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Extrafloral nectar from cotton (*Gossypium hirsutum*) as a food source for parasitic wasps

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Summary

1. For many adult nectar-feeding parasitoids food and moisture are essential for survival in the field. Early in the season, when floral nectar is not yet available in cotton, extrafloral nectar (EFN) is already present on young cotton plants.

2. The parasitoid *Microplitis croceipes* (Cresson) can use EFN cotton plants as an only food source. The longevity and reproduction of EFN-fed female wasps was comparable to wasps fed with honey and water provided on nectariless (NL) cotton plants, and was significantly higher compared with wasps kept on NL plants with no additional food source.

3. Wasps that were given preflight experiences on EFN cotton plants choose EFN cotton over NL cotton plants in two choice experiments in the flight tunnel. The parasitoids are more willing to search on an EFN plant at their second and third encounter with a plant previously visited, compared with an NL cotton plant.

4. Wasps can locate EFN from short distances by its odour alone, and find it almost as fast as honey, but much faster than odourless sucrose, which is only found randomly. Experience with EFN increased the retention ability of parasitoids on a flower model.

Key-words: Longevity, *Microplitis croceipes*, parasitoids, searching behaviour

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Introduction

Plants have developed numerous strategies to defend themselves against herbivores. Besides direct defence mechanisms, plants can recruit natural enemies of herbivores, such as predators and parasitoids, as an indirect defence by providing them with cues that aid in location of hosts or food. Cotton plants damaged by insect herbivores have been shown to emit volatiles that attract parasitoids such as *Microplitis croceipes* (Cresson) (Braconidae, Hymenoptera) (Röse *et al*. 1996; Röse, Lewis & Tumlinson 1998). Finding food and moisture is essential for the survival of many parasitoid species to sustain them during their search for hosts in the field (Lewis *et al*. 1998). The availability of nutritional resources may affect the longevity and fecundity of parasitoids (Leius 1961a,b; Hagley & Barber 1992; Olson & Nechols 1995) and their host searching behaviour (Takasu & Lewis 1995). Since some parasitoids have been reported to live several months and distribute their eggs over most of this period, they need to have adequate food to sustain their search. In the field, nectar-feeding parasitoids can exploit several food sources, depending on their nutritional requirements and the availability of the food source during the season. Sugar-rich food sources are floral nectar, honeydew and extrafloral nectar (Leius 1960; Jervis *et al*. 1993; Jervis & Kidd 1996). Floral nectar is easily detectable by its floral fragrance (Raguso 2001, 2004) and very abundant during flowering season, but might not always be accessible for species with short mouth parts (Jervis 1998). A potential source of food for these wasps are extrafloral nectaries on plants. Extrafloral nectaries are nectar-producing epidermal glands that are located on vegetative plant parts (Caspary 1848, as cited in Elias 1983) or on reproductive parts without being involved in pollination (Delpino 1875, as cited in Elias 1983). Early in the season, when floral nectar is not yet available in cotton, extrafloral nectaries are present and provide a predictable nectar source long before the plants begin to flower. About 45–50 days prior to flowering of cotton, EFN is provided by single foliar glands located on the mid-vein of the lower leaf surface. In later stages of cotton growth, extrafloral nectar is also found on three bracteal nectaries at the base of each of the three bracts.

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The defensive function of EFN has mostly been studied for plant–ant interactions (reviewed in Heil & McKey 2003). Although parasitoids have been observed to feed on EFN (Hespenheide 1985; Koptur 1991), few studies have focused on plant–parasitoid interactions (Treacy *et al*. 1987; Pemberton & Lee 1996; Stapel *et al*. 1997). Little is known about the function and ecological role of EFN and its ability to sustain parasitoids sufficiently as the only food source during their search for hosts in the field and how parasitoids locate extrafloral nectar (Patt, Hamilton & Lashomb 1999).

The objective of our study is to determine the role and importance of extrafloral nectaried plants in sustaining and mediating the foraging behaviour of parasitoids. We hypothesize that EFN may provide a suitable food source for foraging parasitoids that prolongs parasitoid life span and enhances reproduction. Here we determined the longevity and the physiological effects on the development of the parasitoid *M. croceipes* foraging on EFN plants. We further investigated how previous experience with EFN plants will affect the subsequent preferences of parasitoids for plants with and without EFN and if wasps are able to locate EFN by its odour alone. We hypothesize that parasitoids can use experience to optimize exploitation of EFN and that volatiles are involved in the location of EFN. We have chosen *M. croceipes* as a model parasitoid because of its ecological role in multitrophic interactions in cotton. *M. croceipes* parasitizes noctuid larvae, *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F) and its effectiveness in parasitizing noctuid larvae appears to depend on the availability of food (Takasu & Lewis 1995).

Materials and methods

PLANTS

Approximately 6-week-old cotton plants, *Gossypium hirsutum* L. (Malvaceae), nectaried var. 'Deltapine acala 90', and nectariless (NL) var. 'Stoneville 825' with six fully developed leaves in addition to the two cotyledons, were used in all experiments. Cotton was grown in a greenhouse in a mixture of compost, peat moss and vermiculite (metro-mix 300, Scotts-Sierra Horticultural Company, Marysville, OH) with natural light, under Florida summer conditions (14:10 h light : dark cycle, 85 ± 10% r.h., and 35 ± 10 °C). Each cotton plant was grown from seed planted in a 16-cm diameter pot and fertilized once at time of planting with 5 ml slow-release formulation fertilizer (Osmocote 14-14-14 N-P-K controlled release fertilizer, Scotts-Sierra Horticultural Products Company).

LEPIDOPTERA LARVAE

Corn earworm *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) larvae were obtained from the US Department of Agriculture (USDA) rearing facilities in Gainesville, FL. Larvae were reared on an artificial diet, based on Pinto Beans, according to the method of King & Leppla (1984). Early third instars were used as hosts for reproduction experiments with parasitoids.

PARASITOIDS

The specialist larval endoparasitoids *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) were reared from cocoons obtained from colonies maintained at the USDA Agricultural Research Service, Insect Biology and Population Management Research Laboratory, Tifton, GA. *M. croceipes* were reared on larvae of *H. zea* fed on corn-soymilk (CSM) diet (Blended Food Product, Child Food Supplement, formula no. 2) as previously described (Burton 1970; Lewis & Burton 1970). Female and male parasitoids were kept together in screen cages (25 cm × 25 cm × 25 cm) in the laboratory to allow mating and were provided with water after emergence. Females were separated in cages according to their date of emergence. Mated females used for flight tunnel experiments and close range bioassays were 3 days old and kept in the laboratory at 24 ± 2 °C, 55 ± 5% r.h., and on a 14:10 h light : dark cycle.

LONGEVITY EXPERIMENTS

To determine the role of EFN plants as a food source in sustaining parasitoids and the effect of different types of food sources on the longevity of parasitic wasps, EFN plants were compared with honey, which is known to increase parasitoid longevity (Schmale *et al*. 2001) and compared with no food. Longevity studies were carried out in Plexi glass cages covered with gauze on two sides of the cage (35 cm × 35 cm × 35 cm) in the laboratory at 24 ± 2 °C, 60 ± 5% r.h., and an 14:10 h light : dark cycle. For each treatment except the water control, two mated females of *M. croceipes* were caged with eight cotton plants (four pots) on the day of emergence. The four different treatments were EFN cotton (Delta Pine 90), NL cotton (Stoneville 825), NL cotton provided with additional honey in a Petri dish, and the control with no plants. In all treatments wasps were provided with additional demineralized water on a cotton ball in a Petri dish. Cotton plants were replaced daily with new plants from the greenhouse and the mortality of the parasitoids was recorded daily. EFN plants from the greenhouse that did not provide visible amounts of EFN on the leaves were excluded from the experiments as preliminary experiments showed that they would not support parasitoids sufficiently.

EFFECT OF EFN ON REPRODUCTION AND DEVELOPMENT

To investigate whether EFN plants as an only food source would affect the reproduction and development

of the parasitoid *M. croceipes*, mated females were allowed to forage on either EFN plants or on honey offered on a Petri dish placed on a NL plant throughout the experiment and were provided with additional demineralized water on a cotton ball in a Petri dish. On days 3, 6 and 9 after emergence, each female was allowed to parasitize 36 third-instar *H. zea* larvae during a 2-h period on each of the three days. *M. croceipes* can parasitize host larvae very quickly (Hopper 1986). However, after 2 h oviposition time, parasitoids in our experiments slowed down considerably and spent longer times preening, independent of the food source. Host larvae were presented to each wasp in a separate Petri dish and after being parasitized by the wasp, each larva was transferred into a separate plastic cup, filled with Pinto Bean diet. The parasitized larvae were checked daily and emergence of the parasitoid larvae from the host larvae, pupation of parasitoid larvae, and emergence of adult parasitoids from cocoons and their sex were recorded. In addition, dead *M. croceipes* larvae that emerged from the host larvae but did not pupate, dead host larvae and host larvae that pupated normally were recorded.

FLIGHT-TUNNEL EXPERIMENTS

Free flight behavioural bioassays were carried out in a flight tunnel (previously described by Röse *et al.* 1998) to investigate how previous experience with EFN plants will affect the subsequent preferences of parasitoids for plants with and without EFN. Starved female parasitoids were divided into five groups and each group was given a different type of preflight experience immediately prior to their release in the flight tunnel. One group of wasps was allowed to search three times for 5 min on an EFN plant with a 5 min interval between experiences, a second group was allowed to search on a NL plant under the same conditions. A third group of wasps was allowed to feed on sucrose three times for 10 s each in a Petri dish to increase their responsiveness. A fourth group of wasps was allowed to feed on sucrose three times for 10 s each provided on a NL plant. Repeated experience of starved parasitoids with food in the presence of an odour cue will increase the probability of learning to associate this cue with food (Lewis & Takasu 1990). Four groups of experienced wasps were compared with naive wasps. The wasps were then given a choice in the flight tunnel between an EFN and an NL plant. The position of the plants in the flight tunnel was switched routinely after each flight to avoid positional bias. Parasitoids were released individually downwind of the plants in a release device previously described (Turlings *et al.* 1991) that was positioned equidistant to both plants and 25 cm above the flight tunnel floor. Each wasp was given three chances to reach a plant in a non-stop flight from 1 m distance. After an incomplete flight, the wasp was returned to the release device to be released again. The choice of each parasitoid after a completed flight was recorded as was the number of parasitoids that did not complete flights.

CLOSE RANGE BEHAVIOUR

To study the close range attraction of female wasps to extrafloral nectar without the effect of the entire plant, a flower model modified from Patt, Hamilton & Lashomb (1997) was used. The modified flower model consisted of a top of a glass Petri dish ($d = 9$ cm) with eight small Teflon rings ($d = 0{\cdot}3$ cm, $h = 0{\cdot}2$ cm) arranged concentrically and equidistant around the edges of the Petri dish to simulate nectaries. Teflon rings were glued to the Petri dish, forming small cups that held the tested EFN, odorous honey or odourless sucrose without being visible to the wasps. Thus, only potential volatiles emitted from the tested cup-fillings could provide a cue for the wasps. In three different experiments, two of the cups on opposite sides were filled with either 1 µl sucrose (20% in water), 1 µl honey (50% in water) or 1 µl EFN. EFN was collected daily with a 10-µl glass capillary immediately prior to the experiments from the leaf nectaries of several cotton plants that were grown in the greenhouse. EFN was pooled in a glass 1 µl equivalents were-vial and gas chromatography taken for the bioassays. EFN from cotton contains approximately 26% glucose, 21% fructose and 7% sucrose (Wäckers & Bonifay 2004). Honey contains on average 38% fructose, 31% glucose, 1·3% sucrose, 7% other disaccharides such as maltose, and trace amounts of vitamins and minerals. Sucrose is a disaccharide that consists of a glycosidically bound glucose residue and fructose residue. The 20% sucrose solution in our experiments (0·58 M) is a compromise between a previously successfully used 30% sucrose solution for conditioning experiments with *M. croceipes* that examined the effect of food availability on odour preference (Takasu & Lewis 1995) and the lower naturally occurring sucrose content in honey and EFN. The differences in sugar concentrations as well as trace amounts of other compounds in honey and EFN may have influence on the odour conditioning. Hymenopteran parasitoids have been shown to live for more that 30 days when provided with solutions of 1 M fructose, glucose or sucrose (Wäckers 2001). Therefore, a broad range of concentrations appears to be suitable to sustain parasitoids.

For each day, half of the wasps (see Table 1) were preconditioned with sucrose to provide an odour-neutral feeding experience to increase their responsiveness prior to their release in the flower model. The responses of sucrose-experienced wasps were subsequently tested to sucrose-, honey- or EFN-filled cups. Sucrose does not induce a strong olfactory response in parasitoids (Lewis & Takasu 1990). The remaining wasps were given prerelease experiences on the same solution that they were tested on subsequently in the arena (either EFN for trials with EFN-filled cups, or with honey for honey-filled cups). Each experience was

Table 1. Close range searching behaviour of wasps in a flower model in response to different cup fillings after different types of experiences. Values are mean ± SE. Maximum observation time is 300 s

Cup filling	Experience	Time to first encounter of filled cups*	Number of contacts with empty cups*	Number of take-offs (with total number of take offs) (%)*	*N* Replicates (with total number of wasps)
EFN	EFN	22·7 ± 3·8 **A**	0·6 ± 0·11 **A**	8·3 ± 0·3 (5) **A**	12 (58)
	Sucrose	28·1 ± 7·1 **A**	1·0 ± 0·28 **A**	29·1 ± 8·3 (16) **B**	11 (54)
Honey	Honey	12·0 ± 1·5 **B**	0·2 ± 0·09 **B**	12·7 ± 4·1 (7) **A**	11 (48)
	Sucrose	19·6 ± 4·2 **A**	0·2 ± 0·01 **B**	7·3 ± 4·1 (4) **A**	11 (52)
Sucrose	Sucrose	73·6 ± 18·7 **C**	2·2 ± 0·27 **C**	42·7 ± 5·7 (23) **B**	11 (53)

*Different capital letters indicate significant differences between different treatments (ANOVA, post-hoc Bonferroni adjustment, $P \leq 0{\cdot}05$).

given three times for 10 s with a 1-min interval in between. One minute after the last experience on EFN, honey or sucrose, the wasps were released in the middle of the Petri dish arena and the time that elapsed until the wasps discovered the 'filled' cups was recorded. In addition, the frequency of touching empty cups was recorded. This gave information as to whether the wasps showed random movement or whether their moves were directed towards the 'filled' cups. Each wasp was given a maximum of 5 min to search the flower model. The number of wasps that took-off without locating a 'filled' cup was recorded.

Results

LONGEVITY EXPERIMENTS

Differences in the longevity of 10 replicates of parasitoids fed on different food sources were analysed by ANOVA and differences of $P \leq 0{\cdot}05$ were considered to be significant. Data of all experiments were analysed with the Statistical Program SYSTAT. Food had a significant effect on longevity (ANOVA, $P \leq 0{\cdot}005$, df = 3, $F = 676{\cdot}226$). Female parasitoids that foraged on EFN plants or foraged on NL plants provided with additional honey were sustained equally well and did not show any differences in longevity (Fig. 1, Tuckey HSD multiple comparisons, $P = 0{\cdot}939$). Wasps given EFN or honey were sustained significantly longer than females that were kept on NL plants with no additional food or females kept in cages with only demineralized water on a cotton ball (Fig. 1, $P = 0{\cdot}005$). No significant differences were detected in the longevity of females kept on NL plants or demineralized water (Fig. 1, $P = 0{\cdot}889$). Thus, EFN plants, even when they are the only available food source for *M. croceipes*, can sustain the wasps for a considerable number of days.

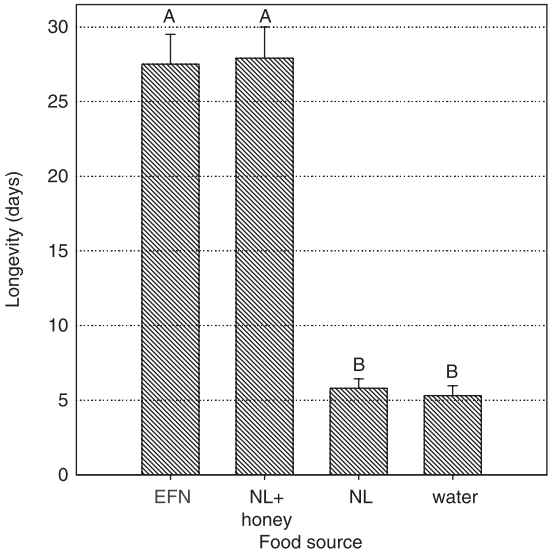

Fig. 1. Longevity of female *Microplitis croceipes* (mean ± SE) fed on extrafloral nectary plants (EFN), or fed with honey provided on a nectariless plant (NL + honey), or females that were kept on nectariless plants with no additional food (NL), or females kept in cages with only demineralized water on a cotton ball (water). Different letters indicate significant differences in longevity between the different food sources (ANOVA, Tuckey HSD multiple comparisons, $n = 10$, $P \leq 0{\cdot}001$).

EFFECT OF EFN ON REPRODUCTION AND DEVELOPMENT

Differences in the reproduction of 12 EFN-fed and 12 honey-fed parasitoids after parasitization of a total of 432 host larvae in 12 replicates for each treatment at each of the time points were analysed by *t*-test. Parasitic wasps that foraged exclusively on EFN plants over a period of 3–9 days showed no physiological difference in terms of reproduction compared to wasps that foraged on honey solution (Table 2). After 3, 6 and 9 days of foraging on either EFN plants or honey solution, the number of parasitoid cocoons that developed from parasitized caterpillars and the sex ratio of adult male and female parasitoids emerging from the cocoons were comparable (Table 2). In addition, no differences were observed between the overall numbers of unsuccessfully parasitized *H. zea* host larvae, as recorded in numbers of dead *M. croceipes* larvae that emerged from their host but did not pupate successfully (Day 1: EFN = 5·3 ± 4·8, honey = 4·9 ± 6·5, df = 22, $P = 0{\cdot}861$; Day 2: EFN = 2·8 ± 1·9, honey = 3·5 ± 2·5, df = 20, $P = 0{\cdot}517$; Day 3: EFN = 4·6 ± 3·2, honey = 4·6 ± 3·5, df = 16, $P = 0{\cdot}999$, 12 replicates,

Table 2. Mean number of cocoons and sex ratio of *Microplitis croceipes* with standard deviation that emerged from 36 *Helicoverpa zea* larvae, parasitized by each adult female *M. croceipes* that was fed on either extrafloral nectar (EFN) or fed on honey for 3, 6 and 9 days after emergence

	Day 3			Day 6			Day 9		
	EFN	Honey	*P*	EFN	Honey	*P*	EFN	Honey	*P*
Coccons	30·9 ± 2·6	31·0 ± 2·3	0·678*	32·2 ± 2·6	31·3 ± 2·3	0·402	29·7 ± 2·7	30·8 ± 3·6	0·466
Female *M. croceipes*	10·4 ± 7·5	6·8 ± 6·9	0·237	12·5 ± 8·6	9·6 ± 7·8	0·416	10·2 ± 6·8	7·6 ± 5·7	0·380
Male *M. croceipes*	15·0 ± 7·4	18·8 ± 92	0·285	16·8 ± 6·4	18·2 ± 9·8	0·705	14·9 ± 7·7	18·7 ± 8·8	0·348

*Differences in the number of cocoons and emerging parasitoids derived from EFN-fed parasitoids and honey-fed parasitoids for 12 replicates with a total of 432 parasitized host larvae for each treatment and day were analysed by *t*-test.

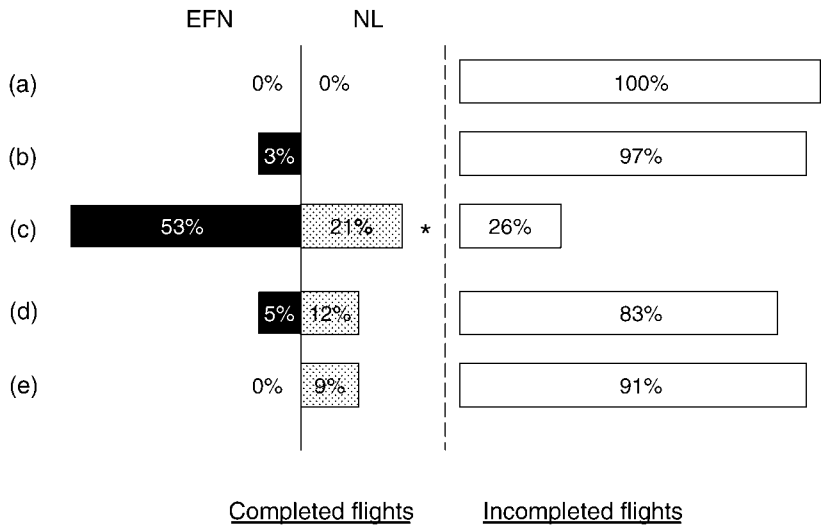

Fig. 2. Flight response in per cent of *M. croceipes* in two-choice experiments to plants with extrafloral nectaries (EFN) compared with nectariless (NL) plants after: (a) no experience, $n = 39$; (b) experience with sucrose, $n = 36$; (c) with extrafloral nectar on plant, $n = 38$; (d) with nectariless plant, $n = 42$; and (e) with sucrose provided on nectarless plant, $n = 33$. The shaded bars indicate the number of wasps that landed on each source, and the open bars show those that did not land on either plant, for each test. Asterisks indicate significant differences in preferences within each pair of odours (Fischer's exact test, $P \leq 0{\cdot}05$).

t-test), or in numbers of dead *H. zea* larvae (Day 1: EFN = 2·6 ± 1·9, honey = 2·8 ± 2·3, df = 22, $P = 0{\cdot}773$; Day 2: EFN = 2·3 ± 2·2, honey = 2·4 ± 2·0, df = 20, $P = 0{\cdot}920$; Day 3: EFN = 2·3 ± 1·4, honey = 2·6 ± 1·4, df = 16, $P = 0{\cdot}744$, 12 replicates, *t*-test) and *H. zea* larvae that pupated normally (Day 1: EFN = 2·3 ± 1·2, honey = 1·8 ± 1·2, df = 22, $P = 0{\cdot}406$; Day 2: EFN = 1·5 ± 0·8, honey = 2·3 ± 1·3, df = 20, $P = 0{\cdot}142$; Day 3: EFN = 4·0 ± 2·7, honey = 2·4 ± 2·1, df = 16, $P = 0{\cdot}197$, 12 replicates, *t*-test).

FLIGHT TUNNEL EXPERIMENTS

For flight tunnel choice tests differences in preferences between each pair of odours were analysed by Fisher's Exact test. Naive wasps showed no response to EFN or NL plants in the flight tunnel (0%; Fig. 2a). Even after priming of the wasps with sucrose provided on a Petri dish, the overall response of parasitoids was low (3%), indicating that there is no innate long range attraction to EFN plants that is mediated by volatiles (Fig. 2b). Wasps that were allowed to forage on an EFN plant prior to their release in the flight tunnel showed clearly overall more completed flights (74%; Fig. 2c), compared with wasps experienced on NL cotton (17%; Fig. 2d) or experienced on sucrose provided on a NL plant (9%; Fig. 2e). Parasitoids that had previous experience on an EFN plant showed a significant preference for EFN plants (71% of responding wasps, $n = 20$ wasps) compared with NL plants (29% of responding wasps, $n = 8$ wasps, $P = 0{\cdot}003$) in the flight tunnel. These results indicate that the wasps are able to distinguish between the two plant varieties and will prefer the variety that provides EFN. This ability is learned through experience. No statistically significant preferences for EFN or NL were found for all other types of preflight experiences (naive: $n = 39$, $P = 0{\cdot}999$; sucrose experience: $n = 36$, $P = 0{\cdot}999$; NL experience: $n = 42$, $P = 0{\cdot}286$; NL & sucrose experience: $n = 33$, $P = 0{\cdot}100$).

CLOSE RANGE BEHAVIOUR

Close range choice tests were analysed by ANOVA after arcsines transformation and post-hoc Bonferroni adjustment. In close range experiments on the flower

model, experience ($P = 0{\cdot}005$, df = 2, $F = 8{\cdot}617$) and cup filling ($P = 0{\cdot}005$, df = 2, $F = 31{\cdot}757$) had a significant effect on the time that the wasps spent searching until they encountered the first filled cup. Wasps found cups that were filled with honey or EFN much faster than they found sucrose filled cups, independent of previous experience (Table 1). The average time necessary to locate honey-filled cups decreased significantly with previous experience on honey compared with experience on sucrose ($P = 0{\cdot}047$, df = 1, $F = 4{\cdot}039$). The time necessary to locate EFN-filled cups decreased only slightly after experience on EFN compared to experience on sucrose ($P = 0{\cdot}098$, df = 1, $F = 2{\cdot}798$). After experience on sucrose, wasps found EFN-filled cups in the same amount of time (statistically) that they found honey that is known to have a distinct odour, and considerably faster than cups filled with sucrose (Table 1). After experience with honey, the time until first encounter with honey-filled cups was significantly shorter than the time necessary to locate EFN-filled cups after experience with EFN, which may indicate that honey is easier to detect or more preferred.

The average number of contacts with empty cups prior to the location of the first filled cup as a measurement of directed movement of the wasps towards an odour source showed a similar picture. Wasps showed the most direct movement towards honey-filled cups, which was independent of experience and almost always contacted honey-filled cups directly without prior contact of empty cups (Table 1, $P = 0{\cdot}877$, df = 1, $F = 0{\cdot}024$). Movements towards EFN-filled cups were also directed, and wasps seldom contacted empty cups prior to the location of EFN-filled cups, independent of experience (Table 1, $P = 0{\cdot}103$, df = 1, $F = 2{\cdot}705$). Cups filled with sucrose were only located after antennating several empty cups, indicating a random searching behaviour of the wasps on the flower model (Table 1).

Although experience had no significant effects on the time to first encounter of EFN-filled cups and on the number of empty cups contacted prior to location of the filled cups, it had a strong effect on the number of take-offs from the flower model. Wasps that had experience on EFN showed a significantly smaller number of take-offs from the flower model with cups filled with EFN than wasps experienced on sucrose (Table 1, $P = 0{\cdot}006$, df = 1, $F = 8{\cdot}211$. This indicated an increase in retention ability after EFN experience that was comparable to honey-filled cups with previous honey or sucrose experience. Wasps experienced on sucrose that were exposed to sucrose fillings showed a very low retention ability on the flower model with more than 42% of the wasps taking off.

These results indicate that there is an odour emitted from EFN that is not as strong as honey odour but strong enough to be detected by parasitoids from a small distance. Although previous experience with EFN did not improve the ability of the parasitoids to find the nectar much faster, it increased the retention of the wasps on the flower model significantly.

Discussion

Cotton plants that provided extrafloral nectar could clearly support parasitoids throughout their life span, even if EFN was the only available food source. Parasitoids that are well fed have been shown to parasitize more host larvae (Takasu & Lewis 1995) and parasitized *H. zea* host larvae in return will consume less of the photosynthetic or reproductive plant tissue (Hopper & King 1984). By sustaining parasitic wasps during their search for hosts, EFN may therefore have an important function in indirect defences of a plant, especially during times when floral nectar is scarcely available.

The ecological role of EFN in indirect defences has been mainly discussed in the context of ant–plant interactions (Janzen 1966; Bentley 1977; Keeler 1977) where plants recruit ants through EFN to guard the plants from herbivores. However, a few studies have also addressed the role of EFN in context to parasitoids (Pemberton & Lee 1996). In cotton patches with different food sources such as EFN, honeydew or artificially applied sucrose, the presence of food increased the number of hosts that were parasitized in those patches (Stapel *et al*. 1997). Although the parasitoid *M. croceipes* can live for up to 30 days in the field, its responsiveness to host odours is greatest within the first 8 days after emergence (Takasu & Lewis 1996). EFN in our experiments was a sufficient food source to sustain parasitoids during parasitization for at least the 9 days tested. No differences were observed in the overall numbers of successfully parasitized host larvae and the developing numbers of offspring from wasps fed on EFN or fed on honey as the only food source. *M. croceipes* is slightly synovigenic (ovigenity index 0·34) and adults emerge with some immature eggs (Jervis *et al*. 2001). Egg production of newly emerged *M. croceipes* is initially low and increases after 1–3 days (Navasero & Elzen 1992). The availability of food may therefore affect life-history traits of the insect life cycle (Boggs 1992; Jervis *et al*. 2001).

Since EFN from cotton plants was able to provide sufficient food for *M. croceipes* to support the parasitoid throughout its life span and during parasitization, the question remains how this food source is located. In our flight tunnel experiments, the overall response of naive wasps to EFN plants was very low even after priming with sucrose and the wasps did not show any preference for EFN. This indicates that the long-range response to cotton plants with EFN is not innate. After experience with EFN, wasps were able to distinguish between plants with and without EFN and preferred the EFN plants. Therefore, EFN plants are detectable for parasitoids from a long distance, but were not attractive to the wasps without any previous experience on EFN plants. Parasitoids can learn to associate food with olfactory cues (Lewis & Takasu 1990; Takasu & Lewis 1996) and may have learned to associate the odour emitted from EFN plants with nectar in our experiments.

Although EFN plants were not innately attractive to parasitoids from a long range and EFN plant odour may have to be learned by association with feeding on EFN, it appears that at a close range, the parasitoid behaviour may be affected by innate attraction to EFN. In close range experiments in our flower model, wasps that were experienced on sucrose found EFN as fast as they found honey, which is known to have a distinct odour. They did, however, take considerably more time to find sucrose when released in the arena. This indicates that there is an odour to EFN that the parasitoids can employ to find the nectar. These results are in accordance with Stapel *et al*. (1997) who found that parasitoids that are released at the tip of a cotton leaf can locate EFN without any previous experience. Our results suggest that for the detection of EFN by the parasitoids at close range the entire nectary gland structure of the leaf is not necessary but that the nectar itself released a detectable odour. The nature of the odour and whether it is the result of microbial degradation of amino acids in the nectar or the result of absorption of volatiles from surrounding tissue as suggested for some floral nectars (Raguso 2004) remains to be investigated.

The presence of food may affect the host-searching behaviour of parasitoids. Starved parasitoids released in patches with herbivore-damaged leaves and food provided on the plant as sucrose or EFN showed a higher parasitization rate compared with parasitoids on patches with no food (Stapel *et al*. 1997). However, not all plants infested by herbivores provide food to parasitoids. Therefore, parasitoids in need of food may have to leave a plant with hosts and may not return to the same plant after being satiated at another location. An herbivore-infested plant that provides both food and hosts may therefore benefit from retaining a parasitoid and increasing its searching efficiency (Takasu & Lewis 1995). Our close range experiments showed that parasitoids with previous experience on EFN would remain on the flower model significantly longer and spend longer times searching the arena than wasps experienced on sucrose. By providing EFN, plants may give hungry parasitoids an incentive to visit plants that do not flower and retain parasitoids on herbivore-damaged plants. Plants may actively increase their EFN production in response to herbivore attack (Wäckers *et al*. 2001) and thereby optimize their indirect defence. This increase in EFN production in response to herbivory is most probably regulated via the jasmonate signalling cascade. Plants treated with jasmonic acid showed also an up-regulation of EFN production (Heil *et al*. 2001).

In cotton, the EFN production can be induced or constitutive, depending on the location of the nectaries. Bracteal nectar is constitutive with a peak production at the time of anthesis and a high level of production during fruit maturation (Wäckers & Bonifay 2004). Foliar nectar production is about 100 times lower than bracteal nectar production, but is induced in response to herbivory (Wäckers & Bonifay 2004). This inducibility of leaf nectar production minimizes the cost of EFN production in absence of herbivores but maximizes the protective benefits to increase indirect defence when herbivory occurs, which is in accordance with the optimal defence theory (McKey 1974, 1979; Rhoades 1979). Our study shows that EFN can function very well as a food source to support parasitoids during their lifetime and will result in a better retention of parasitoids on the plant. This supports the ecological role of EFN in facilitating indirect defence by attracting and sustaining parasitoids that may increase plant fitness.

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