Ant–aphid interactions increase ant floral visitation and reduce plant reproduction via decreased pollinator visitation

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Abstract. For plant species that depend on animal-mediated pollination, reproduction hinges on adequate access to pollinators. Even in the presence of intact pollinator assemblages, negative interactions among floral visitors can compromise pollination services. Ants, for example, visit flowers and can discourage visitation by other insects, but usually do not perform pollination themselves. Effects on plant reproduction that result from this type of interaction may be compounded by factors that increase the likelihood or extent of floral visitation by ants. Interactions between ants and honeydew-producing insects alter the activity and local abundance of ants on plants, but the degree to which these interactions increase floral visitation by ants and in turn disrupt pollination services remains incompletely understood. We manipulated the abundance of cotton aphids (*Aphis gossypii*) on cotton (*Gossypium hirsutum*) over three years to test how the interaction between honeydew-producing aphids and the Argentine ant (*Linepithema humile*) affects floral visitation by ants, floral visitation by bees, and seed production. Increasing aphid abundance increased ant abundance on cotton plants, and floral visitation by ants was positively related to ant abundance on leaves. The duration of visits by honey bees, the most common floral visitor aside from ants, declined with both increasing aphid abundance and increasing ant floral visitation. Two measures of seed production declined with increasing aphid abundance and reductions in the duration of bee visits. Aphid herbivory alone, however, did not affect seed production, which was independent of aphid abundance on plants that were manually cross-pollinated under greenhouse conditions. Our results illustrate that interactions between ants and honeydew-producing insects can enhance levels of floral visitation by ants and in turn disrupt pollination services enough to have measurable effects on plant reproduction.

Key words: ant–aphid interaction; Aphis gossypii; *Argentine ant; biological invasion; cotton aphid;* Linepithema humile; *plant–pollinator interaction; seed production.*

Introduction

More than 85% of flowering plant species rely on animal-mediated pollination for successful reproduction (Delaplane and Mayer 2000, Potts et al. 2010, Ollerton et al. 2011). These services are increasingly jeopardized as a consequence of declining pollinator populations (Winfree et al. 2009, Potts et al. 2010), land-use change and habitat fragmentation (Aguilar et al. 2006), and the establishment of nonnative plants (Morales and Traveset 2009). The interdependence of plants and their pollinators is highlighted by studies that show parallel declines in plant and pollinator populations (Biesmeijer et al. 2006, Pauw and Hawkins 2011). However, pollination services may also be compromised when floral visitors negatively interact with one another.

Negative interactions among floral visitors can affect seed production via pollen limitation, which will negatively affect plant fitness when plant populations are seed limited (Irwin et al. 2001). Negative interactions involving pollinators can include predation by wasps (Dukas 2005, Wilson and Holway 2010, Hanna et al. 2013), spiders (Heiling et al. 2003, Suttle 2003, Dukas and Morse 2013), and other predators (Romero et al. 2011). Nonconsumptive effects such as pollinator harassment (Ness 2006) or predator avoidance by pollinators (Dukas 2001, Abbott and Dukas 2009) can also affect plant reproduction (Gonçalves et al. 2008). In addition to direct interactions like predation, indirect interactions among floral visitors (e.g., exploitative competition) can also discourage visitation by pollinators and negatively impact plant reproduction (Roubik 1982, Irwin et al. 2001).

Negative outcomes for floral visitors may be especially common for interactions that involve ants, which often visit flowers in a wide variety of environments but rarely provide effective pollination services (Hölldobler and Wilson 1990, Lach et al. 2010). Floral visitation by ants can result in damage to floral structures (Palmer et al. 2010), reduced pollen viability (Beattie et al. 1984, Cushman and Beattie 1991, Galen 1999), harassment of other floral visitors (Tsuji et al. 2004, Ness 2006),

PLATE 1. The presence of honeydew-producers on leaves draws Argentine ants to flowers in which they may disrupt visitation by pollinators. Photo credit: K. E. LeVan.

learned avoidance by floral visitors in response to olfactory cues produced by ants (Ballantyne and Willmer 2012), and exploitation or alteration of floral resources (Irwin et al. 2001, Lach 2008*a*, De Vega and Herrera 2013). Floral visitation by ants, however, can in some circumstances benefit plants. In addition to rare cases involving ant-mediated pollination (Gómez et al. 1996, De Vega and Herrera 2013), ants may interact with other floral visitors in ways that deter ineffective floral visitors in favor of effective pollinators (Gonzálvez et al. 2013), or potentially enhance the male component of plant fitness by increasing pollen transfer (Traveset and Richardson 2006). Given the ubiquity of floral visitation by ants, it seems surprising that relatively few studies investigate in detail how this phenomenon affects plant reproduction (Ashman and King 2005, Galen and Geib 2008).

Effects of floral visitation by ants may be amplified when ants interact with honeydew-producing insects, which typically alter the behavior, activity, and local abundance of ants on plants. Honeydew is a highly attractive resource to many ant species (Way 1963,

Völkl et al. 1999, Engel et al. 2001), and ants that tend honeydew-producing insects will commonly consume (or drive off) other insects, often to the indirect benefit of the host plant (Styrsky and Eubanks 2007). Although honeydew-producing insects could potentially distract ants from flowers in a manner similar to that proposed for extrafloral nectaries (Wagner and Kay 2002), enhanced floral visitation by ants may be more common (Lach 2007). Despite the fact that interactions between ants and honeydew-producing insects are widespread and conspicuous, few experimental studies have investigated how such interactions affect floral visitation by ants, how other floral visitors are affected by ants, and how changes in the behavior of floral visitors in turn affect pollen limitation and seed production.

In this study we manipulated an ant–aphid interaction in upland cotton (*Gossypium hirsutum*) to determine whether or not this interaction influences floral visitation by ants, the behavior of floral visitors aside from ants, and plant reproductive performance. We focus on the Argentine ant (*Linepithema humile*), which commonly forages on floral nectar (Lach 2007, 2008*a*, *b*), and the

TABLE 1. Sample sizes for experiments conducted over a three-year period that involved the manipulation of cotton aphid–Argentine ant interactions on cotton.

Year	Location	Cultivation	Mean leaves per plant (SE)	Number of leaves surveyed	Ants vs. aphids (sample size)
2011	field	field	157 (1.9)	all leaves	80 (40, 40)
2012	field	field	108 (3.2)	six mature leaves	50 (24, 26)
2013	field	pot	20 (6.5)	all leaves	48 (15, 33)
2013	greenhouse	pot	19.9 (1.8)	all leaves	

Note: Parenthetical values correspond to sample sizes for plants in the high aphid density aphid treatment (first value) and plants in the low aphid density aphid treatment (second value), unless otherwise indicated. See *Methods: Study system* for aphid treatments. The two right columns refer to the number of plants used to estimate seed production and the mean number of seeds produced per plant by those plants.

cotton aphid (*Aphis gossypii*), which provides honeydew to ants that protect the aphids from their enemies (Kaplan and Eubanks 2005, Powell and Silverman 2010, Styrsky and Eubanks 2010). Previous work has demonstrated that interactions between the cotton aphid and the imported red fire ant (*Solenopsis invicta*) increase cotton reproduction because fire ants consume non-aphid herbivores that are more damaging to cotton compared to aphids (Styrsky and Eubanks 2010). *Solenopsis invicta*, however, rarely forages in cotton flowers (M. D. Eubanks, *personal communication*). Switching the identity of the mutualist partner (i.e., from *S. invicta* to *L. humile*) thus provides a potential opportunity to examine how ant–aphid interactions affect plant reproduction via floral visitation by ants. Negative interactions between the Argentine ant and other floral visitors may affect cotton reproduction because pollinator visitation increases seed production in cotton, and ineffective pollination can result in abnormal fruit development and reduced yield (Delaplane and Mayer 2000). Moreover, bee pollination (Waller et al. 1985), especially by honey bees (Rhodes 2002) and bumble bees (Berger et al. 1988, Van Deynze et al. 2005), can enhance reproduction in cotton up to 38% (Delaplane and Mayer 2000).

In a three-year field study, we manipulated aphid abundance on cotton to test how ant–aphid interactions affect (1) floral visitation by ants, (2) the duration of visits by other floral visitors (especially bees), and (3) two measures of seed production per plant (seed number and seed mass). To our knowledge, this is the first study to manipulate ant–aphid interactions to test how they affect the activity of other floral visitors and in turn plant reproduction. Given the ubiquity of interactions between ants and honeydew-producing insects (Way 1963, Hölldobler and Wilson 1990, Davidson et al. 2003) and the ability of these interactions to affect the composition and structure of food webs on plants (Wimp and Whitham 2001, Styrsky and Eubanks 2007), the implications of our study broadly extend to a wide variety of plant species that rely on animal-mediated pollination. More generally, these experiments provide a novel test of the capacity for positive species interactions to exert effects external to the mutualism itself (Bruno et al. 2003).

METHODS

Study system

Over three field seasons (2011–2013), we manipulated cotton aphid abundance on cotton plants in the presence of the Argentine ant at the University of California San Diego Biology Field Station (32°53′12″ N, 117°13′48″ W). Each year we used a Roundup Ready cultivar (DP167RF) of commercial cotton (obtained from M. D. Eubanks, Texas A&M University). Details of the experimental work differed slightly across years (see descriptions of separate experiments and Table 1), but all cotton plants were treated as follows. Each year, we germinated cotton seeds and grew seedlings in pots in the greenhouse for three weeks. We sorted plants by size and assigned seedlings to one of two aphid density treatments (high aphid density or low aphid density) in an alternating fashion such that each treatment group was initiated with a similar size distribution of seedlings. In 2011 and 2012, we then planted all seedlings ($n = 80$ for 2011; $n = 50$ for 2012) in the ground. We spatially interspersed seedlings with respect to aphid density treatment in a 1×1 m grid. Plants were fertilized once (with 5:1:2 NPK; see Tewolde et al. 2009) and watered with 10 L of water weekly. In 2013 we reared plants ($n = 70$) in pots in the greenhouse until they were just about to bloom, at which point we moved a subset of these potted plants ($n = 60$) into the field in the spatial configuration used for plants in 2011 and 2012. The remainder ($n = 10$) of the 2013 potted plants were kept in the greenhouse to test how aphid herbivory affects total seed production for hand-pollinated plants.

Plants in the high aphid density treatment were inoculated with cotton aphids at the start of the season. Additional aphid inoculations were performed as needed to ensure that plants in the high aphid density treatment maintained relatively large aphid numbers (usually in excess of 25 aphids per leaf [see Kaplan and Eubanks 2005]) throughout the duration of the experiment. Aphids naturally colonized cotton plants in the low aphid density treatment, and plants in this treatment group were checked weekly (or more often as needed) for aphids, which were manually brushed off of leaves. Treatments were maintained throughout each season,

Table 1. Extended.

Ants per flower vs. ants on leaves (sample size)	Number of pollinator surveys (sample size)	Number of plants to estimate seed production (sample size)	Mean seeds per plant (SE)
35 (14, 21)	94 (42, 52)	80 (40, 40)	41.9 (5.5)
50 (24, 26)	116 (54, 62)	50 (24, 26)	84.0 (7.5)
60 (24, 36)	76 (18, 58)	48 (15, 33)	59.9 (4.5)
		10 (5, 5)	29.3 (1.12)

and certain plants were pruned to prevent them from touching adjacent plants.

We sampled ant and aphid abundances on each plant once per week after plants were brought into the field (Kaplan and Eubanks 2005), performing a complete census of all leaves (2011, 2013) or a subset of six leaves (2012) per plant. We wore latex gloves during all surveys and alternated between treatments as we sampled (i.e., to minimize biases that might have resulted from short-term changes in ant abundance). After weekly censuses were performed on each plant, we maintained aphid treatments as just described. To estimate ant and aphid abundances for individual plants, we calculated the mean abundance of each type of insect across weekly observation periods. For these time-averaged counts of abundance, weekly observation periods began when the first cotton flower appeared and concluded at the end of the blooming period. The number of weekly observation periods across which we averaged abundance estimates differed for each year of the study ($n = 6$ for 2011; $n = 8$ for 2012; $n = 4$ for 2013), but ant and aphid abundances were always estimated across the same time interval for all plants in a given year.

Time-averaged counts of per plant aphid abundance differed across aphid density treatments (Appendix A; two-way ANOVA, aphid treatment group $F_{1, 174} = 4.08$, $P = 0.045$; year $F_{1, 174} = 2.37$, $P = 0.12$; interaction $F_{1, 174} = 2.47$, $P = 0.12$). In a given year, plants in the low aphid density treatment had densities of aphids that were, on average, $<18\%$ of those in the high aphid density treatment group (Appendix A). Although aphid removal reduced aphid numbers, the abundance of aphids on individual plants varied over the course of the season in both density treatments and in each year of the study (Appendix A). This variation was likely driven in part by rapid population growth and inter-plant dispersal, which are both typical of cotton aphids (Lombaert et al. 2006). Recognizing that binning aphid abundance data into two experimental groups ignores the substantial variation in aphid abundance within each experimental group, we took a more statistically powerful approach that employed regression (Cottingham et al. 2005). We thus treat time-averaged aphid abundance as a continuous independent variable but indicate on all figures the aphid density treatment to which each replicate belonged.

We used generalized linear models (GLM) to test (1) the effect of aphids on ant abundance, (2) the effect of ant–aphid interactions on the duration of visits by other floral visitors, and (3) the effect of ant–aphid interactions on cotton seed reproduction. We used a Poisson link function for these GLMs because our data showed a strong, positive skew and because this is an appropriate distribution for count data (Quinn and Keough 2002). Unless indicated otherwise, individual plants (or the insects on them) represent the experimental unit in all analyses. All statistical analyses were conducted in R (R Development Core Team 2012; Supplement). As a measure of model fit, we provide Efron's pseudo-R^2 for the main analyses; this statistic is the squared correlation between the predicted and actual values in a GLM (Efron 1978). Although there is no direct equivalent in a GLM framework for the R^2 statistic from a simple linear regression, larger values of Efron's pseudo-R^2 indicate a better model fit and values are bounded by 0 and 1.

Effects of aphids on ant abundance

In each field season we determined how the interaction between ants and aphids affected the degree of floral visitation by the Argentine ant. Methods used to estimate ant and aphid abundances are described in the previous section. To estimate floral visitation by the Argentine ant, we made instantaneous counts of the number of ants per flower (averaged across open flowers on each plant) during weekly censuses of ants and aphids; sample sizes for these surveys are listed in Table 1. We used time-averaged counts to estimate floral visitation by the Argentine ant on individual plants over the course of each season. Time-averaged estimates equal the mean abundance of ants per flower per plant across weekly observation periods. For these time-averaged counts, weekly observation periods began when plants produced their first flower and ended when we harvested plants to estimate total seed production per plant. For each year separately, we used GLMs to compare time-averaged means of (1) per plant ant abundance vs. per plant aphid abundance, and (2) mean ant abundance in flowers vs. per plant ant abundance. In the first analysis, we treat ant abundance as a dependent variable because it was unmanipulated (unlike aphid abundance), but recognize that the presence of ants likely influenced aphid abundance. In the second analysis, we consider ant abundance in flowers to be a

dependent variable because the number of ants present on all of the leaves on a plant always greatly exceeded the number of ants in its flowers.

Effects of ant–aphid interactions on floral visitor activity

To quantify the effect of cotton aphid–Argentine ant interactions on the duration of floral visits by insects aside from ants, we conducted 324 floral visitation surveys (spanning 7562 total minutes of observation) from 2011 to 2013. Table 1 lists the number of individual surveys performed each year. For each plant during each survey, we recorded the number of ants present in a focal flower, the number and identity of other floral visitors, and the duration of non-ant visits over the course of 2 min (2011) or 10 min (2012–2013) intervals. In 2013 a subset of the surveys ran for 120 minutes; this subset was evenly divided across plants in the two aphid density treatments. Floral visitation surveys were always conducted between 1000 and 1500 and were interspersed across aphid density treatments in an alternating fashion. In every year surveys were also spread across the approximately month-long blooming period of cotton at our study site. Individual cotton plants typically had a small number (1–3) of flowers in bloom at any one time. Estimates of floral visitation by ants were based on the total number of ants present in a flower while another floral visitor was present. These counts accurately reflect the level of ant visitation to individual flowers for two reasons: (1) ant workers would typically enter a flower, feed from the nectar well for a short time (0–2 min) and then leave (often with a noticeably distended gaster), and (2) the total number of ants visiting individual flowers was relatively small.

We used the floral visitation survey data described in the previous paragraph to test whether visit duration by bees and other insects was related to the number of Argentine ants in flowers. First, we used a GLM to test the relationship between the number of ants in flowers (as described in the previous paragraph) and the duration of individual floral visits ($n = 146$) by non-ants. We conducted a second GLM to test the relationship between the number of ants in flowers and the duration of floral visits by just honey bees (*Apis mellifera*). In these analyses, each data point represents the cumulative duration of all floral visits to a unique flower, and surveys with no non-ant visitors were included as zeros. Occasional observations of floral visits from the same plant (but to different flowers) were averaged for that plant, and these means were used as data points. Finally, we used contingency table analysis to compare the types of floral visitors (bees vs. flies) that commonly occurred on cotton plants in the two aphid density treatments.

Effects of ant–aphid interactions on plant seed production

To determine whether ant–aphid interactions affect plant reproduction, we evaluated how seed production (total number of seeds, mass of individual seeds) changed with aphid abundance. To estimate each plant's reproductive allocation in each year (i.e., the number of flowers and bolls produced per plant), we harvested and dried bolls (cotton fruit) produced by every plant at the end of each field season. We then counted the number of seeds per boll (and from those data tallied the seeds produced per plant) and estimated the mass of individual seeds produced per boll. Estimates of seed mass were then averaged across bolls within each plant. In 2011, we also determined end-of-season aboveground dry biomass for each plant. For each of the three years of the study, we used a GLM to test for effects of aphid abundance on total seed production.

We also determined the relationship between the duration of visits by putative pollinators and seed production for individual flowers. These measurements were made in 2013 only. We individually marked flowers and then tested how the cumulative visit duration (by insects other than ants) affected seed production. Individual cotton plants had at most one marked flower, and each plant's flower was observed for a 120-minute observational period. In all other respects, these surveys employed methods comparable to those described in the preceding section. Because cotton flowers only remain open for one day and because cross-pollination is known to result in heavier seed masses in cotton (Delaplane and Mayer 2000, Abrol 2012), differences in seed production between individual bolls provide an estimate of the pollen received by those individual flowers. We used a GLM to test the effect of cumulative floral visit duration on seed production per plant.

In a greenhouse experiment we tested the effect of aphid abundance on seed production for 10 plants in a low aphid density treatment and plants in a high aphid density treatment, all of which received supplemental cross-pollination. Aphid treatments were maintained in the same way described previously for all plants in the high and low aphid density treatments, respectively. In this experiment all pollination was achieved manually (i.e., insect pollinators were absent); we removed mature anthers from non-focal plants, placed pollen-bearing anthers directly onto the stigma of focal plants, and ensured thorough pollen deposition. Cotton is partially self-compatible, but produces more seed when cross-pollinated (Delaplane and Mayer 2000). We used simple linear regression to test how aphid density affects (1) the number of seeds produced per plant, and (2) mean seed mass per plant.

RESULTS

Effects of aphids on ant abundance

Argentine ant abundance on cotton plants increased with increasing cotton aphid abundance, and the number of ants present in flowers increased with the number of ants present on leaves. Per plant ant
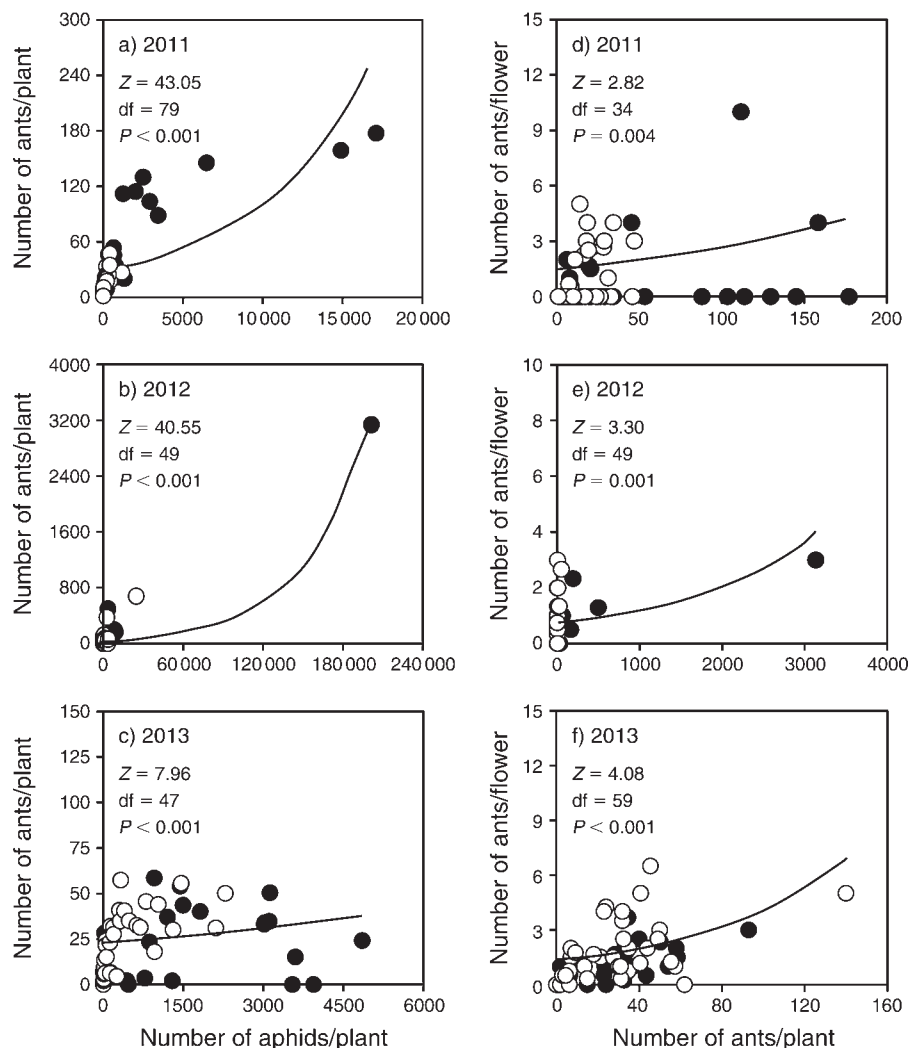
FIG. 1. Per plant abundances of Argentine ants and cotton aphids on cotton in (a) 2011, (b) 2012, and (c) 2013; the abundance of Argentine ants on cotton flowers vs. on leaves in (d) 2011, (e) 2012, and (f) 2013. Data points in each graph (a–f) indicate plants in the high aphid density aphid treatment (solid circles) and those in the low aphid density aphid treatment (open circles). Test statistics and P values are from general linear models.

abundance increased with per plant aphid abundance in all three years of the study (Fig. 1a–c; GLM 2011, Wald $Z = 43.05$, df = 79, $P < 0.001$, pseudo $R^2 = 0.48$; GLM 2012, Wald $Z = 40.55$, df = 49, $P < 0.001$, pseudo $R^2 = 0.92$; GLM 2013, Wald $Z = 7.96$, df = 47, $P < 0.001$, pseudo $R^2 = 0.98$). Removal of the data point in the upper right hand corner of Fig. 1b does not qualitatively affect the results of this analysis. The number of ants visiting cotton flowers also increased with increasing ant abundance on cotton leaves in all three years of the study (Fig. 1d–f; GLM 2011, Wald $Z = 2.82$, df = 34, $P = 0.004$, pseudo $R^2 = 0.17$; GLM 2012, Wald $Z = 3.3$, df = 49, $P = 0.001$, pseudo $R^2 = 0.21$; GLM 2013, Wald $Z = 4.08$, df = 59, $P < 0.001$, pseudo $R^2 = 0.13$).

Effects of ant–aphid interactions on patterns of floral visitation

During floral visitation surveys, we observed 11 insect taxa (excluding the Argentine ant) visiting cotton flowers; 72.6% (106/146) of individual visits were made by bees. Honey bees, the most common floral visitor, made up 26.0% (38/146) of all floral visits and 35.8% (38/106) of all visits by bees. Other bee visitors included *Agapostemon texanus* (18.4% of all floral visits), *Melissodes tessellata* (13.9% of all floral visits), *Lasioglossum incompletum* (12.5% of all floral visits), and *Halictus tripartitus* (5.1% of all floral visits). We also observed 27 visits (18.5% of all floral visits) by two fly taxa (one of these was a hoverfly (Diptera: Syrphidae), whereas the other dipteran remained unidentified), 10
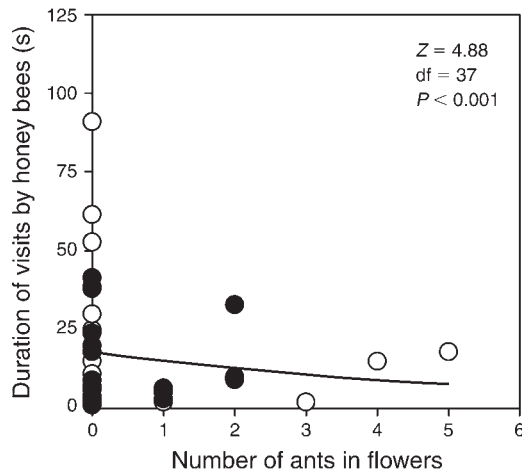
Fig. 2. Floral visit duration by honey bees as a function of the number of Argentine ant workers present in a flower during a bee's visit. Data points in each graph indicate individual floral visits to flowers on plants in the high aphid density aphid treatment (solid circles) and plants in the low aphid density aphid treatment (open circles). Data from 2011, 2012, and 2013 are combined. The test statistic and P value are from a general linear model.

visits (6.8% of all floral visits) by *Diabrotica balteata* (Coleoptera: Chrysomelidae), 4 visits (2.7% of all floral visits) by unidentified thrips, and 1 visit (0.7% of all floral visits) by *Strymon melinus* (Lepidoptera: Lycaenidae).

Floral visit duration by all visitors and by honey bees alone both declined with increasing numbers of Argentine ant workers present in cotton flowers. The duration of visits by all non-ant floral visitors declined with the number of ants present in a flower (GLM, Wald $Z = 10.26$, df $= 284$, $P < 0.001$, pseudo $R^2 = 0.81$). As the number of ants in flowers increased, the duration of honey bee visits also declined (Fig. 2; GLM, Wald $Z = 4.88$, df $= 37$, $P < 0.001$, pseudo $R^2 = 0.91$). Honey bees, for example, reduced the duration of their visits to cotton flowers by 56.3% when ants occupied flowers compared to when no ants were present in flowers (two-sample t test (with Welch's correction for unequal variance): $t = 2.32$, df $= 32$, $P = 0.027$). Bees (all species combined) visited plants in the low aphid density treatment almost twice as often as they visited plants in the high aphid density treatment (70 visits vs. 36 visits), whereas plants in the high aphid density treatment had three times as many visits by flies (mostly syrphids) compared to plants in the low aphid density aphid group (19 visits vs. 6 visits; $\chi^2_1 = 14.7$, $P < 0.001$).

Effects of ant–aphid interactions on plant reproduction

The reproductive performance of cotton declined with increasing numbers of aphids on individual plants. In each year of the study, the total number of seeds produced per plant declined with increasing aphid abundance (Fig. 3, Table 2). In Fig. 3b,

removal of the data point in the lower right-hand corner of the plot does not qualitatively affect the outcome of the analysis. Pseudo-R^2 values for the relationships depicted in Fig. 3a–c are as follows: 0.002 (2011), 0.026 (2012), and 0.02 (2013). In two of three years, the mass of individual seeds produced by individual cotton plants also declined with increasing aphid abundance (Table 2). The lack of a significant relationship between seed mass and aphid abundance in 2012 might have resulted from lower levels of
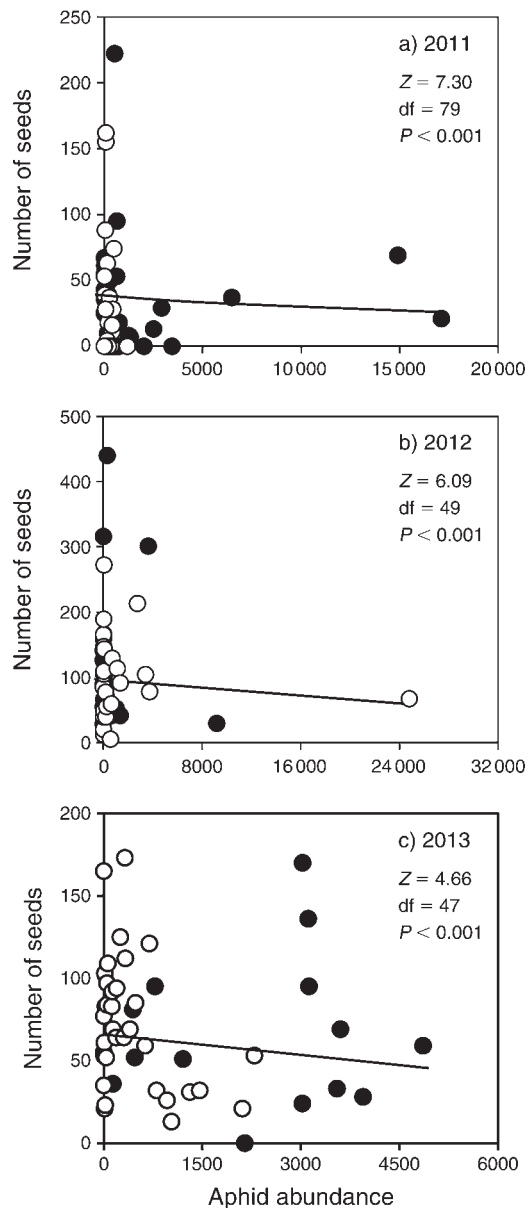

Fig. 3. The number of seeds produced by individual cotton plants in (a) 2011, (b) 2012, and (c) 2013 as a function of cotton aphid abundance on plants. Data points in each graph indicate plants in the high aphid density aphid treatment (closed circles) and those in the low aphid density aphid treatment (open circles). Test statistics and P values from general linear models.

pollinator activity in 2012 compared to 2011 and 2013. Although both measures of seed production declined with increasing aphid abundance, above-ground biomass of cotton plants at the end of the season was independent of aphid abundance (Appendix B).

In floral visitation surveys focused on individual flowers (2013 data), we found that the cumulative duration of visits by putative pollinators enhanced production of seeds produced by those flowers. Seed mass increased with the duration of visits by all putative pollinators (GLM, Wald $Z = 11.8$, df $= 72$, $P < 0.001$) and with the duration of visits by honey bees alone (GLM, Wald $Z = 5.62$, df $= 72$, $P < 0.001$). Thirty-eight percent of observed floral visits exceeded 20 s. These longer visits were associated with greater seed mass (two-sample t test: $t = 2.25$, df $= 276$, $P = 0.025$). For the focal plants used in these analyses, the number of bolls per plant (GLM 2011, Wald $Z = 1.67$, df $= 79$, $P = 0.09$; GLM 2012, Wald $Z = 0.218$, df $= 49$, $P = 0.83$; GLM 2013, Wald $Z = 1.24$, df $= 50$, $P = 0.21$) and number of flowers per plant (GLM 2011, Wald $Z = 0.46$, df $= 79$, $P = 0.65$; GLM 2012, Wald $Z = 0.30$, df $= 49$, $P = 0.76$; GLM 2013, Wald $Z = 1.53$, df $= 50$, $P = 0.13$) were independent of aphid abundance.

Plants in the field produced fewer seeds per plant with increasing densities of aphids (Fig. 3), and this reduction appears to result from compromised pollination services rather than from costs associated with herbivory. In our greenhouse experiment, the number of seeds produced by plants cross-pollinated by hand, for example, was independent of aphid density (Appendix C; simple linear regression, $F_{1,8} = 0.254$, $P = 0.601$). Likewise, seed mass for plants cross-pollinated by hand was also independent of aphid density (Appendix C; simple linear regression, $F_{1,8} = 0.899$, $P = 0.371$).

DISCUSSION

The interaction between Argentine ants and cotton aphids increased levels of floral visitation by ants, and in turn, disrupted pollination services enough to measurably reduce two measures of seed production. In each year of the study, increasing aphid abundance elevated ant abundance on cotton leaves, which in turn increased floral visitation by ants (although ants did sometimes visit flowers on plants with relatively few aphids). Increasing numbers of ants in flowers reduced the duration of visits by all non-ant floral visitors combined and by honey bees alone. Although a variety of factors likely affected seed production by cotton plants, the number of seeds produced per plant and mean seed mass both declined with increasing aphid abundance; mean seed mass also declined with decreasing bee visit duration. Under controlled greenhouse conditions, however, in which plants were hand-pollinated with non-self pollen, per plant seed production and seed mass were independent of aphid

TABLE 2. Results of generalized linear models testing the effect of time-averaged aphid abundance on two measures of seed production.

Response variable	Year	b	SE	Z	P
Number of seeds	2011	−0.0010	0.0001	7.30	**<0.001**
	2012	−0.0004	0.0001	6.09	**<0.001**
	2013	−0.0001	0.0000	4.66	**<0.001**
Mass per seed (mg)	2011	−0.0004	0.0001	3.11	**0.001**
	2012	0.0000	0.0003	0.13	0.89
	2013	−0.0002	0.0000	10.00	**<0.001**

Notes: The number of seeds equals per plant production; mass per seed is averaged across bolls within each focal plant. Sample sizes are provided in Table 1. Parameter estimates (b), standard error (SE), the test statistic (Wald Z), and significance (P) values are reported. Significant P values are in boldface type.

abundance. Moreover, in the field, per plant flower production, per plant boll production, and above-ground biomass were all independent of aphid abundance.

Cotton aphid abundance strongly increased the abundance of ants on leaves, consistent with previous work showing that Argentine ants are strongly attracted to honeydew (Way 1963, Holway et al. 2002, Ness and Bronstein 2004). Increased ant traffic on leaves led to an increased number of ants present within flowers (see Plate 1). Some ant species tend hemipterans without visiting flowers, and thus have little opportunity to disrupt floral visitation (Styrsky and Eubanks 2010), perhaps because of repellent chemicals present in pollen, nectar, or petals (Ness 2006, Nicklen and Wagner 2006, Willmer et al. 2009, Junker et al. 2011). However, these chemicals do not affect some ant species or are not present in high enough concentrations to deter ant floral foraging (Chamberlain and Holland 2008). The modification of ant floral foraging by hemipterans has broad implications, because food-for-protection mutualisms between ants and hemipterans are ubiquitous and ecologically important interactions (Way 1963, Hölldobler and Wilson 1990, Davidson et al. 2003, Styrsky and Eubanks 2007).

The duration of visits by insects (mostly bees) other than ants declined with increasing floral visitation by ants. Lach (2008b) found that honey bees shortened the duration of their floral visits in the presence of the Argentine ant and left flowers following direct encounters with this species. We observed the former phenomenon (Fig. 2), but further research is required to determine the mechanism responsible for reduced duration of bee visits in cotton flowers. Not all floral visitors were less frequent on plants that supported high aphid densities. Syrphids, for example, more often visited plants in the high aphid density treatment group. Syrphid larvae prey on aphids, and we commonly observed syrphids copulating and ovipositing on or near flowers from plants in the high aphid density aphid treatment. Syrphids can be effective pollinators (Branquart and Hemptinne

2000), but in our system adult syrphids typically did not contact the sexual organs of cotton flowers during visits.

Ant–hemipteran interactions may commonly increase the likelihood and degree of floral visitation by ants and in turn reduce floral visitation by other insects, but few studies have assessed the extent to which these interactions affect plant reproduction (Lach 2007). Floral visitation by ants may primarily affect plant reproduction in situations when reduced pollinator access leads to pollen limitation. Plant species that exhibit self-compatibility or that rarely experience pollen limitation are unlikely to be affected by increases in ant floral visitation that result from host plants supporting interactions between honeydew-producing insects and ants. In this study, seed production of hand cross-pollinated plants was independent of aphid density, suggesting that pollen limitation brought about by the interaction between aphids and ants, not by aphid herbivory itself, is the driving force behind reduced seed production. Although we consistently observed that seed production decreased with aphid density in the field, these relationships exhibited a fair degree of scatter (Fig. 3), and it is likely that a variety of factors (degree of self-fertilization, microscale variation in soil conditions), in addition to reductions in the duration of bee visits, affected seed production in the cotton plants examined in this study. Given that the seed production variables examined here often directly affect plant fitness (Baker 1972, Irwin et al. 2001, Moles and Westoby 2004), honeydew-mediated floral visitation by ants may nonetheless negatively affect fitness of plants that host large numbers of honeydew producers.

An alternative explanation for decreased visit duration by putative pollinators under high aphid densities could entail reduced production of floral nectar by cotton plants. Such an effect could cause pollinators to reduce the length of their visits to cotton flowers. Several lines of evidence suggest that this alternative hypothesis fails to explain our results. First, floral visitation by ants, which foraged for nectar in cotton flowers, increased with aphid abundance (Fig. 1); this finding suggests that aphid herbivory does not reduce nectar production. Second, under greenhouse conditions, aphid herbivory did not affect mean seed mass or the number of seeds produced per plant (Appendix C). This finding indicates that aphids do not reduce a plant's allocation to reproduction, at least under controlled circumstances. Lastly, in the field, per plant flower production and per plant boll production were independent of aphid abundance. These latter results provide additional evidence that herbivory by cotton aphids does not affect reproductive allocation in cotton. Moreover, our results are corroborated by other studies showing that herbivory by cotton aphids alone does little to reduce cotton growth and repro-

duction (Rosenheim et al. 1997, Styrsky and Eubanks 2010).

Although numerous studies now indicate that ant–hemipteran interactions enhance host plant growth and reproduction (reviewed in Styrsky and Eubanks 2007), our results illustrate that the manifestation of these positive indirect effects can depend on the foraging behavior of the focal ant species. Positive effects result from ants consuming (or deterring) damaging herbivores (Messina 1981, Skinner and Whittaker 1981, Floate and Whitham 1994, Kaplan and Eubanks 2005); however, if honeydew-producing insects also increase the likelihood or extent to which ants forage in flowers, then the potential exists for plant reproduction to suffer as a result of negative interactions between ants and other floral visitors. Net effects of ant–aphid interactions on host plants will thus depend on the magnitude of benefits resulting from herbivore protection vs. the magnitude of costs resulting from reduced floral visitation by potential pollinators and other negative impacts. Attributes of the participating ant species that will influence these costs and benefits include an ant colony's nutritional needs, which can govern preferences for carbohydrate-rich resources vs. protein-rich resources (Ness et al. 2009, Petry et al. 2012), its tolerance of floral defense compounds, and its ability to reduce visitation by other floral visitors, either directly or indirectly (Lach 2003). The likelihood that ants will disrupt floral visitation also likely depends on external factors that affect local ant density. Moreira et al. (2012), for example, illustrate that local plant diversity can increase the abundance of both ants and the honeydew-producing insects that they tend. If floral visitation by ants discourages visitation by putative pollinators in general (Lach 2007, 2008a, b), then the continued spread of introduced ants will likely lead to additional examples of disrupted pollination services that are made more acute by interactions between ants and the honeydew-producing insects that they tend.

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