Agricultural and Forest Entomology

Agricultural and Forest Entomology (2016), DOI: 10.1111/afe.12159

Ant attendance of the cotton aphid is beneficial for okra plants: deciphering multitrophic interactions

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Abstract

1 Aphids are pest species of many crops and biocontrol methods are often ineffective. Ant–aphid associations can be mutualistic or antagonistic, with ants increasing or reducing aphid numbers. Within-species plant variation or other herbivores may further influence these ant–aphid interactions.

2 Okra is an economically important crop in Cameroon. Several okra varieties are grown here and attacked by the facultatively ant-tended cotton aphid *Aphis gossypii*. We conducted field and screenhouse experiments where plant variety, ant presence and predator access were manipulated to investigate the multitrophic interactions on okra and their effects on okra yield.

3 In the field, ants did not protect aphids from their natural enemies and syrphid larvae reduced aphids by 42%. Additionally, aphid recruitment of ants reduced chewing herbivore damage by 11% and indirectly increased okra fruit set. We also found aphid numbers, aphid predation by syrphids and chewing herbivory to vary across okra varieties. Finally, in the screenhouse, we recorded a 24% reduction in aphid numbers on plants with ant presence.

4 The present study highlights the importance of direct and indirect biotic interactions for pest biocontrol. Tropical agricultural systems are complex and understanding such interactions can help in designing pest control measures in sustainable agriculture.

Keywords Ant–aphid, biocontrol, interactions, multitrophic, plant varieties.

Introduction

Aphids are economically important pests that are responsible for a reduction in yield on many agricultural crops worldwide (van Emden & Harrington, 2007). Pest-resistant plant varieties can help to reduce the impact of aphid outbreaks on crops, although these can be expensive and time-consuming to develop (McCouch *et al.*, 2013). Alternatively, biological control measures can be used to control aphid populations, which usually focus on enhancing aphid natural enemy abundance (Powell & Pell, 2007). The introduction of a novel biocontrol agent is not always successful in the long-term regulation of pest populations, mainly as a result of a mismatch in climate between the native and introduced range of the agent, the lack of an alternate food source and/or predation/parasitism by native fauna of the agent (Stiling, 1993). Native fauna such as ants are known mutualists with aphids and often protect aphids against their natural enemies in return for the aphid honeydew (Way, 1963; Buckley, 1987; Völkl *et al.*, 1990; Kaplan & Eubanks, 2005). Such interactions can hinder biocontrol efficiency and the factors that can maintain or enhance natural enemy populations for pest regulation are still relatively unclear (Rusch *et al.*, 2010).

Other than protecting the aphids from natural enemies, ants can further benefit aphids by removing sticky honeydew and fungal-infected aphid cadavers, which would otherwise support fungal growth, leading to reduced aphid survival (Nixon, 1951; Nielsen *et al.*, 2009). Ants can also benefit aphids by increasing their body size, longevity and reproduction rate (Stadler & Dixon, 1999; Flatt & Weisser, 2000). However, ant–aphid mutualisms do vary from obligate (close) to facultative (occasional) and it is well reported that obligate ant-tended aphids are better protected by ants than facultative ant-tended aphids

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(Stadler & Dixon, 2005). Furthermore, ants do not always benefit aphids and their association with aphids can be antagonistic; for example, when ants negatively affect aphid growth and development (Stadler & Dixon, 1998; Yao *et al.*, 2000; Stadler *et al.*, 2002) or even prey on aphids (Rosengren & Sundström, 1991; Sakata, 1995; Stadler & Dixon, 2005).

More recently, plant genotype has been shown to influence whether ant–aphid associations are mutualistic or antagonistic (Mooney & Agrawal, 2008; Abdala-Roberts *et al.*, 2012). Ant attendance has been shown to increase on higher quality host plants, probably as a result of higher quality honeydew (Stadler *et al.*, 2002). Aphid performance and preference also varies across different plant genotypes or varieties (Zytynska & Weisser, 2016). This could further influence the effect of ants on the aphids, particularly if the interaction is density-dependent, with ant predation being more likely with an increase in aphid numbers (Rosengren & Sundström, 1991; Sakata, 1995). If ants and aphids are influenced by host-plant quality, then other factors that alter host-plant quality could also indirectly mediate ant–aphid interactions. For example, leaf chewers both reduce the biomass of a plant and can induce anti-herbivore plant chemical defences (Walling, 2000). There is strong evidence for the effect of within-species plant variation on its associated invertebrate community (Whitham *et al.*, 2012) and this could further mediate the ant–aphid interaction (e.g. flea beetle abundance has been shown to vary across soybean plant genotypes) (Underwood & Rausher, 2000). Although studies have investigated the effect of plant traits on biocontrol efficiency (Cortesero *et al.*, 2000; Inbar & Gerling, 2008), the effect of plant within-species variation on multitrophic interactions is still understudied in agricultural systems. Furthermore, understanding ant–aphid associations is crucial because these can be keystone interactions influencing the arthropod communities on plants and, in return, influence plant fitness (Styrsky & Eubanks, 2007; Zhang *et al.*, 2012).

Okra (*Abelmoschus esculentus* Moench) is an economically important vegetable crop grown worldwide and is widely consumed in West Africa. In Cameroon, the cotton aphid (*Aphis gossypii* Glover) is one of the dominant pests of okra (Leite *et al.*, 2007; Shannag *et al.*, 2007) and has evolved resistance to pesticides, particularly on cotton plant (Brévault *et al.*, 2008). An annual survey conducted by the International Institute of Tropical Agriculture (IITA) in Cameroon (2011) found that okra farmers grow many different okra varieties, various ant species attend aphids on okra, and ants of genus *Pheidole* were the dominant ant species found attending aphids on okra plants in 75% of the surveyed okra farms (IITA annual survey report, 2011). The cotton aphid is a facultative ant-tended species and therefore its interaction with ants may vary. When suggesting aphid control measures, it is crucial not only to understand the ant–aphid interaction, but also to find varieties with lower pest abundances and a higher yield. Thus, we conducted a field and a screenhouse experiment to test the hypotheses: (i) predators reduce aphid numbers in the field; (ii) ants protect aphids from their predators; (iii) okra variety influences the ant–aphid association; (iv) the ant–aphid association will influence plant growth and okra yield; and (v) the ant–aphid association can affect okra-associated invertebrates (aphid predators and additional okra herbivores), or vice versa, and this in turn can affect okra plants.

Materials and methods

Study site and study species

The experiments were conducted at the IITA research station in Yaoundé, located in the central region of Cameroon (West Africa). We conducted a field experiment and a controlled screenhouse experiment within the research site. Screenhouses are made of a greenhouse frame but are covered with a double layer of fine net to avoid any insects from entering, at the same time as allowing air to circulate from the outside. Our study consisted of cotton aphids (*A. gossypii* Glover) and ants (*Pheidole dea* Santschi) on okra *A. esculentus* Moench.

Okra is mostly grown in humid climate in sandy and clay loam soils and its optimum growing temperature is estimated to be between 24 and 30 °C. The plants are an annual erect herb (height 2–4 m) with lobed and hairy leaves. It is a self-pollinating crop, although insects, especially bees, are attracted to the flowers and hence cross-pollination occurs (Tripathi *et al.*, 2011). Okra plants are attacked by many pests at different growing stages, such as the cotton aphid and beetles (Benchasri, 2012). In our experiment, four varieties of okra were used: *Clemson* (Les Doigts Verts, France), *Bangourain*, *Caffeier* and *Kirikou* (obtained locally from Dschang, Northwest Cameroon). These differ in their growth pattern (*Clemson* and *Kirikou* grow faster than *Caffeier* and *Bangourain*), leaf size (*Caffeier* and *Bangourain* have larger leaf size area than *Clemson* and *Kirikou*) and fruit shape (*Clemson* has longer, slender fruits and the others have broader, shorter fruits) (Akanksha Singh, personal observation). These also vary in their mucilage content (a trait associated with consumer preference), with high mucilage content in *Caffeier* and low content in *Clemson* (Albert Abang, personal communication) and such variation in mucilage content has been studied amongst okra accessions (Ahiakpa *et al.*, 2014).

Aphis gossypii colonizes more than 600 host plants across a wide geographical range and vectors more than 50 plant viruses (van Emden & Harrington, 2007). In tropical climates, this facultative ant-tended aphid undergoes mostly parthenogenetic (i.e. asexual) reproduction, leading to an exponential growth rate at optimal conditions. The aphids were reared on *Clemson* okra in an insectary in IITA Cameroon under a 14 : 10 h light/dark photocycle at 24.1 °C and 71.2% relative humidity prior to use in the experiments.

Pheidole dea are ground-dwelling ants that form large colonies. This species has been recorded in afro-tropical countries such as Cameroon and Uganda and details of their diet are relatively unknown (Fischer *et al.*, 2012). The ants used in the screenhouse experiment were collected from the experimental field site and maintained in field soil, inside plastic containers (depth 8 cm, diameter 14 cm) as a queen and approximately 100 workers. We first applied tape at the rim of the container and then Tanglefoot (a sticky insect barrier; Contech Inc., Spartanburg, South Carolina) was spread on the tape to prevent ants from escaping. Ants were given sugar solution and insect protein (crickets) to maintain the colony.

Seed germination

The seeds were soaked in water in darkness for 24 h. Then one seed per pot (depth 8 cm, diameter 14 cm) was sown in sterilized

soil (25% sand, 25% fowl manure and 50% soil) and left to germinate for 10 days in the open. From the date of sowing, we used 5-week-old plants for the field and 3-week-old plants for the screenhouse experiment. We used older plants for the field experiment because they are more resilient against weather conditions and damage from other invertebrates experienced in the field.

Field experiment

We first conducted a field study to test: whether predators would reduce aphid numbers in the field; whether ants would protect aphids from their predators; whether the ant–aphid association would vary across okra varieties; and the effect of ant–aphid association on okra plants and other okra-associated invertebrates (aphid predators and additional herbivores).

Experimental design. We used a fully factorial randomized block design with 16 treatments including four okra varieties (*Clemson*, *Kirikou*, *Caffeier* and *Bangourain*), two ant treatments (presence and absence) and two cage treatments [open (predator/chewing herbivore presence) and closed (predator/chewing herbivore absence)]. The insect cages were $0.4 \times 0.4 \times 0.7$ m (length × breadth × height), constructed as a frame of polyvinyl chloride piping, covered with a white fine-mesh cotton cloth. Our 'closed' cages were completely covered with mesh, whereas 'open' cages had an opening on all four sides measuring 0.3×0.5 m to allow colonization of the plant by the natural invertebrate community. We used 'open' cages for predator/herbivore presence rather than no cage to ensure that the results were not biased as a result of a cage effect. For ant presence, small V-shaped wooden bridges were constructed connecting the ground with the soil in the pot and, for ant absence, we applied Tanglefoot at the base of the stem of the plants. Each treatment was replicated 10 times ($n = 160$), with one potted plant per cage. We placed these in 10 blocks to control for spatial variation across the field, with one replicate per treatment in each block and treatments randomized within block (4×4 cages). Within a block, each cage was 0.6 m from the adjacent cages, with a distance of 1.6 m between blocks. The field experimental site measured 27×18 m and was surrounded by two plantain fields, an old okra field and fallow land.

Experimental set-up. The experiment started on 1 April 2013. Blocks 1–5 and 6–10 were set up on two consecutive days. Pots were placed on the ground within the cages. After measuring initial plant height and leaf number, 10 aphids (four or five adults and the remainder of earlier ages) were introduced to each plant. Any vegetation around the pots that was touching the experimental pots was removed. Ants and other invertebrates colonized the plants naturally.

Data collection (8 April to 7 May 2013). One week after the experiment was set up, we began to take readings. Data were collected once per plant per week over two consecutive days (one day from blocks 1 to 5 and the consecutive day from blocks 6 to 10) over a period of 4 weeks. The variables recorded per plant were: leaf number, aphid number (total per plant, using a hand tally counter), ant attendance (total number of ants per plant attending aphids during 1 min), ant species, leaf beetle number, foliage remaining (percentage residual leaf tissue after damage from chewing herbivores of all leaves combined, per plant), syrphid larvae number, number of parasitoid mummies and spider number. In the final observation, we also recorded the plant height. A plant was harvested when the first fruit had matured up to a minimum of 7 cm in length. We also recorded the day (number of day after sowing the seeds) on which fruit was collected from each plant. We starting collecting okra fruits on day 78 and fruit collection continued until day 105. Fruits were bagged in paper bags and dried in an oven for 3 days at 60 °C to measure the dry biomass.

We also measured temperature and humidity in open and closed cages using Hobo data loggers (Onset, Cape Cod, Massachusetts). In the open cages, the mean temperature was 24.3 ± 0.1 °C (range 21.0–32.9 °C) and mean humidity was 87.4 ± 0.5% (range 51.6–100%). In closed cages, we recorded a mean temperature of 24.9 ± 0.3 °C (range 20.9–34.7 °C) and a mean humidity of 85.1 ± 0.9% (range 42.9–100%). Mean rainfall during the course of the experiment was 10.3 ± 2.8 mm (range 0–55.9 mm) with a 12 : 12 h light/dark photocycle.

Screenhouse experiment

In the field experiment, aphid predators and herbivory by a leaf beetle could have influenced the ant–aphid interactions on okra. Hence, we also conducted a controlled screenhouse study for a clearer understanding of whether ants benefit aphids in our system or not.

Experimental design. We used a fully-factorial randomized block design with three okra varieties (*Clemson*, *Kirikou* and *Caffeier*) and two ant treatments (presence and absence). In total, there were six treatments with eight repeats per treatment combination (48 plants). We used eight blocks within the screenhouse with one repeat of each treatment in each block. The cages were placed on two separate tables (four blocks per table). Each plant was placed inside entirely enclosed plastic-polypropylene insect cages (1350 µm mesh opening) measuring $30 \times 30 \times 30$ cm (length × breadth × height) (Megaview Science, Taiwan).

Experimental set-up. This experiment was set up on 29 May 2013 and terminated on 13 June 2013. On day 1, one plant was placed inside each insect cage and 10 adult aphids were introduced onto each plant using a fine paintbrush. Ant colonies were introduced 48 h after (on day 3) the introduction of aphids using a small V-shaped wooden bridge connecting the ant colony with plant. In addition, throughout the duration of the experiment, ant colonies were provided with protein (crickets collected from experimental field site) to avoid forced predation on aphids by the ants. Mean temperature in the screenhouse was 25.2 ± 0.2 °C (range 21.3–31.4 °C), mean humidity was 79.4 ± 0.5% (range 37.4–97.8%) and during the 12 : 12 h light/dark natural photocycle, additional lighting was used.

Data collection (29 May to 13 June 2013). On first day, we measured the height and leaf number of the plant. On day 5, 48 h after introduction of ant colonies (day 3), we started our observations. We conducted two different forms of observations of the experimental plants for 9 days. For the first one, all 48 experimental plants were sampled twice each day (morning and evening) and the numbers of ants attending aphids per plant during 1 min were recorded. For the second one, we selected six plants every day (two of each variety) out of the 48 plants and these were also observed twice for 10 min each (morning and evening) to record whether ants were tending the aphids or predating upon them. For these second observations, the same plants were observed morning/evening on the same day but different plants chosen across the 9 days, resulting in two or three observations per plant. On the final day (day 15), we recorded data on aphid colony size, plant height and leaf number.

Statistical analysis

Field experiment. Ants attended aphids on all plants with ant presence treatment except on four plants where ants were never observed throughout the experiment. Similarly, on four plants with ant absence treatment, ants of *Camponotus* and *Pheidole* genus were observed attending aphids as a result of the ants building a soil-bridge to navigate across the tanglefoot barrier. Cages effectively excluded predators from all but two cages in which syrphid larvae were observed; these two plants were removed from our analysis. In addition, we removed the following plants from our analysis: two plants in open cages that died as a result of excessive herbivory during week 1 by individuals of an unidentified grasshopper; a further 41 plants in which aphid extinctions occurred in weeks 1 (88% of the extinctions), 2 and 3; and three plants in which only one aphid was present throughout the experiment. Hence, in total, 48 plants were removed from our analysis, giving us a final sample size of 112 plants, with six to eight repeats per treatment.

Aphid per capita growth rate (aphid GR) was calculated using the formula: $[\ln(N_x) - \ln(N_s)]/t$, where, N_x is aphid number in a particular week, N_s is aphid number at the start of the experiment (i.e. 10 aphids) and t is the duration of the experiment (days). Plant relative growth rate (plant RGR) was used to correct for plant height variation amongst varieties; this was calculated using the formula: [ln(final plant height) – ln (initial plant height)]/total number of days. Linear models were used to analyze the data.

We first tested for the effect of our main explanatory variables cage treatment (predator present and absent), ant treatment (ant present and absent) and plant variety. For the aphid extinctions in week 1 (1, 0; extinction, no extinction) we fitted a generalized linear model (GLM) with quasibinomial distribution. For aphid GR (week 3), plant RGR and fruit biomass, we fitted normal linear models; for day of fruit collection, we fitted GLM with quasipoisson distribution with additional covariates plant RGR and aphid GR, which were included when these were not the respective response variables. Additionally, day of fruit collection was included as a covariate in our analysis for fruit biomass. The main explanatory variables, and their interactions, were included in all five models described above.

Because ant abundance varied across ant-present plants, we also analyzed the effect of ant abundance on aphid GR, plant RGR and fruit biomass. Only data originating from ant-present plants were used. Here, our model included plant variety and cage treatment as the main explanatory variables and ant abundance (mean number of ants recorded per observation) as a covariate. Plant RGR and aphid GR were included as covariates when these were not the respective response variables.

Furthermore, leaf beetle abundance, percentage foliage remaining and frequency of presence of syrphid larvae also varied across plants. Hence, we analyzed the effects of the degree of herbivory and predation by syrphid larvae on aphid GR, plant RGR and fruit biomass. For this, only data originating from open cages were used. Our linear model included plant variety and ant treatment as the main explanatory variables and foliage remaining (percentage residual leaf tissue), leaf beetle abundance (mean number of leaf beetles recorded per observation) and syrphid larvae (presence/absence) as covariates. Plant RGR and aphid GR were included as covariates when these were not the respective response variables.

Finally, we analyzed the effect of our treatments on the secondary response variables ant abundance (week 3) (data used: ant-present plants), syrphid larvae presence/absence, leaf beetle abundance (week 3) and foliage remaining (week 3) (data used: open cages plants). Here, for ant abundance, our model included cage treatment (predator present and absent) and plant variety as main explanatory variables and plant RGR and aphid GR as covariates.

For syrphid larvae (presence, absence), we applied a GLM model with quasibinomial distribution. Foliage remaining data (%) were arcsine transformed before the analysis. Standard linear models were used for foliage remaining and leaf beetle abundance analysis. Ant treatment and plant variety were included as main explanatory variables and aphid GR, plant RGR and ant abundance as covariates. Foliage remaining and leaf beetle abundance were included as covariates when these were not the respective response variables.

Screenhouse experiment. One ant-present plant of *Clemson* variety was removed from the analysis because no ant attendance was observed on it during the experiment. The data were analyzed for two main response variables: aphid GR and plant RGR. For these, we fitted linear models and our main explanatory variables were ant treatment and plant variety. To analyze the effect of ant abundance on our main response variables, data originating from ant-present plants were used. Here, plant variety was our only explanatory variable. Aphid GR and plant RGR were included as covariates for both the total and split data when these were not the respective response variables.

We also analyzed data for ant abundance on a plant, as a response variable and, for this, only ant-present plants were used. The observation time for ant attendance was 2 min per plant per day for focal plants (0.1% of the day) and ants were not always present on the plant during the observation. Thus, we chose the maximum ant number per plant over the full observation period to be included in our analysis as a variable for the effective representation of ant abundance. Here, we applied a GLM model with quasipoisson distribution with plant variety as our main explanatory variable and plant RGR and aphid GR as covariates.

Table 1 The number of extinctions and aphid numbers throughout the field study

	Open (predator present)		Closed (predator absent)	
Data collection week	Number of extinctions	Aphid number	Number of extinctions	Aphid number
Week 1	19	54.4 ± 16.0	17	72.31 ± 9.6
Week 2	2	129.0 ± 23.4	0	336.0 ± 45.2
Week 3	3	77.9 ± 12.9	0	687.5 ± 76.2
Week 4	5	121.9 ± 59.4	0	753.5 ± 101.2

Aphid number is given as the mean ± SE.

All data were analyzed in R, version 3.2.2 (The R Project for Statistical Computing, Austria) using RStudio version 0.98.978 (Rstudio, Boston, Massachusetts). For all the variables that we tested, we used Type I sum of squares; we first fitted a full model with all main effects and all interactions between the main effects. Then, all the nonsignificant effects and interactions (starting from the highest interaction order) were removed for simplification of the final model. Descriptive statistics are reported as the mean ± SE.

Results

Field experiment

Aphid, ant, predator and herbivore observations. Thirty-six plants had no aphids in the first week; out of these, 19 plants (9/19 in ant presence) were in open cages and 17 in closed cages (7/17 in ant presence) (Table 1). The number of extinction events was significantly higher in ant absence than in ant presence ($F_{1,142} = 4.20$, $P = 0.042$) and there was an interaction between plant variety and cage treatment ($F_{3,142} = 2.95$, $P = 0.034$). On *Caffeier* and *Clemson*, aphid extinction events were lower in open cages; on *Bangourain*, these were similar in open and closed cages and, on *Kirikou*, these were higher on plants in open cages (predator present). In the subsequent weeks, there were much fewer extinction events and all occurred in open cages (Table 1). Aphid numbers increased in closed cages from week 1 to week 4 but, in open cages, aphid numbers fluctuated across the weeks (Table 1).

In open cages, we observed predators in 57% (31/54) of the cages. Amongst the predators, we recorded syrphid larvae on 54% (29/54) and spiders on 12% (7/54) of the plants. Aphid parasitoid mummies were found on 5% (3/54) of the plants. *Pheidole dea* was the dominant ant species recorded on ant-present plants and was found attending aphids on 99% (66/67) of the ant-present plants. Other ant species observed were: *Tapinoma carininotum* (Weber) on 22% (15/67) and *Camponotus flavomarginatus* (Mayr) on 6% (4/67) of the plants. We observed 14.1 ± 1.8 ants per sampling effort (i.e. per plant). Ant presence on plants increased from 47% (32/67) on the first week up to 79% (53/67) in the subsequent weeks.

We also observed leaf beetles (*Nisotra uniformis* Jacoby) on 83% (45/54) of the plants in open (chewing herbivore present) cages. Mean leaf beetle numbers per plant increased from 0.4 ± 0.2 at week 1 to 2.1 ± 0.5 and 2.2 ± 0.4 at weeks 3 and 4, respectively. No leaf tissue loss or leaf beetles were recorded in closed (chewing herbivore absent) cages plants. Foliage remaining (percentage residual leaf tissue) also reduced over the weeks from a mean of 89.8 ± 2.1% at week 1 to 78.9 ± 2.8% and 70.8 ± 2.7% at weeks 3 and 4, respectively.

Few predators and ants were observed on the plants in week 1 but, by week 2, most okra plants were colonized by invertebrates and okra fruits had also started to appear by observation week 4. Hence, we report the results from week 3 to explain the effect of our treatments on our response variables. Descriptive statistics are given as the mean ± SE.

Effect of main experimental variables. Aphid GR was lower in open (predator present) cages than in closed (predator absent) cages and differed across okra varieties (Table 2). Overall, aphid GR was higher on larger plants (Table 2). In open cages, the highest aphid GR was observed on *Caffeier* and least on *Bangourain*, whereas, in closed cages, *Caffeier* and *Bangourain* both had the highest aphid GR (Fig. 1). In addition, we found no overall main effect of ant presence/absence on aphid GR (Table 2).

Plant RGR varied amongst the different varieties (Table 2). *Caffeier* and *Clemson* grew the least and *Kirikou* showed the highest growth. Cage treatment affected plant RGR (Table 2) and plants grew more in the closed cages (12.2 ± 0.5 cm) than in open cages (9.3 ± 0.6 cm). This is probably the result of a lack of chewing herbivory.

Table 2 Effect of main experimental variables on aphid growth rate, plant relative growth rate and the resulting biomass of harvested fruit from the okra plants

	Aphid growth rate			Plant relative growth rate			Fruit biomass		
Explanatory variables	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Day of fruit collection							–	–	–
Aphid growth rate				**1,103**	**11.14**	**↑0.001**	–	–	–
Plant relative growth rate	**1,103**	**10.92**	**↑0.001**				**1,100**	**3.98**	**↑0.042**
Ants (P/A)	1,103	1.77	0.186	1,103	0.58	0.447	1,100	0.72	0.399
Cage (open/close)	**1,103**	**76.26**	**<0.001**	**1,103**	**3.54**	**0.050**	1,100	0.03	0.617
Plant variety	**3,103**	**2.94**	**0.036**	**3,103**	**12.51**	**<0.001**	3,100	2.05	0.479
Cage × Ants	–	–	–	–	–	–	**1,100**	**6.48**	**0.013**

–, Term removed from the minimal adequate model because it was not significant. Linear models were used with normal error distribution; all higher-order interaction terms were included in the maximal model. P/A, predator absent. Significant values are given in bold.

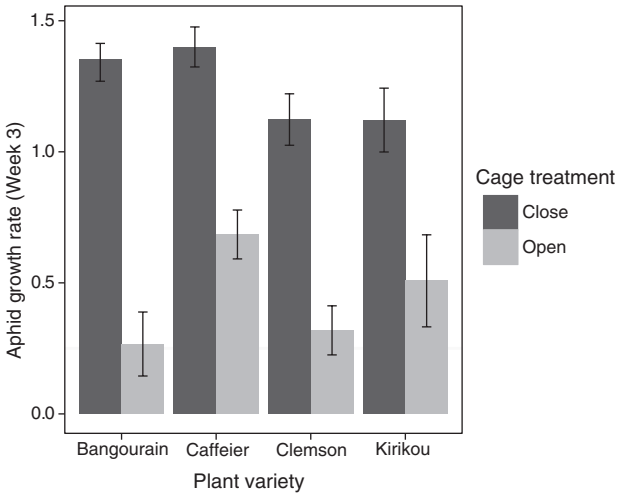

Figure 1 Aphid growth rate on different okra varieties in open and closed cages. Error bars indicate the SE.

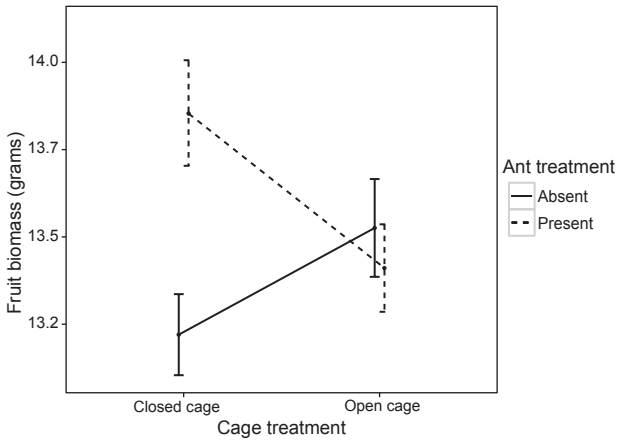

Figure 2 Okra fruit biomass in open and closed cages in the presence and absence of ants. Error bars indicate the SE.

There was no effect of okra variety on fruit biomass (Table 2); however, there was a significant interaction between cage and ant treatment (Table 2), with an increase in fruit biomass in ant presence in closed cages and no such effect in the open cages (Fig. 2). Fruit biomass was recorded to be higher with an increase in plant RGR (Table 2), although there was no effect of aphid GR ($F_{1,98} = 1.25$, $P = 0.266$) on fruit biomass. Plant variety did effect the day of fruit collection ($F_{3,100} = 29.56$, $P < 0.001$) and plants of *Clemson* and *Kirikou* fruited earlier than plants of *Caffeier* and *Bangourain* (see Supporting information, Fig. S1). Ant presence/absence ($F_{1,100} = 0.81$, $P = 0.371$) or cage treatment ($F_{1,100} = 0.49$, $P = 0.485$) did not affect the day of fruit collection.

Effect of ant abundance. Although there was no effect of ants on aphids, we did find a positive association between ant abundance and aphid GR ($F_{1,59} = 19.63$, $P < 0.001$) (see Supporting information, Table S1). Because there was no effect of ant presence/absence on aphid GR (Table 2), we assume that the causal relationship is the result of more aphids attracting more ants. Ant abundance had no effect on plant RGR or fruit biomass (see Supporting information, Table S1).

Effect of degree of herbivory and predation. Aphid GR at week 3 was higher in syrphid larvae presence ($F_{1,45} = 16.20$, $P = 0.002$), suggesting an attraction of syrphids to plants with higher aphid numbers. This effect was mediated by okra variety (aphid GR × okra variety: $F_{3,45} = 3.17$, $P = 0.033$). On three of the four okra varieties, aphid GR was higher with syrphid larvae presence, whereas, on *Caffeier*, it was the opposite (Fig. 3a). To analyze the effect of predation by syrphid larvae on the aphids, we calculated the change in aphid GR from week 3 to week 4 and found that syrphid larvae presence significantly reduced aphids by 42% ($F_{1,48} = 5.15$, $P = 0.027$). Although there was no significant interaction between plant variety and syrphid larvae presence ($F_{3,45} = 0.55$, $P = 0.653$), we did observe that syrphids reduced aphid GR on three of the four okra varieties but not on *Caffeier* (Fig. 3b). The abundance of leaf beetles ($F_{1,43} = 2.39$, $P = 0.129$) or foliage remaining ($F_{1,44} = 0.97$, $P = 0.329$) had no effect on aphid GR.

Plant RGR increased with an increase in foliage remaining (i.e. decrease in herbivory) ($F_{1,48} = 7.75$, $P = 0.007$) but was not affected by leaf beetle abundance ($F_{1,46} = 1.88$, $P = 0.177$). There was also no effect of leaf beetle abundance ($F_{1,44} = 0.17$, $P = 0.683$) or syrphid larvae presence ($F_{1,46} = 1.08$, $P = 0.305$) on fruit biomass.

Effect on ant abundance, syrphid larvae, leaf beetle abundance and chewing herbivory. We found no effect of cage ($F_{1,57} = 0.78$, $P = 0.382$) or plant variety ($F_{3,57} = 0.06$, $P = 0.981$) on ant abundance. As noted above, ant abundance on a plant increased with aphid abundance ($F_{1,57} = 5.46$, $P = 0.023$).

The presence of syrphid larvae was not affected by the presence of ants ($F_{1,48} = 0.57$, $P = 0.452$) or by plant variety ($F_{3,48} = 1.55$, $P = 0.214$). Aphid GR did affect syrphid larvae presence on a plant ($F_{1,48} = 9.98$, $P = 0.002$), probably because more syrphid larvae were attracted to plants with a higher aphid GR.

We found an effect of okra variety on foliage remaining ($F_{3,49} = 3.01$, $P = 0.038$) and leaf beetle abundance ($F_{3,47} = 3.25$, $P = 0.030$). Highest foliage remaining was recorded for *Bangourain* and *Caffeier* and lowest for *Clemson* (see Supporting information, Fig. S2). Leaf beetle abundance was lowest on *Bangourain* and highest on *Clemson* and *Caffeier* (see Supporting information, Fig. S3). There was a moderate negative correlation between leaf beetle abundance and foliage remaining ($r = -0.46$, d.f. = 52, $P < 0.001$) and thus leaf loss was explained only partly by leaf beetle herbivory. Ant presence had a positive effect on foliage remaining ($F_{1,49} = 5.86$, $P = 0.010$), with a higher amount of foliage remaining (i.e. less herbivory) in ant presence ($83.2 \pm 3.8\%$) than in ant absence ($72.3 \pm 3.9\%$). Ant presence/absence had no effect on leaf beetle abundance, although leaf beetle abundance decreased with an increase in ant abundance ($F_{1,47} = 5.38$, $P = 0.024$).

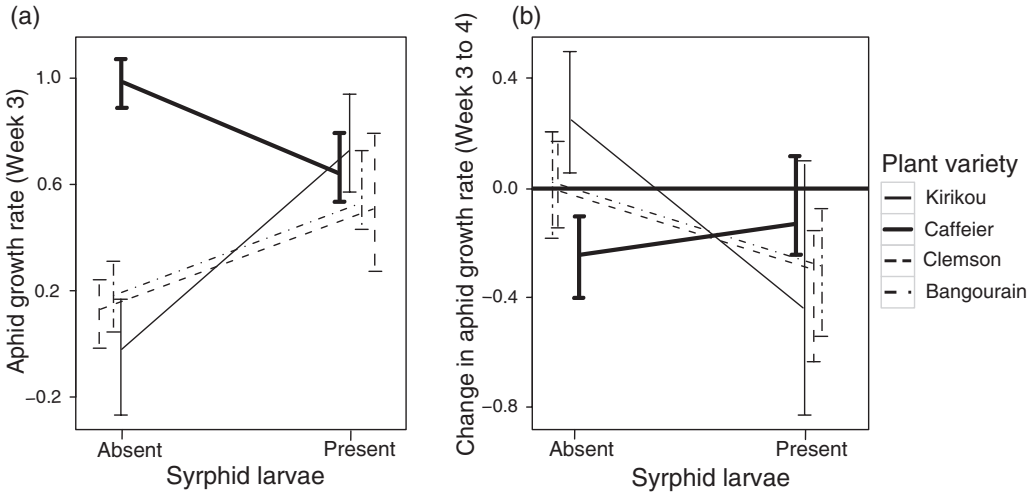

Figure 3 (a) Aphid growth rate at week 3 on different okra varieties in the presence and absence of syrphid larvae in open cages. (b) Change in aphid growth rate from week 3 to 4 on different okra varieties in the presence and absence of syrphid larvae in open cages. The horizontal line in (b) shows no change in aphid growth rate. Error bars indicate the SE.

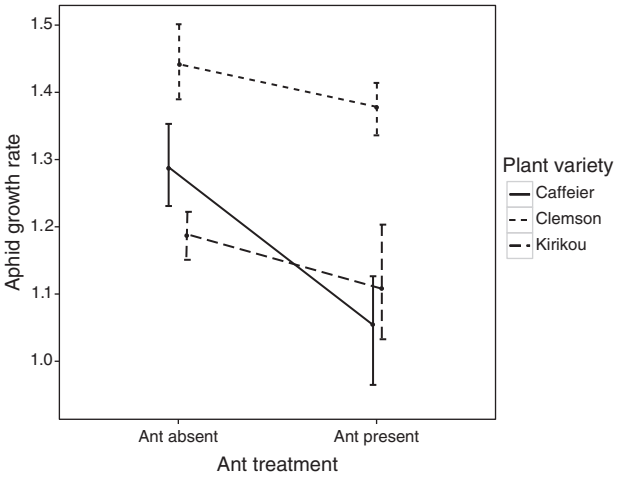

Figure 4 Aphid growth rate in the presence and absence of ants on different okra varieties in the screenhouse. Ten adult aphids were added to the plants and allowed to reproduce. Error bars indicate the SE.

Screenhouse experiment

Plant variety influenced aphid GR ($F_{2,43} = 8.66$, $P = 0.006$) and highest aphid numbers were recorded on *Clemson*, followed by *Caffeier* and *Kirikou*. Ants reduced aphid GR on all okra varieties ($F_{1,43} = 5.19$, $P = 0.023$) (Fig. 4) by 24% and, although there was no significant interaction between ant and plant variety ($F_{2,40} = 1.05$, $P = 0.359$), aphid reduction in ant presence was strongest on *Caffeier* (aphid number: ant presence 216.0 ± 42.9; ant absence 362.3 ± 38.9) (Fig. 4). During one sampling, we observed an ant predating on the aphids on *Caffeier*. Plant RGR did not vary across plant varieties ($F_{2,43} = 1.06$, $P = 0.354$) and was not affected by aphids ($F_{1,18} = 2.19$, $P = 0.156$). However, it was influenced by ants ($F_{1,43} = 4.16$, $P = 0.048$) (see Supporting information, Table S2) and plants grew less in the presence of ants (ant present 14.2 ± 1.0 cm; ant absent 16.4 ± 1.1 cm).

We observed a mean of 4.4 ± 0.7 ants per observation. In accordance with the field experiment results, there was no effect of plant variety on ant abundance ($F_{2,20} = 0.14$, $P = 0.869$). By contrast to the field results, we found no association between aphid GR and ant abundance on the plants ($F_{1,19} = 0.002$, $P = 0.965$).

Discussion

Overall, our results show that ants had neither a positive, nor negative effect on the aphids in the field experiment. However, in the screenhouse, ants had a negative effect on the aphids and ant predation on aphids was observed. Thus, ant–aphid interactions on okra are more complex than a standard model of mutualistic or antagonistic relationships. Similar to our study, previous studies have also found facultative ant–aphid associations to vary, where ants that tend aphids also predate upon them dependent on external food source (Offenberg, 2001), plant genotype (Mooney & Agrawal, 2008) or increasing aphid density (Sakata, 1995). Hence, we argue that, because *A. gossypii* is a facultative ant attended species, the nature of its association with ants can vary and be mediated by a diversity of factors.

In the present study, predators (specifically syrphid larvae) significantly reduced aphids on the plants in the field and were more often present on plants with higher aphid numbers. This suggests that the female syrphid chose to oviposit on plants with more food resource for her offspring (Gripenberg *et al.*, 2010). Aphid extinctions were high in the first week of the experiment, influenced by a cage-by-plant variety interaction, irrespective of ant presence. Because we found little effect of plant variety on predator abundance, this effect may be driven by reduced settling behaviour and acceptance of the plant by aphids (Sauge *et al.*, 1998). From the screenhouse experiment, we know that each plant variety is a suitable host, although potential variation in acceptance could lead to aphids leaving a plant in the field experiment and not returning, thus impacting the future growth and chance of extinction.

Although ants did not protect aphids from predation, their abundance on plants did increase with an increase in aphid abundance suggesting a more opportunistic ant–aphid interaction in our system. Recruitment of ants was beneficial for okra plants because plant herbivory and leaf beetle abundance reduced with an increase in ant abundance. In turn, this indirectly benefited the plant because more ants meant less herbivory, which was associated with a higher plant RGR; fruit biomass was positively correlated with a higher plant RGR. Indeed, an increasing density of flea beetles has previously been shown to reduce okra yield (Pitan & Ekoja, 2011). It is known in many systems that the recruitment of ants by aphids can reduce leaf-herbivory by beetles and caterpillars of the plant (Floate & Whitham, 1994; Styrsky & Eubanks, 2007). Furthermore, in a study on *A. gossypii*, Styrsky and Eubanks (2010) also found that ant attendance of the aphids increased cotton-plant reproduction as a result of a reduction in leaf-chewing herbivores.

We also found that ants benefited fruit biomass; however, this was only apparent in the closed cages and is thus independent of herbivory effects. One possible mechanism might be through efficient removal of honeydew from the aphids by the ants because aphid numbers were higher in the closed cages (possibly leading to higher honeydew production) and this would have attracted more ants. Efficient removal of honeydew by ants will benefit the plant because honeydew left on the plant can encourage the growth of harmful mould (Way, 1963). Okra varieties did not differ in their fruit biomass, although they did differ in the time of reaching fruit maturity (day of fruit collection), which was expected because it known that okra varieties differ in the time that they take to produce mature fruits (Saifullah & Rabbani, 2009).

By contrast to the field study, in the screenhouse, there was no association between ant abundance and aphid numbers. Indeed, ants were observed to prey upon aphids and aphid numbers were reduced on all plants with ant presence. This is in accordance with ant colonies mostly foraging for insect prey (protein source) during their larval growing season (Edwards, 1951). However, we provided an external protein source to our ant colonies and so protein limitation is not considered to explain our results. Possibly, ants preyed upon aphids because of high aphid numbers on the plant resulting in honeydew production that was in excess of the demands of the ant colony (density-dependent predation) (Rosengren & Sundström, 1991; Sakata, 1995). Our experiment did not specifically test for density-dependent predation, although we know from our field experiment that ants colonized plants with a higher aphid growth rate (i.e. *Caffeier* variety).

In the field, plant variety influenced aphid GR and highest aphid GR was recorded on *Caffeier*. Aphids are known to vary in their performance and preference, in various systems, across different plant genotypes or varieties, and this cascades to affect natural enemy abundances (Zytynska & Weisser, 2016). Because more syrphid larvae were observed on plants with a higher aphid GR overall, we might assume that a plant with high aphid performance (i.e. *Caffeier* variety) will also host more syrphid larvae. At week 3, on all varieties except *Caffeier*, we recorded high aphid GR in syrphid larvae presence, suggesting that there was such a high aphid GR on *Caffeier* that syrphid females did not need to particularly seek out plants with high aphid loads. Furthermore, there was little effect of syrphids on the change in aphid GR from week 3 to week 4 on *Caffeier*, whereas there was a negative effect on the other three varieties. This could be explained by such a high aphid performance on *Caffeier* that it negated any effect of predation by the syrphid larvae.

Similar to the field study, plant variety affected aphid GR in the screenhouse; however, here, the highest aphid GR was observed on *Clemson* and lowest on *Caffeier*. Aphid numbers were reduced in the screenhouse in ant presence. In particular, ant presence strongly reduced aphid numbers on *Caffeier* variety. In both studies, ants were observed collecting plant-produced pearl bodies, which have been shown to be produced by plants to attract ants in exchange for protection from phytophagous insects (Dutra *et al.*, 2006; Mayer *et al.*, 2014). The presence of pearl bodies has been little studied in okra, although it may mediate the aphid–ant interactions in our system. For example, the relative suitability of the aphid honeydew versus the plant pearl bodies for resources, and the variable ant preferences for this, could explain why ants might switch between tending the aphids and predating the aphids on okra (Mooney & Agrawal, 2008). Indeed, this might also explain why we recorded a reduced plant growth rate in ant presence in the screenhouse because it is well known that plant fitness can be reduced by investing in defensive compounds (Frederickson *et al.*, 2012; Mayer *et al.*, 2014). However, in the field with multiple food sources and okra-associated invertebrates, we did not record this negative effect of ants on plants or aphids. Controlled studies can only test a limited range of possible outcomes amongst species (Stadler & Dixon, 2005). Hence, in the screenhouse, where ant colonies were restricted to a plant for their nutritional requirements, there was a stronger effect of their presence on aphids and the plant than in the field study.

We also found that herbivory differed across plant varieties, with much less herbivory on *Bangourain* and *Caffeier* than on *Clemson.* A previous study by Underwood and Rausher (2000) found that flea beetles showed a preference for different soybean genotypes. Although we did not specifically test for preference effects, there were fewer beetles on *Bangourain* (with higher foliage remaining) and more on *Clemson* (with lower foliage remaining), suggesting some preference for *Clemson* against *Bangourain*. However, on *Caffeier*, the low rate of herbivory (high foliage remaining) was not explained by low leaf beetle abundance and, indeed, the leaf beetles were most abundant on this variety (see Supporting information, Figs S2 and S3). This suggests that, even though they may be attracted to *Caffeier*, they do not consume as much leaf material, which is potentially explained by a higher nutritional value for the beetle (i.e. low C : N value) (Mattson, 1980), although this remains to be studied. Alternatively, the high aphid GR on *Caffeier* in the open cages would have attracted more ants, which in turn was found to reduce herbivory on the plant (Styrsky & Eubanks, 2007, 2010; Zhang *et al.*, 2012).

Overall, we show that predators significantly reduced aphid numbers on okra and ants did not protect aphids. Furthermore, aphids did not influence okra fruit yield, although there was substantial herbivory on the plants by leaf beetles, which indirectly impacted yield. Aphid recruitment of ants was beneficial for okra plants because they reduced the number of leaf beetles and had an indirect positive effect on fruit yield. We also found aphids

and leaf beetle numbers to vary across okra varieties, although the ant–aphid interaction on okra was not mediated by okra varieties. Despite minimal effects of aphids on plant yield, it is still important to maintain low aphid and leaf beetle population sizes because aphids can potentially transmit plant viruses (Katis *et al.*, 2007) and the spread of okra mosaic virus by beetles is well documented (Pitan & Ekoja, 2011; Benchasri, 2012). Enhancing ant abundance in okra farms can be useful for leaf beetle control (Styrsky & Eubanks, 2007). With respect to biocontrol measures, because ants do not protect aphids, the efficiency of an introduced predator for *A. gossypii* can be higher and syrphid larvae species can be tested to determine suitable species for biocontrol. Finally, understanding the mechanisms behind the negative effect of ants on aphids can further help in the development of efficient biocontrol measures.

Acknowledgements

We thank Raissa Houmgany of IITA, Cameroon, for her assistance in both experiments; Dr Apollin Fotso of IITA for identification of ant species; and BMZ (through IITA) for providing financial support.

AS, SZ, RH and WW designed the experiment, with AS collecting the data. Analysis and interpretation was carried out by AS and SZ. AS, SZ, RH and WW all contributed to the first draft, which was completed by AS and commented on by SZ, RH and WW. All authors read and approved the final manuscript submitted for publication.

Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12159

Table S1. Ant-present plants. Effect of experimental variables on aphid growth rate, plant relative growth rate and resulting biomass of harvested fruit from the okra plants with ant presence (Field experiment).

Table S2. Effect of experimental variables on aphid growth rate and plant relative growth rate (screenhouse experiment).

Fig. S1. Variation in day of fruit collection across okra varieties. Error bars indicate the SE.

Fig. S2. Mean leaf remaining per plant on different okra varieties (open cages). Error bars indicate the SE.

Fig. S3. Mean leaf beetle number per plant on different okra varieties (open cages). Error bars indicate the SE.

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Accepted 9 March 2016