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A comparison of naïve and conditioned responses of three generalist endoparasitoids of lepidopteran larvae to host-induced plant odours

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Abstract—Many parasitic wasps that exploit herbivores as their hosts make use of herbivore-induced plant odours to locate their victims and these wasps often exhibit an ability to learn to associate specific plant-produced odours with the presence of hosts. This associative learning is expected to allow generalist parasitoids to focus on cues that are most reliably associated with current host presence, but evidence supporting this hypothesis is ambiguous. Using a six-arm olfactometer we compared the responses of three generalist larval endoparasitoids, *Cotesia marginiventris* (Hymenoptera: Braconidae), *Microplitis rufiventris* (Hymenoptera: Braconidae) and *Campoletis sonorensis* (Hymenoptera: Ichneumonidae), to the induced odours of three plant species: maize (*Zea mays*), cowpea (*Vigna unguiculata*), and cotton (*Gossypium hirsutum*). We tested the responses of naïve females as well as of females that were first conditioned by parasitising host larvae feeding on one of the plant species. Despite similarities in biology and host range the three wasp species responded entirely differently. Naïve *C. marginiventris* and *C. sonorensis* chose equally among the induced odours of the three plants, whereas naïve *M. rufiventris*, which may have a somewhat more restricted host range, tended to prefer the odour of maize. After conditioning, most *C. marginiventris* females chose the odour of the plant species that they had experienced, but conditioned *M. rufiventris* showed an even stronger preference for maize odours, independently of the plant they had experienced. *Cotesia sonorensis* did not show any change in its preference after conditioning. We speculate that its extremely broad host range allows *C. sonorensis* females to use fixed responses to cues commonly associated with plants damaged by Lepidoptera. These results imply that different generalist parasitoids may employ different foraging strategies and that associative learning is not necessarily part of it.

Keywords: associative learning; herbivore-induced plant odours; host finding; parasitoids.

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INTRODUCTION

Many parasitoids of herbivores use plant odours induced by insect feeding to locate plants that may carry their hosts (Vet and Dicke, 1992; Turlings and Benrey, 1998). Associative learning of the most reliable volatile cues may help the wasps to find such plants more efficiently (Turlings et al., 1993; Vet et al., 1995). Parasitoids can learn to associate a specific odour with a successful oviposition in a host or contact with host faeces. This ability to learn is generally expected to be an adaptive strategy for parasitoids that have a broad host range or which can find their hosts on multiple plant species (Vet and Groenewold, 1990; Vet and Dicke, 1992; Vet, 1999; Steidle and van Loon, 2003). Indeed, generalist parasitoids are often found to exhibit this learning ability, whereas there is a tendency for parasitoids with a more restricted host range (or a limited range of plants on which they find hosts) to show genetically fixed responses to host location cues (Steidle and van Loon, 2003). The general validity of the concept, however, is still unclear (Steidle and van Loon, 2003). More insight may be obtained by comparing the effect of experience in multiple generalist parasitoid species under similar conditions. This was the aim of the current study, which compares the responses of naïve and conditioned females of the three generalist parasitoids *Cotesia marginiventris* Cresson (Hymenoptera: Braconidae), *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) and *Campoletis sonorensis* Cameron (Hymenoptera: Ichneumonidae) to host-induced plant odours.

Cotesia marginiventris and *C. sonorensis* have been extensively studied for their abilities to make use of plant odours to locate their hosts and for their ability to learn such odours. Dmoch et al. (1985) showed that a contact experience with host and/or host by-products enhances the responses of *C. marginiventris* females to frass, silk and feeding damage of one of its hosts, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), on maize leaves. For long-range host finding, *C. marginiventris* relies primarily on volatiles emitted by plants after an attack by caterpillars (Turlings et al., 1990; Turlings et al., 1991). The intensity with which they respond to the plant-provided signals depends on previous experiences and they learn to prefer the odours they encounter in the presence of hosts (Turlings et al., 1990; Hoballah and Turlings, 2005).

Campoletis sonorensis is a solitary parasitoid that co-occurs with *C. marginiventris* and shows a great overlap in the range of host species it attacks (Hoballah et al., 2004). Studies on the flight behaviour of *C. sonorensis* have shown that it orients to microhabitat cues without requiring a prior experience with host or microhabitat stimuli (Elzen et al., 1986; Baehrecke et al., 1990). McAuslane et al. (1991) found that *C. sonorensis* females that were given an oviposition experience in host larvae on a particular plant were subsequently more responsive in flight tunnel assays than naïve females. However, such experiences with a host-plant complex did not change the wasps' innate preference for a particular plant odour, suggesting that *C. sonorensis* does not learn to associate specific odours with host presence (McAuslane et al., 1991).

Studies on *M. rufiventris* have mostly focused on its physiology and development (Altahtawy et al., 1976b; Hegazi et al., 1977; Tawfik et al., 1980; Hegazi and Shabaan, 1984). From previous studies we know that naïve females of *M. rufiventris* are also highly attracted to induced maize odours (Hoballah and Turlings, 2005; Tamò, 2006), but the effect of experience on this behaviour is unclear (Hoballah and Turlings, 2005). The behaviour of some other *Microplitis* species has been studied more extensively and indicates a keen ability of each species to learn to respond to host cues by association (Drost et al., 1986; Hérard et al., 1988; Murray and Rynne, 1994). It is unlikely, however, that the recorded behaviour of a particular species can be extrapolated to other species belonging to the same genus. Different *Cotesia* species, for example, show clear differences in their responses to host cues (Potting et al., 1997; Geervliet et al., 1998). Moreover, different *Microplitis* species have distinctly different host ranges and are therefore likely to have evolved different host searching strategies.

Cotesia marginiventris and *C. sonorensis* are native to the Americas and *M. rufiventris* is mainly found in the Middle East, but these three parasitoid species have much in common. They are generalist solitary larval endoparasitoids that attack the early stages of a wide range of Noctuidae species on many different plants. *Campoletis sonorensis* is an ichneumonid parasitoid with at least 27 species of noctuid larvae recorded as hosts (Lingren et al., 1970). *Cotesia marginiventris*, a braconid, also attacks a wide range of noctuid species, many in common with *C. sonorensis* (Turlings, 1990). The host range of *M. rufiventris*, also a braconid, is less well known and may be more restricted. It has been reported to oviposit and successfully develop in young instars of various *Spodoptera* species and *Heliothis armigera* on many different crop plants (El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Abou Zeid et al., 1978).

Many of the hosts of the three parasitoids belong to the genus *Spodoptera*. *Cotesia marginiventris* and *C. sonorensis* can attack *S. frugiperda*, *S. exigua* and *S. eridania* (Lingren et al., 1970; Turlings, 1990), while *M. rufiventris* attacks *S. littoralis*, *S. litura*, *S. exempta*, *S. mauritia*, *S. latebrosa* and *S. exigua* (Meier, 1929; Thompson, 1946; El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Altahtawy et al., 1976a). Most *Spodoptera* species are polyphagous and attack many plant species including important crops like alfalfa, cotton, cowpea, maize, tomato and soybean (Brown and Dewhurst, 1975; Hodge et al., 1983). Hence, the three parasitoids should be able to locate hosts on a multitude of plant species and can be expected to show similarities in how they forage for hosts. A direct comparison between these species may provide insight into how similar or how variable host searching strategies are among generalist parasitoids.

With this objective, we tested responses of *C. marginiventris*, *C. sonorensis* and *M. rufiventris* to the odours of three different host plants that are readily attacked by *Spodoptera* larvae, and that can be encountered by the parasitoids in their native habitats. The plant species of choice were maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*). The caterpillar-induced odours produced by these plants have already been analysed in various studies (Turlings et al., 1991; McCall et al., 1994; Loughrin et al., 1995; Röse et al., 1996; Paré and Tumlinson, 1997; Hoballah et al., 2002) and show distinct differences in quality (composition) and quantity. The preference of naïve females for the induced volatiles of the three plants was compared with the preference of females that had an oviposition experience on one of the plant-host-complexes. During the behavioural assays part of the emitted odours were collected and subsequent analyses made it possible to correlate the observed behavioural responses to the identities and quantities of the volatiles that the wasps perceived in the olfactometer. Females of all three species were found to be strongly attracted to the induced plant odours and as naïve wasps they showed no clear preferences, but their responses after experience were distinctly different.

METHODS

Plants

Three host plants were used for the experiments: maize (*Zea mays*, var. Delprim), cotton (*Gossypium herbaceum*) and cowpea (*Vigna unguiculata*). For each plant species two seeds were planted per pot (200 ml) with fertilised commercial soil (COOP, Switzerland) and placed in a climate chamber (E15 Conviron, Winnipeg, Canada) set at 23°C, 16 D : 8 L, 60% relative humidity and 50 000 lm/m^2 light intensity. Plants were watered daily. The day before an experiment, two plants of each species were transplanted in glass pots that fit the olfactometer (250 ml, 4.5 cm diam., 11 cm high). Maize and cowpea plants were 8-10 days old and cotton 13-14 days old at day of testing. Plants were induced to emit volatiles by placing 15 2nd instar *S. littoralis* larvae on them on the evening before an experimental day.

Host larvae

Spodoptera littoralis eggs were supplied by the Syngenta rearing facilities (Stein, Switzerland). After emergence the larvae were reared on a wheatgerm-based artificial diet (also supplied by Syngenta) at room temperature. *Spodoptera littoralis* larvae at the L2 stage, the preferred stage for female parasitoids of all three species (Altahtawy et al., 1976b; Isenhour, 1985; Jalali et al., 1987), were used in experiments.

Parasitoids

The rearing strain of *C. marginiventris* came from the USDA-ARS, Biological Control and Mass Rearing Research Unit (Mississippi, USA) and was refreshed with individuals collected in Mexico in 2001. The *M. rufiventris* colony was established in 2000 from individuals collected from parasitised *S. littoralis* larvae in fields in Egypt (Alexandria). The *C. sonorensis* colony was established in 2001 from individuals collected from parasitised *S. frugiperda* larvae in maize fields in Mexico (Poza Rica, Veracruz). All parasitoids were reared on *Spodoptera littoralis* (Noctuidae: Lepidoptera). For the rearing, 25 *S. littoralis* caterpillars were offered to two mated females (4-7 days old) for 3-4 h in a plastic container (9.5 cm diam., 5 cm high). The parasitised caterpillars were then maintained in groups of 100 individuals in transparent plastic boxes (15 × 13 × 5.5 cm) on the wheatgerm-based artificial diet (supplied by Syngenta) until cocoon formation. Cocoons were kept in Petri dishes until adult emergence. Upon emergence, adults were sexed and kept in cages (30 × 30 × 30 cm) (MegaView Science Education Services Co. Ltd., Taiwan) at a sex ratio of 1:2 (male: female). They were supplied with honey droplets as food source and moist cotton. Parasitised larvae and adults of *C. marginiventris* were held until the experimental day in an incubator (E36L Percival, Perry, Iowa, USA) at 25°C and 16 L : 8 D, whereas parasitised larvae and adults of *M. rufiventris* and *C. sonorensis* were held in the laboratory under ambient light and temperature conditions (19-24°C).

Olfactometer set-up

The responses of females of the three parasitoids species *C. marginiventris*, *M. rufiventris* and *C. sonorensis* to volatile chemicals emitted by the three plant species maize, cotton and cowpea were investigated in the six-arm olfactometer described in Turlings et al. (2004). Three arms of the olfactometer had always only clean air and they were alternated with arms with the odour of either two maize seedlings, two cotton seedlings or two cowpea seedlings fed upon by 15 2nd instar *S. littoralis* larvae. For each replicate the odour sources were connected to a different arm, but always with one empty chamber between two chambers that contained two plants of one species. After an experimental day, all parts of the olfactometer were water washed and rinsed with acetone and hexane. The glass parts were then placed in a drying oven (250°C) overnight.

Bioassay procedure

Parasitoid species were tested on separate days in a roughly random order, depending on availability of the wasps. On a given day, four groups of six wasps of one species were released one after the other in random order and choices made within each group were recorded 30 min after release. The four groups of wasps were the following: i) naïve females (no previous contact with hosts or plants); ii) females experienced with maize; iii) females experienced with cotton; and iv) females experienced with cowpea. The experience was given by letting a female parasitise two larvae in a plastic box (9.5 cm diam., 5 cm high) containing a leaf of one of the three plant species, which had been fed upon by 15 2nd instar *S. littoralis* larvae during one night. Within 20 min after six females had had such an experience, they were released in the olfactometer. After their choices had been recorded, the six wasps were removed just prior to the release of the next group. The sequence of the different groups of wasps released in the olfactometer was randomly changed for each replicate day ($n = 12$). It should be noted that in previous experiments we have tested for the possibility that individual females affect each other's choice, as described for *C. marginiventris* by Turlings et al. (2004). In none of the cases did we find such an effect but, interestingly, there is an interspecies interference (C. Tamò, 2006). Even though we feel that we can rule out overdispersion due to interference between the wasps, our statistical analysis corrects for this possibility (see below).

Statistical analyses of the olfactometer data were based on a log-linear model, but in comparison with the method described by Turlings et al. (2004) the statistical analysis was improved by using a stochastic model developed specifically to allow for the significant overdispersion of the olfactometer data relative to that seen in a standard log-linear model. The new model takes into account the censored aspect of the data: some of the wasps did not make a choice within the given time for a release. The number of wasps choosing the ith arm ($i = 1, \ldots, 6$) indicates the relative attractiveness of the corresponding odour source, which is parameterised by λ_i. If only the odour source affects wasp choice, and this effect is the same for all wasp species and experiences, the corresponding model is:

$$\log \lambda_i = \beta_\mathrm{p} \tag{1}$$

where β_p measures the attractiveness of either an empty arm or of the odour source in the ith arm.

As the results for *C. marginiventris* suggested a preference for an experienced odour, we adapted the model to assume that a wasp, which has already encountered a plant may subsequently be more attracted to this plant (fig. 1A):

$$\log \lambda_{\mathrm{e,p}} = \beta_\mathrm{p} + \gamma \mathrm{I} \quad (\mathrm{e} = \mathrm{s} \neq \mathrm{n}) \tag{2}$$

where $\mathrm{I}\,(\mathrm{e} = \mathrm{s} \cdot \mathrm{n}) = 1$ if the odour previously experienced is the same as the odour in the corresponding arm and $\mathrm{I}\,(\mathrm{e} = \mathrm{s} \cdot \mathrm{n}) = 0$ otherwise; here e = experience, s = odour source, and n = naïve.

The parameter γ describes the magnitude of this effect and will differ from zero when previous experience of an odour influences the wasps' attraction to this odour. A significant difference between the fits of models (1) and (2) would indicate that the wasps are indeed more attracted to an odour they previously experienced.

In order to assess whether experienced wasps will, independent from the odour they experienced, always prefer maize, as seemed to be the case for *M. rufiventris* (fig. 1B), we used the model:

$$\log \lambda_{\mathrm{e,p}} = \beta_\mathrm{p} + \gamma \mathrm{I} \quad (\mathrm{e} \neq \mathrm{n}, \mathrm{s} = \mathrm{m}) \tag{3}$$

with m = maize odour.

A significant difference between models (1) and (3) would suggest that experienced wasps always have a higher preference for maize than naive wasps. In all

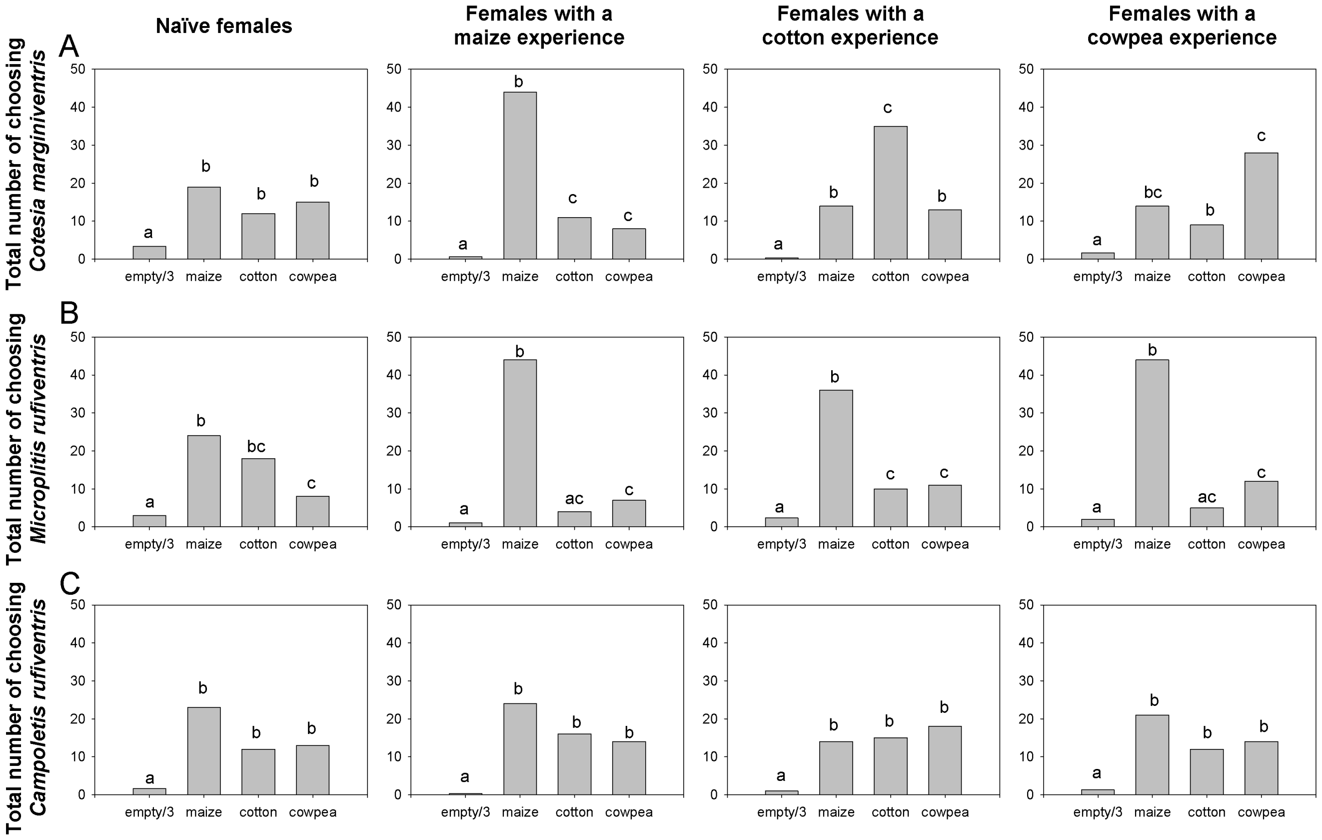


Figure 1. Olfactometric responses of the parasitoids *Cotesia marginiventris* (A), *Microplitis rufiventris* (B) and *Campoletis sonorensis* (C) when given a choice between the odours of *Spodoptera littoralis*-infested maize, cotton and cowpea plants. The wasps were either naïve or had had two oviposition experiences in hosts that were feeding on one of the three plant species. Bars having no letters in common represent significantly different response levels within each graph.

cases, we estimated the parameters by maximising the log-likelihood and compared the different models through likelihood ratio tests.

Volatile collections

During all experiments 50% of the air passing over the odour sources was pulled for 3 h through a trapping filter containing 25 mg of 80-100 mesh Super Q adsorbent (Alltech, Deerfield, Illinois) as described by Turlings et al. (1998, 2004). Immediately after each experiment, the volatiles collected on the filters were extracted with 150 μl of methylene chloride and two internal standards (n-octane and nonyl acetate, each 200 ng in 10 μl methylene chloride) were added to these extracts. The samples were either analysed immediately or stored at -70°C for later analysis. They were analysed by gas chromatography and mass spectrometry as described by Turlings et al. (2004). The purpose of these collections and analyses was to confirm the presence of the typical caterpillar-induced odours for each of the three plant species during each of the experiments.

HP GC Chemstation software was used to quantify all major components by comparison to the known quantity of internal standards. A Kruskal-Wallis ANOVA on ranks and Tukey post-hoc test were used to compare the total quantities of collected volatiles among the plant species.

RESULTS

Wasp behaviour

All naïve and experienced parasitoid females showed a strong preference for the arms containing the odour of infested plants as compared to the control arms (fig. 1). Naïve *C. marginiventris* females had no obvious preference for a particular plant odour and chose equally among the three species ($P = 0.1$). After experience, the majority of *C. marginiventris* females showed a significant preference for the odour that they had experienced ($P < 0.001$; fig. 1A). In contrast, naïve *M. rufiventris* females tended to prefer the odour of *S. littoralis*-damaged maize plants and this preference for maize was greatly enhanced after experience, independently of the plant species used to train the females ($P < 0.001$; fig. 1B). *C. sonorensis* females, whether naïve or experienced, did not show a preference for a particular plant odour produced by one of the three plant species attacked by *S. littoralis* larvae ($P = 0.7$; fig. 1C).

A statistical comparison between the three species may be compromised by the fact that they were tested on different days and to different individual plants. However, as the order of the experiments was more or less random (depending on wasp availability) and there were many replications (12 per experience type, each with six wasps), a day or plant effect seems very unlikely. Moreover, the volatile collections revealed that there was no difference in what the plants emitted when the experimental days were compared for the three wasps species (see below). Using the log-linear model for comparison between the three species showed a highly significant difference ($P < 0.001$) in the effect of experience on responses among the three wasps.

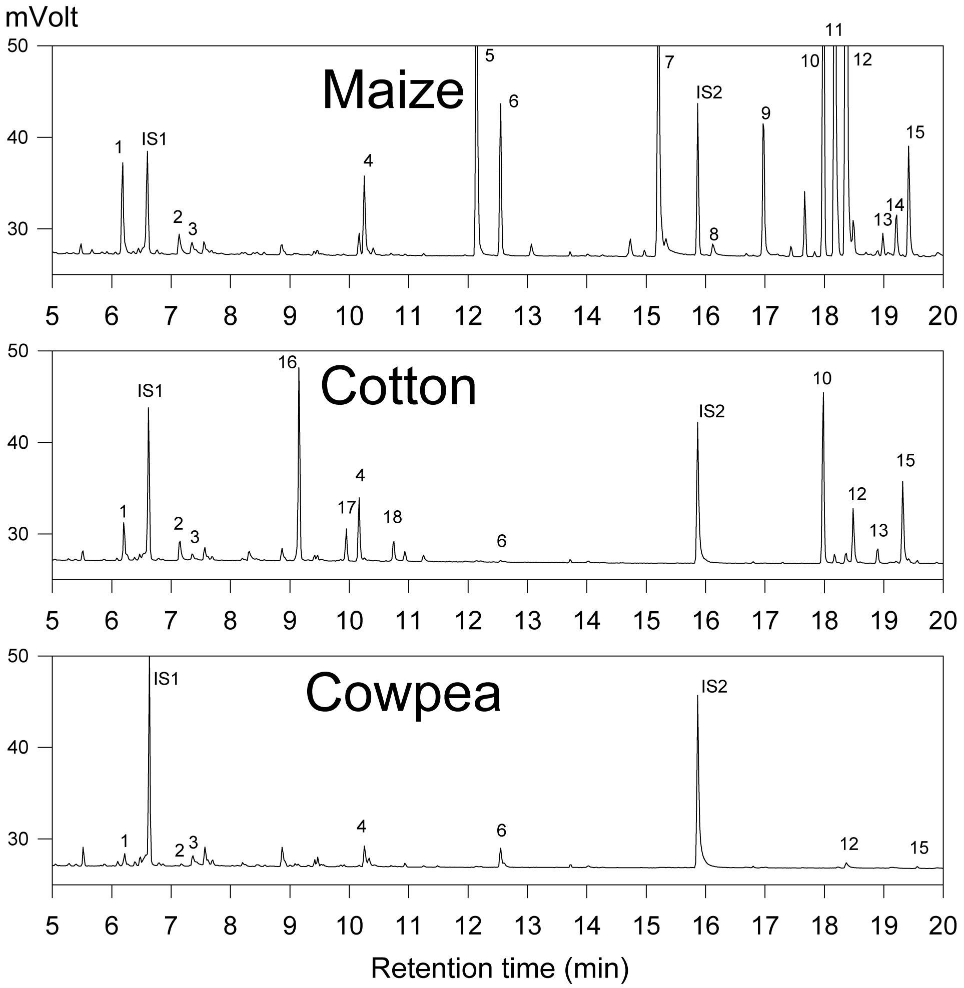
Odour emissions

The analyses of the odours collected during the bioassays produced volatile profiles (fig. 2A) that were very similar to those collected in previous studies (Turlings and Wäckers, 2004). The blends emitted by the three plants differed both in composition and quantity, all of them releasing typical green leafy volatile, but different blends of terpenoids and a few aromatic compounds (fig. 2A). The total quantity of produced volatiles was approximately four times higher for maize than for cotton, and about five times higher for cotton than for cowpea (fig. 2B) and these differences were highly significant (Two-way ANOVA, $F_{2,99} = 93.96$; $P < 0.001$). Comparing between the collections obtained from the experiments with the three wasps species revealed no differences (Two-way ANOVA, $F_{2,99} = 0.257$; $P = 0.774$), implying that they were exposed to similar odours. The quantitative differences between the three plant species were not reflected in the wasps' responses (compare figs 1, 2).

DISCUSSION

The ability of parasitoids to learn by association is well documented (Vet and Dicke, 1992; Turlings et al., 1993; Vet et al., 1995; Steidle and van Loon, 2003) and, according to Steidle and van Loon (2003), is expected to occur mostly in parasitoids with a broad host range. Our comparison of three taxonomically different, but ecologically similar parasitoids shows that such a generality cannot be readily made. Despite similarities in their biology and host range, *C. marginiventris*, *M. rufiventris*, and *C. sonorensis* showed clear differences in how they exploit volatiles that may guide them to their hosts. A recent study on the antennal perception shows that all three perceive most of the volatiles that the plants emit (Guouinguené et al., 2005), but obviously they use this information differently. The three wasps had somewhat similar innate responses, but their odour preferences were entirely different after experiencing a particular odour in association with successful ovipositions.

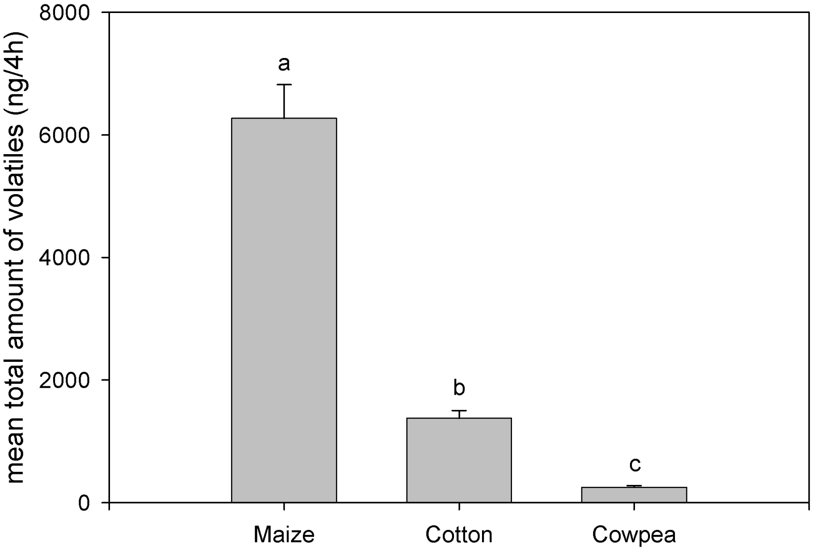
Naïve *C. marginiventris* and *C. sonorensis* chose equally among the induced odours of the three plants, whereas naïve *M. rufiventris* showed a slight preference for maize and cotton odours. After experience, *C. marginiventris* always preferred the odour of the plant species that they had experienced, very much in line with the "varying response model" proposed by Vet et al. (1990). In contrast, experienced *M. rufiventris* increased its preference for maize odour, independently of the plant they had experienced, and *C. sonorensis* showed no change in its preference after experience.



(A)

Figure 2. **A**. Typical chromatographic profiles of the volatiles of the induced plants that were collected during the bio-assays. The labelled peaks are: **1**, (*Z*)-3-hexenal; **2**, (*E*)-2-hexenal; **3**, (*Z*)-3-hexenol; **4**, (*Z*)-3-hexenyl acetate; **5**, linalool; **6**, (*E*)-4,8-dimethyl-1,3,7-nonatriene; **7**, indole; **8**, methyl anthranilate; **9**, geranyl acetate; **10**, (*E*)-β-caryophyllene; **11**, (*E*)-α-bergamotene; **12**, (*E*)-β-farnesene; **13**, (E,E)-α-farnesene; **14**, nerolidol; **15**, (*E,E*)-4,8,12-trimethyl-1,3,7, 11-tridecatetraene; **16**, α-pinene; **17**, β-pinene; **18**, (*E*)-β-ocimene. Two internal standards, n-octane and nonyl-acetate, are labelled with **IS1** and **IS2**, respectively. **B**. The average total amounts of volatiles collected for the three plant species for all experiments. The letters indicate significant differences between the plants.

Thus, *C. marginiventris* showed a clear ability of associative learning, increasing its responsiveness to the odour of the plant-host complex it experienced, as has been observed in previous studies (Turlings et al., 1989, 1990, 1993). Additional studies now show that this parasitoid innately responds to plant volatiles that are associated



(B)

Figure 2. (Continued).

with fresh leaf damage (Hoballah and Turlings, 2005) and not to the truly induced volatiles such as the terpenoids that the plants release only several hours after initial caterpillar attack (D'Alessandro and Turlings, 2005a). The response to the typically induced volatiles apparently only occurs after *C. marginiventris* females have associated such volatiles with the presence of hosts (Hoballah and Turlings, 2005). This should allow a newly emerged wasp to first explore damaged plants of various species for host presence and after successful host location focus its search efforts on the most profitable plant species.

Apparently, *M. rufiventris* and *C. sonorensis* follow different strategies. Experienced *M. rufiventris* females appear to use cues that are closely associated with the host rather than the general plant volatile blend. This is evident from studies whereby naïve females were observed to be strongly attracted to artificially induced maize odours, but after an oviposition experience in the presence of a natural herbivore-induced maize odour blend, their response to the artificially induced blend was much lower and they strongly prefer the natural odour of plants under attack by caterpillars (Hoballah and Turlings, 2005; Tamò, 2005). If indeed they learn to respond to cues that come directly from the host or its by-products (which are missing from the emissions of artificially induced plants) it could be that these host-related cues are best perceived from hosts on maize plants. It should be noted that of the three species tested, *M. rufiventris* appears to have the most restricted host range. To our knowledge only seven host species have been reported for *M. rufiventris* (El-Minshawy, 1963; Hammad et al., 1965; Gerling, 1969; Abou Zeid et al., 1978), but this species has been relatively poorly studied and a broader host range is likely. Nevertheless, it could be considered more of a specialist than the other two, which could explain a more specific use of host location cues (Vet and Dicke, 1992; Steidle and van Loon, 2003). However, more specialized species of the same genus have been shown to be very good learners (Drost et al., 1986; Hérard et al., 1988; Lewis et al., 1991; Murray and Rynne, 1994).

C. sonorensis, on the other hand, is, like *C. marginiventris*, an extreme generalist (Lingren et al., 1970), and the fact that its responses were not affected by experience is therefore in clear contrast to the expected use of learning by generalists. It remains possible that this wasp changes it preferences after more or different types of experiences, or that the absence of an effect is a particularity of the specific strain we used. However, the lack of change in the preference of *C. sonorensis* after experience is consistent with an earlier study by McAuslane et al. (1991), who used an entirely different strain. We cannot rhyme this behaviour with the expected behavioural flexibility in generalists. One could speculate that *C. sonorensis* uses very common plant cues that are nevertheless reliably associated with caterpillar presence. Further studies will have to be conducted with manipulated odour blends to pinpoint the volatile cues this species actually uses.

The analysis of the volatiles collected during the bioassays show that the induced volatiles produced by the three plant species differed both in quantity and in composition. The total amount of produced volatiles was approximately four times higher for maize than for cotton, and about five times higher for cotton than for cowpea (fig. 2B), and the composition of the blends clearly differed among plant species (fig. 2A). As already found by Hoballah et al. (2002), both quantity and composition of the odours affects the response of *C. marginiventris* females. If only the quantity had played a role, than the majority of the females of each species should have chosen the arms containing maize plants. *M. rufiventris* indeed showed a preference for maize volatiles, However, the above mentioned previous experiments (Hoballah and Turlings, 2005; Tamò, 2005) indicate a more complicated effect of experience, whereby it appears that the females increase their responsiveness to cues that are directly related to the host. If this is indeed the case, the observed preference for maize could be due to more of these cues being produced by the host on this plant. The preference for maize in *M. rufiventris* cannot be explained by the origin of the strain used for the experiments, as our lab colony originated from cotton fields in Egypt and host presence and natural parasitisation rates are high on cotton (Hegazi et al., 1973; Ibrahim and Tawfik, 1975).

The considerable differences in responses to host-induced plant odours observed here imply that we cannot readily generalise about how parasitoids have evolved to optimise host microhabitat location. The studied wasps have comparable host ranges and life cycles, but showed considerable differences in how experiences affected their responses to the odours of host-infested plants. This confirms the notion of Steidle and van Loon (2003) that the expectation that generalist parasitoids make use of associatively learning, and that specialists exhibit genetically fixed responses, does not always hold true. Our knowledge on parasitoid host foraging strategies is also largely hampered by the fact that little is known about which compounds are the key attractants in the complex plant-produced blends. Novel behavioral, chemical and molecular techniques may help to resolve this gap in our knowledge (Degenhardt et al., 2003; D'Alessandro and Turlings, 2006b).

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REFERENCES

Abou Zeid, N.A., El-Dakroury, M.S.I., El-Heneidy, A.H. & Abbas, M.S.T. (1978) Biology of *Microplitis rufiventris* Kok. parasitizing *Heliothis armigera* Hb. in Egypt. *Agric. Res. Rev. (Cairo)*, 56, 31-36.

Altahtawy, M.M., Hammad, S.M. & Hegazi, E.M. (1976a) Studies on the dependence of *Microplitis rufiventris* Kok (Hym, Braconidae) parasitizing *Spodoptera littoralis* (Boisd) on own food as well as on food of its host. *Z. Angew. Entomol.-J. Appl. Entomol.*, 81, 3-13.

Altahtawy, M.M., Hegazi, E.M. & Hammad, S.M. (1976b) Convenience of ages and instars of *Spodoptera littoralis* (Boisd) larvae for *Microplitis rufiventris* Kok – Mass rearing and biological-control of Cotton Leafworm. *Z. Angew. Entomol.-J. Appl. Entomol.*, 81, 270-285.

Baehrecke, E.H., Vinson, S.B. & Williams, H.J. (1990) Foraging behavior of *Campoletis sonorensis* in response to *Heliothis virescens* and cotton plants. *Entomol. Exp. Appl.*, 55, 47-57.

Brown, E.S. & Dewhurst, C.F. (1975) The genus *Spodoptera* (Lepidoptera, Noctuidae), in Africa and the Near East. *Bull. Entomol. Res.*, 65, 221-262.

D'Alessandro, M. & Turlings, T.C.J. (2005) *In situ* modification of herbivore-induced plant odours: a novel approach to study the attractiveness of volatile organic compounds to parasitic wasps. *Chem. Senses*, 30, 1-15.

D'Alessandro, M. & Turlings, T.C.J. (2006) Advances and challenges in the identification of volatiles that mediate interactions among plants and arthropods. *The Analyst*, 131, 24-32.

Degenhardt, J., Gershenzon, J., Baldwin, I.T. & Kessler, A. (2003) Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. *Curr. Opin. Biotechnol.*, 14, 169-176.

Dmoch, J., Lewis, W.J., Martin, P.B. & Nordlund, D.A. (1985). Role of host-produced stimuli and learning in host selection behavior of *Cotesia* (= *Apanteles*) *marginiventris* (Cresson) (Hymenoptera, Braconidae). *J. Chem. Ecol.*, 11, 453-463.

Drost, Y.C., Lewis, W.J., Zanen, P.O. & Keller, M.A. (1986) Beneficial arthropod behavior mediated by airborne semiochemicals. 1. Flight behavior and influence of preflight handling of Microplitis croceipes (Cresson). *J. Chem. Ecol.*, 12, 1247-1262.

El-Minshawy, A.M. (1963) *Studies on the morphology and biology of* Spodoptera exigua *Hb. and* Spodoptera latebrosa. Faculty of Agriculture, Dissertation, University of Alexandria.

Eller, F.J., Tumlinson, J.H. & Lewis, W.J. (1992) Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiol. Entomol.*, 17, 235-240.

Elzen, G.W., Williams, H.J. & Vinson, S.B. (1986) Wind-tunnel flight responses by Hymenopterous parasitoid *Campoletis sonorensis* to cotton cultivars and lines. *Entomol. Exp. Appl.*, 42, 285-289.

Geervliet, J.B.F., Vreugdenhil, A.I., Dicke, M. & Vet, L.E.M. (1998). Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomol. Exp. Appl.*, 86, 241-252.

Gerling, D. (1969) The parasites of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) eggs and larvae in Israel. *Isr. J. Entomol.*, 4, 73-81.

Hammad, S.M., El-Minshawy, A.M. & Salama, A. (1965) Studies on *Microplitis rufiventris* Kok. (Hym., Braconidea). *Bull Soc. Entomol. Egypte*, 49, 215-219.

Gouinguené, S., Pickett, J.A., Wadhams, L.J., Birkett, M.A. & Turlings, T.C.J. (2005) Antennal electrophysiological responses of three parasitic wasps to the caterpillar-induced volatiles from maize, cowpea and cotton. *J. Chem. Ecol.*, 31, 1023-1038.

Hegazi, E.M., Elminshawy, A.M. & Hammad, S.M. (1977) Suitability of *Spodoptera littoralis* larvae for development of *Microplitis rufiventris*. *J. Agric. Sci.*, 89, 659-662.

Hegazi, E.M., Hammad, S.M., Altahtawy, M.M. & El-Sawaf, S.K. (1973) Parasites of the larval stage of the cotton leaf-worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Alexandria region. *Z. Angew. Entomol.-J. Appl. Entomol.*, 74, 332-336.

Hegazi, E.M. & Shabaan, M.A. (1984) Competition and the superparasitism of *Spodoptera littoralis* (Boisd.) larvae by *Microplitis rufiventris* Kok. *J. Agric. Sci.*, 103, 181-187.

Hérard, F., Keller, M.A., Lewis, W.J. & Tumlinson, J.H. (1988) Beneficial arthropod behavior mediated by airborne semiochemicals. 4. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson (Hymenoptera, Braconidae). *J. Chem. Ecol.*, 14, 1597-1606.

Hoballah, M.E., Degen, T., Bergvinson, D., Savidan, A., Tamò, C. & Turlings, T.C.J. (2004) Occurrence and direct control potential of parasitoids and predators of the fall armyworm (Lepidoptera: Noctuidae) on maize in the subtropical lowlands of Mexico. *Agric. For. Entomol.*, 6, 83-88.

Hoballah, M.E. & Turlings, T.C.J. (2005) The role of fresh versus old leaf damage in the attraction of parasitic wasps to herbivore-induced maize volatiles. *J. Chem. Ecol.*, 31, 2003-2018.

Hoballah, M.E.F., Tamo, C. & Turlings, T.C.J. (2002) Differential attractiveness of induced odors emitted by eight maize varieties for the parasitoid *Cotesia marginiventris*: Is quality or quantity important? *J. Chem. Ecol.*, 28, 951-968.

Hodges, R.W., Dominick, T., Davis, D.R., Ferguson, D.C., Franclemont, J.G., Munroe, E.G. & Powell, J.A. (1983) *Checklist of the Lepidoptera of America And North of Mexico*. Washington, D.C., The Wedge Entomological Research Foundation, 284 pp.

Ibrahim, M. & Tawfik, K. (1975) *A report on the survey of natural enemies attacking the American bollworm.* Cairo, Agricultural Research Center, Plant Protection Research Institute.

Isenhour, D.J. (1985) *Campoletis sonorensis* Hym, Ichneumonidae as a parasitoid of *Spodoptera frugiperda* Lep, Noctuidae – Host stage preference and functional response. *Entomophaga*, 30, 31-36.

Jalali, S.K., Singh, S.P. & Ballal, C.R. (1987) Studies on host age preference and biology of exotic parasite, *Cotesia marginiventris* (Cresson) (Hymenoptera, Braconidae). *Entomon*, 12, 59-62.

Lewis, W.J., Tumlinson, J.H. & Krasnoff, S. (1991) Chemically mediated associative learning – an important function in the foraging behavior of *Microplitis croceipes* (Cresson). *J. Chem. Ecol.*, 17, 1309-1325.

Lingren, P.D., Guerra, R.J., Nickelse, J.W. & White, C. (1970) Hosts and host-age preference of *Campoletis perdistinctus* (Hymenoptera, Ichneumonidae). *J. Econ. Entomol.*, 63, 518-522.

Loughrin, J.H., Manukian, A., Heath, R.R. & Tumlinson, J.H. (1995) Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.*, 21, 1217-1227.

McAuslane, H.J., Vinson, S.B. & Williams, H.J. (1991) Influence of adult experience on host microhabitat location by the generalist parasitoid, *Campoletis sonorensis* (Hymenoptera, Ichneumonidae). *J. Insect Behav.*, 4, 101-113.

McCall, P.J., Turlings, T.C.J., Loughrin, J., Proveaux, A.T. & Tumlinson, J.H. (1994) Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L) seedlings. *J. Chem. Ecol.*, 20, 3039-3050.

Meier, N.F. (1929) Schlupfwespen die in Russland in den Jahren 1881-1926 aus Schaedlingen gezogen sind (Fortsetzung). *Izv. Otd. Prokl. Entomol.*, 4, 231-248.

Murray, D.A.H. & Rynne, K.P. (1994) Effect of host-plant on parasitism of *Helicoverpa armigera* (Lep, Noctuidae) by *Microplitis demolitor* (Hymenoptera, Braconidae). *Entomophaga* 39, 251-255.

Paré, P.W. & Tumlinson, J.H. (1997) De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol.*, 114, 1161-1167.

Potting, R.P.J., Otten, H. & Vet, L.E.M. (1997) Absence of odour learning in the stemborer parasitoid *Cotesia flavipes*. *Anim. Behav.*, 53, 1211-1223.

Röse, U.S.R., Manukian, A., Heath, R.R. & Tumlinson, J.H. (1996) Volatile semiochemicals released from undamaged cotton leaves – A systemic response of living plants to caterpillar damage. *Plant Physiol.*, 111, 487-495.

Steidle, J.L.M. & van Loon, J.J.A. (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomol. Exp. Appl.*, 108, 133-148.

Tamò, C. (2006) *A comparative study on the plant odour preferences and learning ability of three solitary endoparasitoids of* Spodoptera *species.* Dissertation, University of Neuchâtel.

Tawfik, M.F.S., Hafez, M. & Ibrahim, A.A. (1980) Immature stages of *Microplitis rufiventris* Kok (Hym, Braconidae). *Dtsch. Entomol. Z.*, 27, 39-50.

Thompson, W.R. (1946) *A catalogue of the parasites and predators of insect pests. Section 1: Host-parasite catalogue. Part 7: Parasites of the Lepidoptera.* Commonwealth Institute of Biological Control, Ontario, Canada.

Turlings, T.C.J. (1990) *Semiochemically mediated, host-searching behavior of the endoparasitic wasp* Cotesia marginiventris *(Cresson) (Hymenoptera: Braconidae).* Dissertation, University of Florida.

Turlings, T.C.J. & Benrey, B. (1998) Effects of plant metabolites on the behavior and development of parasitic wasps. *Ecoscience*, 5, 321-333.

Turlings, T.C.J. & Wäckers, F.L. (2004) Recruitment of predators and parasitoids by herbivore-damaged plants. In: R.T. Cardé & J. Millar (Eds.), *Advances in Insect Chemical Ecology*, pp. 21-75. Cambridge, Cambridge University Press.

Turlings, T.C.J., Davison, A.C. & Tamò, C. (2004) A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiol. Entomol.*, 29, 45-55.

Turlings, T.C.J., Lengwiler, U.B., Bernasconi, M.L. & Wechsler, D. (1998) Timing of induced volatile emissions in maize seedlings. *Planta*, 207, 146-152.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J. & Lewis, W.J. (1991) Larval-damaged plants – Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the microhabitat of its hosts. *Entomol. Exp. Appl.*, 58, 75-82.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250, 1251-1253.

Turlings, T.C.J., Tumlinson, J.H., Lewis, W.J. & Vet, L.E.M. (1989) Beneficial arthropod behavior mediated by airborne semiochemicals. 8. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *J. Insect Behav.*, 2, 217-225.

Turlings, T.C.J., Wäckers, F., Vet, L.M., Lewis, W.J. & Tumlinson, J.H. (1993) Learning of host-finding cues by hymenopterous parasitoids. In: D.R. Papaj & A. Lewis (Eds.), *Insect Learning: Ecological and Evolutionary Perspectives*, pp. 51-78. New York, Chapman & Hall.

Vet, L.E.M. (1999) From chemical to population ecology: Infochemical use in an evolutionary context. *J. Chem. Ecol.*, 25, 31-49.

Vet, L.E.M. & Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.*, 37, 141-172.

Vet, L.E.M. & Groenewold, A.W. (1990) Semiochemicals and learning in parasitoids. *J. Chem. Ecol.*, 16, 3119-3135.

Vet, L.E.M., Lewis, W.J. & Cardé, R.T. (1995) Parasitoid foraging and learning. In: R.T. Cardé & W.J. Bell (Eds.), *Chemical Ecology of Insects 2*, pp. 65-101. New York, Chapman & Hall.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. & van Lenteren (1990) A variable-response model for parasitoid foraging behavior. *J. Insect Behav.*, 3, 471-490.

Zanen, P.O. & Cardé, R.T. (1991) Learning and the role of host-specific volatiles during in- flight host-finding in the specialist parasitoid *Microplitis croceipes*. *Physiol. Entomol.*, 16, 381-389.