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Agronomic and seed traits of soybean lines with the Rag1 gene for aphid resistance

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Agronomic and seed traits of soybean lines with the *Rag1* gene for aphid resistance

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CHAPTER 1

INTRODUCTION

The soybean aphid (*Aphis glycines* Matsumura) was first identified in soybean [*Glycine max* (L.) Merrill] in the Midwest during the summer of 2000 (Ragsdale et al., 2004). The aphid quickly spread, and by 2003, 20 states and 3 Canadian provinces reported its presence (Venette and Ragsdale, 2004). The aphid reduces yield by causing stunting, leaf distortion, and reduced pod set (Sun et al., 1990). Soybean aphids also affect yield as vectors for several diseases including the alfalfa mosaic virus and soybean mosaic virus (Hill et al., 2001). Beckendorf et al. (2008) reported a strong negative linear relationship between aphid numbers plant^{-1} and seed yield.

An economic threshold for insecticide treatment of 273 ± 38 aphids per plant for *rag1* cultivars was developed by Ragsdale et al. (2007). Insecticides are currently the most common management tool used by growers in North America for control of the aphid (Olson et al., 2008).

Hill et al. (2004) first reported resistance to the aphid in seven soybean cultivars. Hill et al. (2006) found that a major dominant gene, *Rag1*, in PI 548663 conferred an antibiosis form of resistance to the aphid. This gene has been mapped to linkage group M and simple sequence repeat markers associated with the resistance have been identified including Satt435, Satt463, and Satt540 (Li et al., 2007). This resistance gene provided the opportunity to use host plant resistance as an alternative or supplement to the use of insecticides for management of the insect.

Effective use of the *Rag1* gene for cultivar development would depend on its influence on important agronomic and seed traits. Kim and Diers (2009) examined the

influence of the *Rag1* gene on yield, maturity, plant height, and lodging score in the absence of aphid infestation. They reported no difference in seed yield, plant height, or lodging score; but did find a 2 d difference in maturity between *Rag1* and *rag1* lines. Their study did not report the performance of the lines under aphid-infested conditions. The objective of this study was to compare the agronomic and seed traits of lines with and without the *Rag1* gene that were derived from the same segregating population under aphid-free and aphid-infested conditions.

LITERATURE REVIEW

IMPORTANCE OF APHID RESISTANCE

Increases in the aphid population per plant have a strong negative linear effect on seed yield, pods per plant, seeds per pod, and seed weight (Beckendorf et al., 2008). The soybean aphid reduces photosynthetic ability by sucking phloem sap from leaves and stem tissues, which reduces photosynthesis even at low population levels (Macedo et al., 2003). Aphids are also vectors for several diseases including the alfalfa mosaic virus and soybean mosaic virus (Hill et al., 2001).

The soybean aphid is a heteroecious holocyclic species with various species of buckthorn (*Rhamnus cathartica* L. and *Rhamnus alnifolia* L'Her) as the primary host (Ragsdale et al., 2004). During the growing season, emigration events occur between the primary host and secondary host, soybeans (Ragsdale et al., 2004). Each spring, eggs hatch and reproduce asexually for three generations on buckthorn (Ragsdale et al., 2004). The final generation on buckthorn is winged (alate) and capable of flight (Ragsdale et al., 2004). The alate aphid flies and is carried by the wind to soybean plants (Ragsdale et al., 2004). Once on soybeans, alate aphids reproduce asexually producing apterate, wingless,

aphids, which reproduce asexually within a few days to produce more apterous aphids (Ragsdale et al., 2004). In China, soybean aphids have been observed to have 15 generations on soybeans alone (Lu and Chen, 1993). In autumn, winged females and males are produced on soybeans that fly back to buckthorn and reproduce sexually to produce eggs for overwintering (Ragsdale et al., 2004).

Control of the aphid has been limited to the use of insecticides. In 2003, approximately 1,214,057 hectares of soybeans were sprayed with insecticides to control the aphid in Iowa alone (Rice et al., 2007). Iowa soybean yields suffered a 32% reduction when compared to yields from the previous year (Rice et al., 2007). Pesticide application not only kills aphids, but also destroys beneficial insects and introduces hazardous chemicals into the environment.

GENETIC RESISTANCE TO SOYBEAN APHIDS

An aphid resistance gene, *Rag1*, was found in the cultivar Dowling (PI 548663) (Hill et al., 2006). *Rag1* has a monogenic dominant inheritance (Hill et al., 2006). This inheritance facilitates its integration into elite soybean lines through conventional breeding practices (Hill et al., 2006). This gene has been mapped to linkage group M and simple sequence repeat markers have been developed including Satt435, Satt463, and Satt540 (Li et al., 2007). *Rag1* is expressed constitutively in the plant throughout its life cycle (Hill et al., 2004).

There are three types of resistance; antixenosis, antibiosis, and tolerance (Smith 1989). Tolerance is the plants ability to withstand or recover from damage caused by insect populations equal to those on susceptible cultivars (Smith 1989). Antixenosis is a plants inability to serve as a host to an insect pest that results in forcing a potential pest

insect to select an alternate host plant (Smith 1989). Antibiosis in plants negatively affects insect biology by reducing the quantities of basic metabolites needed for proper insect nutrition or by producing phytochemicals that are toxic to insects (Smith 1989). *Rag1* is known to have a strong form of antibiosis resistance to the soybean aphid (Hill et al., 2004). Unfortunately, *Rag1* is not resistant against all aphid biotypes. Although it confers resistance to the Illinois aphid biotypes, the Ohio aphid biotypes are able to infest plants with this form of resistance (Kim et al., 2008).

The metabolic pathway associated with *Rag1* resistance is not yet known, however, Li et al., (2004) reported that aphids avoided feeding on resistant plants 4 hr after transfer and several aphids died 48 hr after transfer due to the effect of the antibiosis resistance. Li et al., (2008) speculated that hydrogen peroxide may be the compound produced by soybeans to confer resistance by *Rag1*.

CHAPTER 2

Agronomic and seed traits of BC_2F_2-derived soybean lines with the *Rag1* gene for aphid resistance

ABSTRACT

Soybean [*Glycine max* (L.) Merrill] yields can be reduced significantly by infestations of the soybean aphid (*Aphis glycines* Matsumura). A dominant allele, *Rag1*, providing resistance to the aphid was identified in PI 548663. The objective of this study was to compare the agronomic and seed traits of 27 BC_2F_2-derived lines with the *Rag1* allele to those of 27 lines with the *rag1* allele from the same segregating population. The lines were evaluated under aphid-infested conditions at two Iowa environments in 2008 and under aphid-infested and aphid-free conditions at three Iowa environments during 2009. There were significant differences in mean yield between the *Rag1* and *rag1* lines in all the aphid-infested environments. The difference in yield between the two types reached 47.6% at one environment under heavy infestation. Under aphid-free conditions, there was no significant difference in mean yield between the two types. The differences between the two types of lines for maturity, height, lodging, protein concentration, oil concentration, and seed weight were either not significant or sufficiently small to make it possible to develop aphid-resistant cultivars with the *Rag1* gene that were comparable to susceptible cultivars.

MATERIALS AND METHODS

The donor parent used as the source of the *Rag1* allele was the line LD05-16521 developed by the University of Illinois and USDA-ARS. The *Rag1* allele traces to Dowling (PI 548663). LD05-16521 is a BC_3-derived line obtained by backcrossing the *Rag1* allele into the cultivar Loda (Brian Diers, personal communication, 2010). The susceptible cultivar with the *rag1* allele was IA3027 developed by Iowa State University. The cross of IA3027 x LD05-16521 was made at Illinois Crop Improvement Station (ICIA) near Juana Díaz, Puerto Rico, in March 2006. The F_1 seeds and seeds of IA3027 were planted at the Agronomy and Agricultural Engineering Research Center near Ames, IA, during the summer of 2006. Six F_1 plants were confirmed as hybrids by flower color. The F_1 plants were used as the male parents in a backcross to IA3027 and 29 BC_1F_1 seeds were obtained.

The BC_1F_1 seeds and seeds of IA3027 were planted at ICIA in Puerto Rico during October 2006 to obtain BC_2F_1 seed. The BC_1F_1 plants were evaluated with the marker Satt540 by Brian Diers of the University of Illinois to identify plants heterozygous for the *Rag1* allele. Satt540 was used because it was polymorphic between LD05-16521 and IA3027. Crossing was not successful; therefore, the heterozygous plants were harvested individually to obtain BC_1F_2 seeds. The BC_1F_2 seeds and seeds of IA3027 were planted at ICIA in Puerto Rico during January 2007. BC_1F_2 plants were evaluated by the University of Illinois to identify those that were homozygous for the marker Satt540. Five homozygous plants were used as males for backcrossing to IA3027 to obtain BC_2F_1 seed. The homozygous BC_1F_2 plants were harvested individually.

The BC_2F_1 seeds and IA3027 were planted near Ames the summer of 2007. A progeny row of BC_1F_3 seeds also was grown from each of the five homozygous BC_1F_2 plants. Due to adverse conditions during planting, no BC_2F_1 plants survived. To continue the backcrossing program, 16 BC_1F_3 seeds from each of the five homozygous BC_1F_2 plants were planted in the greenhouse in Ames and artificially infested with the aphid. Two of the $BC_1F_{2:3}$ lines were found to exhibit the same level of resistance to the aphid as the donor parent and were used as the males for the backcross to IA3027. The hybrid seeds from the two male $BC_1F_{2:3}$ lines were designated as subpopulations AX21304-1 and AX21304-2. There were 26 BC_2F_1 seeds of AX21304-1 and 36 BC_2F_1 seeds of AX21304-2.

The BC_2F_1 seeds from the two subpopulations were planted at ICIA in Puerto Rico in October 2007. Each BC_2F_1 plant was genotyped by the University of Illinois to identify plants heterozygous for the marker Satt540. All heterozygous plants were harvested individually to obtain BC_2F_2 seed.

The BC_2F_2 seeds from the subpopulations were planted separately at ICIA in Puerto Rico in January 2008. Each plant was genotyped by the University of Illinois with the Satt540 marker to identify plants homozygous for the *Rag1* gene or the *rag1* gene. Based on the marker analysis, 33 of the plants were homozygous for the *Rag1* gene and 57 for the *rag1* gene in AX21304-1 while 29 plants from AX21304-2 were homozygous for *Rag1* and 37 for *rag1*. The homozygous plants were harvested individually to obtain $BC_2F_{2:3}$ lines.

The 90 $BC_2F_{2:3}$ lines from AX21304-1 and 66 from AX21304-2 were planted at the Agronomy Farm and the Burkey Farm near Ames during the summer of 2008. The

soil type at both environments is a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls). Both environments were planted on 21 May. The lines were planted in a randomized complete-block design with one replication at each environment. The plots were one row 0.76 m long and spaced 1.02 m apart with an alley of 1.07 m between the ends of plots. The seeding rate was 15 seeds m^{-1}.

To confirm the genotype of each line for the *Rag1* and *rag1* alleles, 11 leaves from each were bulked and evaluated with the Satt540 marker by the University of Illinois and Iowa State University. Five lines were found to be heterogeneous and were discarded. No insecticide was applied throughout the summer and plants experienced a naturally occurring aphid infestation. The phenotype of each line was scored for aphid abundance on 11 and 18 August at both locations when the aphid infestation was considered to be at its maximum. On those dates, the plants were at the R4 to R5 stage of development (Fehr et al., 1971). The aphid score was a visual rating from 1 (highly resistant) to 10 (highly susceptible) with 5 including the economic threshold level in which an insecticide treatment would have been warranted (Table 1). Aphid scores were rated by estimating the number of aphids present on each plant within a plot and the amount of aphid exoskeletons and sooty mold growth per plant. At low aphid populations of less than 100 per plant, it was possible to count the aphids to differentiate scores from 1 to 3. When the number of aphids exceeded 100 per plant, it was too labor intensive to count them; therefore, a visual estimate of the aphid population was used. A score of 4 was used when the number of aphids exceeded 100 per plant, but was estimated to be below the economic threshold. Scores above the economic threshold were differentiated from each other based on plant damage, sooty mold growth, and

extent of aphid exoskeletons. The time of maturity for each plot was recorded before they were harvested individually with a stationary plot thresher (ALMACO, Nevada, IA). The seed from each plot was weighed and converted to kg ha^{-1}.

To select lines for the 2009 experiment, 27 *Rag1* lines with the lowest aphid scores were matched in maturity to 27 *rag1* lines with the highest aphid scores. The matched pair of lines were from the same subpopulation. Yield was not considered in the selection of the lines. There were 17 pairs of lines selected from AX21304-1 and 10 from AX21304-2.

The two experiments in 2009 consisted of 60 entries, including 27 $BC_2F_{2:4}$ lines homogeneous for *Rag1*, 27 $BC_2F_{2:4}$ lines homogeneous for *rag1*, and two entries each of the parents IA3027 and LD05-16521, and 'IA3045', a *rag1* cultivar developed by Iowa State University that was used to determine the potential of the *Rag1* lines for release as cultivars. In one experiment, the plots were kept aphid-free and in the second experiment the plots were exposed to natural infestation. The two experiments were planted in separate parts of the same fields at Ames, Carlisle, and Rippey, IA. The soil type at Ames is a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls); the soil type at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Agriudolls); and the soil type at Rippey is a Nicollet loam (fine-loamy, mixed, mesic Aquic Hapludolls). Each experiment was planted in a randomized complete-block design with two replications at each environment. The lines were planted in two-row plots 3.05 m long spaced 0.68 m apart within a plot and 0.91 m between adjacent plots. The seeding rate was 30 seeds m^{-1}. Carlisle was planted on 6 May, Ames on 11 May, and Rippey on 21 May.

For the aphid-free experiment, the plots were checked weekly and sprayed with lambda-cyhalothrin (Warrior II®, Syngenta, Wilmington, DE) when the aphid populations reached ~5 aphids per plant. The plots at Ames were sprayed on 30 June, 22 July, and 11 August; plots at Carlisle were sprayed on 14 July and 12 August; and plots at Rippey were sprayed on 22 July and 13 August. For the aphid-infested experiment, the abundance of aphids was scored on 7 August and Carlisle and Rippey on 21 August using the same rating scale as in 2008 (Table 1). Aphid abundance was scored by estimating the number of aphids present on 50 plants within a plot and the amount of aphid exoskeletons and sooty mold growth per plant when the plants were at the R5 stage of development (Fehr et al., 1971).

Each plot was evaluated for yield, maturity, lodging, height, seed weight, protein concentration, and oil concentration. Maturity was recorded as the d after 31 August when 95% of the pods on the main stem had reached their mature color. Lodging was a visual score from 1 (all plants erect) to 5 (all plants prostrate). Plant height was the length in cm from the ground to the terminal node. All plots were harvested with a plot combine (ALMACO, Nevada, IA), and the weight and moisture of the seed were determined. Yields of the plots were adjusted to 130 g kg^{-1} moisture. Protein and oil concentration were determined using an Infratec 1221 near-infrared whole grain analyzer (Tecator AB, Hooganas, Sweden) and adjusted to 130 g kg^{-1} moisture. A sample of ~500 seeds from each plot was used to determine seed weight by counting and weighing the seeds and dividing the weight by the number of seeds.

Table 1. Aphid scoring system used to evaluate phenotypic resistance.

Score	Aphid population and plant description
1	No aphids, plants were normal and healthy.
2	Less the 10 aphids per plant, no colony formation.
3	11 to 100 aphids per plant, plants appeared normal and healthy.
4	101 to 249 aphids per plant, plants appeared normal and healthy.
5	250 to 300 aphids per plant, plants appeared normal and healthy. Score includes the economic threshold level*
6	301-500 aphids per plant, plants appeared healthy.
7	501-800 aphids per plant, leaves slightly curly and shiny, young leaves, and stems covered with aphids.
8	More than 800 aphids per plant, plants stunted, leaves curled, slightly yellow, light sooty mold, and a few exoskeletons.
9	More than 800 aphids per plant, plants stunted, leaves severely curled, yellow, covered with sooty mold and exoskeletons.
10	More than 800 aphids per plant, plants severely stunted, leaves severely curled, yellow-brownish color, covered with sooty mold and exoskeletons, plants dying.

*The economic threshold level is 273 ± 38 aphids per plant; developed for susceptible cultivars (Ragsdale et al., 2007).

STATISTICAL ANALYSIS

For analysis of the data in 2008, the 27 *Rag1* lines and 27 *rag1* lines common to the 2009 experiments were included. The data for IA3027, LD05-16521, and IA3045 were not included in the analyses for either year. The data for both years were analyzed as a randomized complete-block design using the general linear model (GLM) procedure of SAS version 9.1 (SAS Institute, 2003). Environments and replications were considered random effects and the *Rag1* and *rag1* lines were considered a fixed effect. The sums of squares for genotypes were partitioned into *Rag1* lines, *rag1* lines, and the orthogonal contrast between the two types. The mean squares for the genotype × environment interactions were used to test each main effect and orthogonal contrast for significance by an *F*-test for both the2008 and 2009 experiments. The linear additive model used for agronomic and seed trait analyses across all 2009 environments was:

$$Y_{ijk} = \mu + E_i + RP_{(i)j} + G_k + GE_{ik} + \varepsilon_{(ij)k}$$

where,

Y_{ijk} = the observed value at the k^{th} genotype within the j^{th} replication at the i^{th} environment,

μ = the overall mean,

E_i = the effect of the i^{th} environment,

$RP_{(i)j}$ = the effect of the j^{th} replication within the i^{th} environment,

G_k = the effect of the k^{th} genotype,

GE_{ik} = the effect of the interaction between the i^{th} environment and the k^{th} genotype, and

$\varepsilon_{(ij)k}$ = the error of the effect of the ijk^{th} observation.

Table 2. Analysis of variance and expected mean squares for each experiment across three environments in 2009.

Sources of Variation	Degrees of Freedom	Expected Mean Squares
Environments (E)	(e-1)	$\sigma^2_\varepsilon + g\sigma^2_{RP} + rg\sigma^2_E$
Replications (RP)	e(rp-1)	$\sigma^2_\varepsilon + g\sigma^2_{RP}$
Genotype (G)	(g-1)	$\sigma^2_\varepsilon + r\sigma^2_{G \times E} + er\Phi_R$
Resistant (R)	(r-1)	$\sigma^2_\varepsilon + r\sigma^2_{R \times E} + er\Phi_R$
Susceptible (S)	(s-1)	$\sigma^2_\varepsilon + r\sigma^2_{S \times E} + er\Phi_S$
R vs. S	1	$\sigma^2_\varepsilon + r\sigma^2_{(R \text{ vs. } S) \times E} + er\Phi_{R \text{ vs. } S}$
G x E	(g-1)(e-1)	$\sigma^2_\varepsilon + r\sigma^2_{G \times E}$
R x E	(r-1)(e-1)	$\sigma^2_\varepsilon + r\sigma^2_{R \times E}$
S x E	(s-1)(e-1)	$\sigma^2_\varepsilon + r\sigma^2_{S \times E}$
R vs. S x E	1(e-1)	$\sigma^2_\varepsilon + r\sigma^2_{(R \text{ vs. } S) \times E}$
Error	e(rp-1)(g-1)	σ^2_ε
Total	erpg-1	

The linear additive model for the analysis of variance for agronomic and seed trait analyses at an individual environment in 2008 and 2009 was:

$$Y_{ij} = \mu + RP_i + G_j + \varepsilon_{ij}$$

where,

Y_{ij} = the observed value of the j^{th} genotype within the i^{th} replication,

μ = the overall mean,

RP_i = the effect of the i^{th} replication,

G_j = the effect of the j^{th} genotype, and

ε_{ij} = the error of the effect of ij^{th} observation.

Table 3. Analysis of variance and expected mean squares for each experiment at an individual environment in 2009.

Sources of Variation	Degrees of Freedom	Expected Mean Squares
Replications (RP)	(rp-1)	$\sigma^2_\varepsilon + g\sigma^2_{RP}$
Genotype (G)	(g-1)	$\sigma^2_\varepsilon + r\Phi_G$
Resistant (R)	(r-1)	$\sigma^2_\varepsilon + r\Phi_R$
Susceptible (S)	(s-1)	$\sigma^2_\varepsilon + r\Phi_S$
R vs. S	1	$\sigma^2_\varepsilon + r\Phi_{R\ vs.\ S}$
Error	(rp-1)(g-1)	σ^2_ε
Total	rpg-1	

The standard error of the mean (SEM), coefficient of variance (CV), and least significant difference (LSD) at the 0.01 and 0.05 probability levels were calculated as:

$$\text{SEM} = \sqrt{\frac{MSE}{n}}$$

$$\text{CV (\%)} = \frac{\sqrt{MSE}}{\bar{x}} \times 100$$

$$\text{LSD} = t_\alpha \sqrt{\frac{2MSE}{n}}$$

where,

MSE = the error mean square for an individual environment or genotype x environment interaction for the combined analysis,

$\bar{x}$ = the mean of all entries for a trait,

n = the number of observations in each entry mean, and

t = the critical t value at either the 0.01 or 0.05 probability level.

RESULTS AND DISCUSSIONS

The mean yields of the *Rag1* lines in the aphid-free experiment were not significantly different from the *rag1* lines at Ames and Rippey or combined across environments (Table 4). The *Rag1* lines had a significantly higher mean yield than the *rag1* lines at Carlisle. The range in yield among the two types of lines were similar at the individual environments and combined across environments. These results indicated that the *Rag1* gene did not influence the yield of lines in absence of the soybean aphid. Our results confirm those of Kim and Diers (2009) who found no significant difference for yield between *Rag1* and *rag1* lines in the absence of the aphid.

The lines were exposed to natural infestations of the aphid in 2008 and in 2009. In 2008, the mean aphid scores exceeded the economic threshold for *rag1* lines at both environments and *Rag1* lines at the Agronomy Farm. The mean scores were 5.7 for the *Rag1* lines and 8.4 for the *rag1* lines at the Agronomy Farm and 3.2 for the *Rag1* lines and 6.9 for the *rag1* lines at the Burkey Farm. The mean yield of 1241 kg ha^{-1} for *Rag1* lines was 47.6% greater than the 841 kg ha^{-1} for *rag1* lines at the Agronomy Farm and the 2351 kg ha^{-1} for the *Rag1* lines was 24.5% greater than the 1888 kg ha^{-1} for the *rag1* lines at the Burkey Farm. . The orthogonal contrasts between the means of the *Rag1* and *rag1* lines for yield and aphid score were significant at the 0.01 probability level.

The mean yield of the *Rag1* lines in the 2009 aphid-infested experiment was significantly greater than the *rag1* lines (Table 5). The mean aphid infestation ratings at Ames and Rippey for the *rag1* lines were above the economic threshold level of 5.0, but not at Carlisle. The mean rating for the *Rag1* lines did not reach the economic threshold at any of the three environments. The mean yield of the *Rag1* lines was 6.2% greater

than the mean of the *rag1* lines at Ames, 8.8% at Carlisle, and 6.7% at Rippey. These results indicated that the *Rag1* gene has a favorable impact on yield over a range of aphid infestation levels.

The difference in mean maturity between the two types of lines was about 1 d in both experiments (Table 4). This result was expected because similarity in maturity of the two types was a key factor in selecting the lines for the experiment. The difference in the mean height of the *Rag1* and *rag1* lines was 2 cm under aphid-free conditions and 1 cm in the presence of aphid infestation. The differences in lodging scores between the two types were not significant in either experiment. Our results were similar to those of Kim and Diers (2009) who reported no significant differences in mean plant height or lodging between *Rag1* and *rag1* lines.

The mean protein concentrations of the *Rag1* and *rag1* lines differed by 4 g kg^{-1} and the mean oil concentrations differed by a maximum of 1 g kg^{-1} in the two experiments (Table 4). Mean seed weight differed by 3 mg $seed^{-1}$. The ranges among lines for the two types were similar for the three seed traits, which indicated that it would be possible to develop *Rag1* cultivars with protein concentration, oil concentration, and seed weight comparable to that of *rag1* cultivars.

In summary, the *Rag1* gene had no influence on the yield of lines under aphid-free conditions and had a positive impact on yield when there was aphid infestation. The overlap in the distributions of *Rag1* and *rag1* lines for the other agronomic and seed traits evaluated indicated that it should be possible to develop *Rag1* cultivars comparable to those with the *rag1* gene.

Table 4. Mean and range for agronomic and seed traits of 27 *Rag1* and 27 *rag1* lines grown in two experiments across three Iowa environments in 2009.

Trait	Type	Aphid-free		Aphid-infested	
		Mean	Range	Mean	Range
Yield	*Rag1*	3893	3646-4165 ns†	3801	3458-4125*
(kg ha^{-1})	*rag1*	3860ns	3505-4171**	3543**	3097-3827ns
Maturity	*Rag1*	23.6	22-26**	23.9	22-26**
(d‡)	*rag1*	22.9**	21-25**	22.8**	21-26**
Height	*Rag1*	79	71-84*	78	73-85**
(cm)	*rag1*	81**	73-88**	79ns	71-86**
Lodging	*Rag1*	1.7	1.4-2.1**	1.7	1.5-1.9ns
(score§)	*rag1*	1.7ns	1.4-2.1**	1.8ns	1.5-2.2**
Protein	*Rag1*	364	355-375**	363	355-373**
(g kg^{-1}¶)	*rag1*	368**	362-378**	367**	357-374**
Oil	*Rag1*	171	165-177**	171	163-176**
(g kg^{-1}¶)	*rag1*	170**	161-176**	171ns	164-177**
Seed weight	*Rag1*	243	224-257**	238	218-253**
(mg seed^{-1})	*rag1*	246**	205-268**	235*	197-261**
Aphid	*Rag1*	-	-	3.2	2.5-3.8ns
(score#)	*rag1*	-	-	6.0**	4.5-7.0**

*Significant difference at the 0.05 probability level between the means of the two types or among lines within a type.
**Significant difference at the 0.01 probability level between the means of the two types or among lines within a type.
†ns, difference between the means of the two types or among lines within a type were not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).
¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.
#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table 5. Yield and aphid score of 27 *Rag1* and 27 *rag1* lines grown in two experiments at three Iowa environments in 2009.

Trait	Type	Environments					
		Ames		Carlisle		Rippey	
		Mean	Range	Mean	Range	Mean	Range
				Aphid-free			
Yield	*Rag1*	4060	3613-4465ns†	4454	3983-4742*	3163	2814-3665ns
(kg ha^{-1})	*rag1*	4077ns	3661-4448ns	4358*	3824-4639*	3144ns	2540-3654ns
				Aphid-infested			
Yield	*Rag1*	4074	3638-4369ns	4266	3933-4564*	3063	2562-3548ns
(kg ha^{-1})	*rag1*	3835**	3026-4241ns	3921**	3436-4246ns	2872**	2422-3357ns
Aphid	*Rag1*	4.4	3.0-6.0ns	2.3	2.0-3.5ns	2.9	2.0-4.5ns
(score#)	*rag1*	6.6**	4.5-8.5ns	4.4**	3.0-5.5ns	6.9**	4.5-8.0ns

*Significant difference at the 0.05 probability level between the means of the two types or among lines within a type.
**Significant difference at the 0.01 probability level between the means of the two types or among lines within a type.
†ns, difference between the means of the two types or among lines within a type were not significant at the 0.05 probability level.
#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

CHAPTER 3

Agronomic and seed traits of a bulk of BC_3F_2-derived lines with the *Rag1* gene compared with their recurrent parent IA3027

ABSTRACT

Soybean [*Glycine max* (L.) Merrill] yields can be reduced significantly by infestations of the soybean aphid (*Aphis glycines* Matsumura). A dominant allele, *Rag1*, for resistance to the aphid was identified in PI 548663. The objective of this study was to compare the agronomic and seed traits of a bulk of 29 $BC_3F_{2:4}$ lines (*Rag1* bulk) with the recurrent parent IA3027. The two genotypes were evaluated under aphid-free and aphid-infested conditions at three Iowa environments in 2009. The aphid infestation at the three environments was less than the economic threshold for both genotypes. There was no significant difference in yield, plant height, or lodging between the two genotypes at the individual environments or combined across environments. The maturity of IA3027 was significantly earlier than the *Rag1* bulk by 1 d. The results indicated that the *Rag1* bulk could be grown commercially in place of IA3027 without any negative effect on agronomic or seed traits.

MATERIALS AND METHODS

The $BC_3F_{2:4}$ lines included in the *Rag1* bulk were developed through the BC_2 by the procedure described in Chapter 2. In October 2007, BC_2F_1 plants from the two subpopulations AX21304-1 and AX21304-2 and plants of IA3027 were grown at the Illinois Crop Improvement Station (ICIA) near Juana Diaz, Puerto Rico. Each BC_2F_1 plant was genotyped by the University of Illinois to identify plants heterozygous for the marker Satt540, which indicated they had the genotype *Rag1 rag1*. Heterozygous plants were used as the male in the backcross to IA3027 to obtain BC_3F_1 seed. The BC_3F_1 seed produced from AX21304-1 was designated AX21476-1 and that from AX21304-2 was designated AX21476-2.

The BC_3F_1 seeds from both subpopulations were planted at ICIA in Puerto Rico during January 2008. Each BC_3F_1 plant was genotyped by the University of Illinois to identify heterozygous plants using the marker Satt540. All heterozygous BC_3F_1 plants were harvested individually. The harvested plants were designated AX21476-1-3 and AX21476-2-1, -6, -10, -13, -14.

All BC_3F_2 seeds from each BC_3F_1 plant were planted in a progeny row at the Agronomy Farm near Ames, IA, during June 2008. Each BC_3F_2 plant was genotyped by the University of Illinois and Iowa State University using the Satt540 marker to identify all homozygous *Rag1* plants. During the summer of 2008, a natural aphid infestation occurred. Before harvest, each plant was rated twice for resistance to the aphid using the same rating scale as in Table 1 of Chapter 2. The ratings took place on 11 and 18 August. Plants with an aphid score of 4.5 or less and similar maturity to IA3027 were harvested individually to obtain $BC_3F_{2:3}$ seed.

There were 32 BC_3F_2 plants with enough seed for planting in a seed increase at 3rd Millennium Genetics at Santa Isabela, Puerto Rico during October 2008. IA3027, LD05-16521, and IA2053, a high yielding cultivar developed by Iowa State University, also were planted. Three lines originated from AX21476-1 and 29 lines from AX21476-2. Each line was grown in four rows each of which was 3.81 m long and spaced 0.76 m apart with a seeding rate of 26 seeds m^{-1}. The individual plots were threshed with a stationary plot thresher (Swanson, IL) to obtain $BC_3F_{2:4}$ seed. Protein and oil concentration were determined after harvest with an Infratec 1221 near-infrared whole grain analyzer (Tecator AB, Hooganas, Sweden) and adjusted to 130 g kg^{-1} moisture. One line from AX21476-1 and one line from AX21476-2 were discarded due to lower protein concentration than IA3027.

To evaluate the phenotypic resistance of the *Rag1* lines, 10 seeds from each were planted in the greenhouse of the Department of Entomology in Ames, IA, in February 2009. Each plant was infested with 5 aphids at V1 stage of development (Fehr et al., 1971). A week after infestation, the aphid population on each plant was recorded and compared with IA3027. One *Rag1* line was found to have similar aphid populations to IA3027 and was discarded. A similar quantity of seed from the remaining 29 $BC_3F_{2:4}$ lines were bulked for the experiment.

The 2009 experiment originally was designed to develop an economic threshold for *Rag1* cultivars. The experiment consisted of five entries in a split-plot arrangement of a randomized complete-block design (Table 1). There were four whole plots of the *Rag1* bulk and a whole plot of IA3027. Each whole plot consisted of an aphid-free and aphid-infested subplot, which resulted in a total of 10 entries. There were three treatment levels

included as whole plots for the *Rag1* bulk; 8,000 (8k) cumulative aphid days (CAD), 16,000 (16k) CAD, and 32,000 (32k) CAD. There also was a whole plot for the *Rag1* bulk in which the aphid population was to be allowed to increase beyond 32k without spraying (Untreated). Cumulative abundance of the aphid was expressed as CAD using the formula:

$$\text{CAD} = \sum_{i=1}^{n} [(x_i + x_{i-1})/2]*t_i$$

where, n is the number of sample dates; x, is the number of aphids per 10 plants on sample date i; and t, is the number of days since the previous sample. The original plan was to begin spraying as the subplots reached the designated CAD. The only whole plot for IA3027 was one in which one of the two subplots would never be sprayed. The aphids on both IA3027 subplots were never counted; therefore, no CAD was determined for these plots. The experiment was planted in four replications at three environments; Ames, Carlisle, and Rippey, IA. The soil type at Ames is a Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludolls); the soil type at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Agriudolls), and the soil type at Rippey is a Nicollet loam (fine-loamy, mixed, mesic Aquic Hapludolls). Each subplot consisted of four rows 7.32 m long spaced 0.68 m apart within a plot and 1.83 m between adjacent plots. The seeding rate was 30 seeds m^{-1}. Carlisle was planted on 6 May, Ames on 11 May, and Rippey on 21 May.

The aphid populations within subplots were determined by counting the number of aphids on 10 plants in the outside rows of the four row plot once a week. The aphid-free subplots were sprayed as necessary when aphid populations exceeded 1 aphid per

plant. A CAD score was still calculated for these plots to ensure they were really aphid free. The insecticide lambda-cyhalothrin (Warrior II®, Syngenta, Golden Valley, MN) was used throughout the summer as needed (Table 2). None of the aphid-infested subplots ever reached 8k CAD, which meant that original goal of the experiment could not be reached. However, the 10 entries were useful for comparing the *Rag1* bulk with IA3027 for agronomic traits.

Each plot was evaluated for yield, maturity, height, and lodging. All plots were harvested with a plot combine (ALMACO, Nevada, IA), and the weight and moisture of the seed were determined. Yields of the plots were adjusted to 130 g kg^{-1} moisture. Maturity was recorded as the days after 31 August when 95% of the pods on the main stem had reached their mature color. Lodging was a visual score from 1 (all plants erect) to 5 (all plants prostrate). Plant height was the length in cm from the ground to the terminal node.

Table 1. Aphid infestation expressed as cumulative aphid days (CAD) for 10 entries grown across three Iowa environments in 2009.

Entry	Type	Treatment	Aphids/ plant‡	Highest CAD§	Mean CAD¶
755001-8k	*Rag1* bulk	8,000 CAD	37	312	117
755001-8k Free	*Rag1* bulk	Aphid-free	13	112	51
755002-16k	*Rag1* bulk	16,000 CAD	798	6573	653
755002-16k Free	*Rag1* bulk	Aphid-free	19	149	63
755003-32k	*Rag1* bulk	32,000 CAD	150	813	312
755003-32k Free	*Rag1* bulk	Aphid-free	46	901	215
755004-Untreated	*Rag1* bulk	Unlimited CAD	281	2259	542
755004-Untreated Free	*Rag1* bulk	Aphid-free	367	3721	351
755006-Untreated	IA3027	Unlimited CAD	-	-	-
755006-Untreated Free	IA3027	Aphid-free	-	-	-

‡Highest average number of aphids per plant observed of any subplot of an entry at any of the three environments.
§Highest CAD of any subplot of an entry at any of the three environments.
¶Mean CAD of all subplots of an entry across all environments.

Table 2. Insecticide application for 10 entries grown at three Iowa environments in 2009.

			Environment		
Entry	Type	Date	Ames	Carlisle	Rippey
755006-Untreated Free	IA3027	6/26/2009	X		
755006-Untreated Free	IA3027	7/13/2009	X		
755006-Untreated Free	IA3027	7/16/2009		X	
755001-8k Free	*Rag1* bulk	7/22/2009	X		
755002-16k Free	*Rag1* bulk	7/22/2009	X		
755003-32k Free	*Rag1* bulk	7/22/2009	X		
755004-Untreated Free	*Rag1* bulk	7/22/2009	X		
755006-Untreated Free	IA3027	7/22/2009	X		
755006-Untreated Free	IA3027	7/23/2009			X
755001-8k Free	*Rag1* bulk	7/29/2009	X		X
755002-16k Free	*Rag1* bulk	7/29/2009	X		X
755003-32k Free	*Rag1* bulk	7/29/2009	X		X
755004-Untreated Free	*Rag1* bulk	7/29/2009	X		X
755001-8k Free	*Rag1* bulk	8/11/2009		X	X
755002-16k Free	*Rag1* bulk	8/11/2009		X	X
755003-32k Free	*Rag1* bulk	8/11/2009		X	X
755004-Untreated Free	*Rag1* bulk	8/11/2009		X	X
755006-Untreated Free	IA3027	8/11/2009	X	X	X

STATISTICAL ANALYSIS

For analysis of the data, the 10 entries were analyzed as a randomized complete-block design using the general linear model (GLM) procedure of SAS version 9.1 (SAS Institute, 2003). Environments and replications were considered random effects and entries were considered a fixed effect. The mean squares for the genotypes × environment interactions were used to test each main effect for significance by an *F*-test. The linear additive model used for agronomic and seed trait analyses across all environments was:

$$Y_{ijk} = \mu + E_i + RP_{(i)j} + EN_k + EN*E_{ik} + \varepsilon_{(ij)k}$$

where,

Y_{ijk} = the observed value at the k^{th} genotype within the j^{th} replication at the i^{th} environment,

μ = the overall mean,

E_i = the effect of the i^{th} environment,

$RP_{(i)j}$ = the effect of the j^{th} replication within the i^{th} environment,

EN_k = the effect of the k^{th} entry,

$EN*E_{ik}$ = the effect of the interaction between the i^{th} environment and the k^{th} entry, and

$\varepsilon_{(ij)k}$ = the error of the effect of the ijk^{th} observation.

Table 3. Analysis of variance and expected mean squares for 10 entries grown across three environments.

Sources of Variation	Degrees of Freedom	Expected Mean Squares
Environments (E)	(e-1)	$\sigma^2_\varepsilon + en\sigma^2_{RP} + rg\sigma^2_E$
Replications/E (RP)	e(rp-1)	$\sigma^2_\varepsilon + en\sigma^2_{RP}$
Entries (EN)	(en-1)	$\sigma^2_\varepsilon + r\sigma^2_{EN\ x\ E} + er\Phi_{EN}$
EN x E	(en-1)(e-1)	$\sigma^2_\varepsilon + r\sigma^2_{EN\ x\ E}$
Error	e(rp-1)(en-1)	σ^2_ε
Total	erpen-1	

The linear additive model for the analysis of variance for agronomic and seed trait analyses at an individual environment was:

$$Y_{ij} = \mu + RP_i + EN_j + \varepsilon_{ij}$$

where,

Y_{ij} = the observed value of the j^{th} entry within the i^{th} replication,

μ = the overall mean,

RP_i = the effect of the i^{th} replication,

EN_j = the effect of the j^{th} entry, and

ε_{ij} = the error of the effect of the ij^{th} observation.

Table 4. Analysis of variance and expected mean squares of 10 entries grown at an individual environment.

Sources of Variation	Degrees of Freedom	Expected Mean Squares
Replications (RP)	(rp-1)	$\sigma^2_\varepsilon + en\sigma^2_{RP}$
Entries (EN)	(en-1)	$\sigma^2_\varepsilon + r\Phi_{EN}$
Error	(rp-1)(en-1)	σ^2_ε
Total	rpen-1	

The standard error of the mean (SEM), coefficient of variance (CV), and least significant difference (LSD) at the 0.01 and 0.05 probability levels were calculated as described in Chapter 2.

RESULTS AND DISSCUSSION

Due to the low aphid pressure at every environment, all plots were considered to be aphid-free. The mean yields of the eight entries of the *Rag1* bulk and two entries of IA3027 were not significantly different at the individual environments or combined across all environments (Table 5). These results indicated that the *Rag1* allele had no negative affect on seed yield, the same conclusion made from the results presented in Chapter 2. The results also indicated that the *Rag1* bulk of BC_3F_2-derived lines could substitute for IA3027 in commercial production without any yield loss when there was limited aphid infestation.

The mean height and lodging of the entries were not significantly different across all environments or at any individual environment (Table 6 and 7). However, the mean maturities of two of the ten entries were significantly different by 1 d from the other entries when averaged across all environments (Table 8). No significant differences among the 10 entries were found for maturity at any individual environment. These results were similar to those of Kim and Diers (2009) who found a 2 d later maturity for *Rag1* lines. The results indicated that it should be possible to develop *Rag1* cultivars comparable to those with the *rag1* gene for maturity, height, and lodging, which agreed with the results of the study reported in Chapter 2.

In 2009, the 29 BC_3F_2-derived lines in this experiment were evaluated individually at five environments in Iowa for yield, maturity, lodging, height, seed

weight, protein concentration, and oil concentration in comparison with IA3027. No insecticide was applied to this experiment throughout the summer. The same lines were individually grown in a seed increase at Ames. Based on the results of the test, 18 of the lines were considered equivalent to IA3027 for all of the traits. Seed of the 18 lines was bulked to form the cultivar IA3027RA1, which was released to interested growers in November 2009.

Table 5. Mean yield of 10 entries grown at three Iowa environments in 2009.

		Environments			
		Ames ns†	Carlisle ns	Rippey ns	Combined ns
Entry	Type	Yield (kg ha^{-1})	Yield (kg ha^{-1})	Yield (kg ha^{-1})	Yield (kg ha^{-1})
755001-8k	*Rag1* bulk	4135	3960	3808	3968
755001-8k Free	*Rag1* bulk	4069	4300	3515	3961
755002-16k	*Rag1* bulk	4170	4225	3527	3974
755002-16k Free	*Rag1* bulk	4306	4341	3846	4164
755003-32k	*Rag1* bulk	4207	4340	3597	4048
755003-32k Free	*Rag1* bulk	4137	4406	3555	4033
755004-Untreated	*Rag1* bulk	4223	4374	3499	4032
755004-Untreated Free	*Rag1* bulk	4046	4562	3679	4096
755006-Untreated	IA3027	3944	4309	3595	3949
755006-Untreated Free	IA3027	4058	4508	3932	4166

†ns, differences among the means of the 10 entries were not significant at the 0.05 probability level.

Table 6. Mean height of 10 entries grown at three Iowa environments in 2009.

		Environments			
		Ames ns†	Carlisle ns	Rippey ns	Combined ns
Entry	Type	Height (cm)	Height (cm)	Height (cm)	Height (cm)
755001-8k	*Rag1* bulk	85	70	73	76
755001-8k Free	*Rag1* bulk	88	72	73	77
755002-16k	*Rag1* bulk	86	68	72	75
755002-16k Free	*Rag1* bulk	90	68	76	78
755003-32k	*Rag1* bulk	96	73	71	80
755003-32k Free	*Rag1* bulk	90	75	72	79
755004-Untreated	*Rag1* bulk	89	71	72	77
755004-Untreated Free	*Rag1* bulk	87	71	74	77
755006-Untreated	IA3027	87	77	69	77
755006-Untreated Free	IA3027	89	74	73	78

†ns, differences among the means of the 10 entries were not significant at the 0.05 probability level.

Table 7. Mean lodging of 10 entries grown at three Iowa environments in 2009.

		Environments			
		Ames ns†	Carlisle ns	Rippey ns	Combined ns
Entry	Type	Lodging (score‡)	Lodging (score‡)	Lodging (score‡)	Lodging (score‡)
755001-8k	*Rag1* bulk	1.3	1.8	1.3	1.4
755001-8k Free	*Rag1* bulk	1.4	1.8	1.3	1.5
755002-16k	*Rag1* bulk	1.5	1.8	1.1	1.5
755002-16k Free	*Rag1* bulk	1.4	1.8	1.3	1.5
755003-32k	*Rag1* bulk	1.6	1.9	1.1	1.5
755003-32k Free	*Rag1* bulk	1.5	1.9	1.1	1.5
755004-Untreated	*Rag1* bulk	1.3	1.9	1.0	1.4
755004-Untreated Free	*Rag1* bulk	1.3	1.9	1.3	1.5
755006-Untreated	IA3027	1.3	2.4	1.0	1.5
755006-Untreated Free	IA3027	1.5	2.3	1.1	1.6

†ns, differences among the means of the 10 entries were not significant at the 0.05 probability level.
‡Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table 8. Mean maturity of 10 entries grown at three Iowa environments in 2009.

		Environments			
		Ames ns†	Carlisle ns	Rippey ns	Combined*
Entry	Type	Maturity (d‡)	Maturity (d‡)	Maturity (d‡)	Maturity (d‡)
755001-8k	*Rag1* bulk	20.5	25.0	28.5	24.7a
755001-8k Free	*Rag1* bulk	20.3	25.3	28.8	24.8a
755002-16k	*Rag1* bulk	20.5	24.8	28.5	24.6a
755002-16k Free	*Rag1* bulk	20.5	24.5	29.3	24.8a
755003-32k	*Rag1* bulk	20.8	24.8	29.3	24.9a
755003-32k Free	*Rag1* bulk	20.8	24.3	29.3	24.8a
755004-Untreated	*Rag1* bulk	20.3	23.8	28.8	24.3ab
755004-Untreated Free	*Rag1* bulk	20.3	23.5	29.5	24.4a
755006-Untreated	IA3027	20.0	22.5	26.3	22.9b
755006-Untreated Free	IA3027	20.5	23.0	27.3	23.6ab
				LSD 0.05	1.0
				LSD 0.01	1.4

*Entries followed by the same letter were not significantly different at the 0.05 probability level based on the 0.05 least significant difference.

†ns, differences among the means of the 10 entries were not significant at the 0.05 probability level.

‡Days after 31 August.

CHAPTER 4

GENERAL CONCLUSIONS

The *Rag1* gene had no affect on yield under aphid-free conditions and had a positive affect under aphid-infested conditions. The maximum difference in the mean yield between the *Rag1* and *rag1* lines was 32.2% at one environment with a heavy aphid infestation. The overlap in the range among *Rag1* and *rag1* lines for maturity, lodging, height, seed weight, protein concentration, and oil concentration indicated that it should be possible to develop *Rag1* cultivars with similar characteristics to those with the *rag1* gene.

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APPENDIX A

ANALYSES OF VARIANCE AND ENTRY MEANS FOR SEED TRAITS ACROSS ENVIRONMENTS

Table A1. Analyses of variance for the aphid-free experiment across three Iowa environments in 2009.

		Mean Squares						
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})
Environments (Env)	2	45350361.4**	946.3**	6366.0**	29.8**	223.8**	3613.4**	3168.6**
Replications /Env	3	783842.6**	1.9*	133.3**	0.5**	210.8**	42.2**	72.9ns†
Genotypes	53	138620.3**	6.0**	65.9**	0.2**	139.7**	56.6**	617.3**
Rag1	26	105725.5ns	4.9**	55.8*	0.2**	150.5**	56.8**	507.1**
rag1	26	173537.1**	5.5**	65.4**	0.2**	83.7**	55.5**	720.7**
Rag1 vs. *rag1*	1	86044.7ns	48.5**	339.2**	0.1ns	1312.0**	80.0**	794.2**
Genotypes x Env	106	75937.4ns	0.8ns	25.6ns	0.1ns	25.6ns	9.9ns	33.6ns
Rag1 x Env	52	83980.3ns	0.7ns	27.0ns	0.1ns	30.6**	10.2ns	36.5ns
rag1 x Env	52	67379.8ns	0.9ns	23.0ns	0.1*	21.0ns	9.2ns	30.8ns
Rag1 vs. *rag1* x Env	2	89318.1ns	1.4ns	56.3ns	0.1ns	13.4ns	20.5ns	28.9ns
Error	159	69920.4	0.7	27.8	0.1	19.4	9.2	38.3
CV (%)		6.8	3.6	6.6	14.1	1.2	1.8	2.5

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
†ns, not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table A2. Analyses of variance for the aphid-infested experiment across three Iowa environments in 2009.

		Mean Squares							
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})	Aphid (score¶)
Environments (Env)	2	40731970.5**	1066.9**	7412.5**	35.7**	210.8**	3514.1**	1145.7**	140.1**
Replications /Env	3	795772.4**	7.8**	176.1**	0.3**	52.3*	30.1*	754.9**	2.2ns†
Genotypes	53	236122.7**	7.3**	76.6**	0.1**	117.3**	50.0**	652.2**	13.4**
Rag1	26	125510.5**	5.9**	78.9**	0.1**	109.3**	55.4**	521.8**	0.9*
rag1	26	147663.6**	5.7**	75.3**	0.2**	90.8**	45.6**	785.7**	2.2**
Rag1 vs. *rag1*	1	5411975.1**	89.2**	52.2ns	0.3ns	1013.4**	24.4ns	570.2*	627.8**
Genotypes x Env	106	78134.5ns	1.0ns	21.9ns	0.1ns	23.6*	11.4*	106.1ns	1.5ns
Rag1 x Env	52	64887.8ns	0.8ns	18.1ns	0.1ns	19.3ns	10.3ns	120.7ns	0.8ns
rag1 x Env	52	87962.3ns	1.0ns	22.7ns	0.1ns	25.7*	12.6ns	55.4ns	1.0ns
Rag1 vs. *Rag1* x Env	2	167025.2ns	3.5*	98.8**	0.1ns	83.9**	6.0ns	1046.1**	30.9**
Error	159	71453.5	0.7	20.5	0.1	17.0	8.2	94.6	1.3
CV (%)		7.3	3.7	5.7	15.8	1.1	1.7	4.1	24.5

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table A3. Analyses of variance for the aphid-infested experiment in 2008.

		Mean Squares	
Sources of variation	df	Yield (kg ha^{-1})	Aphid (score‡)
Environments	1	31417768.6**	110.0**
Genotypes	53	242671.9**	5.5**
Rag1	26	231252.8ns†	0.5ns
rag1	26	70341.7ns	0.3ns
Rag1 vs. *rag1*	1	5020152.7**	270.8**
Error	53	91335.3	0.7
CV (%)		19.1	13.5

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table A4. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-free experiment across three Iowa environments in 2009.

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852001	*rag1*	3810	23	76	1.4	363	172	250
852002	*rag1*	3844	23	83	1.7	368	174	247
852003	*rag1*	4171	23	88	2.2	368	170	253
852004	*rag1*	3795	23	77	1.8	365	176	259
852005	*rag1*	4030	25	83	1.8	373	166	240
852006	*rag1*	3663	21	77	1.4	367	176	250
852007	*rag1*	4035	23	84	2.0	367	169	252
852008	*rag1*	3613	22	74	1.4	372	169	248
852009	*rag1*	4164	24	81	2.0	366	171	232
852010	*rag1*	4001	23	87	2.0	367	169	253
852011	*rag1*	3903	23	81	1.8	367	170	248
852012	*rag1*	3967	24	83	2.0	369	169	249
852013	*rag1*	3886	23	82	2.0	370	170	251
852014	*rag1*	3509	21	78	1.7	371	173	233
852015	*rag1*	3943	23	85	2.0	369	169	251
852016	*rag1*	3908	25	81	1.8	366	172	240
852017	*rag1*	3986	22	81	1.8	370	169	251
852018	*rag1*	3767	23	81	1.5	370	171	240
852019	*rag1*	3749	22	77	1.8	369	170	243
852020	*rag1*	3505	22	82	1.6	363	172	206
852021	*rag1*	3933	22	82	1.7	366	169	243

Table A4. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}
852022	*rag1*	3899	25	78	1.8	365	170	249
852023	*rag1*	3789	22	81	1.6	373	170	248
852024	*rag1*	3729	22	81	1.8	375	166	251
852025	*rag1*	3999	23	84	1.8	378	161	250
852026	*rag1*	3725	22	80	1.7	364	171	248
852027	*rag1*	3898	23	80	1.7	364	169	268
852028	*Rag1*	3919	24	79	1.7	375	165	248
852029	*Rag1*	3888	23	81	1.8	367	175	243
852030	*Rag1*	3646	23	78	1.8	367	170	254
852031	*Rag1*	3857	23	77	1.8	364	175	238
852032	*Rag1*	3983	24	80	2.0	365	168	246
852033	*Rag1*	3891	23	76	1.6	366	168	228
852034	*Rag1*	3826	23	78	1.8	369	172	230
852035	*Rag1*	3931	24	76	1.5	363	174	252
852036	*Rag1*	4019	23	79	1.8	363	173	246
852037	*Rag1*	3816	24	80	1.7	363	172	228
852038	*Rag1*	4041	24	84	1.7	369	170	246
852039	*Rag1*	3833	24	74	1.5	356	173	241
852040	*Rag1*	4166	24	78	1.9	369	172	241
852041	*Rag1*	3836	22	71	1.6	362	177	224
852042	*Rag1*	3837	25	80	1.6	364	169	257

Table A4. Continued

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852043	*Rag1*	4113	26	80	1.8	357	170	255
852044	*Rag1*	3721	23	79	1.8	365	169	234
852045	*Rag1*	4036	23	82	2.1	359	170	255
852046	*Rag1*	3789	24	83	1.7	371	168	251
852047	*Rag1*	3793	24	79	1.4	362	174	245
852048	*Rag1*	3844	24	81	1.9	361	174	255
852049	*Rag1*	4116	25	80	1.8	366	165	234
852050	*Rag1*	3710	22	77	1.5	374	168	243
852051	*Rag1*	3933	23	73	1.8	355	175	244
852052	*Rag1*	3907	24	80	1.7	360	170	244
852053	*Rag1*	3688	23	81	1.6	360	173	241
852054	*Rag1*	3959	25	84	1.8	363	172	247
SEM		112.5	0.4	2.1	0.1	2.1	1.3	2.4
LSD 0.05		315.4	1.0	5.8	0.3	5.8	3.6	6.6
LSD 0.01		417.3	1.3	7.7	0.4	7.7	4.8	8.8
IA3027	*rag1*	3749	23	82	1.8	369	170	242
IA3045	*rag1*	3914	24	89	2.1	376	172	241
LD05-16521	*Rag1*	3777	25	89	2.0	332	197	208

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

Table A5. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-infested experiment across three Iowa environments in 2009.

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852001	*rag1*	3615	23	80	1.7	359	173	234	6.3
852002	*rag1*	3344	22	79	1.6	366	174	232	6.8
852003	*rag1*	3812	23	82	2.0	369	170	241	5.2
852004	*rag1*	3397	22	73	1.7	363	177	248	6.3
852005	*rag1*	3522	23	74	1.8	367	170	225	6.0
852006	*rag1*	3583	22	77	1.7	369	176	242	6.3
852007	*rag1*	3577	23	80	2.0	371	169	241	5.8
852008	*rag1*	3097	21	71	1.7	364	173	225	6.7
852009	*rag1*	3723	24	75	1.8	364	172	224	6.3
852010	*rag1*	3480	23	81	1.9	367	169	241	5.5
852011	*rag1*	3528	22	79	1.8	369	169	239	6.0
852012	*rag1*	3827	24	82	2.1	369	171	245	5.5
852013	*rag1*	3566	23	82	2.0	367	169	243	5.5
852014	*rag1*	3378	22	77	1.8	372	171	222	6.3
852015	*rag1*	3643	23	83	2.2	368	168	239	4.5
852016	*rag1*	3650	25	79	1.8	368	173	231	6.8
852017	*rag1*	3617	23	82	1.9	368	168	241	5.3
852018	*rag1*	3624	23	83	2.1	368	171	226	6.3
852019	*rag1*	3356	22	77	1.8	366	170	234	6.3
852020	*rag1*	3441	23	86	1.6	357	173	197	6.0
852021	*rag1*	3483	22	77	1.7	366	172	228	6.0
852022	*rag1*	3644	26	77	1.9	367	171	236	6.2

Table A5. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852023	*rag1*	3629	23	80	1.5	372	171	243	5.3
852024	*rag1*	3480	22	79	1.6	372	167	235	5.3
852025	*rag1*	3454	23	82	1.6	374	164	232	7.0
852026	*rag1*	3453	22	78	1.6	361	173	235	6.2
852027	*rag1*	3726	23	83	1.9	363	173	261	5.0
852028	*Rag1*	3912	24	76	1.8	373	163	238	3.7
852029	*Rag1*	3742	23	82	1.8	368	173	240	3.5
852030	*Rag1*	3458	23	73	1.6	365	172	244	3.0
852031	*Rag1*	3888	23	79	1.8	361	172	236	3.2
852032	*Rag1*	4040	25	77	1.9	362	170	244	3.2
852033	*Rag1*	3644	24	74	1.7	365	169	223	3.5
852034	*Rag1*	3812	23	79	1.9	366	174	227	2.7
852035	*Rag1*	4000	25	78	1.6	363	174	248	3.0
852036	*Rag1*	4125	23	82	1.8	359	175	240	3.7
852037	*Rag1*	3867	25	75	1.7	359	173	226	3.5
852038	*Rag1*	3996	24	81	1.7	367	172	243	2.8
852039	*Rag1*	3766	24	73	1.5	355	175	235	3.3
852040	*Rag1*	3846	24	75	1.8	369	172	237	3.5
852041	*Rag1*	3754	23	73	1.8	363	176	220	2.8
852042	*Rag1*	3823	26	85	1.8	363	167	251	3.3
852043	*Rag1*	3898	26	83	1.8	358	170	251	3.8
852044	*Rag1*	3774	23	80	1.9	364	170	232	2.8

Table A5. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852045	*Rag1*	3657	24	79	1.8	363	171	253	2.7
852046	*Rag1*	3810	24	83	1.8	369	170	244	3.0
852047	*Rag1*	3708	24	78	1.8	362	174	236	3.7
852048	*Rag1*	3695	24	83	1.8	359	176	247	2.8
852049	*Rag1*	3864	26	81	1.8	366	166	230	3.7
852050	*Rag1*	3685	22	75	1.7	371	169	237	2.5
852051	*Rag1*	3836	23	77	1.7	357	174	218	2.7
852052	*Rag1*	3597	25	78	1.7	361	170	233	3.5
852053	*Rag1*	3682	23	77	1.6	361	173	237	2.8
852054	*Rag1*	3751	24	83	1.8	361	170	244	3.2
SEM		114.1	0.4	1.9	0.1	2.0	1.4	4.2	0.5
LSD 0.05		320.0	1.1	5.4	0.3	5.6	3.8	11.8	1.4
LSD 0.01		423.3	1.5	7.1	0.4	7.4	5.1	15.6	1.8
IA3027	*rag1*	3501	22	81	1.8	370	170	233	5.8
IA3045	*rag1*	3734	24	89	2.2	376	172	236	5.3
LD05-16521	*Rag1*	3468	26	84	2.0	333	197	202	3.3

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

APPENDIX B

ANALYSES OF VARIANCE FOR SEED TRAITS AT INDIVIDUAL ENVIRONMENTS

Table B1. Analyses of variance for the aphid-free experiment at Ames, Iowa, in 2009.

		Mean Squares						
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})
Replications	1	228215.2ns†	3.3ns	112.0ns	0.4**	80.0*	41.6*	119.3ns
Genotypes	53	102026.9ns	3.3**	52.8ns	0.1ns	52.1**	21.6**	248.4**
Rag1	26	125334.7ns	2.7*	55.0*	0.1ns	55.0**	23.9**	206.0**
rag1	26	82343.6ns	3.0**	39.3ns	0.1ns	31.9ns	19.3*	281.8**
Rag1 vs. *rag1*	1	7786.0ns	26.0**	348.5**	0.1ns	502.7**	24.1ns	480.6**
Error	53	84195.2	1.1	40.7	0.1	17.9	7.3	37.5
CV (%)		7.1	4.9	7.2	13.1	1.2	1.6	2.5

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table B2. Analyses of variance for the aphid-free experiment at Carlisle, Iowa, in 2009.

		Mean Squares						
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})
Replications	1	30382.1ns†	2.3**	79.6ns	0.9**	10.1ns	13.4ns	56.1ns
Genotypes	53	88374.9**	1.7**	28.1ns	0.2**	80.1**	24.0**	237.4**
Rag1	26	73121.2*	1.4**	21.0ns	0.1ns	98.1**	20.8**	214.1**
rag1	26	97526.1*	1.4**	35.7ns	0.2*	43.7*	24.2**	260.5**
Rag1 vs. *rag1*	1	247038.8*	17.4**	14.4ns	0.1ns	555.8**	96.3**	245.7*
Error	53	41322.3	0.3	20.4	0.1	16.7	6.7	53.5
CV (%)		4.6	2.4	6.0	12.6	1.1	1.5	3.0

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table B3. Analyses of variance for the aphid-free experiment at Rippey, Iowa, in 2009.

		Mean Squares						
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})
Replications	1	2092930.5**	0.1ns†	208.3**	0.4**	542.3**	71.7*	43.4ns
Genotypes	53	100093.2ns	2.6**	36.1*	0.1**	58.7**	30.9**	198.6**
Rag1	26	75230.1ns	2.0*	33.8ns	0.1ns	58.7**	32.6*	160.1**
rag1	26	128427.0ns	2.9**	36.4ns	0.1*	50.2ns	30.4**	240.0**
Rag1 vs. *rag1*	1	9856.0ns	7.8**	88.9ns	0.1ns	280.3**	0.6ns	125.8ns
Error	53	84243.6	0.8	22.2	0.1	23.7	13.7	23.9
CV (%)		9.2	3.3	6.2	17.2	1.3	2.2	2.0

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table B4. Analyses of variance for the aphid-infested experiment at Ames, Iowa, in 2009.

		Mean Squares							
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})	Aphid (score¶)
Replications	1	208812.7ns†	19.6**	62.3ns	0.1ns	4.1ns	63.7**	1367.7**	0.3ns
Genotypes	53	131225.2**	2.8**	57.2**	0.1*	54.3**	15.8**	270.8**	4.2**
Rag1	26	73005.7ns	2.8**	39.8ns	0.1*	48.2**	13.3*	220.8**	1.4ns
rag1	26	135093.4ns	2.1**	69.7**	0.1ns	34.8**	18.4**	330.6**	2.1ns
Rag1 vs. *rag1*	1	1544356.9**	19.6**	186.7**	0.1ns	720.8**	14.1ns	15.9ns	133.3**
Error	53	65561.8	0.6	24.7	0.1	13.9	6.2	96.9	2.1
CV (%)		6.5	3.7	5.6	13.8	1.0	1.5	4.2	26.1

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table B5. Analyses of variance for the aphid-infested experiment at Carlisle, Iowa, in 2009.

		Mean Squares							
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})	Aphid (score¶)
Replications	1	55274.5ns†	0.5ns	1.3ns	0.5*	19.6ns	7.8ns	20.4ns	6.3**
Genotypes	53	138121.8**	3.7**	31.1ns	0.1ns	57.8**	19.0**	275.6**	2.8**
Rag1	26	68921.5*	2.1**	41.4**	0.1ns	56.7**	18.1**	156.7**	0.3ns
rag1	26	89033.3ns	3.2**	21.2ns	0.2ns	58.1*	20.6*	309.3**	0.8ns
Rag1 vs. *rag1*	1	3213630.8**	57.8**	25.0ns	0.5*	75.0ns	0.1ns	2489.6**	120.3**
Error	53	52533.6	0.8	24.5	0.1	22.7	8.0	30.2	0.5
CV (%)		5.6	4.0	6.5	12.8	1.3	0.6	2.3	22.2

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table B6. Analyses of variance for the aphid-infested experiment at Rippey, Iowa, in 2009.

		Mean Squares							
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)	Protein (g kg^{-1})	Oil (g kg^{-1})	Seed weight (mg seed^{-1})	Aphid (score¶)
Replications	1	2123230.0**	3.3ns†	464.6**	0.6*	133.3**	18.8ns	876.5*	0.1ns
Genotypes	53	123044.7**	2.8**	32.1**	0.1ns	52.5**	37.9**	318.1**	9.3**
Rag1	26	113359.0ns	2.6**	34.1**	0.1ns	42.9**	44.6**	385.8ns	0.8ns
rag1	26	99461.4ns	2.3**	29.8*	0.1ns	49.2**	31.7**	256.6**	1.3ns
Rag1 vs. *rag1*	1	988037.8**	18.8**	37.9ns	0.1ns	385.3**	22.2ns	156.9ns	436.0**
Error	53	96265.0	0.8	12.3	0.1	14.3	10.5	156.7	1.1
CV (%)		10.5	3.4	4.9	23.3	1.0	2.0	5.4	21.8

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

†ns, not significant at the 0.05 probability level.

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

APPENDIX C

TYPE AND ENTRY MEANS FOR SEED TRAITS AT INDIVIDUAL ENVIRONMENTS

Table C1. Mean and range for agronomic and seed traits of 27 *Rag1* and 27 *rag1* lines grown in the aphid-free experiment at three Iowa environments in 2009.

Trait	Type	Aphid-free					
		Ames		Carlisle		Rippey	
		Mean	Range	Mean	Range	Mean	Range
Yield	*Rag1*	4060	3613-4465ns†	4454	3983-4742*	3163	2814-3665ns
(kg ha^{-1})	*rag1*	4077ns	3661-4448ns	4358*	3824-4639*	3144ns	2540-3654ns
Maturity	*Rag1*	21.5	19-24*	22.5	21-25**	26.9	25-30*
(d‡)	*rag1*	20.6**	19-24**	21.7**	20-23**	26.4**	25-30**
Height	*Rag1*	87	75-98*	75	68-82ns	75	67-84ns
(cm)	*rag1*	91**	83-99ns	75ns	66-82ns	77ns	67-86ns
Lodging	*Rag1*	1.5	1.3-2.0ns	2.3	1.8-2.8ns	1.3	1.0-2.0ns
(score§)	*rag1*	1.6ns	1.3-2.0ns	2.4ns	1.8-3.0*	1.3ns	1.0-1.8*
Protein	*Rag1*	364	355-378**	365	352-378**	363	352-378**
(g kg^{-1}¶)	*rag1*	369**	363-377ns	370**	361-381*	366**	357-378ns
Oil	*Rag1*	171	165-178**	177	172-183**	165	158-174*
(g kg^{-1}¶)	*rag1*	170ns	161-176*	176**	167-181**	165ns	154-173**
Seed weight	*Rag1*	245	224-264**	248	230-266**	238	218-253**
(mg seed^{-1})	*rag1*	249**	205-274**	251*	212-275**	240*	200-255**

*Significant difference at the 0.05 probability level between the means of the two types or among lines within a type.
**Significant difference at the 0.01 probability level between the means of the two types or among lines within a type.
†ns, difference between the means of the two types or among lines within a type were not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).
¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

Table C2. Mean and range for agronomic and seed traits of 27 *Rag1* and 27 *rag1* lines grown in the aphid-infested experiment at three Iowa environments in 2009.

Trait	Type	Aphid-infested					
		Ames		Carlisle		Rippey	
		Mean	Range	Mean	Range	Mean	Range
Yield	*Rag1*	4074	3638-4369ns†	4266	3933-4564*	3063	2562-3548ns
(kg ha^{-1})	*rag1*	3835**	3026-4241ns	3921**	3436-4246ns	2872**	2422-3357ns
Maturity	*Rag1*	21.1	19-24**	23.3	22-26**	27.2	26-30**
(d‡)	*rag1*	20.2**	19-23**	21.8**	20-25**	26.4**	25-30**
Height	*Rag1*	87	79-95ns	76	66-83**	73	64-80**
(cm)	*rag1*	89**	74-102**	77ns	70-83ns	71ns	65-81*
Lodging	*Rag1*	1.6	1.3-2.0*	2.4	2.0-2.8ns	1.3	1.0-1.5ns
(score§)	*rag1*	1.6ns	1.3-2.3ns	2.5*	2.0-3.0ns	1.3ns	1.3-1.8ns
Protein	*Rag1*	361	352-370**	366	352-378**	363	357-375**
(g kg^{-1}¶)	*rag1*	366**	357-374**	367ns	357-376*	367**	357-378*
Oil	*Rag1*	172	167-177*	177	170-182**	166	154-174**
(g kg^{-1}¶)	*rag1*	171ns	166-177**	177ns	172-184*	165ns	153-175**
Seed weight	*Rag1*	235	214-255**	245	223-261**	233	178-247ns
(mg seed^{-1})	*rag1*	234ns	202-270**	235**	196-261**	235ns	193-254**
Aphid	*Rag1*	4.4	3.0-6.0ns	2.3	2.0-3.5ns	2.9	2.0-4.5ns
(score#)	*rag1*	6.6**	4.5-8.5ns	4.4**	3.0-5.5ns	6.9**	4.5-8.0ns

*Significant difference at the 0.05 probability level between the means of the two types or among lines within a type.
**Significant difference at the 0.01 probability level between the means of the two types or among lines within a type.
†ns, difference between the means of the two types or among lines within a type were not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).
¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.
#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table C3. Mean and range for agronomic and seed traits of 27 *Rag1* and 27 *rag1* lines grown in the aphid-infested experiment in 2008.

Trait	Type	Aphid-infested					
		Agronomy Farm		Burkey Farm		Combined	
		Mean	Range	Mean	Range	Mean	Range
Yield	*Rag1*	1241	207-2152	2351	1593-3089	1796	1183-2464
(kg ha^{-1})	*rag1*	841	334-1184	1888	1117-2505	1365	1040-1730
Aphid	*Rag1*	5.7	4.0-7.0	3.2	2.0-4.0	4.5	3.0-5.0
(score‡)	*rag1*	8.4	8.0-9.0	6.9	6.0-8.0	7.6	7.0-8.5

*Significant difference at the 0.05 probability level between the means of the two types or among lines within a type.
**Significant difference at the 0.01 probability level between the means of the two types or among lines within a type.
†ns, difference between the means of the two types or among lines within a type were not significant at the 0.05 probability level.
‡ Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table C4. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-free experiment at Ames, Iowa, in 2009.

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852001	*rag1*	3661	21	83	1.5	364	173	252
852002	*rag1*	4051	20	98	1.5	372	172	251
852003	*rag1*	4246	21	97	2.0	370	167	255
852004	*rag1*	3756	21	84	1.8	366	176	264
852005	*rag1*	4216	24	93	1.8	373	164	241
852006	*rag1*	4057	19	87	1.5	372	174	248
852007	*rag1*	4172	21	93	1.8	370	170	253
852008	*rag1*	3915	19	85	1.5	373	168	249
852009	*rag1*	4382	21	86	1.8	366	173	237
852010	*rag1*	4168	20	96	1.5	363	171	254
852011	*rag1*	4164	22	94	1.5	365	171	254
852012	*rag1*	4387	22	94	2.0	368	167	253
852013	*rag1*	4103	21	94	1.8	372	168	257
852014	*rag1*	3696	19	84	1.5	374	172	237
852015	*rag1*	4094	21	88	1.5	365	170	251
852016	*rag1*	3868	22	88	1.5	367	172	239
852017	*rag1*	4204	20	88	1.5	367	171	254
852018	*rag1*	4218	22	99	1.5	370	170	242
852019	*rag1*	4076	19	90	1.5	369	170	244
852020	*rag1*	3809	21	92	1.5	363	172	205
852021	*rag1*	4151	19	89	1.5	366	170	249

Table C4. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}
852022	*rag1*	4136	23	83	1.5	363	171	254
852023	*rag1*	3904	20	98	1.3	373	171	250
852024	*rag1*	3933	21	97	1.8	376	165	257
852025	*rag1*	4448	20	84	1.5	377	161	252
852026	*rag1*	4171	20	93	1.5	367	172	248
852027	*rag1*	4102	22	87	1.8	369	169	274
852028	*Rag1*	4199	23	93	1.5	371	165	248
852029	*Rag1*	3714	21	85	1.8	367	176	242
852030	*Rag1*	3858	21	86	1.8	369	168	256
852031	*Rag1*	3788	21	96	1.5	362	175	242
852032	*Rag1*	4200	24	94	1.8	364	166	243
852033	*Rag1*	4346	21	94	1.8	368	167	232
852034	*Rag1*	3944	21	94	1.5	370	174	233
852035	*Rag1*	3873	23	84	1.5	366	171	250
852036	*Rag1*	4192	21	88	1.5	364	172	244
852037	*Rag1*	3907	23	88	1.5	359	174	226
852038	*Rag1*	4455	22	88	1.5	366	171	247
852039	*Rag1*	3829	23	99	1.5	356	172	241
852040	*Rag1*	4380	21	90	1.5	366	173	236
852041	*Rag1*	4096	19	92	1.3	359	178	224
852042	*Rag1*	4084	22	89	1.5	366	172	259

Table C4. Continued

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852043	*Rag1*	4465	24	91	1.5	356	170	258
852044	*Rag1*	3613	21	85	1.8	362	170	233
852045	*Rag1*	4076	21	89	1.8	365	168	264
852046	*Rag1*	4080	23	94	1.5	372	166	257
852047	*Rag1*	3776	21	83	1.3	366	173	246
852048	*Rag1*	4118	22	93	2.0	358	175	252
852049	*Rag1*	4455	24	86	1.5	364	166	233
852050	*Rag1*	3706	20	86	1.5	378	167	247
852051	*Rag1*	3922	21	79	1.5	355	175	252
852052	*Rag1*	4161	22	85	1.3	363	171	250
852053	*Rag1*	4002	21	91	1.5	364	171	244
852054	*Rag1*	4390	22	98	1.5	367	169	251
SEM		205.2	0.7	4.5	0.1	3.0	1.9	4.2
LSD 0.05		582.0	2.1	12.8	0.4	8.5	5.4	12.3
LSD 0.01		775.3	2.8	17.0	0.5	11.3	7.2	16.4
IA3027	*rag1*	3910	20	88	1.7	369	171	243
IA3045	*rag1*	4341	22	101	2.1	377	172	244
LD05-16521	*Rag1*	4002	23	93	1.9	330	199	208

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

Table C5. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-free experiment at Carlisle, Iowa, in 2009.

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852001	*rag1*	4596	23	70	1.8	363	179	258
852002	*rag1*	4440	22	72	2.3	369	179	255
852003	*rag1*	4611	23	82	2.8	370	174	259
852004	*rag1*	4451	22	72	2.3	366	180	264
852005	*rag1*	4639	23	78	2.5	375	171	245
852006	*rag1*	3947	20	70	1.8	366	181	252
852007	*rag1*	4422	22	78	2.8	370	174	256
852008	*rag1*	4026	22	66	1.8	370	176	250
852009	*rag1*	4571	22	79	2.5	368	175	231
852010	*rag1*	4427	21	82	2.9	375	173	258
852011	*rag1*	4300	22	73	2.3	370	176	248
852012	*rag1*	4259	22	77	2.8	368	175	252
852013	*rag1*	4330	22	75	2.8	371	178	252
852014	*rag1*	3946	20	73	2.3	374	177	236
852015	*rag1*	4523	23	81	3.0	372	172	255
852016	*rag1*	4420	23	73	2.3	366	180	242
852017	*rag1*	4389	21	78	2.5	372	174	253
852018	*rag1*	4542	23	70	2.0	370	176	252
852019	*rag1*	4330	21	75	2.5	369	177	250
852020	*rag1*	3824	21	79	1.9	367	181	212
852021	*rag1*	4428	21	78	2.3	370	173	244

Table C5. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}
852022	*rag1*	4384	23	70	2.0	361	180	247
852023	*rag1*	4346	21	75	2.4	375	174	250
852024	*rag1*	4145	22	74	2.3	380	170	255
852025	*rag1*	4540	23	81	2.8	381	167	260
852026	*rag1*	4205	21	78	2.3	364	178	253
852027	*rag1*	4635	21	75	2.4	366	175	275
852028	*Rag1*	4399	23	72	2.3	378	172	252
852029	*Rag1*	4685	22	82	2.4	377	177	248
852030	*Rag1*	4026	22	73	2.4	369	175	266
852031	*Rag1*	4298	22	76	2.3	364	183	236
852032	*Rag1*	4609	23	77	2.8	368	177	252
852033	*Rag1*	4416	22	75	2.0	362	177	232
852034	*Rag1*	4499	22	74	2.5	370	176	236
852035	*Rag1*	4742	23	68	2.0	360	182	263
852036	*Rag1*	4593	22	75	2.5	364	179	254
852037	*Rag1*	4421	23	72	2.3	364	179	230
852038	*Rag1*	4420	23	77	2.3	372	175	251
852039	*Rag1*	4675	23	71	2.0	353	180	243
852040	*Rag1*	4453	23	75	2.8	375	177	252
852041	*Rag1*	4534	22	71	2.3	367	179	230
852042	*Rag1*	4614	24	75	1.9	363	177	266

Table C5. Continued

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852043	*Rag1*	4661	25	73	2.3	352	181	257
852044	*Rag1*	4436	22	73	2.5	367	174	239
852045	*Rag1*	4576	23	74	2.5	361	176	254
852046	*Rag1*	4126	23	79	2.4	371	177	249
852047	*Rag1*	4474	23	77	1.8	361	181	253
852048	*Rag1*	4315	23	77	2.5	365	181	260
852049	*Rag1*	4655	23	81	2.3	368	172	238
852050	*Rag1*	4342	21	74	2.0	378	172	247
852051	*Rag1*	4378	21	69	2.4	356	182	240
852052	*Rag1*	4401	23	76	2.5	363	177	243
852053	*Rag1*	3983	22	74	2.0	357	182	241
852054	*Rag1*	4524	23	75	2.3	363	179	252
SEM		143.7	0.4	3.2	0.2	2.9	1.8	5.2
LSD 0.05		407.7	1.1	9.0	0.6	8.2	5.2	14.7
LSD 0.01		543.1	1.4	12.1	0.8	10.9	6.9	19.5
IA3027	*rag1*	4162	22	80	2.5	374	174	242
IA3045	*rag1*	4330	24	83	2.6	380	174	241
LD05-16521	*Rag1*	4162	23	82	2.4	335	202	202

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

Table C6. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-free experiment at Rippey, Iowa, in 2009.

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852001	*rag1*	3173	27	74	1.0	361	164	240
852002	*rag1*	3042	26	79	1.3	364	171	235
852003	*rag1*	3654	27	85	1.8	363	170	246
852004	*rag1*	3180	26	74	1.3	364	172	249
852005	*rag1*	3236	27	77	1.3	372	162	235
852006	*rag1*	2984	25	75	1.0	363	173	251
852007	*rag1*	3510	27	80	1.5	361	164	248
852008	*rag1*	2899	26	70	1.0	373	164	245
852009	*rag1*	3539	28	77	1.8	364	167	228
852010	*rag1*	3407	27	84	1.5	365	164	248
852011	*rag1*	3245	27	77	1.5	366	165	242
852012	*rag1*	3254	27	78	1.3	373	165	243
852013	*rag1*	3225	26	78	1.5	367	165	244
852014	*rag1*	2886	25	76	1.3	365	171	225
852015	*rag1*	3210	27	86	1.5	371	164	246
852016	*rag1*	3437	29	82	1.5	364	165	238
852017	*rag1*	3364	27	76	1.5	371	162	247
852018	*rag1*	2540	26	74	1.0	369	167	225
852019	*rag1*	2840	25	67	1.3	371	164	237
852020	*rag1*	2881	25	75	1.5	359	163	200
852021	*rag1*	3220	25	78	1.3	362	164	237

Table C6. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}
852022	*rag1*	3178	30	79	1.8	371	160	247
852023	*rag1*	3117	26	77	1.0	371	167	244
852024	*rag1*	3109	25	75	1.3	368	163	240
852025	*rag1*	3008	27	79	1.0	378	154	239
852026	*rag1*	2798	27	71	1.3	361	165	244
852027	*rag1*	2956	27	75	1.0	357	163	255
852028	*Rag1*	3158	27	73	1.3	378	158	243
852029	*Rag1*	3266	26	81	1.3	358	171	239
852030	*Rag1*	3054	26	71	1.3	363	167	241
852031	*Rag1*	3486	27	77	1.5	367	168	236
852032	*Rag1*	3138	27	73	1.5	365	161	243
852033	*Rag1*	2912	27	70	1.0	368	161	219
852034	*Rag1*	3037	26	72	1.5	367	167	220
852035	*Rag1*	3179	27	76	1.0	364	171	242
852036	*Rag1*	3272	26	75	1.3	363	168	240
852037	*Rag1*	3120	28	79	1.3	365	164	227
852038	*Rag1*	3249	27	80	1.3	368	165	241
852039	*Rag1*	2994	27	67	1.0	359	167	237
852040	*Rag1*	3665	27	76	1.5	366	167	235
852041	*Rag1*	2878	25	67	1.3	361	174	218
852042	*Rag1*	2814	29	75	1.5	364	160	247

Table C6. Continued

Entry	Type	Yield	Maturity	Height	Lodging	Protein	Oil	Seed weight
		kg ha^{-1}	days‡	cm	score§	g kg^{-1}¶	g kg^{-1}¶	mg sd^{-1}
852043	*Rag1*	3211	30	77	1.5	363	159	251
852044	*Rag1*	3115	26	79	1.3	366	163	229
852045	*Rag1*	3455	27	84	2.0	352	166	248
852046	*Rag1*	3161	27	77	1.3	369	163	247
852047	*Rag1*	3128	28	77	1.3	360	168	237
852048	*Rag1*	3099	27	73	1.3	360	167	253
852049	*Rag1*	3239	28	73	1.5	368	158	230
852050	*Rag1*	3081	27	71	1.0	366	164	234
852051	*Rag1*	3499	27	72	1.5	354	169	241
852052	*Rag1*	3157	28	78	1.3	356	162	240
852053	*Rag1*	3079	26	77	1.3	358	167	239
852054	*Rag1*	2964	29	79	1.5	360	167	239
SEM		205.2	0.6	3.3	0.2	3.4	2.6	3.5
LSD 0.05		582.2	1.8	9.5	0.5	9.8	7.4	9.8
LSD 0.01		775.5	2.4	12.6	0.6	13.0	9.9	13.1
IA3027	*rag1*	3177	26	79	1.4	365	167	241
IA3045	*rag1*	3070	27	84	1.6	371	171	237
LD05-16521	*Rag1*	3167	30	91	1.8	333	191	213

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

Table C7. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-infested experiment at Ames, Iowa, in 2009.

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852001	*rag1*	3781	21	92	1.5	361	174	225	6.5
852002	*rag1*	3634	20	90	1.5	367	175	232	8.5
852003	*rag1*	4241	21	96	2.0	368	170	240	5.5
852004	*rag1*	3713	20	80	1.5	361	176	245	7.0
852005	*rag1*	3513	19	79	1.8	361	174	217	6.0
852006	*rag1*	3656	19	85	1.3	366	177	238	7.0
852007	*rag1*	4179	21	89	1.8	368	170	244	7.5
852008	*rag1*	3026	19	74	1.5	366	172	231	7.0
852009	*rag1*	3981	20	83	1.8	361	172	220	7.5
852010	*rag1*	3877	21	93	1.5	364	169	239	6.0
852011	*rag1*	3808	20	84	1.5	366	170	235	7.5
852012	*rag1*	4022	21	92	1.8	368	170	246	6.5
852013	*rag1*	3879	21	91	1.8	366	169	242	6.0
852014	*rag1*	3880	19	89	1.8	372	172	223	7.0
852015	*rag1*	3890	21	99	2.3	372	166	238	6.0
852016	*rag1*	3907	23	89	1.8	366	175	228	8.5
852017	*rag1*	4026	20	93	1.8	366	169	242	6.0
852018	*rag1*	3927	22	94	1.8	367	171	226	6.0
852019	*rag1*	3948	20	88	1.5	363	173	234	6.5
852020	*rag1*	3884	21	102	1.5	357	175	202	6.5
852021	*rag1*	3563	19	90	1.8	369	172	225	5.5
852022	*rag1*	3890	23	87	1.5	366	168	233	8.0

Table C7. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852023	*rag1*	4131	20	91	1.5	373	169	254	4.5
852024	*rag1*	3655	20	91	1.5	374	166	237	5.5
852025	*rag1*	3449	20	91	1.5	370	166	225	8.5
852026	*rag1*	3865	20	88	1.5	363	172	237	7.0
852027	*rag1*	4217	21	92	1.8	371	170	270	5.5
852028	*Rag1*	4141	21	84	1.5	370	167	235	5.5
852029	*Rag1*	4133	21	94	1.8	366	172	234	5.0
852030	*Rag1*	3638	20	82	1.8	362	173	243	4.0
852031	*Rag1*	4105	20	86	1.8	358	173	233	5.0
852032	*Rag1*	4369	23	84	2.0	360	170	241	3.5
852033	*Rag1*	3887	21	79	1.5	360	171	215	4.0
852034	*Rag1*	4329	21	89	2.0	368	173	217	4.0
852035	*Rag1*	4133	23	88	1.5	364	172	247	4.5
852036	*Rag1*	4339	21	88	1.5	358	173	239	5.0
852037	*Rag1*	4011	21	87	1.3	357	177	214	4.5
852038	*Rag1*	4193	22	87	1.5	362	173	243	3.5
852039	*Rag1*	4027	21	79	1.5	355	175	233	6.0
852040	*Rag1*	3893	20	84	1.5	364	175	223	5.5
852041	*Rag1*	4050	20	83	1.8	366	173	220	3.0
852042	*Rag1*	4256	24	95	1.5	362	167	249	5.0
852043	*Rag1*	4234	24	94	1.5	353	172	244	5.5

Table C7. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852044	*Rag1*	4240	20	90	2.0	362	170	233	3.5
852045	*Rag1*	3760	21	80	1.8	360	171	255	3.5
852046	*Rag1*	4034	22	94	1.5	370	169	249	4.5
852047	*Rag1*	3889	22	86	1.5	365	171	234	5.5
852048	*Rag1*	4084	21	90	1.5	352	177	240	4.0
852049	*Rag1*	4285	23	89	1.5	361	169	232	4.5
852050	*Rag1*	3794	19	85	1.5	369	169	234	3.0
852051	*Rag1*	4153	20	84	1.5	356	174	238	3.5
852052	*Rag1*	3857	22	83	1.3	359	172	234	5.0
852053	*Rag1*	3946	21	89	1.3	358	173	231	4.5
852054	*Rag1*	4224	21	88	1.5	359	169	241	4.5
SEM		181.1	0.5	3.5	0.2	2.6	1.8	7.0	1.0
LSD 0.05		513.6	1.5	10.0	0.4	7.5	5.0	19.8	2.9
LSD 0.01		684.1	2.0	13.3	0.6	9.9	6.7	26.3	3.9
IA3027	*rag1*	3885	20	94	1.6	368	171	225	7.0
IA3045	*rag1*	4305	21	97	2.1	376	173	236	5.8
LD05-16521	*Rag1*	3752	23	95	2.0	331	197	197	5.5

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table C8. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-infested experiment at Carlisle, Iowa, in 2009.

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852001	*rag1*	4151	22	74	2.3	357	182	240	4.5
852002	*rag1*	3782	20	79	2.3	366	181	229	5.0
852003	*rag1*	4040	22	75	2.5	369	175	245	4.0
852004	*rag1*	3918	21	70	2.0	359	184	246	4.0
852005	*rag1*	4161	23	75	2.5	370	176	231	5.0
852006	*rag1*	4246	22	76	2.5	372	176	247	4.5
852007	*rag1*	3895	22	78	3.0	376	174	240	3.5
852008	*rag1*	3436	20	73	2.3	361	180	213	5.0
852009	*rag1*	4161	24	76	2.3	364	176	229	4.5
852010	*rag1*	3727	22	78	2.8	369	174	241	4.0
852011	*rag1*	3975	21	82	2.8	370	174	238	4.0
852012	*rag1*	4103	24	83	3.0	370	177	247	4.5
852013	*rag1*	3918	23	79	2.8	370	174	243	4.5
852014	*rag1*	3558	20	76	2.8	374	176	221	4.5
852015	*rag1*	3775	23	78	2.8	371	172	242	3.0
852016	*rag1*	4241	24	78	2.5	366	180	234	4.5
852017	*rag1*	4023	22	83	2.8	369	173	243	3.5
852018	*rag1*	3687	22	81	3.0	374	176	224	5.5
852019	*rag1*	3697	21	72	2.5	369	176	233	5.0
852020	*rag1*	3800	23	80	2.0	358	182	196	4.5
852021	*rag1*	3926	21	76	2.3	361	180	223	5.0
852022	*rag1*	3933	25	77	2.5	366	178	236	4.0

Table C8. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852023	*rag1*	4029	22	77	2.0	370	179	242	4.5
852024	*rag1*	3996	21	75	2.3	372	175	236	3.5
852025	*rag1*	4147	23	80	2.3	375	174	238	5.0
852026	*rag1*	3625	21	73	2.3	361	178	232	5.0
852027	*rag1*	3922	22	76	2.5	362	180	261	3.5
852028	*Rag1*	4433	24	75	2.3	374	170	246	2.5
852029	*Rag1*	4376	24	81	2.3	371	179	251	2.0
852030	*Rag1*	4173	23	69	2.0	362	181	252	2.5
852031	*Rag1*	4535	23	81	2.5	364	177	246	2.0
852032	*Rag1*	4564	24	74	2.5	363	177	249	2.5
852033	*Rag1*	4244	23	74	2.5	368	174	239	2.0
852034	*Rag1*	4239	23	75	2.5	367	179	238	2.0
852035	*Rag1*	4506	25	73	2.0	362	180	253	2.0
852036	*Rag1*	4493	23	80	2.3	364	180	247	3.5
852037	*Rag1*	4410	25	75	2.3	362	177	236	2.5
852038	*Rag1*	4546	23	77	2.3	370	180	243	2.5
852039	*Rag1*	4419	24	72	2.0	352	182	241	2.0
852040	*Rag1*	4096	24	69	2.5	375	175	246	2.0
852041	*Rag1*	4071	22	66	2.3	364	181	223	3.0
852042	*Rag1*	4127	25	81	2.5	367	173	259	2.0
852043	*Rag1*	4490	26	81	2.5	364	175	261	3.0

Table C8. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852044	*Rag1*	4015	22	74	2.5	369	174	236	2.0
852045	*Rag1*	4108	23	80	2.5	366	177	258	2.0
852046	*Rag1*	4100	24	78	2.3	369	176	237	2.0
852047	*Rag1*	4354	24	76	2.5	360	180	243	2.0
852048	*Rag1*	4146	24	82	2.8	364	180	257	2.5
852049	*Rag1*	4120	25	80	2.5	370	174	236	2.5
852050	*Rag1*	4091	22	69	2.0	378	175	245	2.0
852051	*Rag1*	4216	23	76	2.3	358	178	237	2.0
852052	*Rag1*	4226	23	79	2.5	365	174	234	2.0
852053	*Rag1*	4156	23	74	2.3	363	178	245	2.0
852054	*Rag1*	3933	24	83	2.5	364	176	249	2.0
SEM		162.0	0.6	3.5	0.2	3.4	2.0	3.9	0.5
LSD 0.05		459.7	1.8	9.9	0.6	9.6	5.7	11.0	1.5
LSD 0.01		612.4	2.4	13.2	0.8	12.7	7.5	14.7	2.0
IA3027	*rag1*	3599	21	79	2.6	374	175	237	4.5
IA3045	*rag1*	3822	24	88	3.0	381	174	240	4.3
LD05-16521	*Rag1*	3940	24	79	2.6	339	201	209	2.0

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table C9. Mean performance of 27 *Rag1* and 27 *rag1* entries in the aphid-infested experiment at Rippey, Iowa, in 2009.

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852001	*rag1*	2911	27	74	1.3	360	164	236	8.0
852002	*rag1*	2615	26	67	1.0	367	167	235	7.0
852003	*rag1*	3156	26	75	1.5	370	165	239	6.0
852004	*rag1*	2561	26	70	1.5	369	171	254	8.0
852005	*rag1*	2893	27	68	1.3	370	159	226	7.0
852006	*rag1*	2848	25	69	1.3	370	175	242	7.5
852007	*rag1*	2657	27	72	1.3	370	164	237	6.5
852008	*rag1*	2828	25	65	1.3	366	166	231	8.0
852009	*rag1*	3027	27	67	1.5	368	168	224	7.0
852010	*rag1*	2835	27	73	1.5	368	165	244	6.5
852011	*rag1*	2801	26	71	1.3	371	164	245	6.5
852012	*rag1*	3357	27	72	1.5	369	166	242	5.5
852013	*rag1*	2900	27	77	1.5	367	163	245	6.0
852014	*rag1*	2696	26	67	1.0	369	164	221	7.5
852015	*rag1*	3265	27	73	1.5	362	167	237	4.5
852016	*rag1*	2802	28	71	1.3	372	166	232	7.5
852017	*rag1*	2803	27	71	1.3	369	162	237	6.5
852018	*rag1*	3259	26	75	1.5	362	167	228	7.5
852019	*rag1*	2422	27	70	1.5	367	163	236	7.5
852020	*rag1*	2638	25	76	1.3	357	163	193	7.0
852021	*rag1*	2961	25	65	1.0	370	166	236	7.5
852022	*rag1*	3108	30	67	1.8	371	167	240	6.5

Table C9. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852023	*rag1*	2727	28	72	1.0	373	166	233	7.0
852024	*rag1*	2790	25	70	1.0	369	162	232	7.0
852025	*rag1*	2765	27	76	1.0	378	153	233	7.5
852026	*rag1*	2868	25	72	1.0	359	169	238	6.5
852027	*rag1*	3039	27	81	1.5	357	169	253	6.0
852028	*Rag1*	3164	29	68	1.5	375	154	234	3.0
852029	*Rag1*	2717	26	72	1.5	366	168	236	3.5
852030	*Rag1*	2562	27	68	1.0	372	163	236	2.5
852031	*Rag1*	3026	27	70	1.3	361	165	229	2.5
852032	*Rag1*	3186	28	72	1.3	365	164	242	3.5
852033	*Rag1*	2800	27	68	1.0	367	163	217	4.5
852034	*Rag1*	2867	27	73	1.3	365	169	226	2.0
852035	*Rag1*	3361	27	72	1.3	364	171	245	2.5
852036	*Rag1*	3544	26	78	1.5	357	173	234	2.5
852037	*Rag1*	3180	29	64	1.5	359	166	228	3.5
852038	*Rag1*	3248	27	79	1.3	369	163	242	2.5
852039	*Rag1*	2853	28	69	1.0	359	168	232	2.0
852040	*Rag1*	3548	27	72	1.5	369	167	240	3.0
852041	*Rag1*	3142	27	69	1.5	360	174	219	2.5
852042	*Rag1*	3085	29	80	1.3	362	161	246	3.0
852043	*Rag1*	2970	29	75	1.5	359	163	247	3.0

Table C9. Continued

Entry	Type	Yield kg ha^{-1}	Maturity days‡	Height cm	Lodging score§	Protein g kg^{-1}¶	Oil g kg^{-1}¶	Seed weight mg sd^{-1}	Aphid score#
852044	*Rag1*	3068	27	75	1.3	360	167	226	3.0
852045	*Rag1*	3104	27	77	1.3	365	166	247	2.5
852046	*Rag1*	3295	27	77	1.5	367	166	246	2.5
852047	*Rag1*	2880	28	71	1.3	361	172	231	3.5
852048	*Rag1*	2854	27	77	1.3	360	171	243	2.0
852049	*Rag1*	3187	30	73	1.3	368	156	223	4.0
852050	*Rag1*	3172	26	71	1.5	366	165	232	2.5
852051	*Rag1*	3138	26	70	1.3	357	171	178	2.5
852052	*Rag1*	2709	29	71	1.3	359	165	230	3.5
852053	*Rag1*	2943	26	68	1.3	363	170	234	2.0
852054	*Rag1*	3095	29	79	1.5	362	164	241	3.0
SEM		219.4	0.6	2.5	0.2	2.7	2.3	8.9	0.7
LSD 0.05		622.3	1.8	7.0	0.6	7.6	6.5	25.1	2.1
LSD 0.01		829.0	2.4	9.4	0.8	10.1	8.7	33.4	2.8
IA3027	*rag1*	3021	26	71	1.3	368	165	236	6.0
IA3045	*rag1*	3074	29	82	1.5	370	171	233	5.8
LD05-16521	*Rag1*	2713	30	79	1.7	330	194	201	2.5

‡Days after 31 August.

§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

¶Protein and oil concentration on a moisture basis of 130 g kg^{-1}.

#Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

Table C10. Performance of 27 *Rag1* and 27 *rag1* entries in the aphid-infested experiment in 2008.

2008 Entry	2009 Entry	Population	Type	Environments					
				Agronomy Farm	Burkey Farm	Combined	Agronomy Farm	Burke Farm	Combined
				Yield kg ha^{-1}	Yield kg ha^{-1}	Yield kg ha^{-1}	Aphid (score#)	Aphid (score#)	Aphid (score#)
123003	852001	AX21304-1	*rag1*	982	2231	1606	8.0	7.0	7.5
123008	852002	AX21304-1	*rag1*	659	1714	1187	9.0	6.0	7.5
123009	852003	AX21304-1	*rag1*	888	1946	1417	8.0	7.0	7.5
123013	852004	AX21304-1	*rag1*	646	2072	1359	9.0	7.0	8.0
123016	852005	AX21304-1	*rag1*	762	1929	1345	8.0	8.0	8.0
123017	852006	AX21304-1	*rag1*	853	1612	1232	9.0	7.0	8.0
123018	852007	AX21304-1	*rag1*	1004	1981	1492	8.0	7.0	7.5
123019	852008	AX21304-1	*rag1*	334	1867	1101	9.0	6.0	7.5
123023	852009	AX21304-1	*rag1*	934	1687	1310	8.0	7.0	7.5
123025	852010	AX21304-1	*rag1*	778	1919	1348	8.0	7.0	7.5
123026	852011	AX21304-1	*rag1*	955	2505	1730	9.0	7.0	8.0
123028	852012	AX21304-1	*rag1*	611	2134	1372	9.0	7.0	8.0
123029	852013	AX21304-1	*rag1*	961	2029	1495	9.0	7.0	8.0
123035	852014	AX21304-1	*rag1*	549	1647	1098	9.0	6.0	7.5
123037	852015	AX21304-1	*rag1*	888	2228	1558	9.0	7.0	8.0
123041	852016	AX21304-1	*rag1*	772	1615	1193	9.0	6.0	7.5
123042	852017	AX21304-1	*rag1*	503	1647	1075	8.0	6.0	7.0
123048	852018	AX21304-2	*rag1*	1076	1954	1515	9.0	8.0	8.5
123051	852019	AX21304-2	*rag1*	963	1117	1040	8.0	6.0	7.0
123052	852020	AX21304-2	*rag1*	966	1954	1460	8.0	7.0	7.5
123097	852021	AX21304-2	*rag1*	1103	1493	1298	8.0	7.0	7.5
123099	852022	AX21304-2	*rag1*	1184	2123	1654	8.0	6.0	7.0
123100	852023	AX21304-2	*rag1*	831	1609	1220	8.0	8.0	8.0
123105	852024	AX21304-2	*rag1*	899	2091	1495	8.0	7.0	7.5
123119	852025	AX21304-2	*rag1*	807	1927	1367	8.0	8.0	8.0

Table C10. Continued

2008 Entry	2009 Entry	Population	Type	Environments					
				Agronomy Farm	Burkey Farm	Combined	Agronomy Farm	Burke Farm	Combined
				Yield kg ha^{-1}	Yield kg ha^{-1}	Yield kg ha^{-1}	Aphid (score#)	Aphid (score#)	Aphid (score#)
123123	852026	AX21304-2	*rag1*	910	1593	1251	8.0	6.0	7.0
123126	852027	AX21304-2	*rag1*	888	2357	1623	8.0	7.0	7.5
123058	852028	AX21304-1	*Rag1*	309	2209	1259	7.0	3.0	5.0
123059	852029	AX21304-1	*Rag1*	1798	2949	2373	5.0	3.0	4.0
123061	852030	AX21304-1	*Rag1*	1138	2174	1656	4.0	4.0	4.0
123062	852031	AX21304-1	*Rag1*	1763	1736	1749	6.0	4.0	5.0
123065	852032	AX21304-1	*Rag1*	1407	2608	2007	6.0	3.0	4.5
123070	852033	AX21304-1	*Rag1*	1717	2489	2103	6.0	3.0	4.5
123071	852034	AX21304-1	*Rag1*	1109	2519	1814	7.0	2.0	4.5
123074	852035	AX21304-1	*Rag1*	1437	2118	1777	4.0	2.0	3.0
123075	852036	AX21304-1	*Rag1*	1114	2260	1687	6.0	4.0	5.0
123076	852037	AX21304-1	*Rag1*	207	2180	1193	6.0	3.0	4.5
123080	852038	AX21304-1	*Rag1*	1569	2917	2243	6.0	3.0	4.5
123081	852039	AX21304-1	*Rag1*	503	1862	1183	7.0	3.0	5.0
123085	852040	AX21304-1	*Rag1*	1117	2944	2030	6.0	3.0	4.5
123087	852041	AX21304-1	*Rag1*	1195	1593	1394	6.0	3.0	4.5
123130	852042	AX21304-1	*Rag1*	2153	2774	2464	4.0	4.0	4.0
123135	852043	AX21304-1	*Rag1*	1448	2355	1901	5.0	3.0	4.0
123136	852044	AX21304-1	*Rag1*	1154	2177	1666	5.0	3.0	4.0
123138	852045	AX21304-2	*Rag1*	657	2433	1545	6.0	3.0	4.5
123139	852046	AX21304-2	*Rag1*	1631	2161	1896	6.0	3.0	4.5
123142	852047	AX21304-2	*Rag1*	1719	3089	2404	7.0	3.0	5.0
123144	852048	AX21304-2	*Rag1*	1009	2680	1845	7.0	3.0	5.0
123145	852049	AX21304-2	*Rag1*	1187	1970	1578	5.0	4.0	4.5
123147	852050	AX21304-2	*Rag1*	1235	1916	1576	6.0	4.0	5.0

Table C10. Continued

2008 Entry	2009 Entry	Population	Type	Environments					
				Agronomy Farm	Burkey Farm	Combined	Agronomy Farm	Burke Farm	Combined
				Yield kg ha^{-1}	Yield kg ha^{-1}	Yield kg ha^{-1}	Aphid (score#)	Aphid (score#)	Aphid (score#)
123149	852051	AX21304-2	*Rag1*	1214	2613	1913	6.0	4.0	5.0
123153	852052	AX21304-2	*Rag1*	1666	2024	1845	4.0	3.0	3.5
123155	852053	AX21304-2	*Rag1*	1039	2425	1732	5.0	4.0	4.5
123156	852054	AX21304-2	*Rag1*	1004	2301	1652	6.0	3.0	4.5
SEM	SEM					213.7			0.6
LSD 0.05	LSD 0.05					606.2			1.6
LSD 0.01	LSD 0.01					807.5			2.2
IA3027	IA3027		*rag1*	770	1989	1379	8.8	6.8	7.8
LD05-16521	LD05-16521		*Rag1*	931	2322	1627	7.0	3.5	5.3

‡ Scores ranged from 1 (aphid free) to 10 (>800 aphids plant^{-1}, severe plant symptoms).

APPENDIX D

ANALYSES OF VARIANCE FOR SEED TRAITS OF THE 10 ENTRIES IN CHAPTER 3

Table D1. Analyses of variance for traits of 10 entries grown at three Iowa environments in 2009.

		Mean Squares			
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)
Environments	2	4830029.2**	657.7**	3652.9**	592.5**
Replications /Environments	9	464622.7**	4.4**	112.9**	14.5ns
Entries	9	78222.8ns	4.8*	19.2ns	6.0ns
Entries x Environments	18	84251.1ns	1.4ns	31.8ns	13.3ns
Error	81	72186.5	1.3	30.2	8.8
CV (%)		6.7	4.7	7.1	19.9

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
†ns, not significant at the 0.05 probability level
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table D2. Analyses of variance for traits of 10 entries grown at Ames, IA, in 2009.

		Mean Squares			
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)
Replications	3	605744.8**	0.6ns	50.3ns	15.6ns
Entries	9	43444.7ns	0.2ns	38.0ns	7.6ns
Error	27	60059.7	0.4	48.6	7.8
CV (%)		5.9	3.0	7.9	20.1

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
†ns, not significant at the 0.05 probability level
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table D3. Analyses of variance for traits of 10 entries grown at Carlisle, IA, in 2009.

		Mean Squares			
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)
Replications	3	310834.8*	5.3*	83.6*	27.3ns
Entries	9	107837.7ns	3.3ns	30.2ns	19.5ns
Error	27	102131.1	1.5	18.4	12.5
CV (%)		7.4	5.1	6.0	18.5

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
†ns, not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

Table D4. Analyses of variance for traits of 10 entries grown at Carlisle, IA, in 2009.

		Mean Squares			
Sources of variation	df	Yield (kg ha^{-1})	Maturity (d‡)	Height (cm)	Lodging (score§)
Replications	3	477288.5**	7.4*	204.9**	0.6ns
Entries	9	95442.7ns	4.2ns	14.6ns	5.6ns
Error	27	54368.7	2.1	23.6	6.2
CV (%)		6.4	5.0	6.7	21.4

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
†ns, not significant at the 0.05 probability level.
‡Days after 31 August.
§Scores ranged from 1 (all plants erect) to 5 (all plants prostrate).

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