



# Parasitism of soybean aphid by *Aphelinus* species on soybean susceptible versus resistant to the aphid



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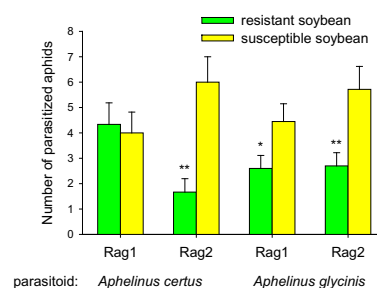
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## HIGHLIGHTS

- Soybean aphid resistance genes, *Rag1* and *Rag2*, affected parasitism by wasp species.
- Neither gene affected oviposition by *Aphelinus glycines* or *Aphelinus certus*.
- Both genes reduced parasitism by *A. glycines*.
- *Rag1* did not effect parasitism by *A. certus*, but *Rag2* reduced parasitism *A. certus*.
- These wasps are nonetheless able to parasitize soybean aphid on resistant soybean.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The soybean aphid, *Aphis glycines*, is native to Asia, but during the last decade it has invaded North America, where it has spread to most soybean growing regions and become the most important insect pest of soybean. Current control of soybean aphid relies primarily on insecticides, but alternatives to insecticidal control are being explored, especially host plant resistance and biological control, which may interact positively or negatively. Research on host plant resistance to the soybean aphid has revealed six genes that affect resistance. We measured the impact of the two most studied resistance loci, *Rag1* and *Rag2*, on two parasitoid species: *Aphelinus glycines*, a recently described species from Asia, which is being introduced into the USA to control the soybean aphid, and *Aphelinus certus*, also from Asia but accidentally introduced into the USA. Resistance did not affect oviposition by either parasitoid species. However, resistance did reduce successful parasitism by *A. glycines*, with each resistance allele causing a two-fold reduction in number of mummified aphids. The resistance alleles did not affect adult emergence, sex ratio, or the size of *A. glycines*. For *A. certus*, the *Rag1* resistance allele had no effect on parasitism, while the *Rag2* resistance allele reduced parasitism four-fold. On the other hand, the *Rag1* resistance allele increased the frequency of males among progeny and decreased female size of *A. certus*. Despite the reduction in parasitism, these parasitoids are nonetheless able to parasitize the soybean aphid on resistant soybean, which means that they should still contribute to the management of soybean aphid on resistant varieties.

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## 1. Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is native to Asia, but during the last decade it has invaded North America (Venette and Ragsdale, 2004), where it has spread to most soybean growing regions (Ragsdale et al., 2011). In Asia, the soybean aphid seldom reaches damaging levels, but in the North America, it has become the most important insect pest of soybean, decreasing yields and incurring large control costs (Kim et al., 2008a; Ragsdale et al., 2011; Song and Swinton, 2009). Furthermore, the soybean aphid has become a major component of an invasional meltdown in North America, involving multiple exotic species, especially *Rhamnus cathartica* L. (Rhamnaceae) which is from Europe and has become a major host plant for overwintering soybean aphid (Heimpel et al., 2010). Current control of the soybean aphid relies primarily on insecticides, and millions of acres of soybeans have been sprayed annually since the soybean aphid invasion. This insecticide use is a major disruption in the ~77 million acres where soybean is grown because insecticides were rarely used on soybean prior to the soybean aphid invasion. Several alternatives to insecticidal control are being explored, especially host plant resistance and biological control, which may interact positively or negatively (Kaplan and Thaler, 2011; McCarville and O'Neal, 2012; Ode and Crompton, 2013).

Research on host plant resistance to the soybean aphid has revealed six genes that affect resistance (for review, see Hill et al., 2012). The mechanism of resistance for the most studied genes, *Rag1* and *Rag2* (named for Resistance to *Aphis glycines*), is primarily antibiosis, although other affects also have been documented, including increased dispersal (Li et al., 2004; Whalen and Harmon, 2012). The *Rag1* resistance allele reduces fecundity and adult longevity and increases nymphal mortality (Li et al., 2004), and the *Rag1* and *Rag2* resistance alleles reduce population growth (Wiarda et al., 2012), presumably because of reduced fecundity, increased mortality or both. Research on the potential for biological control introductions against the soybean aphid has revealed several promising candidates, including aphidiine braconids such as *Binodoxys communis* (Hymenoptera: Braconidae) (Desneux et al., 2009) and aphelinid species in the genus *Aphelinus*, including several new species in the *mali* species complex (Hopper et al., 2013, 2012). The mechanisms of interactions between host plant resistance and biological control may include direct effects on natural enemy survival and development or indirect effects from changes in pest density or distribution that affect attack rates. For *B. communis*, fewer mummified aphids were produced on soybean plants with the *Rag1* resistance allele compared with plants with the susceptible allele (Ghising et al., 2012). This reduction in numbers of mummies resulted from higher mortality of parasitoid larvae, which often did not complete development before their hosts died (Ballman et al., 2012). Adult *B. communis* were also smaller on plants with the *Rag1* resistance allele (Ghising et al., 2012). Another parasitoid of soybean aphid, *Aphidius colemani* (Hymenoptera: Braconidae), also produced fewer mummified aphids on soybean plants with the *Rag1* resistance allele compared with plants with the susceptible allele, and this resulted from higher handling times and lower attack rates on resistant plants, as well as lower survival of progeny (Ode and Crompton, 2013).

The research reported here provides the first results on the effects of soybean aphid resistance alleles on *Aphelinus* species, which differ greatly in biology from the aphidiine braconids. Although like aphidiine braconids, all *Aphelinus* species are internal parasitoids of aphids, *Aphelinus* species have much lower egg loads and develop new eggs throughout life (synovigenic), kill some host aphids by host feeding, search primarily on foot rather than on the wing, have much longer handling times, have longer development times, and live much longer at least in the laboratory. We

measured the impact of the *Rag1* and *Rag2* genes on two parasitoids, *Aphelinus certus* Yasnosh and *Aphelinus glycines* Hopper and Woolley (Hymenoptera: Aphelinidae). Females of *A. glycines* and *A. certus* carry only 11 or 14 mature eggs, respectively, in their oviducts, but like other *Aphelinus* species (e.g., Perng and Liu, 2002; Wu and Heimpel, 2007), produce far more eggs throughout their lives. Adult *Aphelinus* eat nectar and honeydew, but females also feed on aphids to obtain nutrients used for egg production and survival (Papaj, 2000). *Aphelinus* females prefer 2–4th instar hosts for oviposition, but will oviposit in all stages, including alate adults (Rohne, 2002). At 20 °C, wasps develop from oviposited egg to pupation in about 14 days; third instars kill their hosts but leave the host exoskeleton intact, causing it to harden and turn black (Christiansen-Weniger, 1994), a process called mummification. Adults emerge about one week after mummification, chewing a hole through host exoskeleton to escape. *Aphelinus glycines* is a recently described species native to Asia (Hopper et al., 2012), which is being introduced into the USA to control the soybean aphid. *Aphelinus certus* is also native to Asia (Yasnosh, 1963), but was accidentally introduced into the USA at about the same time as the soybean aphid itself (Heimpel et al., 2010). *Aphelinus glycines* has a very narrow host range, being essentially limited to species in the genus *Aphis* and not all of them, whereas *A. certus* has a very broad host range, parasitizing aphid species in four out of five genera tested, distributed across the phylogeny of aphids (unpublished data). Given the larval maturation time for *Aphelinus* and the increased mortality of soybean aphid nymphs on resistant soybean, we expected that *A. certus* and *A. glycines* would produce fewer mummified aphids on resistant compared to susceptible soybean. However, our results were more complicated.

## 2. Materials and methods

### 2.1. Insect sources and rearing conditions

*A. glycines* was collected as 41 mummified soybean aphids on *Glycine max* (L.) (Fabaceae) near Xiuyan, China, during the summer of 2007, and *A. certus* was collected as 25 mummified *A. glycines* on *R. cathartica* (Rhamnaceae) in Allentown, Pennsylvania, USA, during the spring of 2007. Cultures were established at the USDA-ARS, Beneficial Insect Introductions Research Unit, Newark, Delaware, USA. To help maintain genetic variation (Roush and Hopper, 1995), each culture was divided into 4 subcultures, and each subculture was maintained with an adult population size >200 and sex ratio of ~1:1 males:females. Aphids for parasitoid rearing and experiments were from laboratory cultures started from field collections on soybean plants in Newark, Delaware, in 2008. Insects were reared in AR66L plant growth chambers (Percival Scientific, Perry, Iowa) with 500 micromoles light and 16:8 light:dark photoperiod at 20 °C with 40 percent relative humidity on soybean cultivar Pioneer 91M51 (Pioneer Hi-Bred International, Johnston, Iowa), which is susceptible to soybean aphid, in Metro-mix 360 with Suncoir (Sun Gro Horticulture, Agawam, Massachusetts) fertilized at planting with Osmocote Flower and Vegetable Plant Food 14-14-14:N-P-K (ScottsMiracle-Gro, Marysville, Ohio).

### 2.2. Plant varieties

We studied two pairs of backcross lines. The first pair included the aphid susceptible cultivar Dwight (Nickell et al., 1998) and the experimental line LD06-16721, which carries the resistance allele at *Rag1*. LD06-16721 was developed through four backcrosses (BC4) using Dwight as a recurrent parent and 'Dowling' (Craigmiles et al., 1978) as the donor parent of the *Rag1* resistance allele. The second pair includes the aphid susceptible cultivar

LD02-4485 and the experimental line LD09-15087a, which is a BC5 line with *Rag2*, developed using LD02-4485 as a recurrent parent and PI 200538 as the donor of the *Rag2* resistance allele. The full pedigree of LD06-16721 is Dwight(5) × (Dowling × Loda) and the pedigree of LD09-15087a is LD02-4485(6) × (Ina × PI 200538)). Genetic markers linked to each aphid resistance locus were used to select the resistance alleles during each generation of backcrossing. Dwight and LD06-16721 should be over 96% genetically identical for regions outside of the selected interval at *Rag1* and LD02-4485 and LD09-15087a should be over 98% identical for regions outside of the selected interval at *Rag2*.

### 2.3. Differences in parasitism between resistant and susceptible soybean

To measure the effect of soybean resistance to soybean aphid on parasitism, we exposed individual, mated females of *A. glycinis* or *A. certus* that were 1–2 days old to soybean aphid on the four soybean accessions. Females were drawn at random from the replicated sub-populations used for rearing. Seeds were planted in 12 cm diameter × 12 cm tall pots with the same soil mix and fertilizer used for insect rearing and plants and insects were kept under the same conditions as described for insect rearing for 10 days prior to use in experiments. We put each female parasitoid in a cage (polystyrene, 10 cm diameter by 22 cm tall, with eight 2.5 cm holes in the sides and a 12 cm hole in the top covered with fine-mesh screening) enclosing the foliage of 2–3 young plants in a pot with about 100 aphids of mixed instars of the soybean aphid which were placed on the plants to settle and feed two days before the parasitoids were added. Female parasitoids were removed after 24 h and used only once. Because *Aphelinus* in general can parasitize a maximum of about 20 aphids in 24 h, this abundance of aphids and period of exposure allowed parasitoids to use their full egg complement. Furthermore, the density of aphids, amount of plant material, and cage size meant that parasitoids were not limited by search rate.

Ten days after exposure of aphids to parasitoids, we collected mummified aphids and held them for adult parasitoid emergence. After the adults emerged, we recorded the number of mummified aphids and the number and sex of adult parasitoids. We dried the adult progeny of each female at 50 °C for one hour and weighed the sexes separately on a microbalance. Because we scored parasitism after the larval parasitoids killed and mummified their hosts, which occurs during the parasitoid third instar, this measure of parasitism is a combination of acceptance of aphids for oviposition and suitability of aphids for parasitoid survival to third instar.

### 2.4. Differences in oviposition between resistant and susceptible soybean

To determine the mechanisms affecting parasitism in the experiment above and to determine whether resistance alleles affected host feeding, we directly observed behavior of parasitoids exposed to aphids. We observed 8 female parasitoids of each species on each of the four host plant genotypes. We exposed 1–2 day old female parasitoids to 10 2nd–3rd instar aphids on an excised leaf of the appropriate plant species in small arenas (10 mm diameter by 4 mm height). The arenas were formed using two microscope slides with vinyl foam weather-stripping glued to them; on one slide, the weather-stripping had a 10 mm diameter hole bored in it, exposing the slide surface through which observations were made. A leaf freshly cut from a plant was clamped between the slides (weather-stripping against the leaf) and the leaf petiole placed in 1% agar in a small vial to maintain turgor. We placed aphids in the arenas about 0.5 h before beginning observations to allow them to settle and begin feeding on the leaves. We used a

randomized complete-block design with each block done either in the morning or the afternoon.

We observed each female parasitoid and the aphids with which she was enclosed continuously for 25 min under a binocular stereoscope at 10–30× magnification, and we recorded their behaviors using an attached video camera and DVD recorder with digital video tape recorder supplying date and time data. Locations and sequences of behaviors were also noted on maps of the arenas, and these locations and sequences were verified by reviewing the recordings.

On encountering a potential host, *Aphelinus* females stop at about half their body length away from the aphid. Without touching the aphid, females sway from side to side several times with antennae extended (which we will call an approach). They then turn 180° to face away from the aphid, extend their ovipositor, and insert it into the aphid (which we will call a sting). Besides ovipositing in aphids, *Aphelinus* females may also paralyze them, make a wound with their ovipositor, and drink hemolymph from the wound (host-feeding). The aphid individuals paralyzed for feeding die, even if female parasitoids do not actually feed on them, and females do not oviposit in aphids paralyzed for feeding. We tabulated whether an aphid was approached, and if approached, whether stung or host-fed. We dissected all the aphids from a given observation period to determine whether eggs were laid. Aphids were dissected in batches of 5 on glass microscope slides, each batch placed in a small drop of Ringer's solution and covered with a separate cover slip. We counted parasitoid eggs from the dissected aphids at 40–100× magnification. From these data, we tabulated the number of aphids approached and stung, and the number of aphids fed upon or accepted for oviposition. After each observation, we also killed the female parasitoids in ethanol, dissected their ovaries in Ringer's solution mixed with neutral red stain, and counted the number of mature eggs under 40–100× magnification (neutral red does not pass through the chorion of mature eggs and thus they remain unstained).

### 2.5. Design structure and statistical analysis

The experiments on parasitism were designed as randomized complete-blocks with 10 blocks for each parasitoid species: location within rearing chamber was the blocking factor and each cage with a female parasitoid, aphids, and soybean plants was an experimental unit. The experiments on oviposition were also designed as randomized complete-blocks with 8 blocks for each parasitoid species: time of day (morning or afternoon) was the blocking factor and each cage with a female parasitoid, aphids, and soybean plant was an experimental unit. For each analysis, block was included in the initial model but was not significant and so was removed from the final model; we do not report block effects here. Because the dependent variables usually had non-normal distributions with variances proportional to means, we used generalized linear models with appropriate distributions (e.g. binomial, negative binomial or Poisson) for the dependent variable to test for effects of model factors (GLIMMIX procedure in SAS/STAT Version 9.2, copyright 2002–2006, SAS Institute Inc., Cary, NC, USA). We chose the appropriate distribution using the residual deviance divided by degrees of freedom, which should be ~1 for models that fit well (Littell, Milliken, Stroup et al., 1996). For each parasitoid species, we tested the effects of soybean lineage (Dwight versus LD02-4485), allele (resistant *Rag1* or *Rag2* versus susceptible), and their interaction on the number of aphids approached and stung, the number fed upon or accepted for oviposition, the number parasitized (i.e. mummified), the rate of adult emergence from parasitized aphids, progeny sex ratio (proportion males), and progeny size (μg dry mass). For allele effects that were significant, we compared means for resistant versus susceptible alleles within soybean lineage

using step-down simulated probability estimates, corrected for multiple comparisons. For the figures, least square means and standard errors were converted back to the original distributions, using the appropriate inverse link functions.

### 3. Results

The resistance alleles reduced parasitism of the soybean aphid by *A. glycins* (Table 1a), with each resistance allele causing a two-fold reduction in number of parasitized aphids (Fig. 1b). Soybean lineage did not affect parasitism by *A. glycins*, and allele and soybean lineage did not interact in their effects on parasitism by *A. glycins* (Table 1a). Emergence rates from parasitized aphids were high (85–98 percent) and were not affected by resistance allele, soybean lineage, or their interaction (Table 1b). Furthermore, neither progeny sex ratio (Fig. 1c; Table 1c) nor progeny size (Fig. 1d; Table 1d and e) were affected by soybean lineage, allele, or their interactions. The resistance alleles did not affect the number of aphids approached, stung, or fed upon (Table 2a–c), nor did resistance alleles affect the numbers of aphids in which *A. glycins* oviposited (Table 2d; Fig. 1a). The egg load of *A. glycins* females at the start of the behavioral observations was  $11.0 \pm 0.5$ , and females laid  $1.0 \pm 0.2$  eggs during the 25 min exposure period. At this rate, females would exhaust their egg load in about 5 h.

The effects on *A. certus* were more complex: although allele affected parasitism of soybean aphid (Table 3a), this was solely because the *Rag2* resistance allele reduced parasitism four-fold, while the *Rag1* resistance allele had no effect on parasitism (Fig. 1a). This difference gave a strong interaction between soybean lineage and allele (Table 3a). Soybean lineage affected parasitism by *A. certus* (Table 3a): parasitism was 47% higher on LD02-4485 (*Rag2*) than on Dwight (*Rag1*) (Fig. 1b). As with *A. glycins*, emergence rates from mummified aphids were high (79–100 percent) and were not affected by resistance allele, soybean lineage, or their interactions (Table 3b). However, there was a tendency towards lower emergence with the *Rag1* resistance allele compared to the susceptible allele ( $79 \pm 6$  versus  $97 \pm 3$  percent;  $P = 0.06$ ). The presence of the *Rag1* resistance allele increased the proportion of male progeny by 40 percent (Fig. 1b; Table 3c) and decreased the size of female progeny three-fold (Fig. 1c; Table 3d), despite having no

**Table 1**

Analysis of variance for effects of soybean lineage, resistance allele, and their interaction on parasitism of soybean aphid, progeny sex ratio, and progeny size for *Aphelinus glycins*.

Factor	Levels	df	F	P
(a) Parasitism (number mummified aphids)				
Lineage	(Dwight, LD02-4485)	1,32	0.7	0.42
Allele	(resistant, susceptible)	1,32	13.2	0.001
Lineage × allele		1,32	0.4	0.55
(b) Emergence rate (number of adults per mummified aphid)				
Lineage	(Dwight, LD02-4485)	1,18	2.7	0.12
Allele	(resistant, susceptible)	1,18	0.5	0.47
Lineage × allele		1,18	0.1	0.81
(c) Progeny sex ratio (proportion males)				
Lineage	(Dwight, LD02-4485)	1,14	0.4	0.53
Allele	(resistant, susceptible)	1,14	0.6	0.46
Lineage × allele		1,14	2.4	0.14
(d) Female progeny size (μg dry mass)				
Lineage	(Dwight, LD02-4485)	1,13	0.1	0.79
Allele	(resistant, susceptible)	1,13	1.5	0.24
Lineage × allele		1,13	1.7	0.21
(e) Male progeny size (μg dry mass)				
Lineage	(Dwight, LD02-4485)	1,15	1.9	0.19
Allele	(resistant, susceptible)	1,15	0.4	0.54
Lineage × allele		1,15	0.4	0.53

**Table 2**

Analysis of variance for effects of soybean lineage, resistance allele, and their interaction on number of aphids approached, stung, fed upon, and accepted for oviposition by *Aphelinus glycins*.

Factor	Levels	df	F	P
(a) Number aphids approached				
Lineage	(Dwight, LD02-4485)	1,28	1.3	0.27
Allele	(resistant, susceptible)	1,28	1.6	0.21
Lineage × allele		1,28	1.0	0.35
(b) Number aphids stung				
Lineage	(Dwight, LD02-4485)	1,28	1.3	0.26
Allele	(resistant, susceptible)	1,28	0.7	0.42
Lineage × allele		1,28	0.3	0.62
(c) Number of aphids fed upon				
Lineage	(Dwight, LD02-4485)	1,28	0.7	0.42
Allele	(resistant, susceptible)	1,28	1.5	0.22
Lineage × allele		1,28	1.0	0.33
(d) Number of aphids accepted for oviposition				
Lineage	(Dwight, LD02-4485)	1,28	2.9	0.10
Allele	(resistant, susceptible)	1,28	0.0	0.89
Lineage × allele		1,28	0.4	0.54

**Table 3**

Analysis of variance for effects of soybean lineage, resistance allele, and their interaction on parasitism of soybean aphid, progeny sex ratio, and progeny size for *Aphelinus certus*.

Factor	Levels	df	F	P
(a) Parasitism (number mummified aphids)				
Lineage	(Dwight, LD02-4485)	1,33	4.4	0.04
Allele	(resistant, susceptible)	1,33	4.0	0.05
Lineage × allele		1,33	10.7	0.003
(b) Emergence rate (number of adults per mummified aphid)				
Lineage	(Dwight, LD02-4485)	1,24	0	0.97
Allele	(resistant, susceptible)	1,24	0	0.97
Lineage × allele		1,24	0	0.98
(c) Progeny sex ratio (proportion males)				
Lineage	(Dwight, LD02-4485)	1,16	6.0	0.03
Allele	(resistant, susceptible)	1,16	4.5	0.05
Lineage × allele		1,16	2.8	0.11
(d) Female progeny size (μg dry mass)				
Lineage	(Dwight, LD02-4485)	1,12	0.5	0.50
Allele	(resistant, susceptible)	1,12	39.6	<0.0001
Lineage × allele		1,12	9.6	0.009
(e) Male progeny size (μg dry mass)				
Lineage	(Dwight, LD02-4485)	1,18	0.5	0.47
Allele	(resistant, susceptible)	1,18	0.8	0.39
Lineage × allele		1,18	0.4	0.54

effect on parasitism. The *Rag2* resistance allele did not affect proportion of male progeny (Fig. 1b; Table 2c), but did decrease the size of female progeny, although the effect size was only one-third that of the *Rag1* resistance allele (Fig. 1c; Table 2d and e). The resistance alleles did not affect the number of aphids approached, stung, or fed upon (Table 4a–c), nor did resistance alleles affect the numbers of aphids in which *A. certus* oviposited (Table 4d; Fig. 1a). The egg load of *A. certus* females at the start of the behavioral observations was  $14.2 \pm 0.8$ , and females laid  $2.8 \pm 0.3$  eggs during the 25 min exposure period. At this rate, females would exhaust their egg load in less than 3 h.

### 4. Discussion

For *A. glycins*, the results are consistent with our prediction from the effect of soybean resistance on the mortality of soybean aphids. We found no difference in approaches, stings, or oviposition for aphids on resistant versus susceptible soybean. However, we found half as many mummified aphids on resistant compared



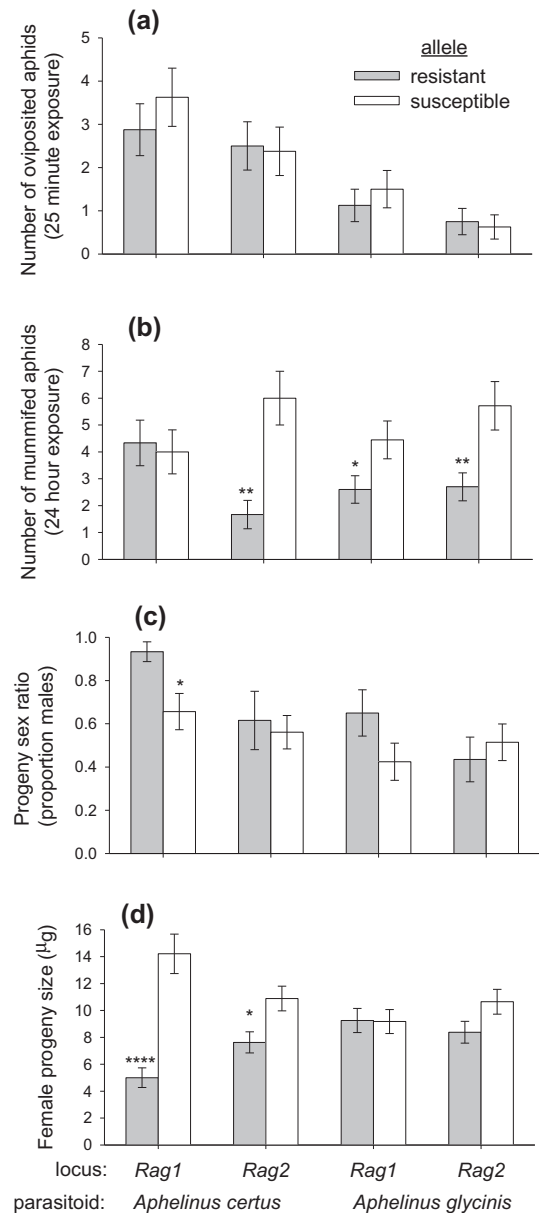
**Table 4**

Analysis of variance for effects of soybean lineage, resistance allele, and their interaction on number of aphids approach, stung, fed upon, and accepted for oviposition by *Aphelinus certus*.

Factor	Levels	df	F	P
<i>(a) Number aphids approached</i>				
Lineage	(Dwight, LD02-4485)	1,28	0.4	0.54
Allele	(resistant, susceptible)	1,28	0.2	0.70
Lineage × allele		1,28	0.4	0.54
<i>(b) Number aphids stung</i>				
Lineage	(Dwight, LD02-4485)	1,28	0.1	0.78
Allele	(resistant, susceptible)	1,28	1.4	0.24
Lineage × allele		1,28	0.1	0.78
<i>(c) Number of aphids fed upon</i>				
Lineage	(Dwight, LD02-4485)	1,28	0.3	0.56
Allele	(resistant, susceptible)	1,28	0.0	0.90
Lineage × allele		1,28	0.0	0.83
<i>(d) Number of aphids accepted for oviposition</i>				
Lineage	(Dwight, LD02-4485)	1,28	1.8	0.20
Allele	(resistant, susceptible)	1,28	0.2	0.67
Lineage × allele		1,28	0.4	0.51

to susceptible soybean, indicating that mortality of parasitoid progeny caused the difference in mummified aphids. Aphid mortality on plants with the *Rag1* resistance allele compared to susceptible plants was 50% higher at 6 days (Li et al., 2004). Given that *Aphelinus* species kill and mummify their hosts about one week after oviposition in them, this level of increased aphid mortality could account for the difference in parasitism between plants with resistant versus susceptible alleles. Because there was no impact on adult emergence rate, progeny size, or sex ratio, it appears that the effects of the *Rag1* and *Rag2* resistance alleles arise only from increased mortality, and perhaps simply from increased mortality of aphids and thus the parasitoid progeny in them.

The effect of the *Rag2* resistance allele on *A. certus* resembles that for both *Rag1* and *Rag2* resistance alleles on *A. glycinis*. However, the difference in parasitism is substantially higher (two-fold between *Rag2* resistant versus susceptible alleles for *A. glycinis*, but four-fold for *A. certus*). There is no difference in larval development time between the parasitoid species and both species of parasitoid show no differences in oviposition in aphids on plants with resistant versus susceptible alleles, so the difference in the effect on parasitism indicates that the *Rag2* resistance allele causes disproportionately high mortality of nymphs parasitized by *A. certus*. On the other hand, the lack of effect of the *Rag1* resistance allele on parasitism by *A. certus* is puzzling and suggests that aphids parasitized by *A. certus* on plants with the *Rag1* resistance allele have a disproportionately low mortality. The increased frequency of male progeny with the *Rag1* resistance allele is consistent with the reduced body size of female progeny with the *Rag1* resistance allele. If size has a larger effect on female versus male fitness, theory suggests that female parasitoids should lay fewer female eggs (i.e. fertilized) in hosts that produce smaller progeny (Charnov et al., 1981). Although the results concerning sex-dependent effects of size on fitness are mixed (Boivin, 2012), size does affect female fitness more than male fitness in many parasitoids (King, 1993), including those that parasitize aphids (Cloutier et al., 2000). However, the increased proportion of male progeny may also arise from higher mortality of female versus male parasitoids (Jarosik et al., 2003). Indeed, the somewhat lower rate of adult emergence on plants with the *Rag1* resistance allele may result from higher mortality of females. Our data do not allow us to distinguish between these hypotheses; to do this, we would need to estimate the mortality rate of progeny of mated versus unmated females (which would allow estimation of male versus female



**Fig. 1.** Effects on two aphid parasitoids, *Aphelinus certus* and *Aphelinus glycinis*, of soybean aphid resistant versus susceptible alleles for two genes (*Rag1* and *Rag2*) in soybean: (a) number of aphids in which eggs were oviposited, (b) number of soybean aphid successfully parasitized (i.e. mummified), (c) size of females among parasitoid progeny, (d) proportion males among parasitoid progeny. Error bars are standard errors; asterisks indicate the difference in means for resistant versus susceptible alleles was significant with  $P < 0.05$  (\*),  $P < 0.01$  (\*\*), or  $P < 0.0001$  (\*\*\*\*) using step-down simulated probability estimates corrected for multiple comparisons.

mortality rate) on plants with the *Rag1* resistant versus susceptible alleles.

Some of our results are similar to those found for the effect of the *Rag1* resistance allele on *A. colemani* and *B. communis*: reduced female body size (Ghising et al., 2012; Ode and Crompton, 2013) and reduced numbers of mummies (Ballman et al., 2012; Ode and Crompton, 2013). However, with the *Rag1* resistance allele, *A. glycinis* suffered no reduction in body size or adult emergence rate, and *A. certus* suffered no reduction in numbers of mummies and a marginal effect on adult emergence rate. Furthermore, unlike *A. colemani*, neither *A. glycinis* nor *A. certus* showed different encounter rates (numbers of approaches) or acceptance rates (eggs

laid per approach) on plants with either resistance allele. Beyond parasitoids, the *Rag 1* resistance allele did not affect adult size of the predators *Orius insidiosus* (Say) (Hemiptera: Anthoridae) and *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), although the latter species had lower adult longevity with the *Rag1* resistance allele (Lundgren et al., 2009). These results suggest different mechanisms for the effects of the *Rag1* resistance allele on different natural enemy groups.

Although our results do show impacts of the *Rag1* and *Rag2* resistance alleles on these *Aphelinus* species, the parasitoids are nonetheless able to parasitize the soybean aphid on soybean with these alleles, which means they may still contribute to the management of soybean aphid on resistant host plants. Indeed, aphelinids were listed among the natural enemies attacking the soybean aphid in experiments on the interaction between biological control and soybean host plant resistance (McCarville and O'Neal, 2012). In these experiments, neither biological control nor each resistance allele alone kept the soybean aphid below economic injury level, but together they provided sufficient control. The *Rag1* and *Rag2* resistance alleles together did provide sufficient control in the absence of natural enemies, although this combination is not yet commercially available. Biotypes of the soybean aphid have already been found that are not affected by the *Rag1* and *Rag2* resistance alleles (Alt and Ryan-Mahmutagic, 2013; Hill et al., 2010; Kim et al., 2008b). Natural enemies may reduce the spread of these biotypes by keeping aphid numbers low on resistant plants. If generalist predators show density-dependent predation through aggregation on prey, many of which are species not shared with specialist parasitoids, soybean plants resistant to the soybean aphid may have less intraguild predation on *Aphelinus* species than susceptible plants because the densities of aphids (both healthy and parasitized) will be lower on resistant plants. Both of these *Aphelinus* species were found on very low-density populations of the soybean aphid in China, which suggests that they should be able to find the soybean aphid at the low aphid densities on resistant soybean. Given that *A. certus* has established accidentally and *A. glycines* is now being released against the soybean aphid, we should be able to test these hypotheses in the future.

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