RESEARCH ARTICLE

Insecticide odour interference with food-searching behaviour of *Microplitis croceipes* (Hymenoptera: Braconidae) in a laboratory arena

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Response to odours plays an important role in resource location by natural enemies, particularly by parasitoid wasps. While a considerable research effort has been dedicated to studying the effects of insecticide intoxication on natural enemy search behaviour, it is yet unknown if the odours themselves interfere with distant chemoreception. We investigated this issue using the food-searching behaviour of *Microplitis croceipes* (Cresson)(Hymenoptera: Braconidae) in laboratory arenas as a model system. Odours of imidacloprid (Genesis®), spinosad (Entrust®), esfenvalerate (Asana®), methamidophos (Monitor®), and vanilla were tested for their ability to interfere with wasp response to the odour of honey. The wasps did not contact the chemicals. Honey odour was generally effective in triggering food-searching behaviour in both honey-fed (and thus conditioned to associate honey odour with food) and unfed, naïve wasps. Mixing honey with imidacloprid and spinosad did not affect wasp responses. The remaining compounds (esfenvalerate, methamidophos, and vanilla) significantly reduced the proportion of positively responding fed wasps, but only methamidophos had such an effect on the unfed wasps. Negative methamidophos effects became completely reversed when wasps were forced to feed on honey in the presence of methamidophos odour. Our results suggest that odours and provision of food may potentially be used to keep beneficial natural enemies away from insecticide-treated areas.

Keywords: chemical control; biological control; integrated pest management; natural enemies; behavioural manipulation

1. Introduction

Advanced integrated pest management implies amalgamation of various control techniques into a unified system capable of keeping pest populations below economically damaging levels. Both pesticides and biological control provide powerful tools for reducing numbers of undesirable organisms. Although the former still dominate commercial agriculture, increased public awareness of their deleterious non-target effects, including those on human health, provides a strong incentive for the use of alternatives. Still, complete pesticide replacement by non-chemical methods is unlikely to happen in the foreseeable future. Therefore, compatibility with chemical control is

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essential for the increased adoption of the alternative techniques, such as biological control, by pest control practitioners.

The behaviour of natural enemies is important to their success in suppressing populations of target pests, and the effects of pesticides on natural enemies' behaviours has been proposed for inclusion into risk assessment schemes (Desneux, Denoyelle, and Kaiser 2006). Much effort has been dedicated to investigating sub-lethal effects of pesticide exposure on a range of natural enemy behaviours, including mobility, orientation, feeding, oviposition, and learning performance (see Desneux, Decourtye, and Delpuech 2007 for review). Although pesticides induced different effects on natural enemies, most of the studies reported disruptions in the investigated behaviours.

Response to odours plays an important role in resource location by natural enemies, particularly by parasitoid wasps (Vinson 1984; Vet and Dicke 1992). Detection of odours is affected by numerous factors, including a background of a larger assortment of chemicals present in the environment. Such volatiles may interfere with the specific olfactory cues through repellent or masking action, or may enhance them by providing a contrasting background against which the informative odours become easier to distinguish (Hilker and McNeil 2008). While many pesticides have odours easily detectable even by a relatively insensitive human nose, little is known about their effect on insect orientation towards otherwise attractive cues. A number of studies reported that pesticides induce abnormal host- and food-searching behaviours in several insect species (Desneux et al. 2007). In most cases, insect ability to locate resources was compromised (Longley and Jepson 1996; Umoru, Powell, and Clark 1996; Stapel, Cortesero, and Lewis 2000; Desneux, Pham-Delégue, and Kaiser 2004; Desneux, Rafalimanana, and Kaiser 2004; Schneider, Smagghe, Pineda, and Vinuela 2004), although on several occasions positive sublethal effects of pesticides on orientation and searching behaviours have also been detected (Rafalimanana, Kaiser and Delpuech 2002; Delpuech, Bardon, and Boulétreau 2005). However, insects in these studies either touched or ingested pesticide residues. It is yet unknown if the odours themselves may interfere with distant chemoreception through repellency, masking, or their combination. A decrease in response to positive olfactory stimuli in the presence of pesticide odours might decrease natural enemy exposure to pesticides.

In the present study, we investigated effects of selected insecticide odours on food-searching behaviour of *Microplitis croceipes* (Cresson)(Hymenoptera: Braconidae), a larval parasitoid of *Heliothis* spp. and *Helicoverpa* spp. This species is an effective biological control agent of these pests in North America (King and Coleman 1989), and its olfactory behaviours have been extensively studied (e.g., Lewis and Takasu 1990; Takasu and Lewis 1993, 1995; Rains et al. 2004; Takasu, Rains, and Lewis 2007). There also appears to be a good correlation between laboratory and field findings (Takasu and Lewis 1993, 1995). Therefore, we considered it to be a convenient model species for our research.

2. Materials and methods

2.1. Insects

Microplitis croceipes were reared in the laboratory as described by Le and Takasu (2005). Newly emerged adults were placed in a transparent Plexiglas cage

(30 × 30 × 25 cm), provided with moistened cotton wicks, and kept at 25°C and 16 h L:8 h D photoperiod for 48 h (Takasu et al. 2007). Approximately 35–40 wasps were kept in one cage. Males and females were kept in the same cage and mating was frequently observed. Between 30 and 45 min before the beginning of experiments, the wasps were transferred into individual glass vials (10.5 × 1.5 cm, long × diameter). New vials were used for each replication. The vials were closed to minimize wasp exposure to outside odours.

2.2. Observation arenas

Observation arenas were modified from those described by Takasu et al. (2007). Tested chemicals (see below) were pipetted into the bottom part of a plastic Petri dish (52 × 14 mm, diameter × deep). The base of the dish was then covered with aluminium foil and sealed by pressing the foil down around the sides of the dish. A circle (18 mm in diameter) of eight equidistant 1-mm holes was made in the centre of each arena to allow diffusion of the tested odours. All behavioural observations were conducted under a fume hood at $25 \pm 2°C$ and light intensity of 1200–1300 lux.

2.3. Experimental procedure

We used a test method originally developed by Rains et al. (2004). Individual wasps were allowed to walk from their vials to the centre of observation arenas inside the circle of holes. When wasps responded positively to the odour diffusing through the holes, they showed a distinct food-searching behaviour (Rains et al. 2004; Takasu et al. 2007) by antennating arena surface around the holes, attaching mouthparts to the surface or trying to insert them into the holes, and frequently changing walking direction to move in a circular pattern around the holes. Unresponsive wasps (or negatively responding wasps, as this methodology does not allow distinguishing between the two) left arenas by walking or by flight without displaying the aforementioned behaviours in the area around the holes. A trial was discontinued once a wasp either displayed the food-searching behaviour, or left the arena. The proportion of wasps positively responding to the odours and the time between wasp release and the onset of food searching behaviour (for the positively responding wasps) or between wasp release and its leaving the arena (for the remaining wasps) were recorded.

2.4. Insecticide interference with food-searching behaviour

The experiment followed factorial randomized complete block design, with parasitoid sex, feeding experience, and odour used as the main factors. Honey, which is known to be an attractive food source for *M. croceipes* (Lewis and Takasu 1990), was selected as a food odour. Half of the males and half of the females used in the experiment fed on a droplet of honey approximately 15–45 min before the beginning of the experiment and thus were conditioned to associate its odour with food intake (Lewis and Takasu 1990). In an earlier study (Takasu and Lewis 1996), there were no differences in *M. croceipes* response behaviours between 5 min and 6 h after conditioning experience. Feeding was done in the arenas similar to the observation arenas described above, but not containing any chemicals. A droplet of

honey was placed on top of the foil in the middle of the arena. Individual wasps were released near the droplet, allowed to feed for 5 s, and then returned to a holding vial. The experience was repeated five times with 30-s intervals and resulted in honey-fed, honey-conditioned wasps. The remaining wasps were left unfed and naïve.

Five different compounds were tested for their ability to interfere with wasp response to the odour of honey. Four of those were commercial formulations of insecticides belonging to four different chemical classes: a neonicotinoid (imidacloprid, Genesis®, Bayer CropScience, Research Triangle Park, NC), a spinosyn (spinosad, Entrust®, Dow AgroSciences, Indianapolis, IN), a pyrethroid (esfenvalerate, Asana®, DuPont, Wilmington, DW), and an organophosphate (methamidophos, Monitor®, Bayer CropScience, Research Triangle Park, NC). The chemicals were chosen because they represented a range of chemical, physical, and biological properties, and were available at the time of the study, not because of their known effects on *M. croceipes* or their use in crop systems where *M. croceipes* is an important biocontrol agent. Imidacloprid and spinosad formulations are virtually odourless to humans, while esfenvalerate and methamidophos have an odour that is easily detectable by humans. The latter two compounds are broad-spectrum insecticides toxic to a wide variety of species, including vertebrates (Ware and Whitacre 2004). They are usually considered to have a low compatibility with biological control. Imidacloprid is somewhat more selective, and has a low mammalian toxicity (Yamamoto et al. 1995). However, it still affects many different insects, including natural enemies (Rogers and Potter 2003; Krischik, Landmark, and Heimpel 2007). Feeding on extra-floral nectar produced by corn plants treated with imidacloprid has been shown to have a negative effect on host-searching behaviour of *M. croceipes* (Stapel et al. 2000). Spinosad is often considered to be relatively safe for natural enemies, although this may vary depending on species and environmental conditions (Jones, Scott-Dupree, Harris, Shipp, and Harris 2005; Arthurs, Lacey, and Miliczky 2007; Ruiz et al. 2008). The fifth compound was undiluted ethanolic vanilla extract, which is characterized by a human-detectable odour, but has no known insecticidal properties and is commonly used to test insect olfactory responses (e.g., Lewis and Takasu 1990). Insecticide formulations were diluted in distilled water to approximate concentrations likely encountered by wasps on fields recently treated with high label rates based on manufacturer recommendations (240 mg ai/L for imidacloprid, 800 mg ai/L for spinosad, 180 mg ai/L for esfenvalerate, 4.72 g ai/L for methamidophos).

Before the beginning of each experimental replication, 6 ml of each of the five tested compounds were thoroughly mixed with 6 ml of honey in a plastic centrifuge tube (Iwaki Co., Tokyo, Japan). That resulted in the concentrations of 120 mg ai/L for imidacloprid, 400 mg ai/L for spinosad, 90 mg ai/L for esfenvalerate, and 2.36 g ai/L for methamidophos. Additionally, 6 ml of distilled water were mixed with 6 ml of honey to obtain positive control. Distilled water alone was used as a negative control. Three ml of the tested mixture were pipetted into observation arenas (our preliminary tests indicated that 1.5 ml of honey elicits a fairly consistent wasp response under such conditions) and wasp behaviour was observed as described above. Both the wasp and the arena where it was tested were discarded after each trial.

The experiment was repeated 10 times, with 10 males and 10 females tested for each treatment combination. Because we were not able to test more than 28 wasps

per day, each replication consisted of a single wasp for each sex by odour by feeding experience combination. Therefore, the data were pooled to calculate proportions of wasps responding to the food odour, and variation among the treatments and between the sexes was used as an error term. Times until the onset of food searching behaviour or until leaving the arena were not pooled. Data normality was tested using Kolmogorov–Smirnov test (PROC UNIVARIATE, SAS Institute 1999). Because of its non-normal distribution ($P < 0.05$), the data on the proportion of responding wasps were transformed using arcsine-square root transformations (PROC TRANSREG, SAS Institute 1999; Zar 1999), while the data on times spent by the wasps from the moment of release until responding or leaving the arenas were transformed using rank transformations (PROC RANK, SAS Institute 1999; Conover and Iman 1981). Transformed data were then analyzed using three-way ANOVA followed by the Duncan mean separation test (PROC GLM, SAS Institute 1999). For the proportion of wasps responding to food odour, wasp feeding experience had a significant interaction with odour exposure, but there was no difference in response between the two sexes (see Section 3). Therefore, we pooled the two sexes together and analyzed the data separately for fed wasps and unfed naïve wasps using one-way ANOVA followed by the Duncan mean separation test (PROC GLM, SAS Institute 1999). Means and standard errors presented in the paper were calculated from the untransformed data.

2.5. Learning to associate insecticide odour with food

Results of the first experiment (see below) suggested that methamidophos odour interferes with food-searching behaviour of *M. croceipes*, especially for the honey-fed, honey-conditioned wasps. Therefore, the second experiment was designed to test if the observed effect could be reduced by training the wasps to associate methamidophos odour with food intake. The experiment followed a factorial randomized complete block design. Wasp sex and odour experience were considered to be the main factors. Three experience groups were created. Wasps from the first group were fed on honey as described above, and then tested in the arenas containing 3 ml of methamidophos solution–honey mix prepared as described above. This recreated the treatment displaying the least amount of food-searching behaviour in the first experiment. Wasps from the second and the third groups were fed on honey as described above, but this time 1.5 ml of methamidophos solution was added to the conditioning arena. Thus, the wasps experienced methamidophos odour diffusing through the holes as they approached and fed on the honey. Rains et al. (2004) and Takasu et al. (2007) showed that wasps conditioned in such a way learn to associate odours with food and respond to them in subsequent tests by displaying food-searching behaviour. Wasps from the second group were then tested in arenas containing 3 ml of methamidophos solution–honey mix, while wasps from the third group were tested in arenas containing 1.5 ml of methamidophos solution alone. Three wasps of the same sex were consecutively tested in each arena, with new foil top used for each wasp. After that, the wasps and the arena were discarded.

Three males and three females were tested for each treatment in a single day, and the proportion responding to odours was recorded. The experiment was repeated four times. Data normality was tested using Kolmogorov–Smirnov test (PROC UNIVARIATE, SAS Institute 1999). Because of its non-normal distribution

($P < 0.05$), the data on proportion of wasps responding to the odours were transformed using arcsine-square root transformations (PROC TRANSREG, SAS Institute 1999; Zar 1999), while the data on times before responding or leaving the arenas were transformed using rank transformations (PROC RANK, SAS Institute 1999; Conover and Iman 1981). Transformed data were then analyzed using two-way ANOVA followed by the Duncan mean separation test (PROC GLM, SAS Institute 1999). Means and standard errors presented in the paper were calculated from the untransformed data.

3. Results

3.1. Insecticide interference with food-searching behaviour

On average, $35 \pm 3\%$ (mean $\pm$ standard error) of wasps showed positive responses in our trials. The odour had a highly significant effect on the proportion of wasps displaying food-searching behaviour in the observation arenas (ANOVA, df $= 6,6$, $F = 27.35$, $P = 0.0004$). Effects of sex (ANOVA, df $= 1,6$, $F = 0.01$, $P = 0.9576$) and feeding experience (ANOVA, df $= 1,6$, $F = 3.13$, $P = 0.1273$) were not significant. The interaction between feeding experience and odour was significant (ANOVA, df $= 6,6$, $F = 6.34$, $P = 0.0204$), but the other interactions were not ($P > 0.05$).

When odour effects were subsequently analyzed separately for each of the two feeding experiences, the results were significant both for the unfed naïve wasps (ANOVA, df $= 6,7$, $F = 14.38$, $P = 0.0013$), as well as for the honey-fed, honey-conditioned wasps (ANOVA, df $= 6,7$, $F = 15.47$, $P = 0.0010$). However, the decline was less pronounced for the former (Figure 1). Of all tested chemicals, only methamidophos significantly reduced the proportion of food-searching unfed naïve wasps. At the same time, methamidophos, esfenvalerate, and vanilla reduced the proportion of food-searching wasps that were previously fed and conditioned on honey.
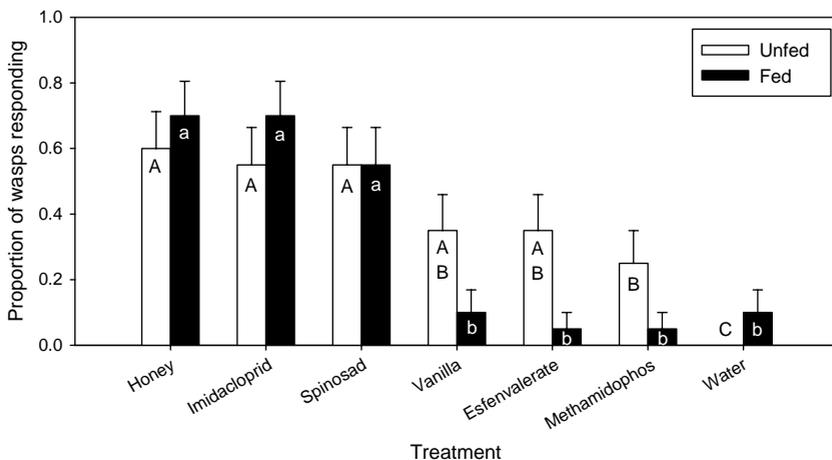

Figure 1. Proportion of *M. croceipes* displaying a characteristic food-searching behaviour in response to selected odours in observation arenas. Honey-fed, honey-conditioned wasps consumed honey before the experiment. Means followed by the same letter were not significantly different from each other (Duncan mean separation test, $P > 0.05$).

Wasps responded to odours after spending 11.9 ± 2.4 s in the observation arenas. None of the factors nor their interactions had a significant effect on the time before positively responding wasps displayed food-searching behaviours ($P > 0.05$). However, both feeding experience (ANOVA, $df = 1,149$, $F = 5.65$, $P = 0.0187$) and odour (ANOVA, $df = 6,149$, $F = 4.26$, $P = 0.0005$) influenced the time before the non-responding wasps left the observation arenas. Although not displaying any food-searching behaviours, unfed naïve wasps spent 24.96 ± 4.14 s within the arenas compared to 11.02 ± 1.23 s spent by the honey-fed, honey-conditioned wasps. The smell of methamidophos appeared to encourage the wasps to leave the observation arenas more quickly compared to the other treatments (Figure 2). There was no difference in the times before leaving between the sexes (ANOVA, $df = 1,149$, $F = 0.07$, $P = 0.7955$), and none of the interactions were statistically significant ($P > 0.05$).

3.2. *Learning to associate insecticide odour with food*

During conditioning, many wasps refused to approach the honey in the presence of a strong odour and needed repeated prodding both in our study and in the study by Takasu et al. (2007). However, that invariably changed after they fed for the first time. Odour experience affected the proportion of wasps displaying a food-searching behaviour in the observation arena (ANOVA, $df = 2,18$, $F = 4.95$, $P = 0.0194$). Consistent with the first experiment, relatively few wasps that fed on honey in an empty arena before beginning of the experiment positively responded to the odour of the mix of methamidophos solution with honey (Figure 3). However, the wasps conditioned with the odour of methamidophos solution readily responded both to
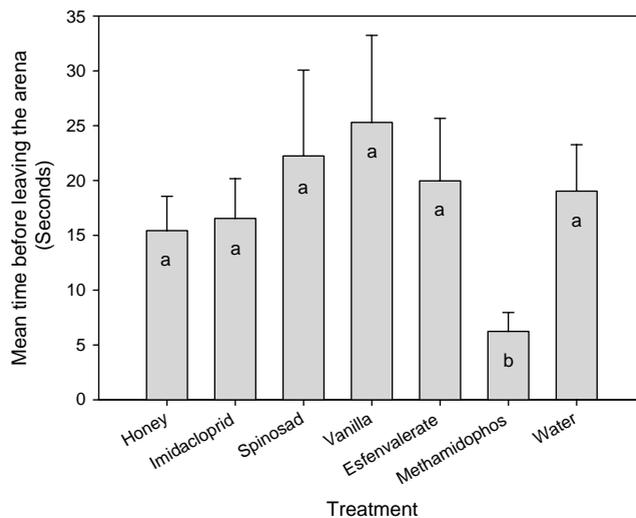

Figure 2. Time spent within observation arenas by *M. croceipes* that did not display a characteristic food-searching behaviour in response to selected odours in observation arenas. Data are pooled for the honey-fed, honey-conditioned and unfed, naïve wasps (see text for details). Means followed by the same letter were not significantly different from each other (Duncan mean separation test, $P > 0.05$).
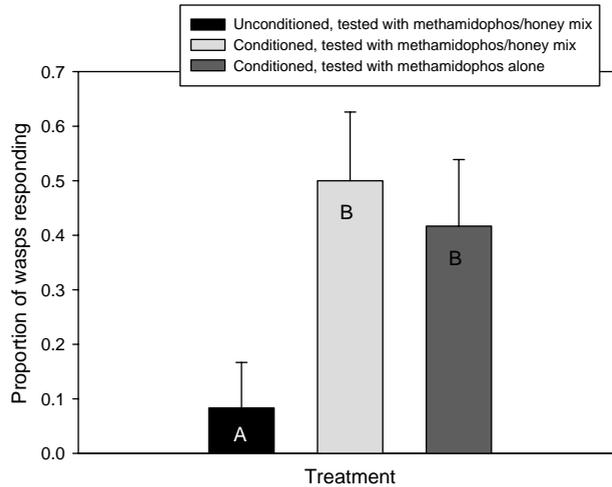
Figure 3.   Proportion of *M. croceipes* displaying a characteristic food-searching behaviour in response to the odours of methamidophos–honey mix or methamidophos alone in observation arenas. All wasps fed on honey. Conditioned wasps experienced methamidophos odour while feeding (see text for details). Means followed by the same letter were not significantly different from each other (Duncan mean separation test, $P > 0.05$).

the odour of the methamidophos solution–honey mix, as well as to the odour of methamidophos solution alone. Both sexes responded at a similar rate (ANOVA, df $= 1,18$, $F = 2.22$, $P = 0.1535$). The interaction between the odour experience and the sex was also not significant (ANOVA, df $= 2,18$, $F = 0.54$, $P = 0.5946$).

Wasps started responding to odours after spending 7.1 ± 1.8 s in the observation arenas. Similar to the first experiment, neither the two tested factors nor their interaction had a significant effect on the time before positively responding wasps displayed food-searching behaviours ($P > 0.05$). Wasps that did not search for food were slightly less eager to leave (ANOVA, df $= 2,30$, $F = 4.26$, $P = 0.0235$) when conditioned with the odour of methamidophos solution and then released in arenas containing methamidophos solution–honey mix (Figure 4). However, the numeric difference among the treatments was relatively small, while the variation was high. No other significant differences were detected ($P > 0.05$).

4. Discussion

Our results indicate that, depending on insecticide considered, insecticide odours could decrease *M. croceipes* response to food odours before parasitoids contact or ingest these insecticides. When occurring, the effect appeared to be less pronounced for the unfed, naïve wasps. However, when wasps were forced to feed while experiencing the odour of an otherwise disruptive insecticide, they learned to associate that odour with food.

Behaviour of the control treatments observed in this study was highly consistent with the previous studies conducted with *M. croceipes* and other parasitoid species (Lewis and Takasu 1990; Wäkers 1994; Takasu et al. 2007; Martínez and Hardie 2009). Honey odour triggered food-searching behaviour in both honey-fed,
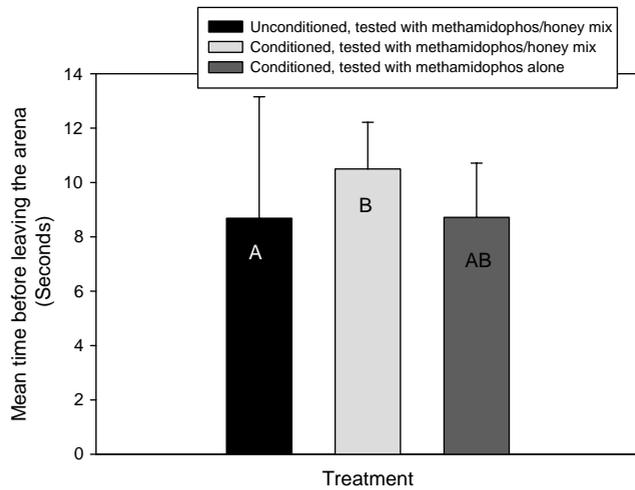
Figure 4. Time spent within observation arenas by *M. croceipes* that did not display a characteristic food-searching behaviour in response to the odours of methamidophos/honey mix or methamidophos alone in observation arenas. All wasps fed on honey. Conditioned wasps experienced methamidophos odour while feeding (see text for details). Means followed by the same letter were not significantly different from each other (Duncan mean separation test, $P > 0.05$).

honey-conditioned wasps and unfed, naïve wasps. There was also a weak positive response to water by honey-fed, honey-conditioned wasps. We did not observe any differences in the behaviour of male and female wasps.

Mixing honey with imidacloprid and spinosad did not affect wasp responses (Figure 1). While insect chemosensory apparatus is often superior to that of humans, none of the two tested formulations apparently produced enough volatiles to interfere with response to the odour of honey. However, the situation was different when the honey was mixed with compounds emitting a human-detectable odour, whether the chemicals had insecticidal properties (esfenvalerate and methamidophos), or not (vanilla). Interestingly, feeding status had a pronounced effect on the wasp response, with honey-fed, honey-conditioned wasps being less likely to react to food in the presence of an additional odour. While all three human-detectable chemicals significantly reduced the proportion of positively responding honey-fed, honey-conditioned wasps, only methamidophos had such an effect on the unfed, naïve wasps (Figure 1). This is not surprising because feeding is very important for adult *M. croceipes*. Wasps that are given only water survive for 3–4 days after emergence, while wasps given both water and honey survive for over 20 days (Takasu and Lewis 1993). Therefore, finding food is a high priority for hungry adults. Similarly, in laboratory and field experiments, poorly fed females were preferentially attracted to food-associated cues over host-associated cues (Takasu and Lewis 1993, 1995). Although the wasps in our study did not become satiated (Takasu and Lewis 1993), the amount of feeding was apparently sufficient to affect their behaviours.

Our experimental protocol did not allow distinguishing between repellent and masking effects of odorous compounds. Wasps that did not show a positive response

abandoned the observation arenas quicker when they contained methamidophos (Figure 2), the insecticide that also had the strongest negative effect on the proportion of the positively responding wasps. Also, we commonly observed that wasps approaching a hole with diffusing methamidophos odour stopped abruptly, made a quick jerking movement backwards as if startled, and then immediately flew away. While we did not quantify that behaviour, it appeared to be virtually absent from other treatments. Therefore, we can speculate that methamidophos might have had a repellent effect on *M. croceipes*. Ecologically, a strong unfamiliar odour potentially indicates a presence of an unknown hazard, while reduced perception of the smell of honey in mixture with another chemical potentially indicates an insufficient food source. Therefore, the two scenarios are similar in a sense that both increase the amount of uncertainty faced by a decision-making wasp. Such a risk is apparently more acceptable to hungry individuals.

Negative methamidophos effects became completely reversed when wasps were forced to feed on honey in the presence of methamidophos odour. Not only did the methamidophos-conditioned wasps dramatically increase their display of food-searching behaviour in response to the methamidophos–honey mix, but they also started associating methamidophos odour itself with the presence of food (Figure 3 and 4). Indeed, associative learning has been repeatedly demonstrated for this species (Lewis and Takasu 1990, Takasu and Lewis 1993, Takasu et al. 2007).

Although behavioural modifications observed in the present study might have important practical implications, it is essential to remember that we used a rather artificial laboratory model system. First, chemicals, their concentrations, and food-to-chemical ratios tested in our study were somewhat arbitrary and probably represented the extreme-case scenarios. Secondly, food is only one of the many resources sought by parasitoids. We do not know how their attraction to hosts, mates, or shelter could be affected. Nor do we know the relative importance of olfactory, visual, and other cues for foraging wasps alighting on plants. Thirdly, the stimuli determining long-distance attraction of parasitoids to host habitats are not necessarily the same as the stimuli triggering food-searching behaviours in our experimental arenas. Finally, our selection of tested chemicals was very limited. Different insecticides or even different formulations of the same chemicals could have profoundly different effects.

Despite the aforementioned issues, our findings provide additional insights towards developing a technology for comprehensively manipulating both parasitoid and environmental components of a cropping system in order to maximize efficiency of biological control as envisioned by Lewis and Martin (1990). First, it might be possible to use appropriate odours to keep beneficial insects away from pesticide-treated areas, thus reducing their mortality and allowing subsequent re-colonization after decay of pesticide residues. Desneux et al. (2005) showed that on encaged deltamethrin-treated canola plants, the aphid parasitoid *Diaeretiella rapae* flew away from treated leaves to the cage walls because of the known repulsive action of pyrethroids on insects (Perera 1982; Bos and Masson 1983; Hoy and Dahlsten 1984). This repellent effect combined with a rapid degradation of the product after treatment may allow a rapid colonization of deltamethrin-treated fields by aphid parasitoids. Behavioural manipulation could be further enhanced by developing new pesticide formulations combining biorational active ingredients with natural enemy-specific repellents. Secondly, our results suggest that in addition to increasing

longevity and improving host-searching efficiency (Takasu and Lewis 1993), feeding might also improve parasitoid survivorship by making them less likely to land on pesticide-treated plants. This lends additional support to providing natural enemies with food-rich untreated refuges in order to improve their efficiency in controlling target pests (Ferro and McNeil 1998). Some separation between such refuges and the main crop may be advisable when strongly smelling insecticides are used on the main crop. Otherwise, parasitoids may learn to associate food encountered in the refuge with insecticide odour diffusing from the main crop, and become preferentially attracted to the treated areas.

Overall, increasing temporal and spatial separation between pesticides and natural enemies is instrumental for their integration in pest management programs (Ruberson, Nemoto, and Hirose 1998). Behavioural manipulation may provide an additional tool for achieving this goal.

Acknowledgements

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