

Combined use of herbivore-induced plant volatiles and sex pheromones for mate location in braconid parasitoids

Hao Xu¹, Gaylord Desurmont¹, Thomas Degen¹, Guoxin Zhou^{1,2}, Diane Laplanche¹, Luka Henryk³ & Ted C.J. Turlings¹

¹Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), Institute of Biology, University of Neuchâtel, CH-2000, Neuchâtel, Switzerland, ²Key Laboratory for Quality Improvement of Agriculture Products of Zhejiang Province, Department of Plant Protection, Zhejiang Agriculture and Forestry University, Lin'an 311300, China and ³Department of Plant Protection and Biodiversity, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 215070 Frick, Switzerland

ABSTRACT

Herbivore-induced plant volatiles (HIPVs) are important cues for female parasitic wasps to find hosts. Here, we investigated the possibility that HIPVs may also serve parasitoids as cues to locate mates. To test this, the odour preferences of four braconid wasps – the gregarious parasitoid *Cotesia glomerata* (L.) and the solitary parasitoids *Cotesia marginiventris* (Cresson), *Microplitis rufiventris* Kokujev and *Microplitis mediator* (Haliday) – were studied in olfactometers. Each species showed attraction to pheromones but in somewhat different ways. Males of the two *Cotesia* species were attracted to virgin females, whereas females of *M. rufiventris* were attracted to virgin males. Male and female *M. mediator* exhibited attraction to both sexes. Importantly, female and male wasps of all four species were strongly attracted by HIPVs, independent of mating status. In most cases, male wasps were also attracted to intact plants. The wasps preferred the combination of HIPVs and pheromones over plant odours alone, except *M. mediator*, which appears to mainly use HIPVs for mate location. We discuss the ecological contexts in which the combined use of pheromones and HIPVs by parasitoids can be expected. To our knowledge, this is the first study to show that braconid parasitoids use HIPVs and pheromones in combination to locate mates.

Key-words: caterpillar-induced plant volatiles; leaf volatiles; mate-finding strategy; plant–insect interactions; tritrophic interactions.

INTRODUCTION

Mate location strategies in parasitoids are mainly based on the use of sex pheromones (Quicke 1997; Ruther 2013). Some parasitoids are able to locate mates at a relatively long distance with the help of highly volatile pheromones, but in some cases, less-volatile pheromones are used at a short range (Ruther 2013), and in other cases, no evidence has been found for the involvement of sex pheromones in mate location (Kimani & Overholt 1995). Therefore, other cues

like host-associated volatiles or host-induced plant volatiles may possibly also aid parasitoids to find mates. In some phytophagous insects, host-plant semiochemicals play a significant role in the biosynthesis or release of sex pheromones or even directly guide herbivores to mating sites (Landolt & Phillips 1997; Reddy & Guerrero 2004), but the use of such signals by parasitoids is unknown.

Newly emerged female parasitoids seem to have two options: Either they search for hosts right away without mating, at the risk of only producing haploid male offspring due to the haplo-diploid sex determination, or they invest time and energy searching for mates before looking for hosts, to ensure that they can produce both male and female offspring (Fauvergue *et al.* 2008; Kant *et al.* 2012; Steiner & Ruther 2009). Previous studies generally advocate the idea that the two foraging strategies are mutually exclusive and the latter is better supported. For example, female virgins of the parasitoid *Cotesia vestalis* are not attracted by host-induced plant volatiles until they mate (Kugimiya *et al.* 2010). The male aphid parasitoid, *Lysiphlebus testaceipes*, are only attracted by conspecific female wasps and not by aphid-induced plants (Auguste & Fauvergue 2015). In the gregarious parasitoid *Cotesia glomerata*, more than half of newly-emerged female wasps leave their natal patch without mating with siblings, but nevertheless, most female wasps find mates and produced both female and male offspring in the field (Gu & Dorn 2003). Generally, once they have mated, female wasps search for hosts rather than for additional mates (Kugimiya *et al.* 2010; Steiner & Ruther 2009; Xu *et al.* 2014). Mate-finding strategies may differ between solitary and gregarious parasitoids. Gregarious parasitoids usually clump their cocoons together on the hosts, and both male and female wasps show synchronized emergence, making mate finding relatively easy (Quicke 1997). For solitary wasps, however, it must be a considerable challenge to find suitable mates due to the more widely dispersed emergence sites. Based on these differences in life history, a possible prediction would be that the quantity and volatility of the sex pheromones may be different between solitary and gregarious parasitoids. However, the situation is even more complex in the gregarious parasitoid *C. glomerata*, as inbreeding and outbreeding

both occur frequently in this species. It is known that a part of female wasps leave the natal patch without mating with male siblings, and those female wasps probably mate with male wasps from other cocoon clusters (Gu & Dorn 2003; Tagawa & Kitano 1981). Therefore, they too should rely on relatively long range cues to find each other. The hosts and host-damaged plants might provide such cues.

Several studies have shown that volatiles originating from the host or the host frass can act as sex kairomones (benefiting the parasitoids, but harmful to the hosts in this case), favouring mate location (Metzger *et al.* 2010; Reddy *et al.* 2002; Steiner *et al.* 2007). Herbivore-induced plant volatiles (HIPVs) have almost exclusively been studied in the context of host location, and their potential role in mate-finding strategies has rarely been discussed. Yet, a number of studies have found that plant volatiles (either from host-induced plants, from fruits or even from intact plants) are also attractive to male parasitoids (Benelli & Canale 2013; Kawaguchi & Tanaka 1999; McAuslane *et al.* 1990; Ngumbi & Fadamiro 2012; Stelinski & Liburd 2005). It has been suggested that this attraction can help the male wasps in their efforts to find female wasps. For example, male *Cotesia plutellae* more easily locate female *C. plutellae* when female *C. plutellae* are presented together with an intact cabbage leaf or a host-damaged cabbage plant, but, as yet, the relative importance of plant volatiles and of sex pheromones in this attraction has not been tested separately (Kawaguchi & Tanaka 1999). In addition, male *Campoletis sonorensis* have been shown to locate female *C. sonorensis* more efficiently when a leaf of the host's food plant is presented together with the female *C. sonorensis* (McAuslane *et al.* 1990). Host-damaged plants are more attractive than intact plants to female and male *Apoanagyrus lopezi*, a parasitoid of mealybugs (van Dijken *et al.* 1989). In several *Microplitis* species, the male and female wasps are both responsive to HIPVs in behavioural or electrophysiological tests (Chen & Fadamiro 2007; Li *et al.* 1992; Ngumbi & Fadamiro 2012; Yu *et al.* 2010). For *Cotesia marginiventris*, both male and female wasps were attracted to certain green leaf volatiles and HIPVs (Ngumbi & Fadamiro 2012). None of these previous studies tested for potential additive or synergistic effects of the combined use of both sex pheromones and HIPVs in mate location strategies of parasitoids.

In the current study, we used four braconid wasps, the gregarious parasitoid *C. glomerata*, the solitary parasitoids *C. marginiventris*, *Microplitis rufiventris* and *Microplitis mediator* to test the respective importance of sex pheromones and HIPVs for mate finding. Parasitoid olfactory preferences were assessed in six-arm and four-arm olfactometer bioassays. The four parasitoids are common natural enemies of important lepidopteran pests, and a good understanding of their foraging

behaviour may help to optimize their use as biological control agents. The plant and insect species involved in each of the four tritrophic systems studied are listed in Table 1.

MATERIALS AND METHODS

Insects

Four parasitoids and their respective hosts were used to test the role of HIPVs and pheromones in parasitoid attraction (Table 1). The four endoparasitoids were reared in our laboratory at the University of Neuchatel, Switzerland. The two *Cotesia* species were reared, following the protocol described by Xu *et al.* (2014). The parasitoid *M. mediator* was reared on first instar caterpillars of cabbage moth *Mamestra brassicae*, which were fed on Chinese cabbage. *M. brassicae* eggs were provided by Forschungsinstitut für biologischen Landbau, Frick, Switzerland. The parasitoid *M. rufiventris* was reared on *Spodoptera littoralis* caterpillars (first instar) kept in square plastic boxes (15 × 13 × 5 cm) and fed with a wheat germ-based artificial diet. The eggs of *S. littoralis* were provided by Syngenta, Stein, Switzerland. In order to get virgin wasps, each parasitoid cocoon was placed in a 1.5 mL of centrifuge tube until the wasp emerged. Then, female virgins and male virgins were kept separately in two Bugdorm-1 cages (30 × 30 × 30 cm, Mega View Science Education Services Co. Ltd, Taiwan). In order to obtain mated wasps, about 50 female and 50 male wasps of the same parasitoid species were put into the same Bugdorm-1 cage. The wasps were provided with honey and moist cotton wool, and the cages were stored in an incubator at 25 °C (LD 16: 8 h) for about 3 d before each test.

Bioassays

The bioassays were carried out in four-arm and six-arm olfactometers described in previous studies (D'Alessandro & Turlings 2005; Turlings *et al.* 2004). Each olfactometer arm had an airflow of 0.6 L min⁻¹ that entered the central release chamber (Fig. 1). The wasps were released in groups of six into an olfactometer. They were allowed to make a choice among treatments for a maximum of 30 min (Xu *et al.* 2014). At the end of this period or as soon as all six wasps had made a choice, the result was recorded, the tested wasps were removed, and a new group of six naive wasps was released. Each replicate included four releases of six wasps. In most cases, we did two replicates on a given day. Each experiment consisted of six replicates (144 wasps in total). Glassware was cleaned, and the positions of the treatments in the olfactometer were changed randomly for each replicate.

Table 1. The four tritrophic systems used in this study

Trophic level	System 1	System 2	System 3	System 4
3rd	<i>C. glomerata</i>	<i>C. marginiventris</i>	<i>M. rufiventris</i>	<i>M. mediator</i>
2nd	<i>P. brassicae</i>	<i>S. littoralis</i>	<i>S. littoralis</i>	<i>M. brassicae</i>
1st	<i>B. rapa</i>	<i>Z. mays</i> (variety Delprim)	<i>Z. mays</i> (variety Delprim)	<i>B. rapa</i>

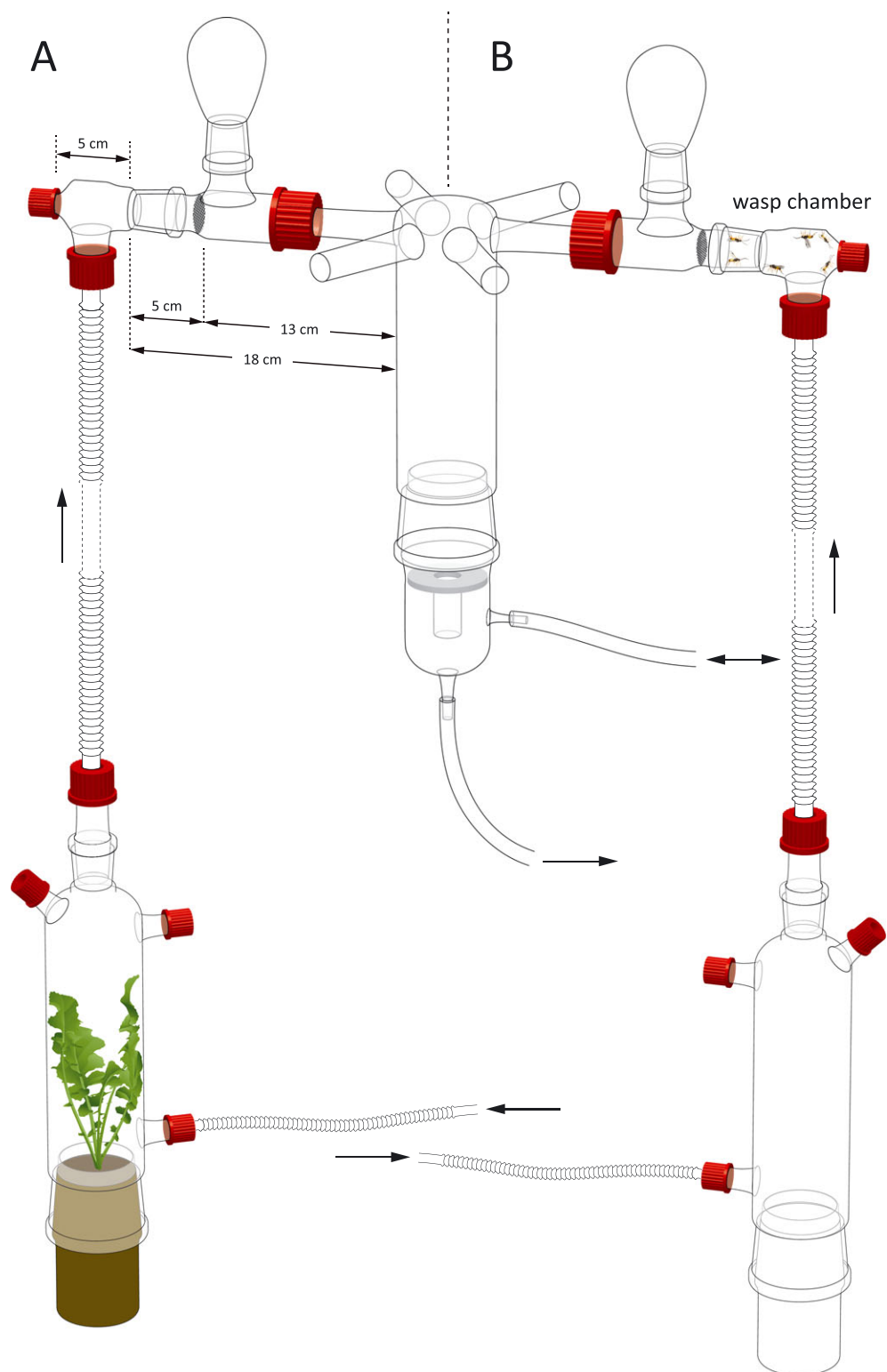


Figure 1. The six-arm olfactometer that was used to test for the attractiveness of plant volatiles (A) and/or sex pheromones (B). To serve as odour sources, plants were placed in glass vessels as indicated and wasps were placed in 'wasp chamber'.

Plants used for the bioassays were 3- to 4-week-old seedlings of wild cabbage *Brassica rapa* and 2-week-old maize *Zea mays* (variety Delprim) grown in plastic tubes (OD = 4 cm, L = 11 cm), kept in a phytotron (25 °C, LD 16: 8 h). These tubes

fitted the size of the glass vessels in the olfactometer systems (Fig. 1A). To induce the release of HIPVs, 20 caterpillars (first instar) were put on their respective host plant seedlings which had been placed into the olfactometer vessels and left feeding

overnight (about 14 h, LD 16: 8 h) before the start of the bioassay. Intact plants were used as the control treatments. Species used in assays were listed in Table 1.

Presence of sex pheromones

To test whether sex pheromones are involved in attraction of the opposite sex of the two *Microplitis* wasps, we used the same six-arm olfactometers as for *Cotesia* parasitoids in a previous study (Xu *et al.* 2014). Six female virgins, six mated female wasps and six male virgins were placed as the three treatments (odour sources) in the olfactometer to test their relative attractiveness to male virgins. Similarly, six male virgins, six mated male wasps and six female virgins were presented as the three treatments in the olfactometer to test their relative attractiveness to female virgins. The groups of six wasps were placed in the three of the arms of the olfactometer as shown in Fig. 1B, and the arms between the three treatments were left empty (Xu *et al.* 2014).

Attractiveness of plant volatiles

To test the attractiveness of plant volatiles, two treatments – a herbivore-damaged plant and an intact plant – were placed in the vessels (Fig. 1A) attached to the opposite arms of a four-arm olfactometer (D'Alessandro & Turlings 2005), and the two other vessels were left empty.

Attractiveness of plant volatiles and sex pheromones in combination

These tests were carried out in a six-arm olfactometer (Turlings *et al.* 2004). Two groups of six male virgins (or mated male wasps) were combined with a herbivore-damaged plant and an intact plant, respectively, to test their attractiveness to female virgins. A herbivore-damaged plant and an intact plant without wasps served as two alternative odour sources, and the two remaining arms were left empty. The wasps (virgin or mated) were placed in the respective arms as shown in Fig. 1B, whereas the plant treatments (herbivore-damaged or intact one) were placed in the odour source vessels (Fig. 1A). In an analogous way, two groups of six virgins (or mated female wasps) were also combined with a herbivore-damaged plant and an intact plant, respectively, to test their attractiveness to male virgins. Two additional treatments were a herbivore-damaged plant and an intact plant, both without wasps, and the two remaining arms were empty. In both experiments, the two empty arms were at opposite positions, and the treatments including the wasps and the treatments including plants treated in the same way were never placed in adjacent arms.

Statistics

Statistical analyses were performed in R 3.0.2 with the package of Lme 4 (Bates 2010). To test whether the differences among

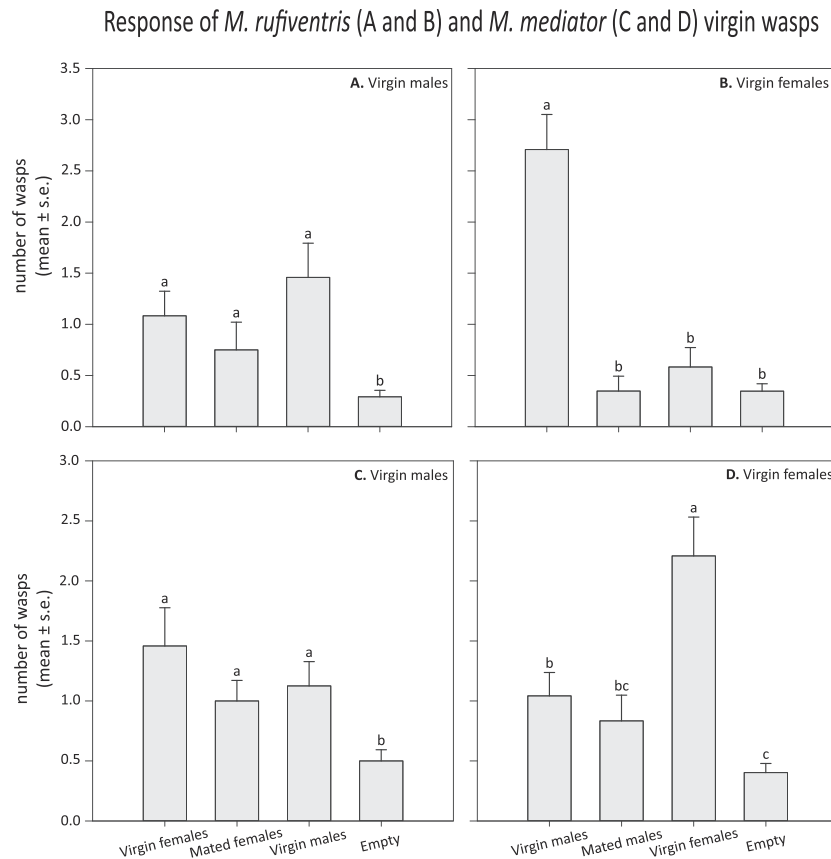


Figure 2. Attraction of virgin *Microplitis rufiventris* (A and B) and *Microplitis mediator* (C and D) to conspecific wasps (virgin or mated).

the responses of the parasitoids to the treatments were significant, we used generalized linear mixed models with Poisson distribution of error. The replicates were treated as the random factor. Tukey's post-hoc test was performed for multiple comparisons (results in Additional file S1). The models were checked with the test of 'overdisp' to estimate the residual deviation of the freedom factor, with considering the possible effects of over-dispersion caused, for instance, by positional biases or wasps affecting each other's responses (Davison & Ricard 2011). Each model was fitted by maximum quasilielihood estimation in the software package R. In the figures, the number of wasps choosing empty arms was divided by the number of empty arms present in the setup to make it comparable to the other treatments. Statistical differences ($P < 0.05$) are indicated with different letters in the bar figures.

RESULTS

For *M. rufiventris*, male virgins were equally attracted to virgin or mated female wasps, as well as to male virgins themselves, but, surprisingly, female virgins were strongly attracted to male virgins only (Fig. 2A,B). For *M. mediator*, we did not find any clear evidence for the use of sex pheromones, but we observed a consistent pattern that female and male virgins were more attracted to the odours of conspecific wasps than to the clean air from the empty arms independent of their sex and mating status (Fig. 2C,D). For both *Cotesia* wasps, it is known that

male wasps are strongly attracted to female virgins, but not to mated female wasps (Xu *et al.* 2014).

The four braconid wasps, irrespective of sex and mating status, were strongly attracted by the HIPVs (Figs 3 & 4). Herbivore-damaged plants were generally much more attractive than intact plants for all parasitoids (Figs 3 & 4), with the exception of mated males of *C. glomerata*, *C. marginiventris* and *M. rufiventris*, which did not discriminate between damaged and intact plants (Figs 3D,H & 4D). Generally, significantly more wasps ended up in arms connected to vessels with intact plants than to arms with empty vessels (Figs 3 & 4).

For the two *Cotesia* species, female virgins were predominantly attracted by herbivore-damaged plants, independently of the presence of virgin or mated male wasps (Fig. 5A,B,E,F). By contrast, male virgins were only strongly attracted by treatments including female virgins, independently of whether they were combined with herbivore-damaged or intact plants (Fig. 5C,G). Male virgin *C. glomerata* were more attracted by herbivore-damaged plant than the combined odours of herbivore-damaged plant and mated female wasps (Fig. 5D), which confirms the intriguing repelled effect of mated female wasps on male wasps (Xu *et al.* 2014). Male virgin *C. marginiventris* were attracted equally by HIPVs and the combined odours of herbivore-damaged plant and mated female wasps (Fig. 5H).

For *M. rufiventris*, the combination of plant odours (either from herbivore-damaged plants or intact plants) and the odour of male virgins was more attractive to the female virgins than

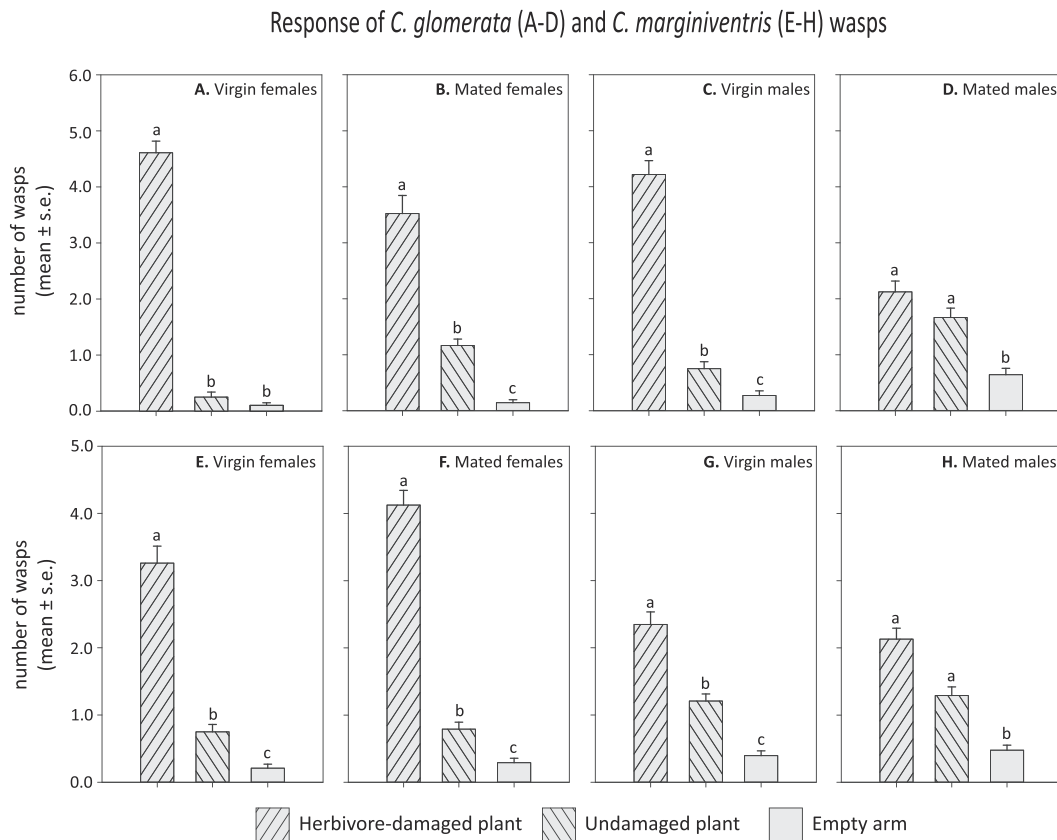


Figure 3. Responses of females (virgin, A and E; or mated, B and F) and males (virgin, C and G; or mated, D and H) of *C. glomerata* (A-D) and *C. marginiventris* (E-H) to the odours of herbivore-damaged plants and undamaged plants.

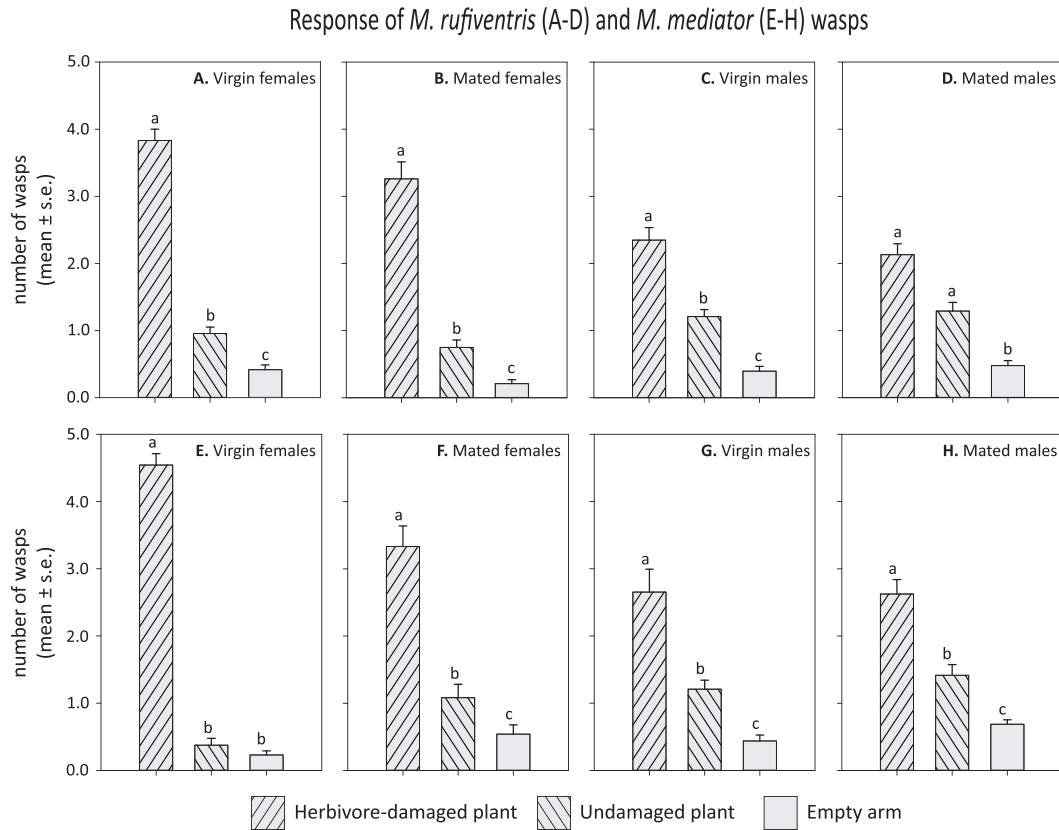


Figure 4. Responses of female (virgin, A and E; or mated, B and F) and male (virgin, C and G; or mated, D and H) *M. rufiventris* (A–D) and *M. mediator* (E–H) to the odours of herbivore-damaged plants and undamaged plants.

respective plant volatiles alone (Fig. 6A). The combination of male virgins and an intact plant was as attractive to the female wasps as the herbivore-damaged plant (Fig. 6A). When exposed to mated male wasps, female virgins predominantly chose the treatments that included the herbivore-damaged plants (Fig. 6B), which suggests that sex pheromones are no longer released by male *M. rufiventris* after mating. Male virgins were strongly attracted by the treatments including HIPVs, no matter whether the female wasps (virgin or mated) were present or not (Fig. 6C,D).

For *M. mediator*, both female and male virgins were strongly attracted by the herbivore-damaged plants independent of the presence or absence of conspecific pheromones (Fig. 6E–H). The male virgins chose the combined odours of intact plant combined with female virgins more than intact plants alone (Fig. 6G). All results are summarized in Table 2.

DISCUSSION

The diverse strategies of mate location in braconid wasps

Mating strategies in parasitoids have received considerable attention, but the respective importance of sex pheromones and plant-produced volatiles for mate location has rarely been examined. In our study, involving four braconid parasitoid species

of leaf-feeding caterpillars, we found that female virgins of the two *Cotesia* species (Xu *et al.* 2014) and male virgins of *M. rufiventris* release sex pheromones that attract conspecific wasps of the opposite sex. Male and female virgins of all four parasitoids were also strongly attracted by herbivore-damaged plants. When sex pheromones and HIPVs were present at the same time, the male *Cotesia* species were predominantly attracted to sex pheromones, irrespective of combining them with odours of herbivore-damaged plants or intact plants (Fig. 5C,G). For *M. rufiventris*, the combination of male virgins and herbivore-damaged or intact plants was more attractive to female wasps than herbivore-damaged or intact plants alone (Fig. 6A), illustrating an additive effect of these two types of cue for this species. This changed when the female virgins were offered odour sources with mated male wasps, in which case they switched their preferences to treatments, including herbivore-damaged plants. This suggests that the mated male wasps stopped releasing their sex pheromone (Fig. 6B). The results also suggest that for *M. mediator*, pheromones are of lesser importance: Both female and male virgins largely chose the treatments including herbivore-damaged plants, irrespective of the additional presence of conspecific wasps (Fig. 6E–H). Thus, HIPVs are likely to be the predominant cues for mate location in this solitary wasp, which was found to not just attract the opposite sex but instead appears to release an aggregation pheromone attractive to both sexes.

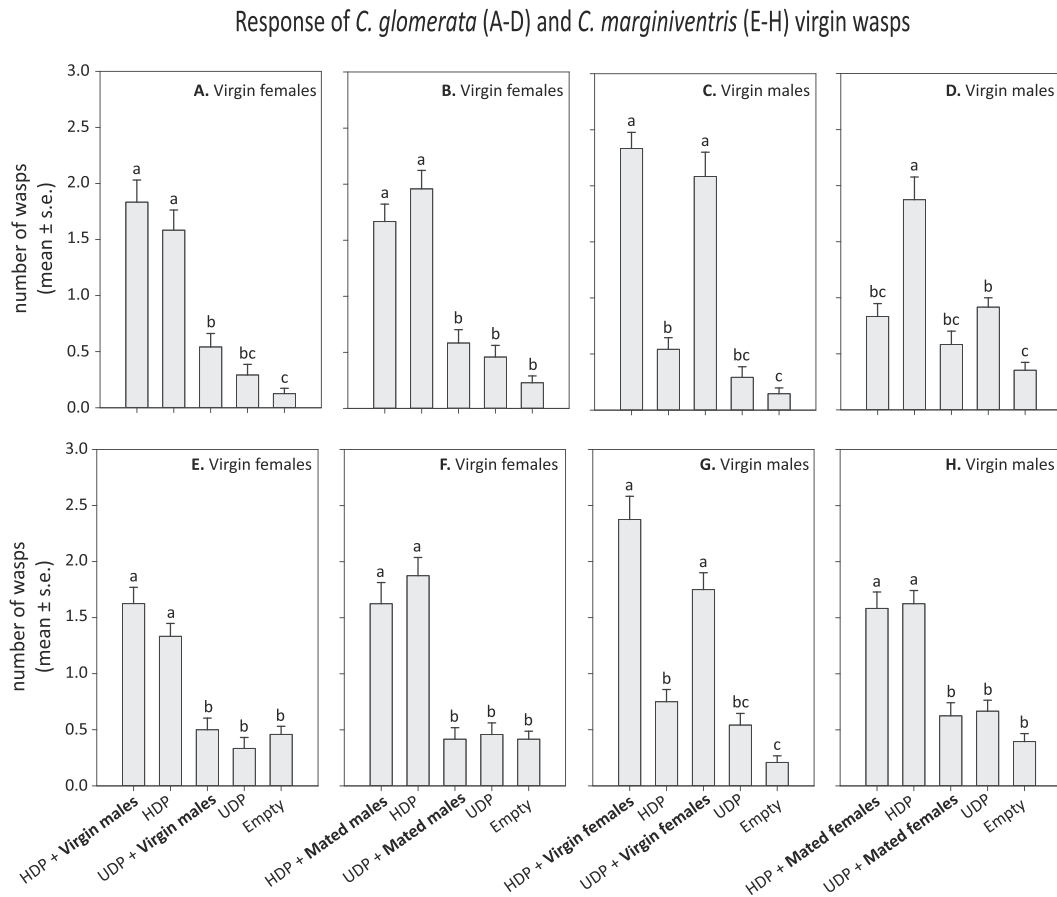


Figure 5. Responses of female virgins (A, B, E, and F) and male virgins (C, D, G, and H) to plant volatiles or combined odours of plants (damaged or not) and potential pheromonal sources (virgin or mated wasps of the opposite sex) by *Cotesia glomerata* (A–D) and *Cotesia marginiventris* (E–H). HDP, herbivore-damaged plant; UDP, undamaged plant.

The male-produced sex pheromone of *M. rufiventris* was not as powerful an attractant as the female sex pheromones in the two *Cotesia* species, whose sex pheromones were more attractive to male wasps than HIPVs alone (Fig. 5C,G). In contrast, the attractiveness of male virgins of *M. rufiventris* increased dramatically when combined with HIPVs, while herbivore-damaged plants alone or the combination of male virgins and intact plants attracted significantly fewer female virgins (Fig. 6A). This implies that *M. rufiventris* relies on both HIPVs and sex pheromones for mate location. Something similar is known for male wasps of the parasitoid *Venturia canescens*, which are considerably more strongly attracted to the combined odours of hosts and female wasps than to the odours of hosts or female wasps alone (Metzger *et al.* 2010).

Male parasitoids are usually able to mate several times during their lifetime (Godfray 1994; Quicke 1997). However, copulating with already mated male wasps can result in male-biased sex ratios of the offspring, possibly as a result of sperm depletion in the male wasps (King & Fischer 2010). In a previous study, we found that mated male *Cotesia* species are less attractive to female wasps (Table 2) (Xu *et al.* 2014). In the current study, the male wasps no longer discriminated between herbivore-damaged and intact plants once they had mated (Figs 3D,H and 4D). This remains unexplained but could mean

that they switch to food foraging in order to restore resources for sperm production. In contrast to male wasps, female wasps generally mate only once during their lifetime (Godfray 1994) and they cease to release their sex pheromone after mating (McClure *et al.* 2007; McNeil & Brodeur 1995; Xu *et al.* 2014). Therefore, the combined use of HIPVs and pheromones (switched on or off by virgin or mated wasps) is probably a reliable strategy for mate location in braconid parasitoids.

Mate and host locations are not two distinct processes

As a consequence of their haplo-diploid sex determination, newly emerged wasps face the dilemma to either search for hosts as soon as possible and producing only haploid male offspring or first search for a mate, after which they would be able to produce both female and male progeny. The latter option has generally been observed to be favoured (Fauvergue *et al.* 2008; Gu & Dorn 2003; Kant *et al.* 2012; Steiner & Ruther 2009). However, we found that both male and female virgins of all species were strongly attracted by HIPVs. Therefore, mating is not a prerequisite for attraction to HIPVs, and HIPVs may not only be indicative of where to find hosts but may also help guiding wasps to potential mates. Attraction towards

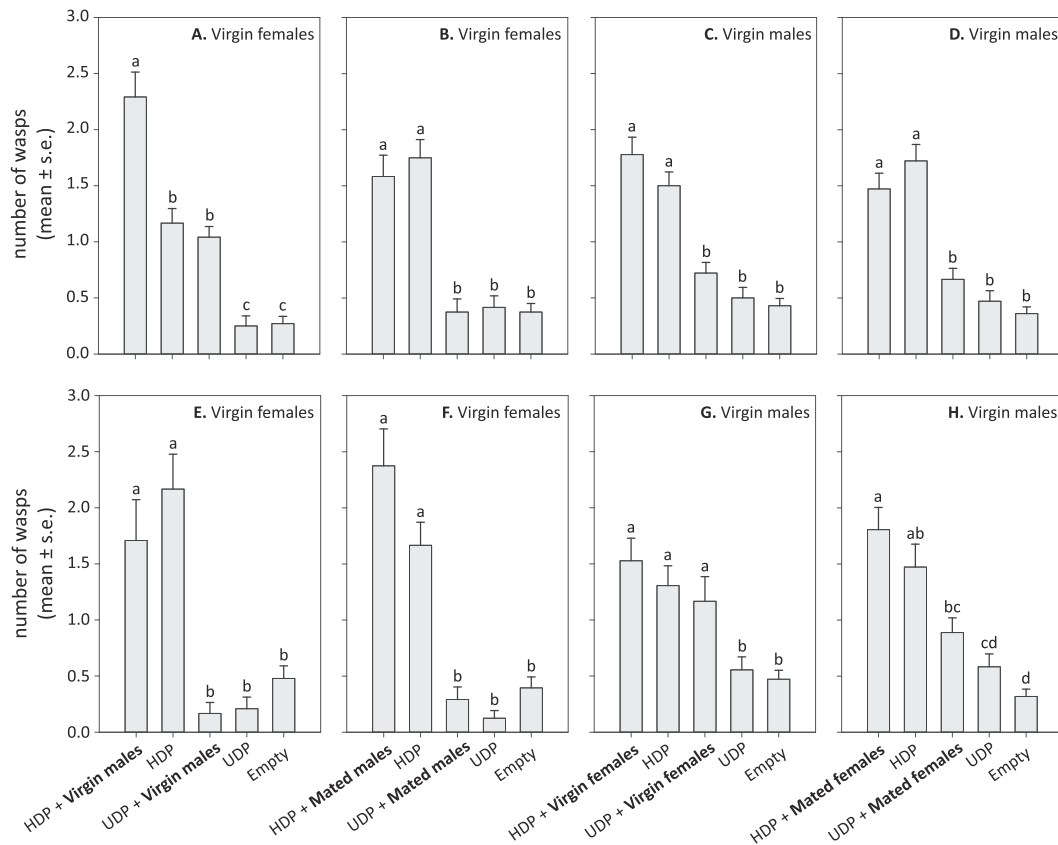
Response of *M. rufiventris* (A-D) and *M. mediator* (E-H) virgin wasps

Figure 6. Responses of female virgins (A, B, E, and F) and male virgins (C, D, G, and H) to plant volatiles or combined odours of plants (damaged or not) and potential pheromonal sources (virgin or mated wasps of the opposite sex) by *M. rufiventris* (A–D) and *M. mediator* (E–H). HDP, herbivore-damaged plant; UDP, undamaged plant.

herbivore-infested plants seemed more pronounced in female virgin *C. glomerata*, *M. rufiventris* and *M. mediator* than in mated individuals (Figs 3A,B and 4A,B,E,F). This implies that herbivore-damaged plants are important mating sites for the wasps. This is also evident from the study on a parasitoid of leaf-miner pests, where both male and female wasps are frequently found together on their host food plant, even if they both emerged at a distance away (Godfray 1994). Fauvergue *et al.* (2008) suggest, based on field observations, that female virgins release their sex pheromone while foraging for hosts. They propose therefore that searching for hosts and for mates are not two exclusive activities, as supported by our results.

HIPVs may serve as long-range cues in mate location by braconid wasps

The synthesis and release of sex pheromones, as well as searching for mates, can be costly for parasitoids (Ruther 2013). Moreover, it has been proposed that the use of sex pheromones by parasitic wasps generally comprises two steps: One attracts the conspecific wasps from a distance and the others involve the subsequent stages of courtship (McClure *et al.* 2007; Quicke 1997). In this context, the use of HIPVs may benefit parasitoids by alleviating the costs of producing a volatile

pheromone for long-range attraction. HIPVs are released in large amounts as a bouquet of semiochemicals with relatively low molecular weight, highly suited for detection at relatively long distances (up to several metres or more; Dicke *et al.* 2009). Once they are in host-damaged plants, short-range visual, wing vibrations and chemical cues may be effective to locate and court the opposite sex (Bredlau *et al.* 2013; Godfray 1994; McAuslane *et al.* 1990; Tagawa & Hidaka 1982). We have evidence that the sex pheromone of female *C. glomerata* is composed of relatively large molecules of low volatility working at a relatively short range (unpublished data). Indeed, their attractiveness to male wasps decreased considerably when female virgins were placed at a longer distance in the bioassay system (Xu *et al.* 2014). The use of HIPVs may therefore be critical to find mates even for this gregarious species because some individuals leave the natal patch without mating with siblings, and thus favour the maintenance of outbreeding in this species (Gu & Dorn 2003; Tagawa & Kitano 1981).

In conclusion, the four braconid wasp species were found to release pheromones, but each in a different way, and virgin individuals of all species were attracted to HIPVs. This implies that gregarious and solitary braconid wasps may rely on pheromones in combination with HIPVs to locate suitable mates. By switching on or off pheromone release, depending on the

Table 2. The responses of four parasitoids of different sexes or mating status to the odours of conspecific wasps, plant volatiles or their combined odours

Parasitoids	<i>C. glomerata</i> ^a				<i>C. marginiventris</i> ^a				<i>M. rufiventris</i>				<i>M. mediator</i>			
	VF	MF	VM	MM	VF	MF	VM	MM	VF	MF	VM	MM	VF	MF	VM	MM
VM	NS		NS		NS		NS		+++		+++		++		++	
MM	-				NS				NS				NS			
VF	NS		+++		NS		+++		NS		+++		+++		+++	
MF			NS				NS				+				+	
HDP	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
UDP	NS	+++	+	+++	++	+	+++	+++	++	+	+++	+++	NS	+	++	++
HDP + VM	+++				+++				+++				+++			
HDP + MM	+++				+++				+++				+++			
HDP + VF			+++				+++				+++				+++	
HDP + MF			NS				+++				+++				+++	
UDP + VM	+				NS				+++				NS			
UDP + MM	NS				NS				NS				NS			
UDP + VF			+++				+++				NS				+++	
UDP + MF			NS				NS				NS				++	

VF, MF, VM and MM refer to virgin females, mated females, virgin males and mated males of each parasitoid species. HDP and UDP refer to the herbivore-damaged and undamaged plants. NS indicates that there is no statistical difference between treatments and empty arms. The symbol '+' or '-' indicates that the responses to the treatments are significantly higher or lower than to empty arms in respective tests (generalized linear mixed models with Tukey's post-hoc test, taking '+' as an example, '+' P 0.05, '+' P 0.01 and '+' P 0.001; details are provided in Additional file S1).

^aThe data showing attraction of *Cotesia glomerata* and *Cotesia marginiventris* to pheromones have been published in a previous study (statistical results are presented in part 2, Additional file S1; Xu *et al.* 2014).

mating status, parasitoids may optimize their foraging efficiency. It will allow female wasps to concentrate their efforts on host location after mating without further harassment by male wasps. We also hypothesize that the HIPVs work at a relatively long range due to their high volatility, while the pheromones are possibly working at a relatively close range and can be expected to be less volatile. The parasitoid *M. rufiventris* was somewhat different in that it responded always better to a combination of pheromones and plant volatiles. Based on our results, we hypothesize that for many parasitoids, host and mate locations are not exclusive processes, and plant odours are important cues for mate location, as has been reported for several phytophagous insects (Landolt & Phillips 1997; Reddy & Guerrero 2004).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Additional file S1. Statistical analyses.