



Electroantennogram and behavioral responses of *Pteromalus cerealellae* to odor stimuli associated with its host, *Callosobruchus maculatus*

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ABSTRACT

Pteromalus cerealellae (Ashmead) (Hymenoptera: Pteromalidae) is a generalist parasitoid of the larvae of many stored-product insects, including the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), which live endophytically in cowpea seeds. The role of host-related semiochemicals in the host location behavior of this parasitoid was investigated using electroantennogram (EAG) and behavioral (olfactometer) techniques. Responses of mated and unmated female *P. cerealellae* were tested to a variety of host-related chemical stimuli including hexane extracts of uninfested cowpea seeds, (bruchid)-infested cowpea seeds, bruchid larvae (whole body or WB), larval frass, adult female bruchids (WB), and adult male bruchids (WB). All of the tested stimuli elicited significant EAG responses in unmated and mated female *P. cerealellae*, with mated females exhibiting a greater EAG response than unmated females to some treatments. Results from Y-tube olfactometer bioassays demonstrated a significant response of mated female *P. cerealellae* to extracts of uninfested cowpea seeds, infested cowpea seeds, adult female bruchids, bruchid larvae, and larval frass, but no significant response was elicited by the extract of adult male bruchids. Pair-wise comparisons of the four most attractive stimuli (i.e. uninfested seeds, infested seeds, bruchid larvae, and larval frass) in a four-way olfactometer showed preference of mated female *P. cerealellae* for the extract of uninfested cowpea seeds compared to larval frass extract. No significant differences were recorded between other paired treatments. These results suggest the relative importance of volatile cues from seeds (host habitat) in mediating host location by *P. cerealellae*.

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1. Introduction

Parasitoids play a major role in sustainable agriculture through their ability to regulate populations of herbivorous insect pests (Godfray, 1994). Many parasitoid species are associated with insect pests of stored products and thus represent potential biological control agents for pests in storage environments (Brower et al., 1996). Recent interest in the potential use of parasitoids for biological control of stored-grain insects has been stimulated by several factors, in particular pesticide regulation and food safety (Schöller et al., 1997; Germinara et al., 2009). Because the success of parasitoids as biological control agents depends on their ability to find and attack their hosts, research on mechanisms of host location by parasitoids has received major attention in the last three decades (Lewis et al., 1982; Noldus et al., 1991; Vet et al., 1991; Vinson, 1991; Sullivan et al., 2000; Mbata et al., 2004; Germinara et al., 2009).

Most of these studies have implicated semiochemical-mediated host location strategies for several species of parasitoids (Noldus and van Lanteren, 1985; Vinson, 1991; van Huis et al., 1994; Steidle et al., 2003; Mbata et al., 2004; Germinara et al., 2004, 2009). The semiochemicals that mediate host location by parasitoids could originate from host insects (i.e. host-specific semiochemicals), hosts' habitat (environment), or be produced from an interaction between host insects and their habitats (Turlings et al., 1991; Mbata et al., 2004; Germinara et al., 2004, 2009).

Host-specific semiochemicals (kairomones) including host pheromones, host body chemicals (e.g., cuticular hydrocarbons) and volatile stimuli from host feces/or frass may represent reliable host location cues for parasitoids of herbivorous insects (Lewis et al., 1982; Noldus and van Lanteren, 1985; Turlings et al., 1991; Lecomte and Thibout, 1993; Cortesero et al., 1993; Steinberg et al., 1993; Agelopoulos and Keller, 1994; Mbata et al., 2004). However, use of host-specific semiochemicals for host location may be particularly challenging for parasitoids of concealed hosts or those whose hosts live within grains (Hawkins, 1994; Vet et al., 1995). Because they may not be directly exposed to host-specific

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semiochemicals in the initial stages of their host location behavior due to the likelihood of chemical masking, parasitoids of concealed hosts may have to increase the number of information sources available to them in order to locate their hosts (Lewis et al., 1982; van Huis et al., 1994; Hawkins, 1994; Vet et al., 1995). Although, semiochemicals associated with the host habitat are non host-specific and less reliable, they are more easily detectable to parasitoids of concealed hosts and may signal host presence (Norlund et al., 1988; Vet et al., 1991; Vet and Dicke, 1992; Sullivan et al., 2000; Germinara et al., 2004; Kalule and Wright, 2004). Indeed, studies have shown that many parasitoids of concealed hosts use non host-specific kairomonal cues, such as semiochemicals from the host habitat, for host location (Lewis et al., 1982; Vet et al., 1995; van Huis et al., 1994; Phillips, 1997; Germinara et al., 2004). Parasitoids of concealed hosts may also use non-chemical cues such as host vibration signals to locate their hosts (Smirnov and Polejaeff, 1937; Kaschef, 1964).

Pteromalus cerealellae (Ashmead) (Hymenoptera: Pteromalidae) is a generalist ectoparasitoid of mature larvae and prepupae of several insect pests of stored products including *Sitotroga cerealella* Olivier, *Lasioderma serricorne* (F.), *Prostephanus truncatus* (Horn), *Sitophilus* spp., and the cowpea seed beetle, *Callosobruchus maculatus* (F.) (Ashmead, 1902; Brower, 1991; Mbata et al., 2005; Onagbola et al., 2007). Females of *P. cerealellae* lay eggs in these hosts, all of which develop inside seeds of cereal grains (plant hosts). Little is known about the cues used by female *P. cerealellae* to locate their concealed hosts. In a previous study, we observed that female *P. cerealellae* were capable of detecting and parasitizing dead (freeze-killed) and live *C. maculatus* larvae alike (Onagbola and Fadamiro, 2009), suggesting that larval vibrations may not be the principal host location cue used by this species. Being a generalist parasitoid, *P. cerealellae* presumably uses host location cues volatile chemicals from different non-related plant-host complexes, as reported for *Lariophagus distinguendus* (Förster) (Hymenoptera: Pteromalidae) (Steidle et al., 2001).

In the first published report on semiochemical-mediated host location mechanisms in *P. cerealellae*, Mbata et al. (2004) reported significant orientation of mated females to various *C. maculatus* (bruchid) host-related odor stimuli including live and body extracts of virgin female *C. maculatus* and extracts of its oviposition marking pheromone, as well as uninfested and bruchid-infested cowpea seeds. The authors further reported that whole body extracts of virgin female bruchids elicited the strongest response in female *P. cerealellae*. These results suggest the use of host-related semiochemicals for host location by *P. cerealellae*.

The present study was carried out to further investigate the role of host-related semiochemicals in mediating host location by *P. cerealellae* and to determine the relative importance of semiochemicals from first (cowpea seeds) and second (bruchid-specific odors) trophic levels in host location. Based on the results of a preliminary experiment showing that female *P. cerealellae* could not differentiate between uninfested and bruchid-infested cowpea seeds (unpublished data), we hypothesized that chemicals from the host habitat (cowpea seeds) are likely to play a relatively more important role in host location. This paper evaluates the electroantennogram (EAG) and behavioral responses of female *P. cerealellae* to a variety of odor stimuli associated with its host, the cowpea seed beetle, *C. maculatus*. Host-related stimuli tested include uninfested and bruchid-infested cowpea seeds (host habitat), and host-specific odors such as larval frass and whole body extracts of bruchid larvae, adult female bruchids and adult male bruchids. Additional tests are conducted in a four-way (choice) olfactometer to determine odor preference.

2. Materials and methods

2.1. Insects

Pteromalus cerealellae was reared in our laboratory on larvae of the cowpea seed beetle, *Callosobruchus maculatus* (host insect). The host insect was reared on cowpea seeds, *Vigna unguiculata* Walpers (California Black-Eyed variety) in 1-L wide-mouthed Mason glass jars. A fresh culture was started every five days by placing ~25 pairs of 3-day-old mated *C. maculatus* in a glass jar containing ~100 g of cowpea seeds held at 30 ± 1 °C, $70 \pm 5\%$ relative humidity (r.h.), and an illumination cycle of LD 12:12 h (Mbata et al., 2005; Onagbola et al., 2007). Beetles were allowed to lay eggs on the seeds for 24 h after which they were removed with an aspirator. Infested seeds were incubated at the conditions specified above until the larvae had reached the fourth instar, which were then provided to *P. cerealellae* for parasitization. The parasitoid was maintained by transferring ~30 adult pairs into a glass jar containing *C. maculatus*-infested cowpea seeds at a stage when most of the bruchid larvae were in the fourth larval instar (this occurred at ~15 days after infestation of cowpea seeds under our rearing conditions). Jars were held at the environmental conditions stated above for *C. maculatus*. Adult *P. cerealellae* were removed from the jars after 5 days of oviposition. Parasitized host larvae were incubated in a growth chamber at the above environmental conditions until the adult parasitoids started to emerge (~11 days after parasitization at the above conditions). Parasitized hosts (still contained within infested seeds) bearing the wasps' pupae were immediately transferred into 10-cm diameter plastic Petri dishes to collect adult parasitoids immediately after emergence.

2.2. Solvent extraction of chemical stimuli

Extraction of chemical stimuli from *C. maculatus* adults and larvae and from cowpea seeds was conducted using a method similar to that described by Shu et al. (1996, 1999) and Mbata et al. (2004). Three hundred, virgin adult females or males (2–3 days old) or fourth instar larvae of *C. maculatus* were soaked in 6 mL of laboratory grade hexane in a 10-mL glass vial for 2 h. To obtain fourth instar larvae of *C. maculatus* for extraction, infested cowpea seeds containing fourth instar larvae (Mbata et al., 2005; Onagbola et al., 2007) of *C. maculatus* were carefully cracked open with a fine knife. The extraction process was repeated 10 times for a total of 3000 adult females, males or larvae. The extracts were stockpiled in glass vials and concentrated under nitrogen to obtain a concentration of ~1 insect equivalent per 0.2 µL (5 insects per µL of extract). Similar procedures were used to extract uninfested and bruchid-infested cowpea seeds and larval frass. Approximately 5 g of uninfested insecticide-free cowpea seeds obtained from a local grocery store were extracted in 6 mL hexane in a 10-mL glass vial for 2 h. Cowpea seeds infested by *C. maculatus* were first cracked open with a fine knife (at ~17 days after oviposition) to remove developing larvae (usually fourth instar) and frass and then extracted (with the larvae and frass removed) as described for uninfested seeds. Larval frass obtained from bruchid-infested cowpea seeds was removed into clean Petri dishes with a fine brush and stored in a freezer at -20 °C until there was enough for extraction. Approximately 4 g of frass were extracted in 5 mL hexane in a 10-mL glass vial for 2 h. Cowpea seeds and frass were weighed on an AR 2140 Adventurer™ balance (OHAUS Corp., Pine Brook, NJ). Extracts of cowpea seeds and frass were concentrated to 5 mg per µL. Each extract was made in three replicates, dehydrated in 2 g of anhydrous sodium sulfate for 12 h and stored in a freezer at -20 °C until used.

2.3. Electroantennogram (EAG) recordings

The EAG responses of 2-d old mated and unmated female *P. cerealellae* were tested to the following seven stimuli treatments: (i) hexane extracts of uninfested cowpea seeds, (ii) bruchid-infested cowpea seeds, (iii) bruchid larvae (whole body or WB), (iv) larval frass, (v) adult female bruchids (WB), (vi) adult male bruchids (WB), and (vii) hexane control. The EAG techniques used in this study were similar to those previously described by Chen and Fadamiro (2007a,b). Glass capillary (1.1 mm I.D.) filled with 0.1 M KCl solution was used for electrodes. The reference electrode was connected to an isolated head of an adult female *P. cerealellae* while the recording electrode was connected to the cut tip of the antenna (flagellum). Chlorinated silver–silver chloride junctions were used to maintain electrical contact between the electrodes and input to the preamplifier. The analog signal was detected through a probe (INR-II, Syntech®, the Netherlands), captured and processed with a data acquisition controller (IDAC-4, Syntech®), and later analyzed with EAG 2000 (Syntech®) software on a computer. A 10-mL aliquot of each solution (stimulus treatment) was applied to a piece (7 × 40 mm) of filter paper strip (Whatman® no. 1). After allowing for solvent evaporation, the impregnated filter paper strip was inserted into a glass Pasteur pipette (~14 cm in length, Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.) which then constituted an odor cartridge. The control stimulus was a similar pipette containing a filter paper strip impregnated with a 10- μ L aliquot of hexane. The tip of the pipette was placed ~3 mm into a small hole in the wall of a metal tube (13 cm long, 8 mm diameter) which was orientated towards the antennal preparation (~0.5 cm away from the preparation). In this way, the stimuli were provided as 0.2-s puffs of air into a continuous humidified air stream at 1000 mL/min generated by an air stimulus controller (CS-55, Syntech®, the Netherlands). At least 2 min was allowed between successive stimulations for antennal recovery. A test series consisting of the above listed stimuli (treatments) was applied to an antennal preparation starting with the hexane control and following a random order thereafter. Recordings were obtained from at least 16 female *P. cerealellae* of different mating status.

Data were first analyzed by using the standard least-squares fit model method (SAS Institute, 2003) to determine the effects of stimuli (treatments), mating, and interactions of both factors on absolute EAGs. Further analysis of the data was by using one-way ANOVA followed by Tukey's HSD comparison test to compare EAG responses of mated or unmated females to the different stimuli ($P < 0.05$; SAS, 2003). The effect of mating on EAG response to each stimulus (treatment) was then compared by using the Student's *t*-test ($P < 0.05$; SAS, 2003).

2.4. Y-tube olfactometer bioassays

A Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) was used to test the attraction of mated female *P. cerealellae* (2-d old) to the stimuli treatments tested in the EAG experiment. The olfactometer system used in this study has been previously described by Chen and Fadamiro (2007a). The system consists of a central tube (13.5 cm long, 24 mm diam.) and two lateral arms (5.75 cm long, 24 mm diam.), which were separately connected to an extending glass tube (14.5 cm long, 19 mm diam.). There is a sieve inlaid in the extending glass tube 5.25 cm away from the connection to prevent escape of insects and to serve as an end point of each lateral arm. Humidified and purified air was passed from an air pump, into each of the extending arms of the olfactometer at a rate 200 mL/min. To minimize visual distraction for the parasitoids, the Y-tube olfactometer was placed inside a white paper box, which was open on the top (for illumination) and on the front side

(for observation). Illumination was provided by vertically hanging an office lamp (20 W, 250 lux) above (~50 cm high) the olfactometer tube.

The experiment was conducted to test the attractiveness of the following host-related stimuli treatments to mated female *P. cerealellae*: hexane extracts of uninfested cowpea seeds, bruchid-infested cowpea seeds, bruchid larvae (WB), larval frass, adult female bruchids (WB), and adult male bruchids (WB). Only mated females were tested in this experiment based on the results of a preliminary experiment, which showed no significant effect of mating on the behavioral response of female *P. cerealellae* to the tested host-related stimuli (unpublished data). Each stimulus was delivered as a 25- μ L sample placed on No. 1 filter paper strips (7 × 40 mm, Whatman® no. 1). This resulted in 125 larvae or adult equivalents or 125 mg of uninfested, infested or frass per loading (25 μ L of each stimulus was used in the behavioral bioassays based on our preliminary data showing minimal response of female parasitoids to a 10 μ L dose of each stimulus). After allowing for solvent evaporation (~15 s), the filter paper strip was inserted into one arm of the Y-tube olfactometer. A similar filter paper strip containing a 25- μ L aliquot of hexane (laboratory grade) was inserted into the second arm (solvent control). Female parasitoids were individually released at the base of the central arm of the Y-tube and observed for a maximum of 5 min. A parasitoid that did not make a choice within this period was removed, discarded and not included in the analyses. Parasitoids that walked to the end of one of the arms and remained there for at least 10 s were recorded as having made a choice between the treatment and the solvent control. After three individual parasitoids had been tested, a fresh odor stimulus was used and the olfactometer arms were reversed (180°). After each subset of 6 parasitoids had been tested, the olfactometer apparatus was rinsed with soap, water and acetone, and then air-dried. Forty females were tested per choice test, and parasitoids were used only once. Bioassays were conducted at 25 ± 1 °C and $60 \pm 5\%$ r.h.

Data obtained on the percentage responses of mated female parasitoids to each stimulus versus control were analyzed by the use of a chi-square (χ^2) test to determine significant deviation ($P < 0.05$, SAS, 2003) from an expected 1:1 response.

2.5. Four-way olfactometer bioassays

The four most attractive odor stimuli treatments from the Y-tube bioassays were further evaluated in a four-way (choice) olfactometer (Analytical Research Systems, Gainesville, FL) to determine the odor preferences of mated female *P. cerealellae*. The four-way olfactometer system used in this study was similar to the system described by Pettersson (1970) and Kalule and Wright (2004). It consists of a central chamber with orifices at the four corners through which purified and humidified air was drawn in, creating 4 potential odor fields, and a central orifice where mixing of the airflow from the arms occurred. Two of the orifices at the corners were designated for odor stimuli treatments and the other two for controls (i.e. only 2 stimuli treatments were compared at the same time). The two controls were hexane (control 1) and charcoal-filtered, humidified laboratory air (control 2). A constant airflow of 0.25 L/min was maintained through each of the 4 orifices at the corners of the olfactometer. Mixtures of air from the control arms and volatile odors from the treatment arms were sucked-out from the olfactometer with a constant airflow of 1.5 L/min, through the central orifice. Glass tubes (1 × 5 cm) containing the test stimuli or controls were attached with Teflon connectors to each of the 4 arms of the olfactometer. Volatile odors were delivered as a 25- μ L sample (resulting in 125 larvae-equivalents or 125 mg of uninfested, infested or frass per loading) placed on Whatman No. 1 filter

paper strips (7 × 40 mm). After allowing for solvent evaporation (~ 15 s), the filter paper strip was inserted into a glass tube, which was connected to an arm of the four-way olfactometer.

The following four attractive host-related hexane extracts were tested in a pair-wise fashion (binary test) for a total of six paired treatments (Table 1): uninfested cowpea seeds, bruchid-infested cowpea seeds, bruchid larvae (WB), and larval frass. A mated female *P. cerealellae* (2-d old) was introduced singly into a glass tube (1 × 5 cm), which was connected to the central orifice of the olfactometer to expose it to the volatile odors/air mixtures in a relatively limited area. Once in the chamber, a parasitoid was given 5 min to make a choice among the 4 air fields (i.e. 2 treatments and 2 control fields). If the parasitoid had not made a choice within this duration, it was removed, discarded and not included in the analyses. Parasitoids that walked to the end of one of the arms and remained there for at least 10 s were recorded as having made a choice for that stimulus. After 5 individual parasitoids had been tested, a fresh odor stimulus was used and the olfactometer was cleaned as previously described. In order to remove any directional bias in the chamber, the olfactometer was rotated after ten parasitoids had been tested. Each binary test (consisting of 2 treatments and 2 controls) was replicated four times (i.e. 4 rotations of the olfactometer arms) with each replicate consisting of 10 females, resulting in a total of 40 females per binary test. All observations were made at ~25 ± 1 °C, 60 ± 5% r.h. under ambient, incandescent light of ~250 lux.

For each binary comparison, the number of female parasitoids which chose the two control arms was minimal (<20%) and not included in the statistical analyses. Data obtained from all four replicates were pooled and analyzed with chi square (χ^2) to test for significant deviation ($P < 0.05$, SAS, 2003) from an expected 1:1 response of female parasitoids to any two paired treatments.

3. Results

3.1. Electroantennogram (EAG) responses

Standard least-squares modeling revealed significant effects of stimuli treatments ($F_6 = 49.70$, $P < 0.0001$), mating ($F_1 = 15.94$, $P < 0.0001$), and treatment × mating interaction ($F_6 = 3.88$, $P < 0.0001$) on the EAG response of female *P. cerealellae*. Based on the significant mating effect, the effect of stimuli on the EAG response was compared separately for unmated and mated females. All of the stimuli elicited significantly greater EAG responses in mated females compared to the hexane control (Fig. 1). However, extracts of uninfested seeds, infested seeds,

bruchid larvae, and larval frass elicited significantly greater EAG responses than extracts of adult female bruchids and adult male bruchids. Similar results were obtained for unmated females (Fig. 1). In general, mated females showed greater EAG response than unmated females, but this was not always significant. Student's *t*-test showed that extracts of infested seeds ($t = 2.31$; $df = 1, 31$; $P = 0.028$) and bruchid larvae ($t = 3.93$; $df = 1, 31$; $P = 0.0005$) elicited significantly greater EAG responses in mated than in unmated females (Fig. 1). In contrast, whole body extract of adult male bruchids elicited a marginally significantly greater EAG response in unmated than in mated females ($t = -2.10$; $df = 1, 31$; $P = 0.044$). No significant differences were observed in the responses of mated and unmated female parasitoids to extracts of uninfested seeds ($t = 1.15$; $df = 1, 31$; $P = 0.259$), larval frass ($t = 1.78$; $df = 1, 31$; $P = 0.084$) and adult female bruchids ($t = 0.22$; $df = 1, 31$; $P = 0.826$).

3.2. Y-tube olfactometer bioassays

Chi square analyses showed a significant olfactometer response of mated female *P. cerealellae* to extracts of uninfested seeds (90%; $\chi^2 = 12.8$; $df = 1, 19$; $P = 0.0003$), bruchid-infested seeds (85%; $\chi^2 = 9.8$; $df = 1, 19$; $P = 0.0017$), larval frass (89.5%; $\chi^2 = 11.8$; $df = 1, 19$; $P = 0.0006$), bruchid larvae (84.2%; $\chi^2 = 8.9$; $df = 1, 19$; $P = 0.0029$), and adult female bruchids (79%; $\chi^2 = 6.4$; $df = 1, 19$; $P = 0.0116$). However, whole body extract of adult male bruchids did not elicit a significant behavioral response in female parasitoids (65%; $\chi^2 = 1.8$; $df = 1, 19$; $P = 0.179$) (Fig. 2).

3.3. Four-way olfactometer bioassays

The results of the pair-wise comparisons (binary test) of the four most attractive stimuli in a four-way olfactometer bioassay are summarized in Table 1. Mated female *P. cerealellae* did not show significant preferences between extracts of uninfested seeds vs. infested seeds ($\chi^2 = 0.03$, $df = 1, 40$, $P = 0.8527$), uninfested seeds vs. bruchid larvae ($\chi^2 = 0.53$, $df = 1, 39$, $P = 0.4652$), infested seeds vs. bruchid larvae ($\chi^2 = 0.81$, $df = 1, 39$, $P = 0.3692$), infested seeds vs. larval frass ($\chi^2 = 1.13$, $df = 1, 39$, $P = 0.2888$), and bruchid larvae vs. larval frass ($\chi^2 = 0.57$, $df = 1, 38$, $P = 0.4497$). However, when an extract of uninfested seeds was paired with an extract of larval frass, female parasitoids showed significant preference for uninfested seeds (53%) over larval frass (23%) ($\chi^2 = 4.8$, $df = 1, 39$, $P = 0.0285$) (Table 1). Furthermore, there was a consistent trend for a preference for cowpea seeds (uninfested or infested) over bruchid-specific stimuli (bruchid larvae or larval frass), although this was not always significant (Table 1).

4. Discussion

The results demonstrated the electroantennogram and behavioral responses of female *P. cerealellae* to various host-related odor stimuli, as have been reported for several other parasitoid species (Elzen et al., 1983; Turlings et al., 1991; Steinberg et al., 1993; van Huis et al., 1994; Röse et al., 1997; Steidle and Schöller, 1997; Kalule and Wright, 2004; Mbata et al., 2004; Germinara et al., 2004, 2009). The data which showed significant EAG responses of *P. cerealellae* to various host-related stimuli suggest the ability of the parasitoid to detect a broad range of volatiles, as reported for another pteromalid, *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) (Germinara et al., 2009). More interestingly, results from Y-tube bioassays showed significant attraction of female *P. cerealellae* to all tested stimuli, with the exception of whole body extract of adult male bruchids. Further evaluation in a four-way olfactometer demonstrated the preference of female *P. cerealellae*

Table 1
Response of mated female *P. cerealellae* to attractive host-related stimuli in a four-choice olfactometer.

Stimuli source	% reacting to		χ^2	P
	T1	T2		
Uninfested seeds (T1) vs. Bruchids infested seeds (T2)	37.5	35.0	0.03	0.8527
Uninfested seeds (T1) vs. Bruchid larvae (T2)	42.5	32.5	0.53	0.4652
Uninfested seeds (T1) vs. Larval frass (T2)	52.5	22.5	4.80	0.0285*
Bruchids infested seeds (T1) vs. Bruchid larvae (T2)	45.0	32.5	0.81	0.3692
Bruchids infested seeds (T1) vs. Larval frass (T2)	47.5	32.5	1.13	0.2888
Bruchid larvae (T1) vs. Larval frass (T2)	40.0	30.0	0.57	0.4497

Table shows percentage choice of female *P. cerealellae* between two stimuli (T1 and T2) sources.

*Indicates significant difference in percentage females' choice (χ^2 , $P < 0.05$).

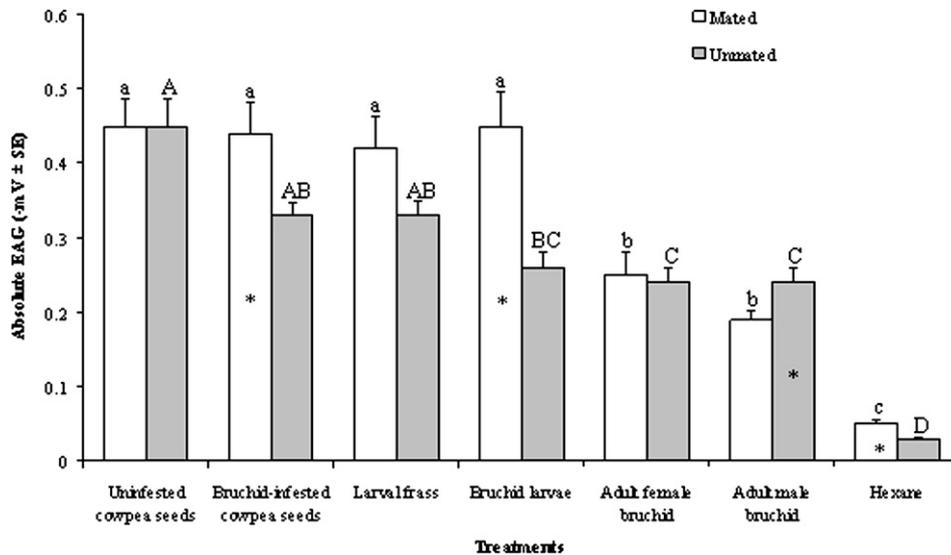


Fig. 1. Electroantennogram (EAG) responses of mated and unmated female *P. cerealellae* to various host-related stimuli treatments. Figure shows mean ($-mV \pm SE$) absolute EAG response of mated (white bars) and unmated (grey bars) female parasitoids to extracts of uninfested cowpea seeds, bruchid (*C. maculatus*)-infested cowpea seeds, and larval frass, and whole body extracts of bruchid larvae, adult female bruchids, and adult male bruchids, as well as the solvent (hexane) control. For each mating group, means accompanied by the same letters (small letters for mated and caps for unmated females) are not significantly different ($P < 0.05$, Tukey's HSD test). White or grey bars containing asterisks (*) indicate a significant difference between responses of mated and unmated parasitoids to the tested stimuli ($P < 0.05$, Student's *t*-test).

for the extract of uninfested cowpea seeds compared to that from larval frass. Overall, these results suggest that semiochemicals from the first trophic level (cowpea seeds) are relatively more important than host bruchid-specific semiochemicals in mediating host location by female *P. cerealellae*.

Females of various parasitoid species, in particular parasitoids of concealed hosts, are known to use volatiles from the habitat (food) of their hosts rather than host-specific semiochemicals for host location (Elzen et al., 1983; Norlund et al., 1988; Tumlinson et al., 1992; Germinara et al., 2004; Kalule and Wright, 2004). For instance, the solitary larval and pupal ectoparasitoid of bruchid beetles, *Eupelmus vuillei* (Crawford) (Hymenoptera: Eupelmidae) (Cortese et al., 1993), an egg parasitoid of *C. maculatus*, *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae) (van Huis

et al., 1994), and the generalist parasitoid, *L. distinguendus* (Steidle et al., 2001) were also attracted to volatiles emanating from uninfested cowpea seeds. Similarly, Germinara et al. (2004) reported on the attraction of another pteromalid wasp, *T. elegans* to volatiles emitted by uninfested wheat kernels.

Several aspects of our results on semiochemical-mediated host location behavior of *P. cerealellae* are in agreement with an earlier report on the same species by Mbata et al. (2004), including observed attraction of female parasitoids to extracts of uninfested and bruchid-infested cowpea seeds and to whole body extract of adult female bruchids. Our results showing greater attraction of female *P. cerealellae* to cowpea seed odor than to host bruchid-specific odor, however, disagree with those of Mbata et al. (2004), which indicated that host bruchid-specific stimuli (e.g., extracts of

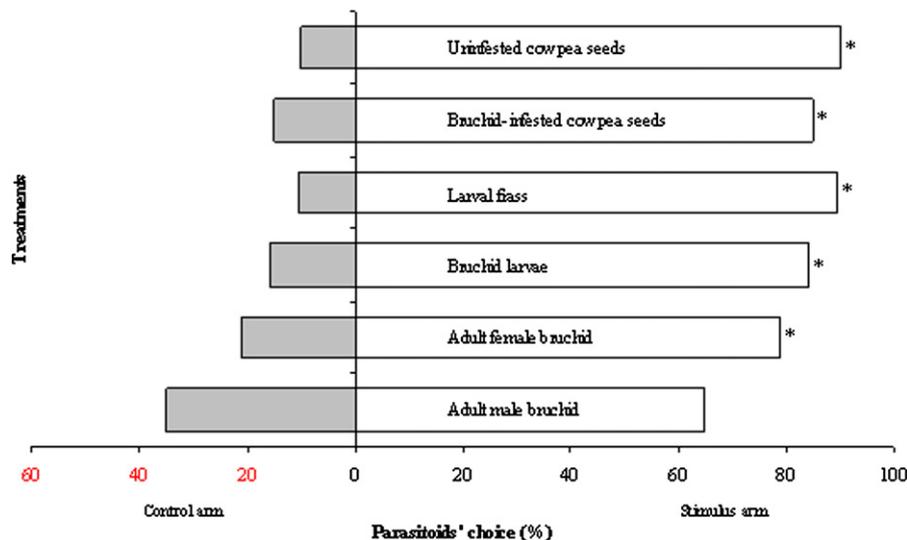


Fig. 2. Response of mated female *P. cerealellae* to various host-related stimuli treatments in a Y-tube olfactometer. Figure shows responses of mated female parasitoids when given a choice between hexane (control) and different host-related stimuli: extracts of uninfested cowpea seeds, bruchid (*C. maculatus*)-infested cowpea seeds, larval frass, bruchid larvae, adult female bruchids, and adult male bruchids. Grey bars indicate the percentage responses to the control; white bars indicate the percentage responses to the tested stimuli. $N = 40$ individuals per choice test. Asterisks (*) indicate significant differences within a choice test ($P < 0.05$, χ^2).

female beetles and infested seeds containing larval frass) were more attractive than extract of uninfested cowpea seeds. Findings similar to those of Mbata et al. (2004) are also common in the literature on parasitoid host location behavior (e.g. Noldus and van Lanteren, 1985; Steinberg et al., 1993; van Huis et al., 1994; Steidle and Schöller, 1997; Eben et al., 2000; Sullivan et al., 2000; Steidle et al., 2001; Reddy et al., 2002). For example, females of *L. distinguendus* were shown to prefer rice and wheat grains infested by stored-grain beetles over uninfested grains (Steidle et al., 2001). Similarly, females of *U. lariophaga* (van Huis et al., 1994), *Rop-trocercus xylophagorum* Ratzeburg (Hymenoptera: Pteromalidae) (Sullivan et al., 2000) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) (Eben et al., 2000) were reported to prefer odors of infested over uninfested hosts' foods. The disparity between some of our results and those reported by Mbata et al. (2004) may be due to differences in experimental design and protocols. For instance, Mbata et al. (2004) made use of head space volatile collection techniques to obtain odors from infested and uninfested cowpea seeds, whereas hexane extracts of infested and uninfested cowpea seeds were tested in the present study.

Attraction of female *P. cerealellae* to whole body extracts of adult female bruchids, as recorded in this study and also by Mbata et al. (2004), is likely mediated by female bruchid sex pheromones (Phillips et al., 1996; Shu et al., 1996). Many parasitoid species, in particular egg parasitoids, are known to use host sex pheromones as host-location cues (i.e. as kairomones) (e.g. Lewis et al., 1982; Noldus and van Lanteren, 1985; Reddy et al., 2002; Mbata et al., 2004). Reddy et al. (2002) demonstrated the attraction of the egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and the larval parasitoid, *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), to the sex pheromones of their host, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Host location in the egg parasitoid, *U. lariophaga* was also reported to be mediated by the host bruchid's sex pheromones (van Huis et al., 1994). Attraction of female *P. cerealellae* to adult female bruchid (*C. maculatus*) may appear illogical (Mbata et al., 2004), given that the mature larvae needed for parasitization may not occur until several weeks after the short-lived adult female bruchids had died.

Contrary to the results of Mbata et al. (2004), which showed greater attraction of female *P. cerealellae* to infested cowpea seed than to uninfested cowpea seeds, we did not record any significant difference in the response of female parasitoids to the two stimuli. Findings similar to ours were reported for female *L. distinguendus*, which also showed no significant preference for bruchid-infested cowpea seeds over uninfested cowpea seeds (Steidle et al., 2001). As a generalist parasitoid, *P. cerealellae* presumably uses general plant odor cues for host location (Steidle et al., 2001). Based on their results, Steidle et al. (2001) proposed a 2-step process for host location by *L. distinguendus*, which may also be applicable to other generalist parasitoids of concealed hosts. First, female parasitoids use general plant (grain) odor cues (host food) to locate their concealed hosts or host habitat, regardless of the plant–host complex. Second, female parasitoids must differentiate between healthy (uninfested) and infested grain seeds. This second step may be accomplished by the use of chemical cues associated with host feces/frass in infested seeds, as demonstrated for *L. distinguendus* on some plant–host complexes (Steidle and Schöller, 1997; Steidle et al., 2001). Our results support the first step regarding the use of general plant odor as host location cues by generalist parasitoids. However, we could not demonstrate the ability of *P. cerealellae* females to differentiate between bruchid-infested and uninfested cowpea seeds using chemical cues. Steidle et al. (2001) also reported that while *L. distinguendus* females showed preference for infested seeds from the complexes rice–*Sitophilus granarius* (L.) and wheat–*Rhy-zopertha dominica* (F.) over uninfested seeds, the parasitoid could

not differentiate between bruchid-infested and uninfested cowpea seeds. The authors proposed that their results may be due to the possible masking of host feces odor in cowpea, compared to rice or wheat. The inability of *P. cerealellae* (this study) and *L. distinguendus* (Steidle et al., 2001) to distinguish between bruchid-infested and uninfested cowpea seeds may suggest that females of both species are primarily attracted to bruchid hosts using cowpea volatiles. Once in the host habitat (a mixture of uninfested and infested cowpea seeds), females may search randomly for infested cowpea seeds (Steidle et al., 2001) or use visual or tactile cues such as those associated with bruchid egg plugs to differentiate between infested and uninfested cowpea seeds. Indeed, we have observed that *P. cerealellae* females drum the tip of their antennae on the surface of cowpea seeds prior to oviposition (unpublished data), possibly as a mechanism to differentiate between infested and uninfested cowpea seeds. It is unlikely that larval vibrations are used as primary cues for this purpose, given a previous study which showed that female *P. cerealellae* were capable of detecting and parasitizing dead (freeze-killed) and living *C. maculatus* larvae alike (Onagbola and Fadamiro, 2009).

These results showed that *P. cerealellae* locates its bruchid host primarily by using innate volatile cues from cowpea seeds, but may use volatile cues associated with adult female bruchids. Further studies are necessary to determine the mechanisms (cues) used by female *P. cerealellae* to distinguish between infested and uninfested cowpea seeds.

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References

- Agelopoulos, N.G., Keller, M.A., 1994. Plant–natural enemy association in the trophic system, *Cotesia rubecula*–*Pieris rapae*–Brassicaceae (Cruciferae). I. Sources of infochemicals. *Journal of Chemical Ecology* 20, 1725–1734.
- Ashmead, W.H., 1902. A new *Catalococcus* on *Sitotroga cerealellae* Oliv. *Psyche* 9, 345.
- Brower, J.H., 1991. Potential host range and performance of a reportedly monophagous parasitoid, *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). *Entomological News* 102, 231–235.
- Brower, J.H., Smith, L., Vail, P.V., Flinn, P.W., 1996. Biological control. In: Subramanyam, B., Hagstrum, D.W. (Eds.), *Integrated Management of Insects in Stored Products*. Marcel Dekker, New York, pp. 223–286.
- Chen, L., Fadamiro, H.Y., 2007a. Differential electroantennogram response of females and males of two parasitoid species to host-related green leaf volatiles and inducible compounds. *Bulletin of Entomological Research* 97, 515–522.
- Chen, L., Fadamiro, H.Y., 2007b. Behavioral and electroantennogram responses of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to red imported fire ant *Solenopsis invicta* odor and trail pheromone. *Journal of Insect Behavior* 20, 267–287.
- Cortese, A.M., Monge, J.P., Huignard, J., 1993. Response of the parasitoid *Eupelmus vuilleti* to the odours of the phytophagous host and its host plant in an olfactometer. *Entomologia Experimentalis et Applicata* 69, 109–116.
- Eben, A., Benrey, B., Sivinski, J., Aluja, M., 2000. Host species and host plant effects on preference and performance of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Environmental Entomology* 29, 87–94.
- Elzen, G.W., Williams, H.J., Vinson, S.B., 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. *Environmental Entomology* 12, 1873–1877.
- Germinara, G.S., Rotundo, G., De Cristofaro, A., 2004. Sostanze volatili dei cereali e localizzazione dell'habitat dell'ospite in *Theocolax elegans*. *Tecnica Molitoria* 55, 324–330.
- Germinara, G.S., De Cristofaro, A., Rotundo, G., 2009. Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae). *Journal of Stored Products Research* 45, 195–200.
- Godfray, H.C.J., 1994. Parasitoids: Behavioral and Evolutionary Ecology. In: *Mono-graphs in Behavior and Ecology*. Princeton University Press, Princeton, New Jersey, 473 pp.
- Hawkins, B.A., 1994. *Pattern & Process in Host–parasitoid Interactions*. Princeton University Press, Cambridge, UK.

- Kalule, T., Wright, D.J., 2004. The influence of cultivar and cultivar-aphid odours on the olfactory response of the parasitoid *Aphidius colemani*. *Journal of Applied Entomology* 128, 120–125.
- Kaschef, A.H., 1964. Further studies of olfaction in *Lariophagus distinguendus* Först (Hymenoptera: Pteromalidae). *Behaviour* 23, 31–42.
- Lecomte, C., Thibout, E., 1993. Pre- and post-imaginal experience in a specialist parasitoid *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Entomophaga* 38, 175–184.
- Lewis, W.J., Nordlund, D.A., Gueldner, R.C., Teal, P.E.A., Tumlinson, J.H., 1982. Kairomones and their use for management of entomophagous insects, XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *Journal of Chemical Ecology* 8, 1323–1331.
- Mbata, G.N., Shu, S., Phillips, T.W., Ramaswamy, S.B., 2004. Semiochemical cues used by *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) to locate its host, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Annals of the Entomological Society of America* 97, 353–360.
- Mbata, G.N., Thomas, A., Fadamiro, H.Y., 2005. Parasitism by *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) on the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae): host density, temperature effects, and host finding ability. *Biological Control* 33, 286–292.
- Noldus, L.P.J., van Lanteren, J.C., 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. & *Mamestra brassicae* L. *Journal of Chemical Ecology* 11, 781–791.
- Noldus, L.P.J., van Lanteren, J.C., Lewis, W.J., 1991. How *Trichogramma* parasitoids use moth pheromones as kairomones: orientation behavior in a wind tunnel. *Physiological Entomology* 16, 313–327.
- Norlund, A.D., Lewis, W.J., Altieri, M.A., 1988. Influence of plant produced allelochemicals on the host and prey selection behaviour of entomophagous insects. In: Barbosa, P., Letourneau, D.K. (Eds.), *Novel Aspects of Insect-Plant Interaction*. John Wiley and Sons, New York, pp. 65–90.
- Onagbola, E.O., Fadamiro, H.Y., Mbata, G.N., 2007. Longevity, fecundity and progeny sex ratio of *Pteromalus cerealellae* in relation to diet, host provision, and mating. *Biological Control* 40, 222–229.
- Onagbola, E.O., Fadamiro, H.Y., 2009. Assessment of frozen larvae of *Callosobruchus maculatus* as host for rearing *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae). *Biological Control* 48, 36–41.
- Pettersson, J., 1970. Studies on *Rhopalosiphum padi* (L.) I. Laboratory studies of olfactometric responses to the winter host *Prunus padus* (L.). *Lantbrukshögskolan Annale* 36, 381–399.
- Phillips, T.W., 1997. Semiochemicals of stored-product insects: research and application. *Journal of Stored Products Research* 33, 17–30.
- Phillips, T.W., Phillips, J.K., Webster, F.X., Tang, R., Burkholder, W.E., 1996. Identification of sex pheromone from cowpea weevil, *Callosobruchus maculatus*, and related studies with *C. analis* (Coleoptera: Bruchidae). *Journal of Chemical Ecology* 22, 2233–2249.
- Reddy, G.V.P., Holopainen, J.K., Guerrero, A., 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28, 131–143.
- Röse, U.S.R., Alborn, H.T., Makranczy, G., Lewis, W.J., Tumlinson, J.H., 1997. Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): role of host- and plant-related volatiles. *Journal of Insect Behavior* 10, 313–330.
- SAS Institute, 2003. *JMP Statistics & Graphics Guide*, Version 5.1 Cary, NC.
- Schöller, M., Prozell, S., Al-Kirshi, A.G., Reichmuth, C., 1997. Towards biological control as a major component of integrated pest management in stored product protection. *Journal of Stored Products Research* 33, 81–97.
- Shu, S., Koepnick, W., Mbata, G.N., Cork, A., Ramaswamy, S.B., 1996. Sex pheromone production in *Callosobruchus maculatus* (Coleoptera: Bruchidae): electro-antennographic and behavioral responses. *Journal of Stored Products Research* 32, 21–28.
- Shu, S., Mbata, G.N., Cork, A., Ramaswamy, S.B., 1999. The chemistry of the sex pheromone of *Callosobruchus subnotatus*. *Journal of Chemical Ecology* 25, 2715–2727.
- Smirnov, E., Polejaeff, W.G., 1937. On the behavior of *Lariophagus distinguendus* Först., a parasitoid of the granary weevil, *Calandra granaria* L. *Zoologiceskij Zhurnal* 16, 999–1012.
- Steidle, J.L.M., Schöller, M., 1997. Olfactory host location and learning in the granary weevil parasitoid, *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Journal of Insect Behavior* 11, 331–342.
- Steidle, J.L.M., Steppuhn, A., Reinhard, J., 2001. Volatile cues from different host complexes for host location by the generalist parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). *Basic & Applied Ecology* 95, 185–192.
- Steidle, J.L.M., Steppuhn, A., Ruther, J., 2003. Specific foraging kairomones used by a generalist parasitoid. *Journal of Chemical Ecology* 29, 131–143.
- Steinberg, S., Dicke, M., Vet, L.E.M., 1993. Relative importance of infochemicals from first to second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *Journal of Chemical Ecology* 19, 47–59.
- Sullivan, B.T., Pettersson, E.M., Seltmann, K.C., Berisford, C.W., 2000. Attraction of the bark beetle parasitoid *Roptocerus xylophagorum* (Hymenoptera: Pteromalidae) to host-associated olfactory cues. *Environmental Entomology* 29, 1138–1151.
- Tumlinson, J.H., Turlings, T.C.J., Lewis, W.J., 1992. The semiochemicals complexes that mediate insect parasitoid foraging. *Agricultural Zoology Reviews* 5, 221–252.
- Turlings, T.C.J., Tumlinson, J.H., Eller, F.J., Lewis, W.J., 1991. Larval damaged plants: source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomologia Experimentalis et Applicata* 58, 75–82.
- van Huis, A., Schutte, C., Cools, M.H., Fanget, P.H., van der Hoek, H., Piquet, S.P., 1994. The role of semiochemicals in host location by *Uscana lariophaga*, egg parasitoid of *Callosobruchus maculatus*. In: Highley, E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds.), *Stored product protection: Proceedings of the 6th International Working Conference on Stored-product Protection*, 17–23 April, 1994, Canberra, Australia. CAB International, Wallingford, Oxon, pp. 1158–1164.
- Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37, 141–172.
- Vet, L.E.M., Wäckers, F.L., Dicke, M., 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. *Netherlands Journal of Zoology* 41, 202–213.
- Vet, L.E.M., Lewis, W.J., Cardé, R.T., 1995. Parasitoid foraging and learning. In: Cardé, R.T., Bell, W.J. (Eds.), *Chemical Ecology of Insects*. Chapman & Hall, New York, pp. 65–101.
- Vinson, S.B., 1991. Chemical signals used by parasitoids. *Redia* 74, 15–42.