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Chemical interaction between the larva of a dipteran parasitoid and its coleopteran host: A case of exploitation of the communication system during the searching behaviour?

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Abstract

The robber fly *Mallophora ruficauda* is one of the principal apicultural pests in the Pampas region of Argentina. As adults, the flies prey on honey bees and other insects; while, as larvae, they parasitize scarab beetle larvae. Females of *M. ruficauda* lay eggs away from the host in tall grasses. After being dispersed by the wind, larvae drop to the ground, where they dig in search of their hosts. It is known that second instar larvae of *M. ruficauda* exhibit active host searching behaviour towards its preferred host, third instar larva of *Cyclocephala signaticollis*, using host-related chemical cues. Furthermore, previous works show that these chemical cues are produced in the posterior body half of hosts. However, the precise anatomical origin of these cues and whether they mediate any behaviour of *C. signaticollis* larvae remains yet unknown. In order to determine the precise origin of the chemical cue, we carried out olfactometer assays with different stimuli of extracts of the posterior *C. signaticollis* body half. Additionally, we tested whether *C. signaticollis* is attracted to any of the same extracts as in the previous experiments. We found that both second instar of *M. ruficauda* and third instar of *C. signaticollis* are attracted to extracts of the fermentation chamber (proctodeum). This is the first report of attraction of conspecific larvae in scarab beetles. We discuss a possible case of system communication exploitation in an immature parasitoid-host system.

Keywords: host location, parasitoid, Asilidae, Scarabaeidae, infochemicals

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Introduction

Chemical compounds play an important role in life of organisms. They are involved in almost every behaviour and physiological responses related to the location of resources as food, mates and oviposition sites (Dicke & Sabelis, 1988; Vet & Dicke, 1992; Vet, 1999; Dicke & Grostal, 2001). The infochemicals are a particular group of chemical compounds that convey information between individuals, which are involved in interactions among individuals of the same (pheromones) or different species (allelochemicals) (Dicke & Sabelis, 1988). Allelochemicals are very important cues used by predators and parasitoids to locate preys in a complex context (Vet &

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Dicke, 1992; Godfray, 1994; Stowe *et al*., 1995; Bottrell & Barbosa, 1998). According to the sources, they are produced directly by prey or indirectly mainly by host plants of herbivorous preys and products derived from prey activities (Lewis & Martin, 1990; Vet & Dicke, 1992; Godfray, 1994; Stowe *et al*., 1995; Vet *et al*., 1995; Bottrell & Barbosa, 1998; De Moraes *et al*., 2000; Steidle & van Loon, 2003). Products of prey activities are weak signals rather than host plants of prey, but they are the most reliable source of allelochemicals that can inform to predators on the presence, identity, density, availability and suitability of the prey (Vet *et al*., 1991; Vet & Dicke, 1992; Stowe *et al*., 1995). For the allelochemicals produced directly from the prey, several sources have been identified: faeces, cuticle, exuviae, honeydew, body scales, hemolymph or body secretions (Vet *et al*., 1991; Vet & Dicke, 1992; Stowe *et al*., 1995).

Pheromones serve as good indicators of the presence of an individual of a species and are involved in behaviours such as aggregation, mate or host location (Dicke & Sabelis, 1988; Stowe *et al*., 1995; Wertheim, 2005; Wertheim *et al*., 2005). Since pheromones mediate the communication between conspecifics, they might be an important source of information for predators and parasitoids that can benefit from exploiting this communication system (Aldrich, 1995; Stowe *et al*., 1995; Wertheim, 2005; Wertheim *et al*., 2005).

Within dipteran parasitoids, pheromones are mainly used as cues in location of hosts (Aldrich, 1995; Stowe *et al*., 1995; Feener Jr & Brown, 1997; Stireman III *et al*., 2006). Moreover, several egg, larval and pupal parasitoids in this group actually use pheromones produced by adults to locate the immature host stages. This strategy is a solution to the reliability-detectability problem, called the 'infochemical detour' (Vet & Dicke, 1992; Wiskerke *et al*., 1993). Particularly, this searching strategy is relevant in those dipteran parasitoids that have a split host location strategy with an active larval stage performing the final location and parasitism of the host (Eggleton & Belshaw, 1992, 1993; Godfray, 1994; Feener Jr & Brown, 1997; Brodeur & Boivin, 2004). Parasitoids with this host location strategy must use reliable cues, such as pheromones, to find them efficiently given their mobility and the potential time-limitation (Brodeur & Boivin, 2004). The use of host-reliable cues enhances the efficiency in host finding and consequently increases the fitness on time-limited parasitoids (Vet *et al*., 1991; Wajnberg *et al*., 2006). However, there are few studies dealing specifically with the origin of the pheromones used as cues by the active larval stage in the host-seeking behaviour (Coulibaly & Fanti, 1992). One of the possible sources of the production is the tissue or the cells that are involved in the production of aggregation or sexual pheromones (Leal, 1998; Tillman *et al*., 1999; Ma & Ramaswamy, 2003; Wyatt, 2003). There is much variability in the anatomic location of this tissue, but the abdomen appears to be the most common location for Blattodea, Coleoptera and Lepidoptera (Leal, 1998; Tillman *et al*., 1999; Ma & Ramaswamy, 2003). The other source of pheromone production is the microorganisms that live in virtually every insect (Hoyt *et al*., 1971; Byers & Wood, 1981; Dicke, 1988). There are microorganisms that are strictly dependent on their hosts and others that can live freely. The location of these microorganisms in the host varies with the species it is associated with (Hoyt *et al*., 1971; Byers & Wood, 1981; Dicke, 1988).

Mallophora ruficauda Wiedemann (Diptera: Asilidae) is a robber fly endemic to the Pampas region of Argentina that inhabits open grasslands near bee farms (Rabinovich & Corley, 1997). As an adult, *M. ruficauda* feeds mainly on foraging honeybees and other flying insects; and, as larva, is an ectoparasitoid of the third instar larvae of *Cyclocephala signaticollis* Burmeister (Coleoptera: Scarabaeidae), which are commonly known as white grubs. Females oviposit on tall grasses or artificial supports, such as wire fences, laying egg-clutches covered by albumin (Copello, 1922; Castelo & Corley, 2004; Castelo *et al*., 2006). After hatching, larvae are dispersed by the wind, falling to the ground, where they start to dig searching for their host (Castelo & Capurro, 2000; Castelo *et al*., 2006). Particularly, it is the second instar larva of *M. ruficauda* that performs an active searching of the hosts (Crespo & Castelo, 2008). According to the biology of the hosts, females of *C. signaticollis* lay isolated eggs in the soil, walking some distance after each oviposition (López *et al*., 1994). After hatching, first instar larvae feed on organic material; and, in the next stadium, they feed on turfgrass and roots of a great variety of plants, consuming a lot of vegetable food (Alvarado, 1980). To find the plants, beetle larvae have to move into the soil and, when the temperature is stable, tend to remain in the upper root zone (Villani & Wright, 1990). During winter, a seasonal pattern of vertical movement apparently associated with soil temperature has been documented in several species of scarab grubs (Villani & Wright, 1990).

Previous works have demonstrated that the sources of the infochemicals involved in this system are associated with the digestive tube of the third instar larva of *C. signaticollis* (Castelo & Lazzari, 2004; Crespo & Castelo, 2008). Nevertheless, the precise anatomic location where these infochemicals are produced is unknown. It is also unknown whether these infochemicals mediate any behaviour of *C. signaticollis* larvae. Previous studies indicate that there are two possible anatomical locations where the allelochemicals might be present: glandular tissues or symbiotic microorganisms inside the digestive tube. According to the morphology and histology of the digestive tube, both hypotheses are valid (Hoyt *et al*., 1971; Bauchop & Clarke, 1975; Byers & Wood, 1981; López-Guerrero & Morón, 1990; Cazemier *et al*., 1997; Egert *et al*., 2005).

In the present work, we study some aspects of the chemical ecology of the host-parasitoid system composed by *C. signaticollis* (the host) and *M. ruficauda* (the parasitoid). The aims of this work were to determine: (i) which part of the posterior intestine of *C. signaticollis* is associated to the attractive chemicals for *M. ruficauda* larvae, and (ii) if the chemicals attractive to *M. ruficauda* larvae mediate behaviours in *C. signaticollis* larvae. For this study, we analyze, by means of behavioural experiments, how the display of different stimuli extracted from body parts of the host affects differentially the orientation response of *M. ruficauda* larvae. We also examine the orientation response of white grub individuals using the same stimuli that were used with *M. ruficauda*. We expect that *M. ruficauda* use host infochemicals mediating conspecific interaction between individuals of *C. signaticollis* as a cue for finding them, when both species show an orientation response towards the same stimulus extract.

Materials and methods

In order to determine both the anatomic production site and whether the infochemicals that mediate the orientation behaviour of *M. ruficauda* larvae also mediate any behaviour

of *C. signaticollis* larvae, we used second-instar larvae of *M. ruficauda* and third-instar larvae of *C. signaticollis* in binary choice tests using different *C. signaticollis* stimuli.

Experimental conditions

Experiments on *M. ruficauda* were conducted during January–March 2009 under laboratory conditions (25.7 ± 1.6°C, 60.0 ± 5% RH), in days with atmospheric pressure between 1012 and 1020 mbar. For the *C. signaticollis* experiments, the tests were made in July–August 2008 under laboratory conditions (22.5 ± 1.3°C, 63.0 ± 15% RH) under atmospheric pressure values between 1005 and 1024 mbar.

Since environmental conditions influence behaviour of insects (Roitberg *et al.*, 1993; Amat *et al.*, 2006), pressure and temperature ranges under which the experiments with *M. ruficauda* and *C. signaticollis* larvae were performed were those in which insects had shown an orientation behaviour in previous experiments (Castelo & Lazzari, 2004; Crespo & Castelo, 2008; Crespo, 2011). In order to keep the experimental conditions similar to natural conditions, all experiments were carried out in darkness, because both insect species in these instars live underground.

In order to guarantee the occurrence of behavioural responses, we used experimental extracts of the host, equivalent to 2.5 white grubs ml^{-1} hexane, for experiments with *M. ruficauda* larvae, which is more than double the concentration used by Castelo & Lazzari (2004). For experiments with *C. signaticollis* larvae, we used experimental extracts of one white grub ml^{-1} hexane to ensure behavioural responses of individuals (Castelo, 2003).

Insects

Larvae of *M. ruficauda* were reared in the laboratory from egg-clusters collected in January–March 2009 on grasslands in Pilar (34°28′S, 58°55′W) and Moreno (34°46′S, 58°93′W), two localities with apiculture activity, in Buenos Aires province, Argentina. In the field, egg-clusters were carefully cut off from their support and were kept individually in Falcon-type tubes until larvae were hatched. In the laboratory, after hatching, the neonate larvae were separated individually in Eppendorf-type tubes with a moistened piece of filter paper as substrate, to keep humidity inside the tube at 100%. Tubes were stored in darkness and at room temperature in the laboratory between 18.6–29.8°C. When the larvae reached the second instar and were 22 to 25 days old, they were used to perform the behavioural experiments.

Scarab larvae were collected at soil depth of 0.30 m in grasslands of Pilar, Mercedes (34°40′S, 59°26′W) and Nuñez (34°32′S, 56°26′W) localities, in Buenos Aires province, Argentina, from May to August 2008. Third-instar larvae of *C. signaticollis* were identified using the taxonomic key of Alvarado (1980), which is based on the morphology of the raster. *Cyclocephala signaticollis* individuals were maintained individually in the laboratory at room temperature (18.6–29.8°C) in black tubes (30 ml) filled with soil and were fed weekly with pieces of fresh carrots.

Extraction of C. signaticollis *stimuli*

Host stimuli used along the experiments were obtained from different body portions of third instar larvae of *C. signaticollis*, following the protocols used by Castelo & Lazzari (2004) and Crespo & Castelo (2008). Immediately after collection, larvae of *C. signaticollis* were dissected in several parts, and each body portion was homogenized using hexane as solvent, obtaining an extract with the host infochemicals. A list of stimuli extracts tested in experiments with *M. ruficauda* and *C. signaticollis* individuals used as experimental individuals are shown in fig. 1. Each type of extract was made only once, and a fraction of the same vial was offered to both insects in the experiments in due time. To determine whether the infochemicals used by *M. ruficauda* in the orientation to *C. signaticollis* individuals mediate any behaviour in the host, we tested the same body portions utilized in Castelo & Lazzari (2004) but with the host as experimental individual: anterior body half (AB), posterior body half (PB), posterior body wall (cuticle) (PC), posterior half of the digestive tube (PDT), faeces (F).

Castelo & Lazzari (2004) determined that the origin of the chemical cues linked to the orientation behaviour of *M. ruficauda* is in the posterior half of the digestive tube of the host. In order to find the specific structure that produces the infochemicals, host extracts were made by dividing the last part of the digestive tube in three portions: posterior mesenteron (M), fermentation chamber (FC) and colon (C). Also, in other experimental series, the content of the posterior digestive tube was separated from the epithelium, to determine if the cue is produced by the gut tissues of the fermentation chamber (López-Guerrero & Morón, 1990) or by the presence of symbionts in the tract (Chapman, 1998). We used two protocols to carry out the extraction of the stimuli of both parts of the gut. In the first protocol, the content of the digestive tube was separated from the epithelium, and the content was homogenized using hexane as solvent. Then, the tissue was washed with distilled water and then homogenized with hexane. For the second protocol, the epithelium was treated as previously, but the chemical cues present in the content of the fermentation chamber were obtained by a solvent extraction using a separating funnel. This technique allowed us to separate the chemical cues from the whole content of the fermentation chamber, dissolving their content in two immiscible liquids (hexane-water). Due to being nonpolar compounds (Castelo & Lazzari, 2004), these substances were extracted in a nonpolar solvent fraction (hexane).

Responses of individuals to host/conspecific stimuli

Experiments to determine the behavioural responses of the insects were performed using similar experimental arenas as in Castelo & Lazzari (2004). We divided the arenas into three equally sized zones (one middle and two laterals) along the long axis. On each lateral zone of the arena, a piece of filter paper impregnated with a volume of either the stimulus or the control extract was placed. At the beginning of each trial, an individual was released at the centre of the arena and allowed to move freely. After a time of experimentation, its position in the arena was recorded. In this way, three possible responses could be obtained: choice for the stimulus (S), for the control (C) or no decision (ND) if the individual remained in the middle zone. After every trial, each individual was discarded and the arena was cleaned with soap and water, and then dried with an air current in order to eliminate possible larval odours. Experimental design and number of replicates for each experiment is detailed in table 1.

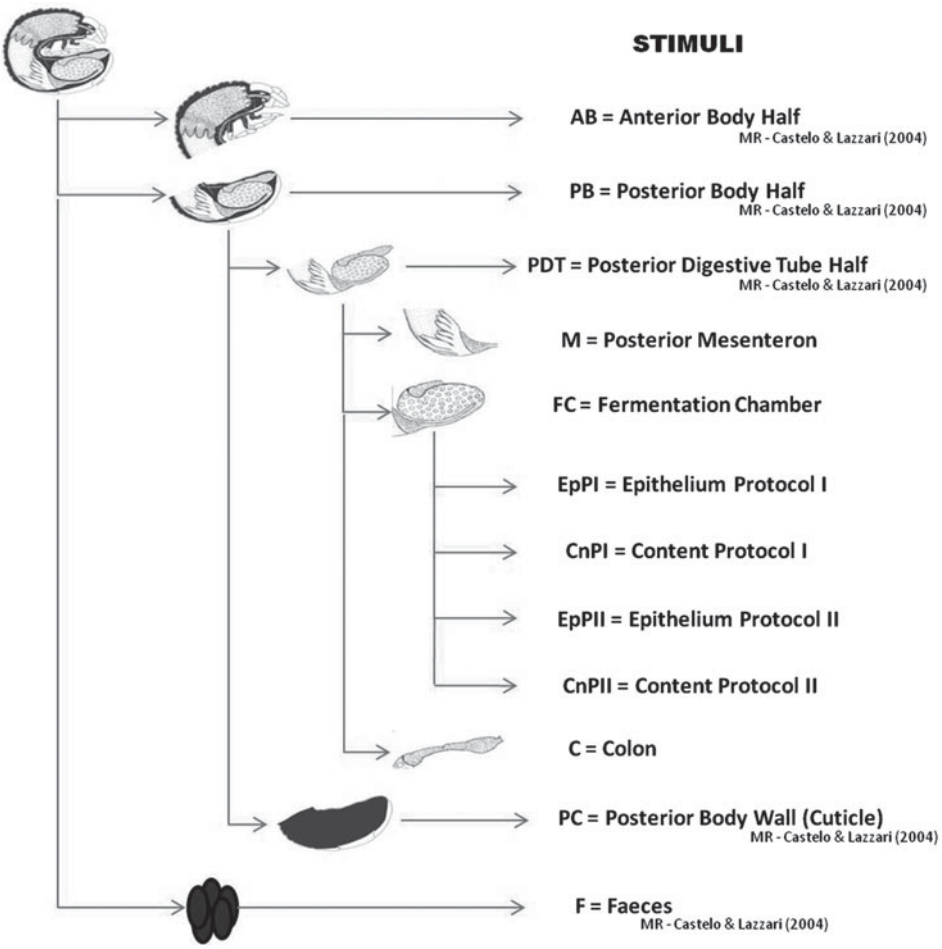

Fig. 1. Regions of the body of *C. signaticollis* larvae from which extracts were used in behavioural assays throughout the experiments. MR – Castelo & Lazzari (2004) indicates previous studies where some of these extracts were tested on *M. ruficauda* larvae.

Responses of M. ruficauda *to host stimuli*

Behavioural experiments with *M. ruficauda* were carried on in an arena of 9 × 6 × 1 cm using a piece of filter paper of 1 × 2 cm impregnated with 10 µl of either the stimulus or the control extract. In each trial, an individual larva was released as experimental individual at the centre of the arena, and after 90 min of experimentation, its position in the arena was recorded (table 1).

Responses of C. signaticollis *to conspecific stimuli*

For behavioural experiments with *C. signaticollis*, we carried out trials with an arena of 13 × 8 × 2 cm. In each lateral side of the experimental arena, a filter paper of 2 × 3 cm impregnated with 40 µl of stimulus or control extract was presented. An individual larva was released at the centre of the arena in each trial as experimental individual; and, after 45 min of experimentation, its position in the arena was recorded (table 1).

Statistical analysis

In the experiments, we tested the influence of *C. signaticollis* stimuli on the orientation behaviour of both *M. ruficauda* larvae and *C. signaticollis* larvae. In both orientation experiments, preference of insects for either side of the experimental arena (stimulus or control) was tested against a random distribution by means of χ^2 tests of goodness of fit (one-way contingency table analysis: Sokal & Rohlf, 1969; Zar, 1984; Rosner, 1995). Individuals that remained in the middle zone of the arena (no decision response) were excluded from the analysis.

Results

Responses of M. ruficauda *to host stimuli*

When second instar larvae of *M. ruficauda* were exposed to *C. signaticollis* third instar larvae odours, experiments revealed that the infochemicals that evoke the positive orientation behaviour of *M. ruficauda* toward the host are associated to the fermentation chamber (table 1, fig. 2). However, larvae distributed at random in the experimental arena when they were exposed to extract of both epithelium and content of the fermentation chamber of its host treated with any of both protocols (table 1, fig. 3). These results did not allow us to determine the precise biosynthesis origin of the infochemicals used by the larvae of *M. ruficauda* during the host-seeking behaviour.

Table 1. Olfactometer experiments carried out to evaluate the response of *M. ruficauda* and *C. signaticollis* larvae to odours from different parts of the body of third instar *C. signaticollis* larvae.

MR, *M. ruficauda*; CS, *C. signaticollis*; AB, anterior body half; PB, posterior body half; PDT, posterior digestive tube half; M, posterior mesenteron; FC, fermentation chamber; EpPI, epithelium protocol I; CnPI, content protocol I; EpPII, epithelium protocol II; CnPII, content protocol I; C, colon; PC, posterior body wall (cuticle); F, faeces; H, hexane (control). Numbers show the replicates for experiments with MR and CS larvae. Between brackets, the total number of individuals that made a choice (left: stimulus; right: solvent) in the experimental arena.

Experiment (stimulus – control)	MR	CS	Description	MR χ^2 ; P	CS χ^2 ; P
AB – H	–	54 (25–21)	Anterior body half extract.	–	0.35 ; >0.5
PB – H	–	55 (33–18)	Posterior body half extract.	–	4.41 ; <0.05
PDT – H	–	54 (34–15)	Posterior digestive tube half extract.	–	7.37 ; <0.01
M – H	100 (37–33)	70 (31–20)	Posterior mesenteron extract.	0.23 ; >0.5	2.37 ; >0.1
FC – H	100 (51–29)	70 (36–20)	Fermentation chamber extract.	6.05 ; <0.025	4.57 ; <0.05
EpPI – H	200 (67–74)	64 (25–22)	Epithelium of FC extract (protocol I).	0.35 ; >0.5	0.19 ; >0.5
CnPI – H	200 (61–59)	64 (28–26)	Content of FC extract (protocol I).	0.03 ; >0.75	0.07 ; >0.75
EpPII – H	200 (58–68)	64 (27–19)	Epithelium of FC extract (protocol II).	0.79 ; >0.25	1.39 ; >0.1
CnPII – H	200 (63–57)	64 (25–20)	Content of FC extract (protocol II).	0.3 ; >0.5	0.56 ; >0.25
C – H	150 (56–43)	70 (33–24)	Colon extract.	1.71 ; >0.1	1.42 ; >0.1
PC – H	–	54 (27–24)	Posterior body wall (cuticle) extract.	–	0.18 ; >0.5
F – H	–	54 (22–25)	Faeces extract.	–	0.19 ; >0.5

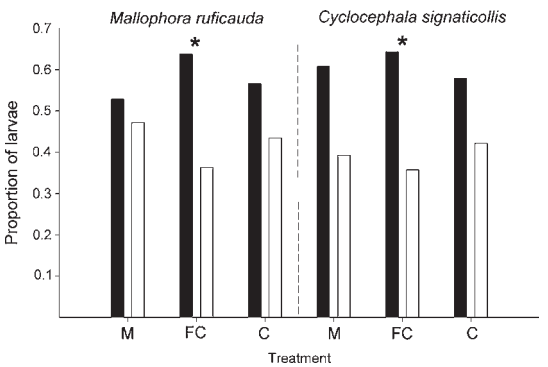

Fig. 2. Response of *M. ruficauda* and *C. signaticollis* to stimuli from three regions of the posterior digestive tube of third instar larvae of *C. signaticollis*. Asterisks denote statistically significant differences (χ^2, $P<0.05$). M, posterior mesenteron; FC, fermentation chamber; C, colon (■, Stimulus; □, Control).

Responses of C. signaticollis *to conspecific stimuli*

We found that *C. signaticollis* larvae showed a positive orientation response towards the extract of the posterior body half of conspecifics, particularly towards of the posterior digestive tube half (table 1, fig. 4). These portions of the host body are the same as those that induced the positive orientation behaviour of the larvae of *M. ruficauda* demonstrated by Castelo & Lazzari (2004).

When we analyzed the orientation behaviour of *C. signaticollis* to extracts of the three morphological portions of the posterior digestive tube half of conspecifics (posterior mesenteron, fermentation chamber and colon), experiments showed that *C. signaticollis* orientated positively to the extract of fermentation chamber (table 1, fig. 2).

Finally, we found that *C. signaticollis* larvae distributed at random in the experimental arena when stimulated with extract of both epithelium and content of the fermentation chamber of conspecifics (table 1, fig. 3). These experiment suggest that the extracts lose their biological activity when we divided the fermentation chamber into content and epithelium.

Therefore, these results suggest that the attraction of *M. ruficauda* and *C. signaticollis* larvae to the same extracts of the body part of *C. signaticollis* is due to the utilization of the same cues in two different contexts: the location of host for the parasitoid and conspecific interaction between *C. signaticollis* individuals.

Discussion

In the present work, we determined which part of the posterior intestine of *C. signaticollis* has the attractive chemicals used by *M. ruficauda* to orientate to its host. Our results show that infochemicals eliciting the orientation behaviour of *M. ruficauda* and *C. signaticollis* larvae are associated with the fermentation chamber but not with the colon or the mesenteron. This result is in agreement with the result found in the study by Castelo & Lazzari (2004), where it was concluded that the origin of the chemical cues involved in the host-seeking behaviour are associated with the posterior digestive tube half. It has been shown that Coleopteran and Dipteran immature parasitoids exhibit a searching behaviour modulated by cues released by their hosts (Wright & Müller, 1989; Godfray, 1994; Feener Jr & Brown, 1997; Brodeur & Boivin, 2004). For *M. ruficauda*, larvae whose entire lifespan is spent underground in a very complex chemical environment, it could be expected that infochemicals triggering the host-seeking behaviour are produced directly by the host.

Regarding the orientation behaviour of *C. signaticollis* to odours from conspecifics, we found that a positive orientation towards the odour fermentation chamber exists. Moreover, this positive orientation was found only to odours from the fermentation chamber. Interestingly, there was no positive response to odours from the colon extract, indicating that these chemicals are not food related volatiles from degradation of metabolites. However, there is a study showing that white grubs, in general, have an aggregated distribution in the field (Castelo & Capurro, 2000). This might indicate that chemicals found in the fermentation chamber could be acting as an aggregation pheromone. The question that arises is how the volatiles in the fermentation chamber get to the outside of the individual. A possibility is that volatiles might be directed somehow towards the cuticle, and reaches the exterior

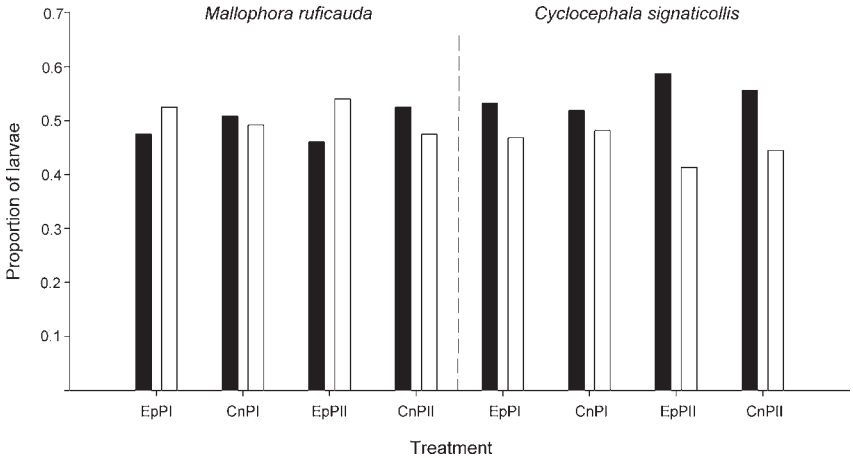

Fig. 3. Response of *M. ruficauda* and *C. signaticollis* to stimuli extracted from two regions of the fermentation chamber of third instar larvae of *C. signaticollis*. EpPI, epithelium protocol I; CnPI, content protocol I; EpPII, epithelium protocol II; CnPII, content protocol II (■, Stimulus; □, Control).

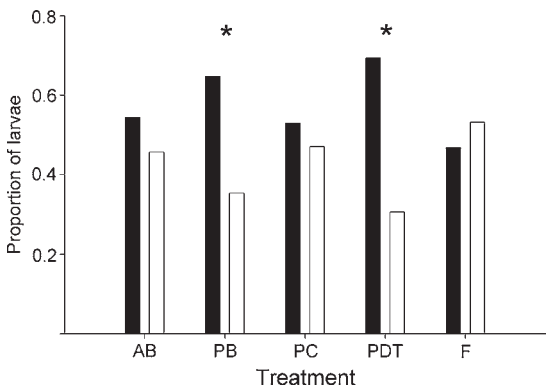

Fig. 4. Response of *C. signaticollis* to stimuli extracted from the different body parts of conspecifics. Asterisks denote statistically significant differences (χ^2, $P<0.05$). AB, anterior body half; PB, posterior body half; PC, posterior body wall (cuticle); PDT, posterior digestive tube half; F, faeces (■, Stimulus; □, Control).

through the tracheal system, as occurs with pheromone gland cells content in others insects (Ma & Ramaswamy, 2003).

This is the first study, to our knowledge, showing active conspecific attraction of scarab beetle larvae by an experimental approach. Nonetheless, there are many reports showing that larvae of insects respond to chemical cues. In those studies, the authors suggested that these chemicals elicited behavioural responses that are indirectly beneficial to the organisms living in groups. Some suggested increases in individual survival, growth or improved development (Ghent, 1960; Stamp & Bowers, 1990; Inouye & Johnson, 2005; Despland & Le Huu, 2006; Jumena *et al.*, 2009). Others proposed an increased efficiency in the exploitation of food or in the defensive ability against natural enemies (Capinera, 1980; Tsubaki & Shiotsu, 1982; Deneubourg *et al.*, 1990; Hunter, 2000; Ruzicka & Zemek, 2008).

Other benefits were proposed to the larval aggregation behaviour. For instance, in the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae), a decrease was recorded in the mating searching time of males after emergence by attraction to cocoon-spinning larvae and to female prepupae allowing them to copulate as soon as the female emerges from the cocoon (Duthie *et al.*, 2003). Species of the genus *Cyclocephala* are univoltine where adults are active only a few weeks per year (Potter, 1981). Hence, it is of extreme importance that both males and females find each other efficiently (Potter, 1981). Therefore, aggregation behaviour between larvae could be expected since more energy could be invested on mating instead of on mate searching, thus increasing their individual fitness.

We also performed experiments to determine whether the infochemicals that attract larvae of *M. ruficauda* are produced either by glands or symbionts from the fermentation chamber. In order to achieve this, we performed two series of experiments, but we were unable to elucidate this. None of the treatments performed (extracts of the content and the epithelium of the fermentation chamber) elicited a response on *M. ruficauda*. The fact that we were unable to obtain a response from this experiment could be indicating a highly volatile chemical cue that was lost during the dissection and manipulation of fermentation chamber and preparation of epithelium and content extracts. Moreover, this fact is indicating that probably both tissues are needed to obtain the attractive cue. There is extensive evidence showing that pheromones are compound blends where a specific proportion of each of them is very important for the blend to have biological activity. Therefore, if the blend composition changes, the biological activity could be lost (Greenfield, 2002). This is probably the reason of loss of activity when we did the extracts. Other possible explanation to the loss of biological activity is the reaction of the immune system of the host to injuries, i.e. the dissection of the fermentation chamber, triggering the synthesis of different compounds that can interact with the infochemical cue modifying their characteristics (Fehlbaum *et al.*, 1994; Bidla *et al.*, 2009). Nevertheless, if this procedure produces injury-based changes on chemicals, the effects would have also been present in the other extracts. Moreover, the insects were killed before performing the dissections, meaning that the immune system could not have produced any injury induced chemicals. Although we were unable to

show where the cue is located, our results indicate that it is possible that a pheromone is involved in conspecific communication of *C. signaticollis* and that the same cue is used by larvae of *M. ruficauda* to locate its host. If this were to be true, then *M. ruficauda* could be exploiting the communication system of its host to locate it.

There are few cases that show that a parasitoid exploits the communication system of its host. This is explained by a reliability-detectability *trade-off* that exists in a complex multitrophic system where very reliable cues have a low delectability decreasing encounters with the host (Vet *et al*., 1991; Vet & Dicke, 1992; Aldrich, 1995; Riba & Blas, 1995; Stowe *et al*., 1995). However, parasitoids such as *M. ruficauda* have a split strategy, where the female would be attracted to less reliable but more detectable cues when laying eggs, whereas the larva seeks and finds the host, orientating to more reliable and specific allelochemicals of the host. This strategy could increase the efficiency of locating a host, augmenting in turn the individual fitness.

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