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Combined effects of host-plant resistance and intraguild predation on the soybean aphid parasitoid *Binodoxys communis* in the field

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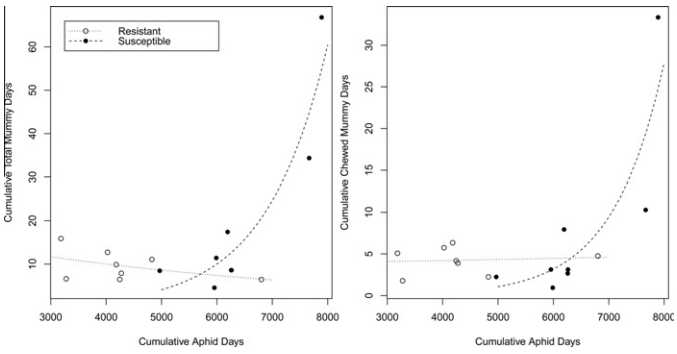
HIGHLIGHTS

- Soybean with the aphid resistance gene *Rag1* harmed parasitoid reproduction.
- *Rag1* reduced parasitoid reproduction by affecting aphid density.
- *Rag1* also reduced parasitoid reproduction through a path not caused by aphid density.
- Predators consumed more parasitoids on susceptible soybean than on *Rag1*.
- *Rag1* thus protects parasitoids from predation while reducing parasitoid reproduction.

GRAPHICAL ABSTRACT



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ABSTRACT

Soybean varieties that exhibit resistance to the soybean aphid *Aphis glycines* have been developed for use in North America. In principle, host-plant resistance to soybean aphid can influence the interactions between the soybean aphid and its natural enemies. Resistance could change the quality of soybean aphids as a food source, the availability of soybean aphids, or resistance traits could directly affect aphid predators and parasitoids. Here, we focus on the effect of soybean aphid resistance on the interactions between soybean aphids, the parasitoid *Binodoxys communis* (Hymenoptera: Braconidae), and predators of these two species. We determined whether host-plant resistance affected within-season persistence of *B. communis* by releasing parasitoids into resistant and susceptible soybean plots. We observed higher *B. communis* densities in susceptible soybean plots than in resistant plots. There were also higher overall levels of intraguild predation of *B. communis* in susceptible plots, although the per-capita risk of intraguild predation of *B. communis* was affected neither by plant genotype nor by aphid density. We discuss these effects and whether they were caused by direct effects of the resistant plants on *B. communis* or indirect effects through soybean aphid or predators.

1. Introduction

Host-plant resistance (HPR) and biological control are two important components of IPM programs. HPR can interfere with or facilitate biological control (Boethel and Eikenbary, 1986). HPR interferes with biological control if morphological or physiological HPR traits harm both the pest *and* its natural enemies. These interactions have been particularly well-documented for tomatoes, their pests, and predators and parasitoids of these pests (Kennedy, 2003). Positive (including synergistic) interactions between HPR and biological control are possible as well – but these are typically mediated by host density. If host-plant resistance to pests is incomplete, leaving lower densities of pests on resistant rather than susceptible varieties, natural enemies that are better able to suppress or stabilize low pest densities are benefited, as could occur

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with biological control agents that exhibit inversely density-dependent attack rates. This relationship between HPR and biological control was first hypothesized by van Emden and Wearing (1965) and first demonstrated experimentally by Starks et al. (1972) for partially resistant barley and sorghum, the aphid *Schizaphis graminum*, and the parasitoid *Lysiphlebus testaceipes*. A pattern of synergism between HPR and biological control has since been found in a number of systems, with much of this work involving aphids and their parasitoids (Kuo, 1986; van Emden, 1986, 1995; Gowling and van Emden, 1994; Fuentes-Contreras and Niemeyer, 2000; Kalule and Wright, 2002; Cai et al., 2009). The principle that density-dependence of natural enemy attack rates can affect how HPR and biological control interact has also been extended to transgenic insecticidal cultivars (Arpaia et al. 1997; Gould, 1994; Neuhauser et al., 2003; Heimpel et al., 2005; White and Andow, 2005).

Another way that HPR and biological can interact is through effects mediated by community-level interactions. Many pests are attacked by many species of biological control agents (Brodeur and Boivin, 2006) that interact and have varying responses to the density of the pest. For example, intraguild predation (IGP) occurs when a pest has a natural enemy (the intraguild predator) that eats another natural enemy of the pest (the intraguild prey) (Rosenheim et al., 1995). IGP and HPR can interact in at least two ways to affect pest densities. If the attack rate of the IG predator is positively density dependent, then HPR should reduce the strength of IGP by reducing pest densities, allowing the IG prey to be more effective. Alternatively, if the attack rate of IG predators is inversely density dependent, then HPR that reduces pest densities may increase the strength of IGP, reducing the effectiveness of the IG prey. Our study examined how HPR against the soybean aphid, *Aphis glycines*, affects reproduction and IGP of one of its parasitoids, *Binodoxys communis*, by a guild of intraguild predators that tend to exhibit positively density-dependent attack rates (Donaldson et al., 2007; Chacón and Heimpel, 2010; but see Noma et al., 2010). Based upon previous field experiments (Chacón and Heimpel, 2010), we hypothesized that reduced aphid densities in resistant soybean plots would reduce aggregation of the intraguild predators, resulting in a lowered risk of IGP to establishing parasitoids.

The soybean aphid has emerged as the most serious arthropod pest of soybeans in North America since its introduction from Asia (Ragsdale et al., 2011). Anthropogenic control is primarily by insecticides, but genotypes resistant to soybean aphid have been developed and started to be commercialized in 2010. In particular, cultivars Jackson and Dowling are resistant to soybean aphid (Diaz-Montano et al., 2006) via the *Rag1* gene (Hill et al., 2004, 2006; Li et al., 2008). Compared to more susceptible soybean genotypes, soybean aphids disfavor (Hesler and Dashiell, 2008), reproduce less upon (Hesler et al., 2007), and feed less efficiently on soybeans bearing *Rag1* (Diaz-Montano et al., 2007; Crompton and Ode, 2010). In addition to HPR, a classical biological control program has recently been implemented for soybean aphid in North America (Heimpel et al., 2004, 2010). The solitary endoparasitoid *B. communis* (Hymenoptera: Braconidae) is native to China and is being released in the northern US in an effort to control soybean aphid (Heimpel et al., 2010; Ragsdale et al., 2011). *B. communis* was recently shown to produce fewer mummies when reared on aphids on *Rag1* soybean. These mummies emerge less often and more slowly and produce adults with smaller metatibiae (Ghising, 2011).

Soybean aphid parasitoids are susceptible to IGP by aphidophagous arthropods that feed on parasitized aphids (Costamagna et al., 2007, 2008; Chacón et al., 2008). This IGP increases on plants with more aphids because of increased predator aggregation (Chacón and Heimpel, 2010). Here, we explore how soybean aphid resistance affects aphid and predator densities, and how these effects interact to influence within-season *B. communis* persistence within soybean fields.

2. Materials and methods

2.1. Plot establishment

We conducted the experiment in a 0.8 ha soybean field on the University of Minnesota experimental station in St. Paul, MN, USA, during summer 2009. The soil was treated with the herbicide Trust (generic Treflan) @ 32 oz/acre, and Pursuit at 1.44 oz/acre on May 12, 2009 to kill sprouting weeds. On 14 May 2009, we planted 16 plots of soybean. Eight of these plots received soybean aphid-resistant seeds containing the *Rag1* gene (var. LD05-16060) and the other eight plots received related, near-isoline soybean seeds not containing the *Rag1* gene (var. SD01-76R). We arranged the plots so that the plant genotypes alternated in each of two rows of eight plots. The dimensions of each plot were 13 × 13 m and each plot contained 16 soybean rows separated by 75 cm of bare ground. Soybean seeds were planted at a rate of approximately 300,000 seeds per hectare. Plots were separated by 10 m of unplanted space. We weeded within each plot by hand throughout the season.

2.2. B. communis release

2.2.1. Soybean aphid rearing to support B. communis

We reared *B. communis* intended for release on laboratory colonies of soybean aphid, which originated from Minnesota-caught apterae in 2008. We maintained aphid colonies on mixed-age potted soybean plants (var. NKS19R5) in plastic-fronted mesh cages (47.5 × 47.5 × 47.5 cm bugdorms from MegaView Science Education Services, Taiwan). Each week, we removed older, heavily infested soybeans from the colony cages and replaced them with younger, uninfested plants.

2.2.2. B. communis rearing

B. communis is a solitary endoparasitoid of soybean aphid originating from East Asia (Desneux et al., 2009a). Females lay a single or rarely two eggs in a host, and the resulting larvae feed internally. Approximately 7 days after oviposition, the host aphid is killed and *B. communis* initiates pupation inside the dead aphid exoskeleton (called a "mummy"). The entire life cycles takes approximately 11–14 days at 25 °C, depending on the instar attacked (Wyckhuys et al., 2008a; Asplen et al., in press). We used *B. communis* reared from laboratory colonies initiated from collections in Harbin, China, in 2002 (see Wyckhuys et al., 2008a for collection details). *B. communis* were reared in cages identical to the ones described above in a greenhouse under natural sunlight supplemented by halogen lamps on overcast days. We used new plants and aphids for each *B. communis* generation and initiated each rearing cage by adding between nine and 16 square pots (5.1 cm diagonal), with each pot supporting 2–4 soybean aphid-infested soybean plants. We then aspirated 15–50 adult *B. communis* from older cages into the new rearing cages. We repeated this process every 12–14 days.

2.2.3. Release methods

We released *B. communis* in the mummy stage. To prepare mummies for release, we first cut soybean plants at the stem from 10-day-old rearing cages (when most mummies had formed but before most adult parasitoid emergence). We divided the plants from each cage equally into sets destined for field plots. From each cage, we counted the number of mummies present on all plants from three pots, noting the number of plants in each sample. This gave us an estimate of the per-plant mummy density from each cage, which we used to estimate the total number of mummies released into the field.

To release the *B. communis* mummies into the field, we created release containers out of 30 quart, shallow, rectangular plastic

tubs. We modified the tubs by cutting away most of the lid and the long sides, and hot-gluing wide (2 mm) mesh over the gaps. This mesh allowed *B. communis* emigration from the release containers but prevented entrance of large predators. Before we filled release containers with *B. communis* mummies, we covered the coarse mesh with fine mesh (<0.1 mm) (No-See-Um Mesh, Quest Outfitters, Sarasota, FL) that prevented *B. communis* emigration. We removed this fine mesh once we placed the release containers into field plots.

To prevent excess handling and to allow emerging *B. communis* to immediately experience the soybean plant they were reared upon, mummies were left on their cut soybean plants during release. We endeavored to reduce the density of unparasitized aphids within release containers in order to reduce attraction of predators and retention of emerged *B. communis* within the containers. This was done by placing six pots containing 2–4 living soybean plants each into each release container prior to adding the cut soybean plants with *B. communis* mummies. We placed the cut sets of soybean plants with mummies into the release containers atop the living soybean plants. This setup was left with the containers closed for 24 h, over which time the cut plants began to desiccate and unparasitized aphids moved onto the living plants below them. During the next morning – the day of the release – we removed the living plants and their pots from the release containers.

We released *B. communis* over three consecutive days to prevent potential release failure due to either isolated weather events or short-term underrepresentation of preferred host stages (Wyckhuys et al., 2008a; Desneux et al., 2009b; Asplen et al., in press). On the first release day, we placed a release container containing its set of desiccated plants with mummies into the center of each field plot. We emptied the contents of release containers for the second and third days of release into the first-day release containers already in the field.

In order to have sufficient aphid abundance in the field for parasitoid persistence as well as sufficient differences in aphid density between plant variety treatments, we used a threshold of five aphids per plant in resistant plots for releasing *B. communis* mummies, coupled with a requirement of statistically significantly greater densities in susceptible than resistant plots. This set of conditions was first observed on 26 June 2009 (see Section 3), when plants had an average of 3.4 fully opened trifoliates. We began the release on 29 June 2009 and continued for the next two days. The release containers were removed from the field on 8 July 2009, at which point all mummies that would emerge should have done so.

Based upon our sub-sampling protocol, we estimate that we released approximately 76,400 mummies in total over the three-day period. Each plot received approximately 1730 mummies on 29 June 2009, 1000 mummies on 30 June 2009, and 2050 mummies on 1 July 2009, totaling approximately 4780 mummies released per plot. Sex ratio of released parasitoids was not estimated.

2.3. Sampling

We were interested in determining how resistant soybeans affected aphid density, parasitism by *B. communis*, predation of *B. communis* mummies, and predator density and diversity. Thus, we monitored each of these variables weekly using non-destructive visual counts on at least 16 plants per plot starting in early June. We distinguished between apterous and alate soybean aphids. We identified adult and late larval coccinellids to species and most other predators to family. Non-feeding stages of predators were not included. When we observed a parasitoid mummy, we used 10× hand lenses to determine whether the mummy was whole, had been chewed by predators, or whether the mummy showed evidence of successful parasitoid emergence (Chacón and Heimpel, 2010). We collected whole mummies into 1.5 ml microcentrifuge tubes and brought the mummies back to the laboratory to determine which parasitoid species emerged. If no emergence occurred, we dissected the mummies for species identification. Finally, we measured the length of one hind tibia of adult parasitoids reared from field-collected mummies to determine whether parasitoid size was affected by plant genotype. We also recorded the number of fully opened trifoliates on observed plants to determine if this was affected by plant genotype. All sampling continued until 10 September 2009, at which point the plants in all plots were senescing.

2.4. An outplant experiment

Measures of the predation of *B. communis* mummies using counts of chewed mummies would underestimate the importance of intraguild predation if mummies are fully consumed by predators. We therefore performed a separate study within our field experiment to assess the rate at which *B. communis* mummies are both chewed and lost from plants in the field. Specifically, we brought potted soybean plants that were manipulated to contain a set number of *B. communis* mummies and soybean aphids into the field plots and followed their fate.

We prepared outplants in the greenhouse, beginning with single soybean plants in 15.25 cm (diameter) round pots. We reared 20 *B. communis* mummies on these plants at the V2 stage using methods described by Chacón and Heimpel (2010) and added aphids to each plant once mummies had formed. Aphid densities on the potted plants were adjusted to match field conditions as we were interested in the influence of current aphid density in the field on mummy predation. Thus, we added aphids to outplants to match the average per-plot aphid densities that we had observed on the most recent sampling date. Outplants were placed into the field and sampled by observing mummies and predators with a 10× lens, coupled with daily removal of emerged or chewed mummies to prevent resampling. Pots were buried in the soil so that the edge of the pot was flush with the soil (as in Chacón and Heimpel, 2010). A single pot was deployed per plot midway between the two middle rows of soybeans on five different dates, starting on the same day *B. communis* was released, 29 June 2009, and then also on 7 July 2009, 16 July 2009, 14 August 2009, and 1 September 2009.

2.5. Statistical analyses

2.5.1. Aphid density and predators at time of release

To decide when to release *B. communis*, we conducted *t*-tests comparing aphid density in the different plant genotypes within single sampling dates. Later, we determined whether predator densities and the predator: aphid ratio differed by plant genotype during the release period. To compare predator densities during parasitoid release, we averaged the per-plant predator density on 26 June and 2 July in each plot and then compared predator density between plant genotypes using a *t*-test on average plot predator densities. To compare the predator: aphid ratios, we averaged the per-plant predator densities on 26 June and 2 July for each plot, divided this by the average per-plant aphid densities on 26 June and 2 July, and then compared the ratios between plot types using a *t*-test.

2.5.2. Initial B. communis reproduction

We were interested in two effects of plant genotype on *B. communis*: how plant genotype affected first-generation reproduction, and how the rest of within-season *B. communis* persistence was affected by plant genotype after the released wasps reproduced. We released *B. communis* starting on 29 June 2009. Based upon the development time of *B. communis*, mummies found between 5 and 10 July 2009 should represent the first generation of offspring

of the released parasitoids (Wyckhuys et al., 2008a). Mummies after this time period could still represent the first generation since female parasitoids can live for a week or more depending on sugar availability (Wyckhuys et al., 2008b). However, *B. communis* mummies sampled on or after 22 July likely represent the second generation. We summed the counts of mummies on all plants on 8 July 2009 and 15 July 2009 within each plot (separately for total mummies, chewed mummies, and the ratio of chewed mummies to total mummies). Then, because we were using count data, we used a generalized linear model with a quasi-Poisson distribution to test how plant genotype and aphid counts affected mummy counts. Slightly different numbers of plants were observed in each plot; thus we offset our analyses by the number of plants observed.

Two of our outplant studies coincided with the initial release and reproduction of *B. communis* (outplants started on 6/29 and 7/7). We analyzed the effects of plant genotype and the aphid density in the plots receiving the outplants on the rate of mummy predation on outplants by ANOVA on arcsin-square root-transformed rate of mummy predation data. The start date of these outplants was tested as a blocking factor in the initial model but was removed due to non-significance (data not shown).

2.5.3. B. communis through the remainder of the season

We were interested in the factors impacting *B. communis* persistence after its initial reproduction in the field. To analyze this, we generated cumulative measurements from our sampling data. The cumulative measurements were determined by

$$\sum_{n=1}^{n} \frac{(x_n + x_{n+1})}{2} \times (d_{n+1} - d_n)$$

where n = the sampling date, x_n = mummy density at time n, and d_n = the number of days elapsed since the start of the experiment. This measure captures insect density variation over time and represents a measure of the area under the population curve (Ruppel, 1983; Ragsdale et al., 2007). We created cumulative measures for aphid density, mummy density (both total mummies and chewed mummies only), and total predator density. These measurements include all sample dates after 15 July 2009, when the first *B. communis* field generation should have been over. We refer to the cumulative measurements as "cumulative (aphid, mummy or predator) days" for the remainder of the paper. Cumulative mummy days will be further specified as either "cumulative total mummy days" for whole, chewed and emerged mummies or "cumulative chewed mummy days" for chewed mummies only. Analyses on mummies did not distinguish by parasitoid species.

We used a generalized linear model with a quasi-Poisson distribution to test how cumulative total mummy days were affected by plant genotype, cumulative aphid days, initial parasitoid reproduction (average of the mean total mummy density on 8 July 2009 and 15 July 2009), the predator/aphid ratio (cumulative predator days/cumulative aphid days), and the interaction of plant genotype with the other variables. In addition, we did a similar test using cumulative chewed mummy days as the response variable. Finally, we tested how cumulative predator days were affected by plant genotype, cumulative aphid days, and the interaction of these two variables.

Three of our outplant studies took place after the first generation of *B. communis* (outplants started 7/16, 8/14, and 9/1). We analyzed the effects of plant genotype and the current aphid density in the plot receiving the outplant on the rate of mummy predation on these outplants as in the initial *B. communis* reproduction outplant analysis above.

2.5.4. Other tests

To determine how plant genotype affected aphid density throughout the season, we conducted a repeated-measures ANOVA on ln(aphids + 1) using plant genotype as the between-plots variable and time as the within-plots variable. To see if plant genotype affected parasitoid size, we did a multi-factor ANOVA testing for main effects and first-order interactions of plant genotype, parasitoid species and sex on the hind tibia length of collected parasitoids. Finally, we conducted a similar test using the average number of fully opened trifoliates per plot as the response variable to determine if plant genotype affected plant size over the season.

3. Results

3.1. Soybean aphid and predator densities over time and predator diversity

There were significantly fewer soybean aphids on resistant than on susceptible plants over the season (Repeated-measures ANOVA: Genotype $F_{(1,13.93)} = 23.92$, $P = 0.0002$; Fig. 1A). Date significantly influenced aphid densities and interacted with plant genotype in a way that resulted in similar aphid densities in resistant and susceptible plots during their population peak (Date $F_{(13,180.10)} = 60.00$, $P < 0.0001$; Genotype∗Date $F_{(13,180.10)} = 2.57$, $P = 0.003$). Aphid densities reached the economic threshold level of 273 aphids per plant (Ragsdale et al., 2007) at their peak but declined thereafter.

Over the course of the season we observed 2209 active predators (i.e. excluding eggs, pupae, or non-predatory adults), including 901 in resistant plots and 1308 in susceptible plots (see Table 1 for identification details, Fig. 2B for predator densities over time and Fig. 2C for the predator/aphid ratio over time).

3.2. Initial parasitoid reproduction

The mean soybean aphid density was significantly higher in susceptible plots than in resistant plots on 26 June 2009 and only slightly below five aphids per plant in resistant plots, so we began the release. Aphid densities were significantly higher in susceptible plots throughout the release phase (between 26 June and 2 July) ($t_8 = 2.6$, $P = 0.01$; Fig. 1A). Predator densities were also higher during the release phase in susceptible plots ($t_8 = 2.0$, $P = 0.04$; Fig. 1B), although the predator:aphid ratio did not differ significantly between resistant and susceptible plots during this period ($t_8 = 0.96$, $P = 0.36$; Fig. 1C).

There were more total parasitoid mummies (whole, chewed, and emerged) in susceptible plots than in resistant plots on dates that corresponded to the first *B. communis* generation (July 8 and July 15, Fig. 2A, B). However, plant genotype did not have a significant effect in the generalized linear model ($t_{(13)} = 0.14$, $P = 0.89$). Instead, aphid counts (which were themselves significantly affected by plant genotype in a separate analysis) positively correlated with total mummies ($t_{(13)} = 5.34$, $P < 0.001$). Plant genotype and aphid counts did not interact in this analysis, so the interaction term was removed for the final analysis. This was also true for the following two analyses.

We observed more chewed mummies in susceptible plots; again, in a generalized linear model this effect was significantly correlated to aphid density but not to plant genotype (genotype: $t_{(13)} = 1.3$, $P = 0.21$, Fig. 2C; aphid density: ($t_{(13)} = 2.22$, $P = 0.04$), Fig. 2D). Finally, the rate of IGP (chewed mummies/total mummies) was not significantly affected by genotype or by aphid density during the first generation of *B. communis* (genotype: $t_{(10)} = 1.16$, $P = 0.28$, Fig. 2E; aphid density: ($t_{(10)} = 0.71$, $P = 0.49$), Fig. 2F).

The rate of IGP on outplants neither differed significantly between resistant and susceptible plots ($F_{(1,26)} = 0.9$, $P = 0.35$) nor was it affected by the aphid density in the plots ($F_{(1,26)} = 0.7$, $P = 0.41$) over the dates corresponding to the first generation of *B. communis*.

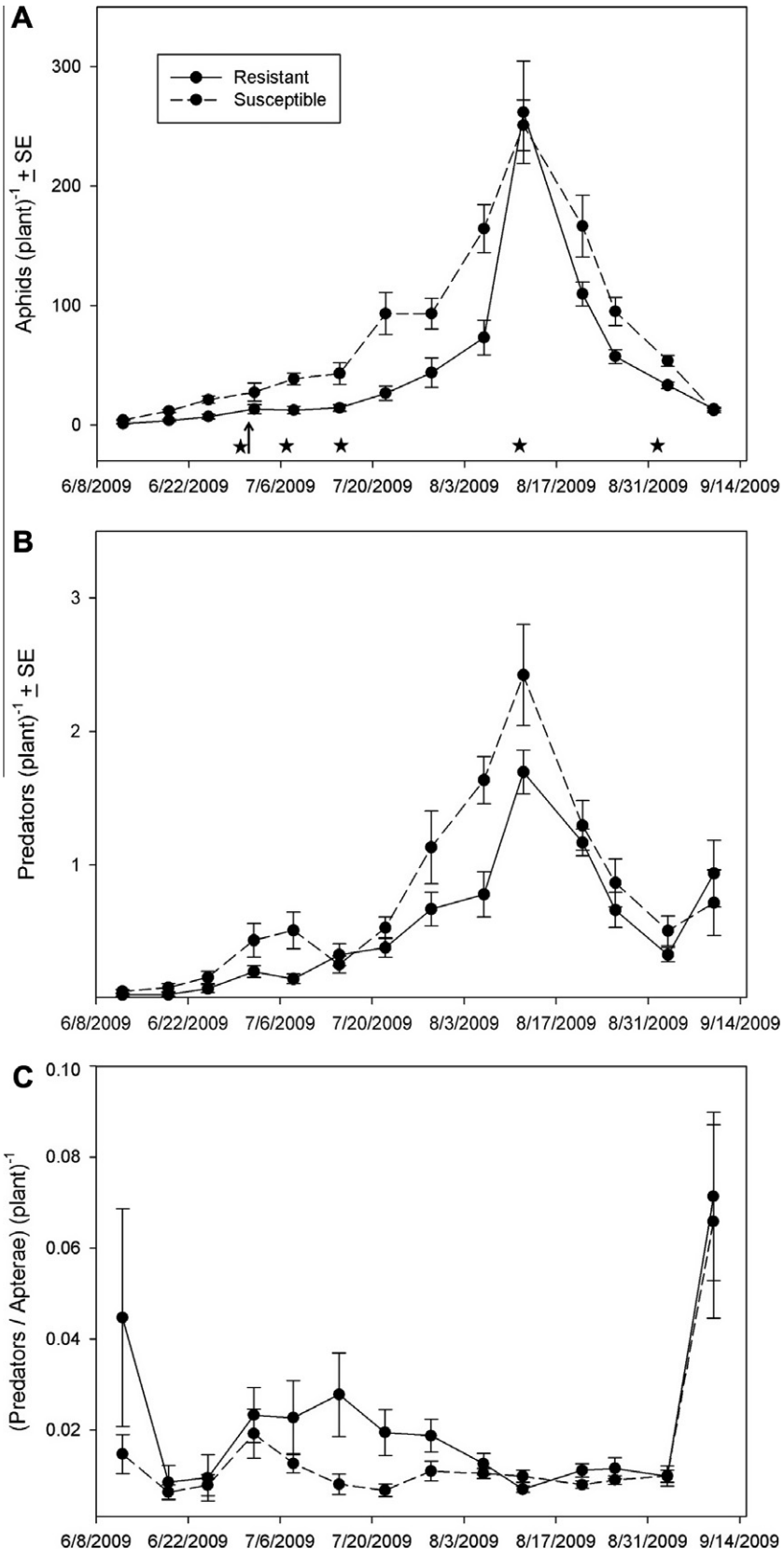

Fig. 1. Time series of the soybean-growing season in experimental plots: (A) average aphid densities, (B) feeding-stage predator densities, and (C) the feeding-stage predator:aphid ratio. The arrow in (A) indicates the first day of *Binodoxys communis* release and the stars indicate when outplants were placed into field plots.

Table 1
The identity and number of predators observed on plants in resistant and susceptible plots summed over the entire experiment. Taxa are listed in order of descending number of observations.

Taxon	Diptera larvae	*Orius insidiosus* adults	*Orius insidiosus* nymphs	Coccinellidae 1st and 2nd instars	*Harmonia axyridis* adults	*Aeolothrips*sp.	*Harmonia axyridis* 3rd and 4th instars	Hemerobiidae larvae
Resistant plots	273	201	148	98	54	42	28	13
Susceptible plots	519	191	160	148	96	61	49	14
Total	792	392	308	246	150	103	77	27
	Araneae	*Hippodamia convergens* adults	*Coccinella septempunctata*	*Chrysoperla* sp.	*Hippodamia tredecempunctata* adults	*Nabis* sp.	*Hippodamia variegata*	*Coleomegilla maculata*
Resistant plots	15	9	5	2	6	2	1	1
Susceptible plots	11	12	12	11	2	4	0	0
Total	26	21	17	13	8	6	1	1

3.3. B. communis, predators and outplants through the remainder of the season

We investigated factors influencing cumulative total mummy days after the initial establishing generation (i.e., following 15 July) using a generalized linear model including the explanatory variables of plant genotype, cumulative aphid days, the predator: prey ratio, and the initial level of parasitoid reproduction, as well as first-order interaction terms between plant genotype and the other variables. The initial level of parasitoid reproduction was not explanatory in any analysis so was removed for the final model. Unlike the effect on initial parasitoid reproduction, the resistant plant genotype significantly reduced cumulative total mummy days over the remainder of the season ($t_{(10)} = 3.76$, $P = 0.004$). While there was not a main effect of cumulative aphid days on cumulative total mummy days, cumulative aphid days did interact with plant genotype (main effect of cumulative aphid days: $t_{(10)} = 0.72$, $P = 0.487$, interaction: $t_{(10)} = 2.47$, $P = 0.03$). Cumulative aphid days in the susceptible genotype had a positive relationship with cumulative total mummy days; no such relationship was evident in the resistant genotype (Fig. 3A). A similar pattern was observed between plant genotype and the predator:aphid ratio; there was no main effect but the predator: aphid ratio interacted with plant genotype (main effect: $t_{(10)} = 0.10$, $P = 0.92$; interaction: $t_{(10)} = 2.47$, $P = 0.03$) (Fig. 3B).

The generalized linear model assessing IGP (measured as cumulative chewed mummy days) did not find significant effects of the predator:aphid ratio on IGP, so this term was removed from the final model. Beyond that, the results for the effects of plant genotype and cumulative aphid days were similar to the effects above, on cumulative total mummy days. The resistant plant genotype significantly reduced IGP ($t_{(12)} = 3.91$, $P = 0.002$) and while there was no main effect of cumulative aphid days ($t_{(12)} = 0.15$, $P = 0.886$), these variables interacted: there was a positive relationship between cumulative aphid days and IGP in susceptible plots but not resistant plots ($t_{(12)} = 3.74$, $P = 0.003$) (Fig. 3C).

Cumulative aphid days and plant genotype affected cumulative predator days as in the analyses presented above. Cumulative predator days were significantly affected by plant genotype as a main effect ($t_{(12)} = 2.85$, $P = 0.015$). While cumulative predator days did not correlate with cumulative aphid days as a main effect ($t_{(12)} = 0.19$, $P = 0.85$), cumulative aphid days interacted with plant genotype to reflect a greater slope in susceptible plots than resistant plots ($t_{(12)} = 3.20$, $P = 0.008$) (Fig. 3D).

We examined predation of mummies on outplants initiated on 16 July, 14 August, and 1 September using ANOVA. The soybean genotype of the plot in which outplants were placed did not significantly affect the proportion of chewed mummies on outplants ($F_{(1,41)} = 0.91$, $P = 0.34$) although there was a significant, but weak, negative correlation between aphid density in the plot and the proportion of mummies that were chewed on outplants ($F_{(1,41)} = 4.2$, $P = 0.05$, slope = -0.0001, $R^2 = 0.007$). However, the proportion of mummies on outplants that were chewed or that disappeared from the outplants was greater in susceptible than in resistant plots ($F_{(1,41)} = 14.89$, $P < 0.001$; Fig. 4). This proportion was not significantly correlated to aphid density in the plot ($F_{(1,41)} = 3.69$, $P = 0.062$).

We collected 190 mummies from the field that were unemerged and unchewed, 57 of which were collected from resistant plots and 133 from susceptible plots. Of these, 114 produced identifiable parasitoids and 99 had measurable hind tibiae (24 from resistant plots, 75 from susceptible plots). We show the number, sex, and species of the parasitoids reared or dissected from collected mummies in Table 2. In a multi-factor ANOVA assessing whether plant genotype, sex of the identified parasitoid, or species of the identified parasitoid affected hind tibia length, only species had a significant effect (plant genotype: $F_{(1,71)} = 0.40$, $P = 0.53$; parasitoid sex: $F_{(1,71)} = 2.20$, $P = 0.14$, parasitoid species: $F_{(3,71)} = 21.47$, $P < 0.0001$).

3.4. Plant size

In a repeated-measures analysis examining soybean plant size in terms of the number of leaves per plant, plant size increased significantly with date ($F_{(13,180)} = 65.40$, $P < 0.0001$), but was not significantly affected by genotype ($F_{(1,13.96)}=1.22$, $P = 0.29$) or the interaction of these effects ($F_{(13,180)} = 1.68$, $P = 0.07$).

4. Discussion

Within-season persistence of the released soybean aphid parasitoid *B. communis* was better in susceptible plots, where aphid density was almost always higher. When we analyzed *B. communis* populations using generalized linear models, whether plant genotype or aphid density was responsible for the effect on *B. communis* persistence depended on whether we were examining *B. communis'* first generation or every generation thereafter. The initial generation was affected by aphid density rather than by plant genotype. Later generations were affected primarily by plant genotype although this interacted with aphid density such that *B. communis* populations scaled with aphid densities in susceptible plots but not in resistant plots. A possible interpretation of these results is that initially, plant genotype had no direct effect on *B. communis,* possibly because the released organisms were reared on susceptible plants, but that as they continued to reproduce a toxic effect of the *Rag1* resistant plants started to affect the wasps

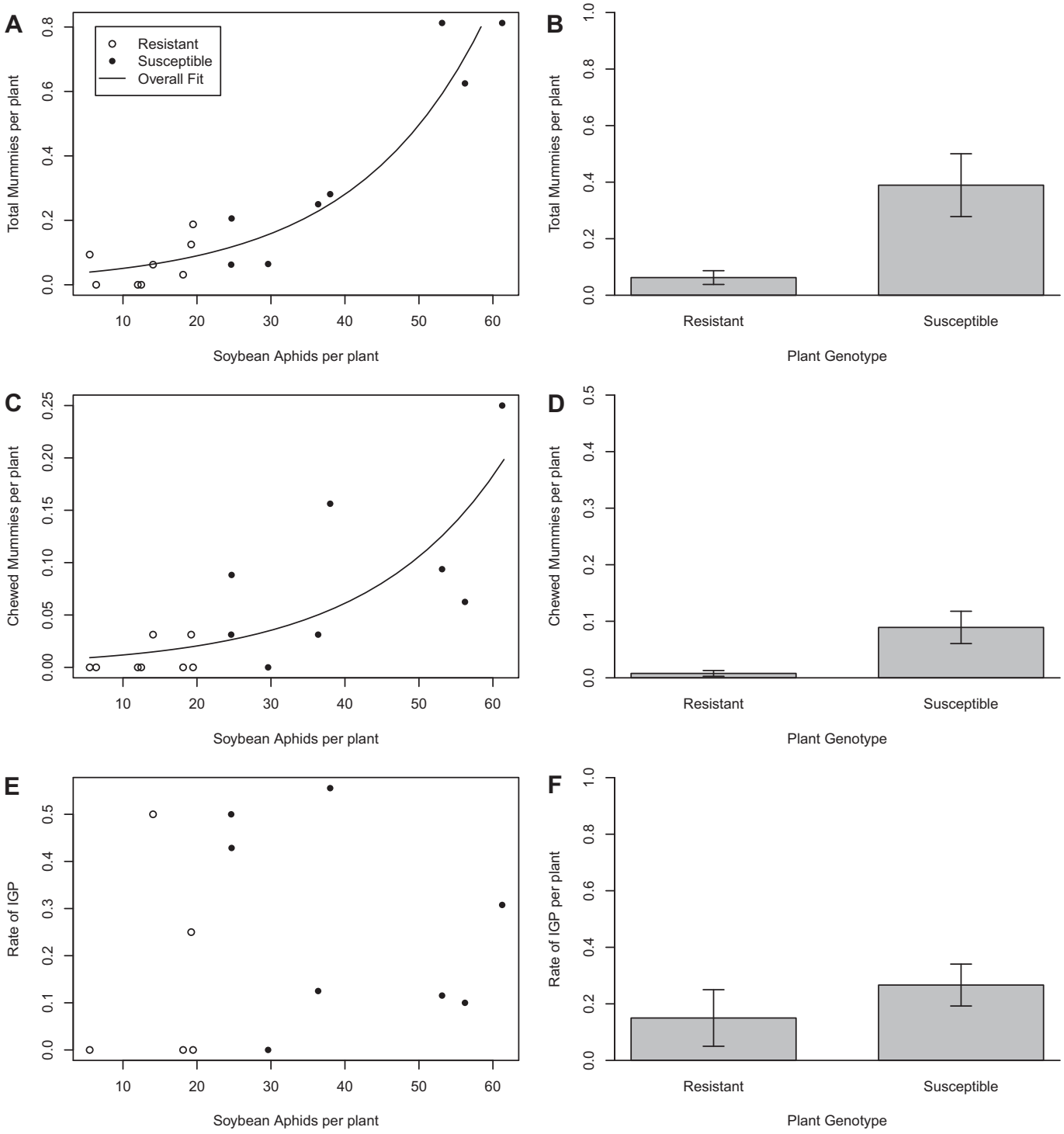

Fig. 2. First generation *B. communis* reproduction and predation of parasitoid mummies as predicted by aphid density and plant genotype. All graphs show data on a per-plant basis, bar graphs are densities ± SE. The fitted lines show significant relationships. None of the relationships depicted with bar graphs were statistically significant. (A) Total mummies per plant (including whole, chewed and emerged mummies) from the resistant and susceptible plots as a function of the number of soybean aphids per plant. (B) Data in (A) expressed as a function of plot type only. (C) The number of chewed mummies per plant as a function of the number of soybean aphids per plant in resistant and susceptible plots. (D) Data in (C) expressed as a function of plot type only. (E) The rate of IGP (chewed mummies/total mummies) per plant in resistant and susceptible plots. (F) Data in (E) expressed as a function of plot type only.

(Ghising, 2011). This explanation is consistent with the effects of plant genotype and aphid density on predator populations, which also appeared to be affected by plant genotype and only scaled with aphid density in susceptible plots.

However, other evidence points to aphid density, rather than an effect of *Rag1* proteins, causing the different densities in resistant vs. susceptible plots. First, the parasitoids we collected from the different plots did not differ in hind tibia length, a proxy of potential fitness for parasitoids in general (Godfray, 1994) and *B. communis* specifically (Dieckhoff and Heimpel, 2010). However, there may be subtle fitness effects at the population level resulting from an effect of the *Rag1* gene that are not captured by hind tibia length

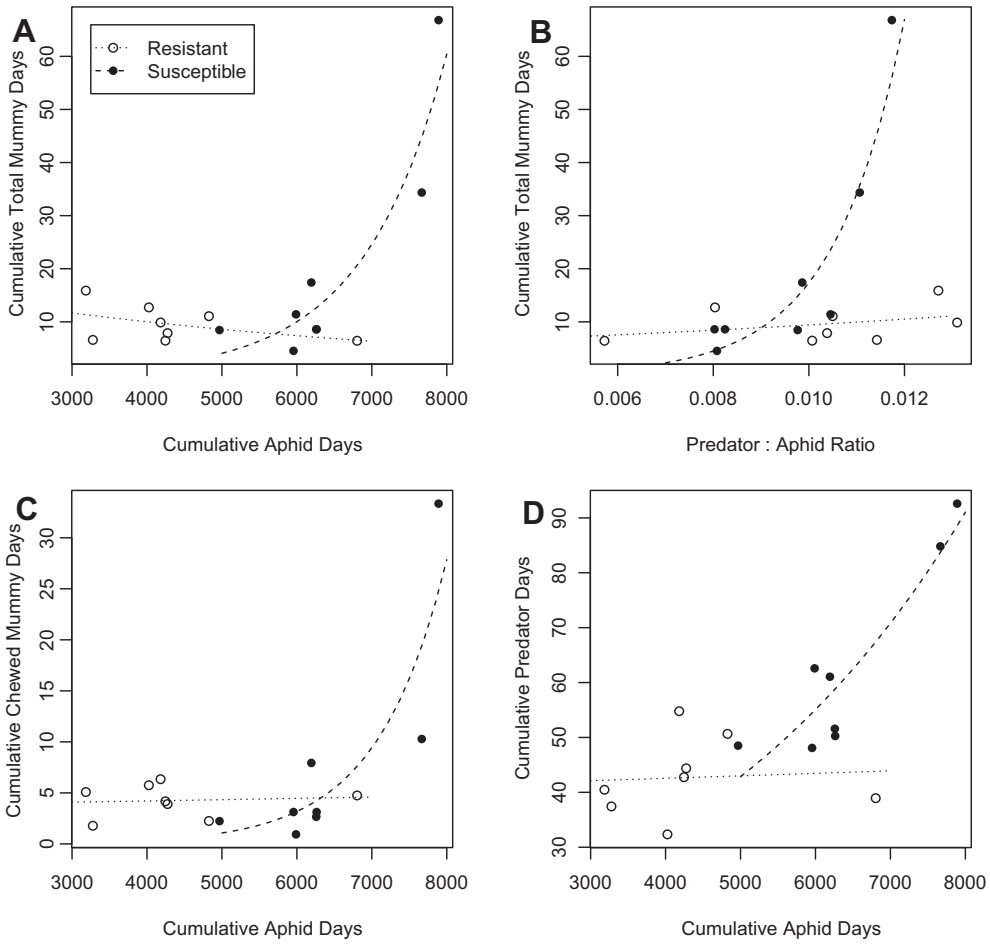

Fig. 3. Relationships among cumulative variables measured after initial *B. communis* reproduction. All graphs show data on a per-plant basis, bar graphs are densities ± SE. Fitted lines show significant relationships. (A) Cumulative total (whole, chewed, and emerged) mummy days from resistant and susceptible plots as a function of cumulative aphid days. (B) Cumulative total (whole, chewed, and emerged) mummy days from resistant and susceptible plots as a function of the predator:aphid ratio. (C) Cumulative chewed mummy days from resistant and susceptible plots as a function of cumulative aphid days. (D) Cumulative predator days from resistant and susceptible plots as a function of cumulative aphid days.

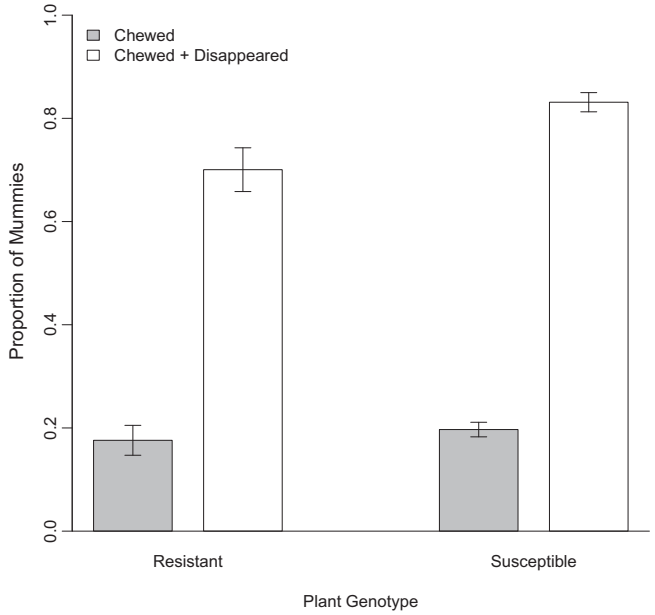

Fig. 4. Predation and predation + complete disappearance of lab-reared *B. communis* mummies on susceptible plants placed into resistant or susceptible fields. The data include outplant experiments started on three separate dates, indicated in Fig. 1 by the last three stars. The data are means ± SE.

data (Rochat, 1997). Additionally, a recent laboratory study did find negative fitness effects resulting from rearing *B. communis* on soybean aphid on *Rag1* soybean, including a difference in metatibia length (Ghising, 2011). Second, while our statistical models point to plant genotype as the cause of reduced *B. communis* populations after their initial reproduction, plant genotype also caused lower aphid densities, potentially confounding these variables. One method of disentangling direct vs. indirect effects in complex systems is to use path analysis with model selection alongside traditional statistics (e.g. Eubanks, 2001, reviewed by Strauss and Irwin, 2004). Unfortunately, accurate path analysis requires larger sample sizes than we had, especially when including a categorical variable. Future experiments using smaller plots to allow for larger samples may take advantage of path analysis. To conclude, we have evidence for both an effect of *Rag1* host-plant resistance on *B. communis* that may be either indirect through aphid toxicity or direct through plant quality effects on the parasitoids (see below) and an indirect effect through aphid density.

Our prediction was that higher aphid densities would increase the risk of IGP of *B. communis* because of density-dependent intraguild predator aggregation. We previously documented this outcome at the per-plant scale in the field (Chacón and Heimpel, 2010), and it is a general pattern predicted by shared predation models (Harmon and Andow, 2004). While the overall frequency of IGP was indeed higher in susceptible plots where aphid density

Table 2
The numbers of identifiable parasitoids reared or dissected from field mummy collections. The number in the first parenthesis is the sex ratio (proportion male) and the number in the second parenthesis is the proportion of that species compared to that row total. *B. communis*, *L. testaceipes*, and *Aphidius* sp. are primary parasitoids. Alloxystinae (Hymenoptera: Eucoilidae), Asaphinae (Hymenoptera: Pteromalidae), and Encyrtidae (Hymenoptera) are hyperparasitoids. The bottom row is the summed data from resistant and susceptible plots. One *Aphidius* sp. and one *Binodoxys communis* were not able to be sexed. Hyperparasitoids were not sexed (x). The unidentified hyperparasitoids were lost between initial identification as hyperparasitoids and subsequent, more specific identification.

Plant genotype	Parasitoid species			
	Binodoxys communis	*Lysiphlebus testaceipes*	*Aphidius* sp.	Alloxystinae
Resistant	6 (0.17) (0.21)	9 (0.67) (0.31)	8 (0.29) (0.28)	3 (x) (0.10)
Susceptible	44 (0.37) (0.52)	18 (0.28) (0.21)	2 (0.00) (0.02)	9 (x) (0.11)
Overall	50 (0.35) (0.44)	27 (0.41) (0.24)	10 (0.22) (0.09)	12 (x) (0.11)
	Asaphinae	Encyrtidae	Unidentified hyperparasitoids	Total
Resistant	2 (x) (0.07)	1 (x) (0.03)	0 (x) (0)	29 (0.41) (x)
Susceptible	7 (x) (0.08)	2 (x) (0.02)	3 (x) (0.04)	85 (0.33) (x)
Overall	9 (x) (0.08)	3 (x) (0.03)	3 (x) (0.03)	114 (0.34) (x)

was higher, the per-capita risk of predation on mummies did not differ significantly with aphid density or with plant genotype. Our previous study did show a per-capita increase in IGP with aphid density (Chacón and Heimpel, 2010). The difference between the outcomes of these two studies may have been related to spatial scale. The current study was done at a substantially larger scale and it is possible that predators are better able to respond to prey density at the level of single plants than larger plots separated by bare soil. Measuring predator movement between plots and how this interacts with prey density could help clarify how scale affected our results (Harmon and Andow, 2004). Another difference between the two studies was that the aphid densities between susceptible and resistant plots over the course of the current study did not differ as much as did the aphid densities between outplants in the earlier study (2 vs. 20 vs. 200) (see Fig. 1A). Thus, the current study may not have generated great enough differences to elicit strong predator responses.

Alternatively, the lack of an aphid-density-dependent effect on IGP may have been due to direct effects of *Rag1* on parasitoids and predators masking aphid-density-dependent effects. The mummies in our outplant study experienced greater per-capita predation when placed in susceptible plots compared to resistant plots, consistent with our predictions (Chacón and Heimpel 2010). This effect came despite no significant difference in the predator : aphid ratios in susceptible vs. resistant plots, and thus may be related to a negative effect of resistant plants on per-capita predation rate, either by a direct effect or indirectly through aphid toxicity. In support of this view, Lundgren et al. (2009) observed a negative effect of *Rag1* on *Harmonia axyridis* even in the absence of prey. A lower predation rate linked to exposure to plant toxins in resistant plots could explain the difference observed on outplants, but if such an effect were occurring we would expect lower IGP in resistant plots within the broader field study as well. However, since removal of mummies was recorded only in the outplant experiment, it remains possible that a greater (and unobserved) mummy removal rate in the susceptible plots obscured a trend for lower IGP in the resistant plots.

5. Conclusions

Whether HPR and biological control cause synergistic pest control depends on many factors including the method of resistance and the biology of the pest, the biological control agents, and their predators (Boethel and Eikenbary, 1986; van Emden, 1995; Agrawal, 2000; Kennedy, 2003). Biological control agents may be more effective on resistant plants if the agents exhibit inversely density-dependent attack rates (van Emden, 1986; Arpaia et al., 1997; Heimpel et al., 2005). This synergism will be enhanced if predation of the biological control agents (IGP) decreases with plant resistance. In our study, reduced *B. communis* persistence occurred in resistant plots and our data suggest that this was due to both an effect of resistance unrelated to aphid density (later in the season) and an indirect effect through aphid density (on *B. communis*' first generation). However, overall rates of IGP were also decreased in resistant plots, providing released parasitoids with some protection from predation.

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