Title: Testing *Rag1* and *Rag2* alone and together: Is one enough?
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Final Report
Submitted October 11, 2013
for the period of February 1, 2010 to July 31, 2013

Goal: Our overall goal is to increase soybean production while reducing the economic and environmental costs of soybean aphid management. We can achieve this goal through the use of soybean aphid-resistant soybean varieties that suppress aphid populations such that insecticides are not needed to protect yield. Our results indicate that this is possible when two aphid resistant genes (in this case *Rag1* and *Rag2*) are combined in a single cultivar.

The specific goals of this project were to determine the yield response of soybeans with *Rag1* alone, *Rag2* alone, *Rag*1 and *Rag2* combined, and an aphid-susceptible isoline when artificially infested with soybean aphids. We achieved this goal by conducting research related to the following two objectives:

1) Determine yield response of soybeans with *Rag1* alone, *Rag2* alone, *Rag*1 and *Rag2* combined, and an aphid-susceptible isoline when artificially infested with soybean aphids in cages (conducted in 2010 and 2011).
2) Determine if additional management of soybean aphid is needed for optimal soybean yield with cultivars that have *Rag1* alone, *Rag2* alone and the two genes combined (conducted in 2011 and 2012).

Major Findings:

- Both alone and combined, aphid resistant genes reduce soybean aphid populations compared to an aphid-susceptible cultivar.
- Combining two aphid resistant genes (*Rag1* and *Rag2*) provides greater protection to soybean aphids than a single gene. During a soybean aphid outbreak both susceptible and single-gene resistant plants experienced yield loss and the pyramid did not.
- A seed-applied insecticide reduced aphid populations on both susceptible and aphid-resistant soybeans. However, including a seed treatment on a pyramid was not necessary to keep aphid populations below economic thresholds, even when artificially infested with soybean aphids.

What does it mean for soybean farmers:

- A pyramid of *Rag1* and *Rag2* can prevent aphid outbreaks to the point that an insecticide is not needed.
- Farmers who suffer repeatedly from soybean aphid outbreaks are recommended to use an aphid-resistant cultivar.
- Commercial sources of aphid resistant soybeans cultivars are available to farmers in Iowa. However, a pyramid line is only available through the ISU Research Foundation (IA3027RA12 and IA3045RA12). A pyramid will not be available for commercial production until 2015, at the earliest.

Background:

Initially, commercially available soybean aphid-resistant varieties included a single gene, *Rag1*, conferring resistance to the aphid. Previous ISA funded research found aphid populations occurred on *Rag1* soybean varieties, often exceeding the economic threshold. When we began this project, it was not clear whether these populations of aphids were capable of reducing the yield of the *Rag1* containing varieties. Also, we did not know if cultivars with two aphid resistant genes (i.e. a pyramid) had greater protection than cultivars with only one gene.

Working closely with Dr. Walter Fehr, soybean breeder at ISU, we tested cultivars that he had developed which had *Rag1* alone, *Rag2* alone, *Rag*1 and *Rag2* combined, and an aphid-susceptible isoline. These four cultivars made it possible for us to determine the value of each gene alone and combined.

We conducted both caged and small plot trials to test the value of aphid resistance. During the summer of 2010, we used cages we could control both the density of aphids on a per plant basis and the timing of their occurrence (Wiarda et al. 2012). During 2011, we again used cages, but included an uncaged treatment to estimate the value that insect predators would have in combination with the resistant cultivars (McCarville and O'Neal 2012). Beginning in 2011 and continuing in 2012, we conducted a field experiment with cages in which the four varieties were grown in small plots, with and without an insecticidal seed treatment (McCarville and O'Neal in press).

Results:

Key findings from these studies are presented below, taken from previously published manuscripts. The complete results can be found in the published manuscripts (see below, Outputs).

From our first cage experiment, we observed a substantial difference in aphid abudance among the four cultivars (Table 3, from Wiarda et al. 2012). When these plants were caged and artificially infested, the aphid-susceptible and single gene varieties supported large populations of aphids that exceeded 1300-3400 aphids per plant. However, the pyramid line had a peak aphid population of only 505 aphids per plant, after being artificially infested twice.

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

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

[a] R1 = *Rag1* present; R2 = *Rag2* present; S1 = absence of *Rag1*; S2 = absence of *Rag2*.
[b] Treatments used to calculate the mean no. of aphids per plant.
[c] Aphid populations were established from a colony found in Iowa and manually infested on 12 July. The resistant genotypes were reinfested on 29 July.
[d] Number of aphids per plant reported were from plots that did not receive insecticide until 18 Aug. This included the combination of two treatments that were assigned to the original experimental design for the aphid resistant genotypes. The aphid susceptible line is represented by the 75K treatment.
[e] Values within a column with the same letter were not significantly different at the 0.05 probability level based on Tukey's honest significant difference (Tukey 1949).

In 2011, we artificially infested the four cultivars with soybean aphids and compared aphid populations on caged and uncaged plants. Again, they were lowest on the pyramid, and almost non-existent when predators were free to feed on the aphids (Figure 1 from McCarville et al. 2012). Combined, biological control and the aphid-resistance genes reduced aphid populations by 99.3%. The presence of natural enemies and one or two aphid resistance genes kept populations below the economic injury level and prevented yield loss (Figure 2). This study demonstrated that biological control and the *Rag* 1 and 2 genes are compatible.

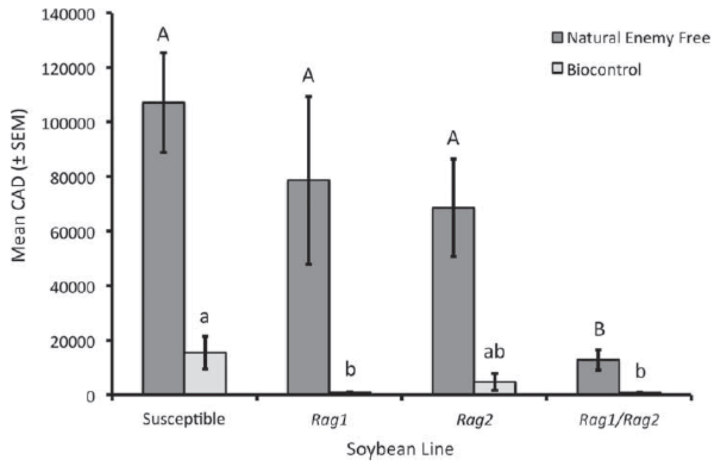

Fig. 1. Mean ± SEM cumulative aphid days (CAD) for the four lines exposed to the natural enemy free and biocontrol treatments. Cumulative aphid days were significantly higher in the natural enemy free treatment compared with the biocontrol treatment ($P < 0.0001$). Letters represent significant differences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

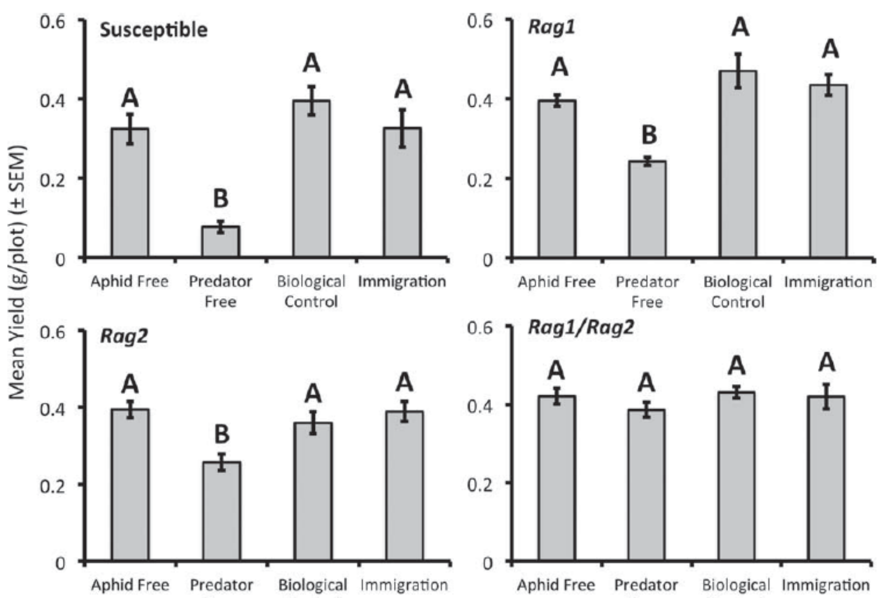

Fig. 2. Yields (Mean ± SEM g/ plot) of four soybean lines exposed to the four aphid treatments. Yield data were analyzed using a test for least significant differences. Letters signify significant differences at $P < 0.05$.

During 2011 and 2012, we tested these four cultivars again in large plots. These plots were split, with each variety grown with our without an insecticidal seed treatment (i.e.

Thiamethoxam, the active ingredient in Cruiser). By artificially infesting plants and caging them we could measure the value of the resistance genes and the seed treatment to prevent aphid population growth. In Fig. 1 (McCarville and O'Neal, in press), we observed the seed treatment contributing negative population growth on lines with Rag1 alone and Rag2 alone. A seed treatment was not needed as we observed consistent negative population growth of aphid populations only on lines Rag1 and Rag2 combined.

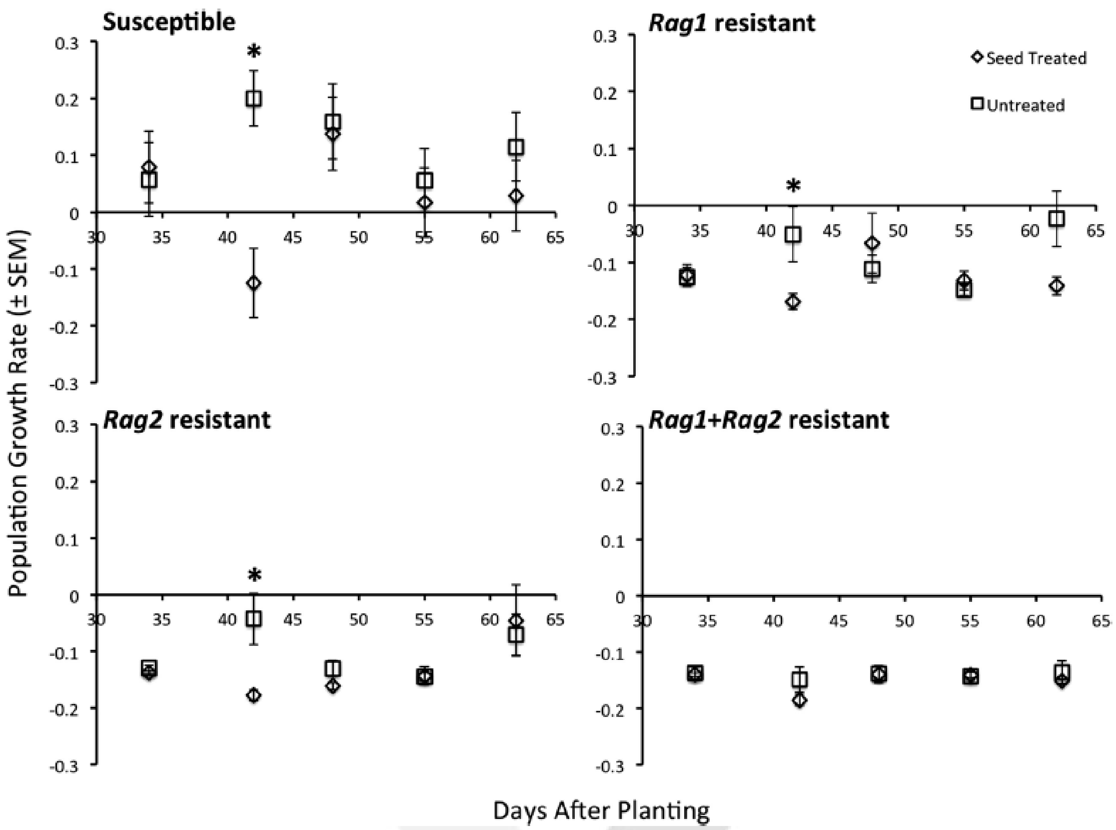

Fig. 1. Soybean aphid population growth rates from both 2011 and 2012 as affected by a thiamethoxam seed treatment over the course of the growing season on four soybean lines. Soybean lines were near-isolines including a soybean aphid susceptible line, a *Rag1* resistant line, a *Rag2* resistant line, 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.

Finally, we have contributed data from this study to a multi-state project funded by the NCSRP. That study uses the same four varieties across 7 states, and plots are left either untreated or treated with insecticide to keep them free of aphids. Although still on-going, the results from this NCSRP project are very promising. The pyramid consistently provides protection across the region (Table 2) and significantly protects yield such that an insecticide would not be needed (Figure 3).

Table 2. Effect of soybean line on cumulative aphid days for 2011 and 2012

Location[a]	Susceptible	*Rag1*	*Rag2*	*Rag1 + Rag2*
2011				
Lamberton, MN	25,100 ± 2,791 a[b]	882 ± 240 bc	5,545 ± 1,305 b	1,189 ± 1,018 c
Volga, SD	25, 949 ± 4,205 a	2,498 ± 331 b	5,001 ± 1,185 b	850 ± 229 c
Ames, IA	5,506 ± 1,315 a	1,023 ± 272 b	963 ± 175 b	242 ± 54 c
Nashua, IA	8,281 ± 1,560 a	2,490 ± 1,169 b	1,137 ± 254 b	287 ± 85 c
Arlington, WI	845 ± 172 a	563 ± 130 ab	594 ± 149 ab	415 ± 80 b
Scandia, KS	332 ± 54 a	20 ± 8 b	57 ± 31 b	11 ± 3 b
Rock Springs, PA	434 ± 75 a	162 ± 25 b	116 ± 31 c	61 ± 15 d
2012				
Lamberton, MN	2,409 ± 1,659 a	559 ± 529 bc	293 ± 102 b	13 ± 6 c
Volga, SD	276 ± 113 a	14 ± 7 c	147 ± 56 b	24 ± 22 c
Ames, IA	51 ± 29 a	25 ± 14 a	1 ± 1 b	1 ± 1 b
Nashua, IA	6 ± 4 a	0 ± 0 b	1 ± 1 ab	0 ± 0 b
Arlington, WI	6 ± 5 a	2 ± 2 a	0 ± 0 a	3 ± 3 a
Scandia, KS	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Rock Springs, PA	152 ± 47 a	61 ± 15 ab	54 ± 21 bc	17 ± 2 c
Wooster, OH	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Prosper, ND	13 ± 3 a	2 ± 1 b	6 ± 3 ab	4 ± 3 b

[a] Cumulative aphid days data from the untreated split-plot by location for each of the four experimental soybean lines.
[b] Different letters represent significant differences among soybean lines within a location at $P < 0.05$.

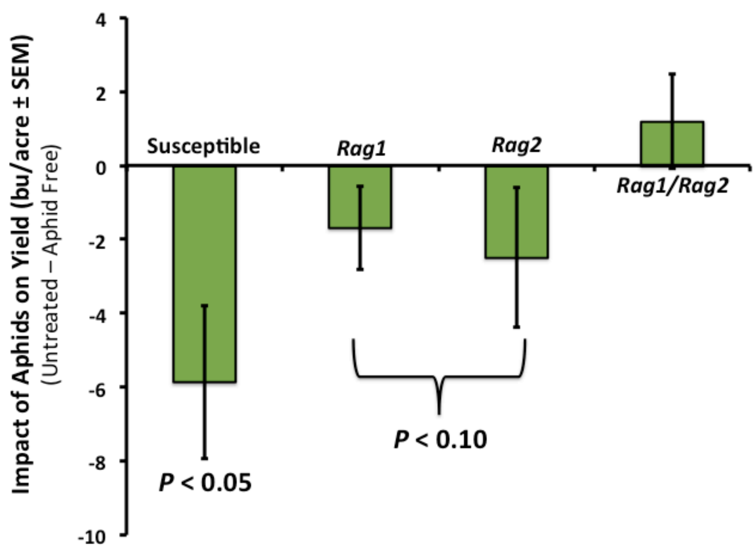

Figure 3. Yield differences among the four soybean lines are depicted from data collected in 2012. Yield data was combined from Minnesota, South Dakota, and Iowa. Soybean aphids reduced yield on the Susceptible line by 5 bu/acre. Yield of the single gene lines, *Rag1* alone and *Rag2* alone, were reduced by approximately 2 bu/acre.

This final result has not been published in a peer-reviewed journal. Field work that contributes to this multi-state project will be completed in 2013 and data analyzed during 2013-2014.

Outputs

These results have been shared with farmers, scientists and agribusiness in several ways. We have published 3 peer-reviewed manuscripts, 5 extension publications and given several presentations on this topic. Also, we share these data and recommendations through our website (www.soybeanaphid.info).

Research Publications:

Wiarda, S.L., W.R. Fehr, and M.E. O'Neal. 2012. Soybean aphid (Hemiptera: Aphididae) development on soybean with *Rag1* alone, *Rag2* alone and both genes combined. J. Econ. Entomol.105: 252-258. DOI*:* http://dx.doi.org/10.1603/EC11020

McCarville, M. T**.,** and M. E. O'Neal. 2012. Measuring the benefit of biological control for single gene and pyramided host plant resistance for soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) management. Journal of Economic Entomology 105: 1835-1843.

McCarville, M. T., and M. E. O'Neal. 2013. Measuring the interaction of two sources of soybean aphid mortality throughout the growing season: host plant resistance and insecticidal seed treatments. Journal of Economic Entomology. *In press.*

Extension Publications:

McCarville, M., E. Hodgson, and M. O'Neal. 2013. Soybean aphid-resistant soybean varieties for Iowa. Iowa State University Extension and Outreach PM 3023.

McCarville, M., E. Hodgson, and M. O'Neal. 2012. New options for soybean aphid host plant resistance. *In* Integrated Crop Management News. 4 December 2012.

McCarville, M., E. Hodgson, and M. O'Neal. 2012. Soybean aphid-resistant soybean varieties for Iowa. Iowa State University Extension and Outreach PM 3023.

McCarville, M.**,** E. Hodgson, and M. O'Neal. 2012. Options for soybean aphid host plant resistance. *In* Integrated Crop Management News. 15 March 2012.

McCarville, M., M. E. O'Neal, and W. R. Fehr. 2011. Assessing the benefits of pyramids and seed treatments for soybean aphid host plant resistance. pp. 67-72. *In* Proceedings: 23rd Annual Iowa State University Integrated Crop Management Conference, Ames, IA. 30 November - 1 December 2011.

Presentations:

1. McCarville, M., M. O'Neal, K. Tilmon, B. Potter, B. McCornack, J. Tooker, E. Cullen, A. Michel, and D. Prischmann-Voldseth. 2012. Is pyramiding resistance the answer for soybean aphid management? Annual Meeting of the Entomological Society of America, Knoxville, TN.
2. McCarville, M., M. O'Neal, K. Tilmon, B. Potter, B. McCornack, E. Cullen, and J.

Tooker. 2012. Are two genes better than one for soybean aphid management? North Central Branch Entomological Society of America Meeting, Lincoln, NE.

3. McCarville, M., M. O'Neal, K. Tilmon, B. Potter, B. McCornack, E. Cullen, and J. Tooker. 2012. Are two genes better than one for soybean aphid management? International Plant Resistance to Insects Workshop, Minneapolis, MN.
4. O'Neal, M. E, and M. McCarville. 2012. Is one enough?: Combining *Rag* genes improves aphid resistance in soybeans. Commercial Soybean Breeders Meeting, St. Louis, MO.
5. McCarville, M., M. O'Neal, and E. Hodgson. 2012. Update on soybean aphid research in Iowa. 2011 S1039 Annual Meeting, Williamsburg, VA.

Finally, funding from this project allowed us to higher 10 undergraduates during the 3 field seasons to help collect data. These data contributed to the M.S. degree of Shaylyn Wiarda (Plant Breeding/Agronomy) and the Ph.D. of Michael McCarville (Entomology). During the course of this project these students were trained in soybean production and pest management. Although it is too soon to tell, previous undergraduate and graduate students who have worked with Dr O'Neal on ISA funded project have continued to work in these fields after graduation.