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Interactions between extrafloral nectaries, aphids and ants: are there competition effects between plant and homopteran sugar sources?

Abstract Broad bean (*Vicia faba*), an annual plant bearing extrafloral nectaries (EFN) at the base of the upper leaves, is regularly infested by two aphid species, *Aphis fabae* and *Acyrthosiphon pisum*. EFN and *A. fabae* are commonly attended by the ant, *Lasius niger*, while *Ac. pisum* usually remains uninfested. Sugar concentration and sugar composition of extrafloral nectar did not change significantly after aphid infestation. The sugar concentration was significantly higher in EFN (*c.* 271 µg µl^{-1}) than in the honeydew of *A. fabae* (37.5 µg µl^{-1}). The presence of small *A. fabae* colonies had no significant effect on ant attendance of EFN, which remained at the same level as that on plants without *A. fabae*. Obviously, there was no significant competitive effect between the two sugar sources. We suggest that the high sugar concentration in the extrafloral nectar may outweigh the higher quality (due to the presence of melezitose) and quantity of aphid honeydew. Ants and the presence of EFN influenced aphid colony growth. While *A. fabae* colonies generally grew better in the presence of ants, *Ac. pisum* colonies declined on plants with EFN or *A. fabae* colonies. We conclude that EFN may provide some degree of protection for *V. faba* against those sucking herbivores that are not able to attract ants.

Keywords *Vicia faba* · Sugar composition · *Aphis fabae* · *Acyrthosiphon pisum* · Mutualistic interactions

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Introduction

When foraging for carbohydrate sources, ants may have a choice between collecting sugar provided by plants in nectaries and extrafloral nectaries (henceforth referred to as EFN), or by animals such as homopterans which produce sugar-rich excretions (Hölldobler and Wilson 1990). In both cases, there also seem to be benefits for the ants' partner. Ant attendance reduces predation and parasitization in many homopteran species (e.g. Nixon 1951; Way 1963; Addicott 1979; Bristow 1984; Völkl 1992; Rozario et al. 1993; Fischer et al. 2001). Similarly, a decrease in damage due to herbivory has been reported on ant-tended plants because ants prey on herbivores or interrupt their oviposition or feeding. In a number of cases, a positive effect of ant attendance on the plant's overall fitness has been demonstrated (e.g. Bentley 1976; Koptur 1979; Messina 1981; Skinner and Whittaker 1981; Warrington and Whittacker 1985; Freitas and Oliveira 1996; but see O'Dowd and Catchpole 1983).

A conflicting situation, however, may arise when both EFN and honeydew-producing homopterans occur simultaneously on the same plant. In the Palaearctic region, such an overlap is frequently found, especially in Fabaceae species, which usually possess EFN and which are often attacked by ant-attended aphid species of the genus *Aphis* (Börner 1952). Becerra and Venable (1989, 1991) and Koptur (1991) hypothesized that nectar produced in EFN may distract ants from the homopteran sugar sources and thus may function as a defence against ant-Homoptera mutualism. They proposed that extrafloral nectar is more predictable in space, time and quality, is easier to sample and will need less extensive care for maintenance. As a consequence, abandoned homopteran colonies would suffer from a higher predation and parasitism, and the damage to the host plant would be either reduced or eliminated. Del-Claro and Oliveira (1993) questioned this hypthesis, demonstrating that simulated EFN on the Brazilian shrub *Didymopanax vinosum* did not distract *Camponotus* workers from tending colonies of the honeydew-producing membracid *Guayaquila*

xiphias. Recent studies have additionally shown that honeydew/nectar composition and quantity may have an important effect on ant behaviour (Völkl et al. 1999; Fischer et al. 2001).

If there is competition between EFN and honeydew-producing homopterans, we would expect an increase in extrafloral nectar production in response to herbivore attack. EFN-bearing plants may respond species-specifically to herbivore attack. In some plant species, there was an increase in extrafloral nectar production following tissue damage by herbivores (Koptur 1989; Wäckers and Wunderlin 1999; Wäckers et al. 2001), while other studies found no such effect (Koptur 1989; F.L. Wäckers, unpublished work). In addition, there may be a change in extrafloral nectar composition after herbivore attack (Smith et al. 1990). This evidence comes, however, from tissue-feeding insects, while there is only poor knowledge of the effect of sucking insects, i.e. competitors of the EFN, on quantitative and qualitative extrafloral production.

In this study, we addressed three questions. First, we investigated the effect of aphid infestation (i.e. of sucking insects) on EFN production and EFN sugar concentration of broad bean (*Vicia faba*). We used two aphid species for our experiments. The black bean aphid, *Aphis fabae*, is regularly tended by honeydew-collecting ants and benefits from this mutualism (e.g. Börner 1952; Banks 1962; Völkl and Stechmann 1998; Fischer et al. 2001), while the pea aphid, *Acyrthosiphon pisum*, remains unattended. Second, we tested for competitive effects between extrafloral nectar and honeydew. In contrast to previous studies that used simulated nectar (Del-Claro and Oliveira 1993), we offered two natural sugar resources (EFN of *V. faba*, *A. fabae* colonies) available on the same plant to colonies of the ant *Lasius niger*, the most common mutualistic ant on *V. faba* and *A. fabae* in Central Europe. Third, we studied the effect of EFN on colony growth of the two aphid species and tested whether there were differences between *A. fabae* and *Ac. pisum*.

Materials and methods

Plant and insect material

V. faba (cv. minor) plants were grown from seeds in plant pots (diameter 15 cm, height 12 cm) in garden soil. They were used for experiments as soon as the first pair of leaves had unfolded. Stock cultures of *A. fabae* and *Ac. pisum* were established from a single virginoparous apterous female (of each species) collected near Bayreuth in June 1999. Aphid cultures were kept on *V. faba* in a climate chamber at 20±1°C, 65% relative humidity (RH), 3,000 lx and 16:8 h light:dark.

Eight nests of *L. niger* (>1,000 workers per nest; all nests contained queens) were established in small terraria (70×35×35 cm) and kept in a climate chamber under the same experimental conditions as the aphid species. The ants were provided with freshly killed crickets (*Gryllus bimaculatus*) as a protein source. Colonies of the aphid species *Metopeurum fuscoviride* on *Tanacetum vulgare* served as a carbohydrate source to avoid conditioning by *A. fabae* colonies or by EFN. All ant nests contained brood when used in the experiments.

Analysis of sugar composition and sugar concentration

Extrafloral nectar was collected directly from the extrafloral nectary using a microcapillary (volume 0.5 μl). We used only plants where the nectar was removed 2 h before collection. By this means, we tried to reduce the bias in sugar concentration resulting from evaporation processes in the nectar droplets. The nectar of all EFN of a single bean plant (between 3 and 6 active EFN per plant) was pooled in one sample. *A. fabae* individuals from an unattended colony feeding on *V. faba* were collected in a Petri dish and stimulated with a brush to release a honeydew droplet. This droplet was collected directly from the aphid's anus, using a microcapillary (volume 0.5 μl). To obtain a sample of 0.5 μl, we needed to pool the honeydew of 10–15 aphids. Thus, one sample represents the average honeydew composition of a single small colony.

After the collection, all samples were stored immediately (in the microcapillaries) at –20°C until they were prepared for analysis. Sugar was analysed by high-pressure liquid chromatography (HPLC), using a CarboPac PA 100, 4×250 mm column. Sample elution was Milli-Q-Water and a gradient with 0.5 M NaOH from 3 to 70% and a flow rate of 1 ml min^{-1} was used. An electrochemical dedector (Dionex ED 40) was used for sugar detection. Borwin Chromatogram software created the respective chromatograms. Typically, 0,1–0.5 μl honeydew was collected in 50 μl of Milli-Q-Water and a 10-μl subsample was injected directly onto the CarboPac column. The chromatogram was calibrated to read out directly the quantity (μg) of each sugar in the 10-μl subsample.

The system was optimized by the injection of sugar standards. The sugar composition of extrafloral nectar/honeydew was based on comparison of retention time to that of standards. Co-elution of sugars in the honeydew with known standards supported this assumption. The sugar concentration in each analysed sample was obtained by a comparison of the peak areas of the standard and the respective sample. The total sugar content in each sample was considered to be 100%.

Quantification of extrafloral nectar production in the presence of ants

The extrafloral nectar production was measured in three situations. In the first trial, we used uninfested beans (*n*=24 plants). In the second trial, *V. faba* was infested by a small *A. fabae* colony (c. 20 individuals) feeding on the upper pair of leaves (*n*=24 plants), while a small *Ac. pisum* colony (c. 10 individuals), also feeding on the upper pair of leaves was used in the third trial (*n*=24 plants).

Workers of a *L. niger* colony (*n*=8 colonies) had access to a single bean plant (and thus to EFN) through an arrangement of wooden sticks. Ants were deprived of carbohydrate for 24 h to ensure that they would have a strong demand for sugars. They were kept off the aphid colonies in trials 2 and 3 by tanglefoot smeared on the leaf stems of the aphid-infested leaves. Each colony was tested on three different plants on three different days. Trials were at least 2 days apart to avoid pseudoreplication.

We measured the extrafloral nectar collected by the ants by comparing the weight differences of ant workers with empty and filled gasters (Herzig 1937; Völkl et al. 1999). First, we counted all ant workers that left the EFN of a plant for 15 min, starting 1 h after the ants had been given access to the plant. Second, we collected 35 ant workers that had just left an EFN (=ants with filled gaster) and additionally 50 ant workers that had just left the nest and entered a wooden stick going in the direction to the aphid colony (=ants with empty gaster). The ants were immediately anaesthetized with ethanol-soaked paper and weighed using a microbalance (Sartorius 2004 MP). Hind tibia length was used as a size measure. We pooled workers collected from all eight *L. niger* nests, since preliminary measurements (25 workers from each colony) had shown that there was no significant size difference between workers of the different nests (one-way ANOVA: *F*=1.14, *df*=7, *P*=0.238).

The average weight differences between similar-sized ants with empty and filled gasters should represent the amount of extrafloral nectar collected by an individual ant. Since extrafloral

nectar of a gland was collected completely by *L. niger* workers and since no division of labor among *L. niger* workers was observed in the experiments, we estimated the extrafloral nectar production of one EFN per hour using the following formula (Völkl et al. 1999):

[(average weight of ants with filled gaster leaving a plant with EFN-average weight of ants with empty gaster)×average number of ants leaving a plant with EFN]/number EFN per plant

Quantification of honeydew production

Standardized *A. fabae* colonies (20 aphids; *n*=8) were established on potted *V. faba* using aphids from the stock culture. Workers of an *L. niger* colony (*n*=8 colonies) received access to the small aphid colony through an arrangement of wooden sticks. We measured the honeydew production of *A. fabae* colonies using the same methods as for EFN. First, we counted all ant workers that left an aphid colony for 15 min, starting 1 h after the ants had been given access to the aphid colony. Second, we collected 35 ant workers that had just left a standardized aphid colony and additionally 50 ant workers that had just left the nest.

As in EFN, the average weight differences between similar-sized ants with empty and filled gaster should represent the amount of honeydew collected by an individual ant. Since all excreted honeydew droplets were collected by *L. niger* workers and since no division of labor among *L. niger* workers was observed in the experiments, we estimated the honeydew production of an individual aphid per hour using the same formula as before:

[(average weight of ants with filled gaster leaving *A. fabae* – average weight of ants with empty gaster) × average number of ants leaving a colony of *A. fabae*] / no. aphids per *A. fabae* colony

Ant attendance on extrafloral nectaries in different situations

The ant attendance of EFN was studied in three situations. In the first trial, we used uninfested beans (*n*=24 plants). In the second trial, *V. faba* was infested by a small *A. fabae* colony (*c*. 20 individuals) feeding on the upper pair of leaves (*n*=24 plants), while a small *Ac. pisum* colony (*c*. 10 individuals), also feeding on the upper pair of leaves was used in the third trial (*n*=24 plants). Ants were kept off the aphid colonies in trials 2 and 3 by tanglefoot. Each ant colony (*n*=8) was used with three different plants on three different days. Trials were at least 2 days apart to avoid pseudoreplication.

Ant attendance of EFN was measured using two different methods one day after *L. niger* had received access to the plants. First, we counted all ants that were found either directly on the EFN or within a diameter of 2 cm. These were assumed to attend the EFN ("direct count"). Since no "guards" (ants which collect no honeydew but guard the carbohydrate source) were observed for *L. niger* in previous studies (Völkl et al. 1999; Mailleux et al. 2000), this value should be a good estimate of the frequency of ant attendance (Fischer et al. 2001). Second, we counted the number of ant workers which left the EFN of a plant for 15 min ("time count"), starting 1 h after the ants had been given access to the plant.

Measurement of ant attendance and aphid colony growth in different situations

In all experiments described in this section, the number of aphids per colony was counted daily for each colony for 6 days. This is a rather short period for the study of aphid colony growth. *Ac. pisum* colonies on ant-attended plants, however, generally disappeared within that period. Therefore, we stopped the experiment at that time because we wanted to study different effects of the presence of EFN on the two aphid species, rather than demonstrate the well-known positive effect of *L. niger* on colony growth of *A. fabae*.

The number of aphid-attending *L. niger*-workers was counted between 8.30 a.m. and 12.00 a.m. to avoid any bias due to potential diurnal patterns in ant activity. In general, all ants that were found either directly within the aphid colony or directly at the EFN, or within a diameter of 2 cm, were assumed to be attending either the aphid colony or EFN.

Aphid colony size usually increased during the experiment because no aphids were removed. Ant numbers per colony can vary considerably with aphid colony size (see also Breton and Addicott 1992; Itioka and Inoue 1996). However, there was a linear positive correlation between aphid colony size and the number of attending ants (*r*=0.329, *n*=385, *P*<0.001).To obtain a parameter for the intensity of ant attendance that was independent of aphid colony size, we divided the number of ants by colony size (ants per aphid=relative ant attendance), as justified by the positive correlation of ant attendance with aphid colony size (Völkl et al. 1999; Fischer et al. 2001).

We set up eight different experiments, each of them with eight replicates: experiment 1=empty plants (EFN removed) only; experiment 2=*V. faba* plants with EFN; experiment 3=*V. faba* plants with EFN+*A. fabae*; experiment 4=*V. faba* plant with *A. fabae* but EFN removed; experiment 5=*V. faba* plants with EFN+*Ac. pisum*; experiment 6=*V. faba* plant with *Ac. pisum* but EFN removed; experiment 7=*V. faba* plants with EFN+*A. fabae*+*Ac. pisum;* experiment 8=*V. faba* plant with *A. fabae* and *Ac. pisum* but EFN removed. EFN were removed by carefully cutting off the side leaves bearing EFN. To avoid an effect on ant attendance, plants were not used for experiments during the following 3 days.

In each replicate, initial colonies of *A. fabae* and *Ac. pisum* colonies consisted of 9–11 individuals (third and fourth instar larvae). A surplus number of aphids (15–18 individuals) obtained from the stock culture was placed on a bean plant 1 day before the start of the experiment. Surplus aphids were gently removed 2 h before the first counts to avoid disturbing the ants during the counts. *L. niger* workers of a particular nest had access to eight *V. faba* plants (one plant of each treatment) through an arrangement of sticks between the plant pots and the terraria. By this means, we were able to control for ant attendance in all experiments.

All experiments were carried out during a period of low *L. niger* activity (on average fewer than 15 ants leaving the nest via sticks during 10 min) because we hypothesized that ants would respond much more selectively to differences in resource quality if their carbohydrate demand was low (Fischer et al. 2001). All experiments were carried out in a greenhouse at 22±1°C, 75% RH, 8,000–10,000 lx and 16:8 h light:dark.

The first experiment showed that the effect of ant attendance on the colony growth of *Ac. pisum* may vary with the degree of ant activity. Therefore, we repeated all treatments involving the effect of EFN or *A. fabae* on *Ac. pisum* in situation when ant activity was much higher (on average more than 40 ants leaving the nest via sticks during 10 min). *Ac. pisum* colonies in the second design initially consisted of 24–26 individuals. Additionally, we ran a control experiment where ants were excluded from the plant.

Competition between EFN and *A. fabae*

In this experiment, we tested whether EFN and *A. fabae* competed for ant attendance when they occurred simultaneously but on different plants (Fischer et al. 2001). *L. niger* (*n*=8 colonies) had access to five *V. faba* plants with EFN only as described above (4 EFN per plant; *n*=8 replicates; each colony was used only once). The relative ant attendance (ants/EFN) was counted 1 day after the ants were given access to the bean plants. The next day, five plants with colonies of *A. fabae* (*c*. 20 individuals), but with EFN removed, were placed close to the plants with EFN only (distance 20 cm). The ants of a particular nest were given access immediately. Ant attendance was counted again the next day when *L. niger* had accepted the new carbohydrate source. The experiment was repeated in the same design in the reverse sequence, beginning with ant access to *A. fabae* first and adding plants with EFN only 1 day later.

Results

Sugar composition and concentration of extrafloral nectar and honeydew

Extrafloral nectar samples from uninfested beans, *Ac. pisum*-infested beans and the majority of *A. fabae*-infested beans consisted of fructose, glucose and sucrose only, with glucose and fructose being the dominant sugars (Table 1). In a few extrafloral nectar samples from *A. fabae*-infested plants ($n=4$, data not included in Table 1), we also detected small amounts of melezitose, maltose and erlose, which made up less than 10% of the total sugar content. In these samples, *A. fabae* colonies were feeding close above the EFN, and extrafloral nectar was probably contaminated by *A. fabae* honeydew.

The total sugar concentration of the extrafloral nectar varied between *c*. 251 µg sugar µl^{-1} nectar (uninfested plants) and *c*. 298 µg sugar µl^{-1} nectar (*A. fabae*-infested plants) (Table 1). Due to the high variability between samples, there were no significant differences between the three treatments, either in total sugar concentration, or in the concentrations of glucose, fructose and sucrose (Table 1). Differences in the proportions of the various sugars also did not differ significantly between treatments.

In contrast to extrafloral nectar, the honeydew of *A. fabae* regularly contained additional sugars which comprised more than 65% of the total sugar content (Table 1). Melezitose was the dominant sugar in *A. fabae* honeydew from *V. faba*. Additionally, trehalose was regularly found in high amounts in *A. fabae* honeydew. Glucose and fructose, the two dominant sugars in extrafloral nectar, were found in significantly lower concentrations and in much lower proportions in honeydew (Table 1). The total sugar concentration of honeydew was significantly lower than that of extrafloral nectar, reaching only between 14% and 17% of the latter (Table 1).

Quantitative extrafloral nectar and honeydew production

Nectar prodcution per EFN on uninfested plants averaged 246±37 µg h^{-1}. The total estimated extrafloral nec-

tar production of four EFN (the average number of secreting EFN per bean plant) was 988 µg plant^{-1} h^{-1}. EFN on *A. fabae*-infested (328±40 µg EFN^{-1} h^{-1}) or *Ac. pisum*-infested plants (257±26 µg EFN^{-1} h^{-1}) did not differ significantly from each other or from uninfested plants (Kruskal-Wallis test: $\chi^2=1.883$, $df=2$, $P=0.390$). A single *A. fabae* individual (feeding in a colony of 20 individuals) produced on average 133±23 µg honeydew per hour, i.e. significantly less than one EFN (Kruskal-Wallis test: $\chi^2=18.83$, $df=3$, $P=0.0390$). The total estimated honeydew of an *A. fabae* colony of 20 individuals, as used in this study, is *c*. 2,620 µg colony^{-1} h^{-1}, i.e. more than twice as much as one EFN.

Ant attendance on extrafloral nectaries in different situations

If *L. niger* had no access to aphid colonies, the number of honeydew-collecting ants feeding directly at the EFN during the counts ("direct count") was significantly higher if plants were not infested by aphids (Fig. 1). There was, however, no difference between plants that were infested by either *A. fabae* or *Ac. pisum*. By contrast, the situation was different if ant attendance per time unit ("time count") was taken as a measure (Fig. 1). In this case, the most intensive ant attendance on EFN was observed on *A. fabae*-infested beans. However, there were no significant differences in ant attendance per time unit between the three treatments.

Ant attendance on *V. faba* in the presence of different sugar resources

The absolute number of foraging ant workers per *V. faba* plant was significantly higher on plants with EFN than on plants without EFN in three treatments (without aphids, infested by *A. fabae*, infested by *Ac. pisum*). Only when both aphid species were present simultaneously was there no significant difference between plants with and without EFN (Fig. 2). Also, there were more ants present on plants with *A. fabae* than on plants with EFN

Table 1 Sugar composition and sugar concentration in extrafloral nectaries (*EFN*) of *Vicia faba* in different treatments (with or without *Aphis fabae* or *Acyrthosiphon pisum*), and in *A. fabae* feeding on *V. faba*. Sugar concentrations are presented as µg sugar µl^{-1} fresh honeydew (*Fru* fructose, *Glu* glucose, *Suc* sucrose,

Mal maltose, *Tre* trehalose, *Mel* melezitose, *Erl* erlose, *Xyl* xylose, *n* number of samples). All *values* represent means±SE Within each *column*. Means of sugar concentrations that share the same *letter* do not differ significantly at $P<0.05$ (Kruskal-Wallis ANOVA and Mann-Whitney *U*-test)

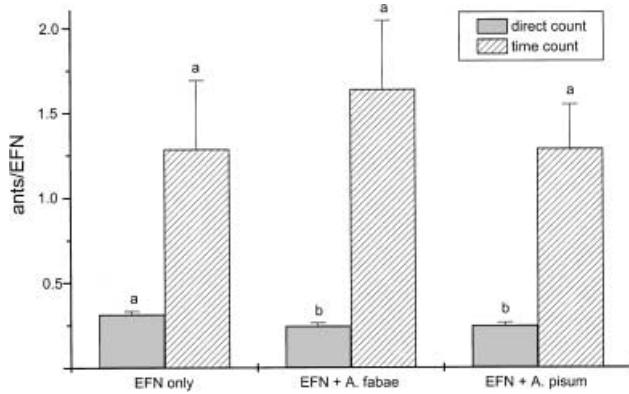|	Fru	Glu	Suc	Mal	Tre	Mel	Erl	Xyl	Units	Total sugar concentration
EFN ($n=10$)	99.7±23.1	119.7±22.7	32.5±6.5	–	–	–	–	–	µg/µl	251.2±50.2[a]
	38.7±2.7	47.6±2.1	13.7±2.7	–	–	–	–	–	%	
EFN (+*A. fabae*) ($n=10$)	126.1±16.7	131.0±12.2	43.0±10.2		–	–	–	–	µg/µl	298.7±32.1[a]
	41.2±2.3	44.6±2.2	13.9±2.3		–	–	–	–	%	
EFN (+*Ac. pisum*) ($n=10$)	102.8±15.1	127.0±7.2	32.5±6.5	–	–	–	–	–	µg/µl	265.2±23.6[a]
	38.1±3.1	50.1±3.4	11.8±2.5	–	–	–	–	–	%	
A. fabae ($n=15$)	5.3±1.3	3.5±0.7	1.8±0.7	1.4±0.4	1.9±0.4	22.9±5.1	0.4±0.1	0.2±0.1	µg/µl	37.5±7.1[b]
	14.4±2.6	12.4±2.8	4.4±1.0	4.0±0.9	7.3±1.7	55.1±5.3	0.9±0.3	1.4±0.9	%	

Fig. 1 Ant (*Lasius niger*) attendance on extrafloral nectaries (*EFN*) of *Vicia faba* without and with aphid (*Aphis fabae* and *Acyrthosiphon pisum*) infestation in "direct counts" (ants/EFN) and "time counts" (ants/EFN×h). *L. niger* had no access to aphid colonies. All *values* give mean±SE. Within each counting method, means sharing the same *letter* do not differ significantly at *P*<0.05 (Mann-Whitney *U*-test with Bonferroni correction)
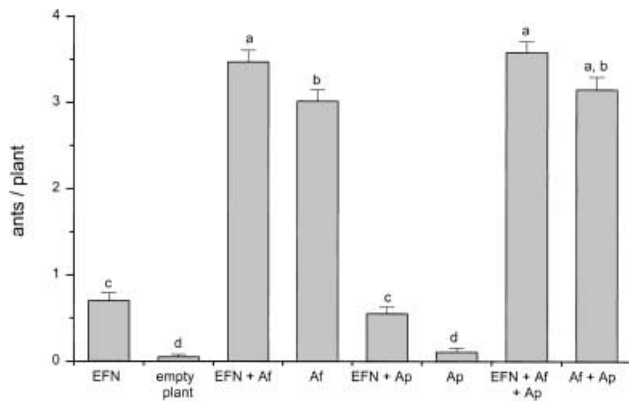

Fig. 2 Ant attendance on *V. faba* in eight different treatments (mean±SE) (*EFN* extrafloral nectary, *Af Aphis fabae*, *Ap Acyrthosiphon pisum*). Means sharing the same *letter* do not differ at *P*<0.05 (Wilcoxon test)

but without *A. fabae*. Plants infested only by *Ac. pisum* (but with EFN removed) did not differ significantly from empty plants without any sugar resource (Fig. 2).

There was, however, no difference between the various treatments when the relative ant attendance (corrected for EFN number and *A. fabae* individuals) on the two different sugar sources was considered. Neither the average number of ants visiting one EFN nor the average number of ants visiting one *A. fabae* differed significantly between treatments, and they varied only slightly (Kruskal-Wallis ANOVA: EFN, χ^2=0.582, *df*=3, *P*=0.900; total mean for all treatments: 0.12±0.08 ant/EFN; *A. fabae*, χ^2=4.375, *df*=3, *P*=0.224, total mean for all treatments: 0.12±0.02 ants/aphids).

Competition between EFN and *A. fabae*

Ant attendance on EFN dropped considerably after the addition of *A. fabae* colonies (Fig. 3) in day 3 (Wilcoxon test: *Z*=–2.051, *n*=20, *P*=0.040), indicating that there was
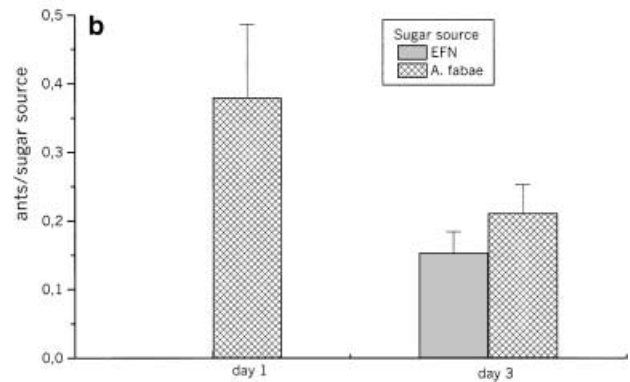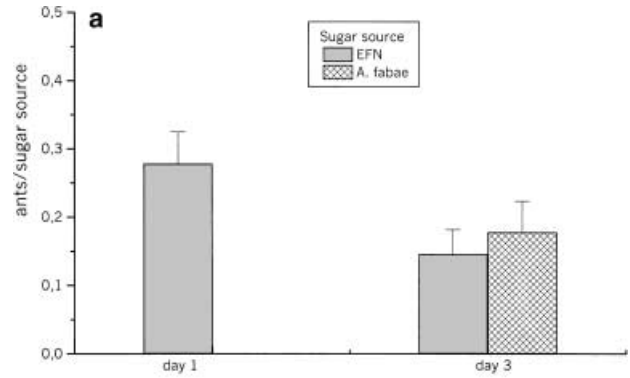

Fig. 3a, b Ant attendance on *V. faba* (mean±SE) when a second sugar source was available after 2 days. **a** EFN were available first, **b** *A. fabae* colonies were available first

a surplus sugar supply for the small *L. niger* colonies. There was, however, no difference between ant attendance on EFN and *A. fabae* colonies within day 3 (Wilcoxon test: *Z*=–0.114, *n*=20, *P*=0.909). The same pattern was found when *A. fabae* colonies were available first (Fig. 3). Ant attendance of *A. fabae* dropped after the addition of the plants with EFN although the difference was not significant in this case (Wilcoxon test: *Z*=–0.681, *n*=20, *P*=0.496). There was also no significant difference within day 3 (Wilcoxon test: *Z*=–0.986, *n*=20, *P*=0.324). This result demonstrates that *L. niger* had no preference for aphid colonies or EFN.

Effect of EFN and ant attendance on aphid colony growth

A. fabae colonies increased in number both in the presence and absence of EFN and/or *Ac. pisum* (Fig. 4). In all experiments, the mean colony size was significantly higher after 6 days. The significantly higher colony sizes of *A. fabae* when *Ac. pisum* was present simultaneously resulted from a higher number of fourth instar larvae in initial colonies.

By contrast, the effect of ant attendance on colony growth of *Ac. pisum* varied between the two different designs. During low ant activity, *Ac. pisum* colonies de-
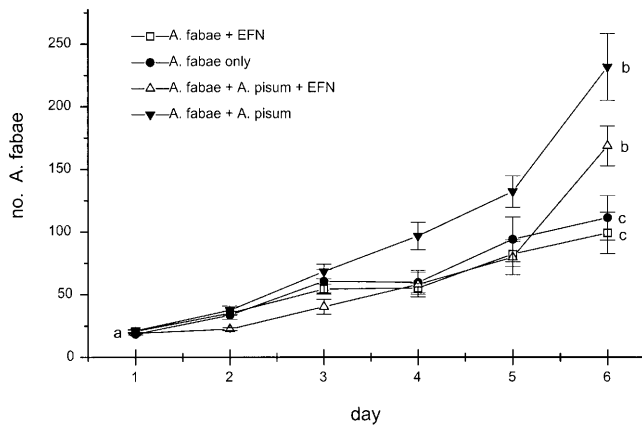
Fig. 4 Growth of ant-attended *A. fabae* colonies (mean±SE) over 6 days in the presence/absence of EFN and/or *Ac. pisum*. Within each experiment, means sharing the same *letter* do not differ at *P*<0.05 (Mann Whitney *U*-test) when day 1 and day 6 are compared

creased significantly in the presence of *A. fabae,* although few individuals of *Ac. pisum* survived the first 6 days in all colonies (Fig. 5a). In the absence of *A. fabae, Ac. pisum* colonies increased significantly, irrespective of EFN presence (Fig. 5a). Predation of *Ac. pisum* by *L. niger* was observed only in the presence of *A. fabae*. A completely different pattern appeared during the period of high ant activity (Fig. 5b). *Ac. pisum* colonies generally decreased if ants had access to the plant, and only colonies where ants were kept off succeeded in growing. *Ac. pisum* colonies on plants with attended sugar sources (EFN, *A. fabae*) generally went extinct during the first 4 days due to high predation by *L. niger*. There was also a significant decrease of *Ac. pisum* on plants without sugar sources, but few individuals survived for longer than 6 days in this treatment (Fig. 5b).

Discussion

For many plant species, extrafloral nectaries (EFN) are believed to serve to attract ants, which reduces herbivory and thus enhances the overall fitness of the plant (e.g. Bentley 1976; Skinner and Whittaker 1981; Warrington and Whittaker 1985; Freitas and Oliveira 1996). Especially in annual plants, EFN are often located near growing or sensitive tissue (e.g. flowers, young growing leaves) which is most vulnerable to phytophagous insects (Deuth 1977; Rico-Gray and Thien 1989). Such a situation is found in *V. faba*, where on average four EFN per plant were active, all of which were situated on the upper part of the plant near the growth point (Engel 2000). Extrafloral nectar is an aqueous mixture of various sugars, amino acids, lipids and other organic components, with sugars representing the dominant solutes (Baker and Baker 1975). In *V. faba*, it consists of the monosaccharides glucose and fructose and the disaccharide sucrose (Table 1), while some other sugars (e.g. arabinose, galactose, maltose, mannose, raffinose) were
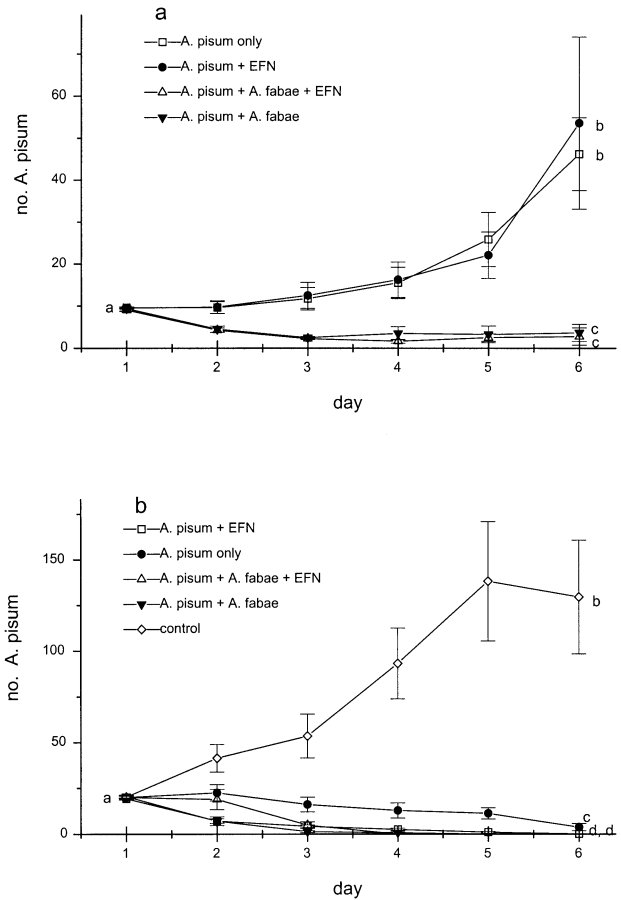

Fig. 5a, b Growth of *Ac. pisum* colonies (mean±SE) on ant-attended *V. fabae* plants over 6 days in the presence/absence of EFN and/or *Ac. pisum*. **a** Low ant activity, **b** high ant activity. Within each experiment, means sharing the same *letter* do not differ at *P*<0.05 (Mann Whitney *U*-test) when day 1 and day 6 are compared

also found in minor proportions in other plants (Baker and Baker 1983).

Recent studies have shown that plants may be able to increase their EFN production and composition after attack by chewing herbivores (Koptur 1989; Smith et al. 1990; Wäckers and Wunderlin 1999). This response may increase the effectiveness of recruiting ants that protect the plant against the herbivore. In *V. faba*, we found no effect of phloem-feeding by *Ac. pisum* or *A. fabae* on sugar composition or concentration. We detected only three sugars (glucose, fructose, sucrose) in uninfested and infested plants, and neither the concentration of particular sugars nor the total sugar concentration differed significantly. A few samples from plants attacked by *A. fabae*, however, also contained – irregularly and in low proportions – melezitose, maltose and erlose. The occurrence of these sugars may indicate contamination of extrafloral nectar by excreted honeydew droplets of *A. fabae* individuals feeding close to the EFN. Also, the volume of EFN production in *V. faba* remained unaffected by sapfeeding by *A. fabae* and *Ac. pisum* if the nectar was regularly collected by ants, similar to observations after tissue feeding (F.L. Wäckers, unpublished work).

Both extrafloral nectar and aphid honeydew represented major sugar sources for the ant, *L. niger* in the present study. However, they differed significantly in sugar composition and sugar concentration. While nectar was dominated by glucose and fructose, the trisaccharide melezitose was the major component in *A. fabae* honeydew. This sugar, which is preferred by *L. niger*, was not recorded for *A. fabae* feeding on another host plant, *Tanacetum vulgare* (Völkl et al. 1999). Its lack on *T. vulgare* may be explained variation in honeydew sugar composition in polyphagous homopterans in relation to host plant (Nemec and Starý 1990; Hendrix et al. 1992). Additionally, the total sugar concentrations were *c*. sevenfold higher in extrafloral nectar.

In our experiments, we found no evidence that EFN may distract *L. niger* from *A. fabae* colonies, or vice versa, and thus there was no impact of EFN on the colony growth of *A. fabae*. This result supports the view of Del-Claro and Oliveira (1993) who claimed that there may be no competition between EFN and honeydew-producing homopterans. Our results may explain the significant influence of sugar composition and sugar concentration on the ant's foraging decisions (Sudd and Sudd 1983; Fischer et al. 2001). Völkl et al. (1999) showed that *L. niger* strongly prefers melezitose-rich honeydew, which might point to a preference for *A. fabae* colonies (with a considerable proportion of melezitose in the honeydew) over EFN. However, the much higher sugar concentrations in extrafloral nectar seem to outweigh this disadvantage. If we roughly estimate the sugar supply of four EFN and of an *A. fabae* colony of 20 individuals, there is 2.5-fold higher sugar supply in four EFN per hour (227 μg sugar h^{-1}) than in an aphid colony of 20 individuals (83 μg sugar h^{-1}). Thus, the higher quality of honeydew sugar (high melezitose content) may be outweighed by the higher quantity of sugar in nectar, so that the two sources are almost equally attractive to *L. niger*.

However, in the case of *Ac. pisum* there was a significant effect of EFN and *A. fabae* on colony growth of *Ac. pisum*, similar to that shown for the species pair *Aphis craccivora/Megoura crassicauda* (Sakata and Hashimoto 2000). *Ac. pisum* is never attended by *L. niger* (or other ant species), probably due to a very low level of honeydew production per time unit (Cloutier and Mackauer 1979; Völkl et al. 1999). On *A. fabae*-infested plants, *Ac. pisum* number always declined significantly within 6 days, independent of low or high ant activity. One reason for this asymmetric competition may be the high absolute ant numbers of such plants (Fig. 2). The frequent physical contacts with ants induced *Ac. pisum* to drop off the plant, and the ant activity on the stem prevented recolonization of the plant. Second, there is a considerable predation of *Ac. pisum* during periods of high activity when *L. niger* covers its protein demand by hunting unattended aphids (Pontin 1963). These "hunting ant workers" were also attracted by *A. fabae*. By contrast, the effect of EFN on *Ac. pisum* colony growth depended on the degree of ant activity. During periods of high activity, with a high protein demand of the ant colony, *Ac.*

pisum number even declined on plants where the EFN were removed. However, the presence of EFN enhanced this effect, leading to a quicker extinction of *Ac. pisum* colonies. No such effect was found during periods of low ant activity. Obviously, the significantly lower ant numbers did not disturb *Ac. pisum* or induce dispersal.

Thus, extrafloral nectaries may provide *V. faba* with some degree of protection against sucking herbivores early in the season when there is a shortage of sugar supply, combined with a simultaneous high sugar and protein demand by the ants. However, since *V. faba* is not native to Europe, we cannot exclude further effects in the area of origin (South America), where the plant is visited by different ant species that may display a completely different foraging behaviour, and where aphid infestation may be less important than in the agroecosystems of Europe.

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