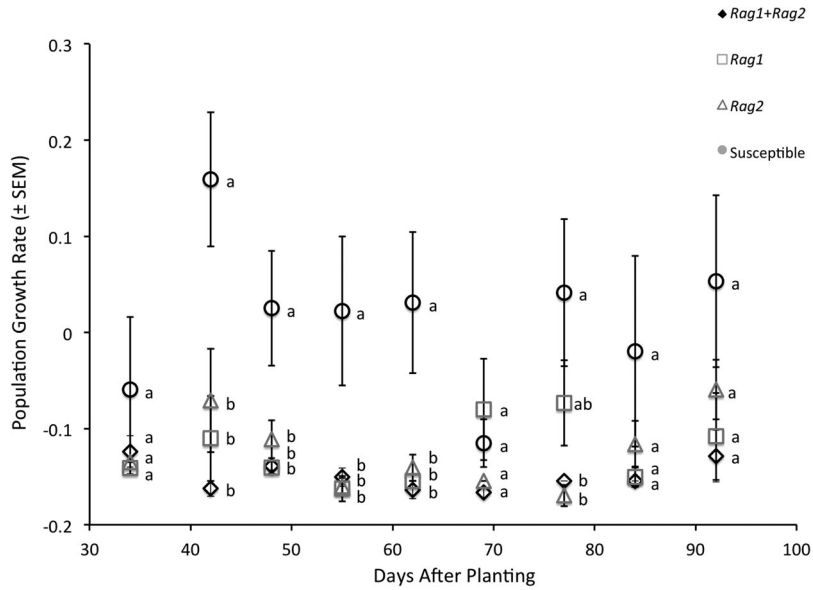

Fig. 3. Soybean aphid population growth rates in 2012 as affected by four near-isoline soybean lines, a susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, and a fourth line containing both the *Rag1* and *Rag2* genes. Five soybean aphids were placed on different caged soybean plants weekly from 34–69 dap and population growth was tracked for 9 to 12 d after infestation. Letters indicate significant differences ($P < 0.05$) among soybean lines within a single sampling point.

significantly reduced soybean aphid growth rates on the susceptible line, we were unable to measure any impact of the seed treatment on soybean aphid populations on the *Rag1*+ *Rag2* pyramid. The lack of an effect of the seed treatment on the *Rag1*+ *Rag2* line was likely because of the design of our assay and the already high efficacy of the pyramid line, which resulted in almost complete soybean aphid mortality from the pyramid line in the absence of a seed treatment. Aphid population growth, however, was still significantly reduced on both single gene lines. This result suggests that while insecticidal seed treatments may provide protection to soybean aphid-susceptible varieties, and also soybean aphid-resistant varieties carrying a single resistance gene, they are likely unnecessary at this time for varieties with multiple resistance genes. This conclusion may not be valid in the future if with increased use of *Rag* genes, the frequency of virulent soybean aphid biotypes increase in the environment.

As for aphid resistance, we observed significantly reduced population growth compared with the susceptible line that extended to at least 55 dap for single-gene resistance and 63 dap for the two genes combined. In addition, at only three sampling periods between 2011 and 2012 was there a positive rate of population growth measured on a resistant soybean line (42, 62, and 69 dap in 2011) and at none of these three sampling points was there a positive rate of growth on the *Rag1*+ *Rag2* pyramid line. However, in both 2011 and 2012, we observed temporal variability in the performance of at least one resistant line. In 2011, both the *Rag1* and *Rag2* lines displayed significant temporal variability in soybean aphid population growth rates, while in 2012 the *Rag1*+ *Rag2* pyramid displayed temporal variability. In neither year did soybean aphid population growth rates significantly vary with time on the susceptible line, indicating that the variations observed on the resistant lines occurred independently of overall host plant quality. In 2011, the variation in efficacy for the *Rag1* line and *Rag2* line was because of elevated soybean aphid growth rates at the 63 dap (*Rag1* and *Rag2*) and 70 dap (*Rag2*) time points. In 2012, the variation in efficacy for the *Rag1*+ *Rag2* pyramid was because of exceedingly high rates of soybean aphid mortality at the 42, 63, and 70 dap time points. Combined the 2 yr of this study suggests there is little seasonal variation in resistance expression for either the *Rag1* or *Rag2* genes.

As a whole this study shows soybean aphid-resistance to provide greater and more consistent soybean aphid control throughout the growing season when compared with an insecticidal seed treatment. These results also suggest insecticidal seed treatments could be used in addition to single gene resistance to improve early season soybean aphid control. The management tactic of pairing host plant resistance with insecticidal seed treatments may have unintended consequences though. Seagraves and Lundgren (2012) showed thiamethoxam seed treatments can reduce the overall population of generalist predators in the field. McCarville and O'Neal (2012) demonstrated the large impact pyramided resistance has on plant exposure to aphids when paired with biological control. In their experiment the soybean plant's seasonal exposure to aphids was reduced by over 99% when soybean aphids were exposed to a *Rag1*+ *Rag2* pyramid and natural enemies compared with a natural enemy free susceptible plant. Therefore, care should be taken when pairing insecticidal seed treatments with soybean aphid host plant resistance.

How the reductions in soybean aphid population growth we measured affect soybean aphid abundance in the field specifically related to the current economic threshold and economic injury level will depend on factors such as soybean aphid arrival time and immigration rates, the occurrence of virulent biotypes, and natural enemy populations. All of these factors are likely to vary among locations and years within the north central United States; therefore, a multi-location regional field study is warranted to investigate the effects of both host plant resistance and insecticidal seed treatments on soybean aphid abundance.

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